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Renal protection by ellagic acid in a rat model of glycerol-induced acute kidney injury

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Abstract

Studies conducted on animal models have shown that the administration of glycerol can lead to kidney tissue damage and impaired renal function. This is believed to be caused by oxidative stress and inflammation, which in turn can result in elevated levels of blood urea nitrogen (BUN) and creatinine. These metabolites are commonly used as indicators of renal function. The aim of the current experimental research was to investigate the protective efficacy of ellagic acid in a rat model of rhabdomyolysis induced by glycerol. Sixty healthy adult male *Wistar* rats weighing between 250 - 300 g were divided into five equal groups including control, rhabdomyolysis (administered 8.00 mL kg^{-1} of glycerol), and three rhabdomyolysis plus various doses of ellagic acid (25.00, 50.00 and 100 mg kg^{-1} *per* day; 72 hr after receiving glycerol for 14 days successively) groups. Serum levels of BUN, creatinine, lactate dehydrogenase, alkaline phosphatase, electrolytes and inflammatory cytokines were evaluated in all rats. Histopathological studies were also performed on kidney tissues from all groups. The administration of ellagic acid resulted in a significant increase in renal function biomarkers compared to the rats with acute kidney injury. This increase was consistent with notable reductions in tumor necrosis factor-α levels and increases in interleukin-10 levels observed in blood samples. Furthermore, the improvement in histopathological indices observed in rats received ellagic acid confirmed its nephroprotective role. The results of the current experimental study suggest that ellagic acid can improve kidney damage following glycerol injection, potentially by modulating the inflammatory process.

Introduction

Kidney and urinary tract disorders can have serious health consequences, and in some cases can lead to the significant mortality rates.[1] Recent studies have shown that acute kidney injury (AKI) is prevalent around the world, with a rate of approximately 34.00%. In hospitalized patients with AKI, the mortality rate is as high as 62.00%.[2] Acute kidney injury is a common occurrence in the population,[3] with an incidence rate reported to be 4.30% for contrast-associated AKI and 2.10% for hospitalized AKI, according to a study by Wonnacott *et al*.[4] The study found that both types of AKI share similar risk factors. The AKI is characterized by a sudden decline in renal function, as evidenced by a rise in serum creatinine levels of more than 0.30 mg dL^{-1} within 2 days, or a decrease in urine output of ≥ 0.50 mL kg^{-1} *per* hr within 6 hr.[5] However, it's important to note that neither serum creatinine nor urine output is a specific factor of AKI.[6]

Acute kidney injury can be caused by several factors. Pre-renal causes include congestive heart failure, pulmonary thromboembolism and pericardial tamponade. Intra-renal causes may include acute glomerulonephritis, malignant vasculitis, nephrolithiasis, thrombotic microangiopathies, high blood pressure, renal vessel damage and acute tubular necrosis. Post-renal causes may involve extra-renal obstruction, ureteral narrowing or coagulation and kidney stones.[6]

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Rhabdomyolysis is a pathological disorder characterized by the destruction of muscle cells, leading to the leakage of myoglobin and other proteins as well as electrolytes into the systemic circulation.[7] This can cause AKI, with a reported prevalence of 13.00 - 50.00%.[6] Following rhabdomyolysis, myoglobin, creatine phosphokinase and lactate dehydrogenase (LDH) are released, filtered by renal glomeruli and can cause obstruction in renal tubules, inflammation, tubular damage, renal vasoconstriction, and ultimately AKI.[8] Studies have shown that myoglobin released from muscle damage is the most important cause of nephropathy in rhabdomyolysis.[9] During this pathological process, myoglobin diffuses in the systemic circulation and can deposit into the renal tubules, contributing to the development of AKI.[9]

Previous studies have shown that myoglobin can cause oxidation of lipid membranes in kidney cells, resulting in the production of arachidonic acid derivatives such as malondialdehyde and isoprostanes. These biomolecules are potent vasoconstrictors and can lead to organ ischemia.[10] The heme component of myoglobin has been identified as a key factor in membrane peroxidation and tissue damage.[11] Additionally, the breakdown of myoglobin within renal tubules can result in the release of iron and production of free radicals, exacerbating ischemic damage. However, even in the absence of free iron release, lipid peroxidation can occur in kidney tissue. Severe increases in free radical production due to myoglobinuria and oxidative stress can ultimately lead to renal failure.[12-14]

The primary goal of treatment strategies for rhabdomyolysis is to reduce risk and damaging factors that can lead to renal failure, such as hypovolemia, intra-tubular obstruction, metabolic acidosis, and coagulation in the renal vascular system. Also, hyperkalemia is a common electrolyte disorder associated with rhabdomyolysis, which can be caused by muscle damage or acute kidney failure. This disorder may lead to cardiac arrhythmia and should be treated promptly to prevent complications.[15,16]

Various *in vivo* and *ex vivo* experimental models using laboratory animals have demonstrated that glycerol injection can induce acute renal failure through rhabdomyolysis.[15,17] Glycerol leads to the disintegration of muscle cell membranes, releasing iron-containing proteins into the extra-cellular environment.[17] The accumulation of protein-containing cells in renal tubules, along with narrowing of renal blood vessels in response to adenosine, can cause damage to proximal and distal convoluted tubules in the renal cortex[17], resulting in acute renal failure.

The LDH, a glycolytic enzyme, is widely distributed in almost all tissues of the human body. The majority of renal nephrons contain LDH, and its levels increase in response to tissue damage.[18] Clinical observations indicate that patients with kidney disease exhibit a significant increase in serum LDH and its isozymes levels. Similarly, alkaline phosphatase (ALP) is another enzyme whose levels are increased in patients with chronic kidney disease (CKD) without liver disorders. Modulating this biomarker is a therapeutic goal in patients with CKD. Elevated ALP levels have also been found to be associated with mortality from coronary artery calcification in patients suffering from CKD. This underscores the importance of monitoring these enzymes as a part of routine clinical management of CKD.[19]

Over the years, therapeutic supplements extracted from plant-derived active compounds have been utilized in treating various illnesses using a therapeutic approach. Among these bioactive components, polyphenols stand out for their potent anti-oxidant properties.[20]

Ellagic acid, with a molecular formula of $C_{14}H_6O_8$ and a molecular weight of 302 g mol^{-1}, is a bioactive compound known for its medicinal properties. This compound is abundant in several fruits, including pomegranate, strawberry, raspberry and grape. It is known for its potent anti-oxidant and anti-inflammatory properties. Various experimental studies have demonstrated that this compound can induce apoptosis and cell death in cancer cell lines; thereby, preventing tumor growth.[21,22] It is a versatile compound that finds application in various medicinal uses such as anti-oxidant, anti-cancer, anti-allergic, anti-malarial and anti-inflammatory activities. It has been extensively studied for its therapeutic potential and has shown promising results in treating several health conditions. Ellagic acid exhibits potent anti-oxidant properties, being proven to be effective in the treatment of AKI. Its anti-oxidative action helps maintain normal levels of blood urea nitrogen (BUN) and creatinine; thereby, playing a crucial role in managing AKI.[23]

According to the literature, various factors such as traumatic kidney damage, drug and toxin side effects, infections, muscle ischemia, and electrolyte and metabolic disorders contribute to the development of rhabdomyolysis. In light of this information, the aim of this experimental study was to assess the potential anti-inflammatory effect of ellagic acid in the treatment of rhabdomyolysis induced by glycerol.

Materials and Methods

Animals. This study utilized a total of 60 male *Wistar* rats, with weights ranging from 280 - 300 g. The rats were obtained from the Animal Care and Breeding Centre of Jundishapur University of Medical Sciences in Ahvaz, Iran, and housed under standard conditions with a temperature range of 22.00 - 24.00 ˚C, 50.00 - 55.00% humidity, and a 12-hr light/dark cycle. Throughout the experiment period, the rats had free access to food and water. All experimental procedures were conducted in accordance with the institutional guidelines set forth by the Experimental Animal Ethics Committee of Ahvaz Jondishapur University of Medical Sciences, Ahvaz, Iran (Ethics Code: IR.AJUMS.REC.1399.051).

Experimental design. Initially, all rats were randomly divided into five groups of equal size including control group which received 5.00 mL kg^{-1} dimethyl sulfoxide (DMSO) 5.00% as a solvent for ellagic acid (Sigma Chemical Co., St. Louis, USA) orally for two consecutive weeks, AKI group which given DMSO 5.00% (5.00 mL kg^{-1}, orally), followed by intra-muscular 8.00 mL kg^{-1} glycerol (Sigma Chemical Co.) after 72 hr to induce AKI, and treatment groups which received three concentrations of ellagic acid (25.00, 50.00 and 100 mg kg^{-1} *per* day respectively by gavage) for 14 consecutive days. After 72 hr, they were orally administered 8.00 mL kg^{-1} glycerol.

Renal function assay. Under deep anesthesia induced by a mixture of 80.00 mg kg^{-1} ketamine (Akorn, Decatur, USA) and 8.00 mg kg^{-1} xylazine (NexGen, Weatherford, USA), blood samples were collected from the heart of the rats and centrifuged at 3,000 rpm for 10 min. The supernatant samples were then evaluated to determine the levels of various biochemical parameters such as serum BUN, creatinine, potassium and sodium. In addition, levels of LDH and ALP were determined using assay kits (Span Diagnostics, Surat, India) and a spectrophotometer.

Inflammatory cytokines measurement. After confirming deep anesthesia, the kidneys were quickly removed from all rats and homogenized using 100 mg of tissue *per* 1.20 mL of cold phosphate-buffered saline with a protease inhibitor cocktail. The resulting mixture was then centrifuged at 10,000 *g* for 20 min at 4.00 ˚C, yielding supernatant samples. The levels of two cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-10, were measured in these samples using enzyme-linked immuno-sorbent assay (ELISA) kits from ZellBio GmbH (Lonsee, Germany), according to the manufacturer's guidelines for the assay (TNF-α: Sandwich ELISA, wavelength: 570 nm; IL-10: Sandwich ELISA, wavelength: 630 nm) using ELISA Micro Plate Reader (Dynex, Chantilly, USA).

Histological assessment of kidney tissue. At the end of the experiment, the kidneys were collected and washed before being fixed in a 10.00% buffered formalin solution. For histopathological examination, they were stained with hematoxylin and eosin (H & E) and evaluated under light microscopy (Olympus, Tokyo, Japan). The H & E staining is a commonly used technique for visualizing cellular structures and identifying any abnormalities or damage in tissues. Moreover, the grading scale method was used to score the kidney tissue damage. The scale graded the histopathological injuries as follows: 0: No injuries; 1+: Changes affecting < 25.00% of the tissue; 2+: Changes affecting 25.00 - 50.00% of the tissue; 3+: Changes affecting 50.00 - 75.00% of the tissue; 4+: Changes affecting > 75.00% of the tissue.[24]

Statistical analysis. The data were presented as Mean ± SEM, and the normality of the data was evaluated using the Shapiro-Wilk Kolmogorov test. Statistical analysis was performed using GraphPad Prism Software (version 6.0; GraphPad Software Inc., San Diego, USA). One-way ANOVA followed by Tukey's *post-hoc* test was used to analyze the data and *p*-values less than 0.05 were considered statistically significant. The ANOVA is a statistical test used to determine whether there are any statistically significant differences among the means of three or more independent groups. Tukey's *post-hoc* test is used to compare all possible pairs of means following significance in ANOVA.

Results

Effect of ellagic acid on renal function. To confirm the presence of rhabdomyolysis and kidney damage in the experimental rat model, concentrations of creatinine, BUN, LDH and ALP were measured in the blood samples of all groups. The AKI group rats displayed a noteworthy increase in creatinine, BUN, LDH and ALP concentrations in comparison with control rats ($p < 0.001$). Conversely, in the rats treated with ellagic acid, there was a substantial reduction in the concentrations of creatinine, BUN, LDH and ALP compared to the AKI group (Figs. 1A-D). The highest reduction in concentration was observed in the group receiving a dose of 100 mg kg^{-1}, which was statistically significant.

Effect of ellagic acid on serum electrolytes. The AKI group exhibited a significantly lower serum sodium level than the control rats ($p < 0.05$). However, ellagic acid treatment caused a notable increase in serum sodium levels compared to the disease model group. In contrast, the serum potassium level in the AKI group showed a significant increase ($p < 0.05$) compared to the control group. Nonetheless, treatment of AKI rats with various doses of ellagic acid for 14 days led to a noteworthy decrease in serum potassium levels compared to the AKI rats ($p < 0.05$; Fig. 2).

Inflammatory cytokines in kidney tissue. As shown in Figure 3A, levels of TNF-α in the AKI group displayed a significant increase compared to the control group ($p < 0.001$). Interestingly, administration of ellagic acid to AKI rats resulted in a dose-dependent remarkable reduction of TNF-α ($p < 0.05$, $p < 0.01$). On the other hand, IL-10 levels in the AKI rats were significantly decreased compared to the control group ($p < 0.001$). However, treatment of AKI rats with 50 and 100 mg kg^{-1} of ellagic acid caused a significant elevation in IL-10 levels compared to the AKI group *(*$p < 0.05$, $p < 0.01$; Fig. 3B).

Histological changes. Figure 4 displays an analysis of histopathological indicators obtained from kidney tissue of control group and other experimental groups. The findings revealed significant damage in both renal cortex and medulla regions among AKI rats, including renal cell and tubular necrosis, as well as vacuolation (Fig. 4). However, treatment with all doses of ellagic acid resulted in a notable reduction in the severity of renal tissue injuries.

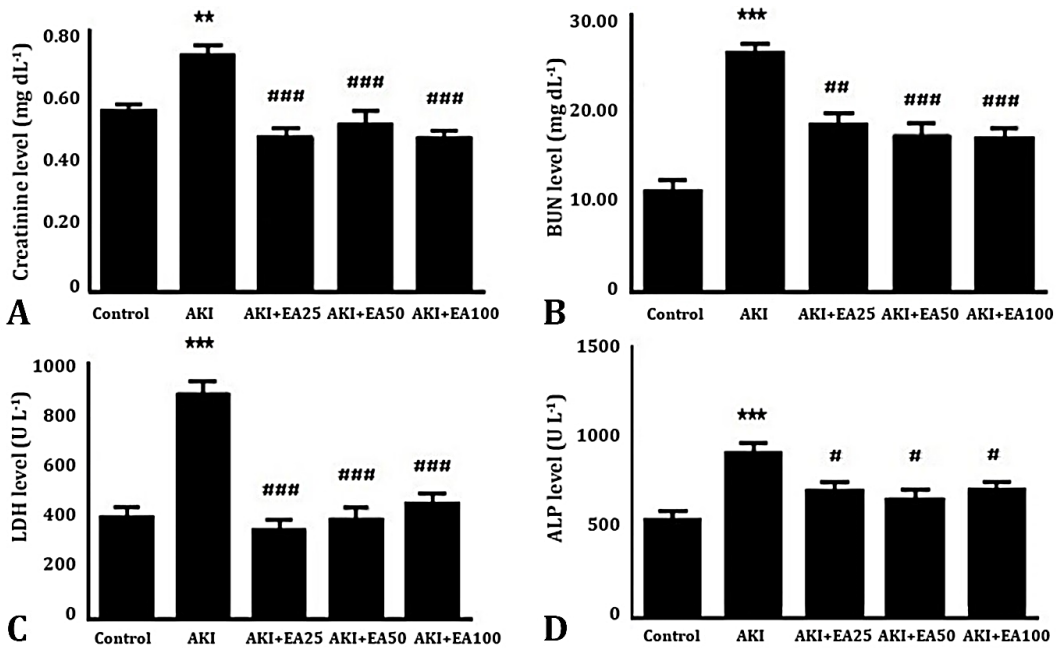

Fig. 1. The effect of ellagic acid (EA) on the levels of serum **A)** creatinine (Cr), **B)** blood urea nitrogen (BUN), **C)** lactate dehydrogenase (LDH) and **D)** alkaline phosphatase (ALP). Results showed that glycerol administration led to a significant increase in Cr, BUN, LDH and ALP biomarkers compared to the control group. The *p*-values for these differences were $^{**}p < 0.01$ and $^{***}p < 0.001$. Notably, there was a significant decrease in the levels of these biomarkers in the EA-treated groups compared to the acute kidney injury (AKI) one, with corresponding *p*-values of $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$.

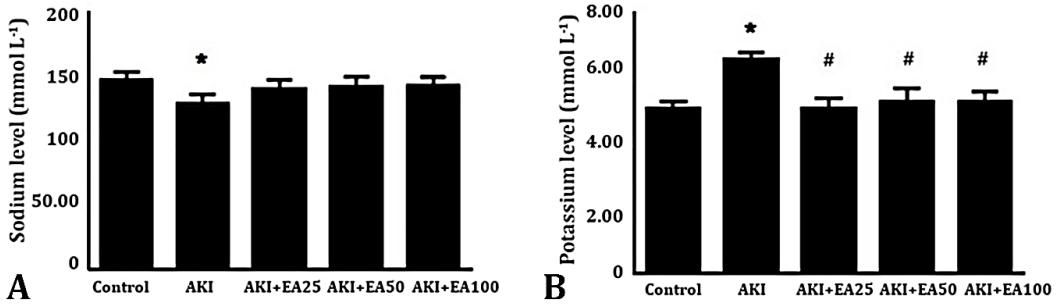

Fig. 2. The effect of ellagic acid (EA) on serum **A)** sodium and **B)** potassium. Results showed that glycerol administration led to a significant decrease and increase in serum sodium and potassium levels compared to the control group, respectively. The *p*-value for this difference was $^{*}p < 0.05$. Notably, there was a significant decrease in serum potassium levels following EA treatment compared to the acute kidney injury (AKI) group, with a corresponding *p*-value of $^{\#}p < 0.05$.

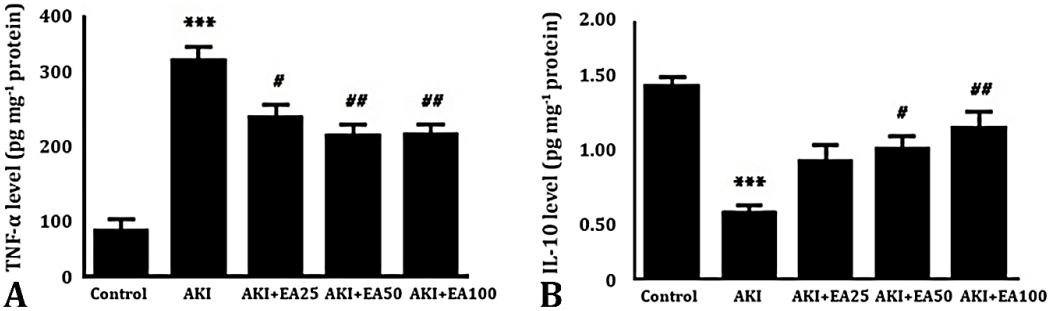

Fig. 3. The effect of ellagic acid (EA) on **A)** tumor necrosis factor (TNF)-α and **B)** interleukin (IL)-10. Results showed that glycerol administration resulted in a significant elevation in TNF-α levels compared to the control group, with a corresponding *p*-value of $^{***}p < 0.001$. Notably, there was a significant higher TNF-α levels in the acute kidney injury (AKI) group compared to the EA-treated groups, with corresponding *p*-values of $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$. Similarly, glycerol administration significantly reduced IL-10 levels compared to the control group, with a corresponding *p*-value of $^{***}p < 0.001$. There was also a significant lower IL-10 levels in the AKI group compared to the EA-administered groups, with a corresponding *p*-value of $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$.

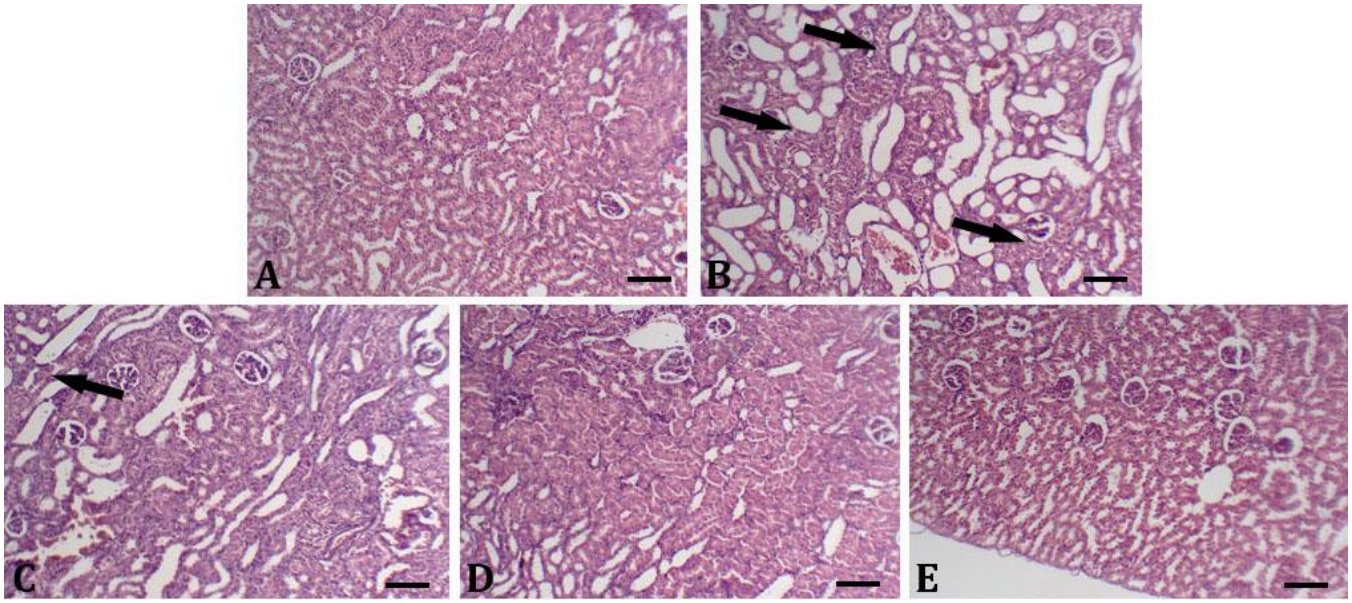

Fig. 4. The effect of ellagic acid (EA) on renal tissue. **A)** Control group; **B)** Acute kidney injury group: Widespread damage is obvious, being evidenced by tubular dilatation and vacuolation in the renal tubular lumen, as well as severe interstitial inflammatory cells infiltration; **C, D** and **E)** Treatment groups which received three concentrations of EA (25.00, 50.00 and 100 mg kg^{-1}, respectively). The EA treatment markedly mitigated the severity of renal injuries. The black arrows indicate inflammatory cells accumulation (Hematoxylin and Eosin staining, bars = 50.00 μm).

These results demonstrated the effectiveness of ellagic acid in preventing widespread renal damage. Also, the evaluation of quantitative scores following H & E staining of the kidney showed that the histological damage was significantly higher in glycerol-treated rats compared to the other groups (Fig. 4B).

Discussion

The data obtained from the current experimental model of acute kidney failure indicate that ellagic acid has anti-inflammatory properties that can prevent the harmful effects of glycerol-induced kidney injuries.

Rhabdomyolysis is a pathological process occurring in muscle cells, causing the release of cellular contents into the extra-cellular fluid. One of the main destructive agents in this process is myoglobin. Studies have shown that this condition is associated with renal dysfunction in approximately 2.00 - 5.00% of patients referred to the emergency department.[25] Rhabdomyolysis is responsible for causing acute renal injury in 10.00 - 40.00% of all cases of renal failure. The breakdown of myoglobin inside the renal tubules leads to the release of free iron, inducing free radicals production, exacerbating renal ischemic damage.[26] Myoglobinuric renal failure is also associated with the involvement of reactive oxygen species (ROS).[27] The primary aim of therapeutic strategies is to prevent or treat glomerular filtration rate reduction, inflammation, tubular obstruction, aciduria and free radical release exacerbation. An imbalance between the ROS productions can cause oxidative stress and lead to chronic inflammation. This oxidative stress can activate various transcription factors, resulting in significant up/down-regulation of genes involved in inflammatory pathways.[28]

Various researchers have used glycerol administration to establish an experimental rhabdomyolysis model leading to renal failure in rodents.[29] In this context, Park *et al*. reported the association of kidney inflammation and oxidative reactions in rhabdomyolysis induced by single injection of glycerol in rats.[30] Karam *et al*. conducted a study that aimed to clarify the effectiveness of endothelin in acute renal failure secondary to rhabdomyolysis. Intra-muscular injection of glycerol (50.00%) was administered to rats, and after 72 hr, acute renal failure was established.[31] Consistent with previous studies, the results of the current study indicate that acute renal failure can be induced by intra-muscular injection of 50.00% glycerol at a dose of 8.00 mL kg^{-1} in rats.

Significant decreases in kidney biomarkers, such as creatinine and BUN, are important indicators of renal function. Decreases in sodium and potassium levels also suggest the establishment of acute renal failure due to the damage caused by glycerol-induced rhabdomyolysis in rat leg muscles. One possible explanation for the increase in renal damage following rhabdomyolysis is the destructive effects of ROS and inflammatory processes on kidney cells.[32,33] Alleviating the inflammatory process is a desirable target for the treatment and prevention of kidney injuries. A study by Reis *et al*. confirmed that an increase in inflammatory cytokines during rhabdomyolysis reactions may lead to the renal dysfunction.[34]

The current experimental study investigated the protective effect of ellagic acid, a natural supplement, in preventing the destructive effects of glycerol on the kidney. Ellagic acid as a polyphenolic agent is found naturally in several fruits and vegetables and known for its efficacy in reducing lipid peroxidation and inhibiting the inflammatory cascade.[35] A study by Rosillo *et al*. has demonstrated that ellagic acid reduces colon inflammation *via* leukocyte secretion suppression and anti-inflammatory cytokines activation.[36] A study by Craig *et al*. reported that ellagic acid can down-regulate some cell signaling pathways involved in the inflammation process.[37] Our data are in line with the mentioned findings, confirming the protective role of ellagic acid against glycerol-induced renal dysfunction through improving renal tissue histopathology. The current study documented that treatment with different doses of ellagic acid resulted in a decrease in pro-inflammatory cytokines and an increase in IL-10, an anti-inflammatory factor.

In summary, our findings indicate that ellagic acid, an anti-oxidant and anti-inflammatory compound, may be considered as a potent strategy to prevent rhabdomyolysis and subsequent AKI through inflammation and oxidative stress modulation in the kidney.

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Conflict of interest

The authors declare no conflicts of interest.

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