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Research Article
Thymoquinone and Oleuropein Combination Ameliorates Renal Ischemia-Reperfusion Injury by Attenuating Oxidative Stress in Rats

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Abstract

Background and Objective: Ischemia-reperfusion-induced acute renal injury is a common clinical problem. The current article investigates the effects of oleuropein and thymoquinone combination in the prophylaxis of renal ischemia-reperfusion-induced injury in rats. **Materials and Methods:** Thirty rats were divided into five groups, G_1: Sham operation group, G_2: IR group, G_3: IR+Thymoquinone (10 mg kg^{-1}, p.o.) G_4: IR+Oleuropein (50 mg kg^{-1}, p.o.) and G_5: IR-TQ (10 mg kg^{-1}, p.o.)+OP (50 mg kg^{-1}, p.o.). A pre-treatment was given for three weeks before IR surgery. The blood urea nitrogen (BUN), uric acid and serum creatinine were analyzed in serum. The homogenate of kidney tissues was prepared for the estimation of the anti-oxidant parameters like Glutathione Peroxidase (Gpx), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA). **Results:** The rats in G_2 (IR) showed high levels of creatinine (4.11±0.18 mg dL^{-1}), BUN (131.76±10.56 mg dL^{-1}) and uric acid (1.72±0.14 mg dL^{-1}) as compared to G_1 (sham-operated rats). Further, the increased levels of MDA (10.54±0.77 nmol mg^{-1} protein) and low levels of SOD (78.92±3.46 U mg^{-1} protein), CAT (23.49±1.93 U mg^{-1} protein) and GSH-Px (26.53±0.64 U mg^{-1} Protein) activity was evidenced in the IR group. The combination treatment group G_5 (IR-TQ-OP) showed the best nephroprotective activity among all the treatment groups, as the kidneys were least affected by the IR-induced renal inflammation and oxidative injuries. It was followed by IR-TQ and then IR-OP. All these observations were corroborated by the findings of histopathological investigations. **Conclusion:** Simultaneous administration of TQ and OP significantly altered the experimentally induced renal function due to ischemia/reperfusion.

Key words: Kidney, ischemia, oleuropein, reperfusion, thymoquinone, nephroprotective activity, urological operation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The renal Ischemia and Reperfusion (IR) induced injury is caused due to a sudden temporary loss of the blood flow to the kidney, which in turn is one of the main causes of acute kidney failure (AKF). Moreover, AKF might be implicated in the progression and development of several chronic renal diseases[1]. Several functional and structural changes take place in Ischemia/reperfusion injury. IR injury is commonly encountered in several clinical situations like hemorrhagic shock, partial nephrectomy, kidney transplantation, urological operation and hypotensive conditions, etc.[2,3]. Post-ischemic kidney normally undergoes pathophysiological remodelling, including inflammation, tissue proliferation, apoptosis and interstitial fibrosis. Renal ischemia causes oxidative stress, consequently, a protracted systemic inflammatory response occurs, which is assumed to be responsible for the death of renal cells post-reperfusion[2]. The ischemia-reperfusion in the kidney is the leading cause of Acute Kidney Injury (AKI), which can histologically be manifested as acute tubular necrosis[4-6]. This is also a well-known fact that a high amount of highly ROS (reactive oxygen species) is generated in the ischemic kidney or any other organ after the reperfusion. ROS exerts a variety of cytotoxic effects on ischemic organs, which includes induction of apoptosis, DNA damage, lipid peroxidation, oxidation and nitrosylation of proteins[7]. The growing number of ROS during renal reperfusion threatens the integrity of the glomerulus epithelium and renal tubules, which is considered one of the reasons for the progress and development of acute necrosis in the renal tubules[8]. The treatment strategy for ischemia-reperfusion injury is based on the depletion of free radicals, which prevent the post-ischemic tissue injury in a patient of organ transplantation[9]. Collectively, ROS is the main cause of impairment of renal functions and, ROS also induces apoptosis of renal cells. Hence, antioxidant therapy has been recommended in several research studies to improve organ functions and prevent apoptosis[10]. Increasingly, several herbal medicines have been used to prevent and/or cure numerous clinical diseases. The protective effects of natural antioxidants have received greater attention. The endogenous antioxidant defense against ROS is strengthened by natural antioxidants and restored to an optimal balance by neutralizing the reactive species. Natural antioxidants are gaining huge significance due to their potential role in the prevention of many diseases. In recent years, researchers have seriously considered various antioxidants from natural sources to prevent damage due to different factors[11,12].

A wide variety of plants with medicinal properties exist and 70-80% global population appears to believe in various traditional medicine to remain healthy, according to the World Health Organization. The essential oil obtained from *Nigella sativa* Linn. (Family: Ranunculaceae), has been reported to exert a large number of biological activities[13,14].

The essential oil from *Nigella sativa* contains several active phytochemicals like 4-terpineol (2-7%), sesquiterpene (1-8%), P-cymene (7-15%), carvacrol (6-12%), tanethole (1-4%) and very high amount of biologically active molecule thymoquinone (30-48%)[13,14]. Pharmacologically, the most important components of the black cumin are thymoquinone and its derivatives[15-18]. Thymoquinone has been shown to act, since its first extraction in 1963, as a potent free radical and scavenger of super oxides[16,19]. Moreover, by inhibiting the accelerated production of several cytokines and growth mediators, thymoquinone has proved to be an anti-inflammatory agent[20]. In many conditions, including renal conditions, the therapeutic effects of thymoquinone have been studied[21-23]. The seeds of *Nigella sativa* and their oil have been traditionally used in the prevention and treatment of several diseases and disorders like arthritis, asthma, bronchitis, cough, diabetes, dizziness, eczema, fever, gastrointestinal disorders, headache, hypertension, tumours, impotence, flu and painful menstruation in the world, most commonly in Arab countries, Europe, India and Iran[24-26].

There are several health benefits of the olive tree and its products like fruit and fruit oil. They have been frequently consumed as food for nutritional purposes and also used as medicines. The olive tree possesses antioxidant properties due to its high polyphenolic contents[27]. The main active constituent of the olive is oleuropein, which is the major polyphenolic compound. Oleuropein exhibited a wide spectrum of therapeutic and pharmacological potential[28-31]. Several studies have established the biological and pharmacological activities exhibited by Oleuropein, including but not limited to, antioxidant[32], anti-inflammatory[33], anti-apoptotic[34], neuroprotective[35] and reno-protective activities[36]. Currently, there is no specific therapy available for ARF and acute kidney injury[37], making it essential to identify potent nephroprotective agents with minimum side effects. Hence, this study protocol was undertaken to find out the combinatorial effect of oleuropein and thymoquinone against kidney injury caused by renal reperfusion ischemia. The biomarkers of renal functions, antioxidant parameters and histopathological alterations in the kidney tissues were estimated.

MATERIALS AND METHODS

Study area: The study was conducted at the PG Research Laboratory of Siddhartha Institute of Pharmacy, Siddhartha

Table 1: Experimental group design

Groups	Experimental groups with their respective treatment for three weeks
Group 1	Sham operation group, 0.25 mL corn oil
Group 2	Ischemia and reperfusion group (IR), 0.25 mL corn oil
Group 3	Pretreatment group I (IR-TQ), TQ (10 mg kg^{-1} *p.o. in 0.25 mL corn oil)
Group 4	Pretreatment group II (IR-OP), Oleuropein (50 mg kg^{-1} **b.wt./day p.o.)
Group 5	Pretreatment group III (IR-TQ-OP), TQ (10 mg kg^{-1}) + Oleuropein (50 mg kg^{-1})

*(Hammad & Lubbad[61]) and **(Yin *et al.*[33])

Group of Institutions, Dobachi, Sahastradhara Road, Dehradun, Uttarakhand. The study was carried out in June-December, 2018.

Drugs and chemicals: The thymoquinone and oleuropein were procured from a chemical company Sigma-Aldrich (St. Louis, MO, USA). The analytical grades of reagents and chemicals have been used in the presented study. Pentobarbital sodium and Ketamine hydrochloride were procured from Sigma Life Science, USA.

Animals: The healthy male Wistar rats (180-200 g) were selected for the study protocol. The whole experiments were carried out under standard conditions by strictly adhering to the recommended experimental protocols and good laboratory practices. The study protocol was carried out according to the institutional ethical guidelines on animal care. The animals acclimatized for seven days before the commencement of experimentation and dosing.

The humidity (55±5%), room temperature of 25±2°C and the 12 hrs dark and 12 hrs light cycle were maintained to provide standard laboratory conditions to all experimental animals. All rats received normal drinking water *ad libitum* and unlimited access to a standard pellet diet.

Experimental groups: The male Wistar rats (30 nos.) were arbitrarily segregated into five experimental groups (n = 6) and these rats were then kept in standard animal cages. The rats in different groups were given specific treatments as per Table 1.

The rats were given an intraperitoneal injection of Ketamine HCl (70 mg kg^{-1}, i.p.) along with pentobarbital sodium (20 mg kg^{-1}, i.p.) in an aseptic environment to induce anaesthesia. After anaesthesia, an incision was made on the left flank to expose the left renal artery, which was subsequently blocked using a microvascular bulldog clamp to induce the considerable ischemic condition. After 60 min of occlusion, the clamps were removed to allow reperfusion in the left kidney. Moreover, a simple surgery is made on the sham-operated rats under the same conditions, which was considered the normal operation control[24].

The rats' abdomen was then sealed after removing the clamp. In every group, the rats were held for 24 hrs in metabolic cages to collect urine and to assess the intake of water.

The blood sample was obtained from each rat after completing 24 hrs and the obtained blood was then subjected to centrifugation (4°C, 1000 g for 15 min).

Following the collection of blood, rats were decapitated to harvest the kidneys. The kidneys were weighed to calculate the kidney weight to body weight ratio (KBR)[24]. Subsequently, the kidneys were perfused in ice-cold saline solution (hypertonic) and the homogenate (10%) of kidney tissues was made in the potassium chloride (1.15% w/v) to measure anti-oxidant activity. Further, some kidney tissues were also transferred to the neutral-buffered formalin solution (10%) to fix the tissues for histopathological investigations.

Evaluation of markers of kidney functions: The level of Blood Urea Nitrogen (BUN), uric acid and creatinine (Scr) in serum were estimated by standard assay kits to assess the kidney functions.

Assessment of antioxidant activities: The homogenate of kidney tissue was prepared in buffer (1.15% KCl) with the help of a Teflon homogenizer. The homogenate was then centrifuged (7000 g) for 10 min at 4°C. Supernatant from the homogenate was separated. The reduced glutathione (GSH) and Malondialdehyde (MDA) were estimated in the collected supernatant to assess oxidative stress. In addition, antioxidant enzymes like Glutathione Peroxidase (GSH-Px), Catalase (CAT) and Superoxide Dismutase (SOD) were also assessed. The estimation of GSH, GSH-Px, CAT, SOD and MDA was determined by commercially available kits as per the instruction of the manufacturer's protocol.

Histopathological investigation: The renal tissue samples were fixed by immersing in buffered formalin solution (10%). It was then embedded in paraffin and subsequently cut into 5 µm thick sections and was finally stained using hematoxylin-eosin. The renal tissue sections were observed blindly under a microscope for discriminating histopathological changes between different groups.

Statistical analysis: The statistical software SPSS (version 26.0) was used to perform the statistical analysis of the obtained data and results. The final results were articulated as Mean±SD. One-way ANOVA was used to compare variables between two groups and then followed by Tukey's posthoc test to the comparison of various experimental groups. The value of $p<0.05$ was regarded as a significant difference.

RESULTS

Combinatorial effect of TQ and OP on renal biomarkers: The effects of various given treatments on the serum SCr, BUN and uric acid levels have been shown in Fig. 1. The level of serum SCr ($p<0.001$), uric acid ($p<0.001$) and BUN ($p<0.001$) in the IR injury group were increased significantly as compared to the sham-operated control group, which asserted the considerable glomerular dysfunction mediated by ischemic renal injury. The IR-TQ and IR-OP groups, which received pre-treatment of TQ and OP respectively showed significantly lowered serum levels of SCr ($p<0.001$), uric acid ($p<0.001$) and BUN ($p<0.001$) as compared to the rats of IR group. Additionally, the IR-TQ group showed better results than the IR-OP group, nevertheless, the serum levels of these parameters were still found significantly higher as compared with the sham-operated control group. The IR-TQ-OP group showed near-normal results as the serum levels of all the renal biomarkers were reduced ($p<0.05$) significantly.

Efficacy of treatment on body weight, water intake and urine excretion: Urinary excretion was markedly reduced to 3.85 mL in the IR group ($p<0.001$) in comparison to the sham group (12.47 mL) (Table 2). This reduction can be attributed to the low water intake by the rats of the IR group (18.25 mL) as compared to the Sham group (35.6 mL). Contrastingly, the water intake in the groups IR-TQ and IR-OP was significantly restored to normal i.e., 30.23 mL ($p<0.001$) and 27.19 mL ($p<0.01$), respectively, in comparison with the IR operated group (18.25 mL). Moreover, the urinary excretion in groups IR-TQ (9.24 mL) and IR-OP (7.95 mL) was also much better as compared with the IR group ($p<0.001$). In group IR-TQ-OP, the water intake (35.42 mL) and urine excretion (11.38 mL) was almost indifferent to the Sham group, while it was significantly ($p<0.001$) different from the IR group. A similar trend was seen in the Kidney/Body Weight ratio (KBR), significant elevation (0.0055) was noticed in the IR group ($p<0.001$) as compared to the Sham group (0.0036). This ratio was observed to incline towards normal in the pretreatment groups IR-TQ (0.0041), IR-OP (0.0045) and IR-TQ-OP (0.0039), which was noticeably different from the IR group ($p<0.001$).

Effect of treatment on antioxidant activity: A significant reduction in the level of GSH ($p<0.001$) and anti-oxidant enzymes viz CAT, GSH-Px and SOD ($p<0.001$) activities in kidney tissue was seen in the IR group as compared to the Sham group. In the IR-TQ, IR-OP and IR-TQ-OP groups, levels of GSH and antioxidant enzymes were significantly restored ($p<0.001$) in comparison to the IR injury group (Fig. 2 and Fig. 3).

Pre-treatment with TQ and OP results in an insignificant increase in the levels of the antioxidant enzyme as compared to the Sham-operated group. Predictably, the MDA level was significantly ($p<0.001$) increased in the IR group in contrast to

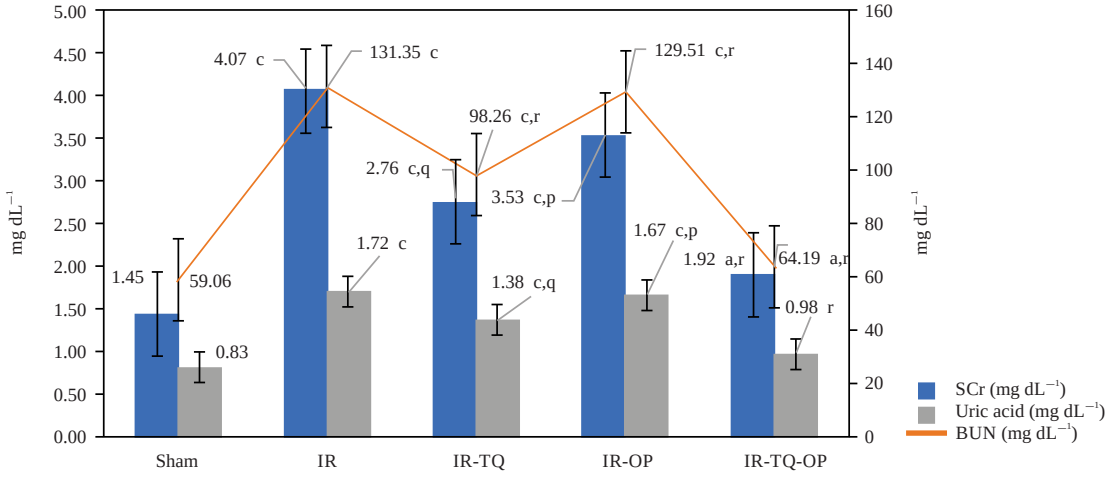

Fig. 1: Effect of TQ and OP pretreatment on SCr, BUN and uric acid levels in experimental rats

Values are expressed as Mean±SD (n = 6), groups as compared to Sham: a ($p<0.05$), b ($p<0.01$), c ($p<0.001$), groups as compared to IR p ($p<0.05$), q ($p<0.01$) and r ($p<0.001$)

the Sham group. However, the level of renal MDA was remarkably restored in the pretreatment groups ($p<0.001$ for all groups) in comparison to the IR group. The combined effect of TQ and OP in the IR-TQ-OP group showed the best efficiency in alleviating ischemic injury by restoring GSH, MDA, SOD, CAT and GSH-Px to near normal.

Histopathological findings: The treatment of TQ and OP revealed several histopathological changes in the renal tissues of different groups (Fig. 4). In the Sham group, normal renal architecture was visible with distinct glomeruli and tubular structures (Fig. 4a). In the IR group, injury such as tubular degeneration, renal cell necrosis, irregular glomeruli and interstitial oedema was seen which asserts the ischemic effect (Fig. 4b). Contrastingly, in the IR-TQ group, a considerable alleviation of the ischemic effect was observed as there was mild interstitial oedema and mild haemorrhage but irregular glomerular morphology was also seen which implies incomplete recovery in this group (Fig. 4c). Similarly, in the IR-OP group, an improvement in ischemic condition was noted with slight tubular degeneration and moderate renal cell necrosis, but the irregular glomeruli, haemorrhage and tubular dilatation implicated incomplete recovery (Fig. 4d). Nevertheless, the combination treatment group IR-TQ-OP showed only mild irregularity in glomerular morphology and insignificant interstitial oedema (Fig. 4e) substantiating the potent combinatorial effect of TQ and OP in the prophylaxis of renal injuries.

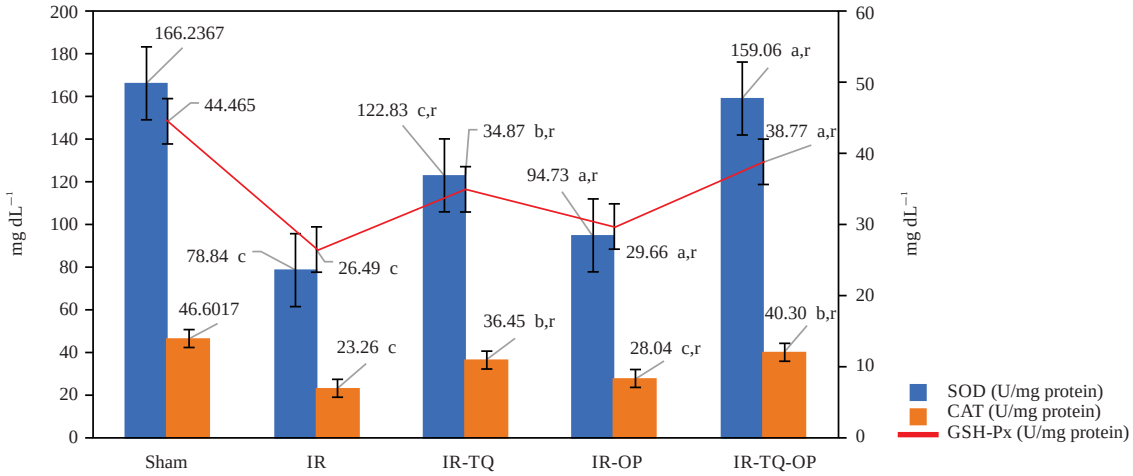

Fig. 2: Effect of TQ and OP pre-treatment on renal MDA and GSH levels in experimental rats

Values are expressed as Mean±SD (n = 6), groups as compared to Sham: a ($p<0.05$), b ($p<0.01$), c ($p<0.001$), groups as compared to IR p ($p<0.05$), q ($p<0.01$) and r ($p<0.001$)

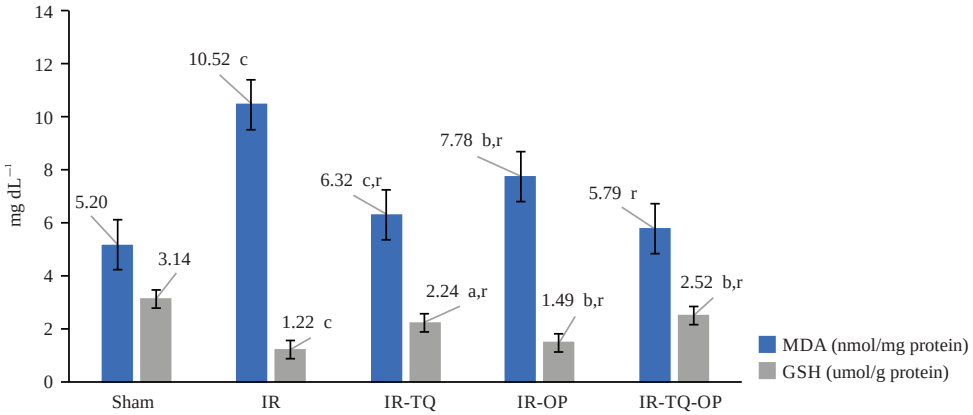

Fig. 3: Effect of TQ and OP pre-treatment on SOD, CAT and GSH-Px activities in experimental rats

Values are expressed as Mean±SD (n = 6), groups as compared to Sham: a ($p<0.05$), b ($p<0.01$), c ($p<0.001$), groups as compared to IR p ($p<0.05$), q ($p<0.01$) and r ($p<0.001$)

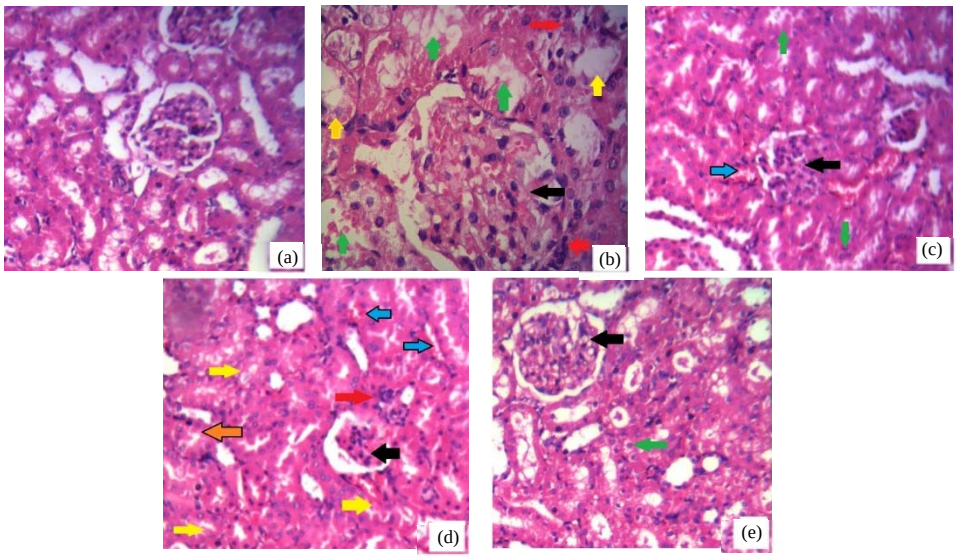

Fig. 4(a-e): Microscopic characteristics of renal tissues (H&E, stained), (a) Sham group showing the normal structure of renal cells such as Glomerulus, distal convoluted tubule (DCT), (b) IR group shows ischemic effects like tubular degeneration (yellow arrow), renal cell necrosis (red arrow), irregular glomerular morphology (black arrow) and interstitial oedema (green arrow), (c) IR-TQ group shows mild interstitial oedema (green arrow), irregular glomerular morphology (black arrow) and mild haemorrhage (blue arrow), (d) IR-OP group shows slight tubular degeneration (yellow arrow), moderate renal cell necrosis (red arrow), irregular glomerular morphology (black arrow), haemorrhage (blue arrow) and tubular dilatation (orange arrow) and (e) IR-TQ-OP group shows only mild irregularity in glomerular morphology and mild interstitial oedema

Table 2: Effects of TQ and OP pretreatment on water intake and urinary excretion rates and kidney-body weight ratio in experimental rats

	Water intake (mL)	Urinary excretion (mL)	Kidney to body weight ratio
G_1: Sham	35.6±1.13	12.47±2.05	0.0036±0.0001
G_2: IR	18.25±0.54^a	3.85±0.82^c	0.0055±0.0001^c
G_3: IR-TQ	30.23±2.16	9.24±1.35^r	0.0041±0.0001^r
G_4: IR-OP	27.19±1.72^q	7.95±0.71^r	0.0045±0.0001^r
G_5: IR-TQ-OP	35.42±2.01^r	11.38±1.44^r	0.0039±0.0001^r

Values are expressed as Mean±SD (n=6), groups as compared to Sham: ^{a}(p<0.05), ^{b}(p<0.01), ^{c}(p<0.001), groups as compared to IR ^{p}(p<0.05), ^{q}(p<0.01) and ^{r}(p<0.001)

DISCUSSION

The results obtained in the presented study substantiate that pretreatment of rats with TQ and OP showed protective effects on renal ischemic-reperfusion injury, as evidenced by renal function biomarkers, oxidative stress indicators and histological examination. Moreover, it has been reported that SCr, BUN and uric acid are the established biomarkers for glomerular filtration rate[38]. In this study, post-renal ischemic-reperfusion surgery, IR group animals exhibited injury of renal tissue characterized by increased SCr (4.07±0.76 mg dL^{-1}), increased BUN (131.35±0.49 mg dL^{-1}) and increased Uric acid (1.71±0.024 mg dL^{-1}) which were significantly contrasting (p<0.01) to the Sham-operated group. The prophylactic action of TQ and OP has been reported in several former studies involving the treatment of IR with TQ and OP[2,8,16,20,22,24,34]. In the pre-treatment groups, the levels of serum SCr, uric acid and BUN were significantly reduced (p<0.05) as compared to the IR group (Fig. 1). IR-TQ group showed better results than the IR-OP group as evident from Fig. 1, the serum levels of SCr, uric acid and BUN were increased in the latter group, which indicates better efficacy of TQ in ameliorating IR than OP. IR-TQ-OP group, which received the combination of TQ and OP showed the best efficiency in resisting the ischemic condition as the serum levels of renal function biomarkers were near normal at the end of the study. The outcomes of this study also revealed a significant (p<0.01) increase in KBR, decreased water intake and reduced urine production in the IR group, which signifies the ischemic injury encountered by the renal tissues which are found to be in line with previous findings[24,39]. The increase in the KBR can be attributed to interstitial oedema, which occurred after renal reperfusion in

the IR group and was absent in the Sham-operated group. Moreover, tubular necrosis causes oliguria by different mechanisms including urine leakage from injured renal tubules. Additionally, the activation of the Renin-Angiotensin System (RAS) causes decreased blood flow in the glomeruli and subsequently decreases the formation of urine[40]. Therefore, it is evident that the pathological changes in the IR group of the presented study are due to the renal injury caused by ischemia and enhanced by reperfusion[41]. On the other hand, the pretreatment groups endured such pathological changes to a significant level, as these changes were almost negligible in the combined treatment group IR-TQ-OP, however, the IR-OP group showed the least resistance to these changes. This incidence can be attributed to the prophylactic activity of TQ and OP in improving tubular oedema and counteracting the ischemic condition[19,42].

The ischemic stress generated by the surgical obstruction of renal arteries induces several histopathological changes like renal tissue inflammation, tubular damage, cellular necrosis and obstruction with cellular debris[2]. Most of these changes occur during IR surgery, occlusion of renal blood supply is followed by anoxia and the generation of ROS which is a major contributor to reperfusion injury[43,44]. The kidney has a high content of polyunsaturated fatty acid which increases the propensity to oxidative injury generated by ROS[45]. Subsequently, cell death occurs due to ROS-induced lipid peroxidation of the cell membranes[46-48] and can be determined using MDA[41,49,50]. The generation of ROS induces diverse cytotoxicity such as protein oxidation, lipid peroxidation, nitrosylation and apoptosis[51]. The renal protective potential of TQ and OP was assessed by determining their individual and combined effects on lipid peroxidation (LPO) measured as MDA. IR group exhibited a significant increase ($p<0.001$) in the MDA level (Fig. 2) and thus weakened the pool of antioxidant enzymes. Contrastingly, pretreatment groups IR-TQ-OP encountered this oxidative stress remarkably ($p<0.01$), however, the IR-TQ group showed lower and IR-OP showed the least resistance to these pathological changes. This indicates that combined pretreatment of TQ and OP prevents protein oxidation and lipid peroxidation during and after the IR surgery.

Moreover, renal ischemic reperfusion is known to deplete GSH and also diminishes the activity of SOD, GSH-Px and CAT[52,53]. Hence in this study, these antioxidant parameters were reduced ($p<0.001$) significantly in the IR injury group which further substantiates the occurrence of renal injury. Oppositely, the pretreatment groups deterred any major change in the antioxidant activity, this may be attributed to the antioxidant potential of TQ and OP (Fig. 3). Thus, the IR-TQ-OP group showed better results followed by IR-TQ and IR-OP groups, the latter being the least protective.

It is understood that the RAS is responsible for regulating blood pressure and Renin in this system forms angiotensin-II[54] and stimulates the excess generation of ROS like hydrogen peroxide and superoxide anion that consequently damages the kidneys[55]. Ultimately, it leads to oxidative stress due to increased production of ROS and/or reduced ROS scavenging ability[24]. When ROS gets attached to the poly-unsaturated fatty acids of the lipids of the cell membrane and it causes lipid peroxidation, structural and functional disorganization of cells take place. After the renal reperfusion, a huge number of superoxide anions in mitochondria are generated due to the imbalance between mitochondrial respiratory function and restoration of oxygen supply[24,56,57]. In such circumstances, the defensive mechanism of antioxidant enzymes begin to fail in preventing the discharge of ROS in the mitochondria and to other cellular organelles. This sequence of events is termed reperfusion injury and therefore its effect is evident in the form of histopathological changes in the renal tissue samples of the IR group (Fig. 4b). These outcomes were following the previous reports[58-61] and further substantiate the renal injury caused by the IR surgery. Moreover, a graded pattern of morphological mutilation was seen in the pretreatment groups, which again indicates that TQ and OP have potent nephroprotective potential.

CONCLUSION

In this study, reperfusion injury increased oxidative stress and decreased the activity of SOD, CAT, GSH-Px and GSH. But the outcomes indicate that pre-treatment with TQ and OP protected the kidneys by preventing lipid peroxidation. In a nutshell, it can be said that the concomitant use of TQ and OP in the prophylaxis of nephrotoxicity has produced significant results. Nevertheless, further studies on a larger scale are essential to establish the concurrency of results.

SIGNIFICANCE STATEMENT

This study investigated the prophylactic efficacy of a novel combination of Thymoquinone and oleuropein which was found to synergistic reduce the renal ischemia-reperfusion injury associated with oxidative stress due to hypoxia. This study will be added value to the researchers, who are willing to discover the critical situation of loss of nephroprotection in the presence of different comorbidities

of the kidneys and cardiovascular system. Therefore, this novel combination of thymoquinone and oleuropein might prove useful in the prevention and prophylaxis of renal ischemia-reperfusion injury.

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