The protective effect of silymarin on the antioxidant system at rat renal ischemia/reperfusion injury model

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The aim of this study is to reveal the protective effects of silymarin (SM) treatment on the generation of oxidative stress with rat renal ischemia/reperfusion (I/R) injury model. Thirty-two (32) Sprague-Dawley rats were evaluated in four groups. Group I (Sham), Group II (renal I/R), Group III (renal I/R injury + SM 100 mg per kg) and Group IV (renal I/R injury + SM 200 mg per kg) were designed to evaluate dose-dependent effects of SM in renal I/R injury on the morphological and biochemical parameters changes. Renal I/R significantly decreased the enzymatic activity of catalase (CAT) and superoxide dismutase (SOD), whereas the malondialdehyde (MDA) levels increased. After renal I/R injury, significant tubular dilatation, tubular necrosis, glomerular necrosis, tubular vacuolization, hyaline casts, interstitial inflammation and necrosis of epithelium due to I/R injury was observed. In the Groups III and IV, in which the rats were treated with SM before renal I/R, tubular dilatation, tubular necrosis, glomerular necrosis, tubular vacuolization, hyaline casts, interstitial inflammation and necrosis of epithelium due to I/R injury were observed to be significantly protected with the treatment. The results of this study have demonstrated that SM significantly prevents the generation of oxidative stress and renal I/R injury induced renal changes in the rat.

Key words: Kidney, oxidative stress, pathology, rat, silymarin, morphology.

INTRODUCTION

Renal ischemia/reperfusion (I/R) injury, which occurs in many clinical during the course such as partial nephrectomy, renal artery angioplasty, trauma, shock, major vascular surgery, sepsis and renal transplantation, is associated with increased mortality and morbidity rates due to acute renal failure (ARF) (Thadhani et al., 1996; Takada et al., 1997; Matin and Novick, 2001; Avlan et al., 2006). Reperfusion of the ischemic tissue may produce reactive oxygen species (ROS), which are known to have deleterious effects such as increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration and necrosis (Granger and Korthuis, 1995). In ischemic, ARF leads to a complex cascade of events which are also known to include the activation of nuclear factor kappa B (NF-κB), which controls cytokine, chemokines and adhesion molecules (Rodrigo and Bosco, 2006). Oxidative stress is a relative excess of oxidants caused by increased free radical production and/or decreased antioxidant defense systems that impairs cellular function and contributes to the pathophysiology of many diseases (Karimi et al.,

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2005; Zhao, 2005). The antioxidant defense systems, none enzymatic free radical scavengers (vitamin E, vitamin C, uric acid and bilirubin) and the antioxidant scavenging enzymes, [catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)] protect cells and tissues against oxidative injury (Granger and Korthuis, 1995; Marubayashi and Dohi, 1996; Zhao, 2005). Naturally occurring flavonoids have antioxidant effects due to their phenolic structures and have been reported to inhibit some free radical-mediated processes (Havsteen, 1983; Mora et al., 1990; Zhao, 2005). Silymarin (SM) is a mixture of three isomeric flavonolignans extracted from the milk thistle *Silybum marianum*. SM has been used in most of the remedies for liver disease. Hepatoprotective effects of SM have been attributed to its scavenging ROS, reduction of lipoperoxidation of cell membranes (Farghali et al., 2000). However; very few studies have been performed on oxidative stress with SM in relation to the kidney (Turgut et al., 2008). SM has also been reported to have beneficial effects to protect acute cisplatin nephrotoxicity (Karimi et al., 2005). In our previous study, we demonstrated that SM significantly prevents renal I/R injury induced histopathological changes in the rat kidney (Senturk et al., 2008). This study aimed to re-investigate the possible protective effect of SM against oxidative stress-induced during kidney I/R injury, by determining biochemical parameters and evaluating histopathological examinations.

**MATERIALS AND METHODS**

The experimental protocols were approved by the Institutional Animal Ethics Committee. Animals were obtained from Medical and Surgical Experimental Research Center (Eskisehir-TURKEY) and all experiments were carried in same center.

**Animals**

Thirty-two (32) adult male Sprague-Dawley rats weighting 220 to 250 g were used in the experiment. Rats were housed in polycarbonate cages in a room with controlled temperature (22 ± 2°C), humidity (50 ± 5%), and a 12 h cycle of light and dark; they were fed with laboratory pellet chows and water was given ad libitum. The experiment was performed after a stabilization period in the laboratory for 5 days.

**Experimental design**

Four groups were designed. Group I (Sham) was designed as the control group. Group II (renal I/R) was designed to renal I/R injury. Groups III (renal I/R injury + SM 100 mg per kg) and Group IV (renal I/R injury + SM 200 mg per kg) were designed to evaluate SM on the morphological and biochemical changes in the rats kidney in renal I/R injury.

**Right nephrectomies**

Right nephrectomies were performed under xylazine (10 mg per kg) and ketamine (70 mg per kg) anesthesia in all rats in all Groups (I to IV). Thereafter, rats were let to recover for 15 days in the standard laboratory.

**Drug administration**

Seven (7) days prior to I/R induction, 0.5 ml of 100 and 200 mg/kg SM solution (Sigma-Aldrich, S0292-50G, Italy) were administered orally (p.o) to the rats in Groups III and IV, respectively. Rats in Groups I and II received 0.5 ml normal (0.9%) saline p.o for 7 days prior to sham operation and I/R induction, respectively.

**Induction of renal I/R injury**

All surgical procedures were performed under xylazine (10 mg per kg) and ketamine (70 mg per kg) anesthesia. Renal I/R injury were induced with left renal pedicle occlusion with a vascular clamp for 45 min followed with reperfusion for 6 h through a median laparotomy under anesthesia. Sham procedures were same beyond vascular occlusion in the Group I. After induction of I/R injury in Groups II, III and IV, left kidneys were dissected for both biochemical and histopathological examinations.

**Histopathological evaluation**

Left kidneys specimens were processed routinely in 10% formalin solution, and embedded in paraffin. Tissue sections of 5 μm were obtained and stained with hematoxylin and eosin (H&E). Histopathological examinations were performed under a light microscope (NIKON, Japan). All histopathological examinations were performed by the same pathologist of the institute, who was blinded to all the tissue specimens. A minimum of 10 fields for each kidney slide with minimum ×50 magnification were examined to assign the severity of these morphological changes. The morphological changes were scored on a scale of none (−), mild (+), moderate (+++) and severe (++++) damage in order to perform a comparison between the groups.

**Biochemical analysis**

**Postmitochondrial supernatant preparation (PMS)**

After sacrificing the animals, isolated areas of the nephron of their kidneys were quickly removed and wash immediately with ice-cold normal saline, and homogenized in chilled potassium chloride (1.17%) using a Potter Elvehjem homogenizer. The homogenate was centrifuged at 800 × g for 5 min at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 × g for 20 min at 4°C to get the PMS which was used to assay malondialdehyde (MDA), CAT and SOD activity.

**The protocols of lipid peroxidation and enzyme activities measurement**

**Determination of lipid peroxides (measured as MDA)**

MDA, a reactive aldehyde, that is, a measure of lipid peroxidation, was determined according to the method of Uchiyama and Mihara (1978). The adducts formed following the reaction of tissue homogenate with thiobarbituric acid in boiling water bath, were extracted with n-butanol. The difference in optical density developed at two distinct wavelengths, 535 nm and 525 nm was a measure of the tissue MDA content. Tissue MDA content was
Figure 1. Effect of SM treatment on kidney tissue content of MDA, SOD and CAT. Rats in sham and I/R groups were administered normal saline 7 days prior to I/R induction. Rats in Groups III and IV were administered 100 and 200 mg/kg SM 7 days prior to I/R induction. Data were expressed as means ± SD. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Renal I/R significantly decreased the enzymatic activity of CAT and SOD, whereas the MDA levels increased. This enzymatic activity levels was significantly improved by treatment with both SM 100 (Group III) and 200 (Group IV) mg (Figure 1).

Light microscopic evaluation revealed that normal renal morphology in the Group I (Sham), and some of the histopathological findings, which were observed in renal I/R injury in Groups II, III and IV (renal I/R; renal I/R injury + SM 100 mg per kg; and renal I/R injury + SM 200 mg per

**Statistical analysis**

All statistical analysis was performed with the computer program “SPSS for Windows” (SPSS Inc; Release 11.5; Sep 6, 2002). All of the data were expressed as means ± SD. Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests. The significance was tested at p > 0.05, p < 0.05, p < 0.01 and p < 0.001.
Table 1. Effect of SM (100 and 200 mg per kg, per oral) treatment on morphological changes as assessed by histopathological examination of kidneys of the rats exposed to renal I/R.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular necrosis</th>
<th>Glomerular necrosis</th>
<th>Tubular dilatation</th>
<th>Necrosis of epithelium</th>
<th>Hyaline casts</th>
<th>Interstitial inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Sham)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Group II (I/R)</td>
<td>+++</td>
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<td>+++</td>
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<tr>
<td>Group III (I/R + SM 100 mg)</td>
<td>-/+</td>
<td>-/+</td>
<td>-</td>
<td>-/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Group IV (I/R + SM 200 mg)</td>
<td>-/+</td>
<td>-</td>
<td>+/+</td>
<td>-/ +</td>
<td>+/+</td>
<td>+/+</td>
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</tbody>
</table>

Silymarin, SM; (-), none; (+), mild; (+/−), mild/none; (++), moderate; (+++), severe.

Figure 2. (A), Control group: Renal corpuscle and tubules were observed normal histological structure. (Glomerulus (*), urinary space (US), proximal convoluted tubules (PCT) and distal convoluted tubules (DCT), original magnification × 100. (B), Group II (renal I/R); some tubules were desquamation of its epithelial cells (arrow head) and tubular dilatation (thin arrow). Displacement and shrinkage of glomerular tuft is also seen in this figure (thick arrow). (B1), Glomerular tuft was observed shrinkage and degeneration (arrow). Inflammatory cells were observed in the intertubular spaces of this figure (arrow head). (B2), Some renal tubules were observed desquamation of its epithelial cells (arrow head) and tubular dilatation (arrow). Inflammatory cells were observed in the intertubular spaces of this figure (*).

In Group II (renal I/R injury), significant tubular dilatation, tubular necrosis, glomerular necrosis, tubular vacuolization, hyaline casts, interstitial inflammation and necrosis of epithelium were observed due to renal I/R injury. In the Groups III and IV, in which the rats were treated with SM 100 and 200 mg per kg before renal I/R, tubular dilatation, tubular necrosis, glomerular necrosis, tubular vacuolization, hyaline casts, interstitial inflammation and necrosis of epithelium due to I/R injury were observed to be protected with the treatment (Figures 2 and 3).
Figure 3. (B3), Widespread tubular necrosis and necrotic cells of the proximal tubules were observed in this figure (arrow). (B4), Hyaline casts were observed tubular structure (arrow). (B5), Epithelial cells of renal tubules were observed desquamation. Necrotic cells were seen in tubule lumen (arrow head). Also, some tubules were observed tubular dilatation (arrow). (C), Kidney section of SM (100 mg per kg, per oral) + renal I/R treated rat showing normal renal corpuscle and tubules.

DISCUSSION

Oxidative stress plays an important role in kidney I/R injury (Granger and Korthuis, 1995). Thus, increasing the kidney antioxidant capacity could be a promising therapeutic approach. Despite improvements in organ preservation and surgical techniques, I/R injury remains a significant clinical problem, and there is considerable interest in its prevention.

Several studies have been reported on the protective effects of antioxidants in different organ and renal I/R injury (Huang et al., 1995; Sehirli et al., 2003; Sener et al., 2004; Sener et al., 2006). Recent approaches advocated to control the production of ROS, which may directly lead to per oxidation of cell membrane lipids and permanent cellular damage, have been generally designed as the therapies including antioxidants such as n-acetylsystein (NAC), revesatrol, vitamin E, and others (Sener et al., 2006; Thurman, 2007).

Tissue ischemia not only leads to the over production of ROS which directly induces tissue damage, but also triggers an aggravated local and systemic inflammatory response that causes multiple organ failure. Several studies demonstrated a recruitment of the neutrophils into post ischemic tissue, but activated neutrophils are also reported to be a potential source of ROS (Zimmerman et al., 1990; Granger and Korthuis, 1995). Chemokines, such as Interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNF-α) released from cellular elements, nitric oxide synthase (NOS) which modulates nitric oxide (NO) levels, adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and NF-κB have been recently studied in several organ I/R injury models (Thurman, 2007). Results of these studies suggest that their treatment and even protection of tissue and organ damage due to I/R may be possible with modulation of
The active constituents of milk thistle are flavonolo-
lignans including silybin, silydianin and silychristine, 
collectively known as silymarin. Medical use of milk thistle 
as a liver protecting herb dates back to the earliest Greek 
references to the plant. Hepatoprotective effects of SM are 
mainly attributed to its antioxidant, anti-inflammatory 
and anti-fibrotic activity (Ferenci et al., 1989; Luper, 1998). 
Also, recently, it has been reported that, induction of NF-
κB with TNF-α and IL-1β was mediated through 
intracellular calcium but not ROS. The same report has 
showed that SM inhibited TNF-α-induced calcium-
dependent NF-κB activation irrespective of its antioxidant 
effect in human mesangial cells (Chang et al., 2006).

These protective effects may be related to different 
mechanisms such as the scavenger activity of the free 
radicals that induce lipid per oxidation (LP), and also 
stimulating antioxidant regeneration through increased 
protein synthesis (Sonnenbichler and Zetl, 1986). In 
experimental hepatic injury models, SM was reported to 
be effective on LP which mainly leads to destruction of 
plasma membrane (Moscarella et al., 1993; Farghali et al., 
2000).

Further study is needed to overcome the limitations of 
this current study and to verify the significance of the 
results. The authors believe that the limitation of this 
study is quantitative measurement of apoptosis and may 
add some objective supporting data to our results to 
clarify the effect of SM in renal I/R injury. I/R injury 
caused an impairment in renal function (increased serum 
creatinine and blood urea nitrogen (BUN) levels along 
with significant decrease in creatinine clearance), in our 
study was not evaluated in this issue.

The protective effects of SM on the primary 
inflammatory cell, renal tubular epithelium, in renal I/R is 
thought to be both due to the inhibition of NF-κB and anti-
oxidative activity of SM. Further specific study is needed 
clarify this issue such as measurement of tissue 
myeloperoxidase activity (MPO). SM has been reported to 
be safe to use in various conditions with minimal 
adverse effects (Jacobs et al., 2002). However, the 
adverse effects and the safety of SM were not in the 
scope of our study. The protective effects of SM was 
observed in even with 100 mg/kg in Group III (SM 100 
mg/kg + renal I/R), with increased dose of SM in Group 
IV (SM 200 mg/kg + renal I/R) prevent the morphological 
changes in all rats. However, SOD and CAT levels 
suggested that the higher levels of SM may be more 
effective in preventing oxidative injury.

Conclusion

The results of our study have demonstrated that SM 
significantly prevents renal I/R injury-induced renal 
changes in the rat. The clinical implications of these 
results merits further experimental and clinical studies to 
be performed.

REFERENCES

Avlan D, Tamer L, Ayaz L, Polat A, Oztürk C, Ozturhan H, Camdeviren 

Proinflammatory cytokine-induced NF-kappaB activation in human 
mesangial cells is mediated through intracellular calcium but not 

Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR 
(1999). Early kidney TNF-α expression mediates neutrophil infiltration 
and injury after renal ischemia-reperfusion. Am. J. Physiol. 277:922- 
929.

effects on intracellular calcium and cytotoxicity: a study in perfused 
rat hepatocytes after oxidative stress injury. Pharmacol. Res. 41:231- 
237.

Ferenci P, Dragosics B, Dittrich H, Frank H, Benda L, Lochs H, Meryn 
S, Base W, Schneider B (1989). Randomized controlled trial of 
silymarin treatment in patients with cirrhosis of the liver. J. Hepatol. 
9:105-113.


Granger DN, Korthuis RJ (1995). Physiological mechanisms of 

Havsteen B (1983). Flavonoids, a class of natural products of high positive 

oxotocin in male rats infused with hypertonic NaCl. Am. J. Physiol. 
268:634-640.

thistle for the treatment of liver disease: a systemic review and 

and protection by milk thistle extract in rats. Evid. Based Complement 


Marubayashi S, Dohi K (1996). Therapeutic modulation of free radical-
mediated reperfusion injury of the liver and its surgical implications. 

Matin SF, Novick AC (2001). Renal dysfunction associated with staged 
bilateral partial nephrectomy. The importance of operative 

Mihara M, Uchiyama M (1978). Determination of malonaldehyde 
86:271-278.

relationships of polymetoxyflavones and other flavonoids as inhibitors 
of non-enzymatic lipid peroxidation. Biochem. Pharmacol. 40:793- 
794.

Moscarella S, Giusti A, Marra F, Marena C, Lampertico M, Reili P, 
effects of silibyn phophadilcholine complex in chronic liver disease: 

Rodrigo R, Bosco C (2006). Oxidative stress and protective effects of 
polyphenols: Comparative studies in human and rodent kidney. A 
review. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 142:317- 
327.

effect of Nacetylcyesteine on the renal ischemia/reperfusion injury in 
the rat. J. Nephrol. 16:75-80.

Mercaptobenothane sulfonate (MESNA) protects against burn-induced 

Resveratrol improves ischemia/reperfusion-induced oxidative renal 


