

Original Research Article

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: The study was 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 ^{space} 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-VI were induced with diabetes using streptozotocin. Group II was untreated, while groups III -VI were treated with leaf, stem bark, root extracts and standard drug respectively. The remaining groups VII-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.

10 Keywords: Alanine aminotransferase; aspartate aminotransferase; total protein; serum

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13 1. INTRODUCTION

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15 Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medicinal

16 policy issues [1]. Diabetes is characterized by metabolic dysregulation primarily of carbohydrate

17 metabolism, manifested by hyperglycemia resulting from defects in insulin secretion, impaired insulin

18 action, or both [2]. The prevalence of diabetes is projected to rise to 366 million in 2030 [3]. According

19 to the International Diabetes Federation (IDF) the number of individuals with diabetes in 2011 already

20 crossed 366 million with an estimated 4.6 million death each year [4]. Diabetes is presently

21 considered among the top ten leading causes of death globally [3]. It results in raised level of ALT (the

22 most sensitive marker of liver cell damage) and elevation in serum ALT is an indication of hepatic

23 damage [5]. Type 2 diabetes mellitus (T2DM) is associated with non-alcoholic fatty liver disease

24 (NAFLD) [5] and the close association of ALT with hepatic steatosis has made it a commonly utilized

25 epidemiologic biomarker of non-alcoholic fatty liver disease [6]. Higher levels of ALT also increase the

26 risk to have T2DM [6]. In diabetics there is usually increase in serum total proteins as buttressed by

27 Gul et al. [7] and Nazki et al. [8].

28 The problem of handling diabetes and other associated issues arising from the disease has made it a

29 disease of public health concern that requires a multi-purpose approach. The use of traditional

30 medicinal approach has to an extent impacted well in addressing the malaise of diseases as plants

31 like *Jatropha curcas* have played major role in the treatment of various diseases, including bacterial

32 and fungal infections [9], [10], [11].

33 *J. curcas* has been shown to be a potential source of chemotherapeutic compounds [12] and the use

34 of *Jatropha curcas* extract has shown promising results as an antidiabetic and antioxidative agent S

35 [13], but the potential of ethanol-methanol extracts of the leaf, stem bark and root in addressing

36 increased levels of AST, particularly ALT and total protein levels; the biomarkers associated with

37 diabetic hepatocellular damage is yet to be exploited. Hence, it is imperative to assess the possible

38 effect of *J. curcas* extract on these selected hepatocellular damage biomarkers of diabetic-induced

39 rats.

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41 2. MATERIAL AND METHODS

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43 2.1 Chemicals and Reagents

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

47 48 **2.2 Plant Material**

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

53 54 **2.3 Extract Preparation**

55
56 The fresh leaf, stem bark and root of *Jatropha curcas* were collected and air dried at room
57 temperature at the Medical Biochemistry Laboratory, Cross River University of Technology
58 (CRUTECH), Okuku, Cross River State, Nigeria. The dried leaf, stem bark and root were pulverized
59 after which 200 g each were soaked in 1000 mL of mixture of ethanol and methanol (1:1) and
60 agitated, then allowed to stay for 72 hours at 40°C. The mixtures were first filtered with cheese cloth,
61 ~~then with~~ ^{followed by} Whatman filter paper 1 (24cm). The filtrates were separately concentrated using water bath
62 (Model -WBHL6/FL, Serial No-Y6M094, china) to 10% of its original volume at 40°C.

63 64 **2.4 Animals**

65
66 Male Wistar rats weighing between 150-200g were obtained from Animal house, Department of
67 Medical Biochemistry, Cross River University of Technology, Okuku, Cross River State, Nigeria. The
68 animals were kept in plastic cages, placed in well-ventilated room at temperature and relative humidity
69 of 28±2°C and 70% respectively with 12-hour day/night cycles and fed with standard rat pelleted diet
70 (vital feed, Jos) and water *ad libitum*. The principles of animal care were also dully followed.

71 72 **2.4.1 Induction of diabetes**

73
74 Diabetes mellitus was induced by single intraperitoneal administration of 50mg/kg of streptozotocin
75 (dissolved in 0.1M fresh cold citrate buffer, pH 4.5) into 12 hour-fasted rats. On the third day of STZ –
76 injection, the rats were fasted for 6 hours and blood was taken from tail artery of the rats [14]. Rats
77 with moderate diabetes having hyperglycemia (that is, with blood glucose of 250-400 mg/dL) were
78 taken for the experiment. The diabetic rats were divided into different groups.

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82 **2.5 Experimental Design**

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

86 Group I: Normal control (NC) animals were given normal feed and water only ✓

87 Group II: Diabetic control (DC) animals were induced with diabetes using STZ and left untreated ✓

88 Group III: Diabetic animals treated with leaf extract (DTLE) of *Jatropha curcas*. ✓

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

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

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

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

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

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

98 The administration of the plant extract lasted for fourteen days ^S ~~14 days~~ ^{and} after which the animals were
99 subjected to an overnight fast of 14 h penultimate to the day of sacrifice after anaesthesia. Principles
100 of laboratory care according to ETS-123 [15] was applied. All experiments and procedures were
101 examined and approved by the Faculty of Basic Medical Sciences, Cross River University of
102 Technology (CRUTECH) Research and Ethics Committee. ✓

103

104 **2.6 Biochemical Assay**

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

109

110 **2.7 Statistical Analysis**

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112 Data obtained was analysed using Microsoft Excel 2007 and expressed as mean $\pm$ SEM. The
113 statistical package SPSS version 17 was used to establish statistical significance at $P < 0.05$

114 **3. RESULTS AND DISCUSSION**

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116
117 The serum levels of AST, ALT and TP of male Wistar rats are given in Table 1.
118 The serum AST showed a significant ($P<0.05$) increase in groups II, IV, V, VI and VIII but none in
119 group III compared with normal control, while groups III, V, VII, VIII and IX were significantly ($P<0.05$)
120 decreased, groups IV and VI were significantly($P<0.05$) increased compared with diabetic control
121 (DC).
122 The serum ALT was significantly ($P<0.05$) increased in group II (DC) compared with the normal
123 control, while groups IV, VII, VIII and IX were significantly ($P<0.05$) decreased, groups III and V
124 showed no significant difference compared with the normal control. All the test groups showed a
125 significant ($P<0.05$) decrease in serum ALT compared with diabetic control (DC).
126 There was no significant ($P>0.05$) difference in serum total protein (TP) of all the test groups
127 compared with the normal control, however, compared with the diabetic control, only groups IV and
128 VII showed no significant difference; the rest were significantly ($P<0.05$) decreased.

129
130 **Table 1. Leaf, stem-bark and root extracts of *Jatropha curcas* on serum aminotransferases**
131 **and total proteins in Wistar rats**

132

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

133
134 Values are mean ± SEM (n=6)
135 ^{a,b,c,d,e,f,g} Values with different superscripts are significantly different at $P<0.05$.

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

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141 **4. DISCUSSION**

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143 The liver plays a key role in the metabolic process of itself as well as other tissues in maintaining the

144 internal body environment (homeostasis). Hepatic injury due to some xenobiotics and failure to
145 eliminate toxic metabolic products by the liver often results in marked distortion of the normal function
146 of the liver [16]. Increased levels of aspartate amino transferase (AST) and alanine aminotransferase
147 (ALT) in the serum are often associated with hepatocellular injury or damage [17]. These serum
148 enzymes are also sensitive detectors in biliary cirrhosis, hepatitis and biliary obstruction [18].

149 The present work showed that STZ - induced diabetes may have caused hepatocellular damage,
150 which is another characteristic change in diabetes as evidenced by the increase in AST and ALT of
151 the DC. This in line with works by Saligram et al [5] and Ko et al. [6]. There was also an increase in
152 AST levels of DTSBE, DTRE and DTSD compared with the NC. However, the increase in AST levels
153 of DTSBE and DTRE are comparable to the standard drug (DTSD) but the serum activity of ALT was
154 reduced in all the test groups except the standard drug group (DTSD) compared with the control. It
155 appears that the DTLE impacted better on the liver as normal levels of AST and ALT were
156 maintained.

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

160
161 **5. CONCLUSION**

162
163 Biochemical evaluation of aminotransferases (AST and ALT) as biomarkers of diabetic effect on the
164 liver and the use of ethanol-methanol (1:1) extracts of the leaf, stem bark and root of *Jatropha curcas*
165 to alter diabetic states on the liver were studied. It appeared that this plant extracts might confer
166 protection against diabetic - induced hepatocellular damage as evidenced by normal serum levels of
167 total protein and ALT of diabetic treated groups. The ethanol-methanol leaf extract of the *Jatropha*
168 *curcas* plant appeared to have exhibited an all-round protection against hepatocellular diabetic-
169 induced damage than the stem bark and root.

Suggestion
more explanation
of the
extracts ?
↓
especially
different
parts of
the plant
used !

ethanol-methanol mixture
vs ethanol alone / methanol
alone ?
↓
any
effect ?

170 **CONSENT**

171
172 Not applicable

173
174 **ETHICAL APPROVAL**

175
176 Author hereby declares that "Principles of laboratory animal care" (NIH publication No. 85-23, revised
177 1985) were followed, as well as specific national laws where applicable. All experiments have been
178 examined and approved by the Faculty of Basic Medical Sciences, Cross River University of
179 Technology (CRUTECH) Research and Ethics Committee.

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