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Possible protective effects of gallic acid against hepatic ischemia-reperfusion injury in rats

Sıçanlarda karaciğer iskemi-reperfüzyon hasarına karşı gallik asitin olası koruyucu etkisi

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SUMMARY

AIM: The protective effect of gallic acid (3,4,5- trihydroxybenzoic acid), which is a phenolic substance with well-known antioxidant characteristics, against liver ischemia reperfusion damage was investigated in this study.

METHODS: Female Spraque-dawley rats were divided into four groups. Group I was set as control group and 60 minutes of reperfusion after 45 minutes of ischemia was applied to the other three groups. Gallic acid was administered through gavage method to each of the animals in Group III and Group IV for seven days prior to Ischemia/Reperfusion. Blood samples for biochemical examinations and liver tissue samples for histologic examinations were collected.

RESULT: When Group II was compared with other groups in terms of ALT and AST serum values, a significant increase was observed in Group II serum amount. ALT levels of Group III (50 mg/kg gallic acid) and Group IV (100 mg/kg gallic acid) among treatment groups were found lower compared to Group II (I/R group) ($p<0.05$). When AST serum values were considered, the serum amount pertaining to group III was found to be significantly lower when compared with other groups ($p<0.05$). Histological findings are in line with these results.

CONCLUSION: In accordance with the obtained results, we may state that gallic acid protects against liver ischemia reperfusion damage.

Key words: Liver, ischemia/reperfusion, gallic acid, free radical, rat.

ÖZET

GİRİŞ: Bu çalışmada antioksidan özelliği iyi bilinen, fenolik bir madde olan, gallik asitin (3,4,5- trihidroksibenzoik asit) karaciğer iskemi reperfüzyon hasarına karşı koruyucu etkisi araştırıldı.

YÖNTEM: Dişi Spraque-dawley sıçanlar dört gruba ayrıldı. Grup I kontrol grubu olarak belirlendi ve diğer üç gruba 45 dakika iskeminin ardından 60 dakika reperfüzyon uygulandı. İskemi/Reperfüzyondan önce yedi gün boyunca Grup III ve Grup IV'deki hayvanların her birine gallik asit gavaj yolu ile verildi. Biyokimyasal incelemeler için kan örnekleri, histolojik incelemeler için karaciğer doku örnekleri alındı.

BULGULAR: ALT ve AST serum değerlerinde Grup II ile diğer gruplar karşılaştırıldığında, Grup II serum miktarında anlamlı olarak artış olduğu gözlendi. Tedavi gruplarından Grup III (50 mg/kg gallik asit) ve Grup IV (100 mg/kg gallik asit) ALT düzeyinin ise Grup II (İ/R grubu)'ye göre daha düşük bulundu. AST serum değerlerine bakıldığında ise grup III'e ait serum miktarı diğer gruplar ile karşılaştırıldığında anlamlı derecede düşük bulunmuştur ($p<0.05$). Histolojik bulgular bu sonuçlar ile paralellik göstermektedir.

SONUÇ: Elde edilen sonuçlara göre gallik asitin karaciğer iskemi reperfüzyon hasarına karşı koruduğunu söyleyebiliriz.

Anahtar kelimler: Karaciğer, iskemi/reperfüzyon gallik asit, serbest radikal, sıçan.

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INTRODUCTION

In clinical environment during surgical operations the blood flow of the entire liver or a part thereof should be stopped (Pringle maneuver) in order to take hemorrhaging under control and ischemia reperfusion (IR) damage becomes inevitable [1-3]. Several mechanisms as cytokines, mediators (TNF-α), transcription factors, ROS (reactive oxygen species), kupffer cell activation, mitochondria permeability and pH play role in I/R damage [4-6]. There are several studies which report that ROS are effective in tissue damage post ischemia reperfusion (IR) [2, 7, 8]. The most basic free oxygen radicals are generalized as superoxide anions, hydroxyl radicals, hydrogen peroxide and peroxinitrite [9]. ROS may be inactivated through endogenous antioxidant such as CAT, SOD and GPx [10] along with antioxidants received externally such as polyphenols [11,12]. Polyphenols are molecules, which are abundant in fruits and vegetables, and have antioxidant, anticancer and antimutagen characteristics that provide protection against various diseases [13]. The antioxidant effect of phenolic compounds is related to the hydroxyl molecules they contain in their structure and the number of such molecules changes their binding position to aromatic ring activities [12]. The gallic acid (3,4,5-trihydroxybenzoic acid) used in this study is a phenolic compound that contains three hydroxyl molecules in its structure. Gallic acid is present in red wine [14-16], green tea [14,15] and pomegranate. The most important source of gallic acid is tea [17]. Gallic acid is a powerful and natural antioxidant. In previous studies, it was reported that gallic acid counteracts superoxide, hydroxyl [16], nitric oxide, peroxynitrite and nitroxyl [18] radicals.

The protective effect of orally administrated gallic acid against liver ischemia reperfusion damage has not been researched in any study. The possible protective effect of gallic acid against liver ischemia reperfusion damage was researched in this study.

MATERIAL AND METHODS

Twenty eight female Spraque-dawley rats, weighting 200-250 g were used after a week of adaptation. They were housed in polycarbonate cages and rats were housed in a controlled environment at 22±2 ^{o}C under a 12-h light/ 12-h dark cycle. Food and water were available *ad libitium* and they were fed laboratory pellet chows. The experimental protocols were approved by the Institutional Ethical Committee for Animal Care and Use at Eskisehir Osmangazi University, Eskisehir, Turkey (protocol number: 180/2010).

Experimental Protocol

The rats were randomly divided into four groups, each consisting of 7 animals. Group I was the sham operated group, group II was the I/R group, group III was the I/R + 50 mg/kg gallic acid, group IV was the I/R + 100 mg/kg gallic acid.

Animals from Groups I, II, III and IV under xylazine (10 mg.kg^{-1}) and ketamine (70 mg.kg^{-1}) anesthesia [19] had laparotomy performed to hepatic artery, portal vein and bile duct visible by clearing the tissues around them. Group I and II animals received orally 1ml saline solition, Group III animals received orally 50 mg/kg gallic acid dissolved in 1ml saline solution and Group IV animals received 100 mg/kg gallic acid dissolved in 1 ml saline solution once a day for seven consecutive days. At the end of treatment, abdomen of each animal was opened through a midline incision to make hepatic artery, portal vein and bile duct visible and Group I animals were sacrificed after 105 min. Group II, III, IV animals were performed 45 min ischemia and 60 min reperfusion by using sterile atraumatic microvessel clamps. After the reperfusion period, all the animals were intracardially sacrificed by hemorrhage.

Biochemical Analysis

Blood samples and the liver tissue samples that were collected from all the rats were stored in deepfreeze (-80 ^{o}C) conditions in order to examine aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Serum levels of ALT and AST were determined using commercially available kits.

Histological Analysis

Liver parts were fixed in 10% neutral formalin for histological investigations. 5µm thick sections in paraffin taken from each liver sample were stained with standard H&E. Samples were investigated by light microscopy by using the Spot Advanced Software (V. 3.2.4; Diagnostic Instruments, Sterling Heights, USA). Sections were digitally photographed using a Spot Insight Color 3.2.0 diagnostic camera.

Stastical Analysis

The results were expressed as the mean ± SD of seven animals per group. The One way analysis of variance (ANOVA) and TUKEY tests were used for the analysis and comparison of data within and

between groups (SPSS 11.0 for windows). Differences were considered significant at $p < 0.05$.

RESULTS

Biochemistry

ALT and, AST serum amounts pertaining to Group I, II, III, IV are given in (Table 1). Group II (I/R group), Group III (50 mg/kg gallic acid) and Group IV (100 mg/kg gallic acid) were found statistically significantly different than the control group in terms of serum AST level ($p < 0.05$). While the increase in AST level compared to the control group was high in Group II (I/R group), there was a significant decrease in Group III (50 mg/kg gallic acid) and Group IV (100mg/kg gallic acid). Especially, AST level in Group III (50 mg/kg gallic acid), which is the treatment group, was found significantly lower than Group II (I/R group).

Table 1. Average values ±SD of AST, ALT amounts determined in the blood serum samples of animals in the trial groups (n=7)

Groups	AST (U/L)	ALT (U/L)
I	186,5±38,04	59.5±10.9
II	1108,9±142,8[a]	886.2±148.6[a]
III	826,4±100,2[ab]	698.7±124.1[a]
IV	1068,9±153,3[a]	855,4±176,5[a]

P< 0.05 There is a significant difference from a: Group I; b: Group II. Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase

Liver Histology

No pathological conditions struck out in the liver tissues of Group I, which is the sham group (Figure 1). In Group II expansion of sinusoidal area, hyperemia, vacuolization and severe nuclear infiltration due to I/R damage were observed (Figure 2). The images obtained from liver tissues of Group III were deemed similar with the images from the sham group. Accordingly, while hepatocyte appeared to be normal, vacuolization or nuclear infiltration was not observed. However, hyperemia, even though slight, was observed in sinusoidal area (Figure 3). The integrity of hepatocyte cells in Group IV could not be maintained and hyperemia and expansion were observed in the sinuses (Figure 4).

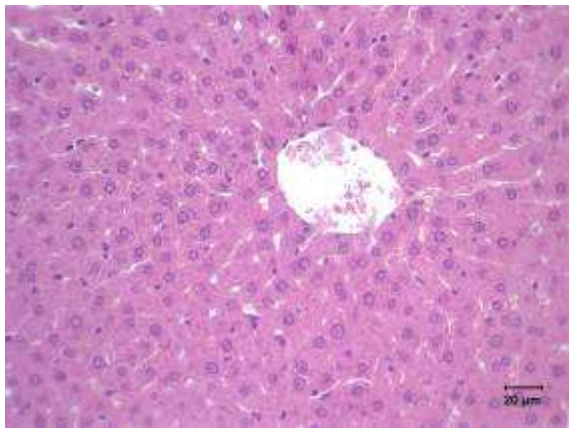


Figure 1. Hematoxylen&Eosin staining of liver tissue in Group I.

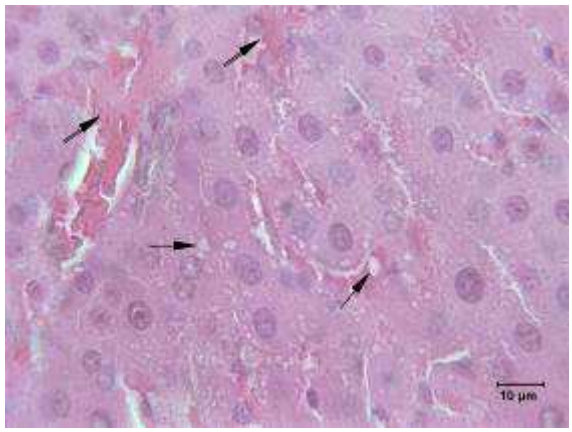


Figure 2. Hematoxylen&Eosin staining of liver tissue in Group II (↗ :vacuolization, ↗ :hyperemia)

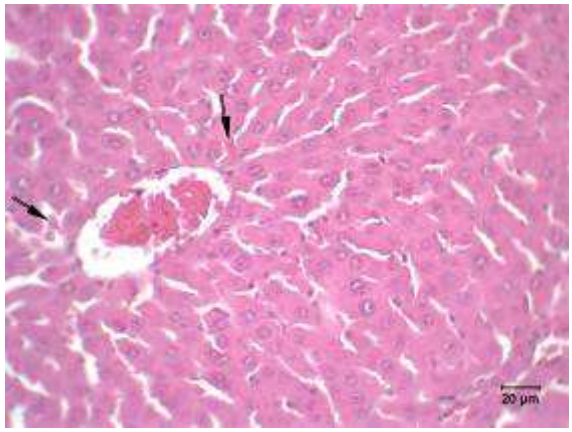


Figure 3. Hematoxylen&Eosin staining of liver tissue in Group III (↗ :slight hyperemia)

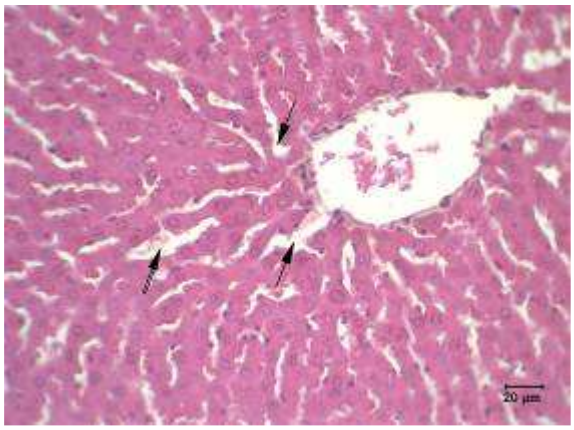


Figure 4. Hematoxylen&Eosin staining of liver tissue in Group IV. (↗ :expansion in the sinuses, ↗↗ :hyperemia).

DISCUSSION

When the blood flow running to an organ is interrupted, sufficient amounts of oxygen and energy cannot reach to that organ and this condition constitutes the ischemia process. Enabling recovery of the blood flow to the ischemic organ constitutes the reperfusion process. It is known that the reperfusion process has a role in occurrence of pathological condition [20]. There are many factors leading to I/R damage, and ROS is considered as the most important one among all other factors [20, 21]. ROS causes several function disorders in the cell [12].

It is known that ALT and AST enzyme activities increase due to liver damage. In many studies, it was reported that the increase in ALT and AST levels can be due to the tissue damage resulted from the free radicals to arise after I/R in the liver [22]. In our study, significant differences were observed in the measured serum ALT levels when Group I (control group) and Group II (I/R group) were compared ($p<0.05$). ALT levels of Group III (50 mg/kg gallic acid) and Group IV (100 mg/kg gallic acid), treatment groups, were found lower compared to Group II (I/R group). However, there was a statistically significant decrease in AST level of Group II compared to Group III, which is the treatment group ($p<0.05$). We may infer from these results that 50 mg/kg gallic acid is effective against ischemia reperfusion damage.

In the study carried out by Canbek et al [23] liver protective effect of carvacrol, which is an antioxidant, against the I/R damage to occur after 45 mins ischemia and 60 mins reperfusion periods were investigated. As a result of the study, they reported that serum ALT and AST levels increased in the I/R group but a decrease was observed in the carvacrol group. This result shows parallelism with our study. Similar results were observed in the study carried out by Gupta et al [7] as well. In this study, carnosine and melatonine were used as antioxidant substance. I/R periods were determined as 60 mins/60 mins and the protective effect on the liver was examined. As a result, it was reported that while ALT and AST enzyme amounts were found the highest in the I/R group, decreases were observed in the treatment groups. In similar studies, ALT and AST values increase time-dependently in damage cases due to the liver I/R when compared to the control groups [24-26].

In the histological examinations (HE) performed, hepatocytes display a straight line in the tissue samples from Group I which is the control group. In the liver samples of Group II which have I/R damage and to which any protective substance have not been given, vacuolization, expansion and hemorrhage in sinusoids and disruptions in hepatocyte cords were observed. When livers from Group I and Group II are histologically compared, the result that ischemia reperfusion damage creates can be found. In the histological examinations of the livers from Group III to which 50 mg/kg gallic acid was given, hemorrhaging was observed from place to place, although sinusoids and hepatocyte lines were normal. In the liver tissue samples from Group IV to which 100 mg/kg gallic acid was administered, protection level was lower. Expansion in sinusoids and bleeding in these areas show that there is no protection.

No scientific record in respect of gallic acid being used as an agent which eliminates the effects of the ROS in prevention of I/R damage has been found. However, there are several studies in which gallic acid, a phenolic substance, is used as antioxidant against ROS.

Tung et al. (2009) reported that gallic acid is a strong protector against chronic liver damage due to CCl_4 in rats [27]. Fei Que et al. (2007) reported that gallic acid which is extracted from yellow asphodel plant reduces lipid peroxidation in the liver and blood of the rats [28]. In addition to these, Jadon et al. (2007) reported that gallic acid is remedial against the liver and kidney damage due to CCl_4 in albino rats [29]. In their studies, Yeh and Yen (2009) found that phenolic acids (gallic acid, gentisic acid, ferulic acid, p-coumaricacid) increase antioxidant enzyme activity and showed that oral intake of phenolic acids improve antioxidant defense system in the rat liver in early times [30]. Krajka-Kuzniak and Baer-Dubowska (2003) found out in their study that tannic

acid increases cytochrome P450 activity in the rat liver and kidney. Furthermore, in respect of phenolic acid intake in the rats, mRNA expression of antioxidant enzymes were higher than the control [31]. In several studies, different antioxidants were found to be effective against oxidative stress due to I/R damage [7, 23, 32, 33].

Natural phenolic acids being taken through diet (fruit, vegetable) are very important in order to trigger antioxidant enzymes and many chronic diseases (for example, cancer and diabetes) can be prevented in this way [34]. Polyphenolic compounds play a fundamental role in enhancing antioxidant systems, and they act just as metal chelatagents and enzyme modulators in sweeping free radicals (scavengers) [35]. It is well known that hydroxyl groups, especially the hydroxyl group in para-position, in composition of phenolic compounds are effective in sweeping free radicals [36]. Antioxidant characteristic of gallic acid, which is a phenolic acid, may be due to this fact. Some studies reported that gallic acid protects cells by minimizing deformation of membrane structure and thus organs maintain their normal functioning [29, 36].

Jadon et al. (2007) reported that 200 mg/kg of gallic acid, among 50 mg/kg, 100 mg/kg, is protective against liver and kidney damage due to CCl_4 in albino rats [29]. In another study by Hsu and Yen (2006), it was reported that negative effects of obesity are reduced by administering 50 mg/kg and 100 mg/kg of gallic acid along with diet [34]. In a study by Padma et al. (2011), it was observed that 50 mg/kg (p.o.) of gallic acid remedies the rat liver of toxic effects of lindane, which is a pesticide [36]. Another study performed by 50 mg/kg (i.p) of gallic acid may prevent gastric mucosal damage. In our study, 50 mg/kg of gallic acid was observed to be more protective [37]. Differences in effective doses detected by these studies may be due to differences in metod, time, type and ischemia models. Therefore, we may attribute the less than expected protective effect of 100 mg/kg o.p dose to administering seven days instead of one single dose.

As a conclusion, when biochemical and histological analysis data is considered, it may be stated that 50 mg/kg oral dose of gallic acid which is a phenolic acid has a protective effect before liver I/R damage.

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