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Studies on Hypoglycemic and Hypolipidemic Effect of Methanolic Extract of *Stachytarpheta angustifolia* (Mill) Plant in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The hypoglycemic and hypolipidemic effects of methanolic extract of *Stachytarpheta angustifolia* (MESA) were evaluated in Streptozotocin (STZ) induced diabetes in albino rats. The rats were treated with 1000 and 1300 mg kg^{-1} b.wt. (mg kg^{-1} b.wt.) of MESA for 28 days. The changes in body weight and fasting blood glucose level were measured in 5 days interval. After experimental period of 28 days, rats were sacrificed by cervical decapitation, blood sample was collected and the following biochemical parameters plasma glucose, total hemoglobin, glycosylated hemoglobin, Glucose 6 phosphatase (Glc 6-phosphatase), lipid Peroxides (LPO), triacylglycerols, cholesterol, LDL-cholesterol and HDL-cholesterol, were estimated. Administration of MESA to STZ-induced diabetic rats caused a significant decrease ($p<0.05$) in the levels of plasma glucose, glycosylated hemoglobin, Glc 6-phosphatase, LPO, triacylglycerols, cholesterol, LDL-cholesterol with a significant increase ($p<0.05$) in bodyweight, plasma insulin and HDL-cholesterol level Glibenclamide 600 μg kg^{-1} b.wt. was used as standard in this study. These results show that the oral administration of MESA plant prevents the progression of Diabetes-associated symptoms in STZ induced diabetic albino rats and suggest that MESA could be useful in the management of diabetes mellitus.

Key words: *Stachytarpheta angustifolia*, Streptozotocin, hyperglycaemia hyperlipidemia, hypoglycemia, hypolipidemia

INTRODUCTION

Diabetes Mellitus (DM) is a major degenerative disease in the world today affecting many people both in the developed and developing countries (Ogbonnia *et al*., 2011). It is a metabolic disorder of carbohydrate and fat metabolism, which is due to lack of insulin or production of ineffective insulin and characterized by hyperglycaemia and hyperlipidemia (Sharon and Marvin, 1975; Walter, 1977). According to the World Health Organization (WHO), about 150 million diabetic patients have been recorded worldwide by the year 2000, which is expected to project to 221 million people in 2010 and 300 million in 2025. Hyperglycemiais a condition in which an excessive amount blood plasma. This is a glucose level higher than 200 mg dL^{-1}. Organ damage is brought about when blood glucose level exceeds 125 mg dL^{-1}) for a long period of time. Hyperlipidemia is a metabolic disorder associated with diabetes mellitus (Yoshino *et al*., 1996). It

is characterized by elevation in plasma triacylglycerols, cholesterol, low density lipoprotein-cholesterol (LDL-c) as well as very low lipoprotein cholesterol (VLDL-c). It has been speculated to be the most prevalent indicator for susceptibility to atherosclerosis (Maruthapan and Shree, 2010). In clinical practice the drug based method for the treatment of Diabetes mellitus poses a number of problems amongst which are failure after a variable period of time and the associated side effects and toxicity (Alarcon-Aguilara *et al*., 2000). Therefore, there is a need to look for more effective and safe oral hypoglycaemic agents.

Stachytarpheta angustifolia (Mill) Vahl Verbenaceae is a shrub that is widely distributed in the temperate and subtropical regions of the world. The plant is commonly called Wutsiyar bera in Hausa and IruAlangba in Yoruba. It is widely distributed in some part of Nigeria (Jinju, 1990). The aerial part of *Stachytarpheta angustifolia* in Asia and America is boiled and used to remedy diarrhea, intestinal parasite, skin ulcer and as an Abortificient agent (Eldridge, 1975). It has been used by a number of traditional medical practitioners in the treatment of diabetes with tremendous success recorded in some patients. In the present study, the hypoglycemic and hypolipidemic effect of methanolic extract of *Stachytarpheta angustifolia* (MESA) on STZ induced diabetic rats has been examined.

MATERIALS AND METHODS

All Chemicals and reagents used were of analytical grade.

Plant material: The fresh plant (herb) of *Stachytarpheta angustifolia* (Verbanaceae was collected during the month of March, 2012 from the space of botanical garden, Ahmadu Bello University, Zaria, Nigeria and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University.

Preparation of plant extract: Two hundred and fifty grams of powdered plant materials were soaked in 1 litre of methanol at room temperature in a conical flask for 48 h. The suspension was filtered using cloth with fine pore and the filtrates were then concentrated in a crucible using a water bath set at 40°C.

Animals: Male wistar albino rats weighing between 180-210 g were obtained from the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The rats were 11-12 weeks of age at the time of this study. They were kept at the animal house in Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria and were allowed free access to commercial grower's mash (vital feed, Grend cereal PLC, Bukuru, Jos,Plateau Nigeria) and water *ad libitum* for a period of 2-3 weeks in before initiation of experiment.

LD_{50} determination: This was carried out according to the method of Lorke (1983). It involved two phases of eighteen rat three groups of six rats each in the first phase, they were given 10,100 and 1000 mg kg^{-1} b.wt. of the extract orally as single doses. In the second phase, twelve rats were grouped into three groups of four rats each and received 1500, 2900 and 5000 mg kg^{-1} b.wt. of the extract. After the administration of the extract animals were observed for mortality over 24 h.

Induction of experimental diabetes: The rats were fasted for 18 h and made hyperglycemic by a single intraperitoneal injection of STZ (Sigma, USA) dissolved in 0.05 M of citrate buffer

(pH 4.5), at a dose of 65 mg kg^{-1} b.wt. (Ganda *et al*., 1976). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with normal saline alone. One week was allowed for the development of diabetes, rats with moderate diabetes having blood glucose level above 250 mg dL^{-1}) were considered as diabetic and were selected for the experiment (Pari and Satheesh, 2004). The methanolic extract of *Stachytarpheta angustifolia* was administered orally through a gavage at a concentration of 1000 and 1300 mg kg^{-1} b.wt. rats day^{-1} for 28 days.

Experimental design: The rats were divided into 5 groups of six rats each after the induction of STZ. Group 1: Normal rats (Negative control) Group 2: Diabetic control, Group 3: Diabetic rats were given glibenclamide 600 μg kg^{-1} b.wt. day^{-1}, Group 4: Diabetic rats were given 1000 mg kg^{-1} b.wt. day^{-1} of MESA orally and Group 5: Diabetic rats were given 1300 mg kg^{-1} b.wt. day^{-1} of MESA orally for 28 days.

Collection of blood sample: At the end of 28th day, the animals were deprived of food overnight and sacrificed by decapitation. Fasting blood sample was collected in two different fresh vials containing sodium fluoride and potassium oxalate (for glucose estimation) and EDTA and Lithium heparin (for lipid and enzyme studies) and was then centrifuged at 3000 g for 10 min to obtain plasma and was used for the determination of glucose and lipid profile of the rats.

Evaluation of biochemical parameters: Plasma glucose level was determined by O-toluidine method (Sasaki *et al*., 1972). Total hemoglobin was estimated by cyanomethemoglobin method (Drabkin and Austin, 1932). Glycosylated hemoglobin was determined by the method of (Bannon, 1982). Plasma insulin was assayed by ELISA kit (Boeheringer-Mannheim kit). Glucose-6-phosphatase activity was assayed by method of Koide and Oda (1959). The plasma LPO was assayed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method (Walls *et al*., 1976). Plasma triacyglycerol and cholesterol levels were assayed by using commercial diagnostic kits (Ranbaxy Diagnostics, New Delhi, India). Plasma phospholipids levels were determined by the spectrophotometric method of (Chen *et al*., 1956). LDL cholesterol was assayed by using commercial diagnostic kits (Ranbaxy Diagnostics, New Delhi, India). The LDL-cholesterol was calculated using the formula of Friedewald *et al*. (1972).

Statistical analysis: The results were expressed in Mean±standard deviation. Statistical analysis was carried out by using one way (ANOVA) Duncan multiple comparison test in standard statistical Software Package of Social Science (SPSS).

RESULTS

Diabetic rats (group 2) showed a significant decrease ($p<0.05$) in weight when compared to group 1 normal control, but the weight significantly increased ($p<0.05$) upon administration of 1000 and 1300 mg kg^{-1} b.wt. of MESA in group 4 and 5 when compared with group 2 as shown in (Table 1). Fasting Blood Glucose significantly increased ($p<0.05$) to 248.0±0.25 in group 2 when compared with group 1, how ever it showed a significant decrease ($p<0.05$) to 104.0±0.25 and 96.80±0.46 upon administration of the extract in group 4 and 5. There is no significant difference ($p<0.05$) between the standard drug (group 3) and 1300 mg kg^{-1} b.wt. of MESA with respect to the decrease in glycosylated Hb concentration in group 4 and 5 (Table 1). The concentration of Plasma

Table 1: Changes in body weight, total hemoglobin, plasma insulin, glycosylated hemoglobin, fasting blood glucose and in the activity of glucose 6 phosphatase in normal and experimental rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Body wt. (g)					
Initial	181.3±2.08^{a}	202.0±1.00^{d}	202.0±1.00^{d}	190.0±1.00^{b}	199.0±1.00^{c}
Final	211±0.1.52^{d}	171.3±1.50^{a}	204.0±1.00^{c}	199.3±1.52^{b}	202.3±1.52^{c}
Fasting blood glucose (mg dL^{-1})	68.43±0.21^{a}	248.0±0.25^{d}	96.20±0.25^{b}	104.0±0.25^{c}	96.80±0.46^{b}
Plasma insulin (µL mL^{-1})	15.86±0.06^{d}	4.186±0.02^{a}	12.36±0.04^{c}	8.88±0.01^{b}	8.950±0.05^{b}
Hemoglobin(g dL^{-1})	12.30±0.02^{e}	9.73±0.03^{a}	11.5±0.02^{d}	10.50±0.10^{b}	10.9±0.03^{c}
Glycosylated Hb(mg g^{-1} Hb)	0.326±0.03^{a}	0.653±0.01^{d}	0.383±0.01^{b}	0.396±0.01^{c}	0.396±0.01^{c}
Glc 6 phosphatase µ mol	0.190±0.005^{a}	0.256±0.006^{d}	0.196±0.005^{b}	0.23±0.10^{c}	0.203±0.005^{b}

All values are expressed as Mean±SD of three replicates, Values with different alphabet superscripts along the row are significantly different at the level of ($p<0.05$)

Table 2: The levels of plasma lipid Peroxides, triacylglycerol, total cholesterol, phospholipids and LDL-Cholesterol and HDL-Cholesterol in normal and experimental rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Lipid peroxides	24.14±0.04^{a}	58.40±0.10^{d}	26.58±0.32^{b}	31.60±0.10^{c}	26.83±0.12^{b}
Triacylglycerol (mg dL^{-1})	79.76±0.15^{a}	147.5±0.26^{e}	98.25±0.15^{b}	109.6±0.21^{d}	102.7±0.65^{c}
Total cholesterol (mg dL^{-1})	85.00±0.10^{a}	201.8±0.152^{e}	94.83±0.25^{b}	101.3±0.10^{d}	97.93±0.305^{c}
Phospholipids (mg dL^{-1})	80.13±0.06^{a}	150.3±0.10^{e}	89.26±0.21^{b}	107.3±0.36^{d}	90.20±0.10^{c}
LDL-cholesterol (mg dL^{-1})	40.20±0.10^{a}	107.4±0.20^{d}	47.33±0.15^{b}	50.56±0.15^{c}	47.33±0.15^{b}
HDL-cholesterol (mg dL^{-1})	20.20±0.10^{e}	14.43±0.20^{a}	18.23±0.15^{d}	16.40±0.20^{b}	17.86±0.152^{c}

All values are expressed as Mean±SD of three replicates, Values with different alphabet superscripts along the row are significantly different at the level of ($p<0.05$)

insulin and Hb significantly decreased ($p<0.05$) to 4.186±0.02 in group 2 when compared with group 1.However administration of the extract significantly increased ($p<0.05$) the levels to 8.88±0.01 and 8.950±0.05 in group 4 and 5 compared to 2 (Table 1). And there is no significant difference ($p<0.05$) between the two doses of the extract with respect to increase in plasma insulin in group 4 and 5. Glycosylated hemoglobin significantly increased ($p<0.05$) in group 2 to 0.653±0.01 when compared with group 1, however it showed a significant decrease ($p<0.05$) upon administration of MESA to 0.396±0.01 and 0.396±0.01 in group 4 and 5. There is no significant difference ($p<0.05$) in the two 1000 and 1300 mg kg^{-1} b.wt. of MESA with respect to the decrease in glycosylated Hb concentration in group 4 and 5 (Table 1). A significant increase ($p<0.05$) in the activity of Glucose 6-phosphatase activity was shown to be 0.256±0.006 in group 2 compared to group 1 as shown in Table 1, however administration of MESA and glibenclamide significantly increased ($p<0.05$) its activity to 0.196±0.005, 0.23±0.10 and 0.203±0.005 in group 3, 4 and 5 compared with 2.

The levels of Lipid Peroxides, triacylglycerol, total cholesterol, Phospholipids and LDL-Cholesterol were found to significantly increase ($p<0.05$) in group 2 (diabetic group) when compared with group 1 (Table 2). However, administration of the MESA resulted in significant decrease ($p<0.05$) in the level of Lipid Peroxides, Triacylglycerol, Total cholesterol, phospholipids and LDL-Cholesterol in group 4 and 5. The level of HDL-Cholesterol significantly decreased ($p<0.05$) in group 2 when compared to group 1 as shown in (Table 2). However, the level significantly increased ($p<0.05$) upon the administration of the extracts in group 4 and 5 when compared with group 2.

DISCUSSION

The emergence of diabetes mellitus, a growing metabolic disorder calls for the need to look for more appropriate therapy. A number of plants and herbs are known through folklore for hypoglycemic effects but modern method of pharmacological testing has to be carried out before their introduction into modern therapy. The Study of such medicines might offer a natural key to unveil the mysteries behind the control of diabetes mellitus.

STZ-induced experimental diabetes is an important experimental model to study the activity of hypoglycemic agents (Szkudelski, 2001). Streptozotocin, is a cytotoxic agent that affects pancreatic β-cell and thus induces chemical diabetes in a wide variety of animal species, it does so by damaging the pancreatic-cells, leaving less β-active cells and resulting in a diabetic state. A number of diabetic complications, such as myocardial, nervous, vas deferens, gastrointestinal, kidney and urinary bladder dysfunction are exhibited by STZ-diabetic animals majorly through oxidative stress.

In this study, the methanolic extract of the plant *Stachytarpheta angustifolia* (Verbanaceae at the doses of 1000 mg kg^{-1} b.wt. could produce significant hypoglycemic and hypolipidemic effects with the extract being more efficacious at the dose of 1300 mg kg^{-1} b.wt. The hypoglycemic and hypolipidemic effects of the extract could be exploited on the control of blood glucose levels in both insulin and non insulin dependent diabetes mellitus. It is very difficult to explain the mechanism of action of the extract in blood glucose level reduction. The possibility is that the residual pancreatic β cell function might be activated by the extract or by enhancing the utilization of glucose peripherally via extra pancreatic mechanism to induce hypoglycemia (Farjou *et al*., 1987).

The increase in body weight after the treatment with the extract could be due to the its ability to prevent massive body weight loss by reducing hyperglycemia.

The increased plasma insulin level following the administration of the extract might be due to stimulation of insulin synthesis and secretion as well as prevention of insulin degradation since many compounds of plant origin are capable of producing these effects (Venkateswaran and Pari, 2003). It could also be due regeneration of damaged β-cells of pancreas there by stimulating the release of insulin. Insulin level in circulation is known to be increased by some medicinal plants (Torres and Suarez, 1980; Farjou *et al*., 1987). The observed increase in glycosylated Hb and decrease in total hemoglobin in group 2 (diabetic rats) indicate poor glycemic control mechanism (Koenig *et al*., 1976). The decrease in glycosylated Hb and decrease in total hemoglobin in diabetic rats treated with the extract (group 4 and 5) signifies its ability to improve glycemic control mechanism and thus prevents the process of glycosylation.

There is adverse effect in the activity of hepatic glucose metabolic enzymes, especially glucose-6-phosphatase in diabetic state (Shieh *et al*., 2004) and the gluconeogenic process is much more favoured than glycolysis. So, antidiabetic agent is therefore expected to decrease the flux through the gluconeogenic pathway by reducing the activity of glucose-6-phosphatase a very important enzyme that hydrolyses glucose 6 phosphate to free glucose. The significant decrease ($p<0.05$) in its activity in the extract treated groups (4 and 5) could lead to a decrease in flux through the gluconeogenic pathway.

Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk of coronary heart disease. LPO is produced when free radicals react with polyunsaturated membrane fatty acids. When the concentration of endogenous peroxides increases, it may initiate uncontrolled LPO which leads to cellular infiltration and damage to the islet cells in diabetes (Metz, 1984). The ability of the plant extract to reduce TBARS levels shows its antioxidant

property. The higher lipid levels seen in diabetic rats (group 2) could be due to increased mobilization of free fatty acids from peripheral tissue and also due to hormone induced lipolysis (Ei-Soud *et al*., 2007; Nikkila and Kekki, 1973). However, administration of methanolic extract of *Stachytarpheta angustifolia* (Verbanaceae significantly affected the lipid profile in group 4 and 5 by reducing triacylglycerol, total cholesterol phospholipids, LDL-cholesterol and increasing HDL levels significantly (Table 2). This effect may be due to reduced activity of cholesterol biosynthetic enzymes and or low level of lipolysis which are under the control of insulin (Sharma *et al*., 2003). Phospholipids are important components of biological membrane and therefore involved in the transport of triglycerides (Draznin and Eckel, 1993). In STZ-diabetic rats, the elevation in the levels of Free Fatty Acids (FFA) causes the elevation of the level of phospholipids (Frayn, 1993) and TC, which can promote the synthesis of phospholipids (Marsc *et al*., 1996). In diabetic rats fed extract, the decreased level of phospholipids may be due to decreased levels of TC and Free fatty acids.

CONCLUSION

It can be concluded that the oral administration of MESA plant prevented the progression of Diabetes-associated symptoms in STZ induced diabetic albino rats, and suggest that MESA could be useful in the management of diabetes mellitus.

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