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Protective effect of *Coriandrum sativum* extract against inflammation and apoptosis in liver ischemia reperfusion injury

Effect of *Coriandrum sativum* against inflammation and apoptosis

A. Kükner¹, G. Söyler², P. Toros³, G. Dede⁴, F. Meriçli⁵, S. Işık⁶, O. Edebal⁷, C. Özoğul⁸

¹Department of Histology and Embryology, Faculty of Medicine, Near East University, Nicosia, Cyprus
²Koç University, Graduate School of Health Sciences and School of Medicine, Istanbul, Turkey
³Department of Histology and Embryology, Faculty of Medicine, Near East University, Nicosia, Cyprus
⁴Department of Histology and Embryology, Faculty of Medicine, Abant Izzet Baysal University, Bolu, Turkey
⁵Department of Phytotherapy, Faculty of Pharmacy, Near East University, Nicosia, Cyprus
⁶Department of Analytic Chemistry, Faculty of Pharmacy, Near East University, Nicosia, Cyprus
⁷Department of Biochemistry, Medical Hospital, Near East University, 99138 Nicosia, Cyprus
⁸Department of Histology and Embryology, Faculty of Medicine, Girne University, Kyrenia, Cyprus

Address for correspondence: Prof. Aysel Kükner MD, Near East University, Medicine Faculty, Histology and Embryology Department, 99138, Nicosia- Cyprus, tel: +90 533 873 4460 (Mobile); +90 392 675 1000 - Ext 3012 (Office), e-mail: akukner@hotmail.com

Abstract

Background: The aim of this study was to investigate the anti-inflammatory and antioxidant effects of *Coriandrum sativum* extract on liver ischemia reperfusion injury at light microscopic and biochemical levels.

Material and methods: Sham, ischemia/reperfusion injury (IRI), IRI+*Coriandrum sativum* extract and only *Coriandrum sativum* extract groups were formed. Sixty minutes of ischemia and 60 minutes of reperfusion were performed. In the treatment group, 300 mg/kg/day *Coriandrum sativum* was given by gavage. Hepatic tissues were fixed in 4% paraformaldehyde. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes were measured. Nuclear factor- kappa beta (NF-kappa B), Tumor necrosis factor-alpha (TNF-α) and Caspase-3 (Cas-3)
immunohistochemistry staining was performed. Microscopic scoring was performed in terms of sinusoidal congestion, vacuolization, and necrosis.

**Results:** Sinusoidal enlargement and diffuse congestion, Kupffer cell increase, neutrophil increase in necrotic areas, and vacuolization in hepatocytes, and bile duct proliferation in the portal triad were observed in ischemia/reperfusion hepatic tissue. Very rare, necrotic areas were observed in the *Coriandrum sativum* treatment group, while congestion and vacuolization and bile duct proliferation were decreased compared to the ischemic group. The AST and ALT levels were increased in the IRI and IRI+*Coriandrum sativum* groups. When compared to the IRI group, the AST and ALT levels of the *Coriandrum sativum* were considerably decreased. The IRI and IRI+*Coriandrum sativum* groups had statistically significant differences in ALP compared to that of the *Coriandrum sativum* and Sham groups. There was no significant difference between the ALP levels of the IRI and IRI+*Coriandrum sativum* groups. TNF-α, NF-kappa B and Cas-3 immune positive stained hepatocytes were numerous and widely observed in the injury group. There were positive TNF-α immunohistochemical staining Kupffer cells in the IRI group. In the group treated with *Coriandrum sativum*, Kupffer cells were not stained, while TNF-α, NF-kappa B and Cas-3 expressing hepatocytes were found to be decreased compared to the IRI group. When the expression values of the TNF-α, NF-kappa B and Cas-3 groups were evaluated statistically, it was seen that there was a significant decrease in the group treated with *Coriandrum sativum*.

**Conclusions:** It was found that *Coriandrum sativum* extract decreased proinflammatory cytokine TNF-α and apoptotic cell death and liver enzymes in liver ischemia reperfusion injury.

**Key words:** liver ischemia, *Coriandrum sativum*, TNF-α, NF-kappa B, Cas-3

**INTRODUCTION**

Liver ischemia/reperfusion injury (IRI) is a clinical problem that increases morbidity and mortality after trauma, hepeatectomy or liver transplantation [1, 2]. The ischemic period is described as a blockage of oxygen and nutrient transportations which, in turn, obstructs the cellular metabolism. Once the blood supply is restored by reperfusion, the sudden increase in the blood flow to the tissue increases the concentration of the free oxygen radicals and results in inflammatory cell infiltration at the site of the damage [1, 3]. The acute phase of the reperfusion lasts between three to six hours. During
this time, T-cell and Kupffer cell activation as well as the activation of adhesion molecules are increased. The late (subacute) phase is 18 to 24\textsuperscript{th} h of reperfusion, and neutrophil infiltration reaches its peak level during this time. The activated Kupffer cells and other hepatic cells also initiate secretion of inflammatory mediators, such as Tumor necrosis factor-alpha (TNF-\(\alpha\)), Interleukin 6 (IL-6), increasing the expression levels of Intercellular Adhesion Molecule (ICAM), Vascular cell adhesion protein (VCAM) and P-selectins [4, 5]. TNF-\(\alpha\) expression is regulated by Nuclear factor kappa beta (NF-kappa B). NF-kappa B is a transcription factor that plays an important role in liver IR damage, inflammation response, and the protection and regeneration of hepatocytes [6]. Hepatic apoptosis is directly proportional to the severity of IRI [7, 8]. Necrotic cell death in hepatic cells with apoptosis may also occur [9]. The pathophysiology of IRI has numerous determinative factors and it is essential to understand and identify these factors to develop new therapeutic strategies in preventive medicine [1]. \emph{Coriandrum sativum} is an important medicinal plant that originated in the Eastern Mediterranean and subsequently spread to India, China and is now cultivated all over the world. \emph{Coriandrum sativum} is frequently used in medicines, natural treatment, and the food preservative industry [10, 11, 12, 13]. To the best of the authors’ knowledge, this is the first study to extensively investigate the biochemical and anti-inflammation and anti-apoptotic effects of \emph{Coriandrum sativum} use on hepatic IR in a rat model. The aim of this study was to investigate the biochemical and microscopic effects of methanol extract of \emph{Coriandrum sativum} on liver ischemic reperfusion injuries in rats.

**MATERIALS AND METHODS**

**Plant Material and Extraction**

Leaves of \emph{Coriandrum sativum} were collected from the Alayköy region in North Cyprus and then dried. Dried leaves were extracted with methanol using the maceration method and the extract was evaporated. According to the HPLC analysis (realized using Agilent Technologies 1200 series HPLC and separated with Eclipse XDB-C18 column (150 mm x 4.6 mm, 5\(\mu\text{m}\)). As mobile phases, (A): water with 0.5% formic acid and (B): Methanol were used at a flow rate of 1 mL/min. The gradient was initially 90-70\% A for 18 min, then 55-80\% A for 5 min and finally, 90\% A for 5 min. The detection wavelength was set at 254 nm. The injection volume was 10 \(\mu\text{L}\) for each sample and standard solutions, and according to the results of data validation, the percentage of isoquercitrin was found to contain 139.25 ppm (0.21\%).
Animals and Experimental Design

All experimental protocols were performed according to the Guide for Care and Use of Laboratory Animals and were approved by the Animal Care and Use Local Ethics Committee of Near East University (2019/01-57). Six – seven old female Wistar Albino rats (450-500 gr weight) were housed at a constant room temperature (22 ± 2°C) under a 12-hr light/ dark cycle. They were fed standard rat chow (210 kcal/100 g/day) and drank tap water ad libitum. All surgical interventions were performed between 9:00 AM and 12:00 PM to minimize diurnal effects.

Animals were randomly divided into four groups:

- **Sham group** (n=10): A midline incision was made, and the hepatic pedicle (contains ductus hepaticus, hepatic portal vein and proper hepatic artery) was mobilized. No blood supply blocked.
- **Ischemia/reperfusion (IRI) group** (n=10): Ischemia was achieved by clamping the hepatic pedicle with a vascular clamp for 1 hour after the ischemic period, the clamp was opened and 1hour reperfusion of the liver was provided.
- **IRI+ Coriandrum Sativum group** (n=10): Coriandrum sativum extract was given by gavage 300 mg/kg/day for 3 days before ischemia and 1 hour before ischemia [14].
- **Coriandrum Sativum group** (n=10): Coriandrum sativum extract was given by gavage 300 mg/kg/day for 3 days.

Ischemia/Reperfusion

All animals were given ketamine HCl 90 mg/kg (Ketalar R, Pfizer Drug Company, Istanbul, Turkey) + xylazine hydrochloride 10 mg/kg (Rompun R 23.32 mg/ml, Bayer Drug Company, Istanbul, Turkey) after 15 hours fasting. The animals were placed on the operating table in the supine position, immobilized at four points, and a midline abdominal laparotomy was performed to expose the abdominal cavity. Hepatic IRI was performed by Pringle Maneuver. After detaching of liver from the ligaments and hepatic pedicule (ductus hepaticus, hepatic portal vein and proper hepatic artery) was clamped with an atraumatic microvascular clamp and hepatic ischemia in the lobus hepatis mediana and lobus hepatis sinister. After 60 minutes of ischemia, the clamp was removed and 1 ml NaCl 0.9% was administered intraperitoneally, then the abdomen was closed and we waited 1 hour for reperfusion [15]. The other hepatic lobs had taken whole
portal and arterial blood supply. Color change appeared in the ischemic lobes, however the color of the lobes returned to normal in reperfusion. Rats were kept on heated tables during ischemia and reperfusion. In groups ischemia/reperfusion and IRI+Coriandrum sativum, rats were under anesthesia for 60 minutes with reperfusion duration and no substance was given. At the end of the reperfusion period, blood was taken from the hearts of the rats and euthanized with the exsanguination method. Tissue samples were taken from the reperfused lobus hepatis sinister for histological examinations. Rats in Sham and Coriandrum sativum groups were euthanized by the same method and tissue samples were taken from lobus hepatis sinister. During the reperfusion period, 3 rats from the IRI group and 2 rats from the IRI+Coriandrum sativum group died.

**Blood Sample Analyses**

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) measurements were made by using an Abbott Architect c8000 clinical chemistry analyzer (Abbott Instruments–Abbott Diagnostics, Abbott Park, IL, A.B.D).

**Light Microscopy**

All rats were euthanized, and tissue samples were fixed in paraformaldehyde 4% for 48 hrs, embedded in paraffin, and cut into 5μm sections. Hematoxylin-eosin staining was carried out to assess the general structure of the liver. Tissue sections were examined, with 10 fields per section, and scored from 0 to 4 for vacuolization, sinusoidal congestion, and hepatocyte necrosis, which were evaluated semi-quantitatively according to the modified Suzuki scoring system [16].

**Immunohistochemical Staining**

Immunohistochemical evaluation of the hepatic tissue samples were performed by the following stains: Cas-3 (Anti Cas 3 antibody ab 2302, abcam), TNF-α (Anti-TNF alpha antibody ab183896, abcam), NF-kappa B p65 (Anti–NF-kappa B p65 antibody–ChIP Grade ab7970, abcam). The cells stained positively with Cas-3, TNF-α and NF-kappa B were counted in a light microscope with a magnification of 40x in 20 different areas.

**Statistical analyses**
All the statistical analyses were performed with SPSS 17.0. Data obtained from the groups were compared using the non-parametric Kruskal - Wallis test within each group, whereas the groups were compared with each other using the Mann - Whitney U test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Biochemical results

The AST and ALT levels were increased in the IRI and IRI+Coriandrum sativum groups. When compared to the IRI group, the AST and ALT levels of the Coriandrum sativum were considerably decreased. The IRI and IRI+Coriandrum sativum groups had statistically significant differences in ALP compared to that of the Coriandrum sativum and Sham groups. There was no significant difference between the ALP levels of the IRI and IRI+Coriandrum sativum groups (Table 1).

Light microscopic results

Congestion, vacuolisation, and necrosis were observed to be considerably higher in the IRI group when compared with the results of the Sham and Coriandrum sativum groups. The IRI+Coriandrum sativum group showed decreased of congestion, vacuolisation, and necrosis. Nevertheless, the levels were still higher than those of the Sham and IR groups (Table 2). Compared to the sham group (Figure 1a), dilatation and congestion of sinusoids (Figure 1b), increased number of Kupffer cells (Figure 1c), necrotic areas with hepatocyte degeneration and increased number of neutrophils (Figure 1d), vacuolisation of hepatocytes especially around the portal triads (Figure 1e), and proliferation of the bile ducts (Figure 1f) were confirmed in the IRI group. The IRI+Coriandrum sativum group showed decreased congestion (Figure 1g), decreased Kupffer cell activation and, although lessened, a still discernible proliferation of the bile ducts (Figure 1h). TNF-α, NF-kappa B and Cas-3 expressions were very common and there was strong expression in hepatocytes in the IR group compared to the sham groups. It was observed that there was no TNF-α, NF-kappa B and Cas-3 immunohistochemical staining in the liver sham group (Figure 2a), but there were positive TNF-alpha immunohistochemical staining Kupffer cells (Figure 2b) and hepatocytes (Figure 2c) in the IRI group. In the group treated with Coriandrum sativum, Kupffer cells were not stained, while TNF-alpha expressing hepatocytes were found to be decreased compared to the IRI group (Figure 2d). It was found that there was NF-kappa B staining in the IRI
group (Figure 2e). The severity of immunohistochemical staining and number of stained cells decreased in the group treated with *Coriandrum sativum* (Figure 2f). It was observed that many hepatocytes were expressed Cas-3 in the IRI group (Figure 2g), and the Cas-3 expression was very low in the *Coriandrum sativum* treatment group (Figure 2h). When the expression values of the TNF-α, NF-kappa B and Cas-3 groups were evaluated statistically, it was seen that there was a significant decrease in the group treated with *Coriandrum sativum*, in which the expressions increased in the IRI group. These results were positive correlated with the microscopic findings (Table 3).

**DISCUSSION**

The results obtained throughout this study indicate that *Coriandrum sativum* has therapeutic effects on the histological and biochemical outcomes of liver IRI during the acute phase. During liver transplantations or surgeries, IRI can have a negative impact on regeneration. Many different factors can trigger this, and one of them is the duration of the ischemic period [17]. The preventive or therapeutic effects of various plant extracts or components in IRI have been studied. The blockage of endothelial adhesion molecules, reduction of cytokines is released from cells, free oxygen radicals and hepatocyte apoptosis have been discussed [18]. The *Coriandrum sativum* plant has antioxidant and anti-inflammatory effects and the protective effect of *Coriandrum sativum* on liver damage caused by various hepatotoxic substances has been reported [14, 19, 20, 21]. Studies have shown that *Coriandrum sativum* extract dosage is not toxic up to 3000 mg/kg [22]. In our study, we have identified the anti-inflammatory effect of *Coriandrum sativum* sourced from the Cyprus-Nicosia region at low doses in rats treated with 300 mg/kg. It has been determined that *Coriandrum sativum* extract obtained from this region contains 139.25 ppm isoquercitrin as the active substance. Isoquercitrin (quercetin-3-O-β-d-glucopyranoside) is a one of the major glycosidic form of quercetin and founds commonly in many medicinal plants. It has antioxidant, anti-inflammatory, antiapoptotic effects [23]. It is the major bioactive component of the Coriander (*Coriandrum sativum* L.) and it is commonly found in coriander leaves and roots. In recent years, isoquercitrin has a great potential due to the discovery of new biological activities and its ability to be obtained by enzymatic modification of rutin [24].

Upon reperfusion injury during the first few hours, Kupffer cells are activated and they release cytokines. There is a positive correlation between Kupffer cell activation and
liver IRI [25, 26]. On the other hand, neutrophils play the main role in the stimulation process of the inflammatory cascade mechanisms [27]. During reperfusion, selectin receptors on the endothelial cells increase in number, leukocytes adhere to the endothelial cells and then migrate to the site of the injury [3, 25]. Nuclear pyknosis of hepatocytes, scattering of the hepatic cords and hepatic sinusoid dilation can be observed. It was observed that diffuse local necrosis in reperfusion, vacuolization in hepatocytes and inflammatory cell increase were significantly increased; these findings decreased on the 3rd day and decreased to the minimal level on the 7th day [25]. Although the cause of hepatocyte fats due to IRI is not fully understood, may be multifactorial and involve both parenchymal and nonparenchymal dysfunction, a large amount of lipid accumulation occurs in hepatocytes due to metabolic changes in ischemia during the reperfusion stage. Intracellular triglycerides accumulate due to deterioration of hepatocyte metabolism and vacuolization is observed on a light microscope [28]. The presence of steatosis is associated with an increased mortality risk between 2% and 14%, following liver resection surgery [29].

In our study, congestion of sinusoids was very prominent and widespread in the IRI group. Areas of necrosis were not common but numerous necrotic hepatocytes and vacuolization were detected. Bile duct proliferation in portal areas was detected as a result of ligation of the bile duct in the hepatic pedicle. In the IRI+Coriandrum sativum group, there were a few mitotic hepatocytes around the portal area. Kupffer cell activation was observed in fewer areas. Decrease in the Kupffer cells affected metabolic changes and the necrotic hepatocyte count. In addition, the decreased vacuolization due to lipid accumulation in the hepatocytes in the treatment group depends on the hypolipidemic effect of Coriandrum sativum.

TNF-α is the primary inflammatory cytokine secreted from Kupffer cells in IR injuries. TNF-α and NF-kappa B levels increase significantly in the reperfusion stage after ischemia. Excessive increase of TNF-α activity in ischemia and reperfusion injurie stimulates cell death or apoptosis by direct toxic effects on the mitochondria [30, 31]. NF-kappa B is thought to act as a proinflammatory. Therefore, inhibition of NF-kappa B is expected to suppress the inflammatory response [5, 32]. The expression of NF-kappa B was seen in the hepatocyte cytoplasm after warm ischemia (35-37ºC), there was no nuclear staining [32]. Our study results are compatible with the literature. A positive correlation was observed between increased TNF-α expression and increased NF-kappa B
expression in the IRI group. In IRI group, the increase in neutrophils is very evident. It was determined that inflammation markers and inflammation decreased in the group treated with *Coriandrum sativum*. In the *Coriandrum sativum* group, NF-kappa B reduction was significantly detected in hepatocytes and Kupffer cells due to decreased TNF-α expression. As our experimental model is a warm ischemia model, NF-kappa B staining was seen in hepatocyte cytoplasm. In addition to inflammation in liver IR injuries, apoptotic cell death and necrosis are observed in hepatocytes [33, 34]. In our study, both necrotic and apoptotic cell death were observed. It was determined that the number of apoptotic and necrotic cells decreased in the *Coriandrum sativum* treatment group, and this was due to decreased neutrophil caused by the anti-inflammatory effect of *Coriandrum sativum*.

In ischemia reperfusion studies, AST and ALT values increased in IRI [14, 35, 36]. AST and ALT levels were significantly increased in the ischemic group and decreased in the *Coriandrum sativum* treated group but did not reach the control level in our study. In the ALP level, no significant difference was observed between IRI and IRI+*Coriandrum sativum* groups. One of the main causes of the ALP increase is bile duct obstruction and proliferation. While bile duct proliferation was common in portal triads in IRI, proliferation was found to be less in the IRI+*Coriandrum sativum* group. This result explained the high ALP level.

There is a complex series of complicated events involving parenchymal and non-parenchymal cells in hepatic IR injuries resulting from trauma, cancer surgery, and transplantation. TNF-α and NF-kappa B factors, inflammatory factors, increase and NF-kappa B causes an increase in inflammation-associated cell adhesion molecules, cytokines, anti-apoptotic and pro-apoptotic proteins. Therefore, it is very important to prevent inflammation to prevent and/or reduce IR damage. Our aim, in this study, was to investigate the possible protective effect of *Coriandrum sativum* against hepatic IRI-induced inflammation, oxidative stress, apoptosis, and hyperlipidemia in the hepatic tissue. In the acute phase, 300 mg/kg *Coriandrum sativum* was used to reduce the damage, decrease liver enzymes, inflammation, and apoptosis. The hepatoprotective effect of *Coriandrum sativum* could be partly due to inhibition of the expression of pro-inflammatory cytokines. Anti-inflammatory and anti-apoptotic effect of *Coriandrum sativum* was demonstrated immunohistochemically. In addition, lipid accumulation in the hepatocytes of the group treated with *Coriandrum sativum*, vacuolization decreased. This
finding was an indication of the anti-lipidemic effect of *Coriandrum sativum*. Isoquercetin, the active ingredient in *Coriandrum sativum* extract, is thought to play an important role. According to the literature review, no previous study has used *Coriandrum sativum* against liver IR injuries. To the best of the authors knowledge, this is the first time such data has been provided.

The limitation of the study is the absence of oxidative stress data. Also, showing protein expressions with Western Blotting method will strengthen our study results. Increasing the dose used and prolonged use before ischemia, prolonging the reperfusion period and continuing *Coriandrum sativum* use will provide more effective results. Study of the effects of *Coriandrum sativum* on other cytokines at a molecular level will increase the knowledge on this subject. These study results will be the source of the studies to be done with *Coriandrum sativum*. In particular, the antioxidant effect of a certain amount of *Coriandrum sativum* consumed in daily life will prevent or delay the emergence of some diseases.

**Conflict of interest:** The authors declare that there are no conflicts of interest

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Table 1. The mean values of liver enzymes in groups.

<table>
<thead>
<tr>
<th></th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>36.33 ± 10.19a</td>
<td>74.00 ± 14.93a</td>
<td>46.50 ± 10.21</td>
</tr>
<tr>
<td>Coriandrum sativum</td>
<td>39.33 ± 3.77b</td>
<td>73.17 ± 17.75b</td>
<td>54.50 ± 9.57</td>
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<tr>
<td>IRI</td>
<td>1297.50 ± 357.30c</td>
<td>1127.83 ± 177.09c</td>
<td>71.67 ± 22.38a</td>
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<tr>
<td>IRI+Coriandrum sativum</td>
<td>801.83 ± 241.86c</td>
<td>765.17 ± 242.87c</td>
<td>66.33 ± 14.71a</td>
</tr>
</tbody>
</table>

p value | 0.000* | 0.000* | 0.048* |

*Kruskal Wallis significant value p < 0.05

abc Significance according to Mann Whitney U Test (p < 0.05)

a Significance between Sham group and IRI, IRI + Coriandrum sativum.
b Significance between Coriandrum sativum group and IRI, IRI + Coriandrum sativum.
c Significance between IRI group and IRI + Coriandrum sativum.

Table 2. Injury scoring in groups.

<table>
<thead>
<tr>
<th></th>
<th>Congestion</th>
<th>Vacuolisation</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
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<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
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<tr>
<td>Coriandrum sativum</td>
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<td>0.00 ± 0.00b</td>
</tr>
<tr>
<td>IRI</td>
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<td>1.50 ± 0.55abc</td>
<td>0.67 ± 0.52abc</td>
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<tr>
<td>IRI+Coriandrum sativum</td>
<td>0.83 ± 0.75c</td>
<td>0.33 ± 0.52c</td>
<td>0.17 ± 0.41c</td>
</tr>
</tbody>
</table>

p value | 0.001* | 0.000* | 0.000* |

*Kruskal Wallis Test significant value p < 0.05

abc Significance according to Mann Whitney U Test (p < 0.05)

a Significance between Sham group and IRI group.
b Significance between Coriandrum sativum group and IRI group.
c Significance between IRI+Coriandrum sativum and IRI group

Table 3. TNF-α, NF-kappa B and Cas-3 expression in groups. p < 0.05

<table>
<thead>
<tr>
<th></th>
<th>TNF-α</th>
<th>NF-kappaB</th>
<th>Cas-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Coriandrum sativum</td>
<td>IRI</td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
<td>--------------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>0.05 ± 0.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.10 ± 0.31&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>11.05 ± 2.82&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.00 ± 0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>10.45 ± 3.35&lt;sup&gt;ac&lt;/sup&gt;</td>
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<td>5.30 ± 2.60&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
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</table>

*p value* 0.000*** 0.000*** 0.000***

* Kruskal Wallis Test significant value p < 0.05

<sup>abcd</sup> Significance according to Mann Whitney U Test (p < 0.05);

<sup>a</sup>:Significance between Sham group and IRI group
<sup>b</sup>: Significance between Sham group and IRI+Coriandrum sativum group
<sup>c</sup>:Significance between Coriandrum sativum group and IRI group
<sup>d</sup>: Significance between Coriandrum sativum group and IRI+Coriandrum sativum group
<sup>e</sup>: Significance between IRI group and IRI+Coriandrum sativum group

**Figure 1.** Liver tissue in experimental groups. Sham group (1a). Dilatation of sinusoids, congestion (Figure 1b), increased Kupffer cells (Figure 1c), necrotic area and increased neutrophil number (Figure 1d), vacuolization in hepatocytes around the portal triads (Figure 1e) and bile duct proliferation are observed in IRI group (Figure 1f). Congestion is less common than IRI group, when vacuolization is not common (Figure 1g), and bile
duct proliferation continues to exist in portal triads as decreased (Figure 1h) in *Coriandrum sativum* treated IRI group. Hematoxylin Eozine x40.

**Figure 2.** TNF-α, NF-kappa B and Cas-3 were negative in the immunohistochemical stained liver tissue belonging to Sham group (2a). It appears to be TNF-α positive in Kupffer cells (2b) and hepatocytes (2c) in the IRI group. In the *Coriandrum sativum* treated IRI group, there is no staining in Kupffer cells and it is observed that hepatocytes expressing TNF-α are decreased compared to IRI group (2d). Commonly in hepatocytes NF-kappa B expression appears to be positive in the IR group (2e). The severity of NF-kappa B immunohistochemical staining and the number of stained cells decreased in the *Coriandrum sativum* treated IRI group (2f). Cas-3 is expressed in a large number of hepatocytes in the IRI group (2g), it is seen that the number of immune positive hepatocytes decreased in the *Coriandrum sativum* treated IRI group (2h). TNF-α, NF-kappa B and Cas-3 immunohistochemistry staining x40.