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Hesperidin improves warm ischemia/reperfusion-induced oxidative renal injury in rats

Chintan Gandhi, Rishit Zalawadia and R Balaraman*

Pharmacy Department, Faculty of Technology and Engineering, M. S. University of Baroda, Kalabhavan, Baroda-390001, Gujarat, India

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SUMMARY

Ischemia/reperfusion injury, which is commonly seen in the field of renal surgery or transplantation, is a major cause of acute renal failure. Previous studies showed that antioxidant treatments attenuated renal ischemia/reperfusion injury. The objective of this study was to examine the role of hesperidin in modulating reactive oxygen species induced inflammation and apoptosis after renal ischemia/reperfusion injury. Rats were subjected to right nephrectomy, 15 days later 45 min of renal ischemia and 24 h reperfusion with or without treatment with hesperidin. Renal function, inflammation and apoptosis were compared at 24 h after reperfusion injury. Hesperidin improved the renal dysfunction and reduced inflammation and apoptosis after ischemia/reperfusion injury. In conclusion, hesperidin shows potent anti-apoptotic and anti-inflammatory properties due to antioxidant property. These findings may have major implications in the treatment of human ischemic acute renal failure.

Key words: Antioxidant; Hesperidin; Ischemia reperfusion injury; Renal ischemia

INTRODUCTION

Postoperative acute renal failure in consequence of ischemia and reperfusion (I/R) injury can occur after kidney transplantation (Bouchier-Hayes *et al*., 1999). Ischemic cell injury in the kidney occurs during cardiovascular surgery, renal transplantation as well as the early allograft rejection subsequent to renal transplantation (Manuela, 2003). Excessive reactive oxygen species (ROS) generation occurs in I/R is proved in many biochemical and immunohistochemical studies. Generation of ROS, leading to dysfunction, injury, and renal cell necrosis (Chatterjee *et al*., 2000; Prabal and Chatterjee, 2007). Defense against free radical injury is provided by enzymatic (catalase, superoxide dismutase and glutathione peroxidase) and non-enzymatic (alpha tocopherol, vitamin C, allopurinol, dimethyl sulphoxide.) free radical scavengers (Mark *et al*., 1991; Devinder and Kanwaljit, 2004b). The protection provided by these free radical scavengers against ROS produced during injury further supports the hypothesis, ROS are involved in the cellular pathogenesis of I/R injury.

Thus, research efforts designed to prevent or ameliorate tissue injury have centered on inhibiting free radical generation during I/R injury.

*Correspondence: R Balaraman, Pharmacy Department, Faculty of Technology and Engineering, M. S. University of Baroda, Kalabhavan, Baroda-390001, Gujarat, India. Tel: +02652434187; Fax: +02652418927; E-mail: rbalaraman2000@yahoo.com

Hesperidin is a major and active flavanone glycoside mainly isolated from citrus fruits (Cho, 2006). It is reported to possess antiallergic, radio protective and anti-oxidant activities (Naveen *et al*., 2005a; Hosseinimehr and Nemati, 2006). Moreover, hesperidin is shown to possess immunomodulator (Chia-Chou *et al*., 2007), and antihypertensive activities (Garg *et al*., 2007). When hesperidin is administered orally, it is hydrolyzed by intestinal microflora to yield a major active metabolite hesperitin (Cho, 2006).

So far, there are no findings to prove that treatment with hesperidin could improve the survival rate after renal warm I/R injury. In this study, we examined whether treatment with hesperidin improve the survival rate in a renal warm I/R injury using a rat model.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats of either sex with body weight between 230 and 260 g were housed in an air-conditioned room with 12 h light and dark cycles, with free access to food and water *ad libitum* during the experiments. The institutional animal ethics committee approved the experimental protocol. All the experiments were conducted as per norms of CPCSEA.

Grouping of animals

The rats were divided into four groups each consisting of six animals.

Group 1: sham operated animals

Group 2: (I/R) on the day 1stanimals subjected to right nephrectomy, later underwent 15 days vehicle treatment (0.5% sodium Carboxy Methyl Cellulose (CMC)), on the 16th day, animals subjected to 45 min of left renal ischemia followed by 24 h reperfusion.

Group 3: (I/R+HSP) on the day 1stanimals subjected to right nephrectomy, later underwent 15 days Hesperidin treatment (100 mg/kg, p.o.) (Naveen *et al*., 2005b) on the 16th day, animals subjected to 45 min of left renal ischemia followed by 24 h reperfusion.

Group 4: (I/R + Vit.E) on the day 1stanimals subjected to right nephrectomy, later underwent 15 days Vitamin E treatment (100 mg/kg/ day, p.o) (Uma and Rao, 2005), on the 16th day, animals subjected to 45 min of left renal ischemia followed by 24 h reperfusion.

Surgical procedure

The progress of the experiment

Day 1	15 days	Day 16
Unilateral right nephrectomy	Drug treatment	45 min ischemia (left kidney) + 24 h reperfusion

After ketamine (100 mg/kg, i.p.) as general anesthesia to animals, which were starved for 12 h prior to surgical procedure, right nephrectomy was performed through a right flank incision (2 cm) 15 days before the ischemic procedures in the contralateral kidneys. In the sham-operated rats, right nephrectomy and left laparotomy were performed without making the left kidney ischemic.

Renal ischemia required performing a left flank incision and dissecting the left renal pedicle to expose the renal vessels. Nontraumatic vascular clamps were used to stop blood flow (artery and vein) during 45 min. Reperfusion was established by removing the clamp.

The abdominal wall (muscular layer and skin) was closed with 4.0 mononylon suture.

At the end of reperfusion period, blood samples were collected and used for the measurement of renal function and TNF-α. The abdomen was opened and the kidneys were collected for further analysis.

Measurement of blood pressure

During treatment schedule Systolic blood pressure

(SBP) was measured from tail vein by tail cuff instrument (latica) on the day 1 (on the day of unilateral nephractomy), 7, 15 (before ischemia) and 17 (after 24 h of reperfusion).

Renal function

After treatment schedule, blood was collected from all the animals. Serum samples were assayed for blood urea nitrogen (BUN) (DAM method) and serum creatinine (Jaffe's method) by using standard diagnostic kits (Span Diagnostics, Gujarat, India).

TNF-α quantitation by ELISA

Levels of TNF-α in serum were determined using an enzyme-linked immunosorbent assay (ELISA) (Endogen, Mouse TNF-α kit, USA) according to the manufacturer's instructions.

MPO activity

MPO (Myeloperoxidase) activity was measured in tissues in a procedure similar to that documented by Hillegas *et al*. (1990). Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0) and centrifuged at 41,400 *g* (10 min); pellets were suspended in 50 mM Phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles with sonication between cycles, the samples were centrifuged at 41,000 *g* for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM Phosphate buffer, o-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as mOD/min.

Biomarkers of oxidative stress

GSH was estimated by the method of Moran *et al*. (1979). MDA was estimated by the method of Slater and Sawyer (1971). SOD was estimated by the method of Misra and Fridovich (1972).

Tissue NO levels

The level of nitric oxide (NO) was estimated by the method of Lepoivre *et al*. (1990). To 0.5 ml of tissue homogenate, 0.1 ml of sulphosalicylic acid was added and vortexed well for 30 min. The samples were then centrifuged at 5,000 rpm for 15 min. The protein-free supernatant was used for the estimation of nitrite levels. To 200 ml of the supernatant, 30 μl of 10% NaOH was added, followed by 300 ml of Tris-HCl buffer and mixed well. To this, 530 ml of Griess reagent was added and incubated in the dark for 10 - 15 min and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

Histopathology

For light microscopic evaluation, kidneys were fixed in 10% phosphate buffered formalin. Parafinembedded specimens were cut into 6 mm thick sections and stained with hematoxylin & eosin (H & E). The kidneys were examined under a light microscope (Olympus Bioxl) for the presence of tubular changes and interstitial inflammatory cell infiltration, by an observer blinded to the animal treatment group.

DNA fragmentation

Genomic DNA was extracted from renal cortices using DNA extraction kit (DNeasy kit, Axygen). Ten micrograms of DNA were electrophoresed on a 2% agarose gel. Fragmented DNA was visualized by ethidium bromide under an UV light source.

Statistics

All the data are expressed as mean ± SEM. 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

Measurement of blood pressure

Fig. 1 shows Systolic Blood Pressure (SBP) of different groups of animals was measured on the day 1, 7, 15 (before ischemia) and 24 h after reperfusion. On the day of unilateral nephrectomy (day 1st), the sham operated animals showed mean SBP values of 121.3 ± 1.64 mmHg, which did not significantly changed after reperfusion. Animals underwent I/R showed significant rise ($P < 0.001$, $n = 6$) in mean SBP (151.2 ± 2.81 mmHg) after ischemia reperfusion in comparison to their mean SBP on the day 1. However, hesperidin treatment had significantly ($P < 0.001$) prevented rise in the mean SBP in comparison I/R group. Treatment with Vit.E in rats subjected to renal warm I/R prevented the rise in SBP, but not significant as hesperidin.

Renal function

To determine the beneficial effects of hesperidin on renal function, we examined serum creatinine and BUN levels in various groups of animals. The serum creatinine and BUN were significantly increased

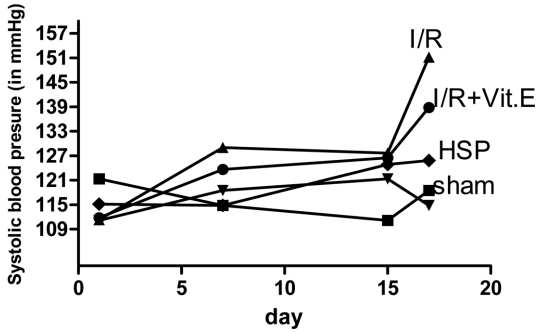

Fig. 1. Effect of hesperidin on systolic blood pressure in rats subjected to 45 min of ischemia in renal artery and vein followed by 24 h reperfusion. Unit: mmHg Values are expressed as mean ± S.E.M. for 6 animals in the group. Comparisons are made between: sham and I/R, I/R + HSP, I/R + Vit. E. The symbols represent statistical significance:* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

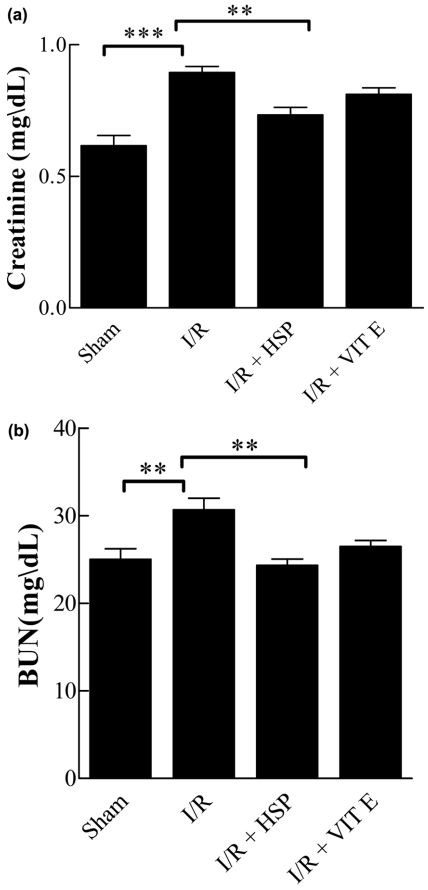

Fig. 2. (**A**) Effect of hesperidin on serum creatinine in rats subjected to 45 min of ischemia in renal artery and vein followed by 24 h reperfusion. Unit: mg/dl. Values are expressed as mean ± S.E.M. for 6 animals in the group. Comparisons are made between: sham and I/R, I/R + HSP, I/R + Vit. E. The symbols represent statistical significance:*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (**B**) Effect of hesperidin on BUN levels in rats subjected to 45 min of ischemia in renal artery and vein followed by 24 h reperfusion. Unit: mg/dl. Values are expressed as mean ± S.E.M. for 6 animals in the group. Comparisons are made between: sham and I/R, I/R + HSP, I/R + Vit. E. The symbols represent statistical significance:*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

by 45.29% and 20% respectively in animals that underwent I/R animals as compared to sham operated group (Figs. 2A and B), indicating a significant degree of glomerular dysfunction mediated by renal I/R. Treatment with hesperidin produced a significant reduction in serum creatinine ($P < 0.01$) and BUN ($P < 0.01$) in comparison to I/R group.

Treatment with Vit. E in rats subjected to renal warm I/R improved renal function, but not significant as hesperidin.

Markers of inflammation

To determine the beneficial effects of hesperidin in prevention of inflammatory reactions, we examined serum level of TNF-α and MPO activity in kidney tissues. The serum TNF-α level was significantly (P < 0.001, n = 6) increased by 3 folds (171.00 ± 11.08 pg/ml) in animals that underwent I/R animals as compared to sham operated group (64.67 ± 5.38 pg/ml) (Fig. 3). Also, MPO activity was significantly (P < 0.001, n = 6) increased (4.63 fold) in animals that underwent ischemia compared to sham-operated animals (Table 1). Treatment with hesperidin significantly reduces serum TNF-α level (P < 0.01, n = 6) and MPO activity (P < 0.05, n = 6) in comparison to I/R group. Treatment with Vit. E in rats subjected to renal warm I/R reduced the tissue MPO activity and serum level of TNF-α, but not significant as hesperidin.

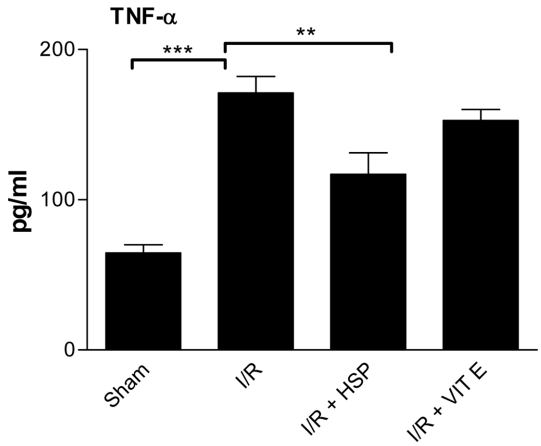

Fig. 3. Effect of hesperidin serum TNF-α levels in rats subjected to 45 min of ischemia in renal artery and vein followed by 24 h reperfusion. Unit: pg/ml. Values are expressed as mean ± S.E.M. for 6 animals in the group.Comparisons are made between: sham and I/R, I/R + HSP, I/R + Vit. E. The symbols represent statistical significance:*P < 0.05, **P < 0.01, ***P < 0.001.

Table 1. Effect of hesperidin on kidney MPO and NO levels in rats underwent renal ischemia reperfusion

Groups	**MPO** (mOD/min)	**NO** (nM/g tissue)
Sham	1.80 ± 0.18	156.7 ± 5.87
I/R	8.35 ± 1.57***	123.8 ± 9.56*
I/R + HSP	4.46 ± 0.63*	174.1 ± 5.08***
I/R + Vit.E	6.48 ± 0.45^{NS}	121.0 ± 11.01^{NS}

Values are expressed as mean ± S.E.M. for 6 animals in each group. Comparisons are made between: sham and I/R, I/R + Hesp,I/R + Vit. E. The symbols represent statistical significance:*P < 0.05, **P < 0.01, ***P < 0.001.

Biomarkers of oxidative

Renal I/R produced a significant increase in MDA levels (147.80 ± 4.71 nM/mg of tissue), as compared to sham operated animals (87.00 ± 4.05 nM/mg of tissue). Treatment with hesperidin produced a significant reduction in MDA level (111.50 ± 6.83 nM/mg of tissue, P < 0.01, n = 6) in renal I/R + HSP group animals in comparison to I/R animals. Renal I/R significantly (P < 0.001, n = 6) decreased the antioxidant enzymatic activity of GSH (278.30 ± 4.24 μg/g of tissue), CAT (1141 ± 21.08 nM of H_2O_2 consumed/mg of tissue) and SOD (56.50 ± 2.59 U/mg of tissue). This reduction was significantly improved by treatment with hesperidin (345.20 ± 11.87 μg/g of tissue of GSH, 1283 ± 42.98 nM of H_2O_2 consumed/ mg of tissue of CAT, and 86.53 ± 3. U/mg of tissue of SOD, respectively) in comparison to I/R group (Fig. 4(A) and 4(B)). Treatment with Vit. E in rats subjected to renal warm I/R improved tissue levels of biomarkers of oxidative stress, but not significant as hesperidin.

Tissue NO levels

Renal I/R resulted in a significant decrease in the tissue levels of nitrite (123.8 ± 9.56 nM/mg tissue, P < 0.05, n = 6) in comparison with values obtained from the tissue of sham-operated animals (156.7 ± 5.87) (Table 1). However, decreased nitrite levels mediated by renal I/R were increased significantly (174.1 ± 5.08, P < 0.001, n = 6) after administration of hesperidin in comparison to I/R animals (Table 1). Treatment with Vit. E in rats subjected to renal

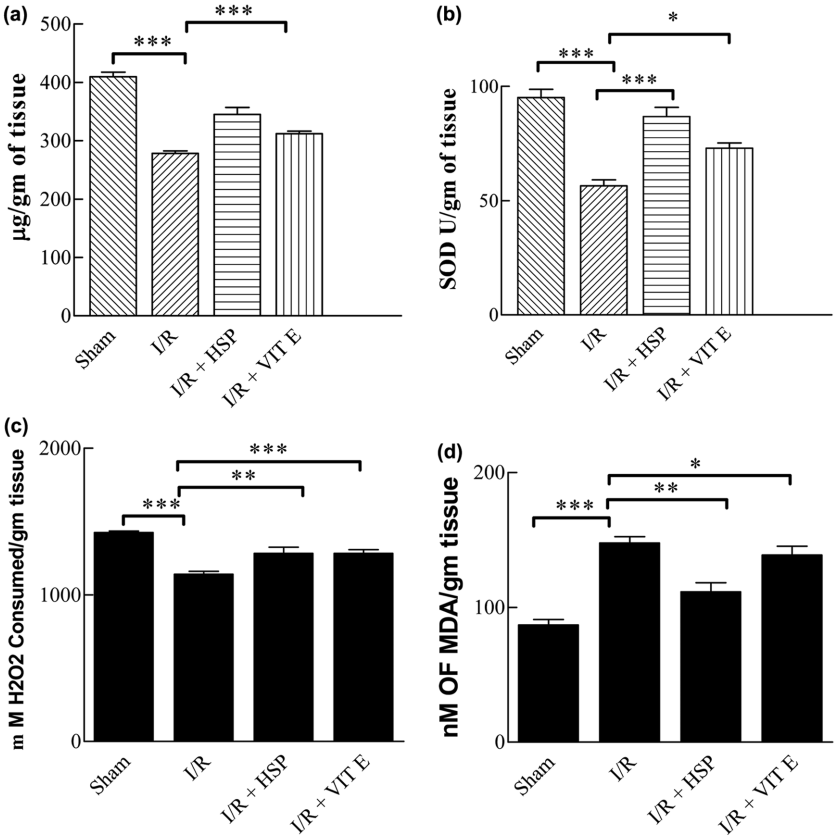

Fig. 4. (**A**) Effect of hesperidin on kidneys GSH levels in rats subjected to 45 min of ischemia in renal artery and vein followed by 24 h reperfusion. Unit: µg/g of tissue.Values are expressed as mean ± S.E.M. for 6 animals in each group.Comparisons are made between: sham and I/R, I/R + HSP, I/R + Vit. E. The symbols represent statistical significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. (**B**) Effect of hesperidin on kidneys SOD activty in subjected to 45 min of ischemia in renal artery and vein followed by 24 h reperfusion. Unit: SOD U/mg of tissue. Values are expressed as mean ± SEM. for 6 animals in each group. Comparisons are made between: sham and I/R, I/R + HSP, I/R + Vit. E. The symbols represent statistical significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. (**C**) Effect of hesperidin on kidney CAT level in rats subjected to 45 min of ischemia in renal artery and vein followed by 24 h reperfusion. Unit: mM H_2O_2 Consumed/g tissue. Values are expressed as mean ± S.E.M. for 6 animals in each group. Comparisons are made between sham and I/R, I/R + HSP, I/R + Vit. E. The symbols represent statistical significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. (**D**) Effect of hesperidin on kidney LPO level in rats subjected to 45 min of ischemia in renal artery and vein followed by 24 h reperfusion. Unit: nM of MDA/g tissue. Values are expressed as mean ± S.E.M. for 6 animals in each group. Comparisons are made between: sham and I/R, I/R + HSP, I/R + Vit. E. The symbols represent statistical significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

warm I/R improved tissue NO level, but not significant as hesperidin.

Histopathology

Light microscopic evaluation of the sham-operated groups revealed a regular morphology of renal parenchyma with well-designated glomeruli and tubuli (Fig. 5A). In I/R group, the interstitial hemorrhage, dilated tubuli and prominent glomerular degeneration followed by atrophy revealed that I/R caused a severe glomerular, tubular and interstitial damage. Tubular dilation was present throughout the tissue (Fig. 5B). In the hesperidin-treated I/R group, there was a significant regeneration in all

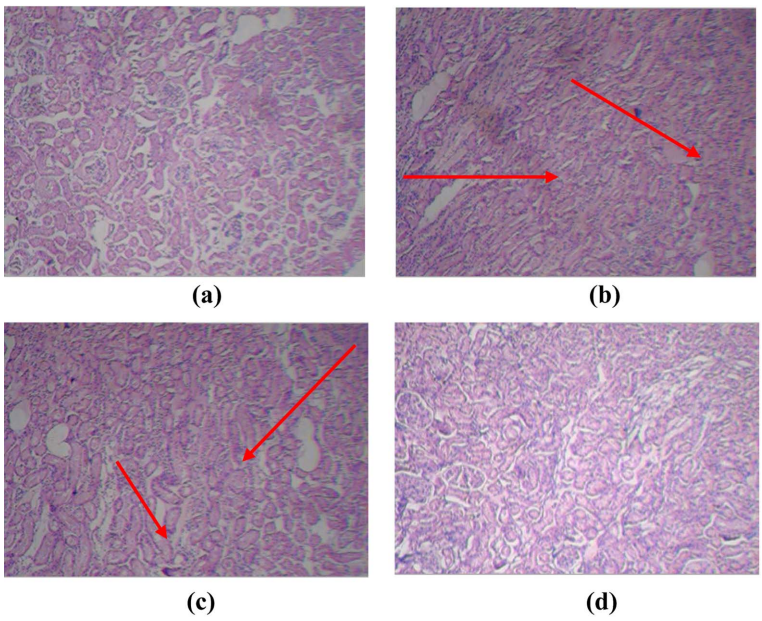

Fig. 5. Microscopic observations of kidneys tissue sections with BIOXL light microscope showing morophological changes. Images were taken under light microscopy using hematoxylin and eosin (×10). (A) sham-operated (B) I/R (C) I/R + HSP.

features of the injury. Reduced tubular dilation, loss of interstitial hemorrhage and glomerular atrophy were the regenerated features (Fig. 5C).

DNA fragmentation

Necrosis was evaluated by DNA fragmentation analysis. The typical DNA laddering activity was observed in I/R group, which indicates cell necrosis. Treatment with hesperidin decreased I/R-induced DNA fragmentation. Treatment with Vit. E in rats subjected to renal warm I/R was failed to protect tissue necrosis as compared to hesperidin (Fig. 6).

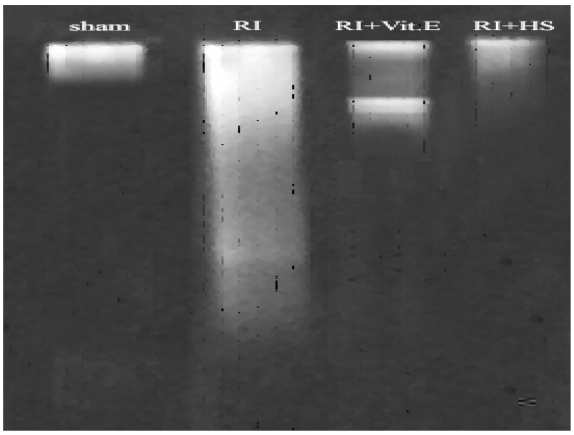

Fig. 6. DNA fragmentation analysis revealed typical laddering of fragmentated DNA in I/R group. Hesperidin treatment decreased the laddering pattern.

DISCUSSION

In renal transplantation, the problem is the onset of I/R, when the transplantation requires a long interval as a consequence of using a brain dead donor's kidney. The production of ROS during I/R of the kidney is one of the major causes contributing to acute renal failure (Devinder *et al.*, 2004; Abdurrahman *et al.*, 2006). Acute renal failure produced by I/R is characterized by major declines in glomerular filtration rate, accumulation of toxic metabolites, disturbance of electrolyte homeostasis, extensive tubular damage, inflammatory cell infiltration and tubular cell necrosis (Devinder and Kanwaljit, 2004; Abdurrahman *et al.*, 2006b). Moreover, free radicals cause DNA scission and

base modification, lipid peroxidation, protein damage and inactivation by their chemical modification (Devinder and Kanwaljit, 2004b; Sadk *et al.*, 2005).

In this *in vivo* study, renal I/R caused significant increase in the renal MDA levels as an indicator of lipid peroxidation and depleted the anti-oxidant enzyme such as reduced glutathione, catalase and superoxide dismutase. Increase in lipid peroxidation can lead to nerve and smooth muscle membrane damage (Seiji *et al.*, 2005). These alterations can increase tubular permeability with loss of membrane exchange functions with consequent impairment of the renal function (Ernani *et al.*, 2002). Similar findings have also been seen in our study. Impaired renal function leading to significant increase in serum creatinine and BUN levels in I/R group compared to sham operated group. Previous experimental studies demonstrated that, antioxidant treatment can improve antioxidant enzyme pool, reduces ROS and protect against reperfusion injury (Devinder and Kanwaljit, 2004a; Kent *et al.*, 2004; Seiji, 2005; Matsuyama, 2006; Zehra *et al.*, 2007). Studies also proved that, the phenolic –OH group of bioflavonoids reacts with lipid peroxide radicals which terminate the lipid peroxidation chain reaction (Husain *et al.*, 1987). Our study also shows hesperidin treatment significantly improved antioxidant enzyme pool, improved renal function and decreased MDA levels in treated animals compared to I/R group alone. Suggesting anti-oxidant properties of hesperidin, which may plays a significant role in protecting the renal microvasculature after I/R.

In our study, there was a significant rise in systolic blood pressure (SBP) during reperfusion period in I/R rats in comparison to sham operated animals. Studies proved that ROS are also implicated as the cause of damage to endothelial cells and hypertension (Esra *et al.*, 2006). Endothelial cells produce less bioactive NO in presence of higher oxidative stress. Studies also proved that gene like, NOX-2 reduces endothelium derived relaxations and increases ROS generation simultaneously, in blood vessels of mice (Christopher, 2002). Ang II is a known stimulant for generation of ROS (Lilach *et al.*, 2001). Moreover, ROS stimulates renin release from the Juxtaglomerular apparatus by means of oxidized low-density lipoproteins and lipoproteins (Jan *et al.*, 1997; Christopher, 2002). This provides a potential positive feedback loop whereby Ang II could stimulate oxidative stress that would release renin to generate more Ang II. Collectively leads to progressive rise in blood pressure and vasoconstriction. Studies show that, increase in hypertension severely changes morphology of kidney (Jean-Jacques *et al.*, 2003). It is also confirmed by our histopathological results. However, administration of anti-oxidant like melatonin reduces hypertension in renal I/R rats (Esra *et al.*, 2006). In this study, hisperidin treatment significantly prevented rise in hypertension during reperfusion period in treated animals compared to I/R animals.

A large number of work in animal models as well as some pathologic analysis of human biopsies demonstrate that ischemia is marked by a robust inflammatory response in tissues and contributes to the resultant tissue injury (Joshua, 2007). The renal tubular epithelium also generate mediators like IL-6, IL-1, TGF-β, cytokines and chemokines that potentiate inflammation following ischemic injury (Joseph and Anna, 2008). We measured the inflammatory response as tissue MPO activity and serum concentration of TNF-α. There was a significant increase in MPO activity of kidney tissue and serum TNF-α level in I/R group in comparison to sham-operated group. Increased MPO activity further produces ROS and hypochlorous acid, which exert a strong destructive effect on kidney tissues. Inflammatory agent such as TNF-α induces activation of NF-κB. Deregulation of NF-κB and its dependent genes has been associated with toxic shock, graft rejection and cancer (Ahmet *et al.*, 2004). Moreover, TNF-α induces secreation of Monocyte chemoattractant protein (MCP)-1. MCP)-1chemotactically recruits monocytes to sites of inflammation, which may further enhance MPO

activities (Jian *et al*., 2008). However, Perianayagam *et al.,* demonstrated that antioxidants like melatonin reduce TNF-α production (Zehra *et al.,* 2007).

In this study also, we observed that increased markers of inflammation (MPO activity and serum TNF-α) were significantly reduced by hesperidin treatment. Thus, our data indicates that hesperidin reduce inflammatory responses after renal I/R.

Inflammatory reactions increase the activity of iNOS mRNA in epithelial tubular cells. Studies showed that elevated expression of iNOS is accompanied by reduction in the number of cells that express eNOS (Manuela, 2003; Yagmurdur *et al*., 2008). NO is generally beneficial, but in presence of oxidative stress, it is potentially toxic. Under oxidative stress conditions, NO reacts with superoxide to produce peroxynitrite (Walker *et al*., 2000). In our study there was a significant decrease in the levels of NO in kidney tissues of rats underwent I/R compared to sham-operated group. If the ratio ROS/ NO increases, which further activates tissue phopholipase A_2 and thereby synthesizing inflammatory mediators (Ernani *et al.,* 2002). In response to ROS the outer membrane of mitochondria becomes permeabilized, resulting in the translocation of Bax from cytosol to the mitochondria and the release of cytochrome c occurs. Release of cytochrome c into the cytosol leads to form the apoptosome which stimulates the activation of procaspase-9 and procaspase-3. Active caspase-3 activates the caspase activated DNAase, leading to DNA fragmentation (Manuela, 2003; Hui *et al*., 2008). Studies also proved that, high-level ROS cause necrotic cell death, in which the cellular contents are released into the surrounding environment, and some of the products released can induce inflammation, which is consistent with our results (Makiya, 2008). In our study, high degree of cell necrosis was observed in DNA samples of I/R group as compared with sham-operated group. However, animals treated with hesperidin showed high levels of NO after I/R. But, significant reduction in levels of inflammatory markers (MPO and TNF-α) and inhibition of cell necrosis (DNA fragmentation) caused by I/R was observed in animals treated with hesperidin in comparison to I/R group, shows that hesperdin inhibits the formation of peroxinitrite by inhibiting ROS.

In conclusion, ROS levels increase while SOD, catalase, and GSH levels decreases in renal ischemia reperfusion. Because of ROS peroxinitrite, blood pressure, inflammatory reactions and cell necrosis is also increases in renal ischemia reperfusion. Hesperidin lessens oxidative stress by increasing the levels of SOD, catalase and GSH. Besides, the use of hesperidin as an antioxidant drug can protect kidneys against renal ischemia reperfusion injury, which is an important issue in renal transplantation. However, it is also possible to speculate on many other mechanisms, such as changes in the level of other substances that attenuate oxidative stress but are to be detected.

REFERENCES

Abdurrahman K, Emine T, Cebrail G, Erkan T, Ersin F. (2006) Prevention of renal ischemia/reperfusion-induced injury in rats by leflunomide. *Int. Urology* **13**, 1434-1441.

Ahmet G, Ferah A, Semsettin S. (2004) Protective role of a-tocopherol and caffeic acid phenethyl ester on ischemia–reperfusion injury via nitric oxide and myeloperoxidase in rat kidneys. *Clin. Chim. Acta* **339**, 33-41.

Bouchier-Hayes DM, Fitzpatrick JM, Grace PA, Mathie RT. (1999) Ischemia-reperfusion injury. *BlackWell Science* 71-81.

Chatterjee PK, Cuzzocrea S, Brown PAJ. (2000) Tempol, a membranepermeable radical scavenger, reduces oxidant stress-mediated renal dysfunction and injury in the rat. *Kidney Int.* **58**, 658-673.

Chia-Chou Y, Shang-JK, Chih-Che L, Shulhn-DW, Ching-JL, Shung-Te Kao. (2007) The immunomodulation of endotoxin-induced acute lung injury by hesperidin in vivo and in vitro. *Life Sci.* **80**, 1821-1831.

Cho J. (2006) Antioxidant and neuroprotective effects of hesperidin and its aglycone hesperetin. *Arch. Pharm. Res.* **29**, 699-706.

Christopher SW. (2002). Reactive oxygen species: Roles in blood pressure and kidney function. *Curr. Hyp. Rep.* **4**, 160-166.

Devinder Singh, Kanwaljit Chopra (2004b). Effect of trimetazidine on renal ischemia/reperfusion injury in rats. *Pharm. Res.* **50**, 623-629.

Devinder Singh, Kanwaljit Chopra (2004a). The effect of naringin, a bioflavonoid on ischemia-reperfusion induced renal injury in rats. *Pharm. Res.* **50**, 187-193.

Devinder Singh, Vikas Chander, Kanwaljit Chopra (2004). The Effect of Quercetin, a Bioflavonoid on Ischemia/Reperfusion Induced Renal Injury in Rats. *Arc. Med. Res.* **35**, 484-494.

Ernani LR, Cláudia RR, Márcio LL, Luiz PL, Cláudio Z, Adriane BK. (2002) The role of nitric oxide pathway in the renal ischemia–reperfusion injury in rats. *Transplant Immun.* **10**, 277-284.

Esra D, Neriman C, Aysel S. (2006) Melatonin attenuates renal ischemia–reperfusion injury in nitric oxide synthase inhibited rats. *Acta histochemica* **108**, 303-309.

Garg A,Garg S, Zaneveld LJ, Singla AK. (2007). Chemistry and pharmacology of the citrus bioflavonoid hesperidin. *Phytother. Res.* 15655-15669.

Harry H, Abraham. (1968) Estimation of Creatinine by the Jaffe Reaction A Comparison of Three Methods. *Clin. Chem.* **14**, 222-238.

Hillegas LM, Griswold DE, Brickson B. (1990) Assessment of myeloperoxidase activity in whole rat kidney. *J. Pharmacol. Method* **8**, 52.

Hui C, Xiuheng L, Bingyan Z. (2008) Ozone oxidative preconditioning inhibits inflammation and apoptosis in a rat model of renal ischemia/reperfusion injury. *Eur. J. Pharm.* **581**, 306-314.

Hosseinimehr SJ, Nemati A. (2006) Radioprotective effects of hesperidin against gamma irradiation in mouse bone marrow cells. *Brit. J. Rad.* **79**, 415-418.

Husain SR, Cillard J, Cillard P. (1987) Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* **26**, 2486-2491.

Hyo JK, Ki WL, Mi-Sung K, Hyong JL. (2007) Piceatannol attenuates hydrogen-peroxide- and peroxynitrite-induced apoptosis of PC12 cells by blocking down-regulation of Bcl-XL and activation of JNK. *J. Nut. Biochem.* **2**, 181-190.

Jan G, Alexandra H, Susanne S, Christoph W. (1997) Effect of HDL and atherogenic lipoproteins on formation of O2- and renin release in juxtaglomerular cells. *Kidney Int.* **51**, 253-260.

Jean-JB, Ying L, Sandrine P, Adam S, Jean-CD, Christos C. (2003) Regression of Renal Vascular and Glomerular Fibrosis: Role of Angiotensin II Receptor Antagonism and Matrix Metalloproteinases. *J. Am. Soc Nephrol.* **14**, 1132-1144.

Jian Zhu, ing Yu, Inghuan LV. (2008) Anti-inflammatory effect of resveratrol on TNF-a-induced MCP-1 expression in adipocytes. *Biochem. Biophys. Res. Commun.* **369**, 471-477.

Joseph V, Anna Z. (2008) Ischemic acute renal failure: An inflammatory disease? *Forefronts Nep.* **3**, 480-485.

Joshua MT. (2007) Triggers of inflammation after renal ischemia/reperfusion. *Clin. Immunol.* **123**, 7-13.

Kent D, Yoshifumi S, Akihide N, Toshiro F, Eisei N. (2004) Radical scavenger edaravone developed for clinical use ameliorates ischemia/reperfusion injury in rat kidney. *Kidney Int.* 1714-1723.

Lepoivre M, Chenais B, Yapo A. (1990) Alterations of ribonucleotide reductase activity following induction of nitrite-generating pathway in adenocarcinoma cells. *J. Biol. Chem.* **6**, 141-149.

Lilach OL, Karl AN, Martin RP. (2001) Increased Oxidative Stress in Experimental Renovascular Hypertension. *Hypertension* **37**, 541-546.

Matsuyama M, Hayama T, Funao K, Tsuchida K, Takemoto Y, Sugimura Y, Kawahito H, Sano T, Nakatani Y, Yoshimura R. (2006) Treatment With Edaravone Improves the Survival Rate in Renal Warm Ischemia-Reperfusion Injury Using Rat Model. *Transplantation Proc.* **38**, 2199-2200.

Makiya N. (2008) Reactive oxygen species in tumor metastasis. *Cancer Letters.* **18**, 53-59.

Manuela A. (2003) Oxidative stress and kidney dysfunction due to ischemia/reperfusion in rat: Attenuation by dehydroepiandrosterone. *Kidney Int.* **64**, 836-843.

Mark SP, Thomas VN, Emil YK, Marsha P. (1991) Reactive oxygen species and rat renal epithelial cells during hypoxia and reoxygenation. *Kidney Int.* **40**, 1041-1049.

Naveen T, Sangeeta P, Anurag K, Kanwaljit C. (2005b) Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharma.* **5**, 1-8.

Prabal KC. (2007) Novel pharmacological approaches to the treatment of renal ischemia-reperfusion

injury: a comprehensive review. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **376**, 1-43.

Sadk Go, Özlen TB, Gürbüz P. (2005) Protective effect of L-carnitine on renal ischaemia–reperfusion injury in the rat. *Cell Biochem. Funct.* **23**, 151-155.

Seiji M, Tadashi H, Nobuhiro Y, Shimizu T, Sugiyama HU, Robert ML. (2005) Edaravone protects against ischemia/reperfusion-induced functional and biochemical changes in rat urinary bladder. *Urology* **66**, 892-896.

Uma Maheswari, Rao P. (2005) Antihepatotoxic effect of grape seed oil in rat. *Indian J. Pharm.* **37**, 179-182.

Walker LM, WP, Imam SZ, Ali SF, Mayeux PR. (2000). Evidence for peroxynitrite formation in renal ischemia–reperfusion injury: studies with the inducible nitric oxide synthase inhibitor L-N (6)-(1-Iminoethyl) lysine. *J Pharmacol. Exp Ther*. **295**, 417-422.

Yagmurdur H, Ayyildiz A, Karaguzel E, Akgul T, Ustun H. (2008) Propofol reduces nitric oxide-induced apoptosis in testicular ischemia–reperfusion injury by downregulating the expression of inducible nitric oxide synthase. *Acta Anaesthesiol Scand*. **52**, 350-357.

Zehra K, Elif O, HaticeOzbilge, Fusun B, Hakim C, Mehmet RG. (2007) Melatonin protects from ischemia/reperfusion-induced renal injury in rats: this effect is not mediated by proinflammatory cytokines. *J. Pineal Res.* **43**, 172-178.