ANTI-DIABETIC AND ANTI-OXIDANT ACTIVITIES OF *Enantia chlorantha* STEM BARK ON STREPTOZOTOCIN-INDUCED DIABETES ON THE LIVER OF ADULT WISTAR RATS FED ON HIGH FAT DIET

BY

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SCHOOL OF BASIC MEDICAL SCIENCE
COLLEGE OF MEDICAL SCIENCE
UNIVERSITY OF BENIN

JULY, 2021

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IN FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF BACHELOR OF SCIENCES DEGREE IN
MEDICAL BIOCHEMISTRY

SUPERVISEDBY:
DR. ORIAKHI KELLY

JULY, 2021

CERTIFICATION

We undersigned hereby certify that ORJI KALU RHODA (BMS1601773) carried out this work in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the award of Bachelors of Science Degree (B.Sc) in Medical Biochemistry.

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(HEAD OF DEPARTMENT)

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EXTERNAL EXAMINER **DATE**

DEDICATION

This project work is dedicate to Almighty God for seeing me through the course of writing this work. This work is also dedicated to my supportive family and my amiable Department.

ACKNOWLEDGEMENT

My sincere gratitude goes to God Almighty for giving me the gift of life and seeing me through this process. I acknowledge the assistance of my supervisor Dr. Oriakhi Kelly for his support during the course of the work.

I wish to thank the lecturers of the Department of Medical Biochemistry who helped equip me with the knowledge to carry out this research and in other areas.

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ABSTRACT

There has been a notable increase in the use of herbal medicine over the past twenty years. Moreover, there is still a tremendous lap of proper research data in the field of traditional herbal medicine. *Enantiachlorantha*is a plant belonging to the Annonacoae family. Its common name is Africa Yellow Wood. It contains some phytochemicals which confer anti-diabetic properties. The aim of this study is to evaluate the anti-diabetic and anti- inflammatory effects of *Enantiachlorantha* stem bark on STZ induced type 2 diabetes in adult wistar rats. Forty-nine (49) male Wister rats of 170-180g weight were used for this experiment. They were grouped into seven groups; Group 1 (Normal control), Group 2 (HFD+STZ), Group 3(HFD+STZ+ metformin 500mg/kg bwt), Group 4 (HFD +STZ+ 200mg/kg bwt crude hydro-ethanol extract), Group 5 (HFD + STZ+ 400mg/kg bwt crude hydro-ethanol extract), Group 6 (HFD + STZ+ 200mg/kg bwt ethyl-acetate fraction), Group 7 (HFD+STZ+ 400mg/kg bwt ethyl-acetate fraction). The results showed a marked decrease in blood glucose level in thr treated groups. Rats administered the extract crude and ethyl acetate fractions showed high expression of Nrf-2.In group 4, at 200 mg/kg body weight of thereextract crude and ethyl acetate fractions was increased activity of superoxide dismutase and catalase enzyme.This study concluded that *Enantiachlorantha*has positive anti-diabetic and anti- inflammatory effects.

CHAPTER ONE

INTRODUCTION

1.0 Background of Study

Medicinal plants in general are plants that are rich in phytochemicals which possesses therapeutic properties to the animals and human body and can be used in drug production. In addition to the vital role they play in the field of drug production some of these plants serve as a great source of nutrition, due to these reasons some these plants are in high demand and they include Gingko, Ginseng, Ginger, Garlic, Rosemary, Aloe-vera, Spinach etc. These plants have different phytochemicals and antioxidants that are used for the treatment of a wide range of diseases like malaria, diarrhea, tuberculosis, asthma etc.

This plants and its therapeutic properties are the backbone of most traditional medicine practices which is acknowledged and practiced in some developed/well developed countries like China, India, Thailand, Japan etc. About 40% of the total medicine in China alone is attributed to traditional medicine. While the fruits and leaves of some plants are used for medicinal purposes, the barks, stems and roots of others are being used e.g*Enantiachlorantha*(African yellow wood),*Enantiachlorantha* (family- Annonaceae), common name African Yellow Wood is a medicinal plant that is distributed along the coasts of West and East

Africa and it is very common in the forest regions of Nigeria (Adesokan*et al.,* 2007).

Several further studies and experiments has shown that the stem bark of *E. chlorantha*posseses wide range of antimalarial and antimicrobial properties (Adesokan, 2007; Odugbemi*et al.,* 2007). Different extracts of the stem bark are used in treatment of large range of diseases like urinary tract infections (Adjanhoun*et al.,* 1996), hypoglycemia, typhoid fever, (FAO, 2001).

Streptozotocin (STZ) is a naturally occurring chemical derived from Streptomyces achromogen agents that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. STZ is used as an antineoplastic agents to induce diabetes in experimental animals. It is a nitrosourea alkylating agents which selective uptake into pancreatic beta cells because of the similarities of it to glucose in structure. It enters the beta cells via a glucose transporter (GLUT 2) and causes alkylation of DNA, it also liberates toxics amount of nitric oxides that inhibits aconitase activity an participates in DNA damage, as a result of this active beta cells experiences necrosis resulting to hypoinsulinemia and hyperglycemia.

1.1 JUSTIFICATION OF STUDY

This disease has shown a great prevalence with a demographic transition in its epidemiology in recent years. According to the latest figure published by the

International Diabetes Federation (IDF) shows that 425 million persons are living with diabetes mellitus worldwide with about 50% of this population undiagnosed.(IDF, 2017).Asia and Africa contributes a significant fraction of the figures. Alongside the increase in the prevalence of this disease is the rising burden of diabetes mellitus related complications.(Uloko*et al.,* 2012). Though there has not been a nationwide diabetic survey in Nigeria in recent years.

(1.2) AIMS OF STUDY

1. The aim of this study is to evaluate the anti-diabetic and anti- inflammatory effects of *Enantiachlorantha* stem bark on STZ induced type 2 diabetes on the liver of the adult wistar rats fed on high fat diet.
2. The specific objective is to investigate the effect of STZ on the liver of the type 2 induced diabetes in the adult wistar rats fed on high fat diet.

CHAPTER TWO

LITERATURE REVIEW

2.0 MEDICINAL PLANTS

There has been a notable increase in the use of herbal medicine over the past twenty years. Moreover, there is still a tremendous lap of proper research data in the field of traditional herbal medicine, this is to say that amongst large varieties of this medicinal plants, only a few of them has been properly researched. Though other medicinal plants are known but they are phytochemical compositions still remain unknown due to lack of research.(Bandaranayake,2006).

Several studies have shown that some of this already researched plants can be multifunctional but have not been exhaustively and extensively researched to be able to ascertain all their phytochemical constituents, anti-oxidant properties and possible pharmacopoeia characteristics.(Jakubowski and Bartosz, 2017). The contributions of plants in some industries are remarkable as it serves as a source of raw materials in the production of cosmetics, fine chemicals and drugs. Medicinal plants play a vital role in the discovery and development of a new drug. Plants are not indispensable in health care as they are seen as the best hope and source for save future medicine and this calls for an urgent need to research, discover and develop new therapeutic agents with little or no side effects from plants. (Gao*ei al*., 2007).

2.1 History of *Enantiachlorantha*

*Enantiachlorantha*is a plant belonging to the Annonacoae family. Its common name is Africa Yellow Wood, it is called Awopa, Osu papa (Yoruba), Dokita (Igbo), Osomolu (Ikale), Erumeru (Nigeria) Kakerin (Boki), Yellow moambi (English) and MoambiJaune (French). (AdesokanAndAkanji, 2003). It is an ornamental tree of about 30m high with a dense foliage and spreading crown.(Akpuku, *et al.,* 1994).It has a thick bark and a fluted stem with a darker brown outer space and a lighter brown inner space.(Agbaje and Onabanjor, 1993).The leaves display parallel secondary nerves and pairs of prominent lateral veins. It is found in the East and South forest of Cameroon, South part of Nigeria, DRC, Angola and Gabon.(Adesokan and Akanji, 2003).

2.1.1 Phytochemicals

*E.chlorantha*contains many bioactive compounds which justifies its numerous medicinal properties. Phytochemical screening conducted by Dawodu*et al.,*shows the presence of saponins, flavonoids, alkaloids, phenols, reducing sugar, glycoside and tannins,(Adesokan and Akanji, 2003) which are responsible for the pharmacological properties.

2.1.2 Medicinal Benefits

*E.chlorantha*has been reported to be beneficial and possess some relevant pharmacological properties which includes;

1. Enhance male fertility

A study was conducted and aimed at evaluating the effect of aqueous extract of stem bark of *Enantiachlorantha* on the sperm parameters and the histoarchitecture of the testicles of rats. Some of this rats (Group 1, 2 and 3) were successively treated with lead (IV) oxide and *Enantiachlorantha*extract, while negative control rats (group 4) received lead IV only. Testicles histoarchitecture showed the spermatogonia as well as the spermatids and spermatocytes that were quite obvious in the rats treated with extract as compared with those of group 4. The interstitial spaces were abnormally widened and the leydig cells were destroyed in group 4 rats. The sperm quality (sperm count, mobility, viability and progressivity) of group 1, 2 and 3 rats were very good, while those of rats in group 4 were weak. They finally suggested that in addition enhancing fertility, this plant's extract can play a protective and a regenerative role against destroying agents when used as prophylactic (Oyewopo*et al.,* 2001).

Another study also showed that the extract of*Enantiachlorantha* significantly increases sperm mobility and viability in a dose-dependent manner, but they did not notice any important increase in the sperm accounts. Following the results

obtained in these studies, we can say that, low doses of this plant extracts could improve sperm quality, hence male fertility (Salman *et al.,* 2008).

2. Anti-convulsion and anti-inflammatory activity

An investigation of the effect of boiled and evaporated extracts of *Enantiachlorantha* in reversing bicucculine-induced convulsions and carrageenan induced inflammation in rodents. The results showed that, while the evaporated aqueous herbal drug increased the latency of convulsion in all the treated animals, the aqueous extract did not, behaving rather similar to the control mice given distilled water.*Enantiachlorantha* did not compare well with phenobarbitone (2.0–6.0 mg/kg) which protected all the animals from seizure. On the other hand, a dose dependent anti-inflammatory action of evaporated extract of *Enantiachlorantha* (50.0– 250.0 mg/kg) in carrageenan induced inflammation was obtained showing a better efficacy than the boiled a queous preparation and compared favorably with aspirin. *Enantiachlorantha* showed statistically significant activity at doses of 100 and 250 mg/kg, exhibiting 67% and 90% inhibition respectively post six hours induction of inflammation. No inhibition was observed in the control group. They concluded that *Enantiachlorantha*, especially the evaporated extract, exhibited significant anti-inflammatory effect on carrageenan-induced inflammatory edema in rats. They added that this effect is more gradual and more sustained than a similar effect of aspirin (Agbaje*et al.,* 2006).

3. Anti-viral activity

A study was conducted to evaluate the antiviral effects of bark extract of *Enantiachlorantha* on yellow fever virus (YFV) in vitro, using Vero cell line, to determine the minimum tolerance dose of these plants extract on Vero cells, and to justify in this regard, the administration of this medicinal plant by the traditional practitioners. This study showed that aqueous extracts exhibit antiviral activities against yellow fever virus.*Enantiachlorantha* resulted in inhibition of YFV at MICs of 0.025 mg/mL. The result of the study revealed that the water extract of this plant showed significant antiviral activity. Based on this experimental evidence, the authors suggested that the extracts were considered effective against YFV as they completely inhibited the infectivity of YFV as evident in complete absence of cytopathic effects. These researchers added that, the broad-spectrum antiviral activity of the plant extracts is possible due to the identified alkaloids (Taiye*et al.,*2011).

The antiviral activity of extracts from dried stem bark of*Enantiachlorantha* was also noted by (Wafo*et al.,* 1999).

4. Anti-oxidant activity

A study was conducted in order to determine the in vitro antioxidant activity of the solvents (methanol, n-hexane, chloroform, ethyl acetate and water) extracted material from stem bark of*Enantiachlorantha*, using models of DPPH

(2,2diphenyl-1-picrylhydrazyl) radical scavenging activity, nitric oxide scavenging property, ferric reducing property (FRAP) and hydroxyl radical scavenging property. Methanol extract revealed the highest flavonoids and phenolic contents followed by aqueous, ethyl acetate, chloroform, n-hexane respectively. As the phenolic and flavonoid contents rose, free radical scavenging potential of the extracts was high. From this result, we can suggest that this plant's antioxidant property justify its uses in the traditional medicine for the management of malaria, cough and wounds which are all stress related diseases (Olanlokun*et al.,* 2013).

5. Anti-helicobacter pylori activity

A study was carried out on the in vitro and in vivo anti-Helicobacter/Campylobacter activity of aqueous extract of *Enantiachlorantha* stem bark. The in vitro activity was dose-dependent, and they recorded the same antimicrobial parameters (MAQ = 0.63 mg; MIC = 0.39 mg/mL; MBC = 1.56 mg/mL; ET (100) = 8 h) for both H. pylori and C. jejuni or C. coli. *Antral mucus* sample cultures from mice treated with*Enantiachlorantha* extract (500 and 1000 mg/kg for three days) did not yield any growth. The results finally revealed that, apart from its in vitro effects,*Enantiachlorantha* aqueous extract also possesses in vivo antibiotic activity against H. pylori (Tan *et al.,* 2010).

6. Analgesic and anti-pyretic activity

Aqueous extract of the bark of *Enantiachlorantha* administered intra-peritoneally into healthy adult albino mice at doses of 1.0 and 5.0 g/kg resulted in elevation of pain threshold. *Enantiachlorantha* extract was about 20 times less potent than morphine, a reference drug. However, a dose of 15.0 g/kg given orally to rabbits infected with Klebsiellasp was capable to relieve the pyrogenic induced fever whereas no such effect was mentioned in the control group that was given simple water (Agbaje*et al.,* 1998).

7. Gastro-protective activity

A scientific study was conducted to determine the gastroprotective effects of *Enantiachlorantha* against induced- gastric ulcers in rats. First of all gastric ulcers were induced by administering 1 ml of absolute ethanol and 30 mg/kg of indomethacin, separately to two groups of rats. On the other side, two other groups of rats were pretreated with the ethanol extract (300 mg/kg) of *Enantiachlorantha* 30 minutes before the ulcerogenic agents. After this, the ulcer indices were compared. The results showed that rats pretreated with extract before the administration of the ulcerogenic agents were well protected. The inhibition effect of extract against ethanol-induced ulcers was most effective than indomethacin-induced ulcers. These researchers suggested that the extract acts particularly as a

cytoprotective agent, but also by inhibiting the secretion of gastric acid (Siminialayi and Agbaje, 2005).

Anti-microbial and anti-bacterial activity

A research work conducted and aimed to determine the in vitro and in vivo antimicrobial activity of an ointment formulated with a purified alkaloid isolated from *Enantiachlorantha*, on rats infected with fungi. The result showed the effectiveness of this ointment against *Trichophytontonsurans* and *Candida glabrata*, while *Trichophytoninterdigitali* and *Candida albicans* was less sensitive. 50 mg/mL ointment had even a better percentage inhibition than Tioconazole cream 1%, used as reference drug. They concluded that the alkaloid fraction of *Enantiachlorantha* stem bark as well as the formulated ointment exhibited significant in vitro and in vivo antifungal activities against different species of candida, dermatophytes and plants fungi (Nyong*et al.,* 2015).

Another study conducted on antimicrobial effects of *Enantiachlorantha* extracts and it was revealed that, the ethanolic one showed antimicrobial activity on all the seven isolates tested (*Staphylococcus aureus*, *Escherichia coli, Salmonella typhi, Enterococcus faecalis, Shigellasonnei, Proteus vulgaris* and *Candida albicans*), with zones of inhibition in the range of 5 mm to 33 mm, and a minimum inhibitory concentration (MIC) between 1.56 and 12.5 mg/mL (Atukpawu and Ozoh, 2014).

The antibacterial activity of aqueous extracts of *Enantiachlorantha* stem bark was also investigated. The zones of inhibition on bacterial isolates (*Staphylococcus aureus* and *Bacillus substilis, Escherichia coli, Salmonella typhymurium* and *Pseudomonas aeruginosa*) were proportional to the concentration of the plant extract. Gram-positive bacteria were more sensitive to the extract than gram-negative one. They suggested that the identified alkaloid might be responsible of the antibacterial activities (Adesokan*et al*, 2007).

Another study also noted the antibacterial activity of this plant extracts. Palmatine chloride and jatrorrhizine chloride have also been identified as the major antimicrobial constituent of the plant extracts (Atata*et al.,* 2013) and (Moody *et al.,* 1995).

Anti-malarial activity

There are several reports on the antimalarial potential of *Enantiachlorantha*. Antimalaria activities of *Enantiachlorantha* and *Rauwolfiavomitoria* extracts were carried out in rodent's malaria (Agbaje*et al.,*2006).

The antimalarial activities of the aqueous extracts of *Rauwolfiavomitoria* and*Enantiachlorantha*, employed separately and in combination in *Plasmodium berghei* infected male albino mice were investigated. Results showed that the prophylactic group, which received the extract prior to challenge with malaria parasite, recorded a suppression of infection compared with the control but no total

protection was offered in all the case investigated. *Enantiachlorantha* extract like *Rauwolfiavomitoria* extract was found to be efficient against *Plasmodium berghei* as compared to the control group were they recorded deaths from day-7, but combination therapy produced a better result than either extract used alone. Another study of three plants traditionally used in the treatment of malaria in the south-eastern part of Nigeria was investigated to determine their efficacies as antimalarial compounds. Ethanolic extracts from the roots of *Salacianitida*, *Nauclealatifolia* and stem bark of*Enantiachlorantha* (Oliv.) were assessed for antimalarial activity against chloroquine sensitive *Plasmodium berghei* in mice using a 4-day suppressive test procedure. The extracts had intrinsic antimalarial properties that were dose dependent. The comparison analysis indicated that 250 mg/kg body weight of the root of S. nitida produced 71.15% suppression of parasitemia and the 500 mg/ kg body weight of the stem bark of E. chlorantha, roots of S. nitida, N. latifolia and the three herbs combined, produced 75.23, 73.28, 71.15 and 77.46%, respectively, compared with chloroquine with 71.15% suppression. The results were significant at ($p < 0.05$) when compared to a placebo and support the traditional use of these plants for the treatment of malaria (Ogbanna*et al.,* 2008) .

Two other studies, conducted by (Kimbi*et al.,* 1996) and (Vennrstrom*et al.,* 1998) respectively, also revealed the presence of two alkaloids in this plant, possessing antimalarial properties.

2.2 Diabetes

Diabetes Mellitus is a complex chronic disease disorder where there is an increase on the level of blood glucose (Wang and Lo, 2018). Insulin is the hormone produced by the beta cells of the pancreas that helps the uptake of glucose into the cells where it is used to generate energy (Viswanath and McGavin, 2013). In a diabetic condition, there is a decrease in the body's ability to respond to insulin and in some cases this condition leads to a decrease in the production of insulin which leads to abnormalities in the metabolism of carbohydrates, proteins and fats (Nentwich and Ulbig, 2015). Symptoms often include frequent urination, increased thirst and increased appetite (WHO, 2013).

A prolonged diabetic condition may result to acute complications which may include, diabetic ketoacidosis, hyperosmolar hyperglycemic state of death (Kitabchi, *et al.,* 2009). Serious long time complications may include, chronic kidney disease, foot ulcers, damage to the eyes, cardiovascular disease and damage to nerves (Saidi, *et al.,* 2016).

2.2.1 Types of Diabetes

There are four major types of diabetes namely;

1. Type 1 Diabetes Mellitus
2. Type 2 Diabetes Mellitus
3. Pre –Diabetes Mellitus
4. Gestational Diabetes Mellitus

Type 1 Diabetes Mellitus

It is an autoimmune condition where the insulin producing cells of the pancreas (beta cells) are damaged.(Curtis, *et al* 2009, Henry and Norman 2015). In this condition, the pancreas produces little or no insulin, so there is low or no uptake of glucose from the blood into the cells. It is the most common type of diabetes for people under the age of 30 but it can occur at any age. People do not inherit type 1 diabetes itself, but they inherit a genetic predisposition towards developing a Type 1 Diabetes Mellitus (Carpenter and Coustan, 2012). This is mostly caused by genetic factors and immunology factors (autoimmune diseases) etc

Treatments

a. It is treated by medication uses (oral hypoglycemia agents).
b. Administering insulin injections (Wang and Lo, 2018)

Type 2 Diabetes Mellitus (Adult onset Diabetes)

In this condition the pancreas produces insulin which is not enough or the insulin does not work properly. This means that the cells of the body not responding properly to the insulin produced.(Shoback and Gardner, 2011). That stimulates the pancreas to produce more insulin until it can no longer keep up with the demand. Insulin production decreases which leads to high blood glucose level. (O'Sullivan and Mahan, 2014).

This is mostly caused by;

a. Genetics
b. Lack of Exercise
c. Obesity (Picot et al 2009)
d. Age (the risk increases with age)

Treatments

a. Dieting (eating right)
b. Insulin injections
c. Oral intake of anti-diabetic drugs

Pre-Diabetes Mellitus

It occurs when blood sugar is higher than normal range, but not high enough to be diagnose. It can make an individual more likely to get Type 2 Diabetes and heart diseases.

It is mostly caused by;

e. Various illnesses
f. Pancreatic diseases (infections/tumors)
g. Hormonal disorders
h. Accidents and Surgeries
i. Drugs such as glucocorticoids and oestrogen containing preparations.

Gestational Diabetes Mellitus

This occurs when there is high blood glucose level during pregnancy, this is as a result of production of insulin blocking hormones caused by hormonal changes during pregnancy. Because a woman's blood sugar travels through their placenta to the baby it's important to control gestational diabetes to protect the baby's growth and development. Glucose level returns to normal after childbirth. (Cash Jill, 2014).

This is mostly caused by;

a. Overweight during pregnancy
b. Age (pregnant women over 35 years old)
c. Women having family history of diabetes mellitus
d. Women having history of Polycystic Ovary Syndrome (PCOS)

Treatment

a. Careful meal planning that comprises enough nutrients and less fat and calories

b. Daily Exercise to keep weight gain under control

c. Taking insulin to control blood sugar level if needed

Complications during pregnancy

High blood sugar levels during pregnancy can harm mother and child and increases the risk of:

a. High blood pressure
b. Preeclampsia
c. Miscarriage or still birth
d. Child defects
e. Difficulty during child birth due to excessive weight gain by the baby

2.2.2 General Symptoms of Diabetes

1. Excessive taste and hunger

2. Frequent urination

3. Drowsiness Or fatigue

4. Dry, itching skin

5. Blur vision

6. Slow healing process

7. Weight loss
8. Frequent yeast infection or urinary tract infection

2.2.3 Prevalence of Diabetes Mellitus

This disease has shown a great prevalence with a demographic transition in its epidemiology in recent years. According to the latest figure published by the International Diabetes Federation (IDF) shows that 425 million persons are living with diabetes mellitus worldwide with about 50% of this population undiagnosed.(Foweler, 2008). Asia and Africa contributes a significant fraction of the figures. Alongside the increase in the prevalence of this disease is the rising burden of diabetes mellitus related complications. (Uloko, *et al.,*2012). Though there have not been a nationwide diabetic survey in Nigeria in recent years (Foweler, 2008).

2.2.4 Pathophysiology

Diabetes Mellitus is a syndrome with disordered metabolism and inappropriate hyperglycemia due to either a deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate. Type 1 diabetes

is due to pancreatic islet beta cells destruction predominantly by an autoimmune process, and these persons are prone to ketoacidosis. While Type 2 Diabetes is the more prevalent form and results from insulin resistance with a defect in compensatory insulin secretion. Diabetes can lead to serious complications resulting in multiple diseases or disorder that affects multiple systems that may result in premature death.(Tripthi and Srivastava, 2006).

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with IDDM. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessivesecretion of glucagons in IDDM patients. Normally,hyperglycemia leads to reduced glucagons secretion, however, in patients with IDDM, glucagons secretion is not suppressed by hyperglycemia (Raju and Raju, 2010).The resultant inappropriately elevated glucagons levels exacerbate the metabolic defects due to insulin deficiency. The most pronounced example of this metabolic disruption is that patients with IDDM rapidly develop diabetic ketoacidosis in the absence of insulin administration. Although insulin deficiency is the primary defect in IDDM, there is also a defect in the administration of insulin. There are multiple biochemical mechanisms that account for impairment of tissue's response to insulin. Deficiency in insulin leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which

suppresses glucose metabolism in peripheral tissues such as skeletal muscle (Raju and Raju, 2010). This impairs glucose utilization and insulin deficiency also decreases the expression of a number of genes necessary for target tissues to respond normally to insulin such as glucokinase in liver and the GLUT 4 class of glucose transporters in adipose tissue (Ozougwu*et al.,* 2013 .0 mg/dl). The individuals with impaired glucose tolerance have hyperglycemia inspite of having highest levels of plasma insulin, indicating that they are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus, the level of insulin declines indicating that patients with NIDDM have decreased insulin secretion. Insulin resistance and insulin deficiency are common in the average NIDDM patients (Holt, 2004). Insulin resistance is the primary cause of NIDDM, however some researcher contend that insulin deficiency is the primary cause because a moderate degree of insulin resistance is not sufficient to cause NIDDM (Raju and Raju, 2010). Most patients with the common form of NIDDM have both defects. Recent evidence has demonstrated a role for a member of the nuclear hormone receptor super family of proteins in the etiology of type 2 diabetes (Raju and Raju, 2010).

2.3 Streptozotocin (STZ)

Streptozotocin (STZ) is a naturally occurring chemical derived from Streptomyces achromogen agents that's particularly toxins to the insulin-producing beta cells of the pancreas in mammals (Brentjens and Saltz, 2011). STZ is used as an antineoplastic agents to induce diabetes in experimental animals. It is a nitrosourea alkylating agents which selective uptake into pancreatic beta cells because of the similarities of it to glucose in structure. It enters the eta cells via a glucose transporter (GLUT 2) and causes alkylation of DNA, it also liberates toxics amount of nitric oxides that inhibits aconitase activity an participates in DNA damage, as a result of this active beta cells experiences necrosis resulting to hypoinsulinemia and hyperglycemia (Schnedl*et al.,*2014).

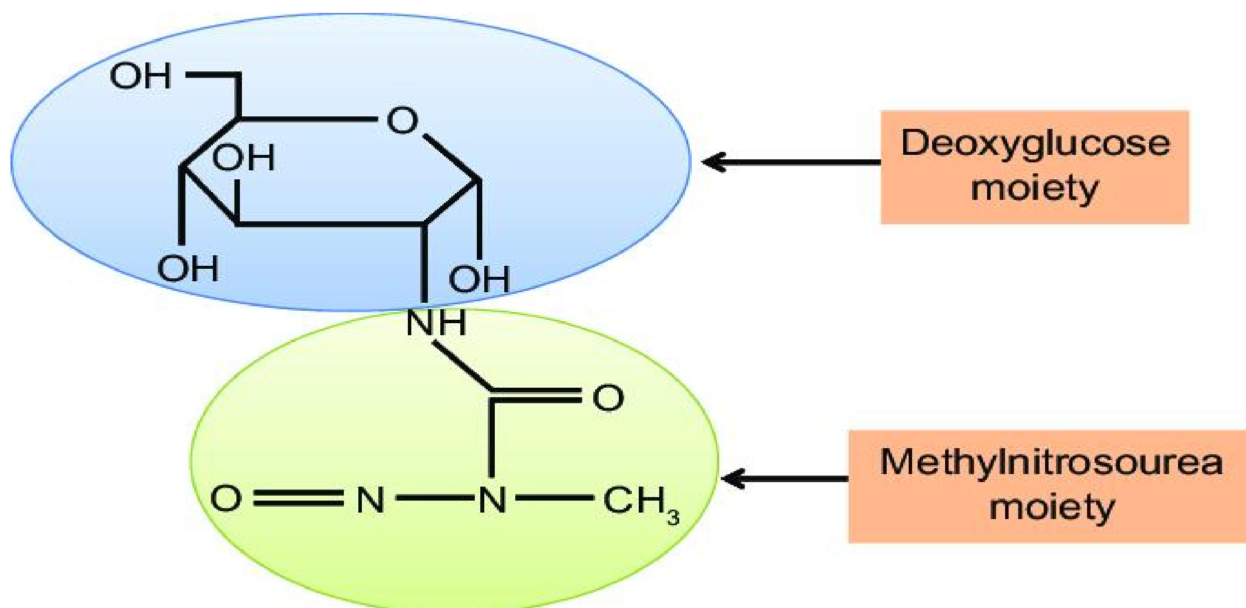


Figure 2.1: Chemical Structure of Streptozotocin.

Source: Researchgate.net.

2.4 Anti-oxidant enzymes

Catalase gene

The catalase gene is the gene that provides instruction for the making of subunits of enzyme called catalase – a key antioxidant enzyme in the defense against oxidative stress caused by reactive oxygen species (ROS). It catalyses the decomposition of hydrogen peroxide to water and oxygen (Deisseroth and Dounce 2010, Chelikani., 2004), thereby mitigating the toxic effects of hydrogen peroxide. It is a very important enzyme in reproductive reactions and also as one of the highest turn-overs numbers of all the enzyme. One catalase molecule converts millions of hydrogen peroxide to water and oxygen each second (Goodsell, 2004).

It is a tetramer of four polypeptide chains, with each chain having over 500 amino acids long (Boon, *et al.,* 2007). It contains four porphyrinheme (iron) groups that enables the enzyme to react with hydrogen peroxide. Catalase has a pH range that varies from 4 – 11 depending on the species (Brenda, 2009). The optimum pH for humans is 7,(Maehly and Chance, 1954), and the rate of the reaction does not change appreciably at pH between 6.8 and 7.5 (Aebi, 1984). Likewise, the optimum temperature also varies according to the specie. (Toner *et al.,*., 2007).

The polymorphism of this gene has been associated with decrease catalase activity thereby resulting to excess accumulation of ROS – main causes of oxidants stress.

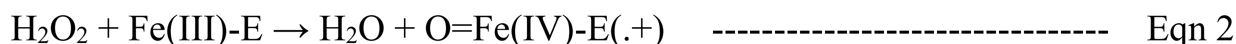
Oxidative stresss play a vital role in the development and progression of many chronic diseases like diabetes, systemic lupus, rheumatoid Asthma, cancers, Alzheimer's disease and arthritis..

The catalase reduces the substrate by the following decomposition reaction;

$2H2O2 \rightarrow 2H2O + O2.$ -- Eqn 1

2..4.1 Molecular Mechanism

Although the complete mechanism of catalase is not currently known (Aebi, 1984), the reaction is believed to occurs in two stages.



$H_2O_2 + Fe(III)\text{-}E \rightarrow H_2O + O{=}Fe(IV)\text{-}E(.+)$ ------------------------------- Eqn 2

$H_2O_2 + O{=}Fe(IV)\text{-}E(.+) \rightarrow H_2O + Fe(III)\text{-}E + O_2$[14] ------------------------ Eqn 3

Here Fe()E represents the Iron centre of the heme group attached to the enzyme. Fe(iv) -E (.+) is a metabolic form of Fe(v) meaning the Iron is not completely oxidized to +v but receives some stabilizing electron density from the heme ligand which is then shown as a radical cation (.+).

Catalase can also catalyse the oxidation (by hydrogen peroxide) of various metabolites and toxins including phenols, formaldehyde, formic acid, acetaldehyde and alcohol according to the following reaction.

$H_2O_2 + H_2R \rightarrow 2H_2O + R$ -- Eqn 4

The exact mechanism remains unknown.

2.4.2 Cellular role

Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani*et al.,* 1996). Hydrogen peroxide is a harmful by product of many normal metabolic processes to prevent damage to cells and tissues.

Mice that has been genetically engineered to lack catalase are initially phenotypically normal, however the diffiency in mice may increase the like hood of developing obesity, fatty liver and type 2 diabetes.

2.4.3 Acatalasaemia

This is a condition also known by the absence of very low level of catalase in an organism.. It is primarily caused by mutations in catalase gene that encoded catalase. A deficiency in a catalase activity results in excessive accumulation of hydrogen peroxide due to insufficient decomposition. This often leads to mouth ulcers and gangrene in human.

Catalase also plays a role in graying process If human hair this is as a result of hydrogen peroxide interfering with the production of melanin - the pigment that gives hair it's colour. (Chelikani*et al,*2004 Wood, *et al.,* 2009).

2.4.4 Superoxide Dismutase gene (SOD)

Superoxide dismutase is a protein coding gene located at chromosome 21. It encodes an enzyme called superoxide dismutase which is abundant in cells throughout then body. Superoxide dismutase binds to the molecule of Copper and Zinc to break down toxic charged oxygen molecules called superoxide radicals (McCord and Fridovich, 1969, Barber, *et al.,* 1993). The under expression of efficiency is associated with a disease known as Amyotropic Lateral Sclerosis (ALS). (Rosen, *et al.,* 1993), it is also know as motor neuron disease (MND) OK Lou GE hi ing's disease. The natural SOD Levels in the body drops as the body ages, hence, one becomes more prone to oxidative stress-related diseases as one ages.(McCord and Fridovich, 1969, Inan, 2001).

2.4.5 Role in Oxidative stress

Superoxide dismutase is a good antioxidant defense against reactive oxygen species (ROS) mediates diseases.(Younus, 2018). Present studies describes the therapeutic effects of sod in various physiological and pathological conditions such

as cancer, diabetes, inflammatory diseases, cystic fibrosis, ischemia, aging, rheumatoid, neurogenerative disease etc.(Richardson *et al.,* 2015, Riley, 1999).

2.4.6 Role in inflammation

SOD serves as an inhibitory agents of neutrophils-mediated inflammation and may stand for a novel therapeutic approach for the Reactive Oxygen Specie (ROS) dependent tissue damage induced by neutrophils-mediated through several mechanisms (Yasui and Baba, 2006).

Radiation induced fibrosis of the breast was significantly reduced by Cu, Zn SOD. Proapoptotic agents induced appoptosis in cystic fibrosis but not in control cells that were reduced by treatment with sod mimetic.(Tainer*et al.,* 2013, Campana, 2004).

2.4.7 Role of in Ischemia

The removal of ROS and peroxynitrate by SOD mimetic helps in the prevention of cellular energetic failure and tissue damage related with ischemia and perfusion.

2.4.8 SOD and Neurodegenerative

Oxidative stress has been shown to be involved in the pathophysiology of several neurodegenerative diseases. The affected region of patients having Alzheimer's

disease have reduced activity of antioxidant enzyme such as SOD, catalase and gluthathione peroxidase. (Borgstahl*et al.,* 2012, Papilla, 1992, Zemlan, 1989).

2.4.9 NuclearErythroid 2-related Factor 2 (Nrf2).

This is an emerging regulator of cellular resistance to oxidants. It is encoded by the NFE2L2 gene and this gene may be dysregulated during the aging process and that this dysregulation may contribute to the development and progression of fibrosis.(Hecker*et al.,* 2014). It is a transcription factor that controls the basal and induced expression of an array of antioxidant response element-dependent genes to regulate the physiological and pathological outcomes of oxidants exposure thereby maintaining cellular reduction-oxidation homeostasis. (Shobha*et al.,* 2016, Al-sawaf*et al* 2015).

2.5 Oxidative resistance and Signaling pathway role.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the major types of reactive oxidants. They're produced from numerous sources in multiple compartments within the cell, either normally or as a result of exposure to toxic or pathological situations. (Janssen-Heininger, 2008).

Mitochondria are considered a primary site for the production of ROS, while RNS are formed in n cells starting with the synthesis of nitric oxide (.NO) by NO synthase, they're produced intentionally or as byproducts.

2.6 Inflammatory and toxicity role

Nrf2 regulates the expression of several oxidant signaling proteins to impact a number of programmed cellular functions. It as inhibits inflammation by inhibiting proinflammatory cytokine productions. (Dinkova-Kostova*et al*, 2005).

A fully functional Nrf2 genotype is required to protect smokersagainsy acquiring lung emphysema (Blake, 2010)

It is also needed in protecting the body against cancer by endogenous and pharmacological anticancer agents. It is persistently activated in some tumours, resulting in a prosurvival phenotype to promote tumour growth and resistance to oxidants and anticancer drugs. (Ma Q, *et al.,* 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

(1.0) 3.1 MATERIALS

The following materials were used during the research study;

1. Cheese cloth
2. *Enantiachlorantha*
3. Aluminum foil
4. Wistar rats
5. Cages
6. Ceramic plates
7. Steel plates
8. Gloves
9. Cotton wool
10. Methylated spirit
11. Nose mask
12. EDTA container
13. Lithium heparin container
14. Plain container
15. Masking tape
16. Universal bottle

17. Test tubes racks
18. Lancet
19. 250ml and 1000ml beakers
20. Blade and scissors
21. Syringe (2ml and 5ml)
22. Insulin syringe
23. Pipette
24. Mortar and pestle
25. Spatula
26. Ependof tube

(1.1) APPARATUS

1. Separating funnel
2. Glass jar
3. Glass rod
4. Measuring cylinder
5. Dissecting set
6. Test tubes
7. Micro-centrifuge tubes
8. Beakers

9. Cuvette

(1.2) EQUIPMENT/ INSTRUMENTS

1. Glucometer	Sigma, Germany
2. pH meter	Sigma, Germany
3. Retort stand	Sigma, Germany
4. Pipette(micro-pipette)	Sigma, Germany
5. Weighing balance	Sigma, Germany
6. Oral gavage	Sigma, Germany
7. Spectrophotometer	Sigma, Germany
8. Centrifuge	Sigma, Germany

(1.3) CHEMICALS AND REAGENT USED

The following chemicals and reagent were used during the research study;

1. Ethyl-Acetate	Sigma, Germany
2. Sodium citrate	Sigma, Germany
3. Citric acid	Sigma, Germany
4. Ethanol	Sigma, Germany
5. Potassium hydroxide	Sigma, Germany
6. Distilled water	Sigma, Germany

7. Picric acid Sigma, Germany

8. Chloroform Sigma, Germany

9. Cholesterol reagent kit Sigma, Germany

10. Triglycerides reagent kit Sigma, Germany

11. Total protein reagent kit Sigma, Germany

12. HDl-Cholesterol reagent kit Sigma, Germany

3.3 Preparation of Plant Extracts

Enantiachlorantha stem bark which is the crude extract was gotten, washed with just water and dried under room temperature and not an oven to avoid lost of so important phytochemicals. When well dried, it was pulverized and soaked in hydroethanol with a ratio of ethanol to distilled water 80:20 respectively, (800ml of Ethanol and 200ml of distilled water) in a jar. The mixture was stirred using a glass rod until there was no friction anymore. It was then allowed to stand for three days where it was stirred with the class rod each day and after the last day of day 3, the marc was separated from the crude extract by sieving using fish net firstly and then using a white unused handkerchief for ultra filtration. The crude extract gotten were poured into clean jars and labeled. The marc was resoaked in hydroethanol with the same ratio of ethanol to distilled water, 80:20 in a jar for another three days. This process is termed exhaustive extraction, the idea behind it is to exhaustively extract all constituents of the *Enantiachlorantha*. The crude extract

were then sent to pharmaceutical chemistry staff research laboratory for freeze drying in a time frame of 24hours.

3.4 Preparation of the Animals Used

Male Wistar rats of four months old (16weeks) were purchased and kept in clean and serene cages and left to acclimatize for two weeks by feeding them *ad lebitum*with normal poultry feed called grower mash. After acclimatization, the Wister rats were divided into 7 groups with six animals in each group and kept in separate cage. The animals were then weighed on a weighing balance to determine their various body weight and each rat was stained on various body parts such as their tails, head, stomach and back using picric acid for easy identification purposes.

3.5 Diet Formulation

Both High fat diet and Normal diet (HFD & ND respectively) were prepared. The content of the high fat diet includes; corn starch from a carbohydrate source, fish meal from a protein source, soybean oil from fat and oils, butter from fat and oil, sucrose from a simple sugar, cellulose gotten from a fiber source, vitamin mix from vitamins and mineral mix from minerals. The normal diet involved all the contents of the high fat diet with an exception of butter. The cornstarch was dried

for a period of three days (3) consecutively, before the feed was formulated, after the drying process the content of the High fat diet and Normal diet were measured, using this measurement provided in the table below, these contents were thoroughly mixed together and pelleted, it was then left to dry for a few hours. The feeds were weighed and 100mg of both high fat diet and normal diet were administered to various groups of animals,

Table 3.1: **Formular for normal diet content**

RAW MAERIALS	SOURCE	WEIGHT(grams)
Cornstarch	Carbohydrate	2,562.5
Mineral mix	Minerals	62.5
Soya bean oil	Fat and oil	625
Vitamin mix	Vitamins	218.75
Sucrose	Simple sugar	62.5
Mineral mix	Fiber	875
Fish meal	Protein	312.5

Table 3.2: **Formular for high fat diet content**

RAW MATERIALS	SOURCE	WEIGHT (grams)
Mineral mix	Mineral	437.5
Soybean oil	Fats and oil	625
Butter	Fats and oil	2,687,5
Fish meal	Protein	1,750
Cellulose	Fiber	625
Corn starch	Carbohydrates	5,125
Vitamin mix	Vitamin	125
Sucrose	Simple sugar	1,250

3.6 Fractionation Using Ethylacetate and Hydroethanol

Separation technique was employed and the principle behind this fractionation is solvent to solvent partition (by like constituents into them) it was carried out via a separating funnel. The marc gotten after freeze drying was weighed and mixed with distilled water, stirred to dissolve and ethanol was added to aid complete dissolving. The ratio of ethanol to water was 4:1. The marc was poured into the separating funnel and ethylacetate was added to it to get the ethylacetate fraction. The ethyl acetate collects polar component from it and layer of ethylacetate fraction was formed above due to it being less denser than the hydro-ethanol solution of the crude extract and its fraction was collected by separating the marc

which drains out through the valve leaving behind the less dense acetylacetatefraction. This process was repeated at an interval of 15 minutes until there was a colorless layer formed indicating that the ethylacetate had completely taken the polar components from the marc. The marc was poured back into the separating funnel and hydro-ethanol was added to it to collect the remaining constituents in the marc. The Ethylacetate fraction and hydro-ethanol fraction were then sent to pharmaceutical chemistry staff research laboratory for freeze drying. The freeze dried fractions were sent to a lab for phytochemistry analysis.

3.7 Experimental Design

The male wistar rats were arranged into seven groups with the weights of those in a group being representative of the weight range of all the rats, such that the average weight of all the groups at the onset of the experimental period was 175.0±1.0g and the groups are

- **Group 1 :-** This is the normal control, they were feed with normal diet(100mg) and water only for four weeks throughout the duration of the study. Diabetes mellitus was not induced and no treatment of any form was administered.
- **Group 2:-** This is the diabetic control, they were feed with high fat diet (100mg)) and water only for four weeks and a single dose of streptozotocin,

(35 mg/kg) and no treatment of any form was administered throughout the duration of the study.

- **Group 3 :-** This is the positive control, they were feed with high fat diet (100mg)) and water only for four weeks and a single dose of streptozotocinand standard diabetic mellitus drug called metformin (200mg/kg bw) administered orally for 14 days.
- **Group 4 :-** They were feed with high fat diet (100mg)) and water only for four weeks and a single dose of streptozotocinand Crude hydroethanol extract of *Enantiachlorantha*(200mg/kg bw) was administered orally daily for 14 days.
- **Group 5 :-** They were feed with high fat diet (100mg)) and water only for four weeks anda single dose of streptozotocin, and Crude hydro-ethanol extract of *Enantiachlorantha* (400mg/kg bw) was administered orally for 14 days.
- **Group 6 :-** They were feed with high fat diet (100mg)) and water only for four weeks and a single dose of streptozotocin, and Ethyl acetate extract (200mg) was administered orally for 14 days.
- **Group 7 :-** They were feed with high fat diet (100mg)) and water only for four weeks.Diabetes mellitus was induced using low dose of streptozotocin and Ethyl acetate extract of *Enantiachlorantha* (400mg/kg bw) was administered orally for 14 days.

3.8 Inducing of Diabetes Mellitus Using Streptozotocin (STZ)

The animals were feed for nine weeks and their weight was being monitored weekly until they were obsessed**.** The streptozotocin drug was administered through intra-peritoneal route using an insulin syringe and the fasting blood glucose was checked after three days by collecting blood samples from the tail tip of the wistar rat. And a rat was said to be diabetic until the fasting blood glucose was above 200mg/dl.

3.9 Fasting Blood Sugar

The wistar rats were starved overnight and fasting blood was obtained from the tail tip very early in the morning. Methylated spirit was used to clean the tail tip and a lancet was used to prick the tail tip, then some drops of blood was placed on the glucose test strip that is already inserted into the glucometer device and within 15 seconds the value for the blood glucose level was displayed on the LED screen using in mg/dl. All of this is usually done before 10:00am in the morning. This was done usually every three days immediately after diabetes mellitus was induced.

3.11 Preparation of Standard Treatment Drug to be Administered

Two tablets each of metformin (500mg) was crushed to powder using a mortar and pestle then was dissolved into 10ml of distilled water and stirred until it dissolved completely. The volume to be administered depends on the weight of the wistar rat.

3.14 Euthanization and Sample Collection from Wistar Rats

After 14days of administration of standard drug (metformin), Ethyl acetate and hydro-ethanol extracts, the wistar rats were euthanized then blood samples and organs samples were harvested from each animal into well labeled test tubes. The fasting blood glucose was taken one last time and the rats were placed into a container with cotton balls soaked with chloroform and after few minutes the rats would lose consciousness. Immediately the rats are inverted and a section of the peritoneum cavity is cut open and extended towards the thoracic cavity of the wistar rat using a dissecting blade. Blood was collected using a 5ml syringe by piercing the left ventricle of the heart and blood was drawn and placed into EDTA container, lithium heparin containers and micro-centrifuge tubes and labeled boldly accordingly to the label of each animal.

The liver, sample was collected and preserved with formalin inside test tubes and kept in a freezer. The duration of feeding, inducing diabetes and treatment of the wistar rats was 11 weeks.

3.18 GENE STUDIES

3.19 Total RNA isolation

Total RNA was isolated from whole tissues following a method described by Omotuyi*et al.* 2018. Briefly, tissues were homogenized in cold (4 °C) TRI reagent (Zymo Research, USA, Cat:R2050-1-50, Lot: ZRC186885). Total RNA was partitioned in chloroform (BDH Analytical Chemicals, Poole, England Cat: 10076-6B) following centrifugation at 15,000 rpm/15 min (Abbott Laboratories, Model: 3531, Lake Bluff, Illinois, United States). RNA from the clear supernatant was precipitated using equal volume of isopropanol (Burgoyne Urbidges& Co, India, Cat: 67-63-0). RNA pellet was rinsed twice in 70% ethanol (70 ml absolute ethanol (BDH Analytical Chemicals, Poole, England Cat: 10107-7Y) in 30 ml of nuclease-free water (InqabaBiotec, West Africa, Lot no: 0596C320, code: E476-500ML)). The pellets were air-dried for 5 min and dissolved in RNA buffer (1 mM sodium citrate, p^H 6.4).

3.20 cDNA conversion:

Prior to cDNA conversion, total RNA quantity (concentration (µg/ml) = 40 * A_{260}) and quality (≥ 1.8) was assessed using the ratio of A_{260}/A_{280} (A=absorbance) read

using spectrophotometer (Jen-way UV-VIS spectrophotometer model 6305, UK). DNA contamination was removed from RNA was removed following DNAse I treatment (NEB, Cat: M0303S) as specified by the manufacturer. 2 µl solution containing 100 ng DNA-free RNA was converted to cDNA using M-MuLV Reverse transcriptase Kit (NEB, Cat: M0253S) in 20 µl final volume (2 µl, N^9 random primer mix; 2 µl, 10X M-MuLV buffer; 1 µl, M-MuLV RT (200 U/µl); 2 µl, 10 mMdNTP; 0.2 µl, RNase Inhibitor (40 U/µl) and 10.8 µl nuclease-free water). The reaction proceeded at room temperature O/N. Inactivation of M-MuLV Reverse transcriptase was performed at 65°C/20 min.

3.21 PCR amplification and agarose gel electrophoresis.

PCR amplification for the determination of genes whose primers (Primer3 software) are listed below (table 3.1) were done using the following protocol: PCR amplification was performed in a total of 25 µl volume reaction mixture containing 2 µl cDNA (10 ng), 2 µl primer (100 pmol) 12.5 µl Ready Mix Taq PCR master mix (One Taq Quick-Load 2x, master mix, NEB, Cat: M0486S) and 8.5 µl nuclease-free water. Initial denaturation at 95 °C for 5 minutes was followed by 20 cycles of amplification (denaturation at 95 °C for 30 seconds, annealing (see TM values for each primer pair on table 1.0) for 30 seconds and extension at 72 °C for 60 seconds) and ending with final extension at 72 °C for 10 minutes. In all experiments, negative controls were included where reaction mixture has no cDNA.

The amplicons were resolved on 1.5% agarose gel (Cleaver Scientific Limited: Lot: 14170811) in Tris (RGT reagent, china, Lot: 20170605)-Borate (JHD chemicals, China, Lot 20141117)-EDTA buffer (pH 8.4).

3.22 AMPLICON IMAGE PROCESSING

In-gel amplicon bands images captured on camera were processed on Keynote platform as previously reported Omotuyi*et al.* 2020 and quantified using image-J software. All graphs were plotted as mean +/- SEM using graph-pad prism.

Rattusnorvegicus nuclear factor, erythroid 2-like 2 (Nfe2l2/NRF2), superoxide dismutase 1 (Sod1) and catalase (Cat), mRNA

PRIMERS	FORWARD SEQUENCE	REVERSE SEQUENCE
Nrf-2	TGTAGATGACCATGAGTCGC	TGTCCTGCTGTATGCTGCTT
SOD	TGGCCGTACTATGGTGGTCC	GGCAATCCCAATCACACCAC
CAT	TGCTCCCAACTACTACCCCA	AGAATGTCCGCACCTGAGTG

CHAPTER FOUR

RESULTS

(4.0)Antidiabetic effects of *Enatiachlorantha* stem bark extract

The antidiabetic activity of extracts of EC stem bark in type 2 diabetes model is shown in Table 1. The rats induced with type 2 diabetes showed significant increase ($p<0.05$) of the blood glucose level, when compared to the normal control group on day three. Also there was a dose-dependent reduction in blood glucose levels in the diabetic treated (200mg/kg and 400 mg/kg body weight) rats on days 7 and 14 when compared to diabetic control. Similarly administration of the standard drug, metformin significantly lower the increased glucose levels progressively throught the experiment.

Table 4.0 Antidiabetic effects of *Enatiachlorantha* stem bark extract

Group	Fasting Blood Glucose Level (mg/dl)			
	Day 0	Day 3	Day 7	Day 14
Group 1	80.00 ± 2.06[a]	71.00 ± 2.66[a]	97.00 ± 2.06[a]	85.62 ± 3.09[a]
Group 2	[a]88.00 ± 1.19*	[b]297.50 ± 1.46*	[c]449.00 ± 7.04*	[d]454.00 ± 7.80*
Group 3	[a]79.50 ± 0.84	[b]357.00 ± 4.90**	[c]103.50 ± 1.46**	[d]84.5.80 ±1.46**
Group 4	[a]80.00 ± 1.19	[b]383.00 ± 3.36**	[c]223.00 ± 2.06**	[d]195.00 ±3.76**
Group 5	[a]78.00±1.18	[b]313.50±1.46**	[c]289.5±6.00**	[d]177.00±3.14**
Group 6	[a]80.5±1.46	[b]317.5±0.84**	[c]366.50±0.84**	[d]390.5±4.03**
Group 7	[a]82.50±1.85	[b]351.50±0.84**	[c]173.00±4.44**	[d]81.5±2.22**

Values are expressed as mean ± SEM (n=7); values with different alphabet letters are significantly different ($p < 0.05$) from one another. *mean is significant ($P < 0.05$) when compared with the control; ** mean is significant ($P < 0.05$) when compared with diabetic control group.
Group1: Normal Control; Group 2 (Diabetic Control): high fat diet for 6 weeks and low dose of STZ (35 mg/kg) only for ; Group 3; high fat diet for 6 weeks and low dose of STZ + Metformin (200 mg/kg bw) Group 4; high fat diet for 6 weeks and low dose of STZ + 200 mg/kg bw of hydroethanol extract of EC stem bark Group 5; high fat diet for 6 weeks and low dose of STZ + 400 mg/kg bw of hydroethanol extract of EC stem bark; Group 6; high fat diet for 6 weeks and low dose of STZ + 200 mg/kg bw of ethyl acetate fraction of EC stem bark Group 7: 400 mg/kg bw of ethyl acetate fraction of EC stem bark .

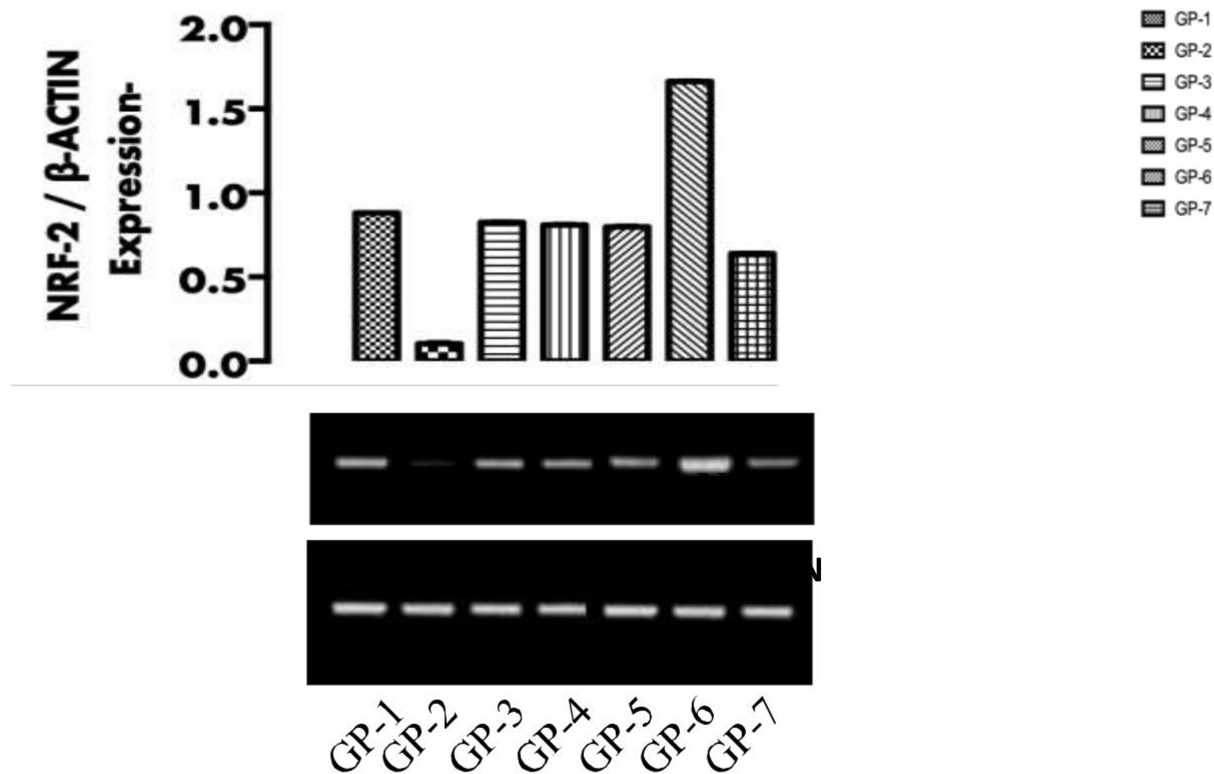


Figure 4.1: *E. chlorantia* stem bark modulate liver Nuclear factor-erythroid factor 2 related factor 2 (NRF2) in STZ induced diabetic rats. Data are represented as mean±SEM, n =4

The antioxidant and anti-inflammatory activity of EC stem bark in type 2diabetes model is shown in Fig 4.1 above was observed that there was a decrease in the expression of the Nrf-2 in the group that was administered STZ only (Group 2) when compared to other the normal control (Group 1). However rats administered the extracts (both hydro-ethanol and ethyl acetate fractions) showed high expression of Nrf-2 compared to group 2. From the results, rats treated with 200mg/kg per body weight of ethyl acetate shows highest expressions of Nrf-2.

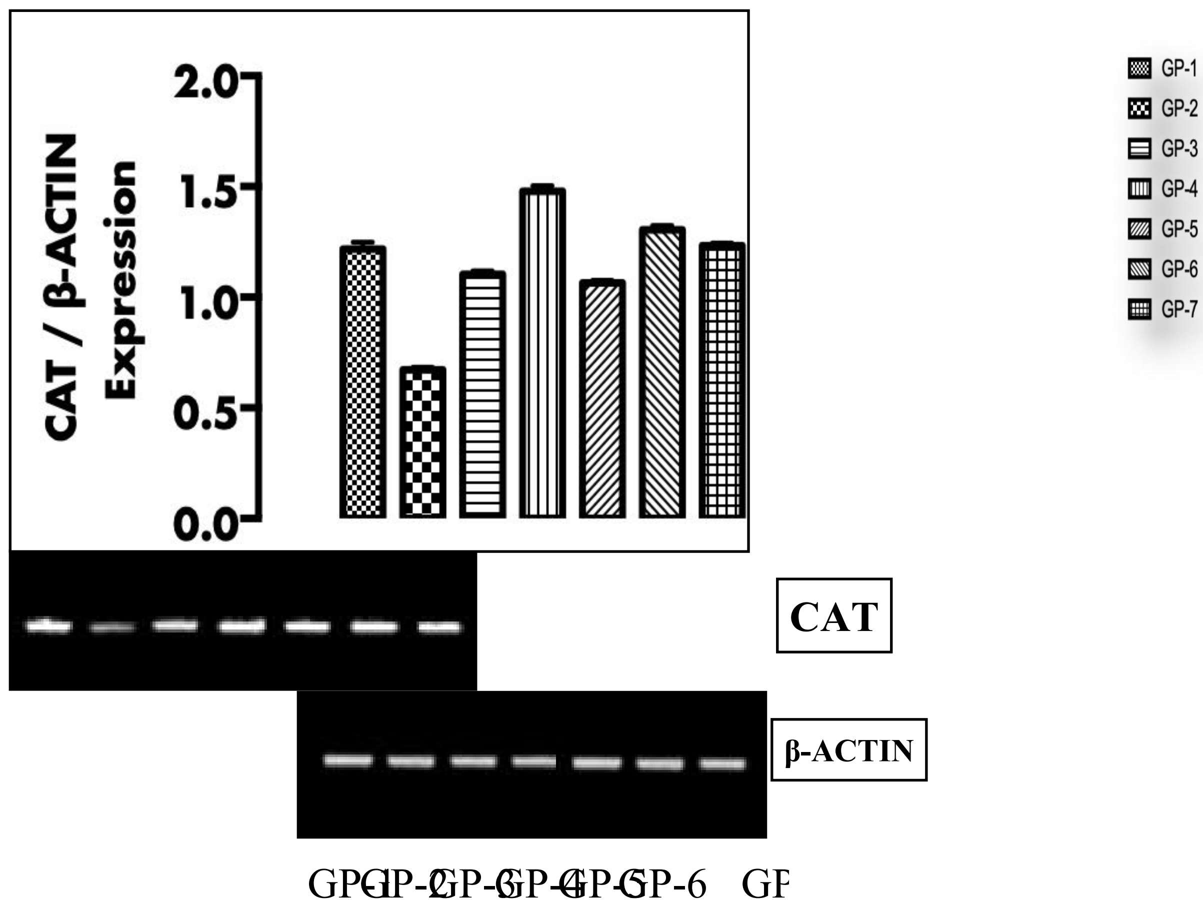


Figure (4.1)*E. chlorantia* stem bark modulate liver Catalase (CAT) gene with reference to gatekeeper gene β-ACTIN in STZ induced diabetic rats. Data are represented as mean±SEM, n =4

The antioxidant and anti-inflammatory activity of EC stem bark in type 2diabetes model is shown in Fig 4.2 above was observed that there was a decrease in the expression of the CAT gene in the group that was administered STZ only (Group 2) when compared to rats in under the normal control (Group 1). However rats administered the extracts (both hydro-ethanol and ethyl acetate fractions) showed high expression of CAT gene when compared to group 2. From the results, rats treated with 200mg/kg per body weight of hydro-ethanol extracts showed highest expressions of CAT gene.

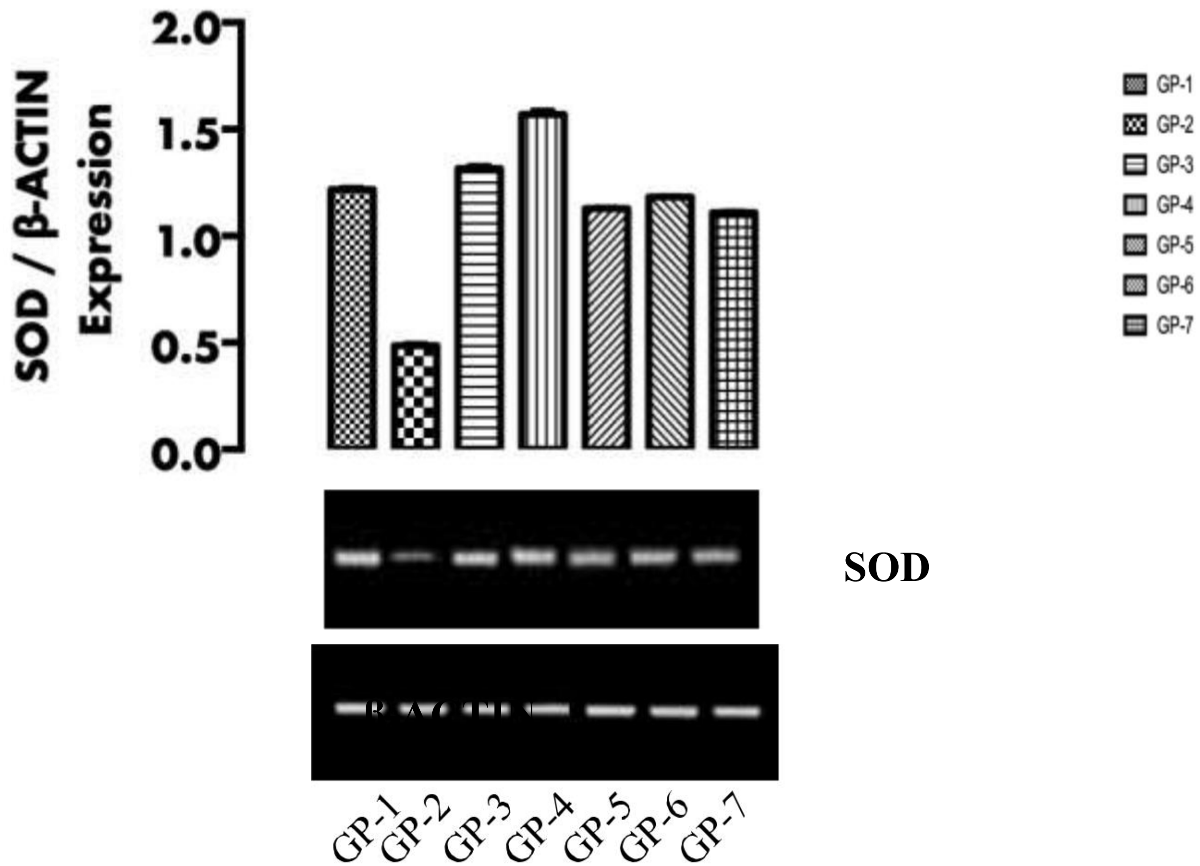


Figure 3*E. chlorantia* stem bark modulate liver Superoxide Dismutase (SOD) gene in STZ induced diabetic rats. Data are represented as mean±SEM, n =4

The antioxidant and anti-inflammatory activity of EC stem bark in type 2diabetes model is shown in Fig 4.2 above was observed that there was a decrease in the expression of the SOD gene in the group that was administered STZ only (Group 2) when compared to rats in under the normal control (Group 1). However rats administered the extracts (both hydro-ethanol and ethyl acetate fractions) showed high expression of CAT gene when compared to group 2. From the results, rats treated with 200mg/kg per body weight of hydro-ethanol extracts showed highest expressions of SOD gene.

DISCUSSION

The present study investigated the antidiabetic and anti-oxidant effects of hydro ethanol extract and ethyl acetate fraction of *Enantiachlorantha*stem bark in Streptozotocin (STZ)-induced diabetes on Wistar rats. Table 4.1 displays the effect of the extract on *E. chlorantha*stem bark on the serum glucose on STZ induced Wistar rats. It was observed that rats induced with STZ caused significant ($p<0.05$) increase in serum glucose level compared to the rats in the group 1(normal control) indicating that the rats were hyperglycemic. This is as a result the degeneration of β cells in rats caused by STZ. The high fat diet that the rats were fed with resulted to significant weight gained observed in the rats resulting to hyperglycemia. This is in agreement with the work done by Gupta *et al*(2017)and also similar to the findings of Ibrahim et a (2020) who stated that *E. chlorantha* stem bark extracts displayed antidiabetic potentials and it also contains some bioactive compounds which they hypothesized might be responsible for the observed antidiabetic activities. It was observed that the rats administered 200 mg/kg and 400mg/kg of hydro ethanol extract respectively showed a dose dependent decrease in serum glucose levels. However, the rats administered 400mg/kg of ethyl acetate fraction showed the highest decrease in serum glucose level. Rats administered with metformin also showed drastic decrease in serum glucose level (Table 4.1)

Diabetes mellitus is a chronic metabolic disorder that affects the metabolism of carbohydrates, fats and proteins (Ibrahim *et al.,* 2020). It is marked by hyperglycemia resulting from either the pancreas not producing enough insulin (type 1) or cells of the body not responding properly to the insulin produced (type 2)(Shoback and Gardaner, 2011). Type 2 diabetes mellitus which is more predominant is often caused by obesity as a result of high fat diet or genetics and exposure to chemicals which also causes Oxidative stress. Studies have shown that the production of reactive oxygen species (ROS) is increased in diabetic state, which also weakens the antioxidant defenses (Yaunus, 2018) system. The onset of diabetes is closely associated with Oxidative stress (Rosen *et al.,* 2001). Oxidative stress is caused by imbalance of free radicals and antioxidants in the body which can lead to tissue damage.(Janssen-Heininger, 2008). Free radicals maybe either Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS) formed under normal physiological conditions in cellular membrane and can be harmful and cause damage to the cellular membrane, but antioxidant prevent the deleterious effect that may arise (Fang *et al.,* 2012, Janssen-Heininger, 2008).

Catalase gene (CAT gene) is a protein coding gene that provides the instructions for the making of subunits of enzymes called Catalase - a key antioxidant in the defense against oxidative stress caused by hydrogen peroxide (Nagababu*et al.,* 2003). From the results obtained, it was observed that CAT gene was compromised

and was down regulated in group 2 which was administered STZ only when compared to group 1(Normal control), however, administration of metformin, crude hydro ethanol extract and ethyl acetate fraction to groups 3, 4, 5,6 and 7 elicited the up-regulation of the CAT genes, indicating that *E. chlorantha*stem bark extracts has antioxidant properties. Group 4 that was administered 200mg/kg body weight showed the highest expression of CAT gene. (Fig 4.2)

The results gotten from the superoxide dismutase protein coding gene (sod gene) located in chromosome 21 encodes superoxide dismutase enzyme - another antioxidant enzyme that posseses antioxidant defense against ROS mediated-diseases (Younus, 2008). It plays an anti-inflammatory role as it inhibits neutrophils-mediated inflammations (Yasui and Baba, 2006) and also reduce radiation induced fibrosis drastically (Campana*et al.,* 2013). The results revealed that rats administered STZ only showed decrease expression of SOD gene when compared to the Normal control (group 1), but administration of the antidiabetic drug metformin, extracts from EC stem bark up regulated the SOD gene with rats given 200mg/kg per body weight of hydro ethanol extracts appearing to be the highest in the expression of SOD gene(Fig 4.3).

Nuclear Erythroid 2-related Factor 2 (Nrf2) is an emerging regulator of cellular resistance of oxidants. It is encoded by the NFE2L2 gene (Hacker *et al.,* 2014). It maintains the reduction-oxidation homeostasis by controlling the basal and induced

expression of an array of antioxidant response element-dependent genes (Shobha*et al.,* 2016). Nrf2 inhibits inflammation by inhibiting proinflammatory cytokines productions (Dinkova-Kostova*et al.,* 2005). It was observed from the results rats induced with STZ only showed decline expression in Nrf2 gene when compared to the group 1(Normal control). Post administration of the extracts from EC stem bark up regulated the Nrf2 gene when compared to rats given STZ only. Group 6 that's was administered 200 mg/kg of ethyl acetate fraction of *Enantiachlorantha* showed the highest expression of Nrf2 gene. (Fig 4.1)

The antidiabetic and anti-inflammatory effects of *Enantiachlorantha* stem bark extracts was evidential as the groups that were administered both extracts of showed decreased serum glucose concentration and increased expression of the antioxidant and anti-inflammatory genes when compared to the group 2 that was administered STZ only. The result is in agreement with the findings of Olanlokun and Akomolape, (2013), which states that the antioxidant properties of *E. chlorantha* is attributed to the presents of phytochemical like phenols, flavonoids and saponins constituents. It is also in agreement with the outcome of the research carried out by Agbeje*et al*. (2006) that stated that *E. chlorantha* especially the evaporated extracts exhibited significant anti-inflammatory effects of Carageenan-induced inflammation edema in rats.

CONCLUSION

The results confirmed that hydroethanol and ethyl acetate extracts of Enantiachlorantha stem bark exhibited anti-diabetic antioxidant and anti-inflammatory activities against STZ induced diabetes on the liver of adult Wistar rat. The therapeutic activities of the extracts are attributed to the presence of bioactive constituents or phytochemicals in them.

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APPENDIX

Preparation of Streptozotocin (STZ) to be Administered

0.03 was multiplied against the weight of each wistar rat the cumulative figure was summed up and the amount of STZ weighed was in correlation to that figure. The STZ was weighed using a sensitive weight balance and it was dissolved into 150ml of cold citrate buffer with a pH of 4.5

The STZ drug is light sensitive needs to be cold; the beaker that houses the STZ is always wrapped inside a foil paper to prevent light from contacting the STZ.

Then the volume of STZ to be administered was calculated using;

$$\text{Volume of STZ} = \frac{(0.03 \text{ x weight of wistar rat}) \text{ x2.0mls}}{\text{Summed up Cumulative figure}}$$

Preparation of Cold Citrate Buffer

2.989g of sodium citrate and 2.1g of citric acid was weighed. It was then dissolved with 50ml of cold distilled water and transferred into a measuring cylinder, where it was topped up to 100ml with cold distilled water. The pH was taken at 4.5. The solution was slightly acidic to allow the streptozotocin carry out its destructive effects on the pancreas of the animal leading to diabetes mellitus. Due to environmental factor the pH reduced and was corrected using a base potassium hydroxide.

Preparation of Crude Hydroethanol Extracts to be Administered

- **For The 200mg Dosage Of Crude Hydroethanol Extract**

After crude hydro-ethanol extract was freeze dried 6.0g was dissolved into 60mls of distilled water inside a beaker and it was stirred continuously to ensure complete dissolution.

a. To get amount of crude hydroethanol extract in (mg)

$$\text{Crude hydro-ethanol extract in (mg)} = \frac{200\text{mg x Bodyweight of animal (g)}}{1000\text{g}}$$

b. To get volume of crude extract to be administered per body weight (g) of animal

$$\text{Volume of crude extract (ml)} = \frac{60\text{ml x Crude hydroethanol extract in (mg)}}{6000\text{mg}}$$

c. To get amount of crude hydroethanol extract in (mg)

$$\text{Crude hydro-ethanol extract in (mg)} = \frac{400\text{mg x Bodyweight of animal}}{1000\text{g}}$$

d. To get volume of crude extract to be administered per body weight (g) of animal

$$\text{Volume of crude extract (ml)} = \frac{60\text{mls x Crude hydroethanol extract in (mg)}}{6000\text{mg}}$$

Preparation of Ethyl Acetate Extracts to be Administered

For The 200mg Dosage Of Ethyl Acetate Extracts

After ethyl acetate extracts was freeze dried 6.0g was dissolved into 60mls of distilled water inside a beaker and it was stirred continuously to ensure complete dissolution.

To get amount of ethyl acetate extract in (mg)

$$\text{Ethyl acetate extract in (mg)} = \frac{\text{200mg x Bodyweight of animal (g)}}{\text{1000g}}$$

To get volume of ethyl acetate extract to be administered per body weight (g) of animal

$$\text{Volume of ethyl acetate extract (ml)} = \frac{\text{60ml x Ethyl acetate extract in (mg)}}{\text{6000mg}}$$

For The 400mg Dosage Of Ethyl Acetate Extracts

To get amount of ethyl acetate extract in (mg)

$$\text{Ethyl acetate extract in (mg)} = \frac{\text{400mg x Bodyweight of animal}}{\text{1000g}}$$

To get volume of ethyl acetate extract to be administered per body weight (g) of animal

$$\text{Volume of ethyl acetate extract (ml)} = \frac{\text{60mls x Ethyl acetate extract in (mg)}}{\text{6000mg}}$$

NB: Ethyl acetate extract poorly dissolved in distilled water; hence double the volume of ethyl acetate extract was administered through the use of an oral gavage to the animals.