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The Study of the Protective Effects of Fargesin on Experimental Ischemia-Reperfusion Injury of Liver in Male Rats

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ABSTRACT

Reperfusion following ischemia can lead to metabolic and structural damage to the liver. Fargesin, known for its anti-inflammatory properties, holds potential applications in the development of drugs targeting inflammatory disorders. Most recent studies on fargesin have focused on its anti-inflammatory effects. This study aimed to evaluate the effects of fargesin on liver function, as well as its antioxidant and inflammatory status, following the induction of ischemia-reperfusion injury in the liver of rats. For this purpose, 40 male Wistar rats were randomly divided into four groups of 10: (1) Sham group: rats without any surgical intervention, (2) Surgical control group: rats subjected to surgery without ischemia-reperfusion, (3) Ischemia-reperfusion group: rats exposed to ischemia followed by 45 minutes of reperfusion, and (4) Ischemia-reperfusion with fargesin treatment group: rats that received fargesin at a dose of 50 mg/kg P.O via gavage for 12 weeks post-ischemia. Blood and liver tissue samples were collected, and the animals were sacrificed. Levels of serum alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase were measured. In the liver tissue homogenates, levels of malondialdehyde and the activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase were assessed. Histopathological examination of liver tissue was performed using light microscopy. In group 4, fargesin significantly ($p < 0.05$) reduced elevated levels of liver damage marker enzymes, decreased lipid peroxidation, and restored diminished antioxidant levels in the liver. Additionally, histopathological changes in the livers of fargesin-treated rats were significantly ameliorated. The results suggest that fargesin, with its anti-inflammatory properties, exhibits protective effects against ischemia-reperfusion-induced liver injury and could be a potential therapeutic agent for managing ischemia-reperfusion-related liver damage.

Introduction

Reperfusion following liver ischemia can lead to significant metabolic and structural damage, often resulting from conditions such as trauma, infection, liver transplantation,[1] or hepatic vascular occlusion during liver surgeries.[2] Ischemia-reperfusion injury (IRI) poses a critical challenge for liver surgeries and transplantation procedures, limiting their success.[3] Although ischemia itself disrupts tissue integrity, reperfusion exacerbates the damage by triggering programmed cell death (apoptosis) through the generation of reactive oxygen species (ROS), such as superoxide and hydroxyl radicals. These ROS, produced during the restoration of blood flow, activate macrophages in vascular walls releasing of inflammatory mediators and lipid peroxidation.

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Consequently, ROS play a pivotal role in structural damage, inflammatory responses, and cell death associated with IRI.[4-7]

ROS play a central role in the pathophysiology of IRI, particularly in the liver. During the reperfusion phase, the sudden restoration of oxygen leads to an overproduction of ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals.[8] These reactive molecules disrupt cellular homeostasis by damaging proteins, lipids, and DNA, and they also trigger signaling pathways that lead to apoptosis and necrosis. In hepatic IRI, ROS are key mediators of endothelial dysfunction, activation of Kupffer cells and neutrophils, and the release of pro-inflammatory cytokines, all of which amplify tissue damage. Given their central role in mediating oxidative stress and inflammation, targeting ROS generation and activity has become a major therapeutic strategy to mitigate IRI. Therefore, understanding the mechanisms of ROS production and their pathological effects is essential for developing effective interventions against liver IRI.[9]

Fargesin, one of the primary components of *Magnolia fargesii*, has traditionally been used to treat sinusitis and inflammation.[10] Additionally, the anti-inflammatory effects of fargesin are attributed to its ability to inhibit NF-κB (nuclear factor-kappa B) signaling and reduce nitric oxide production in various cell types. Recent studies have shown that fargesin can suppress malignancies in mice by modulating the P38/MAPK signaling pathway. Research indicates that fargesin exhibits anti-inflammatory properties in THP-1 monocytes by suppressing the PKC (protein kinase C) pathway and its downstream targets, including JNK (c-Jun N-terminal kinase), nuclear factors AP-1 (activator protein-1), and NF-κB. These findings highlight the potential of fargesin as an anti-inflammatory agent for developing drugs targeting inflammatory disorders.[11]

Beyond systemic inflammatory diseases such as cancer and arthritis, fargesin's ability to modulate oxidative and inflammatory pathways suggests its potential application in hepatic contexts, particularly IRI. Fargesin modulates macrophage polarization from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, partially preventing cartilage degeneration by downregulating p38/ERK MAPK (mitogen-activated protein kinases) and p65/NF-κB signaling. Given the abnormal production of ROS during ischemia-reperfusion injury, it is not surprising that inflammation plays a significant role in this condition. With its potent anti-inflammatory effects, fargesin has the potential to protect tissues against ischemia-reperfusion injury.[12]

Although no previous studies have specifically examined the protective effects of fargesin against hepatic IRI, evidence from other experimental models highlights its potent antioxidant and anti-inflammatory properties. These mechanisms are particularly relevant to hepatic IRI, which is characterized by excessive generation of ROS and subsequent inflammatory responses. Therefore, this study aims to mechanistically evaluate the hepatoprotective potential of fargesin by targeting oxidative stress and inflammation—key contributors to liver damage during IRI. To assess this, we will measure serum biomarkers of liver injury (ALT, AST, and LDH), lipid peroxidation levels (MDA), and the activity of key antioxidant enzymes (SOD, CAT, GPx, and GR) in liver tissue. Histopathological analysis will also be performed to evaluate the structural integrity of hepatic tissue. This comprehensive approach may provide foundational evidence supporting the use of fargesin as a potential antioxidant therapeutic agent for mitigating hepatic IRI.

Materials and Methods

Animal

For this study, 40 adult male Wistar rats (approximately 200 g, 9 weeks old) were housed under identical conditions with a 12-hour light/dark cycle, a controlled temperature of 21 ± 2 °C, and free access to standard food and water. After a one-week acclimatization, the rats were randomly divided into four groups: (1) Control: no surgical intervention or liver manipulation; (2) Sham: underwent the same surgical procedure and anesthesia as the I/R group without hepatic ischemia or reperfusion; (3) ischemia-reperfusion: underwent 45 minutes of ischemia followed by 45 minutes of liver reperfusion; (4) ischemia-reperfusion with fargesin treatment: underwent ischemia and reperfusion as in the I/R group and then received fargesin (Sigma Aldrich, USA) orally at a dose of 50 mg/kg via gavage for 12 weeks, while other groups followed their standard diet. The duration for ischemia was based on Gedik *et al*.[13] All protocols adhered to ethical guidelines approved by the Laboratory Animal Ethics Committee.

Data analysis

Rats were anesthetized using intraperitoneal injections of ketamine hydrochloride (100 mg/kg) and xylazine (20 mg/kg). Following midline abdominal incisions and hepatic artery and portal vein occlusion for 45 minutes using non-traumatic vascular clamps, reperfusion was carried out for another 45 minutes. Blood samples (5 ml) were collected from the abdominal aorta to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) levels using standard biochemical methods.[14,15] Liver tissues were harvested, washed with cold saline, and homogenized (10% homogenate) in Tris-

HCl buffer (pH 7.8) containing magnesium acetate, potassium chloride, and sucrose. The homogenates were centrifuged at 7,000 rpm for 10 minutes at 4 °C, and the supernatants were used to assess lipid peroxidation (malondialdehyde, MDA) and antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), using commercial kits (Nanjing Jiancheng Bioengineering Institute, China). MDA levels were determined colorimetrically via TBARS.[16] SOD activity was measured by the nishikimi method Then it is adjusted by the Kakkar method.[17] CAT activity was assessed via hydrogen peroxide decomposition,[18] GPx activity was evaluated by the Rotruck *et al*. method,[19] and GR activity was measured based on NADPH consumption.[20] Histopathological analysis was performed on formalin-fixed liver tissues, prepared as 5 µm sections and stained with hematoxylin-eosin. Liver damage was graded on a semi-quantitative scale from 0 (no damage) to 4 (severe damage) based on Frei *et al*.[21] using a Nikon ECLIPSE E200 microscope.

The obtained data were presented as mean ± standard error of the mean (SEM), and significant differences between the groups were analyzed using ANOVA followed by Tukey's post hoc test at a significance level of $p < 0.05$, utilizing SPSS version 17 statistical software. Histological sections of liver tissue were stained with Hematoxylin and Eosin (H&E) and observed under a light microscope at 400× magnification. A scale bar representing 50 µm has been included in each image.

Results

In the Sham group (group 2), no notable alterations were observed in any of the measured parameters relative to the Control group (group 1). In contrast, rats in the ischemia-reperfusion group (group 3) exhibited elevated serum levels of alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase ($p < 0.05$), alongside decreased activities of hepatic antioxidant enzymes, including superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase ($p < 0.05$). Additionally, MDA levels increased, reflecting enhanced oxidative stress ($p < 0.05$). Treatment with fargesin in the ischemia-reperfusion with fargesin treatment (group 4) effectively countered these effects: liver injury markers remained at levels similar to the Control group, and the decline in antioxidant enzymes and glutathione levels, as well as the rise in MDA, were all prevented ($p < 0.05$), indicating the protective potential of fargesin against hepatic ischemia-reperfusion injury (Tables 1 and 2).

Histopathological examination of liver tissue in rats from group 1 showed normal and healthy lobular and cellular structure (Figure 1A). No specific pathological changes were observed in the liver tissue of rats from group 2, where the liver tissue structure appeared completely normal (Figure 1B). In the liver of rats from group 3, hepatocyte necrosis accompanied by moderate infiltration of inflammatory cells around the central vein and severe swelling in the portal area, as well as scattered foci of necrosis in various parts of the hepatic lobules, were observed (Figure 1C). In group 4, fargesin treatment significantly prevented pathological changes in liver tissue, with the only observable damage being vacuolization of hepatocyte cytoplasm, particularly around the central vein (Figure 1D). Quantitative histopathological observations of the liver tissue in the studied rat groups are presented in Table 3.

Table 1. The effect of fargesin on serum biochemical parameters in hepatic injury induced by ischemia-reperfusion in rats.

Group	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Lactate dehydrogenase (U/L)
Control	79.33 ± 3.17[a]	143.62 ± 5.34[a]	635.11 ± 20.42[a]
Sham surgery	81.42 ± 3.65[a]	150.74 ± 5.82[a]	647.82 ± 23.19[a]
Ischemia-reperfusion	177.82 ± 5.26[b]	215.33 ± 7.61[b]	1105.84 ± 2890[b]
Ischemia-reperfusion with fargesin treatment	83.60 ± 3.94[a]	154.66 ± 4.92[a]	695.31 ± 2684[a]

The values are presented as mean ± SEM for 10 rats in each group. a,b: Non-identical letters in each column indicate a significant difference ($p < 0.05$).

Table 2. Effect of fargesin on hepatic antioxidant activity in rats with liver injury induced by ischemia-reperfusion.

Group	Malondialdehyde (nmol/g protein)	Superoxide dismutase (U/mg protein)	Catalase (U/mg protein)	Glutathione peroxidase (U/mg protein)	Glutathione reductase (U/mg protein).
Control	4.33 ± 0.14[a]	77.10 ± 4.92[a]	77.10 ± 4.92[a]	16.27 ± 1.20[a]	134.11 ± 4.22[a]
Sham surgery	4.52 ± 0.16[a]	70.95 ± 3.52[a]	70.95 ± 3.52[a]	15.49 ± 1.11[a]	129.46 ± 3.75[a]
Ischemia-reperfusion	5.86 ± 0.19[b]	9.64 ± 0.80[b]	53.48 ± 2.61[b]	9.75 ± 0.84[b]	80.12 ± 1.49[b]
Ischemia-reperfusion with fargesin treatment	4.30 ± 0.11[a]	16.73 ± 0.54[a]	69.88 ± 3.57[a]	14.88 ± 1.04[a]	125.33 ± 2.66[a]

The values are presented as mean ± SEM for 10 rats in each group. a,b: Non-identical letters in each column indicate a significant difference ($p < 0.05$).

Table 3. The effect of fargesin on hepatic tissue damage in rats induced by ischemia-reperfusion injury.

Groups	Histological damage grading	Control	Surgical control	Ischemia-reperfusion	Ischemia-reperfusion + fargesin
Congestion and inflammation in portal area	Grade 0	10	10	0	6
	Grade 1	0	0	0	3
	Grade 2	0	0	0	1
	Grade 3	0	0	6	0
	Grade 4	0	0	4	0
Necrosis	Grade 0	10	10	0	8
	Grade 1	0	0	0	1
	Grade 2	0	0	1	1
	Grade 3	0	0	6	0
	Grade 4	0	0	3	0
Interstitial inflammatory cell infiltration	Grade 0	10	9	0	7
	Grade 1	0	1	0	2
	Grade 2	0	0	2	1
	Grade 3	0	0	6	0
	Grade 4	0	0	2	0

Grade 0: No damage, **1**: Minimal damage, **2**: Mild damage, **3**: Moderate damage, and **4**: Severe damage (10 samples per group presented).

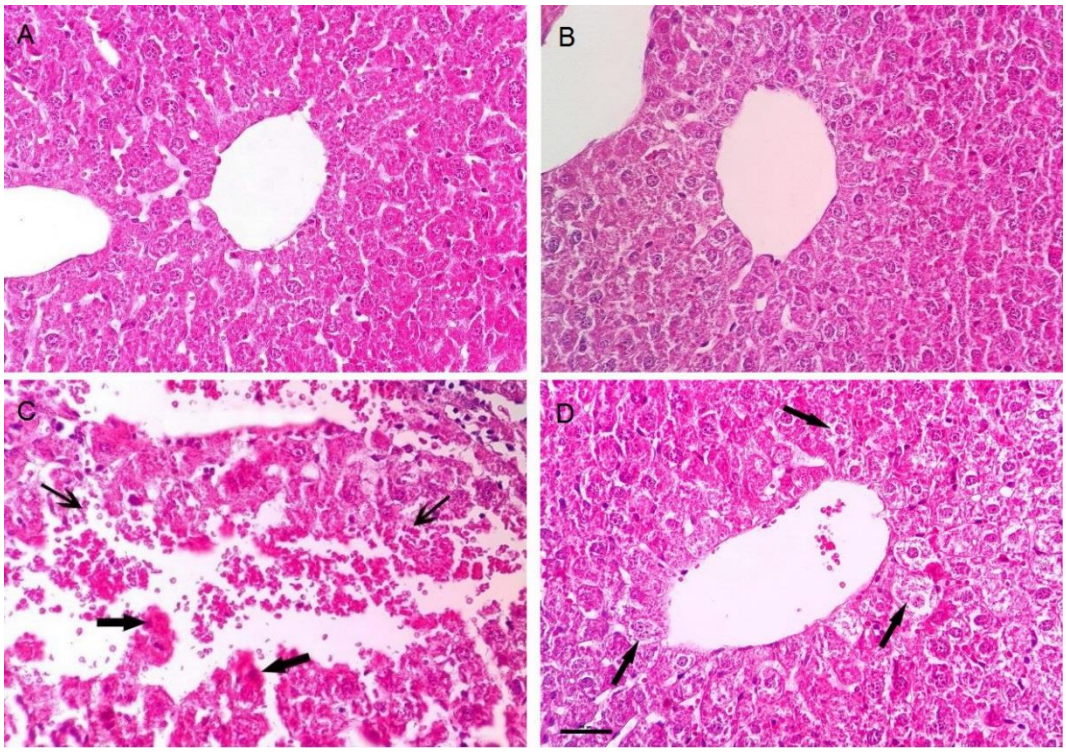

Figure 1. (A) Microscopic view of the liver tissue from the control group of rats, showing a completely healthy and normal histological structure, (B) Microscopic view of the liver tissue from the sham-operated group of rats, appearing healthy with no significant pathological alterations, (C) Microscopic view of the liver tissue from the ischemia-reperfusion group of rats, showing degenerative changes and hepatocellular necrosis around the central vein (thick arrow), along with scattered hemorrhages (thin arrows) in various regions of the hepatic lobules, (D) Microscopic view of the liver tissue from the ischemia-reperfusion plus fargesin-treated group of rats, demonstrating degenerative changes (arrows) in the form of cytoplasmic vacuolization of hepatocytes around the central vein.

Discussion

Given its known anti-inflammatory properties, fargesin appears to be a promising candidate for reducing ischemia-reperfusion (I/R) injury. In contrast to earlier studies that administered antioxidants prior to ischemia or reperfusion, our approach involved post-reperfusion treatment. This timing better reflects clinical scenarios and enables assessment of its therapeutic potential following injury onset. Administration of fargesin led to a marked reduction in the serum activities of ALT, AST, and LDH, all of which are indicative of hepatocellular damage. These reductions suggest that fargesin helps preserve liver cell membrane integrity and limits enzyme leakage

during reperfusion-induced stress.[22]

Liver injury in I/R models is often attributed to oxidative damage, particularly lipid peroxidation initiated by free radicals generated upon reoxygenation. In animals treated with fargesin, only mild cytoplasmic vacuolization and degenerative changes were observed histologically, without evidence of necrosis. These findings support its hepatoprotective role. This protection may stem from the compound's antioxidative and anti-inflammatory mechanisms, which limit ROS production, stabilize cellular structures, and suppress inflammatory cascades.[23]

Oxidative stress is known to activate signaling pathways that promote the release of pro-inflammatory cytokines and expression of adhesion molecules. As a result, ROS accumulation following hepatic I/R can drive systemic inflammation and promote leukocyte infiltration. Our biochemical analyses revealed elevated MDA levels in untreated I/R rats, confirming lipid peroxidation as a major contributor to hepatic injury. These results align with studies by Yildiz *et al*.[24] and Gedik *et al.,*[13] which documented similar oxidative damage and inflammatory responses in I/R models. The observed biochemical and histological improvements following fargesin treatment reinforce its potential as a protective agent against hepatic I/R injury.

Reduced SOD activity, a key enzyme in the enzymatic antioxidant defense system, is a sensitive indicator of hepatic cell injury. SOD eliminates superoxide anions by converting them to hydrogen peroxide, thereby reducing their toxic effects.[25] In the present study, SOD levels significantly decreased in the ischemia-reperfusion group due to the overproduction of superoxide anions. This decrease was followed by a significant reduction in the activities of hydrogen peroxide-scavenging enzymes, such as catalase and glutathione peroxidase. It appears that the inactivation of SOD by excessive superoxide anions leads to the subsequent inactivation of catalase and glutathione peroxidase. Catalase protects tissues against highly reactive hydroxyl radicals by decomposing hydrogen peroxide. Thus, reduced catalase activity may exacerbate damage caused by superoxide radicals and hydrogen peroxide.[26] Glutathione reductase, a hepatic cytosolic enzyme, plays a role in regenerating reduced glutathione from its oxidized form, a product of glutathione peroxidase activity.[27]

In our research, fargesin administration prevented the ischemia-reperfusion-induced reduction in SOD, catalase, and glutathione peroxidase activities. This effect may be attributed to the anti-inflammatory properties of fargesin, which preserved the activities of these enzymes. Furthermore, ischemia-reperfusion caused a significant reduction in glutathione peroxidase activity, limiting the availability of substrates for glutathione reductase and subsequently reducing its activity. It appears that fargesin treatment restored glutathione reductase activity in ischemia-reperfusion injury, enabling the regeneration of reduced glutathione and enhancing the detoxification of active metabolites through conjugation with reduced glutathione. The results of this study corroborate previous reports on the antioxidant and free radical-scavenging properties of fargesin.

Based on the aforementioned points and the findings of the present study, fargesin likely protects the liver of rats against oxidative stress induced by ischemia-reperfusion injury due to its anti-inflammatory properties. Therefore, following successful clinical trials and obtaining positive results, fargesin could be considered a potential therapeutic agent for preventing oxidative injuries caused by ischemia-reperfusion in cases such as trauma, infection, liver transplantation, or hepatic pedicle clamping during liver surgeries. Nevertheless, further investigation is needed to determine the effects of varying doses and to elucidate the precise molecular and cellular mechanisms involved in its pharmacological activity.

Conflict of Interest

The authors declare no conflict of interest.

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