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Berberine improves liver injury following renal ischemia reperfusion in rats

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Abstract

This study investigated the effect of berberine on the hepatic dysfunction and histological damage induced by renal ischaemia/ reperfusion (I/R) at an early stage. There were four groups (n=7). In Ber+I/R group, rats received berberine (Ber; 15 mg/kg/day) orally for 7 days before induction of ischemia. I/R group received distilled water orally for 7 days. In sham and Ber+sham groups in which arteries were not occluded, distilled water and berberin (15 mg/kg/day) respectively were administered orally for 7 days before surgery. Renal ischemia was induced by occlusion of both renal arteries for 45 min followed by 24 h of reperfusion. Blood samples were collected for biochemical analysis, and finally liver samples were preserved for future histological examination. The renal ischaemic challenge resulted in major histological damage of the liver, which was associated with increased levels of creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALK) during reperfusion period. In Ber+I/R group, the histological damage to the liver was improved along with increase in plasma creatinine, BUN, ALT, AST, LDH and ALK being smaller than those of the non-treated rats. Berberine exhibited a hepatoameliorative effect against renal ischemia/reperfusion-induced lesions.

Keywords*:* Berberine; Renal ischaemia/reperfusion; liver; Alanine aminotransferase; Aspartate aminotransferase; Lactate dehydrogenase

1. Introduction

Renal ischemia/reperfusion (I/R) injury is the major cause of acute renal failure in both native and transplanted kidneys (Kelly and Molitoris, 2000). Inflammation contributes to renal I/R injury, potentially causing renal dysfunction. One of the main constituents of the inflammatory infiltrate are neutrophils, which are deleterious for the renal tissue (Rouschop et al. 2005). Although reperfusion is essential for the survival of ischemic tissue, there is evidence that reperfusion itself causes additional cellular injury (Weight et al. 1996). Massive influx of neutrophils mediates the development of postischemic renal failure through the release of cytotoxic proteases and oxygen-derived radicals (Rouschop et al. 2005).

In the kidney, inflammatory process is initiated by both endothelial and tubular cell dysfunction. A number of different proinflammatory cytokines, such as IL-1, -6, and -8, TGF-β, and TNF-α, are released into the renal tissue and finally in the circulation (Kielar et al. 2005; Ramesh and Reeves, 2004). According to Park et al. (2011) ischemic renal injury initiates IL-17A generation in the small intestine resulting in the small intestinal and liver inflammation, apoptosis and necrosis.

In modern system of medicine, valuable drugs are not available to safeguard the liver against various damages (Pattanayak et al. 2011). Thus, the hepatoprotective activity of plants were explored using a variety of toxicants in experimental animals. Generally, some bioactive compounds found in plants were responsible for protecting the cells from oxidative stress via prevention or detoxification of free radicals and helped to prevent various disfunctions.

Berberine, an alkaloid isolated from rhizomes, roots, and stem bulk of the plants such as the Berberidaceae family has gained much attention in recent years for its anti-inflammatory, antioxidant, anticancer, antiviral, and antibacterial activities (Imanshahidi and Hosseinzadeh 2008; Kuo et al. 2004; Kettmann et al. 2004; Stermitz et al. 2000; Racková et al. 2003; Iwasa et al. 1996; Erdogan et al. 2006).

This research evaluated the possible therapeutic potential of berberine as a preventive agent in hepatic damages induced by ischemic acute renal failure in rats.

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1. Materials and methods

Experimental procedure

Male Wistar rats (260–310 g) were obtained from Razi institute, Shiraz, Iran. The animals were grouped and housed in polyacrylic cages and maintained under standard laboratory conditions (temperature, 25±2 °C) with a 12:12 h light/dark cycle. They were allowed free access to a standard pellet diet and water ad libitum. The local ethics committee approved the study. The rats were divided into four groups: Sham (n=7), I/R (n=7), Ber+I/R (Berberine, 15 mg/kg/day during 7 days; n=7), Ber+Sham (Berberine, 15 mg/kg/day during 7 days; n=7). After 7 days of distilled water/berberine treatment, both renal arteries were occluded for 45 min followed by 24 h of reperfusion in I/R performed groups. In sham and Ber+Sham groups, the renal arteries were not occluded and animals received distilled water and berberine (Fluka) respectively for 7 days before surgery. Rats were anesthetized with ketamine (60 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) before I/R operation. At the end of reperfusion period, blood sample was collected from heart ventricles under anesthesia and rats were sacrificed and the liver was quickly isolated and preserved.

Biochemical analysis

Plasma samples were assayed for creatinine and urea nitrogen in milligram per deciliter using an autoanalyser (RA 1000; Technicon Instruments, NY, USA). Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) and (ALK) activities in plasma samples were measured by commercially available kits.

Histopathological examinations

Liver samples were fixed in buffered 10 % formaldehyde (Merck, USA). After dehydration through a graded alcohol series, the samples were cleared in xylol. Then, liver samples were embedded in paraffin and 5 µm sections were obtained by microtome (Erma, Japan). Routine staining with hematoxylin and eosin was done for each liver section. In a blinded fashion, each section was examined in at least 10 randomly selected non-overlapping fields under light microscope. In each section, we examined the degree of the presence of congestion and cellular degenerative changes. The level of each pathological manifestation was graded according to the observed changes as follow: none with 0, less than 20 % with 1, 21–40 % with 3, 61–80 % with 4, and greater than 80 % with 5. The sum of all numerical scores in each group was taken as the total histopathological score.

Statistical analysis

Data are presented as mean±SEM. They were assessed by one-way analysis of variance followed by Duncan's post hoc for comparison between groups. The histopathological scores were statistically compared between groups by nonparametric Kruskal–Wallis multiple comparison test. All data analyses were performed using SPSS ver. 11.5 software (SPSS Software, Chicago, IL, USA) and significance was taken at $P \leq 0.05$.

2. Results

As it can be seen from Fig. 1, plasma creatinine and blood urea nitrogen (BUN) levels were statistically higher in I/R group compared to sham and Ber+sham groups ($P<0.001$). Berberine-treated group showed significant reduction in creatinine (Fig. 1a) and BUN (fig 1b) levels in comparison with I/R group ($P<0.001$).

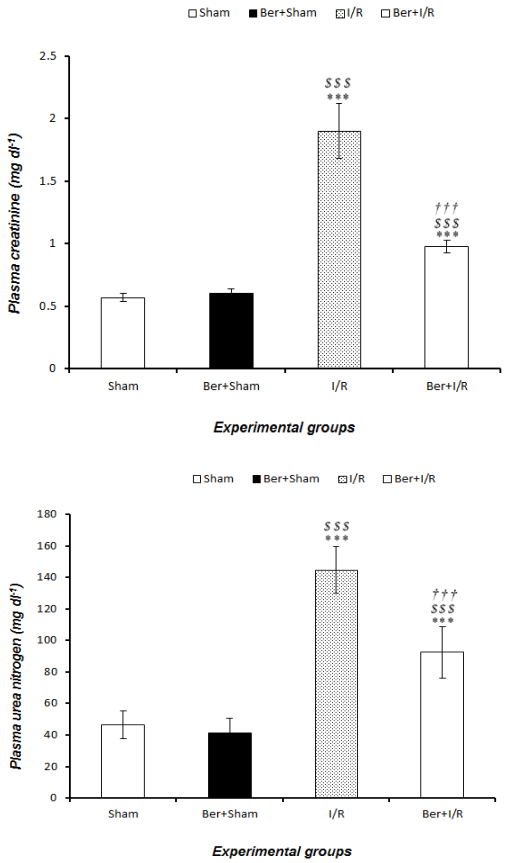

Fig. 1. The levels of plasma creatinine (a) and plasma urea nitrogen (b) at the end of reperfusion period in rats subjected to sham-operation that received distilled water (sham group), or berberine (Ber+Sham group), or to ischaemia/reperfusion that received distilled water (I/R group), or berberine (Ber + I/R group). *P < 0.05, **P < 0.01, ***P < 0.001 vs sham group; $^{\$}$P < 0.05, $^{\$\$}$P < 0.01, $^{\$\$\$}$P < 0.001 vs Ber+sham group; †P < 0.05, ††P < 0.01, †††P < 0.00, vs I/R group

There was a significant increase in the plasma levels of AST and ALT in I/R group compared to sham and Ber+sham groups (P<0.01). Berberine treatment reduced the levels of AST (Fig. 2a) and ALT (Fig. 2b) in Ber+I/R group in comparison with I/R group (P<0.01).

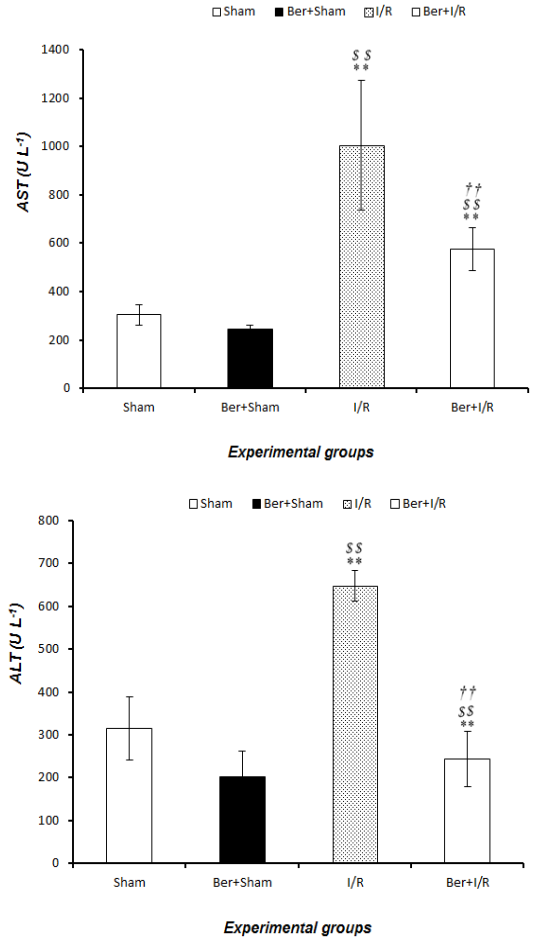

Fig. 2. The levels of plasma AST (a) and plasma ALT (b) at the end of reperfusion period in rats subjected to sham-operation that received distilled water (sham group), or berberine (Ber+Sham group), or to ischaemia/reperfusion that received distilled water (I/R group), or berberine (Ber + I/R group). **P < 0.01, vs sham group; $^{\$\$}$P < 0.01, vs Ber+sham group; ††P < 0.01, vs I/R group

In the I/R group, there was marked increase in the plasma level of LDH with respect to its level in sham and Ber+sham groups (P<0.001). LDH level was reduced in Ber+I/R group in comparison with I/R group (P<0.001), while it was the same in sham and Ber+sham groups (Fig. 3a).

We also showed that plasma level of ALK in I/R group (Fig. 3b) was statistically higher compared to sham and Ber+sham groups (P<0.01). Berberine-treated group showed significant reduction in ALK level in comparison to I/R group (P<0.01).

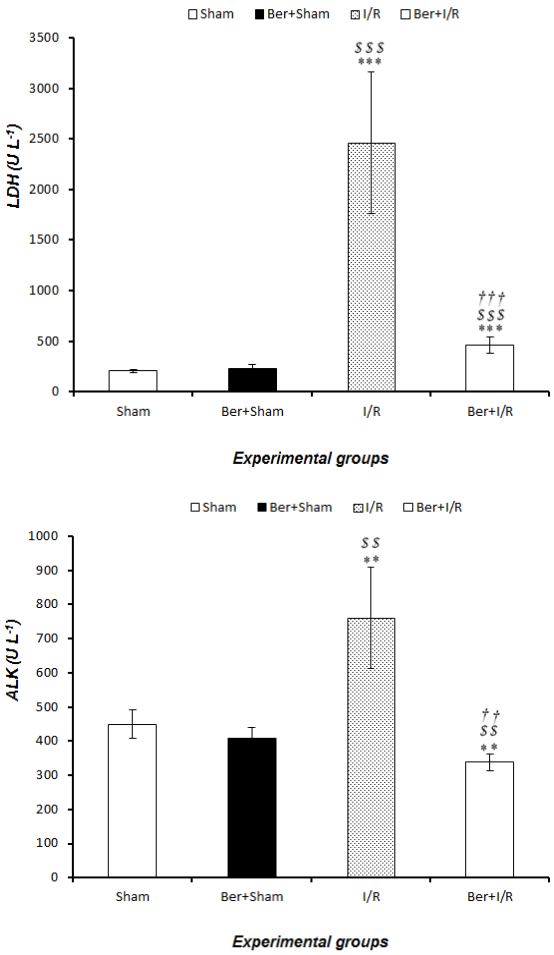

Fig. 3. The levels of plasma LDH (a) and plasma ALK (b) at the end of reperfusion period in rats subjected to sham-operation that received distilled water (sham group), or berberine (Ber+Sham group), or to ischaemia/reperfusion that received distilled water (I/R group), or berberine (Ber + I/R group). **P < 0.01, ***P < 0.001 vs sham group; $^{\$\$}$P < 0.01, $^{\$\$\$}$P < 0.001 vs Ber+sham group; ††P < 0.01, †††P < 0.001, vs I/R group

Histology

Results from the histological studies were in agreement with the measured activities of plasma enzymes. There were no abnormalities or histological changes in the livers of sham and sham+Ber groups (Fig. $4a_1$, $4a_2$). In the I/R group (Fig. $4b_1$, $4b_2$), the most prominent lesions were vascular congestion in the central vein (grade 3), infiltration of inflammatory cells in the portal space (grade 5) and apoptosis of the hepatic cells (grade 5). In the Ber+I/R group less intense lesions were noticed in comparison with I/R group (Fig. $4c_1$, $4c_2$). The sum of histopathological grades, marking the changes described above, is shown in Table 1.

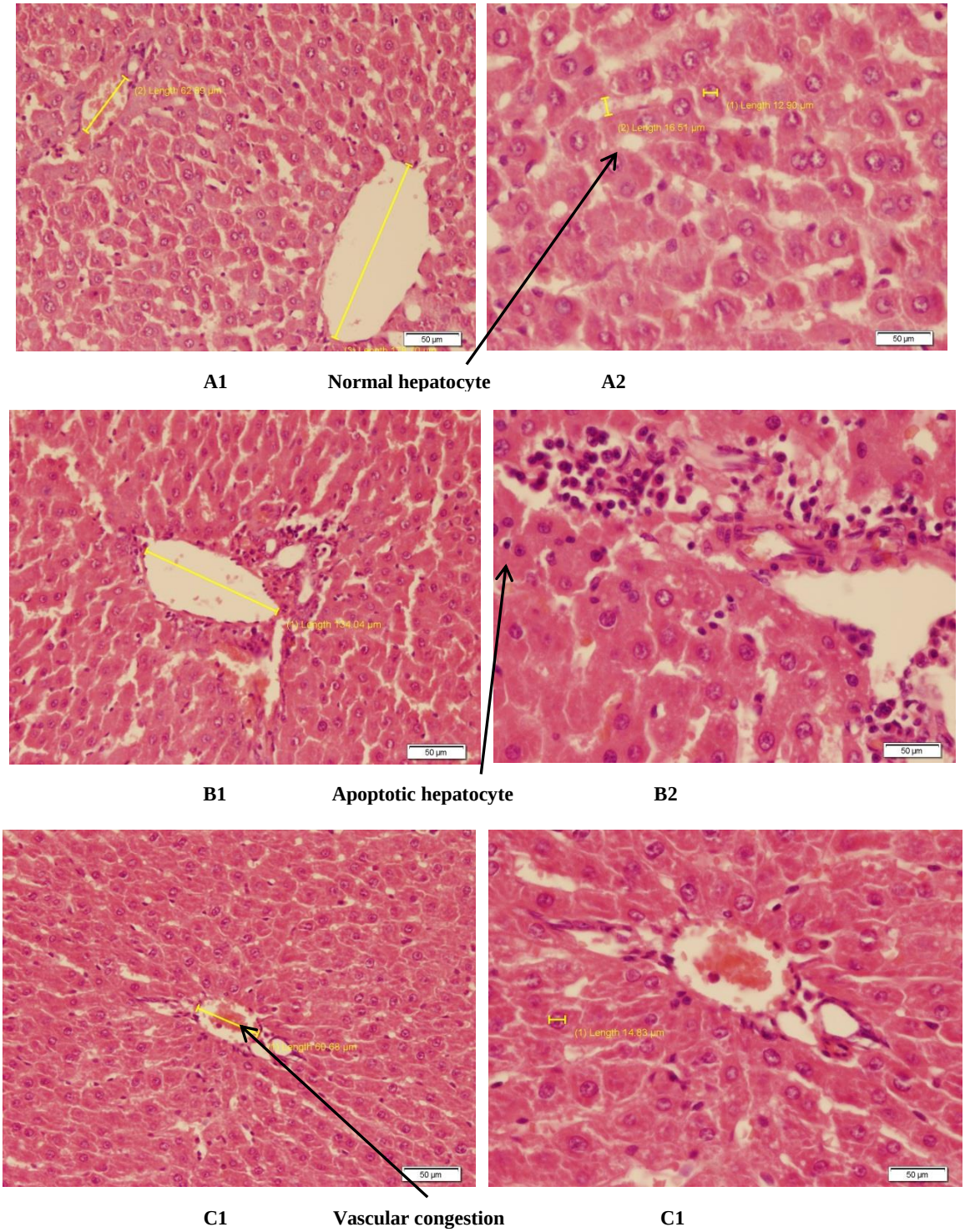

Fig. 4. Representative light microphotographs of the liver obtained from sham group (a_1, a_2) and I/R group (b_1, b_2), or Ber + I/R group (c_1, c_2). (haematoxylin–eosin staining; scale bar= 50 µm)

Table 1. Histopathological score in Sham, Ber+ Sham, I/R and Ber+I/R groups (each n=7) at the end of reperfusion period of ischemia/reperfusion-(I/R)-induced acute renal failure (mean±SEM)

Group	Histopathological score
Sham	0.0 ± 0.00
Ber+Sham	0.0 ± 0.00
I/R	12.42 ± 0.20***
Ber+I/R	9.85 ± 0.34†††

***P<0.001 Vs Sham
†††P<0.001 Vs I/R

3. Discussion

Liver injury is one of the distant organ damages induced by kidney IR. Acute renal failure leading to liver disease is a commonly encountered clinical problem of varied etiology. It is believed that IR injury induces inflammatory response, causing tissue damage in a number of organs in which reactive oxygen species play a key role in the pathophysiology of renal IR injury (Erdogan et al. 2006; Kelly, 2003). It is demonstrated that renal IR injury might cause liver oxidative stress and increase lipid peroxidation in liver tissue (Yildirim et al. 2003). It is reported that liver tissue of rat decreases antioxidant enzyme activities after renal I/R (Sural et al. 2000). It has been shown that the vital steps in the prevention of renal I/R-induced liver diseases include the removal and neutralization of deleterious metabolites like ROS (Canbek et al. 2011; Uyanoglu et al. 2011) . Natural antioxidants could prevent the deleterious effects of toxic agents by scavenging free radicals and other reactive oxygen species or by modulation of the inflammatory response (Grimble, 1994; Domitrovi´ c et al. 2009). In the present study, the changes in the function and histology of the liver were examined at the early phase of reperfusion. In addition, it was determined that berberine pretreatment could modulate ARF-induced liver dysfunction.

In the rats subjected to I/R, the reduction in GFR was attenuated after berberine treatment as indicated by decrease of plasma level of creatinine and urea nitrogen. The most perceptive markers engaged in the finding of hepatic injury caused by renal I/R include plasma AST, ALT, ALK and LDH (Uyanoglu et al. 2011; Kadkhodaee et al. 2009; Wang et al. 2010; Vaghasiya et al. 2010; Giannini et al. 2005). The results of current study showed that the plasma levels of AST, ALT, ALK and LDH increased significantly after renal I/R (45 min /24 h). When these cytosolic enzymes are released into the circulatory system as a consequence of hepatocellular damage, the activities of these enzymes increase in plasma. The enhanced activities of these plasma markers in I/R group correspond to the extensive liver damage induced by the renal I/R. The rise in ALT activity is usually accompanied by the rise in AST following hepatocellular damage, proliferation, or degeneration (Ravikumar et al. 2005). Moreover, the ALK increases in plasma reveal the liver cell membrane damage (Plaa and Hewitt, 1989). LDH is a cytosolic enzyme mainly present in periportal hepatocytes and released when the cells are lysed. In the present study, berberine prevented the alterations in the status of these markers to normal levels, possibly by maintaining the hepatocellular membrane integrity. Recovery towards normalization suggests that berberine causes parenchymal cell regeneration in liver, thus protecting membrane fragility, thereby, decreasing enzyme leakage.

Light microscopy for I/R group showed increased vascular congestion in central vein, infiltration of inflammatory cells into the portal space, and apoptosis of the hepatic cells. Thus, the combination of these factors were likely to be responsible for the reduced liver function following the ischaemia in I/R group. A number of animal studies have shown that ischemia/reperfusion is correlated with the generation of reactive oxygen species (ROS) (Bhalodia et al. 2010). ROS are associated with the inflammatory response. They frequently contribute to the tissue damaging effects of inflammatory reactions (Pawliczak, 2003; Cuzzocrea et al. 2000; Leiro et al. 2004). Moreover, they are important mediators of programmed cell death induced by TNF (Los et al, 2002; Lin et al. 2004). Furthermore, at intermediate concentrations ROS induce apoptosis whereas at higher concentrations it induces necrotic cell death (Takeda et al. 1999; Renz et al. 2001). Neutrophils play a crucial role in the development and manifestation of inflammation and they are the major source of free radicals at the site of inflammation. Free radicals induce lipid peroxidation and impair antioxidant status. On the other hand, Park et al. (2011) showed that both ischemic and non-ischemic renal injury initiates IL-17A generation in the small intestine resulting in small intestinal and liver inflammation, apoptosis and necrosis. They demonstrated crucial roles for TNF-α, IL-17A and IL-6 in generating these injuries. Also, they provided evidence that small intestine derived IL-17A causes further cytokine generation to induce hepatic injury and systemic inflammation. TNF- α is a pleiotropic cytokine associated with a variety of physiological and pathological conditions (Beyaert and Fiers, 1998). TNF- α seems to be responsible for regulating products that stimulate inflammation and fibrosis (Simeonova et al. 2001). The results of the studies

on several cell lines have shown that, independently from the kind of inflammatory stimulus, berberine effectively suppresses the expression of proinflammatory cytokines, including TNF- α, subsequently inhibiting downstream mediators of inflammation, such as iNOS and COX-2 (González-Amaro et al. 1994; Hsiang et al. 2005; Jeong et al. 2009). TNF- α is the key mediator in many experimental liver injury models. It induces iNOS and stimulates production of nitric oxide (NO•), contributing to nitrosative stress. NO• may react with superoxide (O2 •−) in the mitochondria to produce peroxynitrite (ONOO−), both of which are important mediators of cell dysfunction. Overexpression of iNOS has been seen in many acute and chronic diseases (Nussler and Billia, 1993). COX-2 is an inducible form of the prostaglandin synthase enzymes, which catalyse the committed step in the prostaglandin production pathway (Dubois et al. 1998). COX-2 expression is increased in inflammatory conditions as a result of induction by several different stimuli, including proinflammatory cytokines TNF- α, IL-1β, and EGF (Akarasereenont et al. 1995). The hepatoprotective effect of berberine through inhibition of COX-2 has been shown in CCl4-induced liver damage (Domitrovi´ca et al. 2011). Thus, we suggest that renal I/R- induced liver injury may be induced by the generation of ROS.

4. Conclusion

In summary, administration of berberine before a period of 45 min ischemia/24 h reperfusion markedly offset the hepatic tissue damage, possibly due to inhibition of inflammatory events. It also blunted the renal disfunction and the disturbed liver function, and consequently attenuated the increases in $[Cr]_P$, $[UN]_P$, $[AST]_P$, $[ALT]_P$, $[LDH]_P$ and $[ALK]_P$. These findings suggest that the anti-inflammatory and anti-oxidant properties of berberine may alleviate liver damages induced by ischemic ARF at the early phase.

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