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Effects of *Clitoria Ternatea* on renal ischemia and reperfusion injury in rats

Efectos de la *Clitoria Ternatea* en la lesión renal por isquemia y reperfusión en ratas

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ABSTRACT

Ischemia-reperfusion (I/R) injury refers to damage caused by the temporary interruption of blood flow into an organ/tissue and the restoration of circulation. This injury, which affects many vital organs, is still an important clinical and surgical problem. This study aims to investigate the effect of antioxidant and anti-inflammatory effects of *Clitoria ternatea* on renal I/R injury in rats. Twenty-four male Wistar albino rats were clustered into 4 groups, 6 rats in each (Control, I/R group, Clitoria ternatea group, I/R group + Clitoria ternatea group). At the end of the experimental procedures, all groups were euthanised in accordance with ethical rules. Blood and tissue samples were taken and subsequent biochemical (MDA, SOD, CAT, GSH-Px, BUN, Na, K, creatine), histopathological and immunohistochemical analyses demonstrated that *Clitoria ternatea* effectively prevented I/R-induced renal injury in rats. Based on these findings, *Clitoria ternatea* may be considered a potential therapeutic agent for the prevenstion of I/R-induced kidney damage.

Key words: Kidney; ischemia-reperfusion; rat; clitoria ternatea; therapeutic

RESUMEN

La lesión por isquemia-reperfusión (I/R) se refiere al daño causado por la interrupción temporal del flujo sanguíneo en un órgano/tejido y el restablecimiento de la circulación. Esta lesión, que afecta a muchos órganos vitales, sigue siendo un importante problema clínico y quirúrgico. Este estudio pretende investigar el efecto antioxidante y antiinflamatorio de la Clitoria ternatea sobre la lesión renal por I/R en ratas. Se agruparon 24 ratas albinas Wistar macho en 4 grupos, 6 ratas en cada uno (Control, grupo I/R, grupo Clitoria ternatea, grupo I/R + grupo Clitoria ternatea). Al final de los procedimientos experimentales, se practicó la eutanasia a todos los grupos de acuerdo con las normas éticas. Se tomaron muestras de sangre y tejidos y los posteriores analisis bioquímicas (MDA, SOD, CAT, GSH-Px, BUN, Na, K, creatina), histopatológicas e inmunohistoquímicas demostraron que la *Clitoria ternatea* prevenia eficazmente la lesión renal inducida por I/R en ratas. Basandose en estos resultados, la Clitoria ternatea puede considerarse un agenta terapeutico potenscial para la prevencion del dano renal inducido por I/R.

Palabras clave: Riñón; isquemia-reperfusión; rata; Clitoria ternatea; terapeutico

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INTRODUCTION

Injury renal I/R refers to the damage resulting from the temporary cessation of blood flow into an organ/tissue and then the restoration of circulation. This injury is still a significant clinical and surgical concern, affecting several organs including the kidneys, liver, heart, lungs, brain, and intestines. Renal I/R injury may originate from conditions such as sepsis, hydronephrosis, open kidney surgeries, shock, kidney transplantation, partial nephrectomy, hemorrhage, and resuscitation efforts [1 , 2].

During kidney transplantation, various degrees of I/R injury may develop on the graft, potentially resulting in postoperative graft dysfunction or loss. Similarly, vascular clamping, commonly performed in tumor resection surgeries, facilitates a bloodless operative field but can also induce ischemic damage in healthy renal parenchyma due to temporary arterial occlusion. Therefore, implementing effective prophylactic treatments to prevent I/R injury in such surgical scenarios may increase success rates [3].

Therefore, there are ongoing studies on pharmacological agents protecting kidney tissue from I/R injury. In addition to immunosuppressants and corticosteroids, antioxidants have been increasingly utilized. Recent studies emphasized the effects of antioxidants on scavenging free radicals generated by I/R and reducing the resultant damage [1 , 4]. *Clitoria ternatea* is widely recognized in traditional Indian medicine for its multifaceted pharmacological properties. It has antibacterial, anti-inflammatory, diuretic, antipyretic, analgesic, local anesthetic, platelet aggregation inhibitory, antidiabetic, and smooth muscle relaxant effects [5 , 6]. Safhi *et al.* [6] reported the anti-inflammatory and antioxidant properties of *Clitoria ternatea* and its ability to prevent kidney damage and dysfunction in rats induced by L-NG-Nitro-Arginin-Metilester (l-NAME). Given this information, this study aims to investigate the effect of antioxidant and anti-inflammatory effects of *Clitoria ternatea* on renal I/R injury in rats.

MATERIALS AND METHODS

Preparation of Clitoria ternatea extract

In the present study, *Clitoria ternatea* extract was prepared by following the guidelines established by Vidana-Gamage *et al*. [7] and Saraç [8].

Experimental animals

The approval was granted by Bingöl University's Animal Experiments Local Ethics Committee (25.04.2022, No: 2022/02) and carried out at the Animal Experimentation Center of the same University. Twenty-four Wistar albino rats (*Rattus norvegicus*) with weights of 200-280g (Denver Instrument SI-234, Germany) were involved [3 , 9] The rats were given a 12-h light/dark cycle and ad libitum access to pellet feed and water. They underwent a one-week acclimatization period in their cages before experiments were started. They were not fed for 12 h (h) before experiments, but there was water available during the entire experiment process.

Experimental design

Control (n=6): Only right nephrectomy, **I/R group (n=6):** right nephrectomy, followed by 30min. ischemia and 2h reperfusion [9], ***Clitoria ternatea* group (n=6):** rats were administered 200 mg/kg *Clitoria ternatea* flower extract orally for 7 d [10], **I/R group + *Clitoria ternatea* group (n=6):** rats received 200 mg/kg *Clitoria ternatea* extract for 7 d. On the final d, right nephrectomy and left kidney I/R (30 min ischemia, 2 h reperfusion) were performed. All groups were anaesthetized 10mg/kg Xylazine hydrochloride (Rompun 2% Bayer Türk Kimya San. Ltd. Şti., İstanbul, Turkey) + 60mg/kg Ketamine hydrochloride (Ketasol 10% Richter Pharma AG, Wels, Austria) by intraperitoneal injection and fixed in the supine position. Right kidneys were removed in all groups. In order to induce ischaemia in the left kidney (in I/R and I/R + *Clitoria ternatea groups*), the renal arteries and veins of this kidney were ligated for 30 min. and this condition was maintained until discolouration was observed in the left kidney. The kidneys were reperfused for 2 h after the ligature was released. At the end of reperfusion, while anesthetized 10mg/kg Xylazine hydrochloride (Rompun 2% Bayer Türk Kimya San. Ltd. Şti., İstanbul, Turkey) + 60mg/kg Ketamine hydrochloride (Ketasol 10% Richter Pharma AG, Wels, Austria), a cardiac puncture was performed to collect blood samples, and kidney tissue samples were taken. After the samples collection the animals were euthanized by decapitation technique in accordance with ethical rules.

Antioxidant enzyme activities in kidney tissue

Kidney tissue preparation

The samples were homogenized utilizing an IKA (Ultra-Turrax T25, Germany) and a glass-porcelain homogenizer at 20000 rpmfor 5 min. Following this process, the centrifuge (Hettich Rotina 420 R, Germany) was performed at 9692 G for 30 min at +4°C [11]. Antioxidant enzyme activity analyses were conducted using clear supernatants [12].

Antioxidant enzyme estimation

The clear supernatant was utilized to analyze SOD, catalase, and GSH-Px. Tissue SOD activity was investigated utilizing the technique introduced by Sun *et al*. [13], while that of GSH-Px was examined employing the procedure introduced by Gallo and Martino [14]. Estimation of catalase activity was performed following the technique reported by Aebi [15].

MDA analysis

MDA level measurement was performed using the technique introduced by Ohkawa *et al.* [16]. The determination of total protein content was performed employing the standard method introduced by Lowry *et al.* [17] with bovine serum albumin.

Serum Analysis

The serum was separated and kept in covered polypropylene tubes. Sodium (Na), Potassium (K), Creatine, Urea, and BUN levels were measured following standard procedures with the Roche Modular P 800 auto-analyzer. (Roche Diagnostics, Model P 800, Germany).

Histopathological and immunohistochemical investigations

Kidney tissue samples after necropsy were fixed at 10% neutral formalin for 72 h. The samples were processed through alcohol and xylol series in an automatic machine (Leica ASP6025, Leica Biosystems, Germany) according to routine tissue tracing procedures and then embedded in paraffin. Sections of 5 µm thick were taken from the blocked tissues in a microtome (Leica RM 2255, Germany). Haematoxylin eosin (H&E) staining process was conducted following the routine process. The evaluation criteria included degeneration and necrosis in the tubular epithelium, desquamation, tubular atrophy, intratubular hyaline casts, inflammation, and congestion. The results were (BX51 dp72 camera, Olympus, Tokyo, Japan) as none (0 lesions), mild: + (degeneration), moderate: ++ (degeneration+necrosis), and severe: +++ (degeneration+ diffuse necrosis) [18 , 19].

For immunohistochemical analysis, samples prepared at 5 µm and put on poly-L-lysine coated slides were subjected to standard avidin–biotin–peroxidase method (ABC) by the manufacturer's protocol against anti-caspase-3 antibody (sc-56053). All the sections were incubated with DAB (Histostain-Kit, Invitrogen, Camarillo, CA, USA) and counterstained using Mayer's hematoxylin. The staining intensity was independently analyzed by a pathologist. The anti-caspase-3 staining intensity was rated as no staining (0), poor staining (+1), moderate staining (+2), and strong staining (+3) [20].

Statistical analyses

This process was conducted utilizing SPSS (SPSS for Windows, version 20.0). Differences between the groups were examined employing a nonparametric test (Kruskal–Wallis). Paired comparisons in case of significant differences were conducted utilizing the Mann–Whitney U-test (P<*0.05*).

RESULTS AND DISCUSSION

The level of MDA, a lipid peroxidation product, was found to be significantly higher in I/R group in comparison to control (P<0.05). This increase indicates lipid peroxidation and cellular damage. However, the MDA level of I/R + *Clitoria ternatea* group was observed to decrease in comparison to the I/R group (FIG. 1). Examining the SOD enzyme activity, it was observed that activity significantly decreased in the I/R group in comparison to control (P<0.01). However, comparing the I/R+*Clitoria ternatea* group to the I/R group, there was an increase in enzyme activity (P>0.05) (FIG. 1). CAT enzyme activity, one of the key antioxidant enzymes, was determined to significantly decrease in the I/R group in comparison to control (P<0.05). In the I/R+*Clitoria ternatea* group, enzyme activity showed a non-significant increase in comparison to the I/R group (FIG. 1). The GSH-Px enzyme catalyzes the oxidation of GSH to GSSG. GSH-Px activity decreased significantly in the I/R group in comparison to control due to the damage induced (P<0.001). Moreover, the enzyme activity values of the *Clitoria ternatea* and controls were found to be similar (P>0.05). Enzyme activity increased in the *Clitoria ternatea* (P<0.001) and I/R+*Clitoria ternatea* (P>0.05) groups in comparison to I/R (FIG. 1).

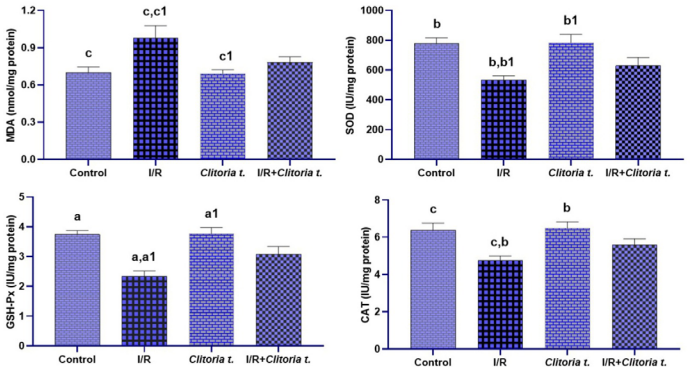

FIGURE 1. CAT, SOD, GSH-Px (EU/mg protein), and MDA in the kidney tissues by groups. Significant differences between the groups due to different letters (a:p<0.001, b:p<0.01, c:p<0.05). CAT (Catalase), GSH-Px (Glutathione Peroxidase), SOD (Superoxide Dismutase), MDA (Malondialdehyde)

Ischemic injuries in the kidneys occur in various conditions such as sepsis, hydronephrosis, open renal surgeries, kidney transplantation, shock, partial nephrectomy, and renal tumors [4 , 21 , 22]. Consequently, many studies are now being carried out to mitigate or prevent renal I/R injury. These studies employed various approaches, ranging from altering I/R duration to administering therapeutic agents either before or after the I/R process, to achieve effective results [23]. One such approach involves the use of antioxidants. It was reported that oxidative stress can be directly or indirectly mitigated by the external administration of antioxidants when endogenous antioxidants are insufficient. Accordingly, several agents were employed both experimentally and clinically to protect tissues and organs from I/R injury. These include pentoxifylline, melatonin, silymarin, astaxanthin, prostaglandin E1, vitamin E, L-carnitine, quercetin, vitamin C, and deoxycoformycin [4 , 24 , 25 , 26 , 27].

The flower of *Clitoria ternatea* contains flavonols like quercetin, myricetin, and kaempferol derivatives, as well as anthocyanins. Extracts of *Clitoria ternatea* were reported to exhibit various therapeutic potentials, including antioxidant and antimicrobial activities. Moreover, it also has various pharmacological properties [28 , 29].

Previous studies indicated that MDA levels increase due to kidney cell membrane damage during IR and that MDA measurement is a critical parameter for diagnosing I/R or subsequent renal failure [30 , 31]. Examining the activity of *Clitoria ternatea* in treating cisplatin-induced acute kidney injury, cisplatin administration was shown to elevate MDA levels, whereas groups treated with both cisplatin and *Clitoria ternatea* exhibited significant reductions in MDA levels [6]. In this study, the MDA level in I/R group was higher in comparison to control (P<0.05). Similar to the previous results [30 , 31], this increase confirms the successful induction of I/R injury and the associated rise in MDA levels. Furthermore, the reduction observed in MDA level following the administration of *Clitoria ternatea* supports the results reported by Safhi *et al*. [6]. The decrease in MDA level may be related to the antioxidant properties of flavonols and anthocyanins present in *Clitoria ternatea*.

The kidneys are among the organs affected by I/R injury the most. Inflammatory cell infiltration and oxidative stress are key mechanisms in the pathogenesis of I/R injury [32 , 33]. It

has been reported that oxidants in ischemic tissue accelerate the inactivation of CAT, GPx, and SOD, rendering cells more vulnerable to oxygen radicals generated during reperfusion Several I/R studies reported significant reductions in SOD, CAT, and GPx activities, accompanied by increased lipid peroxidation levels [34 ,35]. In a study investigating the influence of *Clitoria ternatea* ethanol extract on acetaminophen-induced toxicity in rats, significant reductions (P<0.05) were observed in renal CAT, GPx, SOD, and GSH levels in the acetaminophen-administered group. However, the extract-treated group exhibited remarkable increases (P<0.05) in the levels of these enzymes [36]. Similarly, in this study, the I/R group exhibited decreases in SOD (p<0.01), CAT (P<0.05), and GSH-Px (P<0.001) activities in comparison to the control, corroborating the results reported in previous studies [34]. Moreover, enzyme activities in the I/R + *Clitoria ternatea* group were higher in comparison to the I/R group, but this increase was not significant. This outcome may be related to the higher doses (250 and 500 mg/kg) of *Clitoria ternatea* used in other studies [36].

The analysis of serum sodium levels revealed an increase in the *Clitoria ternatea* group in comparison to control (P<0.01). Furthermore, a partial increase in sodium levels in the I/R+*Clitoria ternatea* group in comparison to the I/R group was found to be significant (P<0.05). No significant difference was found between the other groups (FIG. 2). Serum potassium levels were significantly increased in the I/R (P<0.01) and *Clitoria ternatea* (P<0.05) groups in comparison to the control (FIG. 2). BUN and serum creatinine levels increased in the I/R (P<0.01) and I/R+*Clitoria ternatea* (P<0.05) groups in comparison to control. There was a reduction in BUN and creatinine levels only in the *Clitoria ternatea* group (P<0.01) in comparison to the I/R group. Besides, BUN and creatinine levels increased in the I/R+*Clitoria ternatea* group (P<0.05) in comparison to the *Clitoria ternatea* group (FIG. 2).

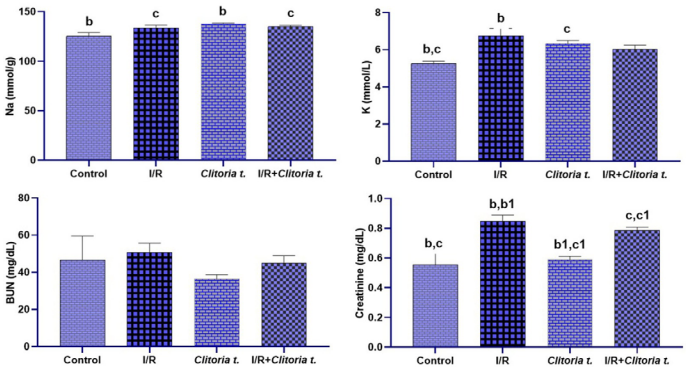

FIGURE 2. Na, K, BUN, Creatinin levels in serum samples of groups. Significant differences between the groups due to different letters (b:p<0.01, c:p<0.05)..Na (Sodium), K (Potassium), BUN (Blood Urea Nitrogen)

I/R was reported to lead to increased production of ROS, loss of selective permeability of cell membranes, and disruption of cellular ion balance [37]. Loss of kidney function can result in disturbances in blood electrolyte levels and acid-base balance [38]. In a study on the effect of hydralazine using a renal ischemia-reperfusion model in rats, it was reported that the difference in sodium (Na) levels between the control and I/R groups was not significant, whereas sodium excretion via urine decreased in hydralazine-treated rats in comparison to the I/R group [39]. In the present study, while no difference was found in Na levels between the control and I/R groups (P>0.05), an increase was determined in the *Clitoria ternatea* (Clitoria t.) and I/R + Clitoria t. groups when compared to control (P<0.05). This increase may be due to reduced urinary excretion in the groups treated with Clitoria t. Renal I/R injury was reported to elevate serum potassium (K) levels [40 ,41]. I/R injury leads to direct damage to the cells responsible for potassium secretion in the distal tubules and collecting ducts [42]. As reported in previous studies [40 ,41], serum K levels in the I/R group were significantly elevated (P<0.05) in the present study. Furthermore, K levels in the Clitoria t. and I/R + Clitoria t. groups exhibited an increase in comparison to the control (P<0.05) but a numerical decrease in comparison to the I/R group (P>0.05).

Acute kidney injury (AKI) is characterized by the sudden loss of kidney function, accompanied by a rise in BUN and serum creatinine levels. Conditions that impede adequate renal perfusion, such as renal ischemia, are among the pre-renal causes of AKI [43]. It was reported that a rise in serum creatinine levels is a better indicator of AKI than tracking BUN levels [44].

In a study investigating the efficacy of calcium dobesilate administered prophylactically for renal I/R injury, rats in the sham and I/R groups were observed without any treatment, whereas rats in the treatment group were administered 100 mg/kg daily dose of calcium dobesilate dissolved in 0.5 mL of drinking water for 10 days (d). It was concluded that there was no significant difference in urea levels among the experimental groups [45].

A rise in serum creatinine levels, a marker of renal (glomerular) dysfunction, after I/R injury was reported to indicate functional impairment in renal proximal tubule cells [46]. In a study on the activity of silymarin on oxidative stress-induced damage in the kidneys of rats with renal I/R injury, serum creatinine levels in the I/R group were higher than control (P<0.05), whereas there was no significant difference between the I/R group and the groups given 50 mg/kg and 100 mg/kg silymarin (P>0.05) [26]. Safhi *et al.* [6] examined the role of *Clitoria ternatea* in conjunction with mesenchymal stem cells in the treatment of cisplatin-induced AKI in rats and reported that this procedure could adjust creatinine, uric acid, and urea levels. In the present study, consistent with results reported by other studies, no statistical significance was found in BUN levels across all experimental groups.

However, serum creatinine levels in the I/R and I/R + Clitoria t. groups were significantly higher compared to the control and Clitoria t. groups. Moreover, even though the creatinine level in the I/R + Clitoria t. group was lower than in the I/R group, the difference was not significant.

Histopathologically, the kidney tissues in the control and *Clitoria ternatea* groups exhibited a normal appearance in the tubular epithelial cells and glomeruli (FIGS. 3a and 3c). Diffuse degeneration, necrosis, and desquamation in the tubular epithelium cells, tubular dilatation, expansion of Bowman's space, and atrophic glomerulus with inflammation were observed in the I/R group (FIG. 3b). Comparing the I/R and I/R+ *Clitoria ternatea* groups, it was determined that the findings observed in the group using *Clitoria ternatea* were much milder in terms of severity and incidence. Overall, the tubules and glomeruli appeared close to normal (FIG. 3d).

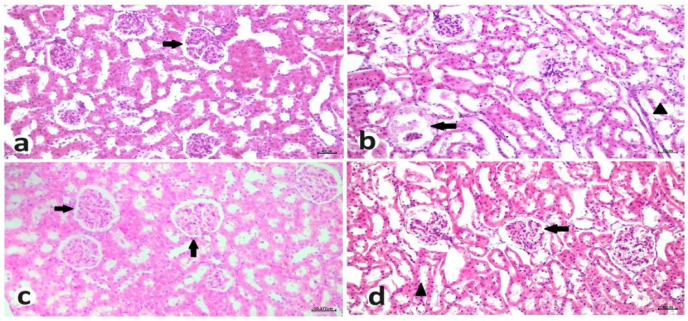

FIGURE 3. a) Control, normal histological appearance of kidney tissue (H&E, 40 µm). b) I/R group, glomerular atrophy (arrows), necrosis, and desquamation in the tubular epithelium cells (arrowhead), (H&E, 40 µm). c) *Clitoria ternatea* group, normal histological appearance of the glomerulus of kidney tissue (arrows), (H&E, 40 µm). d) I/R+ *Clitoria ternatea* group, enlarged bowman spaces (arrow), degenerated tubular epithelium (arrowhead) (H&E, 40 µm)

The histopathological scores indicating the degree of kidney damage in different groups and demonstrating the protective influence of *Clitoria ternatea* on I/R are presented in TABLE I, while the microscopic results showing the distribution of severity across groups, ranging from mild to severe, are detailed in TABLE II.

TABLE I.
Degree of kidney damage by groups

No	Control	I/R	*Clitoria ternatea*	I/R+*Clitoria ternatea*
1	0	3	0	1
2	0	2	0	1
3	0	4	0	0
4	0	3	0	1
5	0	2	0	1
6	0	3	0	0
X±SD	0.00±0.00[b]	2.83±0.75[a]	0.00±0.00[b]	0.67±0.52[b]

TABLE II.
Distribution of microscopic kidney tissue results by groups demonstrating the protective effect of *Clitoria ternatea* against ischemia-reperfusion

Parameters	Control	I/R	*Clitoria ternatea*	I/R+ *Clitoria ternatea*
Tubular dilatation and desquamation	0/6	6/6	0/6	4/6
Mild	*	*	*	3
Moderate	*	3	*	1
Severe	*	3	*	*
Degeneration	1/6	6/6	0/6	3/6
Mild	1	*	*	2
Moderate	*	2	*	1
Severe	*	4	*	*
Tubular epithelial necrosis	0/6	6/6	0/6	2/6
Mild	*	1	*	1
Moderate	*	3	*	1
Severe	*	2	*	*
Inflammatory cell infiltration	0/6	6/6	0/6	1/6
Mild	*	3	*	1
Moderate	*	2	*	*
Severe	*	1	*	*

*: none P<0.0001

In the immunohistochemical analysis of caspase-3 immunoreactivity in the kidney tissue, no staining was detected in the control or the *Clitoria ternatea* group (FIGS. 4a and 4c). Strong and diffuse positive immunoreactivity of caspase-3 was found in I/R group (FIG. 4b). The frequency and intensity of positive reactions in the I/R + *Clitoria ternatea* group were significantly lower than in I/R group. This indicates that the number of positive cells decreased proportionally with the administration of *Clitoria ternatea* (FIG. 4d).

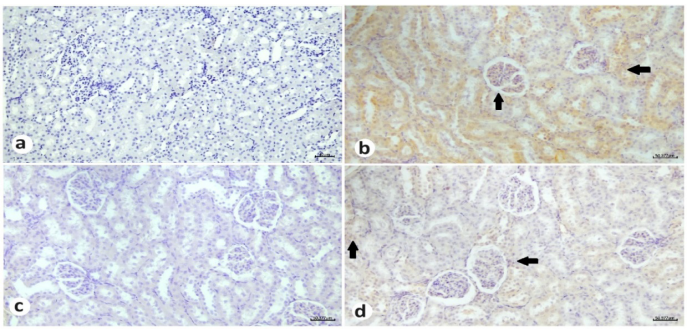

FIGURE 4. a) Control kidney tissue, Caspase -3 negativity (IHC, 40 µm). b) I/R group. Strong Caspase -3 positivity (IHC, 40 µm). c) *Clitoria ternatea* group, Caspase -3 negativity (IHC, 40 µm). d) I/R+ *Clitoria ternatea* group, poor Caspase -3 positivity compared to I/R group (IHC, 40 µm)

Ischemia-reperfusion injuries in the kidneys primarily occurs in the tubules due to tubular necrosis caused by ischemia [47]. Acute kidney failure may develop following ischemia, characterized by a reduction in glomerular filtration rate, edema in tubular epithelial cells, acute tubular necrosis, infiltration of inflammatory cells, congestion, and increased vascular resistance in the kidneys [48]. Reperfusion of ischemic tissue is necessary for cellular regeneration and removal of toxic metabolites. However, reperfusion can cause more severe damage than the initial ischemic injury. I/R injury triggers a series of pathological events, including the production of ROS, apoptosis, infiltration of inflammatory cells, necrosis, and release of active mediators that cause tissue damage [49].

Histopathological studies using routine hematoxylin and eosin staining revealed that I/R-induced renal damage includes degeneration and necrosis in the tubules and glomeruli, hemorrhage, tubuler precipitates, endothelial and epithelial cell damage, vascular damage, and inflammatory cell infiltration. These results vary depending on the cause, duration, and severity of I/R injury. Furthermore, the extent and impact of ischemia-reperfusion injury are also examined immunohistochemically with a focus on apoptosis [50].

In their study involving rats, where 1-hour ischemia followed by 1-hour reperfusion was applied to the left renal artery, Ozturk *et al*. [50] reported significant renal lesions in the I/R group, including tubular dilation, vascular congestion, marked necrosis, and intratubular cast accumulation. Mousavi [21], who applied 1-h ischemia followed by 24-h reperfusion, additionally reported inflammation and cellular vacuolization in the I/R group, extending the results reported by Ozturk *et al.* [50]. Another study reported flattened tubular epithelial cells, marked necrosis, vacuolization, and dilation, along with diffuse tubuler precipitates in tubular lumens and congestion in glomeruli and vascular structures. These results were noted to be consistent with previous studies [22]. In this study, consistent with the results mentioned above, degeneration and necrosis were predominantly observed in the tubules and glomeruli, while vascular damage and inflammation were mild.

Melatonin, as studied by Sahna *et al*. [25] and Rodríguez-Reynoso *et al*. [51], significantly reduced tubuler precipitates, tubular epithelial degeneration, and necrosis in efforts to protect kidneys from I/R injury. Similarly, Sener *et al*. [52] highlighted that melatonin application notably reduced inflammatory cell infiltration and other renal lesions related with I/R injury. In their experimental kidney ischemia study, Demirtaş *et al*. [26] reported a significant histopathological improvement in a group treated with pentoxifylline. Şentürk *et al*. [27] investigated the antioxidant properties of silymarin to determine whether exogenous antioxidant compounds have protective activities against renal I/R injury.

Their results emphasized that silymarin, at doses of 50 and 100 mg/kg, yielded results comparable to the control and effectively inhibited ROS effects. However, studies carried out by Aktoz *et al*. [53] and Unal *et al*. [24], which evaluated the protective effects of exogenous antioxidant vitamins like vitamin E and combined vitamin C+E against I/R injury, noted that while some degree of renal protection was observed, these effects were not significant. The results of histopathological evaluation of renal tissues were consistent with previous studies, except for those carried out by Aktoz *et al*. [53] and Unal *et al*. [24].

CONCLUSION

Reviewing the literature, it was determined that the influence of *Clitoria ternatea* extract on experimental renal I/R injury in rats has not been previously studied. To our knowledge, this is the first experimental study investigating the preventive effects of *Clitoria ternatea* extract in renal I/R injury. This study demonstrated that *Clitoria ternatea* administration showed a protective effect against renal I/R injury in rats. A decrease in oxidative stress markers, histopathological and immunohistochemical improvement in renal tissue and a significant decrease in serum creatinine and BUN levels were observed after administration. These findings suggest that *Clitoria ternatea* may protect the kidney tissue against I/R injury thanks to its antioxidant and anti-inlammatory properties. However, this study is based on preliminary findings and furher studies with a larger number of animals, comparing different doses and investigating moleculer mechanisms are needed to understand the therapeutic potential of *Clitoria ternatea* more clearly. In addition, in order to determine whether this effect is dose-dependent, it is recommended that experimental models including different dose levels be applied in the future.

Conflict of Interest

The authors declare there is no conflict of interest.

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