Protective Effect of Taxifolin in The Prevention of Cardiac Tissue Damage in Liver Ischemia and Reperfusion Injury: Experimental Study

Hüseyin Bilge[1], Ibrahim Yildizhan[2], Burak Veli Ulger[3], Ulas Aday[3], Omer Basol[1], Kadriye Cicek[4], Eda Yildizhan[5]

1 University of Health Sciences, Diyarbakir Gazi Yaşargil Health Research Center, Department of General Surgery, Diyarbakır, Türkiye
2 Iğdir University, Iğdir Faculty Of Agriculture, Agricultural Biotechnology, Iğdir, Türkiye
3 Department of General Surgery, Faculty of Medicine, Dicle University, Diyarbakır, Diyarbakır, Türkiye
4 Faculty of Medicine, Dicle University, Diyarbakır, Türkiye
5 Department of Histology and Embryology, Faculty of Medicine, Dicle University, Diyarbakır, Türkiye

Abstract

Aim: Liver ischemia and reperfusion (I/R) is a serious, irreversible health problem in clinical practice. Taxifolin (Tax) is an easy to obtain and use agent found in maritime pine bark, Douglas fir bark and Siberian larch wood. In this study, we examined the protective efficacy of Taxifolin in the correction of cardiac tissue damage that may develop in liver I/R damage.

Methods: In our study, a total of 28 Wistar Albino rats, 8-10 weeks old, weighing 250-300 grams, were used. Group 1 (n=7): control group, Group 2 (n=7): Tax group with 50 mg/kg dose orally for 3 weeks, Group 3 (n=7): Liver I/R group for 30 minutes ischemia and 120 minutes of reperfusion were performed. Group 4 (n=7): Tax+Liver I/R group.

Results: In our study, MDA analysis was performed to evaluate oxidative stress. In the statistical analysis of MDA values, we observed that there was a statistically significant difference between the serum MDA values of the Tax group and the Tax+Liver I/R group, and the MDA level of the Tax group was lower ($p<0.05$). In myocyte damage scoring, we observed that the liver I/R group had the highest damage score, while the damage score of the Tax+Liver I/R group was significantly lower than the I/R group ($p<0.05$).

Conclusion: As a result of our study, we observed that there was an increase in serum MDA levels as a result of liver I/R and histopathological changes occurred in the heart tissue. However, Taxifolin has been successful in ameliorating this situation.

Key Words: *Taxifolin, Oxidative Stress, Liver ischemia and reperfusion, Heart*

1. Introduction

Ischemia and reperfusion consist of two stages. While hypoxia and cell damage occur during ischemia, oxygen is provided again during reperfusion[1]. For example, it occurs during liver transplantation (cold ischemia period after resection until reanastomosis is performed) and liver resection, after which many mechanisms are activated[2]. Numerous cellular components and mediators are involved in its mechanism. These include reactive oxygen species (ROS), neutrophil infiltration and microcirculatory failure[3,4]. The resulting toxic products cause damage in distant organs such as the lung, heart, brain and kidneys and may lead to multiple organ failure that may require long-term intensive care follow-up[5,6]. Liver ischemia and reperfusion (I/R) may cause irreversible serious health problems in clinical practice[7]. Flavonoids are widely used plant-based agents due to their wide distribution and strong antioxidant properties[8]. Dihydroflavonols, which are among flavonoids, are effective in the elimination of ROS. Taxifolin is one of the dihydroflavonols[9]. Taxifolin (Tax) is an easy-to-obtain and easy-to-use agent found in maritime pine bark, Douglas fir bark, Siberian larch bark, citrus fruits, grapes, olive oil and onions[10]. In this study, we investigated the protective efficacy of Taxifolin in the correction of cardiac tissue damage that may develop in liver I/R injury.

* *Corresponding Author: Huseyin Bilge*
e-mail: dr.huseyinbilge@hotmail.com
Received: 23.05.2023, Accepted: 27.07.2023, Available Online Date: 31.08.2023
Cite this article as: Bilge H, Yildizhan I, Ulger BV, et al. Protective Effect of Taxifolin in The Prevention of Cardiac Tissue Damage in Liver Ischemia and Reperfusion Injury: Experimental Study. J Cukurova Anesth Surg. 2023;6(2):272-5. doi:10.36516/jocass.1300968

2. Materials and methods

For this study, ethics committee approval was obtained from Dicle University Animal Experiments Local Ethics Committee (DÜHADEK) with protocol number 2023/03.

2.1. Liver I/R Surgery Protocol

The rats were anesthetized with 90 mg/kg intramuscular Ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 10 mg/kg Xylazine (Rompun; Bayer, Istanbul, Turkey) under aseptic conditions before surgical procedures in the liver I/R group. The abdominal hair of the rats was shaved and whisked with iodine solution. After midline laparotomy, the portal triad (hepatic artery, portal vein and common bile duct) was clamped with a microvascular clamp. Complete hepatic ischemia was maintained for 30 minutes and then the lamps were removed and 120 minutes of reoxygenation (reperfusion) was ensured[11-13].

2.2. Preparation of Taxifolin

Taxifolin (Evalar, Russia) was obtained and administered orally with a dose of 50 mg/kg in 1 cc saline for 21 days[14].

2.3. Setting of Experimental Groups

A total of 28 Wistar Albino rats with an average age of 8-10 weeks and weighing 250-300 grams were used in our study. The rats were housed in steel cages with 12 hours of light and 12 hours of daylight and were fed freely without feed and water restriction[15].

Group 1 (n=7): Control group; rats were given saline orally by 1 cc gavage for 3 weeks.

Group 2 (n=7): Tax group; 50 mg/kg dose in 1 cc saline was administered orally by gavage for 3 weeks,

Group 3 (n=7): Liver I/R group; On the first day of the experiment, liver tissues were subjected to 30 minutes of ischemia followed by 120 minutes of reperfusion by applying the appropriate surgical protocol.

Grup 4 (n=7): Tax+Liver I/R group; Taxifolin was administered orally by gavage with a dose of 50 mg/kg in 1 cc saline for 3 weeks. At the end of the 3rd week, liver tissues were practiced to 30 minutes of ischemia followed by 120 minutes of reperfusion by applying the appropriate surgical protocol.

At the last of the 3rd week, all experimental animals were sacrificed by exsanguination of the heart. The heart tissues were placed in 10% formaldehyde solution and sent to the Histology laboratory. Blood samples were centrifuged at 4500 rpm for 5 minutes, separated into serum and sent to the biochemistry laboratory for biochemical analysis.

2.4. Measurement of Serum Malondialdehyde (MDA) Values

Serum MDA analysis was performed as shown in the study by Kei S[16]. MDA consequence was defined as nmol/mg protein.

2.5. Histopathological examinations

Heart tissues were fixed in 10% Formaldehyde, washed in tap water for 12 hours after fixation and the tissue samples were passed through increasing series of alcohol (50-70-80-96% alcohol) for dehydration. After embedding in paraffin blocks, 5 µm thick sections were taken, stained with Hematoxylin & Eosin (H&E) and visualized with a rotary micro-tome (Leica Biosystems, USA).

Detected myocardial degeneration was evaluated between 0 and 3 points[17]. The severity of damage was graded as follows: 0 (normal): no degeneration of myocytes, 1 (mild): few degenerated myocytes, 2 (moderate): around 50% myocyte degeneration, 3 (severe): more than 50% myocyte degeneration.

2.6. Statistical Analysis

Statistical analysis of the data was performed using SPSS for Windows version 20 (SPSS Inc., Chicago, IL, USA) statistical program.. Kruskal Wallis-H test was used to the scoring values that did not show normal distribution, and Mann Whitney-U test was maked between paired groups for the discriminations between the significant variables. A value of $p<0.05$ was considered significant.

3. Results

3.1. MDA Analysis

In our study, MDA analysis was performed to evaluate oxidative stress. The graphical change obtained as a result of this is given in Figure 1.

In the statistical analysis of MDA values, we examined that there was a markedly significant difference between the serum MDA values of the Tax group and the Tax+Liver I/R group, and the MDA level of the Tax group was lower ($p<0.05$). We observed that administration of Tax for 21 days before liver I/R positively affected the serum MDA levels of rats (Figure 1).

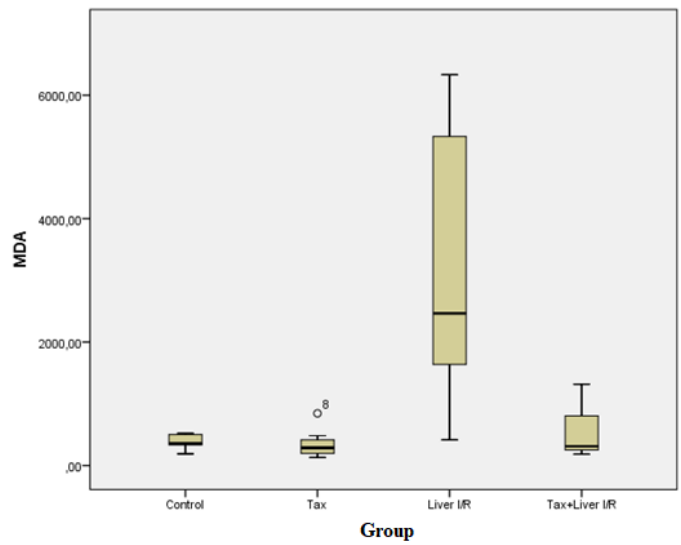

Figure 1

Graphical distribution of the mean MDA analysis of the groups.

3.2. Histologic Evaluation

Mean±standard deviation values of myocyte damage detected by light microscopic examination of heart tissues are given in Table 1.

In myocyte damage scoring, the highest damage scoring was observed in the liver I/R group, while the damage scoring of the Tax+Liver I/R group was markedly lower than the I/R group ($p<0.05$).

Table 1

Statistical difference of myocyte damage between groups and Mean±Standard deviation values of damage scoring.

Groups	Myocyte Injury Scoring
Control	0.28±0.48c,d
Tax	0.42±0.53c,d
Liver I/R	2.71±0.48a,b,d
Tax+Liver I/R	1.57±0.53a,b,c

Tax; Taxifolin group, I/R; Ischemia and Reperfusion group, a; different from control group, b; different from Taxifolin group, c; different from liver I/R group, d; different from Taxifolin+ Liver I/R group ($p<0.05$).

Nevertheless, there was no significant difference between the damage scoring of the control group and the Tax group ($p>0.05$) (Table 1).

When we researched the histopathologic changes in the heart

tissue, the light microscopic images of the control group and Tax group were found to be normal. In the heart tissues of the liver I/R group, hemorrhage, congestion, damage to myocytes and pyknotic appearance were observed. However, in the Tax+Liver I/R group, these histopathologic changes decreased but did not completely disappear. In terms of statistical scoring, it had a lower score than the Liver I/R group (Figure 2).

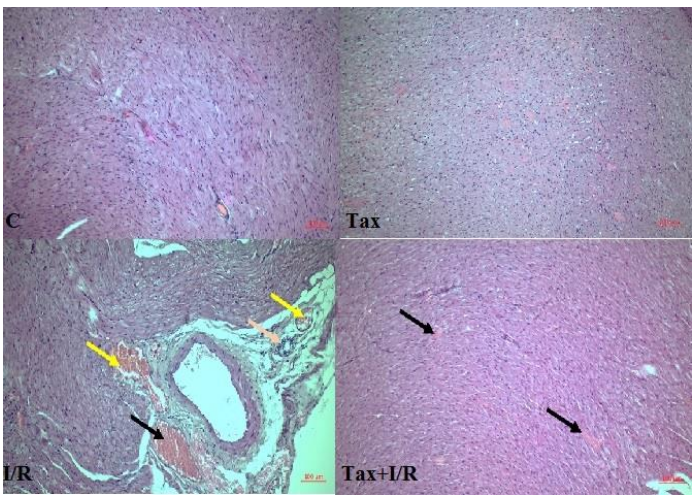

Figure 2

C; Control, I/R; Liver ischemia and reperfusion, Tax; Taxifolin group. Heart tissues of the control group and Taxifolin group appear normal. Mononuclear cell infiltration (orange arrow), congestion (black arrow), hemorrhage (yellow arrow) and pyknosis and degeneration of myocytes in the tissues of the liver I/R group (H&E, 100µm).

4. Discussion

Since there is no substance with a proven effect in reducing oxidative stress due to liver I/R injury, studies on this subject have been ongoing for a long time[18]. Free radicals released due to increased oxidative stress cause various diseases[19]. The pathologic conditions that occur induce the electron transport chain of mitochondria and lead to the formation of large amounts of superoxides, which in turn cause cardiomyocyte damage and increase the likelihood of developing acute myocardial infarction[20,21].

Seker U et al. showed that MDA levels in the testicular I/R group were higher than the other study groups in their study[22]. Similarly, Hüseyin O et al. observed that the MDA levels of the group in which brain trauma was induced were higher than the other study groups[23]. In our study, serum MDA levels in the group that underwent liver I/R were higher than the other study groups.

Taxifolin is a flavonoid found in milk thistle plant and onion and is an antioxidant and anti-inflammatory agent with protective effect against liver I/R injury[24,25]. In one of the studies on Tax, cerebral I/R was induced and it was examined that it inhibited the free radicals released during the apoptosis phase[26]. It is also known to have anticancer and neuroprotective effects[27]. Zhou et al. reported that Taxifolin has high antioxidant capacity in their study[28]. In another study, Eken H. et al. It was shown that there was an improvement in serum MDA levels in the taxifolin-treated group against liver I/R injury and that there was a difference between the taxifolin group and the sham group[29]. In our study, Taxifolin showed efficacy in successfully correcting MDA levels, which is a free radical marker released due to liver I/R injury. Moreover, our findings were consistent with the histopathologic results. Taxifolin showed protective activity in the successful recovery of liver I/R-induced myocyte injury in cardiac tissue.

5. Conclusions

In this study, it was examined that serum MDA levels increased and histopathologic changes occurred in the heart tissue as a result of liver I/R. However, Taxifolin has successfully shown efficacy in correcting this condition.

Acknowledgements

None.

Statement of ethics

The study was approved by the University of Toros University Ethics Committee (September 2022/156) and was conducted in accordance with the Declaration of Helsinki.

Conflict of interest statement

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Funding source

The authors received no financial support for the research, authorship, and/or publication of this article.

Author contributions

All authors contributed to the design and writing of the study. All authors reviewed and accepted the final version of the study.

References

1.Peralta C, Jiménez-Castro M.B, Gracia-Sancho J. Hepatic ischemia and reperfusion injury: Effects on the liver sinusoidal milieu. J. Hepatol. 2013;59(5):1094–106. https://doi.org/10.1016/j.jhep.2013.06.017

2.Fu P, Li W. Role of nitric oxide in hepatic ischemia-reperfusion injury. World J Gastroenterol. 2010; 16(48): 6079–86. https://doi.org/10.3748/wjg.v16.i48.6079

3.Jaeschke H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am. J. Physiol. Gastrointest Liver Physiol. 2003; 284(1): G15-26. https://doi.org/10.1152/ajpgi.00342.2002

4.Montalvo-Jave EE, Escalante-Tattersfield T, Ortega-Salgado JA, et al. Factors in the pathophysiology of the liver ischemia-reperfusion injury. J. Surg. Res. 2008; 147(1): 153-9. https://doi.org/10.1016/j.jss.2007.06.015

5.Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. J. Pathology. 2000; 190(3): 255-66. https://doi.org/10.1002/(SICI)1096-9896(200002)190:3<255::AID-PATH526>3.0.CO;2-6.

6.Peralta C, Fern´Andez L, Pan´es J, et al. Preconditioning protects against systemic disorders associated with hepatic ischemia-reperfusion through blockade of Tumor necrosis factor–induced P-selectin up-regulation in the rat. Hepatology. 2001; 33(1): 100-13. https://doi.org/10.1053/jhep.2001.20529

7.Gracia-Sancho J, Villarreal Jr G, Zhang Y, et al. Flow cessation triggers endothelial dysfunction during organ cold storage conditions: Strategies for pharmacologic intervention. Transplantation. 2010; 90(2): 142-9. https://doi.org/10.1097/TP.0b013e3181e228db

8.Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Scientific World Journal. 2013; 2013: 162750. https://doi.org/10.1155/2013/162750

9.Mülek M, Seefried L, Genest F, et al. Distribution of constituents and metabolites of maritime pine bark extract (Pycnogenol®) into serum, blood

cells, and synovial fluid of patients with severe osteoarthritis: a randomized controlled trial. Nutrients. 2017; 9(5): 443.
https://doi.org/10.3390/nu9050443
10.Slimestad R, Fossen T, Vågen IM. Onions: a source of unique dietary flavonoids. J Agric Food Chem. 2007;1 2(55): 10067– 80.
https://doi.org/10.1021/jf0712503
11.Ali F, Abo-Youssef A, Messiha B, et al. Protective effects of quercetin and ursodeoxycholic acid on hepatic ischemia-reperfusion injury in rats. Clin Pharmacol Biopharm. 2014; 4: 1.
https://doi.org/10.4172/2167-065X.1000128
12.Chaves JC, Neto FS, Ikejiri AT, et al. Period of hyperbaric oxygen delivery leads to different degrees of hepatic ishemia/reperfusion injury in rats. In: Transplantation proceedings, Elsevier. 2016; 48(2): 516-20.
https://doi.org/10.1016/j.transproceed.2015.11.035
13.Kamel EO, Hassanein EHM, Ahmed MA, et al. Perindopril Ameliorates Hepatic Ischemia Reperfusion Injury Via Regulation of NF-κB-p65/TLR-4, JAK1/STAT-3, Nrf-2, and PI3K/Akt/mTOR Signaling Pathways. Anat Rec (Hoboken). 2020; 303(7): 1935-49.
https://doi.org/10.1002/ar.24292
14.Bedir F, Kocatürk H, Yapanoğlu T, et al. Protective effect of taxifolin against prooxidant and proinflammatory kidney damage associated with acrylamide in rats. Biomedicine&Pharmacotherapy. 2021; 139: 111660.
https://doi.org/10.1016/j.biopha.2021.111660
15.Tunik S, Aluclu MU, Acar A, et al. The effects of intravenous immunoglobulin on cerebral ischemia in rats: An experimental study. Toxicol Ind Health. 2016; 32(2): 229-34.
https://doi.org/10.1177/0748233713498461
16.Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta. 1978; 90(1): 37-43.
https://doi.org/10.1016/0009-8981(78)90081-5
17.Tokatli F, Uzal C, Doganay L, et al. The potential cardioprotective effects of amifostine in irradiated rats. Int J Radiat Oncol Biol Phys 2004; 58(4): 1228-34.
https://doi.org/10.1016/j.ijrobp.2003.09.071
18.Glantzounis GK, Salacinski HJ, Yang W, Davidson BR, Seifalian AM. The contemporary role of antioxidant therapy in attenuating liver ischemia-reperfusion injury: A review. Liver Transplantation 2005; 11(9): 1031-47.
19.Günel E, Cağlayan F, Cağlayan O, et al. Treatment of intestinal reperfusion injury using antioxidative agents. J Pediatr Surg. 1998; 33(10): 1536-9.
https://doi.org/10.1016/s0022-3468(98)90492-4
20.Sawyer DB, Colucci WS. Mitochondrial oxidative stress in heart failure : "oxygen wastage" revisited. Circ Res. 2000; 86(2): 119-20.
https://doi.org/10.1161/01.res.86.2.119
21.Perrelli M, Pagliaro P, Penna C. Ischemia/reperfusion injury and cardioprotective mechanisms: role of mitochondria and reactive oxygen species. World J Cardiol. 2011; 3(6):186-200.
https://doi.org/10.4330/wjc.v3.i6.186
22.Seker U, Nergiz Y, Aktas A, et al. Trolox is more successful than allopurinol to reduce degenerative effects of testicular ischemia/reperfusion injury in rats. Journal of Pediatric Urology. 2020; 16(4): 465.e1-465.e8.
https://doi.org/10.1016/j.jpurol.2020.05.008
23.Ozevren H, Irtegün S, Deveci E, et al. Ganoderma Lucidum Protects Rat Brain Tissue Against Trauma-Induced Oxidative Stress. Korean J Neurotrauma. Korean J Neurotrauma. 2017; 13(2): 76-84.
https://doi.org/10.13004/kjnt.2017.13.2.76
24.Wang Q, Wang L, Gaiping L, et al. 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.
https://doi.org/10.1016/j.talanta.2016.11.045
25.Topal F, Nar M, Gocer H, et al. Antioxidant activity of taxifolin: An activity–structure relationship. J Enzyme Inhib Med Chem. 2016; 31(4): 674-83.
https://doi.org/10.3109/14756366.2015.1057723
26.Maksimovich NY, Dremza IK, Troian EI, et al. The correcting effects of dihydroquercetin in cerebral ischemia-reperfusion injury. Biomed Khim. 2014;60(6):643-50.
https://doi.org/10.18097/pbmc2014600643
27. Manigandan K, Manimaran D, Jayaraj RL, et al. Taxifolin curbs NF-κB-mediated Wnt/β- catenin signaling via up-regulating Nrf2 pathway in experimental colon carcinogenesis. Biochimie. 2015; 119: 103-12.
https://doi.org/10.1016/j.biochi.2015.10.014
28.Zhou S, Shao Y, Fu J, et al. Characterization and Quantification of Taxifolin Related Flavonoids in Larix olgensis Henry Var. koreana Nakai Extract Analysis and its Antioxidant Activity Assay. Int J Pharmacol. 2018; 14 (4):534–45.
https://doi.org/10.3923/ijp.2018.534.545
29.Eken H, Kurnaz E. Biochemical and histopathological evaluation of taxifolin: An experimental study in a rat model of liver ischemia reperfusion injury. J Surg Med. 2019; 3(7): 494-7.
https://doi.org/https://doi.org/10.28982/josam.587598