# EVALUATION OF THE EFFECT OF ETHANOLIC LEAF EXTRACT OF SOLENOSTEMON MONOSTACHYUS ON BLOOD GLUCOSE AND LIVER ENZYMES IN STZ INDUCED DIABETIC RATS

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**Abstract**

Diabetes mellitus as a metabolic disorder is known to affect the metabolism of carbohydrate, lipids as well as protein. The myriad devastating symptoms arising from this endocrine disorder is of paramount concern to many scientists who believe that traditional medicine could be the solution to the many years of looking for an antidiabetic drug that is easily accessible, reliable, cost effective with minimal side effect for the treatment of diabetes mellitus, hence, this study was designed to unravel the effect of ethanolic leaf extract from a medicinal plant: *Solenostemon monostachyus* on blood glucose and liver enzymes in STZ-induced diabetic albino wistar rats. A total of 24 rats were used for the experiments and were divided into four groups (i.e. diabetic control (DC), normal control (NC), Insulin treated (INS) and *Solenostemon monostachyus* (SM) treated groups), with 6 rats each. The extract, 250 mg/kg body weight, was administered twice daily for 21 days. The serum glucose level in mg/dL was 70.00 ± 10.50 for SM treated groups, 256.00 ± 15.00 for DC. There was significant decrease at $p < 0.05$ in blood glucose level of rats administered with the extract within this period. The results of the ALT and AST levels in U/L were 13.37 ± 2.08 and 12.38 ± 5.10 for DC, and 7.20 ± 0.19 and 6.50 ± 1.46 for SM treated groups respectively, hence, SM significantly reduced at $p < 0.05$ the level of AST and ALT when compared with the control group. Therefore, it may be concluded that the ethanolic leaf extract of SM may have hypoglycaemic and hepatoprotective properties when administered to rats.

**Keywords:** Solenostemon monostachyus, blood glucose, liver enzymes, diabetes mellitus, dimethylsulfoxide, hepatoprotective

## Introduction

One of the most dangerous and life threatening diseases in the world today, involving the pancreas, is diabetes mellitus (Asanga et al., 2013[a]; Verbrugge et al., 1989). It is a condition primarily defined by the level of hyperglycaemia giving rise to risk of micro-vascular damages (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macro-vascular complications (ischaemic heart disease, stroke and peripheral vascular disease) and diminished quality of life (World Health Organization, 2006). The World Health Organization (WHO) estimates that diabetes mellitus affects over 366 Million people worldwide, and many without efficacious diabetes care. A recent revelation by the WHO indicates that diabetes has tripled in the last two decade globally with the highest prevalence rates found in developing countries. The WHO report indicates that in extreme cases, up to 30 - 50% of the adult population in some developing countries have been afflicted with diabetes. The report further alerted that diabetes will continue to be a major threat to public health beyond the year 2030 and is set to increase worldwide without appropriate prevention strategies.

Chinenye (2015) reported that in Nigeria, with the population of over 140 million people (2006 census), an estimated six million people have full blown diabetes mellitus. The 1992 National prevalence study on Non-communicable diseases conducted by the Federal Ministry of Health in 13 states of the Federation indicated a prevalence rate of 2.7% with a prevalence of 2.6% in adult males and 2.8% in adult females. Reports from subsequent studies as hospital records indicated an alarming increase in both prevalence and incidence of diabetes among all ethnic groups and social classes in Nigeria (Chinenye, 2015). Indeed, diabetes has in the last two decades become a household disease and has robbed this country of an increasing number of distinguished leaders and scholars of high repute.

Plants are the basis for the development of modern drugs and medicinal plants have been used in many years in daily life to treat diseases all over the world (Agbor et al., 2007). Also, diets rich in fruits, cereals and vegetables have been shown to play a crucial role in the prevention of a lot of disorders such as cardiovascular diseases, certain cancer types and even ageing (Miller et al, 2000; Joshipura et al., 2001; Aruoma, 2003). Although, efforts have been made towards the elucidation of bioactive potentials of many medicinal plants, however, this has not translated to the conventional

utilization of these plants coupled with the fact that the bioactive potentials of a lot of them are yet to be investigated.

Ethno botanical studies have revealed ten anti-diabetic plants used to treat diabetes mellitus and the leaves of *Solenostemon monostachyus* (SM) was proposed to have this ability (Olabanji et al., 2008). *Solenostemon monostachyus* sp. Beauv (Lamiaceae), common name: African dead nettle, Efik name: Ntorikwot, Awakmmon is an important herb that is widespread in West and central Africa. It occurs as an annual weed in anthropogenic habitat and rocky savannahs. It is slightly succulent, aromatic and grows up to 100cm in height (Mba and Menut, 1994). It has been reported that the plant has been traditionally used in the past for ritual purposes related to pregnancy (Leung et al., 1986). The decoction of the leaves is also taken as a diuretic (Koffi et al., 2006). Research has also shown that the leaves possess antimicrobial activity (Ekundayo and Ezeogu, 2006). The health promoting properties of plants are ascribed to the possession of various phytochemicals especially phenolics and this beneficial activity is related to their antioxidant activity (Heim et al., 2002).

Therefore, this study was designed to investigate the acclaimed anti-diabetic abilities of *Solenostemon monostachyus* (SM) of leaves extract macerated in 80 % ethanol as well as evaluating some biochemical parameters of liver function often implicated in diabetes in albino wistar rats.

## Materials and methods

### Identification and preparation of Plant Materials

Fresh leaves of *Solenostemon monostachyus* were collected from local garden at the University of Calabar, Cross River State, Nigeria. The sample of the plant specimen was identified and authenticated by a Botanist from the botanical garden of the University. The leaves were sorted to eliminate any dead matter and other unwanted particles, air-dried for 2 weeks, blended with a manual hand blender, 150g of the plant powder was weighed and soaked in 700 mL of 80% ethanol. The mixture was then placed in a water bath at about 60 – 80 $^0$C for 10 minutes for thorough extraction of the plant active components then allowed cooling. The extract was then filtered with a chess material and later a Whatmann no. 1 filter paper to obtain a homogenous filtrate. The filtrate was then concentrated at temperature 37 – 40 $^0$C to dryness in a water bath. The extract was refrigerated at 2 – 5 $^0$C until when used (Asanga *et al*., 2013[b]). Appropriate concentration of the extract was subsequently made by dilution with 5% DMSO and distilled water into 250 mg/kg body weight and administered to the animals.

**Handling and treatment of Animals**

A total of 24 adult male albino rats weighing between 150 – 250 g obtained from the disease free stock of the animal house, Biochemistry department, College of Medical Sciences University of Calabar, Cross River State, Nigeria, were used for the study. The animals kept in cages were housed with a 12 hr light-dark cycle prior to the experimental protocols at room temperature (25$^{o}$C), humidity 46 %, fed *ad libitum* with commercial vital feed pellets, UAC, Nigeria and drinking water. All experiments on rats were carried out in compliance with the University of Calabar graduate school ethical guide for care and use of laboratory animals for graduate research. The rats were divided into four groups with six rats each, as follows:

Group A: normal control group received distilled water as placebo.
Group B: diabetic control group received distilled water as placebo.
Group C: insulin group received insulin (5 i.u/kg bodyweight ).
Group D: diabetic test group received *Solenostemon monostachyus* leaves ethanol extract (250 mg/Kg body weight).

| S/n | Groups | Number of rats | Treatment/vehicle |
|---|---|---|---|
| 1. | Normal control (NC) | 6 | 5% dimethylsulfoxide and distilled water |
| 2. | Diabetic control (DC) | 6 | 5% dimethylsulfoxide and distilled water |
| 3. | Insulin treated group (INS) | 6 | Insulin (5 i.u/kg b.w) |
| 4. | *Solenostemon monostachyus* treated group (SM) | 6 | Extract (250 mg/kg b.w) |

The dose employed during administration was based on the predetermined LD50 values obtained from preliminary studies. The treatment period lasted for 21 days and the experiments were conducted between the hours of 7.00 a.m and 7.00 p.m daily.

**Induction of experimental diabetes**

Prior to diabetes induction, the rats were subjected to 12 hours fast, and diabetes was induced by intra-peritoneal injection with streptozotocin (50 mg/kg b.w.) (Sigma Chemical Co., St. Louis, MO, U.S.A) dissolved immediately before administration in freshly prepared 50 mM citrate buffer (pH 4.0) reconstituted in 5% dimethylsulfoxide. STZ was given to the SM and INS treated groups of rats on day 0. The injections were given at 1600h (Thulesen et al., 2013). The normal control animals received 5% DMSO only, 3 days after STZ treatment, diabetes was confirmed in STZ treated rats with a fasting blood sugar (FBS) concentration ≥ 200 mg/dL. This was

estimated using One Touch Glucometer (Lifescan, Inc. 1996 Milpas, California, U.S.A) with blood obtained from the tail vein of the rats (Asanga et al 2013[a]).

**Collection and analysis of blood**

All the animals were anaesthetized with chloroform vapour, twenty-four (24) hours after last extract administration, and dissected for blood collection. Blood samples were collected by cardiac puncture into a set of plain and fluoride oxalate sample bottles. Blood samples were allowed to clot after which they were centrifuged at 7000 rpm for 10 min to obtain serum. Serum was stored in a refrigerator at -4°C until used for analyses. Their absorbances were measured using AJ 122 Chemistry analyzer (spectrophotometer).

Biochemical tests were carried out, which included liver enzymes: alanine aminotransferase and aspartate aminotransferase according to the method described by Reitman and Frankel (1957). Serum alkaline phosphatase according to the method Klein et al using (Randox test kit, UK), bilirubin (Randox, UK), Total protein (Randox, UK), Albumin (Randox UK), Globulin kit (Randox UK), Glucose oxidase kit (Randox UK). All chemicals were of analytical reagent grade. .

**Statistical Analyses**

The results obtained from this study were analyzed by one-way analysis of variance (ANOVA), followed by Student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups using SPSS windows. A significant change was considered acceptable at $P < 0.05$.

**Results**

Table 1: Effect of treatment on glucose levels in blood and serum of the rats.

| Group/ Treatment | Initial FBG (Mg/dL) | Final FBG (Mg/dL) | Serum Glucose (Mg/dL) |
|---|---|---|---|
| NC | 74.83 ± 8.92 | 90.50 ± 6.20 | 57.14 ± 8.12 |
| DC | 539.33 ± 14.13* | 323.00 ± 8.58* | 256.00 ± 15.00* |
| INS | 433.33 ± 32.24[*a] | 82.33 ± 2.47[a] | 54.93 ± 2.32[a] |
| SM | 297.25 ± 57.69[*a] | 191.33 ± 4.94[a] | 70.00 ± 10.50[a] |

Values are expressed as mean ± SEM
*significantly different from NC at $p < 0.05$
a = significantly different from DC at $p < 0.05$
b = significantly different from INS at $p < 0.05$

Table 2: Effect of the treatment on serum biochemical indices of liver function

| Group | AST (U/L) | ALP (U/L) | ALT (U/L) | TP (g/dL) | Albumin (g/dL) | Globulin (g/dL) | Bilirubin (g/dL) |
|---|---|---|---|---|---|---|---|
| NC | 3.08 ± 1.39 | 3.49 ± 0.83 | 7.01 ± 0.90 | 7.08 ± 0.93 | 4.83 ± 0.44 | 2.26 ± 1.18 | 5.46 ± 0.19 |
| DC | 13.37 ±2.08* | 12.62 ±0.30* | 12.38 ±5.10* | 3.62 ±0.20* | 2.38 ± 0.20* | 1.24 ± 0.04 | 6.11 ± 0.81 |
| INS | 11.21 ±1.30* | 8.31 ±4.14 | 13.36 ± 2.59 | 3.76 ±0.80* | 2.80 ±0.59* | 0.96 ± 0.23 | 5.19 ± 0.22 |
| SM | 7.20 ± 0.19 | 5.32 ± 4.12 | 6.50 ± 1.46 | 7.60 ± 1.90 | 3.10 ±0.93* | 4.50 ± 0.95 | 4.77 ± 0.18 |

Values are expressed as mean ± SEM
*significantly different from NC at $p < 0.05$
a = significantly different from DC at $p < 0.05$

The results of serum glucose (Table 1) showed that the serum glucose levels in mg/dL was (70.00 ± 10.50) for SM treated group. This showed significant decreases ($P < 0.05$) when compared with DC group (256.00 ± 15.00).

Table 2 above, showed the levels of AST (13.37 ± 2.08 U/L) and ALT (12.38± 5.10 U/L) in the diabetic control (DC) group and the normal control (NC) group with AST (3.08 ± 1.39) and ALT (7.01 ± 0.90 U/L) and SM treated group with AST (7.20 ± 0.19) and ALT (5.32 ± 4.12).

Treatment with extracts of *Solenostemon monostachyus* (SM) caused reduction in AST and ALT values of the diabetic treatment group with respect to diabetic control (DC).

Insulin treated diabetic group (INS) showed an increase in levels of AST and ALT which was only significant ($P < 0.05$) for AST (11.21 ± 1.30) when compared to normal control (NC).

Alkaline phosphatase activity showed insignificant change ($P > 0.05$) both in treated diabetic and normal control rats, except a significant increase ($p < 0.05$) in DC (13.37 ± 2.08) when compared to NC.

Treatment with extracts of SM (7.60 ± 3.90) resulted in significant increase ($P < 0.05$) in total serum protein compared to DC (3.62 ± 0.20). This also applied to insulin group. Albumin levels in g/dL of the DC group (2.38 ± 0.20), normal control (NC) (4.83 ± 0.44), showed significant decrease ($P < 0.05$) when compared to normal control. Albumin levels for all the treatment groups showed insignificant change ($P > 0.05$) following treatment. The levels of bilirubin in g/dL of diabetic group (DC) (6.14 ±

0.81) was not insignificantly higher ($P > 0.05$) as compared to normal control (NC) ($5.46 \pm 0.19$).

**Discussion**

Serum enzyme levels are the most commonly used biochemical tools for the assessment of hepato-cellular injury. Whereas, increase in amino transferases (ALT and AST) generally reflects liver cell damage, that of ALP is more specific for cholestasis. This study revealed the elevation of all assayed enzymes in the diabetic untreated rats compared to the normal control group. These elevations were seen to be ameliorated by administration of the ethanol extract of *Solenostemon monostachyus* (SM). Chronic and untreated diabetes tends to induce liver injury as a result of increased free radical generation which affects the integrity of the cell membranes. Free radical induced lipid peroxidation of cellular membrane alters membrane integrity leading to increased membrane permeability and loss of cellular content into the circulation (Litoto and Frei, 2006).

There are however several report on the activity of enzymes such as AST, ALT, ALP, and many more (Udosen and Ojong, 1998; Premalatha et al., 2003). The activities of AST, ALT, and ALP were determined in serum of experimental rats because analyses of liver function enzymes are indicators of biochemical changes in response to treatment.

The significant reduction ($P < 0.05$) in AST and ALT levels (Table 2) is an indication of non toxic and protective activity of this extract. Damage to liver cells with necrosis causes the release of intracellular constituents into the blood stream. From Table 2, alkaline phosphatase activity showed insignificant change ($P > 0.05$) both in treated diabetic and normal control rats, but a significant increase ($P < 0.05$) in DC when compared to NC, probably, due to lesser damages on the animals' organs.

Alkaline phosphatase has been reported to be involved in the following roles: transport of metabolites across the cell membranes, protein synthesis, secretory activities and glycogen metabolism (Sharma et al., 1996). Therefore the gradual rise in the serum alkaline phosphatase activity may be due to disturbance in the secretory activity of cell or the transport of metabolites or may due to altered synthesis of certain enzymes as in other hepatotoxic conditions. In this work, we realised that the ethanolic crude extract of *Solenostemon monostachyus* was capable of reducing blood glucose and the activities of AST and ALT caused by diabetes induction in albino wistar rats. Therefore, the result compared favourably with the result of similar studies by Asanga et al., ($2013^{c}$) on the effect of Nauclea *latifolia* leaves in managing hyperglycemia in rats.

Moreover, the total protein levels in the treatment groups showed significant increases ($p < 0.05$) when compared with the diabetic control

group, this result was similar to the reports by Manchester (1972); Venkarleswarlu (1993) and Asanga et al. (2013) on the ability of methanol fraction of *Salacia macrosperma* roots in improving serum level of total protein in diabetic rats well as fractions of Nauclea *latifolium* doing same.

The complexity of crude extract of medicinal plants is a major factor in the variability of enzymes activities. The multiple mechanisms whereby enzymes are regulated include: increased transcription and post-transcriptional modulation, which are apparently mediated through generation of reactive oxygen species and reduced glutathione (GSH) conjugate formation respectively. Antioxidants protection of cells and tissues prevent free-radical injury and facilitates repair of damaged tissues (Chakraborty et al., 1994; Cadena et al., 1995; Feher et al., 1997). Plants materials contain many antioxidants, including vitamin E, C, and beta-carotene, while serum contains in addition to the above, antioxidants such as transferrin, uric acid, protein, and ceruloplasmin (Vervaat and Knight, 2004; Abdel-Baset et al., 1997). Antioxidants act protectively to oxidative stress; hence, there is likelihood that *Solenostemon monostachyus* leaf extract may not pose serious problems to the users.

**Conclusion**

From the results, the extract of *Solenostemon monostachyus (*SM) significantly reduced the glucose levels in diabetic rats. The serum liver enzymes level also decreased significantly when compared with the diabetic control group.

Hence, this plant leaves will be useful in the management of diabetes mellitus because it possesses some antidiabetic and hepatoprotective abilities.

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