Antidiabetic and Antioxidative Effects of *Jatropha curcas* Extracts in Streptozotocin-induced Diabetic Rats

A. A. Asuk1*, K. Dasofunjo1, A. I. Okafor1 and F. A. Mbina1

1Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus, P.M.B 1123 Calabar, Cross River State, Nigeria.

Authors’ contributions

This work was carried out in collaboration between all authors. Author AAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KD and AIO managed the analyses of the study. Author FAM managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aims: To ascertain the antidiabetic and antioxidative effects of ethanol-methanol extracts of leaf, stem bark and root of *Jatropha curcas* on the blood and liver tissue of streptozotocin-induced diabetic albino wistar rats

Place and Duration of Study: Department of Medical Biochemistry, Cross River University of Technology, Okuku campus between August 2013 and March, 2014.

Methodology: Fifty four (54) male albino wistar rats weighing 150-200g were randomly assigned into nine study groups (n=6). Group I was the normal control, 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 Glibenclamide (standard drug) respectively. The remaining groups VII–IX were not induced with diabetes but were treated with leaf, stem bark and root extracts respectively. The administration of these extracts lasted for 14 days after which the animals were sacrificed. The liver tissue was collected and homogenized and the supernatant used for the
estimation of SOD, CAT and MDA activities.

**Results:** The result of blood glucose level on the fourteenth day of the plant extracts administration, showed further decrease in the groups treated with the plant extracts to the extent that group IV was significantly \( P<0.05 \) decreased compared with the normal control. The blood glucose level of the standard drug (Glibenclamide) treated group (VI) was further decreased however it remained significantly \( P<0.05 \) increased compared with the normal control and about three or more times that of the groups treated with the plant extracts. Result of the liver tissue SOD for the test groups showed significant \( P<0.05 \) difference except for groups IV and VII when compared with the normal control. There was no significant \( P \geq 0.05 \) difference in the SOD activities of test groups compared with the diabetic control. The liver tissue CAT showed general increase for the test groups, but group III produced a significant \( P<0.05 \) increase compared with the normal control, while groups III and V showed significant \( P<0.05 \) increase compared with the diabetic control. The liver tissue MDA showed significant \( P<0.05 \) increase for groups II and IV but a significant \( P<0.05 \) decrease for group V compared with the normal control. However, compared with group II (diabetic control) all the test groups showed a significant \( P<0.05 \) decrease.

**Conclusion:** The present research suggest that the leaf, stem bark and root of ethanol-methanol (1:1) extracts of *Jatropha curcas* possess anti-hyperglycemic and antioxidant activities but their response to the liver tissue enzyme systems of SOD and CAT vary in either to playing compensatory role or boosting the activities of the antioxidant enzymes. The plant parts also appear to possess the potential for reversing the tissue oxidative damage caused by diabetes as seen by their abilities to prevent lipid peroxidation.

**Keywords:** Diabetes mellitus; *Jatropha curcas*; leaf; stem-bark; root; antidiabetic; antioxidant activities.

1. **INTRODUCTION**

Traditional plants contain a wide variety of chemical compounds used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have effect on long-term health when consumed by humans, and can be used to effectively manage human diseases, such as malaria, diarrhea, typhoid, rheumatism, and even diabetes [1]. Plant-derived compounds have great potentials to cure and control diabetes, apart from being safer and cost effective [2]. Most of the researches carried out are usually to evaluate the therapeutic effect of plants along with their mode of action [2].

Plants have shown anti-diabetic activity with varying mechanisms, for example; in the alteration of glucose metabolism, improved glucose tolerance, reduced absorption of glucose from intestine, enhanced insulin signal pathway, hypoglycemia through increased glucose uptake and glucose synthesis, inhibition of \( \alpha \)-glucosidase and \( \alpha \)-amylase, as well as reduction of insulin [3]. Traditional medicine using plant extract continues to provide health coverage for over 80% of the world’s population, especially in the developing world [4]. Plants have been used for medicinal purposes long before recorded history. Many of the pharmaceuticals currently available to physicians have a long history of use in herbal remedies, including opium, digitalis and quinine.

Diabetes mellitus is a metabolic disorder of the endocrine system. The disease is found in all parts of the world and is rapidly increasing worldwide. People suffering from diabetes cannot produce or properly use insulin, so they have high blood glucose (hyperglycemia). The causes of diabetes may be associated with both genetic and environmental factors [5]. Diabetes triggers oxidative stress in the liver, which is characterized by increased concentration of reactive oxygen species (ROS) and significant reduction in its antioxidant defenses [6]. The increased production of ROS during diabetes mellitus is injurious to the liver including vessels, retina, kidneys and nerves [6], resulting in the alterations of the activities of antioxidant enzymes such as SOD and CAT [7].

Balaji et al. [8] reported that methanol extract of *Jatropha curcas* could protect the liver against the aflatoxin B1-induced oxidative damage in rats. Several plants used as anti-diabetic agents have also exercised antioxidant effects [9] however, many oral anti-diabetic agents have a number of serious adverse effects including hepatic and nephritic. Thus, managing diabetes without any side effect is still a challenge [10].
The search for more effective, cost effective and safer agents with pharmacological/therapeutic activity has continued to be an important area of investigation. There is a correlation between diabetes and tissue damage arising from free radicals release, hence the determination of the antidiabetic and antioxidant effects of the ethanol-methanol extracts of the leaf, stem bark and root of *Jatropha curcas* on the blood and liver tissue of streptozotocin-induced diabetic albino wistar rats, will give a strong indication of the use of these plant parts in the trado-medical treatment of diabetes.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Streptozotocin (STZ) was purchased from sigma chemical, (St. Louis, USA); potassium, chloride, sodium chloride from loba chemical Ltd, India; sodium Azide, hydrochloric acid, sodium dihydrogen phosphate, di-sodium hydrogen orthophosphate anhydrous were products of BDH Company U.K. All other chemicals and reagents used in this research were of analytical grade.

2.2 Plant Material

Fresh (leaves, stem bark and roots) of *Jatropha curcas* were collected from Bedia in Obudu local government area of Cross River State, Nigeria. The plant was identified and authenticated at the herbarium unit, Department of Botany, University of Calabar, Calabar.

2.3 Extract Preparation

The fresh (leaves, stem bark and root) of *Jatropha curcas* were collected and air dried at room temperature at Medical Biochemistry Laboratory, Cross River University of Technology (CRUTECH), Okukwu, Cross River State, Nigeria. The dried (leaves, stem bark and roots) were pulverized after which 200g each were soaked in 1000mL 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, and later with Whatman filter paper No. 1 (24cm). The filtrates were separately concentrated using water bath (Model- WBH6/FL serial NO –Y6M094, China) to 10% of its original volume at 40°C.

2.4 Animals

Male albino wistar rats weighing between 150-200g were obtained from the animal house, Department of Medical Biochemistry, CRUTECH, Okukwu, Cross River State, Nigeria. The animals were kept in a well-ventilated laboratory cages with 12-hour day/night cycles and fed with standard rat pelleted diet (vital feed, Jos) and water ad libitum. The principles of laboratory animal care were also duly followed.

2.4.1 Induction of diabetes and measurement of blood glucose levels

Diabetes mellitus was induced by single intra-peritoneal administration of 50 mg/kg of streptozotocin-STZ (dissolved in 0.1M fresh cold citrate buffer, pH 4.5) into 12 hours 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 [11]. The One touch ACCU-CHECK advantage glucometer was used for the determination of blood glucose levels. Rats with moderate diabetes having hyperglycemia (that is, with blood glucose of 250-400mg/dl) were taken for the experiment. The diabetic rats were divided randomly into different groups.

2.5 Experimental Design

In the experiment, a total of 54 male albino rats were used. The rats were divided into nine (9) groups of six (6) rats each.

- **Group I**: Normal control (NC) animals was given normal feed and water only
- **Group II**: Diabetic control (DC) animals was induced with diabetes using STZ and 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 - Glibenclamide (DTS)
- **Group VII**: This group was not induced with diabetes and was given normal leaf extract administration (NLEA) of *Jatropha curcas*.
Group VIII: This group was also not induced with diabetes but was given normal stem bark extract administration (NSBEA) of *Jatropha curcas*.

Group IX: This is the last group that was not induced with diabetes but was given normal root extract administration (NREA) of *Jatropha curcas*.

The administration of the plant extract lasted for fourteen days (14 days). The animals were sacrificed 24 hours after the last administration. "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 appropriate ethics committee.

2.6 Preparation of Liver Homogenate

The liver tissue was homogenized in 1:5 of 0.9% sodium chloride (ice cold). The supernatant obtained was centrifuged at 3500rpm for 20 minutes (4°C) to get the supernatant which was used to assay SOD (superoxide dismutase), CAT (catalase) and MDA (malondialdehyde) activities.

2.6.1 Determination of catalase (CAT) activity

Catalase activity was assayed by the method of Sinha et al. [12]. Briefly, the assay mixture consisted of 1.96 mL phosphate buffer (0.01 M, pH 7.0), 1.0 mL hydrogen peroxide (0.2 M) and 0.04 mL of homogenate in a final volume of 3.0 mL. About 2 mL dichromate acetic acid reagent was added in 1 mL of reaction mixture, boiled for 10 minutes and cooled. Changes in absorbance were recorded at 570 nm.

2.6.2 Determination of superoxide dismutase (SOD) activity

The levels of SOD activity in the tissues were determined by the method of Martin et al. [13]. Briefly, 920µL of 0.05 M phosphate buffer (pH 7.8) was added to 40µL of assay buffer or sample, mixed and incubated for 2 minutes. Then 40µL of hematoxylin was added to start auto–oxidation reaction that yields increase in absorbance at 560nm. After mixing quickly, changes in absorbance was recorded at 560nm immediately at 60 seconds interval for at least 5 minutes.

2.6.3 Determination of malondialdehyde (MDA) activity

A breakdown product of lipid peroxidation, thiobarbituric acid reactive substances (TBAR) was measured by the method of Buege and Aust [14]. One volume of the test sample and two volume of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25N HCl) were mixed in cooked test tube and heated for 15 minutes on boiling water bath. After cooling at room temperature, the precipitate was removed by centrifugation at 2500rpm for 10 minutes and the absorbance of the supernatant was measured at 532nm against blank.

2.7 Phytochemical Analysis

Test for alkaloids, glycosides, tannins, phlobatannins were according to Trease and Evans [15], while that for flavonoids, polyphenols were according to Cuilei, [16]. Test for Saponin was according to Sofowora, [17]. The test for the remaining phytochemicals (coumarins, terpinoids, steroids, triterpinoids, carotenoids, polyacetylated compounds were according to Edeoga, Okwu and Mbaebie [18].

2.8 Statistical Analysis

Data obtained were analyzed using SPSS 16.0 for one way ANOVA (Analysis of variance) and then post hoc (LSD) for significant values. Values were expressed as mean±SEM and statistical significance was accepted at \( P<0.05 \).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of Leaf, Stem Bark and Root of *Jatropha curcas*

The phytochemical screening of leaf, stem bark and root of *Jatropha curcas* are given in (Table 1). The results showed presence of polyphenols, flavonoids, alkaloids, cardiac glycosides, coumarins, saponins, terpinoids, steroids, triterpenoid saponins, carotenoids, phlobatannins and tannins. Polyacetylated compounds were conspicuously absent.
Table 1. Phytochemical screening of the ethanol-methanol extracts of leaf, stem bark, and root of *Jatropha curcas*

| Phytochemicals      | Leaf | Stem bark | Root |
|---------------------|------|-----------|------|
| Polyphenols         | +++  | ++        | ++   |
| Flavonoids          | +++  | ++        | +++  |
| Alkaloids           | +    | +         | +    |
| Cardiac glycosides  | +    | +         | +    |
| Coumarins           | +    | +         | +    |
| Steroids            | +    | +         | +    |
| Triterpenoid saponins | +    | +        | +    |
| Polyacetylated      | -    | -         | -    |
| compounds           |      |           |      |
| Carotenoids         | +    | +         | +    |
| Phlobatannins       | +    | +         | +    |
| Tannins             | +    | +         | +    |

+++Very Strong Presence; ++Strong Presence; +Slight Presence; -Absent

3.2 Result of Blood Glucose Levels of Normal, Diabetic and Diabetic-treated Animals with Leaf, Stem Bark and Root Extracts of *Jatropha curcas*

The results showed that at day 0, there was no significant ($P\geq0.05$) difference in blood glucose level between the test groups and the control. But at day 3 after inducing diabetes and the second day of the plant extracts administration, there was a significant ($P<0.05$) difference between the diabetic-induced groups and the normal control as the blood glucose level was increased in the diabetic-induced groups. However, the blood glucose levels of the non diabetic-induced groups were not altered compared with the normal control. Groups IV and V of the diabetic-induced groups were already showing significant ($P<0.05$), decrease in blood glucose levels after the second day of the plant extract administration compared with the diabetic control. This trend was also seen on day 5; the fourth day of the plant extracts administration. But at day 8; the seventh day of the plant extract administration, the diabetic-induced groups were still significantly ($P<0.05$) increased except group V which showed no significant ($P\geq0.05$) difference compared with the normal control. The blood glucose level of the diabetic-induced groups III, IV and V except group VI were significantly ($P<0.05$) decreased compared with the diabetic control.

At day 11; the tenth day of the plant extracts administration, all the diabetic-induced groups treated with the plant extracts (groups III, IV and V) showed no significant ($P\geq0.05$) difference compared with the normal control. Though group VI (treated with standard drug) still showed a significant ($P<0.05$) increase compared with normal control, it was significantly ($P<0.05$) decreased compared with the diabetic control.

At day 15; the fourteenth day of the plant extracts administration, there was further decrease in the blood glucose level of the groups treated with the plant extracts to the extent that group IV was significantly ($P<0.05$) decreased compared with the diabetic control. The blood glucose level of the standard drug treated group (VI) was further decreased however it remained significantly increased compared with the normal control and about three or more times that of the groups treated with the plant extracts (Table 2).

3.3 Effect of Leaf, Stem Bark and Root Extract Administration of *Jatropha curcas* on Anti-oxidative Parameters

The liver tissue SOD, CAT and MDA activities of male albino Wistar rats are given in (Table 3).

Result of the liver tissue SOD for the test group showed significant ($P<0.05$) difference except for groups IV and VII when compared with the normal control. There was no significant ($P\geq0.05$) difference in the SOD activities of test groups compared with the diabetic control.

The liver tissue CAT showed general increase for the test groups, but group III produced a significant ($P<0.05$) increase compared with the normal control, while groups III and V were significantly ($P<0.05$) increased compared with the diabetic control.

The liver tissue MDA showed significant ($P<0.05$) increase for groups II and IV but a significant ($P<0.05$) decrease for group V compared with the normal control. However, compared with group II (diabetic control) all the test groups showed a significant ($P<0.05$) decrease.
Table 2. The effect of administration of leaf, stem bark and root extracts of Jatropha curcas on blood glucose level of albino rats

| Group | Day 0  | Day 3  | Day 5  | Day 8  | Day 11 | Day 15 |
|-------|-------|-------|-------|-------|--------|--------|
| I (NC) | 72.0±6.35 | 78±5.04 | 75.3±5.34 | 75.75±5.367 | 75.75±5.98 | 75.3±5.57 |
| II (DC) | 69.3±6.19 | 365.5±21.77 | 378.5±25.98 | 381.5±18.50 | 381.5±11.39 | 387.5±6.08 |
| III (DTEL) | 58.75±3.40 | 345±27.33 | 312.3±33.68 | 178.75±13.27 | 93±4.14 | 68.5±3.69 |
| IV (DTSBE) | 62.25±4.17 | 312.3±12.93 | 280.3±7.98 | 159.5±15.66 | 82.5±6.49 | 52.5±5.25 |
| V (DTRE) | 68.8±0.00 | 272.5±9.60 | 219.75±10.55 | 110±6.12 | 88.75±4.66 | 72±4.43 |
| VI (DTSBE) | 57±3.26 | 364.75±13.44 | 338.3±15.14 | 235±14.79 | 191.25±8.73 | 85.5±7.42 |
| VII (NLEA) | 85±3±4.13 | 83.3±3.70 | 83.3±4.7 | 84±1±11 | 85.3±7.42 | 82.75±5.87 |
| VIII (NSBEA) | 59±1.83 | 86±3.33 | 87.5±2.77 | 86.25±3.47 | 84.5±4.66 | 82.75±5.87 |
| IX (NREA) | 67±5.97 | 78.5±9.00 | 77.75±6.64 | 77.75±3.97 | 77.25±2.84 | 77±2.85 |

Values are mean±SEM (n=6); *values are significant at P<0.05 compared with NC.
Values are significant at P<0.05 compared with DC

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

4. DISCUSSION

The ethanol-methanol extracts of leaf, stem bark and root of Jatropha curcas used in this work showed strong anti-hyperglycemic effect as the blood glucose level was normalized within two weeks of administration. The ability of these plant parts to reverse high blood glucose level to normal, appear to be about three times faster than the standard hypoglycemic drug (glibenclamide). Glibenclamide has long been used to treat diabetes, stimulate insulin secretion from the pancreatic β-cells. Polyphenolic compounds present in these plant parts have been associated with anti-diabetic, anti-hypertensives effects and favourable to cardiovascular health [19]. It can also be assumed that the leaf, stem bark and root extracts of Jatropha curcas may have caused regeneration of the β-cells to produce insulin as the glucose level of the diabetic treated rats dropped to normal, while that of the untreated remained high and unchanged. The observed insignificant difference between the NLEA, NSBEA and NREA and the normal control is another pointer to the fact that the leaf, stem bark and root extract used to treat diabetes possess anti-hyperglycemic or anti-diabetic properties.

There is a correlation between diabetes and oxidative tissue damage arising from free radical release [7]. Diabetes causes increased free radical release which results in the alterations of liver tissue superoxide dismutase (SOD) and catalase (CAT) [7]. The administration of plant extract with antioxidant potentials was meant to create a balance between free radical release and free radical elimination [7] in the liver tissue of albino wistar rats.
SOD is an enzyme that has been known to promote the rejuvenation and repair of cells, while reducing the damages caused by free radicals. Superoxide dismutase is found in our skin and it is essential in order for our body to generate adequate amounts of skin-building cells called fibroblasts [20]. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular.

Reduced activity of SOD and CAT in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals (•O2-) and hydrogen peroxide [23]. In this study, the administration of Jatropha curcas leaf, stem bark and root of ethanol-methanol extracts resulted in the reduced activities of liver tissue SOD, probably due to the compensatory role played by the rich phytochemicals such as flavonoids and polyphenols present in these plant parts. This is in agreement with Abd Hamid et al. [24] who reported decreased SOD activities when supplemented with vitamin E antioxidant. The CAT activities were rather increased contrary to the SOD activities. This increased activity was prominent for DTLE but not for NLEA as it was not different from the normal control. This therefore brings one to the assumption that the extracts responded differently to the enzyme systems of liver tissue SOD and CAT. It is also possible that this increased level in liver CAT activity in the diabetic-treated groups was in response to the diabetic-induction by the phytochemicals present in the extracts to prevent tissue damage and reverse the diabetic state to normal. Flavonoids, coumarins and other polyphenols present in the leaf, stem bark and root extracts of Jatropha curcas are well known for their strong antioxidant properties which play important roles in protecting cells and organs from oxidative damage [25].

CAT is a common enzyme found in nearly all living organisms exposed to oxygen. It is found in the peroxisome and is a very important enzyme in protection of the cell against oxidative damage by reactive oxygen species (ROS) [21]. It catalyzes the decomposition of hydrogen peroxide to H2O and O2 [22].

It has been observed that insulin secretion is closely associated with lipoxygenase derived peroxides [26]. Low levels of lipoxygenase peroxides stimulates the secretion of insulin, but when the concentration of endogenous peroxides increase it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet cell damage in type 1 diabetes [27].

MDA is a naturally occurring product of lipid peroxidation and prostaglandin biosynthesis that is mutagenic and carcinogenic. Reactive oxygen species (ROS) react with polyunsaturated lipids to form MDA which in turn reacts with DNA to form a M1G adduct that is the major cause of cancer [28]. MDA is found in tissue sections of joints from patients with osteoarthritis [29]. Wilson et al. [23] have also reported that the concentration of lipid peroxides increases in the liver of diabetic rats. There is also experimental evidence that intra-peritoneal administration of α-lipoic acid to STZ-induced diabetic wistar rats normalizes MDA levels in plasma, retina, liver and pancreas [30]. Increased concentration of MDA was observed in liver of diabetic control (DC) when compared to the normal and diabetic treated groups, but the DTLE and DTRE were able to produce a further decrease compared with the normal control, giving a clear indication of the stabilizing anti-oxidative activities of the leaf, stem bark and root extracts of Jatropha curcas.

5. CONCLUSION

The present research suggest that the ethanol-methanol (1:1) leaf, stem bark and root extracts of Jatropha curcas possess anti-hyperglycemic and antioxidant effects against diabetes, but their response to the enzyme systems of liver tissue SOD and CAT vary in either playing compensatory role or boosting the activities of the antioxidant enzymes. The plant parts also appear to possess the potential for reversing possible tissue oxidative damage caused by diabetes as seen by their abilities to prevent lipid peroxidation.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare 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 appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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