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Renoprotective Effects of the Ethanol Leaf Extract of *Nymphaea lotus* (Linn.) in Glycerol-Induced Acute Kidney Injury in Rats

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ABSTRACT

Background: Acute kidney injury (AKI) is a serious complication with high mortality rates, characterised by oxidative stress and inflammation. Early intervention is crucial to prevent progression to chronic kidney disease or death. *Nymphaea lotus* L. (Nymphaeaceae) is a medicinal plant used in traditional medicine for various inflammatory conditions. Pharmacological studies have established its antioxidant and anti-inflammatory properties.

Objectives: This study aims to investigate the therapeutic roles of ethanol leaf extract of *N. lotus* against glycerol-induced AKI in rats through the evaluation of renal biochemical parameters, oxidative stress markers, inflammatory cytokines and histopathological changes.

Method: Thirty-six Wistar rats were divided into six groups, each consisting of six rats. AKI was induced by intramuscular glycerol administration (8 mL/kg). The baseline group received distilled water only orally (10 mL/kg), the negative control group received glycerol only (8 mL/kg), the treatment groups received ethanol extract of *N. lotus* (100, 200, and 300 mg/kg p.o.), and pioglitazone (10 mg/kg) for 7 days. At the end of the experimental period, the rats were anaesthetised with ketamine (80 mg/kg, i.p.) and humanely sacrificed. Blood was collected through cardiac puncture, for the estimation of urea and creatinine levels, and the kidneys were collected for the determination of MDA, SOD, GSH, TNF-α, and IL-1β, as well as for histological analysis

Results: Glycerol significantly increased the levels of urea, creatinine, MDA, TNF-α, and IL-1β, and reduced CAT, SOD and GSH levels ($p < 0.05$). *N. lotus* pretreatment significantly reduced urea, creatinine, MDA, TNF-α, and IL-1β levels, while increasing the levels of SOD and GSH at all doses ($p < 0.05$). Histopathological evaluation of the kidney revealed that *N lotus* administration caused a marked reduction in capillary congestion, tubular damage and glomerular distortion compared to the glycerol group. The highest protective effect was seen at the dose of 300 mg/kg.

Conclusion: *N. lotus* extract ameliorates AKI by modulating oxidative and inflammatory responses and renal function biomarkers. It shows potential as a nephroprotective agent

INTRODUCTION

Acute kidney injury (AKI) is a commonly occurring pathological condition with serious implications. It is characterised by a rapid decline in kidney function, which results in the retention of metabolic waste products, electrolyte imbalances, and fluid dysregulation[1]. AKI is associated with increased morbidity and mortality and can be caused by reduced blood flow to the kidney or direct damage to the kidneys. High blood pressure, congestive heart failure, infections, toxins, medications, rhabdomyolysis etc., are conditions that can cause or contribute to kidney damage[2–4].

One major experimental model used to mimic human AKI caused by rhabdomyolysis in animals is glycerol administration. Administration of glycerol causes degradation of skeletal muscle, which leads to myoglobin release into the bloodstream, oxidative stress, and inflammatory responses in renal tissue. Serum creatinine and blood urea nitrogen are waste products normally filtered by the kidneys. In AKI, their levels rise significantly in the blood due to impaired filtration[5]. The kidneys are especially prone to oxidative damage due to their high oxygen consumption rate and exposure to blood-borne toxins[6,7]. Reactive oxygen species are generated when there is a disruption in mitochondrial function and the electron transport chain, which leads to oxidative stress. Overproduction of ROS overwhelms the antioxidant defence mechanisms, causing lipid peroxidation, protein modification, and DNA damage. Concurrently, pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) are upregulated, contributing to further renal injury[8,9].

Medicinal plants are a promising and cheaper source of nephroprotective agents. *Nymphaea lotus*, (Linn.) Willd. (Nymphaeaceae) an aquatic plant widely used in African and Asian folk medicine, has been reported to possess antioxidant, anti-inflammatory, and cytoprotective properties due to its rich phytochemical content, including flavonoids, tannins, and saponins[10,11]. This study aims to evaluate the protective role of the ethanol leaf extract of *N. lotus* on oxidative stress parameters and inflammatory cytokines in a glycerol-induced model of AKI in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Pioglitazone (Actos® Takeda Pharmaceutical, Tokyo Japan), distilled water, ethanol (Sigma-Aldrich, St Louis, MO, USA), carboxylmethyl cellulose (Martindale Pharmaceuticals, Ramford, Essex, United Kingdom), glycerol (Sigma-Aldrich, St Louis, MO, USA), ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Plant materials

Leaves of *N. lotus* were collected at Ile-Ife, Osun State. The plant was identified and authenticated by Mr. I.I. Ogunlowo at the Medicinal Plants Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife (Code FPI). A voucher specimen (FPI 2665) has been deposited at the herbarium, registered in the Index Herbariorum under the code FPI

The fresh leaves of *N. lotus* were washed, separated from other plant parts, and air-dried under shade to constant weight and then ground into a fine powder. 500 g of the powdered material was extracted with 2.5 L of 90% ethanol by cold maceration for 72 hours, with the mixture shaken three times daily. The extract was filtered, and the filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator. The resulting concentrate was freeze-dried, weighed, stored in an airtight container, and kept at 4 °C until use.

Animals

Thirty-six Wistar rats of either sex weighing 200-250 g were obtained from our facility, the Animal House of the Department of Pharmacology and Therapeutics, Osun State University, Osogbo, Nigeria.

The animals were allowed to acclimatise for two weeks before the commencement of the experiment. They were maintained in a well-ventilated room at room temperature with a 12-hr light and dark cycle and fed with standard rat chow (New Hope Feeds, Abeokuta, Ogun State, Nigeria).

Ethical Approval

All experimental procedures and protocols were as outlined by the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health[12]. Ethical approval to conduct the study was obtained from the UNIOSUN Health Research Ethics Committee with an ethical reference number UNIOSUNHREC 2025/PHA/007

Experimental Design

The rats were randomly divided into six groups of six animals each. Baseline group received distilled water only (10 mL/kg p.o.); animals in Group 2, (negative control) were given distilled water for seven days before being given intramuscular glycerol (8 mL/kg i.m.); Group 3 received pioglitazone (10 mg/kg p.o.) once daily for seven days; Groups 4, 5, and 6 received *N. lotus* extract orally at 100, 200, and 300 mg/kg, respectively once daily for seven consecutive days. These doses were chosen based on previous doses of the plant extract used in the literature. On the eighth day, AKI was induced by administering 50%

intramuscular glycerol (8 ml/kg) into both hind limbs using a modification of the method described by Rizk *et al*.[13] after a seven-day pretreatment period. Twenty four hours post-induction, the rats were anaesthetized with ketamine (80 mg/kg, i.p.) and humanely sacrificed by cervical dislocation. Blood was collected by cardiac puncture and kidney tissues were collected. Biochemical assays for urea and creatinine, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) were conducted. Tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) levels were determined using ELISA, and histopathological analysis of the kidneys was also carried out.

Biochemical Analysis

Assessment of Kidney Function Biomarkers

Levels of kidney function biomarkers, urea and creatinine, were measured in the serum using Randox/Laboratory (Crumlin, U.K.) kits according to the manufacturer's instructions.

Evaluation of Oxidative Stress Markers

Levels of malondialdehyde (MDA), a marker of lipid peroxidation, were measured using the thiobarbituric acid (TBA) reactive substances assay method described by Ohkawa *et al.*[14]. Superoxide dismutase activity was determined using the nitroblue tetrazolium (NBT) reduction method described by Kakkar *et al.*,[15]. GSH contents were calculated using Ellman's reagent, and the formed yellow chromogen was measured at 412 nm[16,17].

Evaluation of Inflammatory Cytokines

Kidney tissue was homogenised in ice-cold phosphate-buffered saline (PBS, pH 7.4). Homogenates were centrifuged at 12,000 × g for 15 min at 4 °C, and the supernatants were collected for cytokines assay. Renal TNF-α and IL-1β levels were measured using sandwich ELISA kits specific for rat TNF-α and IL-1β (Elabscience® Biotechnology Inc., Wuhan, China), following the manufacturer's instructions.

Histopathological Assessments

The kidneys were fixed in 10% neutral buffered formalin for 24 h, after which these were dehydrated and paraffinised. Renal sections were stained with hematoxylin and eosin (H&E), and light microscopy was performed at x 400 magnification.

Statistical Analysis

Data were analysed using the GraphPad Prism (version 10.2) for Windows (San Diego, CA, United States). The results were expressed as mean ± Standard Deviation. Differences among groups were examined using One-way analysis of variance (ANOVA) followed by Tukey's post hoc test.

RESULTS

Effect of *N. lotus* administration on oxidative stress parameters in glycerol-induced acute kidney injury

In this study, as shown in (Figure 1a-b), glycerol administration caused a marked increase in renal MDA levels compared with the normal control ($p < 0.0001$), indicating elevated lipid peroxidation. This was accompanied by significant reductions in GSH, SOD, and CAT levels ($p < 0.0001$), reflecting impaired antioxidant defence. Treatment with *N. lotus* extract produced a dose-dependent improvement in all four parameters of oxidative stress that were assayed. The dose of 300 mg/kg showed the highest effect.

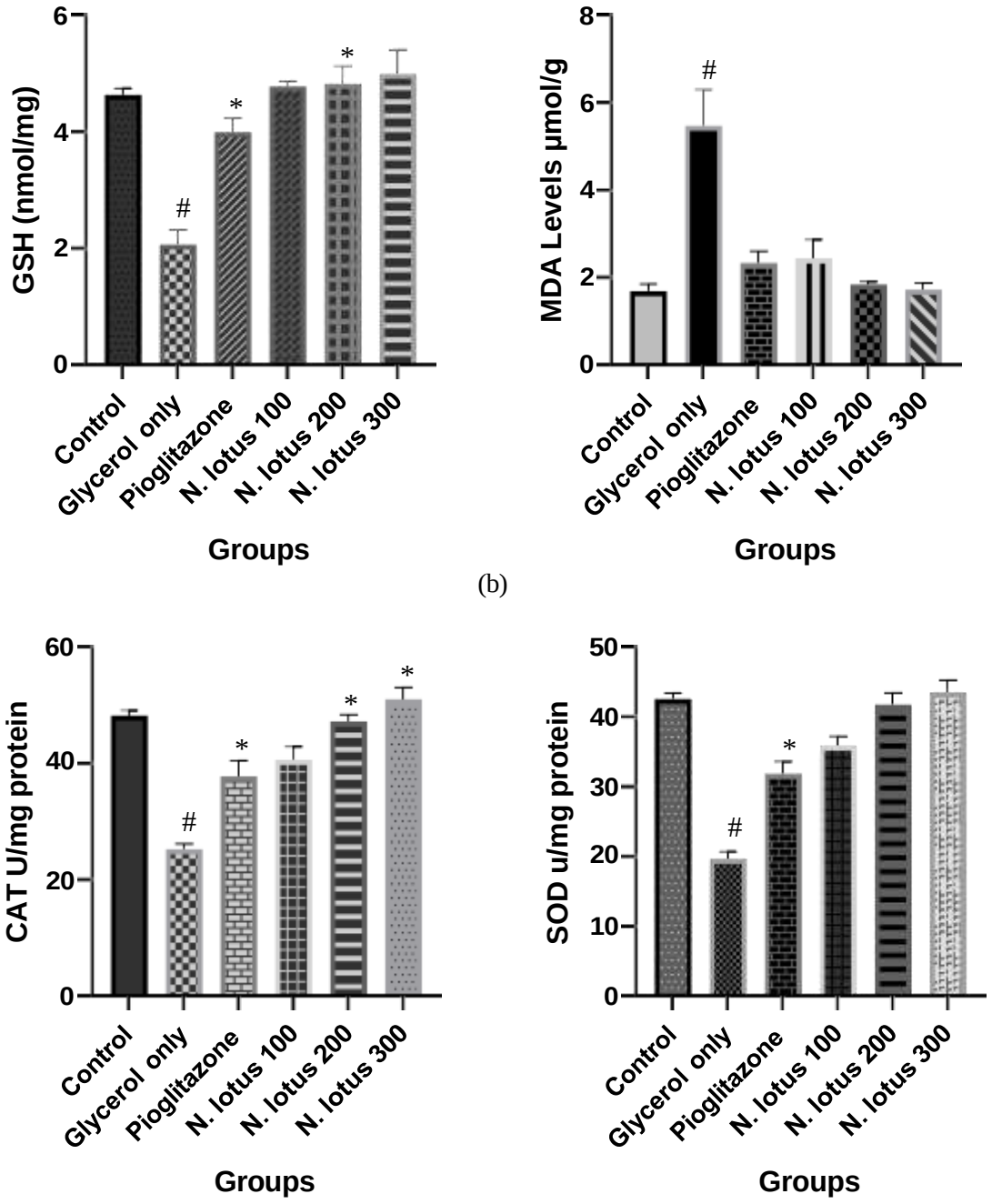
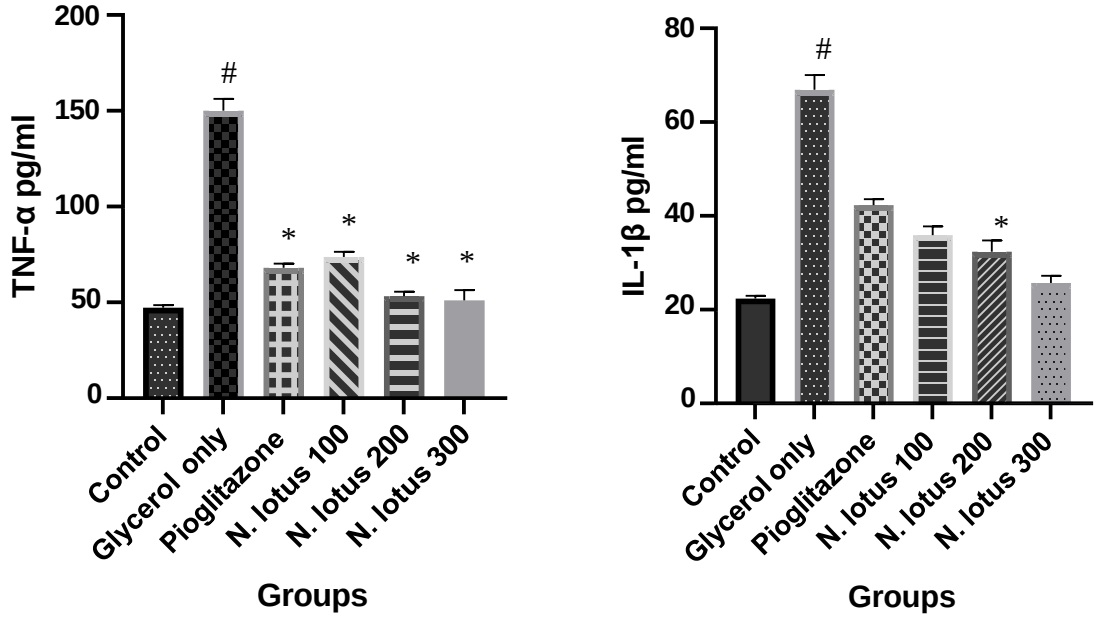


Figure 1 (a –d): Effect of *Nymphaea* lotus treatment on oxidative stress.
 # = p <0.0001 (vs control) * = *p <0.0001* (vs glycerol only)

Effect of *N. lotus* administration on inflammatory cytokines in glycerol-induced acute kidney injury
Glycerol-treated rats exhibited significantly elevated renal IL-1β and TNF-α levels compared with the control group ($p < 0.01$). *N. lotus* treatment reduced both cytokines in a dose-dependent manner. All doses achieved significant reductions ($p < 0.001$ vs. glycerol), but the highest activity was seen at the dose of 300 mg/kg. Pioglitazone also significantly reduced both cytokines, but had a significantly lower effect when compared to *N. lotus* at the doses of 200 mg/ kg and 300 mg/kg (Fig. 2a –b).



(a) (b)

(Figure 2a–b): Effect *N lotus* administration on inflammatory cytokines
 # = p < 0.001 (vs. control) * = *p < 0.0001* (vs. glycerol only).

Effect of *N lotus* administration on renal function indices

As shown in Table 1, administration of glycerol (8 mL/kg) caused significant increases in serum urea and creatinine compared with control (*p < 0.0001*), indicating functional renal impairment. All doses of *N. lotus* significantly reduced both urea and creatinine toward control values (*p < 0.01* vs. glycerol). Treatment with increasing doses of the extract resulted in a dose-dependent reduction in serum urea and creatinine levels, with the highest reduction observed at 300 mg/kg. Pioglitazone also reduced urea and creatinine, but less effectively than *N. lotus*.

Table 1: Effect of *Nymphaea lotus* on renal function in glycerol-induced acute kidney injury in rats

	Urea	Creatinine
Control	21.00 ± 1.10	0.66 ± 0.13
Glycerol only	96.00 ± 5.10	2.30 ± 0.21
G + Pioglitazone	52.00 ± 3.90*	0.98 ± 0.11
G + N. lotus 100 mg/kg	44.00 ± 1.50*	1.60 ± 0.13
G + N. lotus 200 mg/kg	34.00 ± 2.00*	1.00 ± 0.02
G + N. lotus 300 mg/kg	28.00 ± 1.50*	0.84 ± 0.45

*Data are expressed as mean ± SD (n=6). * p < 0.05 was considered significant. Results were analysed byOne way ANOVA with Tukey's post hoc test.* G = Glycerol only 8 mL/kg; G + pioglitazone = Glycerol plus pioglitazone 10 mg/kg.

Histopathological Findings

As shown in Figure 3, H&E-stained kidney sections from the normal control group displayed intact renal cortical architecture with well-defined glomeruli, and normal proximal and distal convoluted tubules. Glycerol-treated rats showed extensive histopathological damage, including glomerular shrinkage/sclerosis, tubular epithelial degeneration with vacuolation, and dilation. Treatment with *N lotus* 100 mg/kg partially restored glomerular definition, and the convoluted tubules are moderately preserved with reduced tubular degeneration. *N lotus* 200 mg/kg also showed moderate restoration of renal architecture. The glomeruli are more prominent and better defined, and the convoluted tubules exhibit a relatively intact epithelial lining. There is a noticeable reduction in tubular vacuolation and interstitial congestion. *N lotus* at the dose of 300 mg/kg showed largely preserved renal cortical structure with glomeruli showing near-normal morphology and minimal sclerosis. The convoluted tubules appear structurally intact and exhibit uniform cellular lining. Interstitial inflammation is minimal or absent.

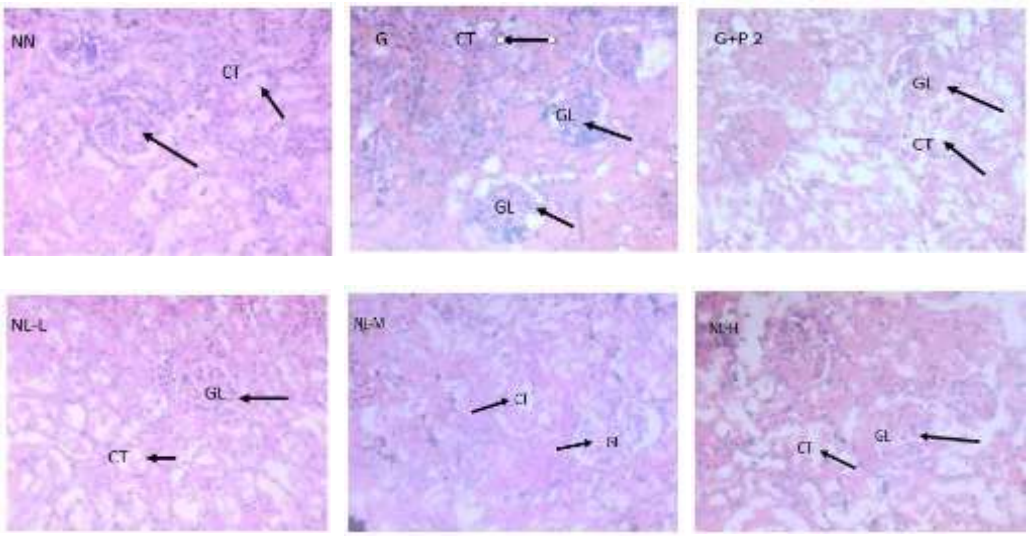


Fig. 3: Photomicrographs of kidney sections (x400 magnification)
Key: NN = Normal control (distilled water 10 mL/kg); G = Glycerol only 8 mL/kg; G+ 2 = Glycerol plus pioglitazone 10 mg/kg; NL-L = *N. lotus* 100 mg/kg; NL-M = *N. lotus* 200 mg/kg; NL-L = *N. lotus* 300 mg/kg; GL = Glomerulus; CT = Convoluted tubule

DISCUSSION

In this study, we evaluated the renoprotective effect of the ethanol leaf extract of *Nymphaea lotus* (100–300 mg/kg) in a glycerol-induced model of acute kidney injury (AKI) in rats. The glycerol group exhibited marked renal dysfunction, oxidative stress, inflammation, and histopathological damage. These findings are consistent with the typical features of rhabdomyolysis-induced AKI. These pathophysiological changes reproduce previously reported glycerol-AKI profiles and validate our model[5,18,19].

Treatment with the ethanol leaf extract of *N. lotus* (100–300 mg/kg) offered significant protection against glycerol-induced acute kidney injury in rats in a dose-dependent manner. Normalisation of urea and creatinine, reduced levels of MDA and pro-inflammatory cytokines (TNF-α and IL-1β), and the concomitant increase in antioxidant markers (CAT, SOD, and GSH) confirm that the extract alleviates oxidative stress and inflammation, which are the two major drivers of AKI pathogenesis. These results are in keeping with multiple reports showing that natural compounds with high antioxidant capacity reduce lipid peroxidation and preserve endogenous antioxidants, leading to improved outcomes in glycerol AKI models[5,13,20]

At 100 mg/kg, there was an observable reduction in tubular damage and capillary congestion, at 200 mg/kg, interstitial and glomerular damage were further ameliorated, and at 300 mg/kg the histoarchitecture was nearly restored to normal, with well-defined glomeruli and tubules. These histological findings align with the biochemical results.

Phytochemical analysis of *N. lotus* has demonstrated the presence of flavonoids, phenolic compounds, triterpenoids, saponins, tannins, and other antioxidant constituents[21–23].

Flavonols like quercetin, rutin, kaempferol, myricetin derivatives, and other unusual flavonoids have been reported to be present in *N. lotus*, and these compounds are known for their radical-scavenging and anti-inflammatory properties. Any or all of them may have mediated the renoprotective effects of *N. lotus* exhibited in this study[11,23,24]

Quercetin has demonstrated robust renoprotective effects in AKI models, reducing blood urea nitrogen and creatinine levels while attenuating oxidative stress and inflammation in animal studies[25,26]. Rutin, the glycosylated form of quercetin, has also been found to have anti-oxidant and anti-inflammatory effects in AKI. In an LPS-induced AKI mouse model, rutin restored levels of SOD, GSH, and catalase, reduced MDA, and normalized NF-κB, TLR4, COX-2, and TNF-α signaling pathways[27,28]. Studies confirm that kaempferol reduces oxidative stress, inflammation, and apoptosis by activating Nrf2 and inhibiting NF-κB, thus countering nephrotoxic insults[29–31].

Pioglitazone, our positive control and a PPAR-γ agonist, generated intermediate protection consistent with its known effects in mitigating oxidative stress and inflammation in AKI models[32,33]. This similarity reinforces the interpretation that *N. lotus* operates through comparable molecular pathways.

CONCLUSION

The ethanol leaf extract of *Nymphaea lotus* significantly mitigates glycerol-induced AKI in rats in a dose-dependent manner, likely through antioxidant and anti-inflammatory activities that preserve renal function. These findings suggest its potential as a therapeutic agent in managing AKI and related renal conditions. Future work should validate molecular targets (Nrf2, NF-κB) and dose-response/safety for translation.

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Declaration of conflicting interests

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Disclosure

The authors confirm that this article has not been published in any other journal, nor is it under consideration for publication in any other journal.

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