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Galngin Mitigates Glycerol-induced Rhabdomyolysis-associated Acute Kidney Injury by Modulating Nf-κb-mediated Cytokine Pathways and Apoptotic Responses

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ABSTRACT

Background: This study investigated the protective capabilities of galangin, a naturally occurring flavonoid found in galangal and propolis, against glycerol-induced acute kidney injury (AKI), a serious complication associated with rhabdomyolysis.

Methods: Rats were pre-treated with galangin (100 mg/kg) for 21 days before receiving a glycerol single injection (50%, 10 mg/kg, intramuscular) to induce AKI. According to the findings, the kidneys in the AKI group showed notable molecular, and functional alterations.

Result: Treatment with galangin notably reduced kidney relative weight and decreased serum levels of urea and creatinine, indicating preservation of renal function. Furthermore, the intervention lowered concentrations of lactate dehydrogenase (LDH) and creatine kinase (CK), key indicators of muscle breakdown associated with rhabdomyolysis. A remarkable finding was galangin's ability to enhance the endogenous antioxidant defence system. Treatment led to increased activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), along with elevated levels of reduced glutathione (GSH). Concurrently, galangin treatment resulted in reduced malondialdehyde (MDA) and nitric oxide (NO) concentrations, demonstrating effective suppression of oxidative stress markers. Similarly, animals receiving galangin exhibited significantly reduced levels of pro-inflammatory mediators, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), myeloperoxidase (MPO), and nuclear factor kappa B (NF-κB). Simultaneously, there was an elevation in anti-inflammatory interleukin-10 (IL-10) compared to the untreated model group, in addition to a significant modulation of apoptotic pathways by decreased expression of pro-apoptotic proteins Bax and caspase-3, and increased in Bcl-2. The comprehensive findings from this study provide compelling evidence for galangin as a promising therapeutic agent against glycerol-induced acute kidney injury. Overall, galangin shows promise as a potential therapeutic agent for preventing or treating rhabdomyolysis-associated acute kidney injury.

Key words: AKI, Antioxidant, Apoptosis, Galangin, Inflammation, Kidney, Rhabdomyolysis.

INTRODUCTION

Acute kidney injury (AKI) is a severe condition characterized by the rapid onset of kidney dysfunction within hours or days, leading to high rates of illness and death (Salem, 2021; Wen *et al*., 2023). Key causes of AKI include reduced kidney blood flow, structural damage to kidney tissues and blocked urine flow. Globally, AKI affects approximately 20% of patients (Li *et al*., 2017), with mortality rates around 20% in those without kidney damage, rising to 59% in AKI patients (Gonsalez *et al*., 2019). The condition disrupts the kidneys' ability to filter waste and maintain fluid and electrolyte balance, leading to toxin buildup in the blood (Olano *et al*., 2024). AKI can also harm other organs, including the heart, brain and lungs. Although kidney function often recovers in survivors, AKI-related deaths remain high (Wu *et al*., 2017) and no effective treatment currently exists to prevent or manage the condition.

Among the multiple etiologies of AKI, rhabdomyolysis represents a major and well-recognized cause that significantly contributes to its incidence and severity. Rhabdomyolysis (RM), the breakdown of skeletal muscle releasing harmful substances like myoglobin into the

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bloodstream, is responsible for 10-40% of AKI cases and is a major contributor to its mortality (Stanley *et al*., 2024). Myoglobin causes kidney damage by constricting blood vessels, clogging kidney tubules and triggering oxidative stress, inflammation and cell death (Hebert *et al*., 2022). It also promotes the production of reactive radicals, activating nuclear factor kappa B (NF-κB), which increases inflammatory mediators like tumor necrosis factor-alpha (TNF-α) and interleukins during AKI. Antioxidants have shown promise in preventing or treating myoglobin-induced AKI (Al-Kharashi *et al*., 2023; El-khadragy *et al.,* 2023).

Investigating plant-based therapeutics has emerged as a *via*ble strategy for reducing the kidney damage linked to AKI, given the increased interest in natural substances as safer substitutes for synthetic medications. *Alpinia officinarum* (*A. officinarum*), a plant native to China and part of the Zingiberaceae family, is widely recognized for its medicinal properties due to its rich content of bioactive compounds like flavonoids, diarylheptanoids and volatile oils (Sun *et al*., 2023; Zheng *et al*., 2024; Wen *et al*., 2024). It contains unique isoflavones such as genistein and galangin, which offer various health benefits (Sharifi-Rad *et al*., 2021). Studies have shown that *A. officinarum* can protect kidney function in animal models (Javaid *et al*., 2021) and short-term use of its proteins has improved kidney function in patients with diabetes or chronic kidney disease (Aladaileh *et al*., 2021). Galangin, a key compound in *A. officinarum*, exhibits antioxidant, anti-inflammatory, antiapoptotic and anticancer properties (Guo *et al*., 2010). It has been shown to protect kidneys from cisplatin-induced damage in mice (Huang *et al*., 2017) and liver from injury caused by toxins (Luo *et al*., 2015). Additionally, galangin has been found to protect heart tissue from hypoxic-ischemic damage by activating the NRF2/HO-1 pathway (Yang *et al*., 2023).

While galangin's kidney-protective effects have been documented, its role in counteracting glycerol-induced kidney injury and the underlying mechanisms involving NF-κB and apoptosis pathways remain unexplored. In this study, glycerol was used to induce AKI in rats to investigate galangin's therapeutic potential. The study focused on evaluating key parameters and uncovering the mechanisms behind galangin's protective effects, including its impact on oxidative stress, inflammation and apoptosis in kidney tissue.

MATERIALS AND METHODS

Chemicals

Glycerol and galangin were procured from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals used in the study were of high analytical grade.

Experimental animals

Male Wistar albino rats were selected for this study due to their wide use in nephrotoxicity models and their availability in the animal facility of the Egyptian organization for biological products and vaccines (VACSERA). This ensured both scientific relevance and logistical accessibility, allowing for standardized handling and reliable replication of experimental procedures, the animals aged 3 months and weighing 180-200 g, were utilized in this study. The rats were sourced from VACSERA (Cairo, Egypt) and housed under controlled environmental conditions, including a 12-hour light-dark cycle, a temperature of 23± 2°C and relative humidity of 50±10%. They were provided with a standard diet and water *ad libitum*. Prior to the experiment, the rats were acclimatized for one week. All animal housing and experimental procedures were conducted in our institutional labs.

Induction of AKI and experimental design

Acute kidney injury (AKI) was induced by intramuscular injection of 50% glycerol (10 mL/kg, single dose) into the hind limbs of the rats after dilution in saline (0.9% NaCl). Rats were deprived of water for 24 hours before glycerol injection, as previously described (Kim *et al*., 2010).

Thirty-two rats were divided into four equal groups (n = 8 per group) as follows:

Group 1 (Negative Control) and was used as a baseline reference for all comparisons: Rats received an intramuscular injection of physiological saline (0.9% NaCl).

Group 2 (Gal): Rats were orally administered galangin at a dose of 100 mg/kg for 21 days, followed by an intramuscular injection of physiological saline (0.9% NaCl). The dosage of galangin was selected based on a previous study by Tomar *et al*. (2017).

Group 3 (AKI): Rats were injected with glycerol (50%, 10 mL/kg, intramuscular).

Group 4 (AKI + Gal): Rats were orally administered galangin at a dose of 100 mg/kg for 21 days, followed by an intramuscular injection of glycerol (50%, 10 mL/kg).

Twenty-four hours after glycerol administration, the rats were anesthetized *via* intraperitoneal injection of pentobarbital (100 mg/kg) and subsequently euthanized. Blood samples were collected from the retro-orbital plexus for biochemical analysis and the kidneys were immediately excised weighed and washed then homogenized in ice-cold 10 mM phosphate buffer (pH 7.4) to producea10%(w/v) homogenate for biochemical analysis. Using bovine serum albumin as a reference protein, the renal protein content was determined using the Lowry *et al*. (1951) technique.

Determination of kidney weight

The following mathematical formula was used to determine the relative kidney weight using the Almeer *et al*. (2019) method:

$$\text{Relative kidney weight} = \frac{\text{Left kidney}}{\text{Body weight}} \times 100$$

Assessment of the intensity of rhabdomyolysis

Using kits provided by Randox/Laboratory, Crumlin, UK, lactate dehydrogenase (LDH, Catalogue Number: LD3842,

measuring ranges 8.8 - 635U/l) and creatine kinase (CK, Catalogue Number: CK3812, measuring ranges 9.16- 2886U/l) were measured in accordance with the manufacturer's procedure. At 2 to 8°C, reagents remain stable until they expire.

Determination of renal function biomarkers

Using kits (Randox/Laboratory, Crumlin, UK), serum levels of urea (Catalogue Number: CR2336, measuring range: 11.4–2460μmol/l) and creatinine (Catalogue Number: UR446, measuring range: 0.866–56.7mmol/l) were determined in accordance with the manufacturer's instructions.

Estimation of renal non enzymatic oxidative stress markers

Using the Ohkawa *et al*. (1979) approach, lipid peroxidation was measured in terms of malondialdehyde (MDA). Furthermore, the Griess reagent was used to assess the amount of nitric oxide (NO) in renal tissues (Green *et al*., 1982). Additionally, Ellman (1959) formula for reduced glutathione (GSH) in renal tissue was used.

Measurement of kidney antioxidant enzymatic activities

The enzymes catalase (CAT) and superoxide dismutase (SOD) were tested in accordance with Aebi (1984) and Nishikimi *et al*. (1972), respectively. Additionally, glutathione reductase (GR) was calculated in accordance with De Vega *et al*. (2002) and glutathione peroxidase (GPx) was evaluated in accordance with Paglia and Valentine (1967).

Assessment of inflammatory related markers

TNF-α (Catalogue Number: NBP1-92681), interleukin-1β (IL-1β; Catalogue Number: NBP1-92702), interleukin-10 (IL-10; Catalogue Number: NBP1-92701) and NF-κB (Catalogue Number: NB100-2176) were measured in accordance with the manufacturer's instructions to assess the renal inflammatory response (Novus Biologicals, Centennial, CO, USA). Myeloperoxidase (MPO) activity was measured using the Bradley *et al*. (1982) methodology.

Assessment of the renal apoptotic markers

The levels of apoptotic proteins (Bax, caspase-3 and Bcl- 2) in renal tissues of all tested groups were measured by ELISA kit (Cusabio (Wuhan, China)) following the manufacturer's information.

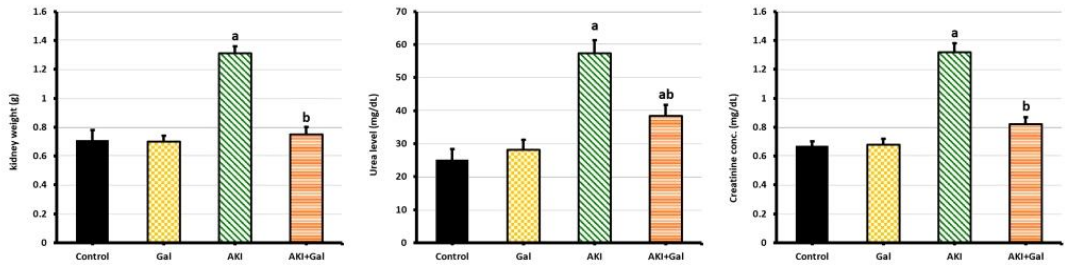
Statistical analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS). The mean±standard error of the mean (SEM) was used to represent the results. The significance was assessed using Duncan's test after one-way analysis of variance (ANOVA). At $p<0.05$, the acceptable threshold of significance was determined.

RESULTS AND DISCUSSION

The results of present study demonstrate the protective effects of galangin on renal function and oxidative stress in a rat model of acute kidney injury (AKI) induced by glycerol. Acute kidney injury (AKI) is marked by a rapid decline in glomerular filtration rate (GFR) and the accumulation of nitrogenous waste in the bloodstream (Zywno *et al*., 2024). Although nephrotoxic agents like gentamicin and cisplatin are commonly used to model AKI in animals, they do not fully capture the pathophysiology of AKI resulting from rhabdomyolysis (RM). RM, a significant contributor to AKI, occurs due to the breakdown of skeletal muscle, leading to the release of muscle enzymes and proteins into circulation (Wang *et al*., 2021). As illustrated in Fig 1, the relative kidney weight in the AKI group was significantly higher (1.31±0.05) compared to the control group (0.71±0.08) at $P<0.05$, indicating renal damage. Elevated plasma creatinine and urea levels further confirmed impaired kidney function in the AKI group. However, treatment with galangin significantly improved renal function biomarkers, suggesting its nephroprotective potential (Fig 1).

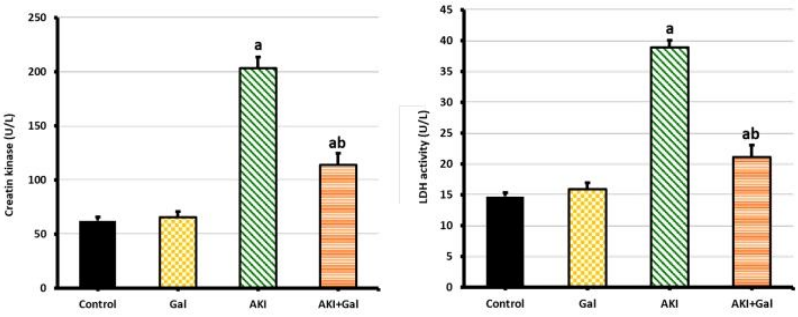
In this study, a glycerol-induced RM model was employed to replicate key clinical features of AKI, including



Statistical significance was set at $P<0.05$. Key findings include: (a) a significant difference ($P<0.05$) between all groups compared to the control group and (b) a significant difference ($P<0.05$) with respect to AKI group. Data are expressed as mean±standard error (SE),(n = 8).

Fig 1: Impact of galangin pretreatment on kidney weight, urea and creatinine levels in a glycerol-induced acute kidney injury (AKI) model in rats.

myoglobin accumulation, tubular obstruction and renal impairment. The administration of galangin demonstrated notable protective effects against RM-induced AKI, as reflected in functional and biochemical improvements. Additionally, serum creatine kinase (CK) (203.38±15.21) and lactate dehydrogenase (LDH) (38.83±7.23) levels, which were markedly increased (P<0.05) in the AKI group as a result of glycerol intramuscular injection. These elevations were significantly reduced by galangin administration at a dose of 100 mg/kg (113.59±10.45) and (21.10±35) respectively, at P<0.05 (Fig 2). These findings highlight galangin's ability to mitigate muscle fibre damage caused by glycerol injection. AKI following RM is characterized by elevated creatine kinase (CK) and lactate dehydrogenase (LDH) levels, indicating muscle damage, in agreement with previous studies (Yin and Wang. 2024). Glycerol-induced RM triggers myoglobin release, which, when exceeding plasma protein binding capacity, accumulates in renal tubules, leading to obstruction and tubular cell death (Qiao *et al*., 2023). The study observed increased kidney weight, along with elevated serum creatinine and urea levels, which are indicative of renal



Statistical significance was set at *P*<0.05. Key findings include: (a) a significant difference (*P*<0.05) between all groups compared to the control group and (b) a significant difference (*P*<0.05) with respect to AKI group. Data are expressed as mean ± standard error (SE), (*n* = 8).

Fig 2: Effect of galangin pretreatment on the rhabdomyolysis-related biomarkers in glycerol-induced acute kidney injury (AKI) model in rats.

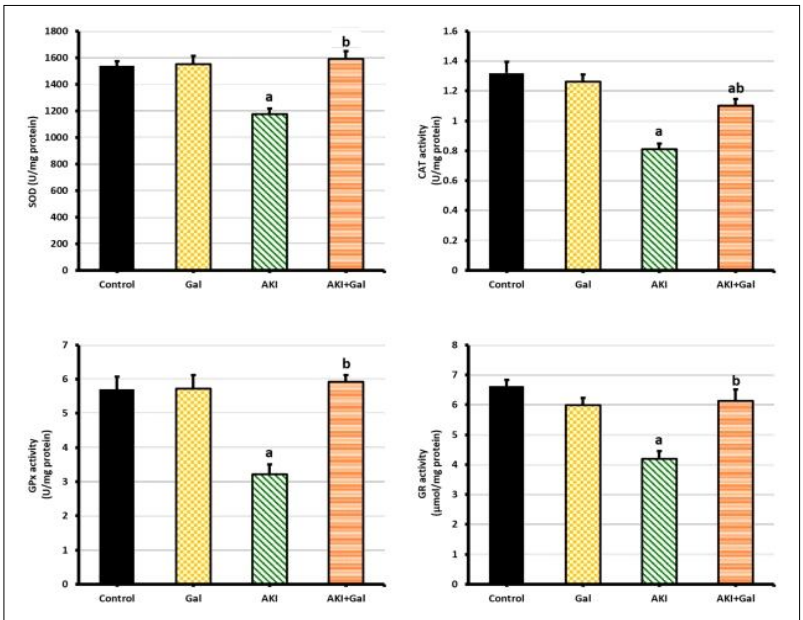


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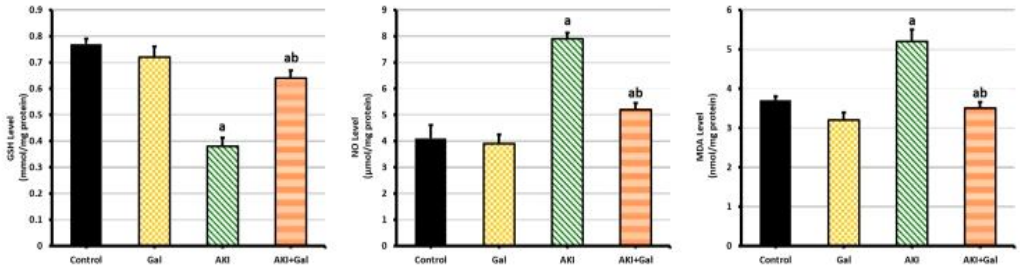
Fig 3: Effect of galangin pretreatment on renal enzymatic antioxidant biomarkers (SOD, CAT, GPx and GR) in glycerol-induced acute kidney injury (AKI) model in rats.

edema and impaired kidney function, consistent with earlier findings (Brookes and Power, 2022; El-Khadragy *et al.,* 2024).

The antioxidant enzymatic activity in renal tissues was evaluated, as shown in Fig 3. Rats treated with glycerol exhibited significantly reduced (P<0.05) activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) compared to the sham group. In contrast, pretreatment with galangin significantly enhanced (P<0.05) the activities of these enzymes, with SOD and GR levels in the galangin-treated group reaching levels comparable to those of the control group. Furthermore, non-enzymatic oxidant biomarkers were analyzed (Fig 4). Glycerol administration led to a significant decline (P<0.05) in glutathione (GSH) levels and a concurrent increase (P<0.05) in malondialdehyde (MDA) and nitric oxide (NO) levels compared to the sham group. Galangin treatment reversed these changes, demonstrating its ability to alle*via*te oxidative stress in renal tissues by enhancing antioxidant defenses and reducing oxidant markers.

The anti-inflammatory effects of galangin were also investigated (Fig 5). Glycerol-induced AKI was associated with a significant increase (P<0.05) in pro-inflammatory cytokines (IL-1β, TNF-α and MPO) and a decrease (P<0.05) in the anti-inflammatory cytokine IL-10. Additionally, nuclear factor-kappa B (NF-κB) levels were elevated (P<0.05) in the AKI group compared to the sham group. Remarkably, galangin administration at 100 mg/kg restored the levels of these inflammatory markers significantly as compared to AKI group. Oxidative stress, primarily driven by reactive oxygen species (ROS), is a key factor in the development of glycerol-induced AKI (Hebert *et al*., 2022). Myoglobin and heme derivatives released during RM contribute to excessive ROS production, which leads to oxidative damage in renal tissues. In this study, glycerol administration was associated with increased levels of malondialdehyde (MDA)



Statistical significance was set at *P*<0.05. Key findings include: (a) a significant difference (*P*<0.05) between all groups compared to the control group and (b) a significant difference (*P*<0.05) with respect to AKI group. Data are expressed as mean±standard error (SE), (*n* = 8).

Fig 4: Effect of galangin pretreatment on GSA, NO and MDA in glycerol-induced acute kidney injury (AKI) model in rats.

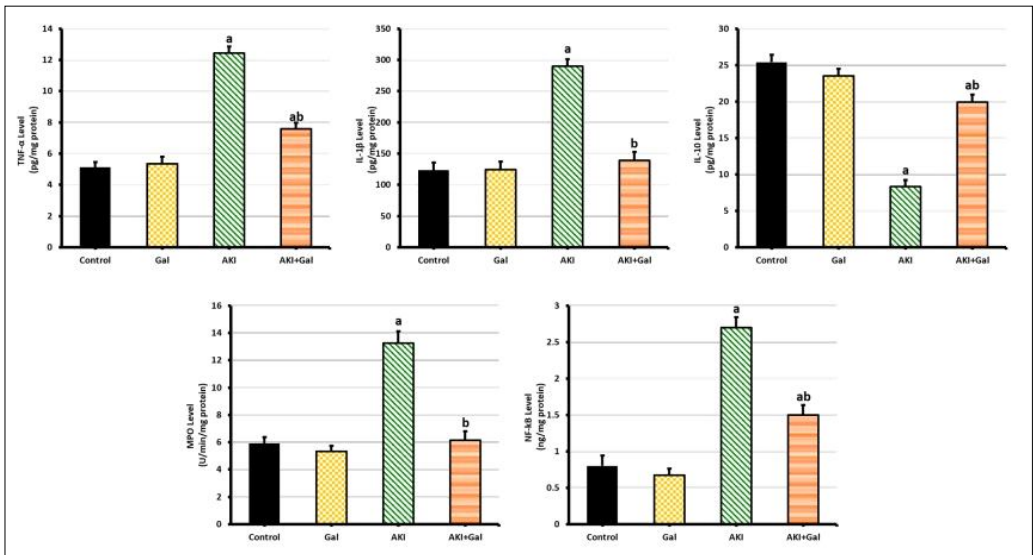


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Fig 5: Effect of galangin pretreatment on inflammatory biomarkers in glycerol-induced acute kidney injury (AKI) model in rats.

and nitric oxide (NO), along with a reduction in glutathione (GSH) and decreased activity of key antioxidant enzymes (SOD, CAT, GPx and GR). These results are in line with previous research (Gyurászová *et al*., 2020; Salem *et al.,* 2023).

Galangin, a flavonoid compound found in Alpinia officinarum improves kidney function in acute kidney injury (AKI) by reducing oxidative stress, thereby preserving glomerular filtration rate (GFR) and enhancing creatinine and urea clearance as observed in the present results (Alsawaf *et al*., 2022). Its antioxidant properties - due to its phenolic structure - neutralize reactive oxygen species (ROS), preventing lipid peroxidation, mitochondrial damage and endothelial dysfunction (Aloud *et al*., 2017). By upregulating the Nrf2/HO-1 pathway, galangin boosts antioxidant defenses, enhances glutathione (GSH) levels and protects renal tubular cells. It also inhibits the NF-| B pathway, reducing inflammation and cytokine production, which helps maintain kidney structure and prevent fibrosis (Khawaja *et al*., 2024; Almohawes *et al.,* 2025). Additionally, galangin restores nitric oxide (NO) bioavailability, improving renal blood flow and preventing vasoconstriction-induced damage (Ahmad *et al*., 2018). By protecting renal cells from oxidative and inflammatory injury, galangin preserves glomerular and tubular integrity, ensuring efficient creatinine and urea excretion, ultimately mitigating AKI progression.

The present study examined apoptotic markers in renal tissues (Fig 6). Glycerol injection resulted in increased ($P<0.05$) expression of pro-apoptotic proteins (Bax and caspase-3) and decreased ($P<0.05$) levels of the anti-apoptotic protein Bcl-2 markedly compared to the sham group. Galangin co-administration effectively suppressed apoptosis in renal tissues, as evidenced by the amelioration of these markers significantly. Inflammation and leukocyte infiltration are characteristic features of RM-induced AKI. Excess ROS production amplifies inflammation by activating the NF-κB pathway, which regulates the expression of pro-inflammatory cytokines such as IL-1β, TNF-α and MPO (McSweeney *et al*., 2021). The current study observed a significant increase in these inflammatory markers following glycerol injection, this is in agreement with previous studied. MPO, released by neutrophils, generates hypochlorous acid, which contributes to lipid peroxidation and oxidative stress, further exacerbating renal damage (Kisic *et al*., 2016).

Galangin's anti-inflammatory effects in acute kidney injury (AKI) are primarily driven by its polyphenolic structure, rich in hydroxyl (-OH) groups, which enable ROS scavenging and modulation of key inflammatory pathways (Ashkar *et al*., 2022). It inhibits NF-κB signaling, reducing the expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, COX-2) and suppressing neutrophil infiltration. Additionally, galangin activates the Nrf2/HO-1 pathway, enhancing heme oxygenase-1 (HO-1) production to counteract oxidative stress and inflammation. It also inhibits iNOS, preventing excessive nitric oxide (NO) production and suppresses the MAPK pathway (p38, JNK, ERK), further reducing cytokine-driven inflammation (Khawaja *et al*., 2024).

The interplay between oxidative stress and inflammation promotes apoptotic cell death in AKI. In this study, glycerol administration led to increased expression of pro-apoptotic markers, including Bax and caspase-3, while reducing the levels of the anti-apoptotic protein Bcl-2. These observations suggest an apoptotic shift, consistent with previous reports (Guttà *et al*., 2020). Bcl-2 and Bax regulate mitochondrial membrane integrity, with Bcl-2 favoring cell survival and Bax promoting apoptosis (Qiao *et al*., 2023).

Galangin attenuates apoptosis in acute kidney injury (AKI) by regulating key pro-apoptotic and anti-apoptotic markers. Its polyphenolic structure enables it to neutralize oxidative stress and inhibit inflammation, both of which contribute to apoptosis (Aladaileh *et al*., 2019). Galangin downregulates pro-apoptotic proteins such as Bax and caspase-3, preventing mitochondrial dysfunction and cell death. Simultaneously, it upregulates anti-apoptotic Bcl-2, preserving mitochondrial integrity and promoting cell survival. Additionally, galangin activates the PI3K/Akt pathway, which enhances cell survival by suppressing apoptotic signaling (Palachai *et al*., 2025). By reducing oxidative damage, inhibiting NF-κB-mediated inflammation and balancing apoptotic regulators, galangin effectively protects renal cells from programmed cell death, aiding in kidney recovery during AKI.

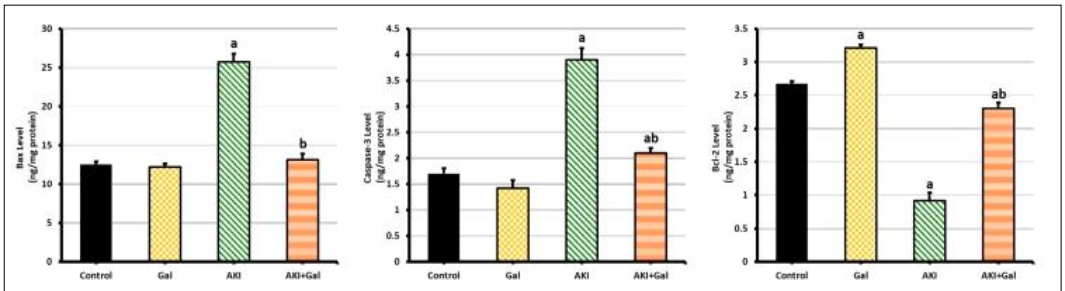

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Fig 6: Effect of galangin pretreatment on Apoptotic biomarkers in glycerol-induced acute kidney injury (AKI) model in rats.

Although this study highlights the renoprotective effects of galangin, its clinical applicability is limited by the pretreatment duration of 21 days. Future research should explore the efficacy of galangin administered post-AKI induction or simultaneously with injury onset to better mimic clinical conditions. Additionally, further investigation is warranted to delineate the precise molecular mechanisms through which galangin modulates oxidative stress, inflammation and apoptosis in AKI.

CONCLUSION

In conclusion, galangin exerts protective effects against glycerol-induced AKI through multiple mechanisms, including the enhancement of antioxidant defenses, reduction of oxidative stress, suppression of inflammation and inhibition of apoptosis. These findings underscore the therapeutic potential of galangin in the management of AKI and provide a foundation for further research into its clinical applications.

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Disclaimers

The views and conclusions expressed in this article are solely of the authors and do not necessarily represent the views of our affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures of these experiments were approved by the Committee of Experimental Animal Care and handling techniques were approved by the University of Animal Care Committee. By the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, 8th edition, all protocols and animal handling at the Department of Zoology, Faculty of Science, Helwan University were approved by the Committee on Research Ethics for Laboratory Animal Care (Cairo, Egypt; approval no. HU2021/Z/RKA0921-01).

Conflict of interest

There are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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