THE EFFECT OF GUM TREE (*Tetrapleura tetraptera)* SAPONINS ON ENZYMATIC OXIDATIVE STATUS OF STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS

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BENIN CITY, NIGERIA.

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A THESIS WRITTEN IN THE DEPARTMENT OF MEDICAL BIOCHEMISTRY AND SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCES IN MEDICAL BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

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CERTIFICATION

We the undersigned certify that Hycent Odion OKO-OBOH presented this Thesis to the Department of Medical Biochemistry, School of Basic Medical Science, University of Benin.

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DEDICATION

I want to dedicate this work to my GOD Almighty for being my source and sustainer. I also want to dedicate this work to my entire Family.

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I am indebted to the following people for their support that resulted in this project's success. I deeply appreciate my project supervisor, Prof A.A. Omonkhua for her patience, support, and guidance in making sure this work is a success. GOD bless you ma. My appreciation also goes to all the lecturers (Dr. Anionye, Dr. Olubodun, Dr. Olumese, Dr. Kelly, and Dr. Agu) in the Department of Medical Biochemistry for the knowledge they impacted me. To all my colleagues, I say a big thank you. GOD bless you all.

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ABSTRACT

A multifaceted metabolic illness termed diabetes mellitus, causes the development of insulin resistance, hampered insulin signaling, malfunction of the beta cells, abnormal glucose and lipid metabolism, inflammation, and elevated oxidative stress. Conventional drugs used for diabetes treatment are associated with drawbacks, such as rigid dosing regimens, high cost, and side effects. Therefore, screening for new anti-diabetic compounds from natural plants used in folk medicine is still attractive for their efficacy, low incidence of side effects, and low cost. Folkloric information that *Tetrapleura tetraptera* has anti-diabetic effect has prompted the use of the root bark of this plant for research into its anti-diabetic effect, and anti-oxidant effect. *T. tetraptera* roots has been shown to be rich in saponins, which led to the design of this study to investigate the effect of saponins extracted from *T. tetraptera* (TTS) on the enzymatic oxidative status of streptozotocin induced diabetic Wistar rats. The study investigated the effect of *T. tetraptera* (TTS) on: serum and tissue total protein; and superoxide dismutase (SOD); catalase; and glutathione-S-transferase activities in the serum and tissues of streptozotocin induced diabetic Wistar rats. *T. tetraptera* saponins (TTS) was administered orally via gavage at different doses of 10, 20, and 40 mg/kg body weight to streptozotocin induced diabetic Wistar rats in group 4, 5, and 6 respectively. The standard drug metformin was administered to group 3, group 1 was the normal control, and group 2 animals were the untreated diabetic group. Treatment lasted for 12 weeks. After treatment, total protein; superoxide dismutase (SOD); catalase; and glutathione-S-transferase was assayed for in the liver, heart, kidney, pancreas, and testis. The result showed a significant increase in total protein concentrations in almost all the tissues especially at lesser dose of 10 mg/kg TTS, while again at 10mg/kg TTS treated group, there was a significant elevation in SOD levels of both serum and the liver. Treatment with saponins from *T. tetraptera* caused significant ($p<0.05$) increase in catalase activities in the serum and kidney. It was lastly observed that administration of saponins from *T. tetraptera* increased GST activities significantly ($p<0.05$) in the serum and kidney. This study has shown that total saponins from *T. tetraptera* especially at 10mg/kg TTS body weight could scavenge free radicals which is very important in the management of diabetes mellitus.

CHAPTER ONE

1.0 INTRODUCTION

A multifaceted metabolic illness termed as diabetes mellitus, causes the development of insulin resistance, hampered insulin signaling, malfunction of the beta cells, abnormal glucose and lipid metabolism, inflammation, and elevated oxidative stress (Kousar, 2019). Spiked levels of sugar in the blood and the presence of sugar in urine discharge characterizes this malady (Nisha, 2010). In medieval texts, several herbal remedies were offered for the therapy of diabetes mellitus (Surya *et al.,* 2014). The vast majority of mankind employs natural herbal therapies as typical treatment methods for a wide range of ailments considering allopathic therapy has a plethora of negative impacts on individuals. Across a number of developing nations, herbal mixtures are pretty commonly welcomed as therapeutic agents for diabetic therapy and certain other anomalies of related nature. They are also widely used as lipid-lowering, hepatoprotective, anti-cancer, antimicrobial, anti-convulsant, emulsifying, contraceptive, and nutritional agents (Atawodi *et al.,* 2014). Gum tree (*Tetrapleura tetraptera*), which is utilized by certain ethnicities much like Igala people of North Central Nigeria, is one of several plants employed in traditional therapy that have been scientifically proven to possess anti-diabetic properties (Surya *et al.,* 2014). Reportedly, the conventional therapeutic methods used to treat diabetes have downsides notably strict dosage schedules, rising costs, and adverse effects (Omonkhua *et al.,* 2014). Hence, due to its potency, minimal likelihood of adverse reactions, and relatively inexpensive, researching potential anti-diabetic agents utilizing plant sources has still been appealing (Omonkhua *et al.,* 2014). Extensive exploration is still being undertaken in this area regardless of the fact that many plants have previously been explored for their anti-diabetic and anti-oxidant activities. This happens because novel plants with any of these capabilities and low toxicity concern are indeed sought after. The utilization of the root bark of this plant for investigations in with its anti-diabetic impact and anti-oxidant action was spurred

by folkloric knowledge indicating *T. tetraptera* (leaves) has anti-diabetic activity. *T. tetraptera* is a member of the Fabaceae family (originally designated as Leguminosea: mimosoideae) and is referred to as "Abogolo" by the Igala people of North Central Nigeria, "Aridan" by the Yoruba ethnic group of South West Nigeria, and "Dawo" by the Hausa people of Northern Nigeria (Atawodi *et al.,* 2014). It is normally prevalent in the forested areas of several African nations, and its fruit, which has a thick pulp and tiny brownish black seeds with a distinctively aromatic and strong scent, is well recognized (Kuate *et al.,* 2015). It is a ubiquitous condiment ingredient in the South-South and South-East of Nigeria, and the fruit is utilized to treat a variety of maladies, notably fever, jaundice, rheumatism, leprosy, flatulence, and type 2 diabetes mellitus (Kuate *et al.,* 2015).

JUSTIFICATION OF THE STUDY

Early literature indicated *T. tetraptera* aqueous root bark extract had an anti-diabetic and antioxidant action in streptozotocin diabetes (Omonkhua *et al.,* 2014). The frothy that *T. tetraptera* roots generate in water, a confirmation of the availability of saponins, is among their most distinctive characteristics. Therefore, the purpose of this study was to determine whether saponins from *T. tetraptera* have antioxidants potential in STZ induced diabetic Wistar rats.

1.1 AIM AND THE OBJECTIVE OF THE STUDY

This study aimed to decipher the effect of saponins extracted from *T. tetraptera* (TTS) on the enzymatic oxidative status of streptozotocin induced diabetic Wistar rats. The specific objectives were to determine the effect of TTS on:

1. Serum and tissues total protein (liver, heart, kidney, pancreas, and testis) of STZ-induced diabetic Wistar rats.
2. Serum and tissue superoxide dismutase (SOD), catalase, and glutathione-s-transferase antioxidant activities of STZ-induced diabetic Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.0 DIABETES MELLITUS

A failure in insulin release by the beta cells of the pancreas, a malfunction in insulin function, or a conjunction of the two is what causes diabetes mellitus, a metabolic malady. Severe hyperglycemia is brought on by insulin deficit alongside issues with the metabolism of protein, fat, and carbohydrates (ADA, 2014). The eyesight, kidneys, nerves, heart, and circulatory arteries are highly susceptible to long-term injury, dysfunction, and failure caused by the chronic hyperglycemia of diabetes (ADA, 2014). The pathogenic events that cause the onset of diabetes are numerous. These events span from defects that lead to resistance to insulin function, to autoimmune death of the beta cells in the pancreas (Kousar, 2019).

Glucokinase phosphorylates sugar to make glucose -6-phosphate (Meisenberg and Simmons, 2006). The ATP-sensitive potassium channel (KATP) is gated by the production of ATP via glycolytic, Kreb's cycle, and the respiratory chain pathways (Meisenberg and Simmons, 2006), permitting uneven Na+ entry. These two occurrences enable the membrane to depolarize and voltage-dependent T-type sodium Na+ and calcium Ca2+ channels to open. This activation raises the Ca2+ levels within cells (Trexler and Taraska, 2017) that prompted the sensory granules carrying insulin to fuse with the plasma membrane, resulting in the first step of insulin release (Trexler and Taraska, 2017). A sequence of metabolism coupling indicators that really can commence and maintain the second phase of insulin release can be generated by sugar metabolism in the beta cell in addition to boosting the ATP and ADP ratio. A few of these coupling elements are involved in mitochondrial metabolism and anaplerosis, making a circle with glutamate, acetyl coenzyme A, NADPH, pyruvate, malate, and 4 citrate (Inigo *et al.*,

2021). Numerous signaling pathways, including the Calcium-Calmodulin-Dependent Protein Kinase II (CaMKII), Protein Kinase A (PKA), Protein Kinase C (PKC), and Protein Kinase G (PKG) pathways, can also influence to the glucose-induced insulin release (de la Vega and Fernandez-Mejia, 2011).

2.1 INSULIN ACTION, INSULIN RESISTANCE, AND DIABETES

Insulin is conveyed to peripheral tissues after being released into circulation, whence it mostly has anabolic effects (Peterson and Shulman, 2018). Insulin commences function via connecting to its receptor, a trans-membrane protein that is a member of the superfamily of protein tyrosine kinase activity receptors and has the ability to autophosphorylate. Thus triggers a chain of processes that involve the phosphorylation of proteins and membrane lipids, linking proteins, and activity of the cytoskeleton (Le Roith and Zick, 2001). P13K, MAPK, and Cb1 constitute the three principal signaling pathways that are triggered in response to insulin receptor phosphorylation. These pathways interact collectively to convert the insulin receptor's signal into biological processes that affect the target organs, such as glucose transport by bringing GLUT vessels to the membrane, protein synthesis, lipid and glycogen synthesis, mitosis, and gene expression (Peterson and Shulman, 2018). Protein phosphorylation turns on various signaling pathways, whereas dephosphorylation turns them off. Several phosphatases can impede insulin signaling, including protein-contain tyrosine phosphatase 1B (PTP1B), phosphatase and tensin homolog (PTEN), SH2-containing tyrosine-protein phosphatase (SHO_2), and inhibitor of cytokine signaling 3 (SOC-3) dephosphorylate (Zick, 2014). Interruption in insulin function results from any changes in the insulin pathway, such as ineffective phosphorylation or a surge in phosphatase activity. This is the biological process that causes insulin resistance (Browlee, 2011).

2.2 TYPES OF DIABETES

Diabetes mellitus can be classified in different ways but one form of classification is according to American Diabetes Association (ADA, 2014).

2.2.1 TYPE I DIABETES MELLITUS

Insulin-dependent diabetes mellitus (IDDM) is the term given to this class of the disease. It results from an autoimmune assault on the pancreatic beta cell that makes insulin, this typically results an utter absence of insulin (ADA, 2014). 5 to 10 % of all diabetic occurrences today are caused by this form of diabetes (Ozougwu *et al.,* 2013). Injury to the nerves (diabetic neuropathy), kidneys (diabetic nephropathy), and smaller blood vessels in the eye (diabetic retinopathy) are indeed the leading causes of a number of health conditions that type I diabetes can generate. Consequently, heart disease and stroke risk are heightened in individuals with this type of diabetes (Schnell *et al.,* 2014). Insulin is administered into the fatty tissue beneath the skin as type I diabetes means of treatment (Ozougwu *et al.,* 2013).

2.2.2 TYPE II DIABETES MELLITUS

Non-insulin dependent diabetes mellitus (NIDDM) is the designation for this kind. Around 90–95% of all diabetic occurrences are type II, which is marked by aberrant insulin production and/or impaired insulin action (IDF, 2013). Type II diabetes has a number of multifaceted origins, comprising obesity, advanced age, ethnicity, and prior experience with the ailment (Defronzo *et al.,* 2015). Regardless of the notion that there is a substantial genetic contribution, genotype only defines an individual's susceptibility to environmental influences and lifestyle factors (Solis-Herrera *et al.,* 2021). When insulin secretion or insulin actions are impaired, type II diabetes occurs. Insulin resistance is a condition in which the metabolic responsiveness of the muscle, liver, and adipose tissue to insulin activity is suppressed or hindered (Zick, 2014).

When the beta cell is unable to release enough insulin in response to metabolic demand, chronic insulin resistance may result in hyperglycemia (Maletkowic and Drexler 2013). It's possible that acquired secretory dysfunction and/or a decline in beta cell mass are to blame for this inability of the beta cells to operate (Maletkowic and Drexler 2013). Every type II person with diabetes has a flaw in their beta cells' capacity to make or secrete insulin (ADA, 2014).

2.2.3 GESTATIONAL DIABETES MELLITUS

This is any type of glucose intolerance that emerges or is first detected during pregnancy. Gestational diabetes is common in the middle or later stages of pregnancy, and since the infant receives glucose via the placenta, it must be managed to safeguard the baby's growth and development (ADA, 2014). Two to ten percent of pregnancies, according to doctors, have gestational diabetes. Oftentimes, it disappears after childbirth. However, type 2 diabetes develops in up to 10% of pregnant women, weeks or even years later (ADA, 2014).

2.3 PATHOPHYSIOLOGY OF DIABETES

After eating, blood glucose levels rise, which prompts the release of more insulin, increasing the amount of glucose that is transported, biotransformed, and stored in muscle and fat cells (Vasudevan and Screekumaric 2007). When fasting, the liver supplies the blood with the glucose that is then utilised by the brain without the need for insulin (Tiwari and Rao, 2002).

2.3.1 PATHOGENESIS OF TYPE I DIABETES MELLITUS (IDDM)

Owing to the autoimmune destruction of pancreatic beta cells in insulin dependent diabetes mellitus (IDDM), there is a deficit of insulin secretion, which causes the metabolic imbalance that is characteristic of IDDM (Ozougwu *et al.,* 2013). The end stage of beta cell destruction is the beginning of the clinical disease contributing to type I diabetes mellitus, in which monocytes, lymphocytes, and a mixture of pseudo atrophic islets with some cells secreting somatostatin, glycogen, and pancreatic polypepetide are infiltrated. As a result, the disease is subsequently brought on by an immunogenic process (Maletkowic and Drexler 2013). Islets cell death is caused by autoimmune responses, genetic makeup, and environmental causes (Anaya *et al.,* 2013).

2.3.2 PATHOGENESIS OF TYPE II DIABETES MELLITUS (NIDDM)

Owing to reduced insulin secretion by the pancreatic beta cell in non-insulin dependent diabetes mellitus (NIDDM), key systems in the control of tissue sensitivity to insulin are altered, which may result in insulin resistance (Defronzo and Farrannini, 2015). In this kind of diabetes, beta cell abnormalities and peripheral tissue insulin resistance are caused by a variety of hereditary disorders as well as particular environmental variables, including obesity (Anaya *et al.,* 2013).

2.4 COMPLICATIONS OF DIABETES

It is hyperglycemia that causes diabetic problems to eventuate. The emergence of diabetic complications is influenced by prolonged exposure to high glucose levels, genetic susceptibility factors, and accelerated factors including hypertension and dyslipidemia (Galicia-Garcia *et al.,* 2013). In addition, hyperglycemia correlates with the onset and progression of blood vessel damage, making reducing blood glucose levels the most crucial objective in managing and mitigating consequences from diabetes. The retina, renal glomerulus, and peripheral nerves are the primary tissues impacted by diabetic problems at the microvascular level (Adeyi *et al.,* 2015). Additionally, the blood arteries that nourish the heart, brain, and lower limbs are more quickly developing atherosclerotic disease in people with diabetes. Likewise, a significant diabetic consequence is diabetic cardiomyopathy (Rask-Madsen, C. and King, 2013).

2.4.1 MICROVASCULAR COMPLICATIONS OF DIABETES MELLITUS

2.4.1.1 DIABETIC RETINOPATHY

Patients report this 10-15 years after their diabetes first manifests. Background retinopathy is characterized by little specks of bleeding in the intermediate layer of the retina (Wang and Lo, 2018). Similarly, lipid deposition and micro aneurism (small vascular dilatation) and edema may appear at the hemorrhage's edges (Sayin *et al.,* 2015). When new blood vessels form on the retina's surface, proliferative retinopathy results, leading to vitreous hemorrhage and external blindness. Owing to their high aldose-reductase content, retinal cells are very susceptible to enhancing the polyol pathway in the face of excessive sugar, which results in a concurrent drop in NADPH (Brownlee *et al.,* 2013). This process produces sorbitol, which increases osmotic stress and has been associated to the development of microaneurysms, thickening of the basement membrane, and pericyte loss. Additionally, it is believed that some retinal cells are

harmed by glycoproteins, especially advanced glycation end products (AGES). Additionally, ROS alone may harm the cell (Wang and Lo, 2018).

2.4.1.2 DIABETIC NEPHROPATHY

This consequence results in the creation of mesangial modules, microaneurisms, and thickened glomerular basement membranes, all of which contribute to proteinuria and, inevitably, renal failure (Adeyi *et al.,* 2015). Elevated polyol pathway activity and the development of AGEs are also part of the harm process. It has been established that AGEs binding to its receptor contribute to diabetes-related nephropathy's kidney injury, fibrosis, and inflammation (Matoba *et al.,* 2020). When the renin-angiotensin cascade is triggered in conjunction with the action of AGEs, multiple cycles that culminate in renal dysfunction might be triggered (Adeyi *et al.,* 2015). Diabetic sufferers, notably individuals who suffer from nephropathy, have weaker antioxidant protections (Matoba *et al.,* 2020).

2.4.1.3 DIABETIC NEUROPATHY

Upon ruling out any potential reasons, this is described as the existence of symptoms and/or evidence of peripheral nerve injury in diabetic sufferers. Diabetes-related peripheral neuropathy can arise in a multitude of manners, including sensory, focal/multifocal, and autonomic neuropathies (ADA, 2013). Whilst the reasons of nerve harm aren't entirely understood, they are probably connected to the polyol pathway, the production of AGEs, and ROS (Adeyi *et al.,* 2015). Moreover, oxidized proteins and lipoproteins engage with receptors on the surface of neurons to start inflammatory signaling processes that harm cellular components, generate ROS, and injure neurons (Vincent *et al.,* 2011).

2.4.2 MACROVASCULAR COMPLICATIONS OF DIABETES MELLITUS

The onset of atherosclerotic anomaly is the fundamental pathogenic cause driving macrovascular issues. The peripheral or coronary vascular system's chronic inflammation and injury to the artery walls culminate in atherosclerosis. Acute arterial infarction is spurred on by the accumulation of oxidized lipids from LDL particles in the endothelial wall of the arteries as a result of this injury (Adeyi *et al.,* 2015). Meanwhile, type 2 diabetes generally causes platelet adhesion and hypercoagulability, therefore increases the danger of arterial occlusion (Fowler, 2008). The immediate inactivation of two anti-atherosclerotic enzymes, endothelial nitric oxide synthase and prostacyclin synthase, as a consequence of elevated superoxide production has been hypothesized to be the foremost and significant mediator of endothelial tissue injury, and the activation of oxidative stress pathways is thought to play a role in the pathogenesis of complications (Giacco and Brownlee, 2010). Aldose-reductase is also plentiful in endothelial cells, making them more exposed to enhanced polyol pathway activation. Furthermore, a vast body of studies backs up the hypothesis that diabetes-related hyperglycemia causes vascular impairments (King and Aubert, 2012; Kolluru *et al.,* 2012; Paneni *et al.,* 2013). PKC action has been tied to vascular changes like higher permeability, contractility, synthesis of extracellular matrix, angiogenesis, leukocyte adhesion, and release and repression of cytokines (Geraldes and King, 2010). The fibrinolytic inhibitor plasminogen activation (PAT-1) is overexpressed as a result of hyperglycemia-induced PKC activation (Adeyi *et al.,* 2015). PKC activity has been proven to suppress insulin-stimulated expression of eNOs in endothelial cells, which is related with lower NO generation in smooth muscle (Geraldes and King, 2010).

2.4.3 ACUTE AND CHRONIC COMPLICATIONS OF DIABETES MELLITUS

If diabetes is not adequately treated, it could bring about acute (quickly occurring) or chronic (developing over time) concerns that might have an impact on a number of organ systems. In Table 2.4, some of these issues are outlined.

Table 2.4 Acute and chronic complications of diabetes mellitus

Acute complications	Chronic complications
Diabetes ketoacidosis	Atherosclerosis
Non Ketotic Hyperosmolar Coma	Retinopathy
Hypoglycemia	Neuropathy

Source: Adeyi *et al.*, 2015

2.5 MANAGEMENT OF DIABETES MELLITUS

2.5.1 TYPE I DIABETES

To aid individuals with type 1 diabetes in leading long, healthy lives, diabetes care and management is the target. The broad managerial techniques to accomplish this goal are as follows:

2.5.1.1 INSULIN

Maintaining an individual's blood sugar levels within the appropriate range hinges on efficient exogenous insulin therapy. This lessens hypoglycemia crises whilst halting the emergence and spread of diabetes sequelae. Levels of hypoglycemia range from level 1 (3.9 to 3.0 mmol/L or 70 to 54 mg/dl), level 2 (3.0 mmol/L or 54 mg/dl), and level 3 (severe events marked by distorted mental and/or physical functioning requiring assistance from another person for recovery). Diabetic ketoacidosis (DKA) episodes should be avoided and should occur, should be treated as necessary (Holt *et al.*, 2021).

2.5.1.2 DIET

Considering diabetes can be managed with diet, proper diet adherence is crucial. The following diet management strategies are adaptable.

a) Consuming high-fiber, minimal-Glycemic index (GI) (<55)(carbohydrate to blood sugar ratio), protein-rich lentils, and vegetables. While taking lengthier time to digest, lean protein, unsaturated fats, and carbohydrates with higher fiber satiate cravings and give you a continuous supply of energy (Kousar, 2019).

b) Because complex carbohydrates require longer to break down into simple carbohydrates and then glucose, blood sugar levels rise more gradually and there is less chance that they will be converted to fat. Blood sugar levels are raised more slowly by solid carbohydrates (nuts like cashews, almonds, walnuts can be taken in limited amounts). Avoid liquid carbs since they quickly breakdown in the blood and raise blood sugar levels. When brown bread (wheat bread) is consumed with butter, blood sugar levels rise more slowly (Butter has butyrate, which interacts with the microbiome in gastro intestinal tract). Choosing brown rice over white rice is healthier (Kousar, 2019).

2.5.1.3 EXERCISE

A healthy weight can be attained or maintained by exercise, which also improves insulin's ability to reduce blood sugar levels. Patients should try to exercise most days of the week for 30 to 60 minutes. Patients should keep glucose tablets on hand while exercising or a carbohydrate-rich food or beverage, like fruit or juice, in case blood glucose levels fall too low. However, if blood sugar levels are higher than 250 mg/dl, they should refrain from exercising since there is not enough insulin and blood sugar levels rise immediately after exercise because muscles convert glycogen to glucose. Checking blood sugar levels before and afterexercise is therefore advised (Rubio-Cabezas *et al.,* 2014).

2.5.2 TYPE II DIABETES

With type 2 diabetes, insulin secretion progressively diminishes across time. When a person is simply receiving diet and exercise as treatment, the use of oral hypoglycaemic agents (OHA) will frequently be essential, and when the sickness worsens and OHAs are insufficient to manage glycemia, further intensification with insulin may be required (Borse *et al.,* 2021).

2.5.2.1 NON-PHARMACOLOGICAL MANAGEMENT

The cornerstones of managing diabetes are a nutritious diet to help attain or keep a normal body weight and frequent bodily exercise (Borse *et al.,* 2021).

a) Diabetics should be encouraged to adopt a healthy, balanced diet that is appropriate for the general public.

b) Individuals who are overweight must be counseled to lose weight by consuming less calories from food.

c) It should be recommended to all individuals to indulge in routine, everyday bodily exercise that is compatible with their physical capabilities (e.g walking). Most individuals should exercise for at least 150 minutes each week at a moderate to strenuous intensity, spaced out across at least three days

d) All individuals should then be counseled to abstain from alcohol and tobacco usage (Borse *et al.,* 2021).

2.5.2.2 PHARMACOLOGICAL MANAGEMENT

a) The first line of therapy for those who are unable to regulate their blood sugar levels with diet and exercise is metformin, which does not induce weight gain or hypoglycemia. In accordance with the diabetes regimen, gradually increase the dosage.

b) When metformin is contraindicated or poorly tolerated, a second-generation sulfonylurea (ideally gliclazide) can be administered as the initial (first-line) treatment. Sulfonylureas might result in hypoglycemia or weight gain.

c) Alternative pharmaceuticals have not been proven to provide better glycemic control and long-term results than metformin or sulfonylurea when used as initial therapy.

d) Therapeutic increase is crucial when metformin alone is unable to normalize glycemia. In individuals with insufficiently regulated glycaemia on metformin, this treatment should be a second-generation sulfonylurea (ideally gliclazide), combined with diet and exercise (WHO, 2020).

2. 6 TRADITIONAL/MEDICINAL PLANTS

The therapy of diabetes mellitus greatly benefits from understanding traditional uses of medicinal herbs (Atawodi *et al.,* 2014). A few of the conventionally utilized medicinal herbs for treating diabetes include:

1. Pawpaw Leaves (*Carica papaya*) (Airaodion *et al.,* 2019)
2. Onions (*Allium cepa*) (Ülger and Çakiroglu, 2020)
3. *Tetrapleura tetraptera* (Omonkhua *et al.,* 2014)

2.6.1 C*arica papaya* (Pawpaw)

Carica papaya is a part of the Caricaceae family, and numerous of its species have been employed as treatments for a wide range of ailments (Airaodion *et al.,* 2019). C. papaya is a perennial plant that was first discovered in southern Mexico and is now widely distributed throughout the entire tropical area. Its fruit is widely distributed and utilized as food or a sort of medicine. Numerous scientific studies have been carried out to assess the biochemical activity of different C. papaya sections, such as fruit, stalk, leaves, seeds, roots, or latex (Otsuki *et al.,* 2010). Papain, chymopapain, cystatin, alpha-ascorbic acid, flavonoids, cyanogenic glycosides, and glycosinolates are just a few of the active substances found in C. papaya leaves that have been demonstrated to lower lipid peroxidation levels and improve blood's total antioxidant activity (Otsuki *et al.,* 2010). Both the fruit and the seed extracts have strong bactericidal capabilities (Airaodion *et al.,* 2019). It has been found that ethanolic papaya leaf extract has antiplasmodial activity against Plasmodium berghei in affected Swiss albino mice (Airaodion *et al.,* 2019). People on the Gold Coast or in Australia ingest its leaf juice due to claims that it has anticancer properties; certain anecdotes of successful cases have been documented in various publications (Airaodion *et al.,* 2019). Indigenous people have traditionally employed the leaf

extracts as a treatment for a variety of ailments, such as cancer and infectious infections. Papain and chymopapain, two significant physiologically active chemicals found in *C. papaya* that are frequently used to treat digestive issues (Airaodion *et al.,* 2019). Additionally, papain, chymopapain, glycerine, and peptidase from *C. papaya* can enhance the state of an acidic pH and the destruction of pepsin (Airaodion *et al.*, 2019). Lipase, a hydrolase that is strongly bound to the water-insoluble portion of crude papain and is therefore regarded as a naturally immobilized biocatalyst, is another active component of *C. papaya* (Dominguez *et al.,* 2006).

2.6.2 A*llium cepa* (Onions)

The Liliaceae family member *Allium cepa* is widely farmed all over the world and is most likely native to south-west Asia (Ülger and Akiroglu, 2020). It is frequently handled as a single household vegetable because of its globose bulb, which is an underground portion of the stem. *A. cepa* has been utilized for healing purposes for many years (Akash *et al.*, 2014). Blood pressure reduction, antibacterial, hypoglycaemic, and hypocholesterolemic characteristics are among its most widely used applications (Akash *et al.*, 2014). Allyl propyl disulfide (APDS), although there are other active sulphurous compounds present, is the active component of *A. cepa* (Akash *et al.,* 2014). For many years, the quest for anti-diabetic drugs will continue to center on plants and other natural resources (Ülger and akiroglu, 2020). The use of herbal items for medical advantages has played an essential role in almost every culture on Earth. According to Ulger and Akiroglu (2020), *A. cepa* has considerable anti-diabetic benefits on both type I and type 2 diabetes and significantly lowers fasting blood glucose levels. Slices of *A. cepa* that had undergone a glucose tolerance test to induce hyperglycemia likewise yielded similar kinds of results (Ülger and Çakiroglu, 2020).

2.6.3 *Tetrapleura tetraptera*

Tetrapleura tetraptera is a well-known medicinal plant that is a member of the Fabaceae family, originally known as the Leguminosae. The Yoruba ethnic group of South Western Nigeria refers to it as "Aridan" or "Aidan," the Hausa ethnic group of Northern Nigeria refers to it as "Dawo," and the Igala people of North Central Nigeria refer to it as "Abogolo" (Aladesanmi *et al.,* 2007). It is typically found in lowland forests in several tropical African nations, and its fruit is noted to have small, brownish-black seeds in a mushy pulp with a distinctively fragrant and pungent aroma (Atawodi *et al.,* 2014). *Tetrapleura tetraptera* has many nutritional and medicinal benefits. It is highly valuable because it contains a high amount of essential phytochemicals and nutrients that are vital for the healthy functioning of the body. It is an excellent source of potassium, iron, calcium, zinc, flavonoids, phosphorous, tannins, alkaloids, saponins, steroids and phenolic compounds (Atawodi *et al.*, 2014).

Its fruit is used to treat convulsions, leprosy, fevers, inflammation, rheumatism, flatulence, jaundice, as well as to manage and control type 2 diabetes mellitus in Southern and Eastern Nigeria (Bella *et al.,* 2011). It is also a common culinary spice there (Aladesanmi, 2007; Bella *et al.,* 2011). An anti-oxidant impact on carbon tetrachloride-induced hepatotoxicity was demonstrated by the methanolic extract of *T. tetraptera* leaves in prior studies (Atawodi *et al.,* 2014), whereas hypoglycemic capabilities were demonstrated by the aqueous extract of the plant's fruits (Bella *et al*., 2011).

2.7 DIABETOGENS

Alloxan and streptozotocin are two of the most well-known compounds in diabetic research that cause diabetes. Both are cytotoxic mimics of glucose. While their cytotoxicity is done in different ways, their processes for choosing beta cells are the same (Lenzen, 2008).

2.7.1 ALLOXAN

Alloxan is a pyrimidine derivative that was created in 1838. (Macdonald *et al.*, 2017). Alloxan first attracted attention in diabetes research in 1943 when Dunn and Meletchine discovered that it might cause diabetes in mice due to the particular necrosis of pancreatic beta cells (Lenzen, 2008). Alloxan diabetes, a condition of experimental diabetes mellitus, is brought on by the ensuing insulinopenia (Lenzen, 2008). Additionally, it has been demonstrated that the reduction product of alloxan to dialuric acid causes the same ultrastructural alterations that are seen in response to alloxan in animals and is diabetogenic (Lenzen, 2008).

2.7.2 MECHANISM OF ACTION OF ALLOXAN

There are two separate pathogenic effects of alloxan. Thus specifically inhibits glucokinase, the beta cell's glucose sensor, preventing glucose from inducing the release of insulin, and it results in an insulin-dependent diabetes state by causing the selective necrosis of beta cells. These two actions can be attributed to the particular chemical characteristics of alloxan, with the beta cell's selective uptake and accumulation of alloxan serving as the common denominator (Lenzen, 2008).

Alloxan is a chemical molecule that is extremely unstable and has a molecular structure similar to glucose (Lenzen, 2008). Because the lipid bilayer of the plasma membrane is hydrophilic and impermeable, neither alloxan nor glucose can cross it. Due to the structural similarity of the alloxan molecule to glucose, the Glut-2 glucose transporter in the beta cell plasma membrane

accepts this glucomimetic and transports it into the cytosol, allowing for unlimited entry into beta cells (Rohilla and Ali, 2012).

Since alloxan does not obstruct the transporter's functionality, it can enter beta cells specifically and without restriction. These cells do not affect insulin-producing cells because they lack this transporter (Lenzen, 2008). The alloxan's half-life is brief (Lenzen, 2008). It must be immediately taken up and stored in the beta cells because it spontaneously decomposes into non-diabetogenic alloxanic acid in aqueous solution within minutes (Rohilla and Ali, 2012). In the first several minutes after receiving an injection of alloxan, it is therefore useless if blood flow to the pancreas is halted (Rohilla and Ali, 2012).

2.7.3 STREPTOZOTOCIN (STZ)

Streptozotocin has been employed as a chemotherapeutic alkylating agent in addition to being an antimicrobial agent (Goud *et al.,* 2015). In 1963. According to reports, the particular necrosis of the pancreatic beta cell in STZ diabetes results in the disease, and ever since then, STZ has been the drug of choice for causing diabetes mellitus in animals (Eleazu *et al.,* 2013). After a decade of research, a comprehensive explanation for the selective toxicity of STZ and alloxan, the two main agents responsible for the development of diabetes, can be offered. (Lenzen *et al.,* 2008).

2.7.4 MECHANISM OF ACTION OF STREPTOZOTOCIN

Antibiotic streptozotocin (STZ) works well against Gram-negative bacteria. Because of its N-nitroso group, STZ causes diabetes by acting as a nitric oxide donor in pancreatic islets (Chaudhry *et al.,* 2013). By alkylating and cross-linking DNA strands, STZ prevents the production of DNA in both microbial and mammalian cells. It also has an impact on all phases of the mammalian cell cycle (Abdollahi and Hosseini, 2014). Instead of causing DNA damage, STZ-induced DNA damage activates poly ADP-ribosylation, which is crucial in the development of diabetes. According to biochemical research, STZ blocks pyridine nucleotides

and the major enzymes involved in glyconeogenesis (Bolzán and Bianchi, 2002; Szkudelski, 2012; Abdollahi, and Hosseini, 2014). The glucose transport protein GLUT-2 is used by STZ to carry glucose into the cell; however, STZ is not recognized by the other glucose transporters. This explains why STZ is relatively toxic in beta cells, which have relatively high levels of GLUT-2 (Abdollahi and Hosseini, 2014).

2.8 ANTIOXIDANT ENZYMES

2.8.1 SUPEROXIDE DISMUTASE (SOD)

It is an antioxidant enzyme that catalyzes the partitioning or dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and regular molecular oxygen (O_2) (Younus, 2018). Superoxide is a by-product of oxygen metabolism that, if unchecked, can result in a variety of cell damage. Additionally harmful, H_2O_2 is broken down by other enzymes including catalase. SOD is a crucial anti-oxidant in almost all live cells that are exposed to oxygen. (Younus, 2018).

SOD catalyses the disproportionation of super-oxide

$$H_2O_2 \longrightarrow O_2 + H_2O$$

Almost all eukarotic cells have a SOD enzyme containing copper and zinc in their cytosols. (Zhang *et al.,* 1999). Commercially accessible Cu-Zn SOD is isolated from red blood cells from cows. The cu-enzyme found in cattle is a homodimer with a molecular weight of 32500. It was the first SOD whose crystal structure in atomic detail was determined (Younus, 2018). SOD isoenzymes have been found in many cell compartments in higher plants. Peroxisomes and mitochondria both contain Mn-SOD. Cu-SOD is localized in the cytoplasm, chloroplast, peroxisomes, and apoplast, while Fe-SOD is primarily found in the chloroplast. Peroxisomes have also been reported to contain Fe-SOD. (Younus, 2018). The majority of chordates, all other animals, and humans all have three different types of SOD. SOD-I, SOD-2, and SOD-3 are all found in the cytoplasm, mitochondria, and extracellular space, respectively. The first has two subunits and is a dimer, whereas the others are tetramers (four subunits). The mitochondrial

enzymes SOD-1, SOD-3, and SOD-2 all contain copper, zinc, and Mn in their reactive centers. (Zhang *et al.*, 1999).

Phytoplankton from the ocean, bovine liver, horse radish, cantaloupe, and certain bacteria are all sources of SOD used for commercial purposes. (Meyrick and Magnuson, 1994). SOD may lessen skin fibrosis after radiation treatment for breast cancer, for example, by reducing free radical damage to the skin (Zhang *et al.*, 1999). SOD has a potent anti-inflammatory effect when used in therapeutic applications. SOD, for instance, is a very efficient experimental treatment for chronic colon inflammation (Chair *et al.*, 1994). SOD therapy reduces the production of reactive oxygen species (ROS) and oxidative stress, which in turn inhibits endothelial activation. As a result, these antioxidants may represent significant new therapeutics for the treatment of inflammatory bowel disease. (Younus, 2018).

2.8.2 CATALASE (CAT)

A common enzyme called catalase is present in almost all living things that are exposed to oxygen, including bacteria, plants, and animals. It catalyzes the conversion of H_2O_2 into water and oxygen (O_2) (Chelikani *et al.*, 2004). It is a crucial enzyme in preventing ROS-induced oxidative cell damage. One catalase molecule may also convert millions of H_2O_2 molecules into water and oxygen per second, making it one of the enzymes with the fastest turnover rates (Sooch *et al.*, 2017). A tetramer comprising four polypetides chains, each over 500 amino acids long, makes up catalase. Its four heme groups, which contain iron, enable the enzyme to interact with hydrogen peroxide. The rate of reaction is not significantly different between pH 6.8 and pH 7.5, while the optimal PH for human catalase is around 7. (Sooch *et al.*, 2017). Catalase is a tetramer containing four subunits, each of which is separated into four domains. Louis Jacques Theonard, who discovered H_2O_2, proposed that an unidentified component is responsible for its breakdown in 1818, which led to the discovery of catalase. Oscar Loew discovered it in numerous plants and animals in 1900 and was the first to name it catalase. James B. Summer

and Alexander Dounce crystallized catalase from beef liver in 1937, and in 1938 they determined its molecular weight. (Sooch *et al.*, 2017).

$H_2O_2 \longrightarrow H_2O + O_2$

Before making cheese, hydrogen peroxide is taken out of milk using catalase in the food business. It can also be used in food wrappers to stop food from oxidizing. Using catalase to remove H_2O_2 from fabrics ensures that they are free of peroxide in the textile industry (Sooch *et al.*, 2017).

2.8.3 GLUTATHIONE-S TRANSFERASE (GST)

The role of the glutathione-S-transferase (GST) family of phase II detoxification enzymes is to defend cellular macromolecules against harm. Glutathione (GSH) is conjugated to a range of endogenous and external electrophilic compounds specifically by the action of GSTs (Salinas and Wong, 1999). These enzymes perform a variety of different tasks in addition to their activity as catalysts for the conjugation of electrophilic substrates to glutathione (GSH). They possess peroxidase and isomerase capabilities, can inhibit the Jun N-terminal kinase (thereby shielding cells from hydrogen peroxide-induced cell death), and can bind a variety of endogenous and foreign ligands non-catalytically (Salinas and Wong, 1999).

The three superfamilies that make up the GST family are the MAPEG (also known as cytosolic, mitochondrial, and microsomal) proteins. On a number of substrates, GSTs catalyze the conjugation of GSH to electrophilic sites via a sulfhydryl group in order to increase the water solubility of the molecules. This process facilitates the breakdown of xenobiotics and detoxifies endogenous substances like peroxidized lipids. Given that diabetes is an oxidative damage-related disease and that diabetic individuals have defective GSH metabolism, GSTs may be a possible target for diabetic medication treatment (Kurutas, 2015).

2.8.4 GLUTATHIONE PEROXIDASE (GPX)

The broad term for an enzyme family with peroxidase activity whose primary biological function is to shield the body from oxidative damage is glutathione peroxide (GPX) (Ighodaro and Akinloye, 2018). Glutathione peroxidase converts lipid hydroperoxides into the appropriate alcohols in their biochemistry, as well as free hydrogen peroxide into water (Ighodaro and Akinloye, 2018). Diverse isozymes with different cellular locations and substrate requirements are encoded by dilute genes. Nearly all mammalian tissues have GPX-I, which is the most prevalent variant and prefers H_2O_2 as a substrate. Lipid hydro-peroxide is a strong predilection of GPX-4. Despite being generated at significantly lesser concentrations, it is found in almost all mammalian cells. While GPX-3 is extracellular and seen predominantly in the plasma, GPX-2 is an extracellular and intestinal enzyme. Eight distinct human GPX isoforms, numbered from 1 to 8, have so far been discovered (Brigelius-Flohé and Maiorino, 2013).

$2GSH + H_2O_2 \longrightarrow GS\text{-}SH + 2H_2O$

Gordon C. Mills first identified glutathione peroxidase in 1957 (Brigelius-Flohé and Maiorino, 2013). Numerous techniques are used to determine the spectrophotometric activity of GPX. Broadly used is a direct assay that measures the amount of NADPH that is converted to NADP by measuring the peroxidase reaction. The alternative method uses Ellman's reagent to measure any remaining GSH in the reaction (Bhabak and Mugesh, 2010).

2.8.5 GLUTATHIONE REDUCTASE (GR)

Glutathione reductase (GR), also known as glutathione disulfide reductase (GSR), is an enzyme that in humans is encoded by GSR gene. Glutathione reductase (EC 1.8.1.7) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydry1 form glutathione (GSH) which is a critical molecule in resisting oxidative stress and maintaining the conducive environment of the cell (Rao and Reddy, 2008).

As a dimeric disulfide oxidoreductase, GR converts one molar equivalent of GSSG to two molar equivalents of GSH by using a FAD prosthetic group and NADPH (Rao and Reddy, 2008).

2.8.6 MALONDIALDEHYDE (MDA)

The chemical formula for malondialdehyde (MDA) is $CH_2(CHO)_2$. It is a colorless liquid that arises in the enol form and is a highly reactive chemical. It happens naturally and serves as an indicator of oxidative stress (Bhabak *et al.*, 2010).

2.8.6.1 STRUCTURE AND SYNTHESIS OF MALONDIALDEHYDE

Malondialdehyde mainly exist as the enol form:

$CH_2(CHO)_2 \longrightarrow HOC(H)=CHO$

The cis-isomer is preferred in organic solvents, but the trans-isomer predominates in water. The equilibrium happens quickly and doesn't matter for many things. In the lab, it can be produced in-situ by hydrolyzing its commercially accessible and shelf-stable acetal 1,1,3,3-tetramethoxypropane, in contrast to malondialdehyde. Malondialdehyde is readily deprotonated to produce the enolate's sodium salt (m.p.245^{0}C) (Tiedge *et al.*, 1997).

2.8.6.2 BIOSYNTHESIS AND REACTIVITY OF MALONDIALDEHYDE

Polyunsaturated fatty acids undergo lipid peroxidation, which produces malondialdehyde. It is a key component in the production of thromboxane A2, which is produced when platelets and a variety of other cell types and tissues metabolize arachidonic acid to prostaglandin H2 using cyclooxygenase 1 or cycloxygenase 2. Thromboxane synthase continues to break down this substance, producing thromboxane A2, 12-hydroxyheptadecatrienoic acid, and malonyldialdehyde (Amed *et al.*, 2005). The concentration of malondialdehyde in tissues can be used to evaluate the level of lipid peroxidation (Jadoon and Malik, 2017).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 PLANT COLLECTION

Tetrapleura tetraptera (Schum and Thonn) Taub was obtained from Akungba-Akoko, Ondo State, South-Western Nigeria and identified in the Department of Microbiology and Botany, University of Ibadan, Nigeria. Herbarium specimens was stored at the Herbarium of the University of Ibadan, Nigeria with herbarium number UIH22320. The plant samples were washed thoroughly under running water, shade dried, and pulverized.

3.2. EXTRACTION OF SAPONINS

The method used to fractionate saponins from *T. tetraptera* stem bark was adapted from Hostettmann *et al,* (1991). One thousand one hundred and thirty four grams (1134g) of the powered plant sample was soaked in 4800ml of methanol (methanol extract) for 72 hours (3 days) in a glass container and covered with foil paper. It was occasionally stirred and at the end of the third day, it was filtered through two layers of cheesecloth and later with a filter paper to completely remove the residues. The filtrate was concentrated by a rotary evaporator at 40°C and then evaporated to dryness by means of a freeze dryer, and dried extract was weighed.

The methanol extract was re-extracted with n-hexane and water in a ratio of 1:2 (v/v) in a separating funnel and left for 48 hours (2 days) on a retort stand. At the end of the second day, the mixture was decanted into two fractions; water layer and hexane layer. The water layer (aqueous extract) was concentrated by a rotary evaporator, evaporated to dryness using a freeze dryer and weighed.

The aqueous extract obtained was re-extracted with ethylacetate and butanol in a ratio of 1:2 (v/v) in a separating funnel, and left for 48 hours (2 days) on a retort stand. At the end of the second day, the mixture was decanted into two fractions; butanol layer and ethlyacetate layer. The butanol layer (butanol extract), which contains saponin was concentrated by a rotary evaporator and then evaporated to dryness by means of a freeze dryer, and weighed. The saponins obtained was then stored in an airtight container in a refrigerator until use. The ethylacetate fraction was also freeze dried to obtain total alkaloid fractions.

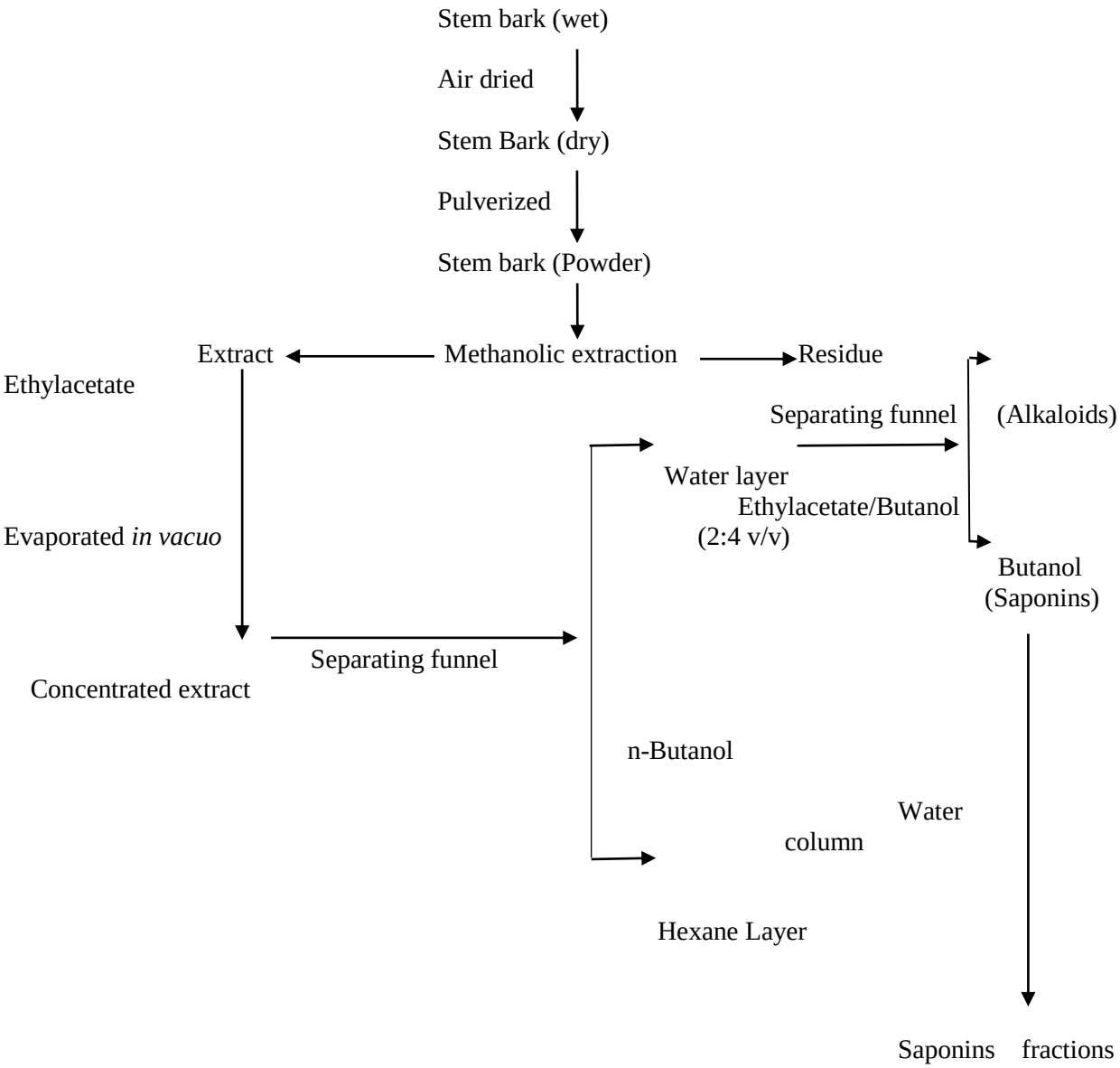

Figure 3.2 Schematics for the extraction of *T. tetraptera* total saponins (TTS) fractions

Source: Hostettmann *et al.* (1991).

3.3 PREPARATION AND ADMINISTRATION OF PLANT EXTRACT

The *Tetrapleura tetraptera* total saponins (TTS) fractions were concentrated under pressure and then freeze dried; the TTS was reconstituted appropriately in distilled water. Different doses of TTS and metformin, at 100 mg/kg body weight was administered orally (by gavage) daily to male Wistar rats for 12 weeks.

3.4 INDUCTION OF DIABETES

Streptozotocin was dissolved in citrate buffer (pH 4.5) and administered to the rats by intra-peritoneal injection, at a dose of 65mg/kg body weight after a 12-hour fast. Diabetes was confirmed after seven (7) days of STZ administration by measuring fasting blood sugar (FBS). After stable diabetes is established (FBS > 180 mg/d1), TTS and metformin administration to rats commenced and lasted for 12 weeks.

3.5 ANIMALS AND EXPERIMENTAL PROTOCOL

The total number of rats used were 42 adult male Wistar rats. They were kept in the animal house in Department of Anatomy. The rooms were aerated with 12 hours' light and the animal was fed daily with standard pelletized feed and water *ad libitum*. The animals were acclimatized for two weeks before the commencement of the study. Treatment of the animals conformed to the guidelines for the care and use of laboratory animals (NAS, 2011). The Faculty of Pharmacy Ethical Committee provided ethical approval for the study, with code, EC/FP/020/19. The animals were divided into the following groups of eight (8) rats each:

a. Normal control (untreated normal rats)

b. Diabetic control (untreated diabetic rats)

c. Positive control (diabetic rats treated with metformin) at 100 mg/kg body weight

d. Diabetic rats treated with 10 mg/kg body weight of TTS

e. Diabetic rats treated with 20 mg/kg body weight of TTS

f. Diabetic rats treated with 40 mg/kg body weight of TTS

The extracts were administered orally for twelve (12) weeks (by gavage), after which the rats were sacrificed. Biochemical and histological investigations were conducted

3.6 BLOOD COLLECTION

Blood for monitoring fasting blood sugar was drawn from the tail vein of each rat once a week and assessed with a glucometer. At the end of 12 weeks, the rats were sacrificed by cervical dislocation; the thoracic/abdominal regions were opened to expose the heart and other organs. Blood was obtained through heart puncture. Blood for glucose assays was collected into fluoride bottles while those for other biochemical analyses were collected in plain bottles. The blood samples were allowed to clot on ice and centrifuged at 1,000 X g for 5 minutes; the serum was separated for analysis.

3.7 TISSUE HOMOGENIZATION

The liver, heart, kidneys, pancreas, and testes were removed and blotted with cotton wool. Portions for tissue homogenization were homogenized in ice cold normal saline 4:1 w/v. The homogenates were centrifuged at 1,000 X g for 5 minutes, and the separated supernatants stored in a freezer until analysis.

3.8 THE BIOCHEMICAL INVESTIGATIONS

The biochemical investigations done included:

1. Serum and tissue total protein determination

This was carried out by means of the Biuret method as described. Potassium iodide was added to the reagent to prevent precipitation of Cu^{2+} ions.

Principle

Proteins form a colored complex with cupric ions in an alkaline solution.

Procedure

0.02ml of serum was mixed with 1 ml of R1 in a test tube and placed in an incubator that the temperature was set at 25 ^{o}C for 30 minutes. The absorbance was taken at 546 nm. The concentration of the total protein was calculated as follows:

$$\text{Protein Mg/100ml} = \frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times \text{concentration of standard}$$

2. Enzymatic antioxidant analysis

a. Superoxide dismutase ((SOD)

Serum and tissue superoxide dismutase assay using the Misra and Fridovich (1972) method.

Reagent

Contents: Carbonate buffer (0.05 mol/l, pH 10.2), adrenalin (0.3 mmol/l)

Assay Protocol

A reaction mixture containing 0.2 ml of serum in 2.5 ml of carbonate buffer (0.05M, pH= 10.2) was equilibrated in the spectrophotometer, this was followed by the addition of 0.3 ml of adrenaline to initiate the reaction. A blank was prepared by the addition of all the reagents a replacing the serum with distilled water, the change in absorbance was read at 480nm for 150 150 seconds at 30 seconds interval.

Mix, read absorbance immediately after adding adrenalin at 0 seconds, then at 30, 60, 90, 120 and 150 seconds, for both blank and sample at 480 nm. For tissue SOD, dilute homogenate 1 + 9 with distilled water.

Calculation

Increase in absorbance /min = A_3-A_0/2.5

Where A_0 =Absorbance after 30secs

A_3 = Absorbance after 150secs

$$\% \text{ Inhibition} = \frac{\text{Increase in absorbance of substrate} \times 100}{\text{Increase in absorbance of blank}}$$

1 unit of SOD activity is given as the amount of SOD to cause 50% inhibition of adrenaline to adrenochrome during 1min.

e.g. if A_0 =0.043

A_3 = 0.046

Increase in absorbance in blank i.e A_{3blank}-A_{0blank} = 0.056

Then

Increase in absorbance / min = 0.046-0.043/2.5= 0.003/2.5= 0.0012

% inhibition = 0.0012 × 100/0.0056 =214.2857143

50% Inhibition= 214.2857143 ×50/100=10.714 unit/ml

b. Serum and tissue Catalase (CAT) assay using the Sinha (1972) method.

Reagent

Contents: Phosphate Buffer (0.1 mol/l, pH 7.0), H_2O_2 (0.2 mol/l), $K_2Cr_2O_7$ (5 g/100ml), Acetic acid

Dichromate/acetic acid solution: Mix 5% $K_2Cr_2O_7$ solution with acetic acid in a ratio 1:3 v/v.

Assay Protocol

Serum Dilute 1 + 9 with distilled water

Tissue homogenate dilute 1 + 49 with distilled water

4.0 ml of 0.2M H_2O_2 was measured into a test tube, followed by the addition of 5 ml of Phosphate buffer. A sample (dilute) of 1.0 ml will then be added to the mixture.

Start timing, transfer quickly 1 ml each into 4 test tubes and add 2.0 ml of dichromate/acetic solution into first test tube after 60 seconds, the 2nd after 120 seconds, 3rd after 180 seconds, and 4th after 240 seconds. Read the absorbance of sample against blank at 570 nm.

Calculation

The catalase enzyme activity was extrapolated from a standard H_2O_2 curve.

Catalase activity = µmole of H_2O_2 decomposed /min/mg protein. Find the average of the changes in H_2O_2 decomposed per minute. Divide by the amount of protein (mg protein).

c. Glutathione – S – transferase (GST) activity.

The cytosolic glutathione S-transferase activity will be determined spectrophotometrically at 37^0c (340nm) by the procedure described by Habig *et al,* (1974).

PROCEDURE

The total reaction mixture will be 3ml containing 0.03ml of 0.1MGHS, 0.15ml of 20mM CDNB (1-chloro-2, 4-dinitrobenzene) and 2.79ml of 0.1M phosphate buffer in the sample test tube. The blank will contain 2.82ml phosphate buffer. The reaction mixture will be pre-incubated at 37^0c for 15min and the reaction mixture will be started by the addition of 0.03ml diluted cytosol and the absorbance will be read at 30 sec, 1min, 2min and 3min interval. The reaction mixture without the enzyme (supernatant) will be used as blank.

CALCULATION

GST activity = $\frac{\text{O.D/min}}{9.6}$ x $\frac{\text{total reaction mixture volume}}{\text{mg/ 0.03ml protein}}$

REAGENT PREPARATION

1. 1-Chloro-2,4-dinitrobenzene (CDNB) (20mM)

   3.37mg of CONB will be dissolved in 1m of ethanol

2. Reduced Glutathione (0.1M)

   30.37mg of glutathione (GSH) will be dissolved in 1ml of 0.1M

3. 0.1M phosphate buffer (PH 6.5)

   4.96g of K_2HPO_4 and 9.73g of KH_2PO_4 will be dissolved in 100ml distilled water and the PH will be adjusted to 6.5

CHEMICALS

1-Chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), Ethanol, Dipotassium phosphate (K_2HPO_4), Potassium dihydrogen phosphate (KH_2PO_4).

3.9 STATISTICAL ANALYSIS

The data were expressed as means of 4 to 7 determinations ± S.E.M. The differences among groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons were done by the Duncan's post hoc test. A value of $P< 0.05$was accepted as significant. IBM SPSS Statistics, version 26 (IBM Corp., Armonk, N.Y., USA) was used for the analysis.

CHAPTER FOUR

4.0 RESULT

Figure 4.1. Shows the effect of *Tetrapleura tetraptera* saponins (TTS) on total protein in the serum, liver, heart, kidney, pancreas and testis of STZ-induced diabetic Wistar rats.

The results showed a significant ($p<0.05$) reduction in the total protein levels of all the tissues, except the heart and serum, in the streptozotocin diabetic control (group 2) when compared with the normal control (group 1).

Treatment with *T. tetraptera* saponins significantly increased the total protein concentrations in almost all the tissues. The lower dose (10mg/kg body weight) of TTS in this current study showed a better effect in the elevation of the total protein levels.

Figure 4.2. Shows the effect of *Tetrapleura tetraptera* saponins (TTS) on superoxide dismutase (SOD) activities in the serum, liver, heart, kidney, pancreas, and testis of STZ-induced diabetic Wistar rats.

There was a non-significant ($p>0.05$) difference in the SOD levels of the diabetic rats (group 2) compared to the normal control (group 1) in almost all the tissues. However, treatment with 10mg/kg body weight of TTS showed a significant elevation in the SOD levels of the serum and the liver.

Figure. 4.3. Shows the effect of *Tetrapleura tetraptera* saponins (TTS) on catalase activities in the serum, liver, heart, kidney, pancreas, and testis of STZ-induced diabetic Wistar rats.

STZ diabetes caused significant ($p<0.05$) reduction in catalase activities in the diabetic rats (group 2) compared to the normal control (group 1) in the serum, liver, and heart. Treatment with saponins from *T. tetraptera* caused significant ($p<0.05$) increase in catalase activities in the serum and kidney.

Figure. 4.4. Shows the effect of *Tetrapleura tetraptera* saponins (TTS) on glutathione-S-transferase activities in the serum, liver, heart, kidney, pancreas, and testis of STZ-induced diabetic Wistar rats.

In the serum and kidney, a significant ($p<0.05$) decrease was observed in the GST activities in the diabetic rats (group 2) compared to the normal control (group 1) Administration of saponins from *T. tetraptera* increased GST activities significantly ($p<0.05$) in the serum and kidney.

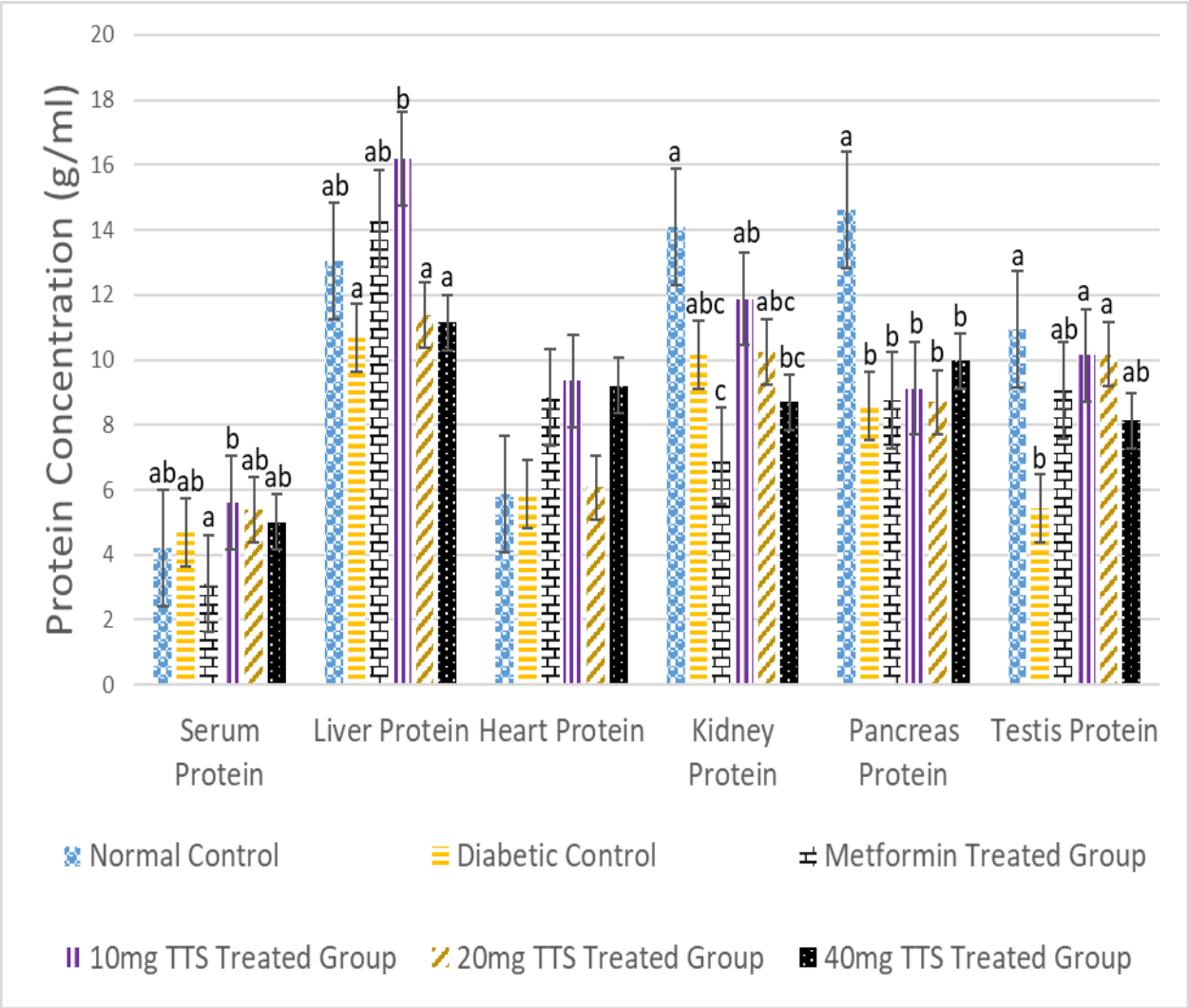

Figure 4.1. Effect of *Tetrapleura tetraptera* saponins (TTS) on total protein in the serum, liver, heart, kidney, pancreas and testis of STZ-induced diabetic Wistar rats. Columns with different superscript are significantly different at $p<0.05$.

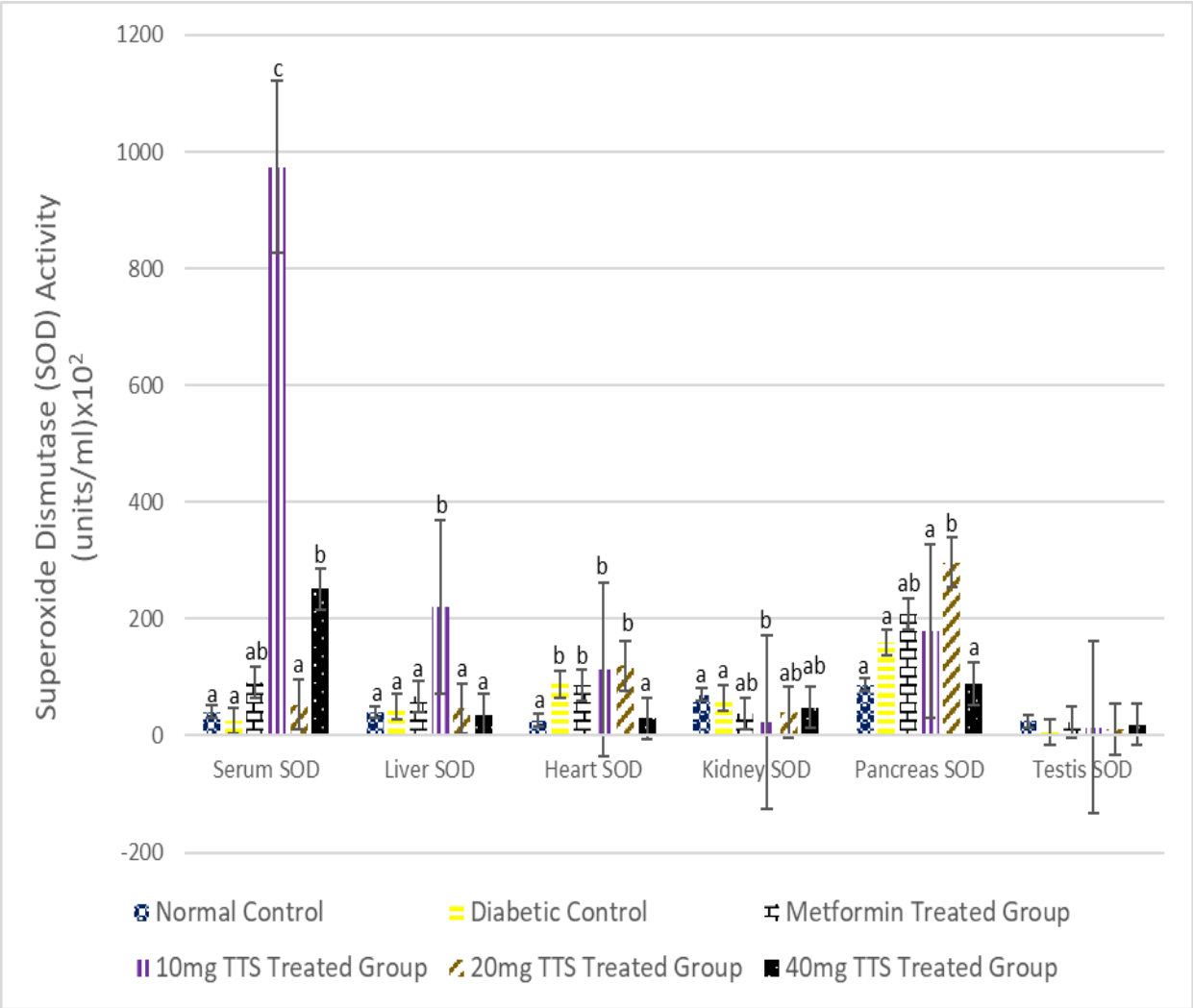

Figure 4.2. Effect of *Tetrapleura tetraptera* saponins (TTS) on superoxide dismutase (SOD) activities in the serum, liver, heart, kidney, pancreas, and testis of STZ-induced diabetic Wistar rats. Columns with different superscript are significantly different at $p<0.05$.

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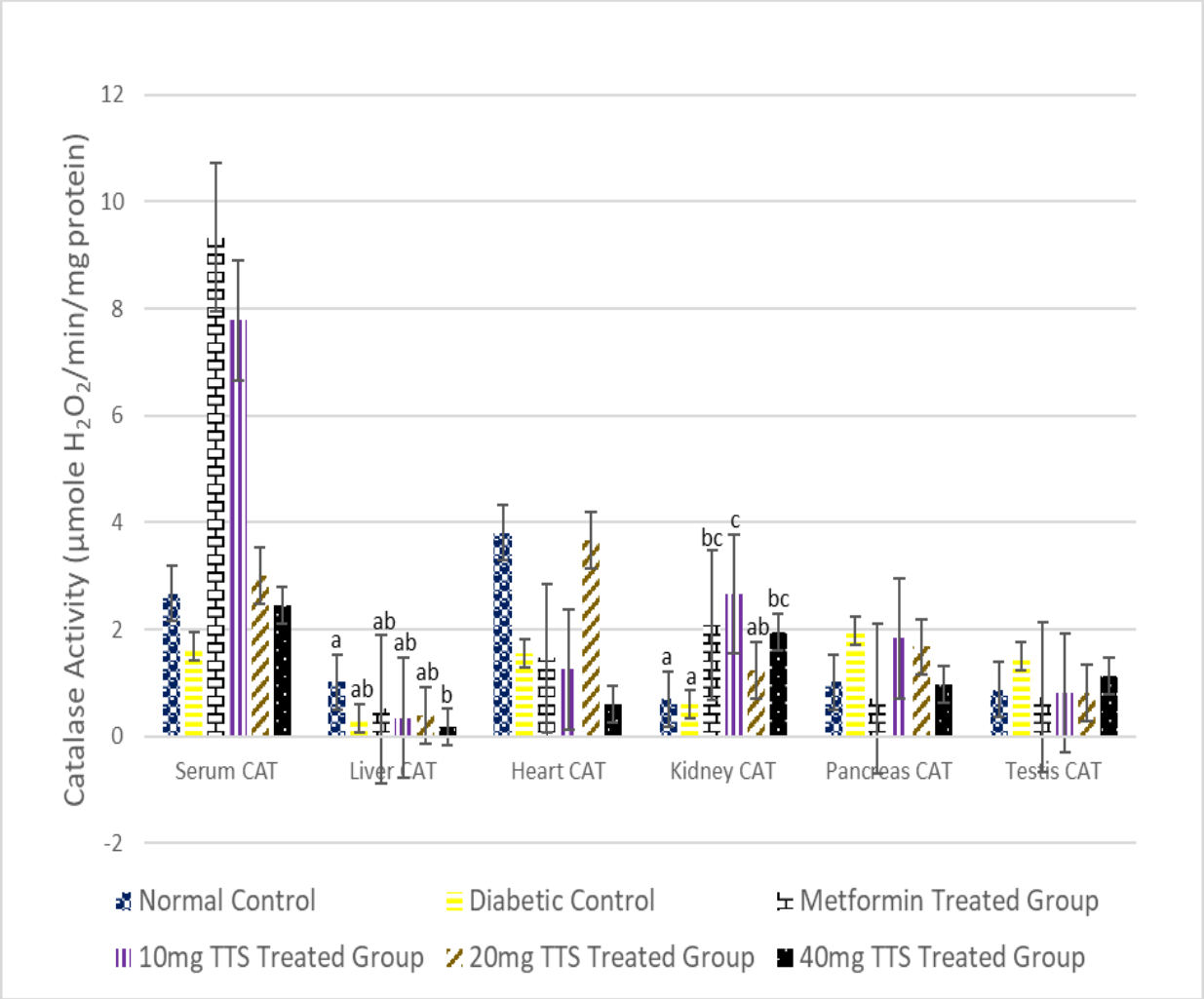

Figure. 4.3. Effect of *Tetrapleura tetraptera* saponins (TTS) on catalase activities in the serum, liver, heart, kidney, pancreas, and testis of STZ-induced diabetic Wistar rats. Columns with different superscript are significantly different at $p<0.05$.

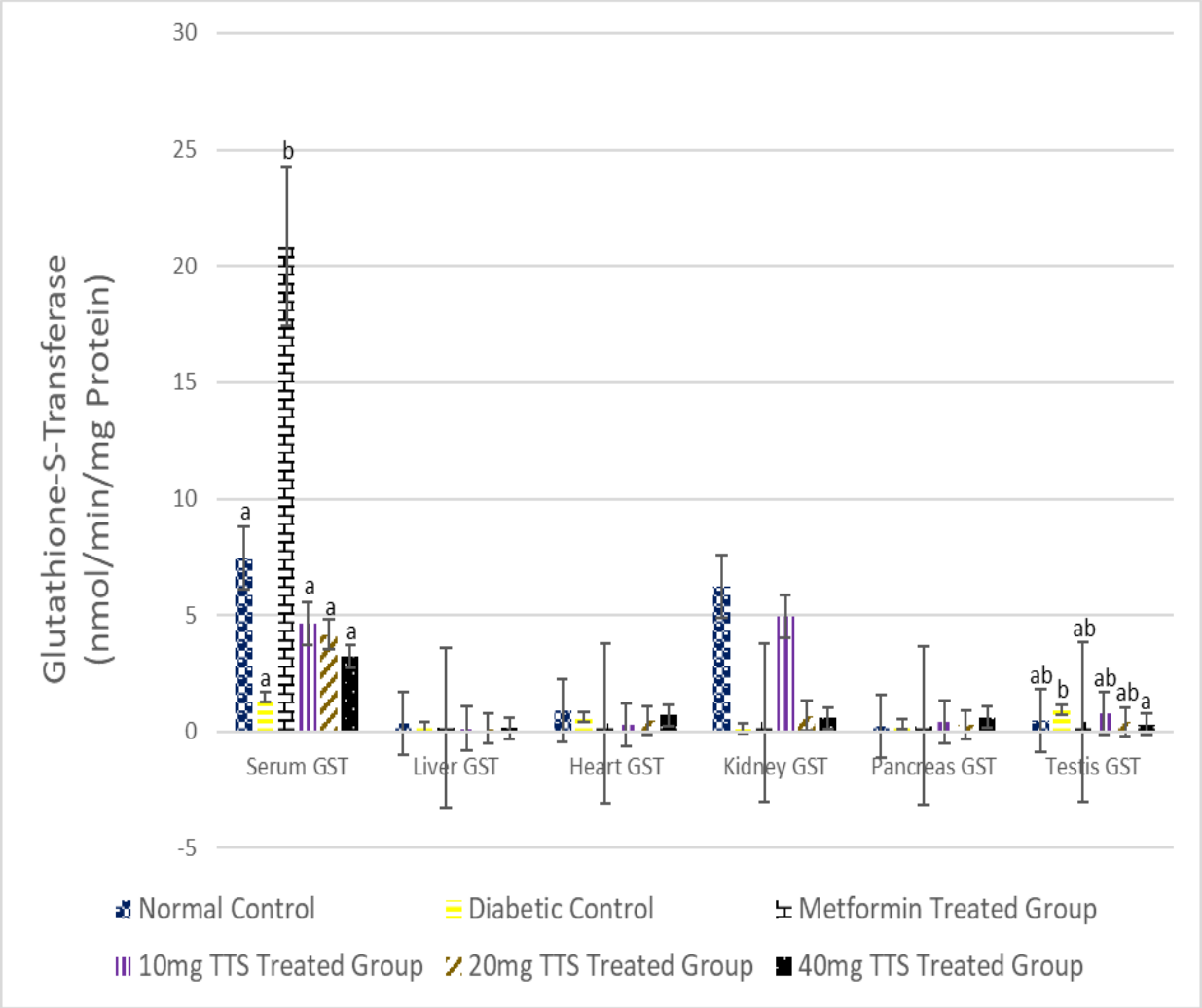

Figure. 4.4. Effect of *Tetrapleura tetraptera* saponins (TTS) on glutathione-S-transferase activities in the serum, liver, heart, kidney, pancreas, and testis of STZ-induced diabetic Wistar rats. Columns with different superscript are significantly different at $p<0.05$.

CHAPTER FIVE

5.0 DISCUSSION

Hyperglycemia usually increases the production of free radicals and decrease tissue anti-oxidative capacity in diabetes thereby resulting in oxidative stress (Pop-Busui *et al*., 2006; Giribabu *et al.*, 2014). The tissue anti-oxidative potential needs to be increased in order to overcome oxidative damage (Giribabu *et al*., 2014). Studies have shown that saponins from medicinal plants could increase the antioxidant potential of tissues such as the kidney, liver, and the heart, which could help reduce free radical formation (Katalinic *et al*., 2006; Elekofehinti *et al*., 2012; Chen *et al*., 2014). As such, this study investigated the effects of TTS on enzymatic markers of oxidative stress in STZ-induced diabetic rats.

The present study showed a significant ($p<0.05$) reduction in total protein levels in the tissues except the heart and serum of the streptozotocin diabetic control (group 2) when compared with the normal control (group 1) and the treated (groups 3-6). This could be due to the fact that hyperglycemia promotes gluconeogenesis and as such, brings about protein degradation, as well as excess nitrogen loss, leading to negative nitrogen balance (Babas *et al*., 2020). This study is similar to that of Babas *et al*., (2020) who stated that treatment of diabetic rats with saponins from the extract of *Biophytum sensitivum* increased the concentration of total proteins. The findings from this study also corresponds with Abubakar *et al*., (2018) who stated that treatment of diabetic rats with hydro-methanolic extracts of *Ziziphus mauritiana* and *Ziziphus spina christi* containing high proportion of saponins resulted in a significant elevation in the plasma total protein levels as compared with their normal control rats.

In this present study, significant ($p<0.05$) decrease in SOD, CAT and GST activities were observed in the untreated diabetic rats. The administration of saponins from *T.tetraptera* improved the STZ-induced reduction in activities of SOD, CAT, and GST in almost all the

tissues under investigation. This study is similar to previous reports that showed that the administration of STZ could decrease GST, superoxide dismutase and catalase activities in untreated diabetic rats when compared to normal control rats (Adeyemi *et al*., 2014; Jiang *et al.,* 2020; Konda *et al*., 2020). The findings from this study also support the claims that saponin fractions from medicinal plants could reduce oxidative stress by increasing the level of catalase, SOD and GST in the tissues of diabetic treated rats (Elekofehinti *et al*., 2013; Gao *et al*., 2016).

CONCLUSION

This study has shown that total saponins from *T. tetraptera* stem bark could scavenge free radicals which is very important in the management of diabetes mellitus as it was able to significantly increase the activities of antioxidant enzymes namely: superoxide dismutase, catalase, and glutathione-s-transferase. The lower dose (10mg/kg body weight) was shown to be more effective in increasing the activities of these endogenous enzymatic antioxidants.

FINDINGS

The findings from this study are as follows:

1. We found out that *T. tetraptera* saponins (TTS) significantly increased the concentration of total protein in streptozotocin induced diabetic Wistar rats especially in the 10 mg/kg treated group.

2. We found out that *T. tetraptera* saponins (TTS) significantly ($p<0.05$) increased SOD, catalase, and GST activities in streptozotocin induced diabetic Wistar rats.

CONTRIBUTION TO KNOWLEDGE

The study has contributed to knowledge in the following ways:

1. By confirming that *T. tetraptera* saponins (TTS) has antidiabetic potentials especially at lesser dose of 10 mg/kg body weight.
2. By showing that *T. tetraptera* saponins has antioxidant capacity by increasing superoxide dismutase, catalase, and glutathione-S-transferase activities, and also increasing the concentration of total protein of streptozotocin induced diabetic Wistar rats.

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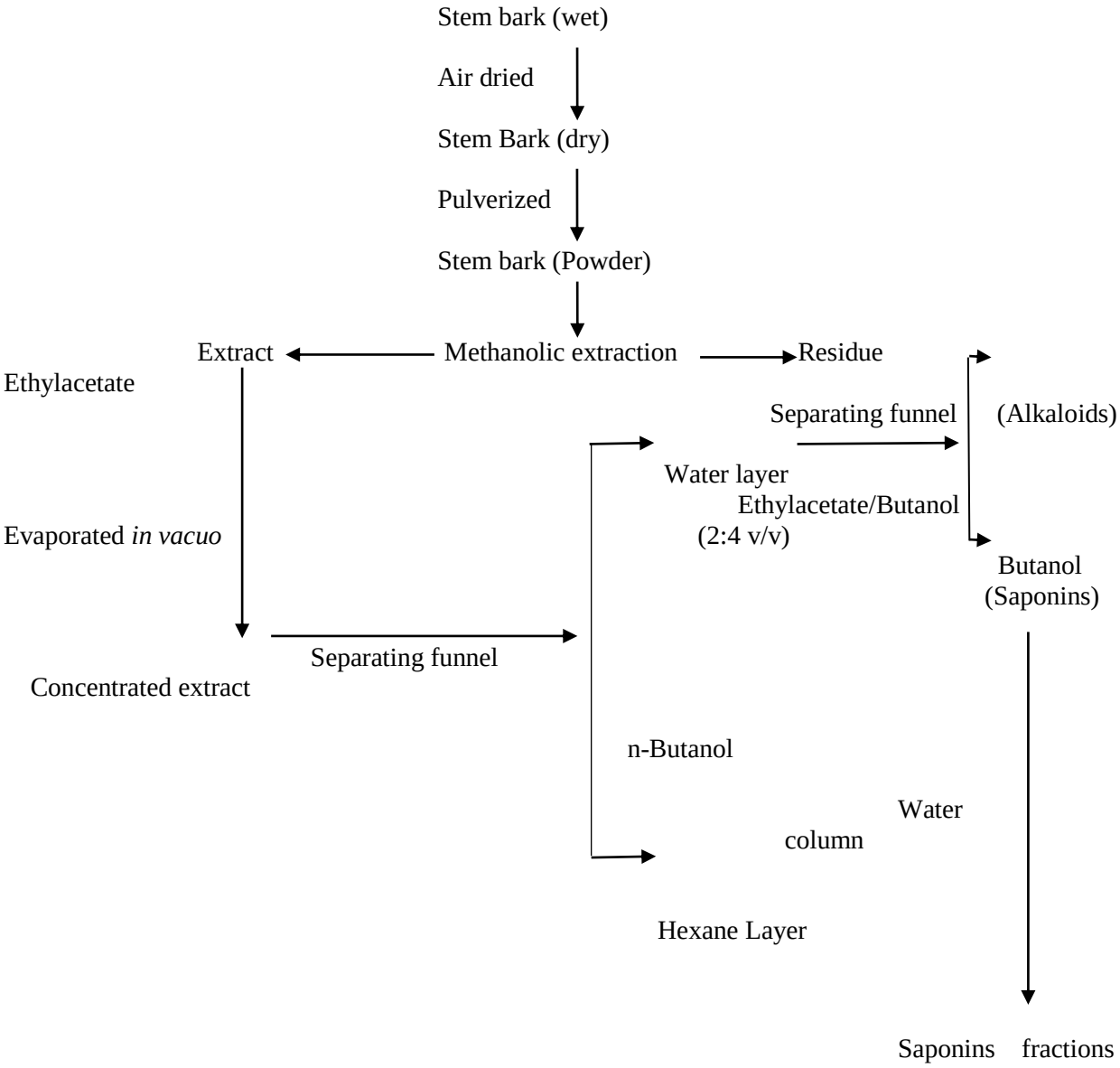
APPENDIX I

Schematics for the extraction of *T. tetraptera* total saponins (TTS) fractions



Statistical analysis

The data were expressed as the S.E.M. of 4 to 7 determinations. The one-way analysis of variance (ANOVA) was used to examine the differences between groups. Duncan's post hoc test was used to assess inter-group comparisons. A significance level of $P < 0.05$ was accepted. IBM SPSS Statistics, version 26 (IBM Corp., Armonk, N.Y., USA) was used for the analysis.

APPENDIX II

ANTI-DIABETIC STUDIES

Serum and tissue superoxide dismutase (SOD) assay using the Misra and Fridovich (1972) method.

Reagent	Contents	Initial Concentration of Solutions
Substrate	Carbonate Buffer Adrenalin	0.05 mol/l, pH 10.2 0.3 mmol/l

Assay Protocol

	Blank	Sample
Distilled H_2O (ml)	0.2	---
Sample (ml)	---	0.2
Carbonate buffer (ml)	2.5	2.5
Adrenalin (ml)	0.3	0.3

Mix, read absorbance immediately after adding adrenalin at 0 seconds, then at 30, 60, 90, 120 and 150 seconds, for both blank and sample at 480 nm.

For tissue SOD, dilute homogenate 1 + 9 with distilled water.

Calculation

Increase in absorbance /min = A_3-A_0/2.5

Where A_0 =Absorbance after 30secs

A_3 = Absorbance after 150secs

$$\% \text{ Inhibition} = \frac{\text{Increase in absorbance of substrate} \times 100}{\text{Increase in absorbance of blank}}$$

1 unit of SOD activity is given as the amount of SOD to cause 50% inhibition of adrenaline to adrenochrome during 1min.

e.g. if A_0 =0.043

A_3 = 0.046

Increase in absorbance in blank i.e A_{3blank}-A_{0blank} = 0.056

Then

Increase in absorbance / min = 0.046-0.043/2.5= 0.003/2.5= 0.0012

% inhibition = 0.0012 × 100/0.0056 =214.2857143

50% Inhibitiom= 214.2857143 ×50/100=10.714 unil/ml

Serum and tissue catalase assay using the Sinha (1972) method.

Reagent	Contents	Initial Concentration of Solutions
Substrate	Phosphate Buffer H_2O_2 $K_2Cr_2O_7$ Acetic acid	0.1 mol/l, pH 7.0 0.2 mol/l 5 g/100ml

Dichromate/acetic acid solution: Mix 5% $K_2Cr_2O_7$ solution with acetic acid in a ratio 1:3 v/v.

Assay Protocol

Serum Dilute 1 + 9 with distilled water

Tissue homogenate dilute 1 + 49 with distilled water

	Blank	Sample
0.2M H_2O_2	4.0	4.0
Phosphate buffer	5.0	5.0
Sample (Diluted)	---	1.0

Start timing, transfer quickly 1 ml each into 4 test tubes and add 2.0 ml of dichromate/acetic solution into first test tube after 60 seconds, the 2nd after 120 seconds, 3rd after 180 seconds, and 4th after 240 seconds. Read the absorbance of sample against blank at 570 nm.

Calculation

The catalase enzyme activity was extrapolated from a standard H_2O_2 curve using the following procedure:

Catalase activity = µmole of H_2O_2 decomposed /min/mg protein.

Find the average of the changes in H_2O_2 decomposed per minute.

Divide by the amount of protein (mg protein).

GLUTATHIONE S-TRANSFERASE ACTIVITY

The cytosolic glutathione S-transferase activity will be determined spectrophotometrically at 37^0c (340nm) by the procedure described by Habig *et al*, (1974)

PROCEDURE

The total reaction mixture will be 3ml containing 0.03ml of 0.1MGHS, 0.15ml of 20mM CDNB (1-chloro-2, 4-dinitrobenzene) and 2.79ml of 0.1M phosphate buffer in the sample test tube. The blank will contain 2.82ml phosphate buffer. The reaction mixture will be pre-incubated at 37^0c for 15min and the reaction mixture will be started by the addition of 0.03ml diluted cytosol and the absorbance will be read at 30 sec, 1min, 2min and 3min interval. The reaction mixture without the enzyme (supernatant) will be used as blank.

CALCULATION

$$\text{GST activity} = \frac{\text{O.D/min} \times \text{total reaction mixture volume}}{9.6 \quad \text{mg/ 0.03ml protein}}$$

REAGENT PREPARATION

4. 1-Chloro-2,4-dinitrobenzene (CDNB) (20mM)

   3.37mg of CONB will be dissolved in 1m of ethanol

5. Reduced Glutathione (0.1M)

   30.37mg of glutathione (GSH) will be dissolved in 1ml of 0.1M

6. 0.1M phosphate buffer (PH 6.5)

   4.96g of K_2HPO_4 and 9.73g of KH_2PO_4 will be dissolved in 100ml distilled water and the PH will be adjusted to 6.5

CHEMICALS

1. CDNB
2. GSH
3. Ethanol
4. K_2HPO_4
5. KH_2PO_4

Effect of *Tetrapluera tetraptera* saponins (TTS) on serum and tissue total protein concentration of streptozotocin (STZ) -induced diabetic rats for group 1 and 2

T-Test

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Serum Protein	Normal Control	7	5.4286	3.82349	1.44514
	Diabetic Control	5	4.7000	1.30384	.58310
Serum Albumin	Normal Control	7	.2529	.22179	.08383
	Diabetic Control	6	.1183	.08280	.03380
Heart Protein	Normal Control	7	5.8571	1.99654	.75462
	Diabetic Control	6	5.8833	2.23644	.91302
Kidney Protein	Normal Control	7	14.0857	4.97743	1.88129
	Diabetic Control	6	10.1667	1.15701	.47235
Pancreas Protein	Normal Control	7	14.6143	3.05856	1.15603
	Diabetic Control	6	8.6000	3.26619	1.33342
Testis Protein	Normal Control	6	10.9333	3.68709	1.50525
	Diabetic Control	6	5.4333	2.19241	.89505

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Serum Protein	Equal variances assumed	.830	.384	.405	10	.694	.72857	1.80014	-3.28238	4.73952
	Equal variances not assumed			.468	7.802	.653	.72857	1.55834	-2.88088	4.33802
Serum Albumin	Equal variances assumed	.915	.359	1.397	11	.190	.13452	.09628	-.07738	.34643
	Equal variances not assumed			1.488	7.861	.176	.13452	.09039	-.07456	.34360
Heart Protein	Equal variances assumed	.067	.801	-.022	11	.983	-.02619	1.17332	-2.60866	2.55628
	Equal variances not assumed			-.022	10.198	.983	-.02619	1.18451	-2.65850	2.60612
Kidney Protein	Equal variances assumed	4.557	.056	1.875	11	.088	3.91905	2.09072	-.68259	8.52068
	Equal variances not assumed			2.020	6.748	.085	3.91905	1.93968	-.70250	8.54060
Pancreas Protein	Equal variances assumed	.002	.963	3.427	11	.006	6.01429	1.75507	2.15139	9.87718
	Equal variances not assumed			3.408	10.430	.006	6.01429	1.76476	2.10403	9.92454
Testis Protein	Equal variances assumed	2.647	.135	3.141	10	.010	5.50000	1.75125	1.59796	9.40204
	Equal variances not assumed			3.141	8.143	.013	5.50000	1.75125	1.47390	9.52610

Effect of *Tetrapluera tetraptera* saponins (TTS) on serum and tissue total protein concentration of streptozotocin (STZ) -induced diabetic rats for group 3 and 4

T-Test

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Serum Protein	Metformin Treated Group	5	3.1000	1.81659	.81240
	10mg TTS Treated Group	3	5.6000	1.38564	.80000
Serum Albumin	Metformin Treated Group	6	.0833	.08430	.03442
	10mg TTS Treated Group	4	1.1675	.96510	.48255
Heart Protein	Metformin Treated Group	6	8.8333	4.73695	1.93385
	10mg TTS Treated Group	4	9.3500	3.38378	1.69189
Kidney Protein	Metformin Treated Group	6	7.0500	1.41386	.57721
	10mg TTS Treated Group	4	11.8750	2.83005	1.41502
Pancreas Protein	Metformin Treated Group	5	8.7600	2.78711	1.24643
	10mg TTS Treated Group	4	9.1250	4.78566	2.39283
Testis Protein	Metformin Treated Group	6	9.0667	3.98932	1.62863
	10mg TTS Treated Group	3	10.1333	1.20554	.69602

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Serum Protein	Equal variances assumed	1.114	.332	-2.031	6	.089	-2.50000	1.23072	-5.51146	.51146
	Equal variances not assumed			-2.193	5.387	.076	-2.50000	1.14018	-5.36866	.36866
Serum Albumin	Equal variances assumed	34.785	.000	-2.824	8	.022	-1.08417	.38391	-1.96946	-.19887
	Equal variances not assumed			-2.241	3.031	.110	-1.08417	.48378	-2.61502	.44669
Heart Protein	Equal variances assumed	.135	.723	-.187	8	.856	-.51667	2.76269	-6.88745	5.85411
	Equal variances not assumed			-.201	7.885	.846	-.51667	2.56949	-6.45705	5.42371
Kidney Protein	Equal variances assumed	3.486	.099	-3.625	8	.007	-4.82500	1.33117	-7.89468	-1.75532
	Equal variances not assumed			-3.157	4.015	.034	-4.82500	1.52822	-9.06189	-.58811
Pancreas Protein	Equal variances assumed	3.238	.115	-.144	7	.889	-.36500	2.53267	-6.35380	5.62380
	Equal variances not assumed			-.135	4.595	.898	-.36500	2.69800	-7.48830	6.75830
Testis Protein	Equal variances assumed	2.600	.151	-.439	7	.674	-1.06667	2.42723	-6.80615	4.67281
	Equal variances not assumed			-.602	6.455	.568	-1.06667	1.77113	-5.32749	3.19416

Effect of *Tetrapluera tetraptera* saponins (TTS) on serum and tissue total protein concentration of streptozotocin (STZ) -induced diabetic rats for group 5 and 6

T-Test

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Serum Protein	20mg TTS Treated Group	3	5.3833	.84311	.48677
	40mg TTS Treated Group	4	3.7500	2.50000	1.25000
Serum Albumin	20mg TTS Treated Group	4	.3175	.50921	.25460
	40mg TTS Treated Group	4	.1425	.09500	.04750
Heart Protein	20mg TTS Treated Group	4	6.0750	4.97418	2.48709
	40mg TTS Treated Group	4	9.2000	1.53406	.76703
Kidney Protein	20mg TTS Treated Group	4	10.2500	1.92959	.96480
	40mg TTS Treated Group	4	8.7000	.92014	.46007
Pancreas Protein	20mg TTS Treated Group	4	8.7000	2.97658	1.48829
	40mg TTS Treated Group	4	9.9750	2.41713	1.20856
Testis Protein	20mg TTS Treated Group	4	10.1750	1.46600	.73300
	40mg TTS Treated Group	4	8.1250	1.89978	.94989

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Serum Protein	Equal variances assumed	2.669	.163	1.065	5	.336	1.63333	1.53407	-2.31011	5.57678
	Equal variances not assumed			1.218	3.846	.293	1.63333	1.34143	-2.15057	5.41723
Serum Albumin	Equal variances assumed	5.112	.064	.676	6	.524	.17500	.25900	-.45874	.80874
	Equal variances not assumed			.676	3.209	.545	.17500	.25900	-.61974	.96974
Heart Protein	Equal variances assumed	4.863	.070	-1.201	6	.275	-3.12500	2.60268	-9.49354	3.24354
	Equal variances not assumed			-1.201	3.566	.304	-3.12500	2.60268	-10.71205	4.46205
Kidney Protein	Equal variances assumed	1.615	.251	1.450	6	.197	1.55000	1.06888	-1.06545	4.16545
	Equal variances not assumed			1.450	4.297	.216	1.55000	1.06888	-1.33844	4.43844
Pancreas Protein	Equal variances assumed	.044	.841	-.665	6	.531	-1.27500	1.91719	-5.96620	3.41620
	Equal variances not assumed			-.665	5.757	.532	-1.27500	1.91719	-6.01452	3.46452
Testis Protein	Equal variances assumed	.211	.662	1.709	6	.138	2.05000	1.19983	-.88587	4.98587
	Equal variances not assumed			1.709	5.638	.142	2.05000	1.19983	-.93223	5.03223

Effect of *Tetrapluera tetraptera* saponins (TTS) on serum and tissue total protein concentration of streptozotocin (STZ) -induced diabetic rats

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Serum Protein	Between Groups	23.816	5	4.763	.759	.589
	Within Groups	131.726	21	6.273		
	Total	155.542	26			
Serum Albumin	Between Groups	3.605	5	.721	4.547	.004
	Within Groups	3.964	25	.159		
	Total	7.569	30			
Heart Protein	Between Groups	77.901	5	15.580	1.407	.256
	Within Groups	276.756	25	11.070		
	Total	354.657	30			
Kidney Protein	Between Groups	182.853	5	36.571	4.502	.005
	Within Groups	203.074	25	8.123		
	Total	385.927	30			
Pancreas Protein	Between Groups	175.674	5	35.135	3.328	.020
	Within Groups	253.356	24	10.556		
	Total	429.030	29			
Testis Protein	Between Groups	110.424	5	22.085	2.649	.049
	Within Groups	191.762	23	8.337		
	Total	302.186	28			

Post Hoc Tests
Homogeneous Subsets

Serum Protein

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
Metformin Treated Group	5	3.1000
40mg TTS Treated Group	4	3.7500
Diabetic Control	5	4.7000
20mg TTS Treated Group	3	5.3833
Normal Control	7	5.4286
10mg TTS Treated Group	3	5.6000
Sig.		.219

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.111.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Serum Albumin

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
Metformin Treated Group	6	.0833	
Diabetic Control	6	.1183	
40mg TTS Treated Group	4	.1425	
Normal Control	7	.2529	
20mg TTS Treated Group	4	.3175	
10mg TTS Treated Group	4		1.1675
Sig.		.420	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.893.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Heart Protein

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
Normal Control	7	5.8571
Diabetic Control	6	5.8833
20mg TTS Treated Group	4	6.0750
Metformin Treated Group	6	8.8333
40mg TTS Treated Group	4	9.2000
10mg TTS Treated Group	4	9.3500
Sig.		.159

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.893.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Kidney Protein

Duncan[a,b]

Group	N	Subset for alpha = 0.05		
		1	2	3
Metformin Treated Group	6	7.0500		
40mg TTS Treated Group	4	8.7000	8.7000	
Diabetic Control	6	10.1667	10.1667	10.1667
20mg TTS Treated Group	4	10.2500	10.2500	10.2500
10mg TTS Treated Group	4		11.8750	11.8750
Normal Control	7			14.0857
Sig.		.119	.122	.058

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.893.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Pancreas Protein

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
Diabetic Control	6	8.6000	
20mg TTS Treated Group	4	8.7000	
Metformin Treated Group	5	8.7600	
10mg TTS Treated Group	4	9.1250	
40mg TTS Treated Group	4	9.9750	
Normal Control	7		14.6143
Sig.		.566	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.764.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Testis Protein

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
Diabetic Control	6	5.4333	
40mg TTS Treated Group	4	8.1250	8.1250
Metformin Treated Group	6	9.0667	9.0667
10mg TTS Treated Group	3		10.1333
20mg TTS Treated Group	4		10.1750
Normal Control	6		10.9333
Sig.		.086	.204

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.500.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Effect of *Tetrapluera tetraptera* saponins (TTS) on serum and tissue superoxide dismutase (SOD), catalase, and Glutathione-s-transferase activity of streptozotocin (STZ) -induced diabetic rats

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Serum Superoxide Dismutase	Between Groups	2196169.750	5	439233.950	32.174	.000
	Within Groups	232082.936	17	13651.937		
	Total	2428252.685	22			
Liver Superoxide Dismutase	Between Groups	104524.168	5	20904.834	2.063	.107
	Within Groups	233053.933	23	10132.780		
	Total	337578.101	28			
Heart Superoxide Dismutase	Between Groups	38083.033	5	7616.607	6.786	.000
	Within Groups	26939.061	24	1122.461		
	Total	65022.094	29			
Kidney Superoxide Dismutase	Between Groups	8153.628	5	1630.726	2.754	.042
	Within Groups	14213.412	24	592.226		
	Total	22367.041	29			
PancreasSuperoxide Dismutase	Between Groups	133221.896	5	26644.379	4.079	.009
	Within Groups	143714.327	22	6532.469		
	Total	276936.223	27			
Testis Superoxide Dismutase	Between Groups	1300.785	5	260.157	1.825	.149
	Within Groups	3136.310	22	142.560		

	Total	4437.095	27			
Serum Catalase	Between Groups	221.255	5	44.251	2.194	.103
	Within Groups	342.899	17	20.171		
	Total	564.154	22			
Liver Catalase	Between Groups	2.260	5	.452	1.784	.156
	Within Groups	5.827	23	.253		
	Total	8.087	28			
Heart Catalase	Between Groups	45.559	5	9.112	1.163	.356
	Within Groups	188.050	24	7.835		
	Total	233.609	29			
Kidney Catalase	Between Groups	17.197	5	3.439	4.828	.003
	Within Groups	17.097	24	.712		
	Total	34.294	29			
Pancreas Catalase	Between Groups	7.196	5	1.439	1.366	.272
	Within Groups	25.284	24	1.054		
	Total	32.480	29			
Testis Catalase	Between Groups	2.340	5	.468	.926	.482
	Within Groups	11.618	23	.505		
	Total	13.958	28			
Serum Glutathione-S-Transferase	Between Groups	1218.158	5	243.632	4.080	.010
	Within Groups	1194.208	20	59.710		
	Total	2412.366	25			
Liver Glutathione-S-Transferase	Between Groups	.199	5	.040	.983	.448
	Within Groups	.971	24	.040		
	Total	1.170	29			
Heart Glutathione-S-Transferase	Between Groups	1.402	5	.280	.783	.572
	Within Groups	8.234	23	.358		
	Total	9.636	28			
Kidney Glutathione-S-Transferase	Between Groups	209.728	5	41.946	2.200	.086
	Within Groups	476.677	25	19.067		
	Total	686.406	30			
Pancreas Glutathione-S-Transferase	Between Groups	.488	5	.098	.685	.639
	Within Groups	3.563	25	.143		
	Total	4.051	30			
Testis Glutathione-S-Transferase	Between Groups	1.388	5	.278	1.963	.123
	Within Groups	3.252	23	.141		
	Total	4.640	28			

Post Hoc Tests
Homogeneous Subsets

Serum Superoxide Dismutase

Duncan[a,b]

Group	N	Subset for alpha = 0.05		
		1	2	3
Diabetic Control	5	24.615385		
Normal Control	4	40.769231		
20mg TTS Treated Diabetic Rats	4	53.134615		
Metformin Treated Diabetic Rats	4	90.384615	90.384615	
40mg TTS Treated Diabetic Rats	3		251.282051	
10mg TTS Treated Diabetic Rats	3			974.358974
Sig.		.491	.078	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.711.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Liver Superoxide Dismutase

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
40mg TTS Treated Diabetic Rats	4	35.769231	
Normal Control	6	39.743590	
20mg TTS Treated Diabetic Rats	4	46.538462	
Diabetic Control	5	49.846154	
Metformin Treated Diabetic Rats	6	66.410256	
10mg TTS Treated Diabetic Rats	4		220.153846
Sig.		.682	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.675.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Heart Superoxide Dismutase

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
Normal Control	6	26.410256	
40mg TTS Treated Diabetic Rats	4	29.615385	
Metformin Treated Diabetic Rats	6		85.384615
Diabetic Control	6		87.692308
10mg TTS Treated Diabetic Rats	4		113.615385
20mg TTS Treated Diabetic Rats	4		120.000000
Sig.		.883	.155

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.800.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Kidney Superoxide Dismutase

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
10mg TTS Treated Diabetic Rats	4	22.692308	
Metformin Treated Diabetic Rats	6	36.923077	36.923077
20mg TTS Treated Diabetic Rats	4	40.000000	40.000000
40mg TTS Treated Diabetic Rats	4	48.076923	48.076923
Diabetic Control	6		64.871795
Normal Control	6		70.102564
Sig.		.151	.069

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.800.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

PancreasSuperoxide Dismutase

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
Normal Control	5	87.076923	
40mg TTS Treated Diabetic Rats	4	88.461538	
Diabetic Control	6	160.256410	
10mg TTS Treated Diabetic Rats	4	178.692308	
Metformin Treated Diabetic Rats	5	207.692308	207.692308
20mg TTS Treated Diabetic Rats	4		296.923077
Sig.		.054	.110

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.557.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Testis Superoxide Dismutase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
Diabetic Control	6	6.600000
20mg TTS Treated Diabetic Rats	4	10.973077
10mg TTS Treated Diabetic Rats	3	13.846154
40mg TTS Treated Diabetic Rats	4	19.230769
Metformin Treated Diabetic Rats	6	22.820513
Normal Control	5	24.246154
Sig.		.064

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.390.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Serum Catalase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
Diabetic Control	5	1.688889
40mg TTS Treated Diabetic Rats	3	2.444444
Normal Control	4	2.666667
20mg TTS Treated Diabetic Rats	3	3.000000
10mg TTS Treated Diabetic Rats	3	7.777778
Metformin Treated Diabetic Rats	5	9.333333
Sig.		.056

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.636.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Liver Catalase

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
40mg TTS Treated Diabetic Rats	4	.175748	
Diabetic Control	5	.342285	.342285
10mg TTS Treated Diabetic Rats	4	.345344	.345344
20mg TTS Treated Diabetic Rats	4	.385833	.385833
Metformin Treated Diabetic Rats	6	.509694	.509694
Normal Control	6		1.010999
Sig.		.374	.080

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.675.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Heart Catalase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
40mg TTS Treated Diabetic Rats	4	.597876
10mg TTS Treated Diabetic Rats	4	1.248754
Metformin Treated Diabetic Rats	5	1.460517
Diabetic Control	6	1.560962
20mg TTS Treated Diabetic Rats	4	3.664021
Normal Control	7	3.800449
Sig.		.131

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.764.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Kidney Catalase

Duncan[a,b]

Group	N	Subset for alpha = 0.05		
		1	2	3
Diabetic Control	6	.598685		
Normal Control	6	.696633		
20mg TTS Treated Diabetic Rats	4	1.235479	1.235479	
40mg TTS Treated Diabetic Rats	4		1.942412	1.942412
Metformin Treated Diabetic Rats	6		2.076684	2.076684
10mg TTS Treated Diabetic Rats	4			2.667930
Sig.		.281	.157	.220

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.800.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Pancreas Catalase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
Metformin Treated Diabetic Rats	5	.703109
40mg TTS Treated Diabetic Rats	4	.978768
Normal Control	7	1.011728
20mg TTS Treated Diabetic Rats	4	1.673811
10mg TTS Treated Diabetic Rats	4	1.833333
Diabetic Control	6	1.982206
Sig.		.101

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.764.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Testis Catalase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
Metformin Treated Diabetic Rats	6	.735547
10mg TTS Treated Diabetic Rats	3	.805068
20mg TTS Treated Diabetic Rats	4	.813021
Normal Control	6	.868298
40mg TTS Treated Diabetic Rats	4	1.131088
Diabetic Control	6	1.499158
Sig.		.167

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.500.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Serum Glutathione-S-Transferase

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
Diabetic Control	6	1.488555	
40mg TTS Treated Diabetic Rats	3	3.240741	
20mg TTS Treated Diabetic Rats	3	4.166667	
10mg TTS Treated Diabetic Rats	3	4.629630	
Normal Control	6	7.444801	
Metformin Treated Diabetic Rats	5		20.833333
Sig.		.345	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.913.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Liver Glutathione-S-Transferase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
20mg TTS Treated Diabetic Rats	4	.136142
10mg TTS Treated Diabetic Rats	4	.142295
Metformin Treated Diabetic Rats	6	.155471
40mg TTS Treated Diabetic Rats	4	.163094
Diabetic Control	5	.190570
Normal Control	7	.347212
Sig.		.165

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.764.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Heart Glutathione-S-Transferase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
10mg TTS Treated Diabetic Rats	4	.281376
Metformin Treated Diabetic Rats	6	.357980
20mg TTS Treated Diabetic Rats	4	.488453
Diabetic Control	5	.629124
40mg TTS Treated Diabetic Rats	4	.698660
Normal Control	6	.908925
Sig.		.169

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.675.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Kidney Glutathione-S-Transferase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
Diabetic Control	6	.119455
Metformin Treated Diabetic Rats	6	.395412
40mg TTS Treated Diabetic Rats	4	.584057
20mg TTS Treated Diabetic Rats	4	.681160
10mg TTS Treated Diabetic Rats	4	4.966144
Normal Control	7	6.241477
Sig.		.063

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.893.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Pancreas Glutathione-S-Transferase

Duncan[a,b]

Group	N	Subset for alpha = 0.05
		1
Normal Control	7	.228456
Metformin Treated Diabetic Rats	6	.261240
20mg TTS Treated Diabetic Rats	4	.292517
Diabetic Control	6	.323157
10mg TTS Treated Diabetic Rats	4	.437395
40mg TTS Treated Diabetic Rats	4	.620693
Sig.		.164

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.893.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Testis Glutathione-S-Transferase

Duncan[a,b]

Group	N	Subset for alpha = 0.05	
		1	2
40mg TTS Treated Diabetic Rats	4	.318968	
Metformin Treated Diabetic Rats	6	.415595	.415595
20mg TTS Treated Diabetic Rats	4	.427210	.427210
Normal Control	6	.467745	.467745
10mg TTS Treated Diabetic Rats	3	.768559	.768559
Diabetic Control	6		.920227
Sig.		.120	.083

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.500.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.