AMELIORATIVE EFFECT OF SELENIUM YEAST ON SOME PHYSIOLOGICAL PARAMETERS IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

BY

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DECLARATION

I declare that the work in the dissertation entitled: "AMELIORATIVE EFFECT OF SELENIUM YEAST ON SOME PHYSIOLOGICAL PARAMETERS IN STREPTOZOTOCIN - INDUCED DIABETIC WISTAR RATS" has been performed by me in the Department of Human Physiology, under the supervision of Dr. Aliyu Mohammed, Dr. Yusuf Tanko and Prof. K.Y. Musa.

All information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

_____ _____

Kabir Ahmed MUHAMMED Date

CERTIFICATION

This dissertation entitled "Ameliorative effect of selenium yeast on some physiological parameters instreptozotocin-induced diabetic Wistar rats" by Muhammed Kabir Ahmed, meets the regulations governing the award of degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty Allah, Who has made it possible for me to attain this level of my life and showing me His mercy throughout the course of this programme and to my lovely parents, my wife (Zainab Adamu Kaura) and my lovely children (Ibrahim and Aisha). You will always mean so much to me.

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ABSTRACT

Oxidative stress and lipid peroxidation are central factors in the metabolic dysfunctions and pathologies associated with diabetes. The results from studies on the benefits of Selenium a trace element with antioxidant, anti-lipidemic and anti-inflammatory properties, in diabetes mellitus have been controversial without prospective outcome and Se appears to be a double-edged sword in the pathologies of diabetes mellitus. It was suggested that selenium could cause glucose disturbance and increase the risk for diabetes mellitus. The present study intends to determine the ameliorative effects of selenium yeast on blood glucose level, oxidative stress and lipid peroxidation biomarkers, and abnormal lipid profile, serum levels of liver enzymes, electrolytes, triiodothyronine and tetraiodothyronine levels in streptozotocin induced diabetes in Wister rats. Thirty five (35) adult male Wistar rats weighing (180 – 200) grams randomly divided into six treatment and one control groups of five rats each (n = 5). Hyperglycemia was induced in all groups except Group IV by single intraperitoneal injection of 60mg/kg of streptozotocin dissolved in 0.1ml fresh cold citrate buffer pH 4.5 into 16 h-fasted rats. In addition, Groups I and II received 0.1 and 0.2 mg/kg/day for 4weeks of selenium yeast respectively, Group III received 1mg/kg/day for 4weeks of glibenclamide, Groups IV and V served as the normal and diabetic control groups respectively and received only 0.9% of normal saline. Groups VI and VII received 300 and 120 mg/kg/day for 4weeks of aspirin and ibuprofen respectively, all treatments were administered via oral route. Blood samples were collected from the tail vein on weekly basis for the period of 4weeks and used for determination of blood plasma glucose levels, and at the end of the fourth week rats were euthanized and blood samples were drawn from the heart by cardiac puncture and used to estimate oxidative stress biomakers (i.e. superoxide dismutase, catalase and gluthation peroxidase) and lipid peroxidation biomarkers (i.e. malondealdehyde),lipid profile, serum levels of liver enzymes, electrolytes, triiodothyronine and tetraiodothyronine levels. Analysis of variance and Turkey's post-hoc test were used to analyze the data obtained. The results showed that there was significant ($P < 0.05$) decrease in blood glucose level at week one and week three with the dose of 0.2 mg/kg of selenium yeast administered, while with the dose of 0.1mg/kg of selenium yeast, there was no significant difference in blood plasma glucose level when compared with the diabetic control group. It was also revealed that the serum liver enzymes aspertate amino transferase and alanine amino transferase were significantly higher ($P < 0.05$) in the groups treated with 0.1 and 0.2 mg/kg of selenium yeast. Also, of the oxidative stress biomarkers assessed, there was significant reduction ($P < 0.05$) in only the malondealdehyde level of the group treated with 0.2 mg/kg of selenium yeast when compared with the diabetic control group. For the lipid profile assessment, the effect of selenium yeast was only seen in the level of triglyceride in the group treated with 0.2 mg/kg of selenium yeast which was significantly lower ($P < 0.05$) when compared to the diabetic control. Sodium and chloride ion levels of the serum electrolytes were significantly lowered ($P < 0.05$) in the group treated with 0.2 mg/kg of selenium yeast when compared to diabetic control group. Serum triiodothyronine and tetraiodothyronine levels did not show any significant difference across all the treated groups when compared to the diabetic and normal control groups. Tissue necrosis factor alpha level in the serum showed a decrease in the groups treated with 0.1 and 0.2 mg/kg of selenium yeast but not statistically significant ($P > 0.05$) when compared with the normal and diabetic control groups. Therefore, selenium yeast possesses hypoglycaemic property that is

comparable to the oral-hypoglycaemic drug glibenclamide. In addition, the effect of the 0.2 mg/kg of selenium yeast on the oxidative stress biomarkers assessed did not provide sufficient evidence to conclude that the selenium yeast used in the study elicited an antioxidant effect. The marked decline in serum triglyceride concentration in the 0.2 mg/kg of selenium yeast treated group was indicative of direct effect of the antioxidant capacity of selenium on oxidation of lipids and lipoproteins.

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LIST OF ABBREVIATIONS

ADP	–	Adenosin diphosphate
ALP	–	Alkaline Phosphatase
ALT	–	Alanine Amino Transferase
ANOVA	–	Analysis of variance
AST	–	Aspertate Amino Transferase
ATP	–	Adenosin triphosphate
BMI	–	Body mass index
CHD	–	Coronary heart disease
Cl^-	–	Chloride
CVD	–	Cardiovascular disease
DFU	–	Diabetic Foot Ulcer
DM	–	Diabetes Mellitus
DNA	–	Deoxyribo nucleic acid
DR	–	Diabetic retinopathy
FFA	–	Free fatty acid
Fox O	–	For head box class O
GBA	–	Glibenclamide
GCK	–	Glucokinase
GDM	–	Gestational diabetes mellitus
GIP	–	Glucose-dependent insulinotropic polypeptide
GLP-1	–	Glucagon-Like peptide 1
GLUT	–	Glucose transporter
GPx	–	Gluthation Peroxidase
GRc	–	Gluthation Redox cycle
Hb	–	Hemoglobin
HNF	–	Hepatic nuclear factor
IAAP	–	Islet Associated Polypeptide
IDDM	–	Insulin dependent diabetes mellitus
IRS1	–	Insulin receptor substrate 1
IV	–	Intravenous

K^+	–	Potassium
LDL	–	Low Density Lipoprotain
LFT	–	Liver function tests
LYP	–	Lymphoid protein tyrosine phosphatase
MDA	–	Malondialdehyde
MODY	–	Maturity-onset diabetes of the young
Na^+	–	Sodium
NADPH	–	Nicotinamide adenine dinucleotide phosphate
OSTB	–	Oxidative stress biomarkers
PARP	–	Procyclic acidic repetitive protein
PNDM	–	Permanent neonatal diabetes mellitus
PTPN22	–	Protein tyrosine phosphatase nonreceptor type 22
RBP4	–	Retinol binding protein 4
ROS	–	Reactive oxygen species
Se	–	Selenium
Se –Y	–	Selenium Yeast
SEM	–	Standard error of mean
SOD	–	Superoxide Dismutase
SOGT	–	Serum Glutamic Oxaloacetic Transaminase
SREBP1c	–	Transcription factors terol regulatory element binding protein 1 c
STZ	–	Streptozotocin
SUVIMAX	–	Supplemental Vitamins Minerals and Antioxidant
T1R2	–	Taste receptor 2
T1R3	–	Taste receptor 3
T_3	–	Triidothyronine
T_4	–	Tetraiodothyronine
TCR	–	T-cell receptor
TNFα	–	Tumor necrosis factor alpha
VEGF	–	Vascular endothelial growth factor
WHO	–	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Diabetes is a common metabolic disorder characterized by hyperglycemia due to an absolute or re1lative insulin deficiency (Lawal *et al.,* 2008; WHO, 2010). It affects essential biochemical pathways of the body including carbohydrate, protein, and lipid metabolisms. The World Health Organization (WHO), estimated that there were 171 million people in the world with diabetes in the year 2008 and this is projected to increase by over a 100% to 366 million by 2030 (WHO, 2010). Diabetes is associated with reduced life expectancy, significant high mortality and diminished quality of life. In 2005 an estimated 1.1 million people died from diabetes and diabetes complications (WHO, 2008). Its prevalence is rising globally, including the rural Nigerian populations (Ime *et al.,* 2011).

Epidemiological reports has highlighted on the fact that low- and middle-income countries will bear the brunt of the increase and that Africa will contribute significantly to this rise. In Africa 40% of people with diabetes live in low and middle income countries causing 5% of the deaths globally each year. This is likely to increase by more than 50% in the next 10 years, if urgent action is not taken (WHO, 2007). The challenges and thus, the solutions in the provision of healthcare that would improve outcome for diabetes in low and middle income countries are many and can be found at multiple levels. Patient-related factors are of extreme importance, these ranges from low levels of self-management practices, lack of adherence to lifestyle changes and medication and lack of faith in the conventional management procedures. Many African populations still regard alternative healing systems as the primary source of healthcare or alternatively, consult both traditional or folk healers

that usually promote the concept that diabetes is curable and have been found to be also reluctant to refer clients to medical practitioners (Peltzer *et al.,* 2001; Otieno, 2006). This, however, is undermined by two key factors; the high cost of drugs and recommended foods as well as the psychosocial burden imposed by the daily oral hypoglycaemic drugs therapeutic routines. Moreover, spiritual causal theories of diabetes such as sorcery and witchcraft are still found in many African populations, particularly in rural communities (Levitt, 2008).

In the clinical setting, the initial management procedure is based on dietary therapy combined with increased physical activity. Pharmacologic therapy with oral hypoglycemic drugs or insulin injectable may be considered in the presence of marked hyperglycemia. Generally therapeutic targets should include establishing optimal, acceptable and non-acceptable values for indicators such as plasma glucose level (fasting and postprandial), glycated hemoglobin (Hb), total cholesterol and triacylglycerol levels, and blood pressure (WHO, 2008a). However, in modern medicine, there is no satisfactory effective therapy yet available to cure diabetes mellitus. Though insulin therapy is used for management of diabetes mellitus but there are several drawbacks like insulin resistance. (Piedrola *et al.,* 2001), anorexia nervosa, brain atrophy and fatty liver (Yaryura-Tobias *et al.,* 2001) along with requirement for refrigeration of the drug and skilled technician as well as of its high cost, which are not affordable in poor economic community. Chronic treatment with oral hypoglycemic drugs such as sulfonylureas and biguanids are also associated with side effects (Rang *et al.,* 1991). Although much research have been done, the pathophysiology of diabetes and its complications is still not well understood and the most appropriate methods to diagnose, treat, or prevent diabetes is still elusive.

However, it appears that *oxidative stress and lipid peroxidation*are central factors and major drivers in the etiology of diabetesand its complications (Sima, 2010) and that reactive oxygen species (ROS)and lipid peroxidation products, operates through multiple pathways to causemetabolic perturbations ofessential biochemical pathways of the body (Sima, 2010).In diabetic condition, elevated levels of blood glucose (hyperglycemia) and insulin (hyperinsulinemia) may provide a pro-oxidant environment (Vijayakumar *et al.,* 2012). Individuals with diabetes do not have sufficient antioxidant defenses (Santini *et al.,* 1997; Martin-Gallan *et al.,* 2003). Hyperglycaemia has been reported to impair their antioxidant defenses (Kashiwagi *et al.,* 1994; Asahina *et al.,* 1995). Under hyperglycaemic conditions, superoxide dismutase is glycated resulting in a decrease in its activity. In addition, the activity of the glutathione redox cycle (GR cycle) is decreased due to the impaired activation of the pentose phosphate pathway (Kashiwagi, 2001). Accordingly, it will be expected that exogenous antioxidant supplementation is desirable in patients with diabetes mellitus but surprisingly available exogenous antioxidants do not seem to confer any decisive benefit (Wiernsperger, 2003; Nathan, 2009; Singh *et al.,* 2009).

However, little is known about the beneficial effect of Selenium (Se), a trace element with both antioxidant and anti-inflammatory properties that offers several health benefits to animals and humans (Thomson, 2004 and Yiming *et al*., 2005; Al-Othman *et al*., 2011).Selenium is found in minute amounts in foods, with the richest sources being from meats, fish, whole grains, and dairy products. The selenium content of vegetables is dependent on the soil in which they are grown (Thomson, 2004; Yiming *et al.,* 2005).

In recent years, selenium (Se) research has attracted tremendous interest because of its important role in antioxidant seleno-enzymes, such as glutathione peroxidase (GPx) and

thioredoxinreductase, which protect against oxidative stress (Tinggi, 2008).The organic form of selenium provided by selenium yeast has been shown to differ in bioavailability and metabolism compared with inorganic (e.g., selenate, selenite) forms of dietary selenium (Schrauzer, 2000). Furthermore, selenium yeast has been used in a wide range of studies aimed at examining the importance of selenium status in the incidence and progression of a variety of infectious and degenerative diseases (Lovell *et al.*, 2009).

Selenium yeast has been reported to be the best bioavailable form being more effective than sodium selenite and selenate in increasing tissue Se retention (Thiry *et al*., 2013). It is employed in the folklore management of diseases associated with oxidative insults due to its antioxidant activities and its ability to inhibit lipid peroxidation (Liao *et al.,* 2012). Selenium yeast both in organic and inorganic products are protective against reactive oxygen species, but there is variability in the level of antioxidant capacity among the products. On the basis of studied data on its antioxidant activities and other beneficial effect, it could be suggested that the consumption of selenium yeast products could render a multi-faceted action that can terminate instant generation of free radicals and sets the human system free from accumulation of radicals (Nouf *et al.,* 2013).

1.2 Statement of Research Problem

Given that enhanced formation of oxidative stress and lipid peroxidation biomarkers are central factors in diabetic associated metabolic dysfunctions and pathologies (Vijayakumar *et al.,* 2012), anti-oxidant and anti-inflammation supplements could be potential therapeutic tools for the prevention and treatment of diabetic associated metabolic dysfunctions and pathologies however, studies on the ameliorative effects of various exogenous antioxidants on oxidative stress and its associated complications in diabetes have been controversial and

are without prospective outcomes (Low *et al.*, 1997; Obrosova *et al.*, 2000; Cadenas and Packer, 2002). Results from human studies on selenium and diabetes are also conflicting. Two studies found lower serum selenium concentrations in diabetic patients than in control subjects (Navarro-Alarcon *et al.*, 1999; Kljai and Runje, 2001), while in the Health Professionals Follow-up Study, toenail concentrations were lower in diabetic men than in non-diabetic controls (Rajpathak *et al.*, 2005). By contrast, higher serum selenium concentration was associated with a higher prevalence of diabetes (Bleys *et al.*, 2007; Laclaustra *et al.*, 2009), higher fasting plasma glucose and glycosylated hemoglobin levels (Laclaustra *et al.*, 2009) in the National Health and Nutrition Examination Survey (Bleys *et al.*, 2007; Laclaustra *et al.*, 2009). Randomized trials again showed discordant findings: in the SUVIMAX trial, despite positive correlations between plasma selenium and plasma glucose both at baseline and at the end of the follow-up, no effect of supplementation with a mixture of antioxidants, including 100 μg/day selenium, on plasma glucose levels was found after 7.5 years of follow-up, was found (Czernichow *et al.*, 2006). In a published analysis of Nutritional Prevention of Cancer Trial data showed that supplementation with 200 μg/d selenium as high-selenium yeast for 7.7 years increased the risk of self-reported type 2 diabetes (Stranges *et al.*, 2007). In consistent with these findings several other studies also reported that long-term Selenium supplementation does not seem to prevent type 2 diabetes, and that it may increase risk for the disease (Stranges *et al.*, 2007).

In contrast, there are studies that demonstrate selenium protection against diabetes. In these studies, it was reported that non-diabetic individuals had higher serum selenium concentrations compared to the diabetic individuals (Navarro-Alarcon *et al.*, 1999; Kornhauser *et al.*, 2008). The mean serum selenium level of diabetics was shown to be

significantly lower (64.9 ± 22.8 μg/L) than normal individuals (74.9 ± 27.3 μg/L) (Navarro-Alarcon *et al.,* 1999). In addition, Se has been shown to be beneficial in diabetic and inflammatory conditions, evidence from in vivo and in vitro studies suggests that selenium could enhance insulin sensitivity by mediating insulin-like actions (Stapleton, 2000; Mueller & Pallauf, 2006) and by acting via several other mechanisms, including detoxifying liver enzymes, exerting anti- inflammatory effects, and providing antioxidant defense to elicits its anti-diabetic and anti-inflammatory effects, however, the mechanisms by which Se exerts these beneficial effects are still not yet fully understood(Thomson, 2004; Yiming *et al.,* 2005; Hafez *et al.,* 2012). Putting the findings together, Selenium appears to be a double-edged sword in the pathologies of chronic diseases (Caroline *et al.,* 2013).

Given these controversies, the present study intends to determine the ameliorative effects of selenium yeast on oxidative stress and lipid peroxidation biomarkers and other metabolic perturbations in DM, including the abnormalities in plasma glucose level and lipid profiles. In addition, the beneficial effects of selenium yeast on DM associated hepatic damage will also be determined.

1.3 Justification of the Study

The body needs small amount of Se for healthy metabolism. Selenium is a co-factor for enzyme glutathione peroxidase. Glutathione peroxidase is a selenium-dependent enzyme. The substrate, for enzyme glutathione peroxidase is reduced glutathione, which is a specific H^+ donor for reduction of H_2O_2, lipid and non-lipid H_2O_2 and protect the membrane lipid and hemoglobin against oxidation by peroxides (Halliwell, 1994). In other words, Se modulates the cellular response and protects against oxidative stress and the production of reactive

oxygen species. Recommended daily intake of selenium in adult humans is 55 μg/day (IMFNB, 2000).

However, precise mechanistic evidence is lacking to explain the clinical and epidemiological data that selenium promotes type 2 diabetes. Thus far, in the literature, there are limited biochemical and laboratory data (Steinbrenner *et al.,* 2011; Yang *et al.,* 2011) that provide evidence of a mechanistic link between type 2 diabetes and selenium, with the most convincing data focusing on specific selenoproteins in glucose metabolism, but not on changes in dietary intake of selenium. One such study showed that type 2 diabetics had higher serum expression of selenoprotein P compared with normal subjects (Misu *et al.,* 2010). In addition, this study provided mechanistic evidence to show that selenoprotein P causes insulin resistance in hepatocytes by decreasing phosphorylation of AMPK, an important kinase in cellular homeostasis and hormone regulation. This suggests that selenium could alter the secretion profile of hepatocytes to one that favors the pro-inflammatory state associated with diabetes. However, there are animal (Iizuka *et al.,* 2010; Ghaffari *et al.,* 2012) and case control studies (Navarro-Alarcon *et al.,* 1999; Kljai and Runje, 2001; Rajpathak *et al.,* 2005) that suggest selenium may improve glucose metabolism. Furthermore, selenium has also been shown to have an insulin-like property (Stapleton, 2000), which qualifies it as a potential anti-diabetic agent.

Oxidative stress and low-grade chronic inflammation play a major role in the etiology, pathogenesis and complications of type 2 diabetes (Maritim *et al.,* 2003; Monnier *et al.,* 2006). Experimental data suggest that supplementation with antioxidants, such as selenium at the nutritional level, could delay the development of type 2 diabetes by decreasing oxidative stress (Steinbrenner and Sies, 2009). In diabetes, many diabetic complications are thought to

be caused by oxidative damage and decreased antioxidant protection. Studies have shown that selenium can protect against oxidative damage attributable to unregulated blood sugar (Naziroglu and Cay, 2001; Guney *et al.,* 2011). In one animal study, hamsters fed a low selenium diet had diabetes-induced weight loss, increased plasma triglyceride and cholesterol concentrations and rapid oxidation of low density lipoprotein, none of which were seen in hamsters fed a high selenium diet (Agbor *et al.,* 2007). Furthermore, selenium's antioxidant capacity has also been shown to help the pathology of type 2 diabetes in other ways, including preventing oxidation of low density lipoprotein (Gebre-Medhin *et al.,* 1988), inhibiting pancreatic calculi (Quilliot *et al.,* 2001), protecting against glomerular lesions (Douillet *et al.,* 1996), helping maintain normal fatty acid concentration (Douillet *et al.,* 1998), correcting expression of glucose and lactate metabolic proteins (Kim *et al.,* 2011), preventing the loss of myofibrils and the size of myocytes (Ayaz *et al.,* 2002), attenuating red blood cell damage (Sheng *et al.,* 2004), reducing malondialdehyde levels (Skripchenko *et al.,* 2003) and increasing the antioxidant and ultrastructure of the liver (Can *et al.,* 2005).

Given these protective capacities of selenium against the pathology of type 2 diabetes, it could be considered a potential therapeutic tool for the prevention and treatment of diabetic associated metabolic dysfunctions and pathologies. In addition, a single dose of streptozotocin (50 mg/kg body weight), a chemical that is toxic to pancreatic beta cells, is enough to cause significant changes in the expression of selenium-containing antioxidant enzymes in the liver of rats (Can *et al*., 2005).

1.4 Hypothesis

Selenium yeast does not treat or ameliorate metabolic complication associated with diabetes mellitus.

1.5 Aim of the Study

The aim of this study is to determine the ameliorative effect of selenium yeast on some physiological parameters in streptozotocin-induced diabetic Wistar rats

1.5.1 Objectives of the study

a. To determine the ameliorative effect of selenium yeast on blood glucose levels of STZ induced diabetic Wistar rats.
b. To determine the ameliorative effects of selenium yeast on serum liver enzymes (ALP, ALT, AST) levels of STZ induced diabetic Wistar rats.
c. To determine the ameliorative effects of selenium yeast on serum electrolytes levels (Na^+, Cl^-, K^+, urea, HCO_3^-) of STZ induced diabetic Wistar rats.
d. To determine the ameliorative effects of selenium yeast on antioxidant enzyme activity; catalase, superoxide dismutase, glutathione peroxidase.
e. To determine the ameliorative effects of selenium yeast on lipid profile, of serum total cholesterol, high-density lipoprotein, triglyceride, Low density lipoprotein.
f. To determine the ameliorative effects of selenium yeast on lipid peroxidation levels of STZ induced diabetic Wistar rats.
g. To determine the ameliorative effects of selenium yeast on serum level of (T_3 and T_4) in STZ induced diabetic Wister rats.
h. To determine the ameliorative effects of selenium yeast on inflammatory marker (TNFα) level in STZ-induced diabetic Wistar rats.

CHARPTER TWO

2.0 LITERATURE REVIEW

2.1 Blood Glucose Homeostasis

The route of entry into the body determines tissue distribution of glucose. For instance, oral intake of glucose in humans leads to 30–40% uptake by the liver while intravenous (IV) administration leads to only 10–15% uptake (Abdul-Ghani *et al*., 2006). In the post-absorptive state or after fasting, insulin levels are low and most glucose uptake occurs in insulin-insensitive tissues; this uptake is matched mainly by endogenous glucose production by the liver and to a smaller extent by the kidney (Stumvoll *et al*., 1997). Oral glucose absorption is partly regulated by the enteroinsular axis which consists of neuronal as well as hormonal factors (Marks *et al*., 1991). Luminal glucose above a threshold triggers a signaling pathway in endocrine cells involving activation of the gut sweet receptor (a heteromeric combination of taste receptor 1 family subunits: T1R2 + T1R3). This receptor functions in association with a-gustducin and other signalling elements to cause secretion of gastrointestinal hormones, so-called incretins (Intestine secretion Insulin; Zunz and La Barre, 1929), which are synthesized in endocrine cells of the gastrointestinal tract and released upon the stimulus of food absorption (Daly *et al*., 2012).

The signals that convey changes in glucose flux may take many potential forms, and these pathways must be taken into account to understand the mechanisms by which tissue-specific changes in glucose flux alter whole-body glucose homeostasis. Glucose enters the islet b cell via facilitated diffusion through GLUT2, the predominant glucose transporter in b cells. In pancreatic b cells, glucokinase, not glucose transport, is rate limiting for glucose metabolism at physiological glucose concentrations (Matschinsky *et al.,* 1998). In addition, glucokinase,

unlike other hexokinases, is not allosterically inhibited by glucose-6-phosphate. Thus, glycolytic flux is proportional to the extracellular glucose concentration. Increases in the ATP/ADP ratio generated as a result of this glucose flux are thought to lead to b cell depolarization via closure of ATP-sensitive potassium channels (KATP channels) and subsequent insulin secretion (Schuit *et al*., 2001). It was demonstrated that ingest food caused a more potent release of insulin than glucose infused intravenously (Perley and Kipnis, 1967). This effect, termed the "incretin effect" suggests that signals from the gut are important in the hormonal regulation of glucose disappearance. Incretin hormones are peptide hormones secreted from the gut and specific criteria have to be fulfilled for an agent to be called an incretin. They have a number of important biological effects, as for example, release of insulin, inhibition of glucagon, maintenance of -cells mass, and inhibition of feeding. Several incretin hormones have been characterized, but currently, GLP-1 (Glucagon-Like Peptide-1) and GIP (Glucose-Dependent Insulinotropic Polypeptide) are the only known incretins. Both GLP-1 and GIP are secreted in a nutrient-dependent manner and stimulate glucose-dependent insulin secretion. Gut hormones are secreted at low basal levels in the fasting state. The secretion of gut hormones is regulated, at least in part, by nutrients. Plasma levels of most gut hormones rise quickly within minutes of nutrient uptake and fall rapidly thereafter mainly because they are cleared by the kidney and are enzymatically inactivated (Drucker, 2007).

Glucose uptake in peripheral tissues such as skeletal muscle, heart and adipose tissue is facilitated by GLUT-1 and GLUT-4 glucose transporters which are co-expressed in these tissues (Wright *et al.*, 1998*a*). The sodium-independent, facilitated-diffusion glucose/hexose transporters (GLUTs) are a family of integral membrane proteins that mediate the transport

of hexoses across plasma membranes (Shepherd and Kahn, 1999). The members of the GLUT family are distinguished by differential affinities for their substrates and tissue-specific expression and regulation (Joost, 2002).

GLUT4, the major insulin-stimulated glucose transporter, is expressed predominantly in skeletal muscle, cardiac muscle, and adipose tissue and is largely responsible for insulin-stimulated glucose transport into these tissues (Shepherd and Kahn, 1999). GLUT4 is sequestered in intracellular vesicles in the absence of insulin. Upon insulin stimulation, GLUT4 vesicles translocate to and fuse with the plasma membrane, permitting increased glucose flux. Glucose transport via GLUT4 into muscle and adipose tissue is the rate-controlling step in insulin-mediated glucose disposal, and this disposal is diminished in insulin-resistant states. GLUT1, which is constitutively present on the plasma membrane and in intracellular membranes, plays a minor role in glucose transport in insulin-responsive tissues, although it plays a major role in other tissues. Important insights into the mechanisms governing glucose homeostasis have been gained from studying the physiological consequences of genetically modifying GLUT expression in specific tissues (Marshall *et al*., 1993).

Transgenic over expression of GLUT1 in skeletal muscle results in a 3- to 4-fold increase in basal glucose uptake in muscle ex vivo (Marshall *et al*., 1993). GLUT1 over expression causes fasting hypoglycemia, reduces glycemia in the fed state, and improves glucose tolerance.

2.2 Diabetes Mellitus

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin production, impaired insulin action or both. It is one of the major non-communicable diseases on the rise worldwide, causing 4.8 million deaths and morbidity in 371 million people every year (IDF, 2013).

It remains an incurable disorder which is associated with poor quality of life (ADA, 2011). The recent statistics shows that the global prevalence of this disorder continues to rise unabated and thus becoming an epidemic (Lam and Leroith, 2012). This is of public health concern due to its social and economic burdens. Even though diabetes has no known cause, complex interplay of several factors including genetic, social, and environmental factors is implicated in its etiology (Imam, 2012). Oxidative stress is thought to be a major risk factor in the onset and progression of diabetes. Many of the common risk factors, such as obesity, increased age, and unhealthy eating habits, all contribute to an oxidative environment that may alter insulin sensitivity either by increasing insulin resistance or impairing glucose tolerance. The mechanisms by which this occurs are often multifactorial and quite complex, involving many cell signaling pathways. A common result of both types of diabetes is hyperglycemia, which in turn contributes to the progression and maintenance of an overall oxidative environment. Macro- and microvascular complications are the leading cause of morbidity and mortality in diabetic patients, but the complications are tissue specific and result from similar mechanisms (Ahmad *et al*., 2005).

With many being linked to oxidative stress, there is a large body of clinical evidence correlating diabetic complications with hyperglycemic levels and length of exposure to hyperglycemia (Lasker, 1993). Although there is a paucity of data on the prevalence of

diabetes in Nigeria and other African countries, available data suggest that diabetes is emerging as a major health problem in Africa, including Nigeria (Mbanya *et al*., 1996).

The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 (Wild *et al.,* 2004). The increase in incidence in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented (Wild *et al.*, 2004). In some of the poorest regions in the world such as Africa, where infectious diseases have traditionally been the focus of healthcare systems, diabetes cases are expected to increase by 90% by 2030 (International diabetes foundation, 2012). At least 78% of people in Africa are undiagnosed and do not know they are living with diabetes (International diabetes foundation, 2012).

Some of Africa's most populous countries also have the highest number of people with diabetes, with Nigeria having the largest number of people (3.0 million), followed by South Africa (1.9 million), Ethiopia (1.4 million), and Kenya (769,000) (International diabetes foundation , 2012). The crude prevalence of DM in a national survey conducted in 1992 in males and females below the age of 45 years was 1.6% and 1.9%, respectively; with a 3-fold increase after the age of 45 years to 5.4% and 5.6% in males and females, respectively (Akinkugbe, 1997). Urban communities had a higher overall prevalence of diabetes (3.3%) when compared with rural communities (2.6%) (Akinkugbe, 1997). The recent IDF data (2011) estimated the prevalence of diabetes in Nigeria to be 4.04%, compared to Reunion (highest in Africa [16.78%]), Benin (1.71%), Ghana (4.09%), Niger (4.36%), Cameroun (5.18%), and South Africa (6.46%) (International Diabetes Foundation, 2012). Over time,

diabetes mellitus (DM) has emerged as a global healthcare problem that has reached epidemic proportions (Alebiosu *et al.,* 2009).

2.2.1 Type 1 diabetes

Type 1 diabetes (T1D) is a chronic lifelong disease in which individuals have high levels of blood glucose due to lack of insulin production (Williams *et al*., 2000).It is perceived as one of the most relevant autoimmune disorders in children and is characterized by selective and aggressive destruction of insulin-producing-cells, orchestrated by autoreactive T cells (American Diabetes Association, 2009). The incidence of type 1 diabetes varies widely on a global level. The highest reported incidence is in Finland, at 57.6 new cases per 100,000 people per year in those 0 to 14 years of age. Other European countries with high incidence are Sweden, Norway and the United Kingdom, all at >25 new cases per 100 000 people per year. Outside Europe, the countries that have large populations of those of European background with the highest incidence are Canada (25.9 per 100,000 per year) and Australia (22.5 per 100 000 per year). Of the non-European-ancestry countries, Saudi Arabia and Kuwait have the highest incidence, at 31.4 and 22.3, respectively. Most African, Asian and South American countries have lower incidence, at less than 8.5 cases per 100,000 per year (International Diabetes Federation, 2013). The overall incidence of diabetes is rising at approximately 3% per year, particularly in those with lower genetic susceptibility, indicating that the role of susceptibility genes is complex and is changing (Patterson *et al*., 2003; Steck *et al*., 2011).

More than 40 genetic loci have been associated with type 1 diabetes, with the human leukocyte antigen (HLA) region accounting for approximately 50% of the genetic risk (Barrett *et al*., 2009). The haplotypes most strongly associated with type 1 diabetes include:

DRB1*03:01-DQA1*05:01-DQB1*02:01 (DR3) and DRB1 *04:01/02/04/05/08-DQA1*03:01-DQB1*03:02/04 (DR4) (Noble and Valdes, 2011). The DRB1*15:01-DQA1*01:02-DQB1*06:02 (DR2) haplotype is dominantly and almost completely protective for type 1 diabetes, (Noble and Valdes, 2011). Of the other associated regions, a region in the regulatory region of the insulin gene, the PTPN22 (protein tyrosine phosphatase nonreceptor type 22) gene, and the interleukin-2 receptor alpha (IL2RA) make the greatest contributions (Barrett *et al.,* 2009). The majority of the genetic loci identified have a function in the immune system, providing new understanding of disease pathogenesis and potential targets for intervention.

A relatively new member of the T1D susceptibility gene set is PTPN22, which encodes the lymphoid protein tyrosine phosphatase (LYP) (Bottini *et al.,* 2004). The same allelic variant mediates risk in several other autoimmune diseases, suggesting the involvement of a crucial signaling axis (Bottini *et al.,* 2006). Indeed, the LYP protein is an important negative regulator of T-cell receptor signaling by way of dephosphorylation of Src family kinases Lck and Fyn, ITAMs of the TCR_/CD3 complex, as well as ZAP-70, Vav, valosin containing protein, and other key signaling molecules (Wu *et al.,* 2006). Explanations for the mechanism are contradicting. A loss-of-function mutation can cause a lower threshold for autoreactive T-cell activation in the periphery. In contrast, a gain-of-function mutation that suppresses TCR signaling during thymic development can allow autoreactive T cells to escape negative selection (Vang *et al.,* 2005).

The pathogenesis of selective β-cell destruction within the islet in type 1 DM is difficult to follow due to marked heterogeneity of the pancreatic lesions. At the onset of overt

hyperglycemia, a mixture of pseudoatrophic islets with cells producing glycogen (a cells), somatostatin (d cells) and pancreatic poly-peptide (PP cells), normal islets, and islets containing both b-cells and infiltrating lymphocytes and monocytes may be seen (Al-Homsi and Lukic, 1992). Lymphocytic infiltration is found only in the islet containing residual β-cells and is likely that the chronicity with which type 1 DM develops reflects this heterogeneity of islet lesions (Al-Homsi and Lukic, 1992).

In contrast to this chronicity in the natural history of the disease, β-cells are rapidly destroyed when pancreas is transplanted from identical twin donors into their long term diabetic twin mates in the absence of immunosuppression. In these cases, massive insulitis develops rapidly with infiltrating T lympocytes indicating an anamnestic autoimmune reaction (Al Homsi and Lukic, 1992). In addition, this observation also indicates that the chronic time course in type 1 DM (but not in a transplanted pancreas) is a consequence of down regulatory phenomena taking part in immunopatho-genesis of the disease (Al Homsi and Lukic, 1992). Activation of islet antigen - specific CD4+ T cells appear to be absolute prerequisite for the development of diabetes in all animal models of type 1 DM (Gill and Haskins, 1993). CD4+ islet specific T-cell clones derived from diabetic NOD mice, when injected into prediabetic or non diabetes prone Fl mice, induce insulitis and diabetes. It was also reported that CD4+ T cells are sufficient to induce insulitis while CD8+ T cells contribute to the severity of the damage (Yagi *et al*., 1992). These findings together with the evidence that insulitis in chronic graft versus host disease may occur in the absence of CD8+ T cells suggest that CD4+ T cells may be the only immuno-competent cells required in the disease process. However, it seems that only one subset of CD4+ T cells are responsible for disease induction. CD4+ T cell bearing alloantigen RT6 are absent in diabetes prone BB rats and appear to protect AO rats

from MLD-STZ induced diabetes (Greineh *et al*., 1987). Down-regulation of diabetogenic autoimmune response by the spleen cells derived from animals treated with adjuvants could also be explained by CD4+ T cell subsets interplay (Ulaeto *et al*., 1992).

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with IDDM. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagons in IDDM patients. Normally, hyperglycemia leads to reduced glucagons secretion, however, in patients with IDDM, glucagons secretion is not suppressed by hyperglycemia (Raju and Raju, 2010). The resultant inappropriately elevated glucagons levels exacerbate the metabolic defects due to insulin deficiency. The most pronounced example of this metabolic disruption is that patients with IDDM rapidly develop diabetic ketoacidosis in the absence of insulin administration. Although insulin deficiency is the primary defect in IDDM, there is also a defect in the administration of insulin. There are multiple biochemical mechanisms that account for impairment of tissue's response to insulin. Deficiency in insulin leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle (Raju and Raju, 2010). This impairs glucose utilization and insulin deficiency also decreases the expression of a number of genes, necessary for target tissues to respond normally to insulin such as glucokinase in liver and the GLUT4 class of glucose transporters in adipose tissue. (Raju and Raju, 2010).

2.2.2 Type 2 diabetes

Type 2 diabetes is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes mellitus (Gonzalez *et al*., 2009). The rise in prevalence is predicted to be

much greater in developing than in developed countries (69 versus 20%) (Shaw *et al*., 2010). It is characterized by the presence of chronic hyperglycemia, which results from resistance to insulin actions on peripheral tissues as well as inadequate secretion of insulin (American Diabetes Association, 2010). It is widely recognized that both insulin secretion and insulin resistance are important elements in the pathogenesis of type 2 diabetes. Subjects with insulin resistance require more insulin to promote glucose up take by peripheral tissues, and genetically predisposed individuals may lack the necessary β-cell secretory capacity. The resulting insulin deficiency disrupts the regulation of glucose production in the liver and is a clue element in the pathogenesis of glucose intolerance (Gastaldelli *et al*., 2004).

Since skeletal muscle accounts for 75% of whole body insulin-stimulated glucose uptake, defects in this tissue play a major role in the glucose homeostasis in patients with T2DM (Bjornholm and Zierath 2005). Insulin receptor tyrosine phosphorylation appears to be normal or reduced in nonobese T2DM (Zierath *et al*., 2000). IRS1 knockout mice demonstrate peripheral insulin resistance and reduced growth; however, these defects are partly compensated by the existence of an IRS1-independent pathway for insulin signal transduction (Araki *et al*., 1994). This compensatory pathway was shown to be mediated by IRS2 (Sun *et al*., 1995). IRS2 knockout mice have a progressive development of T2DM, with insulin resistance in skeletal muscle. Type 2 diabetic subjects have impaired insulin-stimulated tyrosine phosphorylation of IRS1 in skeletal muscle. This is not related to decreased protein expression of IRS1. A similar impairment is observed at the level of PI3K in type 2 diabetic muscle (Zierath *et al*., 2000). Whereas IRS1 and PI3K phosphorylation/ activation is impaired under in vivo and in vitro insulin stimulation in the skeletal muscle from type 2 diabetic subjects, AKT phosphorylation is impaired only under in vitro

conditions (Zierath *et al*., 2000). GLUT4 expression is down-regulated in adipose tissue in patients with T2DM. Given that skeletal muscle is the major site for glucose disposal, the hyperglycemia associated with T2DM cannot be explained by the decreased uptake of glucose into adipose tissue due to down regulation of GLUT4 in adipocytes. Furthermore, adipocyte-selective knockout of GLUT4 (adipose- Glut4K/K) in mice resulted in systemic insulin resistance similar to that induced by muscle-selective Glut4 knock-out mice (Zisman *et al*., 2000, Abel *et al*., 2001). These studies indicate that adipocyte GLUT4 deficiency may result in generations of circulating factors that are responsible for cross organ communication. Retinol-binding protein-4 (RBP4) was recently identified as one candidate for that crosstalk in the adipose tissue of adipose-specific Glut4 knockout mice (Yang *et al*., 2005).injection of recombinant RBP4 protein in normal mice caused insulin resistance, whereas RBP4 knockout mice had enhanced insulin sensitivity (Yang *et al*., 2005). Increased serum RBP4 protein levels induced hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and impaired insulin signaling in muscle. Adipocytes, both visceral and peripheral, secrete a plethora of factors, which may alter systemic insulin action and hepatic glucose production, including adiponectin, resistin, leptin, cytokines IL6 and TNFα, visfatin, RBP4, as well as FFA (Gimeno and Klaman, 2005; Wellen and Hotamisligil 2005).

Considerable current research is focused on trying to determine the pathogenic reason for the lowered beta-cellmass in type 2 diabetes, with several proposed mechanisms. An observation first made at the turn of the century was the occurrence of amyloid plaques in islets from persons with type 2 diabetes. Studies in the late 1980s showed the amyloid developed from a normal beta-cell secreted protein that is co-packaged with insulin in insulin granules, and is

termed Islet Associated Polypeptide (IAPP) (Jack, 2005). Subsequent studies that over expressed IAPP in transgenic mice showed beta-cell destruction and diabetes in some, but not all models (Janson *et al*., 1996). It has been proposed that very small developing intracellular amyloid fibrils cause the beta-cell destruction in the early stages of the disease, that type 2 diabetes may be a form of beta-cell Alzheimer's. Pathological studies of beta cells in type 2 diabetes have reported increased apoptosis as the presumed cause of the lowered beta-cell mass (Butler *et al.,* 2003).

Under diabetic conditions, ROS are induced and involved in the *β*-cell glucose toxicity (Robertson, 2004). *β*-cells express GLUT2, a high-Km glucose transporter, and thereby display highly efficient glucose uptake when exposed to a high glucose concentration. Indeed, it was shown that expressions of ROS markers 8-hydroxy-2-deoxyguanosine (8-OHdG) and 4-hydroxy-2, 3-nonenal (4-HNE) were increased in islets under diabetic conditions (Gorogawa *et al.,* 2004). In addition, *β*-cells are rather vulnerable to ROS due to the relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase (Tiedge *et al.,* 1997). Therefore, it is likely that ROS are involved in *β*-cell deterioration found in diabetes. It was shown that when *β*-cell-derived cell lines or rat isolated islets were exposed to ROS, insulin gene promoter activity and mRNA expression were suppressed. In addition, when they were exposed to ROS, binding of PDX-1 and/or MafA to the insulin gene promoter was markedly reduced. (Gorogawa *et al.,* 2004).

Furthermore, it was shown that the decrease of insulin gene expression after chronic exposure to a high glucose concentration was prevented by treatment with antioxidants (Tanaka *et al.,* 2002; Kaneto *et al*., 1999). Reduction of expression and/or DNA binding activities of PDX-1 and MafA by chronic exposure to high glucose was also prevented by an

antioxidant treatment. These results suggest that chronic hyperglycemia suppresses insulin biosynthesis and secretion by increasing ROS, accompanied by reduction of expression and/or DNA binding activities of two important pancreatic transcription factors: PDX-1 and MafA. Therefore, it is likely that the alterations of such transcription factors explains, at least in part, the suppression of insulin biosynthesis and secretion, and thus are involved in *β*-cell glucose toxicity.

2.2.3 Type 3 (Maturity onset diabetes of the young mody)

Maturity-onset diabetes of the young (MODY) is a heterogeneous form of diabetes characterized by onset of diabetes at an early age, autosomal dominant inheritance and impaired insulin secretion (Fajans *et al*., 2001). MODY represents 1% or more of all diabetes cases, (Pihoker *et al.*, 2013). But it is estimated that around 80% of cases are misclassified as T1D or T2D (Shields *et al.*, 2010).

Furthermore, 5–10% of patients originally classified as having type 1 DM do not carry high risk human leukocyte antigen haplotypes. These patients may indeed have MODY (Moller *et al.*, 1998). To date, molecular defects in six different genes have been identified in MODY patients. Mutations in the glucokinase (GCK) gene, which encodes an enzyme of the glycolytic pathway that modulates insulin secretion in response to glycaemic variations, cause MODY2.The most common types of MODY (in order of frequency in the UK) are due to mutations in hepatic nuclear factor 1 a (HNF1A), glucokinase (GCK), hepatic nuclear factor 4 a (HNF4A) and hepatic nuclear factor 1 b (HNF1B) genes (Shields *et al.*, 2010). Glucokinase, known as the pancreatic b-cell glucose sensor, is a key regulatory enzyme in glucose-stimulated insulin secretion. Heterozygous inactivating mutations cause mild, often subclinical, non-progressive, fasting hyperglycaemia (fasting plasma glucose 5.5 to 8.0

mmol/L, HbA1c 40 to 60 mmol/mol), present from birth (Steele *et al.*, 2013). A unique feature of GCK mutations is that insulin secretion remains intact and regulated, albeit shifted 2 to 3 mmol/L higher than normal. These results in low postprandial glucose excursions compared to other forms of diabetes (Steele *et al.*, 2014). Mutations in HNF1B cause the 'renal cysts and diabetes syndrome' due to the importance of HNF1B in the development of pancreas, kidneys and genitourinary system. HNF1B-MODY accounts for only 6% of cases of MODY in the UK. Renal abnormalities are evident from early gestational life and are frequently the first presentation. Non-diabetic renal failure can occur. Pancreatic atrophy may result, leading to both diabetes and pancreatic exocrine insufficiency in adult life. In contrast to HNF1A-MODY, patients are not SU sensitive and progress to insulin treatment relatively quickly (Bellanne-Chantelot *et al*., 2004).

2.2.4 Type 4 (Gestational diabetes)

Gestational diabetes mellitus (GDM) is defined by glucose intolerance of variable severity with onset of first recognition during pregnancy (WHO, 2012). Hyperglycaemia during pregnancy is found to be associated with various maternal and perinatal adverse outcomes (Metzger *et al*., 2008; Landon *et al*., 2011). The detection of GDM during pregnancy provides an opportunity to identify women at risk of short term and long term complications (Crowther *et al*., 2005).

The quoted prevalence of GDM ranged from 1 to 14% (ADA, 2004). It depended on which population was being studied and which screening strategies and diagnostic criteria were used (Agarwal *et al.*, 2005). Higher prevalence of GDM was noted in African, Asian, Indian and Hispanic women (Carolan *et al*., 2011; Makgoba *et al.*, 2012). Other reported risk factors were advanced maternal age, high parity, obesity, polycystic ovarian syndrome (PCOS),

multiple pregnancy, and family history of diabetes, obstetric history of congenital malformation, stillbirth, macrosomia and previous GDM. In 2008, National Institute for Health and Clinical Excellence (NICE) guideline recommended all women should be assessed for risk factors at the first antenatal visit (Christie *et al*., 2010). Women with body mass index (BMI) > 30 kg/m^2, previous macrosomic baby weighing 4.5 kg or above, previous GDM, family history of first-degree relatives with diabetes or family origin with a high prevalence of diabetes should be offered a diagnostic test using 75g, 2-hour oral glucose tolerance test (OGTT) at 24-28 weeks. Women with history of GDM should receive OGTT at 16–18 weeks and a further OGTT at 28 weeks if the results were normal.

Over the past decade, adipose tissue has been shown to produce numerous factors (adipocytokines), most of them act as hormones. These adipocyte-derived hormones have been implicated in the regulation of maternal metabolism and gestational insulin resistance. Adipocytokines, including leptin, adiponectin, tumor necrosisfactoralpha,interleukin-6, as well as the newly discovered resistin, visfatin, and apelin, are also known to be produced within the intrauterine environment (Catalano, 2010; Briana and Malamitsi-Puchner, 2009). Kirwan *et al.* (2001) reported that TNF-α is a significant predictor of insulin resistance during pregnancy. Together with a small additive contribution from leptin and cortisol, TNF-α exerted a significant influence on insulin-mediated glucose disposal. Circulating TNF-α showed a downward trend during early pregnancy and increased during the third trimester, thus mirroring insulin sensitivity changes during those periods. This observation is consistent with studies showing an increase in plasma TNF-α in late pregnancy (Kirwan *et al.,* 2002; Boyd *et al*., 2007). TNF-α correlates inversely with insulin secretion in normal pregnancy and was significantly higher in GDM group (McLachlan *et al*., 2006). TNF-alpha mRNA and

protein are present in human placenta and uterine cells at both early and late stages of gestation. In maternal obesity, the level of TNF-α is increased in the placenta compared with the non-obese pregnant women (Denison *et al.*, 2010).

2.2.5 Neonatal diabetes

Diabetes presenting in the first 6 months of life is unlikely to be T1DM, based on negative pancreatic antibodies and presence of protective HLA variants (Iafusco *et al*., 2002). NDM is rare and affects 1 in 90,000 -260,000 births. Two distinct subgroups exist: transient neonatal diabetes (TNDM), which usually remits by 12 weeks but may relapse after some years, and permanent neonatal diabetes (PNDM). Mutations in around 20 genes have been associated with NDM.

The most common causes of PNDM (40% of cases) are activating mutations in the KCNJ11 and ABCC8 genes, encoding the Kir6.2 and SU receptor 1 (SUR1) subunits, respectively, of the pancreatic b-cell KATP channel, leading to reduction or lack of insulin secretion (Gloyn *et al*., 2004). Molecular diagnosis of this type of PNDM is important as it can be treated very successfully with high doses of SU (Pearson *et al*., 2006). Some KATP channel mutations cause the more severe syndrome of psychomotor developmental delay, epilepsy and neonatal diabetes (DEND). Insulin (INS) gene mutations are also common causes of PNDM (15% of cases); patients present with ketoacidosis and require lifelong insulin treatment but have no syndromic features (Stoy *et al.*, 2007). Homozygous or compound heterozygous inactivating mutations of GCK are a rare cause of insulin dependent PNDM (Njolstad *et al*., 2001). In TNDM, the most common underlying mechanism is a methylation defect at chromosome

6q24 (ZAC and HYMAI genes). It usually presents in the first week of life and disappears by the twelfth week. In half of all cases, it recurs later in childhood. Insulin treatment is needed, but requirements gradually diminish. (Shield *et al*., 2004). Mutations in KCNJ11, ABCC8 and INS can also present as TNDM (Gloyn *et al.*, 2004).

2.2.6 Idiopathic diabetes mellitus

Some forms of Type 1 diabetes have no known aetiologies (Banerji and Lebovitz, 1989). Patients with this type of diabetes are found to have permanent insulin opening and are prone to keto acidosis, but have no evidence of anti-immunity. Though very few patients with type 1 of the disease are mostly of African and Asian origin and are strongly inherited (Banerji and Lebovitz, 1989). IDDm generally may occur at any age but most commonly it is diagnosed before 30 years of age (Turnbridge and Home, 2001). IDDM is also called juvenile-onset diabetes, keto-prone diabetes and more recently Type 1 diabetes (NDDG, 2007; Turnbrdige and Home, 2001). It is also referred to as insulinopenic diabetes (Karam, 2008) the plasma tryglyceride and free fatty acid concentrations have been reported to be markedly elevated whereas the cholesterol concentrations were only moderately elevated in Type 1 diabetes (Chase and Glasgow, 1976).

2.3 Insulin

Insulin is a key hormone with an important role in the growth and development of tissues and the control of glucose homeostasis (Pirola *et al.*, 2004). Insulin is secreted by pancreatic β-cells as an inactive single-chain precursor, preproinsulin, with a signal sequence that directs its passage into secretory vesicles. Proteolytic removal of this signal sequence results in the

formation of proinsulin. In response to an increase in blood glucose or amino acid concentration, proinsulin is secreted and converted into active insulin by special proteases. The active insulin molecule is a small protein that consists of A and B chains held together by two disulfide bonds (Melloul *et al.*, 2002). The primary role of insulin is to control glucose homeostasis by stimulating glucose transport into muscle and adipose cells, while reducing hepatic glucose production via gluconeogenesis and glycogenolysis. Insulin regulates lipid metabolism by increasing lipid synthesis in liver and fat cells while inhibiting lipolysis. Insulin is also necessary for the uptake of amino acids and protein synthesis (Sesti, 2006).The pleiotropic actions of insulin are all crucial for maintenance of normal cell homeostasis and allow cellular proliferation and differentiation.

2.3.1 Insulin receptor

The insulin receptor is necessary and sufficient to mediate insulin action. Humans and mice lacking insulin receptors are born at term, but do not survive long, suggesting that insulin receptors are essential for postnatal growth and fuel metabolism, but are not required for fetal metabolism (Accili *et al.,* 1999). The complete insulin receptor is a hetero tetrameric membrane glycoprotein composed of two a- and two b-subunits, linked together by disulfide bonds. Insulin binds to the receptor's extracellular a-subunit. Insulin binding presumably brings the two a-subunits closer together. This conformational change enables ATP binding to the b-subunit's intracellular domain. ATP binding activates receptor auto-phosphorylation (Hubbard, 1997). Thus, in turn, enables the receptor's kinase activity toward intracellular protein substrates.

The difference in size was found to be due to exclusion or inclusion of a 12-amino acid segment at the C-terminal end of the IR subunit. Studies on the exon-intron organization of

IR indicated that the 12-amino acid region is encoded by exon 11, spanning 36 bp that could be alternatively spliced, thus generating two IR isoforms (Seino *et al.*, 1989).

2.3.2 Function of insulin receptor

IRS proteins are regulated through multiple reversible posttranslational modifications, most importantly by phosphorylation (Boura-Halfon and Zick, 2009). The amino acid sequences of IRS1 and IRS2 provide a multitude of tyrosine, serine, and threonine residues as potential phosphorylation sites. Multiple sites for tyrosine phosphorylation (p-Tyr) of both IRS1 and IRS2 isoforms have been identified and analyzed, and they are needed for the transduction of insulin's metabolic signaling (Hanke and Mann, 2009). In addition to the tyrosine sites, the function of serine/threonine phosphorylation (p-Ser/Thr) is on sites known to negatively regulate insulin signaling (Greene and Garofalo, 2002).

2.4 Insulin Synthesis and Secretion

2.4.1 Structure of insulin

Insulin is a rather small protein, with a molecular weight of about 6000 Daltons. It is composed of two chains held together by disulfide bonds. Insulin is composed of two peptide chains referred to as the α and β-chain. α and β-chain are linked together by two disulfide bonds, and an additional disulfide is formed within the A chain. In most species, the A chain consist of 21amino acid and the β-chain of 30 amino acids. The figure below shows a molecular model of bovine insulin, with the A chain coloured blue and the larger β chain green. The amino acid sequence is highly conserved among vertebrates, and insulin from one mammal almost certainly is biologically active in another. Even today, many diabetic patients are treated with insulin extracted from pig pancreas (Stumvoll and Gerich, 2001).

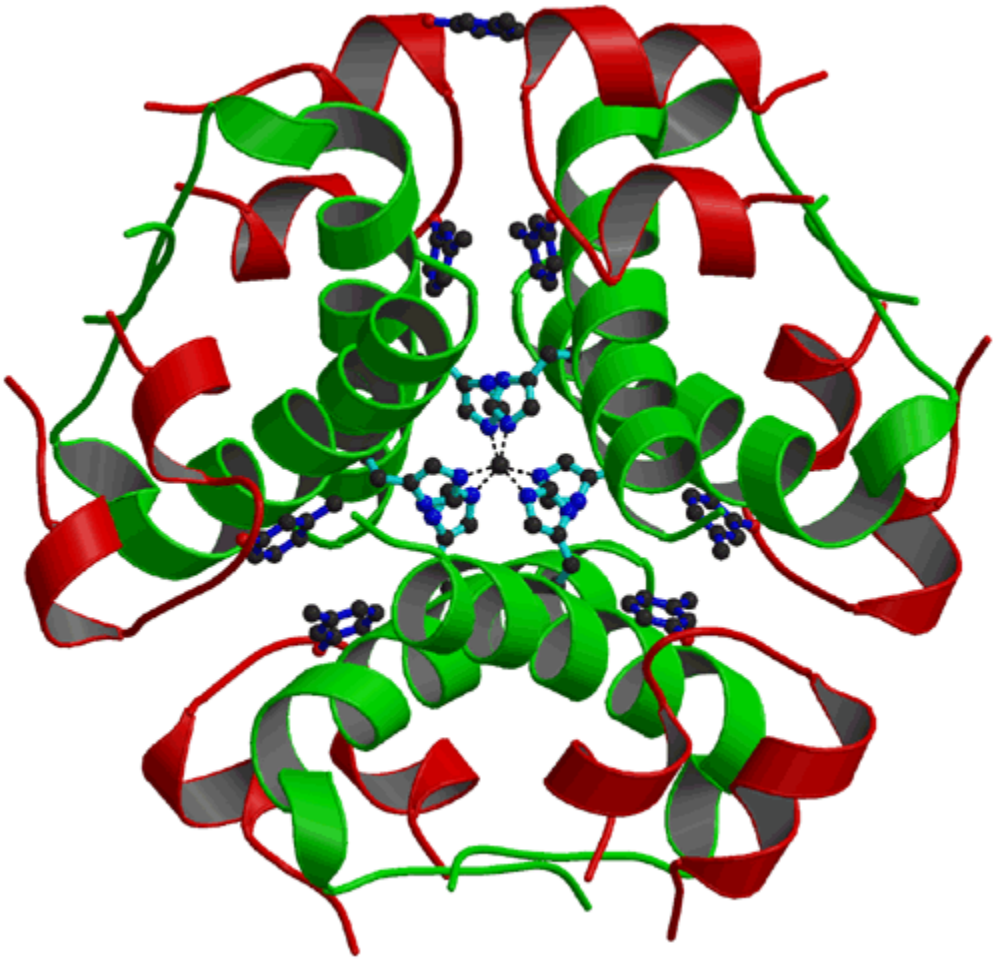


Figure 1: Structure of Insulin (Stumvoll and Gerich, 2001)

2.5 Synthesis of Insulin

Insulin is synthesized in significant quantities only in beta cells in the pancreas. The insulin mRNA is translated as a single chain precursor called preproinsulin, and removal of its signal peptide during insertion into the endoplasmic reticulum generates proinsulin.

Proinsulin consists of three domains: an amino-terminal B chain, a carboxy-terminal A chain and a connecting peptide in the middle known as the C peptide. Within the endoplasmic reticulum, proinsulin is exposed to several specific endopeptidases which excise the C peptide, thereby generating the mature form of insulin. Insulin and free C peptide are packaged in the Golgi into secretory granules which accumulate in the cytoplasm. When the beta cell is appropriately stimulated, insulin is secreted from the cell by exocytosis and diffuses into islet capillary blood. C peptide is also secreted into blood, but has no known biological activity (Al-Zahrani, 2005), as shown in Figure 2.

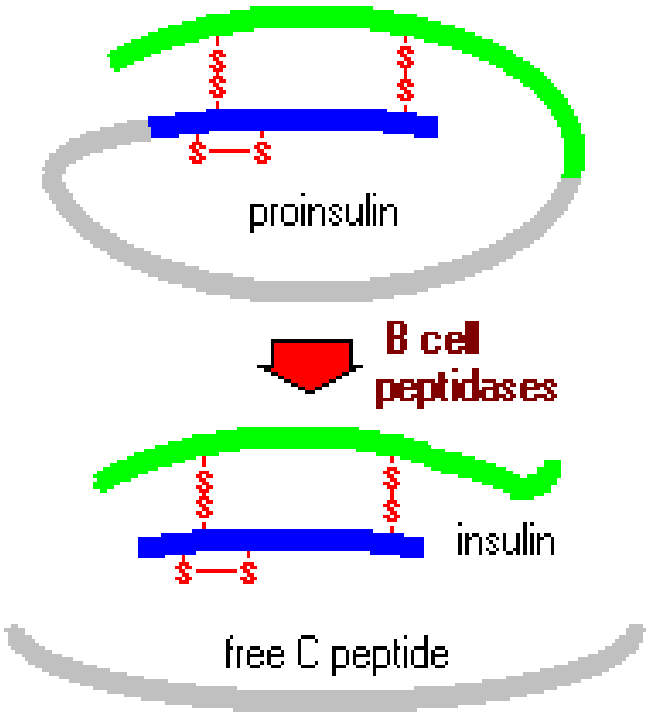


Figure 2: **Schematic diagram showing the mechanism of synthesis of insulin (Al-Zahrani, 2005)**

2.6 Control of Insulin Secretion

Insulin has a key role in the control of carbohydrate and lipid homeostasis, inducing storage of metabolic fuels after food intake. Major effects of insulin on carbohydrate metabolism include the stimulation of glucose uptake in skeletal muscle and adipose tissue and the suppression of hepatic gluconeogenesis and glycogen breakdown. Regarding lipid metabolism, insulin stimulates the biosynthesis of triglycerides from glucose and amino acids in liver and adipose tissue, and it inhibits lipolysis. The metabolic effects of insulin are primarily mediated through activation (phosphorylation) of protein kinase B (Akt) (Burgering and Coffer, 1995). Dysregulation of insulin signaling may result in insulin resistance of liver, adipose tissue and skeletal muscle, representing a hallmark in the patho-genesis of type 2 diabetes mellitus (T2DM) (Schinner *et al.,* 2005). The signaling pathways elicited by insulin have been discussed in detail (Szypowska *et al.,* 2011). Binding of insulin to its receptor induces a series of phosphorylation events, resulting in activation of kinases from three major signaling pathways: Akt, extracellular signal-regulated kinases (ERK1/2) and typical iso- forms of protein kinase C (PKCζ/λ) (Kotani *et al*., 1998).

Akt integrates the metabolic actions of insulin by influencing gene expression, phosphorylation, stability and/or cellular localization of down-stream effect or proteins. Key factors in the insulin-mediated regulation of carbohydrate homeostasis which include for head box class O (Fox O) transcription factors and their coactivator peroxisomal proliferators - activated receptor gamma coactivator 1α (PGC-1α), glycogen synthase kinase 3(GSK3) and glucose transporter 4 (GLUT4) (Schinner *et al.,* 2005). The transcription factors terol regulatory element binding protein 1 c (SREBP1c), which is regulated by insulin at the

transcriptional and post-transcriptional level, plays a pivotal role in the control of fatty acid and triglyceride biosynthesis (Avramoglu *et al*., 2006).

The dose–response relationship for glucose-induced insulin secretion is sigmoidal with a species-dependent stimulatory threshold at 3–7mM and a steep increase, reaching maximum at 15–30mM of the sugar (Henquin *et al*., 2006). Such glucose dependence is consistent with the fact that high *K*m GLUT2 is the dominating glucose transporter in rodent β-cells (Chen *et al.,* 1990; Johnson *et al*., 1990). It was therefore unexpected that human β-cells preferentially express low *K*m GLUT1 (De Vos *et al.,* 1995), whose capacity is close to saturation already at threshold concentrations of glucose. However, consistent with the latter observation, transgenic re-expression of GLUT1 or GLUT2 into β-cells of GLUT2-null mice were equally efficient in restoring normal glucose sensing (Thorens *et al*., 2000).

2.7 Complication of Diabetes Mellitus

Normally properly treated diabetes is symptom less, but continuing hyperglycaemia seen in type 2 diabetes can give rise to chronic complications (United Kingdom Prospective Diabetes Study Group 33 (1998), including retinopathy, neuropathy and nephropathy (Vinik, *et al.,* 2003), and macrovascular complications (Pyorala, *et al.,*1987), which have been related to premature mortality and morbidity (Uusitupa, *et al.,*1993). A common denominator for all microvascular and macrovascular complications is extensive vascular damage. Both conditions are life threatening and may result in an altered mental state, loss of consciousness, and possibly death; therefore prompt medical attention is necessary to avoid adverse outcomes. Microvascular complications comprise changes in the small blood vessels of the eye that result in diabetic retinopathy, in the peripheral nerves, causing neuropathy, and finally in the kidney, causing diabetic glomerulo-sclerosis or diabetic nephropathy.

Consequently, diabetes is the most common cause of blindness, end-stage renal disease (Creager, *et al.,* 2003) and limb amputation (Beckman, *et al.,* 2002).

In macrovascular complications, accelerated atherosclerosis results in cardiovascular disease (CVD) such as coronary heart disease (CHD) and acute myocardial infarction (AMI). Through its effects on cardiovascular disease (70-80% of people with diabetes die of cardiovascular disease), diabetes is also now one of the leading causes of death. While the pathogenesis of these complications has been extensively studied for the past 50 years, no single etiology exists to explain all types of complications. Instead, multiple etiologies exist that are specific to each. The cost to care for patients with DM in the U.S. was approximately $132 billion. Of those costs, $40 billion was indirect medical expenses (disability, work loss, and premature deaths), and $92 billion dollars was direct medical expenses (those attributable to the disease itself, i.e. microvascular and macrovascular complications) (Centers for Disease Control and Prevention, 2003). These are the chronic complications that significantly impact the cost of health care. In fact, approximately 25% of the total Medicare budget is used for the treatment of DM and its complications (Finkelstein, *et al.,* 2003; Finkelstein, *et al.,* 2004).

2.7.1 Diabetic retinopathy

Diabetic retinopathy (DR), a common ophthalmic complication of diabetes, occurs in over 50% of diabetic patients. The manifestations of DR, diabetic macular oedema, and proliferative diabetic retinopathy, can lead to vision loss, as well as blindness (Hutchinson *et al.,* 2001).

Diabetic retinopathy is responsible for approximately 10,000 new cases of blindness every year in the United States alone (Fong *et al.,* 2004). The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia. Development of diabetic retinopathy in patients with type 2 diabetes was found to be related to both severity of hyperglycemia and presence of hypertension in the U.K. Prospective Diabetes Study (UKPDS), and most patients with type 1 diabetes develop evidence of retinopathy within 20 years of diagnosis (UKPDS, 1998). There are several proposed pathological mechanisms by which diabetes may lead to development of retinopathy. Growth factors, including vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor β, have also been postulated to play important roles in the development of diabetic retinopathy. VEGF production is increased in diabetic retinopathy, possibly in response to hypoxia. In animal models, suppressing VEGF production is associated with less progression of retinopathy (Keenan *et al*., 2007).visual deterioration which is part of proliferative retinopathy is characterized by the formation of new blood vessels on the surface of the retina and can lead to vitreous hemorrhage. White areas on the retina ("cotton wool spots") can be a sign of impending proliferative retinopathy. If proliferation continues, blindness can occur through vitreous hemorrhage and traction retinal detachment (Watkins, 2003). The etiology of retinopathy includes hyperglycemia-associated biochemical, anatomical and functional changes.

2.7.2 Diabetic nephropathy

Patients with diabetes have increased glomerular perfusion and plasma filtration owing to decreased resistance in both the afferent and efferent arteriole. The most important early clinical risk factor for diabetic nephropathy is albuminuria, which is caused by hemodynamic

changes and by impairment of the glomerular filtration barrier. Changes to this barrier include thickening and changed composition of the glomerular basement membrane and regression of the cytoplasmic extensions, or foot processes, of podocytes (Dronavalli *et al.*, 2008). Proinflammatory and profibrotic signals from glomerular cells and infiltrating macrophages cause mesangial expansion consisting of accumulating extracellular matrix. Apoptosis of podocytes and glomerular endothelial cells can ensue, with glomerulosclerosis as the most advanced pathological change. Abnormal function of tubules and tubulointerstitial fibrosis develops in parallel with glomerular damage, possibly in response to albuminuria.

Hyperglycaemia is a crucial factor in the development of diabetic nephropathy because of its effects on glomerular and mesangial cells, but alone it is not causative. Mesangial cells are crucial for maintenance of glomerular capillary structure and for the modulation of glomerular filtration via smooth-muscle activity. Hyperglycemia is associated with an increase in mesangial cell proliferation and hypertrophy, as well as increased matrix production and basement membrane thickening. In vitro studies have demonstrated that hyperglycemia is associated with increased mesangial cell matrix production (Heilig, 1995) and mesangial cell apoptosis (Lin, 2006). Mesangial cell expansion seems to be mediated in part by an increase in the mesangial cell glucose concentration, since similar changes in mesangial function can be induced in a normal glucose milieu by overexpression of glucose transporters, such as GLUT1 and GLUT4, thereby increasing glucose entry into the cells (Heilig, 1995). Hyperglycemia is thought to stimulate VEGF expression and, therefore, act as a mediator of endothelial injury in human diabetes (Hohenstein, 2006).

Currently utilized therapies to treat diabetic renal disease largely target systemic blood pressure and/or intraglomerular hypertension. Applied the most widely are interventions which alter the renin-angiotensin system (RAS) which includes angiotensin converting enzyme (ACE) inhibitors (Lewis *et al*., 1993) and angiotensin II (ANG II) receptor antagonists (Brenner e*t al*., 2001), which are considered first line therapies for diabetic nephropathy. Indeed, this strategy is an important component of most national and international treatment guidelines, along with strict glycemic control. It is important to note that early renal disease is a major risk factor for cardiovascular disease in individuals with diabetes (Groop *et al.,* 2009).

2.7.3 Diabetic neuropathy

Diabetic neuropathy is a syndrome which encompasses both the somatic and autonomic divisions of the peripheral nervous system. There is, however, a growing appreciation that damage to the spinal cord (Selvarajah *et al*., 2006) and the higher central nervous system (Wessels *et al.,* 2006) can also occur and that neuropathy is a major factor in the impaired wound healing, erectile dysfunction, and cardiovascular dysfunction seen in diabetes.

Diabetic neuropathy is recognized by the American Diabetes Association (ADA) as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes" (ADA, 2007). As with other microvascular complications, risk of developing diabetic neuropathy is proportional to both the magnitude and duration of hyperglycaemia, and some individuals may possess genetic attributes that affect their predisposition to developing such complications. The precise nature of injury to the peripheral nerves from hyperglycaemia is not known, but may be related to mechanisms such as polyol accumulation, injury from AGEs, and oxidative stress. Peripheral neuropathy

in diabetes may manifest in several different forms, including sensory, focal/multifocal, and autonomic neuropathies (Boulton *et al.,* 2005). Disease progression in neuropathy was traditionally clinically characterized by the development of vascular abnormalities, such as capillary basement membrane thickening and endothelial hyperplasia with subsequent diminishment in oxygen tension and hypoxia. Inhibitors of the renin-angiotensin system and α1- antagonists improve nerve conduction velocities in the clinical context, which is postulated to be a result of increases in neuronal blood flow. Advanced neuropathy due to nerve fiber deterioration in diabetes is characterized by altered sensitivities to vibrations and thermal thresholds, which progress to loss of sensory perception. Hyperalgesia, paresthesias, and allodynia also occur in a proportion of patients, with pain evident in 40–50% of those with diabetic neuropathy. Pain is also seen in some diabetic individuals without clinical evidence of neuropathy (approximately 10–20%), which can seriously impede quality of life (Obrosova, 2009).

Metabolic disorders are the primary cause of diabetic neuropathy. A hyperglycaemic state accompanying diabetes type 1, which is induced through decreased insulin secretion, is responsible for the enhanced activation of the polyol pathway. In the hyperglycemic state, the affinity of aldose reductase for glucose is increased, leading to the increased production of sorbitol. Sorbitol does not cross cell membranes and accumulates intracellularly in the nervous tissue, thus generating osmotic stress. Osmotic stress increases the intracellular fluid molarity as well as water influx, Schwann cell damage and nerve fibre degeneration (Oates, 2002). Furthermore, up regulation of the NADPH oxidase complex results in oxidative stress through reduced glutathione production, decreased nitric oxide concentrations and increased reactive oxygen species concentrations (Matsunami *et al.,* 2011). Free radicals, oxidants, and

some unidentified metabolic factors activate the nuclear enzyme poly (ADP-ribose) polymerase (PARP), which is a fundamental mechanism in the development of diabetic complications, including neuropathy (Drel *et al*., 2010). Moreover, a nitric oxide deficit and increased oxygen free radical activity are responsible for microvascular damage and hypoxia (Miranda-Massari *et al.*, 2011).

However, other than optimization of glycemic control and management of neuropathic pain, there are no major therapies approved in either Europe or the United States for the treatment of diabetic neuropathy. In addition, as is seen with other complications, the mechanisms leading to diabetic neuropathy are poorly understood. At present, treatment generally focuses on alleviation of pain, but the process is generally progressive (Forbes and Cooper, 2013).

2.7.4 Diabetic foot

One of the most distressing of diabetic complication is Diabetic Foot Ulcer (DFU) which affects 15% of people with diabetes (Pendsey, 2010). DFU is prone to infections, chronicity and recurrence which eventually affect the mental health of patients (Carrington *et al.*, 1996). A benign looking ulcer in a patient with diabetes often ends up in amputation. A study in the United States reported that 38% of all the amputations were associated with DM. (Ziegler-Graham *et al.*, 2008). This can lead to severe morbidity and mortality. Therefore DFU puts enormous financial burden on the patient and the health care services, even though it is preventable (Boulton *et al.*, 2004). The aetiology of diabetic foot ulcers usually has many components (Frykberg *et al.*, 2000). A recent multicenter study8 attributed 63 percent of diabetic foot ulcers to the critical triad of peripheral sensory neuropathy, trauma, and deformity.

When the nerve gets injured, the patient is at a higher risk of getting a minor injury without noticing it until it becomes an ulcer. The risk of developing foot ulcers in patients with sensory loss is increased up to seven-fold, compared to non-neuropathic patients with diabetes (Wild *et al.*, 2000). DM also affects the autonomic nervous system, leading to dryness and fissuring of skin, making it prone to infection. Autonomic system also controls the microcirculation of skin. These changes ultimately contribute to the development of ulcers, gangrene, and limb loss (Vinik *et al*., 2003).

2.7.5 Stroke

This is another manifestation of diabetic neuropathy. Diabetes mellitus is an established independent risk factor for atherothrombotic brain infarction at all ages and is responsible for 7% of deaths caused by stroke (Bell, 2004). It is second only to hypertension as a major risk factor for stroke, and as many as 25% of patients dying with diabetes have autopsy findings of cerebrovascular diseases (Bell, 1996).

2.7.6 Cardiovascular complications

Occlusive vascular disease of the lower extremities is common in diabetes mellitus and is a combination of micro anegiopathy and atherosclerosis of large and medium-sized arteries. Diabetes has 20 times the incidence of gangrene of the feet as non-diabetes (Karam, 2008).

2.8 Pathophysiology of Diabetes Mellitus

Insulin production is more or less constant within the beta cells, irrespective of blood glucose levels. It is stored within vacuole pending release via exocytosis, which is triggered by increased blood glucose levels. (Himsworth, 1936), because insulin is the principal hormone that regulates uptake of glucose into most cells from blood (primarily muscle and fat cells,

but not central nervous system cells), deficiency of insulin or the insensitivity of its receptor plays a central role in all forms of diabetes mellitus (Yalow and Berson, 1990).

Much of the carbohydrate in food is converted within a few hours to the monosaccharide glucose, the principal carbohydrate found in blood. Some carbohydrates are not converted. Notable examples include fruit sugar (fructose) that is usable as cellular fuel, but it is not converted to glucose and does not participate in the insulin/glucose metabolic regulatory mechanism; additionally, the carbohydrate cellulose (though it is actually many glucose molecules in long chains) is not converted to glucose as humans and many animals have no digestive pathway capable of handling cellulose. Insulin is released into the blood by beta cells (β-cells) in the pancreas in response to rising to rising leveki07uyikl0s of blood glucose (e.g. after a meal). Insulin enables the body cells (about 2/3 is the usual estimate including muscle cells and adipose tissues) to absorb glucose from the blood for use as fuel, for conversion to other needed molecule, or for storage. Insulin is also principal control signal for conversion of glucose (the basic sugar used for fuel) to glycogen for internal storage in liver and muscle cells. Reduced glucose levels results both in the reduced release of insulin from the beta cells and in the release conversion of glycogen to glucose when glucose levels fall, although only glucose thus recovered by the liver re-enter the blood stream as muscle cells lack the necessary export mechanism.(Bating *et al*., 1992).

Higher insulin levels increase many anabolic ("building up") processes such as cell growth and duplication, protein synthesis and fat storage. Insulin is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction and vice versa. In particular, it is the trigger for entering or leading ketosis (the fat burning metabolic phase) (Borch-Johnson *et al*., 1994). If the amount of insulin available is

insufficient, if the cells respond poorly to the effects of insulin (insulin insensitivity or resistance) or if the insulin itself is defective, glucose will not be handled properly by body cells or store appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, protein synthesis and other metabolic derangements such as acidosis (Himsworth, 1936).

2.8.1 Diagnostic criteria for diabetes mellitus

As with classification, diagnostic criteria for diabetes mellitus has to be modified from those previously recommended (Vandorsten *et al.*, 2013). The vivid criteria for the diagnosis of diabetes mellitus are as follows:

1. When a patient present with signs and symptoms of diabetes, namely, polyuria, polydipsia, polyphagia and unexplained weight cost, plus random plasma glucose concentration ≥ 200 mg/d/ (11.1 mmol/L), and confirmed on a subsequent day, to give a similar outcome, the diagnosis of diabetes mellitus is made.
2. When a patient presents with sign and symptoms of diabetes listed above, with fasting plasma glucose concentration of ≥ 126mg/dl (7.2 mmol/L), also confirmed on a subsequent day, to give similar outcome.
3. When a patient presents with sign and symptoms of diabetes mellitus, with 2 ours plasma glucose concentration of 200 mg/dl (11.1 mmol/L) during an oral glucose tolerance test as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose, dissolved in water. This however, is not routinely used in the diagnosis of diabetes mellitus (WHO, 1985).

An intermediate group of subjects with glucose concentration that do not meet the above criteria for diabetes, and nevertheless, too high to be considered normal exists (ADA 2012).

This group of subjects had fasting plasma glucose level > 110 mg/dl (6.1 mmol/L), but <126 mg/dl (7.0 mmol/L). To avoid the stigmatization attached to the name diabetic, they are classified as subjects with impaired fasting glucose (IFG). With time, these class of subjects may progress to full blown diabetes, or revert to normal plasma glucose concentration.

2.9 Management/Treatment of Diabetes

The cornerstone in the treatment of DM is education- education of both the diabetics and the health workers who manage/treat them. The ultimate aim is to improve and prolong life via the essential goal of normalizing blood glucose levels as far as possible (Guido, 2004). The primary management therapy insulin administration should form an essential part of the education campaign. Rational therapy of DM requires the application of principles derived from current knowledge concerning:

i. The nature of the disease.
ii. The mechanism of action, efficacy and the safety of the available treatment regimens (diet, oral hypoglycaemic drugs and insulin) (Karam, 2008).

Treatment of DM is individualized and patients are treated with:-

- Life-style modification including exercise
- Diet alone
- Diet plus oral anti-diabetic agents
- Diet plus insulin
- Soluble insulin urgently (For patients with Ketoacidosis) (Lewis and Elvin, 1977).

Insulin is required in the treatment of insulinopenic (IDDM) diabetes and is needed to normalize the endocrine and metabolic abnormalities (Karam 2008). Treatment of insulinoplethoric (NIDDM) diabetes will be directed at the cause of insulin insensitivity, e.g. weight reduction in case of obesity and reduction of endocrine hyper- secretion in cases of acromegaly or Cushing's syndrome (Ibid). Thus, oral hypoglycaemic agents are used mainly in the treatments of NIDDM (Evans, 2009) in addition to the conventional regimens; there are a number of hypoglycaemic plants known (Lewis-Lewis and Elvin, 1977), which are currently being studied for hypoglycaemic activities.

2.9.1 Diet and exercise

In all cases, diet will be prescribed individually to meet the needs of each type: caloric restriction for obese patients and regular spaced feedings with a bed– time snacks of patients receiving hypoglycaemic agents, especially insulin (Karam, 2008). In many cases, the type 2 DM, condition can be controlled by a suitable diet and exercise, but if this is not such useful, treatment with oral hypoglycaemic in conjunction with a suitable dietary regimen may prove satisfactory (Evans, 2009). The overall result of exercise in the diabetics is beneficial, if performed mildly (not strenuous) and coupled with an intake of low caloric diet.

2.9.2 Oral hypoglycaemic drugs

These drugs act in a variety of ways:

By stimulating the β - cells to produce insulin

i. By decreasing gluconeogenesis and increasing peripheral utilization of glucose – success is still dependent on some limited production of insulin by the pancreas;
ii. Retardation of carbohydrate absorption from the gut resulting in a reduction of excessive postprandial plasma-glucose concentration (Evan, 2009).The controversy

existing over the safety, in long-term use, of the sulphonylureas and the biguanides led to the recommended discontinuing general use of phenformin (a biguinide) in the USA due to its reported association with lactic acidosis (Karam 2008).

Thiazolidinedione: Trioglitazone, the first agent in these new class of antidiabetic drugs reduce peripheral insulin resistance and appear to lower glucose levels primarily by augmenting insulin mediated peripheral glucose disposal. Although thiazolidinedione require the presence of insulin to work, it does not stimulate insulin secretion and it is unlikely to cause hypoglycaemia (Kettle and Arky, 1998).

Exenatide: Exenatide is one of a new class of medications approval in April (2005) for the treatment of diabetes mellitus type 2. Exinatide is administered as a subcutaneous injection (under the skin) of the abdomen, thigh, or arm, 30 to 60 minutes before the first and last meal of the day (Stephen, 2007).

Mechanism of action: Exenatide is believed to facilitate glucose homoestasis in at least four ways; Exenatide augment pancreas response (i.e. increase insulin secretion) to eating meals, resulting in the release of a higher, more appropriate amount of insulin that help to lower the rise in the blood sugar. Once blood sugar levels decrease close to normal value, the pancreas response to insulin is reduced. However, other drugs (like injectable insulin) are lowering blood sugar, but can "overshoot" their target and cause blood sugar become too low, resulting in the dangerous condition of hypoglycaemia. Exenatide also causes pancreatic release of glucagon in response to eating, which helps stop the liver from overproducing sugar when it is not needed (which prevents hyperglycemia) (Drucker *et al*., 2008).

Exenatide helps slow down gastric emptying and thus decreases the rate at which meal derived from glucose appear in the blood stream. Exenatide reduces appetite, promote satiety via hypothalamic receptors. It reduces liver fat content. Fat accumulation in the liver, (fatty liver disease) is strongly related with several metabolic disorders in particular, low HDL, cholesterol and high triglyceride presence in the patients with type 2 diabetes (Ding *et al*., 2006).

2.9.2 Recent advances in management of diabetes mellitus

Pancreas transplanting: Pancreas transplant are generally performed together with or sometime after kidney transplant. In most extreme cases a pancreas transplant can restore proper glucose regulation. One reason for performing pancreas transplant together with kidney transplant is that introducing a new kidney requires taking immunosuppressive drugs and allows the introduction of new, functioning pancreas to a patient with diabetes without any addition immunosuppressive therapy, however, a pancreas transplant alone can be wise in patients with extremely labile type 1 diabetes mellitus (Donner, 2007).

Islet cell transplanting: In the transplanting of isolated islet from as donor pancreas into another person, it is experimental treatment for type 1 diabetes mellitus. Once transplanted the islets begin to produce insulin actively regulating the level of glucose in the blood. Islets are usually infused into patient liver (Lakey and Burridge, 2003). The patient's body, however, will reject the islet just as it would reject any other introduction of foreign tissue: the immune system will attack the islet as it is would any viral infection amounting to the risk of transplant rejection. Thus, the patient needs to undergo treatment involving immuno-suppression, which reduces immune system activity. Recent studies have shown that islets transplantation has progressed to the point that 58% of the patients in one study were insulin

independent one year after the operation. In the period from 1999 to 2007, 471 patients with type 1 diabetes have received islets transplants at 43 institutions worldwide (Shapiro and Lakey, 2005).

The goal of islets transplanting is to infuse enough islets to control the blood glucose level removing the need for insulin injections. For an average size person (70 kg), a typical transplant requires about one million islets, isolated from two donor pancreases. Because good control of blood glucose can slow or prevent the progression of complication associated with diabetes, such as nerve or eye damage, a successful transplant may reduce the risk of these complications. However a transplant recipient will need to take immunosuppressive drugs that stop the immune system from rejecting the transplanted islet (Shapiro and Lakey, 2005).

2.10 Selenium Yeast

Selenium is an essential element that is fundamental to human health (Steinbrenner and Sies, 2009). Your body needs certain elements in very small amounts. These elements are called "trace elements". Selenium is a trace element. Some people do not get enough selenium and wish to take a selenium supplement (Wasserman, 2013). Selenium supplements can be in two forms: inorganic such as Na selenite (Na_2SeO_3) or organic such as selenomethionine (SeMet), which is the predominant form of Se within seleno yeasts (Se yeast). Selenomethionine, unlike selenite, is actively transported by methionine transporter mechanisms across intestinal membranes during absorption and can be nonspecifically incorporated into body protein (Schrauzer, 2003).

2.10.1 Composition of selenium yeast

Selenium yeast (*Saccharomyces cerevisiae*) supplements contain from about 1,000 to 2,000 micrograms of selenium per gram of supplement. This selenium is in the form of selenomethionine, a specific selenium-containing amino acid. Synthetic selenomethionine supplements are also available, but they are slightly less effective than yeast-selenium (Wasserman, 2013).

2.10.2 Functions of selenium

- Selenium deficiency increases the virulence of viral infection by causing more pro-inflammatory immune response (Beck, 2003), and by boosting T-cell production (Broome, 2004).
- Selenium-containing deiodinase enzymes are critically important for brain development during pregnancy (Kesler, 2004).
- Some studies in patients with diabetes suggest that selenium supplementation may help to prevent vascular complications (Faure *et al.*, 2004).
- A secondary analysis of the NPC trial identified some benefit of selenium supplementation for cancer prevention among participants with baseline plasma selenium levels less than 121.6 ng/mL (Duffield *et al.*, 2002).
- An adequate selenium intake may be essential for cancer prevention (Fleet, 1997).

2.10.3 Safety of selenium yeast

Before it was found to be an essential nutrient (Rotruck *et al.*, 1973). Selenium was considered highly toxic to animals and humans. Selenium is incorporated into selenoproteins as selenocysteine through a complex genetic mechanism encoded by the UGA codon (Papp *et al*,

2007), through selenoproteins, selenium is involved in many biological functions (Rayman, 2000).

The recommended dose for selenium for an average adult weighing 154 pounds is about 350 micrograms. However, even prolonged exposure of up to 850 micrograms has not produced adverse effects. The lowest average daily selenium intake that could cause individuals to develop overt signs of toxicity is believed to be about 1,000 to 2,000 micrograms per day, but only after weeks or months of over-exposure. This would be quite rare and has only been seen with individuals taking inorganic selenium supplements, not with selenium yeast or selenomethionine products (Wasserman, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.0 Scientific Background of the Research

Streptozotocin-induced diabetic rats treated with selenium yeast was used for the present study and blood glucose level, liver enzymes, and serum electrolytes levels were determined using standard test procedures.

3.1 Materials

3.1.1 Animals

Thirty five adult male Wistar rats weighing between (180 – 200) grams were purchased from the animal house Department of pharmacology Ahmadu Bello University Zaria and were used for the study. The animals were housed in polypropylene cages under standard laboratory conditions and had free access to food and water *ad libitum*. Animals were allowed to acclimatize to the laboratory environment over a period of 14 days before the commencement of the experiments. Animal care and use were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Institute of Health (NIH Publications No. 80-23; 1996).

3.1.2 Drugs, reagents and practicals

All drugs and reagents were obtained commercially and were of analytical grades. The drugs and reagents obtained include:

Streptozotocin (Sigma Aldrich), Selenium yeast (Sigma-Aldrich), ELISA kits for the assessment of liver enzymes in LFTs were purchased from Randox Laboratory Ltd,United Kingdom, North West Life Science Specialities, Vancouver Canada. Digital glucometer (Accu-check advantage, Roche Diagnostic, Company USA,). Electronic Weighing balance.

Model: EK 3052, dissecting set, syringes and needles (Sologuard Medical Device P.V.T Ltd., Chema-600 096, India, ML No. 750).

3.2 Methods

3.2.1 Induction of experimental diabetes mellitus

Diabetes mellitus was induced by single intraperitoneal injection of 60mg/kg body weight dose of streptozotocin dissolved in 0.1ml fresh cold citrate buffer pH 4.5 into 16 h-fasted rats. Three days after streptozotocin injection (72 h), blood samples were taken from tail artery of the rats (Burcelin *et al.,* 1995) for determination of blood glucose levels using the glucose-oxidase principle. Rats with blood glucose levels greater than 200mg/dl were considered diabetic and were used for the study. Twenty (20) rats showed streptozotocin-induced hyperglycemia and five norm glycaemic rats served as the negative control group.

3.2.2 Experimental design

After the induction of experimental diabetes in the Wistar rats, the animals were randomly divided into five treatment and two control groups of five rats each. All the animals were fasted for 16-18 hours before the commencement of treatment and the treatment lasted for 4 weeks as follows: Weeks 1, 2, 3 and 4, all treatments were administered via oral route.

Group 1 (n = 5): Diabetic group that received 0.1 mg/kg of selenium yeast only. (Nouf *et al.,* 2013).

Group 2 (n = 5): Diabetic group that receive 0.2 mg/kg of selenium yeast.

Group 3 (n = 5): Diabetic group that received 1mg/kg of glibenclamide (GBA).

Group 4 (n = 5): Normoglycemic group that received 0.9 % normal saline 5 ml/kg.

Group 5 (n = 5): Diabetic control group that receive only 0.9 % normal saline 5 ml/kg.

Group 6 (n = 5): Diabetic group that receive 300mg/kg of moderate dose of Aspirin (Hammadi *et al.,* 2012).

Group 7 (n = 5): Diabetic group that receive 120 mg/kg of Ibuprofen.

3.2.3 Blood glucose level determination

Blood samples were collected from the rat's tail weekly for the period of 4 weeks. Determination of blood glucose level was done by the glucose-oxidase principle (Beach and Turner, 1958), using the digital glucometer (Accu-Check Advantage, Roche Diagnostic, Germany), and results were recorded as mg/dl (Rheney and Kirk, 2000).

3.2.4 Collection and preparation of serum samples for analysis

After the last day of the treatment period, all rats were subjected to light anesthesia by exposing them to chloroform soaked in cotton wool placed in anaesthetic box covered with lid. Blood samples of about 5ml were drawn from the heart of each sacrificed animal from all groups by cardiac puncture after they have been fasted for 16 - 18 h. The samples were collected in Eppendrof tubes and were allowed to clot. Thereafter, the serum sample were separated by centrifugation, using Denley BS400 centrifuge (England) at 1000*g* for 10 minutes. The supernatant was collected and used for the following analysis.

3.2.5 Estimation of serum electrolytes

Serum sodium and potassium ions were estimated by the flame photometry method of Vogel (1960) and bicarbonate ion was determined using the titration method of Segal (1955), Chloride ion was estimated using the method of Schales and Schales (1941).

3.2.6 Estimation of serum liver enzyme levels

3.2.6.1 Serum alanine aminotransferase by the method of Reitman and Frankel (1957).

Principle

L-Alanine + α-ketoglutarate ALT Pyruvate + Glutamate

Alanine Aminotransferase reversibly transfer the amino group from alanine to α-ketoglutarate, forming pyruvate and glutamate. The ALT is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4 – dinitrophenylhydrazine.

3.2.6.2 Serum aspartate aminotransferase by the method of Reitman and Frankel (1957)

Principle

The enzyme Aspartate Aminotransferase, reversibly transfer an amino group from aspartate to α-ketoglutarate. The AST is measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4- dinitrophenylhydrazine.

Aspertate + α-ketoglutarate AST glutamate + oxaloacetate

3.2.6.3 Serum alkaline phosphatase activity by the method of (McComb and Browers, (1972)

Principle

Serum alkaline phosphatase catalyzes the hydrolysis of p-nitrophynylphosphate to p-nitrophenylate ion and phosphate. The substrate was colourless, but the p-nitrophenylate resonates to quinoid form in alkaline solution and strongly absorbs light at 404nm.

3.2.7 Estimation of oxidative stress biomarkers

Glutathione Peroxidase

The NWLSS^{TM} Glutathion peroxidase Assay kit was used which is an adaptation of the method of Paglia and Valentine (1967). Glutathione peroxidase catalyses the reduction of

hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG was then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming $NADP^+$ (resulting in decrease absorbance at 340 nm) and recycling the GSH. Since GPx is limiting, the decrease in absorbance at 340nm is directly proportional to the GPx concentration. The absorbance was read at 1,2 and 3 minutes against reagent blank. The absorbance for blank was subtracted from the sample reading to give the corrected value. Thus, GPx activity was calculated using 8.412 as the extinction coefficient:

GPx(U/L) = 8.412 x Δ A 340/min

U/L = unit activity per liter

Δ A 340/min = change in absorbance at 340 per minute.

3.2.8 Measurement of superoxide dismutase activity

Superoxide dismutase (SOD) activities were measured by the method of *Misra and Fridovich* (1972). Plasma (0.5 mL) was diluted to 1.0 mL with distilled water, and 250 µl of chilled ethanol and 150 µl of chilled chloroform were added. The mixture were shaken and centrifuged. The supernatant were used for the assay of enzyme activity. To 1.2 mL of the supernatant were added 1.5 mL of 0.1 mol/L carbonate-bicarbonate buffer, pH 10.2, containing0.2 mmol/L EDTA. The contents were mixed, and the reaction as initiated by adding 200 µl of epinephrine (pH 3.0, 3 mmol/L) to the buffered reaction mixture. The changes in optical density per minute were measured at 470 nm.

3.2.9 Measurement of catalase activity

Catalase (CAT) activities were assayed by the method of *Sinha* (1972). 0.1 mL of Plasma and 1.5 mL of phosphate buffer were added. To this, 0.4 mL of hydrogen peroxide was added

and the reactions were arrested after 30 and 60 second by the addition of 2.0 mL dichromate acetic acid reagent. A control was also carried out simultaneously. All the tubes were heated in a boiling water bath for exactly 10 min, cooled and absorbance read at 620 nm. Standards in the range of 2-10 mmoles were taken and processed as the test. The activities of catalase were expressed as μmoles of hydrogen peroxide consumed/min/mg of protein (unit per milligram of protein).

3.2.10 Estimation of lipid peroxidation biomarker (MDA)

Lipid peroxidation can be evaluated by the thiobarbituric acid reactive substances method (Gallou *et al*., 1993). Plasma malondialdehyde (MDA) levels were measured by the double heating method of Draper and Hadley (1990) using Malondialdehyde Assay kits from Northwest Life Sciences Specialities (NWLSS^{TM}, product NWK-MDA01). Butylated hydroxytoluene (BHT) in methanol reagent was used as the control. The method is based on the spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA at 532 nm. The MDA formed will therefore be quantified using an extinction coefficient of 1.56×10^5/mole/cm (Yagi, 1987). The amount of MDA formed in the control samples is subtracted from the amount in the experimental samples to obtain the amount of MDA in each sample. Since absorbance is directly proportional to the concentration, thus; concentration of MDA in each sample = Absorbance in sample – Absorbance in control x 10^5 nmol/ml ÷ 1.56×10^5 $M^{-1}CM^1$

3.2.11 Estimation of lipid profile

Serum total cholesterol levels (TC) were assayed by the enzymatic method of Watson (1960). Serum HDL-C was estimated by the method reported by Freidewald *et al.* (1972). Serum triacylglycerol (TAG) were estimated by the method of Fossati *et al.* (1982). Serum low

density Lipoprotein cholesterol (LDL-C) and very low density Lipoprotein cholesterol were calculated according to the Friedewald formula: LDL – C = TC- (HDL-C+TAG/5) (Friedewald *et al.,* 1972).

3.2.12 Estimation of serum triiodothyronine and tetraiodothyronine

Thyroid profile was estimated by using enzymes-linked immunosorbent assay (ELISA) kits (microwell T_3 and T_4) from synthon Bioresearch, Inc, USA. Based on the method of Tahiliani and Kar (1999). The thyroid function was evaluated by determining the serum concentration of (T_3 and T_4). The sensitivity of the kits was 20 ng/l.

3.2.13 Estimation of inflammatory marker

Tumor nectosis factor (TNFα) in serum level were assayed by enzyme-linked immuno-sorbent assay (ELISA) using ready made kit reagent supplied by Thermo Scientific (USA).

3.3 Statistical Analysis

The data obtained were expressed as mean ± standard error of mean (SEM) and data were statistically analyzed using analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The values of $p \leq 0.05$ were considered as significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Blood Glucose Levels of Selenium Yeast Administered in Streptozotocin Induce Diabetes in Wistar Rats

Figure 4.1 showed the result of the changes in blood glucose level treated with different doses of selenium yeast in streptozotocin induced diabetic wistar rats. Plasma glucose levels in the diabetic control group were significantly higher when compared with normal control group through all the 4weeks of treatment. In between groups comparison, 0.1 mg/kg of selenium yeast did not show any significant change in blood glucose level when compared with the normal control group, however blood glucose level were significantly ($p < 0.05$) lower in the 0.2 mg/kg of selenium yeast treated group after week one and week three when compared with control group. Similarly, the diabetic groups treated with Aspirin and Ibuprofen, significantly lower the blood glucose level when compared with control group only after the third week of treatment. The positive control group administered with 1 mg/kg of Glibenclamide showed similar effect with 0.2 mg/kg of selenium yeast at the first and third week of treatment. The 0.2 mg/kg of selenium yeast significantly protect the rise in blood glucose level when compared with the diabetic control group.

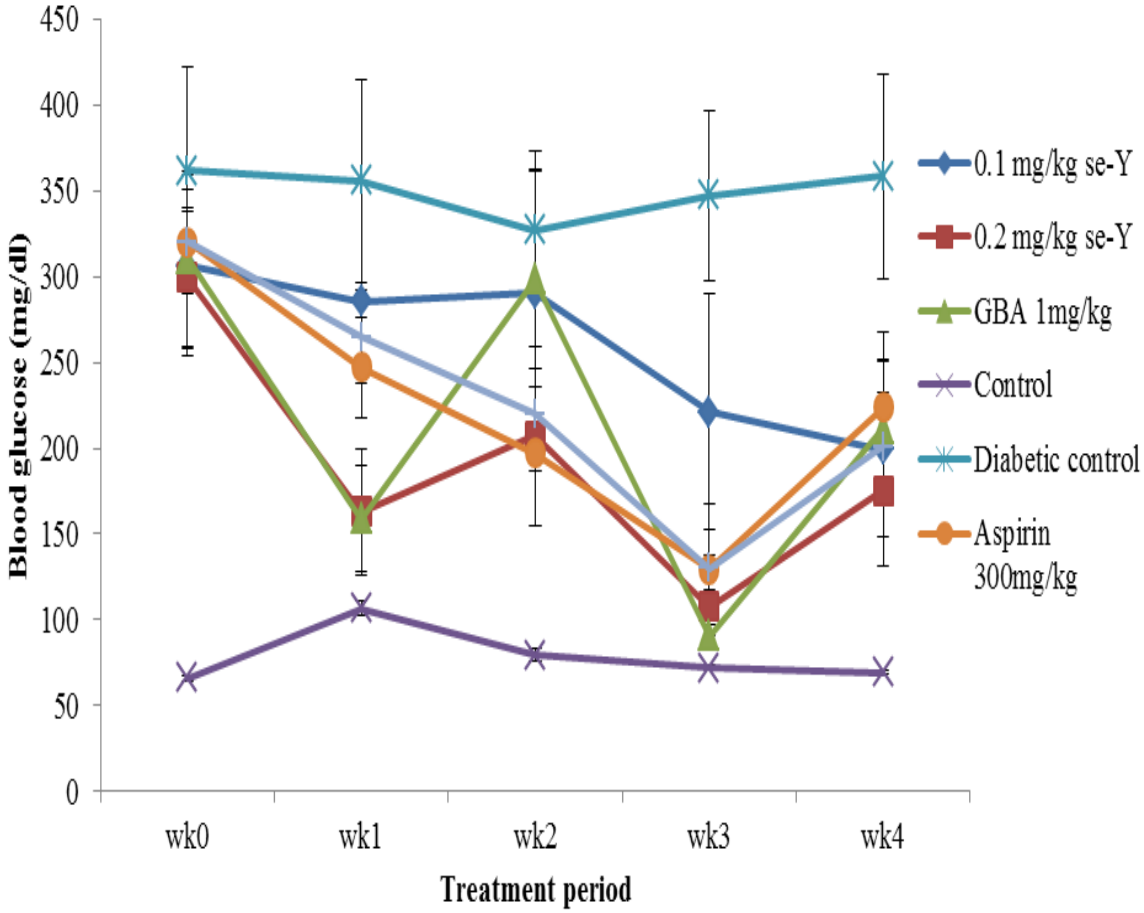


Figure 4.1: Changes in blood glucose levels in 4wks of treatment with different doses of selenium yeast In streptozotocin induced diabetic Wistar rats (n = 5), wk = week of administration.

4.2 Changes in Oxidative Stress and Lipid Peroxidation Biomarkers in Rats Treated with Selenium Yeast for the Period of Four Weeks in Streptozotocin Induced Diabetes

Figure 4.2 showed that superoxide dismutase, catalase, glutathione peroxidase and malondealdehyde were significantly ($P < 0.05$) different in diabetic control group when compared with normal control group, while groups I and II did not show any significant differences when compared with diabetic control group except for malondealdehyde that was significantly ($P < 0.05$) different in group treated with 0.2 mg/kg of selenium yeast when compared with the diabetic control group. Similarly, MDA was significantly deferent in groups III, VI and VII when compared with diabetic control group.

Table 4.1: Changes in oxidative stress and lipid peroxidation biomarkers in rats treated with selenium yeast

	MDA (nmol/L)	SOD (IU/L)	CAT (IU/L)	GPx (IU/L)
Group I (0.1mg/kg Selenium)	1.68 ± 0.12	1.86 ± 0.04	45.60 ± 1.63	40.20 ± 1.49
Group II (0.2mg/kg Selenium)	1.44 ± 0.07●	1.94 ± 0.08	45.40 ± 1.02	40.00 ± 1.45
Group III (1mg/kg GBA)	1.48 ± 0.07‡	1.90 ± 0.07	46.00 ± 0.54	40.40 ± 0.81
Group IV (normal control)	0.96 ± 0.05	2.40 ± 0.05	53.20 ± 0.66	45.60 ± 0.24
Group V (diabetic control)	2.02 ± 0.10†	1.74 ± 0.08†	42.60 ± 0.40†	35.80 ± 0.588†
Group VI (300mg/kg Aspirin)	1.40 ± 0.04 α	1.86 ± 0.05	46.40 ± 0.74	41.20 ± 1.24 α
Group VII (120mg/kg Ibuprofen)	1.28 ± 0.08 β	2.06 ± 0.08 β	47.40 ± 1.02 β	42.80 ± 1.39 β

†$P<0.05$ Group V vs. IV; ● $P<0.05$ Group II vs. V; ‡ $P<0.05$ Group III vs. V; α $P<0.05$ Group VI vs. V; β $P<0.05$ Group VII vs. V; ∞ $P<0.05$ Group I vs. V; ns= non-significant

MDA = malondadehyde
SOD = superoxide dismutase
CAT = catalase
GPx = gluthation peroxidase

4.3 Changes in Serum Electrolytes Levels in Rats Treated with Selenium Yeast for the Period of Four Weeks in Streptozotocin Induced Diabetes

Figure 4.3 showed that only serum chloride level was significantly ($P < 0.05$) different in diabetic control group when compared with normal control group. However, serum chloride and sodium ion levels were significantly ($P < 0.05$) lower in group II and VII when compared with diabetic control group. Urea and HCO_3^- did not show any significant difference across all the groups.

Table 4.2: Changes in serum electrolytes levels (mmol/L) in rats treated with selenium yeast

	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	**Urea** (mmol/L)
Group I (0.1mg/kg Selenium)	141.40 ± 0.60	4.06 ± 0.11	100.40 ± 0.68	24.20 ± 1.36	2.42 ± 0.15
Group II (0.2mg/kg Selenium)	137.60 ± 0.51●	4.46 ± 0.14	98.00 ± 0.63●	23.00 ± 0.70	2.42 ± 0.19
Group III (1mg/kg GBA)	139.80 ± 1.24	4.18 ± 0.18	98.00 ± 0.83‡	23.20 ± 1.86	2.74 ± 0.30
Group IV (normal control)	139.60 ± 0.68	4.22 ± 0.09	98.20 ± 0.66	24.00 ± 0.63	2.70 ± 0.48
Group V (diabetic control)	143.00 ± 0.45	4.18 ± 0.15	103.20 ± 0.58†	25.30 ± 0.50	2.70 ± 0.16
Group VI (300mg/kg Aspirin)	139.60 ± 1.03	4.16 ± 0.23	99.20 ± 1.50	22.80 ± 0.80	2.96 ± 0.30
Group VII (120mg/kg Ibuprofen)	138.60 ± 1.14 β	4.46 ± 0.08	98.20 ± 1.28 β	23.20 ± 0.37	2.74 ± 0.16

†*P<0.05* Group V vs. IV; ● *P<0.05* Group II vs. V; ‡ *P<0.05* Group III vs. V; α *P<0.05* Group VI vs. V; β *P<0.05* Group VII vs. V; ∞ *P<0.05* Group I vs. V; ns= non-significant

Na^+ = sodium ion
K^+ = potassium ion
Cl^- = chloride ion
HCO_3^- = bicarbonate ion

4.4 Changes in Serum Liver Enzymes and the Inflammation Biomarker (TNF-α) in Rats Treated with Selenium Yeast for the Period of Four Weeks in Streptozotocin Induced Diabetes

Figure 4.4 showed that only activities of serum alanine aminotransferase (ALT), and aspartate aminotransferase (AST) and tissue necrosis factor-alpha (TNF-α) level were significantly ($P< 0.05$) different in diabetic control group when compared with the normal control group. AST and ALT activities significantly increased in Groups I, II, III, VI and VII while TNF-α level significantly decreased only in Groups III VI and VII. When compared with diabetic control group, alkaline phosphatase (ALP) activity did not show any significant difference across all the groups.

Table 4.3: Changes in serum liver enzymes (IUl/L) and the inflammation biomarker (TNF-α) in rats treated with selenium yeast

	AST (IU/L)	**ALT**(IU/L)	**ALP**(IU/L)	**TNF-α (**ng/ml)
Group I (0.1mg/kg Selenium)	$76.40 \pm 5.92^{\infty}$	$92.20 \pm 8.90^{\infty}$	94.00 ± 4.22^{ns}	1.30 ± 0.11^{ns}
Group II (0.2mg/kg Selenium)	$80.20 \pm 4.66^{\bullet}$	$98.60 \pm 5.22^{\bullet}$	96.60 ± 5.45^{ns}	1.34 ± 0.09^{ns}
Group III (1mg/kg GBA)	$83.20 \pm 1.01^{\ddagger}$	$98.40 \pm 3.47^{\ddagger}$	92.20 ± 3.81^{ns}	$1.26 \pm 0.09^{\ddagger}$
Group IV (normal control)	52.40 ± 1.03	57.20 ± 0.66	66.20 ± 2.76	0.62 ± 0.05
Group V (diabetic control)	$55.80 \pm 0.58^{\dagger}$	$61.20 \pm 1.11^{\dagger}$	82.20 ± 1.88^{ns}	$1.68 \pm 0.11^{\dagger}$
Group VI (300mg/kg Aspirin)	$85.20 \pm 3.04^{\alpha}$	$101.80 \pm 1.74^{\alpha}$	96.00 ± 7.22^{ns}	$1.16 \pm 0.06^{\alpha}$
Group VII (120mg/kg Ibuprofen)	$77.00 \pm 8.46^{\beta}$	$98.80 \pm 12.33^{\beta}$	89.80 ± 4.80^{ns}	$1.02 \pm 0.10^{\beta}$

†$P<0.05$ Group V vs. IV; ● $P<0.05$ Group II vs. V; ‡ $P<0.05$ Group III vs. V; α $P<0.05$ Group VI vs. V; β $P<0.05$ Group VII vs. V; ∞ $P<0.05$ Group I vs. V; ns= non-significant.

AST = Aspartate amino transferase
ALT = Alanine amino transferase
ALP = Alkaline phosphatase

4.5 Changes in Serum Triiodothyronine, Tetraiodothyroxine and Lipid Profile of Rats Treated with Selenium Yeast in Streptozotocin Induced Diabetes

Figure 4.5 showed that only serum total cholesterol, triglyceride and low density lipoprotein levels were significantly ($P < 0.05$) different in diabetic control when compared with normal control group. There was no significant difference when Groups I, II, III VI or VII are compared with diabetic control group, except for the serum level of triglyceride in Group II when compared to diabetic control group. The result of the serum thyroid hormone level(triiodothyronine, tetraiodothyroxine) showed no significant difference when compared with diabetic control group.

Table 4.4: Changes in serum Triiodothyronine, Tetraiodothyroxine and lipid profile after the treatments with selenium yeast for four weeks

	T3 (ng/ml)	T4 (ng/ml)	TC (nmol/L)	TAG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Group I (0.1 mg/kg Selenium)	0.90 ± 0.09 ^{ns}	70.40 ± 8.44 ^{ns}	2.26 ± 0.05	0.88 ± 0.6	0.72 ± 0.05 ^{ns}	1.36 ± 0.04
Group II (0.2 mg/kg Selenium)	0.84 ± 0.08 ^{ns}	76.80 ± 3.95 ^{ns}	2.26 ± 0.02	0.64 ± 0.2$^{\bullet}$	0.680.04 ^{ns}	1.45 ± 0.06
Group III (1 mg/kg GBA)	1.02 ± 0.10 ^{ns}	66.20 ± 7.69 ^{ns}	2.30 ± 0.07	0.84 ± 0.07	0.84 ± 0.04^{ns}	1.27 ± 0.09
Group IV (normal control)	1.28 ± 0.07	76.60 ± 3.39	1.90 ± 0.04	0.60 ± 0.03	0.64 ± 0.05	1.14 ± 0.07
Group V (diabetic control)	1.10 ± 0.17 ^{ns}	73.20 ± 5.80 ^{ns}	2.42 ± 0.12†	0.96 ± 0.10†	0.72 ± 0.09 ^{ns}	1.51 ± 0.06†
Group VI (300 mg/kg Aspirin)	0.84 ± 0.13 ^{ns}	63.80 ± 6.57 ^{ns}	2.40 ± 0.09	1.00 ± 0.09	0.78 ± 0.12^{ns}	1.40 ± 0.10
Group VII (120 mg/kg Ibuprofen)	0.96 ± 0.15 ^{ns}	67.00 ± 3.96 ^{ns}	2.32 ± 0.09	0.86 ± 0.02	0.90 ± 0.04^{ns}	1.25 ± 0.06

†*P<0.05* Group V vs. IV; ● *P<0.05* Group II vs. V; ‡ *P<0.05* Group III vs. V; α *P<0.05* Group VI vs. V; β *P<0.05* Group VII vs. V; ∞ *P<0.05* Group I vs. V; ns= non-significant

HDL = High density lipoprotein T_3 = Triiodothyronine T_4 = Tetraiodothyroxine
TC = Total cholesterol LDL = Low density lipoprotein TAG = Triacylglycerol

CHAPTER FIVE

5.0 DISCUSSION

The burden of the high morbidity and mortality associated with DM (WHO, 2010) on public healthcare services cannot be over-emphasized. Diabetes mellitus, a common metabolic disorder characterized by hyperglycemia due to an absolute or relative insulin deficiency (Dahiru *et al.,* 2008; WHO, 2010) is associated with a wide-spectrum of metabolic dysfunctions that have been strongly correlated with the elevated morbidity/mortality due to DM. These associated metabolic dysfunctions most often include amongst others; hyperlipidemia and fatty liver, compromised endogenous oxidative stress defense system, and elevated serum level of inflammatory biomarkers and electrolytes imbalance (Sima, 2010).

Several studies have reported on the beneficial effect of selenium in DM but most of the studies have been controversial without prospective outcomes. However, it has been suggested that selenium possesses properties that may provide protection against a wide-spectrum of diabetic complications though; it appears to be a double-edged sword in the pathologies of DM (Rocourt and Cheng, 2013). This study highlights the ameliorative effects of selenium yeast on some of the aforementioned metabolic dysfunctions that have been shown to be central and major drivers in the etiology of DM and its complications (Sima, 2010) in STZ-induced diabetes in Wister rats.

5.0.1 Induction of hyperglycaemia

The data generated from the present study showed that blood plasma glucose levels were significantly higher in all the groups treated with STZ (i.e. Groups I, II, III, V, VI and VII) when compared with the normal control group (i.e. Group IV) at the beginning of WK0

before the commencement of treatment with selenium yeast and the standard drugs used in the study (see figure: 3). This results suggests that the STZ used for the study is potent and of analytic grade and that the diabetes animal model used for the study is suitable for testing the effects of selenium yeast on the biological markers of metabolic dysfunctions in DM assessed in this study. In addition, reviews on the use of animal models in diabetes research have shown that STZ-induced diabetes is appropriate to use when testing drugs or therapies where the main mechanism of action is lowering blood glucose in a non-beta-cell-dependent manner through the amelioration of the metabolic dysfunctions associated with DM (Jederstrom *et al.,* 2005; Sheshala *et al.,* 2009). This further supports the choice of using STZ-induced diabetes animal model to investigate the ameliorative effects of selenium yeast on metabolic dysfunctions in this study.

Streptozotocin (STZ) (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is synthesized by *Streptomycetes achromogenes*. After i.p. or i.v. administration of STZ in animal models, it enters the pancreatic beta cell through the Glut-2 transporter and causes alkylation of the DNA (Szkudelski, 2001) and subsequent activation of PARP which leads to NAD+ depletion, a reduction in cellular ATP and subsequent inhibition of insulin production (Sandler and Swenne, 1983). In addition, STZ is a source of free radicals that can also contribute to DNA damage and subsequent cell death. STZ tends to be administered as a single high dose or as multiple low doses. A single high dose in rats 35–65 mg/kg (Srinivasan and Ramarao, 2007) leads to a rapid ablation of the beta cells and hyperglycaemia (Grossman *et al.,* 2010). In this study, hyperglycaemia was induced by a single intraperitoneal injection of 60 mg/kg body weight dose of streptozotocin dissolved in 0.1ml fresh cold citrate buffer pH 4.5 into 16 h-fasted rats.

5.0.2 Effect of selenium yeast on blood plasma glucose level

The results of the present study showed that blood plasma glucose level in the diabetic control group was significantly higher when compared with the normal control group in the entire four-weeks of the study. The effect of selenium yeast on blood plasma glucose level was determined by comparing the values in the diabetic control group (i.e. Group V) with the values in Group I (i.e. 0.1 mg/kg selenium yeast group) and in Group II (i.e. 0.2 mg/kg selenium yeast group). The results showed that, there was no significant difference in blood plasma glucose levels in Group I when compared with Group V. However, blood plasma glucose levels were significantly lower in the 0.2 mg/kg selenium group (i.e. Group II) at WK1 and WK3 when compared with the diabetic control Group.

Similarly, the lowering effect of glibenclamide on blood plasma glucose level in the study, determined by comparing the values in the diabetic control group with the group that received 1 mg/kg/day/4weeks of glibenclamide (i.e. Group V vs. Group III) showed that glibenclamide significantly lowered blood plasma glucose levels at WK1 and WK3 of the treatments. In addition, the comparison of the blood plasma glucose levels of Group VI that (received 300 mg/kg/day/4weeks of aspirin) and VII that (received 120 mg/kg/day/4weeks of Ibuprofen)with that of diabetic control showed that the anti-inflammatory drugs, aspirin and ibuprofen also possess significant blood plasma glucose lowering effect only at WK3. Furthermore, between groups' comparison showed that there were no significant differences in the hypoglycaemic effect of aspirin, ibuprofen, glibenclamide and selenium yeast. These results put together suggest that 0.2 mg/kg and not 0.1 mg/kg of selenium yeast possesses hypoglycaemic property that is comparable to the hypoglycaemic property of the oral-hypoglycaemic drug, glibenclamide and the anti-inflammatory drugs, aspirin and ibuprofen

but with earlier onset time than the anti-inflammatory drugs. Generally, the hypoglycaemic effect of selenium yeast observed in the study appears to be in disagreement with the results of related studies that suggested that Se could cause glucose disturbance and increase the risk for DM (Bleys *et al.,* 2007; Laclaustra *et al.,* 2009: Rocourt and Cheng, 2013).

Glibenclamide is a well-known oral hypoglycaemic drug that belongs to the family of the sulfonylureas a first generation anti-diabetic drug; it elicits its hypoglycaemic effect mainly by acting on ATP-sensitive potassium channel in pancreatic beta-cells. The pain killer effect of the anti-inflammatory drugs are best known for their effects on the two cyclooxygenase enzymes (COX1 and COX2), but in addition, anti-inflammatory drugs could specifically inhibit the protein I-kappa-β-kinase beta (IKK-beta). This kinase is used for its role in the cascade of signals that activate the nuclear factor kappa-b (NF-kappa-B) family of cellular genes which regulate inflammatory and immune responses. Now, it turns out that IKK-beta also works in another pathway to contribute to insulin resistance by interfering with insulin signalling. Therefore, inhibition of this protein by anti-inflammatory drugs may contribute to the hypoglycaemic property of this class of pain killer (Hammadi *et al*., 2012).

The effect of selenium yeast on blood plasma glucose levels have been reported in several studies on both human and experimental animal, however, the overall results are controversial. Conflicting results were reported on the prevalence of diabetes in correlation with serum selenium levels (Czernichow *et al.,* 2006; Stranges *et al.,* 2007), Similarly, studies on the protective effect of long-term selenium supplementation reported that selenium does not seem to prevent diabetes or improve blood plasma glucose levels and that it may increase risk for the disease (Bleys *et al.,* 2007; Laclaustra *et al.,* 2009). However, evidence from other studies suggests that selenium could enhance insulin sensitivity and improve

blood plasma glucose levels by mediating insulin-like actions (Stapleton, 2000; Mueller and Pallauf, 2006) and by acting via several other mechanisms, including detoxifying liver enzymes, exerting anti- inflammatory effect, and providing antioxidant defense to elicits its anti-diabetic effect, however, the mechanisms by which Se exerts these beneficial effects are still not yet fully understood (Thomson, 2004; Yiming *et al.,* 2005; Hafez *et al.,* 2012; Rocourt and Cheng, 2013). Therefore, the present study further examined the effect of selenium yeast on liver enzymes, inflammatory, oxidative stress and lipid peroxidation biomarkers, and lipid profile in STZ-induced diabetes rat model to determine the probable mechanisms via which Se is eliciting the hypoglycemic effect observed in the study.

5.0.3 Effect of selenium yeast on serum liver enzymes

The doses of selenium yeast used in the present study (i.e. 0.1 and 0.2 mg/kg/day for 4 weeks) were carefully selected within the range of effective doses obtained in related studies that is usually within the ranges of 0.1-1 mg/kg or higher in some cases, how the range of doses used in the present study may affect liver enzymes and function has not been reported, moreover abnormal elevation of certain liver enzyme has been positively correlated with increased incidence of diabetic complications of the liver. Diabetic liver complications are associated with changes in serum levels of the liver enzymes and it is commonly used to predict pathologies of the liver. The enzymes include among others alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) (Harris, 2005; Singh *et al.,* 2011). The activities of the serum liver enzymes were assessed in the present study.

The results of the study showed that there was no significant difference in AST, ALT and ALP in normal control, when compared with diabetic control Group. However, the activities of liver enzymes AST, and ALT, but not ALP, were significantly higher in all the groups

treated with STZ and selenium or standard drugs when compared with Group V (i.e. diabetic control group).

Alanine transaminase (ALT), also called serum glutamic pyruvate transaminase (SGPT) or alanine aminotransferase (ALAT) is an enzyme present in hepatocytes (liver cells) ALT is released into the circulation when hepatocytes are damaged. ALT rises dramatically in acute liver damage such as hepatotoxicity of crude plant extract or viral hepatitis or acetaminophen drug-overdose (Harris, 2005; Singh *et al.,* 2011). Aspartate transaminase (AST) also called serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells, cardiac and skeletal muscle and is therefore not specific to the liver (Harris, 2005; Singh *et al.,* 2011). Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver (Harris, 2005; Singh *et al.,* 2011).

Based on findings of the study, the groups treated with selenium or standard drugs had elevated serum activities of AST and ALT, liver enzymes that are suggestive of liver damage, probably due to the treatment. However selenium has been reported to protect against liver damage (Al-Othman *et al.,* 2011; Yosefi nasab *et al.,* 2012) while the standard drugs used in the study have well defined metabolic pathways with minimal negative side effect on the liver, in cognisance of which it will be difficult to conclusively conclude that the treatments were responsible for the elevated AST and ALT liver enzymes in the study. However, it can be suggested that neither selenium nor glibenclamide or any of the anti-

inflammatory drugs used in the study is protective against the elevation of serum AST and ALT levels in STZ-induced DM.

A plausible explanation for the observed elevated serum AST and ALT levels is the effect of STZ on hepatic function and morphology. In a similar study that used a single dose of STZ (45 mg/kg, body weight.) given intraperitoneally in sodium citrate buffer at pH-4.5 for the induction of hyperglycaemia in rats, increased levels of AST, ALT and ALP were observed in the liver and serum of the STZ-treated rats after 2, 4, 8 and 12 weeks post-treatment (Zafar *et al.,* 2009). Consistent results have also been reported in several other studies (Zhang *et al.,* 1995; Isogai *et al.,* 1997; Salimuddin *et al.,* 2008). It was suggested that STZ is able to produce alteration in hepatic function and morphology by the inactivation of cytosolic AST in diabetic rat tissues via a glycation reaction, accompanied by impairment in glucose utilization in the STZ-induced diabetes animal model.

5.0.4 Effect of selenium yeast on oxidative stress biomarkers (OSTB)

The oxidative stress biomarkers (OSTB) assessed in the present study includes SOD, CAT and GPx. The results of the study showed that all the OSTB assessed were significantly lower in the positive control group when compared with normal control group and were all significantly increased in Group VII when compared with the diabetic control group. However, there were no significant differences in any of the OSTB assessed when Groups I or II was compared with the positive control group.

Oxidative stress (OST) depicts the existence of products called free radicals (molecules possessing an unpaired electron) and reactive oxygen species (ROS), which are formed in normal physiology but become deleterious when not being quenched by a cascade of inherent

antioxidants systems (AOS) that maintains a delicate balance of OST/AOS to ensure physiological homeostasis. Oxidative stress can result from either an overproduction of ROS or from the inactivation of the AOS, thus shifting the OST/AOS balance in favor of stress with overproduction of ROS (Dröge, 2001; Fang *et al.,* 2002). Nevertheless, humans have evolved with antioxidant systems to protect against deleterious productions of free radicals. These systems include some antioxidants produced in the body (endogenous) and others obtained from diet (exogenous) (Kangralkar *et al.,* 2010; Vijayakumar *et al.,* 2012). Endogenous antioxidants systems are of particular importance in DM (Vijayakumar *et al.,* 2012), it comprise of mainly two categories of systems including; enzymatic (e.g. superoxide dismutase SOD, glutathione peroxidase GPx and catalase) and non-enzymatic systems (e.g. glutathione GSH, vitamins A, C and E). Some are located in cell membranes, others in the cytosol and in blood plasma (Culotta, 2000; Wiernsperger, 2003). These biomolecules also referred to as oxidative stress biomarkers (OSTB) are used as measures of the extent of oxidative stress in a biological system (Kangralkar *et al.,* 2010; Vijayakumar *et al.,* 2012).

The low levels of the OSTB observed in the positive control group in the study appear to be consistent with the results of several other studies that showed increased formation of ROS and decreased levels of OSTB in DM (Miyajima *et al.,* 2003; Vijayakumar *et al.,* 2012). Many diabetic complications are thought to be caused by oxidative damage and decreased antioxidant protection. In DM the endogenous AOS is compromised (Santini *et al.,* 1997; Kashiwagi, 2001; Martin-Gallan *et al.,* 2003; Vijayakumar *et al.,* 2012) and there is increased formation of ROS (Ahmed, 2005; Goldlin *et al.,* 2006) and decreased levels of OSTB (Miyajima *et al.,* 2003; Vijayakumar *et al.,* 2012). The (ROS) oxidizes various types of biomolecules and leads to cellular lesions by damaging DNA or stimulating apoptosis for cell

death which eventually results into various types of metabolic perturbations in DM (Ahmed, 2005; Goldlin *et al.,* 2006). Moreover, hyperglycemia increases superoxide anion and other reactive oxygen species (ROS) production in patients with diabetes

Experimental data suggest that supplementation with antioxidants, such as selenium, an essential component of antioxidant enzymes especially in GPx structure (Zachara *et al.,* 2006), at the nutritional level, could delay the development of type 2 diabetes by decreasing oxidative stress via induction of increased expression and activities of endogenous antioxidant enzymes (Can *et al.,* 2005; Steinbrenner and Sies, 2009). The results of the present study did not show any improvement effect of 0.1 or 0.2 mg/kg selenium yeast on OSTB in STZ-induced diabetic rat, it was expected that the activities of the endogenous antioxidant enzymes referred to as OSTB assessed will be increased in the selenium groups, given that selenium's antioxidant capacity has been shown to help the pathology of type 2 diabetes in several ways, including preventing oxidation of low density lipoprotein (Gebre-Medhin *et al.,* 1988) reducing malondialdehyde levels (Skripchenko *et al.,* 2003) and increasing the capacity of endogenous antioxidant enzymes (Gebre-Medhin *et al.,* 1988).

Studies in the past including this present study have mainly focused on the measurement of GPx activity rather than the concentration of GPx and this has led to lack of consistency in the outcomes of several studies. The complexity of the interaction between selenium, plasma GPx concentration and activity, though usually ignored, has made the interpretation of related studies a challenging task and the outcome grossly controversial, this interaction cannot be excluded.

In a more precise context, a condition of sustained high OST as is the case with STZ-induced diabetes, leads to an adaptive up-regulation of plasma concentration of GPx to protect against the high OST insult and the plasma level of GPx increases due to up-regulated synthesis of GPx (Jacobson *et al.,* 2007). Under sustained condition of GPx up-regulation, GPx activity remains relatively static without significant changes (Jacobson *et al.,* 2007). Similar results have been shown in antioxidant status under extreme endurance stress in athletes (Finaudi *et al.,* 2006), in addition GPx activity and Se levels in runners before and after a marathon have been shown to remain relatively unchanged (Rokitzki *et al.* 1994). However, the up-regulation of GPx concentration and the demand for Se results into lower total plasma Se level and at the initial stage, a lower GPx activity (Kadrabova *et al.,* 1996) as was observed in this study. This is usually followed over several days by a slow progressive recovery of plasma Se level and GPx activity (Sandre *et al.,* 2006). Selenium supplementation as earlier mentioned, induces increased expressions and activities of endogenous antioxidant enzymes (Can *et al*., 2005; Steinbrenner and Sies, 2009) has been shown to demonstrate faster recovery and elevated levels of both GPx activity and plasma Se in rats (Sandre *et al.,* 2006). The 0.1 and 0.2 mg/kg of selenium used for this study did not demonstrate significant recovery of GPx activity in the Se groups but the values appears to be higher than the values for the diabetic control though not statistically significant. Two important factors may be responsible for the lack of significant differences: 1) the concentration of Se yeast used in the study 2) insufficient recovery time.

The concentration of Se used in most of the studies that demonstrated the antioxidant capacity of Se in rat subject to oxidative insults was high than the concentration (0.1 and 0.2 mg/kg/b.wt) used in the present study. Both Yosefi nasab *et al.* (2012) and Ghaffari *et al.*

(2011) used 0.5 mg/kg/b.wt for 9 and 5 weeks respectively in their studies and observed significant increases in GPx activity. Haidara *et al.* (2009) and Yanardag *et al.* (2007) also used 0.5 mg/kg/b.wt for 5 weeks in their studies. Though there are studies that used similar doses as was used in this study but different routes of administration of Se. Selenium was administered by either gavage or incorporated into animal food and water, 0.1 mg/kg was used by both *Al-Othman et al.* (2011) and Pavlovic *et al.* (2001), and increase in GPx activity was observed in the treated groups of these studies but the induction of OST in these studies were through the effect of the organophosphate insecticide, malathion and an environmental pollutant Cadmium (Cd) respectively, while in the present study, STZ was used to target beta-pancreatic cells to induce hyperglycemia and its associated OST. Considering the differences in method of induction of OST in these studies the results cannot be comparable. The effect of STZ on the expression of selenium containing enzymes may also contribute to the difference in the results of this study. A single dose of STZ (50 mg/kg body weight) is enough to cause significant changes in the expression of selenium-containing antioxidant enzymes in rats (Can *et al.*, 2005). Finally, an extension of the time allotted for Se supplementation may be helpful in clarifying the implication of insufficient time allowed for recovery from the effect of adaptive up-regulation of GPx concentration. These findings put together does not provide sufficient evidence to conclude that the Se yeast used in the study did not elicit an antioxidant effect however further analysis of the effect of the Se yeast on markers of lipid peroxidation should give an insight into the antioxidant capacity of the Se yeast.

5.0.5 Effect of selenium yeast on the serum levels of lipid profile

The assessment of lipid profile in the study includes the determination of serum levels of TC, TG, LDL and HDL. The results showed that the serum levels of TC, TG and LDL were significantly increased in the diabetic control group when compared with the normal control group and the effect of selenium yeast was only seen in the level of TG in Group II (0.2 mg/kg selenium-Yeast). It was observed to be significantly lower in Group II when compared with the diabetic control group. There was no significant difference in the serum level of HDL across the entire group. The results of the study is consistent with the increase in serum levels of lipid profile reported in several studies on lipid profile changes in STZ-induced diabetes in rats (Prohp *et al.,* 2012; Yakubu *et al.,* 2013).

The increased risk of cardiovascular related diseases (CVDs) and the high mortality rate associated with CVDs in diabetes mellitus consists of multiple factors. Diabetes-related changes in serum lipid levels are among the key factors (Chen and Tseng, 2013; Firdous, 2014). The lipid changes associated with diabetes mellitus is characterized by high plasma triglyceride (TG) concentration, low HDL cholesterol concentration and increased concentration of LDL-cholesterol particles (Chen and Tseng, 2013; Firdous, 2014), which has been attributed to increased free fatty acid flux (lipogenesis) that is secondary to insulin deficiency and insulin resistance (Chen and Tseng, 2013; Firdous, 2014). Exposure of β-cells to elevated lipid levels (lipotoxicity) leads to β-cells failure and contributes to insulin secretion dysfunction as seen to occur during diabetic dyslipidemia, Obesity and peripheral insulin resistance (Kashyap *et al.,* 2003; Muoio *et al.,* 2008; Poitout *et al.,* 2008; Ryan *et al.,* 2011). Therefore, the regulation of serum lipid profile is of high prognostic value in the management of DM and reduction of the risk of cardiovascular related diseases (CVDs) and

the high mortality rate associated with it (Del Pilar and Goldberg, 2005; Chahil and Ginsberg, 2006). However, the precise pathogenesis of diabetic dyslipidaemia is not well known; nevertheless, a large body of evidence suggests that insulin resistance has a central role in the development of this condition (Chahil and Ginsberg, 2006; Mooradian, 2009).

Given that the results of the present study showed that serum TG was significantly lowered by 0.2 mg/kg of Se yeast and low serum TG has been reported to reduce the risk of CVDs while high serum level of TG is classified as an independent predictor of CVDS (Chen and Tseng, 2013), the therapeutic benefits of the anti-lipidemic effect of Se yeast in DM cannot be over emphasized.

Currently, TG is considered a biomarker for CVD, although there is no strong evidence that TG can cause atherogenesis directly (Talayero and Sacks, 2011). Hypertriglyceridemia is known to be associated with increased levels of prothrombotic factors, such as fibrinogen and plasminogen activator inhibitors, and is related to the size and density of the LDL particle (Georgieva *et al.,* 2004). More importantly, hypertriglyceridemia is associated with some atherogenic remnant particles and the association between TG and coronary heart disease (CHD) is strong, probably even stronger than LDL-C and CHD. However, further studies with larger sample size and standard protocol are needed to clarify the role of TG in the development of CVD.

5.0.6 Effect of selenium yeast on serum level of the lipid peroxidation biomarker MDA

The results of the study showed that the lipid peroxidation biomarker assessed in the study, malondialdehyde (MDA) was significantly increased in the diabetic control group compared with the normal control group and significantly reduced in Groups II, III, VI and VII when compared with diabetic control group. Dyslipidaemia is not uncommon in DM both in humans (Singh *et al.,* 2012) and animal models (Shodehinde *et al.,* 2013). Among biological molecules lipids are the most susceptible to oxidative damage or peroxidation. Oxidative deterioration of polyunsaturated fatty acids (PUFA) which are present in abundance in cell membranes, initiates a self-perpetuating chain reaction that yields a wide range of cytotoxic products such as malondialdehyde (MDA), 4-hydroxynonenal, etc. Lipid peroxidation is a free radical-related process, which is potentially harmful because its uncontrolled, self-enhancing process causes disruption of membranes, lipids and other cell components (Mahboob *et al.*, 2005). The free radicals steal electrons from the lipids in the cell membrane, resulting in cell damage. Lipid peroxidation is a late event accompanying rather than causing final cell death. The end products of lipid peroxidation process are aldehydes, hydrocarbon gases and chemical residues including malondialdehyde. MDA is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation (Karataş *et al.,* 2006). Abnormally high levels of lipid peroxidation and the simultaneous decline of antioxidant defence mechanisms can lead to damage of cellular organelles and lead to oxidative stress (Mahboob *et al.,* 2005). Diabetes mellitus is characterised by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation (Mahboob *et al.,* 2005).

A marked decline in serum TG concentration in the 0.2 mg/kg Se yeast treated group is indicative of direct effect of the antioxidant capacity of Se on lipids and lipoproteins oxidation. This is in agreement with the elevated level of MDA in the diabetic control group and its reduction in the Se group observed in this study. As MDA reflects the sensitivity of lipoproteins to oxidation, a lower level of MDA in the Se group compared with diabetic control group would be indicative of the beneficial effect of selenium on the oxidizability of lipoproteins in DM. Finally, the ameliorative effect of Se yeast on the marker of lipid peroxidation in the study provides evidence that 0.2 mg/kg of the selenium yeast used for the study possesses antioxidant capacity and its effect on the other OSTB assessed in the study cannot be overruled.

5.0.7 Effect of selenium yeast on the inflammation biomarker Tissue Necrosis Factor-alpha

The inflammatory biomarker assessed in the study is TNF-α and the results showed that the serum level of the marker is significantly increased in the diabetic control group when compared with the normal control group. There is no significant difference in between Group I or II compared with the diabetic control group. However, TNF-α is significantly reduced in Groups III, VI and VII when compared with the positive control group. The results suggest that, inflammation is a risk factor for the development of DM (Vozarova *et al*., 2002; Thorand *et al*., 2003; Limbert, 2012) and 0.1 and 0.2 mg/kg of Se yeast does not possess anti-inflammatory property. This is in agreement with the elevated level of TNF-α in the diabetic control group and its reduction in Groups VI and VII treated with the anti-inflammatory drugs aspirin and ibuprofen respectively.

5.0.8 Effect of selenium yeast on serum electrolytes levels

The kidneys work to keep the electrolyte concentrations in the blood constant despite changes in the body. The serum concentrations of electrolyte and urea values are usually indicative of the renal functions or dysfunctions. The levels of electrolyte in the blood are the outcome of fine regulatory mechanism of ionic species and osmotic balance. This homeostasis is achieved by an interplay involving the kidney, the lungs and endocrine system (Tilkian *et al.*, 1979).

The concentrations of the serum electrolyte in this study provide good assessment of glomerular and tubular function. However the elevated levels of Na^+, and Cl^+, in the diabetic control suggest that the diabetic state may be associated with hyperosmolar impaired renal function. From the result obtain In untreated diabetes, analysis of result showed a decrease in the serum electrolyte concentrations of (Na^+, K^+, Cl, HCO_3^- and urea). This is consistent with reports of Eteng *et al*. (2008) and Ikpi *et al*. (2009). It is well known that in untreated diabetes (diabetic control) kidney function is compromised (Ikpi *et al*., 2009). Glycosuria, which causes dehydration via glucose osmotic diuresis, is accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates.

Treatment with 0.2mg/kg of Se-Y however, showed significant ($p<0.05$) decrease in plasma electrolyte concentrations (Na^+, Cl^-) caused by streptozotocin-induced diabetes. This is indicative of the ability of Selenium-Yeast to improve on the compromise of the kidneys and restore both acid-base balance and renal functions in experimental diabetes in rats. The present study is in agreement with the report of (Prohp and Onoagbe, 2014). Similarly, the group treated with 0.1 mg/kg Se-Y, showed an increase though not statistically significant when compared to the diabetic control group.

5.0.9 Effect of selenium yeast on serum T_3 and T_4

Thyroid hormones are insulin antagonist; both insulin and thyroid hormone are involved in celluar metabolism. Excess or deficit of any one can result in functional derangement of the other (Singh *et al.*, 2011). In animal, there is evidence from animal studies that the function of the thyroid gland itself may be altered in diabetes mellitus (Bagchi *et al*., 1981). T_3 production from peripheral T_4 mono-denomination is impaired in uncontrolled diabetes mellitus and this impairment is correlated with the impairment of glucose utilization. Plasma selenium levels are low with severe illness (Maehira *et al.,* 2002). Leading to low glutathione complex consisting of a selenium-dependent peroxidase (GSH-Px) activity and a redistribution of selenium occurs away from the liver to the muscles (Bates *et al.*, 200) therefore a decrease production and activity of the selenium-dependent deiodinase has been supposed leading to impaired T_4, and T_3 metabolism. (Mclver and Gorman, 1997).

The result obtain from the present study, shows decrease in T_3 and T_4 levels but it was not statistically significant when compared with the diabetic and normal control groups, also when compared with the different doses of selenium yeast (i.e. 0.1 and 0.2 mg/kg), it was not statistically significant. This result is in agreement with the studies of Rock *et al*. (2001).

The slight decrease in serum T_3 observed in the treated group with two doses of selenium yeast may be due to slight decrease in the T_4 level in the serum, since T_4 has to be converted to T_3 for the biological effect of the hormone to be manifested. In addition, the low T_3 may be due to deficient production of deiodinase enzyme, which plays a significant role in conversion of T4 to more metabolically active T_3 or due to STZ induced pathological damage to the liver source of the enzymes.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

In conclusion, the main findings in the study include:

1. The result of the study is in disagreement with the results of related studies that suggested that Se could cause glucose disturbance and increase the risk for DM. It was observed in the study that 0.2 mg/kg and not 0.1 mg/kg of selenium yeast possess hypoglycaemic property that is comparable to the hypoglycaemic property of the oral-hypoglycaemic drug, glibenclamide.
2. The likelihood of STZ-induced alteration in hepatic function and morphology that leads to increase in serum levels of liver enzymes is considered. However, neither selenium nor glibenclamide or any of the anti-inflammatory drugs used in the study was protective against elevations in serum levels of AST and ALT in STZ-induced DM.
3. The ameliorative effect of Se yeast on the marker of lipid peroxidation in the study provides evidence that 0.2 mg/kg of the selenium yeast used for the study possesses antioxidant capacity and it effect on the other OSTB assessed in the study cannot be overruled, though the results on the effect of the 0.2 mg/kg on the OSTB assessed does not provide sufficient evidence to conclude that the Se yeast used in the study did elicit an antioxidant, moreover the marked decline in serum TG concentration in the 0.2 mg/kg Se yeast treated group is indicative of direct effect of the antioxidant capacity of Se on lipids and lipoproteins oxidation. This is in agreement with the

elevated level of MDA in the diabetic control group and its reduction in the 0.2 mg/kg Se group observed in this study.

4. 0.2 mg/kg of Se yeast is anti-lipidemic in STZ-induced DM. Serum TG was significantly lowered by 0.2 mg/kg of Se yeast and low serum TG has been reported to reduce the risk of CVDs while high serum level of TG is classified as an independent predictor of CVDs (Chen and Tseng, 2013). The therapeutic benefits of Se yeast in DM and associated cardiovascular risk may be considered.

6.2 Recommendations and Future Research

Considering the outcome of the study it can be recommended that:

1. The hypoglycaemic effect of Se is further assessed in more detailed research design that looks at how Se affects a wider spectrum of regulatory molecules of glucose metabolism including not only insulin but also glucagon and intracellular regulators of insulin receptor sensitivity in diabetes animal models.
2. In the assessment of the antioxidant effect of Se, the determination of serum GPx should include not only GPx activity but also serum concentration of GPx as increase in GPx activity may not necessarily translate into an increased antioxidant capacity and vice versa.
3. The assessment of the protective effect of Se on liver enzymes in STZ-induced diabetes animal model may be misleading given the direct effect of STZ of serum levels of liver enzymes in this animal model therefore; it is recommended that the effect of Se on liver enzymes is assessed in diabetes animal models developed from non-chemical induction.

4. The protective effect of Se in diabetes is assessed in more detailed design that includes a wide range of doses of Se and varied routes of administration of Se.
5. The benefit of combined treatment with standard antioxidants such as vitamins C and E may be considered.
6. Considering the implication of the aforementioned adaptive up-regulation of plasma concentration of GPx to protect against the high OST insult as is mostly the case with DM it is recommended that sufficient recovery time is allowed in future research to determine the antioxidant benefits of Se supplementation in DM.

6.3 Contributions to Knowledge

1. From our findings, the selenium yeast significantly ($P < 0.05$) decreased the blood glucose level, when compared to the diabetic control group in streptozotocin induced diabetic Wistar rats.
2. Selenium yeast significantly ($P < 0.05$) decreased triacylglycerol (TG) when compared to the diabetic control group in streptozotocin induced diabetic Wistar rats.
3. Administration of selenium yeast also showed a significant ($P < 0.05$) decrease in lipid peroxidation biomarker, malondealdehyde (MDA) concentration, when compared to the diabetic control group.

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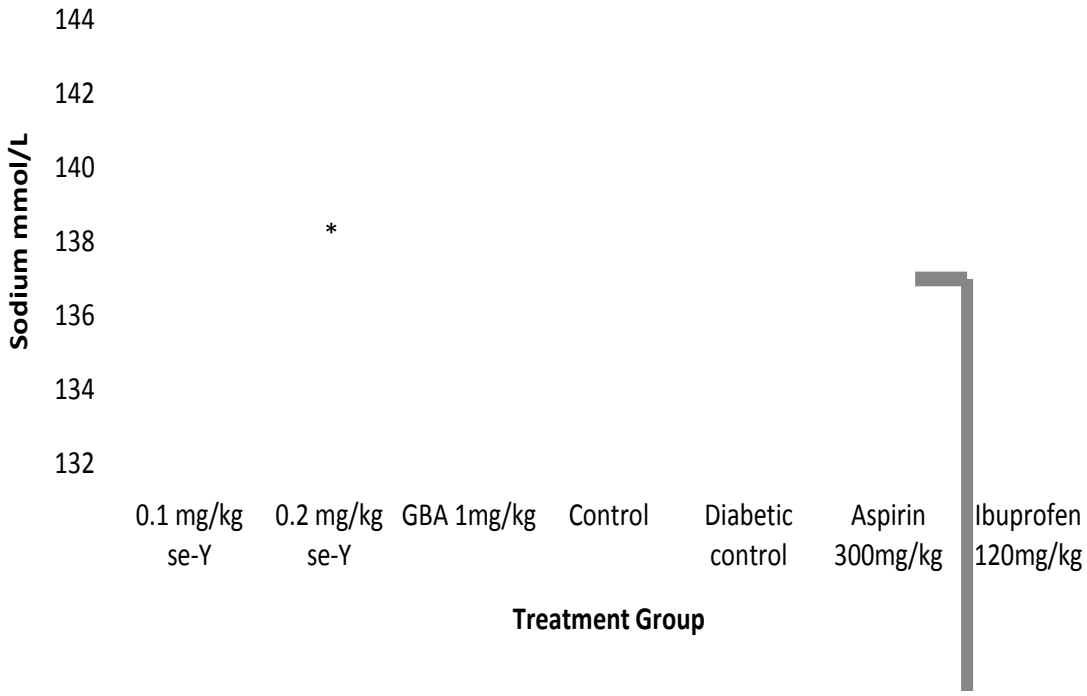
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APPENDICES



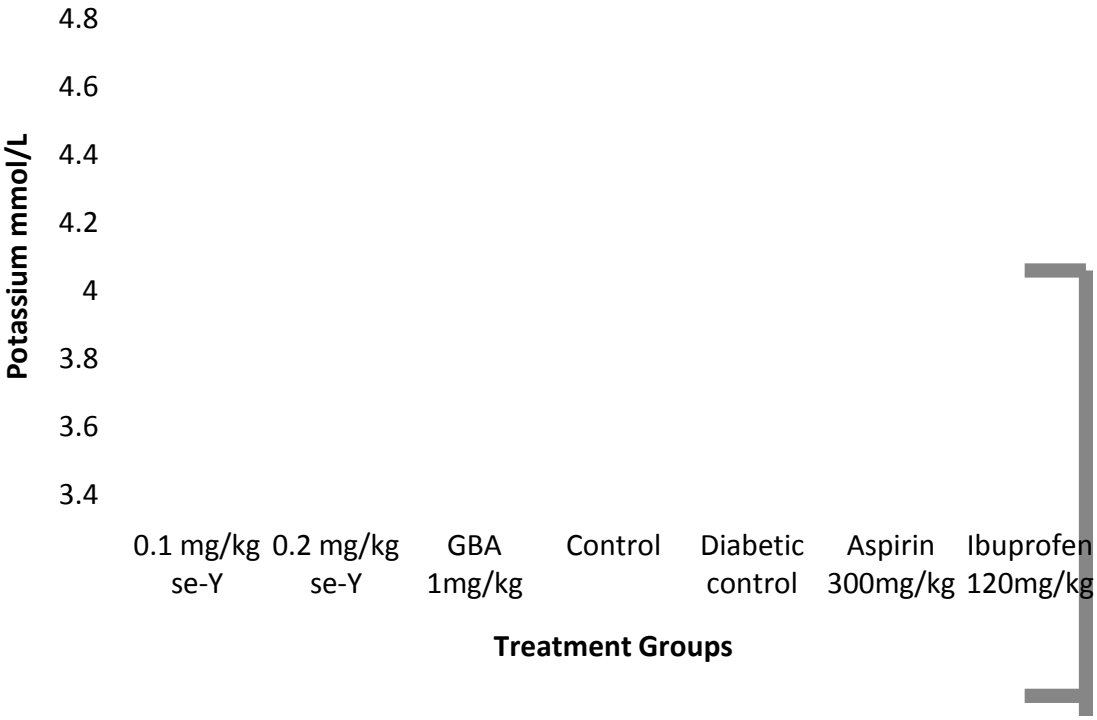
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 1: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on sodium ion concentration

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



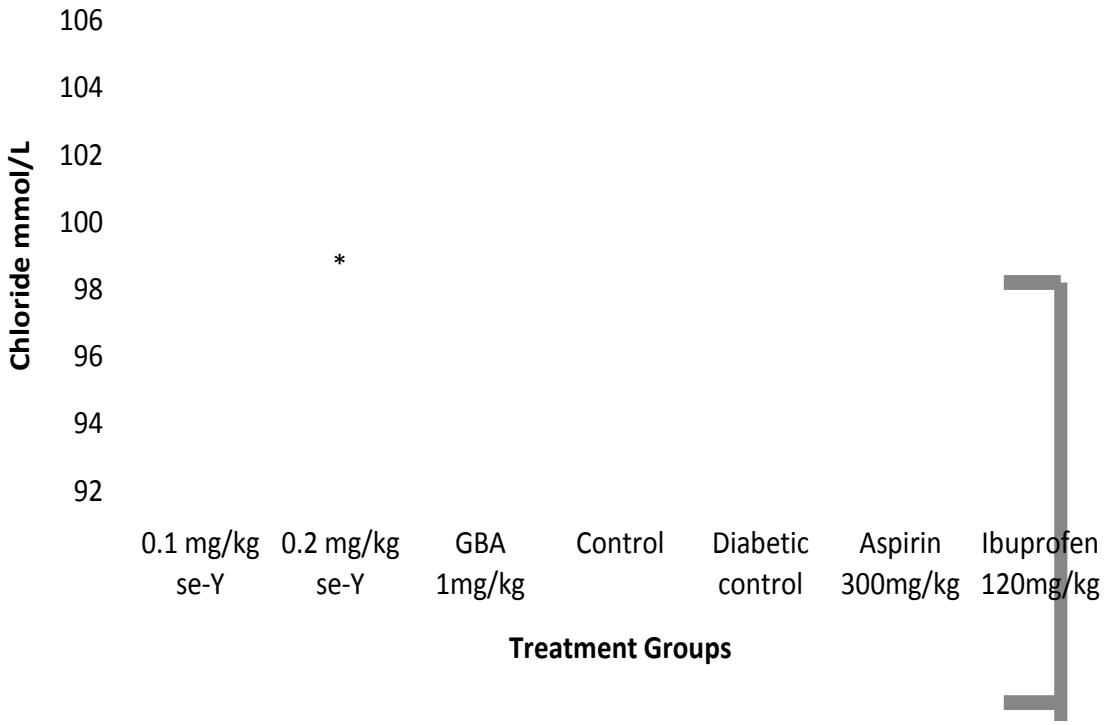
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 2: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on potassium ion concentration

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



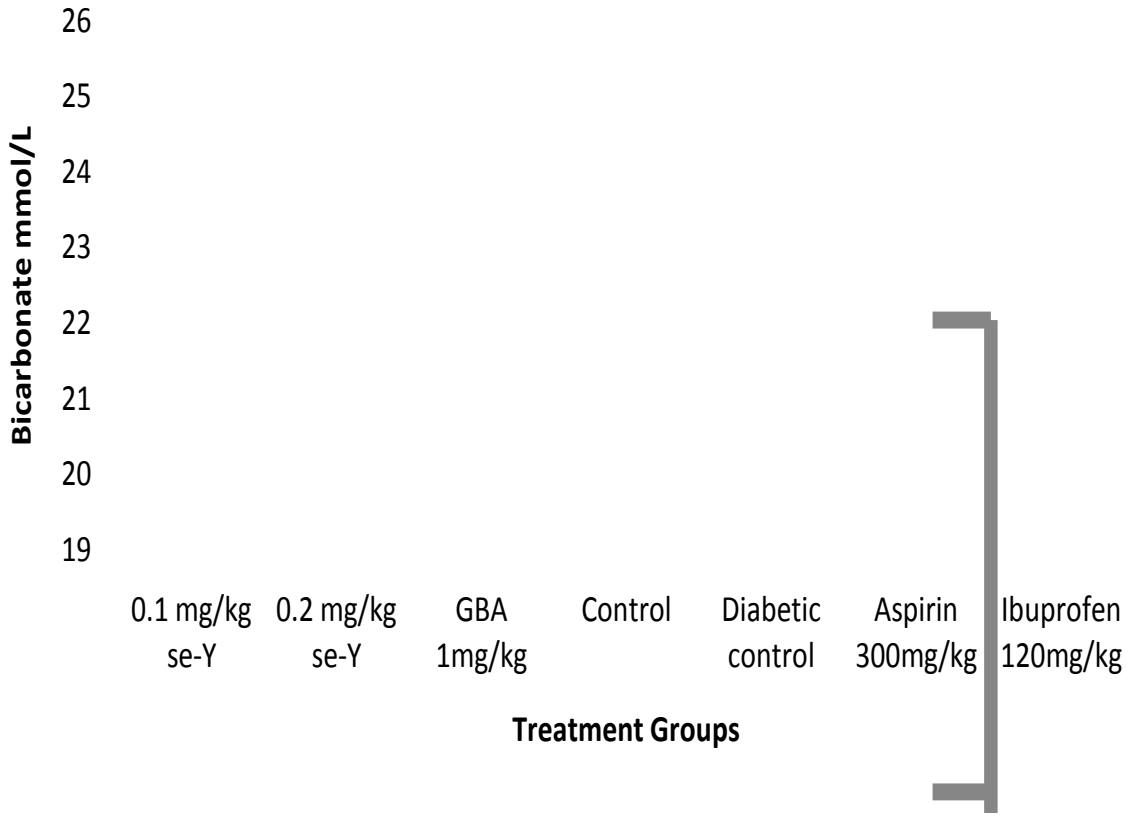
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 3: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Chloride ion concentration

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



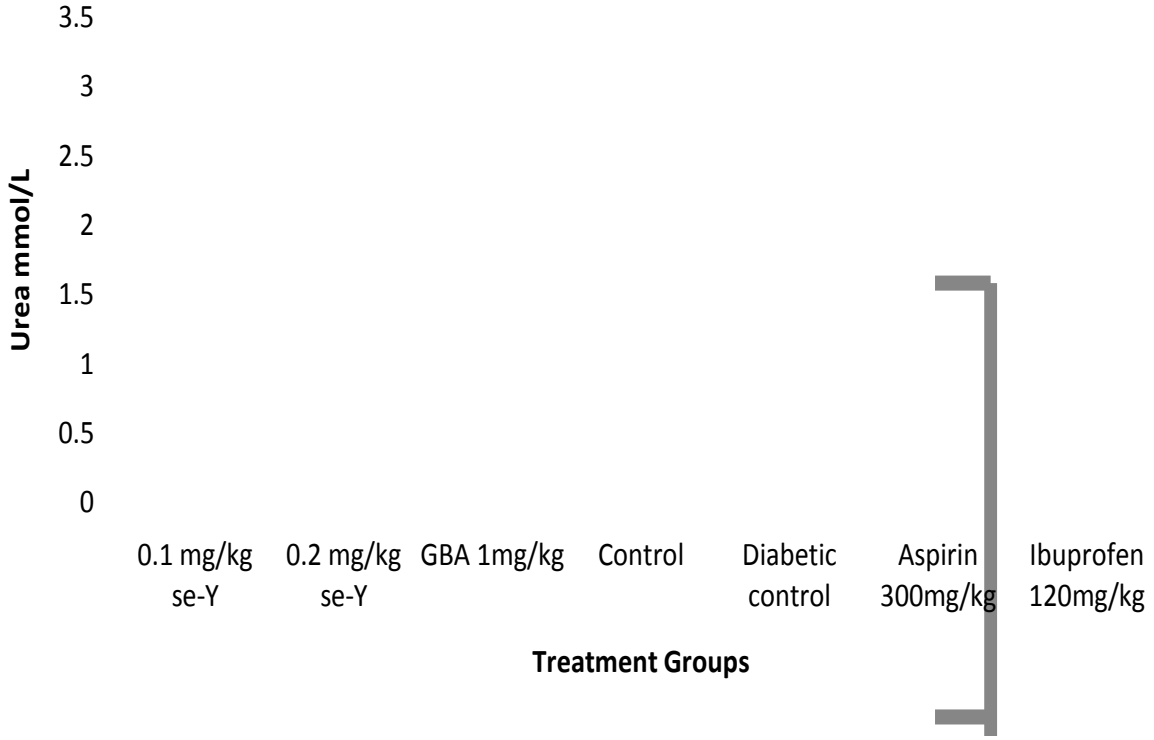
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 4: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Bicarbonate ion concentration

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



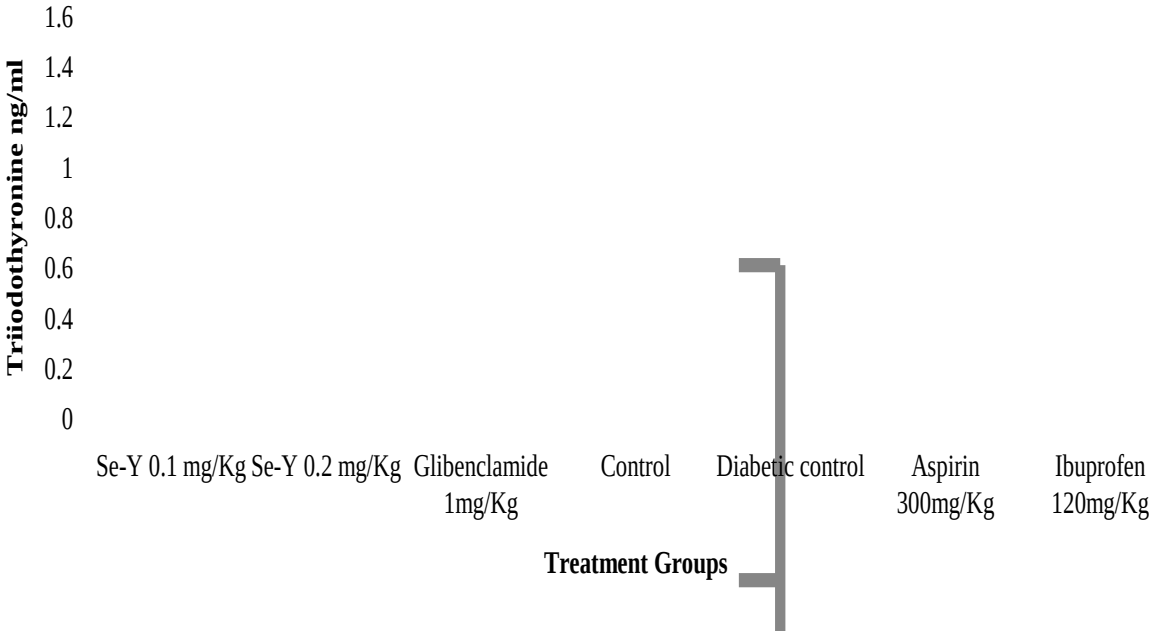
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 5: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Urea concentration

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



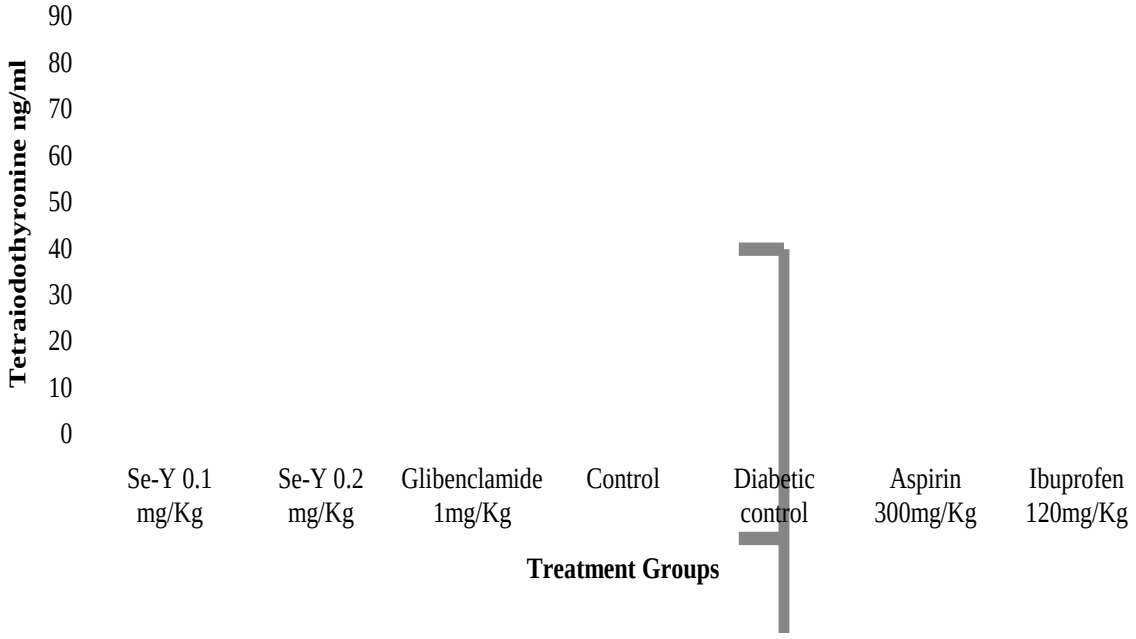
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 6: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Serum levels of T_3

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



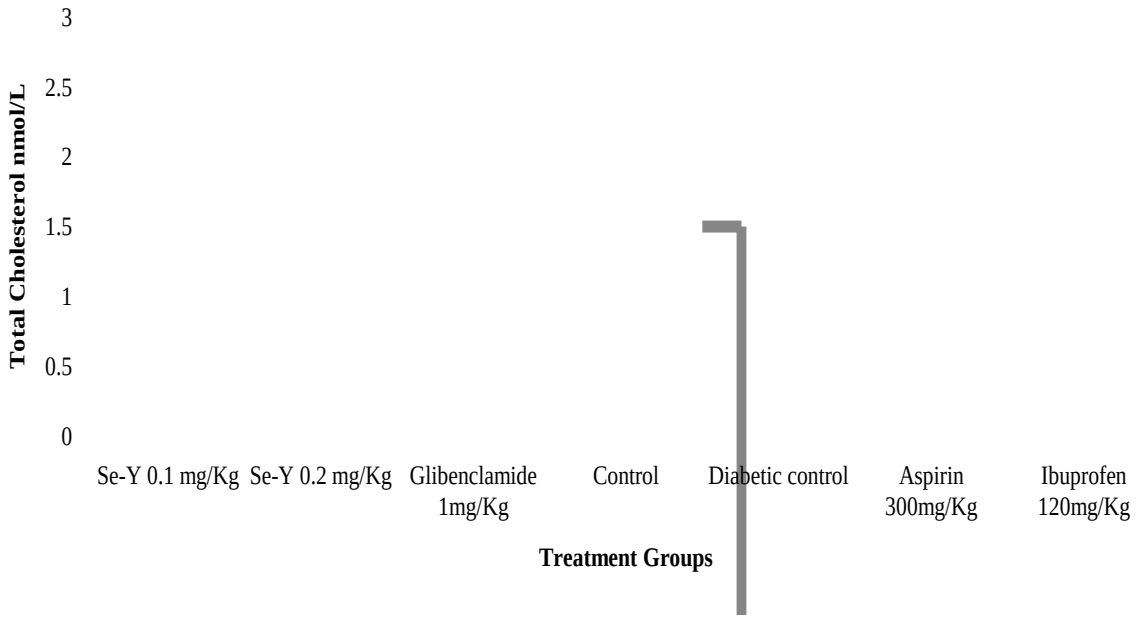
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 7: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Serum levels of T_4

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



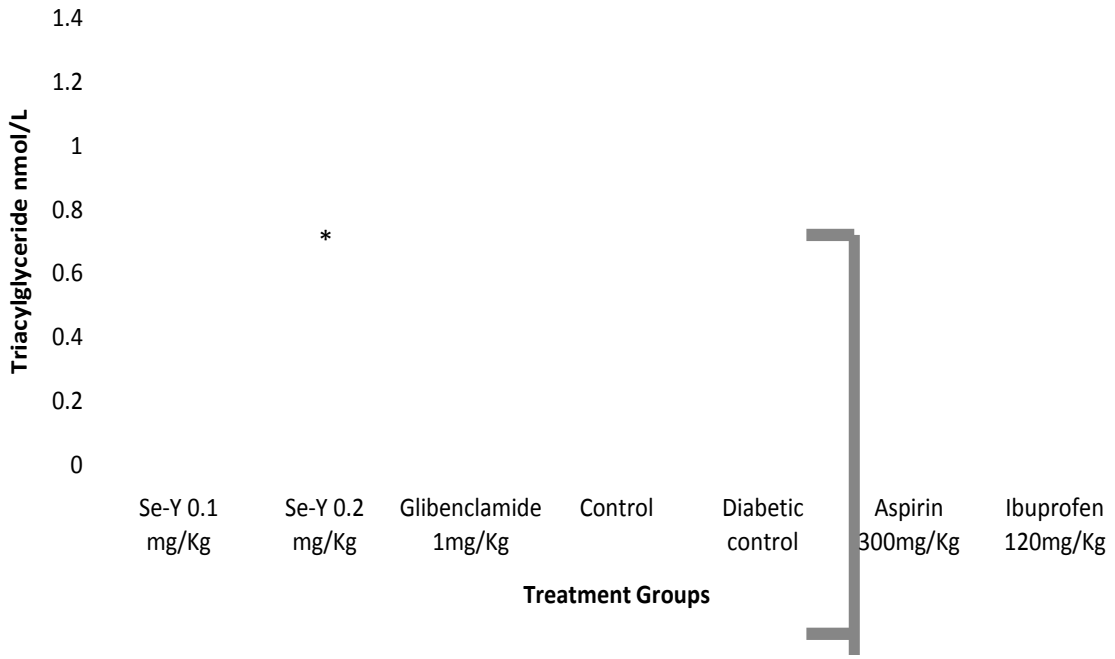
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 8: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Serum levels of Total cholesterol.

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



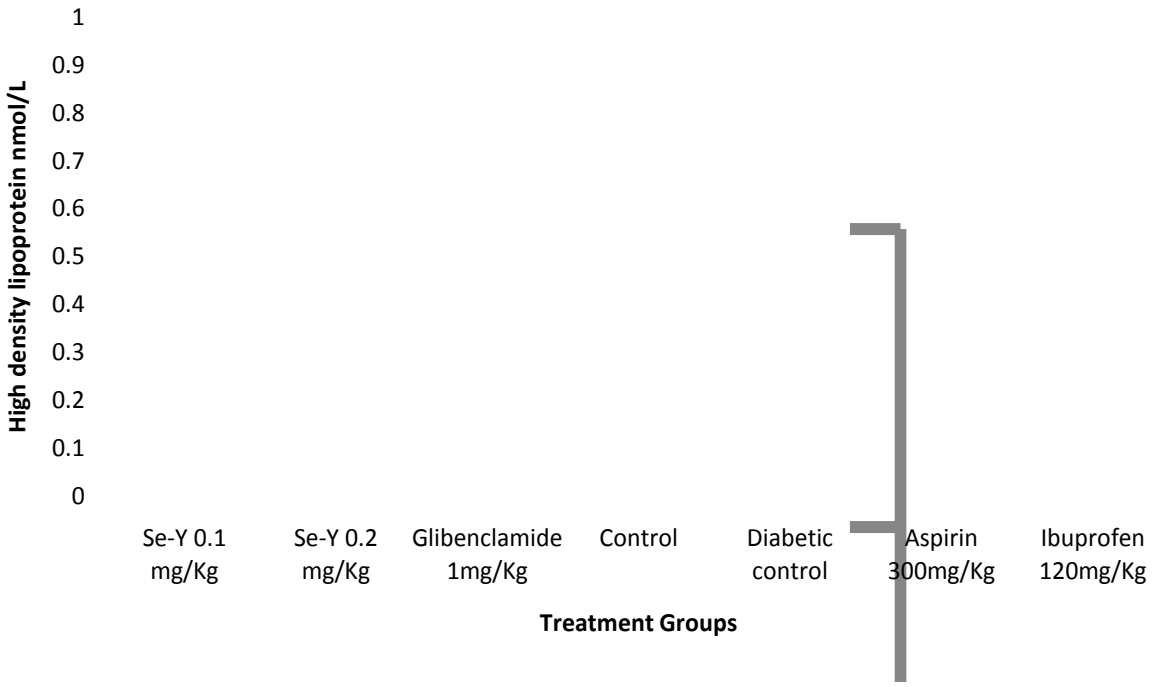
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 9: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Serum levels of Triacylglyceride.

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



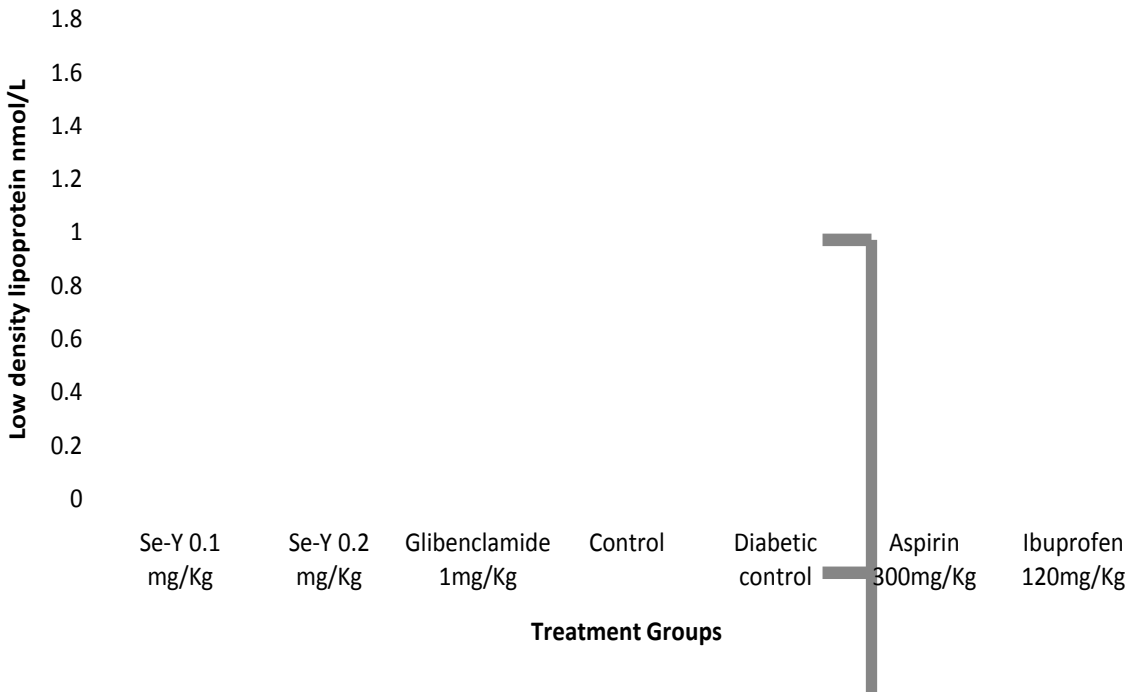
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 10: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Serum levels of High density lipoprotein (HDL)

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



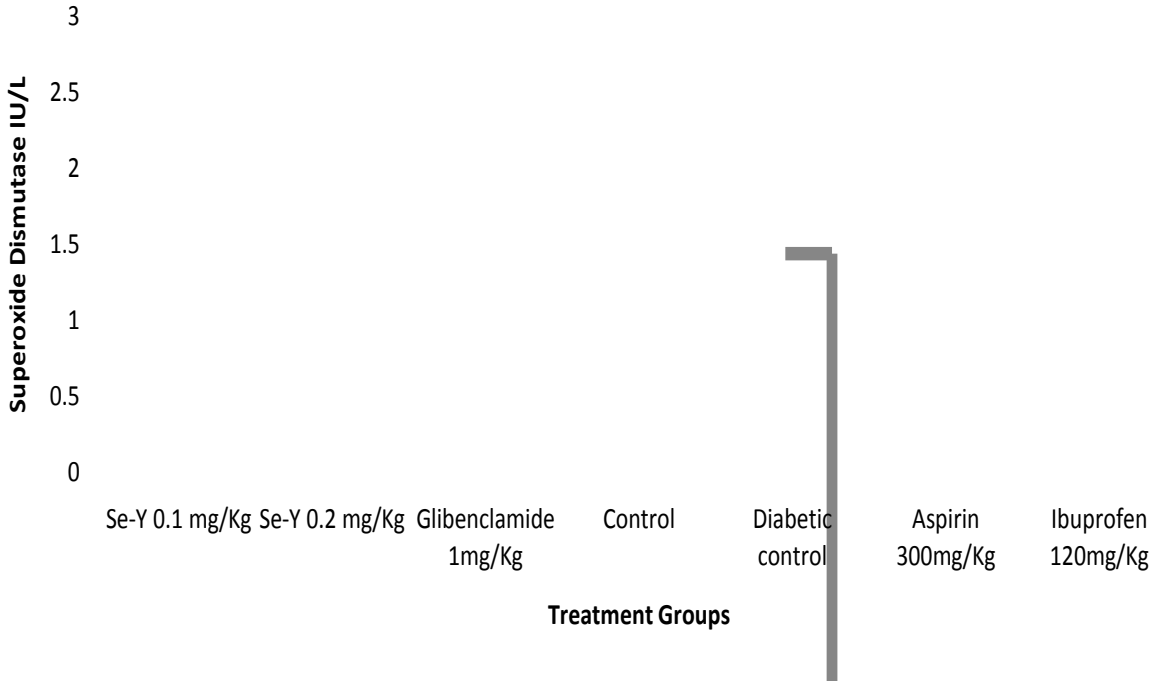
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 11: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Serum levels of Low density lipoprotein (LDL)

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



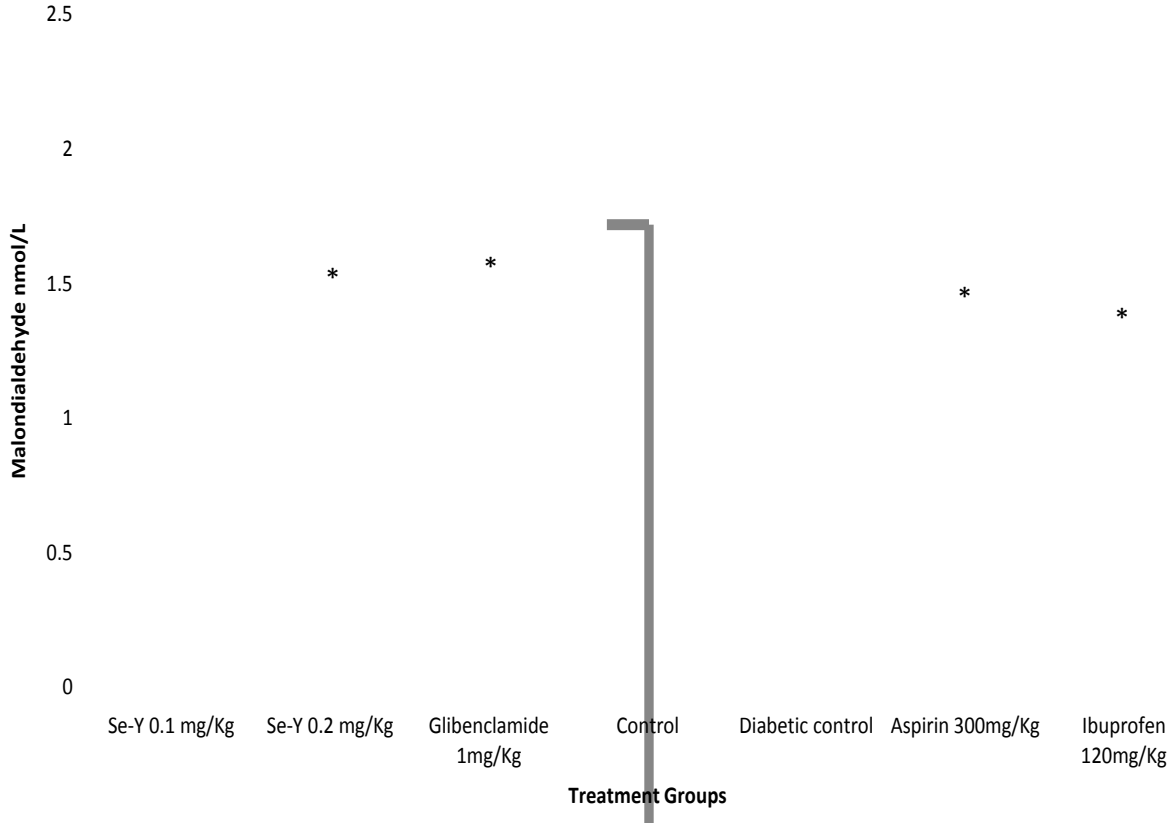
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 12: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Superoxide dismutase activity

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



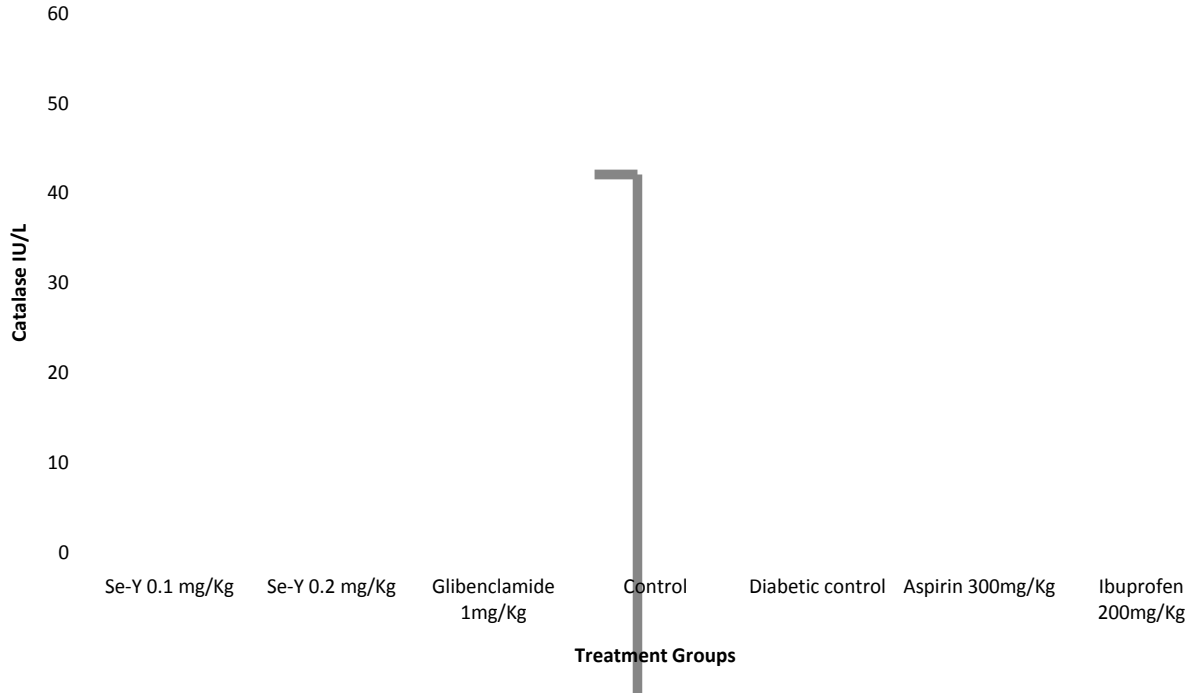
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 13: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Malondialdehyde (MDA)

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



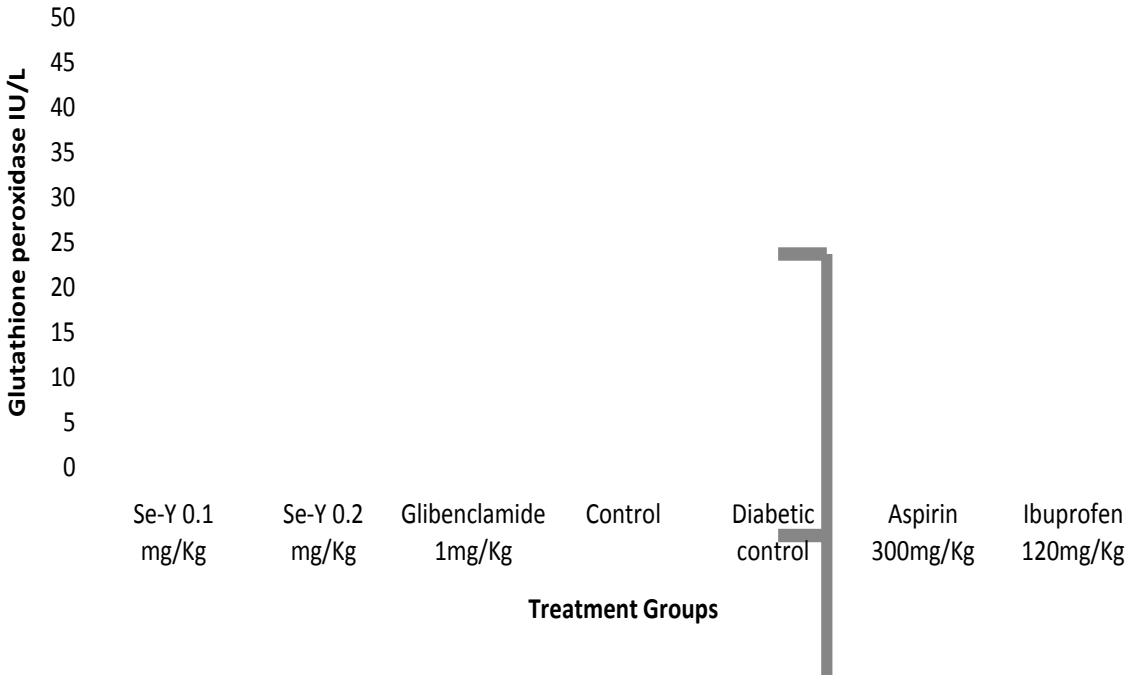
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 14: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Catalase activity

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



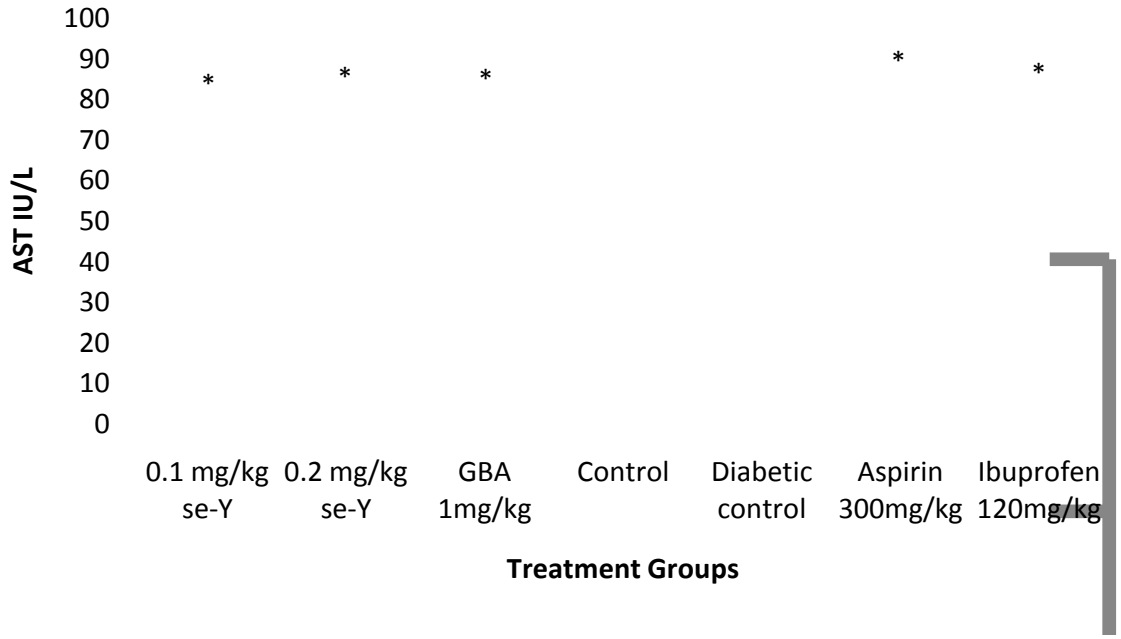
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 15: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Glutathione Peroxidase activity

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



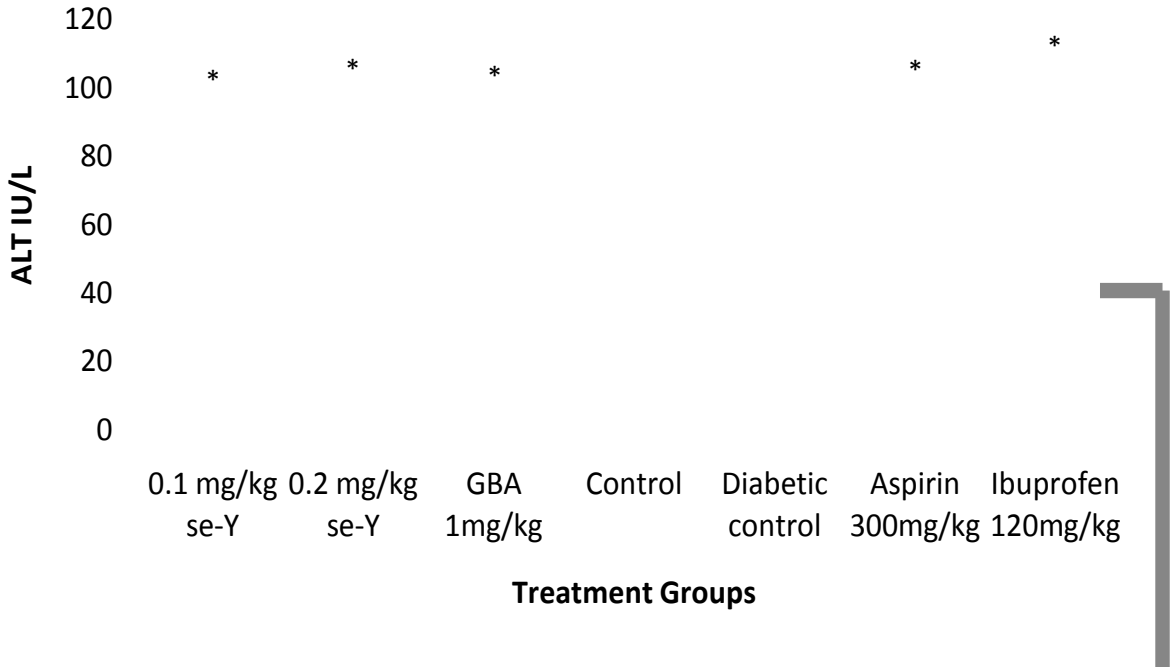
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 16: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Aspartate aminotransferase (AST) activity

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



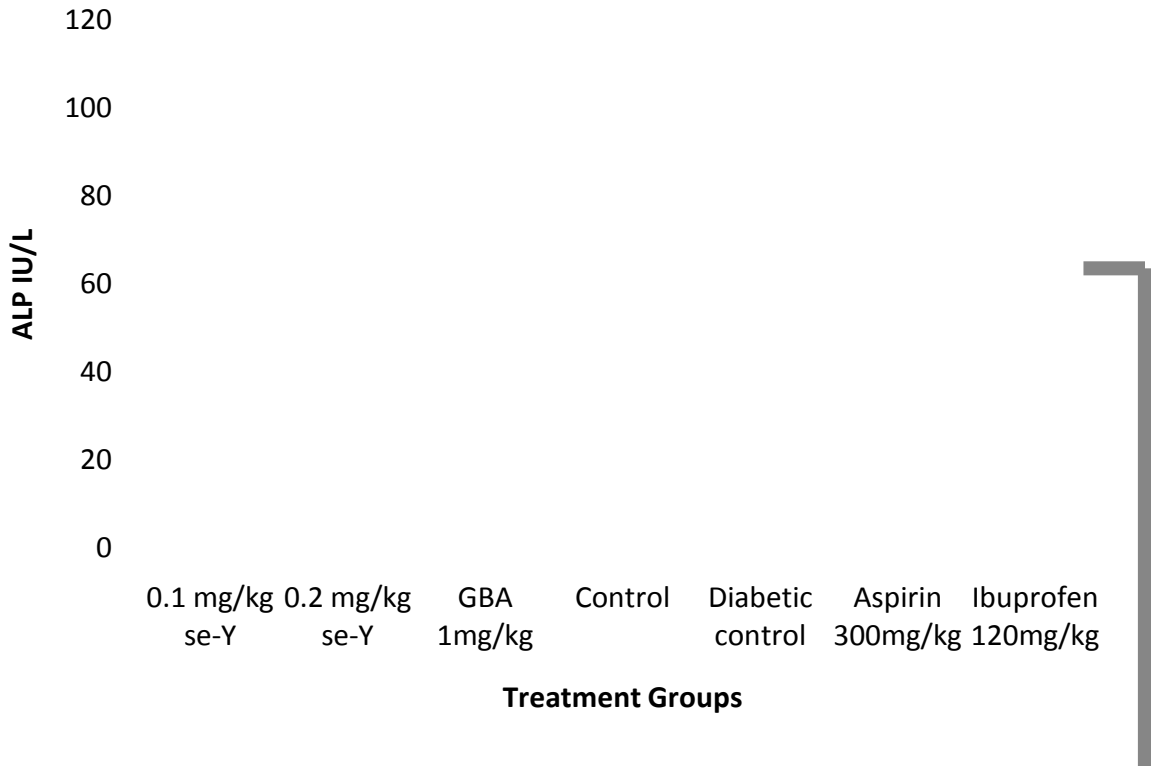
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 17: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Alanine aminotransferase (ALT) activity

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



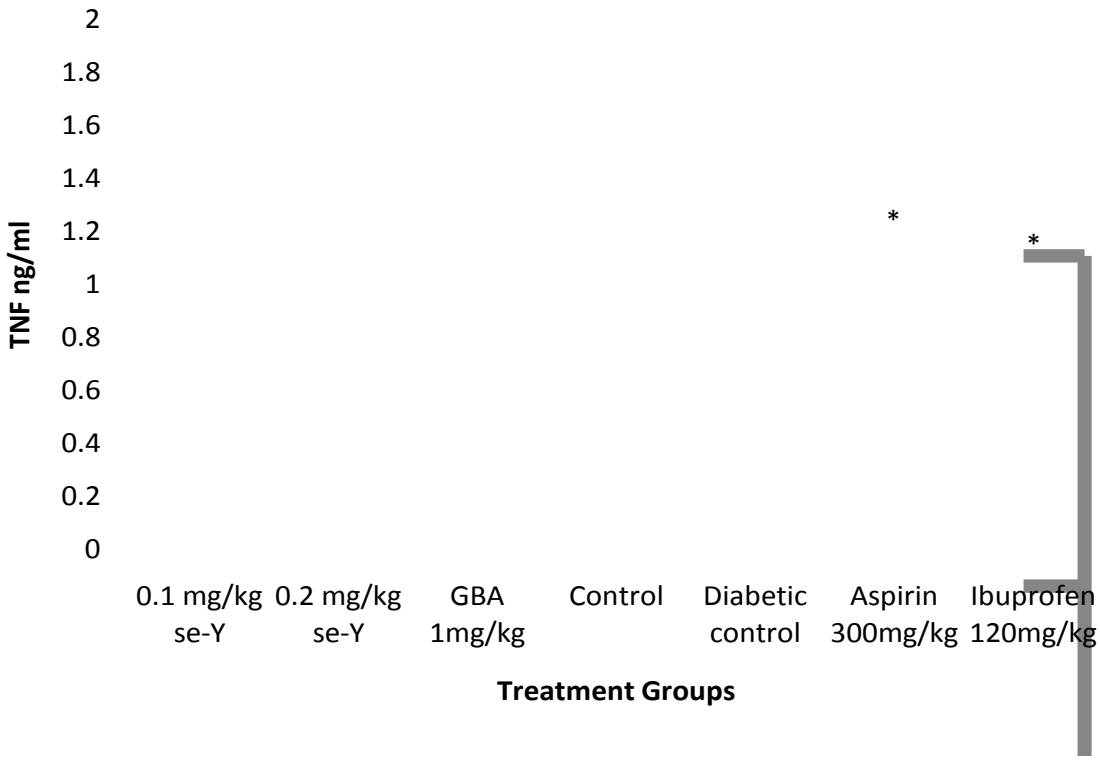
Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 18: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Alkaline phosphatase (ALP) activity

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.



Note: Values are mean ± SEM, n = 5

*P < 0.05 versus diabetic control group

**P < 0.05 versus control group

Appendix 19: Showed the effect of different doses of selenium yeast, Aspirin and Ibuprofen on Alkaline phosphatase (ALP) activity

In STZ Induced diabetic Wistar rats (Mean ± SD) (P < 0.05). Group I: Diabetic rats received 0.1mg/kg Se-Y. Group II: Diabetic rats received 0.2mg/kg Se-Y. Group III: Diabetic rats received 1mg/kg glibenclamide (GBA). Group IV: Normal rats received 1mg/kg normal saline. Group V: Diabetic rats received 1mg/kg normal saline. Group VI: Diabetic rats received 300mg/kg Aspirin. Group VII: Diabetic rats received 120mg/kg Ibuprofen.

Appendix 20: Changes in blood glucose levels (mg/dl) after the treatments with selenium yeast for four weeks

	Wk0 (mg/dl)	**Wk1** (mg/dl)	**Wk2** (mg/dl)	**Wk3** (mg/dl)	**Wk4** (mg/dl)
Group I (0.1mg/kg Selenium)	306.40 ± 52.87^{ns}	285.80 ± 67.80^{ns}	290.40 ± 82.63^{ns}	221.00 ± 68.69^{ns}	199.40 ± 51.29^{ns}
Group II (0.2mg/kg Selenium)	299.20 ±41.33^{ns}	162.80 ± 36.31$^{\bullet}$	207.40 ± 52.35^{ns}	107.40 ± 10.53$^{\bullet}$	175.20 ± 44.32^{ns}
Group III (1mg/kg GBA)	310.60 ± 51.31^{ns}	159.00 ± 31.27‡	298.60 ± 62.54^{ns}	90.80 ± 5.94‡	211.40 ± 40.20^{ns}
Group IV (normal control)	66.20 ± 1.66	106.60 ± 4.02	79.60 ± 4.13	72.00 ± 1.87	69.40 ± 1.03
Group V (diabetic control)	361.80 ± 60.34†	356.00 ± 59.07†	327.40 ± 35.20†	347.20 ± 49.76†	358.60 ± 59.62†
Group VI (300mg/kg Aspirin)	320.20 ± 30.19^{ns}	247.20 ± 29.02^{ns}	197.00 ± 10.07^{ns}	129.40 ± 38.64 $^{\alpha}$	224.00 ± 43.81^{ns}
Group VII (120mg/kg Ibuprofen)	320.40 ± 18.12^{ns}	265.00 ± 26.79^{ns}	220.20 ± 58.04^{ns}	130.20 ± 7.59 $^{\beta}$	200.80 ± 31.26^{ns}

†*P<0.05* Group V vs. IV; ● *P<0.05* Group II vs. V; ‡ *P<0.05* Group III vs. V; **α** *P<0.05* Group VI vs. V; **β** *P<0.05* Group VII vs. V; ∞ *P<0.05* Group I vs. V; ♦ *P<0.05* IV vs. Groups treated with STZ (i.e. I&II&III&VI&VII) at WK0; **ns**= non-significant