Journal of Animal and Veterinary Advances 10 (24): 3244-3248, 2011
ISSN: 1680-5593


# Effects of *Hypericum perforatum* (St. John's wort) Extract on Renal Ischemic Reperfusion Injury in Rats

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**Abstract:** Ischemia/Reperfusion (IR) induces severe tissue injury mainly caused by oxidative stress. A considerable body of recent evidence suggests that oxidative stress and exaggerated production of reactive oxidant species play a major role in several aspects ischemia and reperfusion. *Hypericum perforatum* is a medicinal plant species containing many polyphenolic compounds namely flavonoids and phenolic acids. Because polyphenolic compounds have high antioxidant potential in this study we evaluated the effect of H. perforatum extract on renal ischemic reperfusion injury. Renal ischemic reperfusion was induced in rats by clamping the renal artery for 60 min. After 7 days of reperfusion. *H. perforatum* extract treatment markedly reduced renal ischemia reperfusion injury in I/R+HP 30 mg $kg^{-1}$ group comparative with other treatment and sham groups.

**Key words:** Renal Ischemia/Reperfusion (IR), *Hypericum perforatum*, acute renal failure, tissue, Iran

## INTRODUCTION

I/R induced Acute Renal Failure (ARF) is a common clinical problem which despite significant advances in critical care medicine is still associated with high morbidity and mortality. The mechanisms underlying renal I/R are complex including ATP depletion, accumulation of intracellular $Ca^{2+}$ and reactive oxygen species, mitochondrial dysfunction, multiple enzyme systems activation and pro-inflammatory cytokine production. Although, reperfusion is essential for the survival of ischaemic renal tissue, it causes additional cellular injury. Together, renal ischaemia and reperfusion initiate a multiple and interrelated sequence of events resulting in the injury and eventually the death of renal cells as a combination of both apoptosis and necrosis (Spandou *et al*., 2009). *Hypericum perforatum* L. (Hypericaceae), popularly called St. John's wort has been used in popular medicine since ancient times for several disorders such as skin wounds, eczema, burns, diseases of the alimentary tract, insomnia and mental illness among others (El-Sherbiny *et al*., 2003). *H. perforatum* extract contains flavonoids such as rutin, quercetin and quercitrin which demonstrate a free radical scavenging activity in a model of auto-oxidation of rat cerebral membrances (Gambarana *et al*., 2001; Zou *et al*., 2004). An antioxidant activity of quercetin has also been demonstrated by inhibition of brain lipid peroxidation as manifested by lowering MDA while elevating phospholipid contents in a rat model of endotoxemia (Benedi *et al*., 2004; Sanchez-Reus *et al*., 2007; Silva *et al*., 2005). Therefore, hypericum extract with a potential antioxidant activity may be of value in dementia among other disorders of senility in which free radical generation is implicated. In addition its antidepressant activities, *H. peforatum* in line with popular credence also possesses antiviral, wound-healing, antimicrobial, analgesic and anti-inflammatory effects (El-Sherbiny *et al*., 2003). Studies with other plants of the same genus have been carried out under the stimulus of great scientific interest and economic value acquired by *H. perforatum*. Thus, antidepressant, analgesic, anti-inflammatory, antioxidant, antimicrobial and wound-healing effects have also been found for other species of the genus *Hypericum* (Gambarana *et al*., 2001; Kerb *et al*., 1996). More recently, *H. perforatum* extract has been reported to efficiently attenuate Interferon (IFN)-y-elicited activation of Signal Transducers and Activators of Transcription (STAT)-l in alveolar A549/8 and colon DLD-l cells (Tedeschi *et al*., 2003). Hypericum extract has been always referred to have a benign side effect profile compared with tricyclic antidepressants and serotonin-specific reuptake inhibitors (Nathan, 2001). There has not been a single fatal intoxication of the extract as a monotherapy reported in

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the literature (Kerb *et al*., 1996). Therefore, Hypericum extract as an efficacious antidepressant medication with a benign side effect profile together with a potential antioxidant activity was hypothesized to be useful in the treatment of pathological situation in which ROS play an important role such us ischemia and reperfusion. Thus, the aim of the present study was to evaluate the effect of *H. perforatum* extract on renal ischemic reperfusion.

## MATERIALS AND METHODS

**Animals:** Studies were performed on male Wistar rats weighing 200-250 g (n = 25). Rats received a standard diet and water *ad libitum* and were housed in a 12 h light/dark cycle. The animals were randomly allocated into three groups: I/R-saline group in which rats were subjected to renal ischemia for 60 mm (n = 10); IR-*H. perforatum* (HP) group in which rats were administered HP (30 mg $kg^{-1}$ and gavages) 7 days prior to I/R and 7 day IR (n = 10); I/R-HP group in which rats were administered HP (60 mg $kg^{-1}$ and gavages) 7 days prior to I/R and 7 days after IR (n = 10), sham-operated group in which rats were subjected to identical surgical procedure without occlusion of both renal pedicles and maintained under anesthesia for the duration of the experiment (n = 5) (Bosco and Schweizer, 1988.

**Surgical procedures:** Animals were anesthetized with Ketamine hydrochloride (Ketamine = 10%, Alfasan, Woerden-Holland = 50 mg $kg^{-1}$) and Xylazine (Xylazin = 2%, Alfasan, Worden-Holland = 5 mg $kg^{-1}$) intraperitoneally. A midline incision was made in each rat and the left kidney became available. Ischemia-reperfusion injury was induced by applying a noncrushing microvascular clamp on the left renal artery for 60 min. After 60 min of ischemia, the clamp was removed and the tissue was closed in layers. The animals were then returned to their cages, reperfusion period was 7 days after surgery. In experimental group, 7 days before ischemia, HP 30 and 60 mg $kg^{-1}$ were administrated as orally form. Sham-operated animals underwent the same operation but without clamping. Animals were sacrificed after 7 days postoperatively under general anesthesia with an injection of over dosage of Thiopental sodium (60 mg $kg^{-1}$) and the left kidney was harvested.

**Histopathology analyses:** Kidneys sunk in 10% formalin buffer during 7 days for fixation then each of those segments were included in paraffin blocks, all specimens were serially sectioned longitudinally at 5 μm intervals and stained with Hematoxylin-Eosin (H and E) method and used for light microscopic examination under a Nikon microscope (ECLIPSE E200, Japan).

**Extract preparation:** Powdered leaves of HP were extracted 3 times with 1 L of 70% methanol (MeOH)/$H_2O$ while being macerated at room temperature for 24 h each time. The hydroalcoholic extracts were combined and concentrated in vacuo to yield dried extract and it was defined by the producer as containing 0.28% hypericin. This hydroalcoholic extract was kept in refrigerator for all experiments.

## RESULTS AND DISCUSSION

Histopathological changes of kidney in IR rats and IR rats pretreated with HP (30 and 60 mg $kg^{-1}$) have been shown in Fig. 1-3. In renal tissue of IR rats, acute tubular lytic necrosis, interstitial lymphocyte infiltration, hyperemia and wide hemorrhages were observed. Dilation of urinary space with hyaline proteinaceouse droplets adhesiveness of visceral and parietal bowmann's capsule, mesenchial moderate hypercellularity with hyperemia and some time the necrosis of squamous cell in parietal wall of bowmann's capsule were also prominent. In addition, hyaline casts in some time with obstruction and dilation of tubules were visible. Pretreatment with HP at a dose of 30 mg $kg^{-1}$ caused considerable reversion of pathological changes. In this group, low pathological injuries consisting of petty hyperemia and slight edema in tubular cells were distinguished. Mild infiltration of lymphocytes in interstitial areas was distinguishable. Pretreatment with

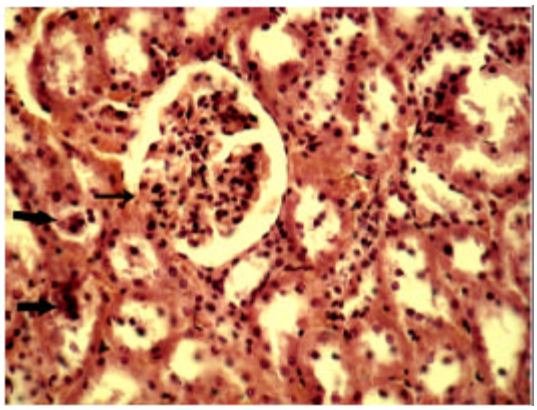

Fig. 1: Microscopic view of the kidney in I/R rat. Acute tubular lytic necrosis of the kidneys featuring detachment of the cells from the basal membrane, cytoplasmic granulation and pyknotic nuclei in both tubuli and glomeruli. Note the cellular debris in the lumens of these tubules (thick arrows). Lymphocyte infiltration in the interstitum is seen. Thickening of glomerular basement membrane, hypercellularity and synechiae (thin arrow) is also prominent (H and E stain 40x)

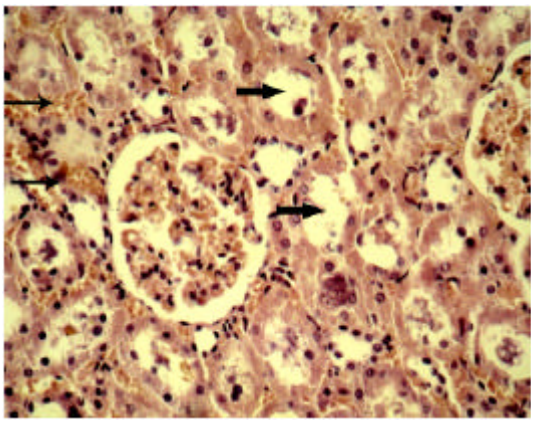

Fig. 2: Microscopic view of the kidney in I/R+HP 30 mg $kg^{-1}$ rat. Enlargement of lining cells of tubules with some tubular dilatation (thick arrows) is visible. There is mild hyperemia (thin arrows) and lymphocyte infiltration in the interstitial areas pathologic changes is mild to moderate comparison with representative sections from I/R rats (H and E stain, 40x)

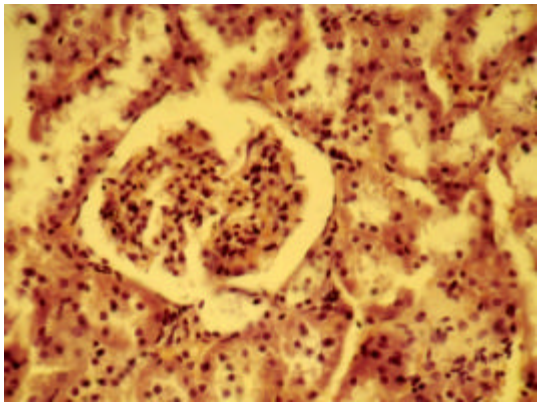

Fig. 3: Microscopic view of the kidney in I/R+HP 60 mg $kg^{-1}$ rat showing normal glomerulus and tubular cells. Pretreatment with HP (60 mg $kg^{-1}$) has reversed pathologic changes to near normalcy (H and E stain, 40x)

HP at a dose of 60 mg $kg^{-1}$ retrieved renal histopathological injuries to near normal. In Sham/IR group, the kidney tissue was normal and remarkably histological differences between them were not observed.

This study provides evidence that *H. perforatum* extract cases substantial reduction of renal ischemia/reperfusion injury in the rat. We speculate that *H. perforatum* extract and related compounds may be useful in the therapy of conditions associated with ischemica/reperfusion. Ischemia-reperfusion injury is a major cause of renal failure and renal graft rejection. Therefore, reducing the extent of this injury in renal transplant patients is important for achieving a good prognosis. Renal Ischemia-Reperfusion (I/R) injury leads to the production of excess Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). These species cause oxidative stress resulting in alterations in the level of mitochondrial oxidative phosphorylation, ATP depletion, increases in the intracellular calcium and activation of protein kinases, phosphatases, lipases and nucleases leading to a loss of cellular function and integrity (Sekhon *et al*., 2003). Therefore, it is important to reduce the levels of these hazardous methabolites in order to improve the patient's outcome. In order to reduce these metabolites, many studies have examined a variety of free radical oxygen scavengers. These include the effects of external supplementation of antioxidants (Gianello *et al*., 1996; Lee *et al*., 2006; Mathews and Gregory, 1997) and the activities of endogenous enzymatic antioxidant defense system in a kidney ischemia/reperfusion injury (Barnard *et al*., 1993; Dobashi *et al*., 2000).

In this study revealed that *Hypericum perforatum* (HP) caused decrease in renal tissue injury due to ischemic reperfusion so that was related to administrated dose of HP. Other researchers results also showed that low dose of *Hypericum perforatum* have more cytoprotective effect than mild doses 30 mg $kg^{-1}$ (Menegazzi *et al*., 2006). *Hypericum perforatum* is effective in treatment of diabetic patients and metabolic disorders (Menegazzi *et al*., 2006). Part of the therapeutic effects of *Hypericum perforatum* is related to antioxidant activation so that nowadays this drug is used in medical research as a stimulating of antioxidative stress pathway (El-Sherbiny *et al*., 2003). There are a few studies on effect of *Hypericum perforatum* on injuries subsequent ischemic reperfusion. De Paola *et al*. (2005) showed that short-term using of *Hypericum perforatum* during a mild ischemia improves cellular performance after ischemia on rats. Tedeschi *et al*. (2003), Hammer *et al*. (2007) showed that *Hypericum perforatum* have a anti-inflammatory reaction with inhibition of human inducible nitric oxide synthase expression by down regulating Signal Transducer and Activator of Transcription-1a (STAT-1) activation and Inhibition of Prostaglandin $E_2$ Production. In all done studies about protective effect of *Hypericum perforatum* as single dose or low dose or short-term usage on injuries subsequent ischemic reperfusion and acute renal failure suggested that activation of antioxidative pathway is as *Hypericum perforatum* action mechanism (Flausino *et al*., 2002). Hunt *et al*. (2001) demonstrated that there is a free radical scavenging effect of *H. perforatum* extract and postulated that this effect could be related to an active

constituent. Likewise the beneficial effects of *H. perforatum* on acute cerebral ischemia-reperfusion could also be attributed to its bioactive constituents (Hunt *et al*., 2001). Benedi *et al*. (2004) and Sanchez-Reus *et al*. (2007) demonstrated that hypericum perforatum reduction of oxidative stress by inhibiting of free radical generation and lipid peroxidation. They also declared that *Hypericum perforatum* by activation of Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and levels of Malondialdehyde (MDA) plays an important protective role on ischemic reperfusion injury (Benedi *et al*., 2004; Sanchez-Reus *et al*., 2007). Anyway, from this and other researches results can be conclude that *Hypericum perforatum* protects of renal ischemia reperfusion injury through inhibition of oxidative stress and inflammatory reactions occurrence in acute renal failure.

## CONCLUSION

Considering the above-mentioned series can be declared that *Hypericum perforatum* can use as a preventive or harmless therapeutic agent against damages of renal ischemic reperfusion after controlled clinical trials. Thus to understanding of accurate sites and action mechanism of *Hypericum perforatum* requires many more expanded studies.

## ACKNOWLEDGEMENT

Researcher thanks for Ahar Branch, Islamic Azad University for support.

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