BIOCHEMICAL AND PHARMACOLOGICAL POTENTIAL OF *Zea mays* L. (POACEAE), *Stigma maydis* ON ACETAMINOPHEN-MEDIATED OXIDATIVE NEPHROPATHY: *IN VITRO* AND *IN VIVO* ASSESSMENTS

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September, 2016

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Submitted in fulfilment of the requirements for the degree of

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GENERAL ABSTRACT

The kidney is tasked with a number of metabolic functions in the body. In its role as a detoxifier and primary eliminator of xenobiotics, it becomes vulnerable to developing injuries. Currently, over 1 million people in the world are living on either of the renal replacement therapies (RRT). These therapies (dialysis and kidney transplantation) are highly sophisticated and generally unaffordable to the average income class and as such most of the patients of kidney disease are left to die because of non-availability of RRT facilities or their inability to afford it. Phytotherapy has emerged as a viable alternative and is being employed to protect renal function and delay progression of renal pathological conditions into end-stage where the last resort is RRT. *Zea mays* L. (Poaceae), *Stigma maydis* is one of several herbs that have been ethnomedicinally advocated to having the capability to improve renal function. This much touted claim was investigated by evaluating its extracts against acetaminophen (APAP)-mediated oxidative nephropathy using *in vitro* and *in vivo* experimental models.

The *in vitro* study revealed that *Z. mays*, *S. maydis* is well tolerated by HEK293 cells (a human kidney cell line) and significantly ($p<0.05$) inhibited calcium oxalate nucleation crystals with the highest dose exhibiting 93.4% potency. This inhibitory effect of the extract had an overall half maximal concentration (IC_{50}) of 256 μg/mL (R^2= 0.9775) with corresponding significant reduction in the degree of turbidity of the treated crystal solution. While the effect elicited may be attributed to its saponin contents, its overall pharmacological effects in this study could in part also be ascribed to its antioxidant activity. These findings have lent credence to the ethnomedicinal significance of *Z. mays*, *S. maydis* as a candidate for the management of nephrolithiasis and renal dysfunctions.

With nephropathy as one of the complications of diabetes, the inhibitory effect of *Z. mays*, *S. maydis* on the specific activities of carbohydrate metabolizing enzymes (α-amylase and α-glucosidase) was evaluated. The results showed that it exhibited potent and moderate inhibitory potential against *α*-amylase and *α*-glucosidase respectively. The inhibition in each case was concentration-dependent with respective IC_{50} values of 5.89 and 0.93 mg/mL. The extract also remarkably scavenged reactive oxygen species like DPPH and nitric oxide radicals, elicited good reducing power and significant metal chelating attributes. The respective uncompetitive and non-competitive nature of the extract on α-glucosidase and α-amylase activity suggests that the phytoconstituents in the extract either assuage substrate level which facilitated their binding and subsequent inhibition of α-glucosidase or bind to a site other than the active site of α-amylase/α-amylase-substrate complex. Consequently, this will reduce the rate of starch hydrolysis, enhance palliated glucose levels, and thus, lending credence to the hypoglycaemic activity of *Z. mays*, *S. maydis*.

Following OECD guidelines for testing of chemicals and extracts, the safety of consumption of the extract was investigated on key metabolic organs of Wistar rats. It was found that at 5000 mg/kg body weight of the extract, no treatment-induced signs of toxicity, behavioural changes or mortality were observed in the animals. Thus, its median lethal dose was estimated to be above 5000 mg/kg. In the repeated dose toxicity study, treatment with the extract also revealed no significant ($p>0.05$) difference in haematological and clinical biochemistry parameters compared to the control group. Similarly, observations from the cage side produced no treatment-related signs of clinical toxicity and histoarchitectural changes. However, there was significant ($p<0.05$) increase in the body weight (22.31%), exploratory ability (28.68%) as well as white blood cell (71.58%) and platelet counts (63.82%) of the 500 mg/kg extract-treated animals compared with

the control group. These observations are consistent with the non-toxic tendency of the extract and suggest that it may be labelled and classified as practically safe within the doses investigated and period of the study.

The results of the role of *Z. mays*, *S. maydis* extract in hepatic biotransformation of APAP showed that, the APAP-induced significant ($p<0.05$) increases in the activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase and the concentrations of bilirubin, oxidized glutathione, protein carbonyls, malondialdehyde, conjugated dienes, lipid hydroperoxides and fragmented DNA were dose-dependently extenuated following treatment with the extract. The extract also significantly ($p<0.05$) improved the reduced activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase as well as total protein, albumin and glutathione concentrations in the treated hepatotoxic rats. These improvements may be attributed to the bioactive constituents as revealed by the GC-MS analysis of the extract. The observed effects compared favourably with vitamin C and are indicative of hepatoprotective and antioxidative attributes of the extract and were further supported by the histological analysis. The overall data from the study suggest that *Z. mays*, *S. maydis* is capable of preventing and ameliorating APAP-mediated oxidative hepatic damage via enhancement of antioxidant defense systems.

The membrane stabilization and detoxification potential of *Z. mays*, *S. maydis* in APAP-mediated oxidative routs in the kidneys of Wistar rats were evaluated over a 14-day period. Nephrotoxic rats were orally pre- and post-treated with the fraction and vitamin C (reference drug). The data obtained revealed that, the APAP-mediated significant elevations in the serum concentrations of creatinine, urea, uric acid, sodium, potassium and tissue levels of oxidized glutathione, protein oxidized products, lipid peroxidized products and fragmented DNA were

dose-dependently assuaged in the extract-treated animals. The extract also markedly improved creatinine clearance rate, glutathione and calcium concentrations as well as activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase in the nephrotoxic rats. These improvements may be attributed to the antioxidative and membrane stabilization activities of the extract. The observed effects compared favourably with that of vitamin C and are indicative of the ability of the extract to prevent progression of renal pathological conditions and preserve kidney function as evidently supported by the histological analysis. Although, the effects were prominently exhibited in the extract-pretreated groups, the general results from the experiment indicate that the extract could prevent or extenuate APAP-mediated oxidative renal damage via fortification of antioxidant defence mechanisms.

Overall, the results from this research have enriched biochemical and pharmacological evidence supporting the ethnomedicinal use of *Z. mays*, *S. maydis* in the management of renal dysfunctions.

Key terms: Acetaminophen; Antioxidant; Bioactivation; Corn silk; Maize; Oxidative nephropathy; Pharmacology; Phytotherapy; Renal replacement therapy; Toxicology

ETHICAL COMMITTEE APPROVAL

The experiments involving the use of animals in this project were conducted subsequent to the approval of the Ethical Committee on the Use and Care of Animals of the University of the Free State, Bloemfontein, South Africa. An approval number (UFS-AED2015/0005) was issued for the study.

COMPLIANCE STATEMENT

No part of this study in any form has been commercialized. The thesis is meant to be used for information dissemination on the biochemical and pharmacological potentials of *Zea mays* L. (Poaceae), *Stigma maydis* to communities where *Zea mays* is a major staple food, the entire Africa continent and the world at large.

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Promoter's signature Student's signature

DECLARATION

It is hereby declared that this Doctoral degree research thesis submitted by me for the Doctoral degree qualification in BIOCHEMISTRY at the University of the Free State is my independent work and has not previously been submitted by me for qualification at another Institution of higher education. The copyright of this thesis is hereby ceded in favour of the University of the Free State.

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CHAPTER ONE

GENERAL INTRODUCTION

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Healing with medicinal plants is as old as mankind itself. The link between man and his quest for medicines in nature dates back to ancient times, when there were convincing evidences from written documents, monuments, and even original plant medicines (Stojanoski, 1999). Specifically, the oldest written evidence of usage of medicinal plants for preparation of drugs was found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 plants (Kelly, 2009). Awareness of medicinal plants usage is a result of the many years of struggles against illnesses which has prompted man to seek medicines in leaves, roots, barks and other parts of plants (Biljana, 2012). The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of health providers to respond to the challenges that have emerged with the spreading of professional services in enhancement of man's life. Until the advent of iatrochemistry in 16^{th} century, plants had been the source of treatment and prophylaxis for many diseases (Kelly, 2009). This is well exemplified in Africa where medicinal plants have always being an integral part of the healthcare system since time immemorial.

The African traditional medicine may be considered the oldest, and perhaps the most assorted, of all therapeutic systems. Africa is considered to be the cradle of mankind with a rich biological and cultural diversity marked by regional differences in healing practices (Gurib-Fakim, 2006). African traditional medicine in its varied forms is holistic involving both the body and the mind. The traditional healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines, particularly medicinal plants to treat the symptoms (Gurib-Fakim *et al.*, 2010). The sustained interest in traditional medicine in the African healthcare system can be

justified by three major reasons. The first one is inadequate access to allopathic medicines and western forms of treatments, whereby the majority of people in Africa cannot afford access to modern medical care either because it is too costly or there are no medical service providers. Secondly, there is a lack of effective modern medical treatment for some ailments such as malaria and/or HIV/AIDS, which, although global in distribution, disproportionately affect Africa more than other areas in the world. The richness and diversity of the fauna and flora of Africa which are an inexhaustible source of therapies for a good number of ailments is another reason for seeking succor in the traditional system of medicine (Sawadogo *et al.*, 2012). Besides, the decreasing efficacy of synthetic drugs coupled with the contraindications of their usage has also re-awakened global attention on natural medicines in recent years (Biljanaa, 2012).

During the last decades, it has become evident that there exists a plethora of plants with medicinal potential and it is increasingly being accepted that the African traditional medicinal plants might offer potential lead compounds in the drug discovery process. In fact, the developed world has also witnessed an ascending trend in the utilization of complementary or alternative medicine (CAM) particularly herbal remedies (Chintamunnee and Mahomoodally, 2012). While over 80% of the population in Sub-Saharan African countries like Nigeria and South Africa use herbal remedies for their primary healthcare, reports from developed countries such as Canada and Germany revealed that more than 70% of their population have tried CAM at least once (Chintamunnee and Mahomoodally, 2012).

The most common traditional medicine in common practice across the African continent is the use of medicinal plants. In many parts of Africa, medicinal plants are the most easily accessible health resource available to the community. In addition, they are most often the preferred option for the patients. For most of these people, traditional healers offer information, counseling, and

treatment to patients and their families in a personal manner as well as having an understanding of their patient's environment (Aone, 2001). Indeed, Africa is blessed with enormous biodiversity resources and it is estimated to contain between 40,000 and 45,000 species of plant with a potential for development and out of which 5,000 species are used medicinally. This is not surprising since Africa is located within the tropical and subtropical climate and it is a known fact that plants accumulate important secondary metabolites through evolution as a natural means of surviving in a hostile environment (Manach *et al.*, 2004). As a result of her tropical conditions, Africa has an unfair share of strong ultraviolet rays of the tropical sunlight and numerous pathogenic microbes, including several species of bacteria, fungi, and viruses, suggesting that African plants could accumulate chemopreventive substances more than plants from the northern hemisphere. For instance, a recent report has it that of all species of *Dorstenia* (Moraceae) analyzed, only the African species, *Dorstenia mannii* Hook.f, a perennial herb growing in the tropical rain forest of Central Africa contained more biological activity than related species (Abegaz *et al.*, 2004). Nonetheless, the documentation of medicinal uses of African plants and traditional systems is becoming a pressing need because of the rapid loss of the natural habitats of some of these plants due to anthropogenic activities and also due to an erosion of valuable traditional knowledge. It has been reported that Africa has some 216 million hectares of forest, but the African continent is also notorious to have one of the highest rates of deforestation in the world, with a calculated loss through deforestation of 1% per annum (Gurib-Fakim and Mahomoodally, 2013). Interestingly, the continent also has the highest rate of endemism, with the Republic of Madagascar topping the list by 82%, and it is noteworthy that Africa already contributes nearly 25% of the world trade in biodiversity.

Indeed, modern allopathic medicine has its roots in traditional medicine, and it is likely that many important new remedies will be developed and commercialized in the future from the African biodiversity, as it has been till now, by following the leads provided by traditional knowledge and experiences. The extensive use of traditional medicine in Africa, composed mainly of medicinal plants, has been argued to be linked to cultural and economic reasons. This is why WHO encourages African member states to promote and integrate traditional medical practices in their health system (WHO, 2008). Plants typically contain mixtures of different phytochemicals (secondary metabolites) that may act individually, additively, or in synergy to improve health. Indeed, medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of the individual constituents. The combined actions of these substances tend to increase the activity of the main medicinal constituent by speeding up or slowing down its assimilation in the body. Secondary metabolites of plant origin might increase the stability of the active phytonutrients, minimize the rate of undesired adverse effects, and have an additive, potentiating, or antagonistic effect. It has been postulated that the enormous diversity of chemical structures found in these botanicals is not waste products, but unique metabolites involved in the relationship of the organism with the environment. For instance, a single plant may, contain substances that stimulate digestion, has anti-inflammatory compounds (that reduce swellings and pain), has antioxidant and antimicrobial effects (phenolics), natural antibiotics (tannins), diuretic substances (that enhance the elimination of waste products and toxins), and that enhance mood and give a sense of well-being (alkaloids) (Sabiu and Ashafa, 2016; Sabiu *et al.*, 2016). Although some may view the isolation of phytonutrients and their use as single chemical entities as a better alternative and which have resulted in the replacement of plant

extracts' use, nowadays, a view that there may be some advantages of the therapeutic use of crude and/or standardized extracts as opposed to isolated single compound is gaining much grounds in the scientific community.

In spite of these huge potentials and biodiversity, the African continent has only few drugs commercialized globally (Atawodi, 2005). Similarly, despite that, the scientific literature has witnessed a growing number of publications geared towards evaluating the efficacy of medicinal plants from Africa which are believed to have an important contribution in the maintenance of health and in the introduction of new treatments; many plants or plant parts with pharmacological potentials have been neglected or underutilized. This is the peculiar case with *Zea mays* L. (Poaceae), *Stigma maydis* (corn silk).

Hence, highlighting the importance and pharmacological potential of corn silk from the African biodiversity which has tendency to be developed as future phytopharmaceutical to treat and/or manage several debilitating disorders is imperative. Paving way for the potential niche market of the botanical is also of utmost significance.

The choice of *Zea mays* L. (Poaceae), *Stigma maydis*

Corn (*Zea mays* Linnaeus), also known as maize, is a member of the family *Poaceae* or *Gramineae*. It is indigenous to Mesoamerica and was domesticated in Mexico some 9,000 years ago, before spreading throughout the American continents (Khairunnisa *et al.*, 2012). Today, it is cultivated worldwide with significant presence in Africa. Global annual production of corn is approximately 785 million tons, with the largest producer, the United States, producing 42% (IITA, 2016). Africa accounts for 6.5% of the production with the largest producer being Nigeria with nearly 8 million tons, followed by South Africa (IITA, 2016).

All parts of corn are utilized, including the silk (CS). The flowers of corn are monoecious in which the male and female flowers are located in different inflorescences on the same stalk (Khairunnisa *et al.*, 2012). While the male flowers (tassel) at the top of the plant produce yellow pollen, the female flowers produce silks which are situated in the leaf axils. The silks (Figure 1b) are elongated stigmas which look like a tuft of hairs. It is a waste product of corn cultivation and available in abundance (Maksimović *et al.*, 2004). The colours at first are usually light green and later turn into red, yellow or light brown depending on the variety. The CS functions primarily to trap pollens for pollination. Each silk is approximately 0.3 m long with a faintly sweetish taste and may be pollinated to produce one kernel of corn. For medicinal purpose CS is harvested just before or after pollination and can be used in fresh or dried form (Figures 1b-1d).

(a)

(b) (c) (d)

Fig. 1: (a) *Zea mays* L. (Poaceae) plant showing the corn with the *Zea mays* L. (Poaceae), *Stigma maydis* (corn silk), (b) Freshly harvested corn silk, (c) Dried corn silk and (d) Powdered corn silk.

Previous reports have lent scientific credence to diverse pharmacological significance of CS and majority of these have been from the Asian continent. Some of these are presented in Tables 1a and 1b.

Table 1a: *In vitro* pharmacological potentials of *Zea mays* L., *Stigma maydis*

Biological activity	Description	Remark(s)	Reference(s)
Anti-glycation effect	Inhibition of AGE formation assay in 80% methanolic extract.	Inhibit non-enzymatic glycation.	(Farsi *et al.*, 2008)
Anti-inflammatory effect	Endothelial-monocyte adhesion assay, molecule expression, treatment of TNF-mediated cytotoxicity, LPS-induced TNF released were evaluated in chloroform, ethyl acetate, butanol and water extract.	Ethanol extract inhibits the expression of intercellular adhesion molecule 1 (ICAM-1) and adhesiveness of endothelial cells.	(Habtenariam, 1998)
	COX-2 determination was conducted on macrophages treated with CS and PGE2 production was determined with PGE2 enzyme immunoassay kit.	CS stimulated COX-2 and secretion of PGE2.	(Kim *et al.*, 2005)
Neuroprotective effect	Acetylcholinesterase (AChE) and butrylcholinesterase (BChE) inhibitions assay were carried out in ethyl acetate extract and ethanol extract	Ethyl acetate extract of *Z. mays* var. intendata strongly inhibit AChE and ethyl acetate extract of *Z. mays* var. everta strongly inhibit BChE	(Kan *et al.*, 2011)
Antioxidant	Methanolic extract of CS were evaluated for antioxidant capacity by lipid peroxidation inhibition in liposomes induced by Fe^{2+}/ascorbate system	Antioxidant activity from matured CS is higher than immature CS.	(Maksimovic and Kovacevic, 2003)
Hypoglycemic effect	CS on glucose uptake by isolated rat hemi-diaphragm	Significant glucose uptake activity	(Ghada *et al.*, 2014)

Table 1b: *In vivo* pharmacological potentials of *Zea mays* L., *Stigma maydis*

Biological activity	Description	Remark(s)	Reference(s)
Antioxidant activity	Exercise induced oxidative stress in mice treated for 28 days	Antioxidant activity against oxidative stress during acute exercise	(Hu and Deng, 2011)
	γ-Radiation induced oxidative stress in mice treated for 10 days.	Antioxidant activity against γ-radiation	(Bai *et al.*, 2010)
Anti-inflammatory effects	Carragenin-induced pleurisy rats were administered orally with CS for 6 h.	Inhibit inflammatory response	(Wang *et al.*, 2012)
Anti-diabetic effect	Streptozotocin-induced diabetic rats were treated intragastrically with polysaccharides from CS for 4 weeks	Shows anti-diabetic activity	(Zhao *et al.*, 2012)
	Adrenaline-induced hyperglycemic mice treated orally with CS extract for 14 and 45 days.	Reduction of blood glucose levels.	(Guo *et al.*, 2009)
Anti-hyperlipidemic effect	Hyperlipidemic rats were treated with CS extract for 20 days	Shows anti-hyperlipidemic effect	(Khairunnisa *et al.*, 2012)
Anti-depressant activity	FST (forced swimming test) and TST (tail suspension test) carried out on 10 male Swiss mice for 6 and 5 min, respectively, 1h after treated with CS extract.	Exhibited anti-depressant activity.	(Ebrahimzadeh *et al.*, 2009)
	Activity times of CS treated mice (normal and diabetic mice) in a black box were observed	Exhibited anti-depressant activity.	(Zhao *et al.*, 2012)
Anti-fatigue activity	Swimming exercise carried out by 10 mice after administration of flavonoid CS for 14 days and loaded with 5% of its body wt. of galvanized wire.	Strong anti-fatigue activity.	(Hu *et al.*, 2010)
Diuresis and kaliuresis effect	Wistar rats were administered with CS extract by orogastric catherer and continuous urine collection for 3 and 5 h.	Exhibition of diuretic and kaliuretic effect	(Velazquez *et al.*, 2005)

Despite the enormous and longstanding ethnomedicinal potentials of CS, its pharmacological significance is just receiving concerted attention in Africa. Lamentably, in Nigeria and South Africa, where corn is a major and common staple food, CS remains hugely underutilized and its therapeutic importance is still significantly unexploited. In view of the foregoing and coupled with the ethnopharmacological advocation of CS in the treatment of kidney disorders, the present study was conceptualized to enrich biochemical information and lend scientific credence to the therapeutic use of CS in oxidative nephropathy.

AIM AND OBJECTIVES OF THE STUDY

The overall aim of this study was to evaluate the biochemical and pharmacological potentials of *Zea mays* L., *Stigma maydis* in acetaminophen-mediated oxidative nephropathy using *in vitro* and *in vivo* methods. The specific objectives were:

(a) Phytotherapy in the management of kidney disorders in Nigeria and South Africa.

Globally, folkloric medicine has been and is still finding relevance in providing preventive and palliative measures against kidney diseases. Phytotherapists with keen interest in renal disorders and those informed of the unawareness and ignorance of majority of the victims are striving not only to educate the world on this 'silent killer' but also proffer suitable solutions to its daunting challenges. Notably, reviews from India and some other Asian countries on nephroprotective medicinal plants revealed appreciable success and have implicated more than 300 plants with remarkable effects against various forms of renal disorder. While previous reports (Ajith *et al.*, 2008; Palani *et al.*, 2009), have demonstrated therapeutic efficacy of *Zingiber officinale* and *Pimpinella tirupatiensis* on drug-induced nephrotoxicity and oxidative stress, others have reported pharmacological significance of medicinal plants in annihilating oxidative insults on

renal tubular cells and preserving overall kidney functions (Gaikwad *et al.*, 2012; Mohana *et al.*, 2012; Talele *et al.*, 2012; Jaya, 2013; Raja *et al.*, 2014).

In Nigeria and South Africa, traditional systems of medicine are also not wanting in offering formulations/drugs for different forms of kidney diseases (Musabayane *et al.*, 2007; Al-Qattan *et al.*, 2008; Usman *et al.*, 2009). Some of the identified renoprotective herbs in these countries are being embraced by physicians, and have been demonstrated to potentiate significant diuretic and antioxidant effect against known toxicants (Simpson, 1998; Kadiri *et al.*, 2015). Plants such as *Carica papaya*, *Vernonia amygdalina*, *Citrullus colocynthis*, *Psidium guajava* and *Ficus mucuso* are commonly cultivated in these countries and have been reported to have nephroprotective capability on the kidneys.

Although, evidences have given credence to nephrophytotherapy in complementary and alternative system of medicine in Nigeria, the concept is just emerging in South Africa. Hence, effort was made in this study to compile a comprehensive list of some selected plants being used as nephroprotective agents in Nigeria and those currently exploited in South Africa.

(b) *In vitro* cytotoxicity, nephroprotective and anti-nephrolithiasis studies

The kidney is tasked with a number of metabolic functions and receives approximately 20% of the cardiac output. In its role as a detoxifier and primary eliminator of xenobiotics, it becomes vulnerable to developing injuries (Schnellmann and Kelly, 1999). Although the most common manifestation of nephrotoxicity is renal failure, the cellular and subcellular targets of toxicity and molecular mechanisms of toxicity varies from agent to agent. For instance, acetaminophen (APAP) nephrotoxicity has been well studied and is characterized by morphologic and functional evidence of proximal tubular injury in humans and experimental animals (Cekmen *et al.*, 2009). Since proximal tubules are the most common site of injury by drugs, screening and

understanding the proximal tubule toxicity effect of drugs is critical in drug discovery. Information on mechanisms of toxicity will further guide structure-activity relationships and minimize risks of clinical renal damage. The use of cells derived from proximal tubules of kidneys like HEK293 is one of the *in vitro* approaches of screening in cytotoxicity assays.

Besides renal toxicity, nephrolithiasis (kidney stones) is another challenge consistent with the kidneys. It is characterized by formation of small, hard, crystalline deposits of mineral and acid salts within the kidney. It is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical, malnutritional (hyperuricosuria), poor diet and genetic risk factors (Rodgers, 2006). Reports have also estimated 1 in every 20 individuals as victim of kidney stones at some point in their life (Bashir and Gilani, 2009; Tyagi *et al.*, 2012). Globally, dietary modification coupled with surgical operation, extracorporeal lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are expensive and recurrence is also common (Prasad *et al.*, 2007). Furthermore, the recurrence rate without preventive treatment ranges from 15-50% within 1 to 10 years (Basavaraj *et al.*, 2007). Despite that various therapies are being used to prevent recurrence, scientific evidence for their efficacy is still less convincing and expected to be nearly 50% (Knoll, 2007; Bashir and Gilani, 2009).

In the traditional systems of medicine worldwide, plants and plant-derived products have proven potential in stemming the recurrence rate of renal calculi with minimal side effects. Corn silk (CS) is one of those herbs that have been advocated in this respect.

Consequent upon the foregoing and couple with the fact that significant number of people in sub-Sahara African countries like Nigeria and South Africa are living on either of the renal replacement therapies (dialysis and transplantation) which are highly sophisticated and expensive

for the average income class, the present study was designed. We evaluated the cytotoxicity and mitigative effects of CS on APAP-induced toxicity in HEK293 cells. The anti-nephrolithiasis potential of CS extract was also investigated.

(c) *In vitro* antioxidant and antidiabetic evaluation

Diabetes mellitus (DM) is a complicated metabolic disorder that has gravely troubled human health and the overall quality of life. It is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago and expressively becoming the third greatest threat to human health after cancer, cerebrovascular and cardiovascular diseases (Vasim *et al.*, 2012). Although, DM is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycemia, glucose intolerance, insulin resistance, and relative insulin deficiency (Mohan *et al.*, 2007), its complications are usually attributable to either microvascular (retinopathy, neuropathy, and nephropathy) or/ and macrovascular (heart attack, stroke and peripheral vascular) ailments (Umar *et al.*, 2010). In 2012, an estimated 1.5 million deaths were directly linked to DM and more than 80% of this occurred in developing countries (Vasim *et al.*, 2012). Recent reports also estimated its global prevalence to be 9.0% among adults and it is projected to be well above 15% before 2025 (Saravanan and Pari, 2015). The geometrical increases in the number of diabetics cannot be divorced from unhealthy life style, urbanization, aging and deleterious impact of free radicals (Wild *et al.*, 2004). Since free radicals have been implicated in the pathogenesis of DM, one of the logical approaches to manage its potential burden may be via antioxidant application. Antioxidants have been shown to prevent destruction of pancreatic β-cells by inhibiting auto-oxidation chain reaction, thereby halting progression to diabetes complications (Sabu and Kuttan, 2004; Liu *et al.*, 2007). Due to their enormous antioxidant and pharmacological significance, medicinal plants are being extensively

explored as therapeutic modality of choice against diabetes (Campos *et al.*, 2003; Aslan *et al.*, 2010). Besides ample advocation by the World Health Organization (WHO) on the relevance of botanicals to manage and treat DM (Wild *et al.*, 2004), the overall increased admiration of phytotherapy for this disorder may be due to the limited efficacy and undesirable adverse effects associated with the orthodox antidiabetic drugs (Marles and Farnsworth, 1994). Interestingly, studies have demonstrated the pharmacological significance of CS as an oral antidiabetic and hypoglycemic agent (Farsi *et al.*, 2008; Zhao *et al.*, 2012), but there is dearth of information on its exact mechanism of action. Hence, this part of the study sought to evaluate the tentative *in vitro* α-amylase and α-glucosidase inhibitory potentials of CS. The antioxidant activities of the herb to consolidate its much touted pharmacological attributes were also evaluated. Beyond evaluating the kinetics of carbohydrate metabolizing enzyme inhibitory potential of CS, it is noteworthy that nephropathy is one of the secondary complications of DM.

(d) *In vivo* toxicological assessments

Medicinal plants offer unlimited opportunities for the discovery of new lead drugs. Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities. In Africa, about 80% of the population depends on this system of medicines for their primary healthcare and is also currently catering for over 30% of healthcare needs of many rural dwellers globally, suggesting its pivotal role in the healthcare and pharmaceutical industries (Adeneye, 2014). More specifically, tropical and subtropical Africa is endowed with approximately 45,000 species of plant with developmental potentials, out of which 5000 species are used medicinally (Jean *et al.*, 2014). The decreasing efficacy of synthetic drugs, non-affordability and the increasing contraindications of their application is an issue of major concern that has also re-awakened peoples' interest on natural medicines in recent years (Sabiu *et al.*,

2015). However, there is paucity of information on the possible toxicity that many of these botanicals may elicit on the overall well-being of key metabolic organs and tissues when administered on humans or experimental animals (Dias and Takahashi, 1994). With drug discovery and development in focus, concerns of all stakeholders including regulatory authorities, healthcare professionals, pharmaceutical companies, patients, and general public with reference to biosafety need to be taken into consideration (Olejniczak *et al.*, 2001). Although there has been considerable success in literatures on medicinal plants and their pharmacological relevance, most of them have never been subjected to exhaustive toxicological tests as normally done for modern pharmaceutical compounds. This might be adduced to common belief that they are safe and toxicity free. However, recent and emerging evidence-based research findings are refuting these anecdotal claims with preclinical and clinical evidence of toxicities being presented to strengthen the counter-claims. Furthermore, lack of standardization and adulteration are other common public health challenges militating against realization of the full potential of phytotherapy. Thus, subjecting medicinal plants and their active metabolites to thorough toxicological evaluation is imperative to ascertaining their therapeutic and pharmacological significance.

More importantly, besides bringing the value of the evaluated agent in terms of safety and efficacy to the global limelight, toxicological screening is a crucial step to standardizing, labelling and classifying plant-derived pharmacological agents. It may also provide comprehensive information on whether a new drug should be adopted for clinical use or not.

In a bid to providing detailed toxicological data as well as pharmacologically label and classify CS, this part of the study was dedicated to evaluating both acute and 28-day repeated dose oral administration of CS on key metabolic markers in Wistar rats.

(e) Hepatic biotransformation of acetaminophen and the role of *Zea mays* L., *Stigma maydis*

Acetaminophen (paracetamol, N-acetyl-*p*-aminophenol; APAP) is a widely used over-the-counter analgesic and antipyretic drug (Bessems and Vermeulen, 2001). At its therapeutic doses, it is well tolerated, believed to be safe, with significant analgesic and antipyretic effects similar to those of aspirin and ibuprofen (James *et al.*, 2003).

APAP was originally introduced as an analgesic by von Mering in 1893, but was not widely used until the 1960s, following the recognition that the structural analog, phenacetin was nephrotoxic in chronic abusers (Hinson *et al.*, 2002). More recently, concern about aspirin-mediated gastrointestinal bleeding and Rye's syndrome has further increased its popularity. Although considered safe at therapeutic doses, at higher doses, APAP produces a centrilobular hepatic necrosis that can be fatal. The mechanism involved a complex sequence of events. These events include:

(i) Cytochrome P_{450} mediates metabolism of APAP to N-acetyl-*p*-benzoquinone imine (NAPQI) which depletes glutathione (GSH) and covalently binds to proteins;

(ii) Loss of GSH with an increased formation of reactive oxygen and nitrogen species in hepatocytes undergoing necrotic changes;

(iii) Increased oxidative stress, associated with alterations in calcium homeostasis and initiation of signal transduction responses, causing mitochondrial permeability transition;

(iv) Mitochondrial permeability transition occurring with additional oxidative stress, loss of mitochondrial membrane potential, and loss of the ability of the mitochondria to synthesize ATP; and

(v) Loss of ATP which leads to necrosis.

Previous studies have established the role of metabolism in APAP hepatotoxicity in animals (Mitchell *et al.* 1973; Potter *et al.* 1989). Their findings showed that APAP was converted by drug metabolizing enzymes to a reactive metabolite that covalently bound to proteins. At nontoxic doses, the metabolite was efficiently detoxified by GSH forming an APAP-GSH conjugate (Mitchell *et al.* 1973). However, at toxic doses, the metabolite depleted hepatic GSH by as much as 80–90% and subsequently covalently bound to protein. The amount of covalent binding correlated with the relative hepatotoxicity.

Subsequently, the reactive metabolite was identified to be N-acetyl-*p*-benzoquinone imine (NAPQI). It was found to be formed by cytochrome P_{450} (CYP_{450}) by a direct two electron oxidation of APAP. The CYP isoforms important in acetaminophen metabolism have been shown to be CYP2E1, CYP1A2, CYP3A4, and CYP2D6 (Bourdi *et al.*, 2002). Reaction of NAPQI with GSH occurs by conjugation to form 3-glutathion-*S*-yl-APAP and by reduction to APAP (Dahlin *et al.*, 1984). The second order rate constant for the reaction of NAPQI with GSH was found to be $3.2 \times 10^4\ M^{-1}\ s^{-1}$. Moreover, the reaction could be catalyzed by glutathione transferase, and NAPQI is one of the best substrates ever described for this enzyme (Coles *et al.* 1988). Thus, detoxification of NAPQI is extremely rapid, and the rapid rate may explain why covalent binding to proteins was not observed in hepatocytes until GSH was almost completely depleted (Mitchell *et al.* 1973).

Although, the events that produce hepatocellular death following the formation of APAP-protein adducts may be poorly understood, one possible mechanism of cell death is that covalent binding to critical cellular proteins results in subsequent loss of activity or function and eventual cell

death and lysis. Primary cellular targets have been postulated to be mitochondrial proteins, with resulting loss of energy production, as well as proteins involved in cellular ion control (Nelson, 1990). Alterations of plasma membrane ATPase activity following toxic doses of APAP have also been reported (Tirmenstein and Nelson, 1989). A number of proteins bound to acetaminophen have been isolated and identified (Cohen and Khairallah, 1997). In addition, loss of mitochondrial or nuclear ion balance has also been suggested to be a toxic mechanism involved in APAP-mediated cell death since either of these losses can lead to increases in cytosolic Ca^{2+}concentrations, mitochondrial Ca^{2+}cycling, activation of proteases and endonucleases, and DNA strand breaks (Salas and Corcoran, 1997). The effect of the addition of NAPQI on isolated mitochondria has been reported (Weis *et al.*, 1992) and inhibition of mitochondrial respiration has been investigated as an important mechanism in APAP toxicity (Donnelly *et al.*, 1994).

Furthermore, several studies have demonstrated the role of macrophage activation in APAP toxicity. Kupffer cells are the phagocytic macrophages of the liver. When activated, Kupffer cells release numerous signaling molecules, including hydrolytic enzymes, eicosanoids, nitric oxide, and superoxide. Kupffer cells may also release a number of inflammatory cytokines, including IL-1, IL-6, and TNF-, and multiple cytokines are released in APAP toxicity (Bourdi *et al.*, 2002). Laskin *et al.* (1995) examined the role of Kupffer cells in acetaminophen hepatotoxicity by pretreating rats with compounds that suppress Kupffer cell function and found that rats pretreated with these compounds were less sensitive to the toxic effects of APAP. This study suggested a critical role for Kupffer cells in the development of APAP hepatotoxicity. However, the report of Ju *et al.* (2002) came to a different conclusion with the number of Kupffer cells in the liver only partially decreased following pretreatment of mice with

gadolinium chloride. Consistent with previous studies, they showed decreased APAP toxicity in the pretreated animals. However, treatment of mice with dichloromethylene diphosphonate completely depleted the liver Kupffer cells, but toxicity was increased and these have raised questions relative to the importance of Kupffer cells in APAP toxicity.

Oxidative stress is another mechanism that has been postulated to be important in the development of APAP toxicity. Thus, increased formation of superoxide would lead to hydrogen peroxide and peroxidation reactions by Fenton-type mechanisms. It has been shown that NAPQI reacts very rapidly with GSH, and there are a number of potential mechanisms that have been suggested to play a role. Under conditions of NAPQI formation following toxic APAP doses, GSH concentrations may be very low in the centrilobular cells, and the major peroxide detoxification enzyme, GSH peroxidase, which functions very inefficiently under conditions of GSH depletion, is expected to be inhibited. In addition, during formation of NAPQI by cytochrome P450, the superoxide anion is formed, with dismutation leading to hydrogen peroxide formation (Dai and Cederbaum, 1995). Also, others have suggested that peroxidation of APAP to the semiquinone free radical would lead to redox cycling between the APAP and the semiquinone. This mechanism may lead to increased superoxide ion formation and toxicity (de Vries, 1981). However, it has been established that the semiquinone reacted rapidly to form polymers and no evidence for reaction of oxygen was observed (Potter *et al.*, 1989). Many reports have pointed to the potential involvement of oxidative stress in APAP toxicity. Nakae *et al.* (1990) reported that administration of encapsulated superoxide dismutase decreased the toxicity of APAP in rats. In addition, the iron chelator, deferoxamine, has been reported to decrease toxicity in rats (Sakaida *et al.*, 1995). Schnellmann *et al.* (1999) have also showed that deferoxamine delayed the rate of development of APAP toxicity in mice, but after 24 h, the

relative amount of toxicity was not affected. These data suggest that an iron-catalyzed Haber-Weiss reaction may also play a role in the development of oxidant stress and injury.

Similarly, immunohistochemical analysis of liver of APAP-treated animals have indicated that nitration occurred in the same cells that contained APAP adducts and developed necrosis (Hinson *et al.*, 1998). This was postulated to have involved a reaction between peroxynitrite and tyrosine that consequently formed nitrotyrosine. Nitration of tyrosine has been shown to be an excellent biomarker of peroxynitrite formation (Kaur and Halliwell, 1994). It is formed by a rapid reaction between nitric oxide and superoxide, and studies have shown increased level of NO synthesis (serum levels of nitrate and nitrite) in APAP toxicity (Hinson *et al.*, 1998). In addition, Gardner *et al.* (1998) have also reported the induction of hepatic iNOS (inducible nitric oxide synthase) in APAP-treated rats. NO and superoxide react to produce peroxynitrite (ONOO*). ONOO* is a species that not only leads to the nitration of tyrosine but is also a potent oxidant that can attack a wide range of biological targets, and under conditions of reduced cellular oxidant scavenging capability, it is highly toxic (Beckman and Koppenol, 1996). Oxidation of important cellular macromolecules (lipids, proteins, or DNA bases) may occur in the phase of ONOO* capacitation. Moreover, it is normally detoxified by GSH or GSH peroxidase, and GSH is depleted in APAP toxicity. Thus, a normal detoxification mechanism for peroxynitrite is impaired. Also, even though acetaminophen itself will detoxify peroxynitrite, the drug is metabolized rapidly in the mouse and concentrations are low at the time when nitration is observed. Similarly, nitrite may be oxidized by heme or free metals, leading to the NO_2 radical (Thomas *et al.*, 2002). Summarily, when the hepatic NO is increased, the superoxide preferentially reacts to form peroxynitrite, which nitrates proteins. In the absence of NO,

superoxide leads to lipid peroxidation. These data indicate the importance of NO in the disposition of superoxide, leading to oxidative stress.

Mitochondrial dysfunction may be another important mechanism in APAP-induced hepatotoxicity. It is known that mitochondrial permeability transition (MPT) occurs with formation of superoxide, and this may be the source of superoxide leading to peroxynitrite and tyrosine nitration. MPT represents an abrupt increase in the permeability of the inner mitochondrial membrane to small molecular weight solutes. Oxidants such as peroxides and peroxynitrite, Ca^{2+}, and Pi promote the onset of MPT, whereas Mg^{2+}, ADP, low pH, and high membrane potential oppose onset. Associated with the permeability change is membrane depolarization, uncoupling of oxidative phosphorylation, release of intramitochondrial ions and metabolic intermediates, and mitochondrial swelling. Studies have shown that addition of NAPQI to isolated rat liver mitochondria caused a decrease in synthesis of ATP and an increase in release of sequestered Ca^{2+}. This release was blocked by cyclosporin A (Weis *et al.*, 1992). This submission is consistent with the hypothesis that NAPQI causes MPT (Palmeira and Wallace, 1997). This is presumably a result of NAPQI-mediated oxidation of the vicinal thiols at the MPT pore. NAPQI is known to be both an oxidizing agent and an arylating agent, and reports have implicated APAP in the oxidation of protein thiols (Tirmenstein and Nelson, 1989). Similarly, Beales and McLean (1996) have also reported that inhibitors of MPT decrease APAP toxicity in rat liver. Hence suggestive of that fact that NAPQI toxicity is mediated by mitochondrial dysfunction resulting in production of reactive oxygen/ nitrogen species.

The summary of the overall involvement of NAPQI in APAP-mediated hepatotoxicity is presented in Figure 2.

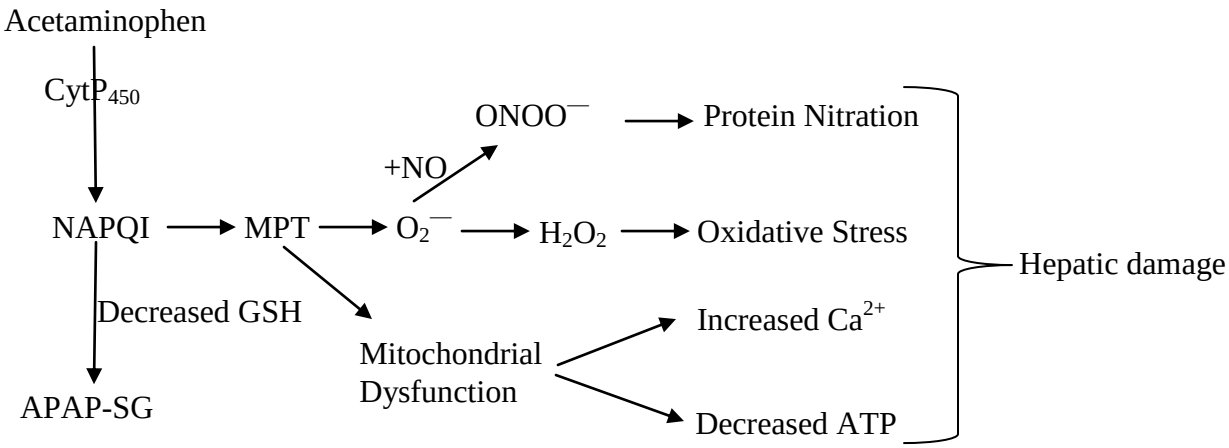

Fig. 2: Acetaminophen bioactivation and the role of NAPQI in hepatic toxicity

Source: James *et al.* (2003).

Bearing the sequence of the events involved in hepatic bioactivation of APAP, this aspect of the study was dedicated to determining the probable role that CS may play in preventing or ameliorating APAP-mediated hepatotoxicity. Thus, the study evaluated the capability of *Zea mays* L. (Poaceae), *Stigma maydis* in preventing and extenuating acetaminophen-perturbed oxidative onslaughts in rat hepatocytes.

(f) Membrane stabilization and *in vivo* nephroprotective activity

The kidney is a highly specialized organ that maintains the body's homeostasis by selectively excreting or retaining various substances according to specific body needs. In its role as a detoxifier and primary eliminator of xenobiotics, it becomes vulnerable to developing injuries. Such injuries have been linked with NAPQI and ROS mediated oxidative stress on renal biomolecules (Ozbek, 2012). Although, the formation of NAPQI by cytochrome P_{450} causes centrilobular necrosis of the liver, NAPQI has also been studied to damage the kidney medulla, which contains low levels of cytochrome P_{450} but relatively high levels of prostaglandin H

synthase (PHS). Hence, PHS may play a significant role in the nephrotoxicity of acetaminophen. The two-electron oxidation of APAP to *N*-acetyl-benzoquinoneimine by PHS has been presumed to involve formation of a one-electron oxidation product (*N*-acetyl-benzosemiquinoneimine radical). Formation of this semiquinoneimine radical by PHS could contribute to the nephrotoxicity of APAP and related compounds, such as phenacetin and 4-aminophenol. PHS can convert aromatic amines to reactive radicals, which can undergo nitrogen–nitrogen or nitrogen–carbon coupling reactions, or they can undergo a second one electron oxidation to reactive diimines. Binding of these reactive metabolites to DNA is presumed to be the underlying mechanism by which several aromatic amines cause renal cancer in humans and dogs. The unified metabolic events involving NAPQI hepatic and renal toxicity is shown in Figure 3.

The response of the kidney to toxicants varies by multiple morphological patterns beginning with tubular or interstitial changes to nephropathy (Silva, 2004). Kidney disorders account for 1 in 10 deaths, making Chronic Kidney Disease (CKD) one of the most sought after public health concerns in recent years (WHO, 2014). The prevalence of the disease is more disconcerting in sub-Saharan Africa countries like Nigeria and South Africa with an estimation of 23% and 40%, respectively (WHO, 2014; NKFS, 2015). Till date, orthodox management therapies for kidney disorders have been embraced and identified to include the use of renal replacement therapy (dialysis and transplantation), applications of angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs) and erythropoietin to slow the progression of loss of kidney function (Ahmad *et al.*, 2014). The affordability, sensitivity, and inherent adverse effects of the aforementioned therapies have undermined their applications in the past. The availability of kidneys for transplantation and cost are other important challenges consistent with renal

replacement therapy (WHO, 2005). Interestingly, traditional systems of medicine have offered effective drugs against kidney pathological conditions and thus can be used to protect renal function and prevent/slow the progression of renal diseases to CKD or end stage renal disease (Naveed *et al.*, 2014). A number of drugs from herbal sources have been shown to be nephroprotective and there is a keen global interest on the development of such. The focus is mostly to protect or prevent injurious insults to the kidney as well as enhance the regeneration of tubular cells (Hamid and Mahmoud, 2013).

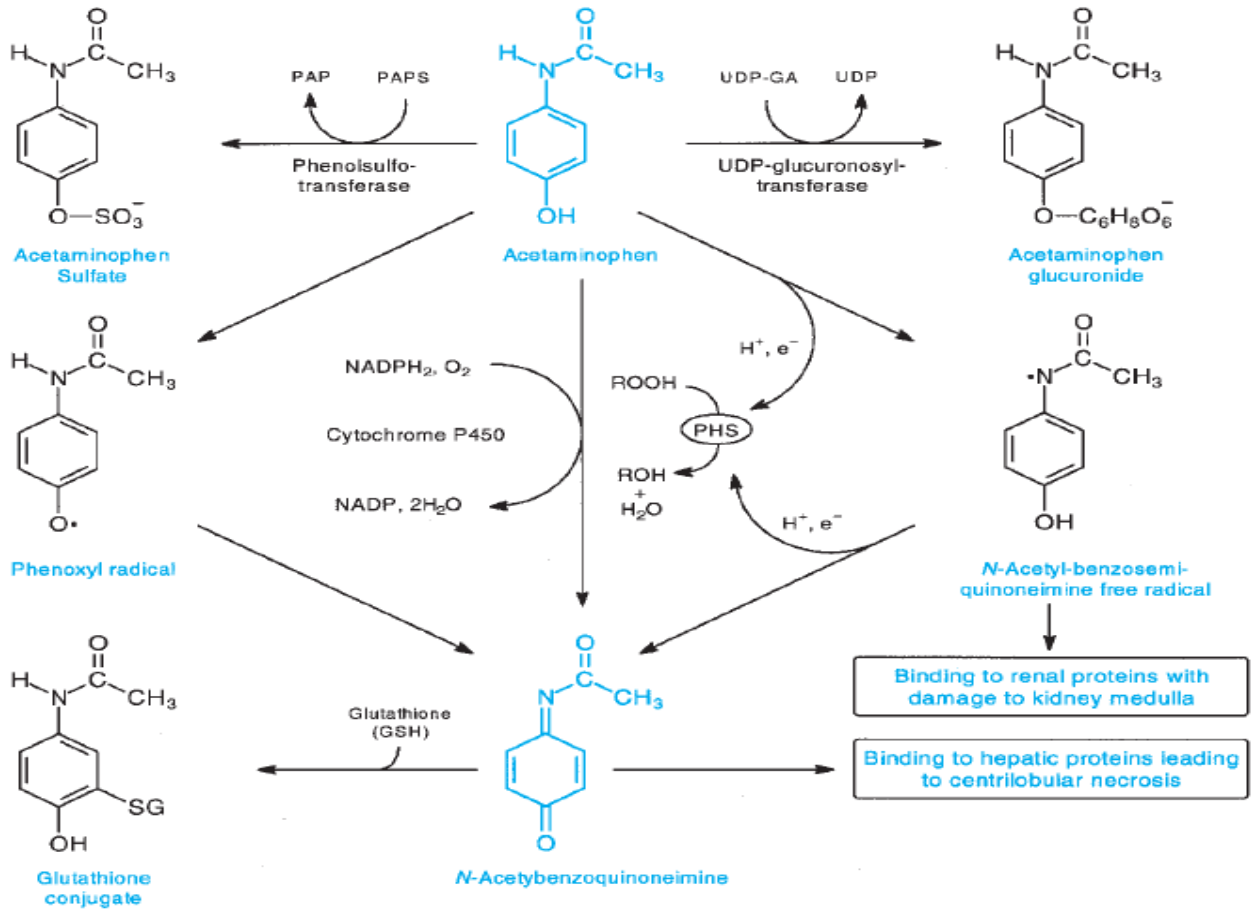

Fig. 3: Activation of acetaminophen by cytochrome P_{450}, leading to hepatotoxicity, and by prostaglandin H synthase, leading to nephrotoxicity. Conjugation with sulfate, glucuronic acid, or glutathione represents detoxification reactions.
Source: James *et al*. (2003)

Besides the underutilized and untapped pharmacological significance of CS in Africa, opinions on its nephroprotective potential are divergent. While Sukandar *et al.* (2013) demonstrated the potency of its ethanolic extract against gentamicin/piroxicam-induced kidney failure, Sepehri *et al.* (2011) submitted that treatment with its methanolic extract did not result in complete reversal of gentamicin-induced alterations in kidney function parameters. More comprehensive research is however imperative in this direction and has prompted the present study with a view to enriching biochemical information on the ability of CS to preserve renal functions and delay/prevent the progression of renal pathological conditions. Hence, this section of the study evaluated CS extract against APAP-induced oxidative onslaughts in the kidneys of Wistar rats. In addition, its membrane stabilization capacity was also investigated.

THE STRUCTURE OF THE THESIS

The thesis consists of contributions in the form of reprints of published articles and submitted articles for publication. The review on the scope of phytotherapy in the management of kidney disorders in Nigeria and South Africa is presented in Chapter 2. While Chapter 3 deals with the *in vitro* cytotoxicity, nephroprotective and anti-nephrolithiasis activities of *Zea mays* L., *Stigma maydis*, its *in vitro* antioxidant and antidiabetic potentials are presented in Chapter 4. The *in vivo* toxicological implications of treatment with CS on key metabolic markers and its role in preserving hepatic functions are presented in Chapters 5 and 6 respectively. The findings of the capability of CS to stabilize biomembrane, prevent oxidative onslaughts on the kidney and preserve renal functions are presented in Chapter 7. In Chapter 8, the general discussion and conclusions to consolidate the overall results obtained from the study is presented.

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CHAPTER TWO

The purview of phytotherapy in the management of kidney disorders: A systematic review on Nigeria and South Africa.

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THE PURVIEW OF PHYTOTHERAPY IN THE MANAGEMENT OF KIDNEY DISORDERS: A SYSTEMATIC REVIEW ON NIGERIA AND SOUTH AFRICA

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Abstract

Background: The kidney is tasked with a number of metabolic functions in the body. In its role as a detoxifier and primary eliminator of xenobiotics, it becomes vulnerable to developing injuries. Currently, over 1 million people in the world are living on renal replacement therapies (RRTs). The case in sub-Sahara African countries like Nigeria and South Africa is not any better than the global trend.
Materials and Methods: A systematic review of medicinal plants used in the treatment of kidney disorders was conducted. Information were gathered from published scientific journals, books, reports from national, regional and international institutions, conference proceedings and other high profile intellectual resources. MeSH words like 'prevalence of kidney disorders in Africa', 'renal replacement therapy', 'nephrotoxins or nephrotoxicants', 'nephroprotective plants', 'nephroprotective plants in Nigeria or South Africa' and 'nephroprotective phytocompounds' were used to retrieve information from online databases (Google, Pubmed, MEDLINE, Science Direct, Scopus and SID).
Results: Interestingly, our findings revealed that phytotherapy has emerged and is being employed to protect renal functions and delay progression of renal pathological conditions into end episodes where the last resort is RRT. In fact, in recent times, Phytotherapists are not only interested in developing relatively safe, more affordable, easily accessible and potent nephroprotective formulations but also increasing awareness on the prevalence of the disease and educating the populace on the probable preventive measures. More importantly, efforts at scientifically elucidating the pharmacological efficacy of the identified nephroprotective plants yet to be validated must be intensified through informed expert opinions. Till date, there is paucity of information on the concept of nephroprotection in most developing countries where kidney disorder is a major threat. Although, the concept is just emerging in South Africa, evidences have given credence to its application in complementary and alternative system of medicine in Nigeria.
Conclusion: This review, therefore, reawaken researchers' consciousness in the continuous search for auspicious nephroprotective plants that could potentially be excellent candidates in developing new lead drugs to manage and treat renal disorders.

Key words: Bioactive principles, Nephroprotective, Nephrotoxic, Renal replacement therapy, Silent killer.

Introduction

The kidneys are a pair of fist-sized organs located outside the peritoneal cavity on each side of the spine. They are highly specialized organs that maintain the body's homeostasis by selectively excreting or retaining various substances according to specific body needs. The importance of urine formation and excretion as a life-sustaining function is highlighted in situations when kidney function is suddenly lost. The complex nature of renal diseases and their progression to renal failure and end stage renal disease (ESRD) makes its management quite difficult. Many cases of renal disease (largely due to chemical exposure, reckless lifestyles, and complication from metabolic ailments) remain unnoticed until they progress to advance stages when the conventional therapeutic interventions are usually not sufficient to provide ample remedy, hence, the name 'silent killer disease'. The major problem with kidney disease however is its progression to a stage when the last resort is the renal replacement therapy (RRT).

In recognition of the importance of the kidney and its affiliated disorders, the second Thursday of every March has been designated as 'World Kidney Day'. It is a day set aside by international bodies to re-awaken human consciousness on the significance of a healthy kidney and the inherent burden of its disorders to health. This review, thus, examines the common nephrotoxins, and the ambit of traditional systems of medicine in providing affordable, relatively safe, and easily accessible renal healthcare in Nigeria and South Africa. In 2014, the World Health Organization (WHO) estimated about 300 million people as victims of Chronic Kidney Disease (CKD) worldwide with 3.4 million attributable death cases and even more in 2012 and 2013 respectively (WHO, 2014). In sub-Saharan Africa countries including Nigeria, the numbers are more disconcerting. An estimated 36.8 million Nigerians (23 percent) were reported to be suffering from various stages of kidney disease. With this figure, one in seven Nigerians is at risk of the different forms of the disease (WHO, 2014). In South Africa on the other hand, inherited hypertension and Type 2 diabetes account for between 60-65% and 20-25%, respectively of kidney failure in adults (NKFS, 2015). With nephropathy as one of the secondary complications of diabetes, the prevalence of renal disorders is even more daunting in South Africa which tops the list with an average of 8.3% and closely followed by Nigeria (4.5%) in Africa (IDF, 2014). This singular fact has placed these countries in the beam light and as requiring concerted and optimized attention in this region of the world.

The two frontiers of RRT (dialysis and kidney transplantation) are highly sophisticated and expensive for the average income class. Only limited fraction of the elite population can take the luxury of such a regimen subject to the availability of this facility. That is why most of the patients of kidney disease are left to die mainly in developing countries because of non-availability of RRT facilities or their inability to afford it. A recent report by Saraladevi (2013) suggests that dialysis treatment rate ranges from < 1 per million populations (pmp) for most sub-Saharan countries, with Nigeria and South Africa at 1.3 and 125 pmp respectively. The

growth in dialysis in South Africa is primarily in the private sector, with improved benefits for dialysis for people on medical insurance. The average cost of hemodialysis in Africa is $100 per session (Saraladevi, 2013). Renal transplantation on the other hand is available in only seven countries in sub-Saharan Africa (South Africa, Sudan, Nigeria, Mauritius, Kenya, Rwanda, and Ghana) with most of the transplants being living donor transplants, except in South Africa, where deceased donor transplants are also encouraged at ratio 2:3, respectively. Lack of legal framework, religious, and social constraints have hindered deceased donor practice in many countries and have led to the low rate of transplantation in these areas. Except with Nigeria and South Africa where at least 70 transplants are performed annually, other nations are challenged with inadequate facilities (Saraladevi, 2013).

The continuous 'brain drain' of healthcare professionals from Africa for greener pasture has also impacted on this menace and has left the continent with inequitable number of qualified nephrologists to patients' population (Eastwood et al., 2005). There are large rural areas of Africa that have no health professionals to serve their populations. Specifically, the proportion of nephrologists per million population for Kenya, Nigeria, Sudan and, South Africa stood at 0.5, 0.6, 0.7, and 1.1, respectively as compared to the United States with reported 16.7 nephrologists pmp (Kletke, 1997; Matri et al., 2008). Orthodox care is funded primarily by private facilities in most African countries, and access to medication is limited by availability of funds, except for Nigeria and South Africa, where public institutions and hospitals are funded by the government to provide these medications to indigent patients (Saraladevi, 2013).

Globally, as at 2010 over 2 million individuals has been treated by RRT at a cost of $1 trillion. More than 100 countries worldwide do not have any provision for RRT and consequently, more than a million individuals can be assumed to die every year from ESRD. Availability of kidneys for transplantation is another important challenge consistent with RRT. Although, expenditure on RRT is costing about $1 trillion but the substantial percentage of patients in need of it, still remains untreated and consequently has out-numbered those receiving the treatment. In the early 90s, it was estimated at about 3.78 million and is expected to be doubled by 2020 (WHO, 2005). In Nigeria, the burden of the disease is also alarming with an estimated 15,000 new patients diagnosed annually. With this statistics, less than 1,000 are on dialysis out of 50,000 patients who should ideally be receiving the treatment and this was largely attributed to the capital intensiveness of RRT. For instance in Nigeria, it cost about $160 (=N=30,000) for a session of kidney dialysis and three sessions are required per week (i.e. =N=90,000 weekly). For a kidney transplant, the cost requirement is put at an average of $22,500 (R265, 000) and $27,500 (=N=5.5 million) in South Africa and Nigeria, respectively (IDF, 2014; NKFS, 2015). The alarming increase in the prevalence of CKD that progresses to ESRD requiring RRT demands huge fund allocation and individuals with kidney diseases are at an increasing risk of other systemic diseases which frequently proves to be fatal (Guildford, 1991). More worrisome is the unimaginable number of mortality recorded daily from kidney failure as terminal end if dialysis or transplant is not performed within two weeks of diagnosis (WHO, 2005). As at 2004, over 1.7 million patients worldwide were receiving treatment for ESRD, of which 77% were on dialysis and 23% had functioning renal transplant, and this number is increasing at the rate of 7% every year (WHO, 2005). With this estimated projection, there is an urgent need for increased awareness among the populace, aimed at preventive measures. Societal practices that seem superficially harmless but nephrotoxic should also be discouraged to stem incidences of the disease and alleviate the associated burdens. Furthermore, since most patients on chronic dialysis cannot be sustained beyond the first 2-3 months due to financial constraints, it can thus be said that it is almost next to impossible to provide replacement therapy to all patients requiring it (Saraladevi, 2013). Therefore, the next option for the physicians is to consider suitable alternatives that will either protect kidney functions or slow the progression of the disease and delay the need of RRT. This will ultimately scale down the prevalence and consequently the financial burden of the disease to a significant level. This latter option could be viewed as paving a way for an envisaged concept that is notably termed as nephroprotection (Ahmad et al., 2014).

Nephrotoxins and Nephroprotective Active Metabolites

The pathological mechanisms of renal disease that compromises its functional capability and the structural integrity has been established to arise mainly through either altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, or thrombotic microangiopathy (Schnellmann and Kelly, 1999; Schectz et al., 2005; Cynthia, 2008). Table 1 shows some of the known nephrotoxic agents and the pathological mechanism involved in their renal pathogenesis.

Table 1: Common nephrotoxicants and the mechanism (s) of action

Class	Agent	Mechanism of renal injury	Reference(s)
Analgesics (NSAIDs)	Acetaminophen, Aspirin	a, b, d and e	(Perneger et al., 1994; Fored et al., 2001; Rossert, 2001)
Antibiotics: *Aminoglycosides Amphotericin B **β-lactam	*Gentamicin **Cephalosporins, penicillins	*f f **a and e	(Prendergast and George, 1993; Rossert, 2001; Markowitz et al., 2003; Graham et al., 2004)
Antihistamines	Doxylamine, Diphenhydramine	F	(Coco and Klasner, 2004)
Antiretrovirals	Adefovir, cidofovir Indinavir	g a, c	(Rossert, 2001)
Cardiovasculars	Statins, Clopidogrel ACE and ARBs	f , h, d	(Pisoni et al., 2001; Palmaer, 2002; Graham et al., 2004; Manor et al., 2004; Perazella, 2005)
Chemotherapeutics	Cisplatin, Mitomycin-C	b and g, g and h	(Appel, 2002)
CNS stimulants	Caffeine, cocaine etc	F	(Prendergast and George, 1993; Markowitz et al., 2003)
Diuretics	Thiazides Triamterene	a c	(Rossert, 2001; Markowitz and Perazella, 2005)
Heavy metals	Cd, Pb, Hg, U	a, d, e and f	(Perazella, 1999; Tarloff, 2001)
Immunosuppresives	Sirolimus, calcineurin inhibitors	a and g	(Olyaei et al., 1999; Mark, 2009)

NSAIDs= Non-steroidal anti-inflammatory drugs, CNS= Central nervous system, a= acute interstitial nephritis, b= chronic interstitial nephritis, c= crystal nephropathy, d= disturbed intraglomerular hemodynamics, e= glomerulonephritis, f= rhabdomyolysis, g= tubular cell toxicity, and h= thrombotic microangiopathy.

A good understanding of the pathogenic mechanism of action of these insultive agents is imperative to spotting and either managing or preventing renal toxicity and the associated disorders.

Following the experimental demonstration that angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and erythropoietin slow the progression of loss of kidney function in models of kidney diseases, a treatment strategy to preserve renal function instead of providing supportive and passive therapy to the patients was conceived (Remuzzi et al., 1993). The concept of nephroprotection thus emerged and strongly stimulated the clinicians to apply such concept in early detection and subsequent prevention of progression of the disease, mainly through lifestyle adjustment and the use of new pharmacological agents (Raja et al., 2014).

Normalization of kidney function following exposure to toxicological agent involves complex processes which enhance kidney's hemodynamics. A drug or agent that can assuage some or all the mechanisms highlighted in Table 1 may be an excellent nephroprotective agent and expectantly aid both restoration of kidney function and improvement in the structural impairment of the kidney, thus, slowing its progression into degenerating episodes (Remuzzi et al., 1993). Despite the fact that the mechanisms of action of some known orthodox drugs are yet to be substantiated, they still merit the criteria of nephroprotection owing to their overall efficacy on the improvement of renal function (Ahmad et al., 2014). However, the challenge even with those with well elucidated mechanisms (like ACE and ARBs) is the associated toxicities and side effects which have undermined their application to a great extent. A drug categorized to be effective as a nephroprotective agent without having liability to produce serious side effects will be the obvious palliative choice for the patients suffering from renal dysfunction. Accordingly, traditional systems of medicine had offered effective drugs against kidney pathological conditions and thus can be used to protect renal function and prevent/slow the progression of renal diseases to CKD or ESRD (Naveed et al., 2014). Medicinal plants are a source for a wide variety of natural antioxidants and are used to treat diseases throughout the world. Their applications are mainly due to being antimicrobial, anticancer, anti-diabetic, anti-lipidemic, immunomodulatory, hepatoprotective and even renoprotective in action. A number of drugs from herbal sources in Nigeria and South Africa have been shown to be nephroprotective and there is a keen global focus on the development of such. Attentions are mostly on protection or prevention as well as accelerating the regeneration of tubular cells against injury to the kidney (Hamid and Mahmoud, 2013). Active principles of plant formulations have proven health benefits and have been elucidated to confer nephroprotection on the nephron of both humans and experimental animal models (Jaya, 2013). While steroid glycosides, terpenoids and flavonoids have been shown to preserve renal cellular macromolecules against oxidative onslaught of reactive metabolites and oxidative stress (Baek et al., 2006; Shelke et al., 2009; Eduardo et al., 2011), the tendency of polyphenols to protect the nephron against glomerulonephritis and altered intraglomerular hemodynamics in cisplatin-mediated nephrotoxic rats has been demonstrated (Subal et al., 2010). Table 2 presents some of these principles and their probable/specific mechanism of action.

Table 2: Bioactive principles implicated in nephroprotective functions

Active principle	Mechanism of action	Reference (s)
Alkaloids	Stems deleterious effect of N-acetyl-*p*-benzoquinoneimine on tubular macromolecules and improves antioxidant defense system in acetaminophen-treated rats	(Palani et al., 2009; Zhao, 2013)
Amino acids	Improves altered glomerulonephritis in cisplatin-induced nephrotoxicity	(Yogesh et al., 2011)
Catechols	Rescues rhabdomyolysis and acute interstitial nephritis in doxorubicin-mediated acute nephrotoxicity	(Ajith et al., 2008)
Carotenoids	Boosts antioxidant status and attenuates cisplatin-induced renal oxidative stress	(Ranjan et al., 2009; Naghizadeh et al., 2010)
Diterpenoids	Repairs and assuages architectural onslaught of reactive metabolites on kidney functions	(Rao, 2006)
Flavonol glycosides	Improves acute nephritis injury	(Shirwaikar et al., 2004)
Flavonoids	Preserves kidney's cellular macromolecules against oxidative insults.	(Shelke et al., 2009; Kannappan et al., 2010)
Glycosides	Restores renal function capacity and speeds up recovery from glomerulonephritis, tubular cell toxicity and altered intraglomerular hemodynamics	(Yadav and Khandelwal, 2009)
Polyphenols	Preserves the renal tubule against glomerulonephritis and altered intraglomerular hemodynamics in cisplatin-mediated nephrotoxic rats	(Subal et al., 2010)
Saponins	Ameliorates renal carcinogenesis and improves kidney function in N-nitrosodiethylamine-treated animals	(Pracheta et al., 2011)
Steroid glycosides	Reduces tubular cell toxicity and crystal nephropathy in cisplatin-induced nephrotoxicity	(Baek et al., 2006)
Sterols	Enhances speedy restoration of renal cellular functions via antioxidative action	(Paterson, 2008; Subal et al., 2010)
Tannins	Prevents and palliates gentamicin-induced nephrotoxic insults on kidney	(Kakasaheb and Rajkumar, 2011)
Terpenoids	Halts ravaging effect of generated oxidants, induced antioxidant enzymes and modulates mitochondrial pathway in chromium-mediated nephrotoxicity	(Eduardo et al., 2011)
Vitamins B and C	Repairs tubular cell toxicity, ameliorates fluoride nephrotoxicity, aids renal excretion of other toxins and improves antioxidant defense system	(Jiménez-Escrig et al., 2001)

With the elucidation and isolation of these active metabolites from medicinal plants, attention is now given to the traditional system of medicine in providing alternative therapies to augment the increasingly expensive orthodox medical services

(Fasola and Egunyomi, 2005). This was well buttressed by the submission of WHO that about 80% of the population in developing nations seek exclusive treatment in folkloric medicine (WHO, 2000). Keeping this in view, researchers across the globe are now focusing on developing herbal renoprotective drugs of plant origin with antioxidant properties for the prevention and cure of kidney disorders (Raja et al., 2014). The exploitation of the antioxidative potentials of these plants is inseparable from the fact that oxidative stress is mostly implicated in episodes of renal tubular necrosis (Hamid and Mahmoud, 2013). Hence, it can be postulated that medicinal plants with excellent antioxidant activities due to the presence of bioactive principles may extenuate the risk of many chronic and degenerative diseases including nephropathy.

Nephroprotective Plants from Nigeria and South Africa

Globally, folkloric medicine has been and is still finding relevance in providing preventive and palliative measures against nephrotoxicity. Phytotherapists with keen interest in renal disorders and those informed of the unawareness and ignorance of majority of the victims are striving not only to educate the world on this 'silent killer' but also proffer extenuating panacea to its daunting effects. Notably, reviews on nephroprotective medicinal plants revealed appreciable success mainly from India and some other Asian countries and have implicated more than 300 plants with remarkable effects against several renal disorders. While Ajith et al. (2008) and Palani et al. (2009) have demonstrated respective therapeutic efficacy of Zingiber officinale and Pimpinella tirupatiensis on drug-induced nephrotoxicity and oxidative stress, others have reported pharmacological significance of medicinal plants in annihilating oxidative insults on renal tubular cells and preserving overall kidney functions (Gaikwad et al., 2012; Mohana et al., 2012; Talele et al., 2012; Jaya, 2013; Raja et al., 2014).

In Nigeria and South Africa, traditional systems of medicine are also not wanting in offering formulations/drugs for different forms of kidney diseases (Musabayane et al., 2007; Al-Qattan et al., 2008; Usman et al., 2009). Some of the identified renoprotective herbs in these countries are being embraced by physicians, and have been demonstrated to exhibit remarkable diuretic and antioxidative effect against known toxicants (Simpson, 1998; Kadiri et al., 2015). Plants such as curcumin, pawpaw, and bitter leaf are commonly and routinely cultivated in these countries and have been reported to have excellent nephroprotective capability on the kidneys. Assertions from some of these studies are highlighted as follows:

***Carica Papaya* Linn (Caricaceae)**

Carica papaya is a dicotyledonous, polygamous, large, tree-like plant, with a single stem of average height 5-10 m. The leaves are large, 50-70 cm in diameter, deep palmately lobed, spirally arranged and confined to the top of the trunk. The flowers appear on the axils of the leaves, maturing into large fruit. A typical fruit of *C. papaya* is ripe when it feels soft and its skin has attained amber to orange appearance. Globally, the fruit is consumed either in its fresh form or the form of juices, jams, and crystallized dry fruit (Nakasone and Paull, 1998). The nephroprotective effect of its aqueous seed extract on CCl_4-perturbed renal damage has been reported (Olagunju et al., 2009). The authors attributed the effect elicited by the plant to its phytoconstituents and opined that it could have been via antioxidative and/or free radical scavenging mechanism(s).

Zea Mays* L. (Poaceae), *Stigma Maydis

Zea mays, *Stigma maydis* (Corn silk) is one of the several plant parts commonly used in the management of kidney stones, bedwetting and urinary infections. GCMS analysis of its aqueous extract revealed the presence of maizenic acid, β-carotene, ascorbic acid, gluten, o-diethyl phthalate, 2-methyl-napthalene, thymol, 3'-o-methyl-maysin, cyanidin, cinnamic acid, hordenine, luteolinidin, pelargonidin and betaine as major adaptogenic phytonutrients (Sabiu et al., 2016a). Although, corn is a common staple food in Nigeria and South Africa, the pharmacological significance of its silk is still hugely underutilized. However, its membrane stabilization and detoxification potential of acetaminophen-mediated oxidative onslaughts in the kidneys of Wistar rats has just been recently reported (Sabiu et al., 2016b).

***Agathosma Betulina* (Bergius) Pillans**

Agathosma betulina is a flowering plant in the family Rutaceae. It is native to the lower elevation mountains of western South Africa, where it occurs near streams in fynbos habitats. It is an evergreen shrub growing to 2 m tall. While its flowers are white or pale pink with five petals, the fruit is five-parted capsule which split open to release its seeds. The leaves are opposite, rounded, about 20 mm long and broad, and are strongly aromatic. The essential oil from its leaves is golden in colour, with a strong-sweetish, peppermint-like odour and rich in isomenthone and diosphenol as major adaptogenic constituents. Leaf extracts of *A. betulina* taste like blackcurrant and is normally used as flavouring agent for teas, and has great reputation for treating kidney and urinary tract diseases (Moolla and Viljoen, 2008).

***Ficus Thonningii* (Blume) (Moraceae)**

F. thonningii is a multistemmed, evergreen tree with a dense, rounded to spreading crown. It is native to Africa with distribution across the upland forests of tropical and subtropical regions, at altitudes of between 1,000-2,500 m (ATD, 2011). While the leaves are rounded or tapering, 4.5-12 cm long, hairless or finely hairy with a prominent midrib, the fruits are round, 10-20 mm in diameter, usually hairy and turn yellowish and rarely pink when ripe (Schmidt et al., 2002). Study of its stem-bark ethanolic extract on blood glucose, cardiovascular and kidney functions of rats, and on kidney cell lines of the proximal (LLC-PK1) and distal tubules (MDBK) revealed remarkable renoprotective activities and presented the plant as a source of probable lead compound in the management of kidney diseases (Musabayane et al., 2007).

***Canarium Schweinfurthii* Engl. (Burseraceae)**

C. schweinfurthii is a tall forest tree growing wild in Africa with characteristic straight and cylindrical bole exceeding 50 m (Orwa et al., 2009). The Leaves are pinnate, clustered at the end of the branches, and may be 15-65 cm long, with 8-12 pairs of leaflets. It produces fruit that is similar in appearance to olives, green in color, and red-purple when fully matured. Okwuosa et al. (2009) have demonstrated the potency of its stem bark extracts against acetaminophen-induced renal injuries in rats. Improved renal function indices and preserved histoarchitectural features were their major assertions.

***Sclerocarya birrea* (A. Rich) Hochst. (Anacardiaceae)**

S. birrea is a highly valued cultural and ethnomedicinal plant in Africa. It is commonly found in semi-arid, deciduous and savannah regions of sub-Saharan Africa. In South Africa, *S. birrea* occurs in the lowlands of KwaZulu-Natal and bears edible fruit that has formed an integral component of the southern African diet. Literatures have indicated the presence of polyphenols, tannins, coumarins, flavonoids, triterpenoids and phytosterols as medicinally-important chemical constituents in its various extracts and standardized fractions. Treatments with stem-bark ethanolic extract of *S. birrea* resulted in decreased plasma urea and creatinine concentrations of streptozotocin-diabetic rats with concomitant increase in glomerular filtration rate (Gondwe et al., 2008).

***Pseudocedrela kotschyi* (Schweinf.) Harms (Meliaceae)**

P. kotschyi is a Savanna woodland plant, chiefly of the Guinea zone on moister heavy soils of valleys. It is commonly 20-30 m high with wide crown. While the crown is rounded with ascending branches, the bark is thick, silvery-grey and fairly regularly fissured into small square pieces. Antioxidative and nephroprotective activities of ethanolic roots extract of *P. kotschyi* against oxidative stress and nephrotoxicity in rats have just been recently documented (Ojewale et al., 2014).

Vernonia Amygdalina, Citrullus Colocynthis, Psidium Guajava* And *Ficus Mucuso

Vernonia amygdalina (Asteraceae), commonly known as bitter leaf is a shrub that grows up to 3 m high in the African tropics. It has petiolate leaves of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and bitter taste. Saponins, alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinone, edotides and sesquiterpenes are the pharmacologically-active constituents of *V. amygdalina*.

C. colocynthis L. (Cucurbitaceae) is a tropical plant, native to Asia and Africa. Its fruit contains bitter glycoside which has found therapeutic significance against an array of diseases. Biologically-active phytonutrients like alkaloids and flavonoids have also been identified and isolated from its various extracts.

P. guajava (Myrtaceae) is a semi deciduous tropical tree commonly called guava. Its antioxidative potentials which are closely associated with its numerous tannins, polyphenolic compounds, flavonoids, pentacyclic triterpenoids, guiajaverin, quercetin, free sugars, vitamins B1, B2, B6 and C have been documented.

F. mucuso (Moraceae) is a tree that usually grows to a height of 30 m on mountains and stands isolated in farmlands. While it flowers in June, the fruits become matured in August. Phytochemical screening of its extracts revealed triterpenes, flavonoids, chromones and alkaloids as major bioactive constituents.

An investigation into the probable nephroprotective attributes of *V. amygdalina*, *C. colocynthis*, *P. guajava* and *F. mucuso* in streptozotocin-induced diabetic animals revealed auspicious submissions and lent support to the nephroprotection concept of medicinal plants. However, *P. guajava* elicited the best and most prominent effect in preventing glomeruli disruption and also preserved nephro-histoarchitectural morphology of the animals (Komolafe et al., 2013).

Though substantive focus is yet to be given to nephrophytotherapy in South Africa, efforts are ongoing in our laboratory in this regard. A comprehensive list of some selected plants being embraced as nephroprotective agents in Nigeria and those currently exploited in South Africa is presented in Table 3.

Table 3: Commonly used nephroprotective plants in Nigeria and South Africa

Plant name	Part used	Implicated active principle(s)	Country	Reference (s)
Adansoni digitata	Leaves, fruit pulp, bark	Alkaloids, tannins, saponins, sterols, flavonoids, vitamin C	Nigeria	(Olowokudejo et al., 2008; Adebisi et al., 2012; Kadiri et al., 2015)
Aerva lanata	Whole plant	Phenols, tannins, saponins, flavonoids, phytosterols	Nigeria	(Olowokudejo et al., 2008; Guarav et al., 2013)
Agathosma betulina Berg.	Leaves	Flavonoids	South Africa	(Posthumus et al., 1996; Simpson, 1998)
Allium sativum L.	Cloves	Phenols, flavonoids	Both	(Al-Qattan et al., 2008)
Annanas comosus	Whole plant	Alkaloids, flavonoids, phenols, tannins, phytosterols, glycosides, amino acids	Nigeria	(Kataki, 2010; Kadiri et al., 2015)
Arachis hypogaea	Nuts, leaves	Glycosides, phenols, saponins	Nigeria	(Borokini et al., 2013; Rajinikanth et al., 2013; Kadiri et al., 2015)
Azadirachta indica	Leaves	Alkaloids, flavonoids, phenols, tannins, saponins	Nigeria	(Harry-Asobara and Samson, 2014; Kadiri et al., 2015)
Bixa orellana L.	Seeds, leaves	Alkaloids, flavonoids, anthraquinones, tannins, steroids, saponins	Nigeria	(Tamil et al., 2011; Dike et al., 2012)
Canarium Schweinfurthii	Stem-bark, fruits, leaves	Tannins, steroids, cardiac glycosides	Nigeria	(Okwuosa et al., 2009; Ayoade et al., 2015)

Canna indica L.	Leaves, root	Alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, saponins, phlobatannins	Nigeria	(Dike et al., 2012; Ali, 2015)
Carica papaya L.	Leaves, seeds	Alkaloids, phenols, flavonoids, tannins, terpenoids, saponins	Nigeria	(Olagunju et al., 2009; Subal et al., 2010; Dike et al., 2012)
Citrullus colocynthis	Seed shaft	Alkaloids, flavonoids	Nigeria	(Dallak and Bin-Jaliah, 2010; Komolafe et al., 2013)
Croton zambesicus Muell Arg.	Root	Alkaloids, saponins, terpenes, tannins, phlobatannins, anthraquinones, cardiac glycosides	Nigeria	(Jude et al., 2011)
Ekebergia capensis	Leaves	Saponins, alkaloids, flavonoids, tannins	South Africa	(Fitzpatrick et al., 1995)
Ficus exasperata Vahl	Leaves	Saponins, steroids, glycosides, tannins	Nigeria	(Shagal et al., 2011; Dike et al., 2012)
Ficus mucuso	Leaves	Flavonoids, monoterpenoids	Nigeria	(Gojayev et al., 2011; Komolafe et al., 2013)
Ficus thonningii	Leaves	Alkaloids, anthraquinones, flavonoids, saponins, tannins	Both	(Musabayane et al., 2007; Usman et al., 2009)
Foeniculum vulgare L.	Leaves	Phytoestrogens	South Africa	(Musabayane, 2012)
Gongronema latifolium	Leaves	Flavonoids, saponins, polyphenols	Nigeria	(Ugochukwu et al., 2003; Ezeonwu and Dahiru, 2013)
Harpagophytum procumbens	Tubers	Phenols, flavonoids	South Africa	(Joffe, 2005; Motlhanka, 2012)
Harungana madagascariensis L.	Root	Glycosides, flavonoids, alkaloids, saponins and tannins	Nigeria	(Okoli et al., 2002; Adeneye et al., 2008)
Helichrysum ceres S.	Leaves	Polyphenols, tannins, triterpenes, saponins	South Africa	(Musabayane et al., 2003)
Ipomoea batatas	Tubers	Triterpenes, steroids, alkaloids, anthraquinones, flavonoids, saponins, tannins, phenols	Nigeria	(Márcia et al., 2011; Borokini et al., 2013)
Launaea taraxacifolia	Leaves	Cardiac glycosides, terpenoids, tannins, saponins, flavonoids, steroids	Nigeria	(Adinortey et al., 2012; Borokini et al., 2013)
Mallotus oppositifolius Mull. Arg.	Leaves	Flavonols	Nigeria	(Iwu, 2000; Dike et al., 2012)
Mangifera indica L.	Leaves, bark	Saponin, steroids, tannin, flavonoid, cardiac glycosides, anthraquinone	Nigeria	(Aiyelagbe et al., 2009; Dike et al., 2012; Kadiri et al., 2015)
Morinda lucida	Leaves, bark	Alkaloids, tannins, saponins	Nigeria	(Chukwuemeka et al., 2013; Kadiri et al., 2015)
Ocimum gratissimum	Leaves	Polyphenols, flavonoids, fatty acids	Nigeria	(Ezeonwu and Dahiru, 2013; Venuprasad et al., 2014)
Olea europaea L.	Leaves	Triterpenes, flavonoids, glycosides	South Africa	(Benavente-Garcia et al., 2000; Bennani-Kabchi et al., 2000)
Opuntia megacantha	Leaves	Phenols, flavonoids	South Africa	(Bwititi et al., 2001)
Parkia biglobosa	Leaves	Tannins, steroids, cardiac glycosides, alkaloids	Nigeria	(Ajaiyeoba, 2002; Kadiri et al., 2015)
Persea Americana Mill.	Leaves	Tannins, saponins, flavonoids, alkaloids, glycosides	Both	(Owolabi et al., 2010; Yasir et al., 2010)
Pseudocedrela kotschyi	Leaves, root	Alkaloids, flavonoids	Nigeria	(Ojewale et al., 2014)
Psidium guajava	Leaves	Glycosides, flavonoids, terpenoids	Nigeria	(Komolafe et al., 2013)
Sclerocarya birrea	Stem-bark	Alkaloids, flavonoids, triterpenoids, vitamin C	South Africa	(Gondwe et al., 2008)
Sida acuta	Leaves, root	Alkaloids, phytosterols, tannins, flavonoids, saponins	Nigeria	(Borokini et al., 2013)
Sutherlandia frutescens	Leaves	Amino butyric acid, flavonol, glycosides, triterpenoid, saponins	South Africa	(Herbert, 2006; van Wyk and Albrecht, 2008)
Syzigium spp.	Seeds	Flavonoids	South Africa	(Mapanga et al., 2009)
Terminalia catapa	Leaves, bark	Alkaloids, saponins, tannins, steroids	Nigeria	(Muhammad and Mudi, 2011; Kadiri et al., 2015)
Uvaria afzelii	Leaves, bark	Tannins, saponins, cardenolides, alkaloids	Nigeria	(Olowokudejo et al., 2008; Temitope et al., 2014)
Vernonia amygdalina	Leaves	Tannins, flavonoids	Nigeria	(Eleyinmi et al., 2008; Komolafe et al., 2013)
Zea mays	Silk	β-carotene, ascorbic acid, thymol, cinnamic acid, betaine	Both	(Sabiu et al., 2016a, b)

Conclusion

Generally, efforts at increasing awareness about kidney disease and its complications in communities and among health professionals should be intensified. This is imperative as many are unaware of the severity of the disorder, which probably serves as a barrier to appropriate preventive measures. Although, thorough clinical trials and expert opinions may be necessary on medicinal plants to gain medical significance as preferred class of drugs, their overall auspicious and remarkable nephroprotective attributes have presented them as excellent candidatures to develop new lead drugs in the treatment and management of renal disorders in Nigeria and South Africa.

Conflicts of Interest

The authors have declared that no conflict of interest exists.

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CHAPTER THREE

In vitro anti-nephrolithiasis, antioxidant and nephroprotective activities of aqueous extract of *Zea mays* L. (Poaceae), *Stigma maydis* in HEK293 cells

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In vitro anti-nephrolithiasis, antioxidant and nephroprotective activities of aqueous extract of *Zea mays* L. (Poaceae), *Stigma maydis* in HEK293 cells

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Abstract

Zea mays L. (Poaceae), *Stigma maydis* is an underutilized waste product of corn cultivation with ethnomedicinal applications against a number of degenerative disorders including urinary and renal dysfunctions. This study investigated the anti-nephrolithiasis, antioxidant and nephroprotective properties of its aqueous extract *in vitro*. While the nephroprotective activity was demonstrated against acetaminophen-induced toxicity in HEK293 cells using the MTT method, its capacity to inhibit calcium oxalate (CaOx) nucleation was evaluated in the anti-nephrolithiasis study. The antioxidant potential was demonstrated against H_2O_2, hydroxyl, and lipid peroxide radicals. The significant ($p<0.05$) acetaminophen-perturbed reduction in the number of viable cells in the nephrotoxic cells after a 24-h incubation period was effectively mitigated with the cell mortality significantly attenuated from 82.0% to 10.77% following treatment with a 125 µg/mL dose of the extract. The observed effect compared favourably with that of vitamin C and is indicative of the nephroprotective capability of the extract as also supported by the MTT phase contrast cytological images. Similarly, the extract significantly inhibited CaOx nucleation crystals in a dose-dependent manner with the highest dose being 93.40% potent. This inhibitory effect by the extract had an overall IC_{50} of 256 µg/mL (R^2= 0.9775) with corresponding significant reduction in the degree of turbidity of the treated crystals solutions. While the anti-crystallization tendency of the extract may be specifically attributed to its saponin contents, its overall pharmacological effects could be due to its antioxidant activity which was concentration-related with the highest dose exhibiting the most potent effect in all the assays (IC_{50}: 250.0–515.0 µg/mL) when compared with vitamin C (IC_{50}: 316.0–511.0 µg/mL). In light of the foregoing, it may be inferred that the present study has demonstrated and lent

scientific support to the ethnomedicinal significance of *Z. mays*, *S. maydis* as a candidate in the management of nephrolithiasis and renal dysfunctions.

Keywords: Calcium oxalate, Corn silk, HEK293 cell, Lipid peroxidation, MTT, Nephrolithiasis,

1.0 Introduction

The kidneys receive approximately 25% of cardiac output and as key excretory and xenobiotic metabolizing organs, are naturally vulnerable to developing injuries resulting from exposure to circulating drugs and chemicals (Schnellmann and Kelly, 1999). Renal toxicity has been reported for diverse agents ranging from microbial, heavy metals, chemicals to drugs (sulfonamides, aminoglycosides and non-steroidal anti-inflammatory drugs (e.g. acetaminophen)) (Sabiu *et al*., 2016a). Although the most common manifestation of toxicity is renal failure, the cellular and subcellular targets of toxicity and molecular mechanisms of toxicity varies from agent to agent. For instance, acetaminophen (APAP) nephrotoxicity has been well studied and is characterized by morphologic and functional evidence of proximal tubular injury in humans and experimental animals (Cekmen *et al*., 2009). Since proximal tubules are the most common site of injury by drugs, screening and understanding the proximal tubule toxicity effect of drugs is critical in drug evaluation. Information on mechanisms of toxicity will further guide structure-activity relationships and minimize risks of clinical renal damage. The use of cells derived from proximal tubules of kidneys like HEK293 is one of the *in vitro* approaches of screening in cytotoxicity assays.

Besides renal toxicity, nephrolithiasis (kidney stones) is another challenge consistent with the kidneys. It is characterized by formation of small, hard, crystalline deposits of mineral and acid salts within the kidney. It is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical, malnutritional (hyperuricosuria), poor diet and genetic risk factors

(Rodgers, 2006). Essentially, varying degrees of calcification (in the form of either calcium oxalate (CaOx) or calcium phosphate) in the kidney, bladder, or urethra are its common features (Tyagi *et al*., 2012). It afflicts both genders but the risk is three times higher in men than women, because of the enhancing capacity of testosterone and the inhibiting capacity of estrogen in stone formation (Selvam *et al*., 2001). Reports have also estimated 1 in every 20 individuals as being affected by kidney stones at some point in their life (Bashir and Gilani, 2009; Tyagi *et al*., 2012). Globally, dietary modification coupled with surgical methods, extracorporeal lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are expensive and recurrence is also common (Prasad *et al*., 2007). Furthermore, the recurrence rate without preventive treatment ranges from 15-50% within 1 to 10 years, respectively (Basavaraj *et al*., 2007). Various therapies are being used to prevent recurrence, however scientific evidence for their efficacy, expected to be nearly 50%, is still less convincing (Knoll, 2007; Bashir and Gilani, 2009).

In the African traditional systems of medicine, plants and plant-derived products are of overall pharmacological significance. In addition, they are very potent in stemming the recurrence rate of renal calculi with minimal side effects. Corn silk (CS) is one of those plant-derived products that have been ethnomedicinally advocated in this respect.

Zea mays L. (Poaceae), *Stigma maydis* (Corn silk) is an underutilized waste product of corn cultivation with therapeutic applications in the management of prostate disorder, bedwetting, urinary infections and kidney stones (Grases *et al.*, 1993). It is phenolics-rich with maysin, maizenic acid, β-carotene, ascorbic acid, cinnamic acid and hordenine as some of its 23 identifiable adaptogenic phytoconstituents (Sabiu *et al.*, 2016b). Although reports from the Asian countries have lent scientific credence to its diverse pharmacological significance (Guevera *et*

al., 2000; Maksimović and Kovacevic, 2003; Kim *et al.*, 2004; Jianyou *et al.*, 2009; Wang *et al.*, 2012; Zhao *et al.*, 2012; Ghada *et al.*, 2014), its medicinal relevance is just receiving concerted attention in Africa (Sabiu *et al.*, 2016a). For instance, in Nigeria and South Africa (where corn is a major and common staple food), CS remains hugely underutilized and its therapeutic importance is still significantly unexploited.

Although, Al-Jawad *et al.* (2012) and Rathod *et al.* (2013) have respectively demonstrated significant diuretic effect that accelerates the excretion of urinary calcium, and anti-urolithiasis efficacy of Asian CS, there is still information gap on the kidney stone management potential of CS of African corn cultivation. Hence, we evaluated the anti-nephrolithiasis and antioxidant potential of aqueous extract of CS via *in vitro* models. The cytotoxicity and mitigative effects of the extract on APAP-induced toxicity in HEK293 cells were also investigated.

2.0 Materials and methods

2.1 Chemicals, drugs and reagents

Sodium dodecyl sulfate (SDS), calcium chloride ($CaCl_2$), sodium oxalate ($Na_2C_2O_4$), L-ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(4, 5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), phosphate buffered saline (PBS), dimethyl sulfoxide (DMSO), Modified Eagle's Medium (MEM) and trypsin were obtained from Sigma Aldrich Co, St Louis, USA, while hydrogen peroxide (H_2O_2) and propanol were products of Merck (Pty) Ltd., Gauteng, South Africa. Antibiotics and fetal bovine serum (FBS) were procured from Biochrom, GmbH, Germany. The water used was glass-distilled and all other chemicals and reagents used were of analytic grade.

2.2 Cell lines and culture medium

HEK293 cell line (from a human embryonic kidney) was a gift from Prof. F Burt of the Medical Microbiology and Virology Department, University of the Free State, Bloemfontein, South Africa. Stock cells of HEK293 were cultured in MEM supplemented with 10% inactivated FBS, and antibiotics in an incubator with humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 1.0% L-glutamate, PSA, NEA, 1.0% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and experiments were carried out in 96-well microtitre plates. All subsequent experiments with cell cultures were carried out at 37 °C in a 5% CO_2 atmosphere.

2.3 Plant collection, authentication, processing and selection

Fresh corn silks were harvested from a corn plantation in the QwaQwa, Phuthaditjhaba, Free State province, South Africa. They were authenticated by Dr. AOT Ashafa of the Plant Sciences Department, University of the Free State, QwaQwa campus, South Africa and a voucher specimen (No. SabMed/01/2015/QHB) was deposited at the Herbarium of the Institution.

The silks were washed to remove foliar contaminants, shade dried to constant weight and subsequently milled (model MS-223; Blender/Miller III, Taiwan, China) to fine powder. A known weight (1.2 kg) of the powdered sample was divided into three portions of 400 g each and extracted exhaustively with regular agitation in 4 L each of ethanol, hydroalcohol and water, respectively. The resulting infusion in each case was filtered (Whatman no. 1 filter paper). The ethanol-containing extracts were evaporated to dryness in a rotary evaporator (Cole Parmer SB 1100, Shangai, China) maintained at 40 °C, while the water extract was lyophilized using Virtis Bench Top lyophilizer (SP Scientific Series, USA). This yielded 25, 34, and 40 g of the ethanol, hydroalcohol and water extracts, respectively.

Exactly 15 µL of each extract was thereafter spotted on silica gel TLC plate and the chromatograms obtained were developed in dichloromethane/methanol (8.5:1.5 v/v) solvent system. Each was later sprayed with 0.2% DPPH in methanol for detection of antioxidant constituents. From the chromatograms, the CS aqueous extract (CSE) prominently bleached the purple-coloured solution of DPPH and was selected for the bioassays.

2.4. Antioxidant assays

2.4.1. Hydrogen peroxide scavenging assay

The method of Ruch *et al.* (1989) was used in this evaluation. Exactly, 1.7 mL of different concentrations (200-1000 µg/mL) of the extract in PBS (pH 7.4) was carefully mixed with about 300 µL of 40 mM H_2O_2 and allowed to incubate (25 ^oC, 10 min). For the standard, 1.7 mL of vitamin C was used. The absorbance in each case was subsequently read at 230 nm and the percentage H_2O_2 inhibitory potential of CSE was evaluated from the expression:

% H_2O_2 scavenged = $A_{control}$ -(A_{sample} – $A_{extract}$)/$A_{control}$] × 100. $A_{control}$ is the absorbance of the mixture without extract. A_{sample} and $A_{extract}$ represent the absorbance of the mixture with the extract and that of the extract alone, respectively. The IC_{50} value was thereafter estimated from the calibration curve.

2.4.2. Hydroxyl radical scavenging assay

The OH radical scavenging capability of the extract was determined as previously described (Smirnoff and Cumbes, 1996). Briefly, 200 µL of either CSE or vitamin C (concentrations 200-1000 µg/mL) were mixed with 600 µL of $FeSO_4$ (8 mM), 500 µL of H_2O_2 (20 mM) and 2 mL of salicylic acid (3 mM) in a test tube. Following a 30 min incubation period at 37^oC, distilled water (0.9 mL) was added and the resulting mixture centrifuged (4472 x *g*, 10 min). The absorbance was subsequently read at 510 nm and the IC_{50} value was calculated from the calibration curve

following estimation of percentage OH* radical scavenging capacity of the extract as per the expression:

% hydroxyl radical scavenged = $A_{control}$ –(A_{sample} – $A_{extract}$)/$A_{control}$] × 100, $A_{control}$, A_{sample} and $A_{extract}$ represent the absorbance of the mixture without extract, mixture with the extract and that of the extract alone, respectively.

2.4.3. Lipid peroxidation evaluation

The assay was performed adopting the method of Oyedemi *et al.* (2012). In brief, 500 µL 10% v/v egg-yolk homogenate in sterile distilled water and 100 µL of varying concentrations of the extract were mixed in different test tubes and each made up to 1 mL with distilled water. To the resulting mixtures, 50 µL of $FeSO_4$ (0.07 M) was added prior to incubation (25^oC, 30 min) to induce lipid peroxidation. Subsequently, the working solution (1.5 mL of 20% acetic acid (pH 3.5), 1.5 mL of 0.8 % w/v TBA prepared in 1.1 % w/v SDS and 0.05 mL of 20 % w/v TCA) was added, vortexed and heated in a boiling water for 1 h. After cooling, n-butanol (5 mL) was added to each tube, centrifuged (3000 rpm, 10 min), and the absorbance of the resulting supernatant was read at 532 nm. For the blank and the standard, 100 µL each of distilled water and vitamin C was used instead of the extract and the IC_{50} values were thereafter estimated.

2.5. Cytotoxicity studies

2.5.1. Preparation of test solutions

For the cytotoxicity studies, CSE was dissolved in sterile distilled water and volume made up with MEM supplemented with 10% inactivated FBS to obtain a stock solution of 1 mg/mL. This was sterilized by filtration (0.2 µm pore diameter) and varying test concentrations were subsequently prepared and used for the cytotoxicity studies.

2.5.2. Cell viability assay by the MTT method

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity evaluations. Microculture tetrazolium (mitochondrial reduction) assay is a colorimetric technique used to evaluate cell vitality. This assay is based on the clue that dead cells or their products do not reduce tetrazolium. However, the assay depends both on the number of cells present and the mitochondrial activity per cell. The principle involved is the cleavage of yellow water soluble tetrazolium substrate (MTT) into an insoluble purple formazan product by the mitochondrial succinate dehydrogenase enzyme. Since this process can occur only in viable, metabolically active cells, the level of activity (which is proportional to the extent of formazan formation) is a measure of cell viability.

For the running of the experiment, the procedure of Pedraza-Chaverri *et al*. (2008) was followed. Briefly, the prepared monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 100 µL of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off and the monolayer washed once with the medium. Subsequently, 100 µL of varying concentrations (62.5-1000 µg/mL) of CSE were added on to the partial monolayer in microtitre plates. The plates were then incubated (37^o C, 5% CO_2 atmosphere) for 72 h, and microscopic examination (Nikon Eclipse TS100, USA) was carried out and observations noted once daily.

Following the 72 h of incubation, the extract solutions in the wells were discarded and 20 µL of MTT (5 mg/mL) in PBS was added. The plates were gently shaken and further incubated (37^o C, 5% CO_2 atmosphere) for 3 h. The supernatant from each well was thereafter removed, 100 µL of DMSO was added and the plates were gently shaken to solubilize the resulting formazan. The

absorbance was subsequently read using a microplate fluorescence reader Spectra-Max M2 (Molecular Devices, USA) with excitation and emission at 540 nm and 620 nm respectively. The percentage growth inhibition was calculated using the expression:

$$\% \text{ Growth inhibition} = 100 - \left[\left(\frac{As}{Ac}\right) \times 100\right]$$

where A_S and A_C represents the mean absorbance of the sample and control respectively. Using a standard dose-response curve, the concentration of CSE needed to inhibit cell growth by 50% (CC_{50} value) was determined.

2.5.3. Nephroprotective activity

This assay was achieved by adopting the method of Mosmann (1983). A preformed monolayer cell culture was trypsinized and adjusted as above (2.5.2). To each well of the microtitre plate, 100 µL of the diluted cell suspension was added. After 24 h (when a partial monolayer had been formed), the supernatant was flicked off and the monolayer washed once with MEM containing 10% FBS. Following this, 50 µL each of the supplemented medium and 1000 µg/mL of APAP in warm (37^o C) PBS (Lijuan *et al*., 2007) and 50 µL of different therapeutic concentrations of CSE and vitamin C (0.25 mM) were added. The plates were thereafter incubated (37^o C, 5% CO_2) for 24 h. Following incubation, the cell supernatants were discarded and 50 µL of MTT solution was added. The resulting mixture in the plates was gently shaken and incubated (37^o C, 5% CO_2) for a further 3 h. The cells in the plates were then swiftly microscopically examined (Nikon Eclipse TS100, USA) for probable morphological alterations prior to flicking off of the new supernatant and addition of isopropanol (100 µL) to solubilize the formed formazan. The absorbance was subsequently read (microplate fluorescence reader Spectra-Max M2) with excitation and

emission at 540 nm and 620 nm respectively. From the estimated % cell viability, the % protection offered by CSE and vitamin C against APAP toxicity was calculated.

2.6. Quantification of saponin

Saponins content of CS was determined as earlier reported (Obadoni and Ochuko, 2001). The powdered sample (20 g) was suspended in 20% ethanol: water (1:4; 100 mL) and vigorously agitated on a Labcon Platform Shaker (Laboratory Consumables, South Africa) for 30 min. The resulting infusion was then heated in a water bath (55 °C) for 4 h prior to filtration (Whatmann No. 1 filter paper). The residue obtained was thereafter re-extracted in 200 mL of the same solvent (20% ethanol: water). Following this, the resulting mixture was concentrated to about 40 mL in a water bath (90 °C). Using a separating funnel (250 mL capacity), the concentrate obtained was then extracted twice with diethyl ether (20 mL) and the ether layer from each was discarded while the aqueous layer retained. Exactly 60 mL of *n*-butanol was added and infusion obtained was concentrated and washed twice with 5% sodium chloride (10 mL). The experiment was performed in triplicate. Following substantial evaporation, the sample was oven-dried (40 °C) to constant weight and the % saponin content was estimated using the expression:

$$\% \text{ saponin} = (FW/IW) \times 100.$$

where FW and IW represents the final and initial weights of the sample respectively. The value obtained was finally converted to g/100 g of the sample.

2.7. Anti-nephrolithiasis study

2.7.1. Nucleation assay

The method of Hennequin *et al*. (1993) with slight modification was adopted for this experiment. Briefly, sterile solutions of $CaCl_2$ and $Na_2C_2O_4$ were prepared at a final concentration of 3.0 mM and 0.5 mM respectively, in a buffer (0.05 M Tris and 0.15 M NaCl (pH 6.5)) and filtered (0.2 μm pore diameter) three times. Exactly, 950 μL of $CaCl_2$ solution was mixed with 100 μL of different concentrations (0.1-1.0 mg/mL) of the extract. Crystallization was then initiated following addition of 950 μL $Na_2C_2O_4$ solution. The control was prepared without the extract solution. The resulting solution in each case was thoroughly but diligently stirred and the working temperature maintained at 37°C. The experiment was performed in triplicate and the absorbance of the solution was subsequently read at 620 nm. The rate of nucleation (as a function of absorbance) was estimated by comparing the induction time (time of appearance of crystals that reached a critical size and thus became optically detectable) in the presence of the extract with that of the control (without extract). For each evaluation, the absorbance obtained with the extract was subtracted from that of the control.

2.8. Data processing and interpretation

Except where stated otherwise, results were presented as mean ± standard error of mean (SEM) of replicate experiments. One way analysis of variance (ANOVA) coupled with Duncan Multiple Range Test using SPSS 16.0 (SPSS Inc., USA) was used to determine significant differences in all the parameters. Values were considered statistically significant at $p < 0.05$.

3.0. Results

3.1. Antioxidant and cytotoxicity

The *in vitro* radical scavenging properties of CSE on H_2O_2, OH^- and lipid peroxidized products are shown in Table 1. The extract dose-dependently inhibited and scavenged the radicals formed

with the highest dose exhibiting the most potent effect in all the assays. The tendency of CSE to scavenge H_2O_2 radical revealed a significant effect judging by the IC_{50} value (250.0 µg/mL) when compared with vitamin C (316.0 µg/mL). For the hydroxyl and lipid peroxidized radicals, the extract also displayed significant radical scavenging effect (with IC_{50} values of 515.0 and 373.0 µg/mL, respectively) and compared favourably with vitamin C (511.0 and 385.0 µg/mL). Their coefficient of determination (R^2) values is also presented (Table 1).

The degrees of cell viability and cytotoxicity of CSE treatment on HEK293 cell lines revealed a concentration-dependent effect with the highest dose-treated cells showing a 22.74% survival tendency compared with 92.43% for those on the lowest dose of the extract (Figure 1 and Table 2). The 72-h CC_{50} value for the extract in HEK293 cells was 435.23 µg/mL (Table 2).

Data obtained with respect to the nephroprotective study showed significant ($p<0.05$) reduction in the number of viable cells in the nephrotoxic groups (APAP-treated cells alone) compared with control (only MEM-treated cells) over the 24-h incubation period (Table 3). In contrast, when compared with the nephrotoxic cells, the CSE-treated groups had significantly ($p<0.05$) higher number of viable and metabolically active cells with the effect elicited by the extract administered at 62.5 µg/mL competing favorably with that of vitamin C. It is also noteworthy that the observed 82.0% cell mortality in the APAP-treated cells was significantly reduced (10.77%) following treatment with the 125 µg/mL dose of the extract (Figure 2).

Microscopic examination of cells from the control group revealed distinct and essentially normal morphological features (Figure 3a). However, while cells treated with MTT following APAP treatment for 24 h showed architectural alterations consistent with apoptotic morphological changes like cyto-constriction, detachment and nuclear condensation (Figure 3b), those treated

along with CSE and vitamin C had significantly lesser morphological infiltrations compared with the nephrotoxic cells (Figures 3c-e)

3.2. Saponin content and anti-nephrolithiasis

The saponin content of *Z. mays*, *S. maydis* was estimated to be 17.11 ± 0.05 g/100 g of its dry sample (Table 4).

The effect of treatments with CSE on the nucleation of CaOx crystals is presented in Table 5. There was consistent decrease in the absorbance with increasing concentration of the extract with the corresponding inhibitory effect of the extract ranging between 47.25-93.41%. The concentration-dependent increase in % inhibition of nucleation of CaOx crystals by CSE was significant with an IC_{50} of 256 μg/mL (R^2= 0.9775). In addition, the degree of turbidity was generally lower in the extract-treated solutions than in the control.

4.0. Discussion

H_2O_2 is an important product of catalytic influence of superoxide dismutase on the superoxide radical. Under the concerted actions of glutathione peroxidase and catalase in the presence of iron or copper ion, it is reduced to hydroxyl radicals which are capable of damaging important macromolecules (membrane lipids, proteins, and DNA base pairs) in biological systems (Sabiu *et al.*, 2016a). Hence, for effective mopping of the hydroxyl radicals, it is imperative for the overwhelmingly H_2O_2 produced to be scavenged. In this study, CSE had very strong potential to annihilate the ravaging effects of both the H_2O_2 and hydroxyl radicals judging by its IC_{50} values that competed well with those of vitamin C. These observations could suggest the capability of the extract to halt cascade of reactions involving these radicals by sacrificing either electron or hydrogen atom thereby making them relatively stable. Consequently, this could prevent probable cellular and macromolecular damage. In addition to being in agreement with the report of

Rahmat *et al.* (2012) on the antioxidant activity of extracts of *Sonchus asper* (L.) Hill, the current assertions are also consistent with our previous report on the *in vivo* capability of CSE to optimize the specific activities of preventive (catalase, glutathione peroxidase) and chain-breaking (superoxide dismutase, glutathione reductase) antioxidants in acetaminophen-challenged hepatotoxic rats (Sabiu *et al.*, 2016a). Another event consistent with hydroxyl radical oxidative damage is lipid peroxidation. Auto-oxidation of membrane-bound lipids may disrupt membrane fluidity and inflict physiological alterations on other metabolically important biomolecules (proteins and DNA). The present study employed egg-yolk homogenate as lipid-bound source and the inhibitory effect of the extract on the seemingly lipid peroxidized products was significant and perhaps indicative of its tenacity to detoxify hydroxyl and other reactive metabolites, which could have prompted and propagated peroxidation of membrane-bound polyunsaturated lipids in biological systems. Furthermore, the tending towards 1.0 of R^2 values of all the antioxidant assays evaluated in this study is another predictive and supporting fact for the excellent antioxidative activity of CSE which could be adduced to the synergistic effect of its reported polyphenolic constituents (Sabiu *et al.*, 2016b).

Consequent upon the ethical and scientific concerns on the use of animals, many *in vitro* techniques for animal toxicity and pharmacological testing have been developed, validated and gained global regulatory acceptance as suitable alternatives to whole animal tests. These techniques have been developed and validated using the 4Rs (Replacement, Reduction, Refinement and Responsibility) approach. One of the most routinely adopted approaches for *in vitro* cytotoxicity evaluation of chemicals or pharmacological agents utilizes various continuous cell lines (Eun-Kee *et al.*, 2007; Soumen *et al.*, 2016). In this study, it was obvious that the HEK293 cells were sensitive to CSE treatments and the degree of sensitivity varied with varying

concentrations. Judging by the CC_{50} value (435.23 µg/mL), it may be inferred that CSE was well tolerated by the cells and holds promising therapeutic and pharmacological potentials. However, the lower concentrations (62.5 and 125 µg/mL) of the extract where the least cytotoxic effects were observed on HEK293 cells with corresponding 92.43 and 71.22% viability were used for the subsequent nephroprotective assay.

APAP-mediated renal tubular damage has been attributed either to translocation of GADD153 (growth arrest- and DNA damage-inducible gene 153) and subsequent proteolysis of caspase-12 (Lorz and Justo, 2004) or involvement of N-acetyl-*p*-benzoquinone imine (NAPQI) (Parameshappa *et al.*, 2012). NAPQI arylates selenium binding protein and glutamine synthetase in the S3 segment of the tubule with consequential depletion of reduced glutathione (Adeneye and Benebo, 2008). This subsequently results in auto-oxidation of renal macromolecules (lipids, proteins and DNA) with associated tubular cell necrosis. Since APAP accumulates in the kidney, HEK293 cell line established from human embryonic kidney cells was used in this study to mimic renal-like features. The decreased number of viable cells following 24 h exposure to APAP could suggests epithelia injury and cell necrosis resulting from free radicals generation. This observation is in agreement with previous studies (Lijuan *et al.*, 2007), where APAP treatment proved lethal to established cell lines. However, the significant and concentration-related increase in the population of viable and metabolically active cells in the CSE-treated groups may be indicative of the capability of the extract to extenuate the toxic influence of APAP on the cells. This assertion may also suggest that the extract at its pharmacological regimens may preserve renal functions and protect against renal pathological conditions. In addition to complementing the cell viability assessment, microscopic examination of the MTT treated cells could provide pertinent information on how pharmacologically good an agent is

against cell necrosis. The significant alterations in the morphological features of the APAP-treated cells could have impaired and facilitated their mortality. However, the apparently prevented onslaughts by APAP on the architectural features of cells in the extract-treated groups could mean that CSE offered considerable protection and stabilized the morphological integrity of the cells. The effect elicited by the extract compared favourably with that of vitamin C and is consistent with the results of the viability tests. Kalaivani *et al.* (2015) also gave similar submissions on the nephroprotective effect of ethanolic root extract of *Boerhaavia diffusa* against cisplatin-induced nephrotoxicity in LLC-PK1 cells. In light of the foregoing, the overall effects elicited by CSE as a nephroprotective candidate against APAP toxicity on HEK293 cells may be adduced to its antioxidative properties which were significantly elicited in effectively scavenging H_2O_2, hydroxyl, and lipid peroxidized radicals in this study. This may also be attributed to its membrane stabilization potential which might have prevented the release of lactate dehydrogenase from the mitochondrial epithelia of the CSE-treated cells into the medium and consequently preventing necrosis. Interestingly, the *in vitro* ability of *Z. mays*, *S. maydis* in stabilizing bovine erythrocytes membrane has just been reported (Sabiu *et al.*, 2016a).

The crystallization of CaOx begins with increased urinary supersaturation, with the subsequent formation of solid crystalline particles within the urinary tract. This is followed by nucleation via which stone-forming salts in supersaturated urinary solution coalesce into crystals that consequently increase in size by accumulation of new constituents (Basavaraj *et al.*, 2007). These crystals then grow and aggregate with other crystals in solution, and are ultimately retained and accumulated in the kidney (Bashir and Gilani, 2009). Renal injury encourages crystal retention and the development of a stone nidus on the renal papillary surface that further worsens crystal nucleation at lower supersaturation levels (Malvinder, 2004; Worcester and Coe,

2008). Therefore, levels of urinary supersaturation correlate with the type of stone formed, and reducing supersaturation is effective in preventing stone recurrence.

In the present study, CaOx crystals were formed from the incubation of $CaCl_2$ and $Na_2C_2O_4$ solutions and their presence were confirmed by the turbid nature of the resulting solutions. Plants or plant parts with good antioxidative and anti-crystallization potentials have been found to be highly anti-nephrolithiatic by disaggregating the mucoproteins which are major promoters of crystallisation in the renal tubules (Atmani and Khan, 2000; Fouada *et al.*, 2006; Rathod *et al.*, 2013). This was evidently shown in this study with the extract at the highest investigated dose conferring a nucleation inhibitory potential of 93.41% against crystals formation. Furthermore, the apparent decrease in the degree of turbidity in the CSE-treated solutions relative to the control was another probable indication that the amount of crystals formed in the presence of the extract was significantly reduced. Aggarwal *et al.* (2010) also gave similar assertions on the *in vitro* CaOx crystals inhibitory effects of *Tribulus terrestris.*

In view of these, it could be logically inferred that CSE is rich in phytoconstituents capable of inhibiting reactions or processes leading to CaOx crystals formation. Studies have implicated several phytocompounds as good anti-nephrolithiatic agents. While saponins are known to disaggregate suspension of mucoproteins (Fouada *et al.*, 2006; Gurocak and Kupeli, 2006), other phytocompounds have found relevance in optimizing urinary excretion which subsequently aids decrease in the concentration of urinary salts that ultimately prevents supersaturation of the crystallizing salts (Bashir and Gilani, 2009). Interestingly, in addition to the quantified saponin content of CS in this study, it has also been reported to be rich in pharmacologically important phenolics (Sabiu *et al.*, 2016c), and these might be responsible for its significantly elicited effects in this study.

5.0. Conclusion

Consequent upon the available data, it may be logically inferred that CSE is endowed with phytoconstituents of utmost significance in radical scavenging events. This might have potentiated its ability to inhibit CaOx nucleation and enhanced vitality of metabolically active HEK293 cells following APAP treatment. Hence, complete characterization of the exact constituent(s) responsible for the elicited effects by *Z. mays*, *S. maydis* in this study is vital. In this direction, efforts are ongoing.

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Conflict of interest

None to declare

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List of figures

Table 1: Reactive oxygen species scavenging and antioxidant properties of *Zea mays*, *Stigma maydis* aqueous extract.

	H_2O_2		OH*		LPO*	
Treatments	IC_{50} (µg/mL)	R^2	IC_{50} (µg/mL)	R^2	IC_{50} (µg/mL)	R^2
CSE	250.0	0.9835	515.0	0.8769	373.0	0.9652
Vitamin C	316.0	0.8999	511.0	0.9183	385.0	0.9431

CSE=Corn silk aqueous extract

Table 2: Cytotoxicity of *Zea mays*, *Stigma maydis* aqueous extract against HEK293 cells

Concentration (µg/mL)	62.5	125.0	250.0	500.0	1000.0
Cytotoxicity (%)	7.56	28.79	54.55	62.13	77.26
CC_{50} (µg/mL)			435.23		

Table 3: % protection offered by *Zea mays*, *Stigma maydis* aqueous extract and vitamin C against acetaminophen toxicity in HEK293 cells

Treatments	Cell viability (%)	Protection offered (%)
MEM (control)	100,000	100[a]
APAP (1000 µg/mL)	18,000	-
APAP + 62.5 µg/mL CSE	75,250	75.25[b]
APAP + 125.0 µg/mL CSE	89,230	89.23[c]
APAP + 0.25 mM vit. C	73,980	73.98[b]

Values bearing different superscripts along the same column for the % protection are significantly different ($p < 0.05$). APAP =Acetaminophen, CSE=Corn silk aqueous extract, vit. C=Vitamin C, MEM= Minimum Essential Medium Eagle.

Table 4: Saponin content of *Zea mays, Stigma maydis*

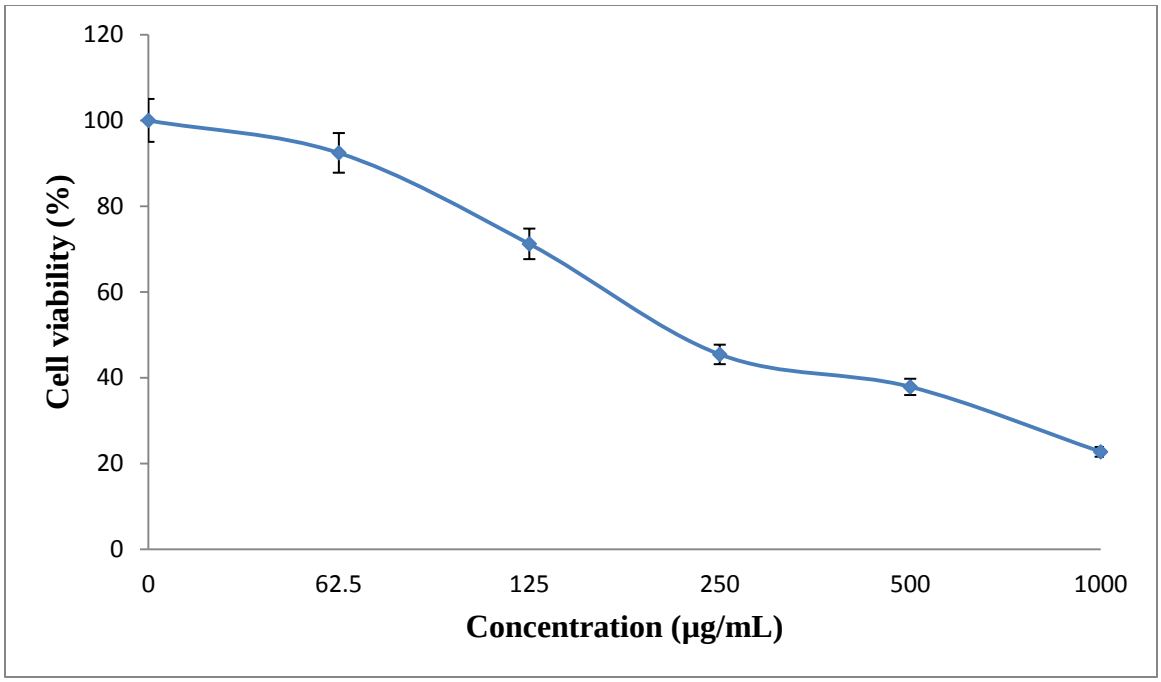
Sample	Saponin (g/100 g sample)
Zea mays, Stigma maydis	17.11 ± 0.05

Value was expressed per 100 g of dry sample and is mean of triplicate determinations ± standard error of mean

Table 5: Inhibitory effect of *Zea mays*, *Stigma maydis* aqueous extract on the nucleation of CaOx crystallization.

	Extract (mg/ml)					
Parameters	Control	0.10	0.20	0.40	0.80	1.00
Absorbance	0.91 ± 0.02^{a}	0.48 ± 0.03^{b}	0.42 ± 0.03^{b}	0.22 ± 0.02^{c}	0.15 ± 0.03^{d}	0.06 ± 0.02^{e}
% inhibition	-	47.25	53.85	75.82	83.52	93.41

[abcde]Values carrying different superscripts across the same row are significantly different ($p<0.05$)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100
80
60
40
20
0
Cell viability (%)
0
62.5
125
250
500
1000
Concentration (µg/mL)

Fig. 1

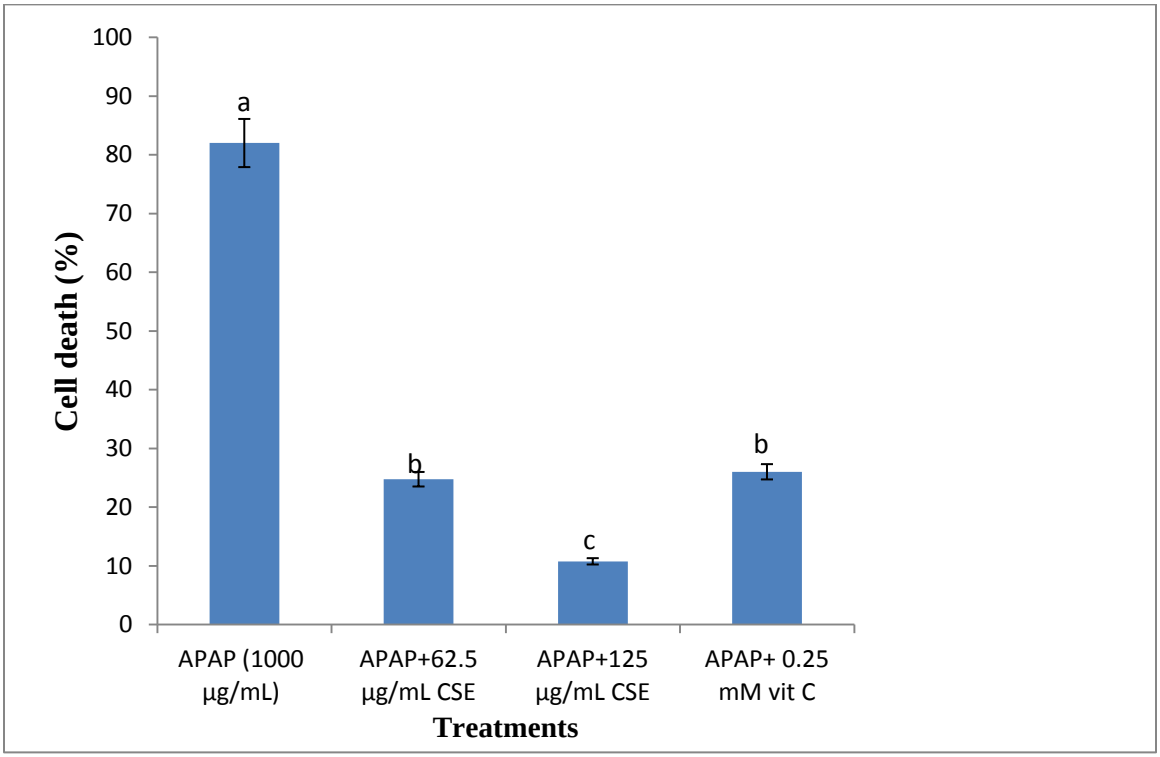
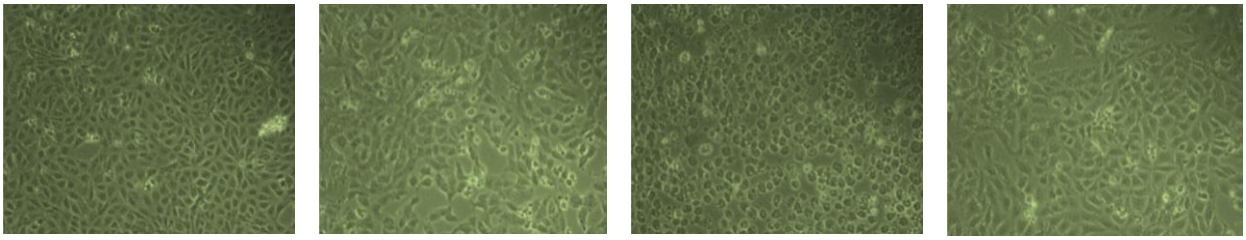
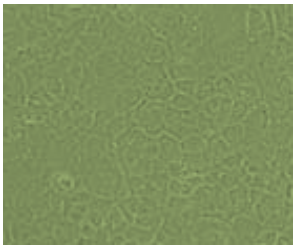

100
90
80
70
60
50
40
30
20
10
0
Cell death (%)
a
b
c
b
APAP (1000 µg/mL)
APAP+62.5 µg/mL CSE
APAP+125 µg/mL CSE
APAP+ 0.25 mM vit C
Treatments

Fig. 2



(a) (b) (c) (d)



(e)

Fig. 3.

CHAPTER FOUR

Kinetics of *α*-amylase and *α*-glucosidase inhibitory potential of *Zea mays* Linnaeus (Poaceae), *Stigma maydis* aqueous extract: An *in vitro* assessment

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Kinetics of α-amylase and α-glucosidase inhibitory potential of *Zea mays* Linnaeus (Poaceae), *Stigma maydis* aqueous extract: An *in vitro* assessment

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ABSTRACT

Ethnopharmacological relevance: Corn silk (*Zea mays* L., *Stigma maydis*) is an important herb used traditionally in many parts of the world to treat array of diseases including diabetes mellitus. Inhibitors of α-amylase and α-glucosidase offer an effective strategy to modulate levels of post prandial hyperglycaemia via control of starch metabolism.
Aim of the study: This study evaluated α-amylase and α-glucosidase inhibitory potentials of corn silk aqueous extract. Active principles and antioxidant attributes of the extract were also analysed.
Materials and methods: The α-amylase inhibitory potential of the extract was investigated by reacting its different concentrations with α-amylase and starch solution, while α-glucosidase inhibition was determined by pre-incubating α-glucosidase with different concentrations of the extract followed by addition of *p*-nitrophenylglucopyranoside. The mode(*s*) of inhibition of the enzymes were determined using Lineweaver-Burke plot.
Results: In vitro analysis of the extract showed that it exhibited potent and moderate inhibitory potential against α-amylase and α-glucosidase, respectively. The inhibition was concentration-dependent with respective half-maximal inhibitory concentration (IC_{50}) values of 5.89 and 0.93 mg/mL. Phytochemical analyses revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins and phytosterols as probable inhibitory constituents. Furthermore, the extract remarkably scavenges reactive oxygen species like DPPH and nitric oxide radicals, elicited good reducing power and a significant metal chelating attributes.
Conclusion: Overall, the non-competitive and uncompetitive mechanism of action of corn silk extract is due to its inhibitory effects on α-amylase and α-glucosidase, respectively. Consequently, this will reduce the rate of starch hydrolysis, enhance palliated glucose levels, and thus, lending credence to hypoglycaemic candidature of corn silk.

1. Introduction

Diabetes mellitus (DM) is one of the most common endocrine metabolic disorders and has caused significant mortality in the world. It is expressively becoming the third greatest threat to human health after cancer, cerebrovascular and cardiovascular diseases (Vasim et al., 2012). Its complications are usually attributable to either microvascular (retinopathy, neuropathy, and nephropathy) or/and macrovascular (heart attack, stroke and peripheral vascular) ailments (Umar et al., 2010). The prevalence of diabetes is increasing annually, affecting more than 150 million (about 4.6%) people globally and the projection is pegged at well above 300 million before 2025 (Ganiyu et al., 2012). With this projection, someone dies from its complications every 10 s and 1 in every 5 individuals may be diabetic by 2025 (Colagiuri, 2010). Recent reports have estimated an increase in these figures, with the global prevalence marching towards 7.8%, representing 438 million people by 2030 (Colagiuri, 2010). Unfortunately, current statistics suggests that South Africa and Nigeria top the list in Africa, with a prevalence of 8.30% and 4.50%, respectively (IDF, 2014). DM not only takes a heavy toll of lives around the world but imposes a serious financial burden on the sufferers and their family members. These geometrical increases in the number of

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diabetics in recent times cannot be dissociated from unhealthy life style, urbanization, aging and ravaging influence of free radicals (Wild et al., 2004). In diabetes, hyperglycaemia enhances formation of reactive oxygen species (ROS)/free radicals which consequently results in auto-oxidation of membrane bound macromolecules (lipids and proteins) and eventually, cellular damage (Dandona et al., 1996; Baynes and Thorpe, 1999; Kajaria et al., 2013). Deleterious impacts of these events are crucial and have been implicated in secondary complications in DM. Since ROS have been implicated in the pathogenesis of the disease, a logical way to manage and extenuate its potential burden may be via antioxidant application. Accordingly, antioxidants have been shown to prevent destruction of pancreatic β-cells by inhibiting auto-oxidation chain reaction and thus stalling progression to diabetes complications (Campos et al., 2003; Sabu and Kuttan, 2004; Liu et al., 2007). However, restriction in the use of synthetic antioxidants is being imposed because of their carcinogenicity, hence the resolve to naturally occurring plant-based antioxidants of relatively low toxicity. Plants containing natural antioxidants can preserve β-cell function and prevent ROS-induced DM (Aslan et al., 2010). Little wonder, the World Health Organization has specifically substantiated utilization of phytotherapies for the management and treatment of diabetes (Bailey, 2003), and in fact an ample proportion of the populace in some African countries including South Africa and Nigeria now relies exclusively on plants as a source of medicine to augment the increasingly expensive orthodox medical services (Fasola and Egunyomi, 2005). Corn silk (CS) is one of the herbs with excellent applications in this respect.

Zea mays L. (Poaceae), *Stigma maydis* (Corn silks) are elongated stigmas from the female flowers of maize. It is a waste product of corn cultivation and available in abundance (Maksimović et al., 2004). The colours at first are usually light green and later turn into red, yellow or light brown. CS functions primarily to trap pollens for pollination and is harvested at the same time as the corn cob. Each silk may be pollinated to produce one kernel of corn. The silks are usually about 30 cm long with faintly sweetish taste. GCMS analysis of its aqueous extract from our laboratory revealed the presence of maizenic acid, β-carotene, ascorbic acid, gluten, o-diethyl phthalate, 2-methyl-napthalene, thymol, maizenic acid, 3′-o-methyl-maysin, cyanidin, cinnamic acid, hordenine, luteolinidin, pelargonidin and betaine as major identifiable adaptogenic phytonutrients. CS has long been used in folkloric medicine as oral anti-diabetic drug by the Chinese (Jianyou et al., 2009; Ghada et al., 2014). Its antibiotics, immune enhancement, and insecticidal activities have also been documented (Guevara et al., 2000; Maksimovic and Kovacevic, 2003; Kim et al., 2004). Formulations of CS have proven benefits in the management of cystitis, edema, kidney stones, prostate disorder, urinary infections, bedwetting and obesity (Grases et al., 1993). Although, studies have demonstrated its pharmacological significance as an oral anti-diabetic and hypoglycemic agent particularly from Asian countries (Farsi et al., 2008; Zhao et al., 2012), there is dearth of information on its exact mechanism of anti-diabetic action. Hence, the present study sought to unravel this by investigating its carbohydrate metabolizing enzyme kinetics through *in vitro* α-amylase and α-glucosidase inhibitory models. In addition, antioxidant activities of CS aqueous extract to consolidate its much touted pharmacological attributes were also evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, rutin, quercetin, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), ferric chloride ($FeCl_3$), potassium ferricyanide, porcine pancreatic α-amylase, rat intestinal α-glucosidase and *p*-nitrophenyl-α-D-glucopyranoside (pNPG) were procured from Sigma Chemical Co., St. Louis, Missouri, USA. Starch, dinitrosalicylic acid (DNS), maltose and sucrose were products of J. T. Baker Inc., Phillipsburg, USA, while acarbose was purchased from Bayer Medical Co. (Germany). Distilled water was obtained from Phytomedicine and Phytopharmacology Research Group Laboratory, Plant Sciences Department, QwaQwa campus, South Africa. All other chemicals and reagents used were of analytical grade.

2.2. Plant collection, authentication and preparation of extract

Corn silks were harvested from a maize plantation in Phuthaditjhaba area of Maluti-A-Phofung, QwaQwa, Free State province, South Africa, between November 2014 and March 2015. They were authenticated (from fresh whole maize plant) at the Plant Sciences Department, University of the Free State, QwaQwa campus, South Africa. Voucher specimen (No. SabMed/01/2015/QHB) was prepared and deposited at the University's Herbarium. The CS was shade dried to constant weight prior to pulverizing with an electric blender (model MS-223; Blender/Miller III, Taiwan, China) to fine powder. The powdered sample (650 g) was suspended in distilled water (6.5 L) for 48 h with regular shaking by labcon platform shaker maintained at 180 rpm. The solution obtained was then filtered and the resulting filtrate lyophilized to give 65.7 g (10.11%) residue. The lyophilized sample (corn silk extract (CSE)) was stored air-tight prior to commencement of the study.

2.3. Qualitative phytochemical analysis

Adopting the procedures of Sofowara (2006) and Prashant et al. (2011), the aqueous extract of corn silk was subjected to qualitative phytochemical screening. Nine active principles were screened for.

2.3.1. Detection of alkaloids

In this screening, the extract was dissolved in dilute hydrochloric acid and filtered. The filtrate obtained was thereafter subjected to Wagner's reagent (2 g of iodine and 6 g of potassium iodide in 100 ml of water) treatment and the presence of alkaloids was confirmed by brown/reddish precipitate formation.

2.3.2. Detection of anthraquinones

Chloroform (2 mL) was added to 0.2 g of the extract and the resulting mixture was vigorously shaken for 5 min prior to filtration. Equal volumes of the filtrate obtained and 10% ammonia solution were thoroughly mixed and the formation of a bright pink colouration in the aqueous layer of the mixture confirmed the presence of anthraquinones.

2.3.3. Detection of glycosides

The extract was hydrolyzed with 10% aqueous hydrochloric acid, and then treated with 2% sodium nitroprusside in pyridine and 20% sodium hydroxide. Formation of pink to blood red colour indicated positive test for cardiac glycosides.

2.3.4. Detection of flavonoids

The extract (0.5 g) was treated with few drops of 10% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, suggested the presence of flavonoids.

2.3.5. Detection of phenols

For this screening, 0.5 g of corn silk aqueous extract was treated with 3–4 drops of 10% ferric chloride solution. Formation of bluish black colour was an indication of phenols.

2.3.6. Detection of saponins

Powdered extract (2.0 g) was boiled in 20 mL of distilled water for 5 min and filtered. The filtrate (10 mL) was mixed with 5 mL of distilled water in a graduated cylinder and shaken vigorously and left for 15 min for persistent frothing. The froth was thereafter mixed with 3–4 drops of olive oil and shaken again for observation of emulsion layer which signifies the presence of saponins.

2.3.7. Detection of tannins

To the extract (0.5 g), 1% gelatin solution containing 10% sodium chloride was added. Formation of white precipitate confirmed the presence of tannins.

2.3.8. Detection of triterpenes

Corn silk extract was treated with chloroform and filtered. The filtrate was then treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour was an indication of triterpenes.

2.3.9. Detection of phytosterols

A portion of the extract was treated with chloroform and filtered. The resulting filtrate was thereafter subjected to few drops of acetic anhydride treatment, boiled and cooled. Following addition of concentrated sulphuric acid, the formation of brown ring at the layer junction indicated the presence of phytosterols.

2.4. Quantitative phytochemical analysis

2.4.1. Total phenolics

Following the reported method of Wolfe et al. (2003), the total phenol contents in the plant extract was determined. Briefly, an aliquot of the extract (1 mL) was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was read at 765 nm using a spectrophotometer (Beckman, DU 7400, USA). Extract was evaluated at a final concentration of 1 mg/mL. Total phenolic content was expressed as mg/g gallic acid equivalent using the equation obtained from a calibration curve of gallic acid.

2.4.2. Total flavonoids

Total flavonoids were estimated using the method of Ordon-ez et al. (2006). In brief, 0.5 mL of 2% $AlCl_3$ ethanolic solution was added to 0.5 mL of the extract. After 1 h at room temperature, the absorbance was read at 420 nm. The development of yellow colour was taking as indication of the presence of flavonoids. Extract samples were evaluated at final concentration of 1 mg/mL. Total flavonoid content was calculated as quercetin equivalent (mg/g) using the equation obtained from the calibration curve.

2.4.3. Total flavonols

The flavonols content was determined by the method of Kumaran and Karunakaran (2007). Twenty gram of $AlCl_3$ and 50 g of sodium acetate anhydrous powder were separately dissolved in little quantity of distilled water and made up to 1.0 L with distilled water respectively. The rutin calibration curve was prepared by mixing 2 mL of varying concentrations of rutin (0.2–0.1 mg/mL) with 2 mL (20 g/L) $AlCl_3$ and 6 mL (50 g/L) sodium acetate. The absorbance at 440 nm was read after 2.5 h at 20 °C. The same procedure was carried out with 2 mL of plant extract (0.1/1.0 mg/mL) instead of rutin solution. All determinations were carried out in triplicates. The flavonols content was obtained from rutin calibration curve and expressed as rutin equivalents (mg/g).

2.5. In vitro antioxidant assays

2.5.1. DPPH radical scavenging assay

The antioxidant activity of CSE was determined by measuring its capacity of bleaching the purple-coloured ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), as described by Turkoglu et al. (2007). Briefly, 1 mL of various concentrations (0.2–1.0 mg/mL) of the extract in methanol was added to 1 mL of a 0.2 mmol/L sample of DPPH in methanol. After a 30 min incubation period at room temperature, the absorbance was read against blank at 516 nm. Inhibition rate (I%) on the DPPH radical was calculated using the expression:

$$\text{Percentage inhibition (I\%)} = [(A_{control} - A_{extract})/A_{control}] \times 100,$$

where $A_{control}$ is the absorbance of the control, $A_{extract}$ is the absorbance of the extract. Using standard calibration curve, the concentration of CSE extract causing 50% inhibition (IC_{50}) of DPPH radical was estimated.

2.5.2. Nitric oxide scavenging assay

Nitric oxide radical stalling activity of CSE was assayed according to the method of Garrat (1964). Briefly, 2 mL of 10 m sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of varying concentrations of the extract, and incubated at 25 °C for 2 h. From the incubated mixtures, 0.5 mL were taken and added to 1 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and further incubated at room temperature for 5 min. Following this, 1 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was introduced into the mixtures and the resulting solution incubated at room temperature for 30 min. The absorbance was taken at 540 nm and the IC_{50} was then evaluated from calibration curve following estimation of percentage nitric oxide radical scavenging capacity of CSE using the expression:

$$\text{Percentage Scavenging (S\%)} = [(A_{control} - A_{extract})/A_{control}] \times 100,$$

where $A_{control}$ is the absorbance of the control, $A_{extract}$ is the absorbance of the extract.

2.5.3. Metal chelating assay

The metal chelating potential of CSE with particular reference to ferrous ion was estimated following the method of Dinis et al. (1994). In brief, 0.1 mL of the extract (different concentrations) was added to 0.5 mL of 0.2 mM ferrous chloride solution. The reaction was initiated by the addition of 0.2 mL of ferrozine (5 mM) and incubated at room temperature for 10 min prior to absorbance readings at 562 nm. Citrate was used as control and measurement of the colour reduction determines the chelating activity of the extract to compete with ferrozine for the ferrous ions and the IC_{50} value was thereafter estimated from the calibration curve.

2.5.4. Reducing power method

The reducing power of the extract was evaluated by adopting the method of Oyaizu (1986). Varying concentrations of CSE (0.2–1.0 mg/mL) were suspended in 1 mL of distilled water and mixed with 2.5 mL of 0.2 m phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50 °C for 20 min prior to addition of 2.5 mL of trichloroacetic acid (TCA). Following centrifugation at 3000 rpm for 10 min, 2.5 mL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance of the resulting solution was then read at 700 nm.

2.6. In vitro anti-diabetic assays

2.6.1. Specific α-glucosidase inhibitory activity on sucrase and maltase enzyme

Adopting the modified method of Elsnoussi et al. (2012), the sucrase and maltase inhibitory activities were evaluated. Briefly, varying concentrations (0.25–8.0 mg/mL) of the extracts were prepared and 50 μL of each extract was mixed with 100 μL of 0.1 m phosphate buffer (pH 6.9) containing 1.0 m of α-glucosidase solution. The mixtures were then incubated in 96-well plates at 25 °C for 10 min. After pre-incubation, 50 μL of 50 mM sucrose and 25 mM maltose solutions in 0.1 m phosphate buffer (pH 6.9) respectively were added to each well at timed intervals for sucrase and maltase assays. The reaction mixtures were incubated at 25 °C for 5 min before introduction of 50 μL of 0.1 m $NaCO_3$ to halt the reaction. The absorbance readings were thereafter taken at 540 nm using a micro-plate reader. The experiments were conducted in triplicate and the sucrase and maltase inhibitory activities were calculated as %inhibition, and the respective concentrations of corn silk extract resulting in 50% inhibition (IC_{50}) of their activity were determined from their calibration curves.

2.6.2. General α-glucosidase inhibition and kinetic methods

The α-glucosidase inhibitory activity was assayed following the method of Elsnoussi et al. (2012). In brief, different concentrations (0.25–8.0 mg/mL) of CS were prepared in distilled water. Then, 50 μL from the stock solution was mixed with 100 μL of 0.1 m phosphate buffer (pH 6.9) containing 1.0 m of α-glucosidase solution. The mixtures were then incubated in 96-well plates at 25 °C for 10 min. Following this, 50 μL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 m phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. The absorbance readings were thereafter taken at 405 nm using a micro-plate reader (Thermomax, USA) and the values compared with a control which contained 50 μL of the buffer instead of the extract. Acarbose (Bayer Medicals, Germany) was prepared in distilled water at same concentrations as the extract and used as control. The experiments were conducted in triplicate and the α-glucosidase inhibitory activity was expressed as %inhibition using the expression:

$$\%\text{Inhibition} = [(\Delta A_{control} - \Delta A_{extract})/\Delta A_{control}] \times 100,$$

where $\Delta A_{control}$ and $\Delta A_{extract}$ are the changes in absorbances of the control and extract sample respectively. Using standard calibration curve, the concentration of corn silk extract causing 50% inhibition (IC_{50}) of α-glucosidase activity was estimated.

For the enzyme kinetics on inhibition of α-glucosidase activity by aqueous extract of CS, a modified method of Dnyaneshwar and Archana (2013) was adopted. Briefly, 50 μL of 5 mg/mL extract was pre-incubated with 100 μL of α-glucosidase solution for 10 min at 25 °C in one set of tubes. In another set of tubes, α-glucosidase was pre-incubated with 50 μL of phosphate buffer (pH 6.9). 50 μL of pNPG at concentrations (0.63–2.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25 °C, and 500 μL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined colourimetrically using a *p*-nitrophenol standard curve. Reaction rates (v) were thereafter calculated and double reciprocal plots of enzyme kinetics were constructed according to Lineweaver and Burk method to study the nature of inhibition. Km and Vmax values were also calculated from Lineweaver-Burk plot (1/v *versus* 1/[S]) (Lineweaver and Burk, 1934).

2.6.3. α-amylase inhibition and kinetic studies

Adopting the methods of Elsnoussi et al. (2012) and Kazeem et al. (2013), the α-amylase inhibitory activity and mode of inhibition were evaluated. Briefly, varying concentrations (0.25–10.0 mg/mL) of the extracts were prepared and 500 μL of each was mixed with 500 μL of 0.02 m sodium phosphate buffer (pH 6.9) containing 0.5 mg/mL of α-amylase solution and incubated in test tubes at 25 °C for 10 min. After pre-incubation, 500 μL of 1% starch solution in 0.02 m sodium phosphate buffer (pH 6.9) was added to each tube at timed intervals. The reaction mixtures were incubated at 25 °C for 10 min and stopped with 1.0 mL of dinitrosalycyclic acid colour reagent. The tubes were then incubated in a boiling water bath for 5 min and subsequently cooled to room temperature. The reaction mixtures were then diluted with distilled water (15 ml), and the absorbance readings were measured at 504 nm using a spectrophotometer (Biochrom WPA Biowave II, Cambridge, England) and the values compared with a control which contained 500 μL of the buffer instead of the extracts. Acarbose was prepared in distilled water at same concentrations as extracts and used as control. The experiments were conducted in triplicate and the α-amylase inhibitory activity was expressed as %inhibition. The concentration of the extract causing 50% inhibition (IC_{50}) of α-amylase activity was estimated from its standard calibration curve.

For the kinetic experiments involving concentration independent inhibition, inhibitor/extract was taken at it IC_{50} value and incubated with α-amylase while the concentration of starch (substrate) was varied from 0.3 to 5 mg/mL and reaction allowed to proceed as highlighted above. The amount of reducing sugars released was determined spectrophotometrically using maltose standard curve and converted to reaction velocities (v). Lineweaver-Burk double reciprocal plot (1/v *versus* 1/[S]) was constructed and the kinetics of α-amylase inhibition by the extract was determined (Lineweaver and Burk, 1934).

2.7. Statistical analysis

Free radical and ROS scavenging activities were expressed in percentage. Other results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) followed by Tukey–Kramer test for differences between means was used to detect significant differences ($p < 0.05$) between each treatment groups in this study using StatPlus, 2011 (AnalystSoft Inc., Alexandria, VA, USA).

3. Results

3.1. Phytochemicals

The qualitative and quantitative phytochemical analyses of aqueous extract of *M. stigma* are presented in Tables 1 and 2 respectively. Alkaloids, flavonoids, phenols, saponins, tannins and phytosterols were detected, while anthraquinones, cardiac

Table 1
Phytochemical constituents of corn silk.

Phytochemicals	Remark
Alkaloids	Detected
Anthraquinones	Not detected
Cardiac glycoside	Not detected
Flavonoids	Detected
Phenols	Detected
Saponin	Detected
Tannin	Detected
Triterpenes	Not detected
Phytosterols	Detected

Table 2
Total phenolic, flavonoid, and flavonol contents of aqueous extract of corn silk.

Phytochemicals	Corn silk aqueous extract
Total phenol (mg gallic acid g-1)	77.89 ± 5.45
Total flavonoid (mg quercetin g-1)	59.65 ± 3.28
Total flavonol (mg rutin g-1)	45.15 ± 2.15

Values were expressed per g of plant extract and are means of triplicate determinations ± Standard deviation.

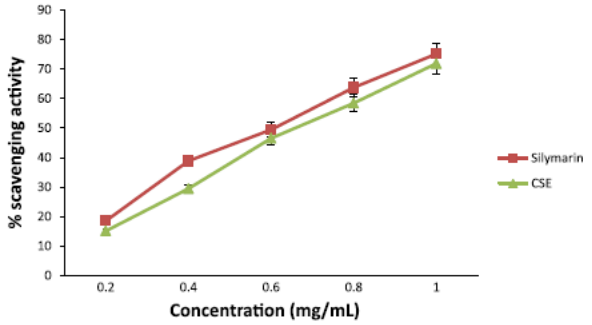

Fig. 1. DPPH scavenging effect of corn silk aqueous extract. Values are mean ± standard deviation (SD) of triplicate determinations. CSE=corn silk aqueous extract.

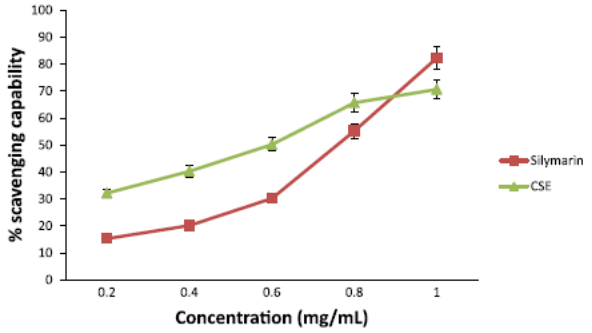

Fig. 2. Nitric oxide scavenging potential of corn silk aqueous extract. Values are mean ± standard deviation (SD) of triplicate determinations. CSE=corn silk aqueous extract.

Table 3
IC_{50} (mg/mL) of DPPH, nitric oxide scavenging and metal chelating capabilities of corn silk aqueous extract.

DPPH	Silymarin	CSAE
IC_{50}	0.61	0.68
R^2	0.987	0.996
Regression equation	y=68.99x+7.75	y=71.20x+1.57
NO scavenging		
IC_{50}	0.71	0.57
R^2	0.917	0.980
Regression equation	y=84.53x−10.07	y=51.23x+21.06
Metal chelating	**Citrate**	**CSAE**
IC_{50}	0.43	0.57
R^2	0.976	0.958
Regression equation	y=58.41x+24.63	y=61.92x+14.48

glycoside and triterpenes were absent. The total phenol, flavonoid and flavonol were 77.89 ± 5.45 mg gallic acid g-1, 59.65 ± 3.28 mg quercetin g-1, and 45.15 ± 2.15 mg rutin g-1 respectively.

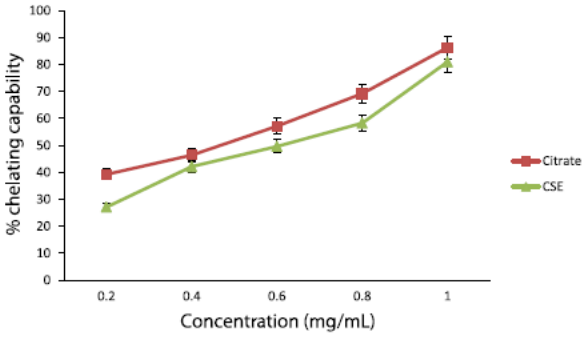

Fig. 3. Metal chelating potential of corn silk aqueous extract. Values are mean ± standard deviation (SD) of triplicate determinations. CSE=corn silk aqueous extract.

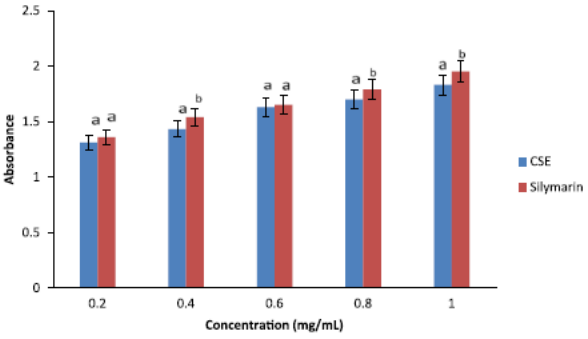

Fig. 4. Reducing power potential of corn silk aqueous extract. Values are mean ± standard deviation (SD) of triplicate determinations. CSE=corn silk aqueous extract.

3.2. Antioxidant activity

The *in vitro* antioxidant potentials of aqueous extract of *M. stigma* are shown in Figs 1–4. The extract dose dependently inhibited/scavenged/chelated the generated radicals/ions/metals in all the assays investigated. The capability of the extract to scavenge DPPH radical revealed a remarkable effect judging by the IC_{50} value (0.68 mg/mL) when compared with silymarin (0.61 mg/mL) (Table 3). Nitric oxide is unstable and reacts with molecular oxygen to form stable nitrate. CS aqueous extract exhibited commendable nitric oxide scavenging effect (with 0.57 mg/mL IC_{50} value) in comparison with silymarin (0.71 mg/mL) (Table 3). Similarly, the metal chelating ability of CSE revealed a significant potential against ferrous ion (Fig. 3), and the respective IC_{50} value when compared with the standard (citrate) is presented in Table 3. Furthermore, the reducing power effect of CSE on ferric ion competed well with that of silymarin in a dose-related manner with the highest dose (1.0 mg/mL) exhibiting the most potent effect (Fig. 4). The respective coefficient of determination (R^2) values and regression equations for the assays are also presented in Table 3.

3.3. In vitro enzyme kinetics inhibitory potentials

The results of α-glucosidase inhibitory assay revealed that the activities of general and specific α-glucosidases were dose-dependently inhibited by CS aqueous extract (Fig. 5a-c), and their IC_{50} values are presented in Table 4. The most potent effect was observed with the extract at the highest dose investigated, while the lowest concentration of 0.25 mg/mL revealed minimum inhibitions. Except for the specific maltase activity, the %inhibition of CSE on α-glucosidases was concentration-dependent and competed favourably with acarbose at lower concentrations (0.25–

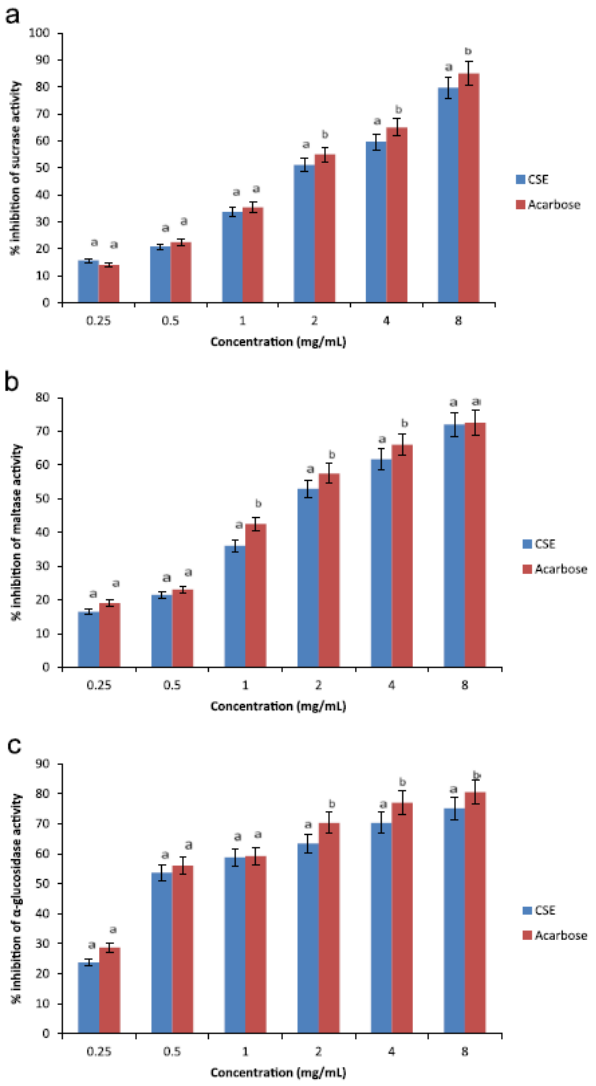

Fig. 5. (a) Inhibitory potential of corn silk aqueous extract on specific sucrase activity. Values are mean ± standard deviation (SD) of triplicate determinations. [ab]Bars with different superscripts for the parameter are significantly different ($P < 0.05$). CSE = corn silk aqueous extract. (b) Inhibitory potential of corn silk aqueous extract on specific sucrase activity. Values are mean ± standard deviation (SD) of triplicate determinations. [ab]Bars with different superscripts for the parameter are significantly different ($P < 0.05$). CSE = corn silk extract. (c) Inhibitory potential of corn silk aqueous extract on specific α-glucosidase activity. Values are mean ± standard deviation (SD) of triplicate determinations. [ab]Bars with different superscripts for the parameter are significantly different ($P < 0.05$). CSE = corn silk extract.

Table 4
IC_{50} (mg/mL) values of CS aqueous extract on specific activities of carbohydrate hydrolytic enzymes.

Parameter	IC_{50} against enzyme activity			
	Sucrase	Maltase	α-glucosidase	α-amylase
Acarbose	3.25	2.99	0.48	3.77
CSE	3.47	3.62	0.93	5.89

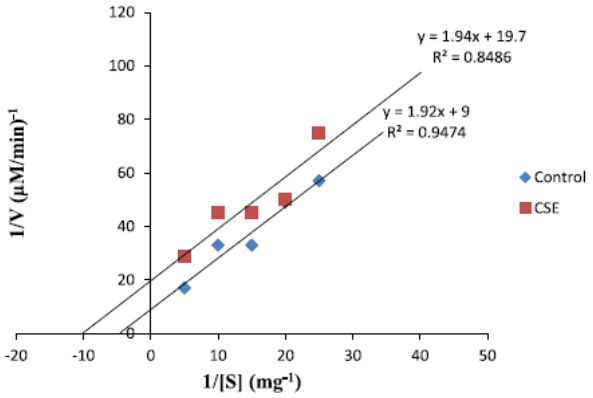

Fig. 6. Lineweaver-Burk plot of corn silk aqueous extract eliciting uncompetitive inhibition on α-glucosidase activity. Results represent mean ± standard deviation; (n = 3); ($p < 0.05$). CSE = corn silk aqueous extract.

1.0 mg/mL) which is in sharp contrast to the observation at higher concentrations with obvious significant differences (Fig. 5a-c). The kinetics study for the probable mode of inhibition of the enzyme showed that CSE exhibited independent inhibition for α-glucosidase. The double reciprocal plot revealed a decrease in both *V*max and Km values for CSE, thereby suggesting an uncompetitive type of inhibition (Fig. 6). For the control, the relative *V*max and Km values were 0.08 μM/min and 0.24 mg, respectively, while they decreased to 0.05 μM/min and 0.10 mg for the extract.

Similarly, the %inhibition by CSE against α-amylase activity produced a concentration-dependent effect (Fig. 7). While the levels of inhibition were significantly ($p < 0.05$) different from the standard at higher doses (1 – 10 mg/mL), the lower doses gave non-significant ($p > 0.05$) effect. The IC_{50} values for the extract and acarbose were also significantly ($p < 0.05$) different with 5.89 mg/mL and 3.77 mg/mL values, respectively (Table 4). Further investigation into the mechanism of α-amylase inhibition by the extract using Lineweaver-Burk double reciprocal plot provided a characteristic non-competitive inhibitory mode (Fig. 8). The respective *V*max values for the extract and the control were 0.04 and 0.05 μM/min, while the *K*m was 1.0 mg.

3.4. Discussion

Generally, the moderation of blood glucose concentrations towards normal range is mainly based on the use of oral hypoglycaemic agents and insulin. However, these treatment options have

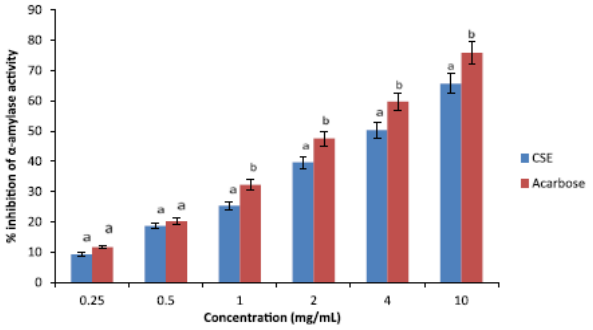

Fig. 7. Inhibitory potential of corn silk aqueous extract on specific α-amylase activity. Values are mean ± standard deviation (SD) of triplicate determinations. [ab]Bars with different superscripts for the parameter are significantly different ($P < 0.05$). CSE = corn silk aqueous extract.

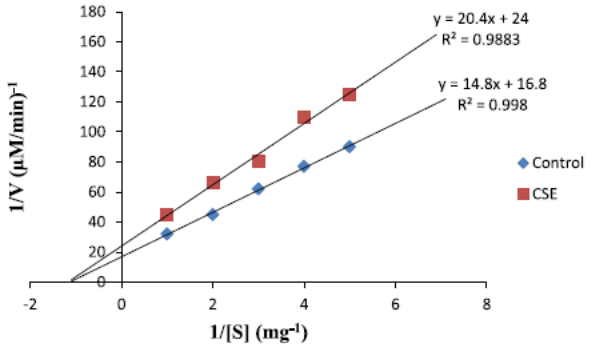

Fig. 8. Lineweaver-Burk plot of corn silk aqueous extract eliciting non-competitive inhibition on α-amalase activity. Results represent mean ± standard deviation; (n=3); ($p < 0.05$). CSE=corn silk aqueous extract.

limited efficacy and are associated with undesirable side effects (Campbell et al., 1996), and have prompted renewed interests in phytotherapy for better, effective and more efficient management of DM. The observed elevation in blood glucose level in diabetics is primarily due to unregulated hydrolysis of starch by pancreatic α-amylase and the subsequent uptake of glucose by intestinal α-glucosidases (Gray, 1975). An effective approach to manage the disorder is to strongly inhibit intestinal activity of α-glucosidases and mildly inhibit pancreatic α-amylase activity, which in turn controls postprandial plasma glucose levels and consequently alleviate potential chronic vascular complications (Krentz and Baile, 2005; Kwon et al., 2008). A strategy for reducing postprandial hyperglycaemia in diabetics is to prevent absorption of carbohydrates after food uptake. Complex carbohydrates must be degraded into individual monosaccharides before absorption in the duodenum and upper jejunum. This digestion is facilitated by enteric enzymes, including pancreatic α-amylase and α-glucosidases attached to the brush border of intestinal cells. Hence, inhibition of these enzymes activities halts digestion of disaccharides to glucose as well as its subsequent rate of entry into systemic circulation. Plant-derived α-amylase and α-glucosidase inhibitors with excellent antioxidative potentials offer attractive alternative in this regard (Kumavat et al., 2012).

The present study demonstrated a commendable inhibitory activity of CSE on α-amylase and α-glucosidase. Inhibition of these enzymes could potentially be employed as an effective approach to control postprandial hyperglycaemia in diabetics. This option has fewer side effects, such as abdominal distention, meteorism and possibly diarrhoea resulting from the abnormal bacterial fermentation of undigested carbohydrates in the colon, as normally observed when potent α-glucosidase inhibitors like acarbose, miglitol and voglibose are used. Results clearly showed that CSE has a significant potent and moderate inhibitory activity at IC_{50} 0.93 mg/mL and 5.89 mg/mL respectively for α-glucosidase and α-amylase when compared with the standard acarbose. Therefore the extract may play important role in the development of neutraceuticals and also in the management of diabetes. This submission is consistent with the findings of Krentz and Baile (2005), Kwon et al. (2008), and Kajaria et al. (2013), where moderate α-amylase inhibition with potent α-glucosidase inhibitory activity was proposed as better therapeutic approach to delay availability of dietary carbohydrate substrate for glucose production in the gut. The uncompetitive and non-competitive nature of α-glucosidase and α-amylase inhibitory activity respectively by CSE suggests that the bioactive principles in the extract either assuages substrate level which facilitate their binding and subsequent inhibition of α-glucosidase or bind to a site other than the active site of α-amylase/α-amylase-substrate complex and perhaps interfering with the action of both.

Antioxidant scavenging effects on DPPH and nitric oxide radicals coupled with modulation of metal chelating and reducing power are models for testing antiradical properties of chemical compounds including plant extracts (Ajiboye et al., 2013). Oxidative free radicals have been implicated in the pathogenesis of diabetes mellitus. It is also believed that the metabolic disorders in diabetes mellitus may be due to enhanced cellular oxidative stress and reduced antioxidant potential (Dandona et al., 1996; Glugliano et al., 1996; Baynes and Thorpe, 1999; Modak et al., 2007). Our results depicted that CSE had good free radical scavenging activity comparable to the standards used in this study. The standard curves of % inhibition/scavenging effects and IC_{50} values of the extract which revealed a decrease in the concentration of these species may be due to the scavenging ability of CSE. This is further buttressed by the closeness of R^2 values to 1.0, suggesting line of best fits for the generated data and accurate predictions for the individual antioxidant assays. The effect noticed compared favourably well with silymarin and citrate (reference standards), and indicative of the extract's capability to halt cascade of reactions involving free radicals. This is facilitated by its ability to either compete with oxygen to react with nitric oxide thereby inhibiting generation of nitrite, or donate hydrogen atom/electron to render them relatively stable, prevent likelihood of cellular damage, and arrests oxidative stress-linked disorders (Sabiu et al., 2015).

The presence of alkaloids, phenols, tannins, flavanoids, saponins and sterols in CSE may be a justifiable reason for its observed hypoglycaemic potential in this study. Their anti-diabetic capability to regenerate pancreatic β-cell and inhibit activity of carbohydrate metabolizing enzymes has been documented (Tadera et al., 2006; Kwon et al., 2008; Chika and Bello, 2010). Phytosterols have been reported to decrease blood sugar in experimental animal models (Suba et al., 2004). Generally, the attributes exhibited by CSE may be linked to its phenolic compounds which are known for their antioxidant activity. Such activity is related to their redox properties in absorbing and neutralizing free radicals and quenching ravaging chain reactions of ROS (Zheng and Wang, 2001; Miliauskas et al., 2004; Gülçin et al., 2007). They are also believed to have inhibitory effect on carcinogenesis. Polyphenolic scavenging action is mainly due to their hydroxyl groups. They are very important plant constituents which can protect body from different types of oxidative stress (Shukla et al., 2009; Jing et al., 2010). Flavonoids as one of the most diverse and widespread groups of natural compounds are probably the most important natural phenolics (Agrawal, 1989). Tung et al. (2007) have reported their wide spectrum chemical and biological activities including radical scavenging properties. Similar attributes have also been documented for flavonols (Miliauskas et al., 2004), and have found profound significance as potent hypoglycemic agents (Hye et al., 2004).

Overall, the present study elucidates the mechanisms of α-amylase and α-glucosidase inhibitory potential of CSE and lends credence to its application as anti-diabetic agent. The search for novel molecules as potential inhibitors of carbohydrate metabolizing enzymes with specific and high affinity has intensified in recent years. Hence, moderation of α-amylase and α-glucosidase activities via the use of high-affinity plant-based inhibitors would be of utmost significance in the management of diabetes.

Authors' contributions

Ashafa AOT and Sabiu S conceptualized and designed the study. Sabiu S performed biochemical estimations, statistical analysis, and drafted the manuscript. O'Neill FH co-supervised the study and was involved in the critical revision of the article for important intellectual content. All authors read and approved the final manuscript for submission.

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CHAPTER FIVE

A 28-day repeated dose toxicological evaluation of *Zea mays* L. (Poaceae), *Stigma maydis* in Wistar rats

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A 28-day repeated dose toxicological evaluation of *Zea mays* L. (Poaceae), *Stigma maydis* in Wistar rats.

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Abstract

Ethnopharmacological relevance: *Zea mays* L. (Poaceae), *Stigma maydis* is a waste product of corn cultivation with good therapeutic applications against several debilitating diseases including nephrolithiasis, hepatotoxicity and diabetes amongst others.

Aim of the study: This study evaluated the safety of its ethyl acetate fraction on key metabolic organs in Wistar rats using OECD guidelines.

Materials and methods: In acute toxicity test, single oral administration of 5000 mg/kg body weight of the fraction was orally given to the animals and was observed for 14 days. The repeated dose toxicity study was conducted by daily oral administration of graded concentrations (50, 100, 200 and 500 mg/kg) of the fraction for 28 days. Clinical toxicity signs, behavioral changes, hematological, and biochemical parameters were thereafter evaluated.

Results: The fraction at 5000 mg/kg produced no treatment-induced signs of toxicity, behavioral changes or mortality in the animals. Thus, its LD_{50} was estimated to be above 5000 mg/kg. In the repeated dose toxicity study, treatments with the fraction revealed no significant difference in hematological and clinical biochemistry parameters. However, at 500 mg/kg, the fraction

significantly increased the body weight (22.31%), exploratory ability (28.68%) as well as serum concentrations of leukocytes (71.58%) and platelets (63.82%) of the treated animals compared to the control group. Cage side observations recorded no treatment-mediated signs of toxicity and macro-histopathological examinations of all the investigated organs also revealed no obvious morphological alterations.

Conclusion: Overall, the effects elicited by *S. maydis* suggest that it is unlikely to be toxic to the investigated tissues and may be labelled and classified as practically non-toxic within the investigation period.

Keywords: Corn silk, Histoarchitectural, Locomotion, Pharmacological, Toxicological.

1.0 **Introduction**

The traditional system of medicine has become a topic of global significance and offers unlimited opportunities for the discovery of new drugs to treat diseases. Over 40,000 species of tropical plants possess medicinal attributes and are currently in use for various clinical conditions (Idu *et al.*, 2008). Although, scientific evidences exist on the pharmacological activities of most of these botanicals, there is paucity of biochemical information on their possible toxicological implications on the general well-being of key metabolic organs and tissues in animals (Dias & Takahashi, 1994). Thus, a thorough assessment of their overall toxicity profile is imperative to validate their safety applications.

Zea mays L. (Poaceae), *Stigma maydis* (Corn silk (CS)) is a waste product of corn cultivation and available in abundance (Maksimović *et al.*, 2004). Each silk is approximately 30 cm long with faintly sweetish taste and functions mainly to trap pollens for pollination. Its pharmacological significance in folkloric medicine as oral anti-diabetic and hypoglycemic agent have been well documented (Jianyou *et al.*, 2009; Zhao *et al.*, 2012; Ghada *et al.*, 2014; Sabiu *et al.*, 2016).

Grases *et al.* (1993) have implicated CS in the management of cystitis, edema, kidney stones, prostate disorder, urinary infections, bedwetting and obesity. Its antibiotics, immune enhancement, and insecticidal activities have also been reported (Guevera *et al.*, 2000; Maksimović & Kovacevic, 2003; Kim *et al.*, 2004). Unfortunately in Africa, particularly, Nigeria and South Africa, where corn is a common staple food, CS is still underutilized and its therapeutic importance is largely untapped.

Previous toxicological study on crude aqueous extract of CS has proven that it is did not show any toxic effect on the hematopoietic systems of Wistar rats (Sabiu *et al.*, 2015a). However, in light of its potential health benefits and coupled with no previous comprehensive and exhaustive reports in scientific literature on its toxicological evaluation on key metabolic organs (heart, kidney and liver), the present study was conceptualized to provide detailed biochemical information on the safety profile of acute and 28-day repeated dose oral administration of ethyl acetate fraction of CS in Wistar rats.

2.0. **Materials and methods**

2.1. **Chemicals, reagents and assay kits**

Assay kits for heart, kidney and liver function parameters were procured from Randox Laboratories limited, United Kingdom. Other chemicals and reagents were all of analytical grade.

2.2. **Plant collection, identification, processing and selection**

Fresh CS were collected between November 2014 and March 2015 from a corn plantation in QwaQwa, Phuthaditjhaba, Free State Province, South Africa. They were authenticated (from fresh whole maize plant) at the Plant Sciences Department, University of the Free State, QwaQwa campus, South Africa. Voucher specimen (No. SabMed/01/2015/QHB) was prepared

and deposited at the Herbarium of the University. The CS was shade-dried to constant weight and thereafter pulverized (model MS-223; Blender/Miller III, Taiwan, China) to smooth powder. The powdered sample (2 kg) was extracted with 70% methanol (10 L) with regular agitation for 24 h. The solution obtained was filtered (Whatman no. 1 filter paper) and the resulting filtrate concentrated to yield 455 g crude extract. Part of the crude extract (400 g) was suspended in distilled water (0.6 L) and subsequently partitioned in succession with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. This yielded 18, 24, 33 and 42 g of the respective fractions. About 10 µL of each (1 mg/mL) was spotted on silica gel coated TLC plate and the resulting chromatograms were thereafter developed in dichloromethane/methanol (8.5:1.5 v/v) system and sprayed with 0.2% 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol for detection of antioxidant metabolites. From the chromatograms, the ethyl acetate fraction of CS (CSEAF) had the highest number of antioxidant spots by prominently bleaching the purple-coloured solution of DPPH and was selected for the toxicity testing. CSEAF was kept air-tight and refrigerated prior to use.

2.3. **Experimental animals**

Healthy Wistar rats (both sex) of weight range 155±4.20 g were obtained from the experimental animal facility of the University of the Free State, Bloemfontein, South Africa. They were housed in clean metabolic cages placed in a well-ventilated house with optimum condition (temperature 23 ± 1°C, photoperiod; 12 h natural light and 12 h dark; humidity; 45-50%). They were acclimatized to the animal house condition for 10 days and had free access to pelleted rat chow (Pioneer Food (Pty) Ltd, Huguenot, South Africa) and water. The cages were cleaned on a daily basis and handling was in accordance with the guidelines of National Institute of Health on the care and use of laboratory animals (NIH, 1985). An approval (UFS-AED2015/0005) was

granted by the Ethical Committee of University of the Free State, South Africa prior to commencement of the study.

2.4. **Acute toxicity testing protocol**

Adopting the Organization of Economic Cooperation and Development (OECD) guideline 420 for testing of chemicals (OECD, 2001), the acute oral toxicity was performed. Twenty rats used in the study were fasted for 18 h prior to randomization into 2 groups of 10 animals each. The control group received only 10% Tween 80 as vehicle, while CSEAF was dissolved in 10% Tween 80 and administered orally (only once) at a single dose of 5000 mg/kg body weight (b.w.) to the animals in the treatment group. Following this treatment, the rats were observed closely for the first 24 h (with pertinent attention paid to the first 4 h) and then every 24 h for the next 14 days. All the animals were weighed and subjected to thorough gross necropsy during this period. Behavioral changes, lethargy, depression, salivation, diarrhoea, muscular weakness, sedation, and ailment signs were also monitored. The LD_{50} was thereafter estimated based on the mortality observed in each group adopting the method of Ajani *et al.* (2014). At the end of the experiment, the animals were humanely euthanized by halothane anaesthetization and the neck area was quickly cleared of fur to expose the jugular vein. The vein, after being slightly displaced, was sharply cut with sterile surgical blade and blood samples were collected into non-heparinized and ethylene diamine tetra acetic acid (EDTA)-coated bottles. The collected samples were thereafter centrifuged at 15000 rpm for 10 min and subsequently used for clinical biochemistry and hematological analyses, respectively. The rats were quickly dissected and the liver, kidney and heart were excised, freed of fat, blotted with clean tissue paper, weighed and their relative organ-

body weight ratios were evaluated. The isolated organs were also fixed in 10% buffered formalin solution for histopathological examination.

2.5. **28-day daily repeated oral dose testing**

Seventy rats randomly distributed into 6 groups were used for this study. The control group had 20 rats that were further designated as normal and satellite controls of 10 animals each. The remaining animals were evenly distributed into 5 treatment groups of 10 rats each. While group 1 served as control and received 1 ml of distilled water in 10% Tween 80, groups 2-5 were administered 50, 100, 200 and 500 mg/kg body weight, respectively of CSEAF (dissolved in 10% Tween 80) daily for 28 consecutive days. Group 6 designated as satellite group comprised animals treated exactly as group 5 but were left without further treatment for another 14 days to observe reversibility, persistence, or delayed occurrence of any probable toxicity. The fraction was freshly prepared and all administrations were done once daily via oral intubation throughout the investigation period. The rats were weighed on weekly basis and also subjected to thorough observations for morbidity, mortality, behavioral changes and possible symptoms of humane end point during this period. This protocol conforms to OECD guideline 407 for testing chemicals and plant extracts (OECD, 2006).

At the end of the experimental period, blood samples were collected from overnight fasted animals via jugular puncture into non-heparinized and EDTA-coated bottles as detailed in acute toxicity testing (see section 2.4) and were also used for clinical biochemistry and hematological analyses, respectively. The liver, kidney and heart were isolated from dissected animals, blotted with clean tissue paper, weighed and processed for macroscopic and histopathological examinations.

2.6. **Locomotion and exploratory evaluation**

Adopting the protocol of Turner (1972), the open-field spontaneous locomotion and exploratory ability of the animals was assessed. In brief, each animal was maintained in an open-field apparatus for 5 min and the square walked was observed and recorded. Observations were made at 0, 7, 14, 21 and 28th day after daily CSEAF dosing and also extended to the 14 days post treatment period.

2.7. **Hematological and clinical biochemistry assays**

Automated Hematologic Analyzer, Sysmex, KX-21 (Japan) was used to analyze hematological parameters, while assay kits were employed for serum analyses of lipid profile, heart, liver and kidney function parameters adopting the procedures described in the kits.

2.8. **Histopathological examination**

This was done following the method of Drury and Wallington (1980). Briefly, tissue sections of the liver, kidney and heart were washed in normal saline and fixed immediately in 10% buffered formalin solution for a period of at least 24 h. They were subsequently dehydrated with graded alcohol and further processed in paraffin embedding using LEICA PT 1020 Automatic Tissue Processor. About 5 μm thick sections of each tissue were stained with hematoxylin and eosin, and observed (x400) for possible histopathological damages. Microscopic features of the organs of CSEAF-treated rats were compared with the control group.

2.9. **Data analysis**

Data were expressed as mean ± standard error of mean (SEM) of replicate determinations and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at $p < 0.05$.

3.0. **Results**

3.1. **Acute oral testing**

Cage side observations indicated no signs of changes in the behavioral patterns, skin, fur, eyes mucous membrane and there were no evidences of tremor, convulsion, salivation, diarrhoea, lethargy, abnormal sleep or coma in the tested rats during the first 4 h of 5000 mg/kg b.w. of CSEAF administration and throughout the 14 days post-treatment period. Neither morbidity nor mortality was observed and the weight gain by the treated rats compared favourably with the normal control (Table 1). Except with a marginal significant difference ($p< 0.05$) in the relative liver weight of the treated animals, there was no significant difference in this parameter for the kidneys and the heart when compared with the normal control group (Table 1). Data obtained with respect to hematological and clinical biochemistry parameters revealed that there were no significant differences ($p> 0.05$) between the 5000 mg/kg body weight CSEAF treated rats and the normal control (Tables 2 and 3). Macroscopic and histopathological examinations also revealed no significant changes in the histoarchitecture of the organs (Figure 1).

3.2. **28-day repeated oral toxicity study**

Repeated daily oral administration of CSEAF for 28 days at all the investigated doses did not induce any treatment-related toxicity in the animals. Neither deaths nor obvious clinical pathological signs were observed and their behavioural activities were essentially normal throughout the investigation period.

Compared with the control group, there were dose-related significant increases in the body weights of the fraction-treated animals (Table 4). Though marginal weight gain occurred in the first 2 weeks of the study, it became significant over the 3^{rd} and 4^{th} weeks of investigation with

most prominent effect noticed at 200 and 500 mg/kg doses of the fraction (Table 4). At autopsy, no abnormal gross findings were found and the relative organ weight of each organ in the treatment groups compared favorably with the normal control (Figure 2).

Effects of 28 days repeated dose treatment with CSAEF at 50, 100, 200 and 500 mg/kg b. w. on the hematopoietic systems of Wistar rats is shown in Table 5. With the exception of white blood cells [leukocytes (WBC)] that increased significantly ($p<0.05$) following treatment with the fraction at 200 and 500 mg/kg when compared with the control, other hematological parameters [red blood cell (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), lymphocytes, monocytes, neutrophils, eosinophil, and basophils] were not significantly altered across all the treated groups. However, administration of CSEAF for 28 days dose-dependently increased the platelet counts when compared with the control.

Except for the significantly increased ($p<0.05$) serum activity of aspartate aminotransferase (AST) at the 100, 200 and 500 mg/kg b.w. of CSEAF when compared with the normal control, other clinical biochemistry indices investigated in this study were not statistically different ($p>0.05$) to treatment with the fraction (Table 6). However, it is noteworthy that the increased activity of AST was swiftly normalized in the 500 mg/kg dose satellite group treated rats during the 14 days post-treatment period (Table 6).

Macroscopic and detailed histoarchitectural examinations of the heart, kidney and liver of CSEAF-treated animals revealed no abnormalities in color and texture of the organs and there were on observable cellular infiltrations. The cellular morphology, nuclear characteristics and

tissue integrity of the organs of CSEAF-treated rats were essentially normal and comparable to the normal control (Figure 3).

Results in respect of 28 days administration of varying doses (50, 100, 200 and 500 mg/kg) of CSEAF on the locomotion and exploratory ability of the experimental rats is presented in Table 9. While the fraction at 50 and 100 mg/kg doses had no statistically significant ($p>0.05$) effect on the locomotion of the animals, increased locomotion were observed in other CSEAF-administered rats from 1st week and was sustained throughout the treatment period when compared with the control (Table 7). However, marginal reductions were noticed in the satellite treated group during no further treatment period when compared with the control. Also noteworthy were the brief periods (about 3-5 min) of excitement and increased physical activities in the fraction-treated animals.

4.0. **Discussion**

Acute toxicity testing and LD_{50} determination have been described as initial steps in the toxicological assessments of unknown substances (Lorke, 1983), and data derived there-in are crucial to providing clues on their classification and labelling (Ukwuani *et al.*, 2012). According to Lorke (1983) and WHO (1998) on labelling of substances on a ≤5 to ≥5000 mg/kg b.w. dose scale (depicting severity of toxicity and X representing LD_{50} value) as follows: X≤5-very toxic; 5<X≤50-toxic; 50<X≤500-harmful; 500<X≤2000-no label; X≥5000-safe and practically non-toxic, CSEAF may therefore be considered non-acutely toxic when administered via oral route and could be adjudged relatively safe for consumption. Additionally, if a dose as high as 5000 mg/kg b.w. of a substance is found to be survivable, no further acute testing will be recommended (NRC, 2006). In this study, the fact that CSEAF at a single oral dose of 5000 mg/kg had no treatment-related adverse effect on the animals up to 14 days observation period is

suggestive of its non-acute toxicity effect. It could also imply that its LD_{50} is approximately above 5000 mg/kg. While the outcome of this test is not intended for determining a precise LD_{50} value, it has given sufficient hint on the no-observed-adverse-effect level of CSEAF dose at which the animals are expected to survive. Furthermore, the no treatment-related alteration on the relative organ weights of the treated animals to this high dose treatment is another justifiable reason supporting the non-toxic potential of the fraction. Although, the significantly increased relative weight of the liver of the treated animals may suggest possible toxic potential of CSEAF on the hepatocytes, it could be regarded as been toxicologically irrelevant as it was not consistent with data from the hematological, clinical biochemistry and histopathological evaluations conducted in this study.

Since deranged clinical signs were not evident during the acute toxicity study, further evaluation was performed to evaluate the repeated dose toxicity of the fraction over a 28-day investigation period. This was conducted with a view to providing comprehensive toxicological data on this underutilized and untapped botanical in Africa. In this study, the fact that continuous 28 days daily dose treatment with CSEAF elicited no clinical signs for morbidity, toxicity or mortality across all the treatment groups may be indicative of its unlikely toxic tendency at the tested doses over the exposure period. A change in body weight is one of the first critical signs of toxicity and may serve as sensitive indication of the overall health status of animals (Sireatawong *et al.*, 2008). The body weight gained by the CSEAF-administered animals relative to the normal control could mean that the fraction enhanced normal metabolism and stimulated the growth and developmental mechanisms of the animals. This may be adduced to enhanced appetite in the rats that could be ascribed to the nutritive constituents in CSEAF as previously reported on its crude aqueous extract (Sabiu *et al.*, 2015a). This submission is also consistent with the finding of

Ashafa *et al.* (2010), where mean body weight gain in experimental rats was attributed to the nutritional constituents of the leaf and berry extracts of *Phytolacca dioica* L. (Phytolaccaceae). The importance of relative organ weight in toxicity studies is germane to unravelling obvious treatment-induced organ weight variations in experimental animals (Wooley, 2003). While an increase in this parameter may either depict inflammation or increased secretory ability of the organ, a reduction could be suggestive of cellular constriction. In addition to defining toxicity as pathological changes observed in organs of interest, the relative organ weight could also be suggestive of organ swelling, atrophy or hypertrophy (Amresh *et al.*, 2008). In this study, the absence of significant changes in the weight of all the investigated organs may indicate that the organs were neither adversely affected nor elicited clinical symptoms of toxicity throughout the 28 days of daily dose treatments with CSEAF. This could suggest that the fraction is unlikely to be toxic to these organs at the investigated doses and was further supported by the preserved architectural features of the organs.

Similarly, the absence of significant difference on the RBCs and Hb following repeated daily dose treatment with CSEAF might be an indication that it may not be toxic to the blood. This suggests that the release of erythropoietin was not activated in the renal cortex of the animals, thus keeping the rates of production and destruction of blood corpuscles at equilibrium (Polenakovic & Sikole, 1996). This further implies that incorporation of Hb into RBCs as well as osmotic fragility and morphology of the RBCs was intact. It could also mean that the oxygen-carrying capacity of the Hb and amount of oxygen delivered to the tissues following treatment with CSEAF was unperturbed (Gruchy, 1976). Analyses on other indices (HCT, MCV, MCH, MCHC and RDW) relating to the status of RBCs may be crucial in defining anemic condition in animals (Sabiu *et al.*, 2015a). The non-significant effect on these indicators in the fraction-

treated rats was suggestive of its non-adverse effect on RBCs' microcytes and Hb weight per RBCs. This suggests that the 28-day repeated daily oral dose treatment with CSEAF does not predispose the animals to anemic condition throughout the evaluation period. This is in agreement with the report of Ashafa and Olunu (2011) who gave similar submission on the non-hematotoxic effect of *M. lucida* (L.) Benth. (Rubiaceae) extract in experimental animals. The concentration of WBCs is a probable reflection of an organism's defensive potential against infections (Aboyade *et al.*, 2009). The significantly increased WBCs at the 200 and 500 mg/kg doses of CSEAF suggest a boost on the immune system and enhanced vascular permeability. Since the lower doses of the fraction also competed well with the normal control on the WBC counts, it could also be logically suggested that the effector cells of the immune system at all the tested doses were not adversely affected and further supported the non-hematotoxic effect of CSEAF. Anofi and Mutiu (2015) also gave similar report on the hematopoietic system of rats following treatment with *Dianthus basuticus* L. (Caryophyllaceae). That CSEAF dose-relatedly increased platelet counts in the animals during the exposure period may be suggestive of its stimulatory effect on thrombopoietin. This may be indicative of its capability to promote thrombopoiesis, repair the minute vascular damage and considerably manage thrombocytopenia in animals (Geddis, 2013). That the fraction elicited the best and most prominent effect on both platelet counts and WBCs at 200 and 500 mg/kg doses could be a tentative submission that the optimum therapeutic dose for CSEAF revolves around these doses.

Clinical biochemistry analyses were performed to evaluate the possible alterations in cardiac, hepatic and renal functions induced by CSEAF administration. CK-MB and LD_1 are isoenzymes of creatine kinase and lactate dehydrogenase respectively and are present in the myocardium that leaks out into the serum under conditions of massive myocardial damage resulting from

disintegration of contractile apparatus and increased sarcoplasmic permeability (Nigam, 2007). The normal serum activity of these enzymes as evident in this study following treatment with the fraction is informative of the normal functional status and no observable cellular damage in the heart of the treated animals. This was closely corroborated by the absence of treatment-induced histoarchitectural infiltrations in the heart of CSEAF-administered rats. Renal and hepatic function tests are germane in toxicity evaluation of plant extracts due to the inevitable involvement of these organs in xenobiotic biotransformation. Electrolytes (sodium, potassium, chloride, calcium, magnesium and phosphate), urea, urate and creatinine are markers of kidney functions and alterations in their serum levels have often been associated with renal damage (Sunmonu & Oloyede, 2006). Specifically, electrolytes imbalance have been implicated in renal failure and if left unchecked may aggravate to secondary metabolic complications. Creatinine, urea and uric acids are major catabolic products of muscle, protein and purine metabolism respectively. Urea and creatinine are waste products which are passed into the blood stream to be removed by the kidney. Increase in the levels of these waste products in the blood is an indication of renal dysfunction (Oloyede & Sunmonu, 2009). In the present study, the non-significant difference in these parameters in the CSEAF-treated animals is suggestive of preserved or normal renal function and further lent credence to the non-toxic tendency of the fraction. Serum concentrations of total protein, globulin and albumin may indicate the state of the liver and the probable nature of hepatic damage (Sabiu *et al.*, 2014). These indices are frequently utilized to assess synthetic and secretory functions of the liver. Thus, non-significant difference in the serum levels of total protein, globulin and albumin in the CSEAF-administered rats does not only indicates that the fraction had no deleterious influence on the synthetic and secretory roles of the liver, but also confirmed its capability to maintain and sustain its overall

hepatocellular functions at the investigated doses. Significantly increased serum activities of ALP, ALT, and AST are closely linked with hepatic damage (Sabiu *et al.*, 2015b). Although, serum AST activity was increased following treatment with the fraction at higher doses, it was however swiftly assuaged during the post treatment period as revealed in the satellite treatment group. This alteration was not only inconsistent with all other liver function indices evaluated in this study, but also insufficient to annul the non-hepatotoxic and pharmacological significance of the repeated daily dose administration of the fraction. Generally, the remarkably normalized serum AST activity of the satellite treatment group during the post-treatment period, absence of treatment-mediated abnormalities in the architectural integrity of the hepatocytes, coupled with the non-significant alterations in the specific activities of ALP, ALT and other liver function parameters in the fraction-treated rats relative to the control group is either implicative of the fact that CSEAF does not affect the hepatocyte function in the rats or the integrity of the liver cells were well preserved. These findings are in agreement with the submission of Ajani *et al.* (2014), where ethanolic leaf extract of *Lagenaria brevifolia* L. (Cucurbitaceae) was reported to be non-hepatotoxic in Wistar rats.

Another important consideration in assessing the safety of a potential therapeutic agent against organ injury is its effects on histology. The effects are manifestations of inflammatory insults on the studied organ and often complement submissions from hematological and clinical biochemistry analyses (Sabiu *et al.*, 2015b). Therefore, the essentially preserved colour and texture of the heart, kidney and liver as evident from macroscopic examinations in this study was another tenable fact that the organs were in good state, void of injuries, and further suggests that CSEAF was not toxic to them at the tested doses. Furthermore, the no treatment-induced infiltrations, inflammations and derangements as revealed in the detailed microscopic scrutiny of

all the organs from CSEAF-administered groups is also supportive of its capability to maintain and sustain histoarchitectural integrity of the organs.

Besides allowing the analysis of locomotion and exploratory capacity of the animals, the open-field method is also used to study emotional behaviour (Prut & Belzung, 2003). A decrease in squares number walked is an indication of reduced spontaneous locomotion which consequently reflects corresponding reduction in excitability (Fernanda *et al.*, 2012). Thus, the significantly increased locomotion elicited by the 200 and 500 mg/kg doses of CSEAF coupled with immediate moments of excitement and improved physical activities following administration of the fraction across all treatment groups relative to normal control is implicative of its potential favorable influence on the animals. However, it is imperative to verify if CSEAF has constituents that can significantly affect the locomotion of the animal beyond the 5 min period of observation as performed in this study. Such test will help to predict if these substances are able to reach the central nervous system via the blood brain barrier and thus, will be considered as either having adverse effects or toxic to the animals. Absence of such effects ultimately signifies safety of its use. This is currently receiving dedicated attention in our laboratory.

Overall, the effects displayed by CSEAF may be adduced to its minerals and secondary metabolites which are known for their pharmacological activities. Besides considerable quantities of phenols, flavonoids and flavonols present in CS, GC-MS analysis of its aqueous extract also revealed 22 active compounds including ascorbic acid, β-carotene, maysin, maizenic acid, and glycolic acid, among others (Sabiu *et al.*, 2016). It has also been reported to be rich in proteins, fixed and volatile oils, vitamins and carbohydrates. Calcium, potassium, magnesium, sodium salts, steroids, phenol, alkaloids, saponins, tannins and phytosterols are also believed to contribute to its pharmacological potentials (Ebrahimzadeh *et al.*, 2008; Sabiu *et al.*, 2016).

Conclusion

The data from this study have shown that the LD_{50} of CSEAF is in excess of 5000 mg/kg in Wistar rats. Consequent upon its repeated dose administration for 28 days in the animals, it can be inferred that it is not toxic at all the investigated doses and did not produce any obvious signs of toxicity. The fraction neither caused morbidity, lethality nor produced any obvious histopathological infiltrations or alterations in clinical biochemistry parameters and thus, may be adjudged relatively safe for consumption. Although, studies are on-going to validate its safety profiles on other systemic organs and tissues in animals, the data from the present study have lent credence to the safety and pharmacological significance of *Zea mays* L., *Stigma maydis* in folkloric medicine.

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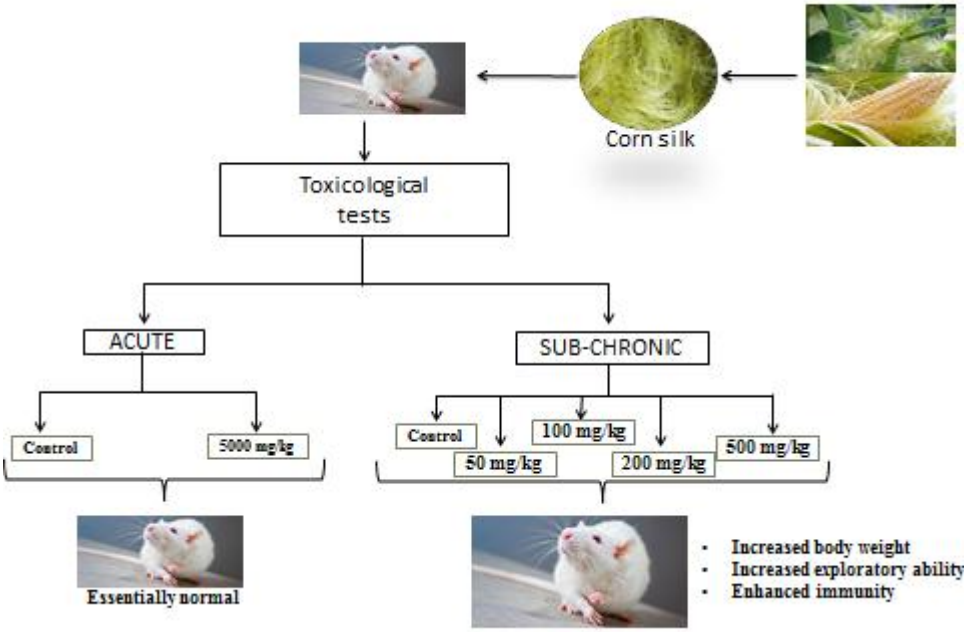
Graphical abstract



List of figures

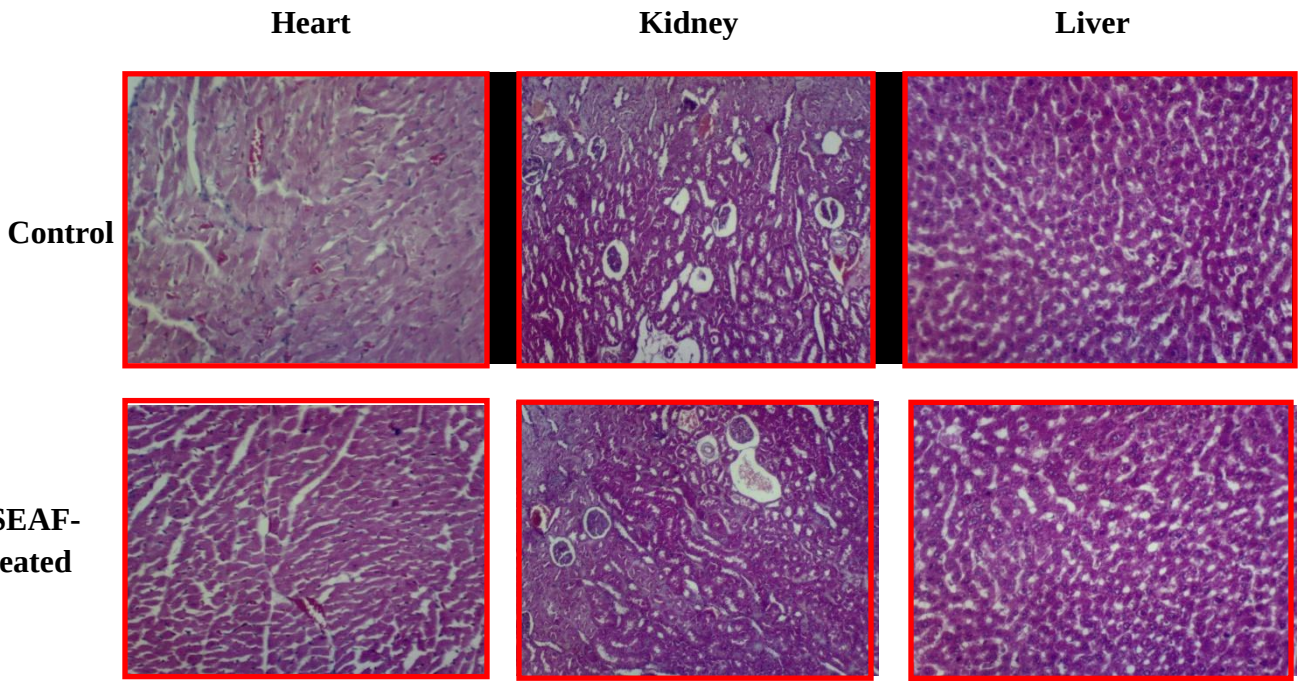


Fig. 1

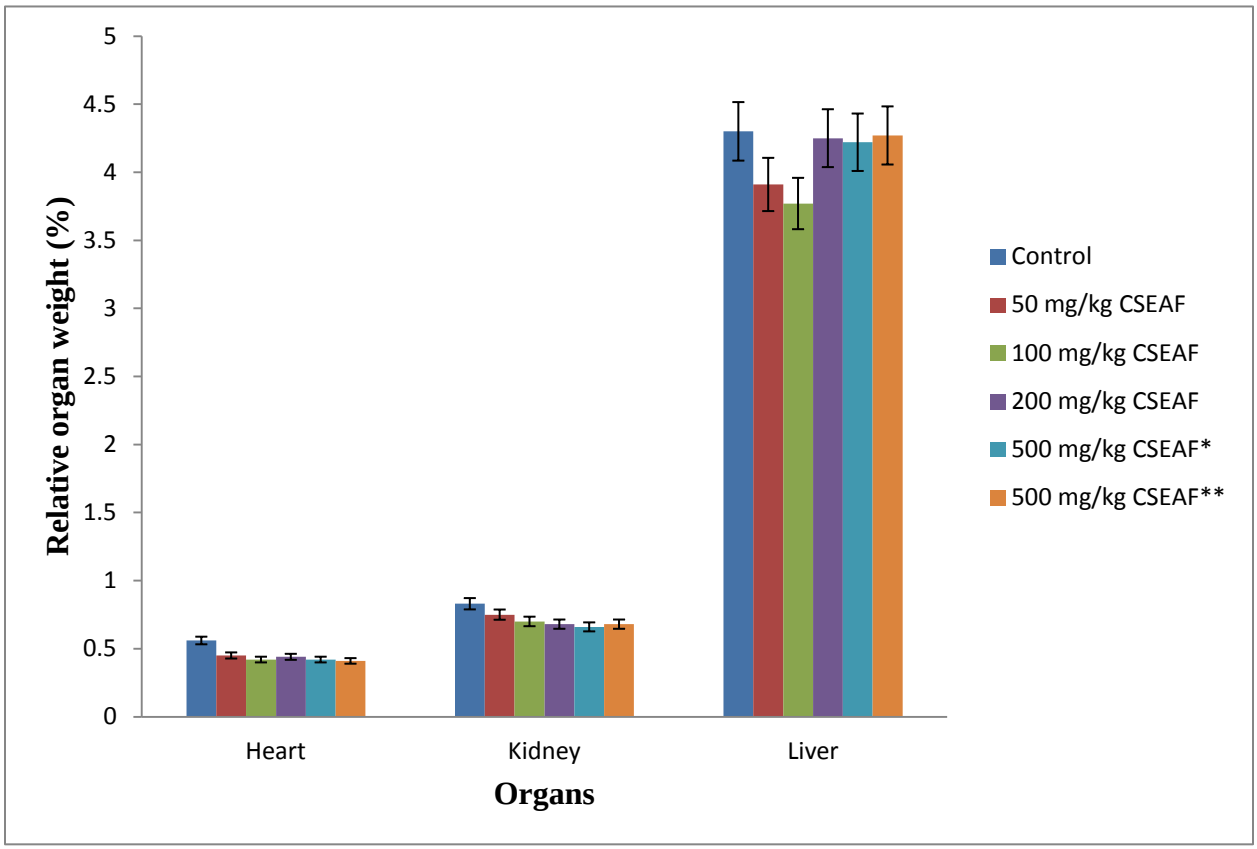

Relative organ weight (%)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Heart
Kidney
Liver
Organs
Control
50 mg/kg CSEAF
100 mg/kg CSEAF
200 mg/kg CSEAF
500 mg/kg CSEAF*
500 mg/kg CSEAF**

Fig. 2

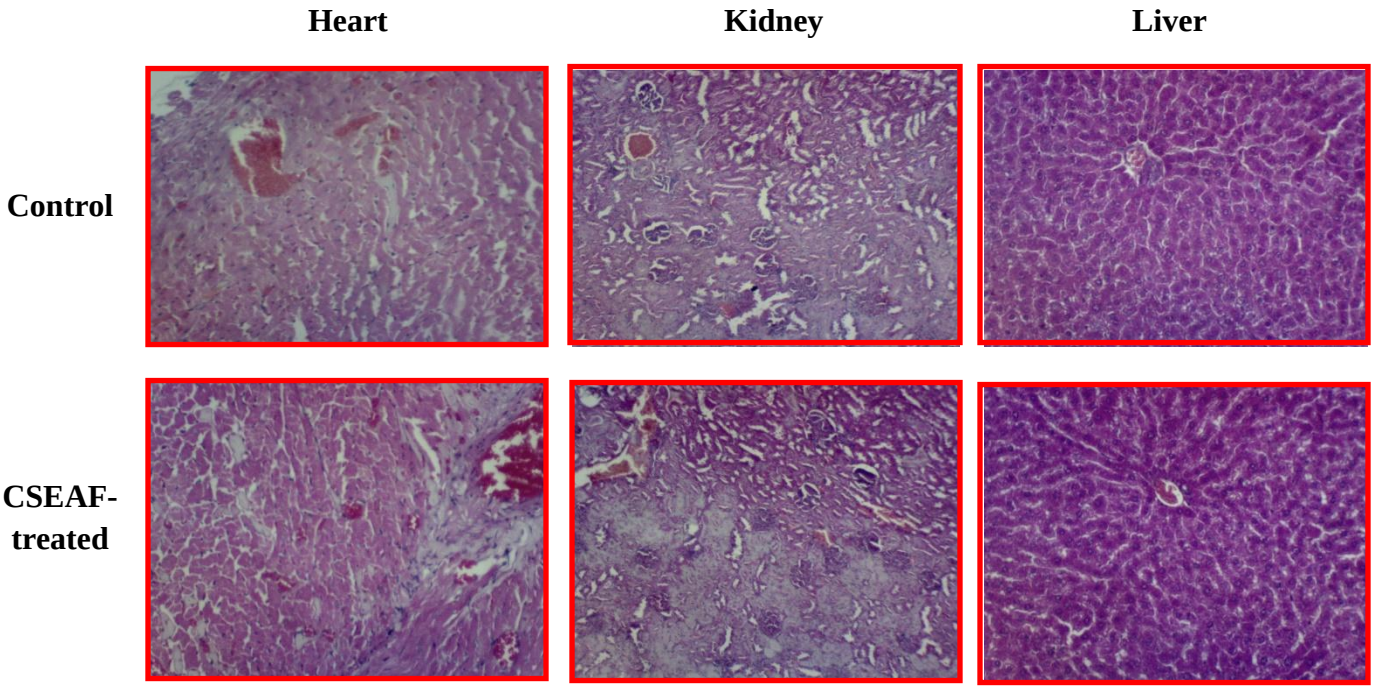


Fig. 3

Table 1: Body weight changes and relative organ weights of the animals following treatment with 5000 mg/kg body weight of corn silk ethyl acetate fraction (n = 10, $\bar{X}$ ± SEM).

Treatments	Weekly weight changes (g)				Relative organ weight (%)		
	0 (initial)	1	2 (final)	% weight gain	Heart	Kidney	Liver
Control	189.15±1.01	190.01±1.15	193.99±1.30	2.50±0.01	0.43±0.03	0.82±0.01	4.37±0.12[a]
5000 mg/kg	210.17±1.01	213.01±1.02	214.91±1.15	2.21±0.01	0.44±0.02	0.81±0.02	5.65±0.15[b]

Values bearing different superscripts along the same column for each parameter are significantly different ($p < 0.05$).

Table 2: Effect of 5000 mg/kg body weight oral single dose administration of corn silk ethyl acetate fraction on haematopoeitic systems of the animals (n = 10, $\bar{X} \pm$ SEM)

Parameters	Control	5000 mg/kg CSEAF
RBC ($x10^{12}$/L)	7.79±0.20	7.68±0.25
Hb (g/dL)	16.55±0.24	16.53±0.33
HCT	0.55±0.01	0.56±0.01
MCV (fl)	70.00±0.10	69.99±0.12
MCH (pg)	21.99±0.14	22.01±0.21
MCHC(g/dL)	30.60±0.22	30.59±0.23
RDW (%)	11.89±0.45	12.04±0.45
WBC ($x10^{9}$/L)	6.35±0.02	5.59±0.03
Lymphocytes($x10^{9}$/L)	5.95±0.05	6.02±0.05
Monocytes ($x10^{9}$/L)	0.04±0.01	0.05±0.01
Neutrophils ($x10^{9}$/L)	0.72±0.01	0.71±0.01
Eosinophils ($x10^{9}$/L)	0.05±0.01	0.06±0.01
Basophils ($x10^{9}$/L)	0.02±0.01	0.02±0.01
Platelet ($x10^{9}$/L)	845.23±2.00	923.57±1.99

Values not sharing superscripts along the same row for each parameter are statistically the same ($p > 0.05$). RBC= red blood cell, Hb= haemoglobin, HCT= hematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin, MCHC= mean corpuscular haemoglobin concentration, RDW= red blood cell distribution width and WBC= white blood cell, CSEAF= corn silk ethyl acetate fraction.

Table 3: Effect of 5000 mg/kg body weight oral single dose administration of corn silk ethyl acetate fraction on some heart, renal, and liver function parameters of the animals (n = 10, $\bar{X} \pm$ SEM)

Parameters	Control	5000 mg/kg CSEAF
CK-MB (IU/L)	414.26±1.03	410.07±1.03
LDH (U/L)	138.25±0.55	135.62±0.50
Sodium (mmol/L)	140.38±0.20	140.31±0.25
Potassium (mmol/L)	5.69±0.02	5.72±0.01
Chloride (mmol/L)	105.50±0.35	106.01±0.35
Calcium (mmol/L)	2.93±0.02	2.88±0.01
Magnesium(mmol/L)	1.12±0.01	1.13±0.01
Phosphate (mmol/L)	3.00±0.01	2.88±0.01
Urea (mmol/L)	10.60±0.03	10.45±0.02
Urate (mmol/L)	0.10±0.01	0.10±0.01
Creatinine (µmol/L)	34.71±0.25	34.62±0.30
Total protein (g/L)	56.15±0.25	56.17±0.25
Albumin (g/L)	33.00±0.04	33.00±0.03
Globulin (g/L)	25.35±0.15	25.50±0.19
ALT (IU/L)	42.35±0.15	43.11±0.14
AST (IU/L)	150.50±0.20	155.35±0.25
ALP (IU/L)	632.25±1.40	645.11±1.35

Values not sharing superscripts along the same row for each parameter are statistically the same ($p > 0.05$). CK-MB= creatinine kinase MB, LDH= lactate dehydrogenase, ALT= alanine aminotransferase, AST= aspartate aminotransferase, ALP= alkaline phosphatase, CSEAF= corn silk ethyl acetate fraction.

Table 4: Changes in body weight (g) of the animals following treatment with corn silk ethyl acetate fraction for 28 consecutive days (n = 10, $X \pm$ SEM)

Week	Fraction (mg/kg body weight)					
	Control	50	100	200	500*	500**
0 (initial)	190.65±1.01	155.01±1.04	173.99±1.02	220.75±1.01	213.03±1.01	210.15±1.00
1	191.99±1.21	153.99±1.07	175.09±1.03	225.08±1.11	215.68±1.00	211.59±1.03
2	193.72±1.09	157.50±1.20	175.17±1.07	231.14±1.04	221.34±1.01	219.99±1.05
3	198.05±1.11	179.32±1.00	194.44±1.16	266.39±1.05	252.78±1.07	248.04±1.21
4 (final)	203.05±1.15	179.59±1.05	204.58±1.05	282.32±1.03	274.23±1.02	265.14±1.10
Weight gain (%)	6.11±0.02[a]	13.69±0.01[b]	14.95±0.02[b]	21.81±0.01[c]	22.31±0.01[c]	20.74±0.02[c]

Values bearing different superscripts along the same row for each parameter are significantly different ($p < 0.05$). *Group treated with 500 mg/kg body weight of corn silk ethyl acetate fraction for 28 days. **Satellite group treated with 500 mg/kg body weight of corn silk ethyl acetate fraction for 28 days followed by no treatment for another 14 days.

Table 5: Effects of 28 days repeated oral dose administration of corn silk ethyl acetate fraction on haematopoeitic systems of the animals (n = 10, $\bar{X}$ ± SEM)

Parameters		Fraction (mg/kg body weight)				
	Control	50	100	200	500*	500**
RBC ($x10^{12}$/L)	7.58±0.25	8.05±0.29	7.74±0.30	8.08±0.24	7.98±0.30	8.05±0.35
Hb (g/dL)	16.45±0.52	16.33±0.45	16.31±0.35	17.00±0.25	16.63±0.28	17.01±0.43
HCT	0.53±0.01	0.53±0.01	0.53±0.01	0.56±0.01	0.55±0.01	0.53±0.01
MCV (fl)	72.00±0.10	68.25±0.15	68.25±0.28	67.90±0.25	70.00±0.22	71.00±0.25
MCH (pg)	21.50±0.15	20.50±0.19	20.75±0.21	21.00±0.22	21.50±0.20	20.66±0.25
MCHC (g/dL)	30.33±0.21	31.00±0.19	31.00±0.11	31.00±0.10	30.00±0.15	31.00±0.21
RDW (%)	11.60±0.44	11.34±0.45	10.91±0.45	11.61±0.35	11.63±0.20	11.62±0.45
WBC ($x10^9$/L)	5.70±0.05^a	5.20±0.09^a	5.40±0.03^a	8.76±0.05^b	9.78±0.03^b	8.85±0.03^b
Lymphocytes($x10^9$/L)	6.31±0.05	5.93±0.09	5.73±0.02	6.18±0.02	6.01±0.01	5.90±0.03
Monocytes ($x10^9$/L)	0.05±0.01	0.04±0.01	0.05±0.01	0.06±0.01	0.05±0.01	0.05±0.01
Neutrophils ($x10^9$/L)	0.63±0.01	0.70±0.01	0.65±0.01	0.71±0.01	0.70±0.02	0.69±0.01
Eosinophils ($x10^9$/L)	0.05±0.01	0.06±0.01	0.05±0.01	0.06±0.01	0.07±0.01	0.06±0.01
Basophils ($x10^9$/L)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
Platelet ($x10^9$/L)	809.52±1.25^a	1090.50±1.89^b	1189.33±1.75^b	1311.15±1.88^c	1326.12±1.99^c	1314.20±1.69^c

Values bearing different superscripts along the same row for each parameter are significantly different ($p < 0.05$). *Group treated with 500 mg/kg body weight of corn silk ethyl acetate fraction for 28 days. **Satellite group treated with 500 mg/kg body weight of corn silk ethyl acetate fraction for 28 days followed by no treatment for another 14 days. RBC= red blood cell, Hb= haemoglobin, HCT= hematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin, MCHC= mean corpuscular haemoglobin concentration, RDW= red blood cell distribution width and WBC= white blood cell.

Table 6: Effects of 28 days repeated oral dose administration of corn silk ethyl acetate fraction on some heart, renal and liver function parameters of the animals (n = 10, $X \pm$ SEM)

Parameters	Fraction (mg/kg body weight)					
	Control	50	100	200	500*	500**
CK-MB (IU/L)	404.30±1.02	406.21±1.05	401.32±1.00	402.31±1.05	406.10±1.10	404.01±1.01
LD_1 (U/L)	127.81±0.52	131.10±0.55	130.09±0.45	129.11±0.51	129.21±0.35	130.15±0.44
Sodium (mmol/L)	141.25±0.22	141.00±0.26	140.00±0.25	140.75±0.20	139.33±0.23	140.99±0.30
Potassium (mmol/L)	5.50±0.01	5.57±0.01	5.88±0.02	6.00±0.02	5.61±0.02	5.55±0.01
Chloride (mmol/L)	102.50±0.35	106.00±0.32	105.75±0.41	104.90±0.32	103.55±0.35	104.16±0.30
Calcium (mmol/L)	2.50±0.01	2.43±0.01	2.40±0.01	2.48±0.01	2.41±0.01	2.51±0.01
Magnesium(mmol/L)	1.12±0.01	1.14±0.01	1.00±0.01	1.08±0.01	1.00±0.01	1.09±0.01
Phosphate (mmol/L)	3.00±0.02	2.89±0.01	2.88±0.02	2.90±0.02	3.01±0.02	2.90±0.01
Urea (mmol/L)	10.60±0.03	11.15±0.02	11.11±0.02	10.93±0.03	10.88±0.01	11.10±0.01
Urate (mmol/L)	0.09±0.01	0.09±0.01	0.09±0.01	0.10±0.01	0.11±0.01	0.10±0.01
Creatinine (µmol/L)	32.67±0.01	34.50±0.01	32.71±0.01	32.00±0.01	33.00±0.01	34.00±0.01
Total protein (g/L)	57.25±0.25	56.67±0.21	55.67±0.19	62.00±0.18	56.33±0.15	57.05±0.19
Albumin (g/L)	33.00±0.03	33.00±0.05	32.00±0.05	35.00±0.05	32.00±0.05	33.00±0.03
Globulin (g/L)	25.00±0.10	25.00±0.11	24.75±0.10	27.50±0.15	24.50±0.19	26.00±0.20
ALT (IU/L)	41.33±0.15	43.33±0.12	45.16±0.18	45.11±0.10	46.01±0.21	43.03±0.25
AST (IU/L)	149.50±0.21[a]	154.33±0.25[a]	234.01±0.23[b]	272.18±0.30[b]	246.05±0.25[b]	152.11±0.11[a]
ALP (IU/L)	634.12±1.35	639.10±1.21	631.50±1.25	627.33±1.32	639.11±1.28	630.10±1.35

Values not sharing superscripts along the same row for each parameter are statistically the same ($p > 0.05$). *Group treated with 500 mg/kg body weight of corn silk ethyl acetate fraction for 28 days. **Satellite group treated with 500 mg/kg body weight of corn silk

ethyl acetate fraction for 28 days followed by no treatment for another 14 days. CK-MB= creatine kinase isoenzyme MB, LD_1= lactate dehydrogenase isoenzyme 1, ALT= alanine aminotransferase, AST= aspartate aminotransferase, ALP= alkaline phosphatase.

Table 7: Weekly open-field performance (square walked (cm)) of the animals following treatment with corn silk ethyl acetate fraction for 28 consecutive days (n = 10, $\bar{X}$ ± SEM)

Day	Fraction (mg/kg body weight)					
	Control	50	100	200	500*	500**
0	76.75±0.10^{a}	71.62±0.19^{a}	75.36±0.10^{a}	75.81±0.12^{a}	76.32±0.15^{a}	76.05±0.12^{a}
7	76.65±0.50^{a}	75.19±0.15^{a}	75.32±0.05^{a}	89.32±0.05^{b}	90.21±0.15^{b}	89.11±0.33^{b}
14	77.63±0.19^{a}	80.99±0.16^{a}	83.25±0.25^{a}	95.32±0.25^{b}	93.62±0.21^{b}	96.99±0.15^{b}
21	76.32±0.15^{a}	80.75±0.15^{a}	85.76±0.18^{a}	97.21±0.15^{b}	99.21±0.25^{b}	98.00±0.12^{b}
28	77.45±0.12^{a}	82.65±0.38^{a}	89.32±0.19^{a}	99.98±0.19^{b}	99.67±0.15^{b}	99.16±0.11^{b}
35	76.50±0.10^{a}	-	-	-	-	90.15±0.35^{b}
42	78.45±0.14^{a}	-	-	-	-	85.60±0.21^{b}

Values bearing different superscripts along the same row for each parameter are significantly different ($p < 0.05$). *Group treated with 500 mg/kg body weight of corn silk ethyl acetate fraction for 28 days. **Satellite group treated with 500 mg/kg body weight of corn silk ethyl acetate fraction for 28 days followed by no treatment for another 14 days.

CHAPTER SIX

Zea mays L. (Poaceae), *Stigma maydis* prevents and extenuates acetaminophen-perturbed oxidative onslaughts in rat hepatocytes

This chapter was presented at the Joint Congress (31 August-3 September, 2015) of South African Society of Basic and Clinical Pharmacology (SASBCP) & Toxicology Society of South Africa (TOXSA) held at University of Witwatersrand, Pretoria, South Africa. It was subsequently published in Pharmaceutical Biology.

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RESEARCH ARTICLE

Zea mays, Stigma maydis prevents and extenuates acetaminophen-perturbed oxidative onslaughts in rat hepatocytes

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ABSTRACT

Context: *Zea mays* L. (Poaceae) *Stigma maydis* is an underutilized product of corn cultivation finding therapeutic applications in oxidative stress-related disorders.

Objectives: This study investigated its aqueous extract against acetaminophen (APAP)-perturbed oxidative insults in rat hepatocytes.

Materials and methods: Hepatotoxic rats were orally pre- and post-treated with the extract (at 200 and 400 mg/kg body weight) and vitamin C (200 mg/kg body weight), respectively, for 14 days. Liver function, antioxidative and histological analyses were thereafter evaluated.

Results: The APAP-induced marked ($p < 0.05$) increases in the activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase and the concentrations of bilirubin, oxidized glutathione, protein carbonyls, malondialdehyde, conjugated dienes, lipid hydroperoxides and fragmented DNA were dose-dependently extenuated in the extract-treated animals. The extract also significantly ($p < 0.05$) improved the reduced activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase as well as total protein, albumin and glutathione concentrations in the hepatotoxic rats. These improvements may be attributed to the bioactive constituents as revealed by the gas chromatography–mass spectrometric chromatogram of the extract. The observed effects compared favourably with vitamin C and are informative of hepatoprotective and antioxidative attributes of the extract and were further supported by the histological analysis.

Conclusion: The data from the present findings suggest that *Stigma maydis* aqueous extract is capable of preventing and ameliorating APAP-mediated oxidative hepatic damage via enhancement of antioxidant defence systems.

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Antioxidant enzymes; auto-oxidation; detoxification; hepatocellular necrosis; peroxidative

Introduction

Acetaminophen (APAP)-mediated hepatotoxicity is a universally established model and has been attributed to the formation of a highly reactive metabolite [*N*-acetyl-*p*-benzoquinone imine (NAPQI)] by the action of hepatic cytochrome P-450 (Lee et al. 2012). Through phase II detoxification, NAPQI is conjugated with reduced glutathione (GSH) to form mercapturic acid, which is eventually excreted in urine (Moore et al. 1985). GSH is an ultimate antioxidant defence system that scavenges reactive metabolites and hepatic free radicals (Kerksick & Willoughby 2005), thereby keeping oxidative onslaughts on the liver under proper checks and preventing subsequent cellular damage. However, when the rate of NAPQI formation overwhelms detoxification by GSH, auto-oxidation of macromolecules such as lipids or sulfylhydride (SH) group of protein occur. This consequently converts GSH to glutathione disulfide (GSSG), resulting in mitochondrial dysfunction and development of acute hepatocellular necrosis (Sabiu et al. 2014).

Apart from the sparse number of sophisticated and functional orthodox medical facilities in most parts of the world, affordability, non-compliance, limited efficacy and potentially life-threatening adverse effects are other issues consistent with conventional drug therapy for liver disorders (Ward & Daly 1999). In contrast, phytotherapies have been used in many quarters of the world for the management and treatment of liver ailments for decades without significant side effects (Rajib et al. 2009; Mahmud et al. 2012; Sabiu et al. 2015). Therefore, it is imperative to keep searching for complementary and alternative medicines in phytotherapy for better efficacious, easily accessible, more affordable and safer treatments for liver diseases (Umer et al. 2010). The search might not be totally divorced from the excellently displayed antioxidative attributes of medicinal plants in preventing undesirable oxidation either by halting ravaging effects of reactive metabolites or simply as reactive oxygen species (ROS) scavengers. In addition to NAPQI, ROS and nitric oxide (NO), which are generated during APAP-activated inflammatory processes may also potentiate liver injury (Luster et al. 2000). Hence, plants or plant-based formulations that offer considerable protection against free radicals and reactive metabolites will be palliative agents of choice in hepatotoxicity-related ailments. *Zea mays* L. (Poaceae), *Stigma maydis* [Corn silks (CSs)] is one of the herbs currently being investigated in our laboratory for pharmacological applications. Though it is a waste of corn cultivation (Maksimović et al. 2004), it is rich in calcium, potassium, magnesium, sodium salts, proteins, fixed and volatile oils, vitamins and carbohydrates. Phytochemical analyses of its extracts have also revealed the presence of steroids, phenol, phytosterols and flavonoids, which are opined to contribute to its

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pharmacological significance (Ebrahimzadeh et al. 2008). Studies have highlighted potential benefits of CS as antioxidant, antifatigue, antidepressant, antidiabetic and hypoglycemic agent (Farsi et al. 2008; Jianyou et al. 2009; Zhao et al. 2012). Its antibiotics, immune enhancement and insecticidal activities have also been reported (Guevara et al. 2000; Maksimović & Kovacevic 2003; Kim et al. 2004). Wang et al. (2012) have also lent credence to its potency against pleurisy and oxidative stress-induced inflammatory diseases.

In view of the commendable antioxidative and anti-inflammatory activities of CS, the present study evaluated the effect of its aqueous extract against APAP-mediated oxidative onslaughts in rat hepatocytes. The gas chromatography–mass spectrometric (GC–MS) analysis of the extract was also performed with a view to providing detailed and comprehensive information on the bioactive constituents present in the extract.

Materials and methods

Chemicals and reagents

APAP and vitamin C were products of Emzor Pharmaceuticals, Lagos, Nigeria. Assay kits were purchased from Randox Laboratories Ltd., Antrim County, UK and Sigma-Aldrich Chemicals Company (St. Louis, MO). Other chemicals and reagents used were of analytical grade.

Plant collection, authentication and extract preparation

Fresh CSs were harvested from a maize plantation in Phuthaditjhaba area of Maluti-A-Phofung, QwaQwa, Free State Province, South Africa. They were authenticated at the Plant Sciences Department, University of the Free State, QwaQwa Campus, South Africa where voucher specimen (No. SabMed/01/2015/QHB) was prepared and deposited at the University's Herbarium. The silks were thoroughly rinsed to remove foliar contaminants, shade dried to constant weight and thereafter pulverized (model MS-223; Blender/Miller III, Taiwan, China) to smooth powder. The powdered sample (1.2 kg) was divided into three portions of 400 g each and extracted exhaustively with regular agitation in 4 L each of ethanol, hydroalcohol and water, respectively. The resulting infusion in each case was filtered (Whatman no. 1 filter paper). While the organic solvent extracts were evaporated to dryness in a rotary evaporator (Cole Parmer SB 1100, Shangai, China), the water extract was lyophilized using Virtis Bench Top lyophilizer (SP Scientific Series Warminster, PA). This yielded 25 g, 34 g and 40 g of the ethanol, hydroalcohol and water extracts, respectively.

About 15 µL of each extract was spotted on silica gel TLC plate and the chromatograms obtained were thereafter developed in dichloromethane/methanol (8.5:1.5 v/v) solvent system and sprayed with 0.2% 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol for detection of antioxidant constituents. From the chromatograms (not shown), the aqueous extract of CS (CSE) revealed most prominent and highest number of antioxidant spots and was selected for the *in vivo* study. CSE was reconstituted in distilled water to give different concentrations [in mg kg^{-1} body weight (b.w.) of the animals] used in this study.

Experimental animals

Sixty healthy Wistar rats (both sex) weighing between 120 and 150 g were obtained from the experimental animal facility of the University of the Free State, Bloemfontein, South Africa. They were housed under hygienic and standard environmental conditions (temperature 23 ± 1 °C, photoperiod; 12 h natural light and 12 h dark; humidity 45–50%), and allowed to acclimatize for 10 days. While the rats had free access to pelleted rat chow (Pioneer Food (Pty) Ltd., Huguenot, South Africa) and water *ad libitum*, the overall treatments were in accordance with the guidelines of National Institute of Health on the care and use of laboratory animals (NIH 1985). An ethical approval (UFS-AED2015/0005) was granted by the Ethical Committee of University of the Free State, South Africa prior to commencement of the study.

Induction of liver damage

Adopting the modified procedure outlined by Sabiu et al. (2014), hepatotoxicity was induced in rats. In brief, the animals were orally administered with 400 mg kg^{-1} body weight (b.w.) of APAP once daily for 14 days. Feed and water were made available to the animals *ad libitum* throughout the induction period.

Animal grouping and protocols

The rats were randomized into nine experimental groups for this study. While the hepatotoxic control group had 12 animals that were further divided into two sets to monitor possible self-recovery effects, the remaining animals (48) were evenly distributed into eight groups of six rats each and treated as follows:

Group 1 served as normal control and were given sterile placebo.
Group 2 comprised animals induced with hepatotoxicity (hepatotoxic rats in two sets) and were not treated.
Group 3 were placed on 400 mg kg^{-1} b.w. of CSE only for 14 days.

Pre-treatment groups

Groups 4, 5 and 6 were pre-treated with CSE (200 and 400 mg kg^{-1} b.w.) and vitamin C (200 mg kg^{-1} b.w.), respectively, for 14 days prior to hepatotoxicity induction with APAP.

Post-treatment groups

Groups 7, 8 and 9 were hepatotoxic rats post-treated, respectively, with the extract (at 200 and 400 mg kg^{-1} b.w. doses) and vitamin C (200 mg kg^{-1} b.w.) for 14 days.

All administrations were done once daily via oral intubation and a transition period of 3 h was observed between the two subsequential treatment periods in both pre- and post-treatment modes.

Serum preparation and excision of liver

Twenty-four hours after the last treatment in each case, the rats were humanely sacrificed by halothane anaesthetization and blood was collected by cardiac puncture into non-heparinized tubes. In preparation for serum collection, the blood was allowed to clot for 10 min before centrifuged (Beckman and Hirsch, Burlington, IO) at 3000 *g* for 15 min. Serum was carefully aspirated and used for liver function tests. The rats were also quickly dissected and the liver was excised, blotted with clean tissue paper, cleaned of fat and sliced into two portions. A portion of the liver was homogenized in Tris–HCl buffer (0.05 mol/L Tris–HCl and 1.15% KCl,

pH 7.4) for antioxidant analyses, while the other portion was used for histological examination.

Liver function indices and antioxidant analyses

Liver function indices were evaluated according to the manufacturer's instructions on the assay kits. Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) as well as albumin, bilirubin, total bilirubin and protein concentrations were assayed. While the homogenate activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GRx) were assayed using the methods of Rotruck et al. (1973), Marklund and Marklund (1974), Aebi (1984) and Thabrew et al. (1987), respectively, levels of lipid hydroperoxides, conjugated dienes and malondialdehyde were evaluated adopting the methods of Reilly and Aust (2001). The reported protocols of Ellman (1959) and Hissin and Hilf (1976) were employed in the determination of reduced GSH and oxidized glutathione (GSSG) concentrations, respectively. Quantification of fragmented DNA and protein carbonyls were done by the procedures of Burton (1956) and Levine et al. (1990), respectively.

Histopathological examination

Following the method of Drury and Wallington (1980), the histopathological examination of the harvested liver was performed. In brief, sliced portions of the liver were washed in normal saline and fixed immediately in 10% buffered formaldehyde solution for a period of at least 24 h. They were subsequently dehydrated with graded alcohol and further processed in paraffin embedding using LEICA PT 1020 Automatic Tissue Processor (SMM Instruments (Pty) Ltd., Randburg, South Africa). About 5-µm thick section of each tissue was stained with haematoxylin and eosin, and observed for possible histopathological derangements. Microscopic features of the hepatocytes of CSE and vitamin C-treated rats were compared with both normal and hepatotoxic control groups. Based on the severity of infiltrations and extent of hepatic damage observed, the liver sections were further evaluated and scored by the histopathologist who was unaware of the experimental treatments. Scores were assigned on a 0–4-scale as follows:

0. Normal hepatocellular tissue with well-preserved architecture;
1. Mild inter-hepatic proliferation characterized by mild evidence of inflammation and degeneration;
2. Moderate hepatocellular degeneration with obvious fat droplets, inflammation and vacuolated cytoplasm;
3. Haemorrhagic sinusoids with clear evidence of necrosis;
4. Complete or almost entire hepatocellular necrosis.

GC–MS analysis of the extract

CSE was subjected to GC–MS analysis using an Agilent Technologies 6890 Series gas chromatograph coupled with an Agilent 5973 Mass Selective detector, driven by Agilent Chemstation software. An eHP-5MS capillary column (Agilent Technologies, Santa Clara, CA) was used (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The carrier gas was ultra-pure helium at a flow rate of 1.0 mL/min and a linear velocity of 37 cm/s. The injector temperature was set at 250 °C. The initial oven temperature was at 60 °C, which was programmed to increase to 280 °C at the rate of 10 °C/min with a hold time of 4 min at each increment. Injections of 2 µL were made in the splitless mode with a split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadruple temperature 150 °C, solvent delay 4 min and scan range 50–700 amu. The compounds were identified by direct comparison of the retention times, mass spectral data and fragmentation pattern with those in the National Institute of Standards and Technology (NIST) library.

Data analysis

Degree of protection offered by the extract on liver function indices was presented as %. Other data were subjected to one-way analysis of variance (ANOVA) using SPSS 16.0 (SPSS Inc., Chicago, IL) and expressed as mean ± standard error of mean (SEM) ($n = 6$). Significant difference between the treatment means was determined at 5% confidence level using Duncan's multiple range test.

Results

Tables 1 and 2 revealed the effects of CSE on the serum activities of ALP, ALT, AST and GGT as well as concentrations of albumin, bilirubin (direct and total) and total protein in the experimental rats. While oral administration of 400 mg kg^{-1} b.w. of APAP for 14 days caused a significant ($p < 0.05$) elevation in the specific activities of these enzymes and level of bilirubin, the concentrations of albumin and total protein were significantly reduced ($p < 0.05$) when compared with the normal control. The observed alterations in these liver function parameters were, however, significantly ($p < 0.05$) and dose-dependently reversed in both extract pre- and post-treated animals. It is noteworthy that the best and most prominent effects that competed favourably with the vitamin C administered groups were observed at 400 mg kg^{-1} b.w. dose of CSE in the pre-treated groups (Tables 1 and 2).

Table 1. Effect of *Zea mays*, *Stigma maydis* aqueous extract on serum activities of liver function enzymes of APAP-treated rats ($n = 6$, Mean ± SEM).

Treatments	ALP (U/l)	ALT (U/l)	AST (U/l)	GGT (U/l)
Sterile placebo (control)	173.13 ± 1.20^{a}	21.95 ± 0.07^{a}	30.51 ± 0.06^{a}	3.91 ± 1.40^{a}
APAP treated	326.18 ± 1.90^{b}	73.51 ± 0.06^{b}	100.81 ± 0.04^{b}	10.12 ± 1.92^{b}
400 mg/kg b.w. CSE	170.12 ± 1.11^{a}	21.99 ± 0.03^{a}	30.50 ± 0.08^{a}	3.96 ± 1.26^{a}
200 mg/kg b.w. CSE, then APAP	215.14 ± 1.44^{c}	42.56 ± 0.04^{c}	50.62 ± 0.07^{c}	5.00 ± 1.80^{c}
400 mg/kg b.w. CSE, then APAP	172.90 ± 1.12^{a}	21.86 ± 0.05^{a}	35.53 ± 0.03^{a}	5.14 ± 1.20^{c}
200 mg/kg b.w. vitamin C, then APAP	172.84 ± 1.36^{a}	31.06 ± 0.07^{d}	33.51 ± 0.01^{a}	4.07 ± 1.22^{a}
APAP, then 200 mg/kg b.w. CSE	225.22 ± 1.13^{c}	42.67 ± 0.08^{c}	53.62 ± 0.01^{c}	7.00 ± 1.10^{d}
APAP, then 400 mg/kg b.w. CSE	173.03 ± 1.10^{a}	21.79 ± 0.03^{a}	49.60 ± 0.05^{c}	5.33 ± 1.11^{c}
APAP, then 200 mg/kg b.w. vitamin C	171.32 ± 1.04^{a}	31.06 ± 0.08^{d}	50.61 ± 0.09^{c}	5.07 ± 1.09^{c}

APAP: acetaminophen; CSE: corn silk extract; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transferase.
[abcd]Values with different superscripts for each parameter are significantly different ($p < 0.05$).

There were no traces of hepatotoxic tendencies in the rats given 400 mg kg^{-1} b.w. of CSE alone, as they compared well with the normal control for these parameters. However, the improvements observed in the extract-administered groups were not evident in the self-recovery groups whose assayed parameters were typical of the hepatotoxic animals. The degree of protection conferred on these parameters by CSE treatments is shown in Table 3.

Observable from Tables 4 and 5 and Figure 1 are the effects of CSE treatments on the antioxidant status of rat liver. Tissue activities of SOD, CAT, GRx and GPx were significantly ($p < 0.05$) induced by CSE in a dose-related manner. These inductions significantly ($p < 0.05$) improved the observed APAP-mediated reduction in their activities and the effect compared favourably with vitamin C (Table 4). Similarly, the APAP-induced significant ($p < 0.05$) reduction in the level of reduced GSH and the increases in the levels of oxidized glutathione (GSSG), protein carbonyls, fragmented DNA, malondialdehyde, conjugated dienes and lipid hydroperoxides were significantly and dose-dependently reversed in the extract-treated animals. Although the extract (at 400 mg kg^{-1} b.w.) competed well with vitamin C in the two treatment modes, better results comparable to those of normal control were observed in the extract-pretreated animals (Table 5 and Figure 1). This was also the case for the rats administered with 400 mg kg^{-1} b.w. of CSE alone.

Table 2. Effect of *Zea mays*, *Stigma maydis* aqueous extract on serum concentrations of albumin, bilirubin and total protein of APAP-treated rats ($n = 6$, mean ± SEM).

Treatments	Albumin (g/L)	Total bilirubin (μmol/L)	Direct bilirubin (μmol/L)	Total protein (g/L)
Sterile placebo (control)	5.10 ± 1.12^{a}	12.48 ± 1.01^{a}	5.95 ± 1.83^{a}	35.72 ± 1.14^{a}
APAP treated	0.61 ± 1.73^{b}	53.76 ± 2.32^{b}	15.32 ± 1.82^{b}	13.78 ± 1.91^{b}
400 mg/kg b.w. CSE	5.00 ± 1.09^{a}	16.19 ± 1.09^{a}	4.99 ± 1.75^{a}	34.80 ± 1.25^{a}
200 mg/kg b.w. CSE, then APAP	2.01 ± 1.32^{c}	33.64 ± 2.97^{c}	7.18 ± 1.89^{c}	25.28 ± 1.90^{c}
400 mg/kg b.w. CSE, then APAP	5.94 ± 1.26^{a}	10.10 ± 1.86^{a}	6.00 ± 1.99^{a}	33.66 ± 1.80^{a}
200 mg/kg b.w. vitamin C, then APAP	5.02 ± 1.70^{a}	11.55 ± 1.65^{a}	6.09 ± 1.51^{a}	31.39 ± 1.91^{a}
APAP, then 200 mg/kg b.w. CSE	3.15 ± 1.08^{d}	12.64 ± 2.97^{d}	12.34 ± 1.09^{d}	23.19 ± 1.23^{c}
APAP, then 400 mg/kg b.w. CSE	2.09 ± 1.12^{c}	36.10 ± 1.86^{c}	6.23 ± 1.56^{a}	33.65 ± 1.45^{a}
APAP, then 200 mg/kg b.w. vitamin C	6.05 ± 1.45^{a}	11.55 ± 1.65^{a}	6.12 ± 1.45^{a}	32.45 ± 1.22^{a}

APAP: acetaminophen; CSE: corn silk extract.
^{abcd}Values with different superscripts for each parameter are significantly different ($p < 0.05$).

Table 3. Mean percentage protection offered by *Zea mays*, *Stigma maydis* and vitamin C against APAP-induced hepatic injury on some liver function parameters.

Treatments	Mean % protection							
	ALP	ALT	AST	GGT	ALB	D-BIL	T-BIL	TP
CSE-treated (200 mg/kg)	69.26*	59.92*	90.50	66.35*	61.68*	60.91*	39.52*	47.65*
CSE-treated (400 mg/kg)	100.11	100.24	100.11	78.66	89.66	99.06	68.48	90.59
Vitamin C-treated	100.69	82.33	100.69	89.37	108.92	97.06	88.84	82.68

Value with (*) along the same column for each parameter is significantly different from vitamin C-treated group ($p < 0.05$). CSE: corn silk extract; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transferase; ALB: albumin; T-BIL: total bilirubin; D-BIL: direct bilirubin; TP: total protein.

Table 4. Effect of *Zea mays*, *Stigma maydis* aqueous extract on specific activities of enzymic antioxidant system of APAP-treated rats ($n = 6$, mean ± SEM).

Treatments	Antioxidant enzymes (nmol min^{-1} mg $protein^{-1}$)			
	SOD	CAT	GRx	GPx
Sterile placebo (control)	55.20 ± 0.10^{a}	45.38 ± 0.22^{a}	63.93 ± 1.80^{a}	252.19 ± 2.10^{a}
APAP treated	22.19 ± 0.11^{b}	13.29 ± 0.19^{b}	25.29 ± 1.82^{b}	96.35 ± 1.00^{b}
400 mg/kg b.w. CSE	69.39 ± 0.09^{c}	60.19 ± 0.25^{c}	70.35 ± 1.16^{a}	272.16 ± 2.15^{c}
200 mg/kg b.w. CSE, then APAP	45.35 ± 0.20^{d}	32.93 ± 0.19^{d}	42.86 ± 1.19^{c}	219.31 ± 2.11^{d}
400 mg/kg b.w. CSE, then APAP	53.98 ± 0.16^{a}	43.62 ± 0.12^{a}	69.72 ± 1.10^{a}	245.03 ± 2.21^{a}
200 mg/kg b.w. vitamin C, then APAP	51.99 ± 0.17^{a}	42.96 ± 0.10^{a}	66.93 ± 1.22^{a}	226.63 ± 2.89^{d}
APAP, then 200 mg/kg b.w. CSE	35.23 ± 0.10^{e}	22.18 ± 0.15^{e}	42.11 ± 1.20^{c}	159.22 ± 1.21^{e}
APAP, then 400 mg/kg b.w. CSE	46.12 ± 0.11^{d}	32.62 ± 0.14^{d}	66.09 ± 1.12^{a}	248.11 ± 2.04^{a}
APAP, then 200 mg/kg b.w. vitamin C	47.17 ± 0.17^{d}	33.96 ± 0.11^{d}	46.91 ± 1.03^{c}	229.12 ± 2.11^{d}

^{abcde}Values with different superscripts for each parameter are significantly different ($p < 0.05$). APAP: acetaminophen; CSE: corn silk extract: SOD: superoxide dismutase; GRX: glutathione reductase; GPX: glutathione peroxidase.

Table 5. Effect of *Zea mays*, *Stigma maydis* aqueous extract on the levels of non-enzymic antioxidant system, protein carbonyl and fragmented DNA of APAP-treated rats ($n = 6$, Mean ± SEM).

Treatments	Reduced glutathione (nmol mg $protein^{-1}$)	Peroxidized glutathione (X)	GSH:GSSG ratio	Protein carbonyl (nmol mg $protein^{-1}$)	Fragmented DNA (%)
Sterile placebo (control)	46.25 ± 1.10^{a}	0.17 ± 0.05^{a}	272.06 ± 0.20^{a}	15.72 ± 0.13^{a}	12.12 ± 0.30^{a}
APAP-treated	10.18 ± 1.13^{b}	2.93 ± 0.07^{b}	5.27 ± 0.08^{b}	58.55 ± 0.12^{b}	79.15 ± 0.30^{b}
400 mg/kg b.w. CSE	33.36 ± 1.88^{c}	0.12 ± 0.10^{c}	278.00 ± 0.32^{a}	15.16 ± 0.12^{a}	12.75 ± 0.20^{a}
200 mg/kg b.w. CSE, then APAP	41.70 ± 1.26^{a}	0.17 ± 0.02^{a}	245.29 ± 0.19^{c}	25.73 ± 0.13^{c}	42.16 ± 0.30^{c}
400 mg/kg b.w. CSE, then APAP	42.80 ± 1.15^{a}	0.15 ± 0.07^{a}	285.33 ± 0.15^{a}	19.10 ± 0.12^{a}	13.19 ± 0.20^{a}
200 mg/kg b.w. vitamin C, then APAP	46.34 ± 1.18^{a}	0.17 ± 0.03^{a}	272.59 ± 0.17^{a}	19.01 ± 0.14^{a}	13.11 ± 0.20^{a}
APAP, then 200 mg/kg b.w. CSE	20.89 ± 1.09^{d}	0.25 ± 0.02^{d}	83.56 ± 0.15^{d}	35.16 ± 0.11^{d}	31.16 ± 0.30^{d}
APAP, then 400 mg/kg b.w CSE	22.88 ± 1.90^{d}	0.19 ± 0.07^{e}	120.42 ± 0.09^{e}	23.09 ± 0.13^{c}	23.19 ± 0.20^{e}
APAP, then 200 mg/kg b.w. vitamin C	21.93 ± 1.65^{d}	0.19 ± 0.03^{e}	115.42 ± 0.10^{e}	25.21 ± 0.11^{c}	22.11 ± 0.20^{e}

APAP: acetaminophen; CSE: corn silk extract; X: nmol mg $protein^{-1}$.
^{abcde}Values with different superscripts for each parameter are significantly different ($p < 0.05$).

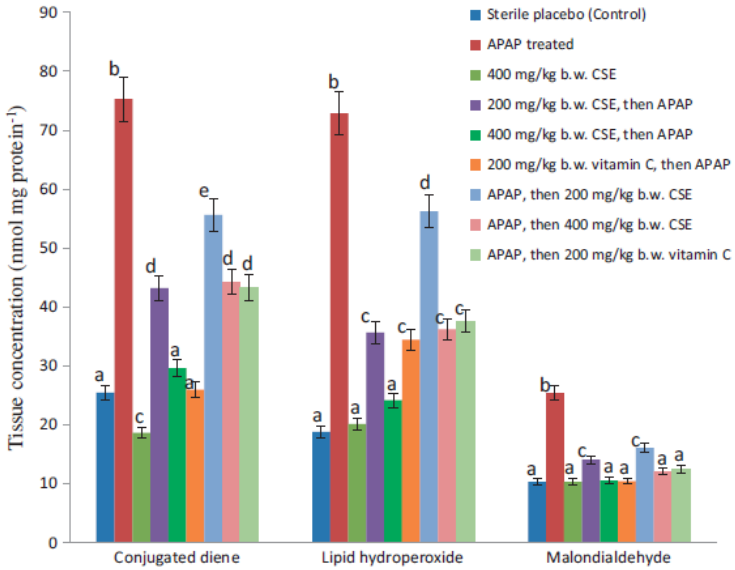

Figure 1. Effect of *Zea mays, Stigma maydis* aqueous extract on tissue concentrations of conjugated dienes, lipid hydroperoxides and malondialdehyde of APAP-treated rats. Values are mean ± standard deviation (SEM) of six determinations. [abcde]Bars with different superscripts for each parameter are significantly different ($p < 0.05$). APAP: acetaminophen; CSE: corn silk extract.

Macroscopic examination of livers from normal control group revealed that they were normal with characteristic dark maroon appearances and smooth texture. While livers from the APAP-intoxicated rats showed changes in colour from maroon to brown with characteristic uneven texture, those of the CSE-treated rats revealed mild spots of brown colour changes. Furthermore, detailed histoarchitectural examination of the liver of the control rats showed normal morphological features with well-preserved cords of hepatocytes, well demarcated sinusoids and no area of infiltration by inflammatory cells (Figure 2(a)). This is in sharp contrast to the features observed in the liver of untreated hepatotoxic rats. Major alterations including haemorrhage, extensive fatty change, distended hepatocytes, vacuolated cytoplasm, compressed sinusoids, infiltration by inflammatory cells and extensive coagulative necrosis were evident in there liver architecture (Figure 2(b)). However, the liver micrographs of the CSE-treated animals in both treatment modes showed mild pathological changes with overall distinct and essentially normal cords of hepatocyte devoid of prominent fatty change (Figure 2(c–i)). In addition, histopathological grading of the liver tissues of CSE-treated groups showed that the obvious necrosis, inflammation and haemorrhage present in the hepatocytes of APAP-intoxicated rats were significantly and dose-dependently extenuated in a manner comparable to the vitamin C administered groups (Table 6).

Results from GC–MS analysis of CSE in comparison with standard mass spectra in the NIST (NIST14 standard version) library revealed the presence of *o*-diethyl phthalate, 2-methyl-naphthalene, thymol, maizenic acid, 3′-*O*-methyl-maysin, cyanidin, cinnamic acid, hordenine, luteolinidin and pelargonidin as major identifiable adaptogenic constituents (Figure 3 and Table 7).

Discussion

Drug-induced liver ailments ensued more often and can be very disconcerting. Oxidative stress occasioned by reactive intermediates (free radicals) has been linked to APAP-mediated hepatotoxicity in rats (Balamurugan 2007). The ROS, NO and NAPQI generated during APAP bioactivation binds covalently and enhanced auto-oxidation of macromolecules (proteins and lipids) of the hepatocytes plasma membranes. This subsequently induces cell membrane damage, perturbs Ca^{2+} homeostasis in the mitochondrial, alters enzyme activities, and consequently results in hepatic injury or necrosis (Hazai et al. 2002). When these happen, cytosolic AST, ALT, ALP and GGT are released into systemic circulation and their measurement can be used to assess the extent of drug-induced hepatotoxicity (Jaeschke et al. 2003). In this study, the elevated activities of these marker enzymes in the APAP-intoxicated rats may be indicative of liver damage and cell necrosis resulting from formation of NAPQI in excess of GSH detoxification capacity. This is in agreement with previous studies (Balamurugan 2007; Kanchana & Mohamed 2011), where APAP administration proved toxic to hepatocytes. However, the significantly and dose-dependently reduced specific activity of these enzymes in rats treated with CSE suggests that it was able to prevent and ameliorate the ravaging effects of APAP. This observation indicates hepatoprotective potential of the extract at the investigated doses.

Albumin is frequently utilized as an index of the hepatocyte's ability to carry out synthetic function. Its serum concentration remains unchanged in mild liver injury but readily declines in the face of sub-massive liver necrosis. In addition, serum levels of total protein and albumin may indicate the state of the liver and the type of damage. Metabolic alterations in their concentrations in the serum have also been employed to assess secretory capability of the liver (Oloyede & Sunmonu 2009). In the present study, the APAP-mediated significant reduction in the serum levels of these parameters may be indicative of diminished synthetic function of the liver (Sabiu et al. 2015). Bilirubin is an important catabolic product of the blood and its biological value has been utilized to monitor excretory role of the liver (Tietz 1995). The significantly increased serum concentration of bilirubin in the hepatotoxic animals could be ascribed to APAP-induced defect in

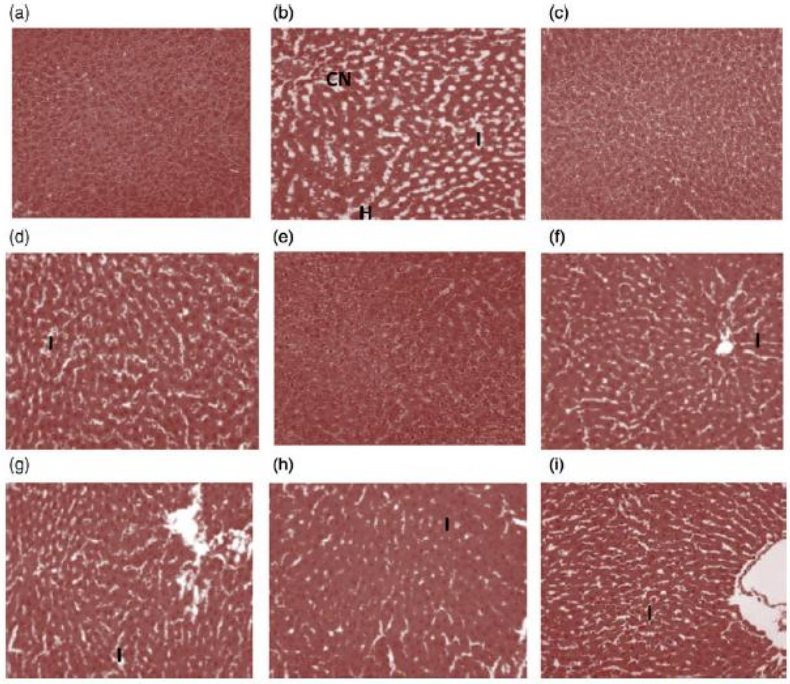

Figure 2. Liver micrograghs (×400, haematoxylin and eosin stained) of (a) normal control rat, (b) hepatotoxic rat, (c) CSE (400 mg/kg b.w.) treated rat, (d) hepatotoxic rat pre-treated with CSE (200 mg/kg b.w.), (e) hepatotoxic rat treated with CSE (400 mg/kg b.w), (f) hepatotoxic rat pre-treated with vitamin C (200 mg/kg b.w.), (g) hepatotoxic rat post-treated with CSE (200 mg/kg b.w.), (h) hepatotoxic rat post-treated with CSE (400 mg/kg b.w.) and (i) hepatotoxic rat post-treated with vitamin C (200 mg/kg b.w.). CSE: corn silk aqueous extract; I: inflammation; CN: coagulative necrosis; H: haemorrhage.

Table 6. Histopathological grading of liver tissue sections of *Zea mays, Stigma maydis* aqueous extract treated animals.

Treatments	Scores				
	0	1	2	3	4
Control	(6)	(0)	(0)	(0)	(0)
APAP-treated	(0)	(0)	(2)	(3)	(1)
400 mg/kg of CSE.	(6)	(0)	(0)	(0)	(0)
200 mg/kg b.w. CSE, then APAP	(4)	(2)	(0)	(0)	(0)
400 mg/kg b.w. CSE, then APAP	(6)	(0)	(0)	(0)	(0)
200 mg/kg b.w. vitamin C, then APAP	(4)	(2)	(0)	(0)	(0)
APAP, then 200 mg/kg b.w. CSE	(3)	(2)	(1)	(0)	(0)
APAP, then 400 mg/kg b.w. CSE	(5)	(1)	(0)	(0)	(0)
APAP, then 200 mg/kg b.w. vitamin C	(4)	(2)	(0)	(0)	(0)

$n = 6$; figure in parenthesis represents number of rats affected in the group. APAP: acetaminophen; CSE: corn silk extract.

the carrier-mediated saturable system at the sinusoidal surface of the hepatocytes that consequently obstruct bilirubin uptake and secretion into bile (Sabiu et al. 2014). Therefore, the dose-dependent and significant improvements in albumin and total protein concentrations coupled with the attenuation in the level of bilirubin in the CSE-administered animals for both treatment modes is informative of enhanced synthetic, secretory and excretory functions of the hepatocytes facilitated by the extract. This does not only suggest that CSE is composed of active principles capable of stabilizing the plasma membrane of the hepatocytes, but also supportive of its preventive and ameliorative potentials on the liver. Our submission is in agreement with the report of Uma et al. (2010). The authors opined that normalization of liver protein systems following APAP intoxication in rats was due to the bioactive compounds in *Moringa oleifera* extracts.

When the body's antioxidant defence system is overwhelmed by catastrophic free radicals events, timely intervention with exogenous antioxidants augments the cellular defence system thereby preventing cell death. The decreased tissue activities of the assayed antioxidant enzymes (SOD, CAT, GRx and GPx) could be due to their excessive mobilization towards detoxification of NAPQI and ROS during APAP hepatotoxicity. This might have led to unguided oxidative attack on cellular macromolecules that consequently results in necrosis (Sabiu et al. 2014). This finding is consistent with the report of Chinnasamy et al. (2011) where similar reductions in activities of ROS detoxifying enzymes were associated with formation of NAPQI and ROS in APAP-mediated hepatotoxicity in rats. Thus, the dose-related significant reversion of APAP-induced reduction in the activities of these detoxifying enzymes by CSE is implicative of its antioxidative activity. This could be ascribed to the tendency of the extract to scavenge NAPQI, ROS and NO or enhance ROS detoxifying enzymes.

In the same vein, the observed attenuation in the level of GSH might be due to depletion of GPx and GR, as well as formation of NAPQI in excess of GSH detoxification capacity (Gini & Muraleedhara 2010). More so, APAP-mediated elevation in the concentration of GSSG may be ascribed to either GSH auto-oxidation or its mobilization towards formation of GPx. The reduction in the ratio of GSH to GSSG caused by APAP intoxication reveals possible oxidative onslaught on the hepatocytes. Conversely, the significantly and dose-dependently improved GSH level coupled with the corresponding high GSH: GSSG ratio and low GSSG levels in the liver of CSE-treated animals relative to APAP-intoxicated rats is informative of the inherent antioxidative

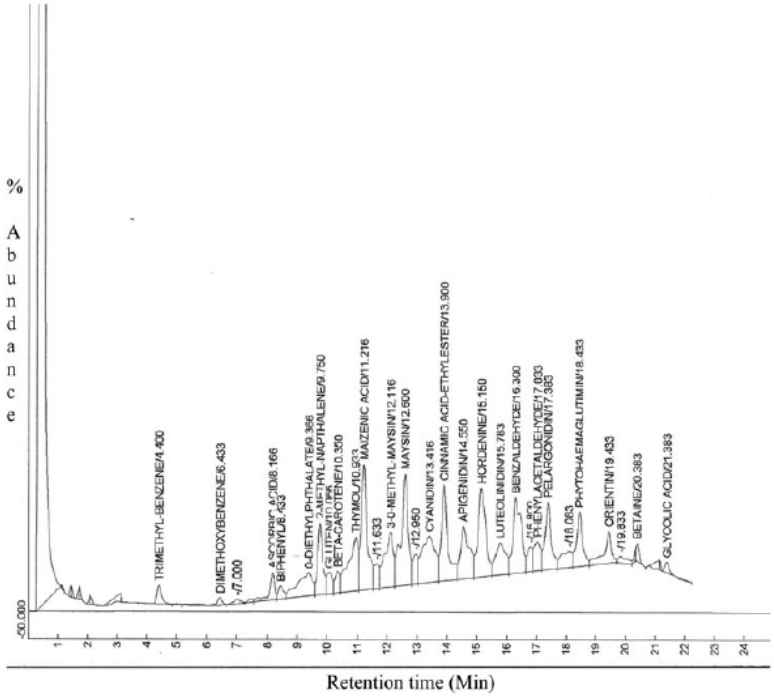

Figure 3. GC–MS chromatogram of *Zea mays*, *Stigma maydis* aqueous extract.

Table 7. Bioactive constituents of *Zea mays*, *Stigma maydis* aqueous extract as revealed by GC-MS chromatogram.

S/N	Active component	Retention time (Min)	Peak area (%)	Molecular formula	Molecular weight (g mol^{-1})
1	Trimethylbenzene#	4.40	1.05	C_9H_{12}	120.19
2	Dimethoxybenzene#	6.43	0.42	$C_8H_{10}O_2$	138.17
3	Ascorbic acid**	8.17	1.47	$C_6H_8O_6$	176.12
4	Biphenyl#	8.43	0.80	$C_{12}H_{10}$	154.21
5	*O*-Diethylphthalate#	9.37	4.03	$C_{12}H_{14}O_4$	222.24
6	2-Methyl-napthalene*	9.75	4.08	$C_{11}H_{10}$	142.19
7	Gluten**	10.07	1.35	$C_{29}H_{37}N_5O_9$	599.63
8	beta-Carotene**	10.35	1.15	$C_{40}H_{56}$	536.87
9	Thymol**	10.93	6.01	$C_{10}H_{14}O$	150.24
10	Maizenic acid*	11.22	8.79	$C_{47}H_{51}NO_{14}$	853.91
11	3-*O*-methyl-maysin*	12.12	5.57	$C_{28}H_{31}O_{15}$	607.50
12	Maysin*	12.60	9.15	$C_{27}H_{28}O_{14}$	576.50
13	Cyanidin*	13.42	7.06	$C_{15}H_{11}O_6$	287.24
14	Cinnamic acid**	13.90	7.33	$C_9H_8O_2$	146.15
15	Apigenidin*	14.55	5.28	$C_{15}H_{10}O_5$	270.24
16	Hordenine*	15.15	7.91	$C_{10}H_{15}NO$	165.24
17	Luteolinidin*	15.78	3.73	$C_{15}H_{11}O_5$	271.24
18	Benzaldehyde#	16.30	7.38	C_7H_6O	106.12
19	Phenylacetaldehyde*	17.03	2.25	C_8H_8O	120.15
20	Pelargonidin*	17.38	4.83	$C_{15}H_{11}O_5$	271.24
21	Orientin*	19.43	2.82	$C_{12}H_{20}O_{11}$	448.37
22	Betaine**	20.38	0.67	$C_5H_{11}NO_2$	117.15
23	Glycolic acid**	21.38	0.40	$C_2H_4O_3$	76.05

#Flavoring agents.
*Antioxidants.
**Antioxidant and hepatoprotective agents.

potential of the extract and further supports that it offered considerable level of hepatoprotection at the tested regimen. This was also evidently supported by the optimized antioxidant status of the animals solely administered with 400 mg kg^{-1} b.w. of the extract. This assertion is in conformity with the report of Gini and Muraleedhara (2010) that administration of plant extracts resulted in improved non-enzymic status in drug-intoxicated rats. Furthermore, APAP has been linked with lipid peroxidation and may facilitate elevated level of peroxidized products (conjugated dienes, lipid hydroperoxides and malondialdehydes) in hepatotoxicity (Muriel et al. 1992). Therefore, the significantly increased levels of these products may depict haphazard oxidative onslaughts of APAP on membrane-bound lipids. This might have disrupted membrane fluidity, orientation as well as modified and

inflicted functional loss on proteins and DNA base pairs (Das 1994; Niki 2009). The attenuation of APAP-mediated increases in these peroxidative products by CSE is suggestive of considerable level of protection on the membrane lipids. This could be adduced to ability of the extract to enhance detoxification of reactive metabolites, which might have initiated and promoted peroxidation of polyunsaturated lipids of the hepatocyte membrane. Additionally, protein carbonyl formation is a useful indicator commonly used to ascertain the level of protein autooxidation in cells. The significant increase in its concentration in the hepatotoxic rats relative to CSE-administered groups might be attributable to covalent binding of NAPQI to mitochondrial proteins (Jaeschke & Bajt 2006). This might have resultantly induced formation of nitrate ion that incapacitates the Ca^{2+} pump of the hepatocyte membrane. By effect, hypercalcemia that further hinders mitochondrial function and ATP production might have occasioned the observed elevated level of carbonyl product. Consequently, that pre- and post-treatments with CSE reversed this trend is a further attestation to its possible potential to incapacitate nitrate ions and other reactive metabolites through induction of antioxidant defence systems of the hepatocyte. Just as in protein carbonyls, calcium ion accumulation and hydroxyl radical mediated oxidative damage are important events in the pathogenesis of DNA fragmentation. These events either promote tissue necrosis or carcinogenesis, which subsequently results in cell death (Cooke et al. 2006). Thus, the significantly increased level of fragmented DNA in the liver of APAP-treated rat is suggestive of genotoxicity. It also suggests probable initiation of carcinogenesis. Jaeschke and Bajt (2006) reported similar increase in damaged DNA due to APAP intoxication. The decrease in the level of fragmented DNA in the liver of APAP-treated rats by CSE also lent credence to its probable antioxidative and antigenotoxic activities. The extract might have mimicked an antigenotoxic agent, thereby augmenting DNA repair or synthesis mechanism (Brahmi et al. 2011), as evidently shown by the rats placed on 400 mg kg^{-1} b.w. of CSE alone.

Besides complementing biochemical analyses, histopathological examination of the hepatocytes may give hints on how therapeutically potent or effective an agent is against hepatic injury. The apparently annulled degenerative threats posed by APAP on the architectural features of hepatocytes in the CSE-treated animals probably suggest that the extract provided a reasonable level of protection and stabilization on the overall histoarchitectural integrity of the liver. In fact, the liver regeneration progress and architectural organization of some of the hepatocytes was almost completely restored to normal with increasing number of viable cells as evidently shown by hepatocyte scores. The effects noticed compared favourably with vitamin C and is consistent with the results of biochemical assays conducted in this study. Our report agrees with the submission of Balamurugan (2007), where recovery towards normalization of serum enzymes and liver histological architecture caused by APAP was attributed to plant extract treatment.

In light of the foregoing, a tentative mechanism of antioxidative and hepatoprotective capabilities of CSE may be proposed as illustrated in Figure 4. This essentially involves induction and optimization of preventive (CAT, GPx) and chain-breaking (SOD, GRx) antioxidants that subsequently increase cellular GSH content, annihilate NAPQI and effectively scavenge ROS (O_2^-, OH^-) (Figure 4). This may also be considered to regulate membrane fluidity and protect hepatocyte membranes from oxidative damage as evident from the histopathological investigations.

Generally, the attributes elicited by CSE in this study might be adduced to its phytoconstituents with known antioxidative activity as revealed by the GC–MS chromatogram. Apart from the preventive and chain-breaking antioxidative properties of these compounds, remarkable hepatoprotective potentials of ascorbic acid, beta-carotene, gluten and cinnamic acid have been well documented

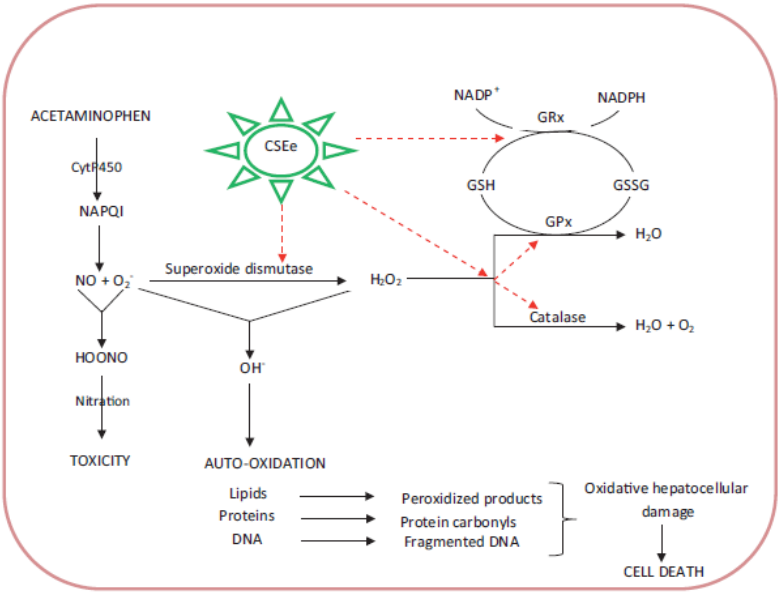

Figure 4. Tentative mechanism of antioxidative and hepatoprotective potentials of *Zea mays*, *Stigma maydis* aqueous extract. The red dotted arrows represent sites of induction and optimization by CSE that consequently promote scavenging of O_2^- and OH^-. This ultimately normalized and increased cellular reduced glutathione (GSH) concentration and facilitates its mobilization towards NAPQI detoxification and by extension stalling nitration. CSE: corn silk aqueous extract; GPx: glutathione peroxidase; GRx: glutathione reductase.

(Fernández-Martínez et al. 2007; Guo-Cai et al. 2012; Morakinyo et al. 2012; Adikwu & Deo 2013). Proven pharmacological significance of thymol, betaine and glycolic acid in enhancing hepatocytes regeneration process have also been reported (Morreale & Livrea 1997; Angulo & Lindor 2001; Janbaz et al. 2003).

Conclusion

The forestalling of oxidative onslaughts inflicted by APAP through treatments with CS aqueous extract is an obvious indication of its potential hepatoprotective and antioxidative capabilities in rats. This in our view was achieved by enhancing detoxification of APAP-mediated hepatic damage via induction of ROS detoxifying enzymes, thereby halting auto-oxidation of cellular macromolecules and hepatocellular injury. Though the effects were prominently exhibited in the CSE-pretreated groups, the overall effects elicited in both treatment modes are commendable and suggestive of an excellent candidature in the management of drug-induced hepatic oxidative disorders.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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CHAPTER SEVEN

Membrane stabilization and detoxification of acetaminophen-mediated oxidative onslaughts in the kidneys of Wistar rats by standardized fraction of *Zea mays* L. (Poaceae), *Stigma maydis*

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Research Article

Membrane Stabilization and Detoxification of Acetaminophen-Mediated Oxidative Onslaughts in the Kidneys of Wistar Rats by Standardized Fraction of *Zea mays* L. (Poaceae), *Stigma maydis*

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This study evaluated membrane stabilization and detoxification potential of ethyl acetate fraction of *Zea mays* L., *Stigma maydis* in acetaminophen-induced oxidative onslaughts in the kidneys of Wistar rats. Nephrotoxic rats were orally pre- and posttreated with the fraction and vitamin C for 14 days. Kidney function, antioxidative and histological analyses were thereafter evaluated. The acetaminophen-mediated significant elevations in the serum concentrations of creatinine, urea, uric acid, sodium, potassium, and tissue levels of oxidized glutathione, protein-oxidized products, lipid peroxidized products, and fragmented DNA were dose-dependently assuaged in the fraction-treated animals. The fraction also markedly improved creatinine clearance rate, glutathione, and calcium concentrations as well as activities of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase in the nephrotoxic rats. These improvements may be attributed to the antioxidative and membrane stabilization activities of the fraction. The observed effects compared favorably with that of vitamin C and are informative of the fraction's ability to prevent progression of renal pathological conditions and preserve kidney functions as evidently supported by the histological analysis. Although the effects were prominently exhibited in the fraction-pretreated groups, the overall data from the present findings suggest that the fraction could prevent or extenuate acetaminophen-mediated oxidative renal damage via fortification of antioxidant defense mechanisms.

1. Introduction

The kidney is a highly specialized organ that maintains the body's homeostasis by selectively excreting or retaining various substances according to specific body needs. In its role as a detoxifier and primary eliminator of xenobiotics, it becomes vulnerable to developing injuries. Such injuries have been linked with reactive oxygen species (ROS) mediated oxidative stress on renal biomolecules [1]. The kidney's response to toxicants varies by multiple morphological patterns beginning with tubular or interstitial changes to nephropathy [2]. Kidney disorders account for 1 in 10 deaths, making Chronic Kidney Disease (CKD) one of the most sought after public health concerns in recent years [3]. The prevalence of the disease is more disconcerting in sub-Saharan Africa countries like Nigeria and South Africa with an estimation of 23 and 40%, respectively [3, 4]. Till date, orthodox management therapies for kidney disorders have been embraced and identified to include the use of renal replacement therapy (dialysis and transplantation) and applications of angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and erythropoietin to slow the progression of loss of kidney function [5]. The affordability, sensitivity, and inherent adverse effects of the aforementioned therapies have undermined their applications in the past. The availability of kidneys for transplantation and cost are other important

challenges consistent with renal replacement therapy [6]. Interestingly, traditional systems of medicine have offered effective drugs against kidney pathological conditions and thus can be used to protect renal function and prevent/slow the progression of renal diseases to CKD or end stage renal disease [7]. A number of drugs from herbal sources have been shown to be nephroprotective and there is a keen global interest on the development of such. The focus is mostly to protect or prevent injurious insults to the kidney as well as enhance the regeneration of tubular cells [8].

Zea mays L. (Poaceae), *Stigma maydis* (corn silk) is one of several herbs commonly used in the management of kidney stones, bedwetting, and urinary infections [9]. GCMS analysis of its aqueous extract from our laboratory revealed the presence of maizenic acid, β-carotene, ascorbic acid, gluten, o-diethyl phthalate, 2-methyl-naphthalene, thymol, 3′-o-methyl-maysin, cyanidin, cinnamic acid, hordenine, luteolinidin, pelargonidin, and betaine as major identifiable adaptogenic phytonutrients [10]. Corn silk (CS) has found therapeutic applications as an insecticide, disinfectant, antioxidant, antibiotic, and immune booster [11–13]. Its pharmacological significance in Asian folkloric medicine as oral hypoglycemic and anti-inflammatory agents has also been reported [14–16]. Lamentably in Africa, particularly, Nigeria and South Africa, where corn is a common staple food, CS is still underutilized and its pharmacological significance is chiefly untapped.

Besides very limited research on the therapeutic importance of CS in Africa, opinions on its nephroprotective potential are divergent. While Sukandar et al. [17] demonstrated the potency of its ethanolic extract against gentamicin/piroxicam-induced kidney failure, Sepehri et al. [18] submitted that treatment with its methanolic extract does not result in complete reversal of gentamicin-induced alterations in kidney function parameters. More comprehensive research is however imperative in this direction and has prompted the present study with a view to providing detailed biochemical information on the ability of CS to preserve renal functions and delay/prevent the progression of renal pathological conditions. Hence, we evaluated its standardized fraction against acetaminophen-perturbed oxidative onslaughts in the kidneys of Wistar rats. In addition, the membrane stabilization capacity of the CS fraction was also investigated.

2. Materials and Methods

2.1. Chemicals, Reagents, and Assay Kits. Assay kits for kidney function parameters, glutathione peroxidase, and glutathione reductase were purchased from Randox Laboratories Limited, United Kingdom. Acetaminophen (APAP) and vitamin C were products of Emzor Pharmaceuticals, Lagos, Nigeria. The water used was glass-distilled and all other chemicals and reagents were of analytical grade.

2.2. Plant Collection and Authentication. Fresh corn silks were harvested from a maize plantation in the Phuthaditjhaba area of Maluti-A-Phofung, QwaQwa, Free State province, South Africa, between November 2014 and March 2015. They were authenticated by Dr. A. O. T. Ashafa of the Plant Sciences Department, University of the Free State, QwaQwa Campus, South Africa. Voucher specimen (number SabMed/01/2015/QHB) was thereafter prepared and deposited at the Herbarium of the University.

2.3. Extract Processing, Standardization, and Selection. The CS was shade dried to constant weight and subsequently milled by an electric blender (model MS-223; Labcon PTY, Durban, South Africa) to fine powder. The powdered sample (2 kg) was extracted with 70% methanol (10 L) with regular agitation for 24 h. The solution obtained was filtered (Whatman no. 1 filter paper) and the resulting filtrate concentrated to a yield of 455 g crude extract. Part of the crude extract (400 g) was suspended in distilled water (0.6 L) and subsequently partitioned in succession with n-hexane, dichloromethane, ethyl acetate, and n-butanol. This yielded 18 g, 24 g, 33 g, and 42 g of the respective fractions. About 10 μL of each (1 mg/mL) was spotted on silica gel TLC plates. The resulting chromatograms were thereafter developed in dichloromethane/methanol (8.5 : 1.5 v/v) solvent system and sprayed with 0.2% 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol for detection of antioxidant metabolites. From the chromatograms, the ethyl acetate fraction of CS (CSEAF) had the highest number of antioxidant spots and was selected for the subsequent biochemical assays. CSEAF was kept air-tight and refrigerated prior to commencement of the study.

2.4. Experimental Animals. This study was approved (UFS-AED2015/0005) by the Ethical Committee of University of the Free State, South Africa, in accordance with the Guidelines of the National Research Council Guide for the Care and Use of Laboratory Animals [19] and principles of Good Laboratory Procedure [20]. Healthy Wistar rats (both sexes) of weight range 200–222 g were collected from the experimental animal facility of University of the Free State, Bloemfontein, South Africa. They were housed in clean metabolic cages placed in a well-ventilated animal house with optimal conditions (temperature 23 ± 1°C, photoperiod; 12 h natural light and 12 h dark; humidity; 45–50%). They were acclimatized to the animal house condition for 10 days and had *ad libitum* access to pelleted rat chow (Pioneer Food (Pty) Ltd., Huguenot, South Africa) and water.

2.5. Nephroprotective Study

2.5.1. Induction of Renal Injury. This was achieved as previously described [21]. Briefly, the animals were fasted overnight for 14 h and a single oral dose of APAP (750 mg/kg body weight (b.w.)) was thereafter administered. These animals essentially represent nephrotoxic rats.

2.6. Experimental Protocol. Fifty rats randomized into 9 experimental groups were used for this study. While the nephrotoxic control group had 10 animals that were further divided into 2 sets (with one of the sets designated as satellite group) to monitor possible self-recovery effects, the remaining animals (40) were evenly distributed into 8 treatment groups of 5 rats each and treated as in Table 1.

Table 1

Groups	Designation	Treatments
1	Control	Given sterile placebo.
2	Nephrotoxic rats in two sets	Animals induced with nephrotoxicity and not treated.
3	CSEAF-treated only	Given 200 $mgkg^{-1}$ b.w. of CSEAF only for 14 days.
4, 5, and 6	Pretreatment	Pretreated with CSEAF (100 and 200 $mgkg^{-1}$ b.w.) and vitamin C (200 $mgkg^{-1}$ b.w.), respectively, for 14 days prior to nephrotoxicity induction.
7, 8, and 9	Posttreatment	Nephrotoxic rats posttreated, respectively, with the fraction (100 and 200 $mgkg^{-1}$ b.w.) and vitamin C (200 $mgkg^{-1}$ b.w.) for 14 days.

Treatments were done once daily via oral intubation between 9.00 and 10.00 a.m. to minimize possible diurnal effects. A transition period of 24 h was observed between the two subsequential treatment periods in both pre- and posttreatment groups.

2.7. Serum Preparation and Kidney Isolation. Forty-eight hours after the last treatment in each case, the rats were humanely euthanized under halothane anaesthetization and blood was collected via cardiac puncture into plain sample bottles. For serum preparation, the blood was allowed to clot for 10 min and subsequently centrifuged (Beckman and Hirsch, Burlington, IO, USA) at 3,000 ×g for 15 minutes. Serum was carefully aspirated and used for kidney function tests. The rats were also immediately dissected and the kidneys were diligently harvested, blotted with clean tissue paper, cleaned of fat, and weighed and the relative kidney-body weight ratios (RKW) were evaluated. The left kidney was thereafter sliced into two portions with one of the portions homogenized in Tris-HCl buffer (0.05 mol/L Tris-HCl and 1.15% KCl, pH 7.4) for antioxidant analyses, while the other was used for histological examination.

2.8. Biochemical Analyses

2.8.1. Kidney Function Parameters. Following the procedures outlined in the assay kits, kidney function parameters were determined. Serum concentrations of creatinine, blood urea nitrogen, uric acid, potassium, sodium, and calcium were evaluated. Creatinine clearance rate (CCR) was estimated as earlier reported [22].

2.9. Antioxidant and Oxidative Stress Assays

2.9.1. Reduced Glutathione (GSH) and Oxidized Glutathione (GSSG). The procedure described by Ellman [23] was employed to determine the level of GSH in the homogenate. Briefly, 1.0 mL of the homogenate was added to 25% trichloroacetic acid (1 mL) and the precipitate was removed by centrifugation at 5,000 ×g for 10 min. Supernatant (0.1 mL) was added to 2 mL of 0.6 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) prepared in 0.2 M sodium phosphate buffer (pH 8.0). The absorbance of the yellow-colored complex was thereafter read at 420 nm and the extrapolated values from the standard calibration curve were expressed as the homogenate concentrations of GSH.

For GSSG level estimation, the described method of Hissin and Hilf [24] was adopted. The homogenate (50 μL) was mixed with 20 μL of 0.04 M *N*-ethylmaleimide (NEM) to prevent oxidation of GSH to GSSG. The mixture was subsequently incubated at room temperature for 30 min prior to consecutive addition of 0.3 M Na_2HPO_4 solution (1.68 mL) and 250 μL of DTNB reagent. The absorbance of the resulting mixture was thereafter read at 420 nm as concentration of GSSG in the homogenate expressed in nmol/mg protein.

2.10. Lipid Peroxidation Products. The homogenate levels of lipid peroxidation products (conjugated dienes, lipid hydroperoxides, and malondialdehyde) were estimated as reported by Reilly and Aust [25].

2.11. Protein Carbonyl and Advanced Oxidation Protein Product (AOPP). The method of Levine et al. [26] based on the reaction of carbonyl compounds with 2,4-dinitrophenyl hydrazine for 1 h and subsequent precipitation with 20% trichloroacetic acid was employed to determine the concentration of protein carbonyl in the renal homogenate. The released carbonyl compounds were measured spectrophotometrically at 380 nm and expressed as nmol/mg protein of the homogenate.

For the AOPP assay, the kidney homogenate (2 mL) was centrifuged at 2500 ×g for 10 min at 40°C. The resulting supernatant was thereafter added to a reaction mixture containing 50% acetic acid and 1.16 mol/L potassium iodide in phosphate buffered saline solution. The absorbance was read at 340 nm and the concentration of AOPP determined from the extrapolated standard curve of serially diluted AOPP standard solution using 500 μmol/L chloramines stock [27].

2.12. Fragmented DNA. The quantity of fragmented DNA in the kidney homogenates was determined using standard protocols [28]. In brief, kidney homogenate was centrifuged at 15,000 ×g, for 15 min at 4°C. While the supernatant was aspirated and treated with 10% trichloroacetic acid (1.50 mL), the resulting pellet was treated with 5% trichloroacetic acid (0.65 mL). The reaction mixture in each case was kept refrigerated (4°C) to precipitate overnight before centrifuging at 2500 ×g for 10 min. Each reaction mixture was subsequently boiled at 100°C for 15 min, cooled to room temperature, and further centrifuged at 2500 ×g for 5 min. Exactly 0.5 mL of the supernatant content was treated with diphenylamine reagent

(1 mL) and incubated at 37°C for 4 h. Absorbance readings for both the treated supernatant and pellet were taken at 600 nm using a spectrophotometer and the % fragmented DNA was calculated using the expression:

$$\% \text{ Fragmented DNA} = 100 \times \left(\frac{A_s}{A_s + A_p} \right), \quad (1)$$

where A_s and A_p represent absorbance of the supernatant and pellet, respectively.

2.13. Glutathione Peroxidase and Glutathione Reductase. Homogenate activities of glutathione peroxidase (GPx) and glutathione reductase (GRx) were also evaluated as per the manufacturer's instructions in the assay kits.

2.14. Superoxide Dismutase. The activity of superoxide dismutase (SOD) in the tissue homogenate was determined as outlined by Misra and Fridovich [29]. In brief, 0.2 mL of the homogenate was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate before addition of freshly prepared 0.3 mM epinephrine (0.3 mL) to commence the reaction. The change in absorbance was measured at 480 nm at 30 s intervals for 150 s. One unit of enzyme activity is defined as 50% inhibition of the rate of autooxidation of pyrogallol as determined by changes in absorbance/min at 480 nm.

2.15. Catalase. The homogenate activity of catalase (CAT) activity was evaluated adopting the method of Aebi [30]. Exactly 50 μL of the kidney homogenate was added to a cuvette containing 2 mL of phosphate buffer (pH 7.0) and 30 mM H_2O_2 (1 mL). Catalase activity was measured at 240 nm for 1 min using a spectrophotometer. The molar extinction coefficient (43.6 M/cm) of H_2O_2 was used to estimate catalase activity.

2.16. Histopathological Examination. Following previously reported standard protocol [31], the histopathological examination of the excised kidney was performed. Briefly, sliced portions of the kidney were washed in normal saline and fixed immediately in 10% buffered formalin solution for at least 24 h. They were further dehydrated with graded alcohol (50–100%) and subsequently processed in paraffin embedding using LEICA PT 1020 Automatic Tissue Processor. About 5 μm thick section of each tissue was stained with hematoxylin and eosin and observed for possible histopathological infiltrations. Microscopic features of the kidneys of CSEAF- and vitamin C-treated rats were compared with both normal and nephrotoxic control groups. Based on the degree of derangements and severity of renal damage observed, the kidney sections were further evaluated and scored by an independent histopathologist on a 0 to 4 scale as follows:

0: normal and well-preserved renal architecture;

1: proximal convoluted tubules dilatation, focal granulovacuolar epithelial cell degeneration, and granular debris in not more than 1% of the tubular lumen;

2: epithelial necrosis and desquamation involving less than 50% of cortical tubules;

3: epithelial desquamation and necrosis involving more than 50% of proximal tubules;

4: complete or almost entire tubular necrosis.

2.17. Membrane Stabilizing Activity

2.17.1. Preparation of Bovine Red Blood Cell Suspension. This was achieved following the method of Oyedapo et al. [32] with slight modification. Briefly, fresh bovine blood samples were collected into ethylenediaminetetraacetic acid (EDTA) bottles and centrifuged (Bench Centrifuge, Beckman and Hirsch, Burlington, IO, USA) at 3,000 ×g for 10 min. The supernatants (plasma and leucocytes) were carefully aspirated with a Pasteur pipette, while the packed red blood cells were washed five times with isotonic buffered solution (154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4)) and centrifuged (3000 ×g, 10 min) each time until the supernatants were clear. The resulting pellet was employed to prepare 2% (v/v) stock suspension of erythrocytes (RBC) that was subsequently used.

2.18. Hypotonic Solution-Induced Hemolysis. Earlier reported methods [32, 33] were adapted for this assay. In brief, 0.5 mL of stock erythrocyte (RBC) suspension was mixed with 4.5 mL of hypotonic solution (50 mM NaCl in 10 mM sodium phosphate buffered saline (pH 7.4)) containing 1.0 mL of either the fraction (0.25–2.0 mg/mL) or ibuprofen (standard drug (0.1 mg/mL)). For the control sample, 0.5 mL of RBCs was mixed with the hypotonic buffered saline alone. The resulting mixture in each case was incubated (20°C, 10 min) and subsequently centrifuged (3000 ×g, 10 min) prior to absorbance reading at 540 nm using a spectrophotometer (Beckman, DU 7400, USA). The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation:

$$\% \text{ inhibition of hemolysis} = 100 \times \left(\frac{A_c - A_s}{A_c} \right), \quad (2)$$

where A_c is absorbance of control (hypotonic buffered saline solution alone) and A_s is absorbance of test sample in hypotonic solution.

2.19. Statistical Analysis. Degrees of protection conferred on the DNA, kidney function parameters, and inhibition of hemolysis by the fraction were expressed as percentages. Other results were subjected to one-way analysis of variance (ANOVA) using SPSS software package for windows (Version 16, SPSS Inc., Chicago, USA) and presented as mean ± standard error of mean (SEM) of five determinations. Significant difference between the treatment means was determined at 95% confidence level using Duncan's Multiple Range Test.

3. Results

3.1. Body and Relative Organ Weight. Data obtained with respect to body weight gain revealed significant ($p < 0.05$)

Table 2: Effect of *Zea mays*, *Stigma maydis* ethyl acetate fraction on the body weight changes and relative organ weights of acetaminophen-treated rats ($n = 5$, mean ± SEM).

Treatments	Weight changes			Kidney weight (g)	RKW (g/100 g b.w.)
	Initial (g)	Final (g)	% weight gain		
Sterile placebo (control)	210.22 ± 0.90	224.01 ± 0.89	6.16[a]	1.61 ± 0.01[a]	0.72[a]
APAP treatment	220.12 ± 0.77	215.00 ± 0.99	(2.34[b])	0.97 ± 0.01[b]	0.45[b]
200 mg/kg b.w. CSEAF	200.05 ± 0.54	220.01 ± 0.86	9.07[c]	1.69 ± 0.01[a]	0.77[a]
100 mg/kg b.w. CSEAF, then APAP	212.21 ± 0.75	223.09 ± 0.76	4.88[a]	1.58 ± 0.02[a]	0.71[a]
200 mg/kg b.w. CSEAF, then APAP	206.15 ± 0.45	227.31 ± 0.69	9.31[c]	1.73 ± 0.01[a]	0.76[a]
200 mg/kg b.w. vitamin C, then APAP	215.05 ± 0.39	228.19 ± 0.50	5.76[a]	1.64 ± 0.02[a]	0.72[a]
APAP, then 100 mg/kg b.w. CSEAF	200.15 ± 0.31	205.99 ± 0.56	2.84[d]	1.49 ± 0.02[a]	0.73[a]
APAP, then 200 mg/kg b.w. CSEAF	219.02 ± 0.97	232.00 ± 0.67	5.60[a]	1.72 ± 0.01[a]	0.74[a]
APAP, then 200 mg/kg b.w. vitamin C	221.09 ± 0.75	227.23 ± 0.45	2.70[d]	1.63 ± 0.01[a]	0.72[a]

Values bearing different superscripts along the same column for each parameter are significantly different ($p < 0.05$).
Parenthesis signifies reduced value for the parameter. RKW: relative kidney-body weight.

Table 3: Effect of *Zea mays*, *Stigma maydis* ethyl acetate fraction on serum concentrations of some kidney function parameters of acetaminophen-treated rats ($n = 5$, mean ± SEM).

Treatments	Creatinine (mg/dL)	BUN (mg/dL)	Uric acid (mg/dL)	CCR (mL/min)
Sterile placebo (control)	0.63 ± 0.02[a]	15.66 ± 0.17[a]	4.09 ± 0.01[a]	10.48 ± 0.01[a]
APAP treatment	2.50 ± 0.04[b]	49.00 ± 0.26[b]	15.99 ± 0.02[b]	2.64 ± 0.01[b]
200 mg/kg b.w. CSEAF	0.62 ± 0.01[a]	14.99 ± 0.43[a]	4.10 ± 0.03[a]	10.65 ± 0.02[a]
100 mg/kg b.w. CSEAF, then APAP	0.99 ± 0.03[c]	22.11 ± 0.23[c]	8.11 ± 0.09[c]	6.67 ± 0.01[c]
200 mg/kg b.w. CSEAF, then APAP	0.57 ± 0.02[a]	15.99 ± 0.13[a]	4.51 ± 0.03[a]	11.58 ± 0.02[a]
200 mg/kg b.w. vitamin C, then APAP	0.56 ± 0.02[a]	16.00 ± 0.12[a]	4.51 ± 0.09[a]	11.79 ± 0.01[a]
APAP, then 100 mg/kg b.w. CSEAF	1.11 ± 0.03[c]	25.12 ± 0.18[c]	8.99 ± 0.06[c]	5.95 ± 0.02[c]
APAP, then 200 mg/kg b.w. CSEAF	0.63 ± 0.05[a]	15.79 ± 0.12[a]	9.01 ± 0.06[c]	10.48 ± 0.01[a]
APAP, then 200 mg/kg b.w. vitamin C	1.25 ± 0.01[c]	16.09 ± 0.23[a]	8.88 ± 0.04[c]	5.28 ± 0.01[c]

[abc]Values with different superscripts for each parameter are significantly different ($p < 0.05$). APAP: acetaminophen, CSEAF: corn silk ethyl acetate fraction, BUN: blood urea nitrogen, and CCR: estimated creatinine clearance rate.

reduction in the body weight of the nephrotoxic rats (APAP-treated) compared to control (Table 2). In contrast, when compared with the nephrotoxic group, the CSEAF pre- and posttreated groups had significantly ($p < 0.05$) higher weight gain with the effect elicited by the fraction administered at 200 mg/kg b.w. competing favorably with vitamin C. However, only marginal variation was observed in this parameter between the 200 mg/kg b.w. fraction-pretreated groups and those placed on 200 mg/kg b.w. of the fraction alone (Table 2). In addition, while there was marked reduction in the RKW of the APAP-treated animals, those of the fraction and vitamin C-administered groups were only marginally different from the normal control group (Table 2).

3.2. Kidney Function Indices. Significantly ($p < 0.05$) elevated serum levels of creatinine, urea, uric acid, sodium, and potassium were observed in the APAP-administered group when compared with the normal control (Tables 3 and 4). However, treatments with the fraction for 14 days dose-dependently and significantly ($p < 0.05$) prevented and extenuated the APAP-mediated increases in these parameters with most prominent effects elicited at the highest investigated dose in the pretreated groups. This is also consistent with the increased CCR and serum calcium levels observed in the fraction-supplemented rats as compared to the significantly ($p < 0.05$) reduced value for this parameter in the nephrotoxic rats (Tables 3 and 4). There were no evidences of nephrotoxic tendencies in the animals given 200 mg/kg b.w. of the CSEAF alone, as they compared favorably with the control for these parameters. However, the marked improvements observed in the fraction-treated animals were not evident in the satellite self-recovery group whose assayed parameters were essentially those of the nephrotoxic animals. The degree of protection conferred on the serum levels of creatinine and blood urea nitrogen by CSEAF treatments is presented in Figure 1. While treatments with 200 $mgkg^{-1}$ b.w. CSEAF compared favorably with vitamin C for overall modulation of blood urea nitrogen, it elicited a better and most prominent effect on creatinine metabolism (Figure 1).

3.3. Antioxidants and Oxidative Stress Markers

3.3.1. Nonenzymic Antioxidants and Oxidative Stress Markers. The effects of 14-day treatment with CSEAF on the nonenzymic antioxidant status and oxidative stress markers of the experimental rats are presented in Table 5 and Figure 2. The APAP-induced significant ($p < 0.05$) reduction in the level of GSH and the elevations in the levels of GSSG, protein

Table 4: Effect of *Zea mays*, *Stigma maydis* ethyl acetate fraction on serum concentrations of selected electrolytes of acetaminophen-treated rats ($n = 5$, mean ± SEM).

Treatments	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mg/dL)
Sterile placebo (control)	135.44 ± 1.35^{a}	3.50 ± 1.01^{a}	8.95 ± 1.01^{a}
APAP treatment	338.22 ± 1.65^{b}	10.11 ± 1.02^{b}	3.33 ± 1.00^{b}
200 mg/kg b.w. CSEAF	145.01 ± 1.21^{a}	3.59 ± 1.00^{a}	9.83 ± 1.02^{a}
100 mg/kg b.w. CSEAF, then APAP	198.22 ± 1.11^{c}	5.55 ± 1.02^{c}	5.99 ± 1.01^{c}
200 mg/kg b.w. CSEAF, then APAP	138.94 ± 1.26^{a}	3.75 ± 1.06^{a}	9.01 ± 1.00^{a}
200 mg/kg b.w. vitamin C, then APAP	143.12 ± 1.70^{a}	3.55 ± 1.01^{a}	9.17 ± 1.01^{a}
APAP, then 100 mg/kg b.w. CSEAF	226.21 ± 1.13^{d}	7.09 ± 1.01^{d}	6.99 ± 1.02^{d}
APAP, then 200 mg/kg b.w. CSEAF	200.12 ± 1.42^{c}	5.99 ± 1.00^{c}	8.99 ± 1.02^{a}
APAP, then 200 mg/kg b.w. vitamin C	139.01 ± 1.22^{a}	3.79 ± 1.02^{a}	9.15 ± 1.01^{a}

[abcd]Values with different superscripts for each parameter are significantly different ($p < 0.05$). APAP: acetaminophen and CSEAF: corn silk ethyl acetate fraction.

Table 5: Effect of *Zea mays*, *Stigma maydis* ethyl acetate fraction on the levels of nonenzymic antioxidant system, protein-oxidized products, and fragmented DNA of acetaminophen-treated rats ($n = 5$, mean ± SEM).

Treatment	GSH (X)	GSSG (X)	GSH/GSSG	PC (X)	AOPP (Y)	F/DNA (%)
Sterile placebo (control)	35.11 ± 1.35^{a}	0.20 ± 0.01^{a}	175.55 ± 0.20^{a}	3.72 ± 0.25^{a}	195.98 ± 1.99^{a}	10.12 ± 0.10^{a}
APAP treatment	7.56 ± 1.09^{b}	1.99 ± 0.02^{b}	3.80 ± 0.08^{b}	15.12 ± 0.22^{b}	501.23 ± 1.59^{b}	65.15 ± 0.19^{b}
200 mg/kg b.w. CSEAF	25.02 ± 1.08^{c}	0.12 ± 0.03^{c}	208.50 ± 0.32^{c}	3.66 ± 0.40^{a}	190.23 ± 1.45^{a}	11.75 ± 0.14^{a}
100 mg/kg b.w. CSEAF, then APAP	30.19 ± 1.11^{a}	0.21 ± 0.02^{a}	143.29 ± 0.19^{d}	8.00 ± 0.25^{c}	275.99 ± 1.40^{c}	30.16 ± 0.15^{c}
200 mg/kg b.w. CSEAF, then APAP	31.01 ± 1.06^{a}	0.16 ± 0.03^{a}	193.81 ± 0.15^{c}	4.67 ± 0.32^{a}	193.01 ± 1.34^{a}	12.01 ± 0.18^{a}
200 mg/kg b.w. vitamin C, then APAP	36.11 ± 1.32^{a}	0.19 ± 0.02^{a}	190.05 ± 0.17^{c}	3.89 ± 0.25^{a}	200.45 ± 1.29^{a}	11.21 ± 0.19^{a}
APAP, then 100 mg/kg b.w. CSEAF	15.99 ± 1.11^{d}	0.22 ± 0.01^{a}	72.68 ± 0.15^{e}	9.04 ± 0.22^{c}	287.32 ± 1.88^{c}	31.00 ± 0.10^{c}
APAP, then 200 mg/kg b.w. CSEAF	16.21 ± 1.00^{d}	0.19 ± 0.05^{a}	85.32 ± 0.09^{e}	9.14 ± 0.35^{c}	203.19 ± 1.25^{a}	12.09 ± 0.11^{a}
APAP, then 200 mg/kg b.w. vitamin C	15.15 ± 1.72^{d}	0.19 ± 0.05^{a}	79.74 ± 0.10^{e}	8.01 ± 0.29^{c}	212.34 ± 1.67^{a}	12.13 ± 0.15^{a}

[abcde]Values with different superscripts for each parameter are significantly different ($p < 0.05$). APAP: acetaminophen, CSEAF: corn silk ethyl acetate fraction, GSH: reduced glutathione, GSSG: peroxidized glutathione, PC: protein carbonyl, AOPP: advanced oxidation protein product, F/DNA: fragmented DNA, X: nmol mg protein^{-1}, and Y: μmol mg protein^{-1}.

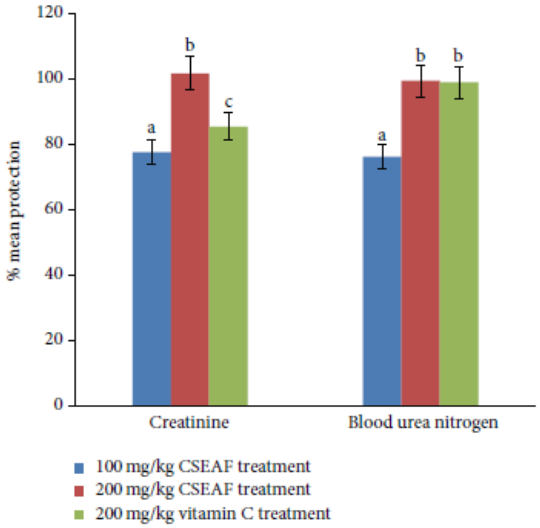

Figure 1: Mean percentage protection offered by *Zea mays*, *Stigma maydis* and vitamin C against acetaminophen-induced renal injury as assessed by serum creatinine and blood urea nitrogen. The percent protection was calculated as follows: 100 × (values of APAP treatment − values of test)/(values of APAP treatment − values of control). [abc]Bars with different superscripts for each parameter are significantly different ($p < 0.05$). CSEAF: corn silk ethyl acetate fraction.

Table 6: Effect of *Zea mays*, *Stigma maydis* ethyl acetate fraction on specific activities of enzymic antioxidant system of acetaminophen-treated rats ($n = 5$, mean ± SEM).

Treatments	Antioxidant enzymes (nmol min^{-1} $mgprotein^{-1}$)			
	SOD	Catalase	Glutathione Rx	Glutathione Px
Sterile placebo (control)	43.12 ± 0.15^{a}	33.99 ± 0.11^{a}	56.11 ± 0.45^{a}	123.15 ± 1.10^{a}
APAP treatment	10.11 ± 0.10^{b}	13.29 ± 0.12^{b}	18.11 ± 0.23^{b}	43.66 ± 1.15^{b}
200 mg/kg b.w. CSEAF	59.21 ± 0.03^{c}	45.35 ± 0.11^{c}	55.19 ± 0.20^{a}	144.11 ± 1.10^{c}
100 mg/kg b.w. CSEAF, then APAP	32.12 ± 0.11^{d}	22.76 ± 0.15^{d}	30.21 ± 0.25^{c}	102.31 ± 1.10^{d}
200 mg/kg b.w. CSEAF, then APAP	41.24 ± 0.12^{a}	32.99 ± 0.19^{a}	53.99 ± 0.23^{a}	125.03 ± 1.11^{a}
200 mg/kg b.w. vitamin C, then APAP	42.07 ± 0.21^{a}	33.09 ± 0.11^{a}	56.04 ± 0.35^{a}	100.19 ± 1.09^{d}
APAP, then 100 mg/kg b.w. CSEAF	32.11 ± 0.12^{d}	22.18 ± 0.13^{d}	31.14 ± 0.31^{c}	98.22 ± 1.15^{d}
APAP, then 200 mg/kg b.w. CSEAF	31.99 ± 0.15^{d}	31.00 ± 0.13^{a}	55.09 ± 0.26^{a}	120.11 ± 1.10^{a}
APAP, then 200 mg/kg b.w. vitamin C	30.98 ± 0.20^{d}	30.01 ± 0.12^{a}	33.04 ± 0.35^{c}	96.12 ± 1.12^{d}

^{abcd}Values with different superscripts for each parameter are significantly different ($p < 0.05$). APAP: acetaminophen, CSEAF: corn silk ethyl acetate fraction, SOD: superoxide dismutase, Rx: reductase, and Px: peroxidase.

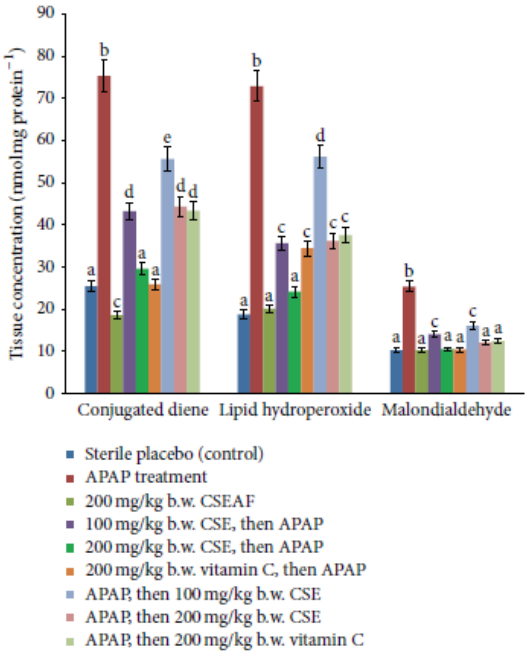

Figure 2: Effect of *Zea mays*, *Stigma maydis* ethyl acetate fraction on tissue concentrations of conjugated dienes, lipid hydroperoxides, and malondialdehyde of acetaminophen-treated rats. Values are mean ± standard error of mean (SEM) of five determinations. ^{abcde}Bars with different superscripts for each parameter are significantly different ($p < 0.05$). APAP: acetaminophen and CSEAF: corn silk ethyl acetate fraction.

carbonyls, AOPP, fragmented DNA, malondialdehyde, conjugated dienes, and lipid hydroperoxides were significantly ($p < 0.05$) and dose-dependently normalized in the CSEAF-treated animals. Although the fraction (at 200 $mgkg^{-1}$ b.w.) compared well with reference drug (vitamin C) in both pre- and posttreatment modes, better results comparable to those of the control were observed in the fraction-pretreated group. However, while other nonenzymic antioxidant parameters were not significantly altered by treatment with 200 $mgkg^{-1}$ b.w. of CSEAF alone, the homogenate level of GSH was significantly increased when compared with the control (Table 5).

3.3.2. Enzymic Antioxidants. Kidney homogenate activities of GRx, GPx, SOD, and CAT were significantly ($p < 0.05$) induced by CSEAF in a concentration-dependent manner in the two treatment models. These inductions markedly ($p < 0.05$) improved the observed APAP-mediated reduction in their activities and at the highest investigated dose the effect compared well with that of vitamin C (Table 6).

3.4. Histopathological Investigation. Macroscopic examination of kidneys from the control group revealed that they were essentially normal with characteristic fine texture and dark maroon appearance. While kidneys from the fraction-administered animals showed mild spots of brown color changes, those of the APAP-intoxicated animals revealed color changes from maroon to brown with characteristic uneven texture. Detailed histoarchitectural examination of the kidney sections of the control and 200 $mgkg^{-1}$ b.w. CSEAF groups showed no histological derangements, as evidenced by the normal and well-preserved renal architecture with characteristic intact glomeruli and tubules (Figures 3(a) and 3(c)). In contrast, kidney sections from the APAP-administered group exhibited altered architecture with extensive destruction of glomeruli and tubular structures, as demonstrated by marked necrotic areas (Figure 3(b)). Hypercellularity in Bowman's capsule indicating leukocyte infiltration, glomerular atrophy, and dilated proximal tubules with loss of the cellular boundary and brush border were also evident (Figure 3(b)). Treatment with CSEAF at the investigated doses in the pre- and posttreatment modes showed dose-dependent protective and ameliorative capabilities in the kidneys that compared favorably with that of vitamin C (Figures 3(d)–3(i)). This was evident by the less severe tubular and glomerular damage, with the best and most prominent effect

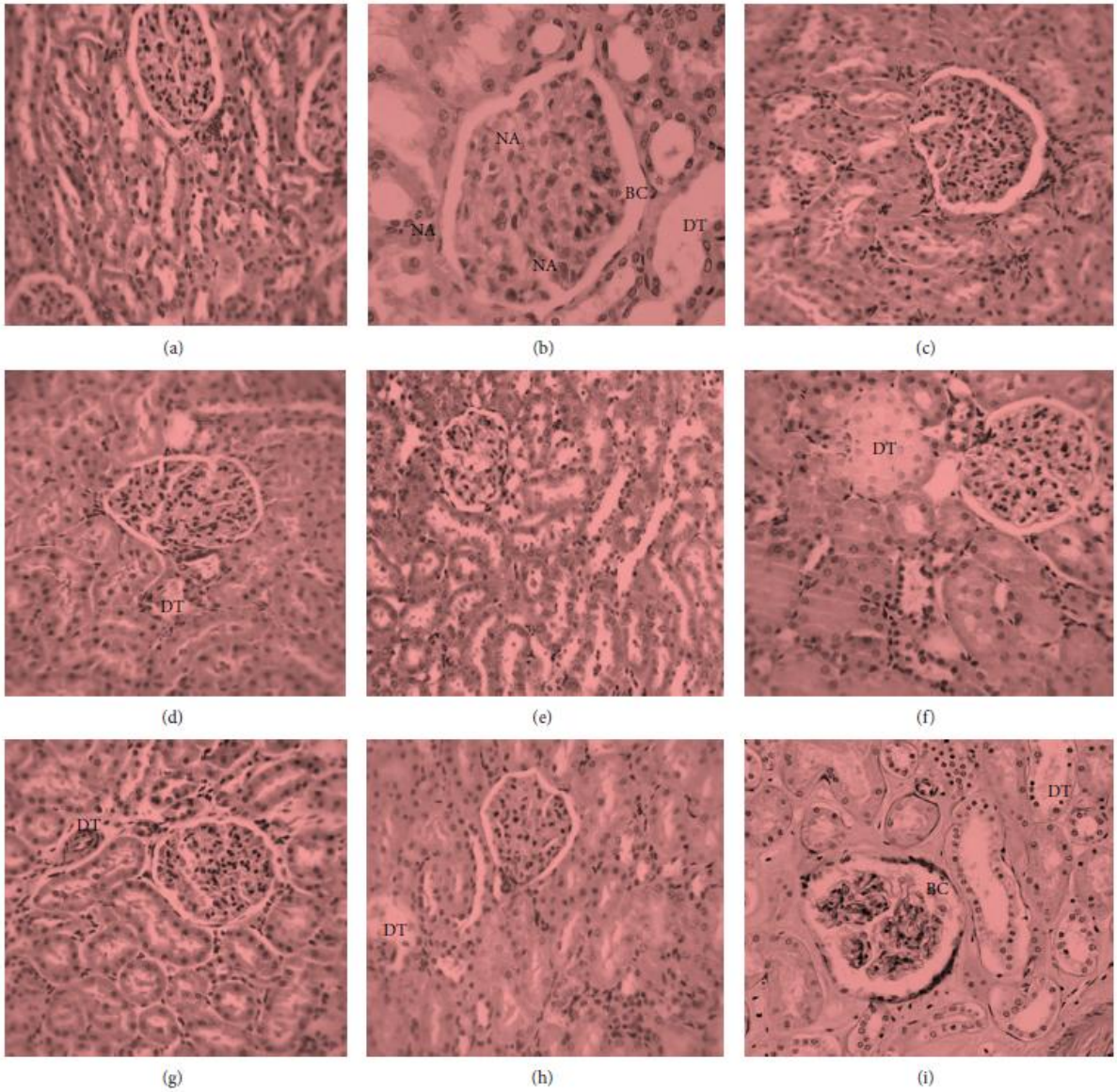

FIGURE 3: Kidney micrographs (×400, hematoxylin and eosin stained) of (a) control rat, (b) nephrotoxic rat, (c) CSEAF (200 mg/kg b.w.) treated rat, (d) nephrotoxic rat pretreated with CSEAF (100 mg/kg b.w.), (e) nephrotoxic rat pretreated with CSEAF (200 mg/kg b.w.), (f) nephrotoxic rat pretreated with vitamin C (200 mg/kg b.w.), (g) nephrotoxic rat posttreated with CSEAF (100 mg/kg b.w.), (h) nephrotoxic rat posttreated with CSEAF (200 mg/kg b.w.), and (i) nephrotoxic rat posttreated with vitamin C (200 mg/kg b.w.). CSEAF: corn silk ethyl acetate fraction, BC: Bowman's capsule showing leukocyte infiltration, DT: dilated proximal tubule, and NA: necrotic area.

observed in the 200 $mgkg^{-1}$ b.w. dose treated groups (Figures 3(e) and 3(h)). Furthermore, histopathological scoring of the kidney sections of CSEAF-treated groups showed that the obvious epithelial desquamation and tubular necrosis present in the kidney sections of the APAP-intoxicated animals were significantly and dose-dependently assuaged in a manner comparable to the vitamin C-treated animals (Table 7).

3.5. Membrane Stabilization. The result of membrane stabilizing activity of CSEAF is shown in Figure 4. Treatment with the fraction dose-dependently protected bovine RBC against hypotonic solution-induced infiltrations. The elicited effect at a 2 mg/mL dose of the fraction compared favorably with ibuprofen (0.1 mg/mL), standard drug used in this study.

Table 7: Histopathological grading of liver tissue sections of *Zea mays*, *Stigma maydis* ethyl acetate fraction-treated animals.

Treatments	Scores				
	0	1	2	3	4
Control	(5)	(0)	(0)	(0)	(0)
APAP treatment	(0)	(0)	(1)	(3)	(1)
200 mg/kg of CSEAF	(5)	(0)	(0)	(0)	(0)
100 mg/kg b.w. CSEAF, then APAP	(3)	(2)	(0)	(0)	(0)
200 mg/kg b.w. CSEAF, then APAP	(5)	(0)	(0)	(0)	(0)
200 mg/kg b.w. vitamin C, then APAP	(3)	(2)	(0)	(0)	(0)
APAP, then 100 mg/kg b.w. CSEAF	(2)	(2)	(1)	(0)	(0)
APAP, then 200 mg/kg b.w. CSEAF	(4)	(1)	(0)	(0)	(0)
APAP, then 200 mg/kg b.w. vitamin C	(3)	(2)	(0)	(0)	(0)

($n = 5$; figure in parenthesis represents number of rats affected in the group). APAP: acetaminophen and CSEAF: corn silk ethyl acetate fraction.

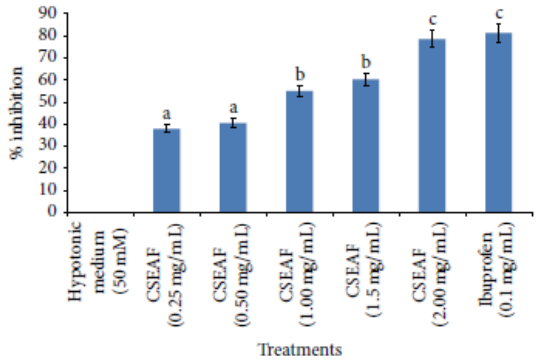

Figure 4: Effect of *Zea mays*, *Stigma maydis* ethyl acetate fraction on hypotonic solution-induced hemolysis of bovine erythrocyte membrane. Values are mean ± standard error of mean (SEM) of three determinations. [abc]Bars with different superscripts for the parameter are significantly different ($p < 0.05$). CSEAF: corn silk ethyl acetate fraction.

4. Discussion

Acetaminophen-mediated oxidative nephrotoxicity has been well documented and is characterized by morphologic and functional evidence of proximal tubular injury in humans and experimental animals [34, 35]. Acute and chronic renal failures in the course of therapeutic APAP administration have also been described in alcoholics and case-control studies [36]. Although molecular studies have linked APAP renal proximal tubular damage to translocation of GADD153 (growth arrest- and DNA damage-inducible gene 153) to the nucleus and subsequent proteolysis of caspase-12 [37], involvement of a reactive intermediate metabolite, N-acetyl-*p*-benzoquinone imine (NAPQI), cannot be excluded from renal damage [21]. NAPQI arylates selenium binding protein and glutamine synthetase in the S3 segment of the proximal tubule with consequential depletion of GSH [38]. This subsequently results in autooxidation of renal macromolecules (lipids, proteins, and DNA) with associated tubular cell necrosis. Tubular cell loss is an important feature of both acute renal failure and chronic renal disease [35] and is accompanied by concomitant increased serum concentrations of creatinine, urea, uric acid, and electrolyte imbalance. Creatinine, urea, and uric acid are major catabolic products of muscle, protein, and purine metabolism, respectively, and their serum concentrations give clues to the functional capacity of the nephrons at the glomerular and tubular levels [39]. These waste products (urea and creatinine) are passed into the blood stream for removal by the kidneys and their increased level in blood is a direct indication of renal dysfunction [40].

In this study, the increased serum concentrations of creatinine, blood urea nitrogen, and uric acid coupled with the attenuated CCR in the APAP-intoxicated animals may be indicative of renal injury and cell necrosis resulting from formation of NAPQI in excess of GSH detoxification ability. This is consistent with previous studies [21, 34], where APAP administration proved toxic to renal tubular cells. However, the significant and dose-dependent reversal in the levels of these parameters in the CSEAF-treated rats suggests that CSEAF was able to prevent or extenuate the deleterious influence of APAP. This observation also indicates that CSEAF at the investigated doses could preserve renal functions and delay progression of renal pathological conditions.

The most common cause of electrolyte imbalance or disturbance is associated with renal failure [40]. Calcium ions play a vital role in muscle contraction and serves as an intracellular second messenger for hormones. Hypocalcaemia is most commonly found in terminal stages of chronic generalized renal failure. Hence, the low level of calcium ion in the serum of APAP-administered rats may be associated with derangement of renal function resulting from interference with ions transport across the renal tubules [41]. The improvement observed in the calcium concentration in CSEAF-treated animals is a probable indication of its nephroprotective tendency. Sodium and potassium are the major extracellular and intracellular cations, respectively, in a living system. Sodium regulates the total amount of water in the body and its transmission across cells plays roles critical to body functions while adequate levels of potassium ions are essential for normal cell function. Many processes in the body, especially in the nervous system, muscles, and renal selective reabsorption, require electrical signals for communication. The movement of these ions is critical in generation of electrical signals [42]. In this study, the concentration-related significant normalization of APAP-mediated increases in serum levels of sodium and potassium ions in the fraction-administered rats is suggestive of its ability to maintain the levels of water and sodium at optimum equilibrium, thereby enhancing selective reabsorption capability of the nephron. Since membrane integrity is vital to signalling, the effect observed may also be due to the potential of the fraction to maintain membrane integrity of the kidney cells. This agrees with a previous report [43] where treatment with plant extracts reversed an acetaminophen-induced electrolyte imbalance in experimental animals.

Changes in the body weight of animals have been used to predict the nature and extent of drug-induced toxicity

and may give important information on their overall health status [44]. It could also dictate the impact of the drug on the overall growth and developmental metabolism of the animals. Therefore, the observed reduction in the body weight of the APAP-treated animals may imply possible impairment in growth-linked metabolic processes. That the CSEAF-administered animals had relatively normal and marginal body weight gain is an obvious indication of the tendency of the fraction to aid normal metabolism and sustain growth and developmental mechanisms in the animals. This could be attributed to enhanced appetite in the animals that may be ascribed to the phytoconstituents in CSEAF as previously reported for the crude aqueous extract of CS [45]. The effect of fraction on the body weight of the animals not only was further supported by the significant weight gain in the rats placed on 200 $mgkg^{-1}$ b.w. dose of CSEAF alone, but also lent credence to the nonnephrotoxic effect of the fraction. This observation is consistent with the finding of Abdul Hamid et al. [46], where administration of standardized leaf extract of *Zingiber zerumbet* was linked to both mean body weight gain and nephroprotective effects in experimental animals. The relative organ weight in pharmacological studies is imperative to understanding crucial treatment-induced organ weight variations in animals [47]. While an increase in the relative organ weight may depict either inflammation or increased secretory ability of the organ, a reduction could be informative of cellular shrinkage. In addition to defining toxicity as pathological changes observed in the organs of interest, the relative organ weight could also be suggestive of organ swelling, atrophy, or hypertrophy [48]. In this study, the sustained kidney weights in all the fraction-administered animals relative to the significantly reduced kidney weight in the APAP-intoxicated rats could imply that the constriction of renal tubular cells caused by the ravaging oxidative insults of APAP was well prevented or extenuated by the fraction. This suggests that CSEAF could protect renal tubular cells against oxidative routs at the tested doses and was closely supported by the histopathological findings where the kidney sections of fraction-treated animals revealed distinct and well-preserved histoarchitectural features.

Studies have implicated NAPQI formation and oxidative stress in APAP-induced nephrotoxicity [49, 50], and their overwhelming effects result in significantly impaired and insufficient levels of both enzymic and nonenzymic antioxidant defense mechanisms in the body [51]. Hence, the observed reduction in the renal level of GSH of APAP-intoxicated rats in this study might be due to depletion of GPx and GR, as well as formation of NAPQI that exceeds GSH detoxification capacity [49]. Also, the APAP-mediated elevation in GSSG levels may be ascribed to either GSH autooxidation or its mobilization towards GPx formation. The reduction in the ratio of GSH to GSSG caused by APAP administration reveals possible oxidative damage on the renal tubular cells. However, the significantly and dose-dependently increased GSH level coupled with the corresponding high GSH: GSSG ratio and low GSSG levels in the kidneys of the CSEAF-treated rats is suggestive of the inherent antioxidative effect of the fraction and further supports that it offered a considerable level of nephroprotection. Palani et al. [52] also reported similar improvement on the nonenzymic antioxidant status in APAP-intoxicated rats following treatment with *Pimpinella tirupatiensis* ethanolic extract.

An elevated level of malondialdehyde in tissues is an obvious indication of cellular damage due to lipid peroxidation resulting from malfunctioning of the antioxidant defense system [53]. Furthermore, APAP has been linked with lipid peroxidation and may facilitate elevated level of peroxidized products (conjugated dienes, lipid hydroperoxides, and malondialdehydes) in nephrotoxicity [46]. Therefore, the significant elevation in the levels of these products could be indicative of uncontrolled oxidative attacks of APAP's reactive metabolites and ROS on membrane-bound lipids. This might have disrupted membrane fluidity as well as modified and inflicted functional loss on the proteins and DNA of the renal tubular cells. The attenuation of APAP-enhanced increases in these peroxidative products by the CSEAF could mean that it was able to offer a considerable level of protection to the renal membrane lipids. This may be adduced to the ability of the CSEAF to aid with detoxification of reactive metabolites, which could have initiated and propagated peroxidation of membrane-bound polyunsaturated lipids of the tubular cells. It may also suggest that the fraction is rich in phytonutrients capable of stabilizing the cellular membranes of renal tubules against oxidative onslaughts of APAP. In the same vein, unguided influence of ROS can trigger protein autooxidation with consequential formation of protein carbonyls and AOPP [54]. These protein oxidative products have been employed as invaluable markers to ascertain the degree of oxidant-mediated protein damage in cells [55]. In this study, the significant increase in the tissue concentrations of these markers in the nephrotoxic animals relative to the fraction-treated rats could be attributable to the capacity of NAPQI to arylate selenium binding protein and glutamine synthetase in the S3 segment of the proximal tubule, thereby facilitating autooxidation of renal proteins [38]. This may also imply covalent binding of NAPQI to mitochondrial proteins which resultantly induced nitrate ion formation that incapacitates the Ca^{2+} pump of the renal membrane. Incapacitation of the Ca^{2+} pump further hinders mitochondrial function and ATP production which could have enhanced the observed elevated levels of AOPP and carbonyl product [54, 55]. The pre- and posttreatments with CSEAF reversed this trend which is a further attestation to its possible potential to incapacitate NAPQI, nitrate ions, and other reactive metabolites through enhancement and fortification of the antioxidant defense mechanisms of the renal tubular cells. Our findings are in agreement with previous assertions [46] that linked restoration to normal of APAP-mediated elevated levels of renal protein-oxidized products with seven-day treatment with ethyl acetate extract of *Zingiber zerumbet*.

Similar to protein-oxidized products, calcium ion accumulation and hydroxyl radical mediated oxidative damage are important events in the pathogenesis of DNA fragmentation. These events promote either tissue necrosis

or carcinogenesis, which subsequently results in cell death [56]. Thus, the significantly increased level of fragmented DNA in the kidneys of APAP-intoxicated rat is indicative of either genotoxicity or probable initiation of carcinogenesis. Jaeschke and Bajt [57] have earlier reported a similar increase in the level of damaged DNA due to APAP administration. The CSEAF-enhanced attenuation in the level of fragmented DNA in the kidneys of APAP-treated rats is a tenable fact that the fraction is endowed with antioxidative and antigenotoxic attributes. The CSEAF might have mimicked an antigenotoxic agent, thereby facilitating DNA repair or synthesis system [58].

During renal damage, superoxide radicals are formed at the site of injury and if their accumulation exceeds the body's antioxidant capacity it could overwhelm the defensive activities of SOD and CAT, thereby aggravating the severity of existing damage [52]. Therefore, exogenous optimization of preventive (CAT, GPx) and chain-breaking (SOD, GRx) enzymic antioxidants that subsequently increase cellular GSH content is not only imperative to annihilating catastrophic influence of free radicals/ROS but also crucial to stalling ravaging impacts of drug-induced oxidative stress. In this study, the decreased tissue activities of the assayed antioxidant enzymes (SOD, CAT, GRx, and GPx) could be due to their excessive mobilization towards detoxification of NAPQI and ROS during APAP-induced nephrotoxicity. This might have led to haphazard oxidative attack on cellular macromolecules that consequently results in necrosis [59]. This report is in agreement with the findings of Aslam et al. [60] and Kadir et al. [61], where the authors also studied drug-mediated renal damage in rats. They noted similar reductions in the activities of ROS detoxifying enzymes that were associated with the formation of reactive metabolites and ROS. Thus, the dose-dependent auspicious reversion of the APAP-induced reduction in the activities of these detoxifying enzymes by the CSEAF is indicative of its antioxidative activity. This could either be attributed to the tendency of the fraction to scavenge NAPQI and ROS or enhance tissue activity of ROS detoxifying enzymes.

In addition to complementing biochemical analyses, histopathological examination of kidney sections may provide invaluable information on how pharmacologically potent an agent is against renal damage. The significant alterations in glomerular structure, the thickening of the glomerular basement membrane, widening of the filtration slits, basal infolding patterns with the presence of cytoplasmic vacuolation, and the increase in collagen deposition around the tubules as observed in the kidney sections of APAP-intoxicated rats could be responsible for their impaired renal function. The consequently decreased glomerular filtration rate with associated elevated serum levels of urea and creatinine was evident in the present study and agreed with a previous report [62]. However, the apparently repudiated oxidative threats inflicted by APAP on the architectural features of renal tubular cells in the fraction pre- and posttreated rats suggest that the CSEAF offered a significant degree of protection and stabilization on the overall histoarchitectural integrity of the kidneys. In fact, the preservation of the tubular cells and architectural organization of some of the kidney sections was almost completely normalized with an increasing number of viable cells. The effects noticed compared favorably with that of vitamin C and are consistent with the results of the biochemical investigations performed in this study. Our observations agree with previous reports [21, 46, 63], where recovery from APAP-mediated derangements towards normalization of serum kidney function parameters and renal histological architecture was attributed to treatment with plant extracts.

During inflammatory events, lysosomal enzymes and hydrolytic constituents are released from phagocytes into the extracellular space. This release consequently inflicts injuries on the surrounding organelles and tissues as well as aggravating the severity of any existing infection [33]. The exposure of an erythrocyte to injurious substances like hypotonic medium and heat results in lysis of its membrane with associated hemolysis and autooxidation of hemoglobin. The autooxidation and hemolysis are direct consequences of the vulnerability of the cells to secondary insults of free radical chain reactions [64]. This hemolytic effect is closely linked to excessive accumulation of fluid within the cell which consequently results in membrane disorientation and disruption. This notion is consistent with the observation that the breakdown of biomembranes generates free radicals that invariably enhance cellular damage as normally observed in renal tubular oxidative damage [61]. Medicinal plants with good antioxidative and anti-inflammatory attributes have been reported to provide succor either by annihilating the ravaging activity of lysosomal enzymes or by enhanced stabilization of biomembranes via maintenance of membrane fluidity and ion gradients [32, 65]. This was evidently displayed in this study with the CSEAF at the highest investigated dose conferring a membrane stabilization potential of 78.55% against hypotonic solution infiltration on bovine erythrocytes. This may be ascribed to either the capability of the fraction to bind tenaciously to the erythrocyte membranes with concomitant prevention of deleterious assaults of the hypotonic solution or its potential in promoting dispersion by mutual repulsion of charges involved in the hemolysis of RBCs. While studies have shown flavonoids to exert stabilizing effects on lysosomes [33], tannin and saponins have been reported as being capable of binding cations, thereby stabilizing the erythrocyte membrane [66]. In addition to the various antioxidative phytoconstituents revealed by GC-MS analysis of CS, alkaloids, sterols, phenols, tannins, flavonoids, and saponins have also been qualitatively and quantitatively evaluated in its crude aqueous extract [67]. Therefore, the remarkable RBC membrane stabilization activity with good protection against hypotonic solution-induced lysis elicited by the CSEAF in this study may be attributed to the presence of these phytochemicals. This might have also enhanced restoration to normal of APAP-perturbed alterations in the assayed biochemical indices and preserved the membrane integrity of renal tubular cells as evidently revealed in both pre- and posttreatment groups in this study.

5. Conclusion

The reduction of oxidative onslaughts posed by APAP via treatments with the ethyl acetate fraction of corn silk is a

manifestation of its capabilities to preserve renal function and delay progression of renal pathological conditions to end stage disease/death. The engendered nephroprotective effect by the fraction could, in rats, be ascribed to its antioxidative and membrane stabilization potential. This is achieved by facilitating detoxification of APAP-mediated nephrotoxicity via induction of ROS detoxifying enzymes, thereby stalling autooxidation of cellular macromolecules and renal tubular damage. Though the effects were prominently exhibited in the fraction-pretreated groups, the overall effects elicited in both treatment groups were remarkable and indicative of an excellent candidate for the management of drug-induced renal oxidative disorders.

Competing Interests

The authors declare that there are no competing interests.

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CHAPTER EIGHT

GENERAL DISCUSSION AND RECOMMENDATIONS

The entire thesis was presented at the 2016 Three Minutes Thesis Competition of University of the Free State, South Africa and adjudged the best (winner) in the Ph.D category

GENERAL DISCUSSION

The use of herbs is staging a comeback and a global herbal renaissance is underway. Today, the herbal products symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been valued for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while (Joy *et al.*, 2001). However, the reliance on synthetics is gradually fading and people are returning to the naturals with expectations of improved safety and security (Joy *et al.*, 2001).

Africa has expansive biodiversity and subsequently a long and impressive list of medicinal plants of which considerable numbers have proven benefits in providing preventive and palliative measures against kidney disorders (Adeneye *et al.*, 2008; Olowokudejo *et al.*, 2008; Kadiri *et al.*, 2015). While some have been demonstrated to have therapeutic efficacy on drug-induced nephrotoxicity and oxidative stress, others have reported pharmacological significance in annihilating oxidative insults on renal tubular cells and preserving overall kidney functions (Ugochukwu and Cobourne, 2003; Musabayane *et al.*, 2007; Al-Qattan *et al.*, 2008). *Zea mays* L. (Poaceae), *Stigma maydis* is one of the several herbs that have been routinely advocated as having the capability to improve renal function. Its other pharmacological importance have also been well studied in other continents besides Africa (Grases *et al.*, 1993; Guevera *et al.*, 2000; Maksimović and Kovacevic, 2003; Kim *et al.*, 2004; Farsi *et al.*, 2008; Zhao *et al.*, 2012).

Regrettably in Africa, especially in Nigeria and South Africa, where corn is the main staple food, CS still remains underutilized and its therapeutic relevance is hugely unexploited. Hence, this study was conceptualized to enrich biochemical and pharmacological data of CSE via *in vitro* (cytotoxicity, anti-nephrolithiasis, antioxidant, membrane stabilization, nephroprotective) and *in*

vivo (toxicological, antioxidant, hepatoprotective, nephroprotective) experimental models. The results from these experiments were hoped to lend scientific credence to the ethnomedicinal significance of CS in the African context and general communities where corn remains a major staple food. In addition, CS as a mere waste product is anticipated to assist less privileged communities with health and wealth.

Nephroprotective plants in Nigeria and South Africa

From the systematic review of medicinal plants used in the treatment of renal disorders in Nigeria and South Africa, it was revealed that phytotherapy has gained prominence and is being employed to protect renal functions and delay progression of renal pathological conditions into advanced stages where the last resort is renal replacement therapy (Chapter 2). In fact, Phytotherapists are currently not only interested in developing relatively safe, more affordable, easily accessible and potent nephroprotective formulations but also increasing awareness on the prevalence of the disease and educating the populace on probable preventive measures. Furthermore, efforts at scientifically elucidating the pharmacological efficacy of the identified nephroprotective plants yet to be validated must be intensified through informed expert opinions. Although, the concept of phytonephroprotection is just emerging in South Africa, evidences have supported its application in complementary and alternative systems of medicine in Nigeria (Chapter 2). To this end, continuous search for auspicious nephroprotective plants that could potentially be excellent candidatures to develop new lead drugs to manage and treat renal disorders is imperative.

Cytotoxicity, nephroprotective, and anti-nephrolithiasis potential of Z. mays L., S. maydis

Following the ethical and scientific frets on the use of animals, many *in vitro* techniques for animal toxicity and pharmacological testing were developed and have gained global regulatory acceptance. They have since remained suitable alternatives to whole animal tests. One of the most routinely adopted approaches in this respect utilizes cultured cell lines (Eun-Kee *et al.*, 2007; Soumen *et al.*, 2016). In this study, HEK293 cells were used and it was obvious that the cells were sensitive to treatments with the extract of CS and the degree of sensitivity was concentration dependent. Judging by the CC_{50} value (435.23 µg/mL), it may be inferred that CSE was well tolerated by the cells and holds promising therapeutic and pharmacological potentials (Chapter 3).

Also, the decreased number of viable cells following 24 h exposure to APAP could suggest epithelia injury and cell necrosis resulting from the generation of free radicals. This observation agrees with the report of Lijuan *et al.* (2007), where APAP treatment proved lethal to established cell lines. However, the significant and concentration-related increase in the population of viable and metabolically active cells in the CSE-treated groups may be indicative of the capability of the extract to extenuate the toxic influence of APAP on the cells. This assertion also suggests that the extract at its pharmacological regimens may preserve renal functions and protect against renal pathological conditions. The effect elicited by the extract compared favourably with that of vitamin C and is consistent with the results of the viability tests. In light of the foregoing, the overall effects elicited by CSE as a nephroprotective candidate against APAP toxicity on HEK293 cells may be adduced to its antioxidative properties which were effective in scavenging H_2O_2, hydroxyl, and lipid peroxidizing radicals in this study (Table 1, Chapter 3). This may also be attributed to its membrane stabilization potential. Interestingly, the *in vitro* ability of *Z. mays*,

S. maydis in stabilizing bovine erythrocyte membranes has just been reported (Sabiu *et al.*, 2016a).

Similarly in the anti-nephrolithiasis assay, CaOx crystals were formed from the incubation of $CaCl_2$ and $Na_2C_2O_4$ solutions and their presence were confirmed by the turbid nature of the resulting solutions. The degree of turbidity was notably reduced following treatment with CSE. This was evidently shown with the extract at the highest investigated dose conferring a nucleation inhibitory potential of 93.41% against crystals formation (Table 5, Chapter 3). In view of this, it could be logically inferred that the extract is rich in phytoconstituents capable of inhibiting reactions or processes leading to formation of CaOx crystals. Interestingly, in addition to the saponin content of CS quantified in this study (Table 4, Chapter 3), it has also been reported to be rich in pharmacologically important phenolics (Sabiu *et al.*, 2016b), and these might also contribute to the observed significant effects in this study.

Antioxidant and carbohydrate enzymes (α-amylase and α-glucosidase) inhibitory potentials of Z. mays* L., *S. maydis

This study demonstrated significant inhibitory activity of the CS extract on α-amylase and α-glucosidase. The results revealed that CSE has a significant potent and moderate inhibitory activity at IC_{50} 0.93 mg/mL and 5.89 mg/mL respectively for α-glucosidase and α-amylase when compared with the standard acarbose (Table 4, Chapter 4). Furthermore, the respective uncompetitive and non-competitive nature of α-glucosidase and α-amylase inhibitory activity by the extract (Figures 6 and 8, Chapter 4) suggests that the bioactive principles in the extract either assuages substrate level which facilitate their binding and subsequent inhibition of α-glucosidase or bind to a site other than the active site of α-amylase/α-amylase-substrate complex and in so doing perhaps interferes with the action of both. The presence of alkaloids, phenols, tannins,

flavonoids, saponins and sterols in CS (Tables 1 and 2, Chapter 4), which are known for their antioxidant activity (Figures 1-4, Chapter 4) may be a justifiable reason for its observed hypoglycaemic potential in this study.

Toxicological implications of treatment with Z. mays L., S. maydis

In this study, the fraction of CS at a single oral dose of 5000 mg/kg had no treatment-related adverse effect on the tested animals within the 14 days observation period. This is suggestive of its non-acute toxicity effect and is indicative that its median lethal dose (LD_{50}) is greater than 5000 mg/kg.

Similarly, continuous 28 days daily dose treatment with graded doses (50, 100, 200 and 500 mg/kg b.w.) of the fraction also elicited no clinical signs of morbidity, toxicity or mortality across all the treatment groups. However, the body weight gained by the fraction-administered animals relative to the normal control could mean that the fraction enhanced normal metabolism and boost the growth and developmental mechanisms in the animals. Also, except for the significantly increased white blood cells at the 200 and 500 mg/kg doses of the fraction (which depict immune system boost and enhanced vascular permeability) as well as the dose-relatedly increased platelet counts (which may be indicative of its capability to promote thrombopoiesis, repair the minute vascular damage and considerably manage thrombocytopenia in animals (Geddis, 2013), other hematopoietic and biochemistry parameters were not affected by treatment with the fraction (Chapter 5). This was well supported by the essentially preserved colour and texture of the studied organs (heart, kidney and liver) as well as the absence of any observed treatment-induced infiltrations, inflammations and derangements as revealed by the detailed microscopic observations of the organs from fraction-administered groups. Furthermore, the significantly increased locomotion elicited by the 200 and 500 mg/kg doses of fraction (Table 7,

Chapter 5) coupled with immediate moments of excitement and improved physical activities following administration of the fraction across all treatment groups relative to normal control is implicative of its potential favourable influence on the animals. However, it is imperative to verify if the fraction has constituents that can significantly affect the locomotion of the animal beyond the 5 min period of observation as performed in this study. Such test will help to predict if these substances are able to reach the central nervous system via the blood brain barrier and thus, will be considered as either having adverse effects or toxic to the animals. Absence of such effects ultimately signifies safety of its use. This is a potential study for the future.

Capability of Z. mays L., S. maydis to prevent and ameliorate APAP-induced oxidative hepatic damage

The release of cytosolic AST, ALT, ALP and GGT into the systemic circulation and subsequent measurement can be used to assess the extent of drug-induced hepatotoxicity (Jaeschke *et al.*, 2003). In the present study, the elevated activities of these marker enzymes in the APAP-intoxicated rats may be indicative of liver damage and cell necrosis resulting from the formation of NAPQI in excess of GSH detoxification capacity. However, the significantly and dose-dependently reduced specific activity of these enzymes in rats treated with CS extract suggests that it was able to prevent and ameliorate the ravaging effects of APAP. This observation is also consistent with the effect of CS on other parameters investigated and is indicative of the hepatoprotective potential of the extract at the investigated doses (Chapter 6). Generally, the attributes elicited by the CS extract in this study might be adduced to its phytoconstituents with known antioxidative activity as revealed by GC-MS analysis (Figure 3, Chapter 6).

Consequent upon the findings from this experiment, the probable mechanism of antioxidative and hepatoprotective capabilities of CS was proposed to involve induction and optimization of

preventive (catalase, glutathione peroxidase) and chain-breaking (superoxide dismutase, glutathione reductase) antioxidants that subsequently increase cellular GSH content, annihilate NAPQI and effectively scavenge reactive oxygen species (O_2^-, OH^-) (Figure 4, Chapter 6).

Membrane stabilization and nephroprotective activities of Z. mays L., S. maydis in APAP-mediated oxidative renal injury

Creatinine, urea and urate are major catabolic products of muscle, protein and purine metabolism, respectively and their serum concentrations give information on the functional capacity of the nephrons at the glomerular and tubular levels (Yakubu *et al.*, 2003). These waste products (urea and creatinine) are passed into the blood stream for removal by the kidneys and their increased level in blood is a direct indication of renal dysfunction (Oloyede and Sunmonu, 2009). In the present study, the increased serum concentrations of creatinine, blood urea nitrogen and uric acid coupled with the attenuated creatinine clearance rate in the APAP-intoxicated animals may be indicative of renal injury and cell necrosis resulting from formation of NAPQI in excess of GSH detoxification ability. The subsequent significant and dose-dependent reversal in the levels of these parameters in the CS-treated rats suggests that CS was able to prevent or ameliorate the deleterious effect of APAP (Table 2, Chapter 7). This observation may be attributed to the membrane stabilization (Figure 4, Chapter 7) and antioxidative activities (Tables 4 and 5, Chapter 7) of CS. Overall, the results from this study presents CS as being able to preserve renal functions and delay progression of renal pathological conditions.

Conclusions and Recommendations

- Healing with medicinal plants is as old as mankind, and a significant proportion of the world's population still seek exclusive healthcare in traditional systems of medicine;
- African traditional medicine may be considered the oldest, and perhaps the most assorted of all therapeutic systems;
- Currently, over 1 million people in the world are living on either of the renal replacement therapies (RRT) and the case in sub-Sahara African countries like Nigeria and South Africa is not any better than the worldwide trend;
- Phytotherapy has emerged and is being employed to protect renal functions and delay progression of renal pathological conditions into end episodes where the last resort is RRT;
- The concept of phytonephroprotection is just emerging in South Africa, scientific evidences have supported its application in complementary and alternative systems of medicine in Nigeria;
- In spite of the large cultivation of *Z. mays* in Africa, the pharmacological potentials of its *Z. mays, S. maydis* is still hugely untapped;
- This study enriches the biochemical and pharmacological information on the ethnomedicinal activities of *Z. mays, S. maydis* in oxidative nephropathy;
- Our findings revealed that:

1. *Z. mays, S. maydis* is endowed with diverse pharmacological attributes;

2. it is well tolerated by HEK293 cells and with significant anti-nephrolithiasis potential;

3. it elicited respective non-competitive and uncompetitive inhibitory effects on α-amylase and α-glucosidase as its hypoglycaemic mechanism of action;

4. it possesses significant antioxidant and reactive oxygen species scavenging activities;

5. it can boost immunity and may be pharmacologically labelled/classified as non-toxic and practically safe. However, its locomotion and exploratory potential still needs further assessment;

6. it can prevent or attenuate hepatic oxidative injuries via optimization of antioxidant defence systems;

7. it can prevent progression of renal pathological conditions and preserve kidney functions through membrane stabilization and fortification of antioxidant defence mechanisms.

- Despite the immense health benefits of *Z. mays*, *S. maydis* and its abundance in Africa, it is still regarded as a waste product of corn cultivation and its therapeutic significance is largely underutilized.
- In view of this, it is recommended that the report of this study be disseminated to communities where corn remains the major staple food in Nigeria, South Africa, the entire African continent and the world at large.
- Focus on *Z. mays*, *S. maydis* is a probable way of creating health/wealth from 'waste'.

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Appendices

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Appendices

1.0. Standard operating procedure in cell/tissue culture laboratory

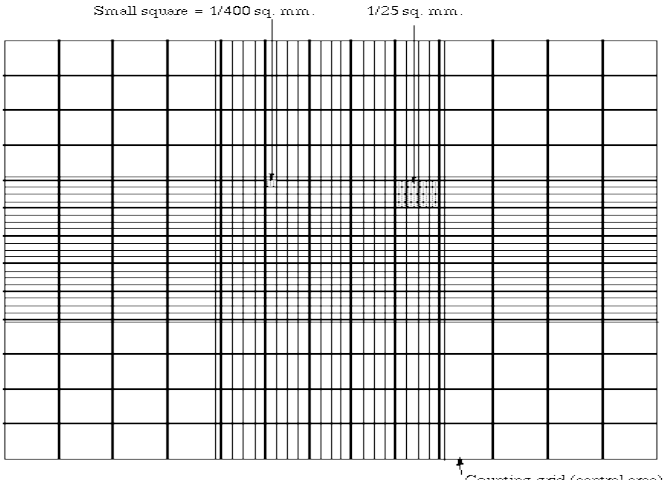
- Laboratory coats must be worn at all times. These coats must not be worn outside of the laboratory. Upon exiting, the coats should be removed and hands must be properly washed
- Gloves must be worn in all cases and especially when handling pathogens and must be removed and properly disposed on leaving the laboratory.
- Good hygiene must be maintained at all times. Spillages must be cleaned up immediately with disinfectant of bleach and disposed into decontaminate equipment and centrifuge buckets.
- All solid biological wastes (plastics) must be inactivated in disinfectant or bleach for at least 30 min, followed by 15 min UV before disposal.
- All liquid wastes must be inactivated in disinfectant or bleach for at least 30 min, followed by 15 min UV before disposal down the laboratory sink.
- Aerosols generating procedures (pouring, vortexing) must be kept to the minimum.
- All work surfaces must be well cleaned after use and should be UV treated for 15 min.
- 70% ethanol, mycoplasma erase spray, cetavlon and disinfectant solution are the normally used to sterilize, disinfect and inactivate.

2.0. Procedure in passaging of HEK293 cell

- The medium (MEM)/ supplement was poured into a disinfectant medium in a beaker. This served as disinfectant solution
- The flask (containing HEK293 cells whose medium has just being emptied) was rinsed with 5 mL of phosphate buffered saline (PBS).
- Exactly 10 mL of a working solution (PBS + trypsin) was then added into the flask and incubated for 3-5 min (depending on cell type) to trypsinize the cells.
- While incubating, a new flask(s) was/were labelled with inscriptions like researcher's name, date, passage time, and cell type.
- Exactly 14 mL of the medium (MEM supplemented) was pipetted into the newly labelled flask and kept capped/ covered. This was kept aside.
- After incubation, cells adherence was checked under the microscope and the content (cells + 10 mL MEM) was poured into a centrifuge tube and additional MEM (5 mL) was added prior to centrifuging (1500 rpm, 4 min).
- The resulting supernatant was discarded and 5 mL of the MEM was added to suspend the cells.
- From the content above, 1 mL was pipetted into the flask kept aside with 14 mL of MEM. The remaining 4 mL could either be discarded or used for other 4 flasks as the experiment and number of splitting may require.
- The passaged or subcultured cells (in the flask) was subsequently incubated (5% CO_2, 37° C)
- The used disinfectant solution was inactivated under UV for 15 min and then discarded.

3.0. Chamber counting of HEK293 cells

- The procedure for passaging was repeated after the cells have confluenced (Well-formed layers of cells).
- Exactly 5 mL of MEM supplement was added to the passaged cells and kept as stock.
- Exactly 100 µL of the cell from the stock was thoroughly mixed with 100 µL of trypan blue.
- A portion from the mixed solution above was then put (pipetted) on both sides of the counting chamber covered with slide.
- Subsequently, the chamber was viewed under microscope and the live cells (those not in blue colour) were counted in the four (4) major squares of the chamber as shown below:



- From the counted cells, the total number of cells in the stock solution was thereafter estimated using the expression:

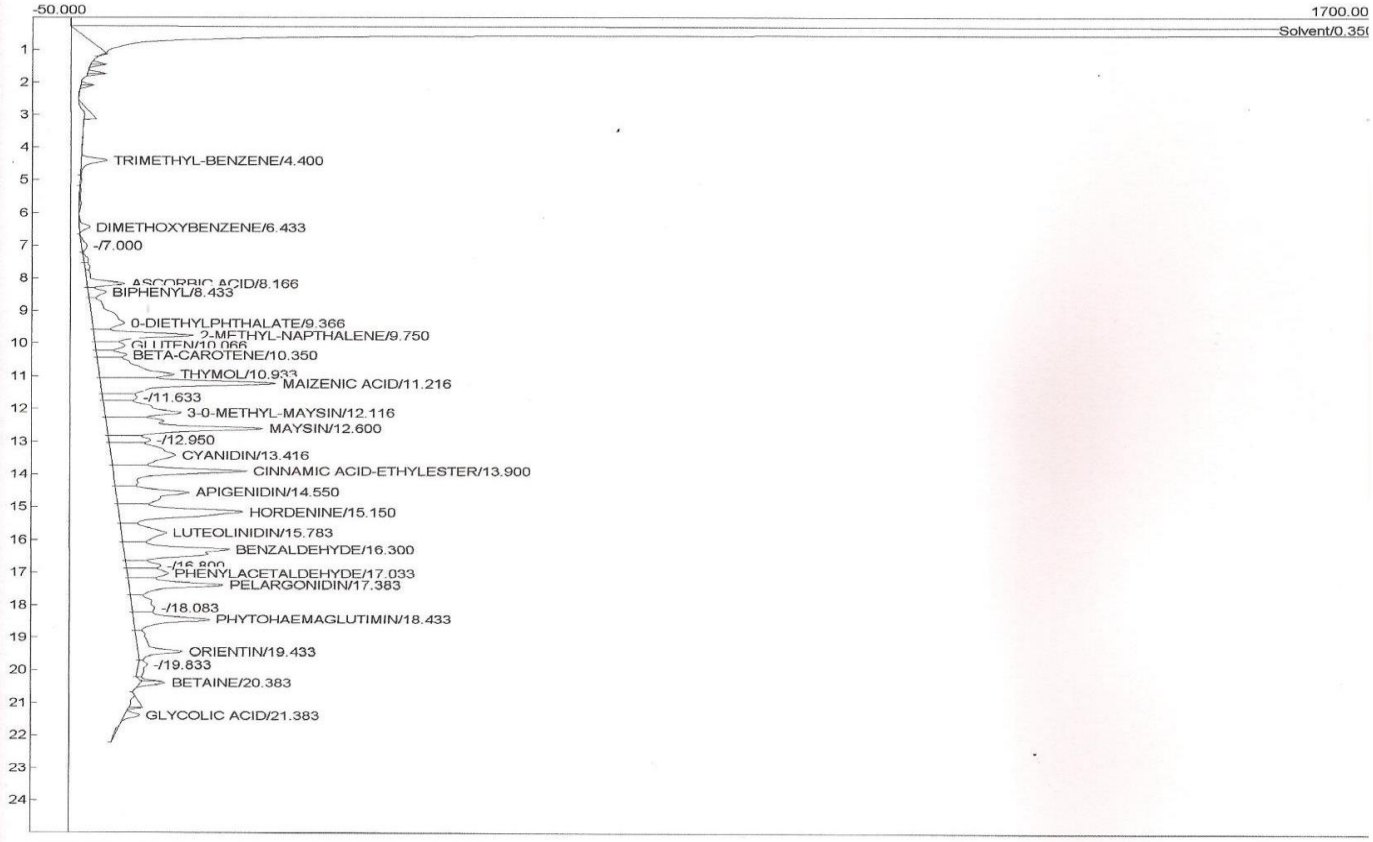
  Total no. of cells in suspension $= \text{Total cells counted} \times \left(\frac{\text{Dilution factor}}{\text{No.of squares}}\right) \times 10^4$

- The value obtained in cells/mL was then adjusted to the required concentration used in the study.

Client: SAHEED ZEA MAYS SILK
Client ID: SAHEED
Method: GC
Description: CHANNEL 1
Column: SE 30
Carrier: NITROGEN
Data file: SAHEED SABIU , ZEA MAYS SILK . PHYTOCHEMICALS . AQUEOUS EXTRACT. 0512Y14.CHR ()
Sample: Z. MAYS SLK. PLNT EXTR
Comments: Plant was dried and grinded to powder,dissolved in Water for 24hrs,filtered and reconcentrated to solid.10.00g Sample extracted with Ethanol and concentrated to 1ml. 1ul injected.

Temperature program:

Init temp	Hold	Ramp	Final temp



Component	Retention	Area	Height	External	Units
Solvent	0.350	67980.4900	5671.230	0.0000	
TRIMETHYL-BENZEN	4.400	384.3330	33.690	0.0000	
DIMETHOXYBENZEN	6.433	150.7225	13.627	0.0000	
ASCORBIC ACID	8.166	542.0420	49.344	0.0000	
BIPHENYL	8.433	293.4680	22.935	0.0000	
0-DIETHYLPHTHALA	9.366	1482.7850	41.881	0.0000	
2-METHYL-NAPTHAL	9.750	1499.3825	129.882	0.0000	
GLUTEN	10.066	496.0805	38.525	0.0000	
BETA-CAROTENE	10.350	422.0615	39.054	0.0000	
THYMOL	10.933	2210.7630	97.084	0.0000	
MAIZENIC ACID	11.216	3233.0850	227.790	0.0000	
3-0-METHYL-MAYSI	12.116	2046.8725	99.187	0.0000	
MAYSIN	12.600	3363.1450	201.819	0.0000	
CYANIDIN	13.416	2595.8520	84.348	0.0000	

CINNAMIC ACID-ET	13.900	TE	2694.6005	174.156	0.0000
APIGENIDIN	14.550		1940.3850	95.405	0.0000
HORDENINE	15.150		2910.0960	161.765	0.0000
LUTEOLINIDIN	15.783		1371.7975	58.688	0.0000
BENZALDEHYDE	16.300		2714.6840	137.099	0.0000
PHENYLACETALDE	17.033		827.9850	53.418	0.0000
PELARGONIDIN	17.383		1774.6990	122.676	0.0000
PHYTOHAEMAGLU	18.433		1478.7540	98.708	0.0000
ORIENTIN	19.433		1037.1940	57.889	0.0000
BETAINE	20.383		245.4600	31.782	0.0000
GLYCOLIC ACID	21.383		148.5050	19.323	0.0000
			103845.2425		0.0000