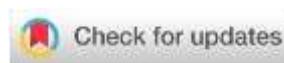


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Research Article

## Effect of Gallic Acid Treatment on Hepatic Tissue in Ischemia-Reperfusion

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### Abstract

**Aim:** Hepatic IR occurs in many clinical conditions such as liver injury, liver hemorrhage and shock, surgical resection, and transplantation. In our study, it is aimed to investigate whether gallic acid (GA) can be used for hepatoprotective purposes, histopathologically.

**Study Design:** 24 Wistar albino male rats were divided into 3 groups (n:8). Surgical procedures were done under general anesthesia. A 4 cm midline abdominal incision were opened to observe hepatoduodenal ligament. The portal vein and hepatic artery was closed with a rubber band and silk suture, and ischemia was performed for 60 minutes. The suture was opened and hepatic reperfusion was performed for 6 hours. 50 mg/kg Gallic Acid was administered to the rats in this group by intraperitoneal route once a day for 14 days.

**Results:** Normal histology of liver was observed with regular vena centralis, endothelial cells, hepatocytes and sinusoid. In IR group, blood vessels were dilated and congested with cell infiltrations. Hepatocytes were degenerated with irregular sinusoides and hyperplastic Kupffer cells. IR+GA group, mononuclear cell infiltration continued with thickening of the basement membrane of the vessels. Vacuolated hepatocyte was observed. GA treatment alleviated the pathologies occurred due to IR injury.

**Conclusion:** It was thought that the cellular damage in the inflammation signal, which started as a result of the cessation of blood flow after ischemia-reperfusion injury, affects the classical lobule of the liver especially around the vena, and may cause necrotic changes by increasing cell apoptosis due to functional nutritional deficiency.

**Keywords:** liver, ischemia, reperfusion, gallic acid, histology

## INTRODUCTION

Ischemia causes a lack of oxygen and impairs cellular metabolism as a result of interruption of blood flow. Reperfusion is the restoration of blood flow and tissue damage occurs in many organs due to previous lack of oxygen and nutrient delivery and accumulation of metabolic byproducts. It causes many clinical complications such as ischemia/reperfusion (I/R) injury in the liver, liver resection, liver transplantation and trauma <sup>1-3</sup>. While vascular occlusion techniques such as the Pringle maneuver and total hepatic vascular exclusion are used to prevent excessive blood loss during liver surgery, liver transplantation involves perfusion of liver grafts with protective fluid prior to cold storage <sup>4-6</sup>. A better understanding of liver I/R injury may lead to improvements in clinical care for many patients, particularly those with prolonged periods of ischemia or those with marginal liver grafts for transplantation. Experimental models of I/R injury have provided a solid basis for the cellular and molecular mechanisms of the hepatic injury response <sup>7,8</sup>.

Today, the use of herbal extracts in addition to various treatments or as an alternative to existing treatments has become widespread. Gallic acid (3,4,5-trihydroxybenzoic acid, GA), a natural phenolic antioxidant, is a biologically active compound produced by shikimic acid in plants. One of the

most important application areas is in the production of trimethoprim, an antibacterial agent used together with sulfonamides in the pharmaceutical industry. In addition, it is used in the production of gallic acid esters such as propyl gallate, which is used as an antioxidant agent, and in the formation of pyrogallol compounds used in leather, cosmetics and photographic paints <sup>9-11</sup>. Gallic acid is abundant in tea, green tea, grapes, eggplant, asparagus, broccoli and fruits. It is widely used in the food, pharmaceutical and cosmetic industries to prevent staleness resulting from lipid peroxidation <sup>12</sup>. Gallic acid has anticarcinogenic, antioxidative, antimutagenic, antiallergic and anti-inflammatory effects <sup>13</sup>. Tang et al. investigated the protective effect of gallic acid against chronic liver damage caused by CCl<sub>4</sub> in rats and reported that it is a strong protector <sup>14-16</sup>. Feique et al. reported that gallic acid extracted from the yellow lily plant in rats reduced lipid peroxidation in the liver and blood of rats <sup>17</sup>. In addition, Jadon et al. investigated the protective effect of gallic acid against liver and kidney damage caused by CCl<sub>4</sub> in albino rats and reported that it is a very effective protector <sup>18</sup>.

In this study, the effects of GA in rat liver ischemia-reperfusion injury will be examined histopathologically and immunohistochemically.

## MATERIAL AND METHODS

### Study design

All protocols were carried out by ethical permission of Dicle University Local Animal Ethical Committee (record number: 2022/38). 24 Wistar albino male rats were used. Experimental animals were divided into 3 groups (n:8). Experimental animals had unlimited access to water and food, and they were kept under control in an environment of 12 hours daytime/12 hours dark, 8:00 am to 8:00 pm, at 23±2°C. Gallic acid (catalog no: 147915) were imported from Merck (Germany)

Before surgical procedures, general anesthesia were provided by injecting 90 mg/kg intramuscular ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey). The abdominal area were shaved and the skin were cleaned with povidone-iodine solution (Betadine). A 4 cm midline abdominal incision was then be made through the peritoneum to the rats. After visualization of the hepatoduodenal ligament, the portal vein and hepatic artery were closed with a rubber band and silk suture, and ischemia were performed for 60 minutes. After a 60-minute ischemic period, the suture were opened and hepatic reperfusion were performed for 6 hours. After these procedures, the abdominal midline were closed by suturing with skin and fascia <sup>19-21</sup>.

**1. Sham group:** No drug administration were done. Midline laparotomy were performed only in the abdomen and the abdomen were closed again.

**2. Ischemia-Reperfusion group (IR):** After creating ischemia by closing the portal vein and hepatic artery of the rats with a rubber band and silk suture for 60 minutes, the band were opened and reperfusion were performed for 6 hours.

**3. Ischemia-Reperfusion+Gallic Acid group (IR+GA):** After creating ischemia by closing the portal vein and hepatic artery of the rats with rubber band and silk suture, the band were opened and reperfusion were performed for 6 hours. Immediately after the experiment, 50 mg/kg Gallic Acid were administered to the rats in this group by intraperitoneal route once a day for 14 days <sup>22</sup>.

### Histological processing

On the 14th day after the end of the experimental phase, the animals were sacrificed by intracardiac blood sampling under general anesthesia. The hepatic tissue of the rats were excised and kept in zinc-formalin solution. After fixation in zinc-formalin for 72 hours, routine paraffin tissue tracing was performed. From the paraffin blocks obtained, 5-micron sections were taken with a rotary microtome and stained with Hematoxylin-Eosin to examine tissue histopathology <sup>23</sup>.

### Staining protocol

Hepatic tissue sections taken from paraffin blocks were placed in a bain-marie set at 37°C. Sections were kept in an oven at 58-62°C for 6 hours to dissolve excess paraffin on the slide. After the sections were deparaffinized in xylene for 3x15 minutes, 10 minutes were passed through the decreasing alcohol series (100%, 96%, 90%, 70%, 50% ethyl alcohol) and brought to distilled water for 5 minutes. Hematoxylin eosin stains were applied to the sections. After the dyeing step, the sections were quickly immersed in increasing alcohol series (through 80%, 90%, 96% ethyl alcohol series) and kept in absolute alcohol for 2 minutes. Finally, the sections were kept in xylene for 3x15 minutes and covered with a coverslip by dripping Entellan onto the tissue.

## RESULTS

### Histopathologic findings

**Control group:** In the transversal section passing through the lobule of the liver, the vena centralis contours are regular, the luminal regular endothelial cells in the blood vessels are flat, and the nuclei of the hepatocytes are arranged in the form of a regular cord. Sinusoid spaces are properly located on the cord. Liver nuclei are regular. In general, the appearance of the liver is normal (Figure 1a).

**IR group:** When the periportal area and the intrahepatic section, together with the classical liver lobule, were examined, ruptures in the lumen of the blood vessels and cell infiltrations with atypical cell appearance were detected in the vena centralis region. An irregular sinusoidal structure was detected in hepatocyte cells with foamy hyalinized cytoplasmic areas. Along with degenerative changes in hepatocytes, hyperplasia was observed in Kupffer cells located in between (Figure 1b).

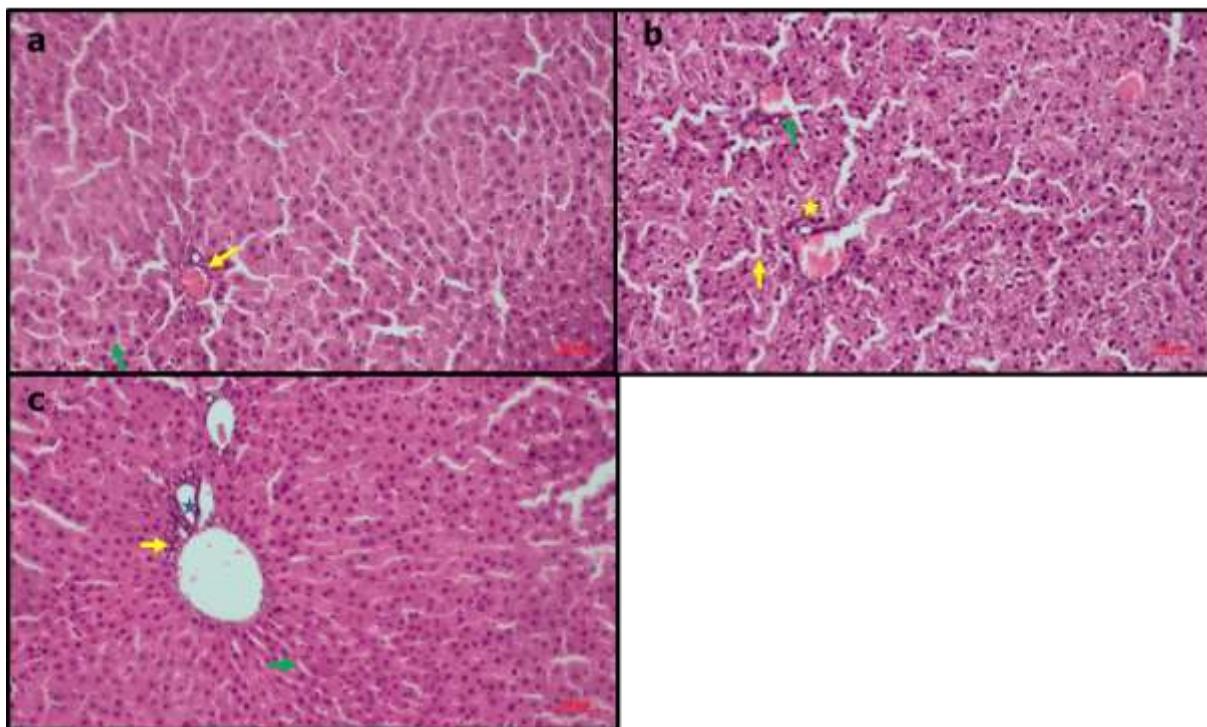
**IR+GA group:** In the outer part of the enlarged central vein, mononuclear cell infiltration in the form of aggregates and sinusoidal spaces were found to be enlarged. A slight thickening of the basement membrane of the vessels was observed towards the periportal area. Vacuolization was observed in the outer hepatocyte cells. Sinusoid contours are generally regular, and liver cells have a double-nucleated appearance. There is a slight siliqueness in the nuclei of some cells. In general, recovery was observed in the classical liver section (Figure 1c).

## DISCUSSION

During liver ischemia, oxygen deficiency in hepatocytes due to depletion of ATP in cell damage causes swelling of mitochondrial degeneration in sinusoidal endothelial cells and Kupffer cells. Activation of Kupffer cells with the production of reactive oxygen species results in upregulation of proinflammatory cytokines, neutrophil-mediated injury. These are the main factors contributing to inflammation-related damage <sup>24-27</sup>. In other models of severe liver damage, when hepatocyte loss is excess and parenchymal proliferative capacity is impaired, activation of hepatic progenitor cells occurs simultaneously with the reaction in the canal system. The expansion of hepatic progenitor cells after liver damage is associated with the severity of hepatocyte loss <sup>28-29</sup>. It has been stated that vacuolization and sinusoidal congestion are indicators of hepatocellular damage and can be reversible, and there is no reversal of necrosis and apoptosis <sup>30-32</sup>. In our study, normal histology of liver was observed with regular vena centralis, endothelial cells, hepatocytes and sinusoid. In IR group, blood vessels were dilated and congested with cell infiltrations. Hepatocytes were degenerated with irregular sinusoides and hyperplastic Kupffer cells. IR+GA group, mononuclear cell infiltration continued with thickening of the basement membrane of the vessels. Vacuolated hepatocyte was observed. GA treatment alleviated the pathologies occurred due to IR injury.

## CONCLUSION

It was thought that the cellular damage in the inflammation signal, which started as a result of the cessation of blood flow after ischemia-reperfusion injury, affects the classical lobule of the liver especially around the vena, and may cause necrotic changes by increasing cell apoptosis due to functional nutritional deficiency.



**Figure 1a)** Normal vena centralis (yellow arrow) and regular hepatocytes (green arrow); **1b)** Tears in blood vessels (green arrow), atypical cell appearance in the vena centralis (yellow star), irregular sinusoidal structure (yellow arrow); **1c)** mononuclear cell infiltration (yellow arrow), irregular sinusoids (blue star), regular sinusoid (green arrow). **Hematoxylin-Eosin staining, Scale bar: 50  $\mu$ m, Magnification: 20X**

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