



Iranian Veterinary Surgery Association

IRANIAN JOURNAL OF VETERINARY SURGERY

Journal homepage: www.ivsajournals.com

ORIGINAL ARTICLE

Hepato- and Reno-Protective Impacts of *Plantago major*: An Experimental Model of Testicular Ischemia/Reperfusion

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Received: 4 February 2020

Accepted: 7 April 2020

Online: 7 April 2020

Keywords:

Plantago major;
ischemia/reperfusion;
liver;
kidney;
Rat.

Abstract

Object- The purpose of this study was to evaluate the protective impacts of *Plantago major* (*P. major*) against the adverse effects resulted from testicular ischemia/reperfusion (I/R) on kidney and liver.

Design- Experimental study.

Animals- Twenty-four male rats.

Procedure- The testicular I/R was induced by firstly anesthetizing rats using ketamine and xylazine and then, incising the skin of testis region. After the stimulation of testicular I/R by rotating the testis in clockwise direction, fixing for 2 hours and then, re-rotating testis. Then, *P. major* was administered through intraperitoneal route at the dose of 50 and 100 mg/kg for 14 days. The liver and kidney samples were used for histopathological investigation.

Results- It was found that testicular I/R negatively affects the kidney and liver as it was demonstrated in histopathological samples. Degeneration of hepatocytes, necrosis, hyperemia in sinusoids and infiltration of inflammatory cells were found in liver. Similarly, the hyperemia, necrosis and infiltration of inflammatory cells were observed in the kidney. Administration of *P. major* diminished significantly these harmful effects with better results at the dose of 100 mg/kg.

Conclusion and Clinical Relevance- It is suggested that treatment of rats with *P. major* is beneficial in terms of reducing the adverse effects of testicular I/R on the kidney and liver tissues and this procedure can be applied in the clinical trials.

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DOI: 10.30500/ivsa.2020.218557.1209



1. Introduction

Ischemia is defined as a condition that cells and tissues are deprived from blood supply.¹ Reperfusion has been proposed to cause tissue injury resulted from ischemia. Accumulating data demonstrates that immediate reperfusion is associated with induction of a number of cellular events leading to the more cellular injury and subsequently, death.² A variety of mechanisms are involved in mediation of adverse effects of ischemia/reperfusion (I/R) injury.³ It seems that enhanced generation of reactive oxygen species (ROS) is the most challenging harmful effect of I/R injury that impairs the function of mitochondria resulting in apoptotic cell death.⁴ During physiological conditions, there is a balance between the concentration of ROS and capacity of antioxidant defense system.^{5,6} The induction of I/R injury interrupts this balance, so that the ROS production exceeds from the capacity of antioxidant defense that negatively affects cell membranes, lipids and genetic materials.⁷⁻⁹ In addition to ROS, neutrophil infiltration and inflammatory responses undergo upregulation under I/R injury resulting in further damage in cells and tissues.¹⁰ It appears that disruption of ion concentrations such as mitochondrial Ca^{2+} overload is another important phenomenon under I/R injury that involves in stimulation of harmful impacts of I/R injury.¹¹⁻¹³

Over the past decades, much attention has been directed towards naturally occurring compounds due to their low cost, minimal side effects and more importantly, great biological and therapeutic effects.^{14,15} *Plantago major* (*P. major*) is a well-known plant-derived chemical in traditional medicine exclusively found in medicinal plants belonging to Plantaginaceae family.¹⁶ This plant-derived chemical has a number of pharmacological effects such as antioxidant,¹⁷ anti-inflammatory,¹⁸ anti-diabetic,¹⁹ hepatoprotective,²⁰ and anti-tumor.²¹ It is held that polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides, and terpenoids are major

components of *P. major* extract that mediate the therapeutic activity of this naturally occurring compound.²² These protective effects of *P. major* have resulted in its extensive application in treatment of pathological conditions.²³ In the present study, we investigated the ameliorative effects of *P. major* extract on the liver and kidney of rats with testis I/R injury.

2. Materials and Methods

Animal Housing

Twenty-four Wistar rats with the weight of 200-220 g were kept at the anatomical department of University of Tabriz. All the rats were kept at a standard condition with 12:12 interval light/dark cycle at 25° C in standard cages. University of Tabriz Research Ethics Committee approved all animal experiments.

Animal Treatment

The rats were randomly divided into four groups including: A) Sham (Sham-operated rats group receiving normal saline (0.9%), B) control group (I/R was induced and the right testicular torsion of 720° lasting two hours was followed by detorsion, C) I/R group receiving 50 mg/kg *P. major*; D) I/R group receiving 100 mg/kg *P. major*. The *P. major* was administered via intraperitoneal route daily for 14 days after detorsion.

The Generation of Testicular I/R

The rats were anesthetized using 80 mg/kg ketamine 5% and 10 mg/kg Xylazine 2% through intramuscular route. The skin of testis region was shaved and scrubbed using betadine solution. A vertical paramedian incision was made on the scrotum to expose testes. Next, tunica vaginalis was opened and the surgery was performed on the right testis. In order to provide ischemia, the right testis was rotated 720° in a clockwise direction. Then, the right testis was kept at the torsion position by fixing to the

scrotum through 4-0 silk suture for 2 hours with the closure of scrotal incision. After 2 hours, the right testis was de-rotated and re-perfused. Then, *P. major* was administered through intraperitoneal route at the dose of 50 and 100 mg/kg for 14 days.

Histopathological Analysis

The liver, kidney, and testis, were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, the tissues were dehydrated in graded ethanol, clearing in Xylol, loading in paraffin wax and sectioning at about 5-6 μm . The resulting samples were stained using hematoxylin and eosin (H&E) and observed by light microscopy (Olympus, BX60). For a semi-quantitative comparison of the structural changes, the abnormalities in the tissue sections were graded from 0 (normal structure) to 3 (severe pathological changes).²⁴

Statistical Analysis

The data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using the SPSS statistical package. *p* values less than 0.05 were considered as statistically significant.

3. Results

Histopathological Profile of Liver

The liver samples of sham group showed no evidence of histopathological alteration (normal structure). The liver samples of control group had a number of features such as necrosis, degeneration, hyperemia of sinusoids and infiltration of inflammatory cells (severe pathological changes) showed in Figure 1. It is noteworthy that liver samples of treatment groups have lower levels of histopathological changes. These changes are more mild in the group D (100 mg/kg) compared to the group C (50 mg/kg) showing the dose-dependent effect of *P. major*.

Histopathological evaluation showed a significant difference between the four groups ($p < 0.05$).

Histopathological Changes of Kidney

A same story occurs in the kidney, so that there is no sign of histopathological alteration in Sham group (normal structure), while there are severe changes in kidney samples of control group such as necrosis, hyperemia and

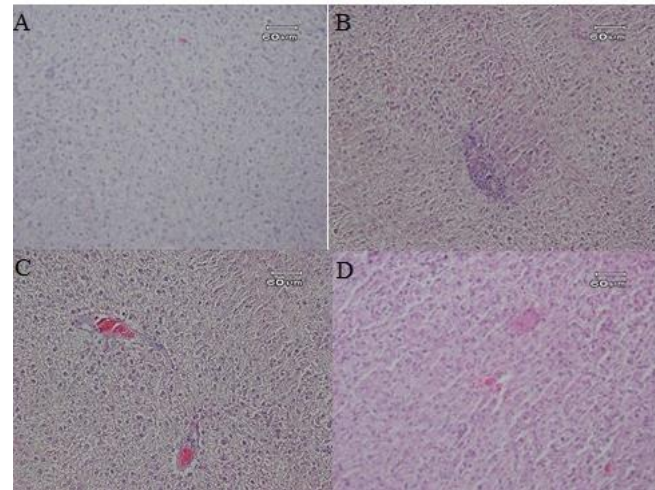


Figure 1. Histological findings of rat liver tissue (H&E). A) Sham group with no sign of histopathological alteration. B) Control group with features including necrosis, degeneration, infiltration of inflammatory cells. C) liver samples of rats treated with 50 mg/kg of *P. major* with moderate degree of changes. D) liver samples of rats treated with 100 mg/kg of *P. major* with mild degree of changes.

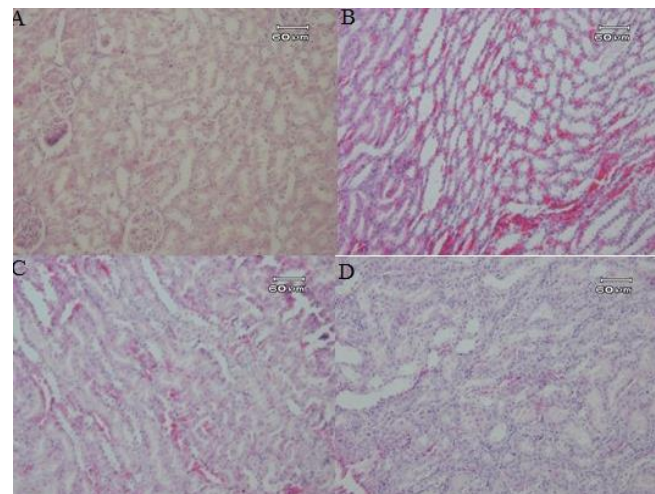


Figure 2. Histological findings of rat kidney tissue (H&E). A) Sham group. B) Control group. C) Treatment with *P. major* (50 mg/kg) with moderate histopathological alterations. D) Treatment with *P. major* (100 mg/kg) with mild histopathological changes.

infiltration of inflammatory cells. The administration of *P. major* is associated with amelioration of these changes with greater beneficial effects in high dose (100 mg/kg) (Figure 2). Histopathological evaluation showed a significant difference between the four groups ($p < 0.05$).

4. Discussion

Accumulating data demonstrates that kidney is an essential organ in secretion and reabsorption of glucose and amino acids, synthesis of active form of vitamin D, modulation of immunological responses and regulation of metabolic proteins. So, it is vital to preserve the homeostasis of this important organ. The main mechanisms that cause mitochondrial dysfunction in IR injury is the production of ROS.^{25,26} The elevated generation of ROS negatively affects the kidney through various signaling pathways. It seems that ROS production is associated with severe inflammation through stimulation of NLRP3 inflammasome.²⁷ Mitochondrial failure and ROS production as key factors of I/R injury induce apoptotic cell death in renal tubular epithelial cells resulting in chronic kidney disease.²⁸ It has been demonstrated that autophagy mechanism undergoes upregulation under kidney injury to reduce ROS-mediated apoptosis.²⁹ *P. major* has demonstrated great potential in amelioration of kidney injury. It appears that the renoprotective impacts of *P. major* are a consequence of its inhibitory impact on the apoptosis. Besides, administration of *P. major* is associated with an improvement in the function of kidney by enhancing the serum albumin level and reducing serum cholesterol concentration and urine protein excretion rate.³⁰ Chemotherapy is still one of the common strategies in cancer therapy.³¹ However, there are drawbacks associated with application of chemotherapeutic agents such as cisplatin. Kidney is one of the main targets of cisplatin.³² Glomerular filtration rate (GFR) failure is one of the complications related to the cisplatin treatment. Administration of *P. major* considerably decreases the

adverse effects of cisplatin on GFR making it a potential candidate in protection of kidney.³³ At our study, we demonstrated that kidney of rats exposed to testicular I/R has some histopathological alterations such as necrosis, hyperemia and infiltration of inflammatory cells that may be associated with malfunction of kidney. Intraperitoneal administration of *P. major* at the dose of 50 and 100 mg/kg ameliorated these adverse effects demonstrating the efficacy of this naturally occurring compound in reducing the negative impacts of testicular I/R on other organs.

In addition to kidney, high ROS concentration harmfully affects liver resulting in its dysfunction. Several studies have elucidated that ROS production and consequently, oxidative damage lead to the genotoxicity, carcinogenicity and tissue toxicity.³⁴ A number of pesticides such as chlorpyrifos exert adverse effects on kidney by enhancing oxidative damage.³⁵ Exposing to high concentration of ROS enhances the risk of liver fibrosis by dissolving cell membrane and induction of damage.³⁶ Notably, *P. major* is capable of improving mitochondrial failure by reducing ROS production.³⁷ Furthermore, *P. major* protects liver against inflammation through decreasing cytokines.³⁸ At the current study, the administration of *P. major* improved the histopathological profile of liver by suppressing the infiltration of inflammatory cells and reducing the degree of degeneration and necrosis. It was found that the higher dose of *P. major* (100 mg/kg) is more advantageous compared to the lower dose (50 mg/kg).

There have been concerns about the negative impacts of testicular I/R injury. It seems that these effects are not limited to testis and other parts of body such as liver and kidney may be influenced. At the present experiment, we examined the potential of *P. major* as being used in alleviation of toxic effects of testicular I/R on liver and kidney. Interestingly, the *P. major* was efficient in both doses (50 and 100 mg/kg) by improving the histopathological profile of kidney and liver tissues. However, more studies are needed to elucidate the findings represented in this study.

Conflict of Interests

The authors declare no conflict of interest.

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