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Effect of Root and Leaf Extracts of *Tetracarpidium conophorum* on Liver Enzyme Levels in Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all the authors. All the authors read and approved the final manuscript.

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ABSTRACT

Aim: *Tetracarpidium conophorum* is a climbing shrub grown principally for its leaf, root, fruit and nut in Southern Nigeria and Western Cameroon. This study was conducted to assess the liver function status in alloxan- induced diabetic rats treated with methanol extracts of leaf and root of *Tetracarpidium conophorum.*
Methodology: The leaf and root extracts of *Tetracarpidium conophorum* were obtained using Soxhlet extractor and diabetes was induced by intraperitoneal injection of alloxan (100mg/kg). Plasma levels of glucose, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using standard diagnostic kits and procedures.
Results: The results from this study show significant (P<0.001) elevation of ALP, AST and ALT levels in alloxan- induced diabetic rats. Oral administration of leaf and root extracts of *Tetracarpidium conophorum* for 14 days significantly (P<0.001) lowered diabetic induced serum liver enzyme levels.

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Conclusion: The present results indicate that the leaf and root extracts of *Tetracarpidium conophorum* possess potent antidiabetic and hepatoprotective activities and could be exploited in the development of antidiabetic and hepatoprotective drugs.

Keywords: Tetracarpidium conophorum; diabetes mellitus; liver enzymes; rats; alloxan; hepatoprotective.

1. INTRODUCTION

Medicinal plants and indeed herbal medicine is the oldest form of health care available to mankind and from antiquity was used by primitive men as food, clothing, shelter and medicine [1]. The World Health Organization consultative group defined medicinal plant as "any plant which in one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs". The World Health Organization (WHO) and individual countries have encouraged the use of plants and plant products for the management of diabetes mellitus as alternative medicine [2].

However, most of the plants used in herbal medicine have not been subjected to scientific research to ascertain the efficacy or otherwise of these plant medicines. Additionally, toxic effects of plants on the human organs have not been ascertained even upon the high intake of the herbal plant infusion and/or decoction in the treatment of various illnesses in man over centuries by different communities of the world. Therefore, it is essential that research into indigenous plant medicines be encouraged and fortified to improve health care of the citizens of these communities.

Diabetes mellitus (DM) is one of the leading causes of death in developed and developing countries today and it is a metabolic disorder of multiple aetiologies, characterized by chronic hyperglycemia, absolute or relative lack of insulin and late complications due to disturbances of carbohydrate, fat and protein metabolism [3]. DM is a syndrome in which the pancreas no longer produces enough insulin or when the cells stop responding to the insulin produced, so that glucose in the blood cannot be absorbed into the cells of the body [3]. Diabetes is one of the leading non communicable diseases affecting mankind with prevalence now reaching epidemic proportion. The World Health Organization described diabetes as a metabolic disorder of multiple aetiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [4]. Diabetes mellitus remains a burden worldwide in spite of the availability but unaffordable numerous antidiabetic drugs, hence the prevalence has continued to increase with many associated deaths and deformities involved with this disease. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss [4]. In its most severe forms, ketoacidosis or a non ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death [4]. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. The abnormalities of carbohydrate, fat, and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin [4].

Aspartate aminotransferase (AST) also known as aspartate transaminase, is an enzyme associated with liver parenchymal cells found primarily in liver. The enzyme is also present in the heart, the kidney, the pancreas and muscles. It is seen in tissue damage especially the heart and liver. The laboratory values are raised in acute liver damage [5]. High AST levels are not specific for liver damage as it is also used as a cardiac marker. A useful tool in the differentiation between causes of liver damage is the ratio AST/ALT [6,7]. Alanine aminotransferase (ALT) is an enzyme present in hepatocytes. Decreased ALT level in combination with increased cholesterol levels is found in cases of congested liver, alcoholism, liver damage, kidney infection, chemical pollutants or myocardial infarction. ALT leaks from damaged cells into blood where it can be measured. ALT rises dramatically in acute liver damage such as viral hepatitis and paracetamol overdose [5]. Alkaline phosphatase (ALP) is an enzyme present in the cells lining the biliary ducts of the liver. It is used extensively as a tumor marker and in injury, pregnancy, skeletal growth with elevated readings. Low levels are found in vitamin deficiencies [5].

This study was aimed at assessing the effect of methanol extracts of root and leaf of *Tetracarpidium conophorum* on liver enzyme levels in alloxan- induced diabetic rats in order to ascertain the hepatoprotective effect or otherwise of this medicinal plant using experimental animal model.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Adult male albino rats were used for this study and were obtained from the Department of Pharmacology animal house of the University of Calabar-Nigeria and were kept in wired cages for two weeks prior to the experiment. They were fed *ad libitum* and allowed free access to drinking water during the whole period of the experiment. The animals were divided into 8 groups of 5 animals per group. The first group was normal control which was placed on water and rat feed only. The remaining seven groups were made up of diabetic untreated rats (DUT), diabetic rats treated with root extract (DRT) at the dose of 50 mg/100g body weight (bwt), diabetic rats treated with leaf extract (DLT) at the dose of 50 mg/100g bwt, diabetic rats treated with glibenclamide (DGT) at the dose of 5 mg/kg bwt, diabetic rats treated with metformin (DMT) at the dose of 500 mg/kg bwt, diabetic rats treated with glibenclamide and leaf extract (DGLT) at doses of 5 mg/kg bwt and 50 mg/100g bwt respectively, and diabetic rats treated with glibenclamide and root extract (DGRT) at doses of 5 mg/kg bwt and 50 mg/100g bwt respectively as shown in Table 1 (Experimental Procedures) and Table 2 depicts Effects of *Tetracarpidium conophorum* root and leaf extracts,oral hypoglycaemic agents on blood glucose levels of diabetic rats . Diabetes mellitus was induced by intraperitoneal injection of alloxan at a dose of 100 mg/kg bwt.

2.2 Place and Duration of Study

This research was carried out in Step-B Anti-Malaria Laboratory, Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Nigeria between December 2013 and June 2014.

2.3 Procurement of Plant Material, Extraction and Preparation of Extracts

The root and leaf samples were harvested from Ikot Nakanda village in Akpabuyo LGA in Cross River State, Nigeria. The plant samples were identified by a plant Taxonomist from Botany Department of the University of Calabar-Nigeria. The samples were dried at room temperature (25 – 30ºC) for two weeks. The dried samples of the root and leaf of *Tetracarpidium conophorum* were crushed into powder and extracted using methods described previously by [8,9]. The resultant dried extracts were labeled and stored in a refrigerator at 4^0C and used for the study.

2.4 Liver Function Test

The liver enzymes were estimated in order to assess the integrity of the liver. Alkaline phosphatase (ALP) was analyzed using method employed by Deutsch Geseiiscchage Furklinische Chemic. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using end point colorimetric diagnostic kit (DIALAB, AUSTRALIA) based on the formulation for the assay of AST and ALT as recommended by International Federation of Clinical Chemistry (IFCC).

Table 1. Experimental procedures

Groups	Parameters	Interpretations
1	Normal control	Normal control
2	DUT	Diabetic untreated rats
3	DRT (50 mg/100gbwt)	Diabetic rats treated with root extracts
4	DLT (50 mg/100gbwt)	Diabetic rats treated with leaf extracts
5	DGT (5 mg/kg bwt)	Diabetic rats treated with Glibenclamide
6	DMT (500 mg/kg bwt)	Diabetic rats treated with Metformin
7	DGLT (5 mg/kg bwt,50 mg/100g bwt)	Diabetic rats treated with Glibenclamide and leaf extracts
8	DGRT (5 mg/kg bwt,50 mg/100g bwt)	Diabetic rats treated with Glibenclamide and root extracts

Table 2. Effect of *Tetracarpidium conophorum* root and leaf extracts, glibenclamide and metformin on blood glucose (mg/dL) levels of diabetic rats

Treatment	Day zero	Day 4	Day 8	Day 14
Control	93.72± 8.70	99.13±7.50	93.26±9.10	94.32±9.00
Diabetic untreated	148.14±11.60	409.68±32.60***	410.38±32.40***	411.50±32.30***
Diabetic root extract (50mg/100g bwt) treated	100..82±6.40	196.24±12.60*	134.18±14.40	105.06±4.40
Diabetic leaf extract (50mg/100g bwt) treated	121.20±10.10	150.28±13.20	115.92±6.50	107.38±5.00
Diabetic glibenclamide (5mg/kg bwt) treated	106.02±11.60	295.90±33.80**	180.95±35.20	150.00±27.60
Diabetic metformin (500mg/kg bwt) treated	124.60±11.80	244.24±12.50**	138.90±7.50	123.32±6.50
$^{\beta}$Diabetic glibenclamide + leaf extract treated	106.60±4.10	305.20±53.80**	142.70±9.50	97.80±4.50
$^{\beta}$Diabetic glibenclamide + root extract treated	115.40± 0.80	172.40±11.60*	116.30±10.30	104.90±7.70

*Results show mean ± SEM of five values. *P = 0.05 vs control and diabetic leaf treated; **P<0.01 vs control and diabetic leaf treated; ***P<0.001 vs control and treated groups; bwt = body weight, $^{\beta}$Glibenclamide and extract doses are as used in other groups*

2.4.1 Measurement of alkaline phosphatase

The analytical method employed was that recommended by Deutsch Geseiischage Fur klinische Chemic (DGKC, 1972).

2.4.1.1 Procedure

The principle is based on the fact that para–nitrophenyl phosphate is hydrolyzed to phosphate and para-nitrophenol in the presence of ALP. The amount of sample added to a test tube was 0.01ml. This was mixed with 0.5ml of reagent containing the substrate para-nitrophenyl phosphate and brought to room temperature. After mixing, the reaction was allowed to stand for 3 minutes and the absorbance read at 405 nm. The activity of alkaline phosphatase was calculated from the formula:

$$\triangle \quad \text{iu/l} = 2757 \times \quad \text{nm/minute micro}$$

Where iu/l = unit of alkaline phosphatase affinity

$$\triangle \quad A = \text{change in absorbance.}$$

2.4.2 Measurement of alkaline (ALT) and asparatate (AST) aminotransferases

Measurement of ALT and AST activities in the serum were done using end point colorimetric diagnostic kit (DIALAB, Australia) based on the formulation for the assay of AST andALT as recommended by the international Federation of Clinical Chemistry (IFCC).

2.4.3 Principle

NADH is oxidized to NAD; the resultant decrease in absorbance at 340nm when measured is directly proportional to the activity of ALT and AST in the sample. The pyruvate that is produced by trans-animation reaction between L-alanine and ketoglutarate reacts with 2,4-dinitrophenyl hydrazine giving a coloured hydrazone useful for the measurement of ALT activity. The oxaloacetate hydrazine formed with 2,4-dinitrophenyl hydrazine is used to measure AST.

2.4.3.1 Procedure

To one test tube, 1ml of working reagent was pipette. Then 0.1ml of the sample was added into the test tube and mixed thoroughly at 37^0C. The initial absorbance against air after 1minute was read. Then reaction was allowed to stand and the absorbance read again after 1, 2 and 3 minutes at 340 nm. Aspartate and Alanine amino-transferases activity was calculated from:

$$\triangle \text{ Activity (iu/l)} = 1745 \times \quad \text{nm/minute micro}$$

Where iu/l = unit of Aspartate and Alanine aminotransferase

$$\triangle \quad A = \text{change in absorbance.}$$

2.5 Statistical Analysis

The results were expressed as Mean ± Standard Error of Mean (SEM). Significant differences

between control and experimental values were assessed using student's t-test and the results were considered significant at P values of less than 0.05 (P = 0.05). Graphical representations were designed using Microsoft Excel (2007).

3. RESULTS

The results of the influence of *Tetracarpidium conophorum* leaf and root extracts on blood glucose of diabetic rats indicates that blood glucose of treated rats significantly (P<0.001) decreased on day 14 compared to diabetic untreated rats. The blood glucose levels of diabetic rats treated with oral hypoglycaemic agents alone or in combination with root or leaf extracts were not significantly (P = 0.05) different from control or extracts treated groups on day 14.

The results of the effect of root and leaf extracts of *Tetracarpidium conophorum* on liver enzyme levels in the diabetic condition are presented in Fig. 1. Diabetic rats exhibited significant (P<0.001) high levels of ALT, ALP and AST. Oral administration of root extract (50 mg/100g bwt) or leaf extract (50 mg/100g bwt) significantly (P<0.001) attenuated the increased liver enzyme levels. Glibenclamide and metformin, or the combination of glibenclamide with root or leaf extract did not yield any significant (P = 0.05) difference in ALT and AST levels when compared with extract treatment. The levels of ALP in extract treated groups were significantly (P = 0.5) lower compared to groups treated with glibenclamide, metformin and combination of glibenclamide with root or leaf extract.

4. DISCUSSION

Serum enzymes commonly used as biochemical tools for diagnostic purposes and also to monitor progress of treatment are alkaline phosphatase (ALP), aspartate aminotransferase (AST) and

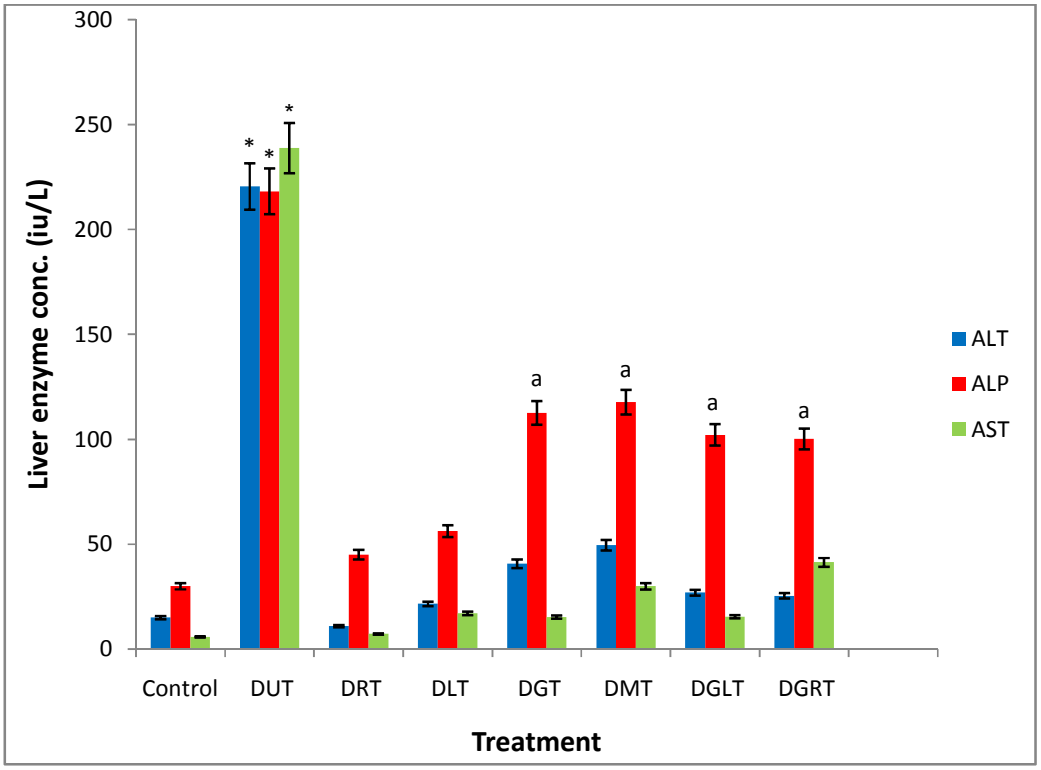

Fig. 1. Effect of *Tetracarpidium conophorum* leaf and root extracts and oral hypoglycaemic agents on liver enzymes

*Results show Mean ± SEM of five values. *P<0.001 vs control and treated groups, [a]P = 0.05 vs control, DRT and DLT. DUT = diabetic untreated, DRT = diabetic root extract (50 mg/100g body weight) treated, DLT = diabetic leaf extract (50mg/100 body weight) treated, DGT = diabetic glibenclamide treated, DMT = diabetic metformin treated, DGLT = diabetic glibenclamide + leaf treated, DGRT = diabetic glibenclamide + root treated. ALT = Alanine aminotransferase, ALP = alkaline phosphatase, AST = aspartate aminotransferase*

alanine aminotransferase (ALT) [10,11]. Liver disease and diabetic patients have higher incidences of liver function abnormalities [10,12]. Specific enzyme activity in the plasma frequently correlate with the extent of liver damage, thus the degree of elevation of a particular enzyme activity in plasma is often used as basis of the state of health or disease state of patient. Elevation of liver enzymes are associated with liver injury [10]. Elevated liver enzymes may indicate inflammation or damage to cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals including liver enzymes into blood stream which can result in elevated liver enzymes on blood test [13]. Diabetes mellitus is also associated with elevated liver enzyme levels [14,15].

The present research work indicated elevation of ALP, AST, and ALT levels in alloxan- induced diabetic rats associated with hyperglycemia. In the diabetic state, it is possible that prolonged hyperglycemia resulted in metabolic complications and release of reactive radicals that interfere with the integrity of liver cells. This interference disrupts liver cell membrane and the resultant damage and leakage of liver enzymes into the serum which is responsible for the elevated levels of AST, ALT and ALP observed in the diabetic rats in the present study.

Oral administration of leaf and root extracts of *Tetracarpidium conophorum* for 14 days to alloxan- induced diabetic rats significantly (P<0.001) lowered the serum liver enzyme levels. *Tetracarpidium conophorum* root and leaf extracts may have reversed the processes of hepatocellular damage due to its antioxidant and antidiabetogenic properties vested in the flavonoids, alkaloids and tannins contents and other phytochemical components [16,17]. This proposition is based on the fact that flavonoids and flavonoid containing herbals possess antidiabetogenic and cytoprotective properties [18,19,20,21]. Recent works have indicated antidiabetic and hypolipidemic potential [22], stress reduction [23], amelioration of diabetic – induced haematological effects [24] and antibacterial potential [25] of some flavonoid and alkaloid containing herbal. The present work supports the fact that medicinal plants possess beneficial health effects and has given the scientific basis for the application of *Tetracarpidium conophorum* root and leaf in the traditional medical practice for the treatment of diabetes.

5. CONCLUSION

The present results indicates that the leaf and root extracts of *Tetracarpidium conophorum* possess antidiabetic and hepatoprotective potentials and could be exploited in the development of antidiabetic- hepatoprotective agents. Extrapolation of the results obtained in laboratory animals to humans will be a welcome idea considering the cost implication of the management of this condition in poor resource countries.

CONSENT

Not applicable.

ETHICAL APPROVAL

All the author's hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

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COMPETING INTERESTS

All authors have declared that no competing interests exist.

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