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# Biochemical and histopathological evaluation of taxifolin: An experimental study in a rat model of liver ischemia reperfusion injury

## Taxifolinin biyokimyasal ve histopatolojik değerlendirilmesi: Rat modelinde karaciğer iskemi reperfüzyon hasarı üzerine deneysel çalışma

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**Abstract**

Aim: Ischemia/reperfusion (I/R) procedure applied during liver resection and transplantation in clinic settings causes liver oxidative damage. The aim of this study was to examine the effects of taxifolin on liver injury induced by I/R in rats.

Methods: Albino Wistar male rats divided into three groups with 6 rats in each group: liver ischemia-reperfusion (LIR), 50 mg/kg taxifolin + liver ischemia reperfusion (TLIR) and sham operation (SHAM). An hour before thiopental sodium anesthesia, 50 mg/kg taxifolin was orally administered to the TLIR group and distilled water to the LIR and SHAM groups. In the TLIR and LIR groups, a clamp was placed in the hepatic artery, portal vein and bile duct, thereby inducing ischemia for one hour, and reperfusion for six hours. In the SHAM group, the abdominal cavity was closed by surgical suture without any procedure. At the end of this period, rats were sacrificed with high dose anesthesia. Liver tissues were removed for biochemical and histopathological examinations

Results: I/R procedure significantly increased MDA ($P$<0.001), ALT, AST levels ($P$<0.001) and decreased tGSH level ($P$<0.001) in liver tissue compared to SHAM group. In the taxifolin treated group, this effect was suppressed. Histopathologically, in the I/R induced group, pathological findings such as dilated congested blood vessel, hemorrhage, destruction in the liver parenchyma and edema observed. Liver tissue in the taxifolin group had near-normal appearance except mild sinusoidal congestion.

Conclusion: Our results indicate that taxifolin is an effective agent in reducing hepatic damage caused by I/R.

**Keywords:** Ischemia, Reperfusion, Rat, Taxifolin

**Öz**

Amaç: Karaciğer rezeksiyonu ve transplantasyon sırasında uygulanan iskemi reperfüzyon (I/R) prosedürü karaciğerde oksidatif hasara yol açmaktadır. Bu çalışmanın amacı ratlarda iskemi reperfüzyonun neden olduğu karaciğer hasarına taxifolinin etkisini incelemektir. Yöntemler: Albino Wistar erkek ratlar her grupta 6 rat olmak üzere üç gruba ayrıldı: karaciğer iskemi reperfüzyon (LIR), 50 mg/kg taxifolin + karaciğer iskemi reperfüzyon hasarı (TLIR) ve sham operasyon (SHAM). Thiopental sodium anestezisinden bir saat önce TLIR grubuna 50 mg/kg taxifolin; LIR ve SHAM grubuna ise distile su uygulandı. TLIR ve LIR gruplarında hepatik arter, portal ven ve safra kanalına bir saat iskemi ve altı saat reperfüzyon oluşturmak üzere klemp yerleştirildi. SHAM grubunda herhangi bir prosedür uygulanmadan karın boşluğu cerrahi dikişle kapatıldı. Bu periyodun sonunda ratlar yüksek doz anestezi ile sakrifiye edildi. Karaciğer dokuları biyokimyasal ve histopatolojik inceleme için çıkarıldı. I/R prosedüründe SHAM grubu ile kıyaslandığında karaciğer dokusunda MDA ($P$<0,001), AST, ALT ($P$<0,001) değerlerinde anlamlı artış ve tGSH ($P$<0,001) değerinde azalma görüldü. Taxifolin uygulanan grupta bu etki baskılandı.

Bulgular: I/R grubunda patolojik olarak dilate konjeste kan damarları, hemoraji, karaciğer parankiminde destrüksiyon ve ödem gözlendi. Taxifolin uygulanan grupta karaciğer dokusu orta derecede sinusoidal konjesyon dışında normale yakın görünümdeydi.

Sonuç: Sonuçlarımız taxifolinin iskemi reperfüzyon sonrası oluşan karaciğer hasarını önlemede etkili olduğunu göstermektedir.

**Anahtar kelimeler:** İskemi, Reperfüzyon, Rat, Taxifolin

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## Introduction

Ischemia/reperfusion (I/R) applied during liver resection and transplantation in clinical settings causes liver damage in the postoperative period [1,2]. I/R is known to cause tissue damage. I/R damage include a number of complex pathophysiological conditions. Therefore, the pathogenesis of I/R injury has not been fully elucidated [3]. However, it is known that reperfusion of ischemic tissue paradoxically leads to more serious conditions than ischemia-related damage. [4]. In addition, high free oxygen radical (FOR) formation from the molecular oxygen presented in abundant amounts to the ischemic tissue by arterial blood is considered to be responsible for reperfusion injury [5]. Uncontrolled production of FORs cause oxidation of cell membrane lipids and proteins, cytokine production and tissue inflammation [6]. Unless it could be presented, it results in liver damage and failure [7]. Therefore, a large number of antioxidants and anti-inflammatory drugs are currently being tested against liver I/R damage [8,9].

Taxifolin (3,3′, 4′, 5,7-pentahydroxiflavanon) which has been examined in this study for its protective effect against liver I/R damage, is a flavanone found in onions, milk thistle, French maritime and Douglas fir bark [10]. The antioxidant and anti-inflammatory activity of taxifolin has been proven [11,12]. This information suggests that taxifolin may be effective and convenient in protecting liver I/R injury. So far there are no studies analyzing the effect of taxifolin on I/R induced liver injury in the literature. Therefore, the purpose of our examination was to analyze the effect of taxifolin on I/R induced liver injury in rats by biochemical and histopathological techniques.

## Materials and methods

Animals

In the study 18 albino rats weighing 260-275 grams were used. The animals were kept and fed in groups at under appropriate conditions prior to the study. Animal experiments were done according to the National Guidelines for the Use and Care of Laboratory Animals and were confirmed (Ethics Committee Number: 75296309-050.01.04-E.1800138985, Dated: 04.05.2018)

Chemicals

In the study thiopental sodium was provided from İ.E ULAGAY (Turkey), and taxifolin was provided from Evalar-Russia.

Animal Groups

Rats were separated into three groups: Liver ischemia reperfusion (LIR), 50 mg/kg taxifolin + Liver ischemia reperfusion (TLIR) and sham operation (SHAM).

Experimental Procedure

Anesthesia

Surgical operations were done under anesthesia by injecting thiopental sodium and xylazine to peritoneum at proper intervals under sterile circumstances. After thiopental sodium injected to the rats, and waited for appropriate surgical time. The moment when the animals were immobilized in the supine position was considered as the appropriate time of anesthesia for surgical examination [8].

Surgical and pharmacological procedures

One hour prior to thiopental sodium anesthesia, the animals in the TLIR group (n-6) were orally given a 50 mg/kg dose of taxifolin. LIR and SHAM groups were treated with distilled water as solvent with the same method. During anesthesia, all rats were placed in the supine position, in front of the abdomen was vertically cut (3.5-4 cm long), and laparotomy was performed. In the SHAM group, abdominal cavity was closed by surgical suture without any procedure. For increase total hepatic ischemia, a clamp was placed in the hepatic artery, portal vein, and bile duct for one hour in the TLIR and LIR groups to induce ischemia for one hour and reperfusion for six hours. At the end of this period, rats were sacrificed with high dose anesthesia. Liver tissues were removed for biochemical and histopathological examinations

Biochemical analyses

Malondialdehyde (MDA) analysis

MDA calculations were based on spectrophotometrical measurement of absorbance of the pink-colored complex formed by MDA and thiobarbituric acid (TBA). The mixture was twisted for a minute and centrifuged for ten minutes at 4000 rpm. The absorbation of the supernatant was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane [13].

Total Glutathione (tGSH) analysis

Glutathione analysis done according to the method defined by Sedlak J [14].

Alanine Aminotransferase (ALT) and Aspartat Aminotransferase (AST) analyses

Serum ALT and AST activities were calculated spectrophotometrically for the liver function tests.

Statistical analysis

All data analyzed using SPSS program (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The results expressed as mean (standard deviation). The differences between groups were obtained using one-way ANOVA test then, Bonferroni test performed as posthoc. Significance level declared at $P<0.05$.

## Results

Biochemical results

As shown in Figure 1, the I/R procedure significantly increased MDA level in liver tissue compared to the SHAM group and decreased tGSH level. Taxifolin administration decreased MDA level compared to the I/R group and the difference between the SHAM group and taxifolin group was not statistically significant. The decrease in tGSH level was suppressed by taxifolin administration and the difference between the I/R group and the taxifolin group was significant (Figure 1).

I/R procedure applied to liver significantly increased serum ALT and AST levels compared to the SHAM group. Taxifolin medication decreased these elevated levels in the I/R group and brought ALT and AST levels to those of the level of SHAM group. The difference between the taxifolin group and the SHAM group was not statistically significant ($P=0.02$) (Figure 2).

Histopathological results

As shown in Figure 3A, the liver tissue of the SHAM group had normal portal region, central vein and liver cell cords. However, I/R treated LIR group showed pathological findings such as dilated congested blood vessel, hemorrhage, destruction in the liver parenchyma and edema in the liver tissue (Figure 3B). In addition, dilated congested sinusoids were detected in the liver (Figure 3C). The TLIR group treated with taxifolin had liver tissue with near-normal appearance except for persistent mild sinusoidal congestion (Figure 3).

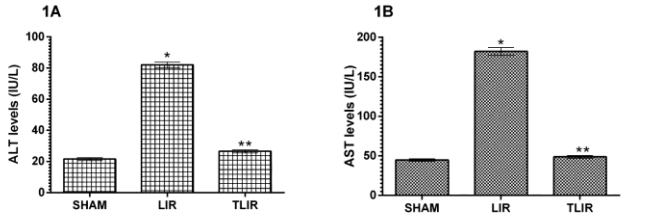


Figure 1: Levels of oxidant antioxidant parameters; 1A: MDA, 1B: tGSH. * *P*<0.001 according to SHAM group, ** *P*<0.001 according to LIR group. MDA- Malondialdehyde, tGSH- Total Glutathione, LIR- Liver ischemia-reperfusion, TLIR-50 mg/kg taxifolin + liver ischemia reperfusion, SHAM- sham operation

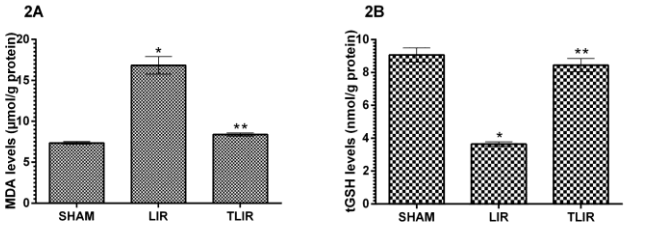


Figure 2: Levels of liver function tests; 2A: ALT, 2B: AST. * *P*<0.001 according to SHAM group, ** *P*<0.001 according to LIR group. ALT- Alanine aminotransferase, AST- Aspartate Aminotransferase, LIR- Liver ischemia-reperfusion, TLIR-50 mg/kg taxifolin + liver ischemia reperfusion, SHAM- sham operation

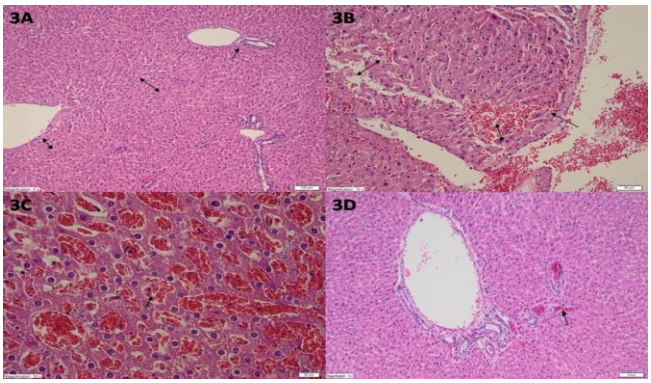


Figure 3: Histopathologic appearence of the liver tissues of experimental groups; 3A: In the liver tissue of SHAM group, normal portal area (straight arrow) , central vein (round arrow), and hepatic cell cords (double arrow) (HEX200), 3B: In the LIR group dilated congested blood vessels (straight arrow), hemorrhage (round arrow), liver parenchymal destruction and edema (double-sided arrow) (HEX100), 3C: Dilated congested sinusoids (arrow) in liver tissue of the LIR group (HEX400), 3D: In the TLIR group, normal portal area (straight arrow), central vein (round arrow), hepatic cell cords (double arrow) are observed (HEX200). LIR- Liver ischemia-reperfusion, TLIR-50 mg/kg taxifolin + liver ischemia reperfusion, SHAM- sham operation

## Discussion

In present study, the effects of taxifolin on I/R induced liver injury in rats were examined biochemically and histopathologically. Our biochemical results revealed that the measure of MDA in liver tissue of animals medicated with I/R showed a significant increase compared to SHAM and taxifolin groups. Elevated levels of MDA in tissue are associated to increased free oxygen radicals in the tissue. The increase in free oxygen radicals (FOR) leads to an increase in lipid peroxidation. MDA is one of the final products of lipid peroxidation [15]. By causing cross-linking and polymerization of membrane components, MDA can inactivate membrane receptors and membrane-bound enzymes, resulting in serious damage to membrane proteins [16,17]. Demiryilmaz et al. [18] reported that I/R procedure caused oxidative damage in liver tissue by elevating MDA levels. Many studies show that MDA is an important parameter in evaluating liver oxidative damage [8,17-19]. As is known, MDA is noted as a marker that reflects oxidative stress in liver I/R injury and I/R injury of different organs [20,21]. In addition, MDA is known to be an efficient parameter in determining the seriousness of damage [20,22]. This information indicates that I/R procedure we induced in liver tissue increases the production of FOR and lipid peroxidation chain reaction occurs. In this regard, our findings are consistent with the literature.

As is known, antioxidants are produced in a continuous and controlled manner in living tissues against FORs. To maintain tissue integrity and function at normal levels, over-produced FORs are neutralized by endogenous glutathione GSH and other enzymatic and non-enzymatic antioxidants. If the antioxidants are insufficient to neutralize the oxidants, the oxidant/antioxidant balanced is disrupted towards the oxidants [23,24]. GSH reacts with the hydrogen peroxide ($H_2O_2$) involved in FOR formation to detoxify $H_2O_2$ and thus protects cells from FOR damage [25]. The results of our experiments, which are consistent with the literature, showed that the amount of tGSH decreased significantly in the liver tissue treated with I/R. This result shows that the balance between oxidants and antioxidants was disrupted in the I/R group in favor of the oxidants. Different studies also show that I/R and toxic substances decrease the amount of tGSH in liver tissue [8,26].

In this study, it has been noted that ALT and AST activities in the I/R group with increased MDA levels and decreased tGSH levels were meaningfully higher compared to the other groups. ALT and AST values are used to determine liver toxicity [27]. Recently, ALT and AST have been used more frequently in evaluating liver I/R injury [28]. The increase in ALT and AST activities is due to an over-increase in hepatocellular necrosis or cell membrane permeability [29]. Previous studies indicate that ALT and ASTs are directly related to the increase in oxidant parameters [8].

In our study, it was revealed that MDA and tGSH levels in the taxifolin group used against liver I/R damage were very close to the SHAM group. This shows that taxifolin prevents the disruption of oxidant-antioxidant balance in advantage of oxidants in I/R-administered liver tissue. Akinmoladun et al. [30] also stated that taxifolin showed antioxidant activity and protected liver tissue from FOR-related oxidative stress. They also reported that taxifolin inhibited MDA and $H_2O_2$ increase and tGSH decrease.

Our histopathological findings also support our biochemical results. Liver tissue of LIR group with increased MDA, ALT and AST levels and decreased tGSH levels showed pathological findings such as significantly dilated congested blood vessels, hemorrhage, parenchymal destruction, dilated congested sinusoids and edema. However, only mild sinusoidal congestion was monitored in the taxifolin-treated liver tissue of the TLIR group.

In the previous study, I/R was found to cause pathological damage like necrosis, hemorrhage, edema, leukocyte infiltration and dilated congested blood vessels in the gastric mucosa [31]. The disappearance of pathological findings

in the taxifolin group may be due to its antioxidant activity. Antioxidant activity has been shown to be important in reducing I/R damage [31,32].

Biochemical results showed that I/R procedure caused oxidative stress in the liver, and this was supported by histopathological findings. We found that taxifolin protects liver tissue from oxidative I/R damage. Our results indicate that taxifolin is an effective agent in reducing hepatic injury caused by I/R.

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## References

1. Jeon BR, Yeom DH, Lee SM. Protective effect of allopurinol on hepatic energy metabolism in ischemic and reperfused rat liver. Shock. 2001;15:112-7.
2. Markmann JF. Relaxing liver ischemia reperfusion injury down 1 notch. Am J Transplant. 2018;18:1587-8.
3. Kong R, Gao Y, Sun B. The strategy of combined ischemia preconditioning and salvianolic acid-B pretreatment to prevent hepatic ischemia-reperfusion injury in rats. Dig Dis Sci. 2009;54:2568-76.
4. Zimmerman BJ, Granger DN. Reperfusion injury. Surg Clin North Am. 1992;72:65-83.
5. Collard CD, Gelman S. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. Anesthesiology 2001;94:1133-8.
6. Zhai Y, Busuttil RW, Kupiec-Weglinski JW. Liver Ischemia and Reperfusion Injury: New Insights into Mechanisms of Innate—Adaptive Immune-Mediated Tissue Inflammation. Am J Transplant 2011;11:1563-9.
7. Jaeschke H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am J Physiol Gastrointest Liver Physiol 2003;284:15-26.
8. Kisaoglu A, Ozogul B, Turan MB Damage induced by paracetamol compared with N-acetylcysteine. J Chin Med Assoc. 2014;77:463-8.
9. Deng WS, Xu Q, Liu Y, Jiang CH, Zhou H, Gu L. Effects of melatonin on liver function and lipid peroxidation in a rat model of hepatic ischemia/reperfusion injury. Exp Ther Med. 2016;11:1955-60.
10. Wang Q, Wang L, Gaiping L, Baoxian Y. A simple and sensitive method for determination of taxifolin on palladium nanoparticles supported poly (diallyldimethylammonium chloride) functionalized graphene modified electrode. Talanta 2017;164:323-9.
11. Topal F, Nar M, Gocer H Antioxidant activity of taxifolin: An activity–structure relationship. J Enzyme Inhib Med Chem. 2016;31:674-83.
12. Gupta MB, Bhalla TN, Gupta GP, Mitra CR, Bhargava KP. Anti-inflammatory activity of taxifolin. Jpn J Pharmacol. 1971;21:377-82.
13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-8.
14. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25:192-205.
15. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clinl Nutr. 1993;5:715-25.
16. Goulart M, Batoréu MC, Rodrigues AS, Laires A, Rueff J. Lipoperoxidation products and thiol antioxidants in chromium exposed workers. Mutagenesis 2005;20:311-5.
17. Sener MT, Suleyman H, Hacimuftuoglu A. Estimating the postmortem interval by the difference between oxidant/antioxidant parameters in liver tissue. Adv Clin Exp Med. 2012;21:727-33.
18. Demiryilmaz I, Sener E, Cetin N Biochemically and histopathologically comparative review of thiamine's and thiamine pyrophosphate's oxidative stress effects generated with methotrexate in rat liver. Med Sci Monit. 2012;18:475-81.
19. Coskun AK, Yigiter M, Oral A The effects of Montelukast on antioxidant enzymes and proinflammatory cytokines on the heart, liver, lungs, and kidneys in a rat model of cecal ligation and puncture–induced sepsis. Sci World J. 2011;11:1341-56.
20. Ingec M, Isaoglu U, Yilmaz M. Prevention of ischemia-reperfusion injury in rat ovarian tissue with the on-off method. J Physiol Pharmacol 2011;62:575-82.
21. Altuner D, Cetin N, Suleyman B Effect of thiamine pyrophosphate on ischemia-reperfusion induced oxidative damage in rat kidney. Indian J Pharmacol. 2013;45:339-43.
22. Polat B, Suleyman H, Alp HH. Adaptation of rat gastric tissue against indomethacin toxicity. Chem Biol Interact. 2010;186:82-9.
23. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology 2003;189:41-54.
24. Kisaoglu A, Borekci B, Yapca OE, Bilen H, Suleyman H. Tissue damage and oxidant/antioxidant balance. Eurasian J Med. 2013;45:47-9.
25. Murray RK, Granner DK, Mayes PA, Rodwell VM. Harper's Biochemistry. 25th ed, McGraw-Hill Press: USA; 2000.
26. Yilmaz I, Demiryilmaz I, Turan MI, Cetin N, Gul MA, Suleyman H. The effects of thiamine and thiamine pyrophosphate on alcohol-induced hepatic damage biomarkers in rats. Eur Rev Med Pharmacol Sci. 2015;19:664-70.
27. Chiu YJ, Chou SC, Chiu CS. Hepatoprotective effect of the ethanol extract of Polygonum orientale on carbon tetrachloride-induced acute liver injury in mice. J Food Drug Anal. 2018;26:369-79.
28. Chies AB, Nakazato PCG, Spadella MA Rivastigmine prevents injury induced by ischemia and reperfusion in rat liver. Acta Cir Bras. 2018;33:775-84.
29. Lott J, Wolf P. Alanine and aspartate aminotransferase (ALT and AST). Clinical Enzymology: a case-oriented approach. 1st ed. Chicago:Year Book Medical Publishers;1986. p. 111-138.
30. Akinmoladun AC, Oladejo CO, Josiah SS, Famusiwa CD, Ojo OB, Olaleye MT. Catechin, quercetin and taxifolin improve redox and biochemical imbalances in rotenone-induced hepatocellular dysfunction: Relevance for therapy in pesticide-induced liver toxicity? Pathophysiology 2018;25:365-71.
31. Çimen O, Çimen FK, Gülapoğlu M. The effect of metyrosine on oxidative gastric damage induced by ischemia/reperfusion in rats. Biochemical and histopathological evaluation. Acta Cir Bras. 2018;33:259-67.
32. Baykara B, Tekmen I, Pekcetin C The protective effects of carnosine and melatonin in ischemia-reperfusion injury in the rat liver. Acta Histochem. 2009;111:42-51.

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