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# Effect of Ethanolic Leaf Extract of *Euphorbia thymifolia* on Some Biochemical Indices of Alloxan-Induced Diabetic Male Albino Wistar Rats.

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**Abstract**

The study investigated the effect of ethanolic leaf extract of *Euphorbia thymifolia* on some biochemical indices of alloxan-induced diabetic male albino wistar rats. Albino rats weighing 106 – 129g were randomly assigned 6 groups of 5 rats each. Diabetes was induced in groups 1-5 rats through intraperitoneal injection of 150mg/kg body weight of alloxan monohydrate. Groups 1, 2, 3 and 4 were treated with 500, 1000,1500mg/kg of *Euphorbia thymifolia* and 50mg/kg of metformin respectively. Groups 5 and 6 served as diabetic control and normal control respectively. Treatment lasted for 21days. All rats were given free access to feed and water *ad libitum*. Animals were sacrificed and blood obtained via cardiac puncture for biochemical assay. Treatment with *Euphorbia thymifolia* extract revealed a significant decrease ($p<0.05$) in blood glucose when compared to the untreated group. CAT, SOD and GPx activities were significantly lower ($p<0.05$) in untreated diabetic rats when compared to the normal. Serum TC, TG and LDL-C showed a significant increase ($p<0.05$) with a concomitant decrease in HDL-C in untreated diabetic rats. Treatment with *Euphorbia thymifolia* significantly ameliorated these biochemical indices. This suggest that *Euphorbia thymifolia* possess therapeutic potentials and could be applied for the management of diabetes and its associated complications.

**Keywords**: *Euphorbia thymifolia*, Diabetes, alloxan, antioxidant enzymes, lipid profile

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple aetiology characterized by elevated blood glucose level as a result of insufficient production of insulin due to pancreatic β-cell damage or inefficiency in its action on the target tissues or both (Satyanarayana and Chakrapani, 2014). DM is also characterized by alterations in carbohydrate, lipid and protein metabolism. Some of the clinical symptoms of diabetes include polyuria, polydipsia, polyphagia, weight loss. Prolonged elevated levels of blood glucose results in microvascular and macrovascular complications which consequently leads to increased morbidity and mortality is linked to diabetes mellitus (Johansen *et al.*, 2005). Some of these complications include hyperlipidemia, atherosclerosis, nephropathy, retinopathy which also aggravate death rate (Behnam-Rassouli *et al.*, 2010). Diabetes mellitus is reported to affect about 8% (150 million) of the world's population in 2000 (Sicree, 2000). According to International Diabetes Federation (IDF), the incidence of diabetes is predicted to skyrocket to 350 million or more by the year 2030 (Menaka *et al.*, 2010). According to the report of Chinenye (2015), in Nigeria, with the population of over 140 million people (2006 census), estimated six million people were affected with cases of diabetes mellitus. In diabetes, disturbances in lipid profiles are common with a concomitant increased susceptibility to lipid peroxidation resulting to oxidative stress, which is responsible for increased incidence of atherosclerosis, a major complication of diabetes mellitus (Uhuo *et al.*, 2019). However, there is need to regulate the level of glucose within the physiological milieu. Management of diabetes without any side effects is still posing challenge in the medical field because current orthodox drugs used for the management of diabetes have one or more adverse effects (Bohannaon, 2002). There is need to continuously seek for hypoglycemic agents with more effective, affordable and less side effect in the form of herbal formulations. Based on World Health Organization comments, investigation of hypoglycemic agents from medicinal plants has gain much attention and importance in the field of medicine (Kumar *et al.*, 2010). More than 400 traditional plants have been reported for the treatment of diabetes and it associated complications (Ramachandran *et al.*, 2011). *Euphorbia thymifolia* Linn is a soft prostrate herb belonging to the family Euphorbiaceae. *Euphorbia thymifolia* are found in waste lands along roadsides and abandoned humid field. *Euphorbia thymifolia* is one of the many medicinal plants with various therapeutic actions such as anti-inflammatory, blood purifier, laxative, sedative homeostatic and diuretic (Muthumai *et al.*, 2016). Despite of the above pharmacological properties, currently, there is paucity of scientific documentation on the anti-diabetic potential of the plant. Therefore, the present study aimed to investigate the effect of ethanolic leaf extract of *Euphornia thymifolia* on the lipid profile, fasting blood glucose and anti-oxidant enzymes activities in alloxan-induced diabetic male albino Wistar rats.

## Materials and Methods

### Collection of Preparation of Plant Extract

Fresh leaves of *Euphorbia thymifolia* were harvested from abandoned farmland in Ikot Osurua, Ikot Ekpene Local Government Area, Akwa Ibom State. The fresh leaves were identified and authenticated by a Botanist in the

Department of Biological Science, Akwa Ibom State Polytechnic, Ikot Osurua. The leaves were detached from the stems, sorted, rinsed with distilled water, sliced and dried under shade. The dried leaves were ground into fine powder using an electric blender. Thereafter, 110g of the powder was extracted in 70% ethanol by stirring intermittently, macerated for 72 hours and filtered through Whatman No. 4 filter paper. The filtrate was concentrated to paste by heating in a water bath at $40^0$C and refrigerated at $4^0$C for analysis.

**Induction of Diabetes**

30 albino Wistar rats weighing 106 – 129g were obtained from the animal house of Department of Biological Science, Akwa Ibom State Polytechnic, Ikot Osurua. The animals were fed *ad libitum* with commercial feed and clean drinking water; and acclimatized for two weeks before experiment. The rats were divided into non-diabetic control group and experimental groups.

Diabetes was induced in the experimental rats by intraperitoneal administration of 150mg/kg body weight of alloxan monohydrates dissolved in 0.9% saline after overnight fast. After induction all rats were allowed free access to feed and clean water. 72 hours after induction, blood sample obtained through the tail tip puncture of the rats were used to confirm diabetes in the rats by testing for hyperglycemia using glucometer. Animals with fasting blood glucose concentration of 200mg/dl and above were considered diabetic and selected for the experiment.

**Experimental Design**

Group 1 - Diabetic rats received 500mg/kg body weight of *Euphorbia thymifolia*

Group 2 - Diabetic rats received 1000mg/kg body weight of *Euphorbia thymifolia*

Group 3 - Diabetic rats received 1500mg/kg body weight of *Euphorbia thymifolia*

Group 4 - Diabetic treated group (received 5mg/kg body weight metformin).

Group 5 - Diabetic untreated group

Group 6 - Normal Control Group

Treatment was administered once a day orally for 21 days.

**Determination of Blood Glucose Concentration**

Fasting blood glucose was determined five times in the course of the study. Firstly, before the induction of diabetes by alloxan. Secondly, after 72 hours of induction of diabetes. The measurement was continued first week, second and third week of treatment. The blood glucose levels were taken by sterilizing the tails of the animals with 10% alcohol, punctured with needles and the exuded blood was allowed to touch the test strip which was inserted into a calibrated glucometer (fine test glucometer). This gave direct reading after 9 seconds in mg/dL.

**Sample Collection for Analysis**

At the end of 21day treatment, rats were made to fast overnight and then euthanized under chloroform vapour and sacrificed. Whole blood was obtained by cardiac puncture into non-heparinized tubes and were allowed to clot for 1 hour 30 minutes. The sample was then centrifuged at 4000rpm for 30 minutes to recover the serum for the various biochemical assays.

**Estimation of Superoxide Dismutase, Catalase and Glutathione Peroxidase Activities**

The activity of superoxide dismutase was assayed in a Daytona analyser using RANDOX SOD kit (Woolliams *et al.*, 1983). The activity of catalase was assayed by hydrogen peroxide method (Luck, 1971). The activity of glutathione peroxidase was assayed in a Daytona analyzer using RANDOX glutathione peroxidase kit (Paglia and Valentine, 1967).

**Determination of Lipid Profile**

Lipids profile was determined according to the methods of Sidhu and Naugler (2012).

**Statistical Analysis**

Data analysis was performed using minitab statistical package. Values were expressed as mean ± standard error of mean (SEM). Statistical significance of the results between groups were determined using ANOVA. Differences between means were considered significant at $P<0.05$.

**RESULTS**

**Table I: Effect of ethanol leaf extract of *Euphornia thymifolia* on the fasting blood glucose of alloxan-induced diabetic rats.**

| Groups | Initial Glucose (mg/dl) | After Induction 72 hours(mg/dl) | Final Glucose (mg/dl) |
|---|---|---|---|
| 1 | 97.76±5.53$^{a}$ | 242.20±20.64$^{a}$ | 145.60±17.23$^{b}$ |
| 2 | 94.35±6.28$^{a}$ | 242.80±17.69$^{a}$ | 124.60±9.04$^{bc}$ |
| 3 | 89.82±5.50$^{a}$ | 221.40±10.40$^{a}$ | 128.40±1.18$^{bc}$ |
| 4 | 90.98±5.77$^{a}$ | 276.00±10.74$^{a}$ | 124.20±9.30$^{bc}$ |
| 5 | 88.22±7.34$^{a}$ | 234.00±4.77$^{a}$ | 258.00±8.88$^{a}$ |
| 6 | 84.28±5.52$^{a}$ | 87.71±4.37$^{b}$ | 84.74±3.88$^{c}$ |

Values with different superscripts are significantly different at $P<0.05$ and are expressed as Mean ± SEM.

**Table II: Effect of ethanol leaf extract of *Euphornia thymifolia* on the Lipid profile of alloxan-induced diabetic rats.**

| Groups | TC (mg/dl) | TG (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) | VLDL-C(mg/dl) |
|---|---|---|---|---|---|
| 1 | 55.13±4.94$^{b}$ | 51.04±4.02$^{a,b}$ | 14.16±1.22$^{b,c}$ | 30.76±5.03$^{b}$ | 10.20±0.80$^{a,b}$ |
| 2 | 51.39±32.9$^{b}$ | 47.08±1.71$^{b,c}$ | 18.88±1.02$^{a,b}$ | 23.09±2.50$^{b}$ | 9.41±0.34$^{b,c}$ |
| 3 | 45.44±4.79$^{b}$ | 37.07±1.84$^{c,d}$ | 23.08±2.91$^{a}$ | 14.94±4.86$^{b}$ | 7.41±0.37$^{cd}$ |
| 4 | 50.19±2.58$^{b}$ | 36.05±2.16$^{d}$ | 21.36±2.65$^{a,b}$ | 21.62±4.31$^{b}$ | 7.20±0.43$^{d}$ |
| 5 | 73.97±4.50$^{a}$ | 57.53±2.37$^{a}$ | 9.72±0.44$^{c}$ | 52.74±4.45$^{a}$ | 11.50±0.47$^{a}$ |
| 6 | 47.01±2.18$^{b}$ | 29.12±0.76$^{d}$ | 24.70±2.30$^{a}$ | 16.27±1.90$^{b}$ | 5.82±0.15$^{d}$ |

Values with different superscripts are significantly different @ P<0.05 and are expressed as Mean ± SEM. TC=Total cholesterol, TG=Triacylglycerol, HDL-C=High Density Lipoprotein cholesterol, LDL-C=Low Density Lipoprotein cholesterol, VLDL-C=Very Low Density Lipoprotein cholesterol

**Table III: Effect of ethanol leaf extract of *Euphornia thymifolia* on Anti-oxidant enzymes Activities of alloxan-induced diabetic rats.**

| Groups | CAT (IU/L) | SOD (IU/L) | GPx (IU/L) |
|---|---|---|---|
| 1 | 46.95±6.88$^{b}$ | 56.31±4.51$^{b}$ | 25.77±5.00$^{a}$ |
| 2 | 50.52±3.53$^{a,b}$ | 62.86±3.42$^{a,b}$ | 28.17±1.89$^{a}$ |
| 3 | 66.26±2.68$^{a}$ | 73.89±6.52$^{a,b}$ | 36.83±2.88$^{a}$ |
| 4 | 60.72±4.02$^{a,b}$ | 66.10±2.57$^{a,b}$ | 28.14±3.75$^{a}$ |
| 5 | 20.82±2.89$^{c}$ | 30.81±1.45$^{c}$ | 10.55±1.07$^{b}$ |
| 6 | 67.65±3.30$^{a}$ | 77.41±3.99$^{a}$ | 38.82±3.61$^{a}$ |

Values with different superscripts are significantly different @ P<0.05 and are expressed as Mean ± SEM. CAT=Catalase, SOD=Superoxide Dismutase, GPx= Glutathione Peroxidase

## Discussion

Diabetes is a metabolic condition accompanied with several clinical complications. Alteration in blood glucose levels out of the physiological milieu is a reflection of impairment in β-cells structural and functional architecture. Alloxan is a diabetogenic agent that impairs insulin secretion through the destruction of pancreatic β-cells (Stanely *et al.*, 2000). The impairment leads to abnormal lipid metabolism characterized by lipid peroxidation and oxidative stress (Ju *et al.*,2008). Effective management of diabetes is crucial as its halts the aggravation of diabetes associated complications such oxidative stress, hypercholesterolemia, hyperlipidemia, retinopathy etc. The result of the present

study revealed a dose dependent significant decrease in the concentration of blood glucose after *Euphorbia thymifolia* administration. The current revelation is in tandem with the report of Parmar *et al* (2017). According to Muthumani *et al* (2017), phytoconstituent analysis of *Euphorbia thymifolia* indicated appreciable amount of flavonoids, carotene, vitamin C, tannins, glycosides etc. The reduction in blood glucose recorded in this study may be attributed to regulation of beta cells of the pancreas by the presence of these antioxidant moieties which may have resuscitated the regeneration of insulin. Insulin activates glucose utilization by increasing key enzymes of glycolysis and suppresses gluconeogenesis (Satyanarayana and Chakranpani, 2014). The restoration of insulin action by the extract is suggested to have boost these series of reactions and thus, stimulate peripheral glucose uptake and consequently the resultant decrease in blood glucose.

Diabetes is reported to reduce plasma levels of both enzymatic and non-enzymatic antioxidant defense machineries leading to the susceptibility of diabetic cells to oxidative stress (Anaduaka *et al.*,2014). It is hypothesized that under severe oxidative stress (such as in diabetes mellitus), there is heavy production of reactive oxygen species which may result in the depletion of protective physiological moieties (Narasmihanaidu and Ponnaian, 2006). The levels of SOD, CAT and GPx were significantly reduced in alloxan induced diabetic untreated rats when compared to the normal control. This corroborates with the report of Kala *et al*. (2012). The antioxidant enzymes such as SOD and CAT have been reported to be inhibited in diabetes mellitus as a result of non-enzymatic glycosylation and oxidation (Al-Azzaawie and Alhamdani, 2006). Furthermore, the decrease in the activities of the antioxidant enzymes may also be due to increased utilization of these enzymes in attenuating the free radicals generated during the metabolism of alloxan (Chioma *et al.*, 2014). Administration of *Euphorbia thymifolia* resulted in a significant elevation in SOD, CAT, and GPx activities in a dose dependent manner comparable to the normal control. The results corroborate with the report of Beltagy *et al.* (2020) who reported elevated level of malondialdehyde (MDA) in diabetic rats treated with *Euphorbia helioscopia* extract. The antioxidant effect of *Euphornia thymifolia* extract obtained in the present study may be explained by two mechanisms. Firstly, it could be that the extract abated protein glycosylation and peroxidation via interaction with free radical and then reduced the damaging effects of the radicals. Secondly, it could be that the extract may have induced protein synthesis of the antioxidant enzymes. In addition, studies have shown that polyphenolic compounds increased expression of SOD and GPx in transcriptional level (Sala *et al.*, 2017). SOD catalysis the dismutation of superoxide radicals and generate $H_2O_2$ which are also decomposed by CAT producing molecular oxygen and water (Materska and Pericka, 2005). Glutathione peroxidase (GPx) catalysis the reduction of hydroperoxide to their corresponding alcohols and reduces free $H_2O_2$ to water.

The results of TC, TG, LDL-C and VLDL-C showed a significant elevation in diabetic untreated rats with a concomitant decrease in HDL-C. This current report is in line with the earlier report of Verma *et al*. (2012). Diabetes is reported to induce hypercholesterolemia, hyperlipidemia and other associated complications. The increased level of TC in the diabetic rats may be due to insufficient insulin, since, insulin is known to inhibit the action of β-hydroxy-β-methylglutaryl CoA (HMG-CoA) reductase, the key regulatory enzyme responsible for *de novo* metabolism of cholesterol (Murali *et al.*, 2002). Under normal condition, insulin decreases the activity of hormone-sensitive lipase

and thus reduces the release of fatty acids from stored fat in adipose tissue (Satyanarayana and Chakranpani, 2014). However, due to the destruction of the pancreas, there is underutilization of glucose which resulted to excessive mobilization of fat from adipose tissues and subsequently discharged into the circulation. This may suggest the basis for the elevated level of triacylglycerol in untreated diabetic rats. However, treatment with ethanol extract of *Euphorbia thymifolia* significantly reduced the levels of TC, TG, LDL-C and VLDL-C with concomitant increase in HDL-C. The results corroborate with the reports of Parmar *et al.* (2017) who reported antilipidemic potential of *Euphorbia thymifolia* on streptozotocin-nicotinamide induced diabetic rats. This suggest that the extract may have contained substance(s) that deactivated the activity of HMG-CoA reductase (Sharma *et al.*, 2003). This may be traced to the synergistic effects of some phytoconstituents in the plant extract. As reported by Muthumani *et al* (2017), the phytochemical analyses of *Euphorbia thymifolia* revealed the presence of alkaloids, saponins, flavonoids, phenols, tannins, anthocyanins, triterpenes, essential oil, vitamin C. Saponins in the extract are known to have hypocholesterolemic activities (Price *et al.*, 1987). The mechanism by which saponins deplete LDL and TC have been suggested to be by the binding of bile acids with cholesterol forming strong insoluble complexes preventing its reabsorption and increase its excretion (Iheanacho *et al.*, 2008). This also could be the reason for the slight reduction in TC and LDL. Vitamin C which is richly found in *Euphornia thymifolia* is also suggested to cause the lipid reduction. However, mechanism for this may be the ability of vitamin C to activate 7 alpha-hydroxylase, the rate limiting enzyme that enhances the conversion of plasma cholesterol to bile acids, thereby decreasing cholesterol levels. This assertion is similar to the report of Mayes (1996) that deficiency of vitamin C inhibits 7alpha-hydroxylase thereby blocking bile acid synthesis resulting in accumulation of cholesterol in the plasma, with subsequent atherosclerosis in Scorbutic Guinea pigs.

## Conclusion

This study concludes that *Euphorbia thymifolia* leaf extract offered ameliorative effects on the blood glucose, lipid profile and antioxidant enzymes in alloxan-induced diabetic rats which suggest that the extract may be applied for the management of diabetes and its associated complications.

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