

Ethanol Leaf Extract of *Moringa oleifera* Mitigates Glycerol-Induced Acute Kidney Damage in Rats through down Regulation of KIM-1 and NF- κ B Signaling Pathways

Adeolu Adedapo^{1*}, Oluwatosin Asunloye¹, Iyanuoluwa Ogunmiluyi¹, Olufunke Falayi¹, Ademola Oyagbemi², Temidayo Omobowale³, Bisi Adeoye¹, Oluwafemi Oguntibeju⁴ and Momoh Yakubu⁵

¹Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Nigeria

²Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria

³Department of Veterinary Medicine, University of Ibadan, Nigeria

⁴Department of Biomedical and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa

⁵Department of Environmental and Interdisciplinary Sciences, College of Science, Engineering and Technology, Texas Southern University, Houston, TX, USA

Abstract

Background: Acute kidney injury is a serious medical condition that usually results in sudden loss of renal function and high mortality rate in the world. The efficacy and the mechanisms of ethanol leaf extract of *Moringa oleifera* in promoting recovery from glycerol induced acute kidney injury (AKI) in rats was investigated in this study.

Methods: Thirty-five adult male Wistar rats were randomly sorted into five groups of seven animals each. Control received distilled water alone, Untreated group received single glycerol (4 ml/kg) intramuscularly, two groups were pretreated orally with 50 and 100 mg/kg of *Moringa oleifera* respectively for seven days and then injected with a single glycerol (4ml/kg) subcutaneously on day eight. Another group received oral administration of *Moringa oleifera* alone at 100 mg/kg for seven days. The animals were later sacrificed through cervical dislocation twenty-four hours after glycerol was administered.

Results: Serum and renal homogenate analysis revealed that glycerol induced significant increases in creatinine kinase renal band, nitric oxide, malondialdehyde, protein carbonyl, myeloperoxidase, advanced oxidative protein products alongside a decrease in superoxide dismutase, reduced glutathione, glutathione peroxidase and an increase in the expression of Kidney Injury Molecule-1 (KIM-1) and Nuclear Factor Kappa Beta (NF- κ B) using immunohistochemistry. Treatment with *Moringa oleifera* at both doses used brought on a reversal of these.

Conclusion: These results support the hypothesis that oral administration of ethanol leaf extract of *Moringa oleifera* is effective in abrogating oxidative stress, attenuating inflammation, and enhancing nephrogenic repair after glycerol-induced AKI.

Keywords: Oxidative stress • Inflammation • Glycerol • Acute kidney injury • *Moringa oleifera*

Introduction

Acute kidney injury (AKI) is a serious medical condition that usually results in sudden loss of renal function and high mortality rate in the world and increased risk for developing chronic renal disease in the survivors. It can result from various causes like ischemia, drug-induced injury, inflammation, or renal obstruction [1-4].

A single intramuscular injection of glycerol in rats causes AKI through induction of rhabdomyolysis [5]. Rhabdomyolysis-induced AKI develops after skeletal muscle trauma related to physical, thermal, ischemic, infective, metabolic, or toxic causes, releasing

lethal doses of myoglobin and other intracellular proteins into the circulation [6-8]. Although the pathogenesis of glycerol-induced AKI is both complex and not well understood [6], apoptosis [9-11], inflammation [12], and oxidative stress [10,13,14] have been implicated. Animal models of glycerol-induced AKI are being used to understand the clinical syndrome and study the mechanisms of AKI in general [15]. In this model, the myoglobin heme induces oxidative stress of the proximal tubular cell and triggers the release of series of mediators as cytokines and chemokines. Subsequently, leukocyte activation and tubular necrosis in the cortical area occurred [12].

*Corresponding author: Adedapo A, Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Nigeria, Tel: +2348162746222; E-mail: adedapo2a@gmail.com

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Although, apoptosis was recognized to be the main form of cell death responsible for renal dysfunction in glycerol induced AKI, studies suggested that other forms of cell death, including, regulated necrosis may also contribute to the pathogenesis of AKI [16]. Necroptosis is a kind of regulated necrosis with similar morphological features of necrosis but molecularly controlled. It depends on receptor-interacting protein kinase 3 (RIPK3) and mixed lineage kinase domain-like pseudokinase (MLKL) [17]. Necroptosis had been suggested to be one of the important mechanisms involved in glycerol induced AKI [18]. In addition, blocking the necroptotic pathways was documented to mitigate renal injuries *in vitro* and *in vivo*, giving a promising outlook in the management of AKI [19].

Moringa oleifera commonly known as horse radish tree is a popular plant in tropical and sub-tropical regions of the world with every part appropriate for both commercial and nutritional purposes [20]. The plant is rich in minerals and phytochemicals such as tannins, saponins and alkaloids, it also contain linoleic, oleic and palmitic acid [21]. *Moringaoleifera* has been regarded as a cure-all, reported to possess various therapeutic properties including antidiabetic, anticancer, neuroprotective and anti-infective properties [20].

Materials and Methods

Extract preparation

The shade dried leaves of *Moringa oleifera* was extracted according to the method of Panovska et al. [22]. The leaves were air-dried for six weeks and grinded into coarse powder using an electric blender. Extraction was carried out by cold maceration of the coarse powder with 100 % v/v ethanol for 72 h, with constant shaken. Each batch of harvested solvent were stored in glass containers and refrigerated at 4°C. The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate was concentrated to dryness *in vacuo* at 40°C using rotatory evaporator to obtain semi-solid crude extract. The ethanol remaining in the extract was finally removed by placing small volumes in porcelain dishes in the oven set at low temperature of 4°C. The extract obtained for the leaves came as semi-solid greenish-black paste. Aliquot portions of the extract were weighed and dissolved in distilled water for use in this study.

Animals

35 male rats were randomly divided into five (5) groups with seven (7) animals in each group. Animals in the control (Group A) received distilled water daily throughout the study. Group B animals were administered 50% Glycerol at 4 ml/kg at day 8 via deep intramuscular route. Group C and D animals were pretreated with 50 mg/kg and 100mg/kg respectively of ethanol extract via oral route for seven days and thereafter were induced with 50% Glycerol (4 ml/kg) on day 8 while Group E received only 100 mg/kg without glycerol for 7 days but distilled water on day. Distilled water was used as a vehicle for dissolving the extract. Ethical approval for the use of animals in this study was obtained from the University of Ibadan use of animal in research committee and the approval number is UI-ACUREC/17/0064.

Induction of acute renal failure in rats

Acute renal failure was induced using the standard method involving administration of 50% glycerol (v/v, 4 ml/kg, intramuscularly). The 50% glycerol was obtained by dissolving glycerol in double distilled water. After 7 days of study period in which the different groups received their specific treatment orally, the required amount of glycerol was administered as a deep intramuscular injection equally distributed to both hind legs. Rats were deprived of food and water for 24 h after glycerol administration [23].

Blood collection

On day 10, about 5 ml of blood was collected from the retro-orbital venous plexus using haematocrit tubes into blood sample bottles for serum chemistry tests. The bloods in sterile plain tubes were left for about 30 min to clot. The clotted blood was thereafter centrifuged at 4000 rpm for 10 min. Serum was decanted into another tubes and kept till when needed.

Preparation of tissue homogenate

The kidney of each rat was carefully removed, perfused immediately with normal saline and blotted with filter paper. It was homogenized in cold potassium phosphate buffer (0.1 M, pH 7.4) using a Teflon homogenizer. The renal homogenate was centrifuged at 10000 rpm for 10 min with a cold centrifuge at 4°C to obtain post mitochondrial fraction (PMF). The supernatant was used as a sample for the biochemical assay.

Biochemical Analysis

Determination of serum markers of renal damage and inflammation

The serum nitric oxide concentrations were measured spectrophotometrically at 548 nm according to the method of Olaleye et al. [24]. The serum myeloperoxidase (MPO) activity was determined according to the method of Xia and Zweier [25]. Creatinine kinase and Urea renal band were carried out using Randox kits according to the manufacturer's instruction.

Renal antioxidants markers

The Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich [26], with slight modification [27]. The increase in absorbance at 480 nm was monitored every 30s for 150 s. The one unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome. Reduced glutathione (GSH) was estimated by the method of Jollow et al. [28]. Glutathione peroxidase (GPx) activity was also measured according to Beutler et al. [29]. Glutathione-S-transferase (GST) was estimated by the method of Habig et al. [30] using 1-chloro-2, 4-dinitrobenzene as substrate. The protein and non-protein thiol contents were determined as described by Ellman [31]. Protein concentration was determined by the Biuret method of Gornal et al. [32], using bovine serum albumin (BSA) as standard.

Renal makers of oxidative stress

Hydrogen peroxide generation was determined according to the method of Wolff [33]. The reaction mixture was subsequently incubated at room temperature for 30 minutes. The mixtures were read at absorbance at 560 nm and H₂O₂ generated was extrapolated from H₂O₂ standard curve. The Malondialdehyde (MDA) content as an index of lipid peroxidation was quantified in the PMFs of renal tissue according to the method Varshney and Kale [34]. The absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation was calculated with a molar extinction coefficient of $1.56 \times 10^5 \text{ M/cm}$. Protein carbonyl (PC) contents in the renal tissues were measured using the method of Reznick and Packer [35]. The absorbance of the sample was measured at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH ($2.2 \times 10^4 \text{ cm}^1 \text{ M}^{-1}$) and expressed as nmoles/mg protein. The advanced oxidation protein product (AOPP) contents were determined as described by Kayali et al. [36]. Briefly, 0.4 ml of renal PMFs was treated with 0.8 ml phosphate buffer (0.1 M; pH 7.4). The absorbance of the reaction mixture was immediately recorded at 340 nm wavelength. The content of AOPP for each sample was calculated using the extinction coefficient of $261 \text{ cm}^{-1} \text{ mM}^{-1}$ and the results were expressed as $\mu\text{moles/mg protein}$.

Histopathology

Small pieces of kidney were fixed in 10% formalin, embedded in paraffin wax, and sections of 5-6 mm in thickness were made and thereafter stained with hematoxylin and eosin for histopathological examination according to the methods as previously described [37]. Thereafter, the sections were examined with light microscopy.

Immunohistochemistry

The immunohistochemistry was described as earlier reported by Oyagbemi et al. [38]. To determine the expression of kidney injury molecule 1 (KIM-1), and Nuclear factor-kappa B (NF- κ B) in the kidney, fixed tissues were embedded in paraffin and sectioned at a thickness of 5 μm . The sections were subsequently deparaffinized in xylene and rehydrated with graded alcohol. Antigen retrieval was carried out by immersing the slides in 10 mM citrate buffer at 95-100 °C for 25 minutes with subsequent peroxidase quenching in 3% H₂O₂/methanol solution. The sections were blocked in goat serum followed by an overnight incubation at 4°C in the rabbit anti- KIM-1 and anti- NF- κ B primary antibodies. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0 $\mu\text{g/mL}$) secondary antibody and subsequently, streptavidin peroxidase (Horse Radish Peroxidase-streptavidin) according to manufacturer's protocol (HistoMark®, KPL, Gaithersburg, MD, USA). Reaction product was enhanced with diaminobenzidine (DAB, Amresco®, USA) for 6-10 min and counterstained with high definition hematoxylin (Enzo®, NY-USA), with subsequent dehydration in ethanol. The sections were subsequently dehydrated in ethanol, cleared in xylene. The slides were covered with coverslips and sealed with resinous solution. The immunoreactive positive expression of KIM-1 and NF- κ B anti-rabbit intensive regions was viewed starting from low magnification on each slice then with 400 \times magnifications using a photo microscope (Olympus) and a digital camera (Toupcam®, Touptek Photonics, Zhejiang, China).

Results and Discussion

In this study, the group that got glycerol administration alone showed significant increases in renal markers of inflammation and oxidative stress such as myeloperoxidase, creatinine, urea and nitric oxide. Treatment with methanol stem bark extract of *Moringa oleifera* resulted in decrease levels of these markers (Figures 1-4).

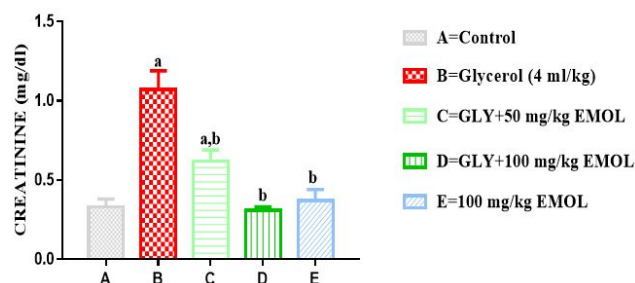


Figure 1: Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean SD, n=7.

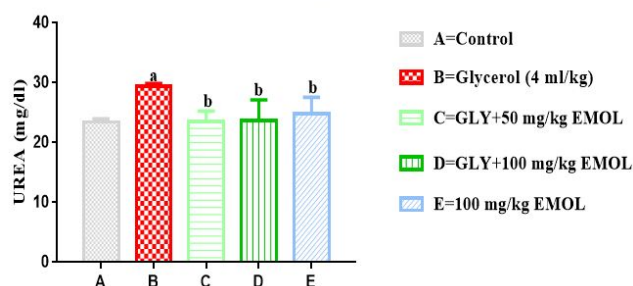


Figure 2: Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean SD, n=7.

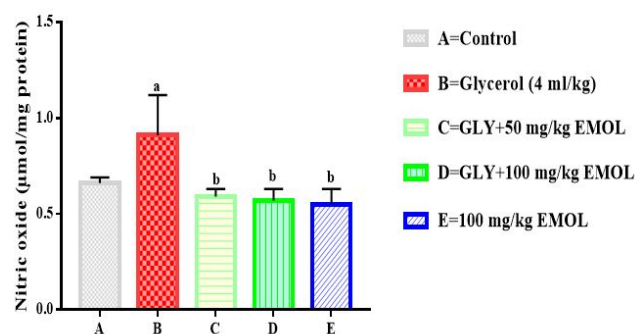


Figure 3: Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean SD, n=7.

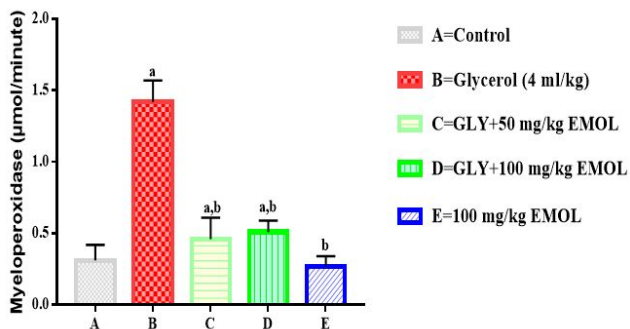


Figure 4: Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean ± SD, n=7.

A significant decrease were observed in the renal SOD, GPx and GST levels in the glycerol alone group when compared with the control and treated groups, whereas treatment with *Moringa oleifera* exhibited an increase in the levels of these enzymatic antioxidant markers (Table 1).

Table 1: Effects of the extracts on the Renal Antioxidant Enzymes (GST, GPx and SOD). A=control, B=Glycerol 4 ml/kg, C= Glycerol 4 ml/kg+50 mg/kg Ethanol extract, D= Glycerol 4 ml/kg+100 mg/kg Ethanol extract, E= 100 mg/kg Ethanol extract. Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D, E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean SD, n=7. SOD (superoxide dismutase; units/mg protein), GPx (glutathione peroxidase; units/mg protein), GST (glutathione-S-transferase; mmole1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein).

Parameters	A	B	C	D	E
SOD	28.96±0.7 2	26.48±1.5 a	30.04± 1.32b	29.07± 1.05b	29.57 ±1.45b
GPx	23.72±1.2 2	18.78±2.1 0a	27.81±1.94a ,b	26.95±1.70a ,b	29.93±0.94a ,b
GST	1.25±0.25	0.97± 0.29a	1.85±0.07a, b	1.53±0.11a, b	1.22 ± 0.17b

We reported significant decreases in renal reduced Glutathione (GSH), protein thiols and non-protein thiol levels in the group treated with glycerol alone when compared with the control and treated groups, whereas treatment with *Moringa oleifera* exhibited amelioration of these non-enzymatic antioxidant markers.

Significant elevations in hydrogen peroxide, malondialdehyde, protein carbonyl, and advanced oxidative protein product were observed in renal homogenate of the glycerol alone group whereas treatment with *Moringa oleifera* caused significant decreases when compared with the group treated with glycerol alone (Figures 5-8).

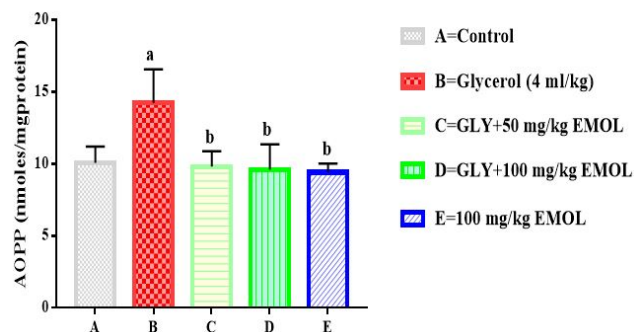


Figure 5: Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean SD, n=7.

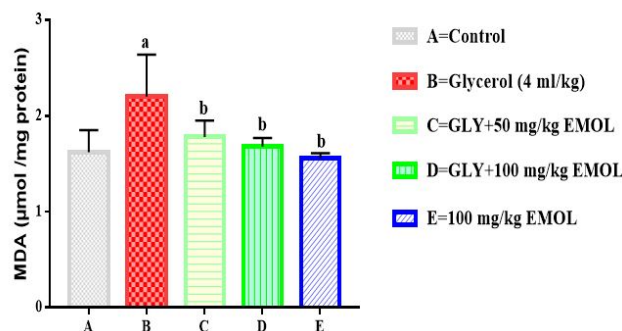


Figure 6: Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean SD, n=7.

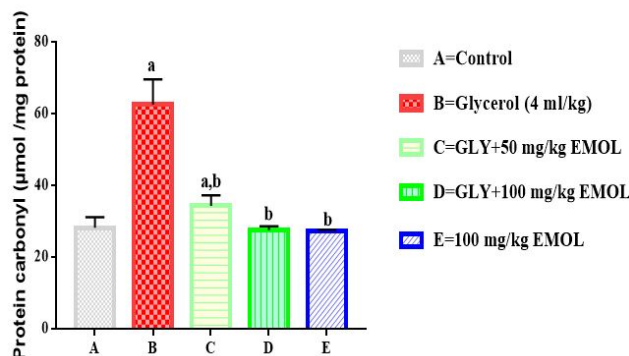


Figure 7: Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean SD, n=7.

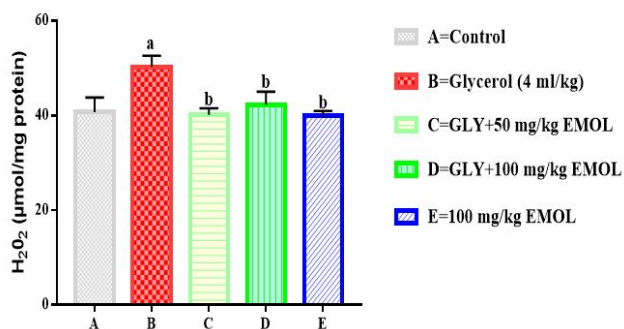


Figure 8: Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D and E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in MeanSD, n=7.

While the control group showed normal glomeruli, bowman capsule and tubules with slight haemorrhagic lesion within the interstitial, glycerol caused focal areas of moderate peritubular and periglomerular inflammation and mild hemorrhagic lesion within the interstitial were observed in the group treated with *Moringa oleifera* (Figure 9).

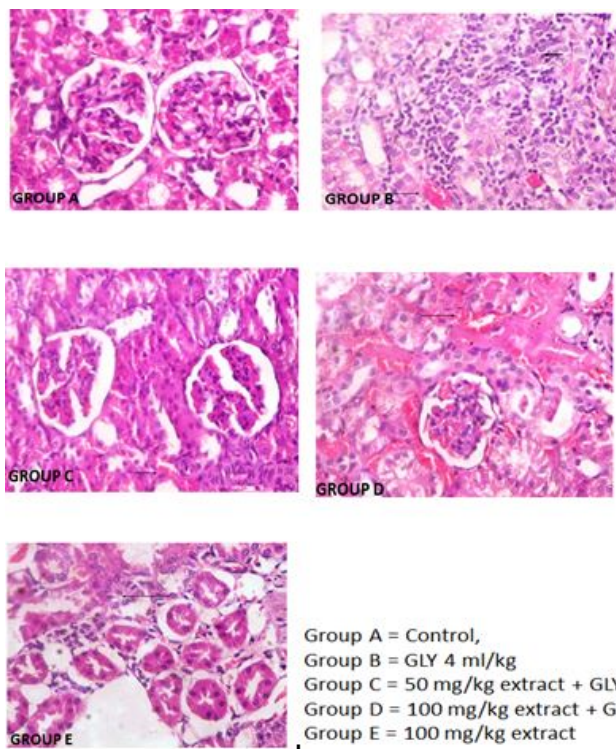


Figure 9: Photomicrographs of kidney plates x 400. Group A shows normal glomeruli, bowman capsule and tubules with slight haemorrhagic lesion within the interstitial (thin arrow). Group B shows focal areas of moderate peritubular (black arrow) and periglomerular (thin arrow) inflammation. Group C shows mild hemorrhagic lesion within the interstitial (thin arrow). Group D shows moderate hemorrhagic lesion (thin arrow). Group E shows slight hemorrhagic lesion (thin arrow).

The control grouped showed least expression of KIM-1 as against the highest expression of KIM-1 in the glycerol alone treated group when compared to other treated groups (Figure 10). Glycerol also caused highest expression NF-KB in the glycerol alone group when

compared to the level of expression of NF-KB observed in other groups (Figure 11).

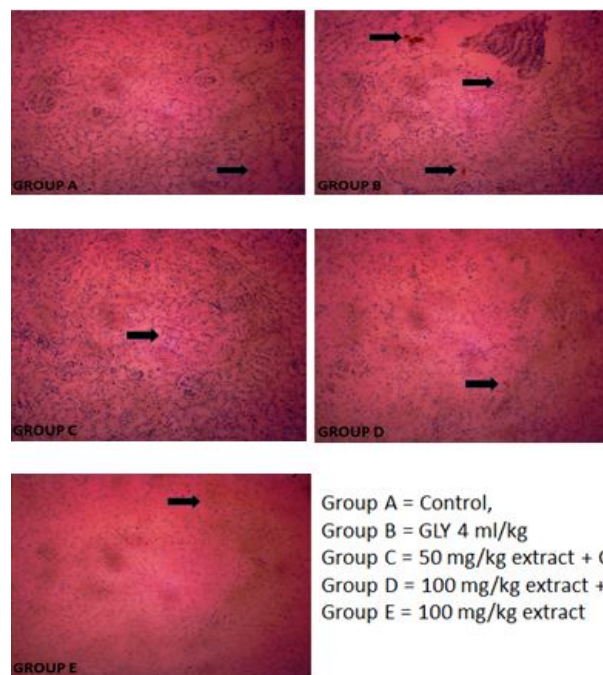


Figure 10 : Group A shows least expression of KIM-1 in the kidney section. Group B shows highest expression of KIM-1 when compared with Groups A, C, D, and E.

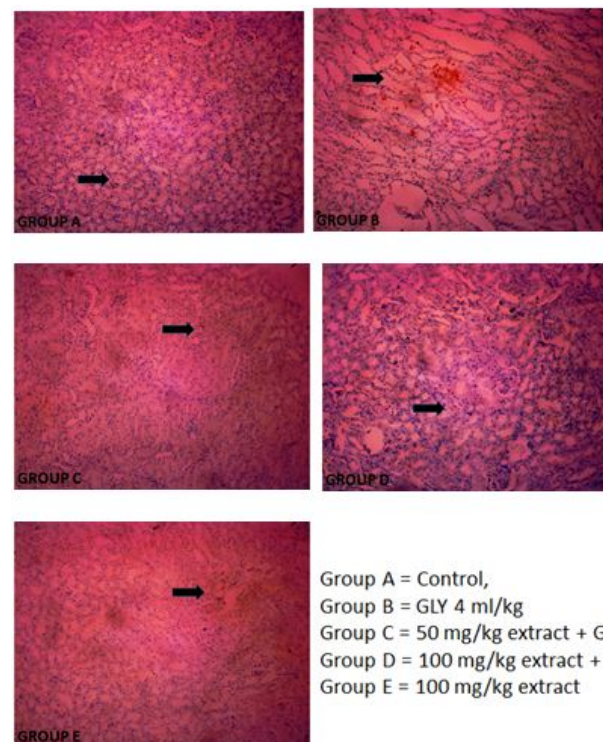


Figure 11: Group A shows least expression of NF-KB in the Kidney section. Group B shows highest expression of NF-KB when compared with Groups A, C, D, and E.

In this study, the protective effects of *Moringa oleifera* against acute kidney injury was elucidated and it was discovered that it nicely prevented the rhabdomyolysis-induced AKI in rats and lowered the markers of oxidative stress and inflammation in both the serum and

post mitochondrial fraction of the kidney. A pivotal aspect of therapeutics is drug delivery, and other major limiting factors that could impede this are adverse effects and bioavailability of drugs. Kidney is one of the targets of organ toxicity due to its function in the excretion of drugs [39].

A model closest to rhabdomyolysis-induced AKI is Glycerol-induced AKI [40]. Basically, glycerol causes damage to muscles as a result of myoglobin release into the blood stream. It also causes hemolysis and hemoglobin is released in the blood. Both myoglobin and hemoglobin contain a catalytic iron metal center. When these hemoglobin and myoglobin go through the kidney, the catalytic iron of both proteins causes lipid peroxidation of the proximal tubular cells of the kidneys. Consequently, oxidative damage occurs followed by activation of inflammatory cascades [41,42]. Therefore, we used this model to study the protective effects of *Moringa oleifera* on the rat kidney.

Biochemical markers play an important role in accurate diagnosis and also for assessing risk and adopting therapy that improves clinical outcome. Serum creatinine and Urea are the most commonly used marker of renal function as they are useful in differential diagnosis of acute renal failure [43]. A significant increase in serum urea and creatinine was observed in the toxicant group, when compared with the control group suggesting the functional impairment of the kidney. Treatment with extract caused a decrease in serum urea and creatinine levels as shown in figures 1 and 2 when compared with the toxicant group. These results tallied with the findings of Siraq [44], who found that serum urea and creatinine were significantly increased in the glycerol-treated group.

In glycerol-induced acute kidney injury, there is increase in renal nitric oxide production due to the expression levels of inducible nitric oxide synthase (iNOS) an indicator of inflammation and stress, activated by tumour necrosis factor- α (TNF- α) [45,46]. There was a significant increase in nitric oxide levels in the positive control group when compared with the negative control group in Figure 3. Moreover, an inappropriately high level of iNOS-mediated NO production correlates with apoptosis and necrosis in the kidney [47,48]. It can then be said that glycerol causes renal damage by eliciting tubular inflammation and necrosis. This inflammatory effect was however blunted by the extracts of this plant thus showing again its anti-inflammatory effect. In addition, in this study the depletion of the levels of SOD results in elevations of superoxide radical. Excess NO reacts with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$), a presumed source of hydroxyl radicals, thus causing renal tubular damage [49,50]. Treatment with extracts resulted in amelioration of serum nitric oxide levels as shown in Figure 3. It thus showed that these extracts have anti-oxidant property.

Myeloperoxidase, a heme protein released by leukocytes plays a crucial role in inflammation and oxidative stress in the cellular level [51]. There is significant increase in neutrophil-derived enzyme, MPO in the kidney of glycerol treated rats as seen in Figure 4. Increased in proinflammatory enzyme MPO concentration in the kidney is an indicator of neutrophil infiltration [52] and it is directly proportional to the tissue inflammation caused by glycerol. Bolisetty and Agarwal [53] showed that neutrophil accumulate in kidney following ischemic insult due to their transmigration into the interstitium. Alteration of epithelial and endothelial cell integrity by neutrophils leads to kidney injury. Takasaki et al. [54] suggested that neutrophil cause kidney

damage through the excessive release of oxygen radicals and proteases. MPO activation has been shown to cause glomerular morphologic changes, endothelial and mesangial cell injury, and activation of platelets as well as glomerulonephritis [55]. In this study there is significant attenuation of MPO activity in the kidney tissue of extract treated rats in Figure 4. This further confirms the anti-oxidative and anti-inflammatory property of this plant.

Glycerol-treated group showed depletion in antioxidant system which was indicated by the significant decrease in the levels of GPx, GST and SOD enzymes indicating a state of oxidative stress as seen in Table 1 and these results are in accordance with Singh et al. [56]. However, the extract treated group protected the severe depletion of antioxidant enzyme pool compared to glycerol-treated group and there was significant amelioration in renal oxidative stress.

SOD catalyzes the dismutation of superoxide radical (O_2^-) to form oxygen and hydrogen peroxide. Thus the depletion of SOD levels causes elevation of superoxide radical and this free radical could react with Nitric oxide to produce peroxynitrite. Peroxynitrite ($ONOO_2$) is a potent oxidant formed by a rapid reaction between nitric oxide (NO) and superoxide anion. Its cytotoxic and also a potent marker of inflammation and renal damage [57,58]. We can then surmise that glycerol also induces renal damage through generation of peroxynitrite.

GSTs are enzymes that catalyze the reaction of glutathione with electrophiles of both endogenous and xenobiotic origins. The main biological roles of GSTs encompass detoxification and protection against oxidative stress. By conjugating glutathione with toxic electrophilic substrates of both endogenous and xenobiotic origins, the resulting molecules generally become less reactive and more soluble, thus facilitating their excretion from cells and the organism [59]. The main biological role of GPx is to protect the organism from oxidative damage. Glutathione peroxidase (GPx) is a selenium-containing antioxidant enzyme that effectively reduces H_2O_2 and lipid peroxides to water and lipid alcohols, respectively, and in turn oxidizes glutathione to glutathione disulfide [60]. There is relationship between oxidative stress and nephrotoxicity, which have been confirmed by different authors [61,62].

Protein thiols in the plasma include the protein sulfhydryl groups and protein mixed disulphides with homocysteine, cysteinyl glycine, cysteine and glutathione [63]. Among all the antioxidants that are available in the body, thiols constitute the major portion of the total body antioxidants and they play a significant role in defense against reactive oxygen species [64]. Presence of oxidative stress in renal failure is well proved and several studies have shown decreased levels of thiol status in renal failure. Increased presence of ROS generated is believed to consume the available thiol groups [65]. There have also been reports of a significant decrease in plasma protein thiol levels in pediatric nephrotic syndrome, primary glomerular diseases, moderate to severe chronic kidney disease, end stage renal disease, with and without nephritis [66-69]. In this study, there is decrease in protein thiol, non-protein thiol and reduced glutathione in the glycerol-treated group in comparison with the control group, while the extract of *Moringa oleifera* significantly

ameliorated the reduction in levels of protein thiol, non-protein thiol and reduced glutathione brought on by glycerol as seen in Table 2.

Table 2: Effects of the extracts on the renal GSH, NPT and PT Level. A=control, B=Glycerol 4 ml/kg, C= Glycerol 4 ml/kg+50 mg/kg Ethanol extract, D=Glycerol 4 ml/kg+100 mg/kg Ethanol extract, E= 100 mg/kg Ethanol extract. Superscripts (a, b) indicate significant difference at pSuperscript (a) indicates significant difference when groups B, C, D, E were compared with group A. Superscripts (b) indicates significant difference when groups C, D, E were compared with group B. Results expressed in Mean SD, n=7. PT (Protein thiol; $\mu\text{mol/L}$), NPT (Non-protein thiol; $\mu\text{mol/L}$), GSH (reduced glutathione; $\mu\text{mol/mg}$ protein).

Parameters	A	B	C	D	E
GSH	2.66 \pm 0.13	2.35 \pm 0.06a	3.07 \pm 0.39a,b	3.07 \pm 0.26a,b	2.72 \pm 0.08b
NPT	53.06 \pm 8.93	46.77 \pm 4.62a	54.53 \pm 4.49b	59.28 \pm 6.20a,b	61.91 \pm 1.02a,b
PT	20.12 \pm 1.85	18.67 \pm 1.15a	24.64 \pm 2.74a,b	22.20 \pm 1.47b	22.75 \pm 1.86b

Glycerol-treated group showed elevation in the levels of AOPP, MDA, Protein Carbonyl and H_2O_2 indicating a state of oxidative stress. However in the extract-treated groups, there was significant reduction in AOPP, MDA, Protein Carbonyl and H_2O_2 levels as seen in Figures 5-8. AOPP is a marker of oxidative stress, inflammation and renal damage. Advanced oxidative protein products (AOPP) were proposed as one of the possible markers of oxidative injury, which originates under oxidative and carbonyl stress and increase global inflammatory activity [70]. Thus it can be said that glycerol induces renal damage via generation of AOPP. Protein carbonyl is also a marker of oxidative stress with elevated level in glycerol-treated group when compared with control and extract treated group. These results are in accordance with Ozturk et al. [71]. It can then be said that glycerol induces renal damage via generation of protein carbonyl.

H_2O_2 is a marker of oxidative stress and glycerol induces renal damage via increased generation of H_2O_2 [72], as the levels were significantly elevated in the toxicant group when compared with the normal and the extract-treated groups. H_2O_2 is converted to water and oxygen by catalase. GSH also reacts with H_2O_2 to produce water and oxidized GSSH. Depleted levels of GSH can thus elevate tissue levels of H_2O_2 and this also results in oxidative stress.

The result of this study also revealed that glycerol induced nephrotoxicity through the release of lipid peroxidation products as evidenced in the elevated level of MDA in the glycerol-treated group when compared with the normal and extract-treated groups. This corresponds to the study conducted by Liu et al. [73] where they found that the administrations of glycerol induced a significant increase in kidney tissue MDA levels.

Histologically, glycerol induced tubular and glomerular inflammation while the extract-treated groups however exhibited recovery from glycerol-induced structural changes in varying degrees as presented in Figure 9. This thus showed that the extract of the plant has nephroprotective property. Histopathological results demonstrating structural changes in renal tissue of glycerol have been reported in previous research by Huang et al [74].

Kidney injury molecule-1 (KIM-1) is an early biomarker of kidney tubular damage and it mirrored well the progression of kidney damage [75]. KIM-1 is a type 1 membrane protein that is not expressed in normal kidney but is markedly upregulated in the injured proximal tubular epithelial cells of the human and rodent kidney in ischemic and toxic acute kidney injury [76,77]. Up regulation of this KIM-1 gene is thought to be associated with proliferative response to toxicity or disease [76] and it is consistent with the histopathological alterations observed following treatment with glycerol. The reduction of the expression of KIM-1 by the extracts of this plant is an indication of its protective effect against glycerol-induced renal tubular injury.

A very complex protein with diverse actions is NF- κ B [78]. Its function in the regulation of cytokine production and cell survival is important [79,80]. Primarily, NF- κ B controls inflammatory process by several mechanisms to affect the duration and degree of the inflammatory reactions [81,82]. Our findings demonstrate elevated level of NF- κ B in the glycerol treated group in comparison to the normal control group. However, *Moringa oleifera* markedly decreased the expression level of NF- κ B. NF- κ B induces the expressions of iNOS and COX-2 genes [83,84] which are key players in inflammation and responsible for initiating the inflammatory processes [84]. Cytokines and other pathological factors produced during renal ischaemia-reperfusion injury are also strong stimulators of NF- κ B. In particular, ischaemia-reperfusion induces the production of TNF- α in an NF- κ B-dependent manner, and TNF- α in turn binds to its receptor to stimulate NF- κ B activation, thereby providing a positive feedback mechanism of NF- κ B regulation [85]. NF- κ B is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, heavy metals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens [79]. Because NF- κ B controls many genes involved in inflammation, it is not surprising that NF- κ B is found to be chronically active in many inflammatory diseases, such as inflammatory bowel disease, arthritis, sepsis, gastritis, asthma, atherosclerosis [86]. The reduction of the expression of NF- κ B by the extracts of *Moringa oleifera* is an indication of its anti-inflammatory property and may find therapeutic usefulness in any of the conditions that lead to over expression of NF- κ B.

Conclusion

From this study, it is clear that ethanol extracts of *Moringa oleifera* play a vital role against glycerol-induced renal damage by reducing the levels of renal markers of oxidative stress, inflammation and tubular necrosis and enhance levels of both enzymatic and non-enzymatic renal antioxidant system. It also showed a decrease in expression of KIM-1 and NF- κ B. All these desirable effects can be due to the presence of various phytochemicals such as Saponin, Tannins, Flavonoids, Terpenoids, Steroids and Alkaloids that were revealed in the photochemical analysis carried out in an earlier study.

It can then be said that ethanol extracts of *Moringa oleifera* ameliorated renal damage through anti-inflammatory, anti-oxidative and anti-cytotoxicity pathways. These results have provided justification for its use in many communities for therapeutic purpose. It could thus be concluded that the extracts of *Moringa oleifera* exhibited nephroprotective property.

Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflicts of interest

The authors have no conflict of interest to declare.

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