The antidiabetic effects and antioxidant activities of *Taverneira abbysinicas'* root crude methanol extracts in STZ induced type 2 diabetic rats

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This is to certify that the thesis prepared by Abbate Araro entitled: *The antidiabetic effects and antioxidant activities of Taverneira abbysinicas' root crude methanol extracts in STZ induced type 2 diabetic rats* and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Diabetes mellitus (DM) is a heterogeneous complex metabolic disorder resulting from defective insulin secretion, resistance to insulin action or both. Broadly it is classified into two categories type-1, and type-2. It is a chronic disease characterized largely by disordered metabolism, glucose urea, negative nitrogen balance, and hyperglycemia. In the long term hyperglycemia has pathologic effects such as atherosclerosis, neuropathy, nephropathy, and others which largely are due to oxidative stress. Since the currently available oral hypoglycemic drugs and insulin have limitations the search for better treatments is going on. Despite the developments of modern treatments the interests in the plant remedies have been renewed. In similar fashion to other countries, in the indigenous system of Ethiopian traditional medicine different plants are used to treat various diseases, such as diabetes mellitus, hypertension, malaria and gastrointestinal disorders of various origins of which *Moringa stenopetala, Melia azedarach* and *Taverniera abyssinica* are the most common. The present study was carried out to investigate the hypoglycemic and antidiabetic effects and antioxidant activities of the methanolic crude root extracts of *Taverniera abyssinica* in STZ induced diabetic rats. The effects of the plants' crude root extracts on blood sugar, the level of plasma MDA, plasma total peroxides, plasma total antioxidant capacity was measured and the level of oxidative stress index was determined.

Results of intraperitoneal glucose tolerance test showed that crude root extracts of *Tavererneira abbysinica* have hypoglycemic effect but not immediate. In the long term treatment the plants' crude root extract exhibited anti-diabetic effects on day 10 ($p<0.001$), day 15 ($p<0.001$), and day 20 ($p<0.001$) at doses of 100mg/Kg and 200 mg/Kg per BW following intraperitoneal administration and was dose dependent with maximum effect observed at a dose of 200mg/Kg. The level of MDA was decreased by 74.07% in *Taverniera abyssinica* treated groups compared to diabetic control receiving 1.0mL of distilled water. . The present study also showed that the level TPP was decreased by 55.12% in *Taverniera abyssinica* treated group compared to diabetic control. But the level of TAC was higher by 5.43% in *Taverniera abyssinica* treated group compared to diabetic control. However, the level of TAC was increased the study showed the level of oxidative stress is decreased by half (50%) in *Taverniera abyssinica* treated groups compared to diabetic control. Generally, this study demeonstrated that the crude root extracts of *Taverniera abyssinica* has hypoglycemic, and antidibetic effects and antioxidant activities.

Key words: diabetes mellitus, hypoglycemic effect, anti diabetic effect, antioxidant activities, methanolic crude root extracts, *Taverniera abyssinica*, intraperitoneal administration.

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Abbrevations

AGEs= advanced glycation end products

CAT= catalase

DM= diabetes mellitus

DMT-1= diabetes mellitus type 1

DMT-2= diabetes mellitus type 2

DNA= deoxyribonucleic acid

GPx= glutathione peroxidase

GSH= glutathione reduced

MDA= malondialdehyde

OS= oxidative stress

PAI-1= plasminogen activator inhibitor-1

PKC= protein kinase-C

RAGEs= receptors of advancerd glycation end products

ROS= reactive oxygen species

SOD= superoxide dismutase

STZ= streptozotocin

TA= *Taverneira abbysinica*

TAC= total antioxidant capacity

TBARS= thiobabituric acid reactive substances

TPP= total plasma peroxides

XO= xanthine oxidase

1. Introduction

1.1. Diabetes Mellitus

Diabetes mellitus (DM) is a heterogeneous complex metabolic disorder resulting from defective insulin secretion, resistance to insulin action or both (Gavin et al, 1997). Broadly it is classified into two categories as type-1 diabetes mellitus (≤ 10%), and type-2 diabetes mellitus (80-90%). Type-1 diabetes occurs as a result of an autoimmune- mediated destruction of pancreatic β-cells, leading to insulin deficiency and patients need insulin treatment for survival. Type-2 diabetes is characterized by insulin resistance and relative, rather than absolute, insulin deficiency. Type 2 diabetes usually occurs in obese individuals and is associated with hypertension and dyslipidemia. It is one of the predominantly occurring diseases in the world affecting 25% of the population and afflicting 150 million people, and the number is estimated to increase to 300 million by 2025 (Vats, 2000).

It is a chronic disease characterized largely by disordered metabolism, glucose urea, negative nitrogen balance, and hyperglycemia. In the long term hyperglycemia has pathologic effects such as atherosclerosis, neuropathy, nephropathy, (Chehade, 2000) and others which largely are due to oxidative stress. Hence, mechanisms that decrease the oxidative stress are essential in minimizing the pathologies which are the outcomes of hyperglycemia in diabetes mellitus. In diabetes, the major causes of oxidative stress have been identified to be enhanced production of oxygen free radicals and steady reduction of antioxidant defenses (Oberley, 1998).

Experimental induction of diabetes mellitus in animal models is important for the progress of our knowledge and understanding of the various features of its pathogenesis and ultimately finding new therapies and cure. Several methods have been used to induce diabetes mellitus in laboratory animals with variable achievements and many difficulties of which surgical removal of the pancreas is effective. Another method that is commonly utilized to induce diabetes is injecting drugs such as alloxan and streptozotocin; that distend and finally degenerate Langerhans islets β- cells. Injection of anterior hypophysis extract is a less reliable method of inducing diabetes (Rastellini et al., 1997) while injection of intraperitoneal or intravenous is the more effective and broadly used.

Streptozotocin (STZ; *N*-nitro derivative of glucosamine) is a naturally existing chemical with a wide antibiotic activity and cyto-toxicity especially to the pancreatic, insulin producing β-cells in mammals (Hayashi et al., 2006; Szkudelski 2001; Takeshita et al., 2006; Weiss, 1982). It is used in the treatment of metastasizing pancreatic islet cell tumor and carcinoid tumors. Inducing diabetes in the rat by streptozotocin injection is very suitable and easy to use (Brosky and Logothetpoulos, 1969; Ito, 1999; Weiss, 1982) since streptozotocin injection causes the degeneration of the Langerhans islets β-cells in particular (Ikebukuro et al., 2002; Smith et al., 1983; Weiss, 1982; Baynes, 1995). Clinically, symptoms of diabetes are observed in rats within 2-4 days after a single intravenous or intraperitoneal injection of 60mg/kg STZ. Therefore, the STZ-induced diabetic animal is taken into account as an animal model of type 2 diabetes mellitus and hyperlipidemia (Suckling et al., 1993). Besides changes in the carbohydrate and lipid metabolism; rats treated with STZ have elevated hepatic and renal thiobarbituric acid reactive substances (TBARS) levels and decreased levels of hepatic reduced glutathione (GSH) concentration, superoxide dismutase (SOD) and catalase (CAT) activities(Zhang and Tan ,2000). These facts indicate the presence of oxidative stress in STZ induced diabetic rats due to decrease in GSH, SOD and CAT which are important anti-oxidant defenses of the body. It seems that the decrement in tissue anti-oxidant status is an indispensable factor in the development of diabetic complications resulting from oxidative stress.

1.2. Oxidative Stress in Diabetes mellitus

Free radicals are the main reasons that cause oxidative stress. They are highly unstable molecules having unpaired (or single) electrons that interact quickly and aggressively with other molecules in our bodies to create abnormal cells. The most commonly produced free radicals can be divided into Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). ROS are a family of active molecules containing free radicals derived from oxygen and involved in the modulation of biological cell functions and represent the most important class of radical species generated in living systems (Millert et al., 1990). ROS comprise molecular oxygen (O_2), superoxide anion radical ($O_2^{\cdot-}$), the peroxide anion radical (O_2^{2-}), singlet oxygen ($^1O_2$), the hydroxyl radical ($^{\bullet}OH$), the hypohlorite ion (OCl^-), and lipid peroxyl ($ROO^{\cdot}$), ozone (O_3). On the other hand, reactive nitrogen species (RNS) include radicals such as nitric oxide (NO•), nitrogen dioxide (NO_2•),

peroxynitrate ($ONOO^-$), nitrous acid (HNO_2), and dinitrogen trioxide (N_2O_3). Reactive oxygen species (ROS), and reactive nitrogen species (RNS), are products of normal cellular metabolism that play a dual role as both deleterious and beneficial species, because they can be either harmful or beneficial to living systems (Valko et al, 2006). They have merits in micromolar concentration and cause oxidative damage to macromolecules when produced in excess (Iuliano et al., 1997; Fang et al., 2002; Cheesman, 2003). In living systems, free-radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, mixed function oxidases, leuckocytes, and xanthine oxidase. Atmospheric pollutants, transition metal catalysts, drugs, smoking and xenobiotics also play a role in the generation of ROS and RNS. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage, in particular, to the immune and nervous systems. Another study revealed Adrenalin and noradrenalin secreted by the adrenal glands under conditions of continuing and excessive emotional stress are metabolized into simpler free radical molecules. Generally speaking the free radicals are continuosly generated from endogenous and exogenous sources within cell and environment (Valko et'al, 2006; Cadenas, 1989; Bagchi and Puri, 1998) and the following table summarizes these.

Table1.1. Sources of free radicals

Internal sources of free radicals	External sources of free radicals
• Mitochondria • Phagocytes • Xanthine oxidase • Reactions involving Fe and other transition metals • Arachidonate pathway • Peroxisomes • Exercise • Inflammation • Ischemia/reperfusion	• Cigarette smoke • Environmental pollution • Radiations • UV light • Certain drugs like anesthetics and pesticides • Industrial solvents and • ozone

The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants are substances that neutralize or mop up free radicals. Antioxidants can be classified as exogenous and endogenous or as enzymatic and non-enzymatic. Some of the enzymatic antioxidants are superoxide (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase while those of non-enzymatic includes vitamin A, vitamin C, vitaminE, polyphenols, and glutathione. Antioxidants, both exogenous and endogenous, whether synthetic or natural, be it enzymatic or non-enzymatic can be effective in protecting free radical formation by trapping them or promoting their decomposition and suppressing such disorders (Cesquini et al., 2003; Kaur and Kapoor, 2002; Halliwell, 2000; Maxwell, 1995). Under normal (or healthier) conditions, the amounts of free radicals generated

are counteracted by antioxidants. It means the rate of free radical production is at equilibrium with the rate of their removal by antioxidants or they are balanced. The following figure illustrates this.

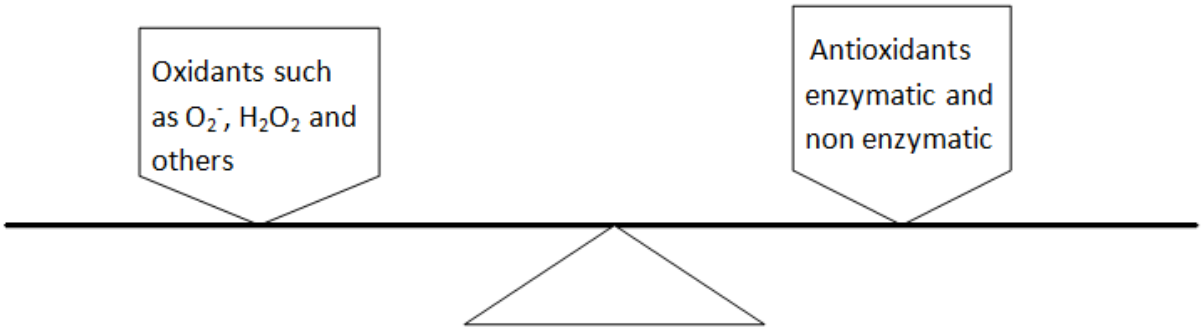


Figure 1.1. Homeostatic state of oxidants and antioxidants

Whenever present in a high concentration free radicals are detrimental to the integrity of biological tissue and mediate their injury. This harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress referring to the effect of ROS and RNS respectively (Kovacic et al, 2001).

Oxidative Stress (OS) is a general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ that can affect a specific organ or the entire organism. It can also be viewed as a harmful condition that occurs when there is an excess of ROS or a decrease in antioxidant levels, causing tissue damage by physical, chemical, and psychological factors that lead to tissue injury in human resulting in different diseases (Tian et al, 2007). Oxidative stress initiated by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite, play a vital role in damaging various cellular macromolecules including DNA molecules, proteins and lipids. The mechanism of damage involves lipid peroxidation, which destroys cell structures, lipids, proteins and nucleic acids. For example they cause damage to cell membranes with the release of intracellular components, leading to further tissue damage (Poli et al, 2004; Halli, 1997). The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Halliwell & Gutteridge, 1999).In line with this studies have shown that metal-induced generation of ROS results in an attack of not only on DNA, but also on other cellular

components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation leading to the formation of peroxyl radicals (Siems et al, 1995).

Researches carried out on amino acids, simple peptides and proteins by exposing them to ionizing radiations exhibited the formation of hydroxyl radicals or a mixture of hydroxyl/superoxide radicals and revealed mechanisms of oxidation of proteins (Stadtman, 2004). Accordingly, side chains of all amino acid residues of proteins, specifically cysteine and methionine are susceptible to oxidation by the action of ROS/RNS that may lead to the reversible formation of mixed disulphides between protein thiol groups (–SH) and low molecular weight thiols, particularly GSH (*S*-glutathiolation). The extent of oxidative stress in a cell is determined by the amounts of superoxide, hydrogen peroxide and hydroxyl radicals that are produced by different cellular mechanisms in response to endogenous and environmental cues.

Several studies established that oxidative stress is not, in and of itself, a disease but one of the major factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others (Heinecke, 2003; Young and Wood, 2001; Halliwell 2000; Metodiewa and Koska 2000; Lang and Lozano 1998; Diaz et al., 1997). When OS occurs, certain by-products such as *oxidized DNA bases*, *lipid peroxides*, and *malonidialdehyde* from damaged lipids and proteins are left behind that are excreted by the body, mostly in the urine. The higher the levels of these various biomarkers, the greater the chance there is of an OS-induced disease, or the worsening and acceleration of an existing one.

Studies have indicated in diabetes mellitus decreased uptake of glucose into muscle and adipose tissue leads to chronic extracellular hyperglycemia resulting in tissue damage and pathophysiological complications, involving heart disease, atherosclerosis, cataract formation, peripheral nerve damage, retinopathy and others (Brownlee and Cerami, 1981). Increased oxidative stress has been assumed to be one of the major causes of the hyperglycemia-induced trigger of diabetic complications. It has been also shown that diabetes mellitus is associated with oxidative stress. Several sources of increased oxidative stress in diabetes mellitus are proposed. The following session discusses some of these sources.

1.2.1. Sources of ROS in diabetes

Several sources of increased oxidative stress in diabetes mellitus are proposed. The following session discusses some of these sources.

A. Hyperglycemia

Several studies have shown that hyperglycemia causes oxidative stress and in turn oxidative stress causes hyperglycemia by inducing insulin resistance and activating transcription factors like NF-κB and others which cause insulin resistance especially in type-2 diabetes mellitus. Hyperglycemia in an organism stimulates ROS formation by activating pathways that give rise to generation of ROS that include oxidative phosphorylation, glucose autooxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P450, monooxygenases, and nitric oxide synthase (NOS). Other studies have also shown that hyperglycemia causes the production of free radicals through; i) increased glycolysis (Vaag et al, 1992); ii) intercellular activation of sorbitol (polyol) pathway where in $\leq$ 30% glucose can be diverted to sorbitol and fructose, (Williamson, 1993); iii) autooxidation of glucose (Wolff et al, 1991) and iv) non-enzymatic protein glycation resulting in increased synthesis of Advanced Glycation End Products (AGEs) v) changes in a receptor for AGEs and (Ceriello et al,1992) vi) increased transcription of genes for proinflammatory cytokines and plasminogen activator inhibitor-1 (PAI-1) vii) activation of protein kinase-C (PKC) leading to several molecular changes.

B. Mitochondrial electron transport chain or complexes

One of the major sources of free radical generation is mitochondria. Several studies revealed that the key sites of superoxide anion formation in the mitochondrial membrane are complex I and the ubiquinone–complex III interface in normal conditions, and were changed in diabetes to complex II (Nishikawa et al., 2000; Kwong & Sohal, 1998). This conclusion comes from the study of a complex II inhibitor, 2-thenoyltrifluoroacetone and an uncoupler of oxidative phosphorylation, carbonyl cyanide *m*-chlorophenyldihydrazone leading to a decrease in ROS formation in various cells exposed to high concentration of glucose (Yamagishi et al, 2001).

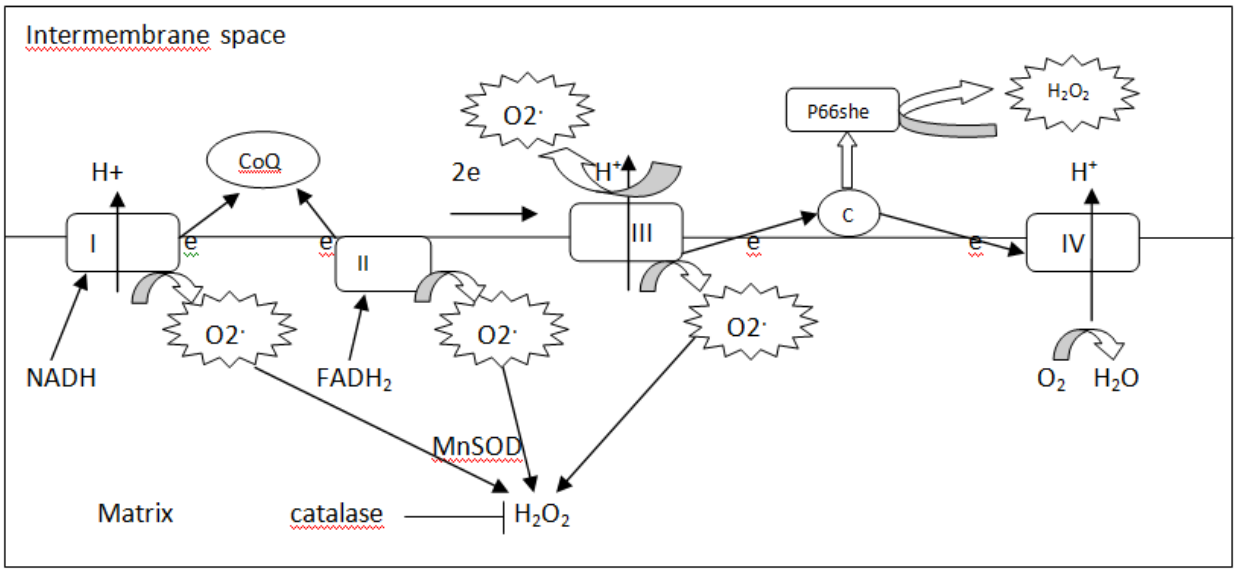


Figure1.2. Generation of ROS in the mitochondrial ETC

C. NAD(P)H Oxidase

Several lines of evidence support that NAD(P)H oxidases are a major source of glucose induced ROS production in the vasculature and kidney cells, confirming that NAD(P)H oxidase as a mediator of diabetic complications. Since hypertension is a common complication of diabetes; studies showing increased angiotensin II labelling in cardiac myocytes and endothelial cells from human diabetic patients (Li & Shah, 2003) assured the expression of NAD (P)H oxidase is regulated similarly in both these disease states. In line with this high glucose-induced formation of ROS and p47phox (cytosolic component of activated NAD(P)H) oxidase can be blocked with Ang II type 1 receptor antagonists, confirming a link between the two pathways of NAD(P)H oxidase activation. Moreover, the NAD (P)H oxidase-mediated production of ROS in diabetes was shown to be suppressed by a variety of PKC inhibitors, implicating this family of kinases in the regulation of hyperglycemia-induced NAD (P)H oxidase activity. In addition the super oxide anion radical generated by induced NAD (P)H oxidase was shown in activating other signaling molecules like mitogen activated protein kinases (MAPK), and inflammatory genes leading to production of more radicals and oxidative stress which in turn induces insulin resistance causing DMT-2.

$2O_2$ + NADPH (or NADH) → 2 $O^{\cdot-}_2$ + NADP (or NAD) + H^+

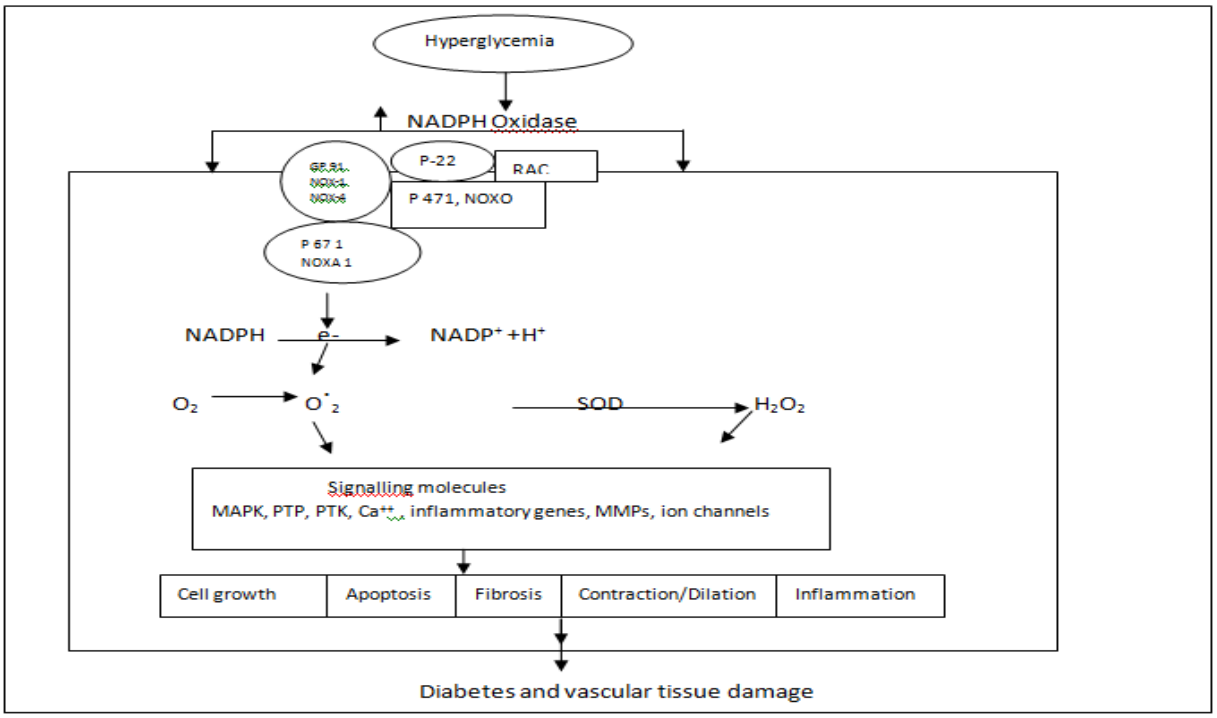


Figure 1.3. Generation of free radicals by NAD(P)H oxidase

D. Glucose autoxidation

The presence of hyperglycemia-induced oxidative stress in non nucleated cells lacking mitochondria and the NAD(P)H oxidase (for example in erythrocytes) is observed and this was accounted by the formation of ROS from glucose auto-oxidation. In addition in vitro experiments revealed that Glucose and its metabolites are known to react with hydrogen peroxide in the presence of iron and copper ions to form hydroxyl radical (Robertson et al, 2003). The following figure illustrates this.

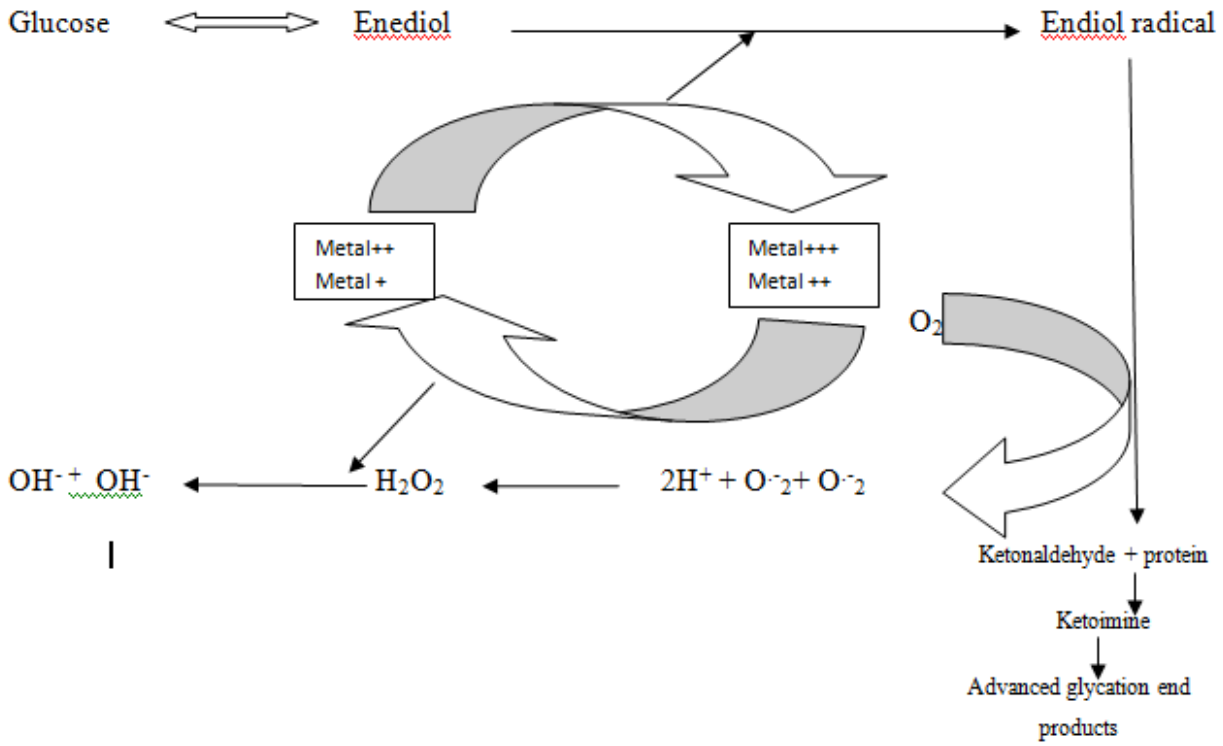


Figure 1.4. Glucose autooxidation and generation of free radicals

E. Lipooxygenases

Lipooxygenases are classes of enzymes that act on lipids. They play great role biologically in activating signaling pathway by acting on arachidonic acid, modifying membrane structure by causing peroxidation of membrane lipids and mobilization of lipids for fuel. For example they catalyse the conversion of arachidonic acid into a broad class of signalling molecules, such as leukotrienes, lipoxins, and hydroxyeicosatetraenoic acid. Similarly the the peroxidation reaction catalyzed by human reticulo type 15-lipoxygenase plays major role in the maturation of red cells, Keratinocyte and lens epithelial cell development physiologically while it was shown to cause oxidation of low density lipoprotein pathologically which is the main cause for atheroschlerosis. It was also demonstrated that diabetes was associated with increased lipoxygenase expression that resulted in eicosanoid formation and the generation of ROS (Brash, 1999) and they use the ferric form of iron the process. The following figure illustrates this.

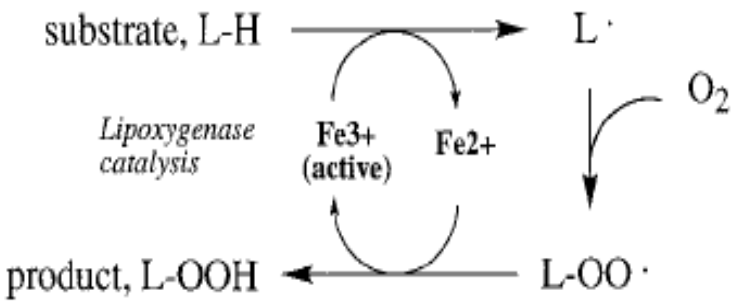


Figure 1.5. Generation of free radical by lipoxygenase and lipid peroxidation

F. Formation of advanced glycation end products

Glucose can react directly with free amine groups on protein and lipids non-enzymatically yielding a diverse group of modifications referred as advanced glycation end products (AGE) (Ling et al., 2001). AGES are observed primarily in long-lived structural proteins, such as collagen and are found in almost all tissues examined from diabetic rats and in human non-insulin dependent patients. Some tissues, such as liver, kidneys, and erythrocytes are more susceptible to AGE formation than others. In addition AGE formation was shown to be associated with the free radical generation. Thus AGE formation is assumed as a significant contributor to the onset of diabetic complications, mainly atherosclerosis by inducing oxidative stress.

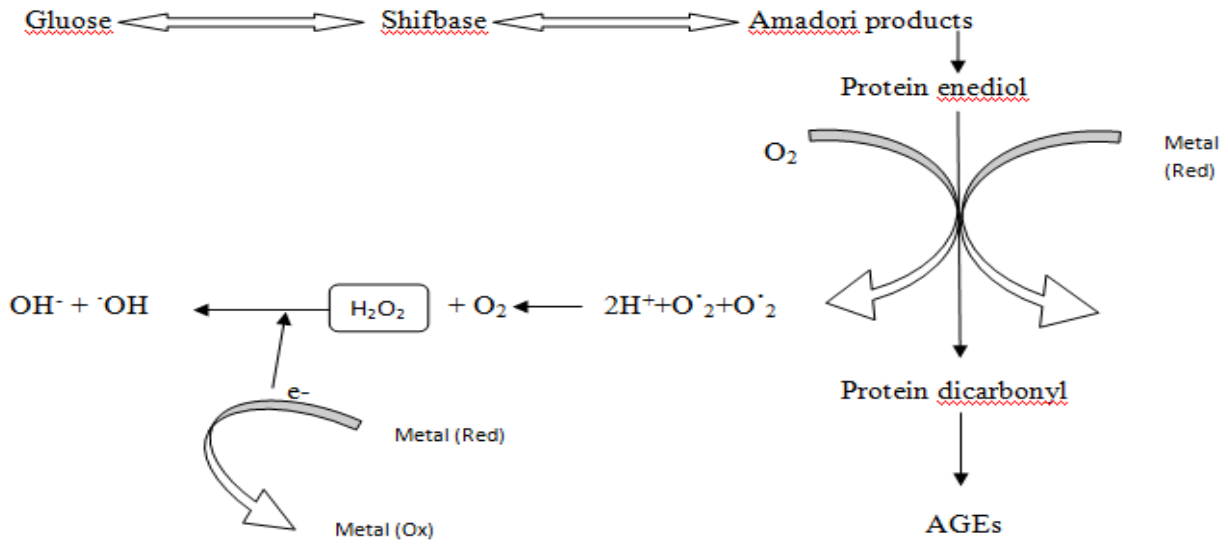


Figure 1.6. Formation of AGEs and ROS generation

The formation of AGEs are also implied in altering receptors of advanced glycation end products (RAGEs) that causes oxidative stress which in turn changes cellular signal transduction leading to activation inflammatory inflammatory genes like interleukin-6 and tumor necrosis factor-α that increase induces insulin resisatance resulting in the development of diabetes mellitus. The following figure shows this.

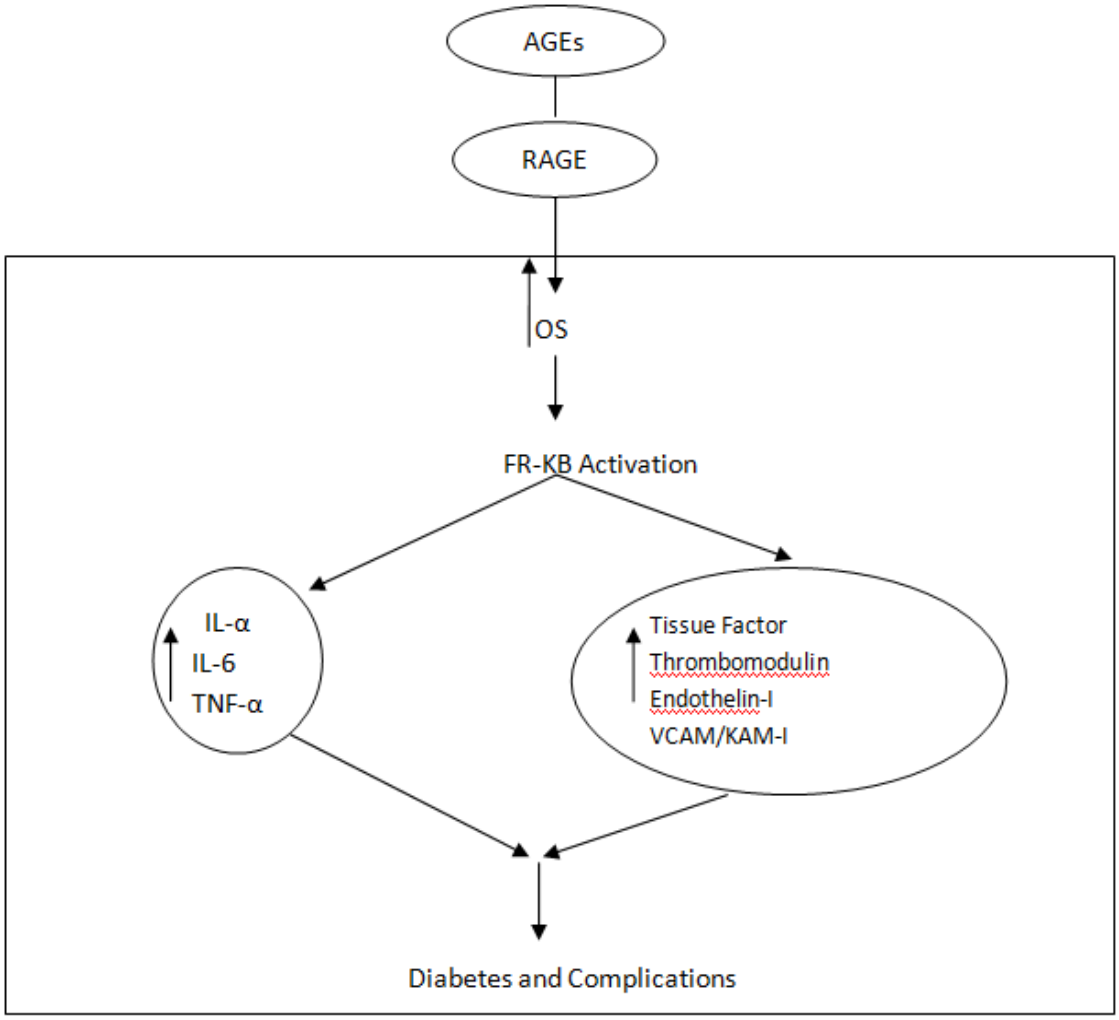


Figure 1.7. Impact of OS that comes from AGE in causing DMT-2

G. Depletion of antioxidants

Other studies revealed that production of ROS and RNS depletes both enzymatic and non-enzymatic antioxidants leading to additional ROS/RNS accumulation causing cellular damage. For example, vitamin E is found depleted in diabetes which has a protective effect mainly

through suppressed lipid peroxidation and vitamin C levels in plasma have also been found to be reduced in diabetic patients. However, the relation between reduced levels of Vitamin C and diabetic complications is unclear. All of these contribute to oxidative stress in diabetes.

Information which suggests that hyperglycemia and diabetes complications affect the regulation of GSH peroxidase expression are available, however the effect to which GSH peroxidase inhibition affects cell health is unclear (Vander et al., 2001). Similarly, hyperglycemic treatment impaired no change in the activity of GSH reductase. Therefore, it does not seem that GSH reductase plays a role in the onset of diabetic complications.

1.3. Diabetes mellitus and herbal medicines

Most of the complications that are associated with diabetes are due to oxidative stress induced by hyperglycemia. Therefore, compounds with both hypoglycemic and anti-oxidative properties would be useful anti-diabetic agents (Baynes, 1995). Both allopathic and herbal medicines can be used to control diabetes, but it is not entirely curable disease by the currently available anti diabetic agents. However, insulin therapy has many side effects such as insulin resistance, loss of appetite, brain atrophy, and fatty liver in chronic use (Piedrola, 2001; Weidmann, 1993); it is the solely satisfactory treatment for diabetes mellitus type 1 in use.

In contrast to allopathic medicine, herbal formulations have low side effects, high efficacy, and are also cost effective i.e. low cost as a result herbal drugs are becoming worth in the treatment of several diseases like cancer, hepatic diseases, and particularly for diabetes mellitus (Parri, 1999). Therefore, currently there is a growing interest toward natural antioxidants of herbal resources (Gazzanio et al., 1998; Velioglu et al., 1998; Larson, 1988). Epidemiological and in vitro studies on medicinal plants and vegetables strongly substantiate the idea that plant constituents with antioxidant activity are able to exert protective effects towards oxidative stress (Eastwood, 1999; Ness and Powel, 1997; Cao et al., 1996; Block and Patterson, 1992). Antioxidants thus play an essential role to prevent the human body from damage caused by reactive oxygen species (Lollinger, 1981; Tutour, 1990).

Literatures on the folk medicine of India indicated that many plants within the country are described to have hypoglycemic effect and are utilized to treat diabetes mellitus. However, most

did not lower blood glucose (Nagarajan et al., 1997) and most were not scientifically ratified (Babu et al., 2002). On the other hand, many studies carried out on traditional medicinal plants revealed a positive result. For example, studies done on medicinal plants such as *Anthocephalus indicus* (family, Rubiacae: Hindi name Kadam) , (Vishnu et al 2009), *Artanema sesamoides;* Benth (Scrophuilariaceae) (Selva et al., 2008), *Bacopa monnieri* (Tirtha et al.,2008), *Pisonia alba (Nyetaginaceae)* commonly known as ‘ lettuce’ tree , *Cinnamomum tamala* (Usha, 2010) and *Fenugreek* (Neveen et al, 2007) have shown that either their root, bark, or leaf extract posses’ both anti diabetic and anti oxidant activity; the needed properties to overcome complications of diabetes mellitus. Those plants contain compounds that belong to flavonoids and isoflavones that have anti diabetic and antioxidant activity but the mechanisms are not well known.

Plants containing flavonoids have been reported to possess strong antioxidant properties (Badami et al., 2003; Raj and Shalini ,1999) . Isoflavonoids have also been attributed a large number of pharmacological and nutraceutical properties including chemoprevention of osteoporosis (Uesugi et al., 2001; Alekel et al., 2000), and other postmenopausal disorders by mimicking the action of estrogen (MerzDemlow et al., 2000). Other studies have also shown isoflavones have antioxidant benefits improving cardiovascular health (Heim et al., 2002; Setchell and Cassidy, 1999; Lichtenstein, 1998), and reducing risks of breast and prostate cancers in humans by inducing prostate cancer cell adhesion, direct growth inhibition, and induction of apoptosis (Venkateswaran, 2003; Lamartiniere, 2000; Adlercreutz, 1998). In general isoflavones supress the development of tumor by inhibiting enzymes that promotes tumor growth. Many isoflavones have estrogen-like properties and structure. Because of favorable side-effect profile, they are ideal alternatives to HRT (hormone replacement therapy). The cardio-protective effects of estrogen replacement therapy are mediated through effects on lipid metabolism including lowering of LDL cholesterol and increasing the level of HDL cholesterol (Lichtenstein 1998, Lissin et al., 2002). For example, Soy protein containing isoflavones significantly reduced total serum cholesterol, LDL cholesterol, and triacylglycerol and significantly increased HDL cholesterol with the changes related to the level and duration of intake and the sex and initial serum lipid concentrations of the subject (de Klein et al., 2005). Isoflavones also influence vascular function and current publications show powerful vasodilator effects similar to that of

estradiol. Most human studies have revealed many effects of isoflavones on arterial circulations; particularly improved systemic arterial compliance, reflecting greater distensibility of large arteries, which is likely to minimize the risk of systolic hypertension and clinical coronary heart disease. Isoflavonoids are a subclass of the more ubiquitous flavonoids, which are naturally occurring polyphenols. Isoflavonoids differ from other classes of flavonoids by their greater structural variability, frequent presence in plants in their free form rather than as a glycoside, and by the greater frequency of isoprenoid substitution. One of the most important chemical properties of isoflavones is the ability to participate in redox processes. Isoflavones act as free radical scavengers (chain-breaking antioxidants) when the phenoxilic head group meets a free radical. Isoflavone metabolites also exhibited higher antioxidant activity than parent compounds in standard antioxidant assays. Studies done on the antioxidant activity of isoflavones showed that they have antioxidant activity in vivo which is effective in higher concentration. Researches carried out on the plant *Taverneira abysinica* showed that it contains compounds that belongs to the class of isoflavonoids.

1.4. The Plant *Taverneira abysinica*.

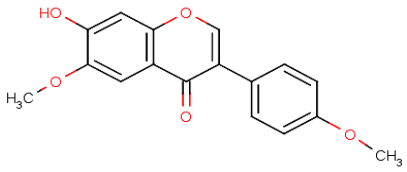
The plant *Taverneira abyssinica* belongs to a relatively small genus that contains only 15 species found in arid regions from Egypt to India (Thulin, 1983) and is indigenous to Ethiopia. It grows up to 2m high at altitudes ranging 1700-2200m and is one of the most widely used plants in traditional medicinal practice of the country.

In the folk medicine of central Ethiopia, the roots of *Taverneira abbyssinica* (Leguminosae)*;* A.Rich (Fabaceae), are used for treating stomach pain and fever (Thulin, 1983). The roots of this plant are chewed and the juice swallowed for the relief of pain and fever. The roots are also known by the local name "Dingetegna" to mean "medicine for sudden illness" in Amharic (Kloos et al, 1978).

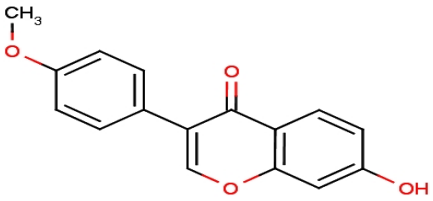
Though no published journal on the antioxidant activity of *T.abbyssinica* extract is available, studies done in Japan *on Astragalus membranaceus BUNGE (Astragali Radix)* showed that isoflavones; afrormosin and formononentin have antioxidant activity (Shizou,1998) . Moreover,

many phytochemicals that contain phenolic moieties have been identified to exhibit antioxidant activity (Kahkonen *et al*., 1999; Frankel *et al*., 1995).

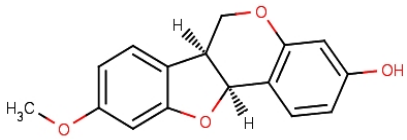
The phytochemical studies carried out on the plant *T.abbysinica* root extract led to the isolation and identification of few of its components of which formononentin, afromosin, the petrocarpans medicarpin and 4-hydroxy medicarpin are the major isoflavonoids (Thulin, 1983).Recent studies done on the T.abbyssinica revealed that its aqueous root extracts posses, significant antipyretic, analgesic activities and anti spasmolytic action (E.Dagne et al., 1990). Several studies done on traditional medicinal plant extracts in other countries like India as discussed above showed that plants that contain isoflavonoid have antioxidant and antidiabetic effects. Even though, no published article on the anti diabetic and antioxidant effects of *T.abbysinica* was found the presence of isoflavonoids in the aqueous root extract have been indicated from the few studies discussed above. Structures of some of isoflavonoids identified from *T.abbysinica* are given below.



Afromosin



Formononetin



Medicarpin

Figure 1.8. Some of the components of the root extracts of *Tavernera abbysinica*.

Thus since some of the extracts of *Taverneira abbysinicca* contains phenol groups they are expected to posses antioxidant activity and anti-diabetic effect. The purpose of this study is to determine the antidiabetic and antioxidant activity of roots extract of *Taverneira abbysinica* in STZ induced diabetic rats.

1.5. Significance of the study

Several studies were done and still are going on in the determination of anti-diabetic, and anti-oxidant activity of traditionally used plant extracts over the world. However, little information is present on the *Taverneira abbysinica,* and despite the effort made no published journal was found on the anti-diabetic and anti-oxidant activity of *Taverneira abbysinica*. This study will contribute a lot to the identification of plants that have both anti-diabetic effects and antioxidant activities in the folk medicine of Ethiopia and particularly to *Taverneira abbysinica*.

1.6. Hypothesis

The methanolic roots extracts of *Taverneira abbysinica* in aqueous solutions have antioxidant activities and posses' hypoglycemic and anti-diabetic effects too.

2. Objective

2.1. General objective

To assess the anti-diabetic effects and antioxidant activities of the aqueous solutions of the methanol extracts of the roots of *Taverneira abyysinica* in rats with streptozotocin (STZ)-induced diabetes mellitus.

2.2. Specific objectives

- To identify the oxidative/antioxidative status in the plasma of STZ induced diabetic Wistar Albino rats after treating with aqueous solution of *T. abyysinica* plant extracts.
- To determine the hypoglycemic effects of the crude extracts of *T. abyysinica* plant extract.
- To evaluate whether the root extracts of *T. abyssinica* plant have anti-diabetic effects or not in STZ induced diabetic rats.
- To compare the effect of concentrating *T. abbysinica* extracts on antidiabetic activity.

3. Materials and Methods

3.1. Study site

This study was carried out in the department of Biochemistry, School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia.

3.2. Plant materials

The plant material used in this study was purchased from Markato, main market in Addis Ababa, Ethiopia from the traditional medicine vendors and the plant material was identified and authenticated by the taxonomists.

3.2.1. Extraction:

One hundred gram of dried root of *Taverniera abyssinica* was ground into a fine powder by means of a regular electrical grinder. The powder was macerated in 1000mL of methanol: water solution (3:1) and left over-night at room temperature. The supernatant was collected and filtered with filter paper to remove residuals. The supernatant was evaporated and dried in lyophilizer and finally 10g of the crude extract was obtained.

3.2.2. Crude extract preparation of *Taverniera abyssinica* .

The crude extract of *T.abbysinica* was dissolved in double deionised water in amount not exceeding 1ml per body weight of each rat for intraperitoneal injection.

3.3. Study Design

In this study, 20 male white albino rats of the same age (12 weeks) were used in four groups each group consisting of five rats. Group I (Diabetic control)-received 1.0 ml distilled water, Group II Metformin treated (200mg/kg), Group III (*Taverniera abbyssinica* treated (100mg/kg) and Group IV *Taverniera abyssinica* treated (200mg/kg). Diabetic rats exposed to streptozotocin (STZ) were utilized for both antidiabetic and antioxidant effect determination in four groups. All the four groups were used for determination of hypoglycemic effect and identification of anti diabetic effect while the first three groups are used for the assessment of antioxidant activities.

3.4. Animal;

3.4.1. Induction of experimental diabetes

Rats were made to fast for 16 hours before the injection of STZ and injected intraperitoneally once with a freshly prepared solution of STZ (in 0.01 M citrate buffer, pH 4.5) at a final dose of 58 mg/kg body weight. The diabetic state was assessed in STZ-treated rats by measuring the non-fasting blood glucose concentration 4 days post STZ injection. Only rats with blood glucose levels greater than 200 mg/dL on repeated measurements were used in the experiments.

3.5. Data Collection

- Blood glucose level was measured on the first day before treatment and every five days after treatment started.
- The intrapritoneal glucose tolerance test was carried out before the treatment and at the 20^{th} day.
- Plasma MDA was determined on the 21th day.
- Plasma total peroxide was determined on the 21th day.
- Pasma total anti-oxidant capacity was determined on the 21th day.

3.6. Laboratory Analysis

3.6.1 Blood glucose concentration

Blood samples were collected from the tail vein. Basal glucose levels were determined prior to STZ injection, using an automated blood glucose analyzer that uses glucose oxidase method to determine blood glucose. Three days after the induction of experimental diabetes, protocol was started and sample collections were made on the fourth days after induction of diabetes. The blood glucose concentrations was measured for the groups and compared with that of diabetic control group.

3.6.2. Intraperitoneal Glucose Tolerance Test (IPGTT)

To determine the hypoglycemic effects of *Taverneira abbysinica* intraperitoneal glucose tolerance was performed on four groups which were STZ induced diabetic rats. Rats were fasted for 12 h before the test and 2 g/Kg glucose solution was administered intraperitoneally. Blood samples were collected by damaging the tip of the tail at 0hr before and at 0.5 h, 1h, 11/2 h and 2h following glucose administration and results were compared.

3.6.3. Determination of Malondialdehyde (MDA)

A.* Principle*: MDA is one of the end products formed when the unstable lipid peroxides break. The reaction of MDA with 2-thiobarbituric acid (TBA) is the most widely used method for determination of MDA. A complex formed during the reaction can be measured both by absorbance and fluorescence. The complex absorbs at 540 nm. Antioxidative substances such as tocopherols and synthetic Butylated hydroxyl toluene (BHT) are added to minimize the artifcial formation of lipid peroxides during boiling in the presence of oxygen usually before the TBA-assay in order not to overestimate MDA (Axel et al, 1996).

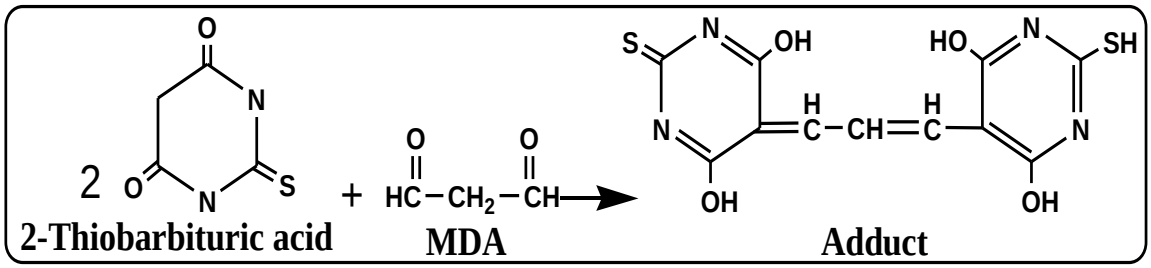


Figure 3.1. Reaction of thiobarbituric acid with MDA

B. Chemicals

BHT, 1,1,3,3-tetraethoxypropane, TBA were purchased from Sigma-Aldrich (Gillingham, UK). Absolute ethanol was from Joseph Mills Denaturants Ltd (Liverpool, UK), phosphoric acid (85%, 15 mol/L) was from Riedel-De Haënag Seezle-Hannover (Germany), sodium hydroxide was from Labort Fine Chem. Pvt. Ltd (Surat, India), sodium chloride was from E. Merk (Darmstadt, Germany), and n-butanol (98%) was from Labmerk Chemicals (Ambala Cantt, India).

C. Reagents preparation:

i. TBA (0.11M): was prepared dissolving 792 mg of TBA in 50 mL of 0.1 mol/L NaOH

ii. BHT reagent **(**0.3 mM**):** Prepared by dissolving 69.108 mg of BHT in 1L of absolute ethanol.

iii. Standard*:* 1, 1, 3, 3-tetramethoxypropane is used as MDA precursor in deionized water. 50 μL of the 1,1,3,3-tetraethoxypropane (10 mM) ,and 9.950 mL HCl (100 mM) was mixed and then incubated for 10 minutes to get 5 μM MDA. Serially it was diluted in deionized water to get: 0.0, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 and 5.0 μM.

D. Procedure:

The procedures are given in the table below.

Table 3.1. Procedures and reaction mixtures for determination of MDA

Reagents	**Sample (μL)**	**Standard (μL)**	**Reagent Blank (μL)**
Sample/Standard/H_2O	200	200	200
BHT	25	25	25
Orthophosphoric acid; vortex for 10 minutes.	200	200	200
TBA reagent; heating 90 ^oC for 45 minutes.	25	25	25
n-butanol; extract.	500	500	500
Saturated NaCl solution, centrifuge.	50	50	50

E. Calculation:

MDA equivalents (TBARS) content of samples was calculated from the equation derived from the standard curve constructed from concentration of the standards and their respective optical densities.

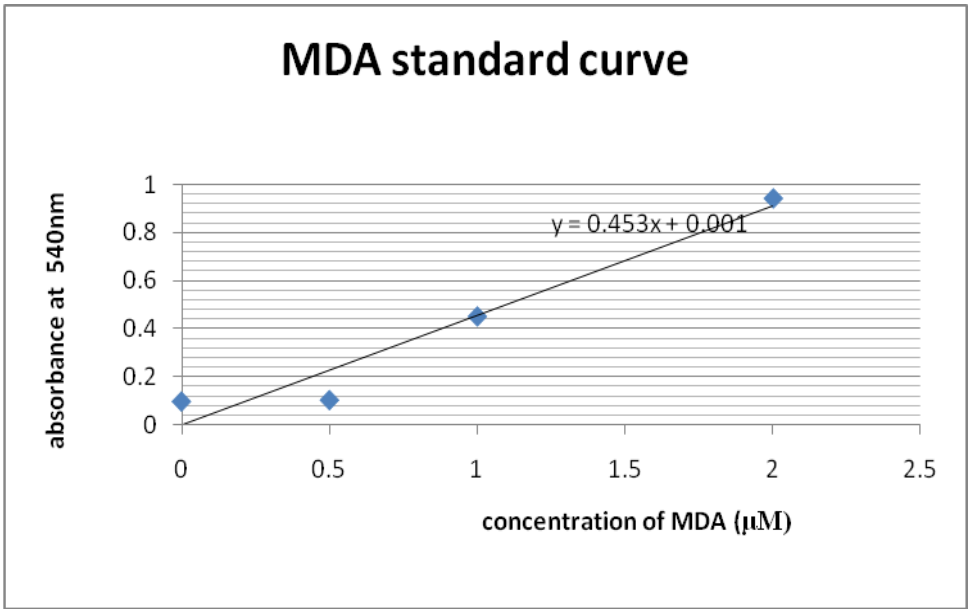


Figure 3.2. Standard curve of MDA

3.6.4. Determination of Total plasma peroxide (TPP) concentration

A. Principle*:* Peroxides (organic and inorganic) oxidize the orange colored xylenol orange into purple colored chromogen that proportionate directly with the peroxide content in presence of the ferrous sulfate as a catalyzer (Harma *et al.*,, 2005). This reaction is used to determine the total peroxides in the plasma of rats.

B. Assay chemicals

Ammonium ferrous sulphate hexahydrate, xylenol orange and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (Gillingham, UK). K_2HPO_4 anhydrous and KH_2PO_4 anhydrous were from Carlo Erba (Milan, Italy) and NaCl from E. Merk (Darmstadt, Germany) and H_2O_2 were from El-Nasr Pharmaceutical Chemical Co. (Cairo, Egypt).

C. Reagents preparation

i. Phosphate buffer saline (PBS) was prepared by dissolving 1.43 gm of K_2HPO_4 anhydrous, 0.25 gm of KH_2PO_4 and 8.5 gm of NaCl in deionized water to 1.0 L.

ii. Chromogen Reagent was prepared by dissolving 9.8 mg of ammonium ferrous sulfate in 10 mL of 250 mM H_2SO_4. This solution was added to 90 mL HPLC-grade absolute methanol containing 79.2 mg butylated hydroxytoluene. Then 7.6 mg of xylenol orange was dissolved in the solution by stirring.

iii. Blank reagent is prepared similarly with the omission of the ammonium ferrous sulfate.

iv. Standard H_2O_2*:* 10 µL of 30% stock reagent was diluted in 250 mL of PBS, pH 7.4, to get 250 µM H_2O_2. The later was diluted serially in the buffer to get: 250.0, 125.0, 62.5, 31.25 and 0.0 µM. The standard was prepared fresh each time.

D. Procedure:

The procedures are given in the table below.

Table 3.2. The procedures and reaction mixture for determination of plasma total peroxides

Reagents	**Sample (µL)**	**Standard(µL)**	**Sample Blank (µL)**
Sample/Standard/ Blank	50	50	50
Chromogen reagent; incubation for 30 min. at RT; centrifugation for 3 minutes at 5,000 rpm	450	450	-
Blank reagent; incubation for 30 min. at RT; centrifugation for 3 minutes at 5,000 rpm; taking 250 µL of the supernatant; read at 560 nm	-	-	450

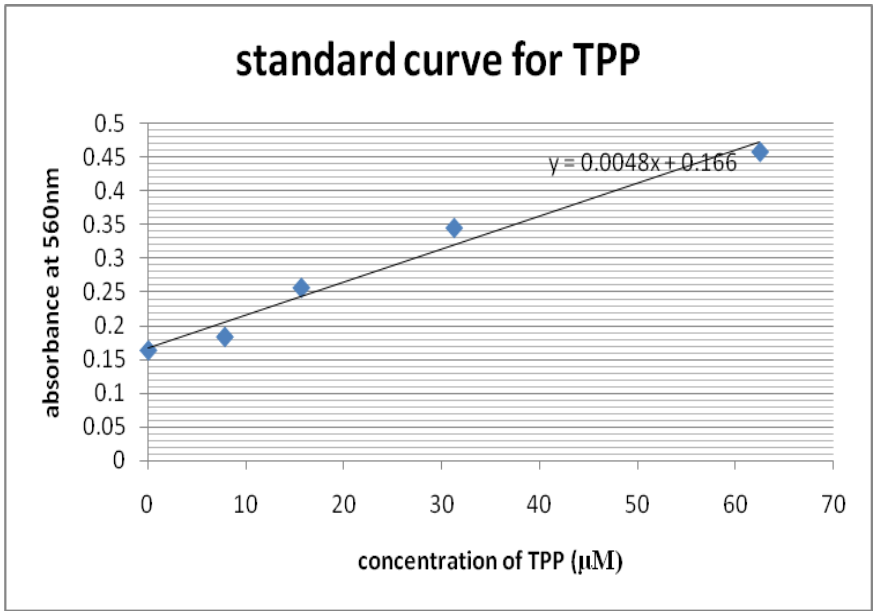


Figure 3.3. Standard curve for TPP

E. Calculation:

Total plasma peroxide concentration in samples was calculated from the standard curve constructed using standard concentrations and readings after subtracting the reagent blank readings for samples.

3.6.5. Determination of Total antioxidant capacity

***A.* Principle**

The reduced 2, 2`-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) molecule is oxidized to ABTS radical cation $ABTS^{\bullet+}$ using hydrogen peroxide in acidic medium (the acetate buffer 30 mM, pH 3.6). In the acetate buffer solution, the concentrated (deep green) $ABTS^{\bullet+}$ molecules stay more stable for a long time. After its dilution in a more concentrated acetate buffer solution with high pH values (the acetate buffer, 0.4 mol/L, pH 5.8), the color is spontaneously and slowly bleached. Antioxidants present in the sample accelerate the bleaching rate to a degree proportional to their concentrations. This reaction can be monitored spectrophotometrically and the bleaching rate is directly related with the TAC of the sample. The reaction rate is calibrated with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) – a water-soluble α-

tocopherol analogue - which is widely used as a traditional standard for TAC measurement assays, and the assay results are expressed in mM Trolox equivalent/L (Erel, 2004).

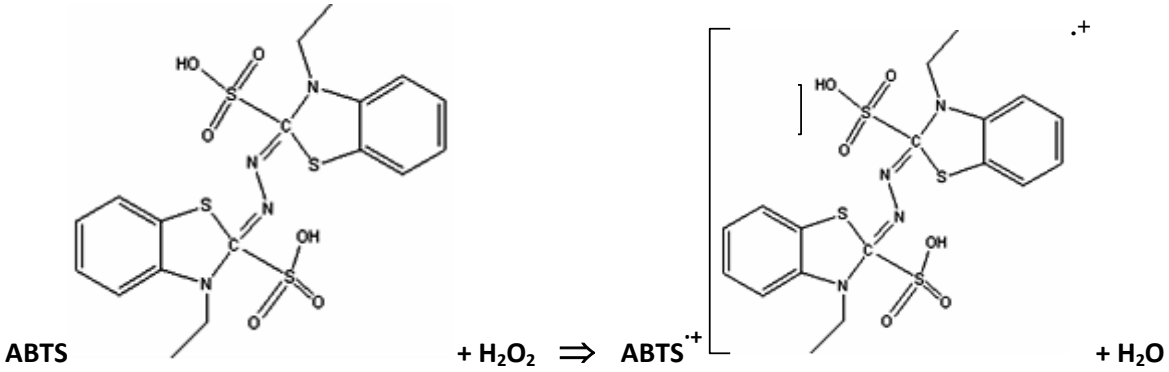


Figure 3.4. Reaction of ABTS with hydrogen peroxide

B. Chemicals;

ABTS and Trolox were purchased from Sigma-Aldrich (Gillingham, UK). Sodium acetate was from May and Baker LTD (Dagenhan, England), glacial acetic acid was from Fischer Scientific UK limited (Leicestershine, UK) and H_2O_2 was from El-Nasr Pharmaceutical Chemical Co. (Cairo, Egypt).

C. Reagents preparation

Reagent 1(R1*):* 0.4 mol/L acetate buffer solution (pH 5.8) was obtained as follows: 32.8 g of CH_3COONa was dissolved in 1000 mL of deionised water (final concentration: 0.4 mol/L). Reagent-grade glacial acetic acid (22.8 mL) was diluted to 1000 mL with deionised water (final concentration: 0.4 mol/ L). The sodium acetate solution (940 mL) was mixed with 60 mL of the acetic acid solution under a pH meter; the pH of the acetic acid–sodium acetate buffer will be 5.8.

Reagent 2 *(R 2)**:*** 30 mmol/L acetate buffer solution, pH 3.6, was prepared as follows: 2.46 g of CH_3COONa was dissolved in 1000 ml of deionized water (final concentration: 30 mmol/L). Reagent-grade glacial acetic acid (1.705 mL) was diluted to 1000 mL with deionized water (final concentration: 30 mmol/ L). The sodium acetate solution (75 mL) was mixed with 925 ml of the acetic acid solution under a pH meter; the pH of the acetic acid–sodium acetate buffer was

adjusted to 3.6. Then 278 μL of commercial H_2O_2 solution (35%) was diluted to 1000 mL with the buffer solution (final concentration, 2mmol/ L). Then 0.549 g ABTS was dissolved in 100 ml of prepared solution (final concentration: 10mmol/L). After 1 h of incubation at room temperature, the characteristic color of $ABTS^{\bullet+}$ was observed.

Standards*:* Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (a water soluble analogue of vitamin E) was used as a standard dissolved in PBS (PH7.2) and diluted in bidistilled water to get: 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 mM concentrations.

D. Procedure:

The procedures are given in the table below

Table 3.3. The procedures and reaction mixture for determination of total antioxidants

Reagents	Sample (μL)	Standard (μL)
Sample/Standard	10	10
Reagent (R1)	200	200
Reagent 2 (R2); read at 660 nm	20	20

E. Calculation

Total antioxidants concentration in samples was calculated from the standard curve constructed using standard concentrations and readings.

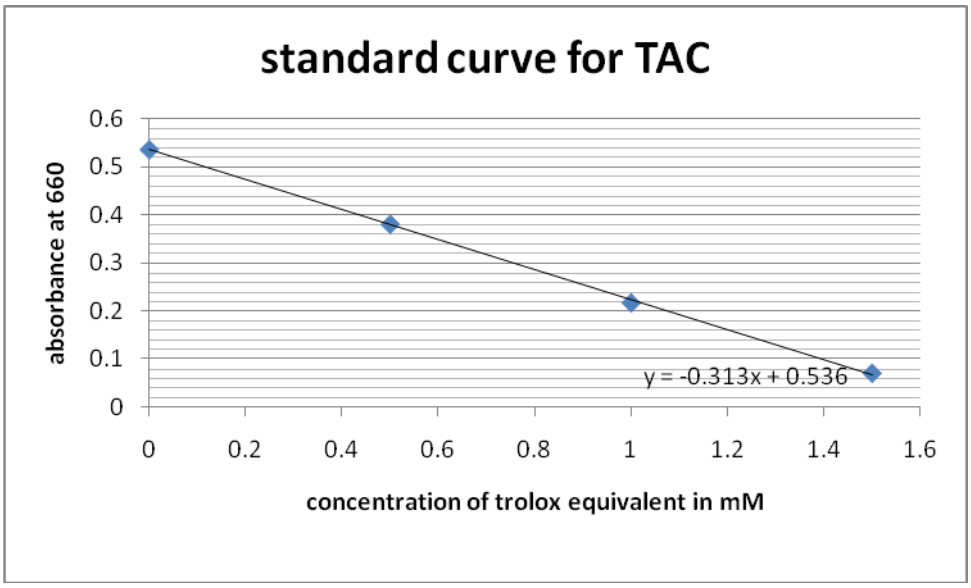


Figure 3.5. Standard curve for determination of TAC

3.6.6. Determination of Oxidative stress index (OSI)

The percent ratio of the TPP to the TAC gives the oxidative stress index, an indicator of the degree of oxidative stress. TAC and TPP in µM Trolox equiv./L were used for calculating OSI according to the following formula (Harma *et al.*,, 2005).

$$\text{OSI} = \frac{\text{TP, µmol/L}}{\text{TAC, µmol Trolox equiv. /L}} \text{ X 100}$$

3.7. Quality control

Proper sampling that minimizes interferences was used. Unavoidable interferences were minimized properly through, e.g., sample blanks. Samples were analyzed in duplicates whenever possible along with standards. Standard curve was constructed to calculate the analyte concentration.

3.8. Data Analysis

Results are expressed as mean values and standard error of mean. Statistical difference among groups and subgroups were analyzed by student T-test, one way ANOVA and two way ANOVA using Graph pad prism software.

3.9. Ethical Clearance

The study proposal was submitted to the department scientific and ethics committee and Institutional Review Board (IRB), school of Medicine, Addis Ababa University and got e ethical clearance.

The study was designed to evaluate the anti diabetic and oxidative/antioxidatve status in streptozotocin induced diabetic rats. Blood was collected by severing the tails with appropriate and sterile scissors. The blood was collected by heart puncture to asses' the level of plasma MDA, plasma TPP, plasma TAC and plasma oxidative stress.

4. Results

4.1. Anti-diabetic effects of *Taverniera abyssinica*

The anti diabetic effect of the crude root extract of *Taverniera abyssinica* was assessed by measuring the blood glucose of the diabetic rats treated with it and comparing the result with the diabetic control groups. The results of blood glucose for diabetic control (mean ±SEM) were 482.6±27.94, 516.6±22.10, 499.6±41.68, 512.2±22.57, and 536.2±8.57 in mg/dL on day 0, day 5, day 10, day 15 and day 20 respectively. The results of blood glucose for metformin treated rats at a dose of 200mg/kg BW (mean ±SEM) were 489.6±37.12, 462±52.17, 154.2±27.54, 213.2±61.74, and 309.2±7.23 in mg/dL on day 0, day 5, day 10 ($p<0.001$), day 15 ($p<0.001$), and day 20 ($p<0.01$) respectively. The blood glucose for metformin treated groups decreased significantly on the 10^{th}, 15^{th}, and 20^{th} days compared to the diabetic control group with the respective dates. The results of the blood glucose for *Taverneira abbysinica* treated rats at a dose of 100mg/kg BW (mean ±SEM) were 494.4±31.72, 370.8±107.14, 325.4±71.08, 230.0±37.65, and 169.8±55.01 in mg/dL on day 0, day 5, day 10 ($p<0.05$), day 15 ($p<0.001$), and day 20 ($p<0.001$) respectively. The blood glucose of the diabetic group treated with *Taverneira abyssinica* crude extract at a dose of 100mg/kg also significanty decreased on the 10^{th}, 15^{th}, and 20^{th} days with the corresponding days of diabetic control group. The results of the blood glucose for *Taverneira abbysinica* treated rats at a dose of 200mg/kg BW the results were (mean ±SEM) (mean ±SEM) were 414.6±65.92, 499.4±27.21, 264.8±51.92, 236.4±6.91, and 149.0±29.06 in mg/dL on day 0, day 5, day 10 ($p<0.001$), day 15 ($p<0.001$), and day 20 ($p<0.001$). In a similar fashion the blood glucose of the diabetic rats treated with 200mg/dL crude extrac *Taverneira abbysinica* was significantly reduced on the 10^{th}, 15^{th}, and 20^{th} day compared to the diabetic control group with respective days. The following figure summarizes the result.

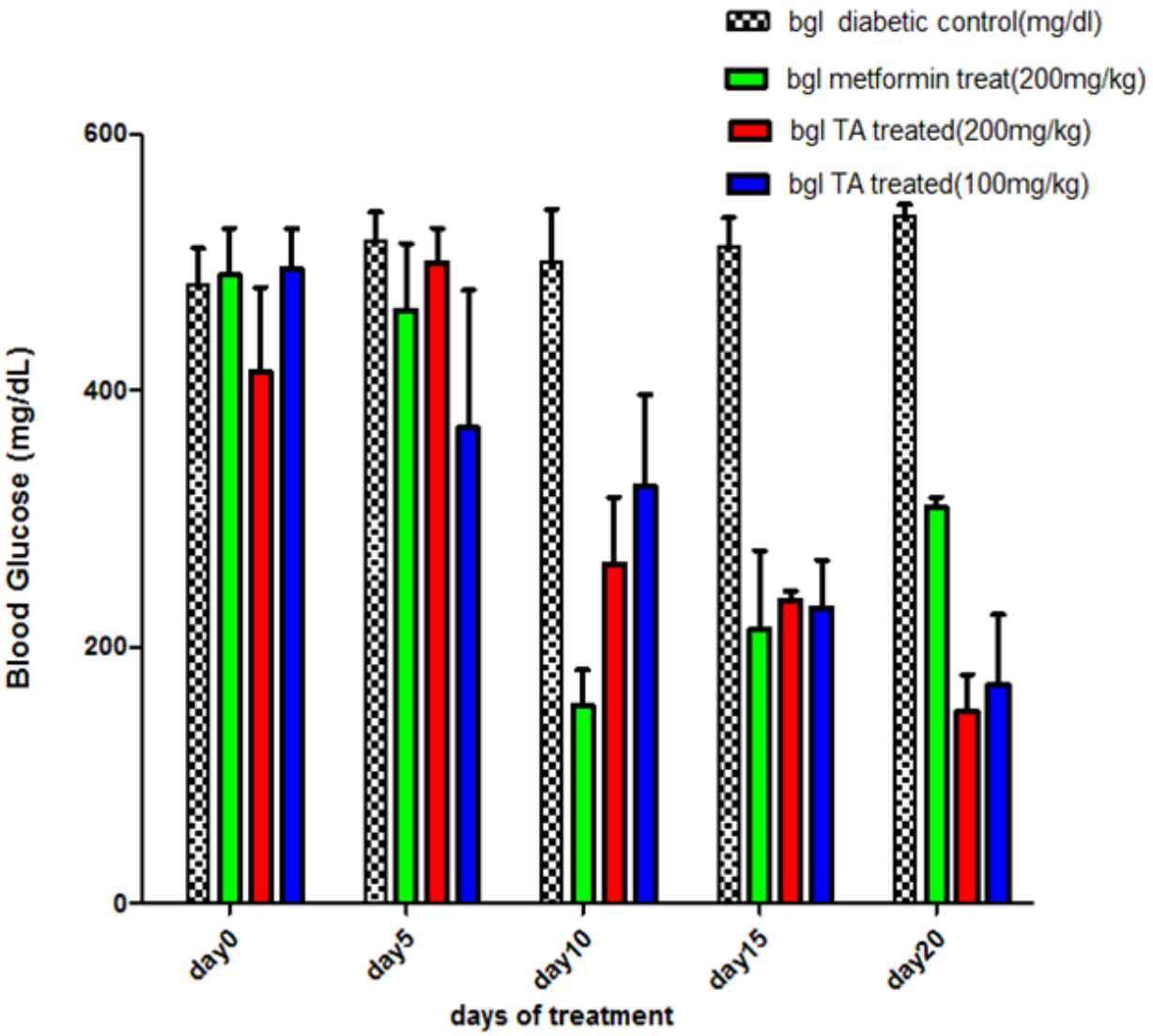


Figure 4.1.The blood glucose of the rats on the corresponding days of treatment

4.2. The Intraperitoneal Glucose Tolerance Test

Before the treatment for the diabetic control group the results of blood glucose were 525.83±9.7, 547.66±1.58, 550±0, 509±32.14, and 521.16±20.52 in mg/dL at times 0min, 30min, 60min, 90min, and 120min respectively for all the groups. On the other hand the results of blood glucose of the diabetic control group after treatment were 478.8±21.86, 547.8±2.2, 550±0, 498.6±33.63 and 414.8±32.23 in mg/dL at times 0min, 30min, 60min, 90min, and 120 min respectively. The measurments of blood glucose for metformin treated group were 469.2±51.94, 530±13.78, 545.6±2.4, 504.2±5.48, and 335±27.99 in mg/dL with the respective times. While the results of

blood glucose for *Taverneira abbysinica* treated rats were 427±29.47, 513.4±28.08, 531.6±18.4, 538.4±4.16 and 434.2±24.74 in mg/dL with the corresponding times respectively. The results revealed that the methanol root extracts of *Taverneira abyssinica* have hypoglycemic effect but not immefdiate. The following figure summarizes the results.

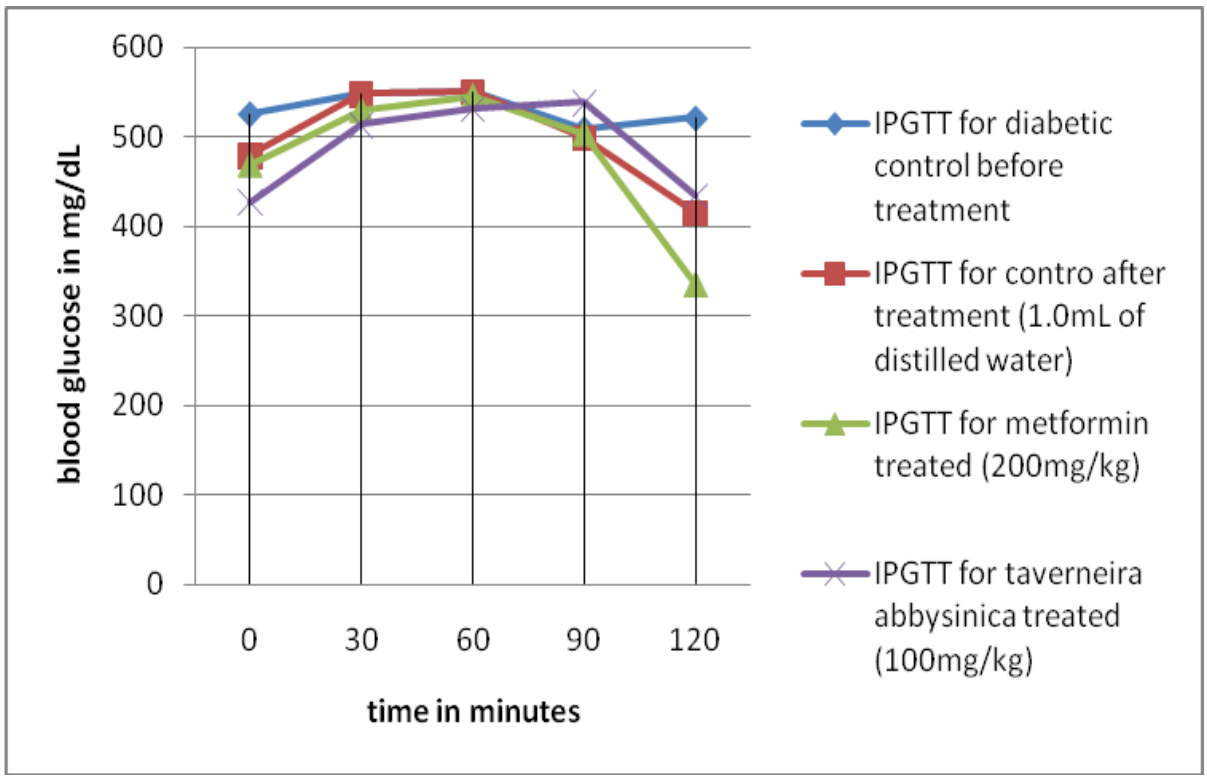


Figure 4.2. The intraperitoneal glucose tolerance test (IPGTT) curve before and after treatment

4.3. The Antioxidant activities of *Taverniera abyssinica* crude extract

The antioxidant activities of *Taverneira abyssinica's* crude root extracts were determined by measuring the level of plasma malondialdehyde, plasma total peroxides, plasma total antioxidant capacity, and calculating the oxidative stress index diabetic rats receiving 1ml of distilled water ip, diabetic rats treated with metformin (200mg/kg) BW ip and rats treated with *Taverneira abyssinica* root extract (100mg/kg) BW ip.

4.3.1. The level of Malondialdehyde

The level of plasma malondialdehyde (MDA) was determined by using the thiobarbituric acid reactive substances. As can been seen from the figure given below the level of MDA was higher in diabetic control (0.27±0.144 in μM). The level of MDA was lower in metformin treated group (0.1283±0.058 in μM) compared to the diabetic control but not significant. Similarly the level of MDA was decreased in *Taverneira abyssinica* treated group (0.0704±0.186 in μM) compared to the diabetic control group however, it was not significant. Though the result was statistically not significant, the level of MDA was decreased by half (50%) in metformin treated group and decreased by 74.07% in *Taverneira abyssinica* treated groups compared to diabetic control.

Table 4.1. The results of the level of MDA among the groups

	Diabetic control (1ml d. water)	Metformin treated (200mg/Kg) BW	Taverniera abyssinica treated (100mg/Kg) BW
Concentration of MDA (μM) as mean±SEM	0.27±0.144	0.1283±0.058	0.0704±0.186
Percent reduction compared to diabetic control	-	50%	74.07%

4.3.2. The level of total plasma peroxides

The level of total plasma peroxides was determined by their reaction with xylenol orange in the presence of ammonium ferrous sulfate. From the figure given below it was obvious that the level of TPP was higher in diabetic control group (4.273±0.665 in μM). The level of TPP in metformin treated group was (1.959±0.549 in μM) lower than diabetic control group but not significant at $p<0.05$. The level of TPP in *Taverneira abyssinica* extract treated group (1.916±1.044 in μM) also decreased but not significant at $p<0.05$ compared with diabetic control. The level TPP was decreased by 54.09% in metformin treated group and by 55.12% in *Taverneira abyssinica* treated group compared to diabetic control.

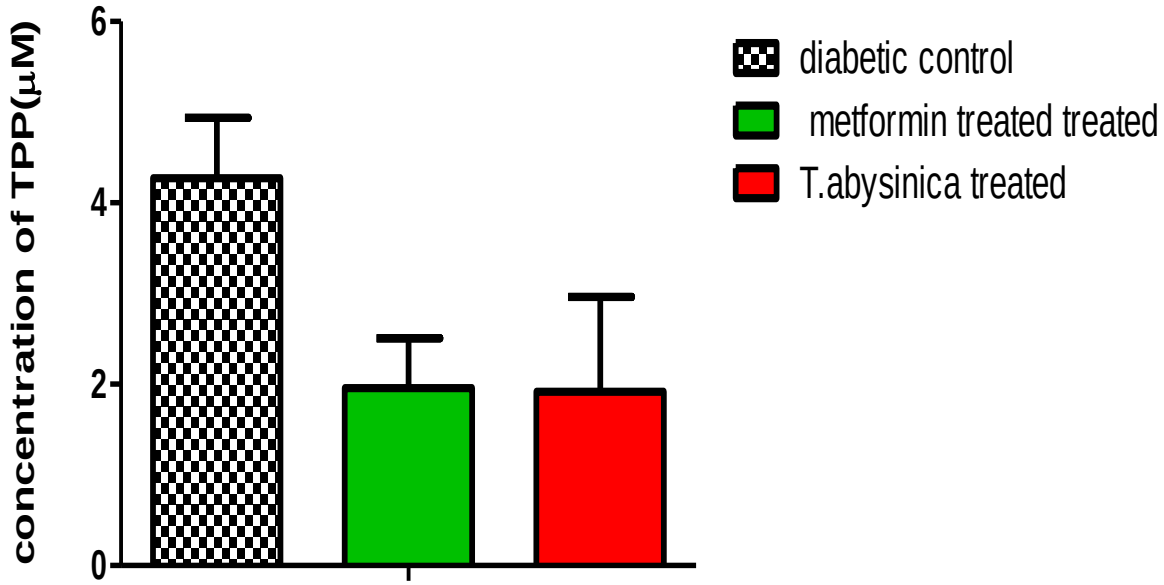


Figure 4.3. The level of TPP

4.3.3. The level of total Antioxidant Capacity

The level of total antioxidant capacity was determined by using the bleaching of $ABTS^{\cdot}$ at higher PH that proportionate with the concentration of TAC. From the figure below the level of TAC in diabetic control group was the lowest however, the degree of decrement was not significant compared to metformin treated and *Taverneira abyssinica* treated groups. The level of TAC was not significantly higher in metformin treated and *Taverneira abyssinica* treated groups compared with the diabetic control group. The level of TAC was higher by 5.43% in *Taverneira abyssinica* treated group compared to diabetic control. The following result was obtained.

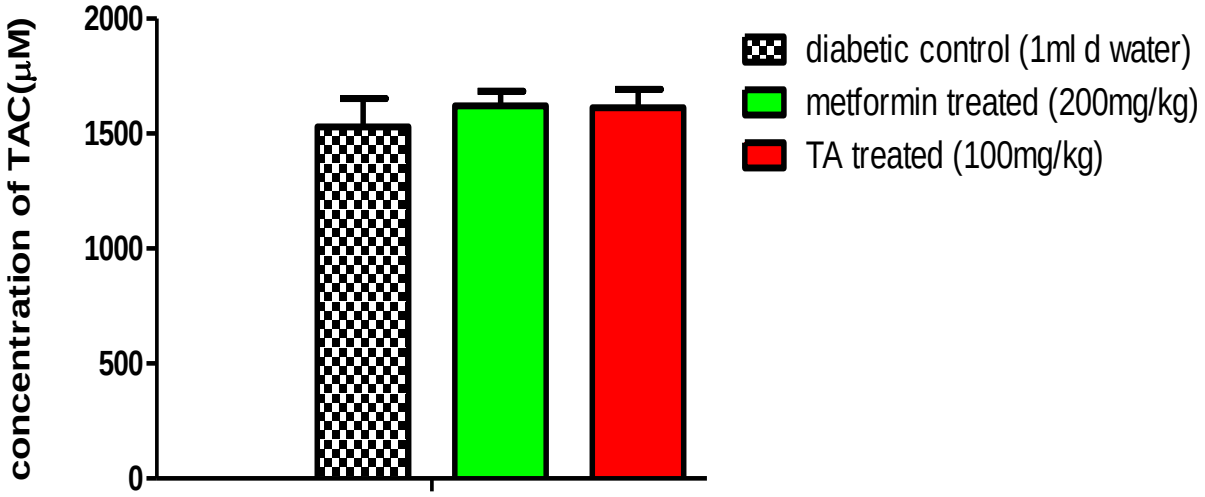


Figure 4.4. The level of TAC

4.3.4. The level of oxidative stress index

The level of oxidative stress index was calculated by using the following formula.

$$OSI = \frac{\text{TP concentraion, µ mol/L}}{\text{TAC, µmol Trolox equiv./L}} \times 100$$

The level of oxidative stress was higher in diabetic control than all other groups. The level of oxidative stress was decreased in metformin treated group compared with the diabetic control. And the level of oxidative stress was also decreased in *Taverneira abyssinica* treated group compared to the diabetic control. The level of oxidative stress is decreased by half (50%) in both metformin treated and *Taverneira abyssinica* treated groups compared to diabetic control. The table given below summarizes the results.

Table 4.2. A comparison of oxidative stress among the groups

	Diabetic control (1ml d. water)	Metformin treated (200mg/kg) BW	Taverniera abyssinica treated (100mg/kg) BW
Concentration of TPP (μM)	4.273	1.959	1.916
Concentration of TAC(μM)	1529	1621	1612
OSI	0.279%	0.12%	0.118%

5. Discussions

Diabetes mellitus is a complex, progressive disease, which is accompanied by multiple complications (Jay et al., 2006). Oxidative stress is significantly increased in DM, due to prolonged exposure to hyperglycemia, disturbances in capacities of the antioxidant defense system including uric acid, scavenging enzymes such as superoxide dismutase and glutathione reductase, and deficiencies of antioxidants such as vitamin C and E (Abou-Seif and Youssef, 2004). Antioxidants have been reported to reduce the complications in DM by arresting free radical damage. In line with this studies indicated that treatment with vitamins C and E could decrease the oxidative stress caused by hyperglycaemia (Kukner et al., 2009).

The present strategies for the management of diabetes are limited and involve insulin and four main classes of oral antidiabetic agents that stimulate pancreatic insulin secretion (sulphonylureas and rapid-acting secretogogous/insulinotropics *e.g.*, glibenclamide, glipizide, rapaglinide), minimizes hepatic gluconeogenesis (biguanides *e.g.*, metformin), delay digestion and absorption of intestinal carbohydrate (α-glucosidase inhibitors *e.g.*, acarbose) or improve insulin action [thiazolidinediones (TZDs) *e.g.*, pioglitazone, rosiglitazone] (Shrinvasan and Ramarao, 2007). These diabetes treatments have the following problems: (i) insulin therapy does not accurately immitate normal *β*-cell function, (ii) oral agents only partially correct the multiple cellular disturbances that characterize type 2 diabetes, (iii) these drugs do not completely normalize or reinstate glucose homeostasis, (iv) insulin resistance is still not responsive treatment and (v) diabetic patients are at risk of chronic morbidity and premature mortality, despite the assiduous use of the available drugs (Balaubramanyam and Mohan, 2001). Thus, there are wide varieties of newer therapeutic strategies being examined for the treatment of diabetes and the search for newer hypoglycemic drugs for the treatment of diabetes is an ongoing process.

In spite of the fact that most of the modern drug development relies upon computer imaginations on the basis of pharmacological data, the need in the utilization of plant remedies is resumed (Davidson 1986). In the Ayurvedic treatment, medicines consist of plant products, either single drug or in combination with others which are considered to be less toxic and free from side effects compared to synthetic drugs (Resmi 2001).

Historical evidences showed diabetes mellitus existed long ago and treated with medicinal plants. Medical plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager. The large number of plants described by Mohammed et al (2006) in his review (176 species belonging to 84 families) clearly demonstrated the importance of herbal plants in the treatment of diabetes. It also showed the effort being carried out to isolate new potential antidiabetic agents. Especially Chinese medicine has demonstrated an interesting practice in the therapy of diabetes because of distinctive medical theory and Chinese herbal medicines (Li *et al.* 2004). The significant role of plant medicine to treat diabetes is exemplified by the development of the anti-hyperglycemic drug metformin (Bailey and Day 1989). Even after the discovery and use of insulin and other modern oral antihyperglycemic agents (Bailey and Turner 1996; Cusi and De Fronzo 1998) the search for safer and more effective drugs of plant origin for the treatment of diabetes has continued (Esmaeili and Yazdanparast 2004). The WHO Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated (Bailey and Day 1989) for the development of new efficient antidiabetic drugs.

World health organization (WHO) estimates that more than 1200 plant species that represent more than 725 genera in 183 families ranging from marine algae to higher plants are used to treat diabetes around the world (Oubre *et al.* 1997) of which only about 350 of them are documented to present hypoglycemic activity (Alarcon-Aguilar *et al.* 2002). Although scant few have been subjected to rigorous scientific evaluation for safety and efficacy in humans (Haddad *et al.* 2001 and Jung *et al.* 2006).

In similar fashion to other countries, different kinds of plants are used to treat various diseases like diabetes mellitus, gastrointestinal disorders and others in the Ethiopian traditional medicine of which *Moringa stenopetala*, *Melia azedarach* and *Taverneira abyssinica* are the most common. Regardless of their wide acceptance and usage, their hypoglycemic and antidiabetic effects are not well established and the scientific publications addressing the biological effects of these medicinal plant both in vitro and in vivo animal models are meagre. The present study was carried out to identify the anti-diabetic effect and antioxidant activities of *Taverneira abyssinica* in animal models.

As described above there are very limited reports on the chemistry and biological activity of *Taverneira abyssinica*. The phytochemical investigation of aqueous root extract on the chemistry of this plant revealed the presence of two isoflavonoids; formononetin and afromosin and two pterocarapans: medicarpin and 4-hydroxymedicarpin (Duddeck *et al.* 1987). Studies on the biological activity of this plant demonstrated that the crude aqueous extracts of roots of *Taverneira abyssinica* posses antispasmolytic action on isolated intestinal smooth muscle of rats , antipyretic and analgesic effects (Noameshi et *al.* ,1990; Dagne *et al.* 1990). These studies justified these effects are due to some of the previously isolated compounds from the roots of *Taverneira abyssinica*. Another study carried out on the aqueous root extracts of this plant by Benjamin *et al.* (1994) exhibited the extract have anti-ulcerative properties in mice and safe to be used.

The results of ip administration of root extracts of *Taverneira abyssinica,* at doses of 100mg/kg and 200mg/kg showed that *Taverneira abyssinica* root extract significantly reduced blood sugar over a period of three weeks in comparison with the diabetic control group. These doses were selected based on the pilot study and previous study. In the pilot study performed the ip administration of the root extract at dose of 50mg/kg did not reduce the fasting blood sugar of normal rats at 1hr, 2hr, 3hr and 4hrs after administration. On the contrary the blood sugar was reduced at doses of 100mg/kg and 200mg/kg at 2hr, 3hr and 4hrs after ip administration the result being better at 4hr. The result was in agreement with the previous study carried out by Daniel et al (2011) unpublished on the hypoglycemic and antidiabetic effects of the methanolic root extract of *Taverneira abbysinica*.

The presence of isoflavonoids-like formononetin, afromosin, genistein, biochanin A, glycintin and diadzein have been identified in *Taverneira abbysinica* root extract which are anticipated to contribute for the insulinogenic effects of the root extract. Therefore, the reduction of blood glucose might be due to the action of these isoflavonoids or other components of *Taverneira abbysinica* which are yet not identified. In addition to this Daniel (2011) unpublished reported that the exposure of islets to formononetin from *Taverneira abbysinica* roots increased insulin secreting ability at basal and high glucose concentrations. The same report showed that formononentin induced insulin secretion significantly at doses of 1μM, 10μM and 100μM with

the maximum effect observed at 100μM in basal glucose. Oppositely at high glucose formononentin had no effect on insulin secretion at all doses except at 100μM. Other study carried out on the mechanism of action of isoflavonoids showed that formononentin induces insulin secretion from existing β- cell and has no proliferative effect on islets cells (Zheng). Another report suggests it could be doing so through its antioxidant activity (Toda and Shirataki 1998; Yu *et al.* 2005). A similar study also revealed that formononentin had a potency of activating peroxisome proliferator activators receptors α/γ (PPARα/γ) comparable with synthesized PPARα/γ agonists, such as ragaglitazar, tesaglitazar and muraglitazar (Shen et al, 2006).

Similarly Daniel et al (2011) unpublished reported that the methanol extracts of *Taverneira abyssinica* induce glucagon secretion at low blood glucose and has no effect at high blood glucose. The same study revealed that formononetin also increased glucagon secretion at low blood sugar and basal glucose and has no effect at high concentration of glucose. This study implies that the reduction of blood glucose after ip administration of the root extract could be accounted by the insulinogenic action of formononentin. In other words it means the root extracts of *Taverneira abyssinica* brings about the regulation of blood sugar by increasing the secretion of insulin and glucagon depending on their demand.

Even though the root extracts of *Taverneira abyssinica* showed a significant reduction over a long period of time the intraperitoneal glucose tolerance test carried out revealed that there was no significant difference on the curve for the diabetic control and *Taverneira abyssinica* treated group. This result shows that the root extracts of *Taverniera abyssinica* has no immediate effect on decreasing the blood sugar.

Regarding the antioxidant activity of the root extracts of *Taverneira abyssinica* no published scientific paper was found to our knowledge. However, the limited scientific publications on *Taverneira abyssinica* revealed that the crude methanolic root extracts contain isoflavonoids. Most of the publications available on flavonoids and isoflavonoids showed that they have antioxidant, anticancer, anti-fungi activities and others. On the basis of properties isoflavonoids this study demonstrated the antioxidant activity of the crude methanolic root extract of *Taverneira abyssinica* in animal model, though not significant. In this study the level of MDA,

TPP, TAC and OSI of the *Taverneira abyssinica* treated groups was measured and the result was compared with the diabetic control group.

The result showed the level of MDA was 74.07% lower in *Taverneira abyssinica* treated group compared to the diabetic control. In a similar fashion the level of TPP was decreased by 55.12% in *Taverneira abyssinica* treated group to the diabetic control group while the level of TAC was increased by 5.45% in *Taverneira abyssinica* treated group than in diabetic control group. The result shows that methanolic crude root extract of *Taverneira abyssinica* have antioxidant activity which could be due to formononentin or other component of the root extract. Similarly this study revealed that the plants extract reduces the oxidative stress with 50% reduction in *Taverneira abyssinica* treated group compared to the diabetic control group.

6. Conclusions

The present study has showed that the crude methenolic root extracts of *Taverneira abyssinica* have hypoglycemic effects but not immediate and also have anti diabetic effects in the long term treatments of three weeks. Thus its utilization in the treatment of sudden illness is helpful giving relief of stresses induced by hyperglycemia in diabetes mellitus in cases where the disease was not identified even though the society did not use the plant specifically to treat diabetes mellitus. This implies that the society is benefiting indirectly to overcome the sudden impacts of DM from the traditional practice.

Regarding the antioxidant activity of the crude methanolic root extract of *Taverneira abyssinica*, this study showed that the plants crude extract posses antioxidant activities by decreasing the level of MDA by 50% , and the level of TPP by 74.4% and increasing the level of TAC by 5%. This suggests that the plants' root extract is rich in antioxidants, and decreases oxidative stress which could also contribute to its anti diabetic effects. This also strengthens the society's practice of utilization of the plant to treat other sudden diseases too as long as sudden illness is associated with inflammations and generation of free radicals and antioxidants play great role in the treatment of diseases like cancer, cardiovascular disease, Alzheimer's disease and other chronic diseases.

7. Recommendations

However, this study demonstrated the antioxidant activities, the hypoglycemic and antidiabetic effects of *Taverneira abyssinicas'* crude methanolic root extract the mechanism of its action was not studied well and the number of sample included in the study was very small. Therefore, further study should be carried out with large sample size and repeatedly in order to;

- ✓ assure whether the plants' methanolic crude root extract exactly have hypoglycemic and antidiabetic effects.
- ✓ identify the unknown components of *Taverneira abyssinica*.
- ✓ demonstrate the mechanism of action and which of the component of *Taverneira abyssinicas'* crude root extract posses' hypoglycemic and anti diabetic effect.
- ✓ confirm the antioxidant activities of *Taverneira abyssinicas'* crude methanol extract and
- ✓ distinguish whether the antioxidant activity of the plants' extract is due to the known components like formononentin or other unidentified components.

8. Limitations of the study

The limitations of this study are;

- it used small sample size, and
- experiments were not repeatedly carried out due to the expensiveness of the cost of the reagents used in the study and little amount have been found and used.

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