CHAPTER 7

IN VITRO ANTIDIABETIC PROPERTIES OF SOUTH AFRICAN MEDICINAL PLANT: *STRYCHNOS HENNINGSII* (Gilg)

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Abstract

Strychnos henningsii Gilg is a plant commonly used in southern Africa traditional medicine for the management of diabetes. However, the mechanism of action of this plant for the management of diabetes has not been elucidated. The plant extract was tested for the potential to induce differentiation in 3T3-L1 preadipocytes using Oil Red O staining. The *in vitro* antidiabetic effect of the extract was screened against differentiated 3T3-L1 cells and Chang liver cells by measuring glucose uptake. We also assessed the therapeutic approach of *S. henningsii* extract against gastrointestinal carbohydrate digestion through inhibition of α - glucosidase and α – amylase. The antioxidant potential and ability of the plant extract to inhibit protein glycation was also investigated. Plant extract significantly increased glucose uptake in the 3T3 L1 cells with a response of 278.63% above the control at 12.5µg/ml but showed no effect on Chang liver cells. No cytotoxicity was recorded for preadipocyte at the concentrations investigated in this study while Chang liver cells exhibited toxic effect at 250 and 500 µg/ml. The extract inhibited the activity of α- glucosidase and α- amylase in a concentration dependent manner with IC_{50} 0.84 and 2.31 mg/ml respectively. The plant extract exhibited weak inhibition of protein glycation relative to aminoguanidine. The FRAP

assay values of the extract was 357.05 µmol Fe (II)/g while that of BHT, catechin and quercetin were 75, 859.32 and 2907 µmol Fe (II)/g respectively. The finding from this study provides some evidences that *S. henningsii* extract may indeed be useful in the treatment of diabetes mellitus.

Keywords: *Strychnos henningsii*; 3T3-L1; Chang liver cells; α –glucosidase; α –amylase; cytotoxicity; glucose uptake; oil red-O; protein glycation; diabetes mellitus

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disorder characterized by hyperglycemia and glucose intolerance as a result of inadequate supply of insulin or impaired effectiveness of insulin to stimulate glucose utilization or both. The impairment of insulin secretion from pancreatic beta cells has been reported to affect glucose utilization as a result of defect in insulin signaling cascade (Zick, 2001; Else et al., 2009). This occurs most importantly in target tissues such as skeletal muscle, adipose tissue and liver cells of type 2 diabetic patients (Baynes, 2004; Guyton, 2005).

Persistent hyperglycaemia in diabetes mellitus induces non enzymatic glycation of protein, hyperlipidemia and oxidative stress which are believed to be pathogenetic in the development of diabetic complication such as retinopathy, neuropathy and nephropathy (Jakus et al., 1999). This could be linked to imbalance of antioxidant defense mechanism to neutralize the formation of excessive free radicals generated in the system (Hazra et al., 1999). Despite the recent surge of new antidiabetic drugs such as insulin releasers, insulin sensitizers and alpha glucosidase traditional healers are heavily reliant upon medicinal plants to treat this disease (Ebbeling and Ludwig, 2001). These plants are known to possess antidiabetic properties due to a wide range of phytochemical compositions with cost effectiveness and lesser side effects (Edwin, 2008). Moreover, the majority of plants use in southern Africa traditional medicine need to be thoroughly explored to understand the mechanism of action underlying their activity.

Strychnos henningsii Gilg (Loganiaceae) is one of the plants used in southern and eastern Africa as a folk remedy for the management of various diseases (Oyedemi et al., 2009). The decoction of the plant (branch, stem and root) has been used in traditional Kenyan medicine for the treatment of rheumatism, gynaecological complaints, chest pain, internal injuries and malaria (Bisset, 1970; Hutching, 1989; Kareru et al., 2007). It is also used in

Tanzania for the treatment of snake bite and hookworm infections. The aqueous bark extract of the plant is used in South Africa traditional medicine for the treatment of stomach ache, colic, dizziness, purgative, nausea and anthelminthic. This plant was discovered recently during our ethnobotanical survey in Nkonkobe Municipality, Eastern Cape, South Africa as herbal mixture for the treatment of diabetes mellitus (Oyedemi et al., 2009). The isolated alkaloids such as holstiine, holstilinne, rindline and strychinine from the stem bark of this plant were shown to possess pharmacological and physiological properties (Ohiri et al., 1983; Tits et al., 1991). These include convulsive, anti-cancer, anti-malarial, hypotensive, anti-inflammatory, anti-spasmodic and cardiac depressant activities (Neuwinger, 1999).

Previously, we have demonstrated the effect of aqueous stem bark extract of *S. henningsii* on fasting blood glucose and glucose tolerance in diabetic induced rats (Chapter 6). The present study was therefore designed to further investigate the antidiabetic properties of the plant extract responsible for its antidiabetic activity using various in vitro models designed to simulate specific known antidiabetic target. This study examined the effect of *S. henningsii* extract on *in vitro* glucose utilization in hepatic (Chang liver) and adipocyte (3T3-L1) cell lines; the two cell lines used in this study were chosen because of the importance of the liver and adipose tissue in the regulation of blood glucose homeostasis. Furthermore they have different glucose transporters, which react differently to insulin stimulation. In addition, preadipocyte differentiation cytotoxicity, inhibition of protein glycation and intestinal carbohydrate digestions were also investigated as potential therapeutic targets.

MATERIALS AND METHODS

Plant material

The bark of *S. henningsii* was collected from a thick forest in February, 2009 by a traditional healer in the Amathole District (Eastern Cape, South Africa). The plant was identified by its vernacular name and later authenticated by Prof. D.S. Grierson of Botany Department, University of Fort Hare. Voucher specimen (Sun MED 2009) was deposited at the Giffen herbarium of the University. The herbal drug was prepared by soaking 3 g of the plant sample in 750 ml of water for the treatment of diabetic patient according to traditional healers interviewed.

Preparation of plant extracts

The bark material was air-dried at room temperature in the laboratory. The dried bark was further grounded into powdery form using an electric blender (Waring Products Division, Torrington, USA). About 80 g of the powdered plant material was extracted in 1000 ml of cold distilled water (4 ^{o}C) maintained on a mechanical shaker (Stuart Scientific Orbital Shaker, SOS1, Essex, UK) for 48 h. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was quickly frozen at -40 ^{o}C and dried for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA) to give a yield of 10.6 g of dry extract. The dried extracts were reconstituted in diluted DMSO, vortexed and left for at least 15 min before further dilution with the respective growth medium for acute exposure. The final DMSO concentration did not exceed 0.25%.

Cell lines, media and chemicals

Chang liver and 3T3-L1 (Preadipocyte) cell lines were obtained from the American Type Culture Collection (Highveld Biological, Johannesburg, South Africa). Glucose oxidase reagent (SERA-PAK Plus, Bayer), and RPMI 1640 medium was obtained from Sigma Aldrich, S. Africa, whereas fetal calf serum (FCS) was purchased from Highveld Biological, South Africa. The chemicals and other reagents used in this study were of analytical grade and were purchased from Merck Chemicals (Pty) Ltd, S. Africa.

Maintenance of cell cultures

All cell cultures were incubated at 37 ^{o}C in a humidified atmosphere with 5% CO_2. The Chang liver cells were fed fresh with growth medium every 2-3 days, consisting of RPMI 1640 (Highveld Biological, South Africa) medium supplemented with 10 % fetal bovine serum. 3T3-L1 cells were cultured in DMEM (1.5g/l $NaHCO_3$) (Highveld Biological, South Africa) with 10 % fetal bovine serum under rigorous aseptic techniques to avoid cell culture contamination. Both cell lines were sub-cultured after 90 % confluence was reached.

Glucose utilization in 3T3-L1 cell line

The 3T3-L1 cells were cultured in DMEM (1.5g/L NaHCO3) with 10% fcs. For glucose utilization determination, 3T3-L1 preadipocytes were seeded at a density of 3 000 cells per well into a 96-well plate and cultured for five days in a growth medium (Erasto et al., 2009). The method described by van de Venter et al. (2008) was adopted to determine the glucose uptake. The cells were left without feeding after seeding to allow glucose depletion from the medium. On day three after seeding, 1 µl of *S. henningsii* extract (12.5µg/ml) was added to give the final concentration of 2.5µg/well of the extracts. On day five after seeding,

the medium was removed and 50 µl of incubation medium (8mM glucose RPMI 1640 + 0.1% BSA) containing the plant extract (12.5µg/ml) was added to the appropriate wells. The control wells contained only incubation medium while the positive control contained 1 µM of insulin. After 90 min of incubation, 10 µl of the incubated medium was removed and placed into a new 96 well plate into which 200 µl glucose oxidase reagent (SERA-PAK Plus, Bayer) was added. After incubation for 15 min at 37 ^{o}C, the absorbance was measured at 492 nm using a Multiscan MS microtitre plate reader (Labsystems). The amount of glucose utilized was calculated by subtracting the amount of glucose left in the medium from the amount given at time 0 (8mM).

Glucose utilization in Chang liver cells

The Chang-liver cells were cultured and maintained in an antibiotic-free growth medium as described above. The cultured cells were seeded into 96 well plates at a density of 6 000 cells per well in a growth medium (RPMI 1640 medium supplemented with 10 % fetal bovine serum) for three days without feeding, prior to glucose utilization assay. On day three after seeding, 1 µl of *S. henningsii* extract (12.5µg/ml) was added to give the final concentration of 2.5µg/well of the extracts. On day five after seeding, the medium was removed and 50 µl of incubation medium (8mM glucose RPMI 1640 + 0.1% BSA) containing the plant extract (12.5µg/ml) was added to the appropriate wells. 1 µM metformin was used as the positive control while the control contained only incubation medium. After 180 min of incubation, 10 µl was removed and placed into a new 96 well plate into which 200 µl glucose oxidase reagent (SERA-PAK Plus, Bayer) was added. After incubation for 15 min at 37 ^{o}C, the absorbance was measured at 492 nm using a Multiscan MS microtitre plate reader (Labsystems). The amount of glucose utilized was calculated by subtracting the amount of glucose left in the medium from the amount given at time 0 (8mM).

Oil - red - O staining

Treated 3T3-L1 cells of were stained with 100 µl of Oil Red O and incubated for 30 min at37°C. The assay was conducted following the method described by Kasturi and Joshi (1982). Excess dye was removed by washing thrice with water and the plate was later dried in an oven at 37 ^{o}C. The dye was further extracted by adding 100 µl of isopropanol followed by measuring the absorbance at 520 nm.

Total phenolics content

The amount of phenolics compound in the aqueous leaves extract of *L. leonurus* was determined with Folin Ciocalteu reagent using the method of Lister and Wilson (2001). To 0.5 mL of plant extract solution (1 mg/mL) was added 2.5 mL of 10 % Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 (2 % w/v). The resulting mixture was incubated at 45 ^{o}C with shaking for 15 min. The absorbance of the samples was measured at 765 nm using UV/visible light. Total phenolics content was expressed as mg/g tannic acid equivalent using the following equation from the calibration curve: $Y = 0.1216x$, $R^2 = 0.936512$, where x was the absorbance and Y was the tannic acid equivalent (mg/g). The experiment was conducted in triplicate, and the results are reported as mean ± SD values.

Ferric reducing antioxidant potential (FRAP)

The method described by Benzie and Strain (1999) was used to determine total antioxidant activity of *S. henningsii* extract**.** The FRAP reagent was prepared by mixing 20 ml acetate buffer (300mM, pH 3.6), 2 ml TPTZ solution, with 2 ml $FeCl_3$ solution and 2.4 ml distilled water. The solution was straw coloured and kept in a water bath at 37 ^{o}C. A volume of 10 µl of the sample was transferred into a 96 well plate with 250 µl of freshly prepared FRAP reagent. The absorbance at 593 nm after 5 min against blank containing distilled water

was taken and recorded. The antioxidant capacity of this plant based on the ability to reduce ferric ions of the extract was expressed as catechin equivalents (mg/g) using the following equation from the calibration curve: $Y = 0.3329x$, $R^2 = 0.9763$ where x was the absorbance and Y the catechin equivalent (mg/g).

Inhibition of Protein glycation

Albumin glycation was determined using the method of Matsuura et al (2002). Bovine serum albumin (10 mg/ml) was incubated with 250 mM of glucose in 200 mM potassium phosphate buffer (pH 7.4; 0.02% sodium azide) for 72 h. During this time, samples were kept in a 37 ^{o}C under 5% CO_2. Results were read using a microtiter plate reader with an excitation/emission wavelength of 370/440 nm. The assay was routinely conducted using 12.5 µl of plant extract in a 1 ml assay. The inhibition of glycation was assumed when fluorescence was reduced to that of albumin in the absence of glucose. All experiments were performed in triplicate and the extract was tested at least twice at a minimum of three dilutions.

Alpha-glucosidase inhibiting activity

Alpha glucosidase solution was prepared from rat intestinal acetone powder. Approximately 100 mg of acetone powder was suspended in 3 ml of 0.01 M phosphate buffer (pH 7.0) and sonicated for 30 s for 12 times in ice bath followed by centrifugation at 3000 rpm, 4 ^{o}C for 20 min. The supernatant containing the enzyme was obtained and kept on ice for further dilution. The enzyme inhibition activity of α-glucosidase was evaluated according to the method previously reported by Sancheti et al (2010). The reaction mixture consisted of 50 µl of 0.5 mM MPNG (dissolved in 0.1 M phosphate buffer pH 7.0), 10 µl of test sample and 25 µl of a glucosidase solution. The glucosidase solution was prepared using

a stock solution of 1 mg/ml in 0.01M phosphate buffer (pH 7.0), which was diluted to 0.1 U/ml with the same buffer (pH 7.0) just before the assay. This reaction mixture was then incubated at 37 ^{o}C for 30 min. The reaction was then terminated by the addition of 100 µl of a 0.2 M sodium carbonate solution. The enzymatic hydrolysis of the substrate was monitored based on the amount of p-nitrophenol released into the reaction mixture by a microtiter plate reader {Multiscan MS microtitre plate reader (Labsystems)} at 410 nm to measure the absorbance. The % inhibition was calculated as follows: (1 - B/A) × 100 where A is the absorbance of control and B is the absorbance of plant extract. All experiments were carried out in triplicate and the results are expressed as the mean ±SD of three determinations.

Alpha- amylase inhibitory activity

The α-amylase inhibitory activity of *S. henningsii* extract was tested based on the method described by Zhizhuang et al. (2005) using microplate-based starch-iodine assay. The reaction was initiated by adding 40 µl (2 g/L) of starch (Sigma S-2630) solution and 40 µl of enzyme in 0.1 M phosphate buffer at pH 7.0 to microplate wells. After 30 min of incubation at 50 ^{o}C, 20 µl of 1 M HCl was added to stop the enzymatic reaction. 100 µl of iodine reagent (5 mM I_2 and 5 mM KI) was added to the mixture. After colour development, 150 µl of iodine treated sample was transferred to a transparent flat bottomed 96 well microplate to measure the absorbance at 580 nm.

MTT cell viability assays

Cell viability in the individual wells of both cell lines treated with plant extract was evaluated using a modified method of Mosman (1983) to determine cytotoxicity and to normalize glucose utilization results. Toxicity assays of both cell lines were done by adding

100µl of MTT (0.5 mg/ml in RPMI1640: 10% fbs) directly into the last three rows of Chang liver and 3T3-L1 cell lines individually with 10µl of plant extracts (12.5 and 25µg/ml). MTT (3-(4,5-dimethylthiazolyl-2)- 2,5-diphenyltetrazoliumbromide) is a yellow water soluble tetrazolium dye that is reduced by metabolically active cells to a purple formazan product that is insoluble in aqueous solution within 2 hrs of exposure. After 2 hrs of incubation (5% humidified incubator) at 37^{o}C, the MTT was removed and the resulting formazan dissolved in DMSO was read spectrophotometrically (Multiscan MS, Labsystems) by measuring the absorbance at 540 nm.

RESULTS

Glucose uptake in 3T3-L1 cells

The plant extract demonstrated high insulin-sensitive glucose uptake of 178.6% at 12.5µg/ml while the cells treated with 25µg/ml were 20.2% over the control (Figure 1). In the positive control, insulin caused a two-fold increase in glucose utilization for 24 h over the basal. However, in the presence of insulin, the extract further increases glucose utilization to 325 % at 12.5 µg/ml and 290 % at 25µg/ml over the control. This result indicates synergistic or additive interaction of the extract with insulin for the induction of glucose utilization in 3T3-L1 adipocytes.

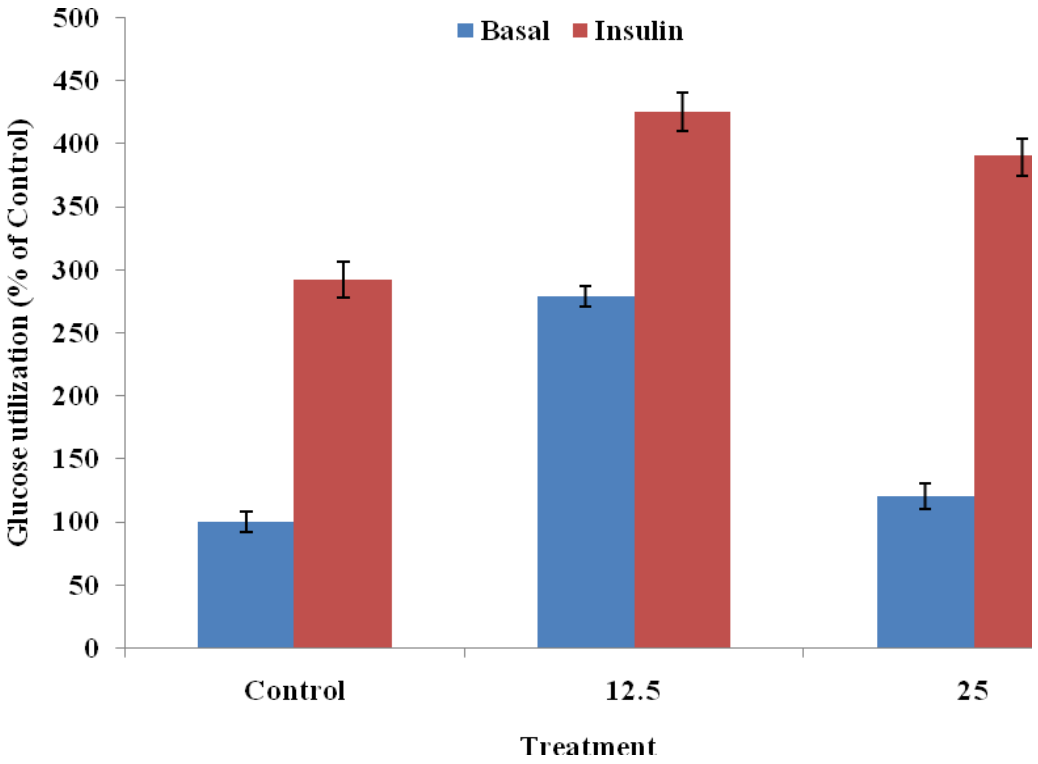

Figure 1: The effect of aqueous extract of *S. henningsii* bark on glucose utilization in the 3T3-L1 cells. Cells were exposed to extract concentration at 12.5, 25µg/ml and insulin (1µM) prior to glucose utilization. Data are represented as % control ± SD of mean (n = 6).

Glucose uptake in Chang liver cells

Figure 2 revealed the effect of *S. henningsii* extract on glucose uptake in Chang liver cells. The extract did not have any significant effect (P>0.05) on glucose utilization in the cells when compared with the control. The percentage glucose uptake by the extract at 12.5µg/ml and metformin at 1uM was 103.5% and 160.24% respectively. Metformin is a popular drug prescribed for the treatment of type 2 diabetes however the precise molecular mechanism remains poorly defined. The glucose lowering effects associated with metformin results mainly from decreased hepatic glucose production via the inhibition of gluconeogenesis, and to a lesser extent due to increased insulin stimulated glucose utilization

in peripheral tissue. It has been shown to inhibit the mitochondrial respiratory chain at complex 1 with concomitant up-regulation of glucose transporters and glycolytic enzymes resulting in increased glycolytic glucose utilization, and hence is used as positive control in the Chang liver assay. Metformin exhibited higher glucose uptake activity with response of 56.74% over the extract. The plant extract appeared to inhibit the action of metformin when incubated together with the Chang liver cells (102.9%).

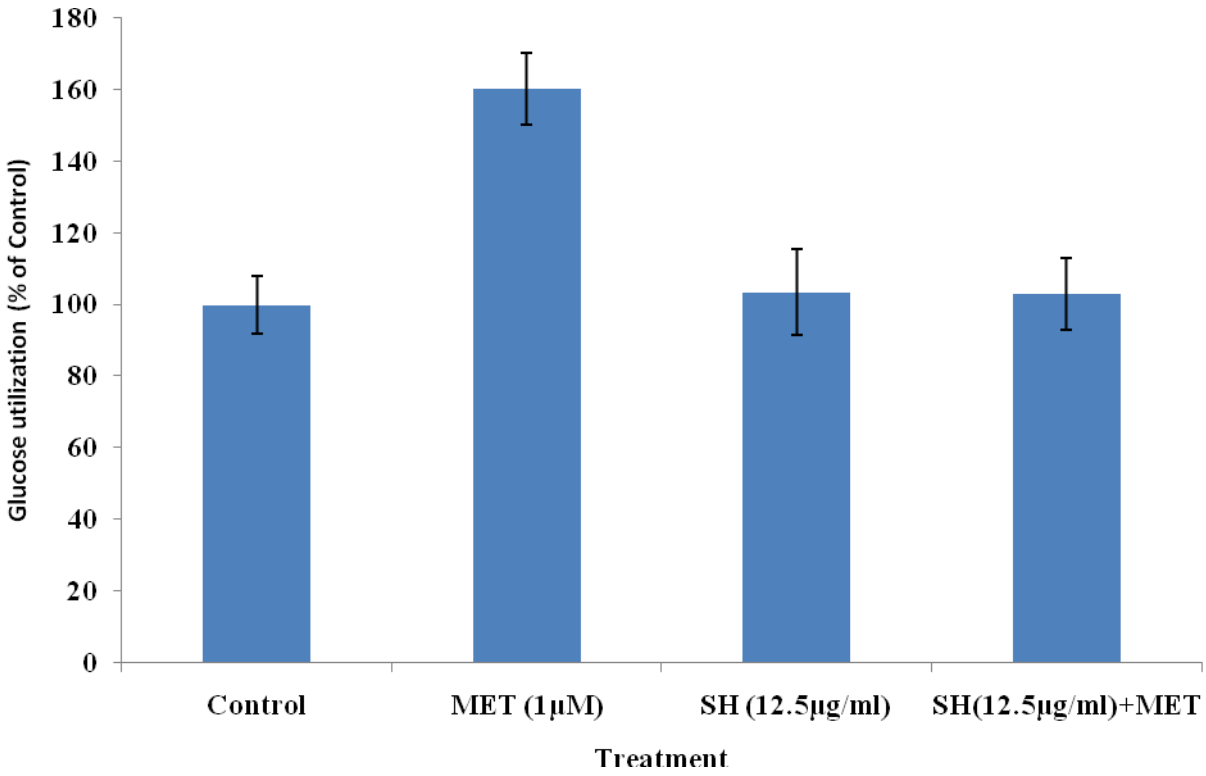

Figure 2: The effect of aqueous bark extract of *S. henningsii* on glucose utilization in the Chang liver cells. Cells were exposed to extract concentration of 12.5µg/ml, metformin (MET) and combination of extract and metformin (SH+MET) prior to glucose utilization measurement. Data are expressed as % control ± SD (n =6).

Cell viability assay

The effect of aqueous stem bark extract of *S. henningsii* on the growth of 3T3-L1 and Chang liver cell lines were examined by MTT assay. The data obtained on Chang liver cells revealed non toxic effect of the extract at both concentrations investigated (Figure 3).

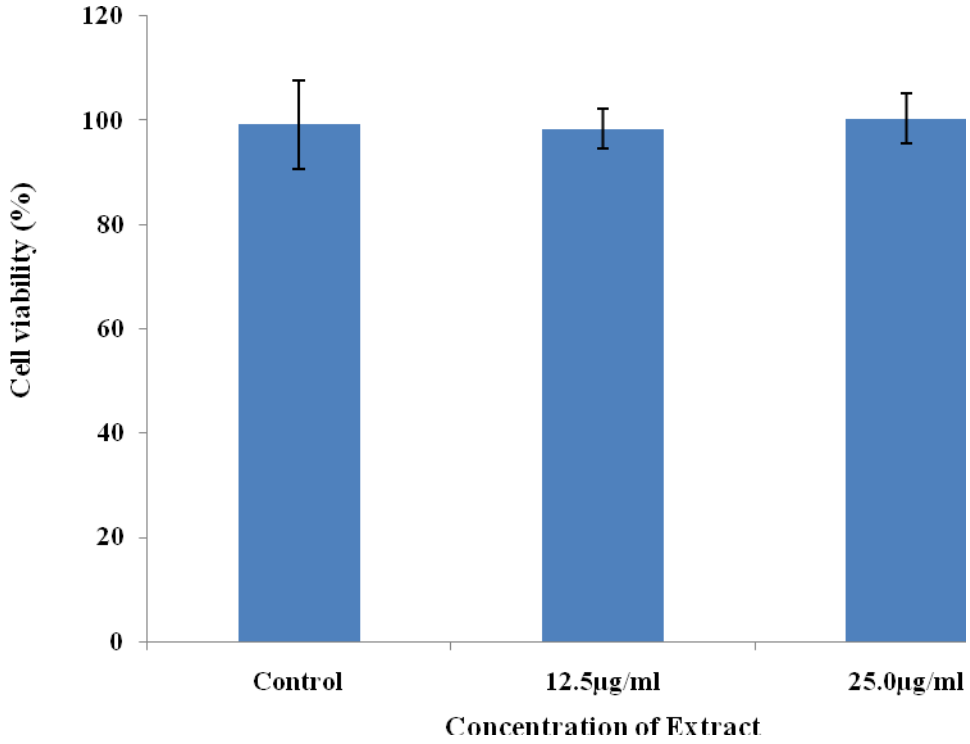

Figure 3: MTT assay of aqueous extract of *S. henningsii* in the Chang liver cells. Data are expressed as % control ± SD (n = 6).

In 3T3-L1 adipocytes, the plant extract reduced the relative cell viability to 90.2 and 85.7% at the dose of 12.5 and 25µg/ml compared with 100 % of the control (Figure 4). These data suggest that *S. henningsii* extract at concentrations investigated have ability to enhance glucose metabolism by the cells with slight toxic effect on 3T3-L1 cell line as shown in this study.

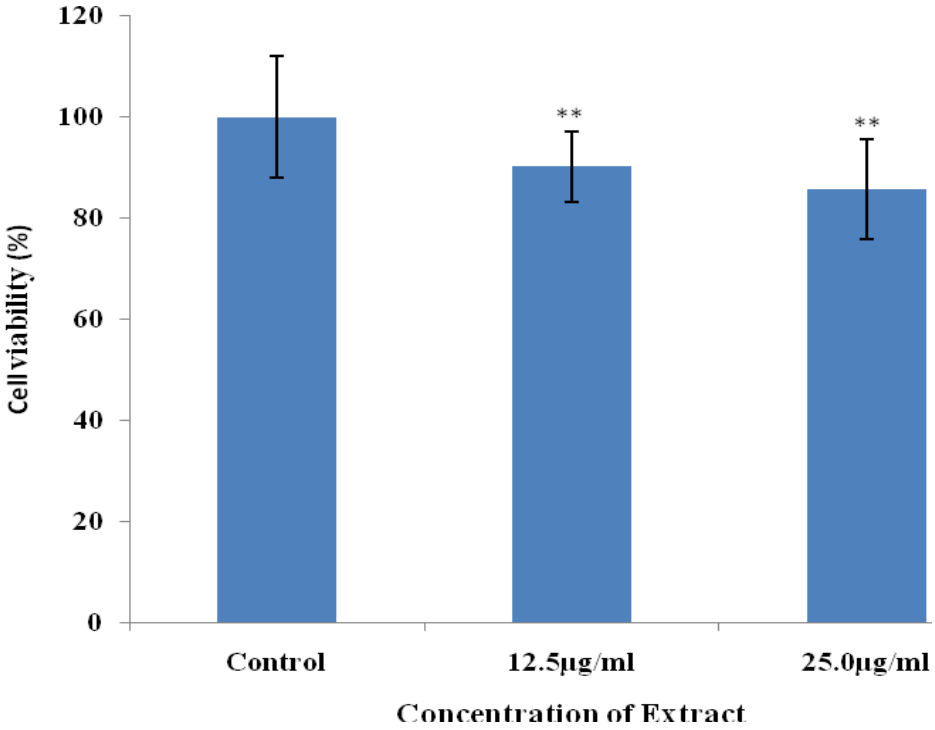

Figure 4: MTT assay of aqueous extract of *S. henningsii* in 3T3-L1 cells exposed to 12.5 and 25µg/ml of the extract. Data are expressed as % control ± SD (n = 6). **P< 0.01 compared to control

Lipid accumulation assay

The effect of plant extract on preadipocyte differentiation was examined using triglyceride accumulation as a marker for adipogenesis. Lipid droplets were quantified by oil-red-O staining (Figure 5). Plant extract did not have any significant effect on the lipid accumulation as compared with insulin treated cells at physiological concentration (12.5µg/ml). The observed results give an indication that the extract might not be a good therapeutic agent in lowering lipid profiles associated with obesity especially in the *in vitro*

studies. Rosiglitazone is a standard antidiabetic drug used as an insulin sensitizer and thus make the cells more responsive to insulin.

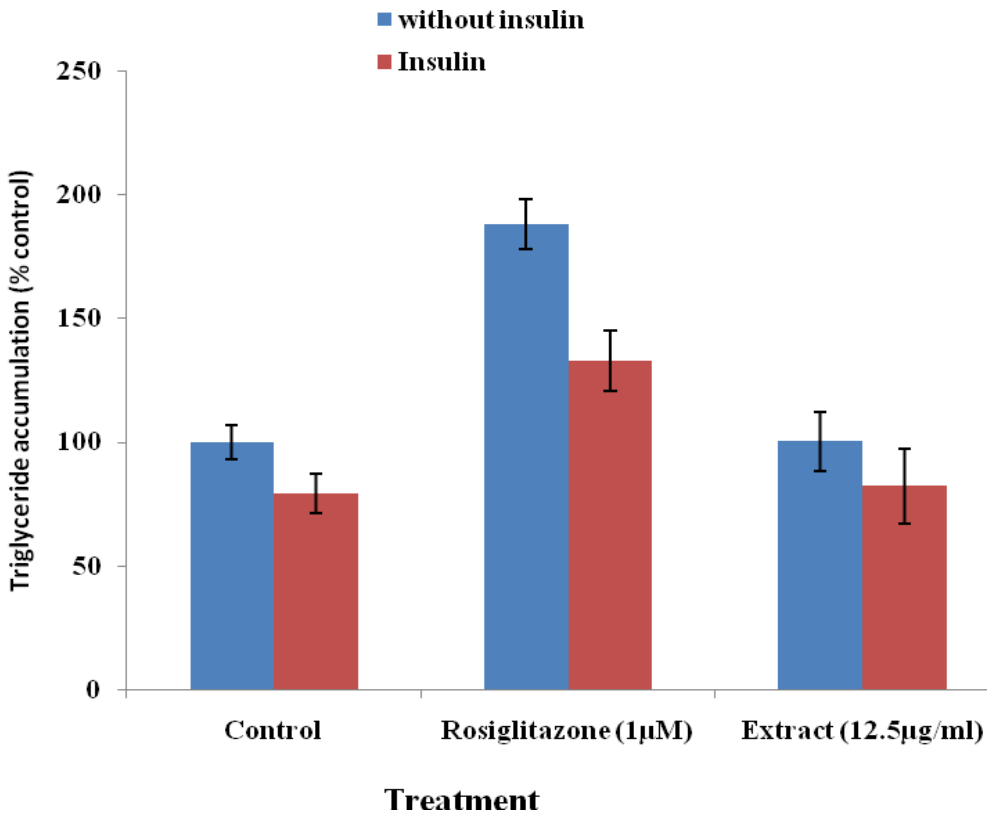

Figure 5: Effect of *S. henningsii* extract on 3T3-L1 preadipocyte triglyceride accumulation.

Protein glycation

In this study, we evaluated the inhibitory activity of plant extract on protein glycation which is implicated as a major pathogenesis process to diabetic complications (Figure 6). The formation of protein glycation was assessed by monitoring the production of fluorescent products at excitation and emission maxima of 370 and 440 nm respectively. The fluorescence intensity was highly increased through incubation of BSA with glucose at 37 ^{o}C compared to the control without glucose. The presence of high molecular weight substances

in the plant extract that may interfere with fluorescence measurements were dialyzed and tested. The plant extract suppressed the intensity of fluorescence in a concentration dependent manner. The percentage protein glycation in the medium was reduced to 86.8, 73.7 and 48.8 % at 3, 6 and 12.5µg/ml when compared with the control while that of aminoguanidine a known inhibitor of protein glycation process was 12.5%. The result obtained demonstrated that *S. henningsii* extract possesses antiglycation activity especially at the concentration of 12.5µg/ml.

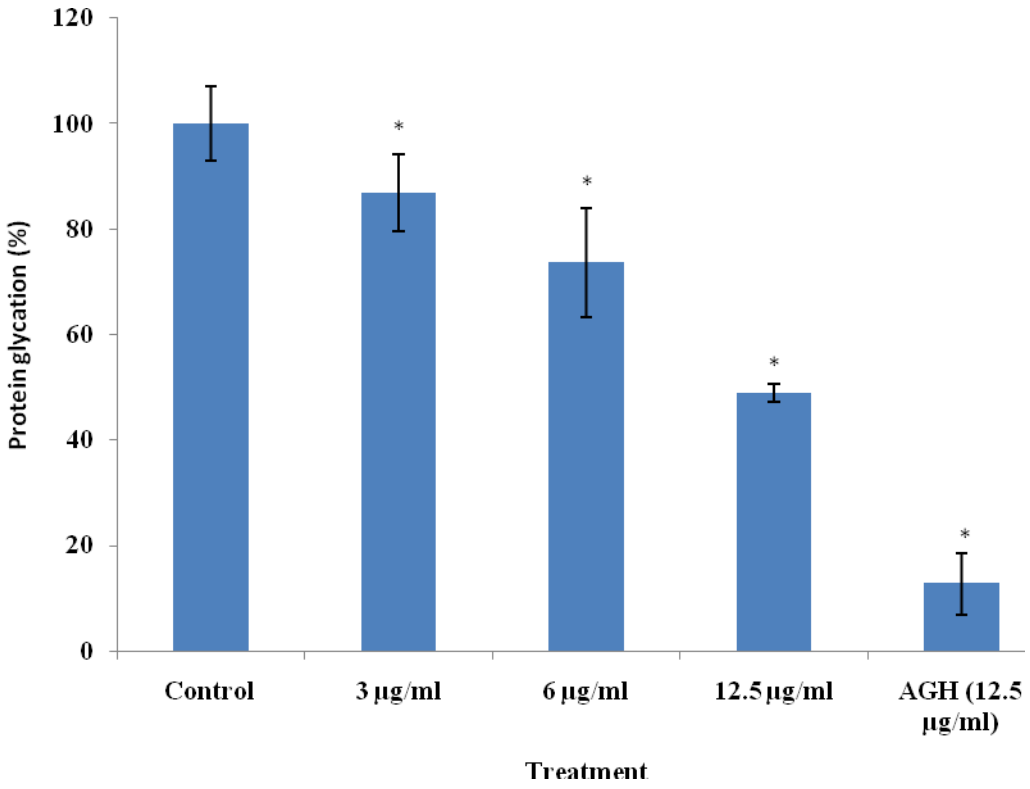

Figure 6: Effect of *S. henningsii* extract and aminoguanidine (AGH) on BSA glycation. Data are expressed as mean ± SD (n = 6). *P<0.01 compared to control

Ferric reducing antioxidant power assay (FRAP)

The quantitative phytochemical analysis in previous studies reported a total phenolics content of 48 mg/g tannic acid equivalent. Phenolics have been documented to possess significant antioxidant activity which may be responsible for the reduction of TPRZ-Fe (III) complex to TPTZ-Fe (II) as shown in this study. The results obtained depicted high antioxidant property of the plant extract (357.05 µmol Fe (II)/g) when compared with BHT (butylated hydroxyltoluene) (75 µmol Fe (II)/g) but significantly lower to that of catechin (859.32µmol Fe (II)/g) and quercetin (2907.25 µmol Fe (II)/g) as depicted in Table 2.

Table 1: Total antioxidant (FRAP) activity of the bark extract of *S. henningsii*

Sample	FRAP
Extract	357.05 ± 9.21[e]
Catechin	859.32 ± 15.67[e]
BHT	75.22 ± 5.16[e]
Quercetin	2907.25 ± 23.67[e]
Total phenolics content (extract)	48 .02 ± 1.52[d]

[e]Expressed in units of µmol Fe (II)/g.

[d]Expressed as Tannic acid equivalent (mg/g)

Alpha glucosidase and α-amylase assay

In vitro α- glucosidase and α- amylase inhibitory activities showed that *S. henningsii* extract had an appreciable inhibitory effect on α – glucosidase but weak inhibitory effect on α -amylase. The percentage inhibitory activity of the extract against α–glucosidase at 0.25, 0.5

and 1 mg/ml was 14.8, 35.27 and 58.04% respectively (Table 1). The value of 0.84 mg/ml was obtained for the extract which is lower than the positive control (Acarbose) which had an IC_{50} of 0.007mg/ml. The extract did not have a significant inhibitory effect on α-amylase (IC_{50} = 2.31 mg/ml) inhibitory activity when compared to that of Acarbose with IC_{50} value of 0.024 mg/ml.

Table 2: The inhibitory effects of aqueous *S. henningsii* extract against α–amylase and α-glucosidase. Data are expressed as the mean (n=3).

Concentration (mg/ml)	% inhibition	
	α–amylase	α-glucosidase
0.25	5.40 ± 0.01	14.80 ± 0.03
0.5	18.70 ± 0.03	35.27 ± 0.02
1.0	31.28 ± 0.12	58.04 ± 0.07
Extract (IC_{50} mg/ml)	2.31 ± 0.02	0.84 ± 0.04
Acarbose(IC_{50} mg/ml)	0. 024 ± 0.31	0.007 ± 0.19

DISCUSSION

Type 2 diabetes is a complex metabolic disorder characterized by abnormal insulin secretion and insulin action caused by impaired cell function and insulin resistance in liver, skeletal and adipose tissues (Pessin et al., 2000). It is also characterized by chronic high blood glucose that causes glycation of body proteins due to defect in postprandial glucose uptake (Rang et al., 1991). The underlying mechanism responsible for this disease and its complication remain unclear though possible events such as activation of series of protein in insulin signaling pathway has been suggested (Giugliano et al., 1996). Other pathway that may be evidenced includes polyol pathway, oxidative stress, non-enzymatic glycation, inhibition of carbohydrate digestive enzymes and glucose uptake (King, 1996). Previously, we have reported the antihyperglycemic and antilipidemic effect of *S. henningsii* extract in streptozotocin-nicotinamide induced diabetic rats (unpublished). The mechanism behind this effect was not fully understood, hence the present study was undertaken to partially assess the possible actions of this plant.

Glucose uptake is a rate limiting step for glucose metabolism which has been shown to regulate increased postprandial blood glucose. Defects in insulin receptors and its signal transduction pathway have been reported in diabetic patients as the underlying cause (Docio et al., 1992). The result obtained in this present study on glucose uptake using differentiated 3T3-L1 cells in the basal state established that *S. henningsii* extract possess the ability to improve glucose uptake in the adipose tissue . The effects of the Plant extract on adipocyte glucose utilization was biphasic with the lower concentration significantly ($P< 0.01$) stimulating glucose uptake in a magnitude comparable to 10^{-6} M of insulin but at the higher concentration basal glucose utilization was strongly inhibited an effect which may at least in part reflect its toxicity towards these cells . The increased basal glucose utilization suggests

the extract may possess insulin mimetic activity; however the precise mechanism needs to be established. In the insulin stimulated state, *S. henningsii* extract exhibited enhanced effect by significantly increased glucose uptake by 325% at 12.5µg/ml and 270% at 25µg/ml over control. The percentage difference of glucose uptake at 12.5µg/ml was 146.4% and that of 25µg/ml was 269.8% which indicate insulin-sensitizing and potentiating activity of the plant extract at higher concentration. The presence of polyphenolics compounds such as phenolics, flavonoids and proanthocyanidins as shown in chapter 4 have been reported to mimic insulin action and thus may be responsible for this observation (Gomes et al., 1995). On this basis, a mechanism of action of *S. henningsii* may be hypothesized which could be linked to activation of series of proteins involved in insulin- mediated glucose transport transduction pathway. This could lead to the translocation of glucose transporter - 4 (GLUT4) to the plasma membrane to facilitate the uptake of glucose from the bloodstream into the cells (Zierath et al., 2002).

Insulin is well known as a regulator of wide variety of metabolic and mitogenic events that involves activation of intracellular signaling pathways (Zierath et al., 2000). The binding of insulin to the insulin receptor provides docking site for several downstream of signaling molecules that transduce to activation of phosphatidylinositol (PI) – 3 kinase (Chen et al., 1996). PI 3-kinase is well recognized as an important step in the insulin signaling to glucose transport through GLUT4 translocation. Increase in enzyme activity of phosphatidylinositide-3-kinase enhances the recruitment of Protein Kinase C (Akt) to bind with the plasma membrane which directly promotes glucose transport and translocation of GLUT 1 and GLUT 4 to the plasma membrane (Eldar et al., 1997; Lodish et al., 2004). In this study, the treatment of 3T3-L1 cells with *S. henningsii* extract at the concentrations investigated enhance glucose uptake and potentiate insulin activity which may be due to the increased expression of PI3K and PKC. Activation of these signaling proteins have been implicated in

several studies to mediate glucose transport due to the presence of polyphenolics compounds (Jorge et al., 2004; Aseervatham et al., 2010; Samane et al., 2006). Thus, the presence of polyphenolics compounds in this plant might therefore account for the up-regulation of these proteins though not investigated in this study (Oyedemi et al., 2010b). Flavonoids and alkaloids have been reported to exert antidiabetic effect through the insulin secretory response and modulation of carbohydrate metabolic enzymes (Hii and Howell et al., 1985; Garcia- Lopez et al., 2004). Owen et al (2008) also demonstrated increase glucose uptake of *Psidium guajava* by activating PPAR in the adipocyte due to the presence of phenolics compounds. It can also be suggested that *S. henningsii* exert indirect benefits on glucose uptake through its strong antioxidant activities (Singh et al., 1993). The results from this study clearly indicate that amelioration of insulin resistance is a potential new field of *S. henningsii* extracts in the treatment of diabetes which warrants further investigation.

The plant extract did not stimulate glucose utilization in Chang liver cells when compared with metformin an antidiabetic standard drug that improves glycemic control primarily by inhibiting hepatic gluconeogenesis and glycogenesis (Gerich, 1989). The extract was also tested with metformin standard drug but did not have synergistic or additive effects. However, reduction of the glucose uptake activity by addition of extract to metformin was observed when compared with metformin alone. The interaction of these agents on the glycaemic control is antagonistic and thus may cause a serious problem for diabetic patients who co-treat diabetes with metformin and *S. henningsii* extract.

The MTT toxicity assay indicated that *S. henningsii* extract was not toxic to the Chang liver and 3T3- L1 cell lines at the concentrations used to determine cellular glucose utilization. Meanwhile, the result exhibited by *Coptis chinensis* (Berberine) on the adipocyte (3T3- L1) as reported by Yin et al. (2008) corroborates with our findings in this study. The cytotoxic effects of this plant extract towards hepatocytes is concerning as this is one of the

main clinical adverse effects reported for use of medicinal plants. Such hepatotoxicity effects are particularly problematic in the treatment of chronic diseases such as diabetes which requires long term medication. Furthermore, the increased prevalence of diseases affecting liver function (hepatitis, obesity and alcohol abuse) and use of hepatotoxic drugs in the treatment of TB and HIV could further increase the potential for toxic side effects. The sub-acute administration of *S. henningsii* barks in our previous studies revealed the relatively non toxic to animals. This is because, there was no apparent damage to some haematological and biochemical parameters used in assessing organ specific toxicity except the white blood count and its relative indices (Oyedemi et al., 2010a). The alterations observed on some parameters suggest parameter and dose selective toxicity. These observations corroborated with the report of Neuwinger (1998) who reported that *S. henningsii* bark produce a marked decrease in toxicity even at high doses in mice due to the opening of lactam and introduction of hydroxyl group at the 17-position of strychnine compound (Neuwinger, 1998).

The adipogenic potential of aqueous stem barks extract of *S. henningsii* on lipid accumulation during differentiation of preadipocytes of 3T3-L1 cells to adipocytes was determined using oil-red-O stained cells. In this present study, the extract did not exhibit any significant effect on the accumulation of lipids in the adipose tissues as compared with the untreated cells but reduced significantly (20 %) when combined with insulin.

Protein glycation is one of the consequences of elevated blood glucose in diabetic patients (Ulrich and Cerami, 2001). This is caused by the non - enzymatic reaction between reducing sugar such as glucose and free amino group of proteins (Singh et al., 2001). This reaction results in the formation of Schiff's base, which can bond covalently to each other to form Amadori products. The rearrangement of this product either by oxidation or reduction further leads to the formation of advanced glycation end-products (AGEs). This product has been reported to cause various complications in patients with diabetes due to loss of collagen

(Kiho et al., 2000). The result obtained in this study demonstrated that *S. henningsii* extract could inhibit protein glycation especially at higher concentration (12.5 µg/ml) although not as efficient as aminoguanidine. The presence of phenolics compounds which have been reported to prevent advance glycation end-product formation could be responsible for this observation (Rice-Evans et al., 1995). The antioxidant potential of this plant as estimated in this study could play a role in inhibiting protein glycation. Several citations of antihyperglycemic and antidiabetic activities of medicinal plants has been done based on their free radical scavenging properties as well as increased glucose metabolism (Zhang and Tan 2000b).

Some antidiabetic drugs act through inhibition of digestion of complex carbohydrates in the gastrointestinal tract (Guyton and Hall, 2000). The plant extract was tested at this level to determine its inhibitory activity against α-amylase and α-glucosidase. These enzymes are well known in the metabolism of carbohydrates. Alpha amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides, which are ultimately converted into monosaccharide by alpha glucosidase. The inhibition of intestinal alpha glucosidase limits postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption, making the inhibitors useful in the management of type 2 diabetes. The ability of *S. henningsii* extract to reduce postprandial glucose level by inhibiting these digestive enzymes was tested to determine its efficacy at this level. The results obtained showed an appreciable inhibitory activity for α-glucosidase but not for α- amylase when compared with Acarbose a standard digestive enzymes inhibitor. Future studies could focus on the isolation of the inhibitory components of this plant and their potential as dietary supplement to control the postprandial glucose level in type 2 diabetes.

It is obvious from the data obtained from this study that the aqueous stem barks extract of *S. henningsii* improves glucose metabolism and insulin sensitivity in the adipocytes. Moreover, the percentage protein glycation and α – glucosidase was moderately improved at the highest concentration (12.5 µg/ml). Therefore, it can be concluded that *S. henningsii* extract exhibits antidiabetic activity through glucose uptake in the adipocytes. Further studies on the insulin signaling pathways are needed to assess the specific mechanism for the observed result.

REFERENCES

Aseervatham, J., Palanivelu, S and Panchanadham, S (2010). Semecarpus anacardium (Bhallataka) alters the energy

Baynes, J and Dominiczak, M (2004). Glucose homeostasis and fuel metabolism. Med Biochem. 2:243-266.

Benzie, F.F and Strain, J.J (1999). Ferric Reducing/Antioxidant Power Assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in enzymology. 299:15-23.

Bisset, NG (1970). The African species of *Strychnos*. The ethnobotany. Lloydia. 33:201-243.

Chen, J., Sadowski H.B., Kohanski, R.A and Wang, L (1996). Stat5 is a physiological substrate of the insulin receptor. Proceedings of the National Academy of Science. 94: 2295-2300.

Docio, S., Riancho, J.A., Perez, A., Olmos, JM., Amado, J.A and Gonzalez-Macias, J (1998). Seasonal deficiency of vitamin D in children: a potential target for osteoporosis preventing strategies? J. Bone Miner. Res. 13:544-548.

Ebbeling, CB and Ludwig, DS (2001). Treating obesity in youth: should dietary glycemic load be a consideration? Adv. Pediatr. 48:179-212.

Edwin, E., Sheeja, E., Gupta, VB and Jain, DC (2008). Fight Diabetes the herbal way review. Express Pharma. 1:41-42.

Eldar-Finkelman, H and Krebs, E.G (1997). Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. Proceedings of the National Academy of Science. 94: 9660-9664

Else, H.J., Per-anders, J., Lars, L., Yuji, M., Tohru, F., Marja-riita, T., Ulf, S and Mette, A (2009). Fat distribution, lipid accumulation in the liver and exercise capacity do not explain the insulin resistance the insulin resistance in healthy males with a family history for Type 2 diabetes. J. Clin Endocrinol and Met. 884:232-4238.

Erasto, P., van De Venter, M., Roux, S., Grierson, D.S and Afolayan, A.J (2009). Effect of leaf extracts of *vernonia amygdalina* on glucose utilization in Chang liver, C_2C_{12} muscle and 3T3-L1 cells. Pharmaceutical Biol. 47:175-181.

Garcia- Lopez, P.M., de la Mora, P.G., Wysocka, W., Maiztegui, B., Alzugaray, M.E., Del Zotto, H and Borelli, M.I (2004). Quinolizidine alkaloids isolated from Lupinus species enhance insulin secretion. Eur J Pharmacol. 504: 139-142.

Gerich, J.E (1989). Oral hypoglycemic agents. N Engl J Med. 321:1231-1245.

Giugliano, D., Ceriello, A and Paolisso, G (1996). Oxidative stress and diabetic vascular complications. Diabetes Care. 19:257-267.

Gomes, A., Vedasiromoni, J.R., Das, M., Sharma, R.M and Ganguly, D.K (1995). Antihyperglycemic effect of black tea (Camelia sinensis) in rat. Journal of Ethnopharmacol. 45:223-226.

Guyton, A.C and Hall, J.E (2000). Digestion and absorption in the gastrointestinal tract, in Text book of Medical Physiology, 10th ed. Guyton AC and Hall JE (Eds). WB saunder Company,UK 753-763.

Guyton, A.C and Hall, J.E (2005). Insulin, glucagon, and diabetes mellitus. In A.C Guyton and J. E Hall (Eds.), *Textbook of Medical Physiology* (10^{th} ed.). Philadelphia: WB Saunders. 961-978.

Hazra, B., Santana, B and Nripendranath, M (2008). Antioxidant and free radicals scavenging activity of *Spondias pinnata*. J Biomed and Med Compl. 8 – 63.

Hii, C.S.T and Howell, S.L (1985). Effects of flavonoids on insulin secretion and 15C2+ handling in rat islets of Langerhans. J Endocrinol. 107:1-8.

Hutchings, A (1989). A survey and analysis of traditional medicinal plants used by the Zulu, Xhosa and Sotho. Bothalial. 19:111-123.

Jakus, V., Hrnciarova, M., Carsky, J., Krahulec, B and Rietbrock, N (1999). Incubation of non enzymatic protein glycation and lipid peroxidation by drugs with antioxidant activity. Life Sci. 65:1991-1993.

Jorge, A.P., Horst, H., De Sousa, EPizzolatti, MG and Silva, FR (2004). Insulinomimetic effects of Kaempfreritrin on glycaemia and on 14C-glucose uptake in rat soleus muscle. Chem. Biol. Interact. 149: 89-96.

Kareru, P.G., Kenji, G.M., Gachanja, A.N., Keriko, J.M and Mungai, G (2007). Traditional medicines among the Embu and Mbeere peoples of Kenya. Afri J. Trad Compl and Alter Med. 4:75-86.

Kasturi, R and Joshi, VC (1982). Hormonal regulation of stearoyl coenzyme A desaturase activity and lipogenesis during adipose conversion of 3T3-L1 cells. J Biol Chem. 257:12224-12230.

Kiho, T., Yamane, A., Hui, J., Usui, S and Ukai, S (2000). Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver. Phytother Res. 14:647-649.

King, G.L., Kunisaki, M., Nishio, Y., Inoguchi, T., Shiba, T and Xia P (1996). Biochemical and molecular mechanisms in the development of diabetic vascular Diabetes. 45: S105-S108.

Lister, E and Wilson, P (2001). Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication). Crop Research Institute, Lincoln, New Zealand.

Lodish, H., Berk, A., Matsudaira, P., Kaiser, C.A., Krieger, M., Scott, M.P., Zipursky, L and Darnell, J (2004). Molecular Cell Biology, Fifth Edition. Freeman books.

Matsuura, N., Aradate, T., Sasaki, C., Kojima, H., Ohara, M., Hasegawa, J and Ubukata, M (2002). Screening system for the Maillard reaction inhibitor from natural products extracts. J. Health Sci. 48: 520-526.

Mosmann, H.M.T (1983). Rapid colorimetric assay for cellular growth and survival: application of ptoliferation and cytotoxic assays. J. Immuninol Methods. 65:55-63.

Neuwinger, H.D (2000). African traditional medicine dictionary of plant use and applications. Medpharm Scientific Publishers, Stuggart.

Neuwinger, H.D (1998). Alkaloids in arrow poisons. In: Roberts, M.C., Wink, M (Eds.), Alkaloids:Biochemistry, Ecology and Medicinal Applications, Plenum Press, New York: 45-84.

Ohiri, F.C., Verpoorte, R and Baerheim, S.A (1983). The African Strychnos species and their alkaloids: a review. J. Ethnopharmacol. 9:167-223.

Owen, P.L., Martineau, L.C., Cares, D., Haddad, P.S., Matainaho, T and Johns, T (2008). Consumption of guava (Psidium guajava) and noni (Morinda citrofolia) may protect betel quid chewing papua New Guineas against diabetes. Asia Pac J Clin Nutr. 17 (4):635-643.

Oyedemi, S.O., Bradley, G and Afolayan, A.J (2009). Ethnobotanical survey of medicinal plants used for the management of diabetes mellitus in the Nkonkobe Municipality of South Africa. J Med Plant Res. 12:1040-1044.

Oyedemi, S.O., Bradley, G and Afolayan, A.J (2010a). *In vitro* and *in vivo* antioxidant activities of aqueous extract of Strychnos henningsii Gilg. Afri J. Pharm and Pharmacol. 4:070-078.

Oyedemi, S.O., Bradley, G and Afolayan, A.J (2010b). Antidiabetic activities of aqueous stem bark extract of *Strychnos henningsii* Gilg. Iranian J. Pharma Res. In press.

Pessin, J.E., Thurmond, D.C., Elmendorf, J.S., Coker, K.J and Okada, S (2000). Molecular basis of insulin-stimulated GLUT 4 vesicle trafficking. J. Biol. Chem. 274:2593 - 2596.

Rang, H.P., Dale, M.M and Ritters, J.M (1991). The endocrine pancreas and the control of blood glucose: In Barbara Simmons, Susan Beasley. Eds. Pharmacology, U.K Longman group Ltd. 403-410.

Rice-Evans, CA., Miller, N.J., Bolwell, P.G., Bramley, P and Pridham, J.B (1995). The relative activities of plant-derived polyphenolic flavonoid. Free radical Res. 22:375-383.

Sancheti, S., Sancheti, S and Seo, S.Y (2010). Evaluation of antiglycosidasae and anticholinesterase activities of *Boehmerianivea.* Pak. J.Pharm. Sci. 23:236-240.

Singh, R.B., Rastogi, S.S., Singh, N.K., Ghosh, S., Gupta, S and Niaz, M.A (1993). Can guava fruit intake decrease blood pressure and blood lipids? J Hum Hypertens. 7:33-38.

Singh, S.N., Vats, P., Suri, S., Shyam, R., Kumria, M.M.L and Ranganathan, S (2001). Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. J. Ethnopharmacol. 76:269-277.

Tits, M., Damas, J., Quetin-Leclercq and Angenot, L (1991). From Ethnobotanical uses of *Strychnos henningsii* to antiinflammatories, analgesics and antispasmodics. J. Ethnopharmacol. 34:261-267.

Ulrich, P and Cerami, A (2001). Protein glycation, diabetes and aging. Recent Prog Horm Res. 56:1-21.

van de Venter, M., Roux, S., Bungu, L.C., Louw, J., Crouch, N.C., Grace, O.M., Maharaj, V., Pillay, P., Sewnarian, P., Bhagwandin, N and Folb, P (2008). Antidiabetic screening and scoring of 11 plants traditionally used in South Africa. J. Ethnopharmacol. 119: 81-86.

Yin, J., Zhanguo, G., Dong, L., Zhijun, L and Jianping, Y (2008). Berberine improves glucose metabolism through induction of glycolysis. Am J Physiol. Endocrinol Metab. 294:E148 - E156.

Zierath, J.R., Krook, A and Wallberg-Henriksson, H (2000). Insulin action and insulin resistance in human skeletal muscle. Diabetologia. 43:821-835.

Zierath, J.R and Wallberg-Henriksson, H (2002). From receptor to effector: Insulin signal transduction in skeletal muscle from type II diabetic patients. Ann NY Acad Sci. 967:120-134.

Zhang, X.F and Tan, B.K (2000b). Antihyperglycemic and antioxidant properties of Andrographis paniculata in normal and diabetic rats. Clin Exp Physiol Pharmacol 27: 358-363.

Zhizhuang, Xiao., Storms, R and Tsang, A (2005). Microplate-based carboxymethyl-cellulose assay for endoglucanase activity. Anal. Biochem. 342:176-178.

Zick, Y (2001). Insulin resistance: a phosphorylations-based uncoupling of insulin signaling. Trends Cell. Biol. 11:437-441.