Efficacy of Taxifolin in The Prevention of Renal Injury Due to Liver Ischemia and Reperfusion

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Abstract

Aim: During surgical procedures such as liver resection and transplantation, ischemia/reperfusion (I/R) injury and related complications may occur at a rate of approximately 10%. Our study, we planned to investigate histologically and biochemically the efficacy of Taxifolin in the prevention of renal tissue damage in liver ischemia reperfusion.

Methods: A total of 28 Wistar Albino rats with an average age of 8-10 weeks and weights of 250-300 grams were used in our study. Group 1 (n=7): control group, Group 2 (n=7): Taxifolin group; Taxifolin was administered orally at a dose of 50 mg/kg for 3 weeks, Group 3 (n=7): Liver I/R group, 30 minutes ischemia and 120 minutes reperfusion was performed. Group 4 (n=7): Taxifolin+Liver I/R group.

Results: Kidney tissues of the liver I/R group showed atrophy, degeneration of tubule epithelium and increased TNF-α expression. In addition, deterioration in renal function tests was also monitored in this group. In the Taxifolin+Liver I/R group, a significant difference was observed on both histologic and biochemical basis compared to the Liver I/R group and a positive effect was observed ($p<0.05$).

Conclusions: As a consequence of hepatic ischemia and reperfusion, impairment in the function and histological appearance of renal tissues was observed and Taxifolin was monitored to be effective in eliminating these adverse effects.

Keywords: *Liver ischemia, reperfusion, taxifolin, histopathology, immunohistochemistry, oxidative stress*

1. Introduction

During surgical procedures such as liver resection and transplantation, blood flow to the liver is temporarily interrupted. This may lead to serious complications such as ischemia/reperfusion (I/R) injury[1]. Liver I/R injury accounts for 10% of graft rejection in liver transplantation[2]. In some patients with acute renal injuries, liver injuries, acute coronary syndrome or organ transplantation, irreversible damage occurs in many organs because blood flow in the vessels stops during ischemia[3]. In addition, the integrity and function of vital organelles such as mitochondria may be disrupted during I/R injury[4]. Recent studies have also reported that mitochondria homeostasis may be disrupted as a result of I/R injury[5]. Flavonoids are a group of secondary metabolic compounds commonly found in plants as essential components of human nutrition[6]. Taxifolin, a dihydroflavone compound commonly found in Larix sibirica Ledeb, is an important substance also known as dihydroquercetin (DHQ)[7]. Flavonoids are extremely popular due to their many positive effects on health[6]. Taxifolin has antioxidant, anti-inflammatory, anti-tumor and antiviral effects, including the prevention of Alzheimer's disease[8]. In a study, it was monitored to be effective in reducing oxidase and reactive oxygen species (ROS) resulting from cerebral ischemia-reperfusion injury[9].

The cause of distant organ damage resulting from ischemia reperfusion is not yet fully understood and efforts are still ongoing to reduce the risk of its occurrence. Our study, we planned to investigate the efficacy of Taxifolin in the prevention of renal tissue damage after liver ischemia reperfusion.

2. Materials and methods

This study was approved by the Animal Experiments Local Ethics Committee (DÜHADEK, 2023/03).

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2.1. Surgical Protocol

General anesthesia was achieved by administering 90 mg/kg intramuscular Ketamine hydrochloride (Istanbul, Turkey) and 10 mg/kg Xylazine (Istanbul, Turkey) to the group to undergo liver I/R before surgical procedures. After the midline of the abdomen of the rats was shaved and laparotomy was performed, the portal triad was clamped with a microvascular clamp. After 30 minutes of hepatic ischemia, clamps were opened and reperfusion was performed for 120 minutes[10-13].

2.2. Preparation of Taxifolin

Taxifolin (Evalar, Russia) suspension prepared as described in the study by Bedir F et al. was given orally by gavage (p.o.) with a dose of 50 mg/kg in 1 cc saline for 21 days[14].

2.3. Formation of Experimental Groups

Group 1 (n=7): It is the control group and 1 cc saline was given to the rats orally by gavage as 21 days. At the last of the 21st day, the animals were sacrificed by exsanguination.

Group 2 (n=7): Taxifolin group, Taxifolin was administered p.o. with a dose of 50 mg/kg in 1 cc saline as 21 days. At the last of the 21st day, the animals were sacrificed by exsanguination.

Group 3 (n=7): Liver I/R group. After applying 30 minutes ischemia and then 2 hours reperfusion to the liver tissues, the rats were sacrificed by exsanguination.on the 1st day of the experiment.

Group 4 (n=7): Taxifolin + Liver I/R group. Taxifolin was administered p.o. a dose of 50 mg/kg in 1 cc saline for 21 days. On 21st day, the rats were administered 30 minutes ischemia and then 2 hours reperfusion to the liver tissues. Then the rats were sacrificed by exsanguination.

2.4. Measurement of Serum Malondialdehyde (MDA) Values

Serum MDA analysis was performed as described by Kei S in his study[15]. MDA results were expressed as nmol/mg protein.

2.5. Evaluation of Renal Function Tests

Blood obtained from the heart was centrifuged at 3000 rpm for 7 minutes and blood urea nitrogen (BUN) and creatinine levels were measured[16].

2.6. Histopathologic Evaluation

Kidney tissues of the rast were fixed in 10% formol for 24 hours. After fixation, the tissue samples were washed in tap water for 12 hours and then passed through increasing series of alcohol for dehydration. Sections obtained after routine histologic tissue follow-up were stained with Hematoxylin & Eosin (H&E)[17].

The scoring method in the study was used to evaluate the histologic changes in renal tissues[18]:

Grade 0: undamaged kidney tissue

Grade 1: tubular cell swelling, margin and loss of 1/3 of tubular integrity.

Grade 2: more loss of tubular integrity in addition to the findings in grade 1

Grade 3: loss of more than 2/3 of tubular integrity[18].

2.7. Immunohistochemical Evaluation

The intensity of TNF-α expression was classified as grade 0: negative, grade 1: mild, grade 2: moderate, grade 3: intense[20].

3. Results

3.1. Biochemical examinations

When the MDA levels of the biochemistry groups were analyzed, it was monitored that the MDA level of the Taxifolin group was lower than the Liver I/R group and the Taxifolin+ liver I/R group. The mean ± standard deviation of the MDA levels of the groups are declareted in Table 1.

When we compared the renal function tests, it was monitored that the BUN values of the Liver I/R group and the Taxifolin+ Liver I/R group were similar, but the BUN value of the Liver I/R group was higher than the other study groups. In terms of creatinine values, the creatinine values of the Liver I/R group and Taxifolin+ Liver I/R group were higher than the other groups. The mean and standard deviations of BUN and creatinine values of the groups are demonstrated in Table 1.

3.2. Histopathological examinations

When the kidney tissues were examined under light microscope; In the kidney tissues of the liver I/R group, glomeruli were atrophied, the epithelium in the tubule structures were degenerated and apoptosis was observed. Vascular dilatation and congestion were also observed. In the taxifolin+ liver I/R group, these histopathologic changes were milder and no congestion was observed. Kidney tissues of taxifolin+ liver I/R group were observed to be healthier. (Figure 1).

When the histopathologic damage in the kidney tissues was scored according to the scoring method in the study of Chatterjee et al. (2000), Kruskal-Wallis Test was statistically significant ($p<0.05$). In the intergroup comparison made by Mann Whitney-U test, it was observed that there was no significant difference between the control group and the Taxifolin group and the mean scores were the same ($p>0.05$). There was a significant difference between the scores of the Liver I/R group and the Taxifolin+ liver I/R group and the mean score of the Liver I/R group was higher ($p<0.05$) (Table 2).

3.3. Immunohistochemical examinations

When the intensity of TNF- α expression in the kidney tissues was equate, it was demonstrated that the most widespread expression was observed in the kidney tissues of the Liver I/R group. In the taxifolin+ Liver I/R group, the expressions were less intense (Figure 2). When the extent of TNF-α expression in kidney tissues was examined under light microscope and scored statistically, it was declareted that TNF-α expression was most intense in the Liver I/R group and the intensity of TNF-α expression was lower in the Taxifolin+ Liver I/R group compared to the Liver I/R group ($p<0.05$), (Table 2).

Table 1

Mean values and standard deviations of MDA, BUN and creatinine values of the groups.

Groups	MDA (nmol/mg)	BUN (mg/dL)	Creatinine (mg/dL)
Control	391,81±124,57	37.14±9.61	0.79±0.27
Taxifolin	355,89±245,85	41.71±6.60	0.94±0.32
Liver I/R	3308,15±2319,50	133.14±27.67	3.16±0.72
Tax+ Liver I/R	560,90±493,85	104.57±12.60	1.69±0.42

Tax; Taxifolin, I/R; Ischemia and reperfusion, MDA; Malondialdehyde

Table 2

Scoring values and standard deviations of histopathologic and immunohistochemical damage of the groups.

Groups	Histopathologic score	Immunohistochemical score
Control	0.14±0.37	0.42±0.53
Taxifolin	0.14±0.37	0.57±0.53
Liver I/R	2.28±0.48	2.28±0.48
Tax+ Liver I/R	1.28±0.48	1.28±0.48

Tax; Taxifolin, I/R; Ischemia and reperfusion.

Figure 1

C; control group, Tax; Taxifolin group, I/R; Liver ischemia and reperfusion group. Atrophy of glomeruli (yellow arrow), degeneration of tubule epithelium (black arrow), congestion (red arrow).

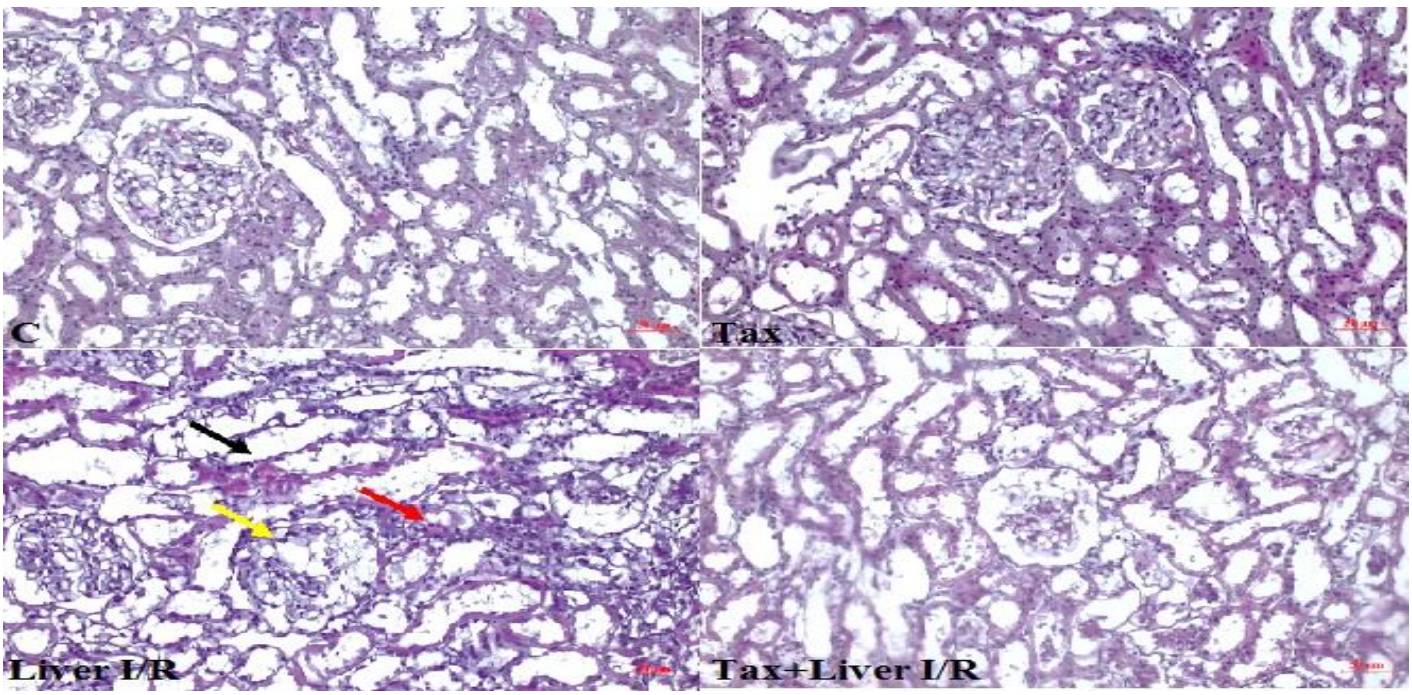
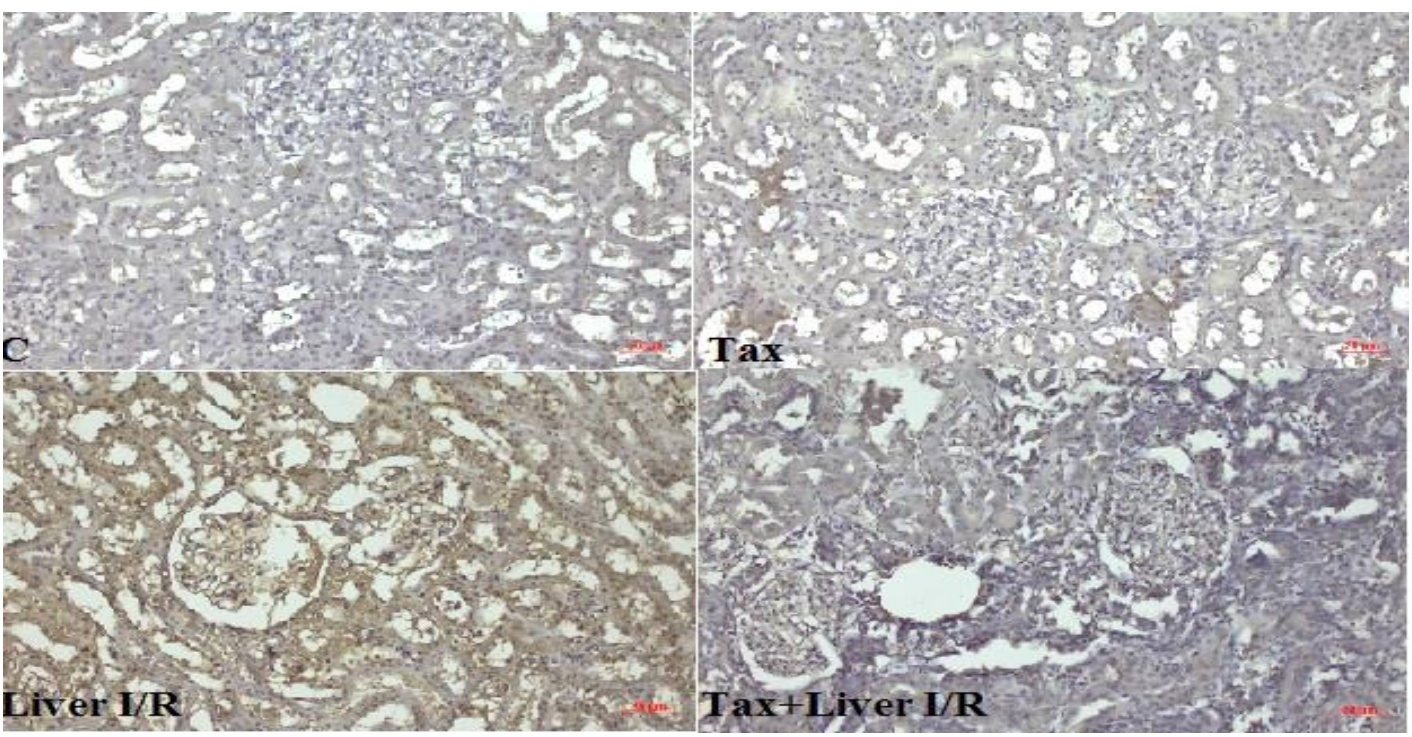


Figure 1

Figure 2

C; control group, Tax; Taxifolin group, I/R; Liver ischemia and reperfusion group.



4. Discussions

Hepatic ischemia and reperfusion injury not only results in liver damage, but also affects many distant organs such as kidneys, lungs, myocardium, adrenal glands and small intestines[21]. Acute kidney injury is a clinical picture of rapid decline in renal function in a short period of time, resulting in high mortality. It usually results in multiple organ failure and distant organ damage[22-24]. Liver I/R injury in experimental animals has been statement to result in acute kidney injury[25]. While liver I/R injury causes damage in the liver parenchyma, it also causes damage in distant organs such as kidney and lung through the production of proinflammatory mediators such as TNF-α, IL-6, IL-1 and free oxygen radicals[26,27]. Among our findings, TNF-α expression was intensely positive in the kidney tissues of the liver I/R group. An increase in serum MDA levels was also observed. Many experimental studies to date have shown that the oxidant-antioxidant status is closely related to proinflammatory cytokines[28,29].

It is also known that increased free radicals leads to an increase in proinflammatory cytokine production[30]. Taxifolin (3,5,7,3′,4′-pentahydroxy-flavanone or 2,3-dihydroquercetin) is a flavonoid with antioxidant properties that positively affects oxidative stress and proinflammatory cytokine production[31]. Previous studies have suggested that Taxifolin has a protective mechanism in the inhibition of important proinflammatory cytokines such as TNF-α and nuclear factor kappa B (NF-κB)[32]. In this study, it was monitored that TNF-α expression was increased in kidney tissue due to liver I/R, but the group that was given Taxifolin beforehand was less affected.

MDA is one of the end products of peroxidation of unsaturated fatty acids in cells. The increase in free radical production in cells leads to an increase in MDA. The level of oxidative stress, disease states, I/R, cancer and some pathologic conditions increase MDA levels[33]. Therefore, measurement of MDA level is very important in I/R studies. Studies have shown that MDA levels were increased when induced by acrylamide and proinflammatory cytokine levels such as IL-1β and TNF-α increased in renal tissues due to oxidative stress, but animals given Taxifolin were less affected or not affected[34]. In our study, in parallel with these studies, there was a significant decrease in MDA level and TNF-α expression in renal tissues of Taxifolin+ liver I/R group.

5. Conclusion

As a result of liver ischemia and reperfusion, renal tissue function is impaired and inflammatory and histopathologic changes occur. In our study, Taxifolin was demonstrated to be effective in eliminating these adverse effects.

Statement of ethics

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by University of Dicle 2023-476064

Conflict of interest statement

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

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