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Comparison of the protective effects of insulin and saponin on renal ischemia-reperfusion injury

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ABSTRACT

Introduction: Oxidative stress induced by renal ischemia-reperfusion (IR) is an effective element in the pathophysiologic renal alterations generated during IR injury. This investigation intended to study the effects of saponin (SP) and insulin (INS) in kidney IR injury.
Methods: Wistar male rats were assigned into four groups: the control, IR, SP + IR, and INS + IR. The rats were nephrectomized unilaterally and went under 45 minutes ischemia followed by 24-hour reperfusion. SP (2.5 mg/kg) and INS (30 U/kg) were intraperitoneally injected prior to ischemia. Following the treatment, a blood sample was taken for the measurement of biochemical parameters, and the left kidney was taken out for the determination of oxidative stress markers and histological changes.
Results: Blood urea nitrogen (BUN), creatinine (Cr), malondialdehyde (MDA) levels, and histopathological scores significantly increased by kidney IR while antioxidant enzymes were significantly decreased. However, the administration of SP and INS significantly decreased BUN, Cr, MDA, and histological scores and improved the antioxidant defense system.
Conclusion: SP and INS exert nephroprotective effects against oxidative damage caused by IR. It seems that SP has a renoprotection similar to INS in renal IR.

Implication for health policy/practice/research/medical education:
Insulin and saponin have antioxidant properties and might be used to protect ischemia reperfusion-induced renal damage.
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Introduction

Kidney ischemia-reperfusion (IR) damage is the principal pathophysiologic component, involving oxidative stress as well as inflammatory and apoptotic reactions. IR injury has been shown to be associated with renal mitochondrial damage, which is one of the major factors responsible for cell death and kidney dysfunction. Renal IR, possessing a high mortality rate, is regarded as an important clinical problem, which is followed by functional, morphological, and biochemical changes such as inhibition of insulin (INS) release. Oxidative stress is a major component in the progression of IR, and innate immunity contributes to the pathogenesis of IR. Free radicals and pro-inflammatory cytokines generated by damaged kidney tissues have been involved as the main contributors to IR (1). Renal IR disturbs the balance between antioxidants and oxidants in the kidney tissues and leads to acute renal failure (ARF) (2).

Additionally, the deleterious effects of free radicals lead to dysregulation of the renin-angiotensin system (RAS) associated with elevated cardiovascular disorders. Angiotensin II, the important effector of RAS, produces superoxide anion and peroxynitrite. These free radicals cause mitochondrial dysfunction with increased oxidative stress and decreased INS secretion due to damage to Beta-cells of the pancreatic islets of Langerhans (3). Lipid peroxidation related to renal IR is a major mechanism resulting in oxidative deterioration of cell membranes, and malondialdehyde (MDA) is a main marker of the level of lipid peroxidation. Endogenous antioxidant defense systems, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) protect

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cells from reactive oxygen species (ROS) toxicity. Also, the levels of antioxidant enzymes are important to show the magnitude of oxidative stress induced by IR injury (4).

INS is an anabolic hormone with numerous metabolic actions, for example increasing the storage of amino acids, glucose, and fatty acids. INS regulates blood glucose and is commonly recognized as an antioxidant factor in IR injury (5). It can protect the kidney from IR damage by the mechanisms implicated in the reduction of kidney cell apoptosis and oxidative stress (2). The reasons for using INS as a therapeutic drug in renal failure might be attributed to the studies indicating that (1) INS binds to the specific receptors on the tubular cells and regulates transport and metabolic processes at tubules (2). The expression of INS is increased in the renal tubules after ARF (3). INS is necessary for the development and growth of nephrons and tubular regeneration after IR injury. Previous investigations have shown that INS administration after IR in animals boosts the recovery rate of kidney function and tubular regeneration. Several explanations for the efficacy of INS after IR damage have been suggested (1). The heightened rate of the glomerular filtration by INS might change the progression of ARF by limiting the injury and the obstruction of tubular epithelium by cellular debris (2). INS is a renotropic agent, decreases protein denaturation, and increases gene expression after ARF in the kidney (3). The release of free radicals and inflammatory cytokines following renal IR can be interrupted by INS treatment (6).

Saponins (SPs) contain bioactive components with various pharmacological properties, such as anti-hyperglycemia, lipid-lowering activities, antioxidant, anticancer, anti-inflammatory, and immune-regulatory effects (7). SPs improve antioxidant capacity in the tissues by scavenging ROS and preventing lipid peroxidation. SPs from *Panax japonicus* (SPJ) can exert hepato-protective and neuro-protective activities (8). Also, the ginseng SPs from the ginseng root decrease catecholamine secretion, which results in the hypotensive action. Furthermore, SPs, when administrated at small doses, have dose-dependent hypotensive effects with reduced plasma renin activity. Some investigators have shown that SPs induce endothelial nitric oxide synthase in animal models as a consequence of nitric oxide production, which may be associated with the hypotensive effect of SPs (9). Therefore, our aim was to assess the effects of INS and SPs on renal IR and their relationship with oxidative stress in rats under ischemic insult.

Materials and Methods

Materials

INS (Sigma-Aldrich) was administered 30 U/kg by IP injection, 30 min prior to ischemia (10). SP powder was purchased from Sigma-Aldrich. The powder was dissolved in 0.9% NaCl at room temperature and administered 2.5 mg/kg IP, 30 min before ischemia (11).

Methods

For the present study, 24 adult Wistar male rats (175-225 g) were placed into four groups (n= 6), randomly. The animals had free access to food and water and were housed under standard conditions (21±2 ºC with 12 hours of light and darkness cycles).

Surgery protocol

The rats were anaesthetized with intraperitoneally (IP) injection of 50 mg/kg ketamine and 10 mg/kg xylazine and. A minimal incision was done under the last right rib, and the right kidney was removed. Then, the left kidney pedicle (artery and vein) was removed. In order to induce ischemia, the left pedicle was occluded by a microvascular clamp for 45 minutes, followed by 24-hour reperfusion. Right nephrectomy in all animals was done, and the ischemia induction was controlled by the observation of the blanched kidney.

Experimental groups

The four experimental groups (n= 6) were:

1. Control group (this group underwent nephrectomy without occlusion).
2. Saline + IR group
3. SP + IR group
4. INS + IR group

Biochemical measurements

Blood samples and left renal tissues were collected following 24-hour reperfusion. The centrifugation of blood samples was done at 3000 rmp for 15 minutes. The levels of blood urea nitrogen (BUN), creatinine (Cr), and glucose in the serum were measured by the original kits using the Auto-analyzer equipment.

Tissue MDA and total antioxidant capacity (TAC) measurement

Tissue MDA assay was performed according to the reaction with thiobarbituric acid, and TAC in the kidney was determined using the ferric reducing antioxidant power (FRAP) method (11).

Tissue GPx and SOD assessment

Kidney samples were homogenized in 1.15% KCl solution. SOD and GPx activities were determined considering the manufacturer protocol and ZellBio kit (Germany) (4).

Histopathological study

To assess the tissue damage, the kidneys were fixed in formalin (10%) and embedded in paraffin blocks after tissue processing. Then, sections (5 µm) were prepared with microtome and placed on the microscopic slides. Lastly, for tissue staining hematoxylin-eosin (H&E) was used. The histological index grading from 0 to 3 (0: none,

1: mild, 2: moderate, and 3: severe damage) was semi-quantitatively calculated (12).

Statistical analysis

The results were revealed as mean ± SEM and analyzed by one-way ANOVA through GraphPad Prism version 8.4.3. *P* value <0.05 shows a significant relationship in statistical investigates.

Results

Effects of ischemia reperfusion

Table 1 reveals a significantly higher levels of BUN, Cr, and glucose levels in IR group compared to those in the control group ($P<0.05$). A significant increase was detected when the mean concentration of IR group MDA with the control group was compared ($P<0.01$, Figure 1). As shown in Figures 2, 3, and 4, the renal levels of TAC, SOD, and GPx in the IR group were respectively declined comparing to the control group ($P<0.05$).

Also, renal histological assessment indicated that there were no morphological changes in the control group (Figure 5A). Whereas, in the IR group, a disruption in the normal organization of the kidney tissues was observed so that after ischemia reperfusion glomerular atrophy, lymphocyte infiltration, acute tubular injury (ATI), and hyaline cast were detected (Figure 5B).

Effects of SP on renal ischemia reperfusion

Serum Cr, BUN, and glucose levels in the SP + IR group were considerably decreased compared with the IR group. Besides, the MDA in the SP + IR group revealed a significant decline in comparison to IR group ($P<0.001$, Figure 1). Antioxidant enzymes (TAC, SOD, and GPx) were also significantly increased in this group in comparison to the IR group ($P<0.01$, Figures 2, 3, and 4, respectively). Marked reduction of histopathological alterations as induced by IR in kidney tissues resulted from administering SP (Figure 5C). Correspondingly, the index related to the renal histology was significantly decreased in the SP group compared to the IR group ($P<0.01$, Figure 6).

Effects of INS on renal ischemia reperfusion

In the INS + IR group, the serum levels of BUN, Cr, and glucose were significantly lower than those of the IR group ($P<0.01$, Table 1). On the other hand, comparing the mean concentration of MDA in this group with the IR group indicated a significant reduction ($P<0.001$). Also, in the INS-treated group, TAC, SOD, and GPx levels significantly increased compared with the IR group ($P<0.01$).

Analysis of renal morphological changes indicated

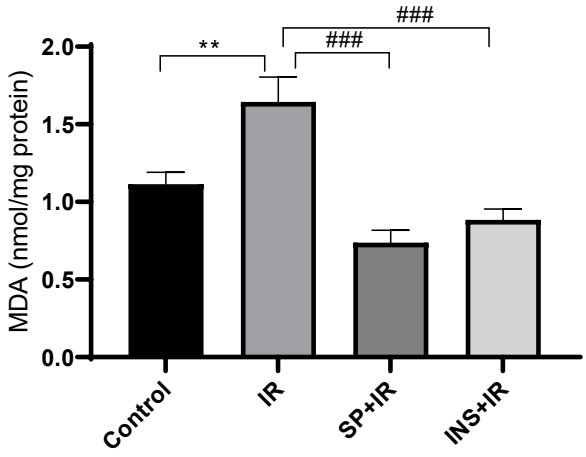

Figure 1. The effects of insulin (INS) and saponin (SP) on the tissue levels of Malondialdehyde (MDA) in treated rats. $^{**}P < 0.01$ versus control group, $^{###}P < 0.001$ versus IR group. IR: Ischemia reperfusion.

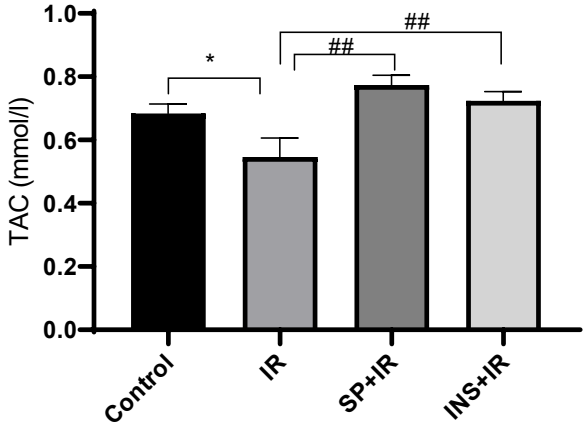

Figure 2. The effects of insulin (INS) and saponin (SP) on the tissue levels of total antioxidant capacity (TAC) in treated animals. $^{*}P < 0.05$ versus control group, $^{##}P<0.01$ versus IR group. IR: Ischemia reperfusion.

Table 1. Biochemical measurements after 24 h of reperfusion

	Control	IR	SP+IR	INS+IR
BUN (mg/dL)	61.00±3.30	84.20±6.87*	57.60±4.20#	56.80±6.88##
Cr (mg/dL)	0.68±0.07	0.97±0.06*	0.77±0.06	0.65±0.04##
Glucose (mg/dL)	199.40±17.40	256.60±9.09*	203.60±20.20	221.40±5.22

Results were expressed as Mean ± SEM.

* Significantly increased when compared with the control group ($P < 0.05$).

#, ## Significantly decreased when compared with the IR group ($^{#}P < 0.05$, $^{##}P < 0.01$).

IR: Ischemia reperfusion, SP: Saponin, INS: Insulin, BUN: Blood urea nitrogen, Cr: Creatinine.

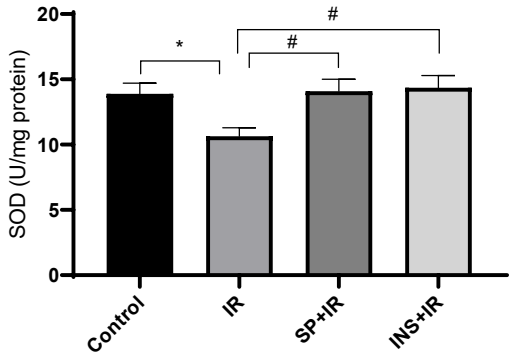

Figure 3. The effects of insulin (INS) and saponin (SP) on the tissue levels of superoxide dismutase (SOD). *P < 0.05 versus control group. $^{\#}P$ < 0.05 versus IR group. IR: Ischemia reperfusion.

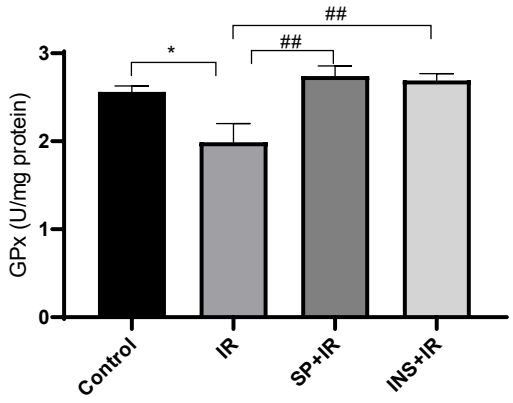

Figure 4. The tissue glutathione (GPx) levels in different groups. *P < 0.05 versus control group. $^{\#\#}P$ < 0.01 versus IR group. IR: Ischemia reperfusion, SP: Saponin, INS: Insulin, GPx: peroxidase.

that INS reduced glomerular and tubular disruption. Glomerular atrophy, ATI, and lymphocyte infiltration were ameliorated by INS treatment (Figure 5D). Also, histological index significantly decreased in this group compared to the IR group (P<0.05, Figure 6). Therefore, the results of this study demonstrated that SP and INS highly reduced the oxidative stress induced by ischemia reperfusion in renal tissues.

Discussion

Acute kidney injury (AKI) is a clinical complication that occurs after kidney IR and transplantation. AKI causes water and electrolyte disorders, edema, hypertension, and disrupts the internal homeostasis. The inflammatory response and ROS generation affect the severity of IR damage. IR-induced mitochondrial dysfunction leads to free radical activation and inflammatory responses (13). Multiple mechanisms are contributed to reperfusion injury; oxidative stress is a major contributor to the onset of IR injury. Oxidative stress triggered by ROS is considered a major initiator of mitochondrial dysfunction, which has been proposed to be associated with ischemic renal

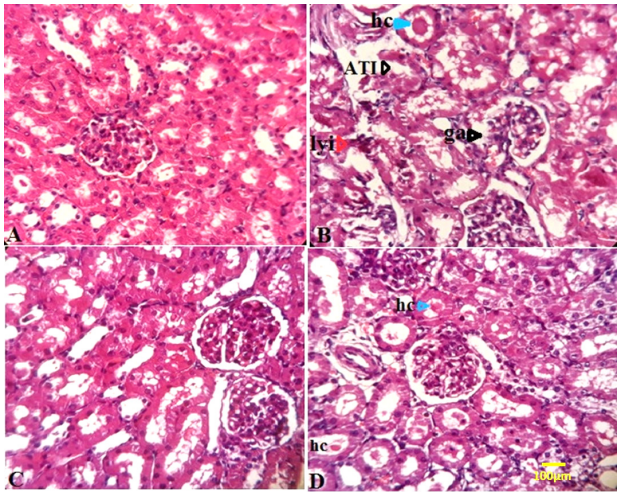

Figure 5. Histopathological assessment of rat kidneys after ischemia-reperfusion (IR). Hematoxylin and eosin (H&E) were used to stain the renal sections. (A) The normal tissue structure, healthy appearance of glomerular and tubular cells in the control group. (B) Acute tubular injury (ATI), glomerular atrophy (ga), lymphocyte infiltration (lyi), and hyaline cast (hc) in the IR group. (C) The normal structure of renal tissue was preserved by saponin treatment. (D) hyaline cast (hc) degree of the insulin group (40× HE). The arrows indicate the disorders on the photomicrographs.

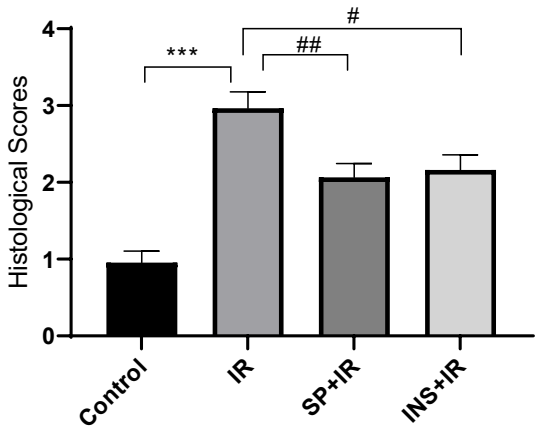

Figure 6. Semi-quantitative analysis of protective effects of saponin (SP) and insulin (INS) against ischemia reperfusion (IR)-induced kidney damage. The histological index grading from 0 to 3 (0: none, 1: mild, 2: moderate, and 3: severe damage) was semi-quantitatively calculated. *** P < 0.001 versus control group. $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 versus IR group.

disease. Under conditions of renal hypoxia and ischemia, mitochondria achieve energy through anaerobic glycolysis, which causes the production of lactic acid. This leads to intracellular acidosis and mitochondrial membrane potential loss, which in turn damages mitochondria (14).

Our findings revealed impaired renal function, manifested by significant elevation of BUN and Cr levels in the IR group, suggesting an ATI, which were significantly decreased after INS and SP treatment. It is shown that prolonged renal ischemia for 45 min can

cause both glomerular and tubular dysfunctions (15). Moreover, administering INS and preceding ischemia attenuated the kidney dysfunction induced by IR injury. INS as a therapeutic drug in AKI comes from research that INS binds to specific receptors on the tubular cells and regulates transport and metabolic processes in the tubules. Also, INS expression is elevated after AKI for the tubular regeneration and development (6).

We reported a significant increase concerning the levels of serum glucose in the IR group, indicating that an impaired pancreatic structure was occurred after IR (16). It has been proposed that hyperglycemia induces oxidative stress and decreases endogenous antioxidant enzymes through different mechanisms. Hyperglycemia, promotes advanced glycation products, the activation of hexosamine, sorbitol, and protein kinase C pathways, leading to INS resistance, reduced INS secretion, and endothelial disruption by ROS production. In particular, glucose variability is related to oxidative stress, prothrombotic events, inflammation, and endothelial dysfunction (17).

The administration of SP and INS decreased the glucose concentration. Previous investigations have confirmed that SPs can ameliorate INS resistance through altering the glucose utilization and uptake. Furthermore, SPs effectively increase INS-stimulated glucose uptake in adipocytes, improve glucose-stimulated INS secretion and INS sensitivity, enhance hepatic INS signaling, glucose homeostasis, and glucose transporter type 4 (GLUT4) translocation into the cell membranes (18).

Our findings suggested that renal IR reduced the tissue levels of SOD, GPx, and TAC significantly and increased MDA level, reflecting elevated lipid peroxidation caused by elevated oxidative stress. Oxidative stress disrupts the oxidant and antioxidant balance of the tissues and increases free radical production that is harmful to cell constituents, in particular, DNA, lipids, and proteins (17). IR-induced oxidative stress in the renal tissues was indicated by decreased antioxidant activities, characterized by reduced SOD, GPx, TAC, and increased MDA. Additionally, the significant elevation of antioxidant defense system and reduction of lipid peroxidation in the SP and INS groups are emphasizing the anti-lipid peroxidation and antioxidant effects of these drugs.

Consistent with the biochemical results, our histopathological evaluation showed that IR caused marked deterioration of both the glomerular and tubular structures depicted by glomerular atrophy, hyaline cast, ATI, and lymphocyte infiltration. SP and INS treatment attenuated the morphological changes related to IR injury, which histopathological index was significantly reduced in these groups compared to the IR group. These cytoprotective effects of SP and INS can be attributed to their powerful anti-inflammatory and antioxidant properties (19,20). Therefore, in animals treated with INS or SP there were signs of restoration of the normal kidney function and structure.

Moreover, these beneficial effects of SPs may be due to maintaining blood flow, regulated metabolism-related glucose enzymes, elevated aerobic metabolism, and energy production. The present study revealed that SPs could prevent renal IR injury by inhibitory effects on oxidative stress, lipid peroxidation, and glomerular and tubular degeneration.

Conclusion

Oxidative stress is an important factor associated with the pathophysiologic tissue changes generated during ischemia reperfusion. TAC activities and antioxidant enzymes prevent free radical generation and scavenge these radicals. SPs and INS treatment highly reduced the oxidative stress induced by IR, which were documented by the decrease in renal histopathological changes and lipid peroxidation and improvement of kidney function, antioxidant capacity and enzymes. However, it seems that SP therapy has similar antioxidant efficacy to the INS treatment. SP application before renal ischemia exerted nephroprotective and hypoglycemic effects similar to INS. In a nutshell, it can be claimed that SP, possessing potent antioxidant activities, deserves to be considered as a potential therapeutic agent in kidney IR damage.

Authors' contributions

Conceptualization: Fardin Rezaei, Mohammad Ghasem Golmohammadi, Hakimeh Saadati, Shokofeh Banaei
Data curation: Fardin Rezaei, Mohammad Ghasem Golmohammadi, Hakimeh Saadati, Shokofeh Banaei
Formal analysis: Shokofeh Banaei
Funding acquisition: Shokofeh Banaei
Investigation: Fardin Rezaei, Mohammad Ghasem Golmohammadi, Hakimeh Saadati, Shokofeh Banaei
Methodology: Fardin Rezaei, Mohammad Ghasem Golmohammadi, Hakimeh Saadati, Shokofeh Banaei
Project administration: Fardin Rezaei, Mohammad Ghasem Golmohammadi, Shokofeh Banaei
Supervision: Mohammad Ghasem Golmohammadi
Visualization: Shokofeh Banaei, Mohammad Ghasem Golmohammadi
Writing–original draft: Shokofeh Banaei, Mohammad Ghasem Golmohammadi
Writing–review & editing: Fardin Rezaei, Mohammad Ghasem Golmohammadi, Hakimeh Saadati, Shokofeh Banaei

Conflict of interests

The authors declare no conflicts of interest.

Ethical considerations

The study was approved by Ardabil University of Medical Sciences Ethical Committee (Ethics code: IR.ARUMS.

AEC.1401.008). The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

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