Preventive effects of turnip *(Brassica rapa L.)* 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 Turnip root ethanolic extract (TREE) 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 (1% and 2%)-treated groups (n = 20 per group). I/R groups' kidneys were subjected to 60 min of global ischemia at 37 °C followed by 30 min of reperfusion. After 24h of reperfusion period, the rats were sacrificed. Kidney function tests and histopathological examination were 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 TREE extracts 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 TREE significantly prevented renal I/R-induced functional and histological injuries.
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1. 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 during the ischemic-reperfusion to wit superoxides and hydroxyls, tissues suffer from structural and functional damages so that more sever pathologic damages cause during the reperfusion process (McCord, 1985). 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 (Kapil and Sharma, 1995). 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 (Hidehisa Kitada, et al, 2002). 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 (Hidehisa Kitada, et al, 2002). Occurrence the ischemic-reperfusion in kidneys is started with producing the free radicals which yield to lipid peroxidation and incidence of acute renal failure (Garcia-Criado, et al, 1998; Paller et al, 1984). 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 (Frei and Higdon, 2003). There are several medicinal herbs with antioxidant properties and it supposes that may be more useful in preventing of damages due to I/R.

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. Many of these plants were used in traditional medicine including the turnip roots.

Brassica rapa species has important varieties such as turnip (Sasaki and Takahashi, 2002). Turnip has active biological compounds such as flavonoids (isorhamnetin, kaempferol and quercetin glycosides), phenylpropanoid derivatives (Romani et al., 2006), indole alkaloids and sterol glucosides (Schonhof et al., 2004). Considering that turnip has antioxidant effects it expects that use of this plant may prevent Ischemia-reperfusion injuries. The main objective of present study was to Histopathological assessment of protective effects of turnip roots on ischemia-reperfusion injury in kidneys of rats by focus on pathological aspects.

2. 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 extract and 4- I/R+high dose extract.

New stems of turnip 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 turnip was taken for 30 days. Groups 1 and 2 were fed by standard diet and groups 3 and 4 were fed by turnip at the dose of 1% and 2%, 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 (Fawcett and Scott, 1960), uric acid (Caraway, 1955) and creatinine (Teitz, 1987). 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 (Lee and Luna., 1988). Slides were prepared and were interpreted by method introduced by Bhalodia et al., 2009.

2.1. Statistical analysis

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

3. Results

3.1. 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+ turnip 1%)	+++	++	++	++
4 (I/R+ turnip 2%)	++	+	+	+

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

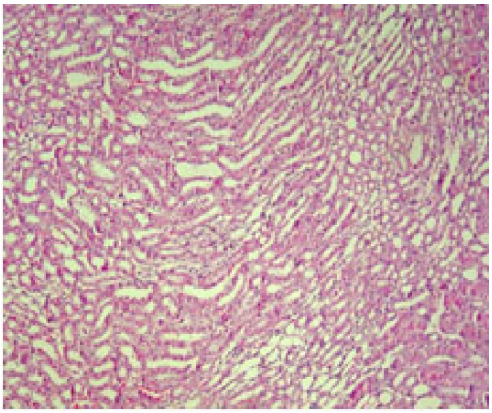

Fig 1: Microscopic view from kidney tissue of a rat belonged to control group. Renal structure is normal and there are no pathologic changes, H&E, 40×.

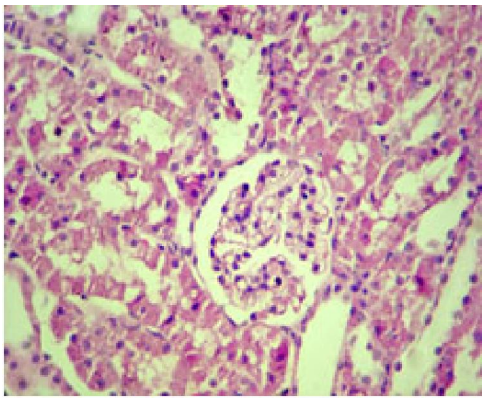

Fig 2: Microscopic view from renal cortex of a rat belonged to control group. Renal cortex is normal and there are no pathologic changes. H&E, 250×.

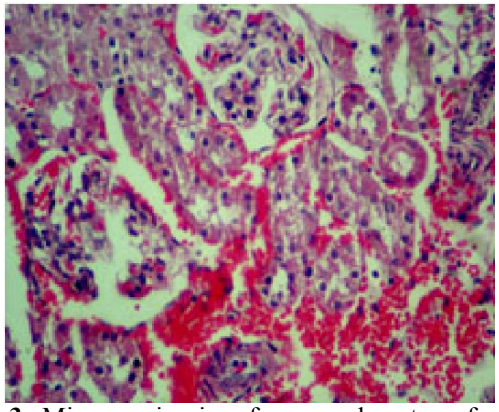

Fig 3: Microscopic view from renal cortex of a rat belonged to group I/R. Hyperemia and sever hemorrhages of glomerulus and renal interstitial tissue with sever degenerative changes and necrosis of tubular epithelium is obvious. H&E, 250×.

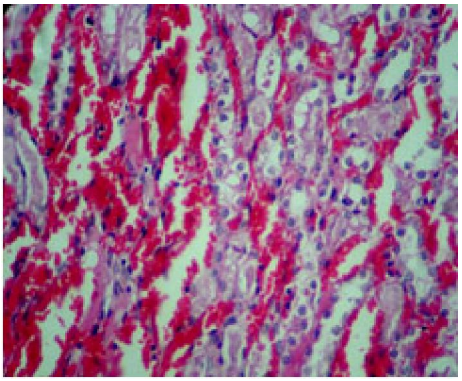

Fig 4: Microscopic view from renal medulla of a rat belonged to group I/R. Hyperemia and sever hemorrhage in the renal interstitial tissue with sever degenerative changes with necrosis of tubular epithelium is obvious. H&E, 250×.

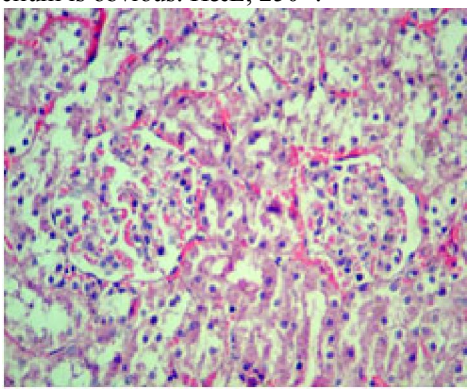

Fig 5: Microscopic view from renal cortex of a rat belonged to group I/R+ Turnip 2%). Moderate hyperemia and hemorrhage of glomerulus and renal interstitial tissue with moderate degenerative changes and mild necrosis of tubular epithelium is obvious. H&E, 250×.

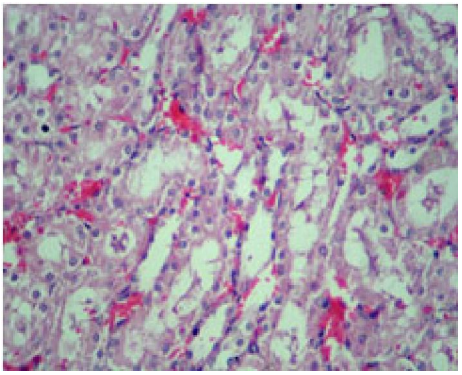

Fig 6: Microscopic view from renal medulla of a rat belonged to group I/R+ Turnip 2%). Moderate hyperemia and hemorrhage of glomerulus and renal interstitial tissue and moderate degenerative changes with mild necrosis of tubular epithelium is prominent. H&E, 250×.

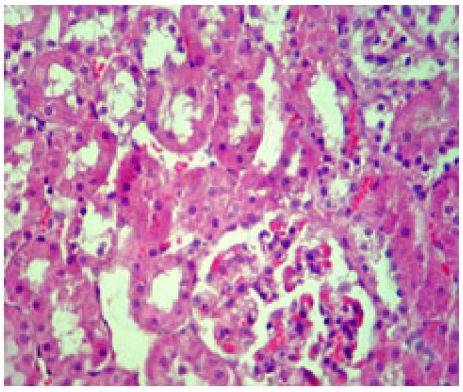

Fig 7: Microscopic view from renal cortex of a rat belonged to group I/R+ Turnip 4%). Moderate hyperemia of renal interstitium with moderate degenerative changes and slight necrosis of tubular epithelium is prominent. H&E, 250×.

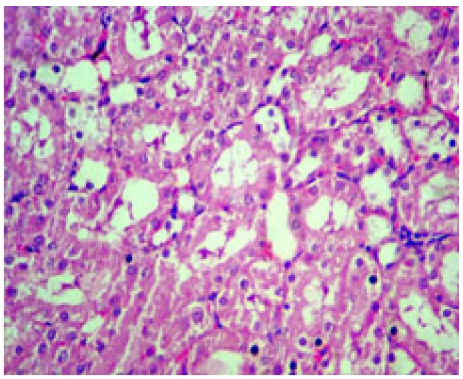

Fig 8: Microscopic view from renal medulla of a rat belonged to group I/R+ Turnip 4%). Moderate hyperemia of renal interstitium with moderate degenerative changes and slight necrosis of tubular epithelium is seen. H&E, 250×.

3.2. Biochemical findings

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

Table 2: effect of turnip on serum biochemical parameters

Parameter / Group	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+ turnip 2%)	2.62±0.11 [b]	103.95±7.32 [b]	1.30±0.12 [b]
4 (I/R+ turnip 4%)	1.80±0.08 [c]	71.35±4.3 [c]	0.88±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.

4. Discussion and conclusion

Use of plants genus turnips is associated with human health reduces risk of chronic diseases such as cardiovascular disorders (Cartea and Velasco, 2008) and cancer (Traka and Mithen, 2008). In studies conducted by Kim et al., (2006) protective turnip root extract against cisplatin nephrotoxicity has been demonstrated. Studies conducted by Rafatullah et al., (2006) have shown that treatment with turnip extract is prevented liver injury by carbon tetrachloride (CCl4). According to their conclusion, protective effect of turnip is probably due to its anti-oxidative properties. In a study done by Choi et al., (2006), anti-oxidant and hepatoprotective effects of turnip ethanolic extract both in vitro and in vivo conditions have been studied. In their study, oral administration of ethanol extract of turnip in d-Galactosamine-induced hepatotoxicity mice resulted in improved liver. Jung et al., (2008) in a study on diabetic rats have shown that the turnip root extract by increasing in glucose and lipid metabolism has anti-diabetic effect in type 2 diabetes. Researches have shown that Isothiocyanates and indole glucosinolate metabolites are two major groups derived from glucosinolates which have anti-cancer effects against wide spectrum of cancers in both in-vivo and in-vitro (Mithen et al., 2003; Zhang et al., 1994). The effects of this plant often is related to itself chemical properties specially glucosinolates (Mithen et al., 2003; Rosa et al., 2006) and phenolic compounds (Hertog, 1996; Duthie et al., 2000). These compounds are divided into the sub groups based on molecular structure and hydroxyl group situation. Flavonoids are common polyphenols. Flavonoids and hydroxycinnamic acid derivatives exist in this plant frequently and are considered as important biological active components of human diet (Cermak et al., 2003; Llorach et al., 2003; Vallejo et al., 2004). These compounds have direct anti-oxidant and free radicals removal effects (Bennett et al., 2006).

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 (Cotran et al., 1989). The results of our study show that pretreatment with turnip 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 turnip 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 turnip. We also found that pretreating with turnip 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 (Gavras and Salerno, 1996). Accumulating evidence suggests that angiotensin-II stimulates intracellular formation of ROS such as superoxide anion and hydrogen peroxide that leads to kidney damage (Sachse and Wolf, 2000). These results show that pretreatment with turnip 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 turnip significantly reduces the damages induced by I/R and have reno-protective effect against I/R. Also, we found that turnip improves the renal functional parameters. However, recognizing the active ingredients or constituents of this herb, determination its exact mechanism of action need further studies.

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