

Evaluation of ethanol-methanol extracts of the leaf, stem bark and root of *Jatropha curcas* on selected liver markers of streptozotocin-induced diabetic rats

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 raised level of 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 root of *Jatropha curcas* on serum aminotransferases (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, V and VI were treated with leaf, stem bark, root extracts and standard drug (Glibenclamide) respectively. The remaining groups VII, VIII and IX were not induced with diabetes but were given normal leaf, stem bark and root extracts respectively. The animals were sacrificed after 14 days and blood collected for 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 ($P\geq 0.05$) 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: In this study, it appeared that *Jatropha curcas* plant extracts might confer protection against diabetic-induced hepatocellular damage as evidenced by normalization 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

1. INTRODUCTION

Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medicinal policy issues [1]. Diabetes is characterized by metabolic dysregulation primarily of carbohydrate metabolism, manifested by hyperglycemia resulting from 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 raised level of 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 utilized 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 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 medicinal approach has to an extent impacted well in addressing the malaise of diseases as plants like *Jatropha 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, had ancient medicinal uses in its center of origin in Latin America. But its ability to grow in the tropics has allowed for different parts of the plant to be used as ethnomedicine in different countries of Africa and Asia spanning from the roots being used in hemolytic 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* has reflected in its being a potential source of chemotherapeutic compounds [13] and research into

the use of *Jatropha curcas* extracts has shown promising results as an antidiabetic and antioxidative agents [14]. Several studies have explored 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 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.

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2. MATERIALS AND METHODS

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2.1 Chemicals and Reagents

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Streptozotocin (STZ) was purchased from sigma chemical, (St Louis, USA). All chemicals and reagents used in this research were of analytical grade.

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2.2 Plant Materials

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Fresh leaf, stem bark and root of *Jatropha 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.

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2.3 Extract Preparation

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The fresh leaf, stem bark and root of *Jatropha 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 pulverized after which 200 g each were soaked in 1000 mL of 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 cheese cloth, followed by Whatman filter paper 1 (24cm). The filtrates were separately concentrated using water bath (Model -WBHL6/FL, Serial No-Y6M094, china) to 10% of its original volume at 40°C.

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2.4 Animals

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Male Wistar rats weighing between 150-200 g were obtained from 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 well-ventilated room at temperature and relative humidity of $28\pm 2^{\circ}\text{C}$ 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 tail artery of the rats [18]. Rats with moderate diabetes having hyperglycemia (that is, with 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 males Wistar rats were used, the rats were divided into nine (9) of groups six (6) rats 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 *Jatropha curcas*

Group IV: Diabetic animals treated with stem bark extract (DTSBE) of *Jatropha curcas*

Group V: Diabetic animals treated with root extract (DTRE) of *Jatropha curcas*

Group VI: Diabetic animals treated with standard drug (DTSD)- Glibenclamide

Group VII: Group not induced with diabetes but was administered normal leaf extract (NLEA) of *Jatropha curcas*.

Group VIII: Group not induced with diabetes but was administered normal stem bark extract (NSBEA) of *Jatropha curcas*.

Group IX: Group was not induced with diabetes but was administered normal root extract (NREA) of *Jatropha curcas*.

The administration of the plant extracts lasted for fourteen days (14days) and the animals were subjected to an overnight fast of 14 h penultimate to the day of sacrifice after anaesthesia. Principles of laboratory care according to ETS-123 [19] was 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 absorbance of the standard and test sample was measured spectrophotometrically at 546 nm against the reagent blank.

2.7 Statistical Analysis

Data obtained was analysed using Microsoft Excel 2007 and expressed as mean $\pm$ SEM. The statistical package SPSS version 17 was used to establish 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 showed a significant ($P < 0.05$) decrease in serum ALT compared with 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.

Table 1. Leaf, stem-bark and root extracts of *Jatropha curcas* on 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.

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 aspartate amino transferase (AST) and alanine aminotransferase (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 showed 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 in line with works 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 the possible reason for the normalization of AST in DTLE than in DTSBE and DTRE. However, the balance between free radical release resulting from diabetic-induced oxidation and their mopng up may have favoured the phytochemical constituents of DTRE over DTSBE hence the decreased serum AST levels of DTRE over DTSBE [14]. It appears that the DTLE impacted better on the liver and possibly some other organ 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 this present work, while that of the treated groups were normalized 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 *Jatropha curcas* to alter diabetic states on the liver were studied. It appeared that this 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 *Jatropha curcas* plant appeared to have exhibited a better protection against hepatocellular diabetic-induced damage than the stem bark and root.

193 **CONSENT**

194 Not applicable

196 **ETHICAL APPROVAL**

197 Author hereby declares that "Principles of laboratory animal care" (NIH publication No. 85-23, revised
200 1985) were followed, as well as specific national laws where applicable. All experiments have been
201 examined and approved by the Faculty of Basic Medical Sciences, Cross River University of
202 Technology (CRUTECH) Research and Ethics Committee.

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