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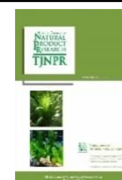

In vitro Antidiabetic Activity and Sub-chronic Toxicity Profile of Ethanol Extract of *Tephrosia bracteolata* Leaves

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ABSTRACT

Tephrosia bracteolata Guill. & Perr. (Fabaceae) has been used crudely in Nigeria for the treatment of diabetes. This study was carried out to determine the antidiabetic activity, possible mechanism of action through the inhibition of the activities of *α*-amylase and *α*-glucosidase, and also to establish its safety. Sub-chronic toxicological studies involved distributing Wistar rats into 4 groups of 6 rats each. Group one served as control and received 5 mL/kg distilled water while groups 2 to 4 received 500, 1000 and 2000 mg/kg of ethanol extract of *Tephrosia bracteolata* (EETB) respectively for 30 days. Food and water intake, and body weight of the rats were monitored for 30 days after which, the rats were sacrificed and blood samples collected for biochemical and haematological analysis. The organs were also harvested for histopathological examination. EETB exhibited concentration-dependent inhibitory effects on both *α*-amylase and *α*-glucosidase. The extract had IC_{50} values of 92.76 and 71.09 µg/mL for *α*- amylase and *α*-glucosidase respectively. These values were higher than that of the standard drug - acarbose which had IC_{50} values of 83.13 and 64.58 µg/mL for *α*-amylase and *α*-glucosidase respectively. The sub-chronic toxicity revealed that EETB had no significant ($p > 0.05$) effects on the biochemical and haematological parameters, and the organs of the rats. Based on these findings, it can be suggested that EETB has antidiabetic activity and possible mechanism of action through the inhibition of *α*-amylase and *α*-glucosidase enzymes. It can also be accepted that is safe due to anecdotal reports.

***Keywords*:** *In vitro,* Antidiabetic, Sub-chronic, Ethanol Extract, *Tephrosia bracteolata*

Introduction

Diabetes is a chronic endocrine disease associated with irregularities in carbohydrate, lipid and protein metabolism. It is characterized by persistent hyperglycaemia as a result of deficiency in insulin secretion and/or increased cellular resistance to insulin.[1] Diabetes has severe effects on the micro- and macrovascular systems, thereby serving as a serious threat to health worldwide.[1] It causes damage and/or dysfunction of several organs and tissues, including the eyes, nerves, heart, kidneys and blood vessels.[1] Approximately, 425 million people live with diabetes and it is projected to rise to 629 million in 2045.[2]
One important factor resulting in a postprandial hyperglycemia is the fast absorption of glucose in the intestine by the action of carbohydrate metabolizing enzymes which breaks down complex carbohydrates to simple sugars such as glucose.[3] Inhibition of these enzymes is therefore an important therapeutic approach to decrease postprandial hyperglycemia.

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At present, a number of conventional drugs are available that competitively and reversibly inhibit *α*-glucosidase from the intestine as well as pancreas. These drugs include acarbose, miglitol and voglibose. The use of these drugs is however, limited due to a large number of side effects such as flatulence, abdominal pain and diarrhea in the patients. These could be due to excessive inhibition of pancreatic *α*-amylase which results in the fermentation of undigested carbohydrates by the colonic flora.[4] Therefore, alternative compounds are necessary for the management of hyperglycemia through strong inhibition of *α*-glucosidase and mild inhibitory effect of *α*-amylase.[5]
Medicinal plants serve as alternative to conventional drugs for the treatment of diseases, as they are more effective and cheaper sources of drugs.[6] The practice of using natural plants as primary treatment of diseases is quite common in Asia, Africa and Latin America. This is especially important in the remote parts of countries, with little or no access to health facilities. Due to the diverse pharmacological properties exhibited by medicinal plants, treatment of diabetes has focused on these plants and their bioactive compounds.[4] In Nigeria, there are diverse medicinal plants with potential antidiabetic potentials.[5,6,7] One of such plants is *Tephrosia bracteolata.*
The plant *Tephrosia bracteolata* Guill. & Perr. (Fabaceae) grows in the open environment and it is wide spread in Tropical Africa.[8, 9] It is common in the Southern and Northern parts of Nigeria, where it is used for various purposes among the local people. In Hausa, the plant is known as 'Kimi' or 'Shege', among the Yorubas, it is referred to as 'Roro'. In the North Central region, The Igalas call it 'Inyomu- ewo' while the Bassas call it 'Kpafutangi'. It is common during the rains but scantly distributed during the dry season. In Tanzania, pounded root of *Tephrosia bracteolata* is administered twice daily to pregnant women who are infected with syphilis[10]. The leaf and roots are subjects of

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charms used in Northern Nigeria against injury by warriors and hunters. Herbal preparations of *Tephrosia bracteolata* are used as analgesics in ethnomedical practices of several tribes of Nigeria. This analgesic, antiinflammatory and antipyretic properties have been reported by Onaolapo *et al.*[11] Its antioxidant and antimicrobial properties have also been demonstrated.[12] *Tephrosia bracteolata* leaf- preparation is also used in Nigeria for the treatment of diabetes as confirmed scientifically by previous studies.[13, 14] This study, therefore, determined the antidiabetic mechanism of *Tephrosia bracteolata* leaves through inhibition of α-amylase and α-glucosidase, and also investigated the safety of the plant.

Materials and Methods

Chemicals and Reagents
Ethanol (Sigma-Aldrich, UK), test kits for liver enzyme activities (Agappe® Diagnostic Inc.), total bilirubin, total protein and albumin test kits (Randox®), test kits for lipid profile (Agappe® Diagnostic Inc.), test kits for oxidative stress biomarkers (BioVision Inc.), standard antidiabetic drug- acarbose® (Bayer).

Enzymes and substrates
The enzymes (porcine pancreatic α-amylase and yeast α-glucosidase) and substrates (soluble potato starch and P- nitrophenyl- α-D-glucopyranoside) used for the study were purchased from Sigma-Aldrich, UK through a local vendor.

Plant collection and Identification
Tephrosia bracteolata leaves were harvested along River Niger Area of Lokoja, Kogi State, Nigeria in May, 2017. The plant was identified and authenticated by Mr. Gbenga Akanni at the Herbarium Unit of the Department of Botany, Federal University, Lokoja, Kogi State, Nigeria, where the voucher number FULH/0765 was assigned and kept for future reference.

Plant Extraction
The leaves were shade-dried for fifteen days and pulverized with an electric blender. A quantity (2 kg) of the pulverized leaves was macerated with 5 L of 70% ethanol for 72 h. The resulting mixture was then filtered with muslin sieve and Whatman filter paper (no 1). The filtrate was thereafter concentrated using rotary evaporator (Buchi) at a temperature of 40 ºC and pressure of 100 mbar to obtain the ethanol extract of *Tephrosia bracteolata* (EETB).

Alpha-amylase inhibitory assay
This assay was performed according to standard procedure.[15] EETB/acarbose (250 µL) at concentrations 10-100 µg/mL and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing 0.5 mg/mL of α-amylase solution were incubated at 25 °C for 10 min. 1% starch solution (500 µL) in 0.02 M sodium phosphate buffer was subsequently added to each tube at timed intervals after pre-incubation. The reaction mixtures were incubated at 25 °C for 10 min, after which the reaction was stopped with 50 µL of dinitrosalicylic acid colour reagent. This was followed by incubating the test tubes in a boiling water bath for 5 min and thereafter cooled to room temperature. Distilled water (10 mL) was used to dilute the reaction mixture and the absorbance taken at 540 nm. The α-amylase inhibitory activity was calculated as follows:

$$\% \text{ inhibition} = \left[\frac{\text{Acontrol} - \text{Aextract}}{\text{Acontrol}}\right] \times 100$$

Alpha-glucosidase inhibitory assay
The α-glucosidase inhibitory assay was performed according to standard procedure.[16]. Fifty (50) µL of EETB/ acarbose (10- 100 µg/mL) and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution were incubated at 25 °C for 10 min. This was followed by the addition of 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) to each tube at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min, after which the reaction was terminated by the addition of 500 µL of 1M sodium carbonate and the volume was made up to 1.5 mL with distilled water. α-glucosidase inhibitory activity was calculated as follows:

$$\% \text{ inhibition} = \left[\frac{\text{Acontrol} - \text{Aextract}}{\text{Acontrol}}\right] \times 100$$

Sub-chronic toxicological studies
This was performed according to standard protocol.[17] Adult male albino rats weighing 120-200 g were used for this study. They were kept in stainless steel cages under standard laboratory conditions, and were maintained on standard rodent feed and tap water *ad libitum* throughout the study. All institutional and national guidelines for the care and use of laboratory animals were followed. Animals were handled in accordance to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the ethical approval was obtained from the Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka (approval number: FVM-UNN-IACUC-2017-05/1037). Rats were randomly divided into 4 groups of 6 rats each. Rats in group 1 served as control and were administered 5 mL/ kg distilled water while rats in groups 2, 3 and 4 were administered 500, 1000 and 2000 mg/kg of EETB respectively for 30 days. All groups were observed for signs of toxicity and mortality for the first 4 hours, and thereafter, daily for 30 days. The daily food and water consumption, weekly body weight changes, hematological indices, liver function, lipid profile, oxidative stress, renal function, electrolytes and relative organ weight were monitored. Histopathological examination of the kidney, liver, heart, lungs and brain of the rats were also carried out.

Daily food consumption
The quantity of food consumed by rats in each group was determined per day as the difference between the quantity of feed supplied and the quantity remaining after 24 h, after which a weekly average was determined.

Daily water consumption
The volume of water consumed daily by each group of rats was determined as the difference between the volume of water supplied and the volume remaining after 24 h, and a weekly average was subsequently determined.

Weekly body weight changes
Rats in the respective groups were weighed weekly, before and during the treatment period and on the day of sacrifice to determine changes in body weight.

Haematological indices
Determination of haematological parameters; red blood cell (RBC) count, haemoglobin (HB) concentration, packed cell volume (PCV), white blood cell (WBC) count, neutrophil (N), lymphocytes (L), monocytes (M) and platelets count were carried out using standard protocol.[18]

Liver function parameters
Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using the method of Reitman and Frankel.[19] Alkaline phosphatase (ALP) activity was determined using the method of Kind and King.[20] Total bilirubin (TB) and direct bilirubin (DB) were determined following the method of Jendrassik and Grof.[21] Total protein (TP) concentration was determined using the Biuret method[22] while albumin concentration was determined through the method of Spencer and Price.[23]

Serum fasting lipid profile
Total cholesterol (TC), triacylglycerol (TAG) and high-density lipoprotein (HDL) concentrations were determined according to the method of Wassan *et al.* [24] while low-density lipoprotein (LDL) levels was calculated using Friedwald equation.[25]

Serum oxidative stress biomarkers
Malondialdehyde (MDA) concentration was determined according to the method of Draper and Hadley.[26] Catalase (CAT) activity was

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assayed following the method of Aebi [27] while superoxide dismutase (SOD) activity was assayed via the method of Xin *et al.*[28]

Renal function indices
Serum urea concentration was determined according to Urease-Berthelot method[29] while creatinine concentration was determined using the method of Perone *et al.*[30]

Serum electrolytes
The serum electrolytes were analyzed using electrolyte analyzer (OPTI® LION electrolyte analyzer, OPTIMedical Systems Inc. Georgia, USA).

Relative organ weight (ROW)
The organs - liver, kidney, heart, lungs and brain were removed, observed for physical signs of abrasions, weighed and the relative organ weight (ROW) calculated as follows:

$$\text{ROW} = \frac{\text{Absolute organ weight}}{\text{Body weight of rat on sacrifice day}} \times 100$$

Histopathological examination of major organs
Following 30 days of administering graded doses of EETB (500, 1000 and 2000 mg/ kg) to Wistar rats, the effect of treatment on the histological appearance of the liver, kidney, heart, lungs and brain of the rats was microscopically evaluated following Hematoxylin and Eosin stain. The organs were fixed in 10% formalin and histopathological examination was carried out according to the method of Drury *et al.*[31]

Statistical analysis
Using SPSS version 20, statistical differences between means were determined by one-way analysis of variance (ANOVA) followed by Duncan's *post–hoc* test for multiple comparison tests. Data were expressed as Mean ± standard deviation, and values were considered significant at $p \leq 0.05$. The IC_{50} value of the EETB and standard drug were determined through a nonlinear regression analysis of the dose-response curve using GraphPad version 5.0.

Results and Discussion

Effect of EETB leaves on alpha-amylase and alpha-glucosidase activities
A concentration-dependent inhibitory effect on α-amylase was observed with EETB and acarbose (Table 1). The Median Inhibitory Concentration (IC_{50}) of EETB for α-amylase was estimated to be 92.76 µg/ mL while that of acarbose was 83.13 µg/ mL (Figure 1). As shown in Table 2, both EETB and acarbose also exhibited concentration-dependent inhibitory effect on α-glucosidase. The IC_{50} of EETB for α-glucosidase was estimated to be 71.09 µg/ mL while that of acarbose was 64.58 µg/ mL (Figure 2).
The high inhibitory activities observed in both assays could be as a result of the high triterpenoid, phenolic, saponin and flavonoid content of the extract as these phytochemicals have been reported to play an important role in modulating the activities of these enzymes.[32-40] EETB had IC_{50} values of 92.76 and 71.09 µg/mL for α-amylase and α-glucosidase respectively, which were higher than those of the standard drug- acarbose. This implies that acarbose may be more potent inhibitor of both α-amylase and α-glucosidase. The significance of this observation is that since the inhibitory effect of EETB on α-amylase is less than that of acarbose, it might have fewer or none of the side effects usually associated with acarbose that usually results from excessive inhibition of pancreatic α-amylase. These findings are similar to previous research[12], who – for the inhibition of α-glucosidase - reported IC_{50} values of 43.95 µg/mL and >50 µg/ml for the ethylacetate and n-hexane extracts of *Tephrosia bracteolata* leaves respectively, compared to acarbose with IC_{50} of 11.31 µg/ml.

Effect of EETB leaves on the food and water consumption of Wistar rats
There was no significant ($p > 0.05$) difference in the weekly food and water consumption of EETB-administered groups compared to the control (Tables 3 and 4). The effect of EETB on the food and water intake of rats, considered as one of the indices of toxicity was evaluated in the sub-chronic toxicity study. EETB has no effect on appetite as there was no significant ($p > 0.05$) difference in the food and water intake of rats at all doses of treatment compared to the control throughout the duration of the studies.

Table 1: Mean percentage inhibition of alpha-amylase produced by Ethanol (EETB) Extract of *Tephrosia bracteolata* leaves and Acarbose

Concentration (µg/mL)	Percentage Inhibition (%) EETB	Acarbose
10	0.88 ± 0.35	2.51 ± 0.53
20	3.82 ± 0.27	5.56 ± 0.53
30	7.31 ± 0.37	10.93 ± 0.79
40	11.11 ± 0.44	19.00 ± 0.37
50	16.37 ± 1.03	25.44 ± 0.80
60	24.72 ± 0.61	32.78 ± 0.84
70	30.46 ± 0.99	41.98 ± 0.62
80	38.95 ± 0.88	47.77 ± 0.62
90	48.07 ± 0.98	53.27 ± 0.83
100	56.84 ± 1.23	60.29 ± 0.99

Values expressed as Mean ± SD, n= 3

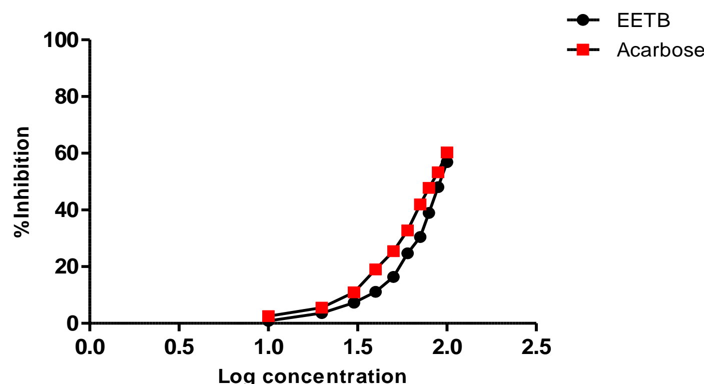

Figure 1: Concentration-inhibition curve and IC_{50} values of EETB leaves and acarbose for alpha- amylase

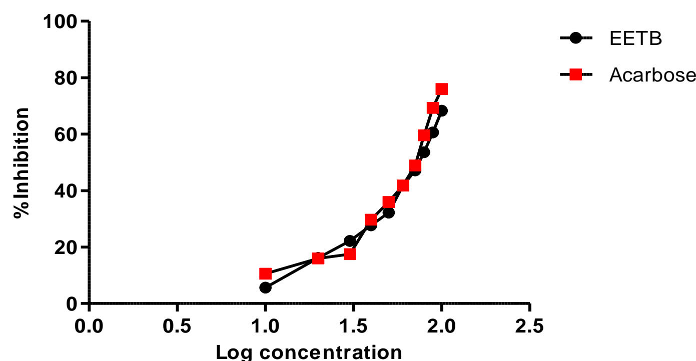

Figure 2: Concentration-inhibition curve and IC_{50} values of EETB leaves and acarbose for alpha- glucosidase

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Effect of EETB leaves on the body weight of Wistar rats

As shown in Table 5, there were no significant ($p > 0.05$) differences in the body weight of EETB-administered groups compared to the control. However, the treated rats showed non-significant ($p > 0.05$), progressive increase in body weight (Table 5), which could be attributed to normal response to growth.

Effect of EETB leaves on hematological parameters of Wistar rats

No significant ($p > 0.05$) difference was observed in the red blood cells, hemoglobin, packed cell volume, white blood cells, neutrophils, lymphocytes, monocytes and platelets of EETB-administered groups compared to control. In toxicological studies, it is also important to evaluate the effect of a substance on the hematological parameters as the substance might be deleterious to the blood cells.[41] reported that anaemia following administration of a substance can be as a result of lyses of blood cells and/or inability to synthesize blood cells as a result of inhibition by the active constituents of the substance. In this wise, the effect of EETB on the hematological parameters of rats was determined. Non-significant changes in the parameters (Table 6) suggest that there were neither lyses of blood cells nor inhibition of blood cell synthesis by the bioactive constituents of EETB.

Effect of EETB leaves on the liver function parameters of Wistar rats

Table 7 showed no statistically significant ($p > 0.05$) difference in serum ALT, AST and ALP activities, total bilirubin and direct bilirubin concentrations, total protein and albumin concentration of EETB-administered groups compared to control. Furthermore, the lipid profile of the rats was not significantly altered as a result of the administration of EETB (Table 8). These results show the relative safety of EETB on the liver.

Effect of EETB leaves on the serum antioxidant biomarkers in Wistar rats

Table 9 revealed no statistically significant ($p > 0.05$) effect on the serum antioxidant markers of EETB-administered groups compared the control. Oxidative stress results from the imbalance between the manifestations of reactive oxygen species (ROS) in the biological system and the system's ability to neutralize the reactive intermediates or repair the ensuing damage.[42] Results of this study showed that the extract did not cause oxidative stress as reflected in the MDA levels of the treated rats and also, the relatively stable activities of the anti-oxidant enzymes were not perturbed compared to the control (Table 9).

Table 2: Effect of Ethanol (EETB) Extracts of *Tephrosia bracteolata* leaves on alpha- glucosidase

Concentration (µg/mL)	Percentage Inhibition (%) EETB	Acarbose
10	5.65 ± 0.59	10.54 ± 0.59
20	16.13 ± 0.48	16.00 ± 0.50
30	22.16 ± 1.43	17.52 ± 0.77
40	27.68 ± 1.11	29.71 ± 0.33
50	32.19 ± 1.01	35.94 ± 0.77
60	41.84 ± 1.09	41.84 ± 0.48
70	47.15 ± 0.60	48.92 ± 0.61
80	53.59 ± 0.98	59.62 ± 0.33
90	60.57 ± 1.19	69.33 ± 0.57
100	68.32 ± 1.08	75.94 ± 0.55

Values expressed as Mean ± SD, n= 3

Table 3: Effect of ethanol extract of *Tephrosia bracteolata* leaves (EETB) on the weekly food consumption (g) of Wistar rats

Treatment	Week 1	Week 2	Week 3	Week 4
Group 1	142.9 ± 26.28[a]	155.7 ± 22.99a	160.0 ± 11.55[a]	180.0 ± 15.28[a]
Group 2	148.6 ± 22.68[a]	164.3 ± 26.37a	155.7 ± 15.12[a]	168.6 ± 23.67[a]
Group 3	144.3 ± 22.99[a]	154.3 ± 13.97[a]	160.0 ± 16.33[a]	178.6 ± 21.16[a]
Group 4	157.1 ± 20.59[a]	157.1 ± 13.80[a]	161.4 ± 13.45[a]	168.6 ± 29.11[a]

Mean values having the same lowercase alphabets as superscripts are considered non- significant (p> 0.05) along the columns. Group 1: Control received 5mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB.

Table 4: Effect of the ethanol extract of *Tephrosia bracteolata* leaves (EETB) on the weekly water intake (mL) of Wistar rats

Treatment	Week 1	Week 2	Week 3	Week 4
Group 1	142.9 ± 26.28[a]	155.7 ± 22.99[a]	160.0 ± 11.55[a]	182.9 ± 14.96[a]
Group 2	148.6 ± 22.68[a]	164.3 ± 26.37[a]	155.7 ± 15.12[a]	168.2 ± 22.68[a]
Group 3	144.3 ± 22.29[a]	154.3 ± 13.97[a]	160.0 ± 16.33[a]	178.6 ± 21.16[a]
Group 4	157.1 ± 20.59[a]	157.1 ± 13.80[a]	161.4 ± 13.45[a]	168.6 ± 29.11[a]

Mean values having the same lowercase alphabets as superscripts are considered not significant (p> 0.05) along the columns. Group 1: Control received 5mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB.

Table 5: Effect of ethanol extract of *Tephrosia bracteolata* leaves (EETB) on the weekly body weight (g) of Wistar rats

Treatment	Post-treatment time in days (d)				
	0	7	14	21	28
Group 1	166.5 ± 28.75[a]	166.7 ± 29.17[a]	170.3 ± 30.36[a]	172.2 ± 30.68[a]	175.0 ± 30.32[a]
Group 2	166.0 ± 23.64[a]	167.2 ± 24.81[a]	170.2 ± 25.55[a]	172.3 ± 24.71[a]	177.7 ± 23.11[a]
Group 3	167.5 ± 21.89[a]	168.8 ± 23.40[a]	172.2 ± 22.53[a]	173.7 ± 23.70[a]	177.3 ± 23.51[a]
Group 4	165.7 ± 19.18[a]	167.3 ± 21.55[a]	170.5 ± 21.48[a]	171.7 ± 20.95[a]	175.2 ± 22.01[a]

Mean values having the same lowercase alphabets as superscripts are considered not significant (p > 0.05) along the columns. Group 1: Control received 5mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB

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Table 6: Effect of Ethanol Extract of *Tephrosia bracteolata* leaves (EETB) on Haematological Parameters of Wistar Rat

Treatment	Parameters							
	RBC ($x10^9$/L)	Hb (g/L)	PCV	WBC ($x10^9$/L)	Neu (%)	Lymp (%)	Mono (%)	Plat ($x10^9$/L)
Group 1	202.33 ± 25.13[a]	11.87 ± 1.24[a]	51.50 ± 3.51[a]	6.22 ± 1.99[a]	49.16 ± 17.50[a]	42.67 ± 17.07[a]	8.17 ± 2.48[a]	860.33 ± 151.30[a]
Group 2	200.20 ± 26.25[a]	12.53 ± 1.73[a]	54.83 ± 4.26[a]	5.52 ± 1.60[a]	50.00 ± 11.52[a]	42.50 ± 9.87[a]	7.50 ± 2.51[a]	885.67 ± 121.36[a]
Group 3	214.17 ± 24.25[a]	13.03 ± 1.86[a]	56.00 ± 7.12[a]	6.47 ± 1.92[a]	57.17 ± 18.08[a]	35.50 ± 17.2[a]	7.33 ± 2.34[a]	870.33 ± 160.01[a]
Group 4	215.33 ± 23.86[a]	12.33 ± 1.85[a]	54.92 ± 6.59[a]	5.97 ± 1.75[a]	48.83 ± 12.54[a]	42.67 ± 11.72[a]	8.50 ± 2.81[a]	895.67 ± 194.21[a]

Mean values having the same lowercase alphabets as superscripts are considered non- significant ($p > 0.05$) along the columns. Group 1: Control received 5mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB.

Table 7: Effect of ethanol extract of *Tephrosia bracteolata* leaves (EETB) on the liver function parameters of Wistar rats

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Tbil (µmol/L)	Dbil (µmol/L)	TP (g/dl)	ALB (g/dl)
Group 1	48.50 ± 8.92[a]	43.06 ± 7.06[a]	30.72 ± 8.70[a]	5.47 ± 1.18[a]	2.61 ± 0.40[a]	73.00 ± 6.19[a]	38.83 ± 1.99[a]
Group 2	39.22 ± 3.43[a]	42.45 ± 10.13[a]	32.01 ± 8.68[a]	5.12 ± 1.10[a]	2.55 ± 0.28[a]	70.17 ± 4.86[a]	38.67 ± 1.52[a]
Group 3	42.78 ± 7.47[a]	42.61 ± 6.65[a]	29.38 ± 9.02[a]	5.70 ± 1.54[a]	2.54 ± 0.22[a]	68.61 ± 6.47[a]	38.77 ± 1.36[a]
Group 4	40.33 ± 3.45[a]	37.95 ± 10.79 [a]	27.83 ± 5.87[a]	4.65 ± 1.33[a]	2.30 ± 0.27[a]	72.94 ± 7.60[a]	37.06 ± 3.21[a]

Mean values having the same lowercase alphabets as superscripts are considered non- significant ($p > 0.05$) along the columns.
Group 1: Control received 5mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB.

Table 8: Effect of ethanol extract of *Tephrosia bracteolata* leaves (EETB) on the serum lipid profile of Wistar rats

Treatment	Tchol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group 1	98.39 ± 13.85[a]	83.33 ± 17.43[a]	45.17 ± 1.94[a]	37.11 ± 14.38[a]
Group 2	96.57 ± 21.49 [a]	84.77 ± 32.86[a]	46.33 ± 10.17[a]	32.93 ± 13.66[a]
Group 3	88.06 ± 10.52 [a]	85.67 ± 15.98[a]	51.67 ± 3.87[a]	32.57 ± 27.65[a]
Group 4	95.56 ± 13.51[a]	87.44 ± 10.85[a]	56.00 ± 4.58[a]	21.51 ± 8.700[a]

Mean values having the same lowercase alphabets as superscripts are considered non- significant ($p > 0.05$) along the columns. Group 1: Control received 5 mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB.

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Effect of EETB leaves on the renal function biomarkers in Wistar rats

No statistically significant ($p > 0.05$) difference in serum K^+, Na^+, Cl^-, HCO_3^-, urea and creatinine concentration of EETB-administered groups was observed compared to the control group (Table 10). Evaluation of the renal function parameters such as urea, creatinine and serum electrolyte levels give useful information about drug-induced renal toxicities.[43] As shown in Table 10, EETB did not exhibit significant changes to these parameters (Table 10), implying its relative safety to the kidneys.

Effect of EETB leaves on the relative organ weights and Histopathology of Wistar rats

No statistically significant ($p > 0.05$) difference in the relative weights of the liver, kidney, heart, lungs and brain of EETB-administered groups were observed compared to control (Table 11). For all the organs, no remarkable changes were also observed in the control group and the treated groups (Figures 3-7). EETB also do not have effect on the relative weights of the organs, which is usually regarded as sensitive indicator of toxicity. This was buttressed by the histopathological studies (Figures 3-7), as EETB did not reveal any remarkable changes in the organs. These observations further lend credence to the findings made in the serum biochemical study of the liver and kidney.

Table 9: Effect of ethanol extract of *Tephrosia bracteolata* leaves (EETB) on the serum antioxidant biomarkers in Wistar rats

Treatment	MDA (nmol/ml)	SOD (U/ml)	CAT (U/ml)
Group 1	14.95 ± 2.60[a]	26.77 ± 3.83[a]	33.40 ± 2.24[a]
Group 2	12.01 ± 2.75[a]	28.15 ± 3.68[a]	33.05 ± 3.79[a]
Group 3	13.92 ± 3.09[a]	28.34 ± 3.60[a]	32.70 ± 2.07[a]
Group 4	12.81 ± 2.77[a]	24.61 ± 4.12[a]	37.63 ± 3.19[a]

Mean values having the same lower-case alphabets as superscripts are considered non- significant ($p > 0.05$) along the columns. Group 1: Control received 5 mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB.

Table 10: Effect of ethanol extract of *Tephrosia bracteolata* leaves (EETB) on the renal function biomarkers in Wistar rats

Treatment	K^+ (µmol/L)	Na^+ (µmol/L)	Cl^- (µmol/L)	HCO_3^- (µmol/L)	Urea (µmol/L)	Creatinine (µmol/L)
Group 1	3.73 ± 0.91[a]	131.00 ± 5.55[a]	106.00 ± 3.58[a]	28.73 ± 4.71[a]	2.67 ± 0.64[a]	59.61 ± 7.02[a]
Group 2	3.80 ± 0.62[a]	132.33 ± 3.08[a]	103.50 ± 1.87[a]	32.63 ± 3.40[a]	2.58 ± 0.66[a]	60.38 ± 1.63[a]
Group 3	3.90 ± 0.27[a]	128.00 ± 5.93[a]	105.00 ± 3.46[a]	28.43 ± 3.05[a]	2.65 ± 0.86[a]	58.66 ± 9.27[a]
Group 4	3.81 ± 0.59[a]	128.50 ± 4.76[a]	103.67 ± 3.72[a]	28.65 ± 3.70[a]	3.16 ± 0.92[a]	52.45 ± 5.48[a]

Mean values having the same lowercase alphabets as superscripts are considered non- significant ($p > 0.05$) along the columns. Group 1: Control received 5 mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB

Table 11: Effect of ethanol extract of *Tephrosia bracteolata* leaves (EETB) on the relative organ weights of Wistar rats

Treatment	Liver	Kidney	Heart	Lungs	Brain
Group 1	3.05 ± 0.57[a]	0.73 ± 0.20[a]	0.34 ± 0.05[a]	0.78 ± 0.12[a]	0.79 ± 0.15[a]
Group 2	3.00 ± 0.25[a]	0.76 ± 0.10[a]	0.28 ± 0.04[a]	0.73 ± 0.18[a]	0.80 ± 0.16[a]
Group 3	3.14 ± 0.49[a]	0.72 ± 0.12[a]	0.32 ± 0.08[a]	0.61 ± 0.14[a]	0.78 ± 0.19[a]
Group 4	3.04 ± 0.23[a]	0.76 ± 0.12[a]	0.35 ± 0.10[a]	0.67 ± 0.12[a]	0.83 ± 0.14[a]

Mean values having the same lowercase alphabets as superscripts are considered not significant ($p > 0.05$) along the columns. Group 1: Control received 5 mL/kg distilled H_2O. Group 2: received 500mg/ kg EETB. Group 3: received 1000 mg/ kg EETB. Group 4 received 2000 mg/ kg EETB.

Conclusion

This study has shown that one of the possible mechanisms of antidiabetic action of EETB is via the inhibition of carbohydrate metabolizing enzymes. As the sub-chronic toxicological studies of the plant did not reveal significant toxicity, it may be concluded that the plant is relatively safe, depicting its relevance in the development of alternative antidiabetic therapy. However, despite the safety in animals, trial in humans is needed so as to establish its safety and efficacy prior to human use.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

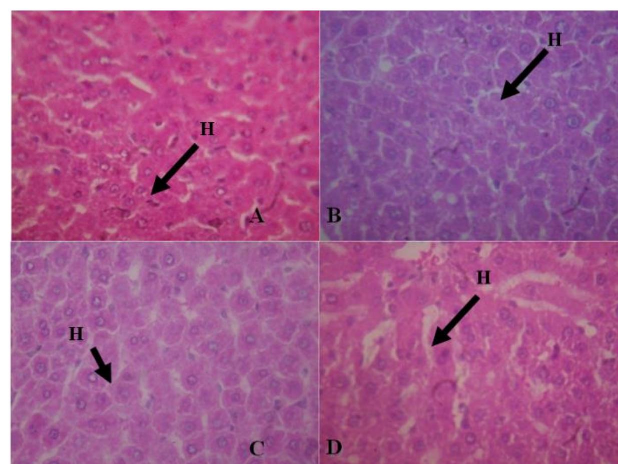

Figure 3: Photomicrograph of a section of the liver of control rat (a), 500 (b), 1000 (c) and 2000 (d) mg/kg EETB-treated rats all showing normal lobular architecture at 30-day post treatment (HE x 250). H= Hepatocytes.

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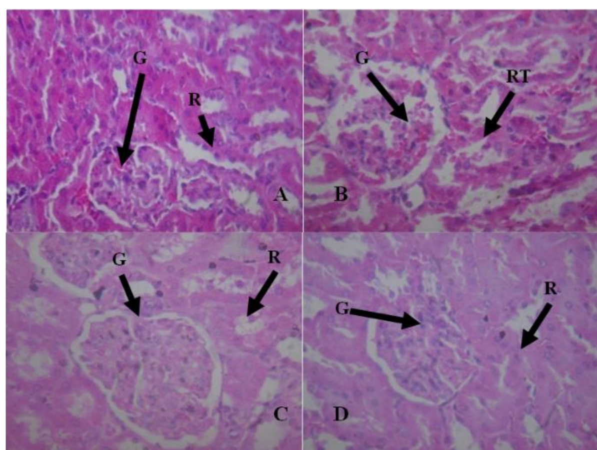

Figure 4: Photomicrograph of a section of the kidney of control rat (a), 500 (b), 1000 (c) and 2000 (d) mg/kg EETB-treated rats all showing normal architecture at 30-day post treatment (HE x 250). RT=Renal tubule, G= Glomerulus.

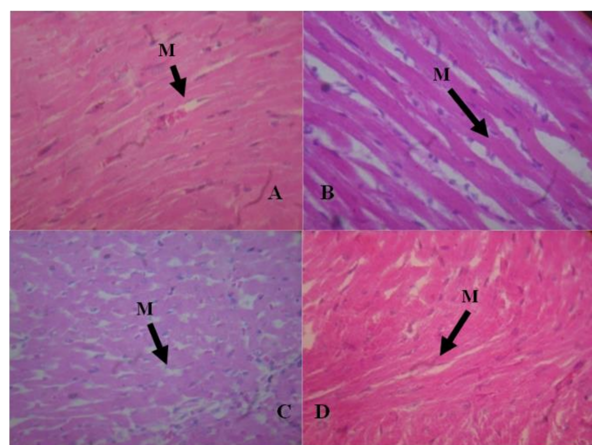

Figure 5: Photomicrograph of a section of the heart of control rat (a), 500 (b), 1000 (c) and 2000 (d) mg/kg EETB-treated rats. No abnormalities seen at 30-day post treatment (HE x 250). M= Muscle fibre.

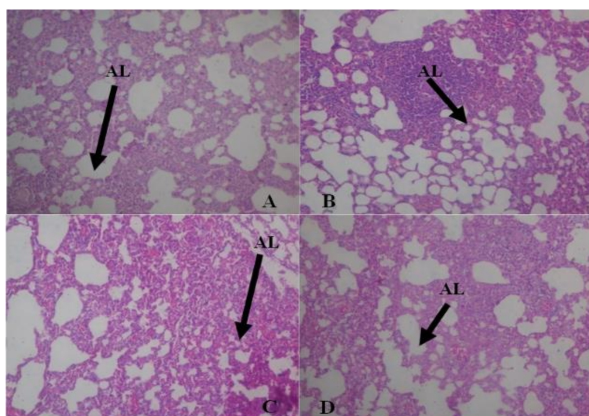

Figure 6: Photomicrograph of a section of the lung of control rat (a), 500 (b), 1000 (c) and 2000 (d) mg/kg EETB-treated rats. No abnormalities at 30-day post treatment (HE x 250). AL= Alveolus

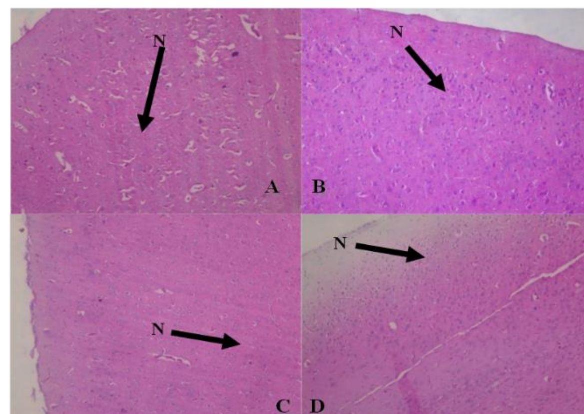

Figure 7: Photomicrograph of a section of the brain of control rat (a), 500 (b), 1000 (c) and 2000 (d) mg/kg EETB-treated rats. No abnormalities at 30-day post treatment (HE x 250). N= Neuron

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