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Ethanol fraction of *Jatropha curcas* reverses renal oxidative stress in diabetic rats

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ABSTRACT

The reversal of renal oxidative stress in the streptozotocin-induced diabetic rat following the administration of ethanol residue fraction of *Jatropha curcas* leaf was investigated. Thirty-two female Wistar rats (100-150g) were divided into four groups (n=8). The groups were treated as follows; Group I: Normal control (NC); non-diabetic rats, given normal saline in 3% Tween-80 (reconstitution solvent). Group II: Diabetic control (DC); non-treated diabetic rats, given normal saline in 3% Tween-80 (reconstitution solvent). Group III: Standard control; diabetic rats treated with 500mg/kg body weight of Metformin (MF) reconstituted with 3% Tween-80. Group IV: diabetic rats treated with 200mg/kg body weight of ethanol fraction (ERF) of *Jatropha curcas*, reconstituted with 3% Tween-80. During the experiment, fasting blood sugar (FBS) was determined at intervals using a one-touch ACCU-CHEK Advantage glucometer. Subsequently, at the end of the 21 days of the administration, diethyl ether (5%) was used to anaesthetize the animals having overnight fasted. The kidneys were surgically excised under aseptic conditions and used for renal oxidative stress determination of SOD, GPx, GSH, GSSG and GSSG/GSH levels. The level of Renal SOD, GPx and GSH showed a significant ($p<0.05$) decrease in the DC compared to the normal control. However, the decrease in SOD was significantly ($p<0.05$) increased when treated with MF and ERF but there was no significant increase in the GPx and GSH levels. The level of renal GSSG and GSSG/GSH showed a significant ($p<0.05$) increase in the diabetic control compared with normal control and the diabetic treated groups. The study demonstrated the use of ethanol residue fraction of *Jatropha curcas* as a potential agent in correcting oxidative imbalance and reversing renal oxidative stress caused by diabetes.
Keywords: kidney, oxidative stress, streptozotocin, diabetic rats, *Jatropha curcas*

INTRODUCTION

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. It has evolved as a pandemic that currently affects 463 million people worldwide and is projected that by 2045 about 700 million will be suffering from diabetes if not addressed [1]People suffering from diabetes cannot produce or properly use insulin, so they have high blood glucose or hyperglycaemia [1]. The causes of diabetes may be associated with both genetic and environmental factors [2] Diabetes triggers oxidative stress in the kidney, which is characterized by increased concentration of reactive oxygen species (ROS) and a significant reduction in its antioxidant defences [3]. Oxidative stress results from the link with the majority of molecular events that underline the pathological process in diabetic nephropathy (DN). It is related to alterations in the redox state caused by the persistent hyperglycaemic state and the increase in (Advanced glycation end products) AGEs [4]. These events affect the renin-angiotensin system and the signalling of the transforming growth factor-beta (TGF-β), producing chronic inflammation and glomerular and tubular hypertrophy. When the redox equilibrium is slightly altered, be it through a prolonged increase in the production of ROS or through inefficient antioxidant mechanisms, it can give way to pathological processes. The slight increase in ROS above the physiological limit can induce significant conformational changes in the lipids, proteins, carbohydrates, and nucleic acids, which leads to distorted interactions of the cellular functions [5]. Oxidative stress in DN can act as a

trigger, modulator, and link within the complex web of pathological events that occur in DN [6]

Multiple intracellular mechanisms are involved in ROS production in the kidney, including xanthine oxidase, cytochrome P450 systems, uncoupled NO synthase (NOS), mitochondrial respiratory chain, and NOXs [7]. However, several antioxidative systems help modulate these intracellular oxidations. For example, GSH effectively scavenges free radicals and other ROS and RNS (e.g., hydroxyl radical, lipid peroxyl radicals, superoxide anion, and hydrogen peroxide) directly and indirectly through enzymatic reactions [8, 9, 10, 11]. The chemical structure of GSH determines its functions, and its broad distribution among all living organisms reflects its important biological role [11]. In particular, it has long been established that the thiol moiety of GSH is important in its antioxidant function in the direct scavenge of radical species. Indeed, the one-electron reduction with radicals is not chemically favourable, because it would generate the unstable thiol radical GS. However, the reaction is kinetically driven in the forward direction by the removal of GS through the following reactions with thiolate anion (GS) and then with oxygen. The first reaction leads to the generation of GSSG, which in the presence of O_2, generates GSSG, and superoxide. Ultimately, the radical chain reactions will be blocked by the antioxidant enzymes superoxide dismutase (SOD) in association with catalase or glutathione peroxidase (GPx) that determines the completely free radicals scavenging [12]. Plants are a rich source of many natural products most of which have been extensively used for human welfare, and treatment of various illnesses both hormonal and metabolic disorders. *Jatropha curcas* (Linnaeus) is a multipurpose bush/small tree belonging to the family of Euphorbiaceae. It is a plant with many attributes. It is a native of tropical America, but now it thrives in many parts of the tropics and sub-tropics in Africa and Asia [13]. Various parts of the plant are of medicinal value, its bark contains tannin and other parts have phytochemicals. Also, like all trees, it removes carbon from the atmosphere, stores it in the woody tissues and assists in the build-up of soil carbon. 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 an effect on long-term health when consumed by humans, and can be used to effectively manage human diseases, such as malaria, diarrhoea, typhoid, rheumatism, and even diabetes [14]. Plant-derived compounds have great potentials to cure and control diabetes, apart from being safer and cost-effective [15]. The increased production of ROS during diabetes mellitus is injurious to the kidney. There is a correlation between diabetes and tissue damage arising from free radical release, hence the determination of the reversal of renal oxidative stress in the streptozotocin-induced diabetic rat following administration of the ethanol fraction of *Jatropha curcas* leaf will give a strong indication of the use of these plant parts in the trado-medical treatment of diabetes.

Aim of the Study

This research aimed to assess the effect of ethanol fraction of *Jatropha curcas* on non-enzymatic antioxidants (glutathione) and enzymatic antioxidants (glutathione peroxidase and superoxide dismutase) of streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Collection of Plant Materials

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 the plant with ID No:67.

Preparation of whole leaf extract

The fresh leaves of *J. curcas* were air-dried at room temperature for one month. After that, the dried leaves were pulverized and 2000 g of which was extracted in 8000 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 cheesecloth, and later with Whatman No.1 filter paper (24 cm). The filtrate was evaporated using a dry air oven (ES-4620, Ecroskhim Ltd, Ecros group of companies, Saint-Petersburg, Russia) at 40 °C until there was no further loss of solvent, giving a constant weight. This semi-solid extract was labelled, ethanol leaf extract (ELE) of *J. curcas*.

Liquid-Liquid fractionation of the whole extract

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 labelled, 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 40°C to dryness, yielding two fractions namely n-hexane fraction (NHF) and ethanol residue fraction (ERF), which were stored in the refrigerator at 4°C until used 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-two (32) female Wistar rats weighing100-150g were obtained from the animal house of the Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus. The animals were kept in well-ventilated laboratory cages and feed standard rat pellets, tap water and standard rat pellets ad libitum.

Induction of Diabetes and Measurement of Blood Glucose Levels

Twenty-four (24) of the rats were overnight fasted and induced with diabetes by single intraperitoneal administration of 50 mg/kg body weight (BW) of streptozotocin (STZ) reconstituted in cold physiological saline. On the third day, approximately 72 h post-STZ injection, fasting blood glucose 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 blood glucose ≥ 200 mg/dL and ≤ 450 mg/dL were considered diabetic and used for the experiment trials.

Experimental design of experiment and treatment plan

Thirty-two rats comprised of 24 diabetic rats and 8 non-diabetic rats were divided into four groups of 8 rats each and treated, thus:

Group I: Normal control (NC); non-diabetic rats, given normal saline reconstituted in 3% Tween-80.

Group II: Diabetic control (DC); non-treated diabetic rats, given normal saline reconstituted in 3% Tween-80.

Group III: Standard control; diabetic rats treated with 500mg/kg BW bodyweight of Metformin (MF) reconstituted with 3% Tween-80.

Group V: Diabetic rats treated with 200mg/kg body weight of ethanol fraction (ERF) of *Jatropha curcas*, reconstituted with 3% Tween-80.

The extract and fractions were administered via oral gavage once per day for fourteen (21) days. Subsequently, diethyl ether (5%) was used to anaesthetize the animals after an overnight fast. The kidneys were surgically excised under aseptic conditions and used for relative organ weights and tissue antioxidant determination.

Homogenisation of kidney

Fresh kidneys (0.2 g) were homogenized in 1.8 mL of the homogenizing medium, using a Teflon homogenizer, to make a 10% homogenate. Unless otherwise stated, the homogenizing medium was 0.1 M phosphate buffer (pH 7.4). After homogenizing, the resulting mixture was centrifuged at 4000 rpm for 10 minutes. The supernatants, collected into appropriately labelled tubes, were used for biochemical assays.

Determination of antioxidant parameters

Reduced glutathione (GSH), glutathione peroxidase (GPx) activity and superoxide dismutase (SOD) activity were determined using standard methods of Misra and Fridovich (1972) [8] for (SOD) and Sato *et al.* (1978) [9] for (GSH) and (GPx).

Preparation of kidney samples for histological examination

The kidney of the rats was quickly removed, washed in normal saline and fixed in 10% formol calcium. The organs were processed as described by Mbaka *et al* (2010) [10]. Sections of 4µm of the organs were cut using a microtome stained with haematoxylin and eosin. This was visualized using the light microscope at x 100 and x 400 magnifications and the photomicrographs were captured.

Statistical Analysis

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

RESULTS

Result of Oxidative stress indicators of diabetic-induced female Wistar rats administered ethanol fraction of *Jatropha curcas* leaf

The result of the SOD, GPX, GSH, GSSG and GSH/GSSG of diabetic-induced Wistar rats administered ethanol fraction of *Jatropha curcas* is presented in Table 1. The result revealed a significant (P<0.05) decrease in SOD, GPX and GSH levels of the diabetic control (DC) and a significant (P<0.05) increase in GSSG and GSSG/GSH ratio compared with the normal control (NC). The result also showed a significant (P<0.05) decrease in GSSG and GSSG/GSH ratio and a significant increase in SOD of groups treated with metformin (MF) and ethanol residue fraction (ERF) when compared with the diabetic control (DC). There was no significant (P>0.05) increase in GPx and GSH levels of groups treated with metformin (MF) and ethanol residue fraction compared with the diabetic control (DC). There are indications of restoration of GPx activity and GSH levels caused by diabetes on the administration of the ethanol residue fraction of *Jatropha curcas* leaf.

Histopathological result of the kidney of diabetic Induced Wistar Rats following the administration of Ethanol residue fraction of *Jatropha Curcas* leaf.

Plates 1-4 are photomicrographs showing the structure and morphology of the kidneys of normal and diabetic induced Wistar rats. The section in Plate 1 is from a non-diabetic control kidney showing normal morphological structures of the renal tubules, glomeruli and basement membrane. Plate 2 is a section from a diabetic control kidney characterized by moderate tubular atrophy and peripheral displacement of nuclei. Plate 3 reveals sections from diabetic kidneys treated with metformin (MF), which showed no observable pathology, improvement and restoration of renal cellular components compared to the diabetic control. Plate 4 reveals sections from diabetic kidneys treated with Ethanol residue fraction of *Jatropha curcas* leaf showing no observable lesion and improvement and restoration of renal cellular components compared to the diabetic control.

Table 1: Effect of ethanol fraction of *Jatropha curcas* on kidney SOD, GSH, GPx, GSSG and GSSG/GSH in streptozotocin-induced diabetic Wistar rat.

Group	SOD (U/L)	GPx (U/L)	GSH (µmol/g)	GSSG (µmol/g)	GSSG/GSH
I (NC)	36.96±2.61[a]	1.97± 0.04[a]	19.66±0.44[a]	13.63±0.77[a]	0.69±0.03[a]
II (DC)	16.71±1.90[b]	1.64±0.02[b]	16.39±0.19[b]	16.04±0.92[b]	0.98±0.07[b]
III (MF)	37.58±1.90[a]	1.71±0.05[b]	17.32±0.42[b]	11.02± 0.64[c]	0.64±0.04[a]
IV (ERF)	38.67±1.62[a]	1.70±0.02[b]	17.00±0.17[b]	11.30±0.81[c]	0.66±0.04[a]

Values are the mean ± SEM (n=4); values with different superscripts (a, b, c) along the columns are statistically significant ($p \leq 0.05$)

Legend: NC = Normal control; DC = Diabetic control; MF = Metformin; ERF = Ethanol Residue Fraction.

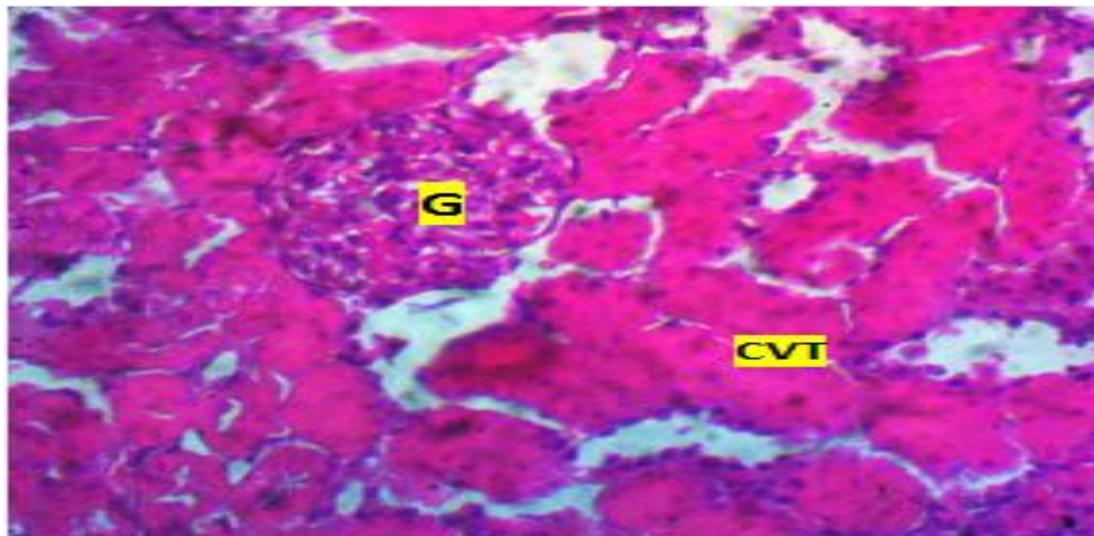

Plate 1: Photomicrograph of the kidney showing a glomerulus (G) with several convoluted tubules (CVT). Parenchyma shows normal cellular arrangements. No pathology was seen.

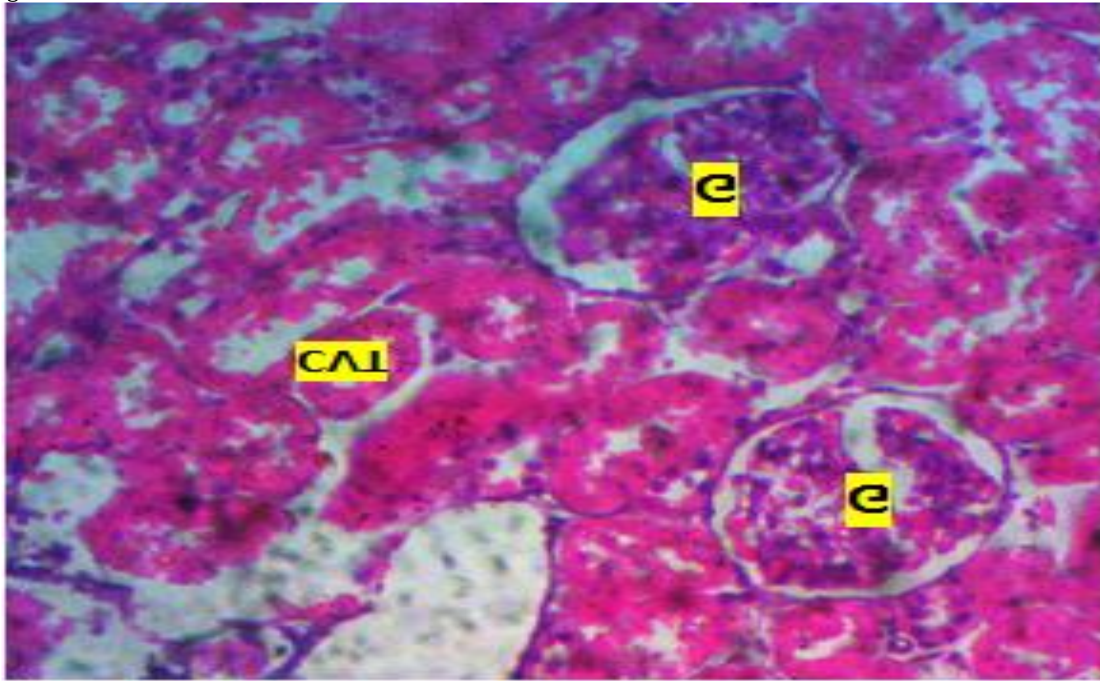

Plate 2: Photomicrograph of the kidney showing two glomeruli (G) interspersed with several convoluted tubules (CVT). Parenchyma shows moderate tubular atrophy and peripheral displacement of nuclei.

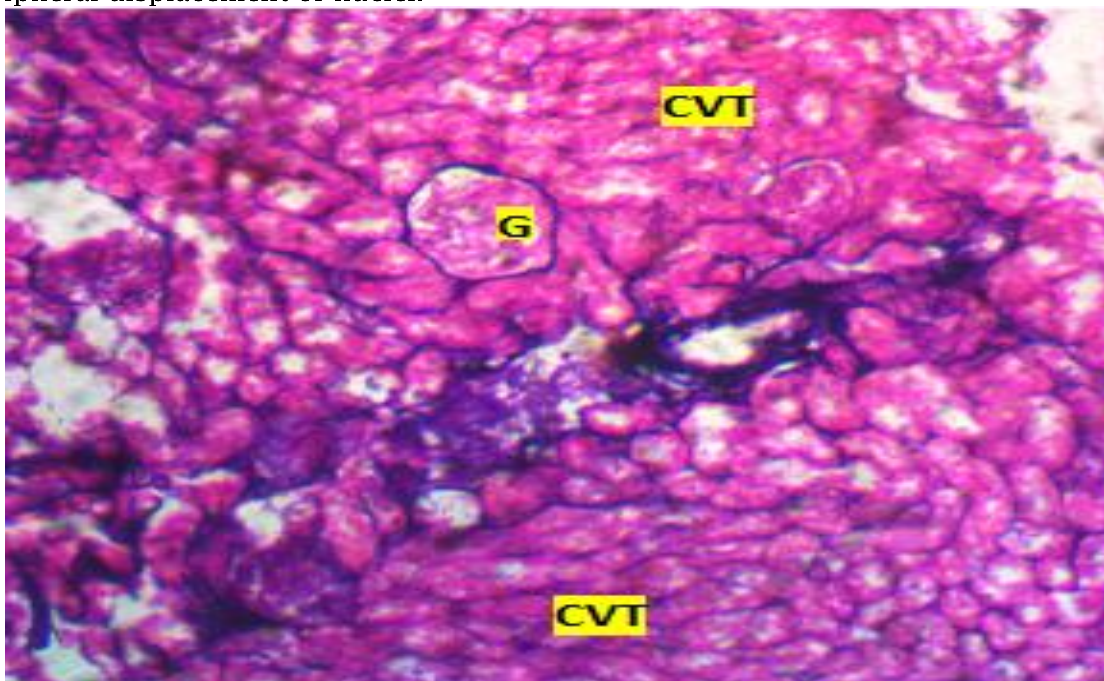

Plate 3: Photomicrograph of the kidney showing a glomerulus with several convoluted tubules (CVT). General tissue microstructure shows no observable pathology.

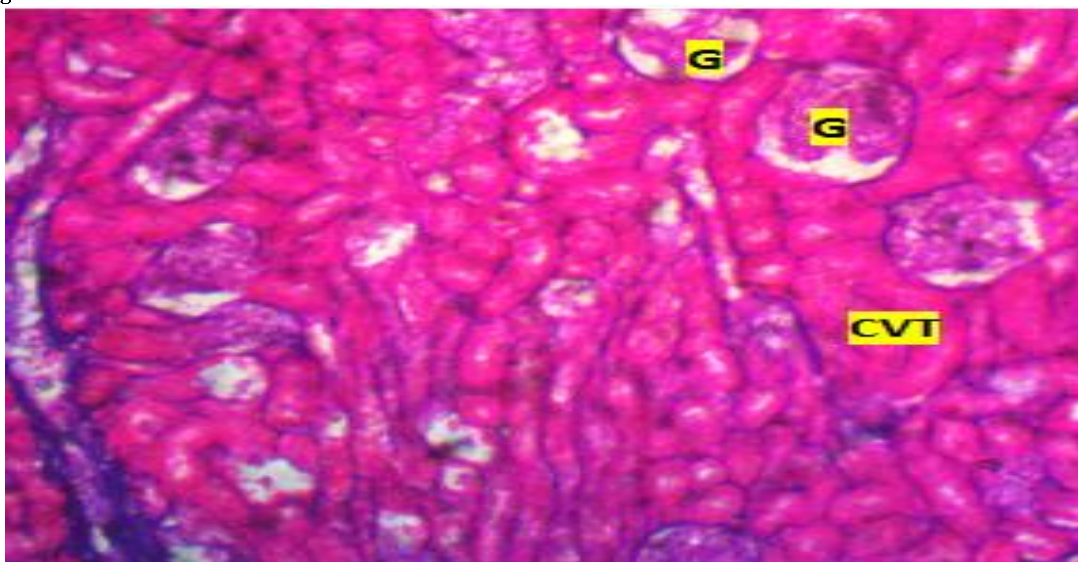

Plate 4: Photomicrograph of the kidney showing glomeruli (G) and several adjoining convoluted tubules (CVT). The general microstructure shows no observable lesion.

DISCUSSION AND CONCLUSION

Diabetes is a major source of morbidity, mortality, and economic cost to society [4]. The prevalence of diabetes is rising worldwide due to population growth, ageing, urbanization, and the increase of obesity due to physical inactivity [16]. Oxidative stress plays a pivotal role in the progression and development of diabetes and its complications [5]. Defence against the reactive oxidants produced during aerobic metabolism is a complex process and is provided by a system of enzymes and antioxidant compounds capable of preventing excess radical production, neutralizing free radicals and repairing the damage caused by them [6]. Long-term complications of diabetes mellitus are supposed to be partially mediated by oxidative stress. Damage caused by free radicals is possibly involved in beta-cell destruction and the pathogenesis of diabetes mellitus [17]. Antioxidant enzymes primarily account for intracellular defence, while several non-enzyme molecules, small molecular weight antioxidants, protect various components against oxidation.

In this study, the effect of *Jatropha curcas* ethanol leaf extract on the kidney SOD, GPx, GSH and GSSG/GSH ratio of streptozotocin-induced diabetic rats were investigated. Enzymatic and non-enzymatic molecules emerged as a way to counter the harm that ROS may cause [18]. SOD is a ubiquitous antioxidant enzyme that converts superoxide radicals to hydrogen peroxide [19], while GPx converts the hydrogen peroxide into water using glutathione (GSH) as a reducing agent [18]. In direct ROS-neutralising reactions, GSH is oxidised and converted from its free thiol form to glutathione disulphide (GSSG) leading to an increase in GSSG/GSH ratio [20]. The GSSG/GSH ratio is one simple way to measure the oxidative/antioxidant balance, where an increase depicts increased oxidative stress [21]. The observed decrease in SOD, GPx and GSH of the diabetic untreated rats which were significant is an indication that hyperglycaemia-induced oxidative stress was pronounced. The significant rise and normalisation of kidney tissue SOD activity as well as improved GPx activity and GSH level, compared with the diabetic control, is a demonstration that administration of ethanol residue fraction reduced oxidative stress and hence maintained the tissue integrity of the kidney. Furthermore, GSSG/GSH ratio which was significantly increased in the diabetic rats strongly supports the fact that oxidative to antioxidant balance was highly altered, causing a significant increase in oxidative stress. This imbalance was corrected by the administration of ethanol residue fraction of *Jatropha curcas*.

CONCLUSION

The study demonstrated the use of ethanol residue fraction of *Jatropha curcas* as a potential agent in correcting oxidative imbalance and reversing renal oxidative stress caused by diabetes, hence maintaining the integrity of the kidney against oxidative assaults.

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