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Hypoglycemic Effect of Ethanol Leaf Extract of *Thuamatococus Daneilli* (ELETD) In Alloxan Induced Diabetic Wistar Rats

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Abstract: *The hypoglycaemic effect of ethanol leaf extract of Thuamatococus daneilli (ELETD) in alloxan diabetic Wistar rat was investigated. Acute toxicity study was also carried out. Animals were assigned into group as follows; Group 1 (Normal control), group 2 (diabetic control) group 3 (diabetic + metformin, 150 mg/kg), group 4 (diabetic + low dose, 200 mg/kg) and group 5 (diabetic + high dose, 400 mg/kg). Experimental diabetes was induced by intraperitoneal injection of alloxan monohydrate (150 mg/kg). After confirmation of diabetes, acute (4 hours) and sub-acute (14-days) hypoglycemic studies were carried out. Serum amylase and glycosylated hemoglobin (HbA1) and fasting blood glucose (FBG) were also assessed at the end of the study. From result of the study, LD_{50} of ELETD was above 6000 mg/kg. Acute and sub-acute treatment with ELETD (200 and 400 mg/kg) caused reduction in FBG, while 14 days treatment with ELETD showed significant ($P<0.05$) decrease in HbA1c and significant increase in serum amylase. Body weight recovery was also observed in ELETD treated groups. This study therefore, validates the traditional use of Thuamatococus daneilli in the management of diabetes mellitus.*

Key words**:** *Thuamatococus daneilli, Glycohemoglobin, amylase, fasting blood glucose, alloxan monohydrate, Wistar rats.*

I. Introduction

Diabetes mellitus, one of the leading causes of death affecting over 100 million people worldwide, is the commonest non-communicable endocrine disease characterized by hyperglycemia and disturbances in carbohydrate, protein, and lipid metabolism, due to absolute or relative deficiency in insulin secretion or insulin action [1].Estimations by the International Diabetes Federation (IDF) showed that in 1985, 30 million people had diabetes, while the figure rose to 150 million in 2000 and 246 million in 2007, it is projected by IDF that by the year 2025, the figure would have risen to 380 million [2].

The persistent state of hyperglycaemia seen in diabetes predisposes the person to diabetes-associated organ dysfunctions, especially in the eyes, kidneys, nerves, heart, and blood vessels [3].

Presently available therapy for diabetes mellitus which includes; insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazones and GLP-I agonists are not without unwanted side effects [4]. This has encouraged the use of medical herbs for treatment of diabetes, especially by local traditional medicine practitioners in many developing countries [3]. This is despite the fact that only a few of these plants with hypoglycemic effects have been scrutinized and received scientific validation [3]. However, the World Health Organization (WHO), encourages the continued research for safer and effective hypoglycemic agents from many indigenous medicinal plants that are more accessible, affordable and relatively safe, compared to synthetic oral hypoglycemic agents [5,6].

Amongst such validated plants with beneficial effects in the treatment of diabetes are; *Annona Sqamosa* (Annonaceae), *Piper longum* (Piperaceae), *Annona Reticulata* L (Annonaceae), *Bauhinia purpurea* (Leguminosae), *Calamus erectus* (Arecaceae), *Momordica Charantia* (Cucurbitaceae) and *Zizyphus nummularia* (Rhamnaceae), among others [5].

Thuamatococus daneilli (commonly called; miracle fruit, miracle berry, serendipity berry in Africa and soft cane in Yoruba), belonging to the family of Marantaceae is a multipurpose rhizomatous, perennial and monocotyledonous plant, native to countries such as Nigeria, Ghana and Cote d'Ivoire [7]. It can also be found in the Princes Islands, Angola, the Central African Republic, Uganda and Indonesia [8]. The sturdy leaf petioles are used as building materials, while the leaves are used for wrapping food as well as for a number of traditional medicinal uses.

The thaumatin that is present in *Thuamatococus daneilli* is is used as a sweetener and flavoring to enhancer food and confectionaries. The nature of sweetener found in thaumatin has been shown to be very ideal for diabetics because it is an intensely sweet protein and not a carbohydrate [9, 8]. In Ivory Coast and Congo, the fruit is used as laxatives while the seed is used as an emetic and for pulmonary problems. The leaf sap is used as antidote against venoms, stings and bites. Leaf and root sap are also used as a sedative and for the treatment of insanity [8].

Recent survey of traditional medicine practice for the treatment of diabetes mellitus in Oyo State, Nigeria, enlisted *Thaumatococcus danielli* as an effective antidiabetic plant [10]. However, with no research information on the efficacy of *Thaumatococcus danielli* in the treatment of diabetes, and in the light of its popularity amongst traditional medicine practitioners in Nigeria, this study therefore intends to investigate the hypoglycemic activity of the ethanol leaf extract of *Thaumatococcus danielli* (ELETD) in alloxan-induced diabetic Wistar rats.

II. Materials and Methods

1.1 Plant material

1.1.1 Collection and authentication of plant material

Fresh leave samples of *Thaumatococcus danielli* were collected from the bank of a stream in Otorho-Abraka, Nigeria in the month of September, 2014. The *Thaumatococcus danielli* leaves were authenticated in the Department of Botany, Faculty of Science, Delta State University, Abraka, Nigeria.

1.1.2 Preparation of extract

Fresh leaves of *Thaumatococcus danielli* were crisply air dried and powdered with the aid of electrical blender. Two hundred gram (200 g) of the powdered leaf was macerated in 800 ml of 70% aqueous ethanol for 72-hours with occasional agitation and stirring. The mixture was filtered with the aid of sieve and Whatman's filter paper (Size No. 1) to obtain a clear filtrate which was further concentrated to dryness with the aid of hot air oven set at 40^0C. The weight of the final extract recovered was 25.27 g, giving a percentage yield of 12.64 %w/w. This was stored in the refrigerator prior to its use for the study.

1.2 Experimental animals

Fifty-four (54) female adult Wistar rats weighing between 130-150 g were procured from the animal facility of the Department of Pharmacology and Therapeutics, Faculty of Basic Medical Science, Delta State University Abraka, Nigeria. The animals were acclimatized for ten (10) days prior to the study, and were maintained under standard husbandry conditions of 12 hour light and 12 hour darkness and relative humidity of 46 ± 6%, and ambient room temperature of 25°C -27ºC. Guidelines followed in the handling of the animals were in accordance with the ethical standards of the Institutional Animals Ethics Committee (IAEC), as adopted by Ethical Committee of the Faculty of Basic Medical Science, Delta State University Abraka, Nigeria.

1.3 Materials, chemicals and reagents

Chemicals used in the research include Chloroform (Kermal), Alloxan monohydrate (Qualikems, Batch no: CE102AK01) and amylase reagent kit (Teco diagnostic), glycohemoglobin reagent kit (Teco diagnostic), Electronic weighing balance (DK-420), rat feed (Savannah Feeds Nigeria Plc), plain tubes, EDTA tubes, Marker pen, Oncall plus glucometer and Spectrophotometer (Spectrumlab, 752S, SN: 752S10043) and Centrifuge (Optimal centrifuge, XC-2000).

1.4 Methodology

1.4.1Acute toxicity study

The acute toxicity (LD_{50}) study was carried out following modified Lorke's method (1983) [11]. Twelve (12) animals were fasted overnight prior to the study.

Phase 1: Rats were placed in three groups (n = 3). Groups 1, 2, 3 and 4 received 100, 200, 400 and 800 mg/kg of ELETD respectively. The animals were observed and the number of death recorded after 24-hours of administration.

Phase 2: Another twelve animals were placed in four groups (n = 3). Groups 1, 2, 3 and 4 received 1000, 2000, 4000 and 6000 mg/kg of ELETD respectively. Animals in each group were also observed for obvious toxic signs and mortality for 24 hours post administration.

1.4.2 Induction of experimental diabetes

Animals were food deprived for twenty-four (24) hours (but with free access to water) and were rendered diabetic by a single dose of alloxan monohydrate (150 mg/kg, I.P.) prepared in stock of 1500 mg/50 ml (30 mg/ml). Two days after induction of diabetes, animals with fasting blood glucose concentration above 200 mg/dl were confirmed diabetic.

1.4.3 Preparation of stock solution

Two grams (2 g) and four grams (4 g) of ELETD were weighed with the aid of electronic weighing balance and constituted in 100 ml of distilled water respectively. This gave stock solutions of 2000 mg/100 ml

(20 mg/ml) and 4000 mg/100 ml (40 mg/ml) for low (200 mg/kg) and high dose (400 mg/kg) respectively. Animals were dosed according to their respective body weights.

1.4.4 Experimental design

Six (6) animals that were not induced with diabetes (non-diabetics) were placed in group 1, while twenty-four (24) other animals that were induced with diabetes and showed evidence of diabetes mellitus were randomized into four groups (group 2 to 5) of six (6) animals per group as follows:

Group 1: Non-diabetic control: Received the vehicle (distilled water), 10 ml/kgper oral.
Group 2: Diabetic control: Received the vehicle (distilled water), 10 ml/kgper oral.
Group 3: Diabetic: Received 150 mg/kg of metformin per oral.
Group 4: Diabetic: Received a low dose (200 mg/kg) of ELETD per oral.
Group 5: Diabetic: Received a high dose (400 mg/kg) of ELETD per oral.

1.4.5 Assessment of acute hypoglycemic effects of ELETD and determination of fasting blood glucose (FBG)

Overnight fasted animals were grouped as shown above and were treated after determining their Initial blood glucose level (0 hour) with the aid of 'Oncall plus glucometer'and values were expressed in mg/dl. Thereafter, their FBGL were determined hourly for four hours.

Percentage change in fasting blood glucose level was calculated as follows:

$$\textbf{Percentage change in fasting blood glucose level} = \frac{FBG\,(T1) - FBG(T0)}{FBG\,(T1)} \times \frac{100}{1}$$

Where FBG (T1) = FBS at present time, FBG (T0) = Initial FBS at time zero.

1.4.6 Blood collection and assay methods

Treatment lasted for two weeks (14-days). On the 15th day, animals were anaesthetized using chloroform. A laparotomy was carried out and blood samples collected from the vena cava were delivered into well labeled plain bottles (for the assessment of serum amylase using Teco diagnostic kit and method described by Wallenfel et al., (1978) [12], after centrifuging whole blood at 4000 rpm for ten minutes). Blood samples delivered in EDTA tubes were used for the assessment of glycosylated hemoglobin concentration using Teco diagnostic kit and method described by Trivelli et al., (1971) [13].

1.5 Data analysis

The result of this study were expressed as mean ± SEM (Standard error of mean) of six replicates, n = 6. Raw data were subjected to one way analyses of variance (ANOVA) followed by post Hoc- Turkey's test for multiple comparisons, using statistical package for social science (SPSS, 20). Statistical significance was declared when $P<0.05$.

III. Results

Acute toxicity test

From the result of $LD_{50,}$ there were no obvious signs of toxicity in both phases (1 and 2) respectively after the administration of ELETD to experimental animals. All the animals survived after twenty four (24) hours of observation. The LD_{50} was estimated to be above 6000 mg/kg (per oral).

Physical observation of diabetic animals

After the induction of diabetes, animals exhibited; increase urination, reduction in body weight, polyphagia and polydypsia. However, these signs were reduced following sub-acute administration of metformin and ELETD for two weeks.

Table 1: Acute effect of ethanol leaf extract of *Thuamatococus daneilli* on fasting blood glucose level of alloxan induced diabetic rats.

	Fasting blood glucose (mg/dl)				
	0 hour	**1 hour**	**2 hour**	**3 hour**	**4 hour**
Group 1: Normal control	100.25 ± 3.88	118.25 ± 6.60	106.50 ± 4.03	128.50 ± 12.55	102.50 ± 3.66
Group 2: Diabetic control	564.00 ± 36.00	538.25 ± 38.04 (-4.78%)	559.00 ± 41.00 (0.89%)	555.00 ± 25.42 (-1.62%)	510.75 ± 51.24 (-10.43%)
Group 3: Diabetic + Metformin	600.00 ± 0.00	596.75 ± 3.25 (-0.54%)	386.50 ± 33.81 (-55.24%)	327.50 ± 28.16 (-83.21%)	479.75 ± 55.65 (-25.07%)
Group 4: Diabetic + Low dose	570.00 ± 30.00	586.25 ± 13.75 (2.77%)	457.50 ± 36.75 (-24.59%)	388.50 ± 20.09 (-46.72%)	448.25 ± 51.24 (-27.16%)
Group 5: Diabetic	394.75 ± 30.00	406.50 ± 70.51	403.50 ± 35.89	335.75 ± 16.18	291.50 ± 14.38

+ High dose	(2.89%)	(2.17%)	(-17.57%)	(-35.42%)

Values are presented as mean ± Standard error of mean (SEM), n = 6. Values in parenthesis indicate percentage change in fasting blood glucose (FBG) relative to zero hour (0 hour).

Effect of ELETD on fasting blood glucose level

1 hour: From table 1 above, there was percentage reduction of -4.78% in diabetic control followed by metformin group (with percentage reduction of -0.54%). However, there was percentage increase of 2.77% in extract low dose and 2.89% in extract high dose.

2 hour: From table 1 above, metformin reduced FBG by -55.24% followed by extract low dose (-24.59%) when compared to diabetic control (0.89%) and extract high dose (2.17%).

3 hour: From table 1 above, administration of metformin caused a reduction (-83.21%) in FBG when compared to extract low dose (-46.72%), high dose (-17.57%) and diabetic control (-1.62%).

4 hour: On the fourth hour, from table 1 above, extract high dose caused percentage reduction of -35.42%, followed by low dose (-27.16%), followed by metformin group (-27.16%) when compared to diabetic control (-10.43%).

Table 2: Sub-acute effect of ethanol leaf extract of *Thuamatococus daneilli* on fasting blood glucose level of alloxan induced diabetic rats.

	Initial FBG (mg/dl)	Final FBG (mg/dl)	Percentage change in FBG (%)
Group 1: Normal control	82.80 ± 3.14	84.00 ± 4.32	1.43
Group 2: Diabetic control	259.20 ± 22.66	295.20 ± 19.40	13.89
Group 3: Diabetic + Metformin, 150 mg/kg	252.60 ± 17.51	229.60 ± 18.54	-10.02
Group 4: Diabetic + Low dose, 200 mg/kg	252.20 ± 6.09	228.00 ± 3.66	-10.61
Group 5: Diabetic + High dose, 400 mg/kg	237.40 ± 8.07	213.80 ± 4.85	-11.04

Values are presented as mean ± Standard error of mean (SEM), n = 6.

From table 2 above, there was -10.02%, -10.61% and 11.04% decrease in FBG level in groups 3, 4 and 5 respectively when compared to 13.89% increase in FBG level in group 2.

Table 3: Effect of ethanol leaf extract of *Thuamatococus daneilli* on serum amylase and percentage glycohemoglobin of alloxan induced diabetic Wistar rats.

	Amylase (IU/L)	Glycohemoglobin (%)
Group 1: Normal control	560.45 ± 16.11	3.49 ± 0.13
Group 2: Diabetic control	322.05 ± 3.27*	7.65 ± 0.44*
Group 3: Diabetic + Metformin	432.34 ± 9.40*[a]	5.20 ± 0.41*[a]
Group 4: Diabetic + Low dose	459.41 ± 5.96*[a]	3.78 ± 0.17*[a]
Group 5: Diabetic + High dose	470.77 ± 6.02*[a]	3.45 ± 0.15*[a]

Values are presented as mean ± Standard error of mean (SEM), n = 6. *P<0.05: Significantly different from normal control. [a]P<0.05: Significantly different from diabetic control.

Effect on serum amylase

From table 3 above, there was significant (*P<0.05) decrease in serum amylase levels of groups 2 to 5 when compared to serum amylase level in group 1 (normal control). Administration of metformin, low and high doses of ELETD caused significant ([a]P<0.05) increase in serum amylase level when compared to serum amylase level in diabetic control.

Effect on percentage glycohemoglobin

From table 3 above, there was significant (P<0.05) increase in percentage glycohemoglobin in groups 2, 3, 4 and 5. However, administration of metformin and ELETD caused significant ([a]P<0.05) reduction in percentage glycohemoglobin when compared to diabetic control.

Table 4: Effect of ethanol leaf extract of *Thuamatococus daneilli* on body weight of alloxan induced diabetic rats.

	Initial body weight (g)	Final body weight (g)	Percentage weight change (%)
Group 1: Normal control	108.00 ± 19.39	132.00 ± 20.96	18.18
Group 2: Diabetic control	102.00 ± 7.44	98.00 ± 8.21	-4.08
Group 3: Diabetic + Metformin	106.50 ± 10.24	112.00 ± 10.03	4.91
Group 4: Diabetic + Low dose	88.00 ± 8.08	91.50 ± 9.00	3.83
Group 5: Diabetic + High dose	101.00 ± 15.80	109.50 ± 11.59	7.76

Values are presented as mean ± Standard error of mean (SEM), n = 6.

Effect on body weight

From table 4 above, there was weight gain of 18.18% in normal control followed 7.76% in high dose extract, followed by 4.91% in metformin group and followed by 3.83% in low dose. However, there was body weight reduction of -4.08% in diabetic control.

IV. Discussion

The present study was carried out to validate the ethnomedicinal role of *Thuamatococus daneilli* in the treatment of diabetes using alloxan-induced diabetes model in Wistar rats.

Absence of death and toxicity signs in both phases of the acute toxicity tests suggest that the ethanol leaf extract of *Thuamatococus daneilli* (ELETD) is relatively safe on short term usage [14].

Alloxan monohydrate (a diabetogenic agent widely used to induce Type 11 diabetes in animals) is selectively toxic to the beta cells of the pancreas, causing cell necrosis [15]. The cytotoxic action of alloxan monohydrate is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to rapid destruction of beta cell which further causes reduction in insulin secretion thereby increasing blood glucose level. This is accompanied by polyuria, reduction in body weight and polyphagia, which could be due to poor glycemic control and excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin deficiency [16]. In diabetes, the obligatory renal water loss combined with the hyperosmolarity tends to deplete intracellular water, triggering the osmoreceptor of the thirst centre of the brain and polydipsia which leads to increase in water intake [2].

In the present study, reduction in FBG level of diabetic rats (Table 1) following single-dose (200 mg/kg and 400 mg/kg) administration of ELETD at 2, 3 and 4 hours as well reduction in FBG following repeated administration for two week (Table 2), suggest its hypoglycemic potential which was supported by reversal of polydypsia, polyphagia, polyuria and body weight loss in diabetic rats, which could be due to insulin like action or peripheral glucose utilization or decreased gluconeogenesis [17].

Increase in glycosylated haemoglobin (HbA_{1c}), an excellent marker of overall glycemic control in diabetic condition is directly proportional to the fasting blood glucose levels [18]. It had been shown that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition, and the level of HbA1c is considered as one of the markers of oxidative stress which is associated with diabetes complications [19, 20]. Measurement of HbA1c is a very sensitive index for glycemic control [21].

In the present study, repeated administration of ELETD daily for two weeks caused significant ($P<0.05$) reduction in glycosylated haemoglobin (HbA_{1c}), compared to diabetic control, which suggests its potential to control long term diabetic condition [18].

Determination of amylase (one of the main enzymes produced in exocrine pancreatic cells that participates in the carbohydrate metabolism of liver, muscle, and other tissues) activity in serum is often performed in the diagnosis of acute pancreatitis, which is associated with diabetes mellitus [22]. Low serum amylase levels had been found in the diabetic condition as compared to non-diabetic state [23]. Decrease in amylase activity may be as a result of decreased stimulating insulin effect on exocrine pancreatic cells [22]. This is supported by this study, where induction of diabetes with alloxan caused significant ($P<0.05$) decrease in serum amylase compared to diabetic control, while repeated administration of ELETD daily for two weeks caused significant ($P<0.05$) increase in serum amylase compared to diabetic control.

Although, the hypoglycemic mechanism of medicinal plants had not been clearly defined, the increase in oxygen free radicals in diabetes could be primarily due to hyperglycemia resulting from the effects of the diabetogenic agent, alloxan [24, 25]. Plants chemical constituents (carbohydrates, phenolics, flavonoids, alkaloids, saponins and glycosides) had been reported to recover diabetic stress by increasing pancreatic secretion of insulin from islet of Langerhans [26]. Terpenoids, flavonoids, alkaloids, tannins, saponins, anthraquinones and cardiac glycosides as well as carbohydrates, trypsininhibitors, proteases, thaumatins, protein, fat and phosphorous present in the ELETD [9, 27, 28] may contribute to its hypoglycemic activity. Thus, in this study we suggest that the possible mechanism of action by ELETD could be increased insulin sensitivity by decreased glycogenolysis and enhance transport of blood glucose to peripheral tissues which could be related to its antioxidants activity that aid to recover from impaired glucose metabolism.

V. Conclusion

The result of the present study showed that the ELETD possess hypoglycemic properties, whose mechanism could be due to increased insulin sensitivity by decreased glycogenolysis and enhance transport of blood glucose to peripheral tissues. Further studies on the fractionation and purification of its active fraction and specific structural elucidation are necessary for the development of hypoglycemic agents from natural sources.

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