Advances in Bioresearch
Adv. Biores., Vol4 (4) December 2013: 40-46
©2013 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr/abr.htm
CODEN: ABRDC3

ORIGINAL ARTICLE

Preventive Effects of Turmeric *(Curcuma longa* linn) on Renal Ischemia-Reperfusion Injury in Rats

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ABSTRACT
*Renal ischemia/reperfusion (I/R) injury is a major cause of acute renal failure (ARF), which is faced in many clinical situations. This study was designed to investigate the effect of pre-treatment with turmeric (Curcuma longa linn) powder on kidney histopathology and function markers in renal ischemia / reperfusion (IR) induced injury in the rats. A total of 80 male Wistar rats were randomly divided into 4 groups: sham, IR model and two I/R+TREE (2%and4%) - treated groups (n=20 per group). I/R groups kidneys were subjected to 60 min of global ischemia at 37*c followed by 30min of reperfusion. After 24h of reperfusion period, the rats were sacrificed. Kidney function tests and Histopathologic examination was also performed. Results were compared with a group of rats with sham operation. High serum creatinine, blood urea nitrogen and uric acid were observed in I/R rats compared to the sham rats. Pre-treatment of turmeric powder for 30 days prior to IR operation improved renal function reduced IR induced renal inflammatory and oxidative injury.The results of this study showed that turmeric powder significantly prevented renal I/R-induced functional and histological injuries.*
Keywords: *Curcuma longa linn, Ischemia-reperfusion, Kidney, Rat.*

Received 22/09/2013 Accepted 11/11/2013

INTRODUCTION

There are several pathologic pathways have been introduced for damaging the body tissues during the ischemic-reperfusion. Free radicals are responsible for causing the injuries due to ischemic-reperfusion in the majority of tissues. By production of reactive oxygen's free radicals, superoxides and hydroxyls, during the ischemic-reperfusion tissues suffer from structural and functional damages so that more sever pathologic damages cause during the reperfusion process [1]. Ischemic damages may appear when the perfusion is stopped to a tissue but pathologic features paradoxically occur when the blood flow reestablish during the reperfusion process [2]. The kidneys are the example of organs which are injure from this clinical syndrome. For example, in consequence of decreasing the perfusion to kidneys due to hemorrhage, shock, great operations or complete interruption blood flow to this organ during the implantation, this condition occurs. The most important reason for delayed performance of implanted organ is damages due to ischemic-reperfusion. Whatever the severity of damage due to I/R be more the rejection rate or functional impairment is increased. So, decreasing the damages during the early I/R will be result in better consequences for the remaining the allograft in the both short and long term. The short term inflammatory signs due to I/R are determined by induction of pre-inflammatory cytokines, expression of attached molecules and cellular infiltration. IL-1 and TNF-α are known as pre-inflammatory cytokines which play the important role during the post-implantation induced I/R [3]. Occurrence the ischemic-reperfusion in kidneys is started with producing the free radicals which yield to lipid peroxidation and incidence of acute renal failure [4, 5].

Use of medicinal herbs for treating the range of diseases is developed increasingly and special attention has been made to the protective effects of antioxidants with natural origin against disease [6]. There are several medicinal herbs with antioxidant properties and it supposes that may be more useful in preventing of damages due to I/R. Turmeric is such herbs that its antioxidant properties has been reported [7]. Pathologic conditions such as cancers, oldness, cardiovascular disease, ischemia and inflammation are involved in production of reactive oxygen species [8, 9]. So, removing the radicals is one

of the defensive ways against several diseases. Antioxidants are agents which may found in the food and body, even in small amounts, can protect the body against various types of oxidative damage caused by reactive oxygen species [10]. Medicinal plants due to ease of access, low side effects and the cost benefits considered as alternatives to synthetic drugs and has been attended by researchers in recent decades. Biologic agents with herbal origin comprise branch of modern pharmacotherapy of disease. Although there are various pharmacological agents for the treatment of various diseases, most patients are not able to tolerate the side effects of chemical drugs. On the other hands, most plants are very few side effects on the patients. The medicinal properties of turmeric powder are assessed for the first time in present research in terms of pathology during I/R. Since Turmeric has antioxidant properties, it is expected that use of it would prevent from I/R. With regard the point that the effect of mentioned plant on damages due to I/R has not been studied histopathologically yet, the aim of present study was to evaluation the protective effects of turmeric powder on I/R induced injury histopathologically in rats.

MATERIALS AND METHODS

Eighty male Wistar rats (200±20 g and 9-weeks aged) were selected for the study. The animals were housed under standard environmental conditions (23 ± 1 °C, with 55 ± 5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. Rats were randomly divided into four equal groups: 1- normal controls; 2- I/R group; 3- I/R+ low dose turmeric powder and 4- I/R+ high dose turmeric powder.

New stems of Turmeric prepared by the Department of Agriculture, Islamic Azad University of Tabriz, and after confirmation by them, completely cleaned, washed by water and then dried, then milled by grinding. Produced powder was kept in the room temperature. Treatment with turmeric powder was taken for 30 days. Groups 1 and 2 were fed by standard diet and groups 3 and 4 were fed by turmeric powder at the dose of 2% and 4%, respectively. After 30 days, for inducing the I/R, all animals were anesthetized by i.p. injection of sodium pentobarbital (50mg/kg). Then an incision was made in the line alba. In control group we induced I/R by manipulation of renal artery but in others, renal artery was caught by non-traumatic forceps. Then the incision was closed by suturing. 24 hours after reperfusion, blood samples were taken from retro-orbital plexus for measurement the urea [11], uric acid [12] and creatinine [13]. Rats were killed by dislocation in the cervical vertebrates. The left kidneys were quickly removed for measurement of damage severity and Histopathologic assessments. Blood samples were centrifuged at 2500 rpm for 15 min at 30˚C [14]. Slides were prepared and were interpreted by method introduced by Bhalodia et al., 2009 [15].

Statistical analysis

Data were presented as mean±SEM. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.05$ was considered statistically significant.

RESULTS

Pathologic findings

Histopathologic graphs of experimental groups are given in the figs 1-8. Also, severity of changes was assayed and data are depicted in Table 1.

Pathologic findings showed that renal structure is normal in the control group and there were not pathologic changes. In the group 2, degenerative changes of tubular cells, acute tubular necrosis, edema, hyperemia and sever hemorrhage were more prevalent. Also, hyperemia and sever hemorrhage of glomerulus was obvious. In group 3, relative improvement was observed in the pathologic changes. Pathologic changes in this group included edema, moderate hyperemia and hemorrhage in the glomerulus and renal interstitial tissue with moderate degenerative changes and mild necrosis of tubular epithelium. Sections prepared from kidneys of group 4 rats' showed a significant improvement in the occurrence of pathologic changes. The only observed pathologic finding was the mild vacuolation of tubular cells and slight hyperemia.

Table 1: Data related to renal damage obtained from experimental groups

Pathologic evidence / Group	Acute cell inflammation	Hyperemia and hemorrhage	Tubular distention	Necrosis of tubular cells' epithelium
1 (Normal control)	-	-	-	-
2 (I/R)	+++	+++	+++	+++
3 (I/R+ Turmeric 2%)	+++	++	++	++
4 (I/R+ Turmeric 4%)	++	+	+	+

- shows absence of pathologic changes, + shows existence of pathologic changes.

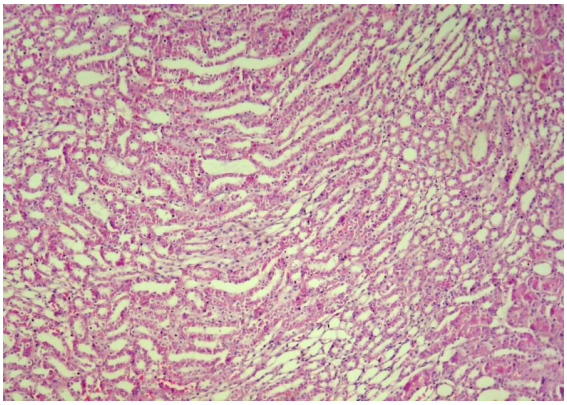

Fig 1: Microscopic view from kidney tissue of a rat belonged to control group. Renal structure is normal and there is no pathologic change, H&E, 40×.

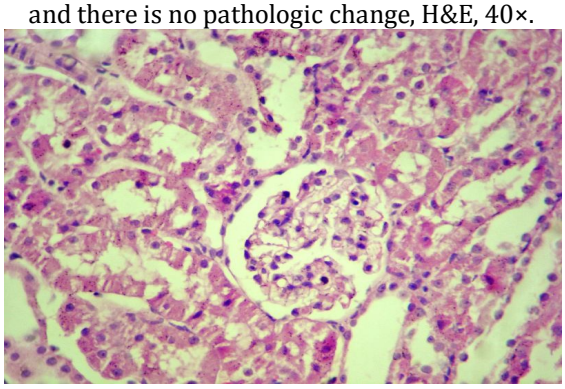

Fig 2: Microscopic view from renal cortex of a rat belonged to control group. Renal cortex is normal and there is no pathologic change. H&E, 250×.

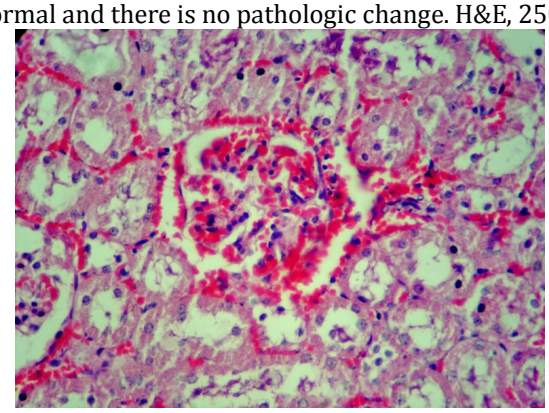

Fig 3: Microscopic view from renal cortex of a rat belonged to group I/R. Hyperemia, sever hemorrhage in the glomerulus and renal interstitium is prominent. Sever degenerative changes and necrosis of tubular epithelium is obvious. H&E, 250×.

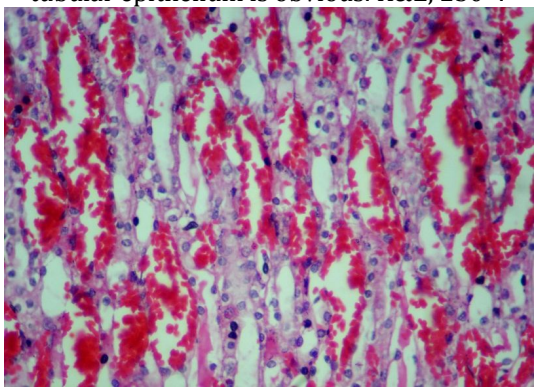

Fig 4: Microscopic view from renal medulla of a rat belonged to group I/R. Hyperemia and sever hemorrhage in the renal interstitium with sever degenerative changes along with necrosis of tubular epithelium is obvious. H&E, 250×.

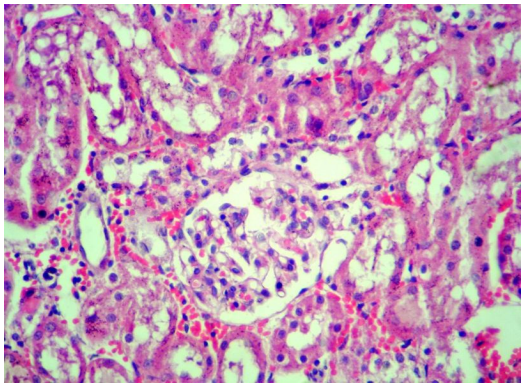

Fig 5: Microscopic view from renal cortex of a rat belonged to group I/R+ Turmeric 2%). Moderate hyperemia and hemorrhage of glomerulus and renal interstitial tissue with moderate degenerative changes and mild necrosis of tubular epithelium is seen. H&E, 250×.

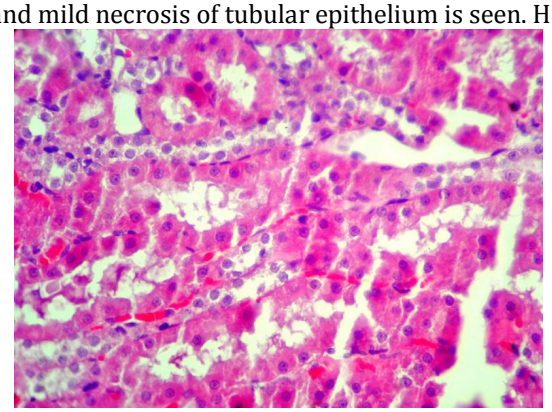

Fig 6: Microscopic view from renal medulla of a rat belonged to group I/R+ Turmeric 2%). mild necrosis of tubular epithelium is prominent. H&E, 250×.

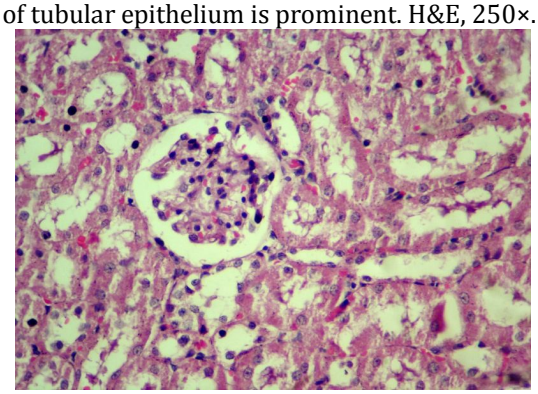

Fig 7: Microscopic view from renal cortex of a rat belonged to group I/R+ Turmeric 4%). Moderate hyperemia of renal interstitium with moderate degenerative changes and slight necrosis of tubular epithelium is visible. H&E, 250×.

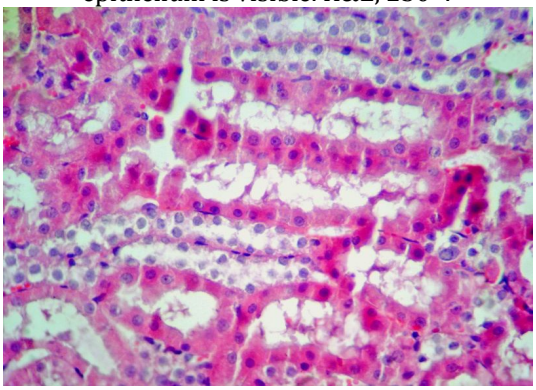

Fig 8: Microscopic view from renal medulla of a rat belonged to group I/R+ Turmeric 4%). Moderate hyperemia of renal interstitium with moderate degenerative changes and slight necrosis of tubular epithelium is seen. H&E, 250×.

Biochemical findings

Biochemical changes in the serum and statistical comparison of groups are given in the table 2.

Table 2: Effect of turmeric on serum biochemical parameters

Group	Parameter		
	Serum creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
1 (normal control)	1.55±0.09	61.32±5.8	0.79±0.11
2 (I/R)	4.23±0.21[a]	142.95±12.75[a]	1.82±0.18[a]
3 (I/R+ Turmeric 2%)	2.60±0.11[b]	103.95±7.32[b]	1.28±0.12[b]
4 (I/R+ Turmeric 4%)	1.85±0.08[c]	72.35±4.3[c]	0.90±0.15[c]

a: P<0.001 in compared with control group; b: P<0.05 and c: P<0.01 in compared with I/R group.

DISCUSSION AND CONCLUSION

Turmeric has been used in traditional medicine to treat respiratory problems such as cough, asthma and allergies, liver disorders, anorexia, rheumatism, diabetic ulcers and sinusitis for centuries [16]. Turmeric therapeutic properties including antioxidant effects [17], anti-inflammatory effects [18-20], anti-cancer and anti-microbial effects [21, 22], hepato-protective effects [23], reno-protective effects [24], thrombo-inhibitory effects [25], cardio-protective effects [26, 27], the hypoglycemic effects [28-30], an anti-inflammatory effects on the rheumatoid arthritis [31], has been confirmed by modern and advanced researches. The most important factor causes the turmeric to be more useful in traditional medicine is its safety and non-toxicity properties so that there is no report about its toxicity on animals [32, 33] and humans [34] so far. It is evident that Turmeric is not toxic even at high doses [35]. In recent years, access to new types of antioxidants with herbal origin has been seriously considered by researchers [36]. Curcumin is a biologically active constituents extracted from stems of Turmeric which has a powerful antioxidant activity [37]. Cell damage is one of the most important issues in Ischemia-reperfusion. Paradoxically, reperfusion induces sever cellular damage. So, in addition to cells that were undergone irreversible damages at the end of the ischemic period, the other cells in the tissue also are lost [38]. The results of our study show that pretreatment with Turmeric have preventive, protective and therapeutic effects on ischemia-reperfusion in the kidney. In our trial, animals that had injuries due to I/R showed renal failure signs such as decrease in the renal function as increase in the serum levels of urea, uric acid and creatinine. It must be noted that serum values of creatinine, urea and uric acid are indicator of glomerular filtration rate. Using turmeric resulted in significant reduction in biochemical and Histopathologic changes due to I/R. So that in present study, serum values of creatinine, urea and uric acid in I/R group was significantly more than control group. Our results showed significant decrease in values of these parameters in treated groups with turmeric. We also found that pretreating with turmeric on preventing of disturbance induced by I/R was useful and dose-dependently. Acute renal failure induced by I/R as shown in Histopathologic graphs demonstrate extensive damage of renal tubules, tubular necrosis, glomerular damage and tubular obstruction with cell cuprous. Most of the tubular and glomerular dysfunction during reperfusion occurs after oxidative burst and reactive oxygen species are produced in large quantities, which is one of the most important factors in damaging the cells. Reactive oxygen species (ROS) are responsible for lipid peroxidation in biological membranes which are leading to cell death. Protection induced by free radicals' removers versus ROS which have been made during I/R shows that free radicals are involved in pathogenesis of cells with I/R. Altogether, the mechanism of the protective effect of GTE on renal I/R injury can be explained by its antioxidant activity. The rennin-angiotensin system plays a pivotal role in regulation of blood pressure. Renin acts on angiotensinogen to form angiotensin-I, which is converted to angiotensin-II with the help of angiotensin-converting enzyme [39]. Accumulating evidence suggests that angiotensin-II stimulates intracellular formation of ROS such as superoxide anion and hydrogen peroxide that leads to kidney damage [40].

These results show that pretreatment with turmeric prevents the damages induced by I/R by preventing the lipid peroxidation and protects the kidneys from accumulation of ROS and discharging the SOD, CAT and GPx.

Anyway, the results showed that turmeric significantly reduces the damages induced by I/R and have reno-protective effect against I/R. Also, we found that turmeric improves the renal functional parameters. However, recognizing the active ingredients or constituents of this herb, determination its exact mechanism of action need further studies.

ACKNOWLEDGMENTS

The authors would like to thank Tabriz Branch, Islamic Azad University for the financial support of this research.

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Citation of This Article

Alireza Monadi Sefidan and Daryoush Mohajeri, Preventive Effects of Turmeric *(Curcuma longa* linn) on Renal Ischemia-Reperfusion Injury in Rats. Adv. Biores. Vol 4[4] December 2013: 40-46