ANTIOXIDANT, ANTIDIABETIC AND CARDIOPROTECTIVE ACTIVITIES OF *Dicoma anomala* (SOND.) USED IN THE BASOTHO TRADITIONAL MEDICINE.

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General Abstract

Dicoma anomala (Sond.) belongs to the Asteraceae family and locally called Hloenya (South Sotho), fever or stomach bush (Afrikaans). The plant is used in the management of various diseases, particularly diabetes mellitus among the Basotho tribe of eastern Free State Province, South Africa. The study evaluates the antioxidant, antidiabetic and cardioprotective potentials of the plant as a way of validating the folkloric usage.

The result of *in vitro* antioxidant assays [2, 2- azino-bis (3-ethylbenzothiazoline-6-) sulfonic acid (ABTS), reducing power, superoxide anion, hydroxyl radicals, 1,1-diphenyl-2-picryl hydrazyl (DPPH) radicals, etc.] as well as phytochemicals (such as total phenol, total flavonoids and total antioxidant capacity) in various concentrations (1.56-25 µg/ml) tested using water, ethanol, hydro-ethanol and methanol extracts of the plant's root revealed that the water extract exhibited the best activity with half maximal inhibitory concentration (IC_{50}: 15.20, 11.70, and 0.84 µg/mL) in DPPH, hydroxyl radical, and superoxide anion radicals respectively. The four extracts also possessed high phenolic contents, total antioxidant capacity with lower total flavonoids content. The effect of treatment with 125, 250 and 250 mg/kg body weight (b.w.) aqueous roots extract of *Dicoma anomala* (AQRED) was investigated *in vivo* in CCl_4- induced hepatotoxic rats in a 15-day curative and prophylactic study. The result revealed that pre-treatment and treatment with AQRED lowers the elevated serum activities of aspartate transaminase (AST), alanine aminotransferase (ALT) and the level of thiobarbituric acid reactive species (TBARS) while restoring the activities of liver antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) towards normal control in a dose-dependent manner. This result proved the antioxidant and hepatoprotective activity of the plant.

The *in vitro* antidiabetic potential of *D. anomala* was investigated via the inhibition of α-amylase and α-glucosidase using same extracts (as above) at the range of 1.56 - 25.00 µg/mL concentrations. All the tested extracts of the plant were active against both enzymes, although, the most potent against α-amylase and α -glucosidase was hydro-ethanol (IC_{50}: 9.00 µg/mL) and water (IC_{50}: 27.41 µg/mL) respectively. Similarly, aqueous extract of the *D. anomala* displayed competitive and non-competitive inhibition of α -amylase and α -glucosidase respectively using Lineweaver-Burk plot. Treatment with AQRED at concentration 125, 250 and 500 mg/kg b.w. in Wistar rats reversed towards control the elevated blood glucose levels, lipid peroxidation, lipid profile, glycosylated haemoglobin and activities of gluconeogenesis enzymes, with concomitant reduction in the activities of enzymatic antioxidants, glycolytic enzymes as well as the high-density lipoprotein – cholesterol (HDL-c) brought about by streptozotocin induction. Thus, the study proved the antihyperglycaemic activity of the plant.

Additionally, AQRED at 125, 250 and 500 mg/kg b.w. was evaluated for its ameliorative activity against isoproterenol (ISP) –induced cardiotoxicity in an animal model. The results from the evaluated biochemical parameters revealed significant ($p<0.05$) elevation in the serum level of AST, ALT, creatine phosphokinase (CPK) and lipid peroxidation while significantly ($p<0.05$) reducing CAT and GPx levels. Treatment with different doses of AQRED significantly reversed towards normal the activities of these enzymes and cardiac lipid peroxidation towards control. The result obtained in the study is suggestive of cardioprotective efficacy of AQRED in ameliorating cardiovascular-related ailments.

The toxicological implications of oral administration of the plant via acute (15 days) and subchronic (90 days) oral toxicity studies was evaluated in Wistar rats (both sexes) using the three concentrations (125, 250 and 500 mg/kg). The findings revealed no mortalities or observed clinical signs of toxicity in all the rats during both investigation periods. In

subchronic toxicity testing similarly, administration of AQRED did not cause any significant ($p > 0.05$) changes in the organ-body weight, haematological parameters and blood chemistry between the experimental animals and the control except in platelet count, alkaline phosphatase (ALP) and sodium levels where a significant increase ($p < 0.05$) in these parameters was observed. The data obtained indicate that the lethal dose (LD_{50}) of AQRED is in excess of 2000 mg/kg and its oral administration for 90 days is unlikely to cause any toxic effects.

In conclusion, the results from this study proved the antioxidant, antihyperglycaemic and cardioprotective potentials of AQRED. The results further validate the folkloric usage of the plant in the management of diabetes mellitus among the Basotho tribe of Eastern Free State Province, South Africa.

Keywords: *antihyperglycaemia, antioxidant, cardioprotection, Dicoma anomala, hepatotoxicity, haematology, phytoconstituents, serum enzymes, toxicity, Wistar rats*

INTELLECTUAL PROPERTY RIGHT AGREEMENT AND ETHICAL APPROVAL

The key players who provided an information or the other with respect to the folkloric use of *Dicoma anomala* (Sond.) were rewarded financially and with the agreement that the research will be a source of providing information on the use, safety, efficacy and toxicity of the plant to the entire Basotho people of Eastern Free State Province, South Africa.

ETHICAL COMMITTEE APPROVAL

The study involves the use of animals and was performed after obtaining the ethical approval on the usage of animals from the University of the Free State Institutional Animal Ethics Committee (UFS-AED 2015/ 0002).

COMPLIANCE STATEMENT

The commercialization of any part of this study in any form has not been done and discouraged. The thesis is written to be a tool for disseminating information to the people of Basotho and the entire Southern Africans on the medicinal uses of *Dicoma anomala*.

Supervisor's signature	**Student's signature**
AOT Ashafa (PhD)	FO Balogun

Declaration

I, Balogun Fatai Oladunni hereby declare that the thesis submitted by me under the supervision of Dr AOT Ashafa for the award of PhD degree at the University of the Free State is my own independent research and has not previously been submitted by me at another University or faculty. I furthermore, cede the copyright of the thesis in favour of the University of the Free State.

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Chapter One

General Introduction

General Introduction

The current trend in the study of free radicals (FR) and reactive oxygen species (ROS) in biology had paved way for a rigourous medical transformation that gives hope to the new age of health and disease control (Aruoma, 2003). Free radicals are molecules with the unpaired electron in their outermost shell; they are highly unstable and capable of independent existence. FR such as hydroxyl radical, superoxide anion, nitric oxide are highly reactive species which are formed when oxygen (an indispensable element for existence) is used by cells for energy production in the form of adenosine triphosphate (ATP) within the mitochondria, thus producing by-products such ROS or reactive nitrogen species (RNS). These species plays a double role; in low concentration, they can be of benefit in cellular responses and immune function while being toxic at a high level leading to a phenomenon called oxidative stress (Pham-Huy *et al.,* 2008).

A number of degenerative diseases such as cancer, aging, autoimmune disorders, diabetes mellitus, cardiovascular diseases have been linked to oxidative stress caused by generation of FR (Hazra *et al.,* 2008) which are associated with destruction of cellular macromolecules such as DNA, proteins, carbohydrates and lipids (Polterat, 1997). However, human cells are endowed with variety of defensive mechanisms via enzymatic (catalase, superoxide dismutase, glutathione reductase) and non-enzymatic (vitamin C, vitamin E) antioxidants (Erasto and Mbwambo, 2009) through which they overcome the overwhelming effect of FR and oxidative stress (Chandra *et al.,* 1994; Ayoola *et al.,* 2011). These antioxidants combat the excesses of these radicals by reacting directly and quenching their catalytic metal ions (Robak and Marcinkiewicz, 1995; Oyedemi *et al.,* 2010). Moreover, antioxidant from natural sources such as medicinal plants are considered excellent candidates (Kolar *et al.,* 2011) since the synthetic ones (like butylated hydroxyl anisole, butylated hydroxyl toluene etc.) are

reported to be toxic to humans and animals (Anagnostopoulou *et al.*, 2006). Perhaps, the antioxidant study of the pharmacological potentials of these medicinal plants may provide an insight to their mechanism of action.

The liver is the largest glandular organ in the body saddled with the responsibility of bile secretion, body protection from various injurious substances and toxic metabolic by-products asides its physiological role (Maher, 1997). While performing its functions, it is exposed to various toxicants which make it susceptible to varying illnesses. In recent times, the prevalence of liver diseases particularly jaundices and hepatitis has continued to increase globally resulting in deaths despite the enormous scientific achievements in the field of hepatology (Shahani, 1999). The use of medicinal plants with good antioxidant activity has been reported to impact hepatoprotection against many of these liver-related diseases (Yeh *et al.*, 2012; Tenkerian *et al.*, 2015).

Diabetes is perhaps the oldest disease known to human dated as far back as 3000 years when it was first reported in Egyptian literature (Ahmed, 2002). Diabetes derived from the Greek to mean 'siphon' or 'to pass through' was first used during the second century AD by a scientist named Aretaeus from Cappadocia when he presented the clinical description of the disease. After him, other scientists such as Thomas Willis and Matthew Dobson had provided astonishing submissions. However, it was not until the 18th century when John Rollo differentiated between 'diabetes mellitus' (honey) and 'diabetes insipidus' meaning tasteless (Holt, 2004).

Diabetes mellitus (DM) is a complex metabolic disorder marked by continuous hyperglycaemia arising from a defect in insulin secretion, insulin action or both. DM is divided into two broad classifications namely; type 1 (also called insulin-dependent diabetes mellitus, IDDM) caused by the immunological destruction of pancreatic beta cells resulting

in insulin deficiency and type 2 (also called noninsulin-dependent diabetic mellitus, NIDDM) which is a consequence of impaired insulin secretion and resistance to insulin action. The classification of these two types of DM made in 1936 based on the report from 'Diabetes mellitus history- from ancient to modern times' revealed the most common form of DM as the type 2, identified as a component of metabolic deficiency. The common features of IDDM include hyperglycaemia, insulin resistance and / or relative insulin disorder which are mostly associated with obesity and hereditary disposition. It may also occur as a result of association among risk factors from genetics, environmental and behavioural factors. The symptoms include excessive thirst (polydipsia), frequent urination (polyuria), excessive food intake (polyphagia), blurred vision and weight loss (WHO, 1999). NIDDM on the other hand, is caused by hyperglycaemia and most of the sufferers are susceptible to both short and long-term damage to vital organs resulting in metabolic complications such as neuropathy, nephropathy, retinopathy, atherosclerosis, hyperlipidemia etc. which ultimately leads to death arising from its insidious onset as well as late recognition especially in most poverty-driving continent like Africa (Azevedo and Alla, 2008). NIDDM may be controlled through dietary therapy, exercise and oral hypoglycaemic agents, OHAs (Afolayan and Sunmonu, 2010).

Diabetes mellitus remains one of the top priority non-communicable diseases (NCD) in the world. The prevalence of the menace continues to grow with increasing population. Information from IDF 2012, estimated the number of sufferers at 366 million with more than 4.3 million deaths in 2011. In 2014, the number of diabetic patients escalated to over 422 million (a 13.3% increase) compared to 108 million in 1980, thus accounting for 8.5% of the total adult population globally (WHO, 2016) with projected figure of 552 million by 2030. In Africa currently, over 25 million people are reported to be living with the disease as against 4 million in 1980 and it is of interest to note that South Africa, as well as Nigeria, top the list of countries in Africa with the highest incidence of DM (IDF, 2014).

According to reports, lifestyle (such as physical inactivity, alcohol intake, smoking of cigarette, obesity, environmental toxins etc.), genetics and sometimes medical conditions are possible factors principally responsible for the development of NIDDM (Hu *et al.,* 2001; Lang *et al.,* 2008; Ripsin *et al.,* 2009). Heredity also plays a role in NIDDM because of the likelihood of strong inheritable links among families. In a recent development, genes such as *KCNJ11, TCF7L2, PPARG, FTO, WFS1, IGF2BP2, JAZF1, SLC30A8* etc. have been linked to the development of type 2 DM (Olokoba *et al.,* 2012). Notable among these genes are potassium inwardly-rectifying channel, sub family J, member 11 (KCNJ11) reported to encode the islet ATP-sensitive potassium channel Kir6.2 and transcription factor 7-like 2 (TCF7L2) responsible for regulation of pro-glucagon gene expression and glucagon-like peptide-1 production (McCarthy, 2010). Worthy to also mention that obesity is inherited with maturity-onset diabetes for the young (MODY) accounting for 5% of such cases in type 2 DM (Camastra *et al.,* 1999; Walley *et al.,* 2006). Hypertension, hyperlipidemia, acromegaly, cancer, thyrotoxicosis etc. are examples of medical conditions that can exacerbate NIDDM (Olokoba *et al.,* 2012).

The management of type 2 DM is not only limited to the usage of OHAs, as lifestyle and diet modifications also have roles to play. Studies have shown that intake of high fibre, unsaturated fat, low-fat diet, low alcohol intake, abstinence from smoking can go a long way in reducing the prevalence of NIDDM (Hu *et al.,* 2001; Yoon *et al.,* 2006; Willi *et al.,* 2007; Boffetta *et al.,* 2011; Chen *et al.,* 2011). Various pharmacological agents such as biguanides (involved in the suppression of hepatic glucose production by increasing insulin sensitivity), sulfonylureas (stimulation of endogenous secretion of insulin), thiazolidinediones (insulin sensitizer), alpha-glucosidase inhibitors, insulin, etc. are synthetic drugs of importance used in the control and management of DM (van Staa *et al.,* 1997; Mutalik *et al.,* 2003; Mayfield and White, 2004; Scheen, 2005; Yoon *et al.,* 2006; Kim *et al.,* 2008; Kawamori *et al.,* 2009).

However, most of these synthetic OHAs come with various side effects, as such; there is a dire need to search for new compounds with little or no adverse effect in the management of diabetes. The current trend in diabetes control is the usage of traditional medicinal plants owing to lower toxicity profile, cost effectiveness and little side effects (Prince *et al.,* 1998; Latha *et al.,* 2013).

Diabetes mellitus is associated with micro and macrovascular complications which include nephropathy, retinopathy, neuropathy and cardiovascular diseases. Cardiovascular disease (CVD) is generally referred to as the disease of the heart and blood vessels. CVD includes coronary artery disease (angina, myocardial infarction), stroke, hypertensive heart disease, cardiomyopathy, congenital heart disease etc. (Shanti *et al.*, 2011). Aside being among the top five NCD in the world, it is ranked the leading cause of death globally except in Africa accounting for more than 17 million deaths in 2013, as against 12.3 million in 1990 (Shanti *et al.,* 2011). In South Africa, CVD accounts for 13.7 % of total deaths in 2008 according to the report from Heart and Stroke Foundation. Although, the underlying mechanism of CVD varies depending on the disease in question, however, high blood pressure, high blood cholesterol, diabetes, obesity and excessive alcohol intake are some of the causes of CVD (Shanti *et al.,* 2011). In fact, diabetes is a major risk factor for CVD because it affects the heart muscles causing both systolic and diastolic heart dysfunction (Dokken, 2008). The management of CVD does not come easy, as a number of pharmacological agents such beta blockers (atenolol), angiotensin converting enzyme inhibitors (Lisinopril), calcium channel blockers, angiotensin receptor blockers, diuretics which are currently being used to control cardiac-related diseases present different side effects. Thus, recent researches are gingered towards developing alternative drugs from medicinal plants with fewer side effects to treat cardiac-related ailments.

Traditional medicine (TM) is a term interchangeably used with alternative medicine or complementary medicine in some countries. TM according to World Health organization (WHO) traditional medicine strategy 2014 – 2023, is defined 'as the total addition of knowledge, skills and practises based on theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the preservation of health as well as in the forestalling, diagnosis, improvement or treatment of physical and mental illness'. The knowledge of TM particularly in issues relating to the health of both humans and animals has continued to emerge in many nations of the world. This is not only due to their proven quality, safety and efficacy but because it is becoming the only source of healthcare for 80% of the entire populations in both developed and developing countries for disease control and management. Common examples are found in India, China, and Africa, where 70%, 40% and up to 90% respectively of the entire populations rely on traditional medicine in meeting their health care needs (WHO 2005). Although this is not limited to developing countries alone, the usage of ethnobotanicals in most of the industrialized countries like United States of America (USA) has also increased during the past twenty years (Ernst *et al.,* 2005; Barnes *et al.,* 2008). This is despite the development and mass production of chemically synthesized drugs which have revolutionized the health care sector in most countries of the world over a century ago.

TM or appropriately, traditional system of healthcare (phytotherapy) assigned to a marginal place within the health planning of many developing countries has also undergone a crucial revolution within the last score of years with every region adopting a form of TM at a particular point in time in their history. Reference can be made to Chinese TM, Arabic TM, African TM, and not also forgetting the Basotho TM. The medicine is said to be traditional based on specific socio-cultural context and the disparity in the approach to health and disease which might vary from one community to another. Although, the ultimate goal of all

TMs is on the state of health of the individual, rather than on the disease which is affecting the individual and the usage of herbs remained an integral part of all TM systems (Conboy *et al.,* 2007; Rishton 2008; Schmidt *et al.,* 2008). In line with the aforementioned, medical community particularly government agencies, pharmaceutical industries and scientists are now paying more attention to the development of newer drugs sources from TM due to the setbacks from orthodox medicines which comes in form of side effects or resistance of the drugs to the agent causing the disease. Notable among the success stories of drugs from TM is the development of artemisinin (antimalarial) from Chinese herb, Qinghao (sweet wormwood), lovastatin (anticholesterol) from traditional Arab, Chinese medicine (Oyster, mushrooms), and quinine (antimalarial) from the bark of Cinchona tree (Rishton 2008; Schmidt *et al.,* 2008; Li and Vederas, 2009).

Traditional medicine according to WHO is described as the surest means to attaining total health care coverage of the global population, because they are currently serving as alternatives for the treatment of mild, acute or chronic conditions and ailments such as diabetes mellitus, cardiovascular disease, prostate problems, depression, inflammation among numerous others as well as in immune system enhancement. In China, traditional herbal medicines had been very useful in the strategy to contain and treat severe acute respiratory syndrome (SARS) in 2003. In Europe (France, Germany etc.), herbal medicines are becoming the leading household products in all the over-the-counter (OTC) sales while the presence of essential oils, herbal extracts, or herbal teas being sold in pharmacies with conventional drugs is the order of the day in most developed countries. In modern day Africa, the usage of ethno-medicine as the treatment alternative to a number of ailments has been reported (WHO, 2002). A common example is a usage of ‘the Africa flower’ in the management of HIV-related symptoms for many years (De Smet, 2005; Tilburt and Kaptchuk, 2008). South Africa as a country is not left out in the use of TM as remedy to a number of diseases (Hutchings,

1996) as approximately 80% of South Africans now adopt the use of TM during a particular stage of their lives (Ojewole, 2006; Makundi *et al.*, 2006; Mander *et al.*, 2007).

South Africa is rich in biodiversity. It accounts for 9% of higher plants (in the world) from more than 30, 000 species (Gericke and van Wyk, 2000). It is however not surprising the integral role medicinal plants plays in the lives of South Africans in food, shelter, fuel, medicine etc. (Meyer and Afolayan, 1995; Pretorius and Marx, 2006).

The Eastern part of Free State Province and Lesotho nationality housed the Basotho tribe whose knowledge and usage of TM cannot be overemphasized. Lesotho is a country with lots of mountains completely landlocked within the borders of South Africa. It has an area of about 11,700 square miles (about 30,350 square kilometers). The Free State is a highland plain, called a *highveld* in South Africa, and it shares a border with Lesotho in the West. The Basotho tribe in South Africa are traditional people with a mixture of the rural and urban populace. They account for more than 5 million people according to the 1995 estimation and most of its rural settings are marked by poverty and inadequate access to health care with the presence of various diseases such as diarrhoea, malaria, diabetes, malnutrition etc. taking the order of the day (*www.everyculture.com/wc/Japan-to-Mali/Sotho.html*). They are noted for their usage of TM and according to Moteetee and van Wyk (2011), more than 300 plant species are currently being used in the management of various diseases with well over 23 species adopted in the treatment of diabetes mellitus (Tshabalala and Ashafa, 2011) as reviewed in the next chapter of this thesis.

The use of TM by the majority of people globally particularly among the rural dwellers are attributed to being affordable, less toxic probably because they are from natural origin, thus perceived safe and well accepted as an alternative form of therapy to conventional medicine. Traditional medicinal plants are rich in numerous compounds, many of which are secondary

metabolites such as aromatic substances (mostly phenols) or their oxygen-substituted derivatives like tannins (Hartmann, 2007; Jenke-Kodama *et al.,* 2008) with limitless pharmacological potentials which includes but not limited to antioxidant, antidiabetic, anti-inflammatory, anthelmintic, antibacterial. Despite the excellent pharmacological activities of these ethnobotanicals, there is a need for continued effort in a number of researches leading to the identification of the active compounds in these traditional medicinal plants to fully elucidate the mechanism of their pharmacological activities (Li and Vederas, 2009).

The choice of *Dicoma anomala* (Sond.) for the study

Dicoma anomala subsp. *anomala* belongs to the family of Asteraceae and locally called 'Hloenya' among the Basotho people. There are over 16 species of the genus *Dicoma* in Southern Africa and *D. anomala* is the second largest. *D. anomala,* a prostrate or erect perennial herb with underground tuber is widely distributed in sub-Sahara Africa with major distribution in most Provinces such as Limpopo, North-West, Gauteng, Mpumalanga, Free State, Northern Cape and KwaZulu-Natal across South Africa. The conservation of the plant is not threatened according to South African National Biodiversity Institute (SANBI) Threatened species program but caution is however encouraged against its over-utilization. The plant grows in summer rainfall areas and found mostly in grassland and stony places (Figure 1A). The leaves are simple, stalkless and alternately arranged; the stem originates from a woody rootstock and the flower heads are terminal, solitary with stiff, narrow sharp-pointed bracts and pink tubular florets (Figure 1B). Ethnobotanically, the leaves and the roots of the plant are widely used in the treatment of numerous diseases such as cardiovascular disorders, constipation, cough, diarrhoea, fevers, infertility, venereal diseases, toothache, sores, wounds, dizziness, dysentery and diabetes mellitus among many others (Gelfand *et al.,* 1985; Roberts, 1990; von Koenen, 2001), though the root is predominantly used (van der Merwe, 2008). Pharmacologically, the plant had been reported to exhibit antibacterial,

antihelmintic, anti-viral, anti-plasmodial, anti-spasmodic, wound healing, analgesic, anti-inflammatory, antimalarial, antioxidant, hepatoprotective and cardioprotective activities (Becker *et al.*, 2011; Balogun and Ashafa, 2016a; Balogun and Ashafa 2016b).

There are four criteria necessary to be considered during the selection or investigation of a plant for drug discovery according to academic science. This includes random collection, collection based on chemotaxonomy, bio-rational collection (based on chemical ecology) and lastly, collection based on traditional knowledge (Rodrigues, 2007). The choice of *Dicoma anomala* in validating the folkloric usage in the management of diabetes mellitus and cardiovascular-related disorders was based on traditional knowledge collection criterion which is the centre of ethnomedicine (Rodrigues, 2007).

Fig.1A Distribution of *Dicoma anomala* (Sond.) in its natural habitat

Mnengwane and Koekenoer, 2007

Fig.1B Pictorial representation of *D. anomala* (Sond.) showing a portion of leaves and flowers

Objectives of the study

The general objective of the study was channelled towards authenticating the traditional usage of *Dicoma anomala* by the Basotho tribe of Eastern Free, South Africa in the treatment of diabetes. The specific objectives are as follow:

Antioxidant and hepatoprotective activity of the plant

A good number of degenerative diseases are linked to oxidative stress arising from excessive free radical formation. The use of antioxidants has been exploited against the ravishing effect of free radicals in order to offer protection against these disorders, particularly liver disease (Subramaniam, *et al.,* 2000). Known antioxidants of synthetic origin most times come with adverse effects despite alleviating or relieving the symptoms of the disease. Hence, the search for probable antioxidants of natural source that can offer outstanding efficacy and safety. Evidences from literature have revealed a number of medicinal plants particularly containing polyphenols with strong antioxidant activity offering excellent hepatoprotection against various toxicants (Zeashan *et al.,* 2008; Upur *et al.,* 2009; Madureira *et al.,* 2011; Yeh *et al.,* 2012; Sabiu *et al.,* 2015). Despite, the wide usage of *D. anomala* in the control of many ailments, there is absolutely no information in our view from the literature, regarding its antioxidant and hepatoprotective activity. Hence, the present study was undertaken with the hope that the information originating from the study might provide additional information on the usefulness of the plant as a traditional medicine. In achieving this objective, the crude extracts of the plant were tested against some free radicals such as ABTS, DPPH, hydroxyl, superoxide, nitric oxide; and total phenol, total flavonoids and total antioxidant capacity of the extracts were also determined *in vitro*. The effect of the extract against carbon tetrachloride-induced oxidative stress and hepatotoxicity was also performed.

Antidiabetic activity of the *Dicoma anomala*

Diabetes is a non-infectious disease whose effect on the global populace holds no bounds because it poses serious health and socioeconomic challenges. It is ranked the third greatest health problem of man following cancer, cerebrovascular and cardiovascular diseases (Vasim *et al.,* 2012). The prevalence is considered to be high affecting more than 1.5 billion people globally (Ganiyu *et al.,* 2012) and it is a major cause of disability and hospitalization with serious financial implications (Nagappa *et al.,* 2003). South Africa and Nigeria are two countries in Africa with the highest incidence of DM with more than 5 million sufferers (IDF, 2012) translating to 12.9% of the total population of the two countries (IDF, 2014). Based on adverse effects of synthetic oral hypoglycaemic agents, the use of phytotherapy from natural sources has recently been advocated by World Health Organization (WHO) in the control and management of many disorders particularly diabetes mellitus (Bailey, 2003). In the light of this, large population of the world, particularly African countries (Nigeria and South Africa) now take solace in medicinal plants as an alternative source of medicine to the very expensive and highly toxic conventional drugs. Literature search indicated that many herbal formulations or extracts have been very effective against diabetes with little or no side effects (Gandhi and Sasikumar, 2012; Daisy and Jeeva Kani, 2012; Anusuya *et al.,* 2013; Nabi *et al.,* 2013; Ahmed *et al.,* 2015). Although ethnobotanical report on *D. anomala* revealed the use of the plant in the management of diabetes mellitus in the Basotho traditional medicine (Tshabalala and Ashafa, 2011; Balogun *et al.,* 2016), this study is then aimed at validating this claim in both *in vitro* and *in vivo* studies. To this end, the inhibition of α-amylase and α-glucosidase enzymes by the crude extracts of *D. anomala in vitro* as well as the determination of blood glucose level among other biochemical parameters in animal model were evaluated to achieve this objective. Glibenclamide (Aventis Pharma), acarbose, *p*- nitrophenyl

glucopyranoside (pNPG), porcine pancreatic α-amylase, rat intestinal α-glucosidase (from Sigma-Aldrich) are standards and enzymes used for the study.

Cardioprotective activity of the plant

Studies on the epidemiology of diseases revealed an alarming prevalence of CVDs all over the world (Lopez and Murray, 1998; Gilski and Borkenhagen, 2005). CVD remains the leading cause of morbidity and mortality accounting for one-third of total deaths in the industrialized countries of the world (Murray and Lopez, 1997; Lopez *et al.,* 2006). The prevalence is reported to be the rise based on increasing global incidence of associated risk factors such as obesity, diabetes and hypertension (Yusuf *et al.,* 2001; Mathers and Loncar, 2006; Lopez *et al.,* 2006). In recent times, due to side effects from orthodox medicine, there is a need for a viable phytotherapy substitute from natural sources to complement or replace conventional drugs with lots of side effects. Revelation from recent research maintained that medicinal plants with good antioxidant potential are able to impact cardioprotection (Ai and Bolling, 2002). Although information on the ethnobotanical properties of *D. anomala* revealed its usefulness in the management of cardiovascular disorders, this study is aimed at validating this claim in Wistar rats.

Toxicological implications of *Dicoma anomala* (Sond.) root extract

The use of medicinal plant has been an age-long tradition in the prevention or treatment of diseases, health promotion as well as for enhancement of span and quality of life. However, there is a paucity of a structured approach to evaluating their safety and effectiveness. Herbal medicine is widely used owing to the perception that they are safe. However in some cases, they may be contaminated, adulterated, and may contain toxic compounds. As such, there is an urgent need for quality control of herbal medicines which should be a direct correlation of their quality, safety, and efficacy (Ernst *et al.,* 2005; Ribnicky *et al.,* 2008). Despite the wide

usage of traditional medicine in recent times, a lot of concerns due to illnesses and fatalities (Veiga-Junior *et al.*, 2005; Park *et al.*, 2010) such as hepatotoxicity (Saad *et al.*, 2006), nephrotoxicity (Colson and De Broe, 2005, Debelle *et al.*, 2008) originating from them have been raised but only a few have been evaluated through various phases of clinical trials (Cheng *et al.*, 2009). It is worth mentioning that notwithstanding the all-inclusive usage of *D. anomala* across a range of ailments, there is a dearth of information regarding its toxicological implications. This study was conducted to evaluate the safety profile of the plant in Wistar rats.

Structure of the thesis

The thesis was compiled as reprints of published articles and submitted manuscripts for publication. The review of antidiabetic medicinal plants used by the Basotho tribe of Eastern Free State, South Africa is presented in chapter two. Chapter three presents the antioxidant and hepatoprotective activity of *D. anomala* aqueous roots extract. The antidiabetic potential of the plant in *in vitro* and *in vivo* assessments is presented in chapter four. The submission on the ameliorative activity of aqueous root extract is presented in chapter five while the report on its toxicological implications is found in chapter six. Chapter seven represents the general discussion and the conclusion drawn from this study in order to strengthen the obtained results from the investigation of *D. anomala*.

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Chapter Two

Antidiabetic medicinal plants used by the Basotho tribe of eastern Free State: A review

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Review Article

Antidiabetic Medicinal Plants Used by the Basotho Tribe of Eastern Free State: A Review

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Diabetes mellitus (DM) belongs to the group of five leading important diseases causing death globally and remains a major health problem in Africa. A number of factors such as poverty, poor eating habit, and hormonal imbalance are responsible for the occurrence of the disease. It poses a major health challenge in Africa continent today and the prevalence continues to increase at an alarming rate. Various treatment options particularly the usage of herbs have been effective against diabetes because they have no adverse effects. Interestingly, South Africa, especially the Basotho tribe, is blessed with numerous medicinal plants whose usage in the treatment of DM has been effective since the conventional drugs are expensive and often unaffordable. The present study attempted to update the various scientific evidence on the twenty-three (23) plants originating from different parts of the world but widely used by the Sotho people in the management of DM. Asteraceae topped the list of sixteen (16) plant families and remained the most investigated according to this review. Although limited information was obtained on the antidiabetic activities of these plants, it is however anticipated that government parastatals and scientific communities will pay more attention to these plants in future research.

1. Introduction

Diabetes mellitus (DM) is an endocrine disorder marked by abnormalities in lipid, carbohydrates, and protein metabolism. It does not only cause hyperglycemia but result in numerous complications which are grouped as acute, subacute, or chronic; these include but are not limited to retinopathy, neuropathy, nephropathy, cardiovascular disorders, hypoglycemia, diabetic ketoacidosis, hyperosmolar nonketotic syndrome, polydipsia, frequent urination, lack of vigour, ocular impairment, weight loss, and excessive eating (polyphagia) [1, 2].

Diabetes mellitus (DM) may be classified based on the etiology and clinical symptoms as type 1 (insulin dependent diabetes mellitus, IDDM) and type 2 (non-insulin dependent diabetes mellitus, NIDDM). It is a typical and very predominant disease which troubles people of different classes and races worldwide [3]. Report from International Diabetes Federation (IDF) revealed that the menace presently affects well over 366 million (M) people globally and that, by 2030, the figure will be reaching 552 M [4]. It is estimated that Nigeria (3.2 M), South Africa (2 M), Kenya (over 0.7 M), and Cameroun (over 0.5 M) top the list of countries with the prevalence of the disease in each subregion of Africa [5]. DM is also considered a vital cause of disability and hospitalization as it results in significant financial burden [6].

Due to the inherent side effects such as hypoglycemia, weight increase, gastrointestinal (GIT) disturbances, nausea, and diarrhoea [7] of common oral hypoglycemic synthetic drugs like sulphonylureas (glibenclamide, e.g., Daonil), biguanides (metformin, e.g., Glucophage), and glucosidase inhibitors like Acarbose, researchers are now intensifying efforts in alternative and complementary medicines to proffer lasting solution or at least stem the burden of this menace [8]. This is partly because herbal remedies are more efficient and have little or no adverse effects and could also be due to the fact that they form a vital component of the health care delivery system in most African nations [9]. World Health Organization (WHO) in one of their submissions advocated the evaluation of medicinal plants (MP) based on their efficacy, low cost, and possession of little or no adverse effects [10]. Similarly, WHO in one of their technical reports [11]

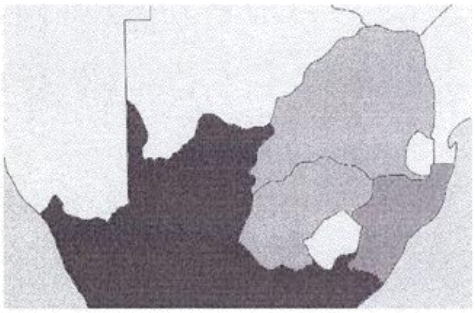

FIGURE 1: Map of South Africa showing the Basotho region (highlighted in orange).

maintained that 4/5th of the citizens in African countries rely on folk medicines particularly herbal remedy for their primary health care requirements [12, 13]. This could be ascribed to the efficacy and availabilities of these plants because they account for 25% of higher plants in the world comprising 5 400 with more than 16 300 medicinal uses [14]. South Africa (SA) accounts for 9% (about 30 000 species) of higher plants in the world [15]. It is therefore not amazing that over 3 500 species of these plants are employed by over 20 000 indigenous healers [16]. Interestingly, about 80% of South Africans use plants for therapeutic purposes [17] mainly because the cost of buying orthodox medicine or conventional treatment continues to increase, thus making affordability impossible.

Herbal drugs with antidiabetic activity are known for their therapeutic potentials within the traditional healthcare system, but despite their pronounced folkloric activity, they have not been commercially formulated as modern medicines. This is despite the fact that their therapeutic properties have been reported to serve as a potential source of hypoglycemic drugs and many of these compounds derived from plants are used in the management of DM. This is confirmed by numerous ethnobotanical surveys conducted on medicinal herbs employed in the control of DM from divergent regions, communities, and tribes within the African subregion [18–27].

Basotho (South Sotho) tribes are the largest population of blacks within SA and they are concentrated in Free State, Gauteng, and Eastern Cape (Figure 1) Provinces. It is worth mentioning that their knowledge and usage of numerous MP in the treatment of various disorders such as DM and hypertension cannot be overemphasized. Tshabalala and Ashafa [28] in the past conducted an ethnobotanical overview of plants utilized for diabetes control by the Basotho people and identified twenty-three (23) plants with such potentials. In this paper, we conducted a comprehensive review of these plants with a view to helping researchers and government agencies to avert the probable extinction of these plants. This review is also intended to serve as a guide for possible future research on the scientifically unproven plants.

2. Methodology

Literature used for this review was obtained through searching the individual botanical names of the plants on Google Scholar. Informative articles used in this study were sourced from scientific databases such as Science Direct, PubMed, and Medicine. The articles mostly cover the period between 2000 and 2015. One hundred and sixteen (116) journals were retrieved, although emphasis was placed on the hypoglycemic and/or hyperglycemic and antihyperlipidemic activities of the plants when keywords such as MP and hypoglycemic were typed in. Various other *in vitro* and *in vivo* pharmacological activities of these plants (Tables 1 and 2) were sourced from 79 of the peer-reviewed articles.

3. Basotho Ethnobotanically Reported Plants with Antidiabetic Potentials

3.1. Eriocephalus punctulatus. Eriocephalus punctulatus is a flowering plant belonging to the Asteraceae (daisy) genus family with over 35 species. It is commonly called wilde roosmaryn, Kapokbos (meaning snowbush in Afrikaans), and wild rosemary (Eng.) and it is widely distributed in SA (in mountain areas of Free State and Western Cape Provinces) and Namibia [29, 30]. Traditionally, the plant is used as diaphoretic and diuretic agents [31] and for the treatment of cold [32], DM, and so forth. *E. punctulatus* contains essential oil called Cape chamomile which comprises over 50 aliphatic esters with 2-methyl butyl-2-methyl propanoate (21.2%), 7-methylbutyl-2-methyl butanoate (5.6%), 2-methylpropyl-2-methyl propanoate (5.3%), 7-methyl-2-octyl acetate (4.5%), linalyl acetate (4.4%), and α-pinene (1.9%) as main compounds [29] reported to be of use in cosmetic toiletries and aromatherapy [29]. The anti-inflammatory, antiallergic, antidepressant, and antiseptic properties of *E. tenuifolius* essential oil, a related species of *E. punctulatus*, have been reported [33]. Njenga et al. [34] reported the antimicrobial activity of *E. punctulatus* with other species of *Eriocephalus* and 113 essential oils. Antioxidant and anti-inflammatory properties were also reported [35] however; a report from chemotaxonomic evidence suggests that Cape chamomile oil is a product of *E. tenuifolius* and not *E. punctulatus* [33] and to date no scientific evidence of its antidiabetic potentials has been reported.

3.2. Hypoxis hemerocallidea. Hypoxis hemerocallidea formerly referred to as *H. rooperi* (African potato) according to Laporta et al. [36] belongs to the Hypoxidaceae (star lily) family. The locally called star flower and yellow star (Eng.); sterblom and gifbol (Afr.); moli kharatsa and Lotsane (South Sotho); or Inkomfe (Zulu) is widely distributed within SA virtually in all the provinces and can be found in other African countries such as Botswana, Lesotho, and Swaziland. There are over 76 species of the genus *Hypoxis* in Africa, 40 of which are found in SA while 16 others are endemic to SA. Traditionally, various parts of the plant are used in the treatment of various diseases such as dizziness, burns, wounds, anxiety, depression or insanity, DM, cancer, polyarthritis, hypertension, and asthma [37, 38]. The formulated and marketed products of the species have been reported to ameliorate benign prostrate hypertrophy (BPH), urinary infections, and immune modulations [39]. Activities of this

Table 1: *In vitro* pharmacological activities of medicinal plants used in the treatment of diabetes mellitus by the Basotho tribe.

Plant name	Family	Local name (South Sotho)	Pharmacological studies				Solvent used	Province	Part used	References
			Antioxidant	*Inflammation*	*Antimicrobial*	*Cytotoxic*				
E. punctulatus	Asteraceae	Kapokbos (Afr.)	DPPH	5-Lipoxygenase enzyme	Disc diffusion assay	*	Acetone		Leaves	[34, 35]
H. hemerocallidea	Hypoxidaceae	Lotsane	DPPH	*	Microdilution assay	*	Ethanol, acetone	Mpumalanga	Leaves and corms	[41]
D. anomala	Asteraceae	Hloenya	DPPH, ABTS, hydroxyl radicals, superoxide anion, metal chelating, reducing power	*	*	*	Water, ethanol, methanol, and 50% aqueous ethanol	Free State	Roots	[56]
X. undulatum	Apocynaceae	Leshokoa	*	*	Microplate dilution method	*	Water, ethanol, ethyl acetate	KZN	Roots	[62]
M. serrata	Myricaceae	Smalblaarwasbessie (Afr.)	*	*	Microplate dilution method	Brine shrimp lethality assay	Hexane, water, methanol, acetone	Free State	Roots	[66]
G. krebsiana	Asteraceae	Botterbloom (Afr.)	DPPH, ABTS, hydroxyl radicals, superoxide anion, metal chelating, reducing power	*	*	*	Water, ethanol, methanol, and 50% aqueous ethanol	Free State	Leaves	[56]
E. elephantina	Fabaceae	Mositsane	DPPH	*		Brine shrimp lethality assay	Hexane, water, ethanol	Gauteng	Rhizomes	[77]
					Microdilution method		Water, DCM/water	Swaziland, South Africa, and Zimbabwe	Rhizomes	[76]
H. scaposa	Asteraceae	Khutsana	*	Cyclooxygenase	Disc diffusion	*	Hexane, methanol, water	Pietermaritzburg	Leaves, roots	[83]
P. prunelloides	Rubiaceae	Sooibrandbossie (Afr.)	DPPH			Brine shrimp lethality assay	Hexane, water, ethanol	Gauteng	Rhizome	[77]
				15-LOX	Microdilution assay		water, 80% ethanol, DCM	KZN	Whole plant	[89, 91]

Table 1: Continued.

Plant name	Family	Local name (South Sotho)	Pharmacological studies				Solvent used	Province	Part used	References
			Antioxidant	*Inflammation*	*Antimicrobial*	*Cytotoxic*				
B. narcissifolia	Asphodelaceae	Serelelile	*	*	Cup plate method	*	Water, acetone, ethyl acetate	KZN	Leave, roots, rhizome	[104]
G. perpensa	Gunneraceae	Qobo	DPPH, ABTS, nitric oxide, hydroxyl radicals, superoxide anion	LOX activity	Microplate dilution method	Brine shrimp lethality assay	Water, ethanol, ethyl acetate	KZN	Roots	[62, 114, 116]
							Methanol	KZN	Rhizome	[115]
Aloe vera	Liliaceae	Barbados Aloe	Peroxyl radical, superoxide		Agar diffusion Agar dilution		Water	Ondo, Nigeria Romania	Leaf, gel Leaves	[121] [128, 129]
E. zeyheri	Fabaceae	Khungoana			Agar dilution		Acetone	Japan	Roots	[134, 135]

*The pharmacological activity yet to be determined.
DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid.
KZN: KwaZulu-Natal; DCM: dichloromethane; LOX: lipoxygenase.

Table 2: List of scientifically investigated medicinal plants with *in vivo* antidiabetic activity used in Basotho traditional medicine.

Plant name	Family	Local name	Type of effect	Model	Medium/part	Dosage (mg/kg)	Province/area	References
H. hemerocallidea	Hypoxidaceae	Lotsane	Cardiodepressant, hypotensive, anti-inflammatory, antioxidant, hypoglycemic	Rat	Aq. stem bark, Aq.	50, 100, 200, 400	KZN	[37]
					Corms	25, 50, 100, 200, 400	*	[38]
E. elephantina	Fabaceae	Mositsane	Analgesic, anti-inflammatory	Rat	Root	50, 100, 200	Eastern Cape	[75]
C. africana	Commelinaceae	Geeleendagsblom	Antihyperglycemia	Rat	Leaves	500	Ibadan, Nigeria	[81]
Aloe vera	Liliaceae	Barbados Aloe	Wound healing, hypoglycemic	Rabbits	Gel	*	*	[130]
			Hypolipidemic	Mouse	Gel	25, 50, 100	S. Korea	[131]
			Antioxidant, hypolipidemic	Rats	Gel	300	India	[132, 136]
			Hypolipidemic	Mice	Gel	350	*	[137]
			Hypoglycemic, hypolipidemic	Humans	Powder	100, 2000	Ludhiana	[138]

*Not indicated.
KZN; KwaZulu-Natal.

plant are attributed to its main bioactive compounds, hypoxoside and its aglycone derivative, rooperol [40]. Katerere and Eloff [41] maintained that the leaves and corms of the plant possess antibacterial and antioxidant activities while the anticonvulsant activity was recently reported by Liu et al. [42]. The cardiovascular activity of *H. hemerocallidea* was reported in experimental animal models [38], antidiarrhoea activity was reported in rodents [43], and the uterolytic effect was found in rats and guinea pigs [44]. The antinociceptive activity (studied in mice) and the anti-inflammatory and antidiabetic activities (rats) have been reported when aqueous extract (50–800 mg/kg) of this plant was administered to rodents induced with a rat hind paw oedema (0.5 mg/kg) and streptozotocin (90 mg/kg), respectively (Table 2). The herb was able to bring about a significant reduction in the fresh egg albumin-induced acute inflammation of the rat hind paw and blood glucose concentrations in these animals [37]. Several other reports of these activities had also been detailed by several authors [40, 45–47].

3.3. Dicoma anomala. *Dicoma anomala* belongs to the Asteraceae family. The plant commonly called fever bush and stomach bush (Eng.); maagbitterwortel, kalwerbossie, koorsbossie, gryshout, and maagbossie (Afr.); Hloenya and Mohasetse (South Sotho); Inyongana (Swazi, Xhosa); or Isihlabamakhondlwane and Umuna (Zulu) is a herbaceous plant that grows in grassland, stony places, hillsides, flat grasslands, and savannah areas within an altitude that ranges from 165 to 2075 m [48]. *D. anomala* is widely distributed within SA in places such as Limpopo, North West, Gauteng, Mpumalanga, Free State, Northern Cape, and KwaZulu-Natal [49] and over 16 species of the genus *Dicoma* exist in SA with *D. tomentosa* and *D. anomala* being the most distributed species in Africa. Traditionally, the plant is used in the treatment of coughs and colds, fevers, ulcers, dermatosis, venereal diseases, labour pains, dysentery, intestinal parasites, stomach pains, toothache, and internal worms which are linked to versed majority of pharmacological activities such as antihelminthic, antispasmodic, analgesic, wound healing, anti-inflammatory, and antimicrobial activities [50–52]. *Dicoma tomentosa* from the same genus was reported to possess antiplasmodial activity [53] while the *in vitro* inhibitory potentials of *D. anomala* against cytochrome p450 enzymes and p-glycoprotein were also reported [54]. The antiplasmodial activity of *D. anomala* *in vitro* [55] as well as the *in vitro* antioxidant activity is recently reported (Table 1) by Balogun and Ashafa [56] but unfortunately, report on the *in vivo* antidiabetic activity of the plant is still awaited in the scientific world.

3.4. Xysmalobium undulatum. *Xysmalobium undulatum* is a member of the Apocynaceae family. The plant is commonly called milk bush, milkwort, Uzura, wild cotton, and wave-leaved *Xysmalobium* (Eng.); bitterhout, bitterwortel, bitterhoutwortel, and melkbos (Afr.); Leshokoa and Poho-tsehla (South Sotho); iyeza elimhlophe, Nwachaba, and Ishongwane (Xhosa); or Ishongwane, Ishongwe, and Ishinga (Zulu). The plant is widely distributed in all provinces within SA as well as Namibia, Botswana, Lesotho, and Swaziland. There are over forty (40) species of the genus *Xysmalobium* in the world and 24 species of these are found in SA. Locally, the usage of the plant is in the treatment of stomach cramps, diarrhoea, colic, afterbirth cramps, headache, wounds, and abscesses [48, 50, 57, 58]. The main compounds as elucidated by Ghorbani et al. [59] are uzarin and xysmalorin with few quantities of allouzarin and alloxysmalorin. Steenkamp et al. [60] reported the *in vitro* antioxidant activity and selective serotonin reuptake inhibitors (SSRI) antidepression activity of the plant reported by Pedersen et al. [61]. Antibacterial and antifungal properties [62], antiplasmodial and central nervous system (CNS) activity [63], antidiarrhoeal activity [64], and inhibition of uptake of serotonin [16] are reported. Still, no scientific work on the antidiabetic activity of the plant was found in the literature.

3.5. Morella serrata. *Morella serrata* belongs to the Myricaceae family and it is locally called Smalblaarwasbessie and Berg-wasbessie (Afr.); lance-leaved strawberry, waxberry, and mountain waxberry (Eng.); Isibhara, Umakhuthula, and Umaluleka (Xhosa); or Iyethi, Ulethi, Umakhuthula, and Umlulama (Zulu). *M. serrata* grows along streams on grassy hillsides as well as on forest fringes. They are widely distributed within SA virtually in all the provinces while they are also occurring in Swaziland, Zimbabwe, and northern Botswana. Traditionally, the plant is used to cure headaches and tuberculosis [65] and for the management of DM. The antimicrobial and antitumor activity of the plant have been reported [66]; however, it is worth reporting that the pharmacological evidence of its antidiabetic efficacy still remains unknown.

3.6. Gazania krebsiana. *Gazania krebsiana* belongs to the family Asteraceae and it is locally referred to as terracotta *Gazania* meaning "beautiful flower" (Eng.); gousblom and botterbloom (meaning) butterflower (Afr.). There are over nineteen (19) species of the genus *Gazania* in Africa and most of these are predominantly found in SA. The plant is distributed in all the provinces of SA from Namaqualand in the west to the Eastern Cape and KwaZulu-Natal (KZN) in the east, through Free State in the north and Gauteng. The plant is used in the management of DM traditionally among the Basotho tribe and recently Balogun and Ashafa [56] reported the antioxidant activity of the plant in *in vitro* study (Table 1) but in our view, there is no scientific evidence to support its antidiabetic efficacy to date.

3.7. Elephantorrhiza elephantina. *Elephantorrhiza elephantina* is a member of Fabaceae or Leguminosae family. The plant is commonly called eland's bean, eland's wattle, and elephant's root (Eng.); baswortel, elands-boontjie, leerbossie, looiersboontjie, and olifantswortel (Afr.); Mupangara (Shona); Mositsane (South Sotho, Tswana); or Intolwane (Xhosa, Zulu). There are over nine (9) species of the genus *Elephantorrhiza* and the species *elephantina* is the most widely spread. It can be found in southern Angola, Namibia, Botswana, Zimbabwe, and Mozambique and in most provinces within SA. Traditionally, the plant is used to treat diarrhoea, dysentery, stomach disorders, haemorrhoids, peptic

ulcer, skin diseases, and acne [58, 67–70]. The anthelmintic activity *in vitro* and *in vivo* was reported by Maphosa et al. [71]. Equally, the antiprotozoal activity has been reported [72, 73]. Mathabe et al. [74] also reported the antibacterial activity while the anti-inflammatory, antinociceptive *in vivo* [75], antimicrobial [76], antioxidant, cytotoxic [77], and immune enhancing as well as anti-HIV [78] activities were similarly reported (Table 1). Despite these various pharmacological reports, there has not been any scientific literature that supports its antidiabetic properties.

3.8. Hermannia pinnata. *Hermannia pinnata* belongs to the family of Malvaceae (Mallow) and is commonly called orange *Hermannia* or doll's rose (Eng.); Kwasblaar and Kruip Poprosie (Afr.). There are over 180 species of the genus *Hermannia* in SA; 162 species of these are widely spread across the country. Traditionally, the Sotho tribe used the plant in the management of DM but the scientific efficacy has not been ascertained.

3.9. Commelina africana. *Commelina africana*, a member of the Commelinaceae family, is commonly called yellow *Commelina* (Eng.); geeleendagsblom (Afr.). Sixteen (16) of the over 170 species of the genus *Commelina* in the world are found in SA. They are mostly distributed in Africa (e.g., Madagascar) as well as other places of the world such as Arabian Peninsula where there are forest, savannah, and grassland. Traditionally, the plant is useful in the treatment of venereal diseases and as medicine for women suffering from menstrual pain and infertility [31, 79, 80]. The hypoglycemic effect of the plant extract had been reported in alloxan (125 mg/kg) induced diabetic rats when aqueous leaves extracts of the plant brought down the increased glucose level of the animals (Table 2). This reduction by the plant not only was attributed to its inhibitory effect on glucose absorption but could probably be due to other mechanisms such as direct stimulation of glycolysis in peripheral tissues, facilitation of glucose entry into peripheral cells, reduced hepatic gluconeogenesis, and reduction of plasma glucagon levels [81].

3.10. Haplocarpha scaposa. *Haplocarpha scaposa* belongs to the family Asteraceae and is commonly called false gerbera (Eng.); melktou (Afr.); Khutsana (South Sotho); or Isikhali (Xhosa). Ten (10) species of the genus *Haplocarpha* existed and five (5) of them occur in central Africa. *H. scaposa* is endemic to Africa and is widely distributed in Mpumalanga, Free State, Eastern Cape within SA, Swaziland, and some part of eastern Africa [82]. Traditionally, the plant is used to reduce menstrual pain and in the management of DM [82]. *H. scaposa* has been reported to exhibit anti-inflammatory activity [83]; however, it is noteworthy to report that research on the antidiabetic activity of the plant has not been validated to date.

3.11. Helichrysum aureum. The plant belongs to the Asteraceae family and is locally called Leabane (South Sotho). According to Flora of Zimbabwe [84], there are over 600 species of the genus *Helichrysum* in the world; about 244 to 250 of these species are found in Africa, particularly SA. The plant is found in submontane grassland and miombo woodland areas with a wide distribution in Mozambique, Zimbabwe, Lesotho, Swaziland, and SA (precisely Eastern Cape and Free State). The areas of the world where *Helichrysum* species predominates include southern Europe, southwest Asia, southern India, Sri Lanka, and Australia [85]. The antimicrobial and cytotoxic activity of the plant had been reported [86].

3.12. Empodium plicatum. *Empodium plicatum* belongs to the Hypoxidaceae family and is locally called golden star (Eng.); Ploegtydblommetjie (Afr.). *E. plicatum* is endemic to SA and widely distributed in Northern and Western Cape. Traditionally, Basotho people use the plant to manage DM although information from the literature at the time of compiling this review reveals no scientific report on the pharmacological activity of the plant.

3.13. Mimulus gracilis. The plant belongs to the family of Scrophulariaceae and is locally called Sehlapetsu (South Sotho). However, the plant is not endemic to SA but is widely distributed in Eastern Cape, Free State, KZN, Limpopo, Mpumalanga, Northern Cape, and North West Provinces. It is also reported to be abundant in Angola, Botswana, Namibia, Nigeria, Sudan, Kenya, Tanzania, Ethiopia, Malawi, Mozambique, Zambia, Zimbabwe, Lesotho, Yemen, India, China, and Australia. Traditionally, the plant is used to treat DM by Basotho people; there is urgent need to determine the antidiabetic activity of the plant for the treatment of DM.

3.14. Pentanisia prunelloides. *Pentanisia prunelloides* belongs to the Rubiaceae family. The plant is locally called wild verbena and broad-leaved *Pentanisia* (Eng.); Sooibrandbossie (Afr.); or Icimamlilo (Zulu). Three (3) of the 15 species of the genus *Pentanisia* are found in SA. Traditionally, various uses of the plant include diarrhoea, dysentery, rheumatism, heartburn, vomiting, fever, toothache, tuberculosis, snakebite, haemorrhoids, burns, and swellings. The *in vitro* anti-inflammatory and antioxidant activity of the plant have been reported [87]. Mpofu et al. [77] established the antibacterial, cytotoxic, and antioxidant activities while the anti-inflammatory, antimycobacterial, antimicrobial, nongenotoxic [88–90], and antioxidant and anti-inflammatory [91] activities *in vitro* had also been reported (Table 1). It is interesting to note that, despite the various *in vitro* activities, there is a dearth of information on the antidiabetic activities of the plant.

3.15. Cannabis sativa. *Cannabis sativa* belongs to the Cannabaceae family. The local names include marijuana (Eng.); dagga (Afr.); Umya (Xhosa); Matekwane or Patse (Northern Sotho); or Nsangu (Zulu). The plant originated from Asia but is presently being cultivated in many countries of the world though naturalized in SA. Three varieties of cannabis are recognized, namely, *sativa* which is commonly referred to as hemp, cultivated for psychoactive cannabinoids, durable fibre, and nutritious seed [92], while the other varieties are

indica and *spontanea*. *Cannabis* is widely distributed in SA and *sativa* variety predominates in Botswana, Limpopo, North West, Gauteng, Mpumalanga, KZN, Western Cape, Eastern Cape, Lesotho while *indica* variety can also be found in Mpumalanga, whereas *spontanea* variety is in Northern Cape. Traditionally, the plant is used as a cure for asthma, bronchitis, headache, flu, epilepsy, cough, and pains. The crude drug and the pure chemical derivatives are used in modern day medicine in the treatment of a migraine, epilepsy, malaria, glaucoma, nausea, acquired immune deficiency syndrome (AIDS), appetite induction for cancer patients, and muscular spasm suppression in multiple sclerosis [93]. Its main active compound is called Δ^9-tetrahydrocannabinol [94, 95]. The antipsychotic activity of the plant has been investigated and reported in rodents and humans [95–97]. The neuroprotective, antioxidative, and antiapoptotic activity [98] and the antibacterial activity of cannabinoids [99] and anticonvulsive, anti-inflammatory, and analgesic activity of Δ^9-tetrahydrocannabinol have been reported [100–102], but till date, the scientific validation of the antidiabetic activity has not been reported.

3.16. Bulbine narcissifolia. *Bulbine narcissifolia* is a member of the Asphodelaceae family. The local names include strap-leafed bulbine and snake flower (Eng.); lintblaar bulbine, geelslangkop, and wildekopieva (Afr.); Khomo-ea-balisa and Serelelile (South Sotho). It has been reported that different names of the plant are adopted based on the appearance and due to the wide use of the genus by all stakeholders or tribes within SA [14, 93]. There are 73 species of the genus *Bulbine*; 67 are predominant in Africa while 6 species are found in Australia. The most common species are *B. frutescens*, *B. abyssinica*, *B. latifolia*, *B. natalensis*, and *B. narcissifolia*. The latter species is widely distributed in Western Cape, Eastern Cape, Free State, KZN, North West, Gauteng, and Limpopo within SA and in Lesotho, Botswana, and Ethiopia. Traditionally, the plant is of utmost importance in wound healing and as a mild purgative [103] and in vomiting, diarrhoea, urinary infections, DM, rheumatism, and blood-related problems. The antibacterial [104] and anticancer and antimicrobial [105] activities of the plant have been reported *in vitro* but till date, there has not been any scientific evidence of antidiabetic properties.

3.17. Rumex lanceolatus. *Rumex lanceolatus* belongs to the Polygonaceae family; the common names include the small dock, smooth dock, and common dock (Eng.); Gladdetong-blaar (Afr.); Idolo Lenkonyane (Zulu); Idolonyana (Xhosa); Khamane, Kxamane, and Molokoli (South Sotho). The plant is not endemic to SA but is widely distributed within SA in Eastern, Western, and Northern Cape, Free Sate, Gauteng, KZN, Limpopo, Mpumalanga, and North West. Ethnobotanically, the root and rather the leaves are used as medicine [106]. The plant serves as a cure for infertility, intestinal parasites [31], internal bleeding [107], and DM. The nonmutagenic activity [108] has been reported while the presence of chrysophanol and related glycosides has been attributed to its laxative activity [107]; however, there has not been any scientific fact about its antidiabetic efficacy in the literature.

3.18. Gunnera perpensa. *Gunnera perpensa* is one of the members of Gunneraceae family. It is locally referred to as river pumpkin and wild rhubarb (Eng.); rivierpampoen and wilde ramenas (Afr.); Qobo (Sotho); Uqobho (Swati); rambola-vhadzimu and shambola-vhadzimu (Venda); Iphuzi lomlambo and Ighobo (Xhosa); Ugobhe and Ugobho (Zulu). Fifty (50) species of the genus *Gunnera* existed and only *perpensa* are found in Africa. *Gunnera* are naturally occurring in central and southern Africa, Madagascar, New Zealand, Tasmania, Indonesia, Philippines, Hawaii, Mexico, and central and southern America. *G. perpensa* is widespread in Sudan, Ethiopia, Zaire, Rwanda, Uganda, Kenya, Zimbabwe, SA (Western and Northern Cape), Swaziland, Lesotho, Namibia, and Botswana [109]. Traditionally, the plant is used to remove placenta in newborn and to relieve menstrual pain [106, 110–112]. The toxic effect of the plant was investigated in rats when aqueous extract of the plant at different concentrations (50–400 mg/kg body weight) was administered and 20% mortality was observed in subacute (400 mg/kg dose) and chronic toxicity (200 mg/kg) tests indicating the toxic effect of the plant over long usage [113]. The antifungal and antibacterial [60, 62, 114], antioxidant [60, 115], antimicrobial [116], anthelmintic [117], and uterotonic activity [118] *in vitro* have been reported (Table 1). Moreover, the lactogenic activity *in vivo* was investigated and reported in female rats over 400–1600 mg/kg concentration ranges [119] but despite the various pharmacological effects of the plant *in vitro*, the antidiabetic activity had been reported.

3.19. Aloe vera. The plant belongs to the Liliaceae family and has its origins in Africa. The plant is commonly called Indian Aloe, True Aloe, Barbados Aloe, Burn Aloe, and so forth [120]. *Aloe vera* is widely distributed in places such as Arabian Peninsula, Morocco, Mauritania, and Egypt. Traditionally, the plant is used in the treatment of various ailments which includes stimulation of cell growth, restoration of damaged cells, restoration of damaged stomach mucous membrane [121], alleviation of various gastrointestinal tract (GIT) disturbances, haemorrhoid treatment [122], wound healing, thermal burn or sunburn [123], and body immune system stimulation [124]. The anti-inflammatory [125–128], modulatory, antiprotozoal, ultraviolet (UV) protective [128], antimicrobial [121], and antifungal [129] activity *in vitro* have been reported (Table 1). The wound healing, hypoglycemic, hypolipidemic, and antioxidant activities of the plant *in vivo* in rabbit and rodents [130–132] have been reported (Table 2).

3.20. Asparagus asparagoides. *Asparagus asparagoides* is a member of Asparagaceae family. The plant is locally called African *Asparagus* fern, baby smilax, bridal creeper, and so forth. *A. asparagoides* is native to SA, Lesotho, and Swaziland and widely distributed in southern Australia and Europe, New Zealand, Hawaii, and California. Traditionally, it is used by Basotho tribe of eastern Free State in the management of DM, though; there has not been any scientific proof for this folkloric use till date.

3.21. Anthospermum ternatum. The plant is a member of Rubiaceae family. It is widely distributed in Angola, Malawi, Zambia, Zimbabwe, and Tanzania. No scientific report of its antidiabetic activity has been reported to date despite its usage by the Basotho people in the management of DM.

3.22. Erythrina zeyheri. Erythrina zeyheri also belongs to Fabaceae family and Faboideae subfamily. The plant is commonly called harrow-breaker and plough-breaker (Eng.); Ploegbreker (Afr.); Khungoana and Motumo (South Sotho); Umnsinsana (Zulu). The plant grows in grassland and moist vleis with clay soils or sandy soils and it is found in colonies. It is widely spread in Mpumalanga, North West, Gauteng, Free State, KZN, and Lesotho. Traditionally, the plant has its usage in asthma, tuberculosis, rheumatism [133], and DM treatments. The antibacterial [134, 135] and anti-inflammatory [133] activity of the plant have been reported, though; scientific evidence on the antidiabetic activity is still awaited.

3.23. Sisymbrium thellungi. Sisymbrium thellungi belongs to the *Brassicaceae* (Cabbage) family and is commonly called African turnip weep. The plant is native to SA and widely distributed in Northern New South Wales, Queensland, and eastern part of SA. The scientific evidence on antidiabetic potentials of the plant has not been submitted to date.

4. Conclusion

Diabetes mellitus (DM) is a major endocrine disorder and its growth or prevalence is attributed to a number of factors that include but are not limited to obesity, social structure, hormonal imbalance, and hereditary. The current trend in the management of DM characterized by hyperglycemia involves the use of herbs since the oral hypoglycemic agents (OHAs) are known to result in unwanted side effects; hence, the need to explore rich and potential plants with antidiabetic activity became necessary. However, from our review, it is evident that the folkloric use of most of these MP has not been adequately explored, thus the need for the government to sponsor or support more research activities in this area so that the potential in these plants to offer lasting solutions to the management of this menace can be ascertained.

Competing Interests

The authors declare that there is no conflict of interests.

Authors' Contributions

All authors read and approved the paper for submission.

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Chapter Three

Antioxidant and hepatoprotective activities of *Dicoma anomala* (Sond.) aqueous root extract against carbon tetrachloride (CCl_4) -induced liver damage in Wistar rats

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EXPERIMENTAL STUDY

Antioxidant and hepatoprotective activities of Dicoma anomala Sond. aqueous root extract against carbon tetrachloride-induced liver damage in Wistar rats

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Abstract

OBJECTIVE: To evaluates the antioxidant and hepatoprotective potentials of Dicoma anomala Sond. (Asteraceae) on body weight, feed and water intake, biochemical parameters and organ histology.

METHODS: Various concentrations (1.56-25 μg/mL) were used in the *in vitro* assays 1,1-diphenyl-2-picryl hydrazyl (DPPH, superoxide anion, hydroxyl radicals, etc.). The effects of treatment with 125, 250 and 250 mg/mL Dicoma anomala aqueous roots extract (DARE) was investigated *in vivo* in the CCl_4-induced hepatotoxic rats during the 15 days study.

RESULTS: Water extract exhibited the best activity (IC_{50}: 15.20 ± 0.03, 11.70 ± 0.10, and 0.84 ± 0.05 μg/mL) *in vitro* in DPPH, hydroxyl and superoxide anion radicals, respectively, when compared with standards. Pre-treatment and treatment with different concentrations of DARE significantly ($P < 0.05$) attenuated the elevated serum activities of aspartate transaminase, alanine transaminase levels while increasing the activities of superoxide dismutase, catalase and glutathione peroxidase. The histopathological evaluations revealed extensive liver damage characterized by severe vacuolar and cytoplasmic degeneration, hepatic necrosis, and cellular infilteration in pre-treated groups while in the treated groups; such liver damages were not observed most especially at 500 mg/kg dose.

CONCLUSION: The results proved the hepatoprotective potential of DARE against CCl_4-induced oxidative stress. Moreover, histopathological examinations revealed better therapeutic advantage of DARE than prophylactic use.

Key words: Dicoma anomala Sond. (Asteraceae); Antioxidant enzymes; Carbon tetrachloride; Hepatoprotection; Lipid peroxidation

INTRODUCTION

Free radicals are implicated in the pathogenesis of several degenerative diseases including liver damage. Their deleterious influence causes oxidative stress which is a reflection of disequilibrium between them and the body's antioxidant defence system. This result in significant damage to important cellular macromolecules (proteins, lipids and DNA) and ultimately cell necrosis.[1] When this happens, swiftly intervention with exogenous antioxidants[2] (which could be easily and readily achieved through consumption of vegetables and fruits) augments the cellular defence system thereby preventing cell death. Foods of plant origin usually contain natural antioxidants that can scavenge free radi-

cals[2] and there is a great deal of interest in edible plants with excellent phytonutrients primarily due to their health benefits.

Dicoma anomala Sond. (Asteraceae) commonly called fever or stomach bush (Eng.) or hloenya (South Sotho) is a prostrate. Decumbent or erect perennial herb with underground tuber. D. anomala is widely distributed in sub-Sahara Africa including most Provinces within South Africa.[3] The plant is ethno-botanically indicated in the treatment of cold and coughs, fever, ulcers and dermatosis. Its pharmacological potentials as antiplasmodial, antibacterial, anthelmintic, antiviral and anti-inflammatory have also been documented.[4] Till date, there is paucity of information in scientific literatures on the hepatoprotective potentials of D. anomala. Therefore, the present study reports on this with focus on its curative and prophylactic models.

MATERIALS AND METHODS

Chemicals

Carbon tetrachloride, 2, 2- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and assay kits were purchased from Sigma-Aldrich (Johannesburg, South Africa). All other chemicals and reagents used were of analytical grade.

Plant collection and extraction

Fresh root stocks of Dicoma anomala were procured in April 2014 from Setsing market, Phuthaditjhaba, Free State Province, South Africa. The sample was confirmed by Dr. AOT Ashafa of Plant Sciences department, University of the Free State, South Africa. A voucher specimen was prepared and deposited at the herbarium. The rootstocks were cut into smaller pieces; oven dried (40 ℃), and pulverized using a Waring commercial blender (Waring Instrument, Torrington, CT, USA) into fine powder. Approximately 10 g each from the powdered materials was exhaustively extracted with 40 mL each of water, ethanol, aqueous ethanol and methanol. They were filtered and organic extracts were concentrated using a rotary evaporator (Cole-Palmer, model SB-1100 Shanghai, China) to obtain dry brown crude extracts. The water extract was dried on a water bath (Memmert W600, Schwabash, Germany) at 45 ℃. The crude extracts were reconstituted in respective solvents to prepare various concentrations used for the *in vitro* antioxidant assays.

Similarly, 200 g of the powdered material was extracted with 2 L of water, filtered and concentrated to dryness on water bath at 40 ℃. The extraction yielded 48.87 g of brown gum (24.435% w/w of dry plant material). The crude extract was reconstituted in water to give various concentrations used for the *in vivo* assays.

In vitro antioxidant assays

The 1,1-diphenyl-2-picryl hydrazyl DPPH radical scavenging activity of the extracts determined using Braca *et al.*[5] method, nitric oxide,[6] reducing power,[7] metal chelating,[8] 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid ABTS,[9] superoxide anion,[10] hydroxyl radical,[11] total antioxidant capacity,[12] total phenolic content[13] and total flavonoids according to Chang *et al.*[14]

In vivo hepatoprotective study

Experimental animals: male and female Wistar rats (Rattus norvegicus, 10 weeks old) weighing 145.00 ± 10.00 g were used in this study. They were procured from the animal house of the University of the Free State, Bloemfontein. They were acclimatized for one week, fed with standard rat feed (Epol mice cubes, Westville, South Africa) and water ad libitum. Ethical clearance (number NR 02/13) to undertake the study was obtained from the Interfaculty Animal Ethical Committee of the University of the Free State prior to the commencement of the study.

Experimental design: the hepatoprotective study was conducted according to Chandan *et al.*[15] method with slight modifications. A total of 66 rats were randomly divided into eleven groups of six animals each (n = 6). Group A animals were orally administered 1 mL normal saline for 15 days. Groups B and C rats received 1 mL/kg CCl_4 (in olive oil; ratio 1 : 1 v/v) i.p on days 1 and 15 and represented curative and prophylactic hepatotoxic rats, respectively. Groups D-G rats were curative hepatotoxic rats treated orally with silymarin (100 mg/kg body weight BW), 125, 250 and 500 mg/kg BW Dicoma anomala aqueous roots extract DARE respectively. Groups H-K represented prophylactic hepatotoxic rats given respective similar treatments as in the curative study and CCl_4 i.p on the 15th day of the experiment.

Serum preparation and organ isolation: at the end of experimental period, all the rats were anaesthetized with halothane and blood was collected by cardiac puncture. An aliquot (2 mL) of the blood collected into ethylenediamine tetraacetic acid EDTA bottle was used for the analysis of haematological parameters, while another 5 mL of the blood collected in non-heparinized bottle was centrifuged at 1285 × g for 10 min and the resulting serum was aspirated and used for other serum bioassays.

The animals were quickly dissected and the liver, kidney, heart and lungs were excised, freed of fat and weighed for evaluation of organ -body weight ratios. The liver was further divided into two portions and a portion immediately fixed in 10% formalin for histopathological examination, while the other was homogenized in ice cold 0.1 mol/L Tris-HCl buffer (pH 7.2), at 10 000 rpm for 15 min and the supernatant obtained was kept at − 80 ℃ freezer prior further bioassays.

Determination of haematological and biochemical parameters: the automated haematologic analyzer (Sysmex, KX-21, Chuo-ku, Kobe, Japan) was used to analyse total protein, bilirubin levels and haematological parameters. The serum levels of total cholesterol, high

density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triglycerides were determined using standard procedures.[16-19]

Antioxidant enzymes assays

Catalase activity in tissues determined based on adapted method of Aebi,[20] glutathione peroxidase activity[21] and superoxide dismutase activity.[22] Lipid peroxidation was assayed by the formation of thiobarbituric acid reactive substances.[23]

Histopathological studies

The fixed liver tissues was dehydrated in graded (50%-100%) alcohol, embedded in paraffin, cut into 4-5 μm thick sections and stained with hematoxylin-eosin. The sections were evaluated for the pathological/rejuvenative changes in the hepatocytes.

Statistical analysis

Data analysis were done by one way analysis of variance, followed by Bonferroni's multiple comparison test and results were expressed as mean of three/six replicates (*in vitro* and *in vivo* assays respectively) ± SEM using Graph pad (Graph Pad software, San Diego, CA, USA). Statistical significance was considered at $P < 0.05$.

RESULTS

In vitro assays

The scavenging activities of the extracts on the evaluated *in vitro* antioxidant assays are presented in Table 1. Judging by the half maximal inhibitory concentration (IC_{50}) values, water extract was the most potent in scavenging DPPH, hydroxyl, and superoxide anion radicals when compared with other extracts and standards. For the nitric oxide and ABTS inhibitions, best effects were elicited by ethanolic and hydroethanolic extracts respectively. With the exception of hydroethanolic extract, others revealed good activity in the metal chelating activity. A steady reducing power effect was also exhibited by the hydroethanolic extract of D. anomala compared with vitamin C (Figure 1). The hydroethanolic extract had the highest amount of total antioxidant and total phenolic contents, while the highest amount of flavonoids was found in the ethanolic extract (Table 2).

Feed and water intake

As observed in this study (result not shown), there was no significant ($P > 0.05$) difference in the feed and water intake on the animals of all the treated groups compared to control.

Body weight

The effect of DARE on body/organ weight of rats is shown in Table 3. In both study, CCl_4 significantly ($P < 0.05$) reduced the body weight of the rats when compared with normal control, pre-treatments and treatment with DARE enhances the body weight gain of the animals. The observed effect was also reflected in the average weight of the various excised organs.

Haematological parameters

Table 4 showed the effect of DARE on haematological parameters. In the both study, CCl_4 raised the level of red blood cells (RBC) and haemoglobin, while it significantly ($P < 0.05$) reduced the concentration of neutrophils, lymphocytes, monocytes and WBC (though indifferent in prophylactic study) compared to normal control. These alterations were significantly ($P < 0.05$) improved towards normal following treatment with DARE and silymarin. Other haematological indices were not affected in the two studies.

Lipid profile

The effect of DARE on lipid profile is shown in Table 5. In the two studies, the significantly ($P < 0.05$) increased levels of triglycerides, total cholesterol and LDL-c coupled with lowered concentration of HDL-c in the hepatotoxic rats were significantly ($P < 0.05$) moderated towards normal following treatment with DARE and silymarin.

Table 1 *In vitro* antioxidant capability of different extracts of Dicoma anomala root ($n = 3$, $\bar{x} \pm s$)

Assay	Extracts (IC_{50} μg/mL)					
	Water	Ethanol	Hydro-ethanol	Methanol	Gallic acid[a]	Vitamin C[β]
DPPH	15.200±0.030[ab]	336.900±41.060[ab]	32.490±0.610[b]	109.300±0.310[a]	33.930±0.400	71.490±0.760
Metal chelating	8.500±0.290[ab]	6.100±0.100[ab]	278.000±0.710[ab]	3.500±0.220[ab]	69.370±9.170	116.100±3.850
Hydroxyl radical	11.700±0.100[ab]	160.900±10.850[ab]	21.710±0.150[ab]	44.830±0.580[ab]	93.770±0.840	280.800±23.910
Nitric oxide	47.000±1.800[a]	0.770±0.070[ab]	39.000±0.130[a]	58.000±1.700[ab]	30.020±1.560	38.000±0.030
Superoxide anion	0.840±0.050[a]	21.000±0.720[ab]	2.900±0.000[a]	0.890±0.140[ab]	131.200±4.510	3.000±2.560
ABTS	1.200±0.100[b]	20.000±0.280[b]	0.600±0.020[b]	12.000±0.200[a]	2.500±0.050	15.560±0.340

Notes: scavenging abilities of different extracts of Dicoma anomala against 1, 1-diphenyl- 2-picrylhydrazyl radical, 2, 2- azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid, hydroxyl radical, nitric oxide, iron chelation and superoxide anion radical. Aliquots of varying concentrations (1.56 - 25 μg/mL) of the extracts (water, ethanol, hydro-ethanol, and methanol) and standards (gallic acids, vitamin C) were tested to assess the scavenging abilities of different extracts of D. anomala. DPPH: 1, 1-Diphenyl-2-picryl hydrazyl; ABTS: 2, 2- Azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid. Significantly different compared to gallic acid [a]$P < 0.05$; compared to βvitamin C, [b]$P < 0.05$.

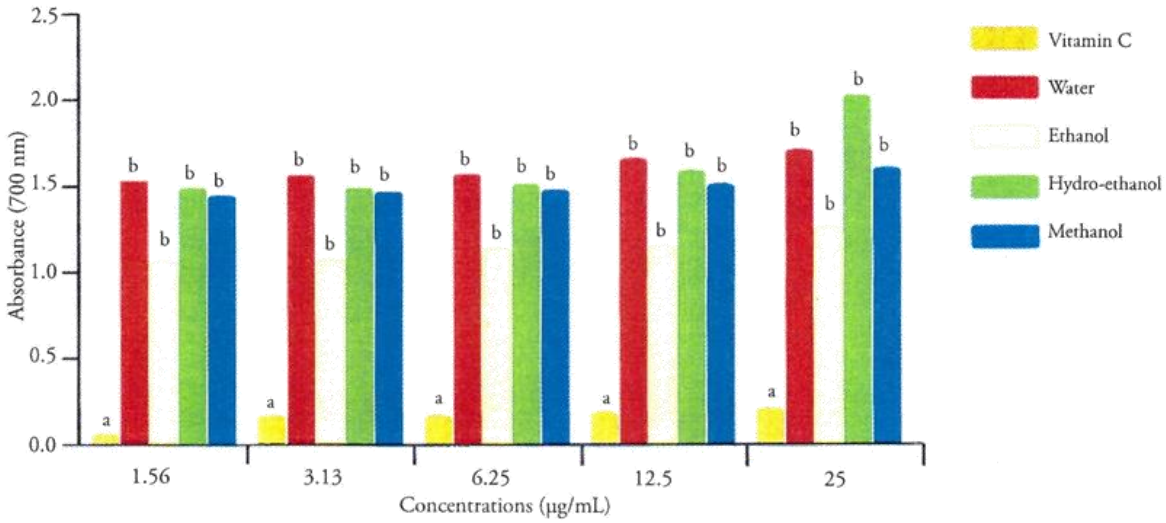

Figure 1 Reducing capabilities of different extracts of Dicoma anomala roots
Values are expressed as means ± standard error of mean (SEM) of triplicate determinations. Bars (or groups) bearing superscript (a, b) for each concentration are statistically significant ($P < 0.05$) compared to the control (vitamin C). Aliquots of varying concentrations (1.56 - 25 µg/mL) of the extracts (water, ethanol, hydro-ethanol, methanol) and standard (vitamin C) were used to assessed the reducing capabilities of D. anomala.

Table 2 Yield, total antioxidant activity and quantitative phytochemical contents of D. anomala root extracts ($n = 3$, $\bar{x} \pm s$)

Extracts	Yield (%)	Total antioxidant (mg GAE/100 g extract)	Total phenols (mg GAE/100 g extract)	Total flavonoids (mg QUE/100 g extract)
Water	23.04	137.20±1.95^a	254.00±0.38^a	46.33±0.14^a
Ethanol	10.97	153.80±3.66^b	145.90±0.19^b	61.24±0.23^b
Hydro-ethanol	36.63	213.40±0.99^c	426.80±0.73^c	50.94±0.14^c
Methanol	28.50	166.90±3.61^d	316.00±0.51^d	56.89±0.14^c

Notes: values with different superscripts (a, b, c, d) along the same column for each parameter are significantly different ($P < 0.05$) compared to each other. GAE: Gallic acid extract; QUE: quercetin extract. Varying concentrations (1.56-25 µg/mL) of the extracts (water, ethanol, hydro-ethanol and methanol) and standards (Gallic acids, Vitamin C) were used to assess the scavenging abilities of different extracts of D. anomala.

Effect on liver function indices and antioxidant marker parameters

Table 6 and 7 present the effect of DARE on liver function parameters and the antioxidant status of the animals, respectively. There was no significant difference ($P > 0.05$) in total bilirubin and total protein levels in both studies. Similarly results from both studies showed that CCl_4 administration significantly ($P < 0.05$) increase the tissue level of TBARS as well as serum activities of alanine transaminase (ALT) and aspartate amino transferase (AST), while the tissue activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were significantly reduced when compared with the normal control. Treatment with different doses of DARE reversed these trends and the effect noticed competed well with silymarin.

Effect on organ histology

The effect of DARE on the microscopic examination of the liver is shown in Figure 2. As observed, 500 mg/kg DARE was able to restore the effect of CCl4 induced hepatotoxicity in the curative study.

DISCUSSION

Plant phenolics are major group of compounds with high antioxidant activity, which is attributed to their ability to absorb, neutralize and quench the activity of free radicals.[24] The highest activity of water extract observed in most of the *in vitro* antioxidant tested assays could possibly suggest the polar nature of bioactive compounds in D. anomala, thus, suggesting the choice of aqueous extract for the hepatoprotective study. The high phenolic content observed could be attributed to the presence of hydroxyl group attached to the aromatic ring structures which confers strong activity to the compounds thus quench the effect of free radicals.[25]

The haematopoietic profile is an important index for determining the physiological and pathological status of humans and animals.[26] The significant increase in red blood cells (RBC) and haemoglobin of the hepatotoxic rats when compared with normal control and their subsequent reduction in the treatment groups was an indication of the extracts' capability to restore RBC's morphology and to prevent alteration in its osmotic fragility. However, increase in white blood cells (WBC) and its related indices in the curative treat-

Table 3 Effect of DARE on body-organ weights of Wistar rats ($n = 6$, $\bar{x} \pm s$)

Parameter	Normal control	Hepatotoxic controls		Curative Silymarin	DARE (mg/kg bw)		
		Curative	Prophylactic		125	250	500
Initial body weight (g)	135.100±1.630	134.000±1.750	135.500±2.740	136.600±1.800	144.900±3.340	145.700±5.810	150.100±5.790
Final body weight (g)	207.400±7.890	171.700±5.730[a]	189.400±3.370	174.900±1.430[a]	227.700±3.280[b]	223.600±4.400[b]	224.400±4.720[b]
% Weight changes	53.500	28.100[a]	38.000[a]	28.000[a]	57.100[ab]	53.500[b]	49.500[b]
Liver weight (g)	8.360±0.010	6.240±0.010[a]	6.680±0.010[a]	6.670±0.010[a]	7.870±0.010[ab]	8.930±0.010[ab]	9.150±0.010[ab]
Heart weight (g)	0.790±0.010	0.630±0.010[a]	0.740±0.010[a]	0.770±0.010[b]	0.800±0.010[b]	0.800±0.010[b]	0.750±0.010[ab]
Spleen weight (g)	0.610±0.020	0.570±0.010[a]	0.480±0.010[a]	0.570±0.010[a]	0.680±0.020[ab]	0.670±0.010[ab]	0.530±0.010[a]
Kidney weight (g)	1.690±0.010	1.200±0.000[a]	1.230±0.010[a]	1.340±0.010[a]	1.480±0.000[a]	1.610±0.010[ab]	1.570±0.000[a]
Lungs weight (g)	1.620±0.050	1.520±0.010[a]	1.620±0.010	1.520±0.010[a]	1.970±0.010[a]	1.390±0.010[a]	1.460±0.020[a]

Parameter	Prophylactic Silymarin	DARE (mg/kg bw)		
		125	250	500
Initial body weight (g)	141.100±2.420	154.200±3.110	146.500±3.550	149.500±6.780
Final body weight (g)	189.200±3.150	243.200±4.160[ab]	236.100±8.390[ab]	240.600±9.580[b]
% Weight changes	34.100[ab]	57.700[b]	61.200[b]	60.900[ab]
Liver weight (g)	7.040±0.010[a]	9.290±0.010[ab]	9.160±0.010[ab]	9.740±0.010[ab]
Heart weight (g)	0.740±0.010[a]	0.850±0.010[a]	0.770±0.010	0.800±0.010
Spleen weight (g)	0.710±0.010[ab]	0.570±0.010[a]	0.600±0.000[b]	0.650±0.010[ab]
Kidney weight (g)	1.660±0.000[ab]	2.190±0.000[ab]	2.200±0.000[ab]	1.930±0.010[ab]
Lungs weight (g)	1.450±0.050[a]	2.030±0.010[a]	1.660±0.010[a]	1.400±0.020[a]

Notes: the curative and prophylactic treatments with D. anomala aqueous extract enhances the body weight changes of CCl_4-induced hepatotoxic rats. Normal control and the hepatotoxic control groups received 1 mL of distilled water while the treatment groups received 1 mL of silymarin (100 mg/kg bw) and DARE prepared in distilled water. DARE: dicoma anomala aqueous roots extract. Significantly different compared to normal control, [a] $P < 0.05$; hepatotoxic controls, [b] $P < 0.05$.

ments reflected non-toxic nature and leucocytic tendency of the extract.

Derangements in metabolism and oxidation of lipid molecules have been implicated in the etiopathogenesis and progression of human diseases.[27] Elevated levels of lipids in serum, particularly cholesterol linked with generation of reactive oxygen species (ROS) are vital in the development of atherosclerosis and coronary artery disease. Moreover, increased risk of atherosclerosis arising from elevated levels of all lipids except HDL-c has been reported.[28] In the present study, DARE suggests hypolipidemic activity as the extracts reduces plasma cholesterol, LDL-c and triglycerides following the curative and prophylactic treatments, this might suggest the beneficial effect (s) of the plant in reducing plasma lipid profiles.

Liver, the largest organ in the body is concerned with the metabolism of foreign compounds, which makes it susceptible to many diseases such as hepatitis, cirrhosis, liver cancer caused due to exposure to various environmental pollutants, drugs such as carbon tetrachloride, paracetamol, thioacetamide etc., thereby resulting in the formation ROS ultimately damaging the liver.[29]

CCl_4 is a hepatotoxin whose action has been attributed to its metabolism by cytochrome P_{450} to trichloromethyl radical (CCl_3-). CCl_3 is not only toxic but act as free radical initiators which increase the hepatic lipid peroxidation products and thus facilitates liver damage. An obvious sign of liver damage is leakages of cellular enzymes via the hepatocytes' membrane into the bloodstream[30] thereby increasing the level of these enzymes in the serum. Estimation of these enzymes in the serum is an important marker to the extent and types of damage on the liver.[31] In this study, the significantly increased serum activities of CCl_4 treated rats was an obvious indication of liver damage. The normal-

Table 4 Effects of DARE on haematological profile of Wistar rats ($n = 6$, $\bar{x} \pm s$)

Parameter	Normal Control	Hepatotoxic controls		Curative Silymarin	DARE (mg/kg)	
		Curative α	Prophylactic β		125	250	500
RBC	6.920±0.010	8.130±0.010[a]	7.070±0.010[a]	7.910±0.010[ab]	7.770±0.010[ab]	7.630±0.010[ab]	7.920±0.010[ab]
Haemoglobin	14.500±0.060	15.800±0.060[a]	14.000±0.010	15.700±0.060[a]	15.200±0.060	14.800±0.060[ab]	15.300±0.060
Haematocrit	0.500±0.010	0.540±0.010	0.470±0.010	0.530±0.010	0.530±0.010	0.520±0.010	0.540±0.010
MCV	72.000±0.580	67.000±0.580	67.000±0.580	67.000±0.580	68.000±0.580	68.000±0.580	68.000±0.580
MCH	21.000±0.580	20.000±0.580	20.000±0.580	20.000±0.580	20.000±0.580	19.000±0.580	19.000±0.580
MCHC	29.000±0.580	29.000±0.580	30.000±0.570	30.000±0.580	29.000±0.570	29.000±0.570	29.000±0.570
RDW	12.400±0.060	12.400±0.060	11.300±0.060[a]	11.500±0.060[ab]	12.200±0.060	13.500±0.060[ab]	13.230±0.090[ab]
WBC	10.800±0.060	8.200±0.060[a]	10.400±0.060	10.000±0.580[b]	10.200±0.060[b]	13.200±0.060[a]	11.500±0.060[b]
Neutrophils	1.320±0.010	1.160±0.010[a]	1.960±0.010[a]	1.150±0.010[a]	2.090±0.010[ab]	2.160±0.010[ab]	1.790±0.010[ab]
Lymphocytes	9.170±0.010	6.800±0.010[a]	8.040±0.010[a]	8.330±0.010[ab]	7.660±0.010[ab]	10.530±0.010[ab]	9.370±0.010[ab]
Monocytes	0.210±0.010	0.120±0.010[a]	0.270±0.010[a]	0.330±0.010[ab]	0.300±0.010[ab]	0.340±0.010[ab]	0.200±0.010
Eosinophils	0.080±0.010	0.080±0.010	0.090±0.010	0.150±0.010[a]	0.100±0.050	0.080±0.010	0.080±0.010
Basophils	0.040±0.010	0.040±0.010	0.050±0.010	0.040±0.010	0.050±0.010	0.050±0.010	0.060±0.010
Platelet	1000.000±0.570	1193.000±0.580[a]	1321.000±0.580[a]	1133.000±0.570[ab]	1189.000±0.580[a]	1287.000±0.580[ab]	1198.000±0.580[a]

Parameter	Prophylactic Silymarin	DARE (mg/kg)	
		125	250	500
RBC	7.170±0.010[a]	8.090±0.010[ab]	8.030±0.010[ab]	6.620±0.010
Haemoglobin	13.700±0.050	15.900±0.060[ab]	15.700±0.060[ab]	13.000±0.580[ab]
Haematocrit	0.480±0.010	0.540±0.010	0.540±0.010	0.470±0.010
MCV	66.000±0.580	67.000±0.580	67.000±0.580	70.000±0.580[a]
MCH	19.000±0.580	20.000±0.580	20.000±0.580	20.000±0.580
MCHC	29.000±0.570	29.000±0.570	29.000±0.570	28.000±0.580
RDW	11.900±0.060[ab]	12.500±0.060[b]	12.200±0.060	11.800±0.060[a]
WBC	7.000±0.580[ab]	10.900±0.060	10.000±0.580	12.000±0.580[b]
Neutrophils	1.490±0.010[a]	3.890±0.010[ab]	3.440±0.010[ab]	2.470±0.010[ab]
Lymphocytes	5.290±0.050[ab]	6.310±0.010[ab]	6.070±0.010[ab]	9.180±0.010
Monocytes	0.110±0.010[ab]	0.560±0.010[ab]	0.380±0.010[ab]	0.260±0.010[ab]
Eosinophils	0.090±0.010	0.100±0.010	0.070±0.010	0.050±0.010
Basophils	0.010±0.010	0.050±0.010	0.050±0.010	0.040±0.010
Platelet	742.000±0.580[ab]	1022.000±0.580[b]	1113.000±0.580[ab]	1213.000±0.580[a]

Notes: curative and the prophylactic treatments with DARE affects the haematological profiles of CCl_4-induced hepatotoxic rats during the 15-day study. Normal control and the hepatotoxic control groups received 1 mL of distilled water while the treatment groups received 1 mL of silymarin (100 mg/kg bw) and DARE prepared in distilled water. Significantly different compared to normal control [a]$P < 0.05$; compared to hepatotoxic controls [b]$P < 0.05$. RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell width; WBC: White blood cells; DARE: dicoma anomala aqueous roots extract.

Table 5 Effect of DARE on lipid profiles of experimental Wistar rats ($n = 6$, $\bar{x} \pm s$)

Parameter	Normal Control	Hepatotoxic controls		Curative Silymarin	DARE (mg/kg)			Prophylactic Silymarin	DARE (mg/kg)		
		Curative	Prophylactic		125	250	500		125	250	500
Triglycerides (mmol/L)	1.360±0.210	1.950±0.350[a]	1.540±0.100[a]	0.840±0.110[ab]	1.480±0.010[ab]	1.550±0.160[ab]	1.750±0.710[ab]	0.420±0.410[a]	0.650±0.520[ab]	0.730±0.010[ab]	0.930±0.230[ab]
HDL-c (mmol/L)	0.800±0.050	0.400±0.020[a]	0.300±0.010[a]	0.800±0.000[b]	0.700±0.100[ab]	0.750±0.010[b]	0.790±0.010[a]	0.420±0.220[a]	0.690±0.010[ab]	0.700±0.110[ab]	0.700±0.010[ab]
LDL-c (mmol/L)	0.600±0.010	0.750±0.110[a]	0.800±0.120[a]	0.500±0.000[a]	0.400±0.010[ab]	0.500±0.010[ab]	0.600±0.110[b]	0.500±0.100[ab]	0.400±0.010[ab]	0.500±0.110[ab]	0.400±0.120[ab]
Total cholesterol (mmol/L)	1.400±0.310	1.900±0.010[a]	1.700±0.010[a]	1.300±0.010[ab]	1.400±0.010[b]	1.500±0.010[ab]	1.600±0.010[ab]	1.200±0.010[ab]	1.100±0.010[ab]	1.200±0.010[ab]	1.300±0.010[ab]

Notes: the curative and the prophylactic treatments with DARE restored the blood lipids profiles of the CCl_4-induced hepatotoxic rats towards normal control. Normal control and the hepatotoxic control groups received 1 mL of distilled water while the treatment groups received 1 mL of silymarin (100 mg/kg bw) and DARE prepared in distilled water. Significantly different compared to normal control [a]$P < 0.05$; compared to hepatotoxic controls [b]$P < 0.05$. HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol; DARE: dicoma anomala aqueous roots extract.

Table 6 Effect of DARE on serum enzymes and liver function indices of experimental rats ($n = 6$, $\bar{x} \pm s$)

Parameter	Normal Control	Hepatotoxic controls		Curative Silymarin	DARE (mg/kg)			Prophylactic Silymarin	DARE (mg/kg)		
		Curative	Prophylactic		125	250	500		125	250	500
AST (IU/l)	210.00±1.16	237.00±1.15[a]	291.00±1.16[a]	205.00±0.58[ab]	201.00±1.16[ab]	178.00±0.58[ab]	186.00±0.58[ab]	368.00±0.58[ab]	193.00±1.16[ab]	185.00±0.57[ab]	178.00±0.58[ab]
ALT (IU/l)	71.10±2.86	147.50±17.18[a]	169.00±5.33[a]	57.60±27.72[b]	61.50±18.46[b]	37.00±10.22[b]	59.10±8.85[b]	17.80±5.41[b]	21.40±17.13[b]	67.20±13.40[b]	75.70±33.60[b]
Total Protein (g/L)	61.00±0.58	63.00±0.58	62.00±0.57	62.00±0.57	61.00±0.58[b]	63.00±0.58	61.00±0.58[b]	62.00±0.57	61.00±0.58	61.00±0.58	63.00±0.58
Bilirubin (μmol/L)	2.00±0.58	2.00±0.58	2.00±0.58	2.00±0.58	2.00±0.58	2.00±0.58	2.00±0.58	2.00±0.58	2.00±0.58	2.00±0.58	2.00±0.58

Notes: treatment and pre-treatments with DARE reverses the elevated activity of the liver serum enzymes during the 15-day experimental study. Normal control and the hepatotoxic control groups received 1 mL of distilled water while the treatment groups received 1 mL of silymarin (100 mg/kg bw) and DARE prepared in distilled water. Significantly different compared to normal control [a]$P < 0.05$; compared to hepatotoxic controls [b]$P < 0.05$. AST: aspartate transaminase; ALT: alanine transaminase; DARE: dicoma anomala aqueous roots extract.

ization in the activities of these enzymes in both treatments with DARE is indicative of its potential in maintaining normal functioning status of the liver. This is in agreement with the finding of Sreelatha *et al* [29] who gave similar observation following treatment of hepatotoxic rats with Coriandrum sativum extract. The total protein and bilirubin level in the serum is an indication of the state of the liver and type of damage.[32] Liver damage arising from low level of protein in the blood (hypoproteinemia) is a consequence of decrease in the synthesis of protein.[33] The insignificant decrease in serum total protein observed in our study for the hepatotoxic rats could be attributed to the damaged hepatocellular functions and the indifference in bilirubin levels compared to normal might suggest the intact state of the biliary tract.

Free radicals generated by CCl_4 damage increase hepatic lipid peroxidation through the generation of lipid peroxide products like malondialdehyde (MDA) and thiobarbituric acid reactive species (TBARS).[34] Our study showed that administration of a single dose of CCl_4 caused liver damage and oxidative stress as revealed by the increase in lipid peroxidation. This observation might have resulted from the inability of the antioxidant defense mechanisms of the rats to prevent excessive free radical formation. However, the significant attenuation in the level of TBARS in the two studies when compared with the hepatotoxic animals is suggestive of antioxidative potentials of DARE, thereby preventing hepatic free radi-

Parameters	Normal Control	Hepatotoxic controls		Curative Silymarin	DARE (mg/kg)	
		Curative	Prophylactic		125	250	500
Catalase (U/mg protein)	145.30±2.13	34.70±1.61[a]	34.20±1.52[a]	109.10±2.66[ab]	198.90±19.44[ab]	207.40±10.04[ab]	245.80±15.35[ab]
Glutathione peroxidase (U/mg protein)	100.80±4.14	12.90±2.44[a]	9.80±2.26[a]	207.00±2.66[ab]	255.90±0.85[ab]	408.00±1.96[ab]	231.30±0.11[ab]
Superoxide dismutase (U/mg protein)	121.40±2.75	30.50±1.52[a]	31.70±1.96[a]	88.80±2.50[ab]	67.30±0.54[ab]	80.30±2.20[ab]	102.00±1.61[ab]
TBARS (mM/ 100 mg tissue)	164.80±3.90	198.40±16.75[a]	194.40±1.39	102.90±6.01[ab]	122.80±18.29[ab]	115.00±2.21[ab]	114.60±2.34[ab]

Parameters	Prophylactic Silymarin	DARE (mg/kg)		
		125	250	500
Catalase (U/mg protein)	207.90±7.00[ab]	146.10±8.52[b]	373.50±2.74[ab]	386.90±2.84[ab]
Glutathione peroxidase(U/mg protein)	379.70±5.13[ab]	204.70±1.67[ab]	207.90±6.22[ab]	283.80±2.15[ab]
Superoxide dismutase(U/mg protein)	54.80±4.44[ab]	35.60±1.39[a]	50.40±2.23[ab]	70.60±1.10[ab]
TBARS (mM/100 mg tissue)	183.60±0.37[ab]	162.40±1.34[ab]	98.70±1.92[ab]	100.50±1.42[ab]

Notes: antioxidant status of the Wistar rats treated and pre-treated with DARE on CCl_4-induced hepatotoxic animals were enhanced during the 2-week study. Normal control and the hepatotoxic control groups received 1 mL of distilled water while the treatment groups received 1 mL of silymarin (100 mg/kg bw) and DARE prepared in distilled water. Significantly different compared to normal control, [a] $P < 0.05$; hepatotoxic controls, compared to [b] $P < 0.05$. TBARS: Thiobarbituric acid reactive substances; DARE: Dicoma anomala aqueous roots extract.

cal formation by directly interfering with cytochrome P_{450}.[35]

SOD is one of the major cellular defense enzymes that dismutate superoxide radical to produce H_2O_2 and oxygen. The observed reduction in the activity of SOD following the CCl_4 administration suggests oxidative stress.[36] DARE ameliorated this effect suggesting its hepatoprotective attribute. GPx is the first line defense against free radicals. It requires a cofactor for maximum efficiency. The ability of the extract to boost the reduced activity of GPx could possibly suggest the presence of elements such as zinc in the extract responsible for the increased synthesis of the enzyme. Catalases are heme-containing proteins concerned with cell protection from toxic effects of ROS. The reduction in the activity of catalase following CCl_4 administration might be an indication of impaired antioxidant status in the hepatocytes of the rats and ultimately membranal liver damage.[37] The ability of DARE to reverse and normalize this effect further corroborates the antioxidant and hepatoprotective effects of the herb. Our submissions are consistent with the findings of Coriandrum sativum L. (Apiaceae)[29] and Strychnos henningsii Gilg. (Loganiaceae)[38] where similar observations were given.

The histopathological study revealed the complete restoration of the liver integrity by DARE following CCl_4 liver injury most especially at the highest concentration of 500 mg/kg in the curative study. This corroborates the results obtained from biochemical assays and further buttresses the hepatoprotective potential of the plant.

The overall effects displayed by DARE may be ascribed to its possession of excellent antioxidant principles as revealed in the present study with respect to total flavonoid and phenol contents. These compounds have been studied to be hepatoprotective [25,32] and thus supportive of the submissions in the present study.

The results from this study revealed that DARE elicited excellent hepatoprotective activity against CCl_4 induced liver damage and may be embraced as neutriceutical in the management and treatment of liver related disorders. Further studies are suggested to isolate, purify and structurally elucidate the exact active principles responsible for the observed effects in this study.

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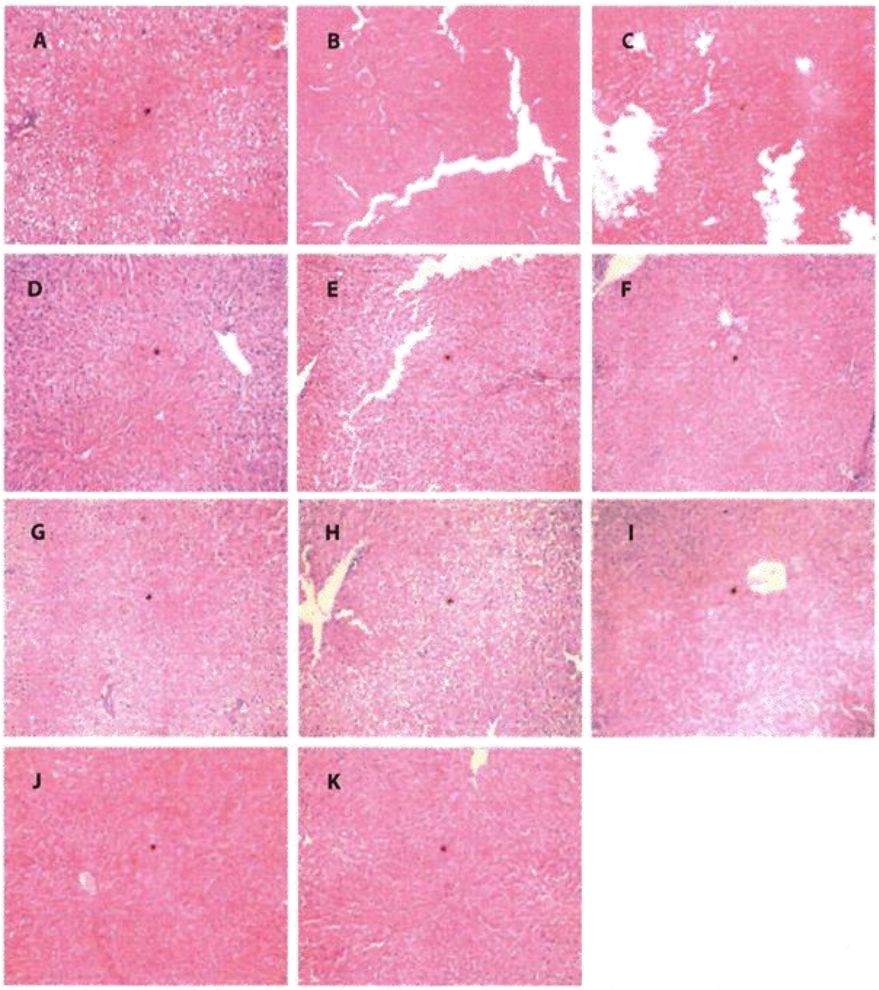

Figure 2 Histopathological micrographs of the liver in normal and hepatotoxic animals (hematoxylin and eosin staining, x 150)
A: sections normal control showing normal hepatocytes with intact portal triads; B: shows hyperchromic hepatocytes with mild cellular infilteration (curative hepatotoxic); C: reveals severe hepatic necrosis and periportal cellular infilteration (prophylactic hepatotoxic); D: shows normal liver architecture with intact portal triads (silymarin 100 mg/kg curative); E, F: revealed hyperchromic and normochromic hepatocytes with mild periportal cellular infiltration (curative 125 & 250 mg/kg DARE respectively); G: shows normal liver architecture (curative 500 mg/kg DARE); H: normochromic hepatocytes with mild vacuolar degeneration and moderate cellular infilteration (prophylactic silymarin 100 mg/kg); I: shows severe and diffuse vacuolar degeneration and hepatic necrosis (prophylactic 125 mg/kg DARE); J: revealed an hypochromic hepatocytes with moderate cytoplasmic degeneration (prophylactic 250 mg/kg DARE); K: normal hepatocytes with moderate periportal cellular infilteration (prophylactic 500 mg/kg DARE). DARE: Dicoma anomala aqueous roots extract.

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Chapter Four

Antidiabetic potential of *Dicoma anomala* Sond. (Asteraceae) root: *in vitro* and *in vivo* evaluations

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Antidiabetic potentials of *Dicoma anomala* Sond. (Asteraceae) root: *in vitro* and *in vivo* evaluations

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Abstract

Ethnopharmacological relevance: Dicoma anomala Sond (Asteraceae) is widely incorporated as a treatment for diabetes mellitus among the Basotho tribe of the Eastern Free State Province, South Africa.

Aim of the study: The study examined the antidiabetic effect of the plant via the *in vitro* inhibition of α-amylase, and α -glucosidase as well as against streptozotocin (STZ) –induced diabetic Wistar rats.

Materials and Methods: The *in vitro* antidiabetic activity of the plant is via the inhibition of α-amylase, and α -glucosidase using water, ethanol, hydro-ethanol and methanol extracts The effect of administration of aqueous root extract of *Dicoma anomala* (AQRED) at 125, 250, and 500 mg/kg bodyweight (b.w.) on water consumption, feed intake, body-weight, blood glucose, carbohydrate-metabolizing enzymes, antioxidant enzymes, glycosylated haemoglobin and lipid profiles were determined in STZ (60 mg/kg b.w.) –induced diabetic rats with comparison with glibenclamide (5 mg/kg b.w.).

Results: While all the extracts of *D. anomala* showed activity against α -amylase and α -glucosidase, water extract revealed the most effective inhibition with an IC_{50} of 101.90 and 27.41 µg/mL respectively. The water extract displayed competitive and non-competitive inhibitors of α -amylase and α -glucosidase respectively. AQRED reversed towards normal control the elevated food/water intake, blood glucose levels, lipid peroxidation, lipid profiles, glycosylated haemoglobin and activities of gluconeogenesis enzymes with a concomitant decrease in body-weight, activities of enzymatic antioxidants, glycolytic enzymes as well as the high-density lipoprotein –cholesterol level brought-about by STZ administration.

Conclusions: The result of our findings proved the antihyperglycaemic activity of the plant and therefore validates the folkloric usage of the herb.

Keywords: antihyperglycaemic, carbohydrate-metabolizing enzymes, lipid peroxidation, α-amylase, α-glucosidase

1.0 Introduction

Diabetes mellitus (DM) is a group of metabolic derangements characterized by inadequacies in the metabolism or regulation of carbohydrates, lipids or proteins caused by defects in insulin secretion, insulin action or both (Khan et al., 2009). It is not only a major cause of hyperglycaemia but results in many other complications such as hyperlipidaemia, diabetic ketoacidosis, retinopathy, nephropathy, neuropathy, cardiovascular-related diseases such as atherosclerosis etc. (Kumar and Clark, 2002; Umar et al., 2010). DM is one of the leading causes of global mortality responsible for more than 150 million deaths worldwide with increasing global prevalence (Aslan et al., 2006). It is estimated that by 2030, the number of people with the menace would be reaching 552 million (M) from 422 M in 2011 (Whiting et al., 2011; WHO, 2016).

The management of DM can be non-pharmacological (such as exercise, diet control or surgery) or pharmacological (such as usage of insulin or oral hypoglycaemic agents, OHAs such as biguanides, sulphonylureas, α-glucosidase inhibitors etc.) approaches. In recent times, due to adverse effects from conventional drugs which are not only expensive and readily unavailable to sufferers, interest in an alternative therapeutic approach of plant origin has become very germane (Mukherjee et al., 2006). This could be attributed to a number of inherent bioactive compounds which acts on various targets by numerous modes and mechanisms are able to impact therapeutic efficacy in complicated disease conditions such as diabetes and its related complications (Tiwari and Rao, 2002).

South Africa accounts for 9% of the higher plant in the world (van Wyk, 2000). As such, it is not surprising with numerous achievements in the development of therapeutic efficacies of medicinal plants origin in the treatment of many ailments have continued to gain publicity. In fact, the usage of plant extracts for medicinal purposes, particularly in the treatment of diabetes, had been advocated by World Health Organization (WHO) to be normal, less expensive with little or no side effects (WHO, 1980; Rice-Evans et al., 1995; Bailey, 2003).

Dicoma anomala Sond. (Asteraceae) commonly called fever or stomach bush (Eng.) or hloenya (South Sotho) is a prostrate, decumbent or erect perennial herb with underground tuber. Stems are erect, thinly covered with hairs and originate from a woody rootstock. Leaves are simple, alternate, stalkless, linear or narrowly lanceolatus, flower heads are terminal, solitary or in small pairs, narrow, sharply- pointed bracts and slender, white, mauve, purple or pink tubular florets (Mnengwane and Koekenoer, 2007). *D. anomala* is widely distributed in sub-Sahara Africa including South Africa in Limpopo, North-west, Gauteng, Mpumalanga, Free State, Northern Cape and KwaZulu- Natal Provinces (Mnengwane and Koekenoer, 2007). The plant had been ethnobotanically implicated for the treatment of various diseases including cold and coughs, fever, ulcers, dermatosis, venereal diseases, diarrhoea and diabetes mellitus (Gelfand, 1985; Roberts, 1990; von Koenen, 2001; Tshabalala and Ashafa, 2011). Studies have also given credence to pharmacological properties of *D. anomala* as antiplasmodial, antibacterial, anthelmintic, antiviral, antidiarrhoea, anti-inflammatory agents (Gwaza et al., 2009; Setsbogo and Mbereki, 2010; Becker et al., 2011) and antidiabetic (Balogun et al., 2016). Similarly, the gas chromatography mass spectrometry (GCMS) analysis of the aqueous extract of the plant had also been reported from our labouratory to possess rich variety of vital phytoconstituents associated with many of the above indicated biological activities of the plant (Balogun and

Ashafa, 2016a). Previous studies on the root extract of the plant revealed antibacterial, anti-inflammatory (Becker et al., 2011) antioxidant, hepatoprotective (Balogun and Ashafa, 2016a) and cardio-ameliorative (Balogun and Ashafa, 2016b) activities but little or no information in our view had been reported on the antidiabetic potential of the herb. In this study, we report on the *in vitro* antidiabetic effect as well as it's amelioration against streptozotocin –induced diabetic Wistar rats.

2.0 Materials and Methods

2.1 Chemicals

Acarbose, *p*- nitrophenyl glucopyranoside (pNPG), porcine pancreatic α-amylase, rat intestinal α-glucosidase, streptozotocin and assay kits were purchased from Sigma-Aldrich (South Africa). Glibenclamide (Daonil, Aventis Pharma, India), other chemicals and reagents used were of analytical grade.

2.2 Plant materials and preparation of extracts

The collection of rootstocks of *D. anomala* was from the wild within Phuthaditjhaba area, Qwaqwa, Maluti- A - Phofung municipality of the Free State Province, South Africa in April 2014. The identity, as well as the authentication of the plant, was done by Dr AOT Ashafa of Plant Sciences department, University of the Free State and a voucher specimen (BalMed/01/2015/QHB) was prepared and deposited in the University herbarium. Approximately 5.2 kg of fresh rootstock was washed to remove the debris, oven-dried (40 °C) and ground with a hammer mill to yield 3.063 kg of finely powdered plant material. Approximately 10 g each of the powdered sample was exhaustively extracted with 40 ml each of water, ethanol, hydro-ethanol (50 %) and methanol. While the organic extracts were filtered using Whatman No 1 filter paper, concentrated at 45°C using a rotary evaporator (Cole-Palmer, model SB-1100 Shanghai, China) to obtain dry brown crude extracts, the water extract also filtered was dried on a water bath (Memmert W600, Germany) at 45°C. The crude extracts were reconstituted in respective solvents and used to prepare 25, 12.50, 6.25, 3.13, and 1.56 µg/mL concentrations used for the *in vitro* antidiabetic as well as the mode of inhibition enzymes' assays.

Similarly, 400 g of powdered material was extracted with 4 litres of distilled water, filtered using Whatman No 1 filter paper and the filtrates pooled together, dried on a water bath to remove all trace of water. The extraction which yielded 64.37 g of brown gummy (24.435% w/w of dry plant material) crude extract was reconstituted in aqueous water to prepare the three concentrations (500, 250 and 125 mg/kg b.w.) used for the *in vivo* assays

2.3 In vitro antidiabetic potentials

The *in vitro* antidiabetic activity of different extracts of *D. anomala* was evaluated by α-amylase and α-glucosidase inhibition assays based on the methods described below.

2.3.1 α-Amylase inhibitory assay

The assay was carried out using a modified procedure of McCue and Shetty (2004). 50 µL of each extract (1.56 – 25 µg/mL) was pipetted into a test tube where 50 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was pre-incubated at 25 °C for 10 min, after which 50 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and subsequently incubated at 25 °C

for 10 min. The reaction was terminated by adding 100 µL of dinitro salicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 1 mL distilled water and the absorbance was measured at 540 nm using a spectrophotometer (WPA Biowave II, Bichrom, England). The control was prepared using the same procedure replacing the extract with distilled water while the activity of the standard was tested by replacing the extract with acarbose (1.56 – 25 µg/mL). The α-amylase inhibitory activity was calculated as percentage inhibition following the expression below:

% Inhibition = [(Abs control-Abs extract)/Abs control] x 100

Where Abs is the absorbance reading of the samples and the concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.3.2 Mode of α-amylase inhibition

The method of Ali et al. (2006) was used to determine the mode of inhibition of the root extract of *D. anomala* using the water extract with the lowest IC_{50}. In brief, 250 µL of the extract (5 mg/mL) was pre-incubated with α- amylase solution (250 µL) for 10 min at 25 ºC in one set of tubes. 250 µL of phosphate buffer (pH 6.9) was also pre-incubated with 250 µL of α-amylase solution in another set of tubes and starch solution (250 µL) of increasing concentrations (0.30 – 5.00 mg/mL) was added to both sets of reaction mixtures to start the reaction. The resulting mixture was then incubated for 10 min at 25°C and then suspended in a boiling water bath for 5 min after addition of 500 µL of DNS to stop the reaction. A maltose standard curve was used to determine the amount of reducing sugars released and converted to reaction velocities. A double reciprocal plot (1/ [V] versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was plotted. The mode of inhibition of the extract on α- amylase activity was thereafter determined using Michaelis-Menten kinetics.

2.3.3 α-Glucosidase inhibitory assay

The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim et al. (2005) using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenyl glucopyranoside (pNPG) (5 mM) was prepared in 0.02 M phosphate buffer (pH 6.9). Briefly, 50 µL of the different concentrations of the extracts (1.56 – 25 µg/mL) was pre-incubated with 100 µL of α-glucosidase (0.5 mg/mL) in a test tube. 50 µL of 5.0 mM (pNPG) as a substrate dissolved in 0.02 M phosphate buffer (pH 6.9) was afterward added to start the reaction. The reaction mixture was incubated at 37 °C for 30 min and terminated by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow coloured para-nitrophenol released from pNPG at 405 nm. Percentage inhibition was calculated thus:

% Inhibition = [(Abs control-Abs extract)/Abs control] x 100

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.3.4 Mode of α-glucosidase inhibition

The modified method of Ali et al. (2006) was used to determine the mode of inhibition of the water extract with the lowest IC_{50} value. Briefly, 50 µL of the (5 mg/mL) extract was pre-incubated with 100 µL of α- glucosidase solution for 10 min at 25 ºC in one set of tubes and α- glucosidase was pre-incubated with 50 µL of phosphate buffer (pH 6.9) in another set of tubes. 50 µL of pNPG at increasing concentrations (0.25 – 2.0 mg/mL) was subsequently added to both sets of reaction mixtures to initiate the reaction. The resulting mixtures were then incubated for 10 min at 25 °C, and 500 µL of Na_2CO_3 was added to terminate the reaction. A p-nitrophenol standard curve and converted to reaction velocities was used to

determine spectrophotometrically the amount of reducing sugars released. A double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α-glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

2.4 In vivo antidiabetic potentials

2.4.1 Experimental animals

Male Wistar rats (150 ± 10.00 g) were used in the study. They were procured from the animal facility of the University of the Free State, Bloemfontein campus, South Africa. The animals were acclimatized for one week while being housed in polypropylene cages (maintained at 25 ± 2 °C under 12 h dark/light cycle and 35 - 60% relative humidity). They were fed with standard rat feed (Epol mice cubes, Westville, Durban, South Africa) and water *ad libitum*. The husks in the cages were renewed daily to ensure proper hygiene and maximum comfort for the animals. Ethical clearance (UFS-AED 2015/0002) for handling the animals was obtained from the Interfaculty Animal Ethical Committee of the University of Free State, Qwaqwa campus prior to the commencement of the study in line with the internationally accepted guidelines of National Research Council for animal care and use (NRC, 2011).

2.4.2 Induction of diabetes and experimental protocol

Diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ (60 mg/kg b.w.) in ice-cold 0.1 M citrate buffer while the control rats were injected with citrate buffer alone. Prior induction, all the animals were fasted overnight for 16 hr. In order to balance the episodes of hypoglycaemic shock arising from STZ administration, the diabetic animals were immediately administered 5% glucose solution orally. After 72 hr of STZ-injection, the rats were fasted for 6 h and blood was taken from the tail of the rats to measure the blood glucose level using (Contour TS glucometer, Bayer Healthcare, NJ, USA). Rats with moderate diabetes having hyperglycemia (blood glucose level ranging between 250–400 mg/dL) were chosen for the experiment. The blood glucose levels outside the specified range were excluded from the study. For the experiment, a total of 36 rats (6 normal; 30 STZ-induced diabetic rats) divided into 6 groups were used. The randomly grouped rats were identified differently with varying colours so as to monitor weekly the fasting blood glucose level of the individual rat throughout the experimental period. The groupings and treatments are as follows:

Group I: as normal control and were orally given 1 mL distilled water once daily for 30 days.

Group II: STZ- induced diabetic rats served as diabetic control and were orally given 1 mL distilled water only once daily for 30 days.

Group III: Diabetic rats treated orally with 1 mL aqueous solution of glibenclamide at a dose of 5 mg/kg b.w. once daily for 30 days

Group IV: Diabetic rats treated orally with 125 mg/kg b.w. (1 mL) aqueous root extract of *D. anomala* (AQRED) once daily for 30 days.

Group V: Diabetic rats treated orally with 250 mg/kg b.w. (1 mL) AQRED once daily for 30 days.

Group VI: Diabetic rats treated orally with 500 mg/kg b.w. (1 mL) AQRED once daily for 30 days.

The food and water intakes of the animals were monitored daily while the body-weight were taken weekly during the experiment.

2.4.3 Oral glucose tolerance test (OGTT)

After 30 days of distilled water, glibenclamide and AQRED treatments, the animals were deprived of food but, only had access to water overnight. Following this on day 31, fasting blood sample was obtained from the tail of each animal dropped on glucose strips at the time intervals of 30, 60, 90 and 120 min in order to determine the oral glucose tolerance test (OGTT) using a glucometer following administration of 2 g/kg b.w. of glucose to the animals.

2.4.4 Preparation of serum and tissues

The animals were anaesthetized in a jar containing cotton wool soaked in halothane after two hours of glucose administration. As soon as the rats became unconscious, their neck region was quickly cleared of fur and skin to expose their internal jugular veins. The veins were slightly displaced (to prevent contamination of the blood with interstitial fluid) with a sterile surgical blade and the head of the rats were then tilted downwards to allow flow of blood, collected in two different tubes, i.e. one tube with anticoagulant (EDTA) for plasma, and plain tube for serum preparation. The blood in the former tube was allowed to clot for 30 min, centrifuged at 3000 rpm for 20 min using centrifuge (BHG Roto Uni, England) and the serum aspirated with a Pasteur pipette for further biochemical assays. The liver tissue was excised, weighed and a portion homogenized and centrifuged at 10 000 *g* for 15 min in ice-cold 0.1 M phosphate-buffered saline (pH 7.4), the obtained supernatant from the homogenization was prepared to a concentration of 50 mg/mL and used for the determination of specific activities of carbohydrates-metabolizing and antioxidant enzymes.

2.4.5 Assessment of biochemical parameters

The serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglycerides (TG) were determined using an auto-analyser (Sysmex, KX-21, Japan). The levels of haemoglobin and glycosylated haemoglobin were determined based on the methods of Drabkin et al. (1932) and Nayak et al. (1981) respectively.

2.4.5.1 Glycogen content estimation

The method of Morales et al. (1973) was used for the determination of glycogen content. Briefly, a wet portion of the liver tissue was cut and alkali digested in boiling potassium hydroxide (30%) for 20 min. 3 mL of ethanol was added to the tube to precipitate the glycogen, kept overnight in a freezer and then centrifuged at 3000 rpm for 40 min. The obtained precipitate was re-precipitated in ethanol after dissolving in warm water and centrifuged. The final precipitate was dissolved in boiling water for 5 min and the aliquot of the sample was mixed with anthrone reagents (4 mL; 0.2% anthrone powder in concentrated H_2SO_4) in a test tube and subjected to heat in a boiling water bath for 20 min. The intensity of the green colour obtained was read at 600 nm using WPA Biowave II (Bichrom, England) spectrophotometer and the amount of glycogen present in tissues were expressed as mg glycogen / 100 mg fresh tissue weight.

2.4.5.2 Hexokinase activity determination

The activity of hexokinase was assayed according to the method of Branstrup (1957). The assay mixture contains 7.5 mM $MgCl_2$, 3.7 mM glucose, 11 mM thioglycerol and 45 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) buffer. In brief, 0.9 mL of the

assay mixture was taken and emptied into the culvert, 0.1 mL of the tissue supernatant was subsequently added following the addition of 0.3 mL of 0.22 M ATP and mixed thoroughly. The absorbance of the mixture was taken at 340 nm using WPA Biowave II spectrophotometer (Bichrom, England) and the determination of hexokinase activity as units/min/mg of protein of tissue was recorded.

2.4.5.3 Glucose-6-phosphatase activity evaluation

The activity of the glucose-6-phosphatase was measured based on the modified method of Koide and Oda (1959). 100 µL 0.1 M glucose-6-phosphate solutions were mixed with 300 µL of 0.5 M maleic acid buffer (pH 6.5) in a test tube and incubated at 37 °C for 15 min. The reaction mixture was stopped by adding 1000 µL 10% trichloroacetic acid (TCA) and chilled in ice. The mixture was then centrifuged at 3000 rpm for 10 min, absorbance measured at the 340 nm in a WPA Biowave II spectrophotometer and expression of the glucose-6-phosphatase at the liberation of the inorganic phosphate units/min/mg of protein of tissue determined.

2.4.5.4 Fructose-1, 6-bisphosphatase activity determination

Fructose-1-6-bisphosphatase activity assay was determined following Gancedo (1971) method. The assay mixture contains 1200 µL of tris-HCl buffer (0.1 M, pH 7.0), 100 µL of substrate (0.05 M), 250 µL 0.1 M $MgCl_2$, 100 µL 0.1 M KCl, 250 µL 1 mM ethylenediamine tetraacetic acid (EDTA) solution and 100 µL enzyme homogenate. The mixture was incubated for 15 min at 37°C and 1 mL 10% TCA solution was thereafter added to stop the reaction. The reaction mixture was centrifuged at 3000 rpm for 10 min and the supernatant collected used for the phosphorous estimation. To 1 mL of the supernatant, 0.5 mL of ammonium molybdate and 0.3 mL of aminonaphthol sulfonic acid (ANSA) were added. The development of blue colouration after 20 min and measurement of the absorbance at 680 nm was achieved using WPA Biowave II (Bichrom, England) spectrophotometer. The activity of the fructose-1, 6-bisphosphatase was measured in units/min/mg of protein.

2.4.5.5 Glycogen phosphorylase activity assay

The activity of glycogen phosphorylase was determined by measuring the release of inorganic phosphate at 30 °C in a reaction mixtures containing 0.1 mL of glucose-1-phosphate (64 mM) mixed with 0.1 mL glycogen (4%) and 0.2 mL liver homogenate according to the method of Cornblath et al. (1963). The reaction was terminated by the addition of 5 mL acid molybdate reagent (5 g ammonium molybdate in 25 mL concentrated H_2SO_4 per litre) after incubation at 30 °C for 10 min. In order to measure photometrically the inorganic phosphate, 1 mL of Elon reducer was added to the mixture followed by the addition of 10 mL distilled water and incubated for 45 min at 30 °C. The activity of glycogen phosphorylase was expressed as µmole inorganic phosphate released per g of protein per min.

2.4.5.6 Catalase activity assessment

The activity of catalase (CAT) was determined based on Aebi (1984) method. Briefly, 1000 µL of the liver homogenate was mixed in a test tube with 1900 µL of phosphate buffer (50 mM, pH 7.4). The reaction mixture was initiated by the addition of 1000 µL 30 mM H_2O_2. A mixture of 2900 µL of phosphate buffer (100 mM) and 1000 µL of H_2O_2 without the liver homogenate served as the blank. The decrease in absorbance due to the decomposition of H_2O_2 was recorded at 240 nm against the blank using a spectrophotometer (WPA BIOWAVE II, England). Units of catalase were expressed as the amount of enzyme that decomposes 1 µM of H_2O_2 per min at 25°C and the activity was expressed in terms of units per milligram of proteins.

2.4.5.7 Glutathione peroxidase activity evaluation

Glutathione peroxidase (GPx) activity was assayed according to the method of Nicolas (1962) respectively. Briefly, 1000 µL of 10 mM potassium iodide solution and 1000 µL of 40 mM sodium acetate were mixed with 500 µL of the liver homogenate; the absorbance of potassium per iodide was then read using a spectrophotometer (WPA BIOWAVE II, England) at 353 nm, which indicated the amount of peroxidase. Then 20 µL of 15 mM H_2O_2 was added to the reaction mixture. This was followed by recording the change in the absorbance for a period of 5 min. Units of the peroxidase activity were expressed as the amount of enzyme required to change the optical density by 1 unit per min. The specific activity was expressed in terms of units per milligram of proteins.

2.4.5.8 Superoxide dismutase activity assessment

Adopting the procedure of Misra and Fridovich (1972), the activity of SOD was evaluated. Briefly, the assay mixture containing 500 µL of hepatic microsomal protein, 1000 µL of 50 mM sodium carbonate, 400 µL of 25 µM nitroblue tetrazolium and 200 µL of freshly prepared 0.1 mM hydroxylamine hydrochloride were mixed with 100 µL of the clear supernatant of liver homogenate. The change in absorbance at 560 nm using WPA Biowave II spectrophotometer was thereafter recorded and taken as the specific SOD activity.

2.4.5.9 Lipid peroxidation

Lipid peroxidation in the liver homogenate was determined by colorimetric measurement of thiobarbituric acid reactive substances (TBARS) as reported by Niehius and Samuelson (1968) method. Briefly, 100 µL liver homogenate was treated with 2000 µL thiobarbituric acid (TBA)-TCA- HCl (hydrochloric acid) reagent (0.37% TBA, 15% TCA and 0.25 N HCl) in ratio 1:1:1. The tubes were placed in a boiling water bath for 30 min and allowed to cool; the amount of malondialdehyde formed was evaluated by taking the absorbance of the clear supernatant at 535 nm against the reference blank. Percentage lipid peroxidation inhibition was calculated using the equation:

$$\% \text{ lipid peroxidation inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the sample and A_1 is the absorbance of the sample extract

2.5 Statistical analysis

Statistical analysis was performed using Graph Pad Prism 5 statistical package (Graph Pad Software, San Diego MA, USA). Data were expressed as means of replicate determinations ± standard error of mean (SEM) and were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Statistical significance was considered at $p < 0.05$

3.0 Results

3.1 In vitro antidiabetic assays

Table 1 revealed the result of inhibitory potentials of α -amylase and α-glucosidase by water, ethanol, hydro-ethanol and methanol extracts of *D. anomala*. It was observed that hydro-ethanol extract exhibited the best activity against α -amylase (9.00 ± 3.46 µg/mL) and water extract was most potent against α-glucosidase (27.41 ± 1.39 µg/mL) judging by the half maximal inhibitory concentration (IC_{50}) result when compared with other extracts and standard (acarbose: 86.40 ± 8.55 and 3.20 ± 0.10 µg/mL respectively). It was also observed

that there was no significant difference ($p > 0.05$) in values obtained from the extracts for both enzymes in all the tested concentrations except at the lowest concentration of 0.16 µg/mL where significant difference ($p < 0.05$) was obtained for percentage inhibition of α-amylase, although the inhibition is dose-dependent for all the extracts (Figure 1a and b). The mode of inhibition by water extract of *D. anomala* obtained through line weaver Burke plot revealed a constant Vmax value of 0.146 mM/min and increase in Km values for the extract from 0.61 to 1.24 $(mM)^{-1}$ for α-amylase suggesting a competitive inhibition while a constant Km value of 1.05 mM for both control and extract with a decrease in Vmax value between the control and the extract from 0.07 to 0.06 mM/min for α-glucosidase suggesting a non-competitive inhibition (Figure 2a and b).

3.2 Effect of AQRED on feed and water of experimental rats

The effect of AQRED on the water consumption of the experimental animals is shown in Figure 3. STZ revealed a significant ($p < 0.05$) increase in water consumption during the 30-day experimental period when compared with the control rats. Treatment with different doses of AQRED and glibenclamide subsided the excessive intake of water by the diabetic control rats. Similar trends were also witnessed in food intake of the experimental rats (Figure 4).

3.3 Effect of AQRED on body weight gain of experimental rats

It was observed that there was a significant ($p < 0.05$) reduction in body weight of diabetic control rats when compared with the normal control. Treatment with different doses of AQRED and glibenclamide enhanced the body weight of the rats with a pronounced effect exhibited by the highest concentration of the extract (Figure 5).

3.4 Effect of AQRED on blood glucose level of experimental rats

The effect of AQRED on blood sugar level of experimental animals is shown in Table 2. It was observed that STZ –induced diabetic control revealed a significant ($p < 0.05$) rise in the blood glucose level when compared with the control rats. However, oral administration of AQRED brought-about a gradual reduction in the sugar level of the animals in a dose-dependent manner during the 30-day study. It was further observed that the extract performed better than glibenclamide in reducing the glucose level (Figure 6). Further observation of the animals after a glucose overload revealed that the peak blood sugar level of the animals was achieved after an hour of glucose load. While the level of glucose for the control, glibenclamide and extract-treated rats decreased significantly ($p < 0.05$) after 2 h, that of the diabetic control rats remained essentially high after 120 min (Figure 7).

3.5 Effect of AQRED on haemoglobin and glycosylated haemoglobin level of experimental animals

Table 3 revealed the effect of AQRED on total haemoglobin and glycosylated haemoglobin levels of control and STZ –induced diabetic rats. It was observed that there was a significant ($p < 0.05$) increase in haemoglobin level with a decrease in glycosylated haemoglobin level of the diabetic animals when compared with the control. Treatment with glibenclamide and AQRED especially at 250 and 500 mg/kg b.w reversed these alterations towards normal control.

3.6 Effect of AQRED on glycogen content and carbohydrate-metabolizing enzymes

The change in the liver glycogen content of the experimental rats as well as the activities of carbohydrate-metabolizing enzymes is presented in Table 4. The results presented in this

study revealed a statistically significant ($p < 0.05$) decrease in glycogen content, hexokinase activity with resulting increase in the activities of glucose -6- phosphatase, fructose -1,6-bisphosphatase and glycogen phosphorylase of the diabetic rats when compared with the control. Treatments with the standard drug and AQRED extenuated the activities of these enzymes and improved the glycogen content at higher concentrations towards normal.

3.7 Effect of AQRED on lipid peroxidation and antioxidant enzymes

Table 5 depicts the effects of AQRED on STZ –induced diabetic rats against hepatic oxidative and lipid peroxidative parameters. STZ exerted a significant ($p < 0.05$) decrease in the activities of SOD, CAT, and GPx with a concomitant increase in the level of lipid peroxides measured by malonaldehyde (MDA) when compared with the control. Treatment with AQRED for 30 days elevated the activities of these enzymes while improving TBARS levels towards normal.

3.8 Effect of AQRED on lipid profiles of the experimental rats

The activity of AQRED on the serum levels of TC, TG, LDL-c and HDL-c against STZ –induced diabetic rats is shown in Table 6. It was observed that STZ administration significantly ($p < 0.05$) increased TC, TG and LDL-c levels with a reduction in HDL-c level when compared with the control. Treatments with AQRED at the three concentrations and Glibenclamide reversed the levels of these blood lipid parameters except the triglycerides level at lower concentrations.

4.0 Discussion

The prevalence of DM as a disease in all the countries of the world and the need to develop an important control mechanism in curbing the spread of the menace requires the prompt development of newer antidiabetic drugs preferably from natural sources since the synthetic ones are known with side effects. This is partly because these medicinal plants are effective, less expensive and most times come with fewer or no side effects. DM caused by hyperglycaemia and epitomized by constant and increased blood sugar levels is aided by the activities of a pancreatic α-amylase enzyme involved in the non-stop breakdown of starch to smaller absorbable units and an intestinal α-glucosidase enzyme which catalyse the end step digestion of polysaccharides to monosaccharides. The inhibitors of these two enzymes such as acarbose, miglitol etc. will go a long way in controlling hyperglycemia, most importantly in non-insulin dependent diabetes mellitus, NIDDM (Gin and Rigalleau, 2000). The action of these inhibitors is found in the delay of carbohydrate digestion by elongating the carbohydrates digestion time thus reducing the absorption of glucose into tissues and ultimately brings down the postprandial glucose elevation in diabetic state (Bhandari et al., 2008). As such, interest in the inhibitors of α-amylase and α-glucosidase from plant origin with minimal side effects have continued to grow since the orthodox drugs when used singly or combination with other oral hypoglycaemic agents (OHAs) displays some varying degree of side effects which includes but not limited to flatulence, diarrhoea etc.

The result obtained from the *in vitro* antidiabetic inhibition of α-amylase and α-glucosidase by the extracts of *D. anomala* showed that all the extracts are active against the two enzymes. This activity as epitomized by the water extract suggests the potential of the plant on the carbohydrates binding sites of the enzymes thus acting as an inhibitor by modulating starch digestion. Additionally, the higher IC_{50} value obtained by the water extract of *D. anomala* against α-amylase (101.90 µg/mL) and lower value (27.41 µg/mL) against α-glucosidase suggests the undesirability of α-amylase inhibitor as confirmed from high IC_{50} values. Pinto

et al. (2009) and Kazeem et al. (2013) maintained that for any hypoglycaemic agents to have an edge over the synthetic drug (such as acarbose) with side effects, it must be able to inhibit α-amylase mildly and α-glucosidase strongly (Sabiu et al., 2016). Furthermore, the mode of inhibition of α-amylase as determined from the double reciprocal plot revealed a competitive inhibition of the enzyme by *D. anomala* extract. This is indicative of the active component of the extract in competing with the substrate at binding at the active site of the enzyme thus retarding the breakdown of oligosaccharides to disaccharides (Shai et al., 2010). However, α-glucosidase mode of inhibition by the extract of the plant indicated a non-competitive inhibition which depicts the binding of the active constituent of the extract at other site asides the active site of the enzyme. Thus, suggest the possibility of the extract at binding with either free enzyme or enzyme substrate complex thereby altering the action of both (Mayur et al., 2010).

Streptozotocin (STZ) is a diabetogenic agent widely used in many animal model type of research involving diabetes. This is partly because it selectively acts on the β-cells of islets of Langerhans of the pancreas causing the breakage of nuclear DNA strands of the islets (Gandhi and Sasikumar, 2012) thereby interfering with the cellular metabolic oxidative mechanism (Papaccio et al., 2000). The choice of STZ for this study and in a number of animal studies was due to some of the manifestations it produces in animals which are similar to those of human diabetes mellitus. Additionally, the development of diabetes has been linked to oxidative stress due to the generation of free radicals. The mechanism of free radicals formation accompanied by antioxidant defense impairment is via glucose oxidation, protein glycation and oxidative degradation of protein glycation.

In the present study, an investigation into the hypoglycaemic potentials of AQRED *in vivo* was part of the assessment at evaluating the antidiabetic efficacy of the plant. The normalization of water consumption and feed intake in the diabetic rats by the AQRED is a pointer to the hypoglycaemic potentials since excessive thirst and constant food consumption are features of diabetic individuals.

An essential feature or symptoms of a diabetic state is the reduction in body weight. The reduction in body-weight in DM is associated with abnormalities in fats and protein catabolism due to excessive muscle wastage and tissue protein loss (Shirwaikar et al., 2004; Shiwarikar et al., 2006; Habibudin et al., 2008). The subsequent improvement of body-weight in the diabetic rats with AQRED suggests the possibility of the extract in restoring the muscle and tissue protein. This corroborates the report of Hussain (2002) for *Curcuma longa*, Daisy and Jeeva kani (2012) for *Cassia auriculata* and Anusuya *et al.* (2013) for *C. raktakantha.*

The low level of fasting blood glucose following its increment by STZ administration and its subsequent inhibition of elevated blood glucose level following oral glucose overload may provide an evidence on the likelihood of AQRED in inhibiting intestinal α-glucosidase enzyme thus laid credence to the antihyperglycaemic activity of the extract in maintaining blood glucose level and subsequent reduction in postprandial hyperglycaemia. Our result is in consonance with the submission of Silawat and Gupta (2013) who reported the hypoglycaemic effect of chebulic acid from *Terminalia chebula* in Wistar rats.

The non-enzymatic glycation arising from hyperglycaemia produces structural and functional impairment to both the soluble and insoluble protein molecules such haemoglobin. In diabetes, excess amount of glucose react with haemoglobin to form glycosylated haemoglobin (Sheela and August, 1992; Nabi et al., 2013) which continues to increase with

time (Bunn et al., 1978) and even induced the formation of oxygen-generated free radicals in DM (Gupta et al., 1997).

The liver is the largest internal organ and it allows for the occurrence of two complementary events (glycolysis and gluconeogenesis) for proper glucose balance in the body (Chakrabarti et al., 2003). Hexokinase, one of the vital regulatory enzymes in glycolysis (Bhavapreya and Godisamy, 2000) and the most sensitive marker of glycolytic pathway in diabetic state (O'Doherty et al., 1999) had its activity reduced in the diabetic control rats in the present investigation, this might be attributed to low synthesis of low level of mRNA coding for the enzyme and low level of insulin. However, following treatment with AQRED, the enzymatic activity was increased which could suggest that the extract activate the mRNA coding the synthesis of the enzyme. Fructose -1, 6 – bisphosphatase and glucose -6- phosphatase are similarly prominent regulatory enzymes during gluconeogenesis. In the diabetic state, the activities of these enzymes may be raised (Vats et al., 2003). The increase in the activities of these enzymes specifically in the liver of STZ –induced diabetic control may be associated with insulin insufficiency (Arathi and Sachdanandam, 2003) and overproduction of glucose (Baquer et al., 1998) due to increased synthesis or activation of the enzymes. However, the curative role of glibenclamide and AQRED in reversing the elevated activities of these enzymes may suggest the inhibition of glycolysis and gluconeogenesis or regulation of 3', 5' cyclic adenosine monophosphate (Senthikumar and Subramanian, 2008). This further buttressed the antihyperglycaemic activity of the *D. anomala*.

The emergence of DM in a way impairs the ability of the liver or muscle to synthesize glycogen. Glucose is converted to glycogen in the liver cells based on extracellular glucose concentration and the availability of insulin. The restoration of reduced level of glycogen obtained in this study for the STZ –induced diabetic rats by AQRED and glibenclamide might suggest the insulinogenic effect of AQRED. Additionally, the *in vivo* mediation of glycogen metabolism is aided by prominent multifunctional enzymes such as glycogen synthetase and glycogen phosphorylase. The reduction in glycogen stores in diabetic rats has been reported to be associated with elevated glycogen phosphorylase (GP) and reduced glycogen synthetase (Senthilkumar and Subramaniam, 2008). However, the reversal in the activity of GP observed in this study by AQRED could also buttress the insulinogenic activity which is in consonance with previous work of Ravi et al. (2003) for *Eugenia jambolana*.

The STZ administration is associated with oxidative stress resulting from overproduction of free radicals resulting in numerous toxic effects (Santhakumari et al., 2003). The reduction in the activities of CAT, SOD, GPx signifies the compromised nature of the antioxidant defense mechanism of the diabetic state which has been reported to be as a result of radical –induced inactivation of glycosylation (Rajasekaran et al., 2005). The weakening of antioxidant defense status is synonymous to enhanced lipid peroxidation. The result from our investigation revealed the reduction in the activities of CAT, SOD, and GPx with a concomitant increase in TBARS (a lipid peroxidation marker) by STZ diabetic control which signifies the compromised antioxidant status of the rats. However, AQRED administration enhanced or improved antioxidant status as revealed from decreased TBARS level of AQRED-treated diabetic rats. This corroborates the antioxidant potentials of the plant as established from previous work on this plant in our labouratory (Balogun and Ashafa, 2015).

Increased postprandial glucose level elevates the risk of cardiovascular diseases (CVD), a common cause of death for diabetic patients. The emergence of CVD has been attributed to abnormalities in lipid profiles. Insulin under normal situation activates lipoprotein lipase and hydrolyses triglycerides but in a diabetic state where insulin is deficient, lipoprotein lipase is

inactivated thus causing hypertriglyceridemia. Under the present investigation, it is revealed that STZ induction inhibited lipid metabolism as evidenced in TC, TG and LDL-c increase with a decrease in HDL-c. However, the reversal in the levels of these serum lipid profiles by AQRED may suggest improvements in insulin levels (Shirwaiker et al., 2006). It might also indicate the hypolipidemic efficacy of the plant (Balogun and Ashafa, 2016a) thus, of benefit in the management of atherosclerosis and cardiovascular-related disorders.

Conclusion

The study revealed the hypoglycaemic potential of water extract *in vitro* as well as in streptozotocin –induced diabetic rats. The activity of the extract was found to compare favourably with glibenclamide, the standard drug. The study laid credence to the use of water mostly as the solvent of extraction by the traditional healers and also validates the folkloric usage of the plant in the management of DM. Further studies are however on-going to isolate the bioactive compounds responsible for the elicited antidiabetic activity.

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Authors' contributions

Balogun and Ashafa conceptualized and designed the study. Balogun carried out biological experimentations, data collection, and analyses, as well as drafting and revising the manuscript for critical intellectual contents. Ashafa supervised the study. All authors read and approved the final manuscript for publication.

Declaration of interest

The authors report no conflicts of interest

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Figure Legend

Figure 1a: Inhibitory potential of extracts of *Dicoma anomala* against α-amylase

Figure 1b: Inhibitory potential of extracts of *Dicoma anomala* against α-glucosidase

Figure 2a: Mode of inhibition of α-amylase by water extract of *Dicoma anomala* root

Figure 2b: Mode of inhibition of α-glucosidase by water extract of *Dicoma anomala* root

Figure 3: Effect of AQRED on weekly average water consumption of the animals

Figure 4: Effect of AQRED on weekly average feed intake of the animals

Figure 5: Effect of AQRED on body weight gain of the control and diabetic rats

Figure 6: Effect of AQRED on blood glucose level of STZ –induced Wistar rats

Figure 7: Effect of AQRED on oral glucose tolerance test of control and diabetic rats

Table 1 IC_{50} values of α-amylase and α-glucosidase inhibition by root extracts of *Dicoma anomala*

Assays	Extracts (IC_{50} µg/mL)				
	Water	Ethanol	Hydro-ethanol	Methanol	Acarbose
α-glucosidase	27.41± 1.39[a]	29.37 ± 1.53[a]	29.37 ± 0.45[a]	39.62 ± 0.41[b]	3.20 ± 0.10[c]
α-amylase	101.90 ± 6.05[a]	34.16 ± 1.44[b]	9.00 ± 3.46[c]	20.70 ± 0.39[d]	86.40 ± 8.55[e]

Values are presented as mean ± SEM (n=3).

[a]Values with different superscript along the same row for the same parameter are significantly different ($p < 0.05$) to each other

Table 2 Effect of AQRED on blood glucose level of the animals

Parameters	Control	Streptozotocin (STZ)	STZ + Glibenclamide 5 mg/kg	STZ + AQRED		
				125 mg/kg	250 mg/kg	500 mg/kg
Week 0	2.62±0.16[a]	22.28±1.08[b]	32.24±0.24[c]	24.78±0.74[b]	26.32±0.93[b]	26.62±1.00[b]
Week 1	3.16±0.20[a]	23.55±1.45[b]	31.13±0.80[c]	24.20±2.00[b]	23.98±1.72[b]	24.65±1.57[b]
Week 2	3.04±0.18[a]	25.70±1.35[b]	28.30±0.85[b]	20.30±0.10[b]	21.35±0.91[b]	22.50±0.94[b]
Week 3	3.18±0.24[a]	29.10±0.93[b]	26.77±1.87[b]	18.25±0.45[c]	19.05±0.61[c]	19.90±0.61[c]
Week 4	3.36±0.14[a]	30.50±0.54[b]	22.50±2.36[b]	17.70±0.80[c]	17.48±0.45[c]	16.68±0.23[c]

Values are presented as mean ± SEM (n=6).

[a]Values with different superscript along the same row are significantly different ($p < 0.05$) to each other

AQRED: Aqueous root extract of *Dicoma anomala*

Table 3 Effect of AQRED on haemoglobin and glycosylated haemoglobin of control and STZ –induced diabetic Wistar rats

Parameters	Control	Streptozotocin (STZ)	STZ + Glibenclamide 5 mg/kg	STZ + AQRED		
				125 mg/kg	250 mg/kg	500 mg/kg
Haemoglobin[α]	15.21±0.33[a]	6.14±0.40[b]	12.35±0.20[c]	6.77±0.06[b]	9.17±0.41[d]	11.79±0.29[c]
Glycosylated haemoglobin[β]	2.19±0.04[a]	8.16±0.18[b]	3.45±0.15[c]	6.59±0.11[d]	5.29±0.12[e]	3.29±0.09[c]

Values are presented as mean ± SEM (n=6).

[a]Values with different superscript along the same row are significantly different ($p < 0.05$) to each other

AQRED: Aqueous root extract of *Dicoma anomala*

[α]Unit: mg/dL

[β]Unit: %HbA_1c

Table 4 Effect of AQRED on glycogen content and carbohydrate-metabolizing enzymes of control and STZ –induced diabetic rats

Parameters	Control	Streptozotocin (STZ)	STZ + Glibenclamide 5 mg/kg	STZ + AQRED		
				125 mg/kg	250 mg/kg	500 mg/kg
Glycogen content[α]	30.78±0.54[a]	20.02±6.54[b]	37.88±1.76[a]	20.42±4.59[b]	31.18±3.00[a]	44.99±1.33[c]
Hexokinase[β]	1.39 ±0.03[a]	0.23 ±0.04[b]	0.95 ± 0.05[c]	0.61 ± 0.03[d]	0.94 ± 0.06[c]	1.31 ± 0.03[a]
Glucose-6-phosphatase[µ]	1.05 ± 0.06[a]	1.20 ± 0.01[b]	1.04 ± 0.05[a]	1.04 ±0.00[a]	1.05 ± 0.06[a]	1.05 ± 0.03[a]
Fructose-1,6-bisphosphatase[µ]	0.97 ± 0.03[a]	1.01 ± 0.05[b]	0.94 ± 0.03[a]	0.98 ± 0.06[a]	0.96 ± 0.07[a]	0.97 ± 0.09[a]
Glycogen Phosphorylase[µ]	0.11± 0.00[a]	0.96 ±0.00[b]	0.09 ± 0.01[c]	0.08 ±0.00[c]	0.17± 0.00[d]	0.08 ± 0.00[c]

Values are presented as mean ± SEM (n=6).

[a]Values with different superscript along the same row are significantly different ($p < 0.05$) to each other

AQRED: Aqueous root extract of *Dicoma anomala*

[α]Unit: mg of glucose/ g of wet tissue

[β]Unit: µmole glucose -6- phosphate formed/hr/mg protein

[µ]Unit: µmole phosphate liberated/hr/mg protein

Table 5 Effect of AQRED on lipid peroxidation and antioxidant marker enzymes of control and STZ –induced diabetic rats

Parameters	Control	Streptozotocin (STZ)	STZ + Glibenclamide 5 mg/kg	STZ + AQRED		
				125 mg/kg	250 mg/kg	500 mg/kg
CAT$^{\alpha}$	104.10±0.52^{a}	58.89±0.55^{b}	100.80±0.48^{a}	65.56±0.72^{c}	74.20±1.47^{d}	100.80±0.74^{a}
GPx$^{\alpha}$	127.00±1.16^{a}	47.41±1.53^{b}	113.00±1.32^{a}	68.87±10.86^{c}	88.88±1.07^{d}	118.30±0.49^{e}
SOD$^{\alpha}$	88.68±0.86^{a}	24.03±0.93^{b}	55.83±0.59^{c}	32.78±1.19^{d}	42.21±1.12^{e}	72.79±1.48^{f}
TBARS$^{\beta}$	59.70 ±0.47^{a}	93.07±2.50^{b}	68.73 ±1.43^{c}	82.13±0.70^{c}	71.67 ±0.58^{c}	63.77±2.20^{a}

Values are presented as mean ± SEM (n=6).

^{a}Values with different superscript along the same row are significantly different ($p < 0.05$) to each other

CAT: catalase; GPx: glutathione peroxidase; TBARS: thiobarbituric acid reactive species; SOD: superoxide dismutase; AQRED: aqueous root extract of *Dicoma anomala*

$^{\alpha}$Unit: U/mg protein

$^{\beta}$Unit: mM/ 100 g tissue

Table 6 Effect of AQRED on lipid profiles of control and STZ –induced diabetic rats

Parameters	Control	Streptozotocin (STZ)	STZ + Glibenclamide 5 mg/kg	STZ + AQRED		
				125 mg/kg	250 mg/kg	500 mg/kg
TC$^{\alpha}$	3.29±0.13^{a}	5.37±0.09^{b}	3.75±0.03^{a}	4.18±0.09^{b}	4.16±0.03^{b}	3.53±0.06^{a}
TG$^{\alpha}$	1.20±0.05^{a}	2.80±0.06^{b}	1.70±0.12^{c}	2.37±0.04^{b}	2.05±0.03^{b}	1.30±0.15^{a}
LDL-c$^{\alpha}$	0.86±0.03^{a}	2.10±0.06^{b}	0.76±0.03^{a}	1.63±0.09^{c}	1.43±0.06^{c}	1.20±0.06^{d}
HDL-c$^{\alpha}$	1.82±0.06^{a}	0.93±0.02^{b}	1.65±0.08^{a}	1.00±0.01^{b}	1.31±0.01^{c}	1.67±0.08^{a}

Values are presented as mean ± SEM (n=6).
^{a}Values with different superscript along the same row are significantly different ($p < 0.05$) to each other
TC: total cholesterol; TG: triglycerides; LDL-c: low-density lipoprotein-cholesterol; HDL-c: high-density lipoprotein-cholesterol; AQRED: aqueous root extract of *Dicoma anomala*
$^{\alpha}$Unit: mg/dL

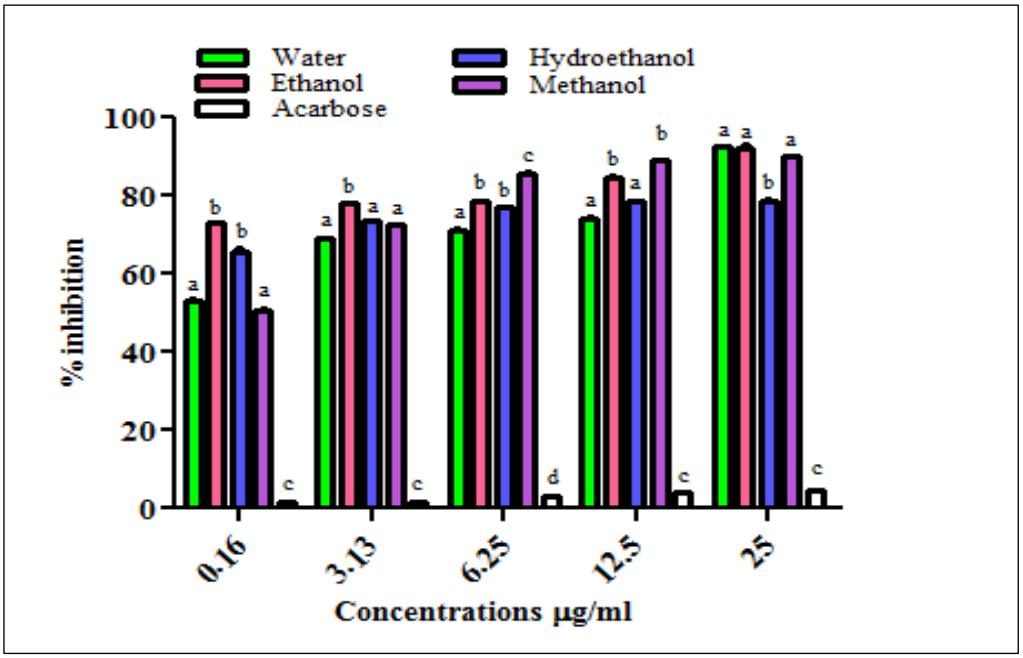
Water
Ethanol
Acarbose
Hydroethanol
Methanol
% inhibition
100
80
60
40
20
0
0.16
3.13
6.25
12.5
25
Concentrations µg/ml

Fig. 1a

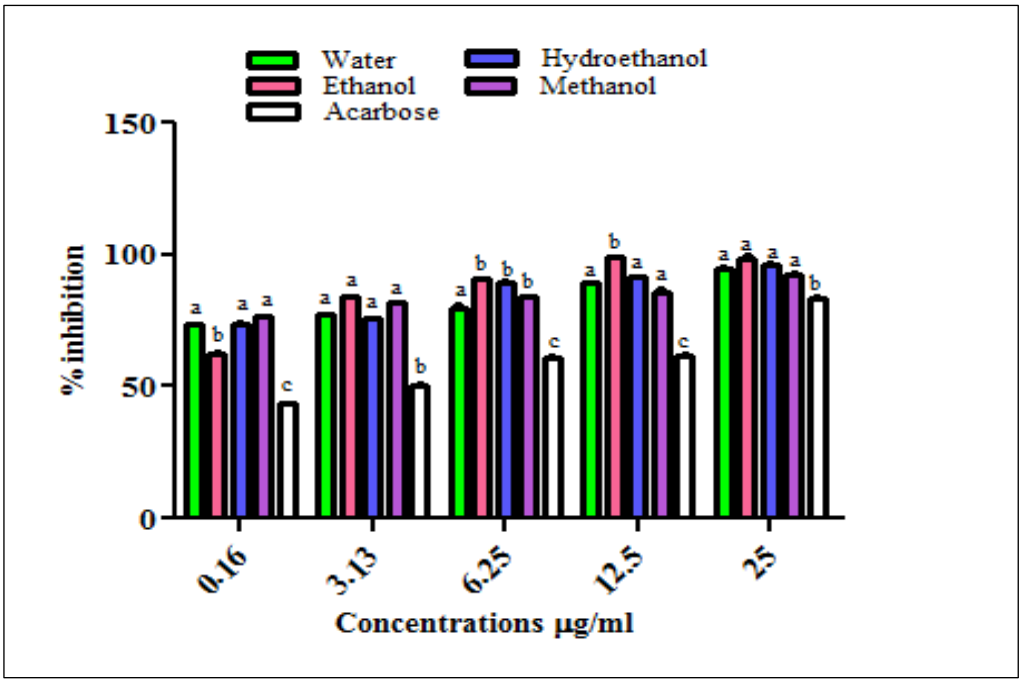
Water
Ethanol
Acarbose
Hydroethanol
Methanol
% inhibition
150
100
50
0
0.16
3.13
6.25
12.5
25
Concentrations µg/ml

Fig. 1b

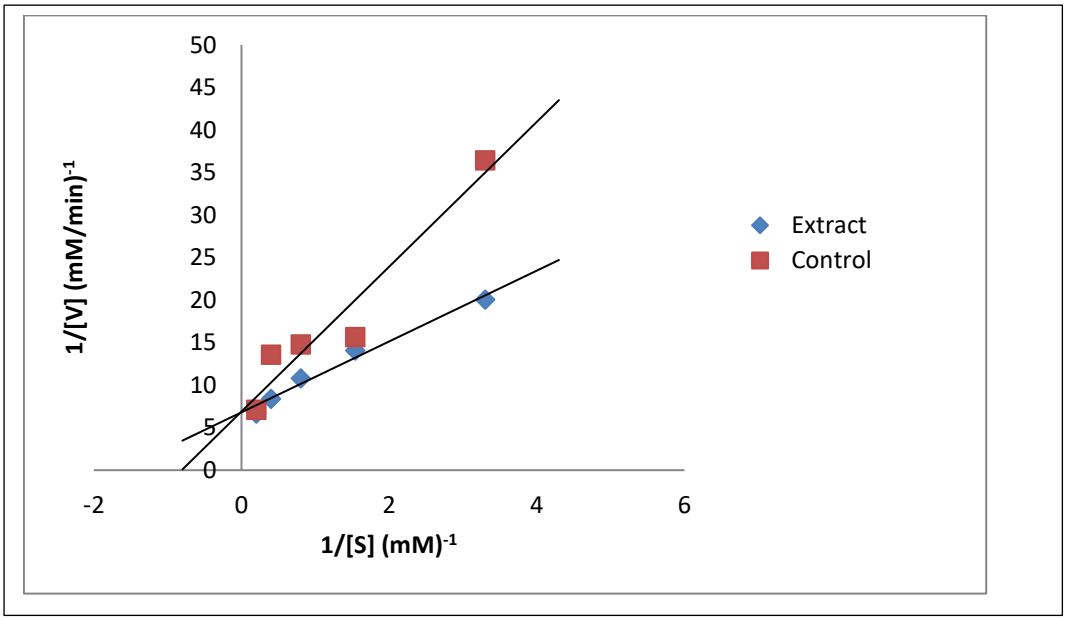
50
45
40
35
30
25
20
15
10
5
0
-2
0
2
4
6
1/[V] (mM/min)-1
1/[S] (mM)-1
Extract
Control

Fig. 2a

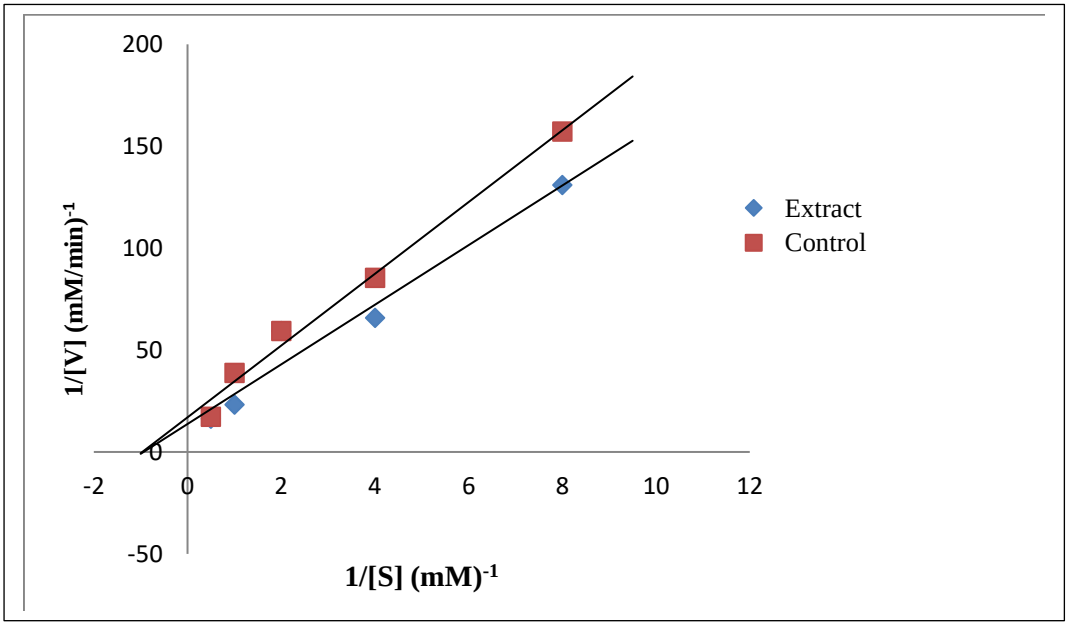
200
150
100
50
0
-50
-2
0
2
4
6
8
10
12
1/[V] (mM/min)-1
1/[S] (mM)-1
Extract
Control

Fig 2b.

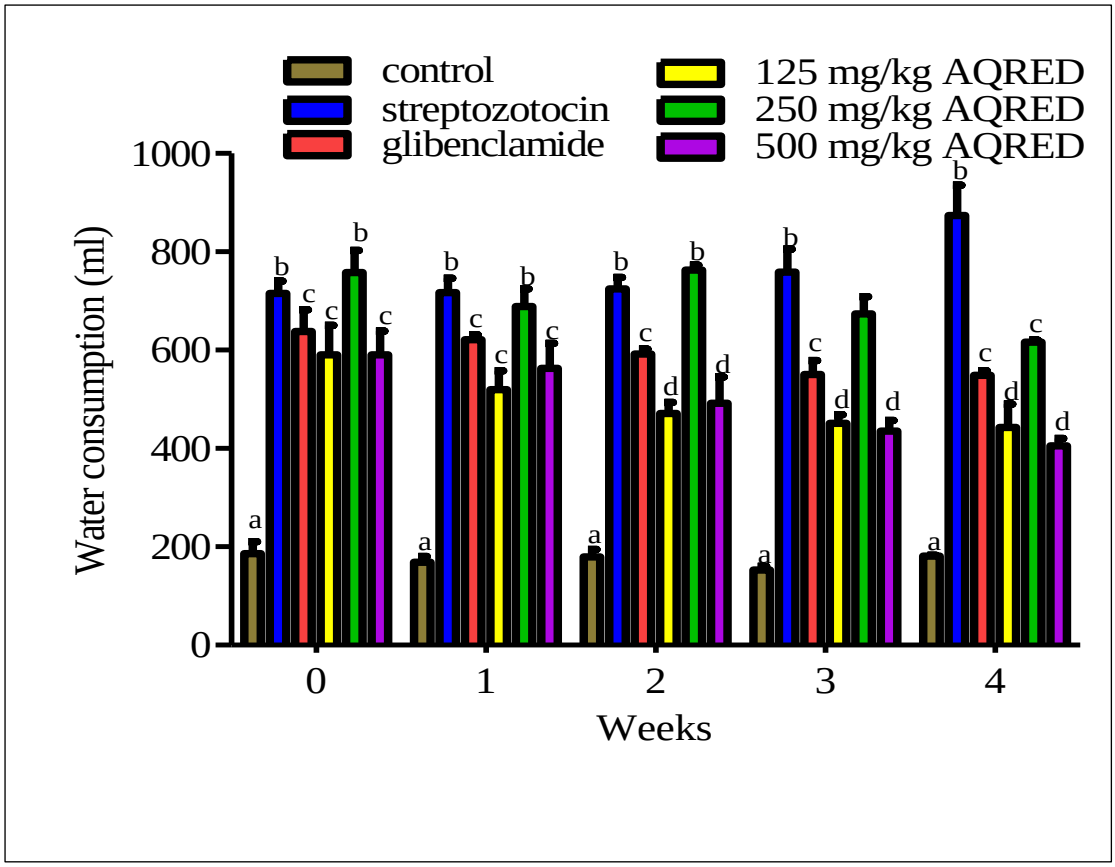
control
streptozotocin
glibenclamide
125 mg/kg AQRED
250 mg/kg AQRED
500 mg/kg AQRED
Water consumption (ml)
1000
800
600
400
200
0
0
1
2
3
4
Weeks

Fig. 3

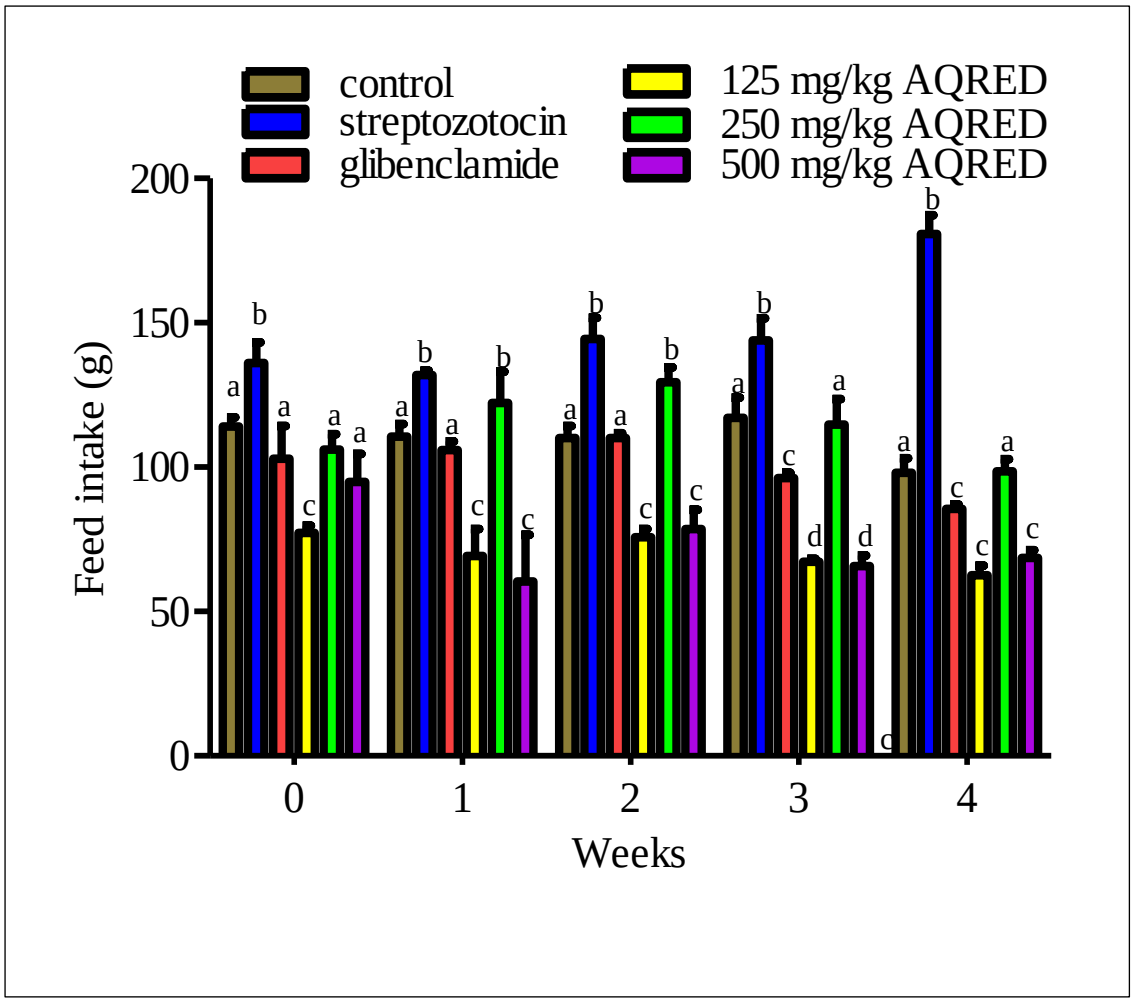
control
streptozotocin
glibenclamide
125 mg/kg AQRED
250 mg/kg AQRED
500 mg/kg AQRED
Feed intake (g)
200
150
100
50
0
0
1
2
3
4
Weeks

Fig. 4

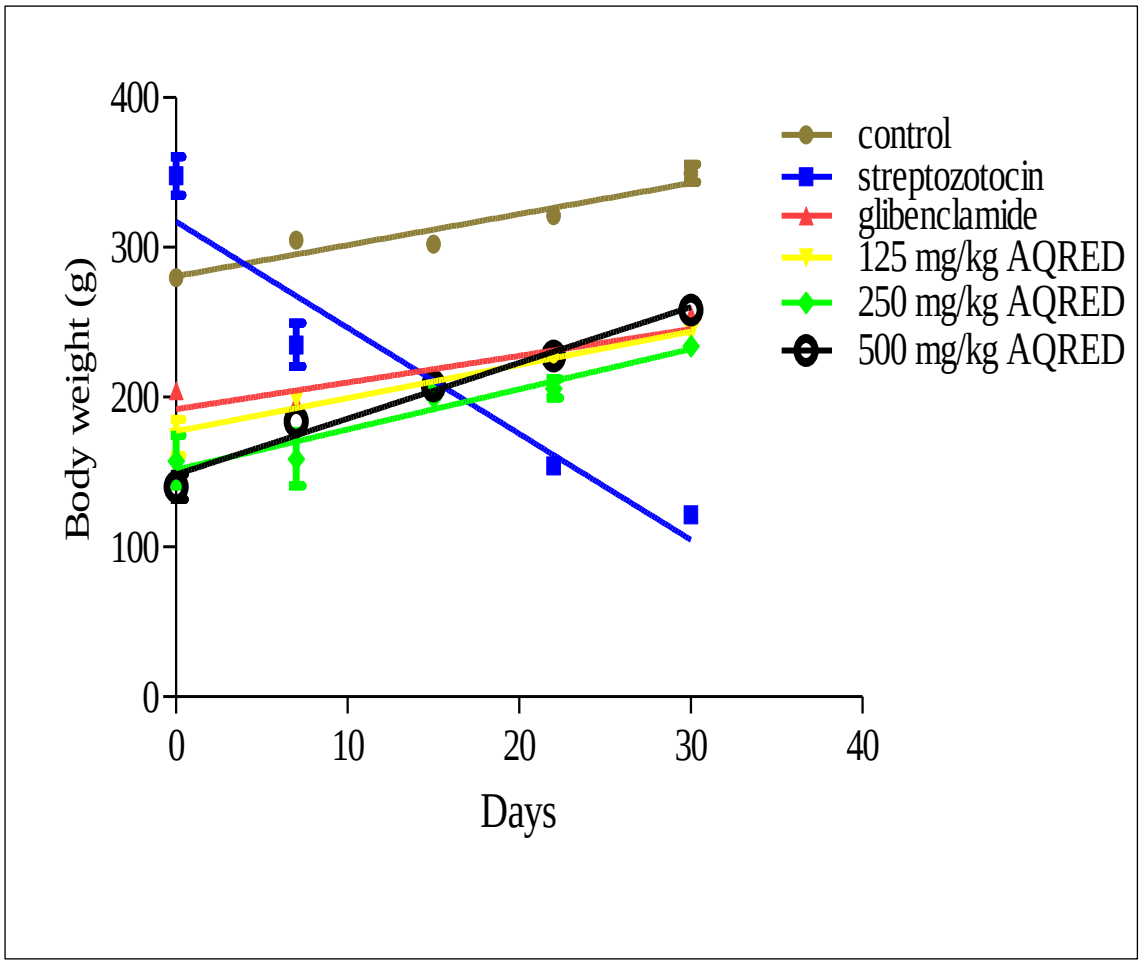
400
300
200
100
0
Body weight (g)
0
10
20
30
40
Days
control
streptozotocin
glibenclamide
125 mg/kg AQRED
250 mg/kg AQRED
500 mg/kg AQRED

Fig. 5

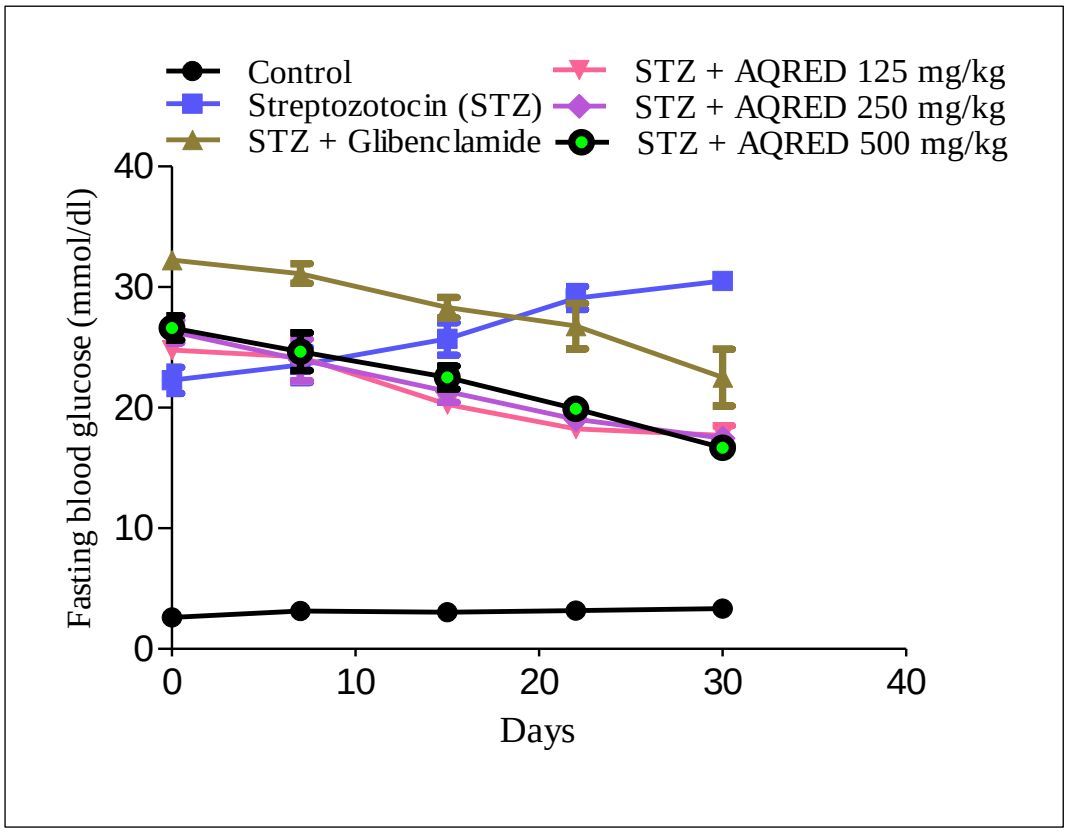
Control
Streptozotocin (STZ)
STZ + Glibenclamide
STZ + AQRED 125 mg/kg
STZ + AQRED 250 mg/kg
STZ + AQRED 500 mg/kg
Fasting blood glucose (mmol/dl)
0
10
20
30
40
Days

Fig. 6

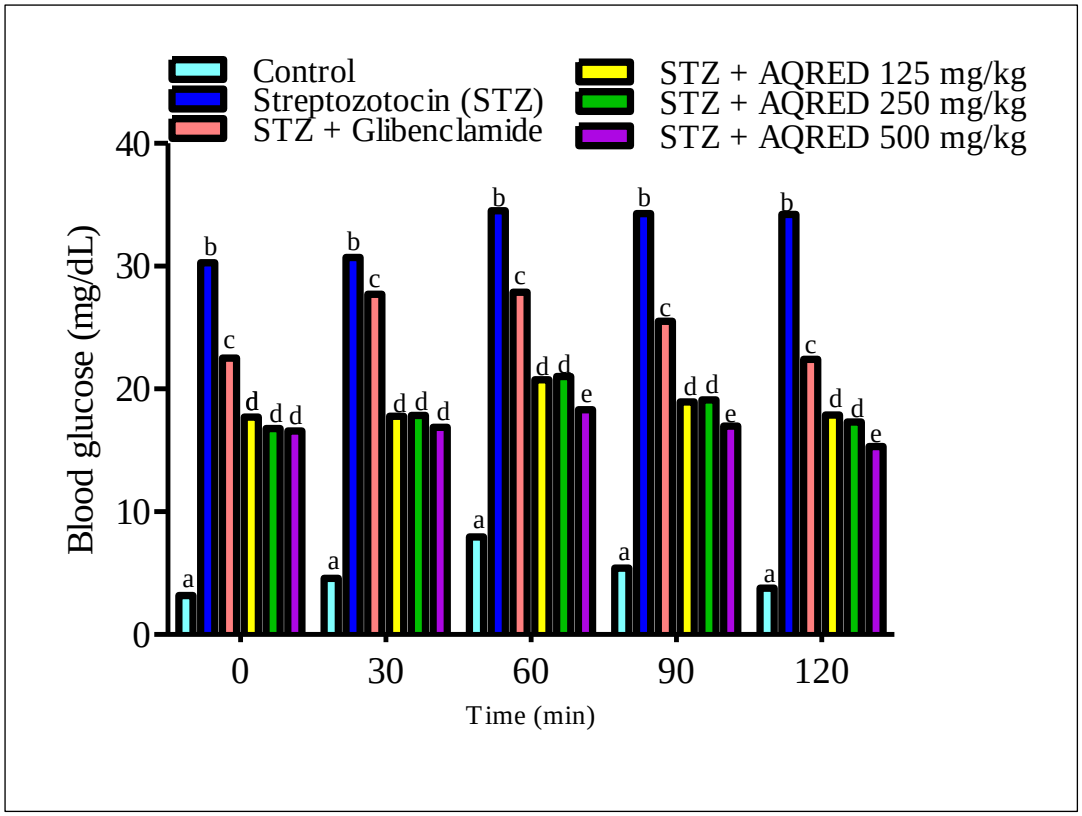
Control
Streptozotocin (STZ)
STZ + Glibenclamide
STZ + AQRED 125 mg/kg
STZ + AQRED 250 mg/kg
STZ + AQRED 500 mg/kg
Blood glucose (mg/dL)
0
10
20
30
40
0
30
60
90
120
Time (min)

Fig. 7

Chapter Five

Aqueous root extract of *Dicoma anomala* Sond. ameliorates isoproterenol–induced myocardial infarction in Wistar rats

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Original Research Article

Aqueous root extract of Dicoma anomala Sond ameliorates isoproterenol–induced myocardial infarction in Wistar rats

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Abstract

Purpose: *To evaluate the protective potentials of the aqueous root extract of Dicoma anomala (AQRED) against isoproterenol (ISP)-induced myocardial damage in Wistar rats.*
Methods: *Myocardial damage was induced in Wistar rats by isoproterenol (60 mg/kg body weight, b.w.) Various concentrations (125, 250, and 500 mg/kg b.w.) of AQRED and their effects on the rats' feed and water intake, body weight changes, serum enzymes, including aspartate transaminase (AST), alanine transaminase (ALT) creatinine phosphokinase (CPK), as well as tissue antioxidant enzymes, including catalase (CAT), glutathione peroxidase (GP) and lipid peroxidation, during a 30-day experimental period were examined.*
Results: *ISP-treated rats showed no significant ($p > 0.05$) effect on the feed, water and body weight but increased significantly ($p < 0.05$) AST, ALT, CPK and lipid peroxidation while significantly reducing CAT and GP levels ($p < 0.05$). Treatment with different doses of AQRED significantly ($p < 0.05$) reversed the activity of these enzymes and cardiac lipid peroxidation towards control levels. Histopathological examination of ISP-induced myocardial rats treated with D. anomala revealed evidence of oedema and myocardial necrosis at 125 and 250 mg/kg b.w. doses, but these alterations were ameliorated or cleared at 500 mg/kg dose, suggesting attainment of maximum efficacy.*
Conclusion: *The findings indicate the ameliorative potential of AQRED in myocardiac disease, and therefore, could be of therapeutic significance in the management or treatment of cardiac-related diseases.*

Keywords: *Antioxidant enzymes, Dicoma anomala, Lipid peroxidation, Serum enzymes*

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INTRODUCTION

Myocardial infarction (MI), the most dreaded among the various forms of ischemic heart disease is the leading cause of morbidity and mortality in developed countries [1]. It is a condition of acute necrosis of the myocardium arising from the imbalance between the coronary blood supply and myocardial demand [2]. This is mostly followed by numerous pathophysiological and biochemical changes such as lipid peroxidation, thrombosis and hyperlipidemia [3], leading to myocardium alteration. MI preceding the development of heart failure is reported to be due to antioxidant deficit and increased myocardial oxidative stress. Hence, prompt intervention with an antioxidant may be of benefit against cardiac damage [4]. In recent times, due to adverse effects from synthetic drugs, interest in conventional medicines of natural sources have continued to increase particularly in developed and developing countries such as China, United States of America. [1,5]. The use of herbal therapies against numerous diseases

including MI has been encouraged with few people doubting the effectiveness of the treatment modality [6].

Isoproterenol (ISP) is a synthetic catecholamine and a β-adrenergic agonist that causes severe stress in the myocardium, leading to an 'infarct-like' necrosis of the heart muscles [3]. ISP forms free radicals and stimulates lipid peroxidation which perhaps cause irreversible damage to the myocardial membrane [6]. Thus, a high production of reactive oxygen species (ROS) may be a unifying mechanism in ischemic injury progression and antioxidants administration may protect against ISP induced cardiac damage. ISP induced myocardial injury is a well-established model used to study the beneficial effect of arrays of drugs on cardiac dysfunction [7,8]. This is because the pathophysiological signs in animals after ISP administration are similar to myofibrillar degeneration, a typical symptom of MI in humans [9].

South Africa is noted for her rich biodiversity in indigenous plants and she account for 9 % of the higher plants globally [10]. There are has been a continued interest in the development of therapeutic efficacies of medicinal plants in the treatment of numerous ailments. Besides being readily available, affordable and with minimal adverse effect, the presence of flavonoids, vitamins and polyphenols in these plants gave credence to their therapeutic significance and is well buttressed by the WHO [11].

Dicoma anomala (Asteraceae) commonly called fever or stomach bush (English) or hloenya (South Sotho) is a prostrate, decumbent or erect perennial herb with underground tuber. *D. anomala* is widely distributed in sub-Sahara Africa including South Africa within North West, Gauteng, Mpumalanga, Free State, Northern Cape and KwaZulu- Natal Provinces [12]. Traditionally, the plant is indicated in the treatment of various diseases such as coughs, cold, fever, ulcers, dermatosis and venereal ailments linked to numerous pharmacological activities including antiplasmodial, antibacterial, anthelmintic, antiviral and anti-inflammatory [13]. Previous studies on the plant revealed the antibacterial, anti-inflammatory and antioxidant activity of the root extracts [13] but little or no information has been reported on its cardioprotective or ameliorative efficacies. In this paper, we present the report on the ameliorative potential of aqueous root extract of *D. anomala* in ISP -induced myocardial infarcted rats.

EXPERIMENTAL

Chemicals

Isoproterenol hydrochloride (ISP), assay kits such as alanine transaminase (ALT), aspartate transaminase (AST), creatine phosphokinase (CPK) were purchased from Sigma-Aldrich (South Africa). While water used was glass-distilled, other chemicals and reagents used were of analytical grades.

Plant collection and extraction

Fresh rootstocks of *D. anomala* were collected from the wild within Phuthaditjhaba area of QwaQwa, Maluti- A - Phofung municipality of the Free State Province, South Africa in April 2014. The identity of this taxon was confirmed by Dr. AOT Ashafa of the Department of Plant Sciences, UFS, QwaQwa campus and the voucher specimen was deposited in the herbarium of University of Free State, South Africa. A total of 5.2 kg of fresh rootstock was washed, oven dried (40 °C) and ground with a hammer mill to yield 3.063 kg fine powdered plant material. Of this, 200 g was extracted with 2 litres distilled water, filtered and concentrated on a water bath to yield 48.87 g of brown gummy (24.435 % w/w of dry plant material) crude extract.

Experimental animals

Male and female Wistar rats (weighing 150 - 200 g) were used in this study. They were procured from the animal facility of the University of the Free State, Bloemfontein. The animals were acclimatized for one week and maintained at (18 ± 2 °C under 12 h dark/light cycle. They were fed with standard rat feed (Epol mice cubes, Westville, South Africa) and water *ad libitum*. The husks in the cages were renewed thrice weekly to ensure proper hygiene and maximum comfort for animals. Ethical clearance for the animal studies was obtained from Institutional Interfaculty Animal Ethics Committee of the University of the Free State (no NR 02/13), prior to the commencement of the study in line with the internationally accepted guidelines of National Research Council for animal care and use [14].

Experimental design

The animals were randomly divided into six groups of 6 rats each. Group A animals served as normal control and were orally administered 1 mL normal saline for 30 days. The rats in Groups B, C, D, E and F were respectively treated with 1

mL normal saline, simvastatin (30 mg/kg b.w.) and 125, 250 and 500 mg/kg b.w. aqueous extract of *D. anomala* for 30 days via oral intubation. The rats in all the groups except group A were given ISP (60 mg/kg b.w.) [15], intraperitoneally on the first day of the experiment prior to administration of test drugs and the extracts.

At the end of the experimental period, all the rats were anaesthetized with halothane and blood was collected from the retro-orbital plexus. About 5 mL of the blood collected into a non-heparinized bottle was centrifuged at 1285 g for 10 min and the resulting serum was used for marker enzymes determination. The heart tissue was excised immediately and divided into two portions; a portion was immediately fixed in 10 % formalin for histopathological studies while the other portion was homogenized in ice-cold 0.1 mol/L Tris-HCl buffer (pH 7.2). The supernatant obtained was used for the antioxidant assays as well as tissue activities of catalase (CAT), glutathione peroxidase (GP) and the level of lipid peroxidation in terms of thiobarbituric acid reactive species (TBARS) was estimated. The daily measurement of food intake and water consumption was recorded.

Assessment of biochemical parameters

The activities of ALT, AST, and CPK in the serum were determined following the procedures described in the Sigma-Aldrich assay kits.

Determination of antioxidant parameters

Glutathione peroxidase activity in tissues was assayed according to the method of Nicholas [16] while catalase activity determined based on the adapted method of Aebi [17]. Lipid peroxidation assayed by the formation of thiobarbituric acid reactive substances according to the method of Niehaus and Samuelson [18].

Histopathological studies

A portion of the heart tissue from each group was fixed immediately in 10 % neutral formalin for a period of at least 24 h, dehydrated in graded (50 – 100 %) alcohol, embedded in liquid paraffin, cut into 4-5 µm thick sections and stained with hematoxylin-eosin. The sections were evaluated and photographed for the pathological/ rejuvenative changes in the myocardial tissue under a canon power shot S72 digital camera (x 200) attached to a light microscope (Amscope, model B409A).

Statistical analysis

Data analysis were done by one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test and results are expressed as mean ± SEM using Graph Pad Prism version 3.0 for Windows software, Graph Pad software, San Diego, California, USA. Statistical significance was considered at ($p < 0.05$).

RESULTS

Effect of AQRED on feed and water intake

The effects of oral administration of AQRED on feed and water intake is presented in Table 1. ISP had no effect on feed and water intake of the experimental rats when compared with control. A significant ($p < 0.05$) increase in average feed and water intake of the cardiotoxic group with a similar increase in food and water consumption in all the treatment groups on days 10, 20 and 30 was observed.

Effect of AQRED on body weight changes

Table 2 presents the effect of AQRED on average body weight changes of the experimental rats. As observed from the results, administration of ISP had no significant ($p > 0.05$) effect on the weight of the animals treated with different doses of AQRED and simvastatin when compared with control group.

Effect of AQRED on serum enzymes

The effect of AQRED on serum enzymes of ISP-induced cardiac damage is presented in Table 3. ISP showed no effect on the activity of ALT when compared with control. However, the ISP - mediated increased serum activities of AST, ALT, and CPK were significantly ($p < 0.05$) and dose-dependently attenuated following treatments with simvastatin and the extracts although, simvastatin tends to restore the activities of these enzymes better towards normal than the extracts except in AST

Effect of AQRED on tissue antioxidant status

Table 4 shows the effects of AQRED on tissue marker enzymes. ISP significantly ($p < 0.05$) decreased the activities of the antioxidant marker enzymes (catalase and glutathione peroxidase) while It increased the level of TBARS when compared with control. Simvastatin and the three concentrations of the plant extract significantly ($p < 0.05$) increased the activities of the enzymes

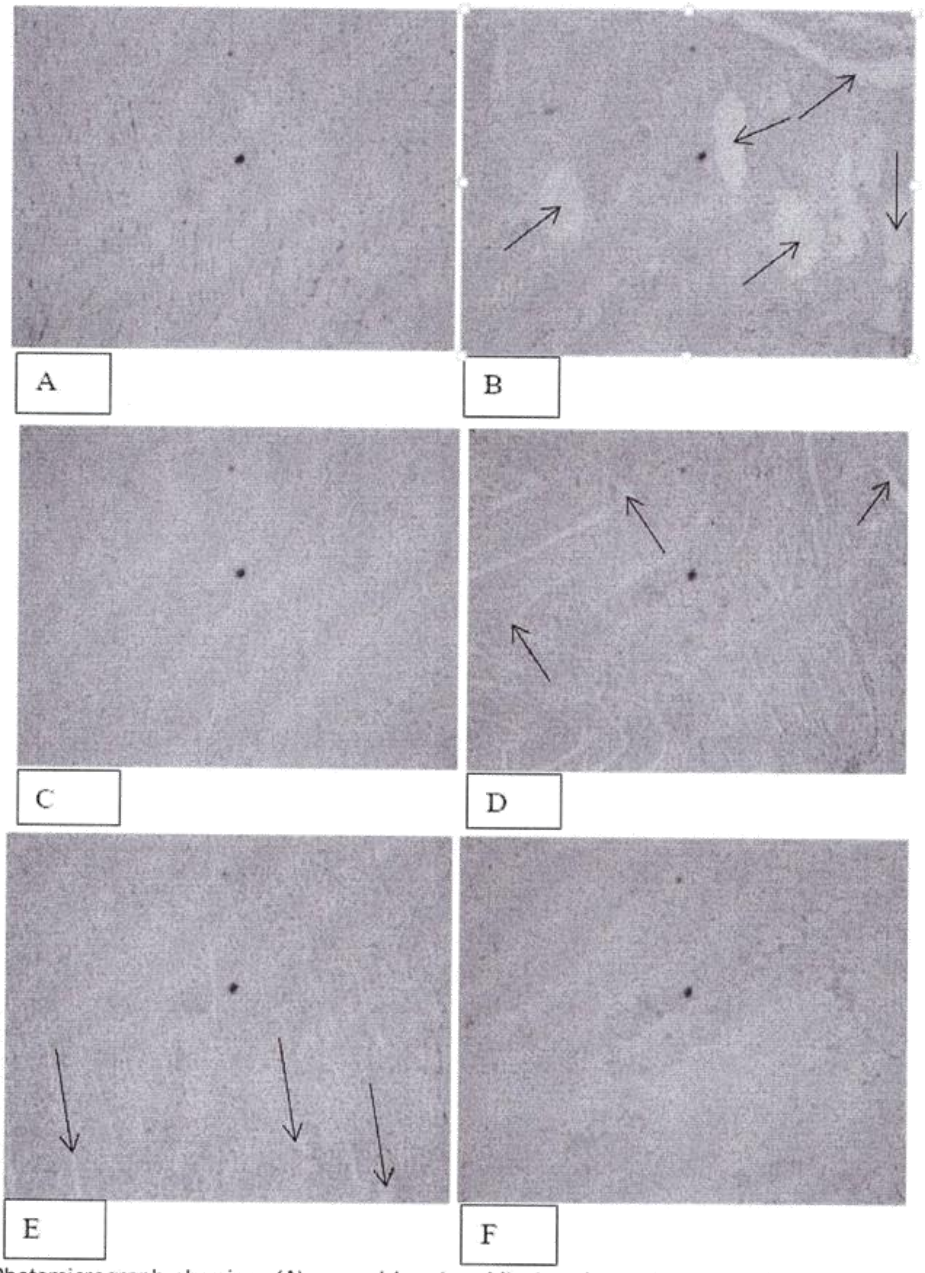

Figure 1: Photomicrograph showing: (A) normal heart architecture in control group (H&E 150x); (B) extensive tissue granulation, myocardial necrosis and coronary congestion (H&E 150x); (C) normal cardiomyocytes with mild oedema (H&E 150x); (D) myocardial necrosis and severe oedema (H&E 150x); (E) myofibres disintegration and oedema (H&E 150 x); (F) normal cardiomyocytes (H&E 150x). Figures are representative of four independent experiments

Table 1: Effect of AQRED on feed and water intake of ISP -induced myocardial infarcted rats

Parameters	Control	Isoproterenol	Simvastatin	125 mg/kg	250 mg/kg	500 mg/kg
AFI (g) Day 1- 10	148.90 ± 3.36^{a}	150.90 ± 9.61^{a}	137.94 ± 8.48^{a}	113.70 ± 7.07^{a}	122.34 ± 8.92^{a}	116.78 ± 7.95^{a}
AFI (g) Day 11-20	148.50 ± 1.62^{a}	167.02 ± 8.54^{b}	178.06 ± 5.71^{b}	137.72 ± 5.60^{b}	157.32 ± 5.15^{b}	147.06 ± 3.99^{b}
AFI (g) Day 21- 30	148.14 ± 1.98^{a}	181.84 ± 5.13^{c}	169.40 ± 4.93^{c}	154.56 ± 1.91^{c}	160.50 ± 3.53^{b}	159.92 ± 1.56^{c}
AWI (ml) Day 1 -10	166.26 ± 4.45^{a}	140.76 ± 5.89^{a}	155.00 ± 5.55^{a}	138.50 ± 11.54^{a}	148.50 ± 9.01^{a}	146.50 ± 8.25^{a}
AWI (ml) Day 11-20	171.20 ± 4.97^{b}	146.00 ± 6.45^{b}	164.80 ± 3.73^{b}	162.20 ± 11.32^{b}	169.00 ± 7.03^{b}	168.00 ± 7.36^{b}
AWI (ml) Day 21- 30	179.20 ± 5.97^{c}	167.80 ± 4.54^{c}	171.80 ± 1.60^{c}	173.60 ± 5.45^{c}	189.40 ± 4.32^{c}	194.80 ± 8.43^{c}

Values with different superscript along the same column for each parameter are significantly ($p < 0.05$) to each other ***Key:*** AFI: Average feed intake; AWI: Average water intake

Table 2: Effect of AQRED on body weight of ISP -induced myocardial infarcted rats

Parameters	Control	Isoproterenol	Simvastatin	125 mg/kg	250 mg/kg	500 mg/kg
Initial weight (g)	141.80 ± 10.55^a	141.90 ± 5.94^a	142.60 ± 11.52^a	140.80 ± 9.52^a	144.10 ± 9.53^a	139.70 ± 6.64^a
ABW (g) Day 1– 10	172.80 ± 12.84^a	168.70 ± 8.51^a	169.20 ± 14.92^a	164.00 ± 12.63^a	162.30 ± 8.37^a	165.90 ± 10.82^a
ABW (g) Day 11 – 20	185.70 ± 14.64^a	179.20 ± 12.57^a	180.10 ± 16.10^a	170.30 ± 8.60^b	174.30 ± 10.34^a	175.40 ± 12.89^b
ABW (g) Day 21 – 30	200.80 ± 16.50^a	194.10 ± 15.90^a	194.10 ± 18.67^a	187.80 ± 13.59^b	192.70 ± 12.76^a	192.40 ± 15.69^a

Values with different superscript along the same row for each parameter are significantly different ($p < 0.05$) to each other; ***Key:*** ABW: Average body weight

Table 3: Effect of AQRED on cardiac marker enzymes of ISP -induced myocardial infarcted rats

Parameters	Control	Isoproterenol	Simvastatin	125 mg/kg	250 mg/kg	500 mg/kg
ALT IU/L	12.96 ± 2.40^a	13.49 ± 0.37^a	7.15 ± 3.30^b	9.91 ± 0.67^b	8.87 ± 0.90^b	7.22 ± 2.24^b
AST IU/L	211 ± 1.16^a	290 ± 1.15^b	207 ± 0.58^a	193 ± 1.16^c	185 ± 0.57^c	178 ± 0.58^d
CPK IU/L	76.07 ± 0.88^a	224.60 ± 12.77^b	83.57 ± 21.48^a	181.00 ± 0.60^c	178.20 ± 9.30^c	140.20 ± 8.56^d

Values are presented as mean ± SEM (n = 6). *Values with different superscript along the same row for each parameter are significantly different ($p < 0.05$); ALT: Alanine transaminase; AST: Aspartate transaminase; CPK: Creatine phosphokinase

Table 4: Effect of AQRED on tissue marker enzymes of ISP-induced myocardial infarcted rats

Parameters	Control	Isoproterenol	Simvastatin	125 mg/kg	250 mg/kg	500 mg/kg
Catalase (U/mg protein)	66.20 ± 0.27^a	7.20 ± 0.56^b	57.28 ± 2.44^a	20.97 ± 0.56^c	21.75 ± 4.08^c	38.97 ± 2.71^d
Peroxidase (U/mg protein)	71.01 ± 0.10^a	27.25 ± 0.13^b	54.83 ± 0.26^c	35.08 ± 0.13^b	36.39 ± 0.11^b	58.51 ± 1.25^c
TBARS (mM/ 100 mg tissue)	90.34 ± 1.93^a	201.40 ± 0.84^b	113.50 ± 1.74^c	104.80 ± 0.97^c	110.10 ± 2.21^c	116.90 ± 0.97^d

Values are presented as mean ± SEM (n = 6). *Values with different superscript along the same row for each parameter are significantly different ($p < 0.05$). TBARS: Thiobarbituric acid reactive substances

while they reduced TBARS level in a dose-related manner by bringing towards normal the cardiac damage done to the rats.

Effect of AQRED on histopathological alterations

The result of histopathological examination of the heart sections from control and cardiotoxic rats is shown in Figure 1. Control rats showed normal cardiomyocytes with no visible lesions (Figure 1A). The ISP-treated rats revealed necrotic cardiomyocytes, marked granulation of tissue characterized by collagenous tissue deposition and presence of coronary congestion (Figure 1B). Simvastatin-treated rats exhibited normal cardiomyocytes with mild oedema (Fig 1C). Treatment with 125 mg/kg AQRED shows multiple areas of myofibres disintegration, presence of necrotic myocytes and severe oedema (Figure 1D). 250 mg/kg AQRED revealed myofibres disintegration and severe oedema (Figure 1E). However, at 500 mg/kg dose, the heart appeared to be normal (Figure 1F).

DISCUSSION

Destruction of myocardial cells arising from myocardial ischemia results in ruptured or damaged cardiac membrane thus, leading to leakage of heart enzymes [19]. MI occurs when there is an insufficient oxygen supply or glucose to the heart resulting in necrosis of the myocardium. CPK and LDH are marker enzymes diagnostically used to measure the level of cardiac damage and the release of these enzymes from the myocardial tissues of the heart into the bloodstream is an indication of myocardial damage induced by ISP. This does not only increase their concentration in the blood [20] but causes increase in heart rate, abnormal electrocardiogram (ECG) pattern [21,22] due to the formation of reactive oxygen species which ultimately leads to tissue degenerative changes resulting in the necrosis of the myocardium. The pathogenesis of MI has not been fully elucidated but insight into studies involving ISP-induced

cardiotoxicity suggests oxidative stress involvement. Moreover, increase in the serum activities of AST and ALT might also be attributed to myocardial injury [19].

The results of our study revealed increased activities of AST, ALT, and CPK following intraperitoneal administration of ISP, this affirms the preliminary prevalence of myocardial necrosis [23] and the leakage of marker enzymes from the heart to the blood. The treatment with different doses of AQRED reduced the level of these enzymes compared to control; this suggests the protective action of the plant in ameliorating cardiac damage arising from ISP. This result corroborates the previous report of Suchalatha and Shyamala-Devi [3] for Arogh as well as Sunmonu and Afolayan [24] for *A. afra*.

Free radical formation and accumulation of lipid peroxides have been established as one of the probable biochemical mechanisms leading to myocardial damage from catecholamine such as ISP [25]. Catecholamine undergoes rapid oxidation and the oxidation products cause the necrosis of the cell and contractile failure in the rat heart [7]. CAT, superoxide dismutase, and GP are free radical scavenging enzymes concerned foremost with cellular defence against oxidative damage, O_2 and H_2O_2 decomposition prior their interaction to form more reactive hydroxyl radical. It is, therefore, essential that equilibrium is maintained among these enzymes for effective removal of oxidative stress within intracellular organelles. It has been reported that glutathione protect the myocardium from free radical-induced injury and reduction in cellular glutathione content could hinder the recovery of ischemia after a short period [19]. The results from the present study indicate that treatment with doses of AQRED increased the level of catalase and glutathione peroxidase suggesting an improved antioxidant status in the system. The result of our study was in line with the submission of Raju *et al* [26] who reported a reduction in the activity of tissue antioxidant enzymes in rats with myocardial damage.

Tissue damages are caused by free radicals; these free radicals attack the cellular membrane of the poly-unsaturated fatty acids (PUFAs) thereby causing lipid peroxidation [27]. Myocardial damage induced by ISP in rats causes lipid peroxidation and this signifies an elevated free radical generation [28]. The results of the present study reveal that rats treated with ISP show a significant increase in TBARS when compared with control group. Treatment with different doses of aqueous root extract of *D. anomala* reduced the elevated TBARS level.

Histopathological analysis of the heart of ISP-induced myocardial disease in rats treated with AQRED revealed the normal structure of the heart with some traces of oedema and necrotic myocytes when compared with the heart of ISP-treated rats. This shows effective protection of AQRED most especially at the highest dose of 500 mg/kg on the heart against ISP-induced cardiotoxicity.

CONCLUSION

The findings of this study indicate the ameliorative activity of AQRED in rats with myocardial disease. Thus, *D. anomala* might is a potential alternative source of medicine for the management and prevention of cardiovascular disease. Further studies are, however, required to identify the active components of the plant as well as elucidate their mechanisms of action.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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Chapter Six

Acute and subchronic oral toxicity evaluation of aqueous root extract of *Dicoma anomala* (Sond.) in Wistar rats

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Research Article

Acute and Subchronic Oral Toxicity Evaluation of Aqueous Root Extract of *Dicoma anomala* Sond. in Wistar Rats

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The present study evaluated the safety of aqueous root extract of *Dicoma anomala* (AQRED) through acute and subchronic toxicity studies. Single oral dose of AQRED at the concentration of 0, 5, 300, and 2000 mg/kg as well as 125, 250, and 500 mg/kg/day was administered to rats for 14-day acute and 90-day subchronic oral toxicity studies. The results revealed no mortalities or observed clinical signs of toxicity in all the rats during both investigation periods. In subchronic toxicity testing, administration of AQRED also did not cause any changes in body weight as well as food and water consumption patterns. The haematological parameters and blood chemistry revealed no significant difference ($p > 0.05$) between the treatment and the control except in platelet count, alkaline phosphatase, and sodium levels where there was a significant increase ($p < 0.05$), although there was also a significant reduction ($p < 0.05$) in alanine transaminase, aspartate transaminase, and creatinine when compared to control. However, these changes were not reflecting the results from histology. Conclusively, the obtained results suggested that the LD_{50} of AQRED is in excess of 2000 mg/kg and its oral administration for 90 days revealed that it is unlikely to be toxic, hence, safe.

1. Introduction

Dicoma anomala belongs to the family of Asteraceae and is locally called Hloenya (South Sotho) or fever or stomach bush (Afr.). This herbaceous plant which grows in grasslands or stony places has erected stems, thinly covered with hairs, and originates from a woody rootstock. The leaves are simple, alternate, stalkless, linear, or narrowly lanceolate. The flower heads are terminal, solitary or in small pairs, narrow, with sharply pointed bracts and slender, with white, mauve, purple, or pink tubular florets [1]. *D. anomala* is widely distributed in sub-Saharan Africa including most provinces such as Limpopo, North West, Gauteng, Mpumalanga, Free State, Northern Cape, and KwaZulu-Natal within South Africa [1]. The plant is traditionally used for the treatment of cold and coughs, fever, ulcers, and dermatosis mostly among the Basotho tribe of eastern Free State. A number of documented pharmacological potentials such as antiplasmodial, antibacterial, anthelmintic, antiviral, and anti-inflammatory have been reported [2]. However, despite the various pharmacological efficacies in relation to numerous health-related benefit of the plant, it is noteworthy not to rule out issues of safety. Moreover, based on documented reports, continuous intake of high dose of medicinal plants and/or associated chemical compounds could alter hepatic and thyroid function adversely [3–6] and to date the potential toxicities of *Dicoma anomala* have not gotten enough attentions when taken in increased concentration despite the recently reported antioxidant activity of this plant by our research group. In this study, we evaluated the toxicity of the aqueous root extract with the dietary administration to male and female Wistar rats for acute 14 days and subchronic 90 days as parts of a safety assessment according to the internationally acceptable guidelines. We also subjected the extract to gas chromatography/mass spectrophotometric (GC/MS) analysis for possible identification of the phytoconstituents present in the plant.

2. Materials and Methods

2.1. Plant Collection and Extraction. Fresh root stocks of *D. anomala* were procured in April 2014 from Setsing market, Phuthaditjhaba, Qwaqwa, Free State province, South Africa. The sample was confirmed by Dr. AOT Ashafa of Plant Sciences Department, University of the Free State, Qwaqwa Campus, Free State, and a voucher specimen with (BalMed/01/2015/QHB) was thereafter prepared and deposited at the Department of Plant Sciences herbarium. A total of 5.2 kg of the root sample was cut into smaller pieces, oven-dried (40°C) for 96 hr, and pulverized using a Waring Commercial blender (USA) to yield 3.063 kg fine powder. Approximately, 600 g of the powdered root material was extracted with 5 L of distilled water on a platform shaker for 5 days. The resulting mixture was centrifuged at 1285 g for 5 min using a BHG Roto-Uni II centrifuge and afterwards filtered using Whatman number 1 filter paper. The filtrates obtained were pooled together and concentrated to dryness on a water bath (Memmert W600, Schwabach, Germany) at 40°C. The extraction yielded 137.27 g of brown gum (22.88% w/w of dry plant material) crude extract. The crude extract was reconstituted in water to afford various concentrations used in this study.

2.2. Experimental Animals. A total of one hundred (both sexes) 5-week-old Wistar rats were purchased from animal facility of University of the Free State, Bloemfontein, and were used after a week of acclimatization. Individual body weights were recorded and detailed physical examinations were performed twice during the acclimation period to ensure the use of healthy animals. Animals were housed in clean metabolic cages placed in a ventilated house and allowed free access to commercial laboratory feed (Epol rat cubes, South Africa) and tap water *ad libitum*. Animals were subjected to housing condition of the controlled system of the light-dark cycle (12-12 h), ventilation (air-exchange rate of 18 times per hour), temperature (23 ± 2°C), and relative humidity (55 ± 5%) during the study. The cages and the chip bedding were exchanged twice weekly and feed intake and water consumption, on a daily basis (by measuring the left-over feed/water and subtracting it from the initial weight or volume) and body weight measurement was taken on a weekly basis. Ethical approval for use of animals was obtained from Interfaculty Ethics Committee of the University of the Free State (NR/02/13) prior to the commencement of the study and the study was performed in accordance with the stipulation for the care and use of laboratory animals, prepared by National Institute of Health, USA (Guide for the Care and Use of Laboratory Animals, 1996).

2.3. Acute Oral Toxicity Study of Aqueous Root Extract of D. anomala (AQRED). The single dose acute oral toxicity study was evaluated following the recommendations by The Organisation for Economic Co-operation and Development (OECD, 2001) [7]. Animals (female rats) were randomly divided into four groups of 5 animals. After overnight fasting (i.e., withdrawal of feeds only and not water for about 12–15 h), they were administered a single oral dose of 0, 5, 300, and 2000 mg/kg body weight AQRED by oral gavage. Distilled water was administered to the control group. Feeding was commenced 4 h after the administration and all animals were observed for clinical signs including mortality and moribundity immediately at 1, 2, 4, 8, and 12 h and then twice daily until day 14. Body weights, food consumption, and water intake were measured on days 0, 1, 5, 10, and 14. On the 14th day, all animals were sacrificed and all organs and tissues were observed macroscopically. The organs were fixed in 10% neutral buffered formalin and observed for histopathological examination.

2.4. Subchronic 90-Day Oral Toxicity Study of AQRED

2.4.1. Experimental Design. The subchronic 90-day oral toxicity study was evaluated following the recommendations by OECD (1998) [8]. Four groups of ten (10) male and female Wistar rats received doses of 0, 125, 250, and 500 mg/kg body weight (BW) of AQRED at daily gavage of 1 mL/100 g (BW) for 90 consecutive days. Observations were made twice daily for mortality and changes in general appearance or behaviour. The body weights were recorded every week and the individual dose administered to the animals was adjusted weekly for the body weight to maintain the target dose level for all rats. Additionally, detailed clinical examination and measurement of food and water consumption were made daily.

2.4.2. Blood Preparation and Organ Isolation. The animals were anaesthetized with halothane at the end of the experimental period (i.e., day 90 of treatment) and blood was collected by clearing the neck region of fur to locate the jugular vein. An aliquot (2 mL) of the blood was collected into ethylene diamine tetra-acetic acid (EDTA) bottle and was used for the analysis of haematological parameters, while another 5 mL of the blood collected in nonheparinized bottle was centrifuged at 1285 g for 10 minutes; the resulting serum was aspirated and used for other serum bioassays. The animals were quickly dissected and the liver, kidney, heart, lungs, spleen, stomach, testes, ovary, brain, and pancreas were excised, freed of fat, blotted with clean tissue paper, and weighed for evaluation of organ-to-body weight ratios. Defined samples of the liver, brain, pancreas, kidney, and testes were placed in 10% neutral buffered formalin for histopathological examination. All organs were visually inspected and weighed directly after dissection to reduce mechanical damage.

2.4.3. Hematology and Blood Chemistry. One blood sample (approximately 20 μL) was treated with EDTA-2K for white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), platelets (PLT), percent of lymphocytes (LY), percent of monocytes (MO), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), platelet haematocrit (PCT), platelet distribution width (PDW), and red blood cell distribution width (RDW) using a hematology analyzer MEK-6318K (Nihon Kohden Co., Ltd.). Serum from blood samples

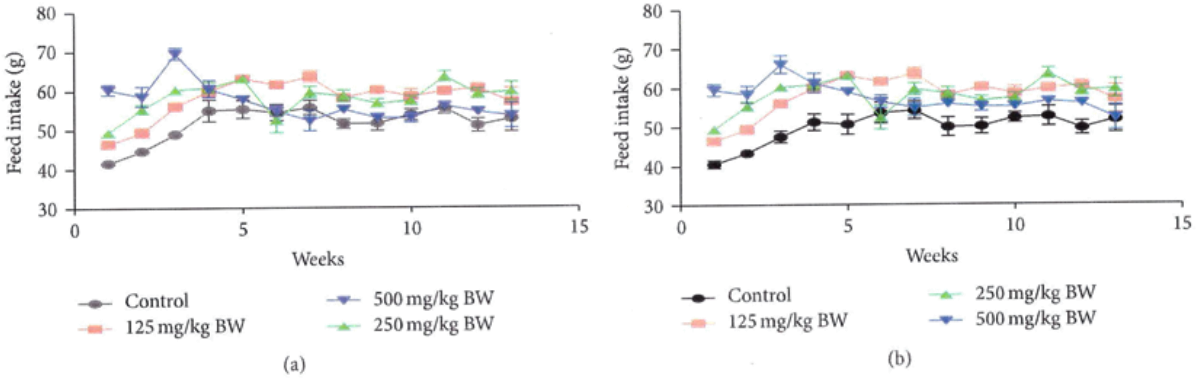

FIGURE 1: Effect of aqueous root extract of *Dicoma anomala* on food consumption for male and female Wistar rats during a 90-day subchronic toxicity study. Values were calculated and data expressed as mean ± (standard error of mean) SEM [BW: body weight].

collected in separator tubes were measured using a BS-200 automatic biochemistry analyzer (Mindray Co., Ltd.) including aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, nitrogen, creatinine (Cr), total cholesterol (TC), triglyceride (TG), total protein (TP), albumin (Alb), and glucose (Glu). Total calcium (TCa) was measured using the 7020 automatic biochemistry analyzer (Hitachi Co., Ltd.) as well as sodium (Na), potassium (K), and chloride (Cl).

2.4.4. Histopathological Studies. Pieces of vital organs from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50–100%) alcohol, cleaned with xylene, embedded in paraffin, cut into 4-5 μm thick sections, and stained with hematoxylin-eosin. The microscopic features of the organs of male and female rats were compared with that of the control group.

2.4.5. Statistical Analysis. Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, San Diego, CA, USA). Data were expressed as means of ten replicates ± standard error of mean (SEM) for all assays and were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Statistical significance was considered at $p > 0.05$.

2.5. Gas Chromatography/Mass Spectrophotometric Analysis. The GC/MS analysis of the extract was performed on a QP-2010 system (Shimadzu, Japan) via electron ionization (EI) at 70 eV. GC separation was achieved on a capillary column (0.25 mm i.d × 30 m, film 0.5 mm) of HP-5MS (Hewlett Packard, USA). The injection of the extract (1 mL) was done using the splitless injection method. The initial column temperature was 50°C, then went up to 250°C at a rate of 10°C/min, and finally held at 250°C for 5 min. The injection port was set at 120°C. Helium gas was used as the carrier at a flow rate of 0.6 mL/min [9]. The mass spectra of the constituents were compared with known spectra stored in Wiley library.

3. Results

3.1. Single Dose Subacute Oral Toxicity Study of AQRED in Wistar Rats. No deaths occurred during the 14-day study. There were no significant differences in body weights and food consumption of rats between the AQRED administrated group and the controls (data not shown). A short time of sluggish movement appeared in some AQRED treated rats immediately after oral gavage at 2000 mg/kg body weight concentration in the first hour, but the rats continued to live afterwards. Aside from this observation, there were no abnormal findings of other clinical signs and autopsy performed in all the experimental and control animals. It was based on these findings that the LD_{50} value of AQRED was assumed to be greater than 2000 mg/kg since no mortality or signs of toxicity were observed in all experimental animals.

3.2. Subchronic 90-Day Oral Toxicity Study of AQRED in Wistar Rats

3.2.1. Clinical Signs and Mortality. There was no mortality attributed to any effect of AQRED during the 90-day administration. Similarly, there was no treatment-related change at the autopsy in the AQRED groups or control group.

3.2.2. Feed and Water Intake. The effect of aqueous root extract of *Dicoma anomala* on feed intake is shown in Figures 1(a) and 1(b). From the results, it was observed that, during the 90-day study, there were no significant differences ($p > 0.05$) in mean food consumption among all the experimental animals. There seems to be an increase in feed intake as the experimental days increased.

The result of the effect of aqueous extract of *Dicoma anomala* on water intake (Figures 2(a) and 2(b)) revealed no significant difference ($p > 0.05$) in water intake during the 13-week study for both sexes of the animals.

3.2.3. Organ Weights. The results of relative organ weight of the animals are shown in Tables 1 and 2. It was observed that

Table 1: Effect of oral administration of aqueous extract of *Dicoma anomala* for 90 days on relative organ-to-body weight ratio of male Wistar rats.

Organ	Dose (mg/kg/day)			
	Control	125	250	500
Initial weight[a]	122.60 ± 2.55	123.00 ± 2.94	117.60 ± 4.09	106.60 ± 5.17
Body weight[a]	234.50 ± 6.92	394.20 ± 17.77*	371.10 ± 15.08*	342.60 ± 4.70*
% weight change	91.27	220.48	215.56	221.39
Liver[a]	5.27 ± 0.19	9.63 ± 0.95	10.22 ± 0.23	8.22 ± 0.39
Brain[a]	1.23 ± 0.05	1.35 ± 0.05	1.28 ± 0.22	3.17 ± 0.16
Heart[a]	0.82 ± 0.03	1.42 ± 0.03	1.42 ± 0.06	1.22 ± 0.12
Lungs[a]	2.02 ± 0.36	3.26 ± 0.62	3.39 ± 0.32	1.95 ± 0.20
Kidney[a]	1.69 ± 0.18	2.45 ± 0.28	2.45 ± 0.10	2.22 ± 0.16
Spleen[a]	0.46 ± 0.03	0.62 ± 0.08	0.69 ± 0.02	0.60 ± 0.07
Stomach[a]	2.65 ± 0.12	2.94 ± 0.44	3.02 ± 0.52	3.17 ± 0.05
Testes[a]	3.16 ± 0.12	4.58 ± 1.16	3.41 ± 0.21	3.33 ± 0.25
Pancreas[a]	0.96 ± 0.03	1.52 ± 0.01	1.64 ± 0.26	1.36 ± 0.18
Liver[b]	2.25	2.44	2.75	2.4
Brain[b]	0.52	0.34	0.34	0.93
Heart[b]	0.35	0.36	0.38	0.36
Lungs[b]	0.86	0.83	0.91	0.57
Kidney[b]	0.72	0.62	0.66	0.65
Spleen[b]	0.20	0.16	0.19	0.18
Stomach[b]	1.13	0.75	0.81	0.93
Testes[b]	1.35	1.16	0.92	0.97
Pancreas[b]	0.41	0.39	0.44	0.40

Mean organ-to-body weight ratio was calculated and expressed as mean ± SEM ($n = 10$).
[a]Unit: g.
[b]Unit: % body weight.
*Statistically significant compared to control ($p < 0.05$).

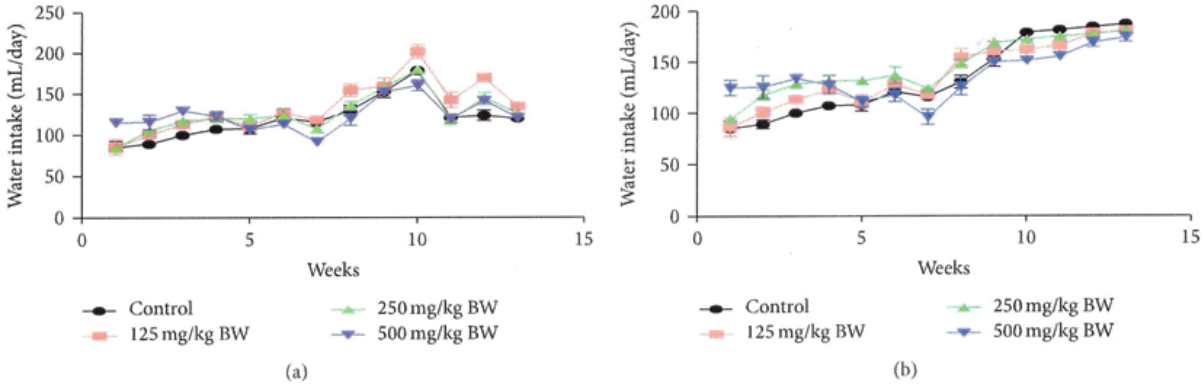

Figure 2: Effect of aqueous root extract of *Dicoma anomala* on water intake of male and female Wistar rats during a 90-day subchronic toxicity study. Values were calculated and data expressed as mean ± SEM.

there was no statistical difference ($p > 0.05$) in all the excised organs as compared with the control group.

3.2.4. Body Weights. The mean body weights of the animals at all concentrations were compared to the values of control (Figures 3(a) and 3(b)). The rats in all AQRED groups had statistically ($p < 0.05$) higher mean body weights (394.20 g, 371.10 g, and 342.60 g in 125, 250, and 500 mg/kg/day group, resp.) of males as compared to the control group (234.50 g). Moreover, there was a significant difference ($p < 0.05$) in body weight of female rats at all the three concentrations 125, 250, and 500 mg/kg body weight (269 g, 299 g, and 306 g, resp.) compared to control (244 g).

3.2.5. Haematology and Blood Chemistry. The results from haematological parameters only revealed statistically

Table 2: Effect of oral administration of aqueous extract of *Dicoma anomala* for 90 days on relative organ-to-body weight ratio of female Wistar rats.

Organ	Dose (mg/kg/day)			
	Control	125	250	500
Initial weight[a]	120.60 ± 2.55	121.00 ± 2.94	119.60 ± 4.09	116.60 ± 5.17
Body weight[a]	244.50 ± 6.94	269.20 ± 0.76	299.40 ± 3.22	306.00 ± 4.91*
% weight change	102.74	122.48	150.33	162.44
Liver[a]	6.87 ± 0.19	5.99 ± 0.95	6.29 ± 0.23	6.22 ± 0.32
Brain[a]	1.01 ± 0.05	1.15 ± 0.05	1.23 ± 0.21	1.17 ± 0.16
Heart[a]	0.52 ± 0.03	0.62 ± 0.03	0.58 ± 0.06	0.60 ± 0.12
Lungs[a]	1.52 ± 0.36	1.36 ± 0.62	1.39 ± 0.12	1.45 ± 0.20
Kidney[a]	1.39 ± 0.18	1.40 ± 0.28	1.45 ± 0.10	1.35 ± 0.06
Spleen[a]	0.32 ± 0.03	0.40 ± 0.08	0.39 ± 0.02	0.41 ± 0.07
Stomach[a]	2.05 ± 0.12	2.44 ± 0.44	2.12 ± 0.52	2.17 ± 0.05
Ovary[a]	3.16 ± 0.12	4.58 ± 1.16	3.41 ± 0.21	3.33 ± 0.25
Pancreas[a]	0.76 ± 0.03	0.78 ± 0.01	1.64 ± 0.26	1.36 ± 0.18
Liver[b]	2.81	2.23	2.10	2.03
Brain[b]	0.41	0.43	0.41	0.38
Heart[b]	0.21	0.23	0.19	0.20
Lungs[b]	0.62	0.51	0.46	0.47
Kidney[b]	0.57	0.52	0.48	0.44
Spleen[b]	0.13	0.15	0.13	0.13
Stomach[b]	1.84	0.91	0.71	0.71
Ovary[b]	1.29	1.70	0.14	1.09
Pancreas[b]	0.31	0.29	0.55	0.44

Mean organ-to-body weight ratio was calculated and expressed as mean ± SEM ($n = 10$).
[a]Unit: g.
[b]Unit: % body weight.
*Statistically significant compared to control ($p < 0.05$).

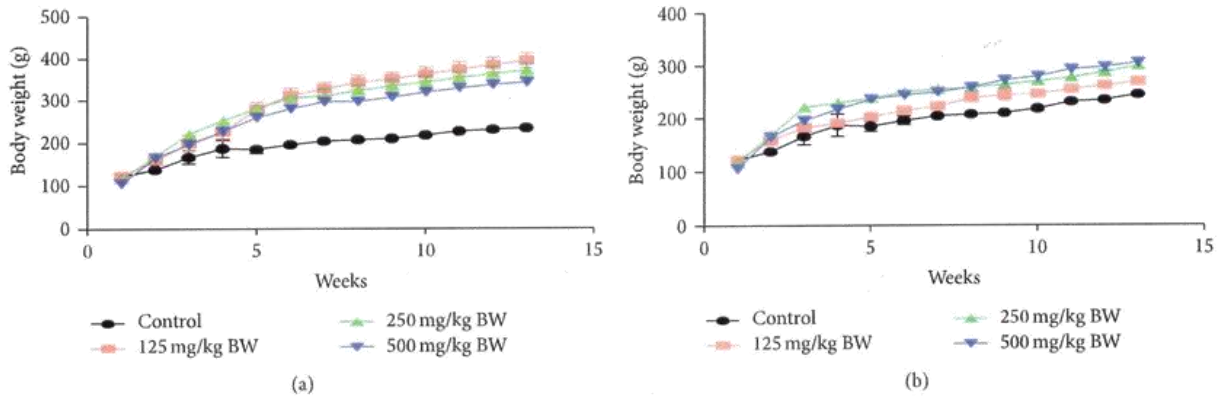

Figure 3: Effects of oral administration of aqueous root extracts of *Dicoma anomala* on mean body weights of male and female Wistar rats. Body weight was calculated and data expressed as mean ± SEM.

significant decrease ($p < 0.05$) in platelet count compared with control groups for both sexes, while there was no significant difference in other parameters compared to the control groups (Tables 3 and 4). Moreover, the result obtained from the blood chemistry showed a dose-dependent decrease ($p < 0.05$) in creatinine level and alanine transaminase activity, while it recorded a dose-dependent increase ($p < 0.05$) in sodium level. Serum activity of alkaline phosphatase also showed a statistical increase ($p < 0.05$) in the entire dose regimen, while the reverse is the case for aspartate transaminase when compared with control groups. Beyond all the reported results, there was no treatment-related or statistically significant ($p < 0.05$) effects observed in all the other tested blood chemistry parameters (Tables 5 and 6).

Table 3: Haematological data for male rats given aqueous root extracts of *Dicoma anomala* for 90 days.

Parameters	Unit	Dose (mg/kg/day)			
		Control	125	250	500
RBC	millions/μL	8.42 ± 0.10	9.44 ± 0.01	9.3 ± 0.05	9.18 ± 0.02
Haemoglobin	g/dL	16.4 ± 0.01	17.6 ± 0.02	16.9 ± 0.01	16.9 ± 0.01
Haematocrit	L/L	0.55 ± 0.05	0.61 ± 0.01	0.58 ± 0.02	0.59 ± 0.00
MCV	fL	65.00 ± 0.00	64.00 ± 0.00	62.00 ±0.00	64.00 ± 0.00
MCH	pg	19.00 ± 0.01	19.00 ± 0.01	18.00 ± 0.01	19.00 ± 0.01
MCHC	g/dL	30.00 ± 0.10	29.00 ± 0.10	29.00 ± 0.10	29.00 ± 0.10
RDW	fL	12.60 ± 0.01	13.40 ± 0.10	14.00 ± 0.00	14.10 ± 0.2
WBC	thous/μL	5.80 ± 0.02	6.50 ± 0.01	6.40 ± 0.02	6.90 ± 0.02
Neutrophils	$\times 10^9$/L	0.22 ± 0.00	0.46 ± 0.01	0.51 ± 0.02	0.62 ± 0.01
Lymphocytes	%	4.58 ± 0.10	4.45 ± 0.20	4.37 ± 0.20	4.89 ± 0.10
Monocytes	%	0.92 ± 0.00	1.43 ± 0.01	1.38 ± 0.01	1.28 ± 0.02
Eosinophils	$\times 10^9$/L	0.07 ± 0.00	0.16 ± 0.02	0.09 ± 0.00	0.08 ± 0.00
Basophils	$\times 10^9$/L	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.01
Platelet	thous/L	979.00 ± 0.50	803.00 ± 0.03*	701.00 ± 0.10*	754.00 ± 0.49*

Values are presented as mean ± SEM ($n = 10$).
*Significantly different compared to control at $p < 0.05$.

Table 4: Haematological data for female rats given aqueous root extracts of *Dicoma anomala* for 90 days.

Parameters	Unit	Dose (mg/kg/day)			
		Control	125	250	500
RBC	millions/μL	7.45 ± 0.10	7.44 ± 0.01	7.53 ± 0.05	7.88 ± 0.02
Haemoglobin	g/dL	14.41 ± 0.01	13.96 ± 0.02	15.09 ± 0.01	15.19 ± 0.01
Haematocrit	L/L	0.50 ± 0.05	0.49 ± 0.01	0.56 ± 0.02	0.59 ± 0.00
MCV	fL	61.00 ± 0.00	60.00 ± 0.00	62.00 ±0.00	63.00 ± 0.00
MCH	pg	16.00 ± 0.01	17.00 ± 0.01	17.00 ± 0.01	18.00 ± 0.01
MCHC	g/dL	27.00 ± 0.10	27.00 ± 0.10	28.00 ± 0.10	28.00 ± 0.10
RDW	fL	10.60 ± 0.01	11.40 ± 0.10	11.09 ± 0.00	11.91 ± 0.2
WBC	thous/μL	5.80 ± 0.02	6.30 ± 0.01	6.70 ± 0.02	6.91 ± 0.02
Neutrophils	$\times 10^9$/L	0.12 ± 0.00	0.16 ± 0.01	0.15 ± 0.02	0.16 ± 0.01
Lymphocytes	%	4.08 ± 0.10	4.05 ± 0.20	4.07 ± 0.20	4.80 ± 0.10
Monocytes	%	0.73 ± 0.00	0.93 ± 0.01	1.08 ± 0.01	1.12 ± 0.02
Eosinophils	$\times 10^9$/L	0.09 ± 0.00	0.12 ± 0.02	0.08 ± 0.00	0.08 ± 0.00
Basophils	$\times 10^9$/L	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.01
Platelet	thous/L	1007.00 ± 0.50	893.00 ± 0.03*	871.00 ± 0.10*	944.00 ± 0.49*

Values are presented as mean ± SEM ($n = 10$).
*Significantly different compared to control at $p < 0.05$.

3.2.6. Histopathological Examination. The result from histological analysis revealed no macroscopic observations considered to be treatment-related. No gross deformities could be considered to be related to the test article for any of the euthanized animals necropsied at the expiration of the 90-day experimental study. Histopathological observation including presence of hyperplasia and lymph nodes was only noticeable in one of the sections of the stomach for the 125 mg/kg body weight male rats as well as presence of renal calculi in the collecting tubes of the kidney for untreated control animals; as such, these changes could not be treatment-related.

3.3. GC/MS Analysis. The GC/MS chromatogram of *Dicoma anomala* extract revealed the presence of rich amounts of eighty phytochemical compounds. The major identifiable constituents are 5-(Hydroxymethyl)-2-furancarboxyaldehyde, 2-(1-methyl-2,5-dioxoimidazolidin-4-yl) acetic acid, n-tridecane, 9-octadecenoic acid, hexadecanoic acid, phosphine, 1,6-anhydro-β-D-glucopyranoside, 3-(Bromophenyl) triphenyl phosphonium bromide, Guanosine, and Xanthosine. The compound name, molecular formula, and peak area (%) of the majority of the constituents are presented in Table 7.

Table 5: Effect of aqueous root extracts of *Dicoma anomala* for 90 days on biochemical parameters of male Wistar rats.

Parameters	Unit	Dose (mg/kg/day)			
		Control	125	250	500
Total cholesterol	mmol/L	2.50 ± 0.23	2.10 ± 0.15	2.20 ± 0.13	2.00 ± 0.20
Triglycerides	mmol/L	0.65 ± 0.00	1.21 ± 0.11	0.87 ± 0.20	0.86 ± 0.01
HDL-c	mmol/L	1.60 ± 0.23	1.30 ± 0.17	1.40 ± 0.25	1.30 ± 0.19
LDL-c	mmol/L	0.90 ± 0.00	0.80 ± 0.11	0.80 ± 0.14	0.70 ± 0.50
ALT	U/L	51.00 ± 0.85	48.00 ± 0.55*	47.00 ± 0.77*	47.00 ± 0.74*
AST	U/L	215.00 ± 0.85	188.00 ± 0.90*	145.00 ± 0.87*	192.00 ± 0.88*
ALP	U/L	185.00 ± 0.87	200.00 ± 0.90*	264.00 ± 0.47*	220.00 ± 0.75*
Glucose	mg/dL	4.23 ± 0.10	3.70 ± 0.11	4.27 ± 0.05	4.47 ± 0.23
Sodium	mmol/L	137.00 ± 0.67	141.00 ± 0.85*	142.00 ± 0.44*	142.00 ± 0.53*
Potassium	mmol/L	5.40 ± 0.01	5.20 ± 0.01	4.80 ± 0.02	5.60 ± 0.00
Chloride	mmol/L	104.00 ± 0.11	103.00 ± 0.21	104.00 ± 0.12	104.00 ± 0.01
Calcium	mmol/L	2.38 ± 0.01	2.31 ± 0.10	2.32 ± 0.14	2.34 ± 0.20
Magnesium	mmol/L	0.99 ± 0.40	0.87 ± 0.02	0.95 ± 0.14	0.88 ± 0.01
Urea	mmol/L	8.10 ± 0.01	7.50 ± 0.00	7.90 ± 0.12	7.50 ± 0.24
Creatinine	μmol/L	44.00 ± 0.23	41.00 ± 0.21*	40.00 ± 0.17*	35.00 ± 0.20*
Albumin	g/L	34.00 ± 0.15	32.00 ± 0.20	31.00 ± 0.10*	32.00 ± 0.05
Total protein	g/L	65.00 ± 0.3	65.00 ± 0.25	63.00 ± 0.33	64.00 ± 2.0
Total bilirubin	μmol/L	3.00 ± 0.11	3.00 ± 0.12	2.00 ± 0.00	2.00 ± 0.15
Conj. bilirubin	μmol/L	1.00 ± 0.1	1.00 ± 0.01	1.00 ± 0.10	1.00 ± 0.01
Unconj. bilirubin	μmol/L	2.00 ± 0.01	2.00 ± 0.01	1.00 ± 0.00	1.00 ± 0.00

Values are presented as mean ± SEM ($n = 10$).
*Significantly different compared to control at $p < 0.05$.

Table 6: Effect of aqueous root extracts of *Dicoma anomala* for 90 days on biochemical parameters of female Wistar rats.

Parameters	Unit	Dose (mg/kg/day)			
		Control	125	250	500
Total cholesterol	mmol/L	2.30 ± 0.23	2.00 ± 0.15	2.20 ± 0.13	2.10 ± 0.20
Triglycerides	mmol/L	0.56 ± 0.00	0.67 ± 0.11	0.85 ± 0.20	0.89 ± 0.01
HDL-c	mmol/L	1.65 ± 0.23	1.15 ± 0.17	1.30 ± 0.25	1.30 ± 0.19
LDL-c	mmol/L	0.80 ± 0.00	0.70 ± 0.11	0.70 ± 0.14	0.60 ± 0.50
ALT	U/L	49.00 ± 0.85	46.00 ± 0.55*	45.00 ± 0.77*	45.00 ± 0.74*
AST	U/L	210.00 ± 0.85	178.00 ± 0.90*	150.00 ± 0.87*	182.00 ± 0.88*
ALP	U/L	165.00 ± 0.87	170.00 ± 0.90*	235.00 ± 0.47*	210.00 ± 0.75*
Glucose	mg/dL	3.23 ± 0.10	3.10 ± 0.11	4.29 ± 0.05	4.74 ± 0.23
Sodium	mmol/L	107.00 ± 0.67	115.00 ± 0.85*	123.00 ± 0.44*	144.00 ± 0.53*
Potassium	mmol/L	5.49 ± 0.01	5.29 ± 0.01	4.99 ± 0.02	5.61 ± 0.00
Chloride	mmol/L	107.00 ± 0.11	106.00 ± 0.21	107.00 ± 0.12	107.00 ± 0.01
Calcium	mmol/L	2.32 ± 0.67	2.30 ± 0.10	2.31 ± 0.14	2.33 ± 0.20
Magnesium	mmol/L	0.80 ± 0.40	0.82 ± 0.02	0.85 ± 0.14	0.88 ± 0.01
Urea	mmol/L	7.99 ± 0.01	7.65 ± 0.00	7.89 ± 0.12	8.05 ± 0.24
Creatinine	μmol/L	43.00 ± 0.23	40.00 ± 0.21*	40.00 ± 0.17*	37.00 ± 0.20*
Albumin	g/L	35.00 ± 0.15	33.00 ± 0.20	32.00 ± 0.10*	34.00 ± 0.05
Total protein	g/L	65.00 ± 0.3	65.00 ± 0.25	65.00 ± 0.33	64.00 ± 2.0
Total bilirubin	μmol/L	2.00 ± 0.11	2.00 ± 0.12	3.00 ± 0.00	3.00 ± 0.15
Conj. bilirubin	μmol/L	1.00 ± 0.1	1.00 ± 0.01	1.00 ± 0.10	1.00 ± 0.01
Unconj. bilirubin	μmol/L	2.00 ± 0.01	2.00 ± 0.01	1.00 ± 0.00	1.00 ± 0.00

Values are presented as mean ± SEM ($n = 10$).
*Significantly different compared to control at $p < 0.05$.

Table 7: Reported biological activities of the identified bioactive compounds from *D. anomala*.

Compound	% Composition	Biological activity
5-(Hydroxymethyl)-2-furancarboxyaldehyde [$C_6H_6O_3$]	40.64	Antimicrobial preservative, antifungal [10], anti-inflammatory [11–13], antihepatotoxic [14] activity
2,3-Dihydro-3,5-dihydroxyl-6-methyl-4H-pyran-4-one [$C_{11}H_8O_4$]	1.52	Antimicrobial, anti-inflammatory activity [15]
n-Tridecane [$C_{13}H_{28}$]	5.56	Antimicrobial activity [16]
1,6-Anhydro-β-D-glucopyranoside [$C_6H_{10}O_5$]	3.48	Nontoxic on cell-cell communication systems [17]
2-(1-methyl-2,5-dioxoimidazolidin-4-yl) acetic acid [$C_6H_8N_2O_4$]	10.90	Nontoxic, antihepatotoxic activity [18]
Acetamide [$C_4H_9NO_2$]	1.85	Anticonvulsant activity [19]
Hexadecanoic acid [$C_{16}H_{32}O_2$]	4.36	Antioxidant, hypocholesteroemic, nematicide, pesticide, lubricant, antiandrogenic, flavour, haemolytic 5-α-reductase inhibitor [15]
9-Octadecenoic acid [$C_{57}H_{104}O_6$]	4.46	Antioxidant, anticancer [20, 21]
Globulol [$C_{15}H_{26}O$]	1.68	Antimicrobial [22, 23], antibacterial, antiglutamatergic, sedative activity [24]
Phosphine [$C_{18}H_{15}P$]	4.69	Antihypertensive, diuretic, uric acid excretion stimulant, saluretic activity [25]

4. Discussion

The recognition of herbal treatment or phytomedicine as the most common form of alternative medicine has been around since time immemorial [26]. This is because a larger percentage of the world's population (about 80% according to World Health Organization's estimation) depends on these plant-based remedies as a viable option to diseased conditions most especially in developing and/or developed countries where conventional or modern drugs are majorly used [27]. Similarly, it is worth mentioning that the popularity, as well as the usage of these traditional medicines, has continued to increase all over the world [28]. Despite this popularity and wide usage, the safety of these herbal therapies has, in recent times, raised a lot of questions as a result of revelations due to illnesses and fatalities [29, 30] such as hepatotoxicity [31] and nephrotoxicity [32, 33] and only a few of them have been evaluated through various phases of clinical trials [34].

Toxicity studies encompass acute, subacute, chronic, or subchronic toxicity. In the present study, acute and subchronic toxicities studies were evaluated on *Dicoma anomala* aqueous root extract that paved the way for the meaningful interpretation of its toxic effect. The single oral dose administration of AQRED to Wistar albino rats at the highest concentration of 2000 mg/kg body weight did not cause any mortality and revealed no clinical signs, like changes in fur and skin, eyes, mucus membrane, respiratory rate, circulatory signs, autonomic effects, and central nervous system. In general, exposure of the animals to a single oral dose of AQRED did not produce any mortality or any treatment-related effect [35]; as such, the lethal dose (LD_{50}) was assumed to be greater than 2000 mg/kg suggesting that the extract could be generally regarded as nontoxic. This report corroborates the work of M. L. Clarke and E. G. C. Clarke [36] who maintained that any drug with oral LD_{50} greater than 1000 mg/kg BW could be regarded as safe and of low toxicity.

The administration of AQRED for 90 days revealed no clinical signs of toxicity or mortality in both sexes of the animals used in this study. There was no significant reduction in feed and water intake of the treated rats in either sex through the 90-day study; this is a pointer to the fact that the diet and water were well accepted by the rats, suggesting that the extracts did not in any way alter the metabolism of carbohydrate, protein, and fats in the rats. It may also signify the fact that the nutritional status (weight gain and appetite stability) which was expected to be seen in prolonged-fed animals was not adversely affected by the extracts. This corroborates the traditional usage of the plant by the oral route.

The body weight changes may reflect the general health status of animals [37]. However, the body weight gain witnessed in all the animals treated with AQRED suggests that the extracts did not interfere with normal body metabolism of the animals as the increment in food and water intake is synonymous to an increase in body weight. The essence of weighing organs in studies relating to toxicity provides facts on their sensitivity to toxicity, physiologic perturbations, induction of enzymes, and acute organ damage [38]. An insignificant difference in the weight of the excised vital organs compared to that of the control obtained for this study was an indication that AQRED on prolonged use or intake might not have an effect on normal growth; and since there was no reduction in body and/or relative organ weight in all the tested doses of the treated rats, it is assumed that the extract is not toxic to the excised and evaluated organs.

The assessment of blood hematology and clinical biochemistry provides an insight to possible damage brought about by the extract in the hepatic and renal functions. In toxicity studies, assessment of liver and kidney functions is germane because both organs are essential for the survival of an organism [39]. Alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) are sensitive enzymes used in assessing the severity of liver damage [40]. Elevated activities of these enzymes are associated with

liver or heart damage [41–43]. The significant reduction in ALT and AST of the treated animals in both sexes compared with normal control could suggest that AQRED may not have hepatotoxic effect [44] and, equally, might not have deleterious effect on the heart [45], while the increase in ALP might suggest obstruction of the biliary tract which may be present in the liver. Evaluation of total protein gives an estimation of the nutritional status and diagnostic measurement of liver and kidney diseases [46]. A reduction in total protein, albumin, and globulin is an indication of impaired hepatocellular function [47]; however, an insignificant difference in serum level of these parameters upon prolonged administration of *D. anomala* when compared with the control further corroborates the fact that the extracts do not destroy the secretory functions of the liver. Measurement of serum urea, creatinine, and uric acid concentration reflects the likelihood of renal problems or dysfunction [48]. The insignificant changes in urea and the significant reduction in creatinine levels obtained in this study as compared to the normal control could suggest an indication of the potentials of AQRED in maintaining a normal renal function [45]. The result was in consonance with the report of Patrick-Iwuanyanwu et al. [49] for Baker cleansers bitters.

Abnormalities in the concentration of major lipids like cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG) can give useful information on the lipid metabolism as well as the predisposition of the animals to atherosclerosis and its complications. The insignificant changes in the value of TC, LDL-c, HDL-c, and triglycerides recorded in this study might signify the intact position of the myocardial membrane [50] and could also suggest the hypolipidemic potentials of the plant [51].

A prominent role in the exchange of gas and the intercompartmental water balance is played by electrolytes. Increase or decrease in the levels of these electrolytes within the serum may be a consequence of the hypo- or hyperfunctioning of the concerned organ or tissue. Sodium, potassium, and chlorides are examples of these clinical electrolytes used in assessing the functioning of the kidney. In the present study, there was no significant change in the treated rats in these parameters when compared with the control; this could be attributed to a normal functioning status of the kidney.

Haematological evaluation is used to determine the extent of the deleterious effect on the blood. It can also explain the blood relating functions of a plant extract or its products [52]. The result from the present study revealed no significant effects on red blood cells (RBC), MCV, and haemoglobin values of the treated rats when compared to the control, suggesting that the erythropoiesis, morphology, or osmotic fragility of RBC are not affected [53]. Similarly, the insignificant changes in neutrophils, lymphocytes, and monocytes witnessed in all the tested concentrations similarly suggested the intact state of the immune system and lack of injury to the tissues, while the significant increase in platelets concentration for all the treated groups compared to control may be attributed to the elevated secretion and production of thrombopoietin, the primary regulator of platelet production [54], by AQRED suggesting its homeostatic property [55]. This result was in line with the report of Patrick-Iwuanyanwu et al. [49]. Generally, the result obtained from the hematology examinations further justified the safety potential of AQRED.

The histological examination of all the analysed (vital) organs revealed that none of the organs from the extract treated rats revealed alteration and there were no abnormalities in the cell structure of these vital organs.

Besides being antioxidative [15, 20, 21], most of the identified compounds from the GC/MS chromatogram of the plant have been implicated in other biological and pharmacological activities. 5-(Hydroxymethyl)-2-furancarboxyaldehyde, 2,3-dihydro-3,5-dihydroxyl-6-methyl-4H-pyran-4-one, n-tridecane, and globulol are reported to possess antimicrobial activity [10, 15, 16, 22, 23]. The presence of 5-(Hydroxymethyl)-2-furancarboxyaldehyde and 2,3-dihydro-3,5-dihydroxyl-6-methyl-4H-pyran-4-one in the phytonutrients is attributed to their anti-inflammatory potentials [11–13, 15]. 2-(1-methyl-2,5-dioxoimidazolidin-4-yl) acetic acid and 5-(Hydroxymethyl)-2-furancarboxyaldehyde have been reported to have antihepatotoxic effect [14, 18]. Some of the other pharmacological activities such as anticancer [20, 21] and antihypertensive [25] exhibited by the phytonutrients are shown in Table 7.

5. Conclusions

In conclusion, the administration of aqueous root extract of *Dicoma anomala* to Wistar rats was not toxic in all the tested doses and did not reveal any toxic signs in the two studied toxicities. The extract did not have a direct impact on the liver and kidney functions as evidenced from the haematological and blood chemistry results and did not cause any change in food intake, water consumption, and body weight and produces no evident histopathological damage in the rats. The presented results might give an insight to exploring the pharmaceutical and therapeutic benefit of AQRED as a viable candidate to conventional drugs with lots of side effects. Moreover, the presence of various bioactive compounds as revealed by the GC/MS result and the confirmation of some of the therapeutic properties justify the usage of the plants for various ailments by traditional healers. Further studies to ascertain the effects of the herb on pregnant animals, animal foetus, and their reproductive capability are however encouraged to fully explore the safety profile of the plant.

Competing Interests

The authors both read the paper and declared that there are no competing interests.

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Chapter Seven

General discussion and recommendations

General discussion

Diabetes is a multifaceted and multisystemic disease whose management through the use of orthodox medicines has not yielded complete therapy without adverse effects. Throughout the world, the use of phytotherapy has been seen as one of the most successful and well-accepted treatment option against diabetes (Hnatyszyn *et al.,* 2002; Kaushik *et al.,* 2010) owing to their ability to interact with the body chemistry with little or no side effects (May *et al.,* 2010).

Africa (particularly South Africa) is a continent enriched with arrays of therapeutically active plants, very effective against myriads of disorders. Aside this, numerous research conducted in the labouratory and in animal models have also revealed the pharmacological potentials of these plants (Magassouba *et al.,* 2007), attributed to different inherent secondary metabolites such as phenolics, flavonoids, tannins, alkaloids, saponins etc. The Basotho tribe (particularly the traditional healers) of Eastern Free State uses *Dicoma anomala* in the management of diabetes (Tshabalala and Ashafa, 2011) in addition to varying number of ailments such as fever, veneral diseases, cough, and colds etc. (Gelfand *et al.,* 1985; von Koenen 2001).

Antioxidants are stable molecules which bind to free radicals to terminate their chain reaction before further damage is done to cells (Halliwell, 1995). A number of degenerative diseases including liver disorders have been linked to overproduction of free radicals in the body. Liver diseases are regarded as one of the leading health problems of the world (Williams, 2006) and the use of medicinal plants with good antioxidant potentials have been reported to be effective in the amelioration of these disorders (Shehab *et al.,* 2015).

The results from the antioxidant and hepatoprotective studies (chapter three) revealed the effectiveness of all the extracts particularly hydro-ethanolic extract exhibited the highest total phenolic capacity. The high phenolic content of the extracts is indicative of good antioxidant potentials of the plant (Duh *et al.,* 1999). Shaikh *et al.* (2014) attributed this activity to the

presence of hydroxyl group attached to the aromatic ring structures which allow them to absorb, neutralize and quench the action of free radicals. The result of this investigation also showed that all the extracts, especially water were able to scavenge free radicals suggesting that most of the bioactive compounds are polar in nature. The hepatoprotective activity of the plant was determined when the aqueous root extract of the plant at 500 mg/kg body weight completely reversed towards normal the reduced activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) while also restoring the liver integrity due to damages (necrosis, cellular infiltration etc.) brought about by CCl_4 –induced hepatotoxicity.

The pancreatic α-amylase and the intestinal α -glucosidase are prominent enzymes concerned with the breakdown of carbohydrates into smaller absorbable units (Manohar *et al.,* 2002; Afifi *et al.,* 2008). Thus, inhibition of these two enzymes will go a long way in reducing the plasma glucose level in postprandial hyperglycaemia (Kwon *et al.,* 2007). The results obtained in this study (chapter four) revealed that all the extracts inhibited the activity of these enzymes dose dependently. The strong inhibition of water extract against α -glucosidase and mild inhibition against α –amylase, as evidenced by the IC_{50} values (27.41, 101.90 µg/mL respectively), is indicative of the potentials of the plant as a good hypoglycaemic agents. The result was in consonance with the submission of Kazeem *et al.* (2013) for *Ficus exasperata* and Sabiu *et al.* (2016) for *Stigma maydis*. Further probe into the mode of inhibition of the two enzymes by the aqueous extract revealed a competitive inhibition against α –amylase and a non-competitive inhibition against α –glucosidase.

Streptozotocin is a diabetogenic agent used in many studies involving animal models to induce diabetes. It acts on the β cells of the islets of the Langerhans of the pancreas to cause necrosis within 24 – 48 hr resulting in hyperglycaemia (Ghandi and Sasikumar, 2012). The aqueous root extract of *Dicoma anomala* (AQRED), especially at the highest concentration

(500 mg/kg body weight b.w.) reversed the elevated levels of plasma glucose level, lipid peroxidation measured through thiobarbituric acid reactive species (TBARS), low-density lipoprotein-cholesterol (LDL-c), triglycerides (TG), total cholesterol (TC), glycosylated haemoglobin and activities of gluconeogenesis enzymes while increasing the activities of antioxidant enzymes such as CAT, SOD, GPx, glycolytic enzyme like hexokinase and the level of high-density lipoprotein-cholesterol (HDL-c) arising due to isoproterenol administration (Vats *et al.,* 2003; Senthikumar and Subramaniam, 2008; Nabi *et al.,* 2013). Similarly, AQRED was able to clear glucose overload in the diabetic animals after 120 min following the attainment of a peak plasma glucose concentration within 1 hr, although, this effect was more pronounced in the 500 mg/kg b.w. treated-animals. The obtained results buttressed the antihyperglycaemic activity of the plant.

Creatine kinase (CK), aspartate transaminase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) are marker enzymes used to assess the fatality of myocardial infarction (Sheela Sasikumar and Shyamala Devi, 2000). As such, an increase in the activity of these enzymes in the serum is a sign of damage to the liver or heart tissues. The results obtained from this investigation (chapter five) revealed that AQRED at the three concentrations (125, 250 and 500 mg/kg b.w.) brought about a reduction in dose-dependent manner the raised activities of AST, ALT, and CPK due to isoproterenol (ISP) induction, although, AQRED at 500 mg/kg expressed satisfactory efficacy in ALT and AST activity assays than the standard drug (simvastatin). Thus, indicating the protective action of the extract against ISP –induced cardiotoxicity. Similar observations were reported by Suchalatha and Shyamala Devi (2004) for Arogh (a polyherbal mixture) and Sunmonu and Afolayan (2010) *for Artemia afra*.

The ameliorative potency of the plant in cardiac-related disorders was also corroborated when AQRED dose-dependently raised the reduced activities of antioxidant enzymes such as SOD

and CAT while concomitantly lowering the elevated level of TBARS. Similarly, AQRED depicted superior activity than simvastatin (standard) in these parameters and justifies the ameliorative activity of the plant on antioxidants. Our results were in accordance with the findings of Thounaojam *et al.* (2011) for *Sida rhomboidea* (Roxb) extract, Abhilash *et al.* (2011) for *Oxalix corniculata* and Patel *et al.* (2012) for *Coriandrum sativum*.

The use of medicinal plants for the treatment of innumerable diseases has gained global acceptance but unfortunately, scores of these plants have not been evaluated for safety (Mehreen *et al.*, 2016). The toxicological profile of AQRED at 125, 250 and 500 mg/kg b.w. was examined through oral acute and subchronic toxicity testing in Wistar rats (chapter six). In the 14-day acute toxicity study, observations of the animals revealed no abnormal findings in the fur, skin, colour of the eyes or even death between the control and the AQRED-treated rats after single oral administration of 5, 300, and 2000 mg/kg b.w AQRED to different groups of the animals. This suggests that AQRED is not toxic and that its lethal dose would be in excess of 2000 mg/kg (Clarke and Clarke, 1967). Moreover, the result from the haematological parameters following the 90 days of administering AQRED revealed no significant difference in the red blood cells (RBC), haemoglobin, haematocrit, mean corpuscular volume (MCV), white blood cells (WBC), neutrophils, etc. in both sexes between the control and the AQRED-treated rats except in platelet count where there is a significant elevation of the three concentrations relative to the control. Elevated platelet count may be attributed to increase in the secretion and production of thrombopoietin (Kaushansky, 1995) by AQRED, indicating its homeostatic property (Olaleye *et al.*, 1998). Above all, the results obtained in this aspect of the research displayed the safety of the plant and is in agreement with the previous work reported by Patrick-Iwuanyanwu *et al.* (2011). Correspondingly, AQRED at the studied concentrations did not reveal any noticeable difference in the blood chemistry results. The levels of serum total protein, albumin, globulin, urea, creatinine, LDL-

c, TG, TC, HDL-c and the activities of AST and ALT in the AQRED-treated Wistar rats were not remarkably affected when compared with the control. This suggests that the extract may not be nephrotoxic and also further justified the hypolipidaemic, hepatoprotective (Balogun and Ashafa, 2016a) and cardio-ameliorative (Balogun and Ashafa, 2016b) potentials of the herb. This is a pointer to the fact that the extract was able to maintain the hepatocellular and renal functions of the animals since alterations in the level or activities of these parameters could be ascribed to impaired hepatocellular function and or renal dysfunction (Yakubu *et al.,* 2003; Crook, 2006; Patrick-Iwuanyanwu *et al.,* 2011). However, the increase in the activity of ALP in all the AQRED doses compared with the control may reflect blockage of the biliary tract in the liver which may not be treatment-related.

In line with the aforementioned, it is not enough to submit that the plant is effective against numerous diseases or active against diabetes, liver or cardiac-related ailments as experimented in this study without assessing the possibility or the likely phytoconstituents in the plants conferring the varying pharmacological activities witnessed in this investigation. The chromatogram from the gas chromatography / mass spectrophotometric (GC/MS) analysis of the water extract of *Dicoma anomala* revealed rich quantity of eighty phytochemical compounds with 5-(Hydroxymethyl)-2 –furancarboxyaldehyde, 2-(1-methyl-2,5-dioxoimidazolidin-4-yl) acetic acid, n-tridecane, 9 –octadecenoic acid, hexadecanoic acid, phosphine, 1, 6 –anhydro-β-D-glucopyranoside, 3- (Bromopheyl) triphenyl phosphonium bromide, Guanosine, and Xanthosine identified as the prominent ones. Literature search indicated the pharmacological and or biological activities of these compounds as antioxidative, hepatoprotective, antihypertensive, anticancer, anti-inflammatory, antimicrobial etc. (Brustum *et al.,* 2005; Xu *et al.,* 2007; Mulyaningsih *et al.,* 2010; Hema *et al.,* 2011; Kaminski *et al.,* 2011; Syeda *et al.,* 2011; Mallikadevi *et al.,* 2012;

Rahbar *et al.,* 2012; Gopalakrishnan and Kalaiarasi, 2013; Silva *et al.*, 2013; Deryabin and Tolmacheva, 2015; Tang *et al.*, 2015).

Conclusion

The use of medicinal plants forms an integral part of the lives of South Africans particularly the rural dwellers because it plays a vital role in therapy within the traditional healthcare system. Based on the results obtained from this study which is hoped to provide baseline information on the medicinal properties of *Dicoma anomala*, the following submissions could be made:

- The four extracts (water, ethanol, hydro-ethanol and methanol) are very effective in scavenging free radicals.
- The aqueous extract was the most active in scavenging free radicals *in vitro*.
- The aqueous root extract at the highest concentration protected the liver against hepatic injury *in vivo*, thus, could serve as a suitable alternative in the treatment of liver-related disorders.
- All the extracts inhibited the activity of pancreatic α-amylase and the intestinal α-glucosidase enzymes *in vitro*.
- Aqueous extract was the most potent inhibitor of α-glucosidase while hydro-ethanol was the most potent against α-amylase.
- The mode of inhibition of α-amylase and α-glucosidase by water extract of *D. anomala* is competitive and non-competitive respectively.
- Aqueous root extract of *D. anomala* (Sond.) suppressed postprandial hyperglycaemia in glucose-loaded rats.
- AQRED possessed antihyperglycaemic activity and thus validates the folkloric use of the plant in the management of diabetes.

- AQRED ameliorated the effect of isoproterenol –induced cardiotoxicity and as such, could be employed in the control or management of cardiac-related diseases.
- Prolonged intake of the herb at least for 90 days in its aqueous form was not toxic to vital organs such as the liver, heart, kidney etc. and thus, may be regarded as safe.
- The best activity displayed by the aqueous extract of the plant in all tested assays justifies the usage of water mostly as the solvent of extraction by traditional healers.

Recommendations

Although, the conservation of the plant is not threatened despite being widely employed as the treatment in the management of various diseases, it is hoped that the information provided in the study would go a long way in guiding against it's over utilization. Further studies to ascertain the effect(s) of the plant on pregnant animals, animal foetus, and reproductive capability are encouraged to fully explore the safety profile of the plant. The need to isolate, purify and structurally identify the bioactive compounds responsible for the elicited pharmacological activities is also encouraged.

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Thank you

Balogun Fatai Oladunni

December, 2016

Dedication

This research is dedicated

To

My Late father

Mr TO Balogun

For being my inspiration and for giving me the best legacy- education

My mother

Alhaja SA Balogun

For her support, care and prayers at all times

My wife

Mrs RO Olawoore-Balogun

For her love, advice, understanding and for taking care of our households

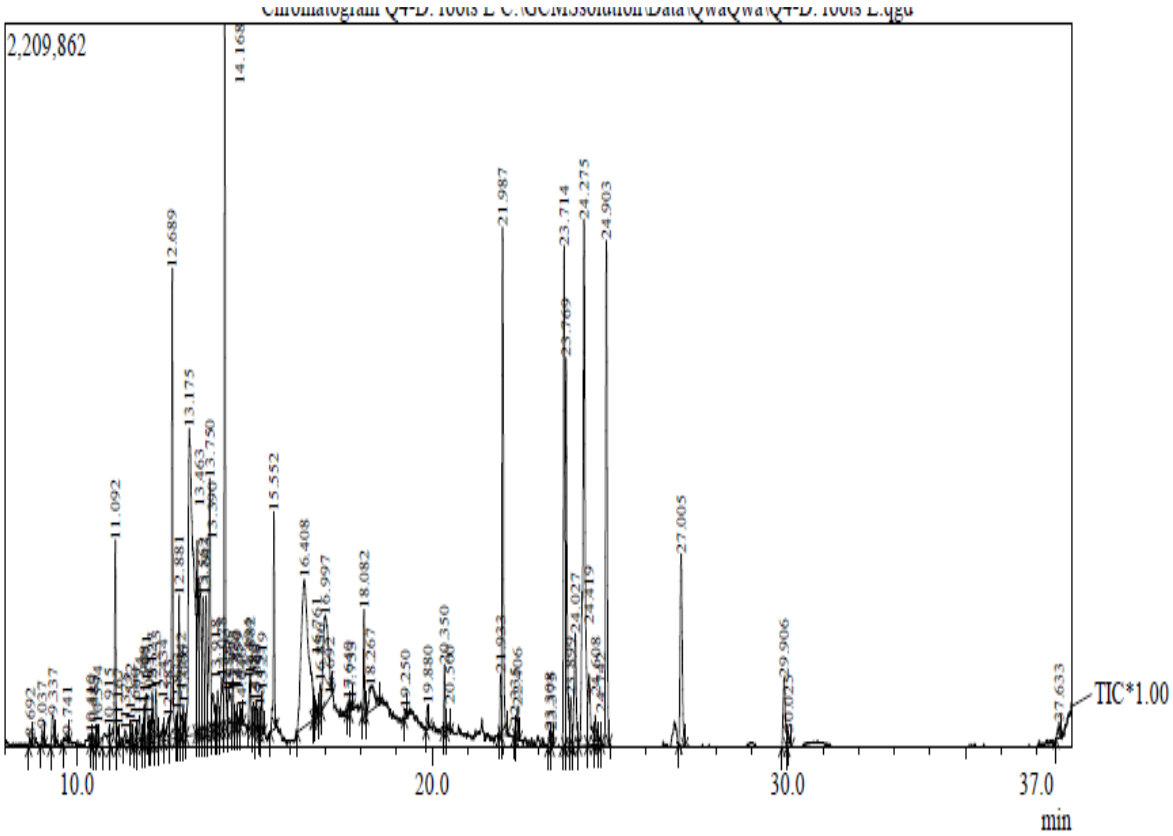
And

To

The seekers of truth and knowledge till the day of accountability

Appendices:

GC/MS chromatogram of the aqueous root extract of *Dicoma anomala* Sond.



Identified bioactive compounds from aqueous root extract of *D. anomala* (Sond.)

Peak area	Retention time	% Area	Chemical formula	Compound name
1	8.267	0.06	$C_{12}H_{22}O$	7E,9E-Dodecadiene-1-ol
2	8.458	0.03	C_6H_9N	1H-Pyrrole
3	8.525	0.03	$C_6H_5D_2N$	β-D2-Δ-Picoline
4	9.328	0.03	$C_4H_6O_3$	2-Keto-butyric acid
5	10.793	0.26	$C_{16}H_{20}F_3NO_2$	4-Methylpiperidine ®-MTPA Amide
6	10.883	0.21	$C_{18}H_{32}N_2$	(2R*3R*)-1-(Cyclohexyl)-3-(cyclohexylamino)-2-methyl-4-methylenepyrollidinebutoxy acetate
7	11.008	0.47	$C_6H_6O_3$	2-Hydroxyacetylfuran
8	11.076	0.42	$C_{10}H_{22}$	Nonane
9	11.192	0.12	$C_6H_{10}N_2O$	1H-Imidazole-1-ethanol
10	11.233	0.25	$C_{13}H_{28}$	n-tridecane
11	11.389	0.36	$C_{38}H_{46}O_2$	3,11-Diotyloxybenzo[c]benzo[a]phenanthrene
12	11.517	0.05	C_6H_9N	2-ethyl-1H-Pyrrole
13	11.600	0.05	C_6H_9N	1H-Pyrrole
14	11.650	0.57	$C_6H_{15}Al$	Triethylaluminium
15	11.800	0.03	$C_{57}H_{104}O_6$	9-Octadecenoic acid
16	11.907	1.52	$C_6H_8O_4$	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one

17	12.125	0.03	$C_{14}H_{14}O_3$	methyl endo-5-hydroxytricyclo[6.4.0.0(2,5)]dodeca-1(12),6,8,10-tetraene-4-carboxylate
18	13.180	40.64	$C_6H_6O_3$	5-(hydroxymethyl)-2-Furancarboxaldehyde
19	13.866	0.05	C_9H_{18}	Z/E-3-Methyl-4-octene
20	13.932	0.02	$C_3H\ N$	Cyanoacetylene
21	13.991	0.02	C_3HF_6N	1,1,1,3,3-Pentafluoroacetone, fluoroimine
22	14.033	0.02	$C_9H_{10}O$	2-[[(methylenecyclopropyl)oxy]methyl]-Thiophene
23	14.092	0.14	$C_7H_{13}I$	6-Iodo-1-heptene
24	14.359	0.04	$C_{10}H_{16}O_2$	(+-)-2-hydroxydec-3-yn-5-one
25	14.441	0.02	$C_{10}H_{17}BrO$	2-Bromo-4,4-di-n-propylcyclobutanone
26	14.685	0.02	$C_5H_6N_2O_2$	4-hydroxy-5-methoxypyrimidine
27	14.767	0.03	$C_{10}H_{17}BrO$	2-Bromo-4,4-di-n-propylcyclobutanone
28	14.856	0.04	$C_9H_{13}F_6NO$	1-(bistrifluoromethylamino-oxy)-1-methylcyclohexane
29	15.448	0.02	$C_6H_7NO_2$	Diacrylamide
30	15.498	0.17	$C_{11}H_{16}N_2O_3$	Vinylbital
31	15.642	0.02	$C_8H_{14}O$	n-Butyl Cyclopropyl Ketone
32	15.700	0.02	$C_8H_{12}O_3$	Methyl (1R,2R,4S)-4-methyl-6-oxabicyclo[3.1.0]hexanecarboxylate
33	15.808	0.03	$C_7H_{12}O$	3-ethoxy-3-methyl-1-Butyne
34	16.544	6.63	$C_{10}H_{12}N_4O_6$	Xanthosine
35	16.750	1.85	$C_4H_9NO_2$	Acetamide

36	17.089	10.90	$C_6H_8N_2O_4$	2-(1-Methyl-2,5-dioxoimidazolidin-4-yl)acetic acid
37	17.349	1.68	$C_{14}H_{20}ClN$	1-dimethylamino-2,3-dimethyl-4-(4-chlorophenyl)-2-butene
38	17.508	1.06	$C_{10}H_{16}Br_2O$	2,2-Dibromo-4-di-n-propylcyclobutanone
39	17.658	0.49	$C_{13}H_{26}O$	6-methylene-4-Dodecanol
40	17.776	1.35	$C_5H_{10}O_2$	n-Butyl-Formate
41	17.992	1.26	$C_{12}H_{24}O_2$	2,4-Dipropyl-5,5-dimethyl-1,3-dioxane
42	18.142	0.64	$C_4H_3NO_2$	1H-Pyrrole-2,5-dione
43	18.376	3.48	$C_6H_{10}O_5$	1,6-Anhydro-.beta.-D-glucopyranose
44	18.667	0.85	$C_8H_{14}O_3$	2-methyl-, anhydride -Propanoic acid
45	18.867	0.96	$C_5H_{12}O$	2-methoxy-2-methyl- Propane
46	19.016	0.58	$C_6H_{12}O_2$	3-methyl- Pentanoic acid
47	19.183	0.44	CH_4N_2O	Urea
48	19.275	0.50	$C_9H_{15}NO_2$	Methyl-3-hydroxy-cis-3a,4,5,6,7,7a-hexahydroisoindolin-1-one
49	19.425	0.83	$C_{21}H_{26}N_2O_8S$	N-Phenacyl-N'-(3,4,6-tri-O-acetyl-2-deoxy-.beta.-D-arabinohexopyranosyl)thiorea
50	19.492	1.03	$C_{21}H_{26}N_2O_8S$	N-Phenacyl-N'-(3,4,6-tri-O-acetyl-2-deoxy-.beta.-D-arabinohexopyranosyl)thiorea
51	19.708	0.52	$C_5H_8O_4$	Pentanedioic acid
52	19.880	0.49	$C_{12}H_{20}N_2O_3$	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-1,3-dimethyl-5-(1-methylpropyl)-
53	19.950	0.08	$C_7H_{12}O$	3-Hexyn-2-ol, 5-methyl-
54	20.010	0.20	$C_{12}H_{13}NO_2S_2$	2-(t-Butoxycarbonyl)thibenzothiazole

55	20.158	0.04	$C_6H_{12}O_2$	(2S,3S)-2,3-Epoxy-1-hexanol
56	20.208	0.03	$C_7H_{13}I$	6-Iodo-1-heptene
57	20.272	0.08	$C_8H_{14}O_3$	2,3,11-trioxabicyclo[6.2.1]undecane
58	20.733	0.02	$C_{16}H_{39}B_2N$ SI	1,1-di(t-Butyl)-2-(t-butylamino]-2-[(trimethylsilyl)methyl] diborane(4)
59	21.220	0.14	$C_{21}H_{23}N_2O_2$	2,2-Dimethyl-4-phenyl-N-(3'-phenylprop-2'-en-1'-ylidene)-1,3-dioxan-5-amine
60	21.391	0.20	$C_{10}H_{17}NO_2$	Ethyl (1S,3R,4R)-2-Methyl-2-azabicyclo[2.2.1]heptane-3-carboxylate
61	21.465	0.06	C_3H_7NO	Isoxazolidine
62	21.767	0.13	$C_{13}H_{17}IOSI$	1-Iodo-2-[4'-(trimethylsilyl)but-2'-ynyloxy]benzene
63	21.977	2.36	$C_{16}H_{32}O_2$	Hexadecanoic acid
64	22.126	0.04	$C_6H_{11}NO$	3-(hydroxymethyl)-pentanenitrile
65	22.167	0.04	$C_7H_{16}O$	3-Pentanol, 2,4-dimethyl- (CAS) Diisopropylcarbinol
66	22.369	0.24	$C_9H_7N_3$	2-Methyl-5(6)-cyanobenzimidazole
67	22.979	0.05	$C_{20}H_{30}O_6S$	2,5,5-trimethyl-2-[6-(tosyloxy)-4,5-epoxyhexanyl]-1,3-oxane
68	23.395	0.05	$C_7H_{11}NO_2$	N-Allyloxymethylacrylamide
69	23.703	1.16	C_9H_{16}	3-methyl-Cyclooctene
70	23.763	1.43	$C_{16}H_{30}$	1-Hexadecyne
71	24.007	0.25	$C_5H_{10}O_2$	2-[(2-Propenyl)oxy]ethanol
72	24.271	4.69	$C_{18}H_{15}P$	(Triphenyl)phosphine
73	24.413	0.53	$C_9H_{11}F_3O_2$	2-(trifluoromethyl)-2-(1-cyclohexenyloxy)oxirane

74	24.508	0.12	$C_{50}H_{52}NO_8S$	N,N'-Bis(carbobenzyloxy)cystine Dibenzyl ester
75	24.690	0.34	$C_{21}H_{19}Br_2ClO_2$	6-{3',5'-Dibromo-4'-(4"-chlorobutoxy)phenyl]methyl}naphthalene-2-ol
76	24.896	2.63	$C_{20}H_{28}O_6$	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis
77	26.795	0.12	$C_4H_6O_3$	2-keto-butyric-acid
78	26.998	2.88	$C_{20}H_{14}$	9-(phenylmethylene)- 9H-Fluorene
79	29.984	0.15	$C_{20}H_{23}N$	1,5-dimethyl-3,3-diphenyl-2-ethylidene- Pyrrolidine
80	37.653	0.19	$C_{11}H_{12}N_2O_3S$	N-(4-Nitro-thiobenzoyl)-morpholine