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Evaluation of Ethanol-methanol Extracts of the Leaf, Stem Bark and Root of *Jatropha curcas* on Selected Liver Markers of Streptozotocin-induced Diabetic Rats

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Aims: Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medicinal policy issues. It is presently considered among the top ten leading causes of death globally resulting in a raised level of alanine aminotransferase (ALT) (the most sensitive marker of liver cell damage). This study was therefore aimed at evaluating the effect of ethanol-methanol extracts of leaf, stem bark and the root of *Jatropha curca*s on serum aminotransferases (aspartate amino transferase (AST) and ALT) and total protein (TP) of streptozotocin - induced diabetic rats.
Methodology: Fifty-four (54) male Wistar rats weighing 150-200 g were assigned according to body weight into nine (9) groups of six (6) rats each. Group I was the normal control and given water and rat chow only, groups II, III, IV, V and VI were induced with diabetes using streptozotocin. Group II served as the diabetic control and was therefore, left untreated, while groups III, IV and V were treated with leaf, stem bark, root extracts of *Jatropha curcas,* respectively and group VI was given a standard drug (Glibenclamide). The remaining groups VII, VIII and IX were not induced with

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diabetes but were given normal leaf, stem bark and root extracts, respectively. The animals were sacrificed after 14 days and blood was collected for the study.

Results: The result obtained showed a significant (p<0.05) decrease in serum AST of groups III, IV, VII, VIII and IX compared with the diabetic control (DC). The serum ALT showed a significant (p<0.05) increase in group II (DC) compared with the normal control, while groups VII, VIII and IX were significantly (p<0.05) decreased compared with the normal control. All the test groups showed a significant (p<0.05) decrease in serum ALT compared with the diabetic control. There was no significant (p≥0.05) difference in serum TP of all the test groups compared with the normal control, however, there was significant (p<0.05) increase in the TP of diabetic control.

Conclusion: This study revealed that *Jatropha curcas* plant extracts might confer protection against diabetic-induced hepatocellular damage as evidenced by normalisation of serum levels of total protein and ALT of treated diabetic groups. The *Jatropha curcas* leaf extract appeared to have exhibited a better protection against hepatocellular diabetic-induced damage than the stem bark and root.

Keywords: Alanine aminotransferase; aspartate aminotransferase; total protein; serum; Jatropha curcas.

1. INTRODUCTION

Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medicinal policy issues [1]. Diabetes is characterised by metabolic dysregulation primarily of carbohydrate metabolism, manifested by hyperglycaemia resulting from the defects in insulin secretion, impaired insulin action, or both [2]. The prevalence of diabetes is projected to rise to 366 million in 2030 [3]. According to the International Diabetes Federation (IDF) the number of individuals with diabetes in 2011 already crossed 366 million with an estimated 4.6 million death each year [4]. Diabetes is presently considered among the top ten leading causes of death globally [3]. It results in a raised level of alanine aminotransferase (ALT) (the most sensitive marker of liver cell damage) and elevation in serum ALT is an indication of hepatic damage [5]. Type 2 diabetes mellitus (T2DM) is associated with non-alcoholic fatty liver disease (NAFLD) [5] and the close association of ALT with hepatic steatosis has made it a commonly utilised epidemiologic biomarker of non-alcoholic fatty liver disease [6]. Higher levels of ALT also increase the risk to have T2DM [6]. In diabetics, there is usually an increase in serum total proteins as buttressed by Gul et al. [7] and Nazki et al. [8].

The problem of handling diabetes and other associated issues arising from the disease has made it a disease of public health concern that requires a multi-purpose approach. The use of traditional medicine to an extent impacted well in addressing the malaise of diseases as plants like *Jatropha curcas* (*J. curcas*) have `played major role in the treatment of various diseases, including bacterial and fungal infections [9,10,11]. *J. curcas* from the family of Euphorbiaceae have ancient medicinal uses in its centre of origin in Latin America. But its ability to grow in the tropics has allowed the plant to be used as ethnomedicine in different countries of Africa and Asia spanning from the roots being used in haemolytic disease to the leaves being used to relieve pains, rheumatism, fever, malaria, wounds and a host of others [12]. These enormous properties of *J. curcas* are responsible for considering this plant as a potential source of chemotherapeutic compounds [13]. Several researches on the use of *J. curcas* extracts have shown promising results as an antidiabetic and antioxidative agent [14]. Several studies have worked on methanol or ethanol extracts of *J. curcas* plant (particularly the leaf) on liver function parameters [15, 16]. A work by Asuk et al. [17] revealed that both methanol and ethanol extracts have similar levels of phytochemical content, but the potential of ethanol-methanol (1:1) extracts of the leaf, stem bark and root in addressing increased levels of aspartate amino transferase (AST), particularly ALT and total protein levels; the biomarkers associated with diabetic hepatocellular damage is yet to be exploited. Hence, it is imperative to assess the possible effect of ethanol-methanol (1:1) extracts of *J. curcas* on these selected hepatocellular damage biomarkers of diabetic-induced rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Streptozotocin (STZ) was purchased from Sigma Chemical, (St Louis, USA). All chemicals and

reagents used in this research were of analytical grade.

2.2 Plant Materials

Fresh leaf, stem bark and the root of *J. curcas* were collected from Bedia farm in Obudu, Obudu Local Government Area of Cross River State, Nigeria. The plant was identified and authenticated (Identification no: 67) by Mr. Frank I. Apejoye of Botany Department, University of Calabar, Calabar.

2.3 Extract Preparation

The fresh leaf, stem bark and root of *J. curcas* were collected and air dried at room temperature at the Medical Biochemistry Laboratory, Cross River University of Technology (CRUTECH), Okuku, Cross River State, Nigeria. The dried leaf, stem bark and root were pulverised after which 200 g each were soaked in 1000 mL of a mixture of ethanol and methanol (1:1) and agitated, then allowed to stay for 72 hours at 40°C. The mixtures were first filtered with cheesecloth, followed by Whatman filter paper 1 (24 cm). The filtrates were separately concentrated using a water bath (Model - WBHL6/FL, Serial No-Y6M094, China) to 10% of its original volume at 40°C.

2.4 Animals

Male Wistar rats weighing between 150-200 g were obtained from the Animal House, Department of Medical Biochemistry, Cross River University of Technology, Okuku, Cross River State, Nigeria. The animals were kept in plastic cages, placed in a well-ventilated room at a temperature and relative humidity of 28± 2°C and 70% respectively with 12-hour day/night cycles and fed with standard rat pelleted diet (Vital Feed, Jos) and water *ad libitum*. The principles of animal care were also dully followed.

2.4.1 Induction of diabetes

Diabetes mellitus was induced by single intraperitoneal administration of 50 mg/kg of streptozotocin (dissolved in 0.1 M fresh cold citrate buffer, pH 4.5) into 12 hour-fasted rats. On the third day of STZ –injection, the rats were fasted for 6 hours and blood was taken from the tail artery of the rats [18]. Rats with moderate diabetes having hyperglycaemia (that is, with a blood glucose of 250-400 mg/dL) were taken for the experiment. The diabetic rats were divided into different groups.

2.5 Experimental Design

A total of 54 male Wistar rats were used, the rats were divided into nine (9) groups of six (6) each.

Group I : Normal control (NC) animals were given normal feed and water only.
Group II : Diabetic control (DC) animals were induced with diabetes using STZ and left untreated.
Group III : Diabetic animals treated with leaf extract (DTLE) of *J. curcas*.
Group IV : Diabetic animals treated with stem bark extract (DTSBE) of *J. curcas.*
Group V : Diabetic animals treated with root extract (DTRE) of *J. curcas* .
Group VI : Diabetic animals treated with the standard drug (DTSD)-Glibenclamide
Group VII : Group not induced with diabetes but was administered normal leaf extract (NLEA) of *J. curcas*.
Group VIII : Group not induced with diabetes but was administered normal stem bark extract (NSBEA) of *J. curcas*.
Group IX : Group was not induced with diabetes but was administered normal root extract (NREA) of *J. curcas*.

The administration of the plant extracts continued for fourteen days (14days) and the animals were subjected to an overnight fasting of 14 h penultimate to the day of sacrifice after anaesthesia. Principles of laboratory care according to ETS-123 [19] were applied. All experiments and procedures were examined and approved by the Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH) Research and Ethics Committee.

2.6 Biochemical Assay

The aminotransferases (ALT and AST) were determined using Randox Laboratory Kits, United Kingdom. The total protein concentration was assayed by the biuret method where the absorbance of the standard and test sample was measured spectrophotometricaly at 546 nm against the reagent blank.

2.7 Statistical Analysis

Data obtained was analysed using the Microsoft Excel 2007 and was expressed as the mean ± SEM. The statistical package SPSS version 17 was used to establish the statistical significance at $p<0.05$.

3. RESULTS

The serum levels of AST, ALT and TP of male Wistar rats are given in Table 1.

The serum AST showed a significant (p<0.05) increase in groups II, IV, V, VI and VIII but none in group III compared with normal control, while groups III, V, VII, VIII and IX were significantly (p<0.05) decreased, groups IV and VI were significantly(p<0.05) increased compared with diabetic control (DC).

The serum ALT was significantly (p<0.05) increased in group II (DC) compared with the normal control, while groups IV, VII, VIII and IX were significantly (p<0.05) decreased. Groups III and V showed no significant difference compared with the normal control. All the test groups recorded a significant (p<0.05) decrease in serum ALT compared with the diabetic control (DC).

There was no significant (P>0.05) difference in serum total protein (TP) of all the test groups compared with the normal control. However, compared with the diabetic control, only groups IV and VII showed no significant difference; the rest were significantly (p<0.05) decreased.

4. DISCUSSION

The liver plays a key role in the metabolic process of itself as well as other tissues in maintaining the internal body environment (homeostasis). Hepatic injury due to some xenobiotics and failure to eliminate toxic metabolic products by the liver often results in marked distortion of the normal function of the liver [20]. Increased levels of AST and ALT in the serum are often associated with hepatocellular injury or damage [21]. These serum enzymes are also sensitive detectors in biliary cirrhosis, hepatitis and biliary obstruction [22].

The present work reveals that STZ - induced diabetes may have caused hepatocellular damage, which is another characteristic change in diabetes as evidenced by the increase in AST and ALT of the DC. This is in line with the works done by Saligram et al. [5] and Ko et al. [6]. There was also an increase in AST levels of DTSBE, DTRE and DTSD compared with the NC. However, the increase in AST levels of DTSBE and DTRE are comparable to the standard drug (DTSD), but the serum activity of ALT was reduced in all the test groups except the standard drug group (DTSD) compared with the control. The increase in serum AST in some groups is not liver specific as it may have resulted from leakage from the muscle or heart tissues in contrast to ALT which is largely predominant in the liver hence making it a more sensitive marker to liver damage. In an earlier work, Asuk et al. [17] reported significant levels of polyphenols and flavonoids in the methanol and ethanol extracts of the leaf of *J. curcas* compared with the stem bark and root. These phytochemicals are known to play important role in antioxidation of tissues, hence, it is the the possible reason for the normalisation of AST in DTLE than in DTSBE and DTRE. However, the

Table 1. Effect of leaf, stem bark and root extracts of *Jatropha curcas* on the serum aminotransferases and total proteins in Wistar rats

Parameters / Group	AST (U/L)	ALT (U/L)	TP (g/dL)
I(NC)	12.46±0.08^{a}	5.91±0.84^{a}	7.18±0.25^{a}
II(DC)	19.21±0.34^{b}	13.12±0.30^{b}	8.35±0.38^{b}
III(DTLE)	12.59±0.08^{a}	5.33±0.34^{ac}	7.22±0.39^{a}
IV(DTSBE)	27.07±0.22^{c}	3.79±0.47^{d}	7.34±0.19^{ab}
V(DTRE)	16.59±0.49^{d}	5.38±0.53^{ac}	7.19±0.40^{a}
VI(DTSD)	24.15±0.54^{e}	7.04±0.26^{a}	6.82±0.33^{a}
VII(NLEA)	9.89±0.44^{f}	3.36±0.38^{d}	7.58±0.15^{ab}
VIII(NSBEA)	17.35±0.40^{d}	3.30±0.43^{d}	6.85±0.36^{a}
IX(NREA)	8.35±0.85^{g}	3.50±0.57^{d}	7.11±0.38^{a}

Values are mean ± SEM (n=6)

^{a,b,c,d,e,f,g} *Values with different superscripts are significantly different at P<0.05.*

Note: NC= Normal Control; DC = Diabetic Control; DTLE = Diabetic treated with leaf extract; DTSBE = Diabetic treated with stem bark extract; DTRE = Diabetic treated with root extract; DTSD = Diabetic treated with standard drug (Glibenclamide); NLEA, NSBEA and NREA = Normal leaf, stem bark and root extracts administration respectively.

balance between free radical release resulting from diabetic-induced oxidation and their mopping up may have favoured the phytochemical constituents of DTRE over DTSBE, hence the decreased serum AST levels of DTRE over DTSBE has been observed [14]. It appears that the DTLE impacted better on the liver and possibly some other organs as normal levels of AST and ALT were maintained.

In diabetics, there is usually increase in total serum proteins as corroborated by Gul et al. [7] and Nazki et al. [8]. The total serum protein of the DC was increased in the present work, while that of the treated groups were normalised by the extracts.

5. CONCLUSION

Biochemical evaluation of aminotransferases (AST and ALT) as biomarkers of diabetic effect on the liver and the use of ethanol-methanol (1:1) extracts of the leaf, stem bark and root of *J. curcas* to alter diabetic states on the liver were examined in the present study. It appears that the plant extracts might confer protection against diabetic – induced hepatocellular damage as evidenced by normal serum levels of total protein and ALT of diabetic treated groups. The ethanol-methanol leaf extracts of the *J. curcas* plant appeared to have exhibited a better protection against hepatocellular diabetic-induced damage than the stem bark and root.

ETHICAL APPROVAL

The author hereby declares that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH) Research and Ethics Committee.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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