J Biochem Technol (2021) 12(3): 84-90
https://doi.org/10.51847/3ChBg8qAJJ
ISSN: 0974-2328

# *Jatropha curcas* Leaf Extract and Fractions Attenuate Hyperglycemia, Tissue Oxidation, and Kidney Dysfunction in Diabetic Rats

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Received: 23 June 2021 / Received in revised form: 21 September 2021, Accepted: 24 September 2021, Published online: 26 September 2021

## Abstract

This research studied the effect of extract and fractions of *Jatropha curcas* leaf, on serum glucose, kidney function and tissue oxidative stress indices of diabetic Wistar rats. The design comprised of 6 groups (n=6) treated: Groups I (NC) and II (DC), the normal and diabetic controls, respectively, received 3% tween-80 and groups III - VI (diabetic rats) received 500 mg/kg bodyweight of Metformin (MF) and 200 mg/kg bodyweight each of ethanol extract (ELE), residue (ERF) and n-hexane (NHF) fractions, respectively. Thereafter, blood, kidney, and liver samples were obtained and used for biochemical analyses. The 14-day oral administration of ELE, ERF, NHF, and metformin lowered serum glucose relative to the DC ($P$<0.05), with ERF and NHF exerting a stronger glucose-lowering action than ELE ($P$<0.05). The bodyweights, as well as absolute and relative organ weights, were not significantly affected. The observed diabetes-induced hyponatremia, hypochloraemia, and hyperkalemia were ameliorated upon the intervention of ELE, ERF, NHF, and MF, which lowered $K^+$ vs DC ($P$<0.05); ELE, NHF, and MF heightened $Cl^-$ vs DC ($P$<0.05). However, only MF improved the lowered $Na^+$ level. The diabetes-induced oxidative stress was also modulated: increased CAT, SOD, and GSH, with a concomitant decrease in MDA in both liver and kidneys relative to the DC ($P$<0.05). Besides, validation of the antidiabetic claim, *J. curcas* leaf modulates hyperglycemia-induced oxidation stress and assuage some worrisome features of kidney dysfunction in diabetes, including hyponatremia and hyperkalemia.

**Keywords:** Diabetes mellitus, Hyperkalemia, Hypochloraemia, Hyponatraemia, Oxidative stress, Serum glucose

## Introduction

Diabetes mellitus describes a group of metabolic disorders with chronic hyperglycemia as the common main feature (Alali *et al.*, 2019; Ahmed *et al.*, 2020). Chronic hyperglycemia, the main distinct and diagnostic feature of diabetes, induces a free radical formation and diminished antioxidant defenses via gluco-oxidation, thereby potentiating oxidative stress, which is concerned in the pathogenesis of diabetes complications, including kidney dysfunction (Kawanami *et al.,* 2016; Wu *et al.,* 2016; Gyuraszova *et al.,* 2020). In hepatocytes, heightened oxidation poses tremendous stress on the cells and their molecular components (Mendes-Braz & Martins, 2018; Newsholme *et al.,* 2019), resulting in the alterations of the activities of antioxidant molecular markers such as catalase (CAT), superoxide dismutase (SOD), and oxidative biomarkers including reduced glutathione (GSH) and malondialdehyde (MDA) (Matough *et al.,* 2012; Yaribeygi *et al.,* 2020).

Also, one of the major pathophysiological features of diabetes is polyuria, i.e. excess urination along with glucosuria (glucose in urine). This feature does impose a metabolic imbalance in body fluid and electrolyte regulations given the highly osmolar nature of glucose (Oguntibeju, 2019). Accordingly, kidney function is altered in diabetes mellitus and accounts for a great majority of the reported deaths due to complications of diabetes. Diabetic kidney disease (DKD) is the main cause of renal failure in the world and the strongest and single predictor of mortality in diabetic patients, occurring in 20%-40% of all diabetics (Reidy *et al.,*2014; Gheith *et al.,* 2016). Approximately 20% of diabetics with end-stage renal disease (ESRD) arising from DKD die annually of complications of the disease (Osman *et al.,* 2018). It is important therefore that any treatment or preventive measure against diabetes mellitus should provide protection against oxidative stress and kidney dysfunction.

Consequently, alternative herbal approaches from traditional systems are becoming increasingly popular in recent times (Choudhury *et al.,* 2018) because they tend to provide a complement of antioxidant bioactives, which directly neutralize the oxidants or indirectly attenuate the processes leading to the generation of oxidants, thereby modulating the risk of kidney dysfunction in diabetes. However, such traditional claims or practices require rigorous investigation to validate their veracity or otherwise and establish sound modes and biochemical mechanisms by which the therapeutic actions are achieved (Atangwho *et al.,* 2012).

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*Jatropha curcas*, commonly called *physic* nut is a flowering plant in the spurge family, Euphorbiaceae. It is indigenous to America's tropics, particularly Mexico and Central America, but now, extensively cultivated in both tropical and sub-tropical regions of the world because of its numerous medicinal and health benefits (Asuk, 2018). Preliminary studies on the use of *J. curcas* have demonstrated promising data attesting to the fact that the plant possesses anti-inflammatory, antidiabetic, and antioxidative properties (Johnson *et al.,* 2014; Asuk *et al.,* 2015; Papalia *et al.,* 2017; Salim *et al.,* 2018; Othman *et al.,* 2019). However, there are no detailed studies (beyond biological activity screening), using the systematic procedures of plant extract preparation with the aim of developing a natural product or herbal medicine in the long-term. Accordingly, this study combined a sequential fractionation of the plant extract with biological activity evaluation, as part of the fundamental complements of procedures leading to the development and standardisation of therapeutic natural products from plant sources.

## Materials and Methods

*Collection of Plant Material*

Fresh leaves of *Jatropha curcas* were collected from Okuku, in Yala Local Government Area of Cross River State, Nigeria. Mr Frank Apojeye at the Herbarium Unit, Department of Botany, University of Calabar, Calabar, identified and authenticated (ID No: 67) the plant.

*Preparation of Whole Leaf Extract*

The fresh leaves of *J. curcas* were air-dried at room temperature for a period of one month. After that, the dried leaves were pulverized and 100 g of which was extracted in 450 mL of absolute ethanol (BDH) at room temperature via maceration. The suspension was agitated and allowed to stand for 72 h, after which it was first filtered with a cheese cloth, and later with Whatman No.1 filter paper (24 cm). The filtrate was evaporated using dry air oven (ES-4620, Ecroskhim Ltd, Ecros group of companies, Saint-Petersburg, Russia) at 45 °C until there was no further loss of solvent, giving a constant weight. This semi-solid extract was labeled, ethanol leaf extract (ELE) of *J. curcas*.

*Liquid-Liquid Fractionation of the Whole Extract*

The fraction procedure was as reported previously (Uti *et al.,* 2020). Briefly, 10-g ELE in a separating funnel was solubilized with an aliquot of ethanol (the extraction solvent), n-hexane was added and agitated vigorously. This thoroughly agitated suspension was allowed to stand until two clear visible layers (fractions) separate based on the differential densities of the two solvents: the denser ethanol fraction (residue) beneath and the less dense n-hexane fraction above. The fraction was collected, and the whole cycle was repeated until all n-hexane-soluble components were collected and pooled into a separate beaker and labeled, leaving the residue. Accordingly, the whole extract was separated into n-hexane fraction and the ethanol extract residue. These two fractions were, oven-dried at 45 °C to dryness, yielding two fractions namely n-hexane fraction (NHF) and ethanol residue fraction (ERF), which were stored at 4 °C for the animal experiments. In the animal experiments, the whole extract (ELE) and the two fractions (NHF and ERF) were reconstituted in 3% Tween-80 before administration via oral gavage.

*Animals*

Thirty-six (36) female Wistar rats weighing 100-150 g were obtained from the animal house of the Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus and kept in well-ventilated laboratory cages and feed tap water and standard rat pellets *ad libitum*.

*Induction of Diabetes*

Thirty (30) of the rats were overnight fasted and induced with diabetes by intraperitoneal administration of 50 mg/kg BW of streptozotocin (STZ) reconstituted in cold physiological saline (Atangwho *et al.,* 2012). On the third day, approximately 72 h post-STZ injection, fasting blood sugar (FBS) level was determined with a One-touch ACCU-CHEK Advantage glucometer (Model – GB 13117699, Roche Diagnostics, Mannheim, Germany), using blood obtained from the tail vein puncture. Rats with FBS ≥ 200 mg/dL and ≤ 450 mg/dL were considered diabetic and chosen for the trial.

*Design of Experiment and Treatment Plan*

Thirty-six rats comprised 30 diabetic and 6 non-diabetic rats were divided into 6 groups of 6 rats each and treated, thus:
Group I: Normal control (NC); non-diabetic rats, given 3% Tween-80 (reconstitution solvent).
Group II: Diabetic control (DC); non-treated diabetic rats, given 3% Tween-80 (reconstitution solvent).
Group III: Standard control; diabetic rats treated with 500mg/kg BW of Metformin (MF) reconstituted with 3% Tween-80.
Group IV: Diabetic rats treated with 200mg/kg BW of ethanol leaf extract (ELE) of *J. curcas*, reconstituted with 3% Tween-80
Group V: Diabetic rats treated with 200mg/kg BW of ethanol residue fraction (ERF) of *Jatropha curcas*, reconstituted with 3% Tween-80.
Group VI: Diabetic rats treated with 200mg/kg BW of n-hexane fraction (NHF) of *Jatropha curcas,* reconstituted with 3% Tween-80.

The extract and fractions were administered via oral gavage once per day for fourteen (14) days. Subsequently, diethyl ether (5%) was used to anesthetize the animals after an overnight fast. Then, whole blood was collected via cardiac puncture into well-labeled plain tubes and allowed to stand for approximately 2h before the blood was centrifuged to separate serum from clotted cells. The serum was used for blood glucose and serum electrolyte assays. The liver and kidneys were surgically excised under aseptic conditions and used for relative organ weights and tissue antioxidant determination.

*Determination of Organ and Relative Organ Weights*

The organs excised, including liver and kidneys were weighed using an electronic weighing balance to obtain absolute wet weights. The wet weights were used to determine the relative organ weights, thus: w/W × 100%, where W = the weight of the rat before the termination of the experiment w = the wet weight of the organ.

*Determination of Serum Glucose and Electrolytes*

Serum glucose was evaluated using Agappe assay kit (Agappe Diagnostics, Switzerland GmbH) following the manufacturers' instructions. Serum electrolytes: sodium, potassium and chloride were evaluated via automated dry chemical techniques, of the Vitros DT 60 II Chemistry System (Serial No. 60035760, Ortho Clinical Diagnostics, New Jersey, USA), according to the manufacturer's instruction. Serum carbon dioxide (bicarbonate) was determined using assay kits obtained from TECO Diagnostics, according to the manufacturer's protocols provided in the insert.

*Preparation of Liver Supernatant from the Whole Homogenate*

The liver and kidney tissue samples were homogenized in 1:9 ratios of tissue (g) to phosphate buffer (PBS) with pH 7.4 manually with a mortar and pestle. The homogenate obtained was centrifuged at 3500rpm for 20min and the supernatant was separated and utilized for antioxidant assays, namely, the determination of SOD and CAT activities and MDA and GSH levels.

*Determination of Superoxide Dismutase (SOD) Activity*

Martin *et al*.'s (1987) method was utilized to measure the SOD activity of the supernatant gotten from the liver and kidney homogenates. Briefly, 920 µL of 0.05M phosphate buffer (pH 7.4) was added to 40 µL of assay buffer (Northwest Life Science Specialties, Product NWK-SOD02) or sample, mixed and incubated for 2 min. The assay buffer was shaken or the bottle was inverted, opened to air, and repeated 4 times before use to saturate with $O_2$. Then, to the reaction mixture, 40-µL hematoxylin was added to start the auto-oxidation reaction that yielded an increase in absorbance at 560 nm. The absorbance changes were immediately recorded at 560nm in 60-second intervals for at least 5 min.

*Determination of catalase (CAT) Activity*

The CAT activity was determined using the method of Sinha (1972). The assay mixture consisted of 1.0 mL $H_2O_2$ (0.2 M), 1.96 mL phosphate buffer (0.01M, pH 7.0), and 0.04mL supernatant in a final volume of 3mL. 2 mL of dichromate acetic acid was added to 1mL of the reaction mixture, boiled for 10min, and cooled. Absorbance changes were recorded at 570nm. The CAT activity was calculated, thus: (the change in absorbance × 100/1) divided by the amount of protein (mg) divided by time (min)= units/mg protein/min.

*Determination of Glutathione (GSH) Concentration*

Rukkumani *et al*.'s (2004) method was used to estimate the GSH level of the supernatant of liver and kidney gotten from whole homogenates. The GSH comprises most instances the bulk of cellular non-protein sulfhydryl groups. Therefore, this method is based on the development of a relatively stable yellow when 5, 5-dithiobis- (2-nitrobenzoic acid) (Ellman's reagent) reacts with sulfhydryl compounds. The chromophoric product caused by the reaction of Ellman's reagent with GSH, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412nm. The concentration of reduced GSH is related to the absorbance at 412nm. The equivalent GSH was estimated from the standard GSH curve.

*Determination of Malondialdehyde (MDA) Concentrations*

Thiobarbituric acid (TBA) reactive substances were determined in tissues based on Fraga *et al.'s* (1988) method in the GenWay Biotech assay kit for MDA. At pH 3.5 and 100 °C, MDA binds with TBA and produces a pink color that can be measured at 532nm. The phosphate buffer (0.01 M, pH 7.0) was prepared by dissolving 0.97 g of the base and 1.046 g of the acid in distilled water the adjusted to appropriate pH. Trichloroacetic acid (10%) was prepared by dissolving 10-g TCA in distilled water and TBA (0.1 M) was prepared by dissolving 2.88-g TBA in distilled water. 0.5 mL of tissue supernatant, 0.5 mL normal saline, 1.0 mL of 10% TCA and 0.3 mL of the buffer in a tube, was centrifuged at 3000rpm for 20 min. Then, 0.25 mL of 0.1M TBA was added to the content and mixed well. The mixture was then incubated for 1 h at 95 °C. After that, the tubes were allowed to cool and the absorbance of the supernatant was measured using a spectrophotometer at 532 nm. The interpolation of the MDA standard curve was used to estimate the concentrations of MDA. The amount of MDA (nmol) from the standard curve was calculated, thus:

$$C\ (nmol/mg) = [(A/(mg)] \times 4 \times D \quad (1)$$

Where: C stands for concentration of MDA in the sample, A - MDA amount from the standard curve, mg - Original amount of tissue used, 4-Correction factor and D - Dilution factor.

*Statistical Analysis*

Data obtained were analyzed using the SPSS v.23 with one-way ANOVA and $P < 0.05$ was considered statistically significant. Data are expressed as the mean ± SEM.

## Results and Discussion

The results of relative organ weights, serum glucose, and electrolytes, and oxidative stress parameters measured in kidney and tissue- liver supernatant of streptozotocin-induced diabetic rats treated with extract and fractions of *Jatropha curcas* are presented below.

*Serum Glucose and Relative Organ Weights*

The result of serum glucose measured in diabetic rats treated with extract and fractions of *Jatropha curcas* is depicted in **Figure 1**.

Streptozotocin-induced diabetes caused a significant increase in serum glucose compared to the normal control rats ($P$<0.05). However, 14-day oral administrations of ethanol leaf extract (ELE), ethanol residue fraction (ERF), and n-hexane fraction (NHF) were all found to cause significant decreases in serum glucose relative to the diabetic control ($P$<0.05). The fractions were found to exert stronger glucose-lowering actions than the whole extract ($P$<0.05): the residue fraction being as potent as metformin and the NHF effect compared well with the serum glucose of the NC.

Moreover, the bodyweight, as well as the absolute and relative weights of the organs most prone to complications in diabetes, namely, liver and kidneys were determined to assess the gross impact of the treatment, and the results are shown in **Table 1**. The 14-day diabetes status was found to lower body weight and increase the relative weight of kidneys in the DC compared to the NC ($P$<0.05). Probably because of the relatively short duration of treatment, our interventions did not significantly modulate these changes, including extracts, fractions, and metformin. Also, within the study duration, both experimental diabetes and treatments exerted null effects on the weights and relative weights of the liver ($P$>0.05).

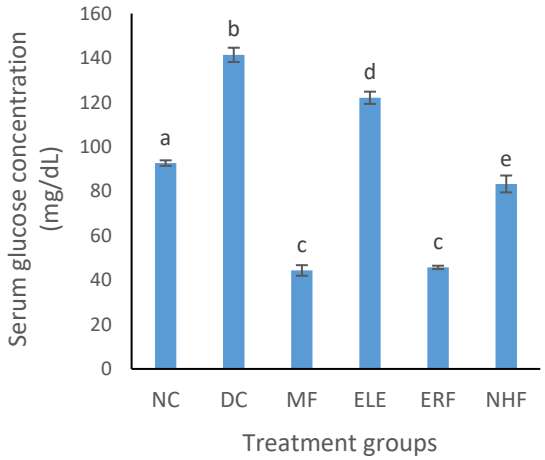


**Figure 1.** Serum glucose level of diabetic rats fed with leaf-extract and fractions of *Jatropha curcas*. Values are as the mean±SEM (n=6). Values with different superscripts (a, b, c and d) are statistically significant ($P$<0.05). MF=Metformin, DC=Diabetic control, NC=Normal control, ELE=Ethanol leaf extract, ERF=Ethanol residue fraction, NHF=n-Hexane fraction.

**Table 1.** Final bodyweights, and weights and relative weights of the kidneys and liver of diabetic rats administered leaf extract and fractions of *Jatropha curcas*

| Group | Weight prior to sacrifice (g) | Liver weight (g) | Kidney weight (g) | Relative Liver weight (%) | Relative kidney weight (%) |
|---|---|---|---|---|---|
| NC | 171.65±4.11$^{a}$ | 5.21±0.26$^{a}$ | 0.75±0.03$^{abc}$ | 3.05±0.20$^{a}$ | 0.44±0.03$^{a}$ |
| DC | 144.15±4.78$^{b}$ | 4.89±0.34$^{a}$ | 0.74±0.03$^{abc}$ | 3.38±0.16$^{a}$ | 0.51±0.02$^{ab}$ |
| MF | 134.77±5.04$^{bc}$ | 4.26±0.39$^{a}$ | 0.72±0.02$^{abc}$ | 3.20±0.33$^{a}$ | 0.54±0.03$^{b}$ |
| ELE | 134.75±3.17$^{bc}$ | 4.47±0.33$^{a}$ | 0.69±0.03$^{c}$ | 3.35±0.29$^{a}$ | 0.51±0.02$^{ab}$ |
| ERF | 140.02±4.41$^{b}$ | 5.21±0.40$^{a}$ | 0.79±0.03$^{b}$ | 3.70±0.20$^{a}$ | 0.57±0.03$^{b}$ |
| NHF | 122.12±4.63$^{d}$ | 4.56±0.48$^{a}$ | 0.67±0.03$^{c}$ | 3.70±0.26$^{a}$ | 0.55±0.01$^{b}$ |

Values are as the mean ± SEM (n = 6). Values with different superscripts (a, b, c, d) along the columns are statistically significant ($P$<0.05). Legend: MF=Metformin, DC=Diabetic control, NC=Normal control, ELE = Ethanol leaf extract, ERF=Ethanol residue fraction, NHF = n-Hexane fraction.

*Serum Electrolytes*

The results of the serum electrolyte profile of STZ-induced diabetic Wistar rats measured after a 14-day administration of extracts and fractions of *J. curcas* are shown in **Table 2**. The results showed that serum $Na^{+}$ and $Cl^{-}$ levels were lower, while $K^{+}$ level was higher in the DC than their corresponding levels in the NC ($P$<0.05), suggesting a dysfunction of the kidneys induced by experimental diabetes (diabetes-induced hyponatremia, hypochloraemia, and hyperkalemia). Upon 14-day intervention, both whole extract and fractions of *J. curcas* caused a positive modulation of diabetes-induced hyperkalemia along with metformin relative to the DC ($P$<0.05). Similar to the effect of metformin, the whole extract and n-hexane fraction ($P$<0.05) effectively modulated the diabetes-induced hypochloraemia. However, only metformin treatment improved the lowered sodium levels. Diabetes or its treatment did not alter serum bicarbonate levels.

**Table 2.** Serum electrolyte profile of diabetic rats administered leaf- extract and fractions of *Jatropha curcas*

| Group | $Na^{+}$ (mmol/l) | $K^{+}$ (mmol/l) | $Cl^{-}$ (mmol/l) | $CO_2$ (mmol/l) |
|---|---|---|---|---|
| NC | 198 ± 2.96$^{a}$ | 2.38 ± 0.06$^{a}$ | 193.33 ± 0.49$^{a}$ | 31.00 ± 0.45$^{a}$ |
| DC | 166.5 ± 2.13$^{b}$ | 4.52 ± 0.03$^{b}$ | 97.83 ± 1.40$^{b}$ | 30.00 ± 1.13$^{a}$ |
| MF | 218.67 ± 2.46$^{c}$ | 1.77 ± 0.07$^{c}$ | 109.00 ± 2.38$^{c}$ | 30.50±0.72$^{a}$ |
| ELE | 165.67 ±5.73$^{b}$ | 1.66 ± 0.04$^{c}$ | 107.17 ± 2.09$^{c}$ | 29.50 ± 1.65$^{a}$ |

| | | | | |
|---|---|---|---|---|
| ERF | 167.67 ± 3.00[b] | 3.85 ± 0.04[d] | 95.17 ± 2.48[b] | 30.00 ± 1.41[a] |
| NHF | 140.33 ± 2.14[d] | 3.23 ± 0.06[e] | 109.00 ± 1.10[c] | 31.17 ± 0.70[a] |

Values are as the mean±SEM (n=6). Values with different superscripts (a, b, c, d. e) along the columns are statistically significant ($P$<0.05). Legend: MF=Metformin, DC=Diabetic control, NC=Normal control, ELE=Ethanol leaf extract, ERF=Ethanol residue fraction, NHF=n-Hexane fraction

*Tissue Oxidative Stress Markers*

**Tables 3 and 4** show results of SOD and CAT activities, GSH and MDA levels of the liver and kidney supernatant from tissue homogenates, measured in test and control rats in the study. From the results, SOD and CAT activities and GSH levels were significantly lower in both liver and kidney tissue of the diabetic control (DC) rats than in normal control (NC) ($P$<0.05), indicating diabetes-induced suppression of free radical defence system of the animals. Also, the level of MDA radical was found to be higher in the tissues (liver and kidneys) of DC than that of NC ($P$<0.05). However, upon a 14-day administration of extract and fractions of *J. curcas*, there was observed a reduction of diabetes-induced oxidative stress thus: increased activities of CAT and SOD and GSH levels, and a concomitant decrease in MDA levels in both liver and kidney tissue of the three extract and fraction-treated groups relative to the diabetic control ($P$<0.05). Notably, the ameliorative effects of the extract and fractions on oxidative stress were comparable to that of metformin, implying that the plant share a similar antidiabetic mechanism with metformin.

**Table 3.** Oxidative stress markers measured in the liver supernatant of diabetic rats administered leaf extract and fractions of *Jatropha curcas*

| Group | SOD (mg/protein) | CAT (units/mg protein/min) | GSH (µg/mg/protein) | MDA (nmol) |
|---|---|---|---|---|
| NC | 113.68±1.54[a] | 20.01±0.62[a] | 129.09±1.71[a] | 3.78±0.28[a] |
| DC | 84.45 ±1.22[b] | 12.53±0.63[b] | 73.88±1.46[b] | 7.83±0.30[b] |
| MF | 107.90±2.42[c] | 16.64±0.38[cd] | 127.75±0.50[ac] | 4.52±0.31[ac] |
| ELE | 96.1±1.46[d] | 17.13±0.30[cd] | 129.40±1.29[a] | 4.83±0.23[cd] |
| ERF | 99.74 ±1.10[e] | 16.51 ± 0.28[c] | 125.90 ± 0.99[c] | 5.13 ± 0.20[cd] |
| NHF | 106.53±2.04[c] | 17.75±0.39[d] | 129.53±1.03[a] | 5.57±0.25[d] |

Values are as the mean±SEM (n=6). Values with different superscripts (a, b, c, d, e) along the columns are statistically significant ($P$<0.05). Legend: DC=Diabetic control, NC=Normal control, MF=Metformin, ELE=Ethanol leaf extract, ERF=Ethanol residue fraction, NHF=n-Hexane fraction

**Table 4.** Oxidative stress markers measured in the kidneys of diabetic rats administered leaf extract and fractions of *Jatropha curcas*

| Group | SOD (mg/protein) | CAT (units/mg protein/min) | GSH (µg/mg/protein) | MDA (nmol) |
|---|---|---|---|---|
| NC | 120.56 ± 0.87[a] | 15.67 ± 0.38[a] | 119.79 ±0.67[a] | 5.88 ± 0.29[ac] |
| DC | 97.23 ± 1.56[b] | 10.84 ± 0.58[b] | 58.34 ±0.42[b] | 10.87± 0.28[b] |
| MF | 118.90±0.78[ad] | 13.70 ± 0.60[c] | 99.54 ±1.29[c] | 5.13 ± 0.31[c] |
| ELE | 109.90 ± 0.78[c] | 12.32 ± 0.28[d] | 102.44 ± 2.38[c] | 5.83± 0.25[ac] |
| ERF | 112.46 ± 1.25[c] | 13.07 ± 0.34[cd] | 100.56 ± 1.10[c] | 7.28 ± 0.32[d] |
| NHF | 116.82 ± 1.35[d] | 14.40 ± 0.59[ac] | 98.96 ± 0.72[c] | 6.72 ± 0.39[ad] |

Values are as the mean±SEM (n=6). Values with different superscripts (a, b, c and d) along the columns are statistically significant ($P$<0.05). Legend: MF=Metformin, DC=Diabetic control, NC=Normal control, ELE=Ethanol leaf extract, ERF=Ethanol residue fraction, NHF=n-Hexane fraction

In this study, extract and fractions of *J. curcas* significantly lowered the blood glucose - antihyperglycemic action. The implication is that phytocompounds in the study plant arrested the free radical generation process caused by STZ, thereby initiating the process of repair of the β-cells of the pancreas. It is also due to the extract and fractions having rebutted the distorted insulin secretion mechanism induced by STZ, a subject for further study. Whichever way, it is germane that the traditional claim of the antidiabetic effect of the plant *J. curcas* is validated in this study.

Oxidative stress markers of the liver and kidney tissue were also studied. There were lowered activities/levels of liver and kidney SOD, CAT, and GSH, with a concurrent higher level of MDA radicals in diabetic control than that of the normal control, suggesting heightened oxidative stress and lipid peroxidation and a tendency for decreased tissue integrity in uncontrolled diabetes. Earlier studies have shown decreased CAT, SOD, and GSH with increased MDA levels in diabetic animals (Li *et al.*, 2015).

However, the administration of the leaf extract and fractions of *J. curcas* impacted a restorative effect by raising the activities of SOD and CAT, increasing GSH, and lowering MDA levels, suggesting a boost in the antioxidant status. Plants are endowed with a rich mix of phytochemicals and antioxidant bioactives with the capacity to exert a potent effect towards mobbing up free radicals or attenuate the generation of such oxidants. These are the case with the extract and fractions from *Jatropha curcas.* Incidentally, our preliminary study and some previous studies have indicated the antioxidant potential of phytocompounds in *Jatropha curcas* (Asuk *et al.,* 2015; Papalia *et*

*al.,* 2017), implying that oxidative stress attenuation is one mechanism by which *Jatropha curcas* exerts its antidiabetic effect.

Diabetic subjects frequently develop a constellation of electrolyte disorders because of the relationship between increased fasting blood glucose and alteration in serum electrolytes, particularly sodium, chloride, and potassium. This study showed a significant reduction in serum sodium and chloride levels with increased fasting blood glucose and increased level of serum potassium, similar to what was also observed in an earlier study by Khan *et al*. (2019).

The extract and fractions failed to ease the diabetes-induced hyponatremia, rather, in treatments such as with the n-hexane fraction, there was a further aggravation of the situation. This situation is difficult to explain, however, a similar occurrence has been observed with some conventional and non-conventional antidiabetic managements. For instance, in one of our earlier investigations on the antidiabetic properties of African bitter leaf, we found that ethanol leaf-extract further aggravated the diabetes-induced hyponatremia, yet a potent antidiabetic plant (Atangwho *et al.,* 2007); but was resolved when the extracts were combined with another antidiabetic plant, *Azadiracta indica* (Atangwho *et al.,* 2009). It is also reported that some conventional hypoglycaemic agents or drugs such as insulin, diuretics, amitriptyline, among others induce hyponatremia (Liamis *et al.,* 2014). Therefore, further studies are suggested for *Jatropha curcas* in relation to plasma sodium regulation.

Also consistent with previous reports, there was observed diabetes-induced hyperkalemia, which was effectively reduced by the extract and fractions of *J. curcas* and restored to physiological levels within the treatment period, demonstrating a partial ability to modulate kidney dysfunction in diabetes. Moreover, diabetes causes a reduced concentration of serum chloride or hypochloraemia (Khan *et al*., 2019) which was also observed in the current study. In tandem with restorative action on serum $K^+$, the altered serum $Cl^-$ levels were also normalized suggesting yet again, an enhanced capacity to modulate kidney malfunction in diabetes. It is, however, a subject for further study, to investigate how $Cl^-$ regulations were achieved independent of sodium concentration.

Serum bicarbonate, a useful biochemical tool for the test of renal function was also studied. However, there was no significant effect on serum bicarbonate. The alteration of bicarbonate levels takes a longer time as the body's mechanisms to ensure that minimal changes in pH are maintained, which may be a suitable reason that there was no change in $CO_2$ or $HCO_3^-$ given that the study lasted for 14 days only. The non-alteration in liver and kidney weights and their relative organ weights supports the unchanged $CO_2$ or $HCO_3^-$ levels, although there was a slight increase in kidney weight and its relative organ weight, justifying the altered $Na^+$, $K^+$, and $Cl^-$ levels.

## Conclusion

Besides validation of the antidiabetic claim, *J. curcas* modulates hyperglycemia-induced oxidative stress and counter some worrisome features of kidney dysfunction in diabetes, including hyponatremia and hyperkalemia.

**Acknowledgments:** The authors of this study wish to appreciate the technical assistance of Dr. Obem O. Okwari of the Department of Human Physiology, Cross River University of Technology, Okuku Campus, Nigeria.

**Conflict of interest:** None

**Financial support:** None

**Ethics statement:** Ethical approval for the study was obtained from the Faculty of Basic Medical Sciences Animal Research Committee of the Cross River University of Technology, Calabar, Nigeria (approval number CRUTECH/FBMS/IREC/2020 – A1101).

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