

PROTECTIVE EFFECT OF *DAUCUS CAROTA* ROOT EXTRACT AGAINST RENAL ISCHEMIA REPERFUSION INJURY IN RATS.

Patel Mital R.¹, Patel Laxman J.², Patel Rameshvar K.²

¹ Nootan Pharmacy College, Visnagar

² S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Mehsana- Gozariya highway, Mehsana, Gujarat.

Summary

This study was designed to investigate the renoprotective activity of *Daucus carota* root extract on renal ischemia reperfusion injury in rats. Renal pedicles of 48 rats were occluded for 45 minutes followed by 24 hours reperfusion. Six days prior to induction of I/R, 12 of the rats received petroleum ether extract, 12 of the rats received fractional methanolic extract and another 12 received direct methanolic extract of *Daucus carota* root (250 & 500 mg/kg, orally). Serum creatinine, urea, and uric acid levels were measured after reperfusion period, the rats were sacrificed. Superoxide dismutase, Catalase, reduced glutathione, and renal malondialdehyde content were determined in renal tissues. Results were compared with a group of rats with sham operated. Renal ischemia reperfusion caused significant impairment of kidney function. Six day administration of *Daucus carota*, however, minimized this effect. Rats with renal I/R only showed significantly decreased activity of superoxide dismutase, catalase, and reduced glutathione compared with the sham operated rats. These declining trends were significantly less in the group treated with petroleum ether, fractional methanolic and direct methanolic extract of *Daucus carota* root compared with those in I/R group. Renal I/R produced a significant increase in malondialdehyde level, while pretreatment with *Daucus carota* extracts was associated with a significantly lower malondialdehyde level. These finding imply that reactive oxygen species play a crucial role in I/R induced kidney injury and *Daucus carota* extracts exerts renoprotective activity probably by the free radical scavenging activity.

Key words: *Daucus carota*, Ischemia-reperfusion injury, Renoprotective, Anti-oxidant

Corresponding Author:

Name: Patel Mital R.

Address: Nootan Pharmacy College, Visnagar.

E-mail: meet_mika206@yahoo.co.in

Introduction

Acute kidney injury (AKI) is a devastating disease with clinical, economic and ethical dimensions and is emerging as a major public health problem globally. More recent prospective studies report an overall incidence of acute renal failure of almost 500 per million per year^[1, 2] and an incidence of acute renal failure needing dialysis of more than 200 per million per year^[3]. The kidney, in particular, is at risk in situations of low-flow ischemia/reperfusion following aortic aneurysm repair. The mechanisms underlying ischemia/reperfusion damage to kidneys are likely multifactorial and interdependent, involving hypoxia, free radical damage and inflammatory responses^[4].

Daucus carota Linn. Commonly known as “Carrot” belonging to family Apiaceae (Umbelliferae) and is cultivated almost all over the world as a useful vegetable. The plant has undergone extensive phytochemical studies and a large number of active ingredients have been isolated^[5]. The phytochemical substances in carrot are composed of phenolic compounds, terpenes, and carotenoids which act as free radical quenchers and antioxidant agents^[6, 7, 8]. As all the free radical scavengers having protective role in renal injury, the purpose of this study was to evaluate the protective effect of *Daucus carota* root extract against Ischemia/ reperfusion induced oxidative stress in rats.

Methods

Plant Material

Fresh root of *Daucus carota* of orange colour were not cultivated in the state of Gujarat and it is procured by the local merchants from outside Gujarat. The collected *Daucus carota* is authenticated from Mehsana Urban Bank Institute of Bioscience. After washing, the root of plant were taken and crushed. The crushed roots were dried under shade. The dried root were finely powdered and stored in polythene bags at room temp.

Preparation of Extracts

The root powder of *Daucus carota* was subjected to extraction using soxhlet apparatus. The powdered materials were exhaustively extracted successively with petroleum ether, chloroform, methanol & water in soxhlet apparatus by continuous hot extraction. Direct methanolic extraction of fresh powder was also carried out and all the extracts were concentrated under reduced pressure.

In-vitro Antioxidant Activity: DPPH Free Radical Scavenging Assay^[9] The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH.

Animals

Healthy female/male wistar rats weighing 200-250g were procured from central animal facility of S.K.Patel College of Pharmaceutical Education and Research. All animals were housed at ambient temperature (22 ± 1 °C) and relative humidity (55 ± 5) with standard diet and water provided *ad libitum*. The care and the use of these animals were in accordance with the guidelines of the CPCSEA. An experimental protocol was approved by IAEC. On the bases results obtained from DPPH free radical scavenging assay renal I/R activity was performed for petroleum ether, fractional methanolic and direct methanolic extracts of *Daucus Carota* root. 48 rats were divided in eight groups, six rats in each group. Except group 1, renal I/R were performed in all groups of animals. Group 1 served as normal sham control.

Group 2 served as normal renal I/R injury control. Group 3 and 4 animals received 250 mg/kg and 500 mg/kg petroleum ether extract of *Daucus carota* root respectively for 7 days before sacrifice. Group 5 and 6 animals received 250 mg/kg and 500 mg/kg fractional methanolic extract of *Daucus carota* root respectively for 7 days before sacrifice. Group 7 and 8 animals received 250 mg/kg and 500 mg/kg direct methanolic extract of *Daucus carota* root respectively for 7 days before sacrifice.

Induction of Renal Ischemia-Reperfusion Injury in Rat

Healthy male/female wistar rats were used. Rats were anesthetized with Ketamin (60 mg/kg i.p.) and Diazepam (5 mg/kg i.p.). Body temperature was maintained throughout surgery at $37 \pm 0.5^\circ\text{C}$ with a lamp. The skin on back was shaved and skin was disinfected with povidone iodine solution. All rats underwent surgical exposure of the left and right renal pedicles via midline incision. To induce renal ischemia, both renal pedicles were occluded for 45 min with vascular clamps. After 45 min. of occlusion, the clamps were removed, and kidneys were observed to undergo reperfusion for 24 hrs. The abdominal muscle layer was closed with an interrupted suture, and the skin layer was closed with a continuous subcutaneous suture. For analgesia, rats received topical lidocaine jelly (2%) to the wound for the 24 hrs and one dose of acetaminophen (6.8 mg/kg pr) as deemed by the Animal Care Staff. All rats were having free access to water and food. After 24 hrs reperfusion for protective study (preconditioning of kidney) rats were killed and kidney was rapidly removed for further analysis.

Serum Analysis and Histopathological Examination

Blood was collected from the rats by retro-orbital puncture at the time of sacrifice and was allowed to clot for 10 minutes at room temperature. Clots were centrifuged at 2500 rpm for 10 minutes to separate the serum. Serum creatinine, urea, and uric acid levels were measured by assay kits (Nicholas Piramal India Pvt Ltd, Mumbai, India) using semiautomatic analyzer (photometer 5010, Nicholas Piramal India Pvt Ltd, Mumbai, India). The kidneys fixed in a 10% neutral-buffered formalin solution were embedded in paraffin and were used for histopathological examination. Five micrometer- thick sections were cut, deparaffinized, hydrated, and stained with hematoxylin-eosin. The renal sections were examined blindly for tubular cell swelling, interstitial edema, tubular dilatation, and moderate to severe necrosis in all treatments.

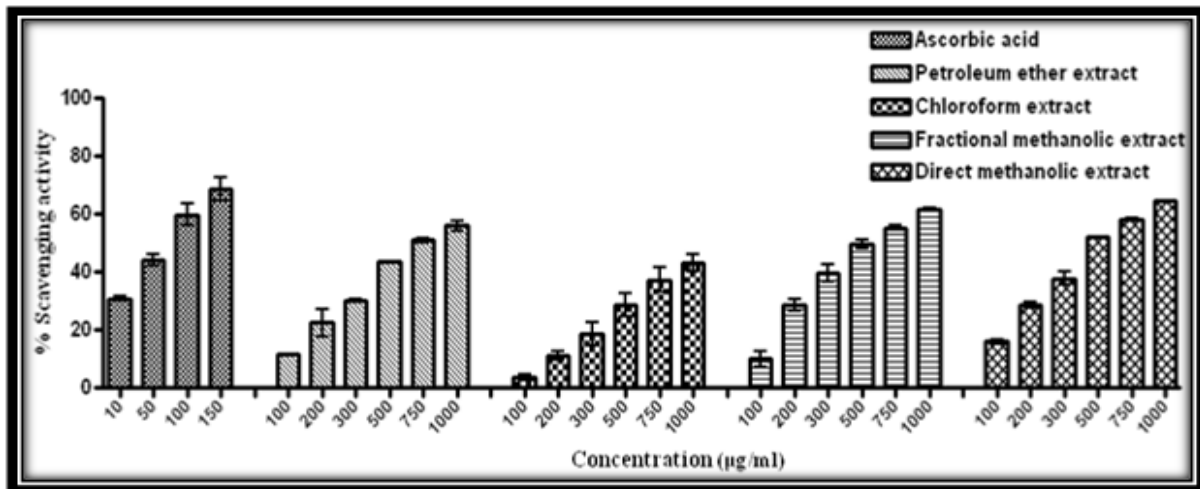
Estimation of Antioxidant Enzymes

After sacrificing the animals, their kidneys were quickly removed, perfused immediately with icecold hypertonic saline solution, and homogenized in chilled potassium chloride (1.17%) using a Potter Elvehjem homogenizer (Remi, Mumbai, India). The homogenate was centrifuged at 10500 g for 20 minutes at 4°C to get the postmitochondrial supernatant, which was used to assay superoxide dismutase^[10], catalase^[11], reduced glutathione^[12] and lipid peroxidation activity^[13].

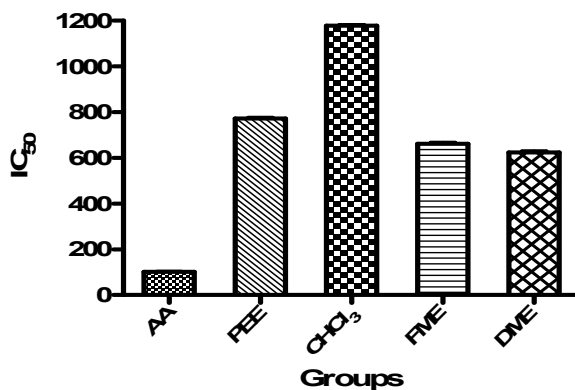
Statistical Analysis

Results were expressed as Mean \pm S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using computer based fitting program (Prism, Graphpad.). Differences were considered to be statistically significant when $p < 0.05$.

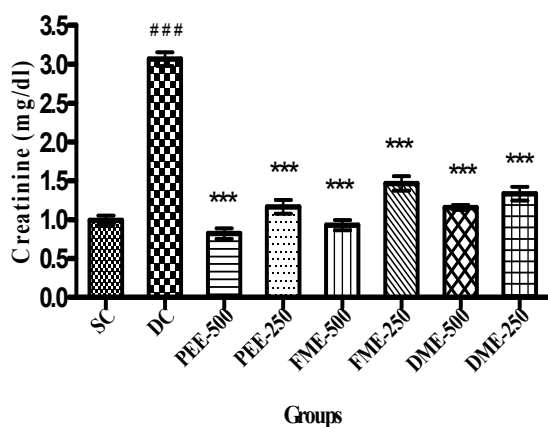
Results



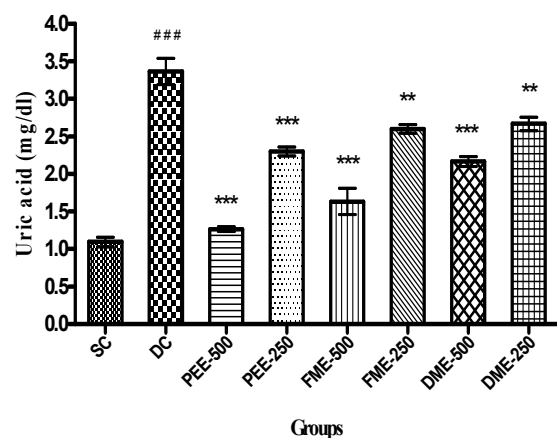
Data showing the comparison of % DPPH free radical scavenging activity of ascorbic acid, petroleum ether, chloroform, direct methanolic and fractional methanolic extract of *Daucus carota* root. Values are expressed in mean±SEM (n=3).



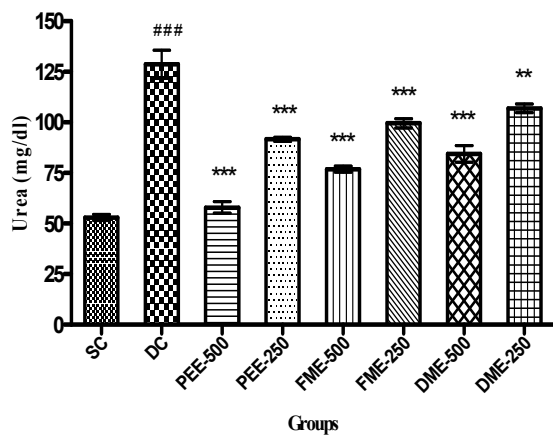
Data showing the comparison of IC₅₀ for DPPH free radical scavenging activity of ascorbic acid (AA), petroleum ether (PEE), chloroform (CHCl₃), fractional methanolic (FME) and direct methanolic (DME) extract of *Daucus carota* root. Values are expressed in mean±SEM. (n=3)



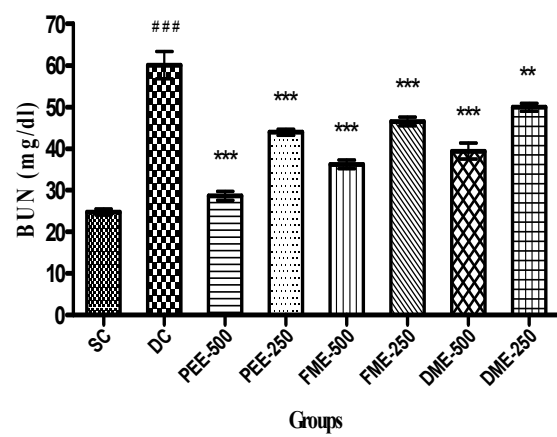
(A)



(B)

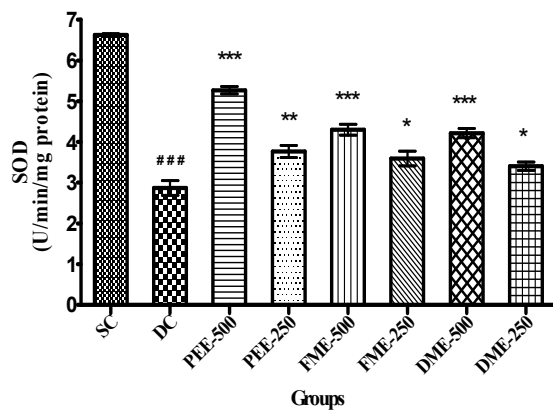


(C)

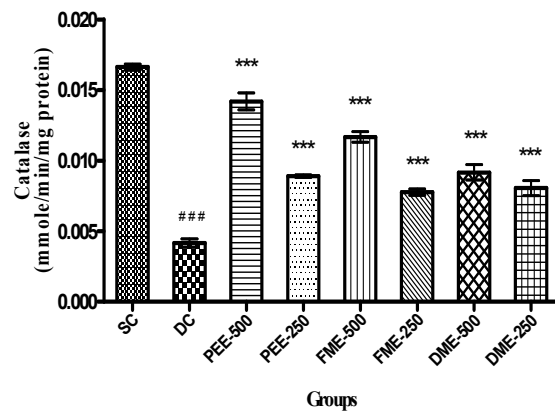


(D)

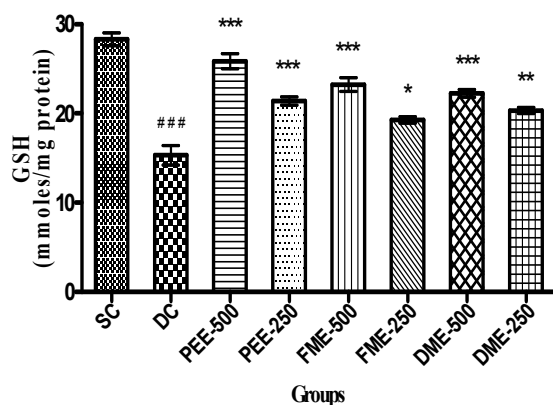
Data showing comparison of serum creatinine (A), uric acid (B), urea (C) and BUN (D) in sham control (SC), disease control (DC), and treated group of petroleum ether extract (PEE 250 & 500 mg/kg), fractional methanolic extract (FME 250 & 500 mg/kg), direct methanolic extract (DME 250 & 500 mg/kg) of *Daucus carota* root. Values are expressed in mean \pm SEM. n=6. ### p < 0.001 Vs sham control, *** p < 0.001 Vs disease control.



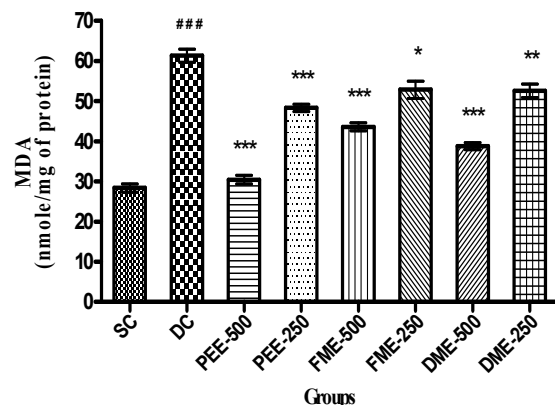
(A)



(B)



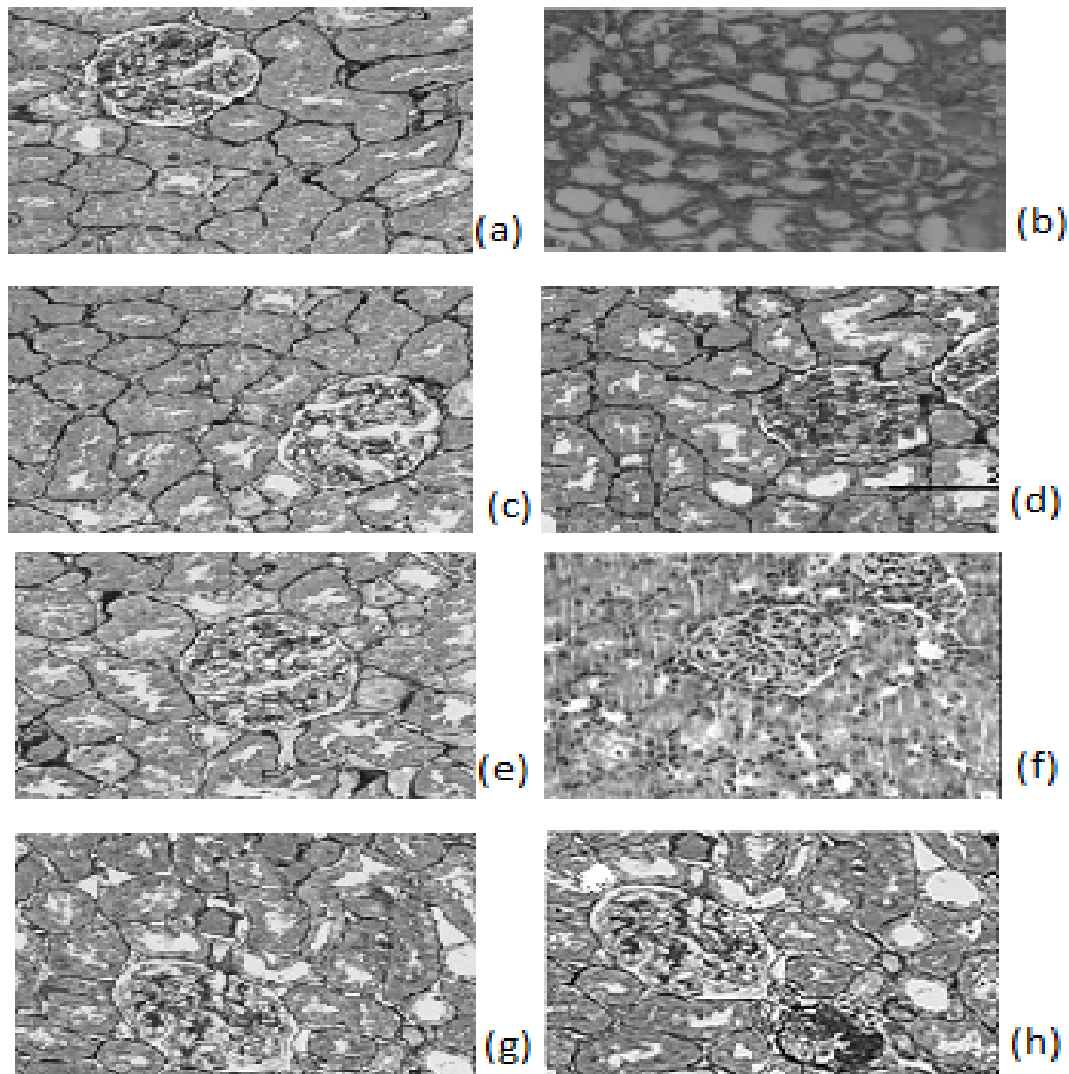
(C)



(D)

Data showing comparison of SOD (A), catalase (B), GSH (C) and MDA (D) level in sham control (SC), disease control (DC), and treated group of petroleum ether extract (PEE 250 & 500 mg/kg), fractional methanolic extract (FME 250 & 500 mg/kg), direct methanolic extract

(DME 250 & 500 mg/kg) of *Daucus carota* root. Values are expressed in mean \pm SEM. n=4-6. ### p < 0.001 Vs sham control, *** p < 0.001 Vs disease control.



Sections of the rat kidney (hematoxylin-eosin, \times 100). (a) kidney section of a rat in the sham control group shows normal glomeruli and tubuli. (b) Kidney section of a rat exposed to renal ischemia/reperfusion shows interstitial hemorrhage surrounding the glomeruli, loss of tubular epithelial cells & lumen of tubule shows cell debris. (c, e & g) Kidney section of the rats with ischemia/reperfusion injury treated with 500 mg/kg PEE, DME & FME respectively, showing moderate congestion of glomeruli and moderate damage to tubules (d,f &h)) Kidney section of the rats with ischemia/reperfusion injury treated with 250 mg/kg PEE, DME & FME respectively, showing mild congestion of glomeruli and damaged tubules.

Discussion

Ischemic pre-conditioning is a phenomenon by which multiple brief exposures to ischemia can reduce the damage caused by subsequent prolonged ischemia. Pretreatment with some ROS scavengers improve renal function after I/R damage, indicating that ROS are involved in renal I/R insults^[14]. Attributed to *Daucus carota* plant, no biochemical studies have been carried out to shed light on the role of *Daucus carota* in renal I/R. In the light of the above, the present study was undertaken to elucidate the effect of *Daucus carota* root extract on serum creatinine, urea, uric acid, and blood urea nitrogen and antioxidant enzymes in I/R rats.

1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable nitrogen centered free radical which can be effectively scavenged by antioxidants^[15]. In present DPPH free radical scavenging study, PEE, FME and DME of *Daucus carota* root showed better scavenging activity (IC₅₀ values 772.3, 662.0 and 624.0 respectively) compare to chloroform extract (1178.0). The results shown by the in vitro antioxidant assay clearly suggest that *Daucus carota* root is showing promising anti-oxidant activity. On the bases of these results, invivo evaluation was performed for PEE, FME and DME of *Daucus carota* root.

In pre-conditioning of kidney study significant increase (P< 0.001) in serum creatinine, uric acid, urea and blood urea nitrogen were found in renal I/R rat as compared to sham control, which indicates that renal I/R leads to generation of oxidative stress, which leads to kidney damage. In pre-conditioning of kidney, significant decrease (P< 0.001) in serum creatinine, urea, uric acid and blood urea nitrogen levels were found in renal I/R rats pre-treated with PEE, FME and DME of *Daucus carota* root at 500 mg/kg, compare to renal I/R control rats, which indicates the protective activity of *Daucus carota* root extract against renal I/R induced oxidative stress in kidney. Pre-treatment of PEE, FME and DME of *Daucus carota* root at 250 mg/kg also showed significant decrease (P< 0.05 or 0.01 or 0.001) in creatinine, urea, uric acid and blood urea nitrogen levels compare to renal I/R control rats but the levels were still high compare to 500 mg/kg pre-treated animals. These shows that at 500 mg/kg dose *Daucus carota* root extract shows best protective activity against renal I/R in rats.

In pre-conditioning of kidney, significant increase (P<0.001 or 0.01)) in SOD, catalase and GSH, while significant decrease (P<0.001) in MDA were found in renal I/R rats pre-treated with PEE, FME and DME of *Daucus carota* root at 500 mg/kg, compare to renal I/R control rats. It can be speculated that pretreatment with *Daucus carota* root extract prevented renal I/R-induced lipid peroxidation and protected the kidneys from severe increasing of ROS products. Pre treatment of *Daucus carota* root extract at 250 mg/kg also showed anti-oxidant activity but it is not as significant as at dose of 500 mg/kg.

Conclusion

The findings imply that in the settings of renal I/R injury, the ROS play a important role and that administration of *Daucus carota* root extract can reduce the renal I/R injury by boosting of antioxidant capacity and free radical scavenging activity. From the results of this study it could be stated that PEE of *Daucus carota* root having best renoprotective and anti-oxidant activity compare to FME and DME. Carotenoids content of PEE may be responsible for variation in activity which was found absent in FME and DME.

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