ORIGINAL ARTICLE

THE POTENTIAL RENOPROTECTIVE EFFECT OF TILIANIN IN RENAL ISCHEMIA REPERFUSION INJURY IN MALE RAT MODEL

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ABSTRACT

The aim: To determine whether Tilianin (TIL) may have Nephroprotective effects on bilateral renal IRI in rats by analyzing kidney function biomarkers U and Cr, inflammatory cytokines like TNF α and IL-1β, antioxidant marker total anti-oxidant Capacity (TAC), anti-apoptotic markers caspase-3, and histopathological scores.
Materials and methods: 20 rats divided into even 4 groups as: Sham group: Rats underwent median laparotomies without having their ischemia induced. Control group: Rats had bilateral renal ischemia for 30 minutes, followed by 2 hours of reperfusion. Vehicle group: 30 minutes prior to the onset of ischemia, rats were given a pretreatment of corn oil and DMSO. Tilianin treated group: Rats administered Tilianin 5 mg/kg for 30 min prior to ischemia induction, then IRI.
Results: The study found that the serum levels of TNF, IL-1, caspase-3, urea and creatinine, as well as TNF and creatinine in the Tilianin group were significantly lower than those of the control and vehicle groups. On the other hand, it revealed that TAC levels are remarkably higher in the Tilianin group than they are in the control and vehicle groups.
Conclusions: This study concluded that Tilianin have a Nephroprotective effect via multiple impacts as anti-inflammatory, anti-apoptotic, and anti-oxidant agents.

KEY WORDS: Tilianin, renal ischemia reperfusion injury, male rat model

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INTRODUCTION

A temporary decrease in blood flow that carrying oxygen to the renal then followed by reperfusion, referred to a renal ischemia reperfusion injury (IRI). During IRI, kidney tissue injury promotes excess production of reactive oxygen species (ROS), which induces oxidative stress. During reperfusion time, restoration of blood flow generates even greater generation of ROS that ends up with apoptosis and cell death [1]. A clinical state known as acute kidney injury (AKI), which is marked by fast renal failure and high mortality rates, is developed as a result of IRI involving renal tissues. Renal IRI pathology involves a number of pathogenic processes, including the generation of reactive oxygen species and the activation of various inflammatory mediators such adhesion molecules and a number of cytokines [2]. When compared to other organs, there are several notable differences in the hemodynamics and oxygenation of the kidneys. 20 percent of the cardiac output is delivered to the kidneys, but while 100 percent of this reaches the cortex, only 15 percent reaches the medulla [3]. Unquestionably, the inflammatory response plays a significant role in the etiology of IRI. Numerous experimental studies have demonstrated that inhibiting inflammatory responses lowers renal IRI while maintaining renal function [4]. It has been demonstrated in numerous organs that the microvascular and parenchymal tissue damage that results from ischemia and reperfusion is predominantly caused by reactive oxygen-free radicals. As a result of a reduction in the synthesis of antioxidant enzymes, post-ischemic reperfused tissue has more damage caused by free radicals [5]. A cytokine called tumor necrosis factor alpha (TNF α) has pleiotropic effects on several cell types. TNF-α has been identified as a critical regulator of inflammatory reactions and is known to have a part in the genesis of a number of inflammatory and autoimmune diseases [6]. Additionally, it induces the production of additional cytokines since IL-1β, a key proinflammatory cytokine, has a variety of purposes in various organs and diseases [7]. Despite being multifaceted, IRI pathophysiology involves inflammation and oxidative stress. During the ischemia phase, a lack of oxygen and nutrients results in the accumulation of hypoxanthine, and induction of pro-inflammatory cytokines. The reperfusion phase causes a rise in iNOS and NOXs in endothelial cells, and the influx of neutrophils [8].

One of the primary signs of oxidative stress is malondialdehyde (MDA), which is formed upon breaking down of lipid peroxyl radicals. The only reliable method for determining an organism's antioxidant capability is through TAC measurement [9]. A decrease in kidney tissue TAC levels was noted during renal IRI [10]. Apoptosis is the process through which a cell ceases to divide and develop in favor of an action that finally results in the cell's controlled death without discharging its contents into the immediate environment [11]. Apoptosis occurs as a result of excessive reactive oxygen species (ROS) generation that causes oxidative stress, which in turn triggers lipid peroxidation and ultimately results in cell death during the process of renal ischemia reperfusion damage [12]. When a cell undergoes apoptosis, caspase-3, the final caspases in the cascade, is activated by both internal and extrinsic mechanisms [13]. Caspase-3 has been extensively examined among the several caspases proteins, and it has been suggested that it plays a major factor in renal dysfunction [14]. A protein kinase signalling molecule called extracellular signal-regulated kinase (ERK) belongs to the MAPK family along with p38 MAPKs, extracellular signal-regulated kinase 5 (ERK5), and c-Jun N-terminal kinases (JNKs). The phosphorylation processes that trigger these enzymes, which are a component of the mitochondrial route for apoptosis, amplify and send signals from the cell membrane to the nucleus. Growing data suggests that the MAPK protein family is essential for ROS-mediated apoptosis [15]. Among the three recognized MAPK signalling pathways, ERK-associated intracellular signal transduction pathways are considered as traditional MAPK pathways. Activation of the P38MAPK and ERK1/2 signal transduction pathways is known to regulate cell growth and differentiation, but accumulating evidence suggests that these pathways also play a role in cell death [16]. An immediate early gene called transcription factor-early growth response-1 (EGR1) is involved in the processes of growth, differentiation, apoptosis, and wound healing [17]. EGR1 was found in the proximal tubule of kidney disease patients and was activated by hypoxic stimuli. EGR1 silencing could improve diabetic kidney disease and prevent renal fibrosis and inflammation [18].

Tilianin, also known as acacetin-7-glucoside, is an active flavonoid glycoside that is obtained from several medicinal plants, most notably *Dracocephalum moldavica*. It was highlighted for a wide range of biological activities, including anti-diabetic characteristics [19], anti-inflammatory, antioxidant, and anti-depressant effects, Cardioprotection [20, 21], and neuroprotection. Tilianin suppresses cell death through the mitochondrial route, making it a potential therapeutic treatment for ischemia reperfusion-induced AKI. This impact is achieved by reducing ERK pathway activation and downregulating EGR1 expression [18].

THE AIM

The aim of this research is to determine whether Tilianin (TIL) may have nephroprotective effects on bilateral renal IRI in rats by analyzing kidney function biomarkers U and Cr, inflammatory cytokines like TNF α and IL-1β, antioxidant marker total anti-oxidant capacity (TAC), anti-apoptotic markers caspase-3, and histopathological scores.

MATERIALS AND METHODS

ANIMAL PREPARATION, TREATMENT, AND SACRIFICE

Adult Spragge-Dawley rats were used in this study weighing between 200 and 350 g at ages 20 to 24 weeks. They were acquired from the Ministry of Health/ Center of Control and Pharmaceutical Research/Baghdad and housed in the animal home at the Animal Resources Center/College of Sciences/University of Kufa. The rats were fed a normal diet made up of food and tap water. All rats were included in the study once the Institutional Animal Care and Use Committee (IACUC) at the University of Kufa gave its consent.

STUDY DESIGN

The rats were randomly separated into an even 4 groups (each group had five rats) for the case control study's case study design:

- Sham group*:* Rats experienced median laparotomy for around 2 hours and 30 minutes, but with no ischemia induction;
- Control group: Rats experienced bilateral renal ischemia for 30 min, then initiate reperfusion through restoring renal blood flow for 2 hours [22];
- Vehicle group: Rats administered intraperitoneal injection of mixture of corn oil and DMSO (Medchemexpress/USA) 30 min before ischemia induction. Then rats experienced 30 min bilateral renal ischemia and 2 hours' reperfusion [22];
- Tilianin treated group: Total of five rats administered intraperitoneal injection of Tilianin 5 mg/kg [23] 30 min [24] before ischemia induction. Then after receiving anesthesia, the rats experienced 30 min bilateral renal ischemia and 2 hours' reperfusion [22].

EXPERIMENTAL STUDY MODEL

Before the experiment, rats must be weighed, for which 100 mg/kg ketamine and 10 mg/kg xylazine are injected intraperitoneally to provide anesthesia. After the rats were fixed with stickers, their chest and abdominal hair were shaved, and the full sedation were ensured. Through a midline laparotomy incision, the intestine was withdrawn, exposing the abdomen and both renal pedicles. The renal pedicles were isolated for the bilateral ischemia model by securing non-trauma micro vascular clamps around the renal arteries and veins [25]. After a few minutes, the color of the kidneys changes from red to dark purple, and light stains start to develop on the surface, indicating that blood flow is being blocked. The pedicles were released from the clamps after 30 minutes, allowing renal blood flow to be restored, and the dark purple color of the kidneys changed to pale red [31], signaling the start of the two-hour reperfusion phase [25]. The kidney was repositioned and the abdominal cavity incision was stitched with three interrupted sutures. Then euthanasia is performed by deep anesthesia. Finally, blood and tissue samples were gathered for the study. It has been demonstrated that ischemia lasting more than 20 minutes causes kidney injury [27].

PREPARATION OF TILIANIN

The pure powder of Tilianin was purchased from Medchemexpress, USA Company. Molecular Formula: $C_{22}H_{22}O_{10}$. Chemical name: acacetin-7-O-β-D-glucopyranoside**.** CAS Number: 4291-60-5. Purity: > 98% (HPLC). Physical description: solubility is more than 2.08 mg/mL (4.66 mM). Administer 2.403 ml/kg intraperitoneally, prepared by dissolving 2.08 mg of Tilianin in a mixture of 10% DMSO and 90% corn oil according to instructions leaflet of manufactured company and prepared immediately before use (Med Chem Express company instructions). The dose of drug that was used is 5mg/kg of rat weight intraperitoneally [23].

PREPARATION SAMPLES OF BLOOD FOR DETERMINING OF KIDNEY FUNCTION PARAMETERS

After two hours of reperfusion, the surgery was completed, and blood was immediately drawn from each rat's heart (3.5-5 ml). The blood sample was placed in a gel tube that had been pre-labeled and left untreated for 30 minutes at a temperature of 37°C. The serum required for determining urea and creatinine was then produced by centrifuging each gel tube at 3000 rpm for 10 minutes [28].

PREPARATION OF TISSUE FOR MEASUREMENT OF TNF-A, IL1-B, AND CASPASES 3 BY ELISA, AND MEASUREMENT OF TOTAL ANTI-OXIDANT CAPACITY BY COLORIMETRIC METHOD

At the end of reperfusion period, the left kidney was removed and rinsed with ice-cold isotonic solution 0.9% to eliminate any blood clots before being divided into two parts. One part undergo homogenization with a high intensity ultrasonic liquid processor in a percent of 1:10 (w/v) phosphate buffered saline that contain 1% Triton X-100 and a protease inhibitor cocktail. Then the homogenate undergo centrifugation at 14000 rpm for 20 min at a temperature 4°C [29]. Thereafter, the supernatant is collected for determination of TNF-α, IL1-β, and Caspase3 by ELIZA technique as well as TAC measurement through colorimetric method.

SAMPLING OF TISSUE FOR HISTOPATHOLOGY ANALYSIS AND GRADING THE SCORE DAMAGE

The remaining left renal tissue was fixed in 10% formalin, dehydrated in a series of alcohols, cleaned in xylene, and then embedded in paraffin to create the paraffin block. Once fixation was complete, a score evaluation utilizing light microscopy was done by a researcher who was unaware of the experimental treatment groups. Renal tubule injury is indicated by tubular epithelial swelling, brush boundary loss, vacuolar degeneration, and cast formation [30]. A magnification of X100 and X400 was used to determine renal damage. The percentage of renal tubular damage was calculated from the score of histological alterations in the kidney tissue segment:

Score 0, represents normal;
Score 1, represent < 25% of damage tubules;
Score 2, represent 26%-50% of damage tubules;
Score 3, represent 51% -75% of damage tubules;
Score 4, represent 76% -100% of damage tubules [31].

STATISTICAL ANALYSIS

According to GraphPad Prism version 7, the experimental results were statistically analyzed using Tukey multiple comparisons. A one-way analysis of variance (ANOVA) was used to determine the significance of group differences. P value ≤ 0.01 was regarded as statistically significant. The values were given as mean ± standard errors of the mean (SEM).

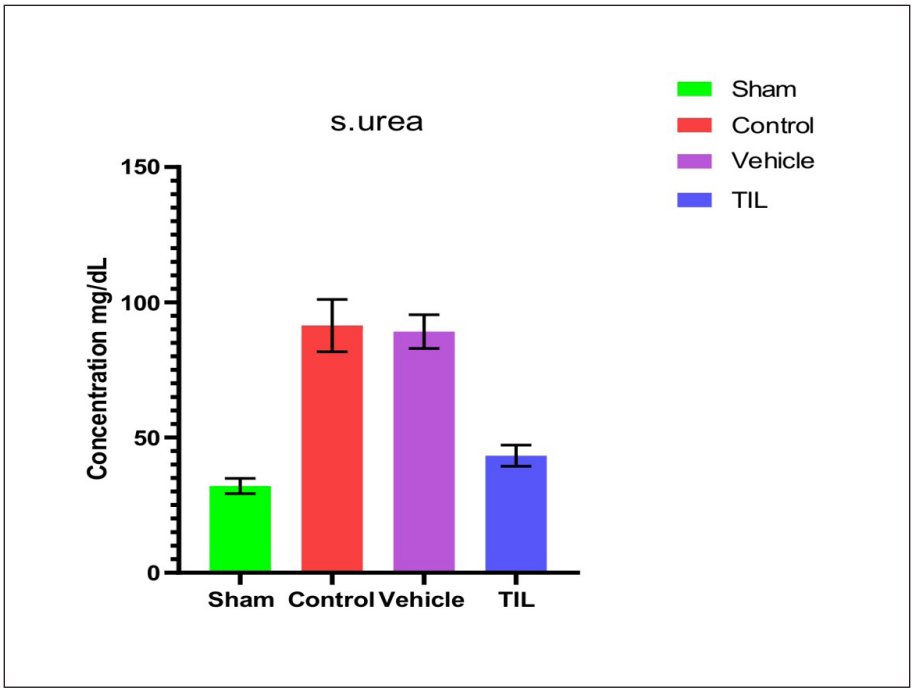

Fig. 1. Mean serum urea (mg/dl) level of the 4 groups after collecting all samples. The data are presented as mean ± SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p-value ≤ 0.01; TIL group vs. Control & Vehicle groups: p-value ≤ 0.01; TIL: Tilianin.

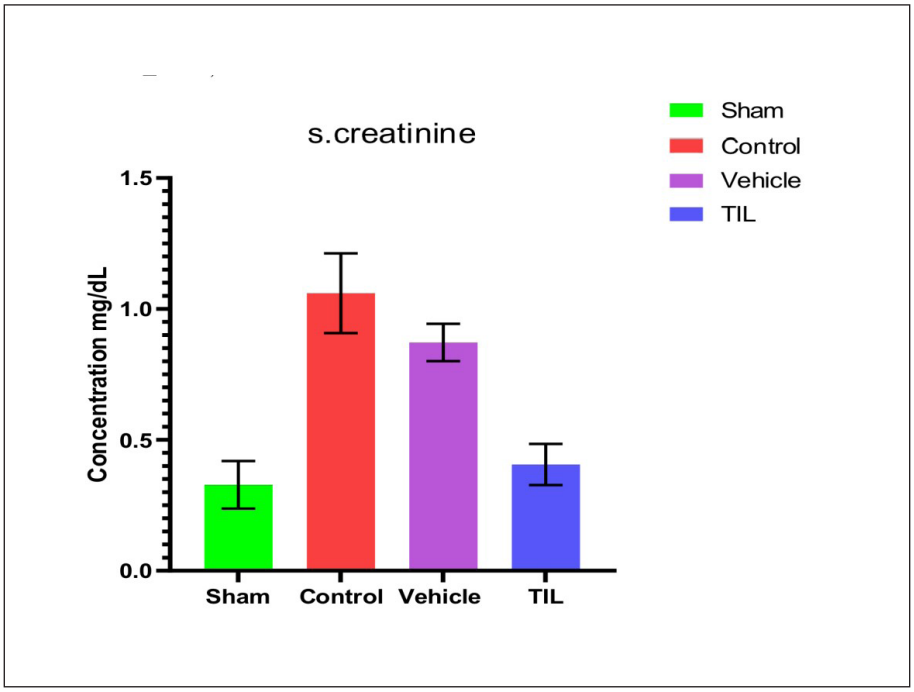

Fig. 2. Mean serum creatinine (mg/dl) level of the 4 groups after collecting all samples. The data are presented as mean ± SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. P value ≤ 0.01; TIL group vs. Control & Vehicle groups: p-value ≤ 0.01; TIL: Tilianin.

RESULTS

Effects of IRI and Tilianin on kidney function parameters (blood urea nitrogen and serum creatinine)

The results of the current study showed that serum levels of urea (U) and creatinine (Cr) were significantly elevated ($p \leq 0.01$) in the control and vehicle groups compared to the sham group. However, there was no significant ($p \geq 0.01$) difference between the control and vehicle groups when compared. However, when compared to the levels in the control and vehicle groups, the Tilianin-treated group demonstrated a substantial ($p \leq 0.01$) decrease in serum levels of urea and creatinine. Mean serum U and Cr level as it shown in figure 1 and figure 2, respectively.

EFFECTS OF IRI AND TILIANIN ON INFLAMMATORY MARKERS TNF-A AND IL1-B

The results of the current study showed that renal tissue levels of Tumor Necrosis Factor (TNF-α) and Interlukine-1β (IL1-β) were significantly elevated ($p \leq 0.01$) in the control and vehicle groups compared to the sham group. However, there was no significant ($p \geq 0.01$) difference between the control and vehicle groups when

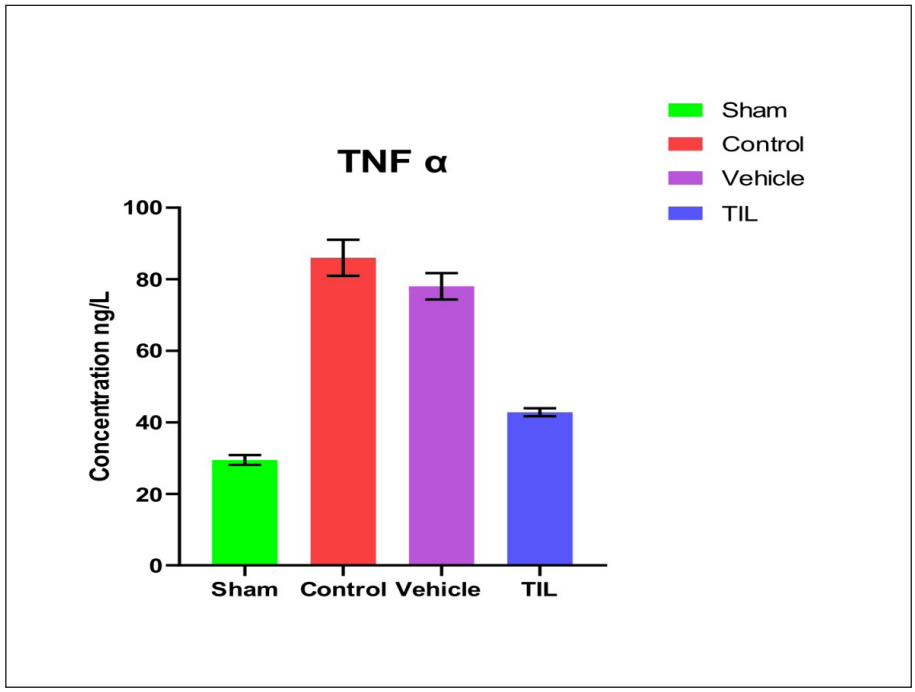

Fig. 3. Mean level of TNFα in renal tissue (ng/l) of the 4 groups after collecting all samples. The data are presented as mean ± SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p-value ≤ 0.01; TIL group vs. Control & Vehicle groups: p-value ≤ 0.01; TIL: Tilianin.

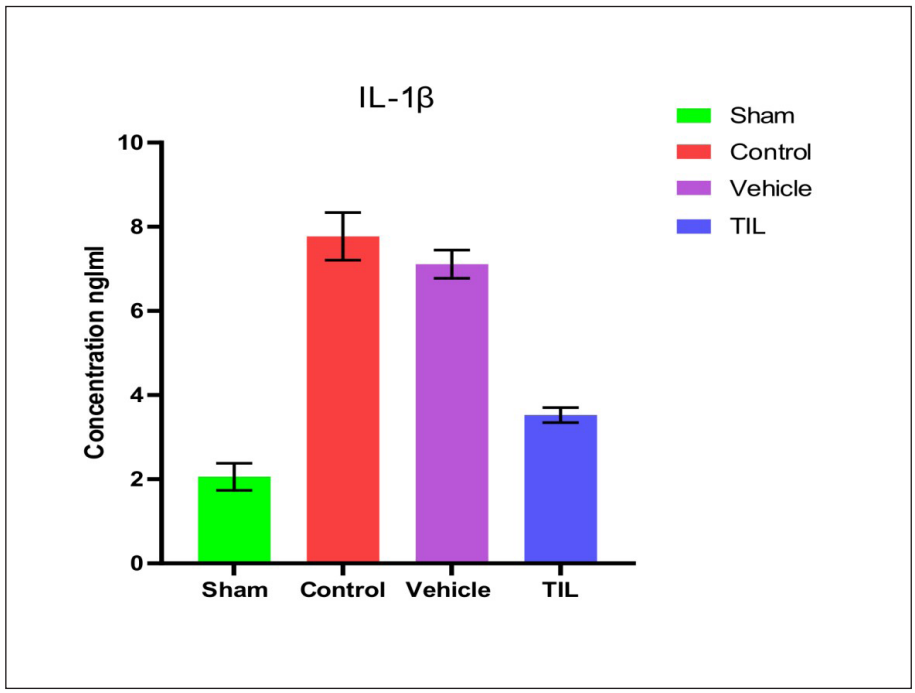

Fig. 4. Mean serum IL-1β (ng/ml) level of the 4 groups after collecting all samples. The data are presented as mean ± SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p-value ≤ 0.01; TIL group vs. Control & Vehicle groups: p-value ≤ 0.01; TIL: Tilianin.

compared. However, when compared to the levels in the control and vehicle groups, the Tilianin-treated group demonstrated a substantial ($p \leq 0.01$) decrease in renal tissue levels of TNF-α, and IL1-β. Mean tissue TNF-α and IL1-β level are shown in figure 3 and figure 4 respectively.

EFFECTS OF IRI AND TILIANIN ON APOPTOTIC MARKER (CASPASE-3)

The results of the current study showed that renal tissue level of caspase-3 were significantly elevated ($p \leq 0.01$) in the control and vehicle groups compared to the sham group. However, there was no significant ($p \geq 0.01$) difference between the control and vehicle groups when compared. However, when compared to the levels in the control and vehicle groups, the Tilianin-treated group demonstrated a substantial ($p \leq 0.01$) decrease in renal tissue levels of caspase-3. Mean tissue caspases 3 level (Fig. 5).

EFFECTS OF IRI AND TILIANIN ON TAC

The results of the current study showed that renal tissue level of total anti-oxidant capacity (TAC) were

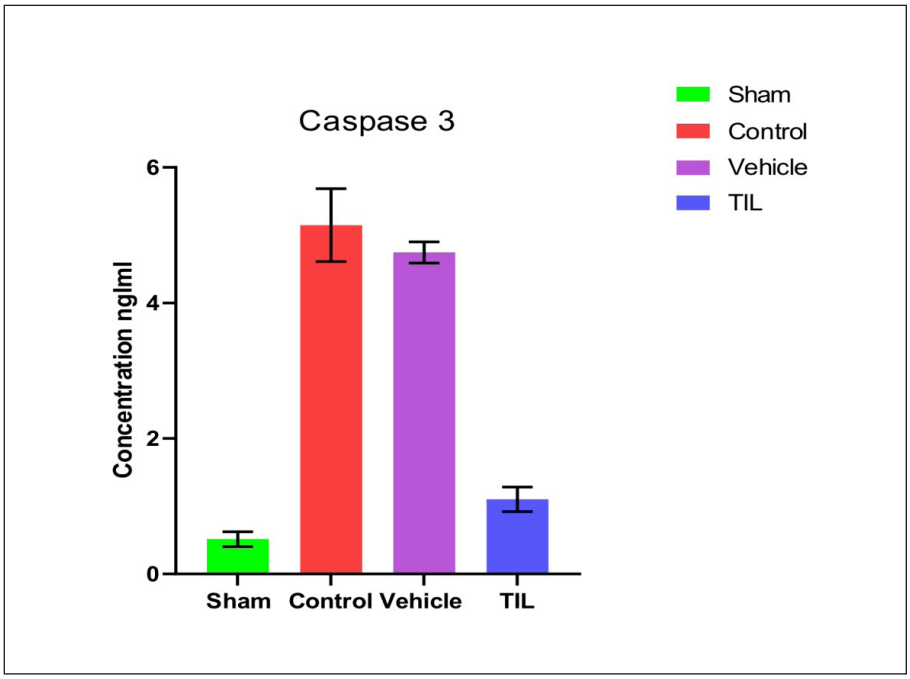

Fig. 5. Mean serum caspase-3 (ng/ml) level of the 4 groups after collecting all samples. The data are presented as mean ± SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p-value ≤0.01; TIL group vs. Control & Vehicle groups: p-value ≤ 0.01; TIL: Tilianin.

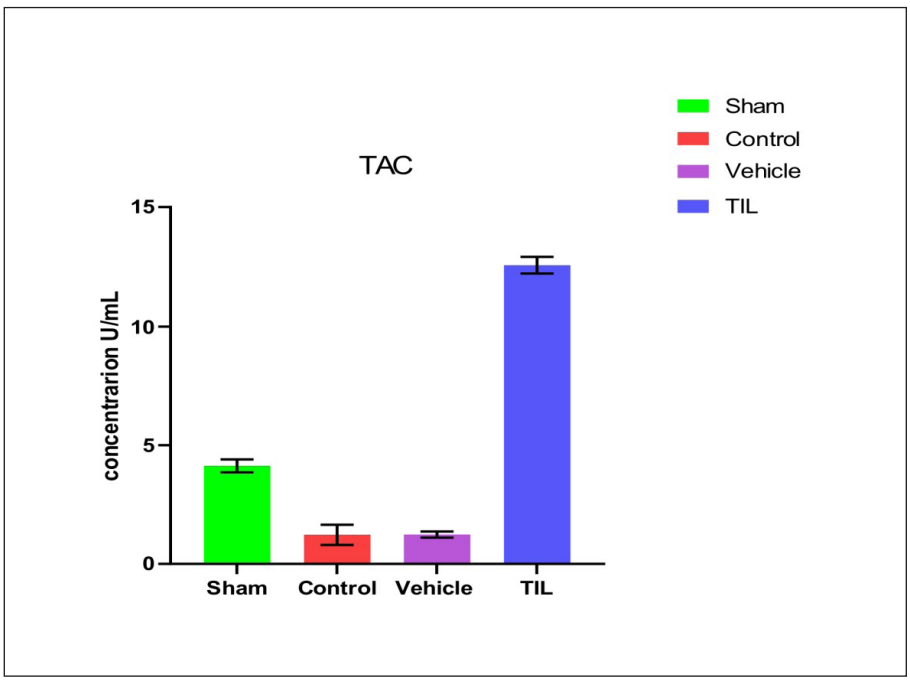

Fig. 6. Mean serum TAC (U/ml) level of the 4 groups after collecting all samples. The data are presented as mean ± SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p-value ≤ 0.01; TIL group vs. Control & Vehicle groups: p-value ≤ 0.01; TIL: Tilianin.

significantly reduced (p ≤0.01) in the control and vehicle groups compared to the sham group. However, there was no significant (p ≥0.01) difference between the control and vehicle groups when compared. However, when compared to the levels in the control and vehicle groups, the Tilianin-treated group demonstrated a substantial (p ≤0.01) increase in renal tissue levels of TAC. Mean tissue TAC level (Fig. 6).

HISTOPATHOLOGICAL EXAMINATION

The damage score and the histology results are displayed in figure 7 and figure 8 at the end of the study. The kidney tissue cross section from the sham group displayed a normal renal structure, glomerulus, and kidney tubules (Fig. 8A). As it shown, the renal tissue cross section from the control group (Fig. 8B), on the other hand, showed aberrant renal structure and severe kidney damage, with the renal tubules being damaged to an extent of 80% and showing enhanced eosinophilia, vacuolated epithelium, and Eosinophilic cast; interpreted in high severity score (severity score mean=4). The kidney tissue of the vehicle group was cross-sectioned, revealing injured kidney structure and severe renal injury (damage of about 80%), including renal tubules dilatation, loss of the brush border, in-

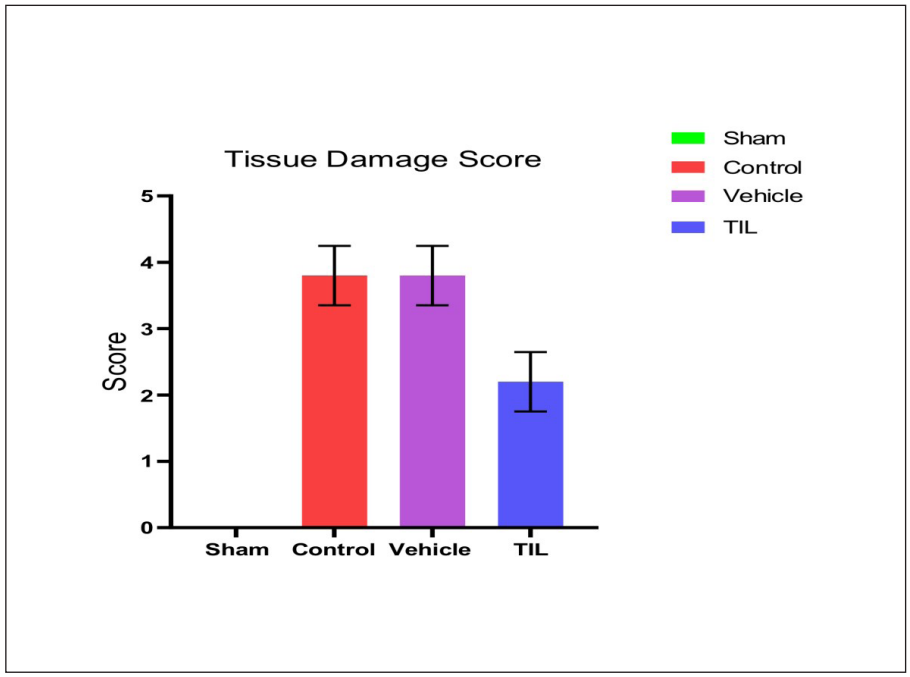

Fig. 7. Severity score mean of histopathological kidney tissue of the 4 groups after collecting all samples. The data are presented as mean ± SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p-value ≤ 0.01; TIL group vs. Control & Vehicle groups: p-value ≤ 0.01; TIL: Tilianin.

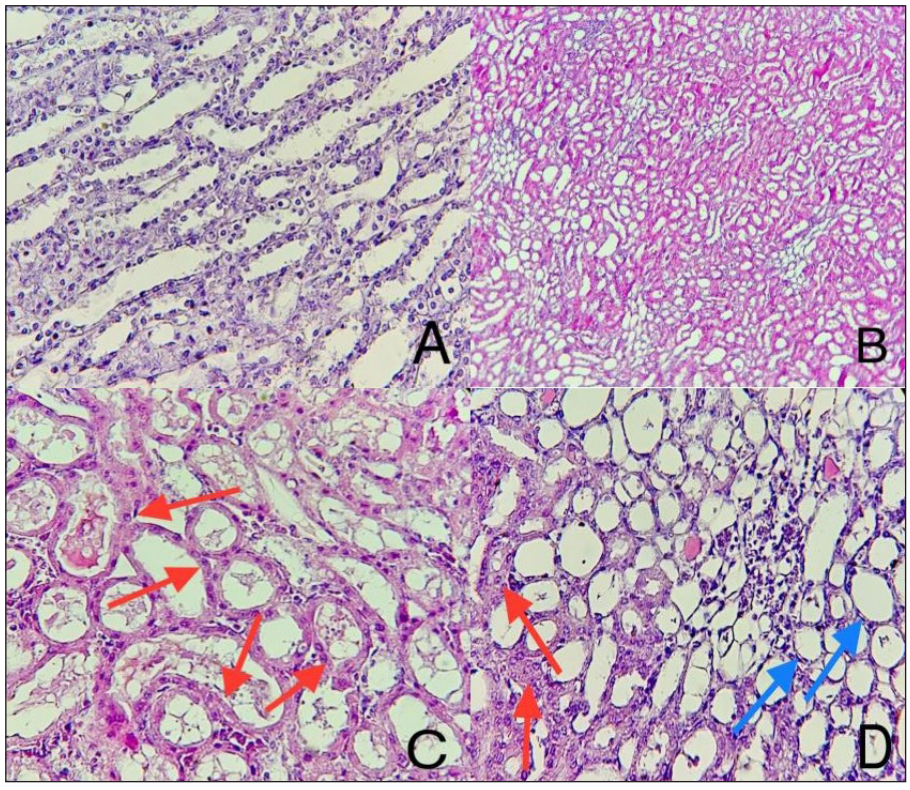

Fig. 8. Renal tissues undergo staining with hematoxylin and eosin A. Sham group. A cross section of left kidney showed normal histology. 400×. B. Control group. Cellular swelling and cytoplasmic eosinophilia, vacuolated epithelium, and Eosinophilic cast. 100×. C. Vehicle group cellular swelling and cytoplasmic eosinophilia (red arrow). 400×. D. Tilianin treated group. A cross section of left kidney revealed score 2. The renal ischemic changes with damage affecting 35% of renal tubules, including cellular swelling and cytoplasmic eosinophilia (red arrows) and presence of normal renal tubules (blue arrow). 400×.

creased cytoplasmic eosinophilia, vascular congestion, and hemorrhage (figure 8C). The kidney tissue cross section of the TIL-treated group showed mild to moderate kidney structural alterations, with damage impacting 35% of the renal tubules (Fig. 8D).

DISCUSSION

The structural and functional alterations take place in response to restoration of blood flow after an ischemia period is referred as "ischemia reperfusion injury" (IRI). In addition to the negative effects of ischemia, restoration of blood flow can also have harmful impacts on the tissue, such as necrosis of irreparably damaged cells, apparent cell swelling, and uneven restoration of blood flow [1]. Acute kidney injury (AKI) brought on by renal ischemia-reperfusion injury (IRI) is still a significant disorder that is accompanied by an unacceptable high morbidity rate. This leads to extended hospitalization, major renal disease, and occasionally even death [4].

The current experimental study showed that following ischemia reperfusion injury, urea and creatinine serum levels were elevated significantly ($p \leq 0.01$) in both the control and vehicle groups compared to the sham group. These results agreed with other studies. In a rat model research using IRI, blood levels of urea and creatinine were significantly elevated in the control group due to renal tubule injury after a 45-minute ischemia period and a 24-hour reperfusion period [32]. Another study that compared the control group to a sham group likewise found that blood urea and creatinine levels were higher in the control group. This increase in serum nitrogenous waste products, including urea and creatinine, was explained by reduction in oxygen and nutrient availability [33]. Tilianin pretreated group showed significant ($p \leq 0.01$) reduction in serum urea and creatinine levels as compared with their levels in control and vehicle groups. This research demonstrates that TIL protects parameters of kidney function after development of renal IRI in a rat model (urea and creatinine). This finding agreed with other studies. One trial involving the renoprotective impact of Tilianin in diabetic rats revealed a significant drop in blood levels of urea and creatinine, which was attributed to the anti-oxidative and anti-inflammatory properties of TIL and led to an improvement in the diabetic rats' renal functioning. The NrF2/Keap1 signaling pathway is the mechanism via which TIL exerts its antioxidant effects [34]. This experimental study found that the control and vehicle groups' mean severity scores for a portion of the left kidney were significantly ($p \leq 0.01$) higher than the score for the sham group. Cellular edema, cytoplasmic eosinophilia, the disappearance of brush borders, desquamation of epithelial cells, formation of casts in tubular lumens, vascular congestion, interstitial inflammation and bleeding are some of these histological alterations. These results had been consistent with those of other studies. According to one of these investigations, the ischemia reperfusion injury can result in epithelial cell vacuolization, tubule casting, tubule dilatation, and loss of brush border [35]. Other studies have demonstrated renal histological deformation due to ischemia reperfusion damage, including cell desquamation, congestion, necrosis, and apoptosis. Another study found that bilateral IRI could significantly damage the renal tubules, reduce the number of normal renal cells, and increase gene expression that causes macrophage accumulation, which in turn affects cell proliferation, leukocyte migration to the site of injury, suppresses cellular immunity, and ultimately activates the inflammatory response that results in additional renal damage [36]. Tilianin pretreated group showed a remarkable ($P \leq 0.01$) reduction in renal damage severity compared to both control and vehicle groups. The score severity mean for the vehicle and control groups indicated severe kidney damage, but the score severity mean for the group receiving Tilianin treatment suggested mild to moderate damage. Our findings in settlement with other studies. One experimental investigation that evaluated Tilianin renoprotective impact after giving it to diabetic rats, showed improvement in glomerular and tubular damage. The fact that TIL improved the renal functions of diabetic rats in the results shows that it has anti-oxidative and anti-inflammatory properties [34]. A different experimental study suggested that Tilianin enhances kidney function following the generation of ischemia-reperfusion damage causes AKI in mice. The tubular damage score dropped to around the half of what was seen in the IRI group after TIL injection, indicating that Tilianin protects kidney function in vivo by suppressing the ERK/EGR1 signalling pathway [18]. This study discovered that after ischemia reperfusion damage, both the control and vehicle groups' levels of TNF-α and IL-1β were considerably ($p \leq 0.01$) higher than those of the sham group. The findings supported those of other studies. According to the study, renal ischemia for 45 minutes followed by reperfusion for 6 or 24 hours induced structural and functional damage to the kidneys, as well as systemic and renal inflammation, which is shown by a rise in TNF-α levels in injured tissue [37]. As for IL-1β, several studies revealed a significant elevation in its level in both control and vehicle groups as compared to sham group.

The development of renal IRI is significantly influenced by the inflammatory response. During the inflammatory process, there is a marked accumulation of neutrophils, and inflammatory factors, chemokines, and adhesion molecules produced as a result of the inflammatory response can further activate neutrophils. Chemotaxis then causes them to infiltrate and aggregate, which exacerbates renal injury [38]. Tilianin pretreated group showed a remarkable ($p \leq 0.01$) reduction in the level of inflammatory mediators (TNFα, and IL-1β) in kidney ischemic tissues as compared with the levels of those inflammatory cytokines in both control and vehicle groups. This result indicates that Tilianin has an anti-inflammatory effect on kidney tissues that underwent ischemia and then had their blood flow restored. Our findings agreed with other experimental studies. According to one study, Tilianin significantly reduced of the gene expression of TNF-α, suggesting that the main cause of its anti-inflammatory actions was the downregulation of the TNF-α/NF-KB pathways [39]. According to a study, pretreatment with Tilianin dramatically reduced the production of the pro-inflammatory mediators IL-1β and TNF-α in a dose-dependent way.

These findings suggested that Tilianin might reduce inflammation. Additionally, this research showed that Tilianin has an anti-inflammatory effect via inhibiting the MAPK/ERK signaling pathway [40]. The current experimental study found that after IRI, that the levels of total antioxidant capacity (TAC) in renal tissues in both the control and vehicle groups significantly decreased ($p \leq 0.01$) compared to the sham group. In comparison to normal tissues, injured kidney tissues (control and vehicle groups) exhibit this downregulation that is explained by an increase in oxidative stress and ROS (sham group). Other studies have endorsed our conclusion. According to one study, reoxygenation of the ischemic kidney causes the creation of ROS, which in turn causes the activation of cytokines and chemokines, which is what causes the large fall in TAC tissue level after IRI. After IRI, the antioxidant defense system deteriorates, and the amount of MDA, a byproduct of lipid peroxidation, increases while antioxidant levels decrease. All of these processes result in cell apoptosis [41]. In comparison to the control and vehicle groups, the Tilianin pretreatment group had a significant ($p \leq 0.01$) increase of TAC expression while a reduction in oxidative stress and the production of free radicals in injured kidney tissues. This suggests that Tilianin has an anti-oxidative effect on damaged renal tissues brought on by ischemia and reperfusion. The effect of Tilianin on total antioxidant capacity in rat or mouse models has not been studied previously. One study, however, examined the cardio-protective action of TIL in a rat model of cardiac ischemia and reperfusion injury and examined the anti-oxidative effect of TIL by assessing malondialdehyde (MDA), an oxidative stress marker, and superoxide dismutase (SOD), an antioxidant enzyme. The outcome confirmed the antioxidant effect of tilianin, showing that it decreased MDA levels and increased SOD activity [20]. The Nrf2 signaling pathway, amplification of Nrf2-regulated genes, and several cytoprotective enzymes, particularly HO-1, are the mechanisms that underlie the enhancement of SOD and the down-regulation of MDA. The activation of the HO-1 enzyme was confirmed to protect cells from oxidative-induced damage [34]. The current experimental investigation showed that following IRI, the levels of caspase-3 in the renal tissues were considerably higher in the control and vehicle groups ($p \leq 0.01$) than in the sham group. This outcome was consistent with findings from prior research. Caspase-3 levels in the sham group were lower than in the control group, according to an experimental investigation that involved bilateral renal ischemia for 60 minutes, followed by 24 hours of reperfusion in a rat study model [10]. A rat model experiment revealed that after 30 minutes of ischemia and two hours of reperfusion, the levels of caspase-3 in the control and vehicle groups were significantly higher than in the sham group [42]. The ROS generation during IR injury, which also causes the initiation of the inflammatory response and the occurrence of AKI, is responsible for the adverse effects of IR injury on the cell. Furthermore, the generation of ROS activates the signaling pathways that lead to cell death and necrosis [34]. According to the results of the current study, pretreatment with the phenolic flavonoid Tilianin before the induction of ischemia can significantly ($p \leq 0.01$) reduce the expression of caspase-3 in injured kidney tissues as compared to the control and vehicle groups. Our result is in settlement with other studies. In one study, the effectiveness of Tilianin against ischemia injury in neuronal cells was demonstrated. TIL administration reduced pro-apoptotic cytochrome c production and caspase-3 activation in vitro. These findings showed that Tilianin effectively controlled mitochondrial malfunction and played a significant role in preventing apoptosis [21]. When used as a pretreatment for oxidative stress and apoptosis in a cellular model of Parkinson's disease, a study indicated that Tilianin had a dose-dependent reduction in the number of apoptotic cells. Additionally, after receiving Tilianin administration, caspase-3 protein expression levels decreased [40].

CONCLUSIONS

Our results show that Tilianin has a significant nephroprotective effect in renal ischemia-reperfusion injury, as demonstrated by improvements in kidney function parameters (urea and creatinine), and a reduction in the expression of inflammatory mediators (TNF-α, and IL-1β), a confirmation of the anti-inflammatory effect, an increase in the TAC of cells against free radicals and ROS in ischemic kidney tissues, an indication of antioxidant activity, and a decrease in the level of the anti-apoptotic impact.

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