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The Effect of Red Reishi Mushroom *(Ganoderma lucidum)* Extract on Carbon Tetrachloride Induced Liver Injury and Cyclooxygenase-2 Immunoreactivity

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ABSTRACT
Carbon tetrachloride (CCl_4) is a xenobiotic compound with toxicological action. It is absorbed by gastrointestinal system, respiratory system, and skin. Studies have reported that many countries have used *Ganoderma lucidum* (GL, Reishi Mushroom) as a medicinal mushroom against liver diseases induced by hepatotoxic agents such as CCl_4 for more than thousands of years and is used for many diseases, including cancer since it has been thought that it increases resistance against them and treats them. In the present study, immunohistochemical localization and expression of cyclooxygenase-2 (COX-2) by administrating carbon tetrachloride and *Ganoderma lucidum* in adult rats were examined. In the study, 32 adult Spraque-Dawley male rats that were 8-10 weeks old were used. Rats were divided into 4 groups as control, CCI_4, *Ganoderma lucidum* (GL), and CCI_4+GL. As a result of the experimental applications, the liver tissue was found to be normal in the control and GL groups, and multifocal necrosis areas, hepatocellular degeneration, cell infiltration, sinusoidal dilatation, and congestion were observed in the central and portal areas in CCI_4 group. In the CCI_4+GL group, decreases were observed in lesion severity and density. COX-2 immunoreactivity was detected as more common in hepatocyte cytoplasm in the area from the central vena to the Kiernan space, while it was observed as sporadic in the hepatocyte nucleus. While CCI_4 caused a decrease in total antioxidant level (TAS) in blood plasma samples, it caused an increase in total oxidant level (TOS), Aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels. It is seen that *Ganoderma lucidum*, which has an important place in alternative and folk medicine, reduces oxidative stress with its hepatoprotective effect and inhibits the inflammatory response in the liver.

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Kırmızı Reishi Mantarı *(Ganoderma lucidum)* Extratının Karbon Tetraklorür ile İndüklenen Karaciğer Hasarı ve Siklooksijenaz-2 İmmunoreaktivitesi Üzerine Etkisi

ÖZET
Karbon tetraklorür (CCl_4) toksik etkiye sahip ksenobiyotik bir bileşiktir. CCI_4, gastrointestinal sistem, solunum ve deri tarafından emilir. Yapılan çalışmalarda *Ganoderma lucidumun* (GL, Reishi Mantarı), CCl_4 gibi hepatotoksik ajanların neden olduğu karaciğer hastalıklarına karşı, birçok ülkede binlerce yılı aşkın süredir tıbbi mantar olarak kullanılmakta olduğu ve kansere varıncaya kadar birçok hastalığa karşı direnç arttırdığı ve tedavi ettiği düşünülerek kullanıldığı bildirilmiştir. Çalışmada yetişkin dönemdeki sıçanlara karbon tetraklorür ve *Ganoderma lucidum* uygulaması yapılarak siklooksijenaz-2 (COX-2)'nin immunohistokimyasal lokalizasyon ve ekspresyonu incelendi. Çalışmada 8-10 haftalık 32 adet yetişkin

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Ganoderma lucidum

Spraque-Dawley ırkı erkek sıçan kullanıldı. Sıçanlar; kontrol, CCI_4, *Ganoderma lucidum* (GL), CCI_4+GL olmak üzere 4 gruba ayrıldı. Deneysel uygulamalar sonucunda kontrol ve GL grubunda karaciğer dokusu normal, CCI_4 grubunda ise sentral ve portal alanda multifokal nekroz alanları, hepatosellüler dejenerasyon, hücre infiltrasyonu, sinuzoidal dilatasyon ve konjesyon gözlendi. CCI_4 ile birlikte GL verilen grupta ise lezyon şiddet ve yoğunluğunda azalmalar tespit edildi. COX-2 immunoreaktivitesi; sentral venadan kierman aralığına kadar olan bölgede hepatosit sitoplazmasında daha yaygın olarak saptanırken, hepatosit nükleusunda sporadik olarak tespit edildi. Kan plazma örneklerinde CCI_4, Total antioksidan seviyede (TAS) azalmaya yol açarken, Total oksidan seviye (TOS), Aspartat aminotransferaz (AST) ve Alanin aminotransferaz (ALT) düzeylerinde ise artışa sebep olduğu gözlenmiştir. Alternatif ve halk tıbbında önemli yere sahip olan *Ganoderma lucidum*'un ise oksidatif stresi hepatoprotektif etkisi ile azalttığı, karaciğerdeki enflamatuar yanıtı inhibe ettiği görülmektedir.

Histopatoloji
İmmunohistokimya

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INTRODUCTION

The liver provides many functions in the body, such as the process of metabolism (Kandimalla et al., 2016). Toxic molecules of foreign origin and xenobiotics are absorbed by the intestine and then pass through the liver and they are detoxified here (Stickel et al., 2002; Elmubarak & Özsoy, 2016). Carbon tetrachloride (CCl_4) is a xenobiotic causing chemical-related injuries both in most living beings and in humans and it has the potential to develop cellular injury (Tekeli & Bildik, 2013). It has a toxic character in the organs such as the heart, testis, brain, and especially liver and kidneys (Abraham & Wilfred, 1999; Elmubarak & Özsoy, 2016). In the metabolism of action of CCl_4, enzymes that are defined as microsomal enzymes metabolizing medicine and chemical substances, play a role. After CCl_4 enters in the liver, it is converted into two free radicals, as trichloromethyl and trichloromethyl-peroxyl, by the microsomal system that is dependent on monooxygenase P-450 (Zhao et al., 2016). The toxic character of CCl_4 emerges after it is converted to trichloromethyl (CCl_3). CCl_4 is first converted to a harmful intermediate metabolite called trichloromethyl radical (-CCl_3) through the cytochrome P-450 enzyme system, and then to trichloromethyl peroxy (-$OOCCl_3$) radical in the presence of oxygen. These reactive free radicals react with polyunsaturated fatty acids and bind either to fats or lipids or start lipid peroxidation. As a result of lipid peroxidation occurring in CCl_4 exposure, more common tissue injury occurs as a result of the septicemia of some cellular enzymes (El-haskoury et al., 2018; Li et al., 2019).

A number of studies have shown that various plant extracts can protect the liver and kidney against oxidative stress caused by CCl_4 by inhibiting lipid peroxidation and increasing antioxidant enzyme activity (Shahjahan et al., 2004; Bellassoued et al., 2018). *Ganoderma lucidum* (GL, Reishi Mushroom) is used as a medicinal mushroom in many countries for more than 2000 years and has been used by considering that it increases resistance against and treats many diseases, including cancer (Kim et al., 2016). In the recent studies conducted with *G. lucidum*, its anti-tumor, anti-inflammatory, hepatoprotective, and anti-microbial effects are reported. Additionally, the components of reishi mushrooms have an antioxidant character. Its antioxidant character is provided by terpene and polysaccharide content (Cör et al., 2018). Triterpenoids, also called as ganoderic acid, carry a carboxyl group and have immunomodulatory and antioxidant properties. Thus, they gain their hepatoprotective properties (Satria et al., 2019).

COX enzyme has two types; constitutive and inducible. COX1, that is the structural cyclooxygenase, and COX-2, the inducible cyclooxygenase, catalyze the same reactions, but differ in structure and function. There are differences in structure and shape between these two enzymes caused by two amino acid changes in the amino acid sequence (Fu et al., 1990; Hawkey, 2001). While COX-1 is present in endoplasmic reticulum (ER), COX-2 is localized in ER and nuclear membrane. Both enzymes convert the arachidonic acid to prostaglandins (Dannhardt & Kiefer, 2001; Kaya Çavuşoğlu et al., 2021). COX-1 protects the tissues generally in terms of physiological reactions, while COX-2 has a completely reverse effect (Hawkey, 2001; Smith et al., 1996). COX-2, activated by inflammatory

agents, is present in inflammatory cells, especially macrophages (Hawkey, 2001). Proinflammatory agents such as cytokines, tumour necrosis factors, growth factors, and bacterial endotoxins cause the release of COX-2. Prostaglandins (PG), which the products of COX-2 and involved in inflammatory reactions, are responsible for the major symptoms of inflammation such as swelling, redness, pain, fever, and loss of function (Smith et al., 1996; Domitrovica et al., 2011). PGs developing by the induction of COX-2 degrade the cellular apoptosis mechanism in some cancer types and provide the metastasis of cancer (Hoffmann, 2000).

While PGs are synthesized via COX-1, the ones causing inflammatory response are only synthesized via COX-2. COX-2 is involved in the regulation of most of the renal functions (perfusion, fluid use, renin production) in both natural and pathophysiological (renal failure, congestive heart failure and liver cirrhosis) cases (Gambaro, 2002).

In the present study, the effects of *Ganoderma lucidum* on general oxidant/antioxidant levels, liver injury markers, histopathological lesions and COX-2 immunoreactivity levels of the rats with carbon tetrachloride-induced liver inflammation were determined.

MATERIAL and METHOD

Study design

Ethics committee approval required for the study was obtained from Animal Experiments Local Ethics Committee of Gaziantep University (GAÜN-DAM, Decision no: 2018/22). In the study, 32 adult (8-10 weeks old) *Spraque-Dawley* male rats weighing approximately 250-300 g, obtained from Gaziantep University Experimental Animals Research Centre were used as the material. Care and material administrations of the rats were also conducted at the research units of Gaziantep University Experimental Animals Research Centre. The rats were kept in an environment at the ambient temperature of 21 °C with a 12-hour light/12-hour dark cycle and were fed with standard rat feed (containing 21% crude protein) and tap water. The subjects included 32 rats in total as 8 in each group. The rats were divided into 4 groups as control, carbon tetrachloride (CCI_4), *Ganoderma lucidum* (GL), Carbon tetrachloride (CCI_4)+*Ganoderma lucidum*. Ganoderma lucidum. The *G. lucidum* extract used in the experiment was commercially obtained from GanoTurk (Seyhan, Adana). Physiological saline at a dose of 2 ml/kg was administered to the control group via gavage for 14 days, *Ganoderma lucidum* extract at a dose of 1000 mg kg^{-1} was administered to GL group via gavage for 14 days, Carbon tetrachloride at a dose of 10 ml/kg was administered to the CCI_4 group as a single dose intraperitoneally only on the first day. After giving *G. lucidum* extract to the CCI_4+GL group via gavage for the first 3 days, CCI_4 was administered in a single dose of 10 ml/kg. Then, *G. lucidum* extract was administered only via gavage for 14 days. At the end of the experiment, the rats were dissected after they have killed via cervical dislocation under ketamine hydrochloride/ xylazine (80/10 mg kg^{-1}) anaesthesia administered intramuscularly (i.m.). All the animals were weighed at the beginning and end of the study. At the beginning of the study, the animals were randomly divided into 4 groups provided that their mean weights were close to each other. The dose of CCI_4 in the study was determined according to Karakus et al., 2011, and the dose of *G. lucidum* extract was determined based on the data of researchers working in this field (Sliva et al., 2012; Lin & Lin 2006; Zhang et al., 2002).

Histological analysis

At the end of the experiment, liver tissue of the rats that were killed and dissected via cervical dislocation under general anaesthesia was fixed in 10% buffered formalin solution (SigmaAldrich, HT501128). After fixation, routine tissue follow-up (graduated alcohols, methyl benzoate, and benzole follow-up) was performed and then the tissues were embedded in paraffin and 5 µm serial sections were taken from the blocks with a microtome on slides previously coated with chrome alum gelatin (CAG). Hematoxylin-eosine, one of the histological staining methods, was applied to the sections and the histopathological changes were examined via light microscopic study (Luna, 1968).

Immunohistochemical analysis

Liver tissues taken from the rats were fixed in 10% buffered formaldehyde solution for COX-2 immunoreactivity and then, they were blocked in paraffin after passing through graduated alcohols, methyl benzoate, and benzoles. Anti-COX2 (Cyclooxygenase 2 antibody ab15191, Abcam) primary antibody was applied to 4-5 µm sections taken from paraffin blocks for one hour at room temperature in a humid environment at a ratio of 1/100. Only PBS (phosphate buffer solution) (Invitrogen™, AM9624) was dropped on the tissue sections for the negative control group. After primary antibody incubation, Streptavidin- biotin peroxidase technique, one of the indirect methods, was used (Shu et al., 1988). Chromogen application was conducted by adding 3-Amino-9-Ethylcarbazole (AEC) (Thermo Fisher Scientific, 1122). After adding AEC solution, the sections were controlled under a light microscope and when immunoreactivity developed, the reaction was stopped with distilled water and contour staining was performed with Mayer's hematoxylin. At the end of the processes, the sections were dried, and water-based adhesive was dropped and closed by using a lamella. Random areas were selected in the preparations and

their photographs were taken after they were examined under a Zeiss Primo Star integrated camera light microscope, assessment was conducted semi-quantitatively and the density was examined according to an immunoreactive score (Zhu, 1989; Seidal et al., 2001; Nur et al., 2015). COX-2 immunoreactivity in the cells was determined by comparing them with each other according to the degree of darkness of the colours.

Biochemical analysis

Blood samples taken for plasma output were centrifuged at 3000 rpm for 10 minutes and stored at -20 °C until the time of analysis. Total oxidant level (TOS) and total antioxidant levels (TAS) (Rel Assay Diagnostics, Mega Medical Industry and Trade Limited Company, Gaziantep) were measured in the blood samples (Erel, 2004; Erel, 2005). Plasma Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity analyses were measured by using an autoanalyzer (HumaStar 600, Germany).

Data analysis

Statistical analysis of the data was conducted in SPSS package program (IBM SPSS Statistics 22). Kolmogorov-Smirnov normality test was employed to reveal the compatibility of the data to normal distribution. For groups showing a normal distribution, one-way analysis of variance (ANOVA), a parametric test, was used. If there was a difference between the means of the experimental groups, the "Anova-Duncan" test was applied to the group averages to determine this difference and the value of $P<0.05$ was accepted as statistically significant.

RESULTS and DISCUSSION

Biochemical findings

When the biochemical data of the study were evaluated, the values in the control and *G. lucidum* groups were normal and close to each other. No statistical difference was observed between TAS, TOS, AST, and ALT values of the control and *G. lucidum* groups. There was no statistical difference between the biochemical data obtained from CCl_4 and CCl_4+GL groups in terms of TAS, TOS, AST, and ALT levels. However, a statistically significant difference was found between these two groups and the control and *G. lucidum* groups in terms of TAS, TOS, and AST levels ($P<0.01$). When ALT level was assessed, there was a statistical difference between CCl_4 group and control and *G. lucidum* ($P<0.05$). However, different from other biochemical indicators, no statistical difference was observed between the CCl_4+GL group and control and *G. lucidum* groups ($P> 0.05$). Data of the groups obtained from the study are shown in Table 1 and Figure 1.

Table 1. TAS, TOS, AST, and ALT values of the groups and statistical significance.
Çizelge 1. Gruplara ait TAS, TOS, AST ve ALT değerleri ve istatistiki önem.

PARAMETERS	GROUPS Control (n:8) Mean±SE	*Ganoderma lucidum* (GL) (n:8) Mean ±SE	CCl_4 (n:8) Mean ±SE	CCl_4+GL (n:8) Mean ±SE	P<
TAS (mmol Trolax eqivalen/L)	1.91 ± 0.045^{a}	1.85 ± 0.042^{a}	1.43 ± 0.045^{b}	1.56 ± 0.035^{b}	**
TOS (µmol H_2O_2 equiv./L)	7.33 ± 0.20^{b}	7.40 ± 0.19^{b}	8.96 ± 0.16^{a}	8.34 ± 0.17^{a}	**
AST (U/L)	158.56 ± 3.4^{b}	165.79 ± 3.75^{b}	203.38 ± 3.7^{a}	194.07 ± 6.19^{a}	**
ALT (U/L)	65.27 ± 4.21^{b}	67.10 ± 2.54^{b}	79.37 ± 1.75^{a}	$74.14 \pm 2.32^{a,b}$	*

*: P<0.05= Statistically significant difference, **: P<0.01= Statistically significant difference, a, b: The difference between the averages of the groups having different letters on the same line is significant. n: number of the subjects in the group, Mean ± SE: Mean±Standard Error.

Histological findings

Liver tissues were embedded in paraffin blocks after routine fixation and tissue follow-up processes. After staining the 5 µm serial sections taken from these blocks via microtome with hematoxylin-eosin, they were examined under a light microscope. In the sections obtained from the groups, the central vena and Kiernan's space were normal, the remark cords were regular, the parenchymal structure was normal, and sinusoids were found between hepatocytes in the control and *G. lucidum* groups. In the portal area, the connective tissue area including the hepatic artery, portal vein, and bile duct had a normal structure (Figure 2a, 2b, 2c). In the CCl_4 group, congestion was observed in the central vein and in the vein in portal area. Multifocal necrosis areas and hepatocellular degeneration were observed between the central vein and the portal region in this group sections. In addition, cell infiltration, vacuole in hepatocyte cytoplasm, irregular remark cords, sinusoidal dilatation, and congestion were observed (Figure 2d, 2e). In the CCl_4+*Ganoderma lucidum* group, focal necrosis areas and hepatic degeneration, congestion, sinusoidal dilatation, and cell infiltration were detected (Figure 2f, 2g, 2h). The incidence of the lesions occurring in this group was detected as close to the CCl_4 group. Thus, *G. lucidum* is not sufficient on its own in terms of the treatment of degeneration in the liver caused by CCl_4 (Table 2). Table 2 shows the tissue change ratings of histopathological lesions occurring in the liver tissue and the frequency ratings were adopted from Bernet et al. (1999).

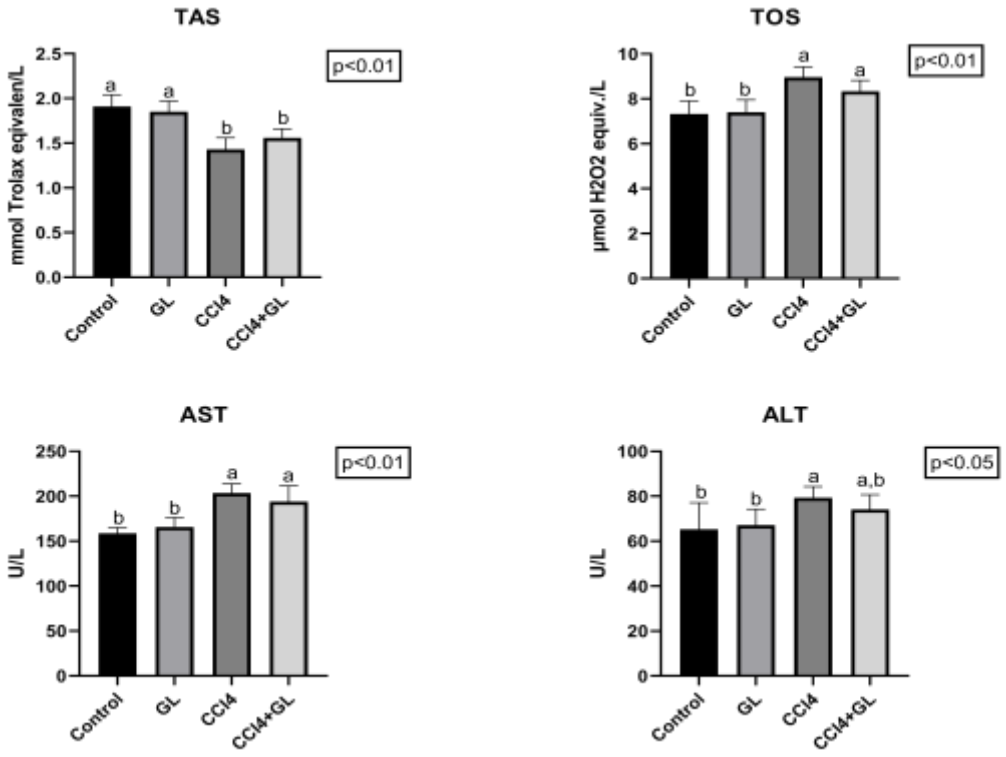
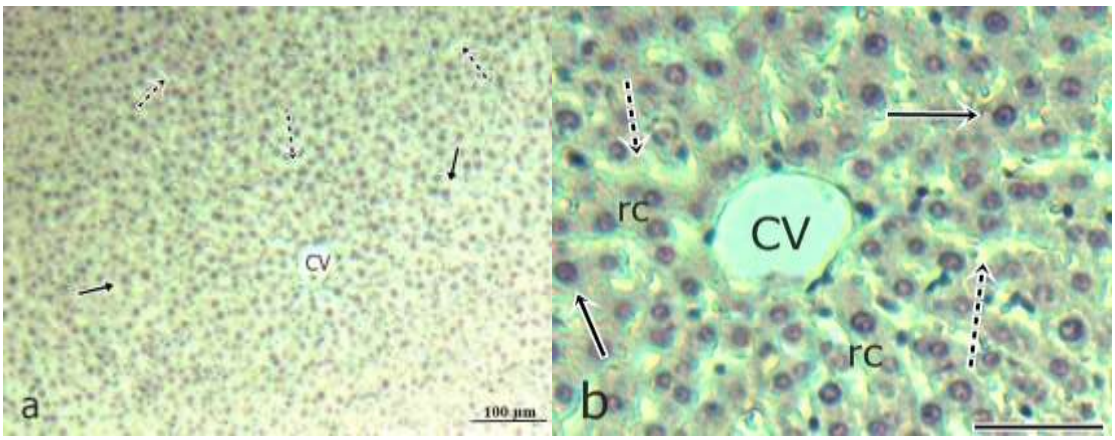


Figure 1. Graphical presentation of TAS, TOS, AST, and ALT values of the groups.
Şekil 1. Gruplara ait TAS, TOS, AST ve ALT seviyelerinin grafiksel gösterimi.

Table 2. Tissue change ratings of the histopathological lesions in the liver tissue.
Çizelge 2. Karaciğer dokusunda histopatolojik lezyonlara ait doku değişim derecelendirmeleri.

Liver lesions	**Groups** Control group	*Ganoderma lucidum* (GL)	(CCl_4)+GL	Carbon tetrachloride (CCl_4)
Degeneration in hepatocytes	-	-	++	+++
Infiltration	-	-	++	+++
Irregularity in remark cords	-	+	++	+++
Vascular degeneration	-	-	++	++
Sinusoidal dilatation	-	-	++	++
Central and portal congestion	-	-	++	++
Necrosis	-	-	++	+++
Sinusoidal congestion	-	-	++	++
Vacuolization	-	+	++	++

-: no abnormality, +: low abnormality frequency, ++: moderate abnormality frequency, +++: high abnormality frequency.



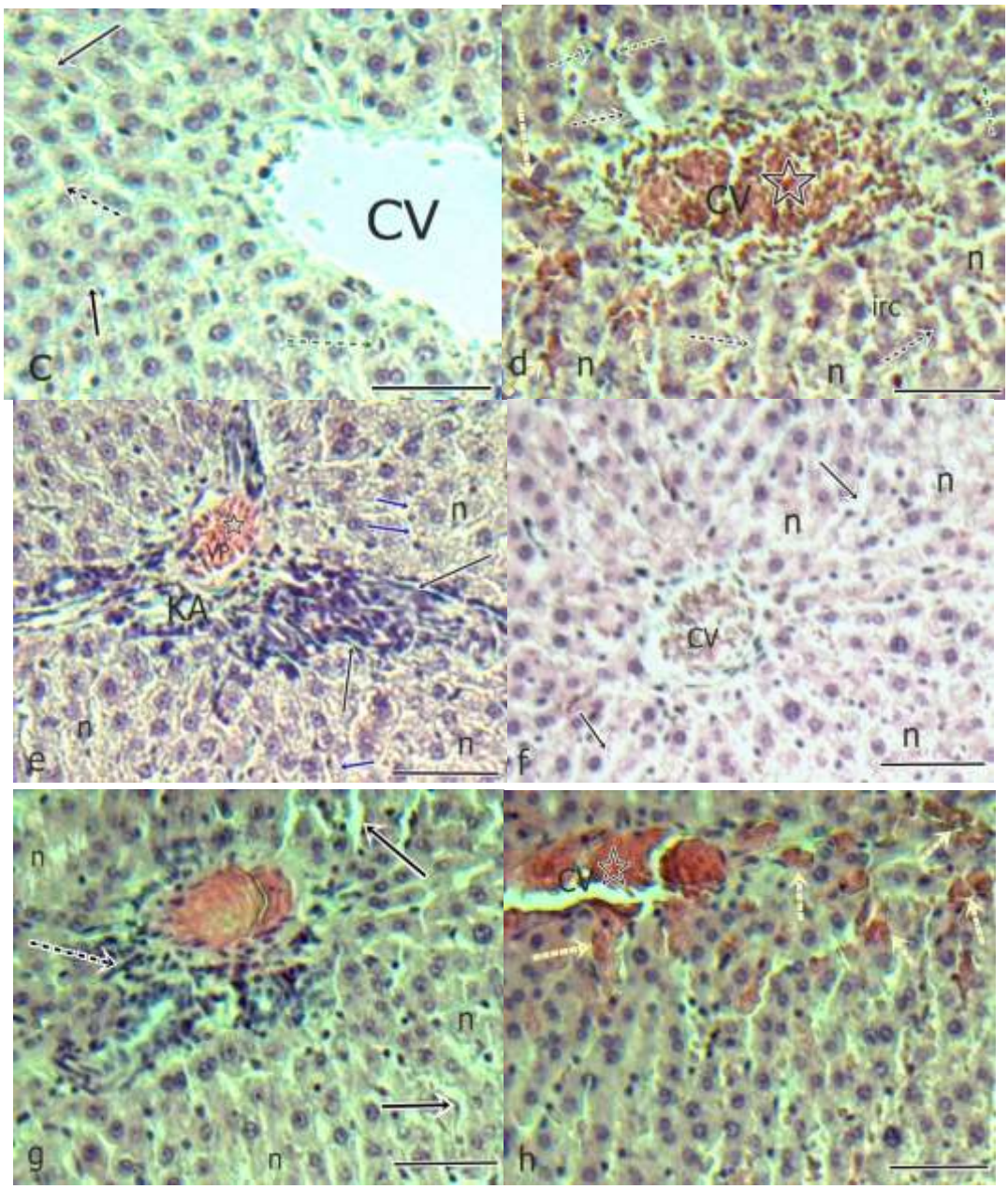


Figure 2. a, b. Liver tissue obtained from the animals in the control group. Hepatocytes and sinusoidal structure have a normal appearance (CV: central vein, arrows: hepatocyte, rc: radially sequenced remark cords, dashed arrows: sinusoid). c. Liver tissue obtained from the animals in the group to which *G. lucidum* was administered at a dose of 1000 mg kg^{-1}. Hepatocytes and sinusoidal structure have a normal appearance (CV: central vein, arrows: hepatocyte, black dashed arrows: sinusoid, green dashed arrows: kupffer cells). d, e. Liver tissue obtained from the animals in the group to which carbon tetrachloride was administered at a dose of 10 ml/kg (CV: central vein, VP: vena porta, asterisk: congestion in central and portal vein, black arrows: Cell infiltration, blue arrows: Vacuole in hepatocyte cytoplasm, black dashed arrows: Sinusoidal dilatation, green dashed arrows: kupffer cells, yellow dashed arrows: sinusoidal congestion, irc: irregular remark cords, n: Multifocal necrosis areas and hepatocellular degeneration). f, g, h. Liver tissue obtained from the animals in the group to which carbon tetrachloride at a dose of 10 ml/kg and *G. lucidum* at a dose of 1000 mg kg^{-1} are administered. vena centralis, black arrows: sinusoidal dilatation, n: Multifocal necrosis areas and hepatocellular degeneration, black dashed arrows: Cell infiltration, asterisk: congestion in central and portal vein, yellow dashed arrows: sinusoidal congestion. H&E. Bar: 100 µm.

Şekil 2. a, b. Kontrol grubundaki hayvanlardan elde edilen karaciğer dokusu. Hepatositler ve sinozoidal yapı normal görünümde (CV: vena centralis, oklar: hepatosit, rc: ışınsal dizilmiş remark kordonları, kesik çizgili oklar: sinuzoid). c. 1000 mg kg^{-1} dozunda G. lucidum uygulanan gruptaki hayvanlardan elde edilen karaciğer dokusu. Hepatositler ve sinozoidal yapı normal görünümde (CV: vena centralis, oklar: hepatosit, siyah kesik çizgili oklar: sinusoid, yeşil kesik çizgili oklar: kupffer hücreleri). d, e. 10 ml/kg dozunda karbon tetraklorür uygulanan gruptaki hayvanlardan elde edilen karaciğer dokusu (CV: vena centralis, VP: vena porta, yıldız: sentral ve portal vende konjesyon, siyah oklar: hücre infiltrasyonu, mavi oklar: hepatosit sitoplazmasında vakuol, siyah kesik çizgili oklar: sinusoidal dilatasyon, yeşil kesik çizgili oklar: kupffer hücreleri, sarı kesik çizgili oklar: sinusoidal konjesyon, irc: düzensiz remark kordonları, n: multifokal nekroz alanları ve hepatosellüler dejenerasyon). f, g, h. 10 ml/kg dozunda karbon tetraklorür ve 1000 mg kg^{-1} dozunda G. lucidum uygulanan gruptaki hayvanlardan elde edilen karaciğer dokusu (CV: vena centralis, siyah oklar: sinusoidal dilatasyon, n: multifokal nekroz alanları ve hepatosellüler dejenerasyon, siyah kesikli oklar: hücre infiltrasyonu, yıldız: sentral ve portal vende konjesyon, sarı kesik çizgili oklar: sinusoidal konjesyon. H&E. Bar: 50 µm (b, c, d ,e, f, g, h), 100 µm (a).

Immunohistochemical findings

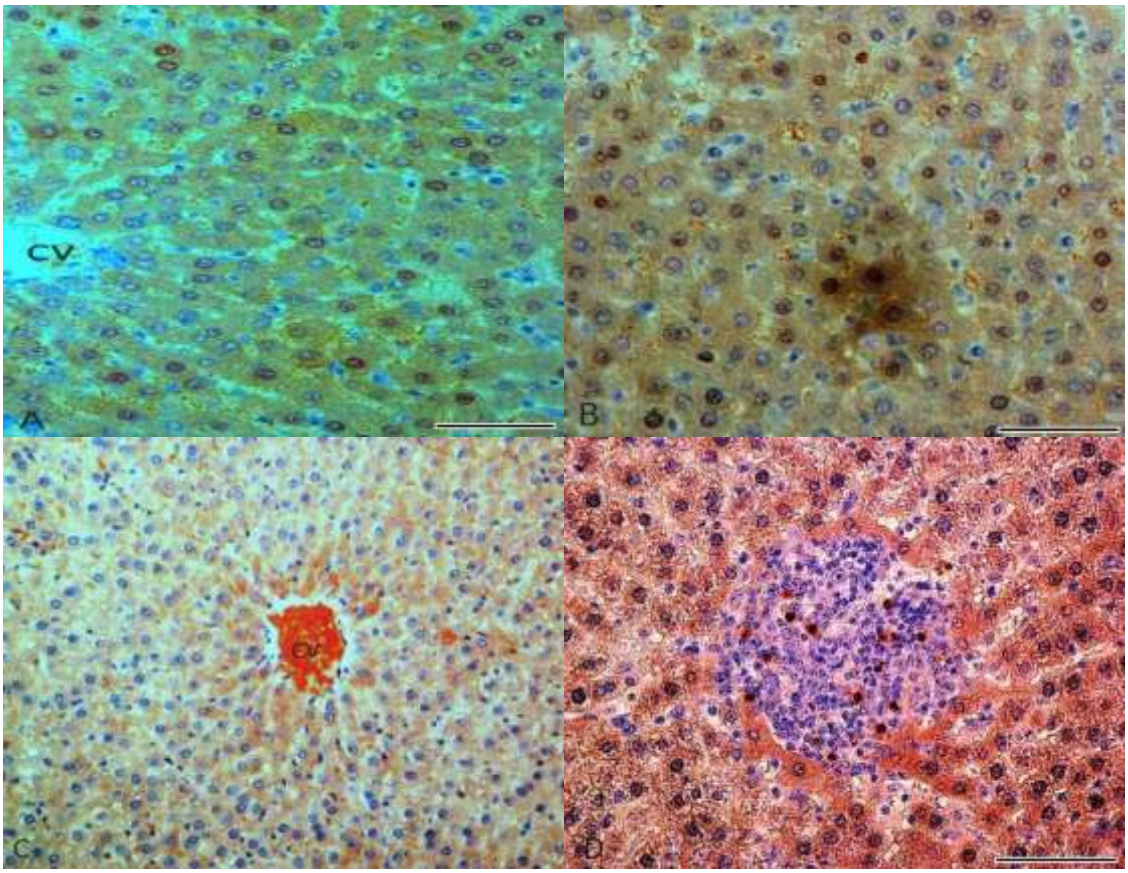
COX-2 immunoreactivity was assessed in the preparations obtained from control, *Ganoderma lucidum*, CCI_4, and CCI_4+*Ganoderma lucidum* groups. In all the groups, COX-2 immunoreactivity was commonly observed in the hepatocyte cytoplasm with moderate intensity in the region from the central vena to the Kierman's space, and sporadically in the hepatocyte nucleus (Table 3, Figure 3). COX-2 immunoreactivity was commonly observed at a higher intensity in the hepatocyte cytoplasm both in centrilobular and in perilobular area in the CCI_4 group when compared to other groups.

Carbon tetrachloride is a toxic material that is widely used by researchers to induce liver injury (El-haskoury et al., 2018; Li et al., 2019). It is known that contact with this toxic chemical induces oxidative stress with the formation of free radicals and causes tissue damage (Ganie et al., 2011). Injuries induced by the substances such as CCl_4 cause apoptosis formation in the cells. Various studies have revealed that natural antioxidants have organ-preserving potential against toxic materials such as CCl_4 (Said et al., 2018; Satria et al., 2019). In a study in which CCl_4 and locust honey there against were used, it was stated that 1 mg kg^{-1} dose of CCl_4 administration caused an increase in the

Table 3. COX-2 immunoreactivity density in the control and application groups. (+++) very dense, (++) moderately dense, (+) less dense, (-) no reaction.

Çizelge 3. Kontrol, ve uygulama gruplarında COX-2 immunoreaktivite yoğunluğu. (+++) çok yoğun, (++) orta derecede yoğun, (+) az yoğun, (-) reaksiyon yok.

Cyclooxygenase	Structure showing immune reaction	Control group	*Ganoderma lucidum* group	CCI_4 group	*Ganoderma lucidum*+CCI_4 group
COX-2	Centrilobular area	+	+	+++	+
	Hepatocyte cytoplasm	+	+	+++	++
	Hepatocyte nucleus	+	+	+	+
	Perilobular area	+	+	+++	++



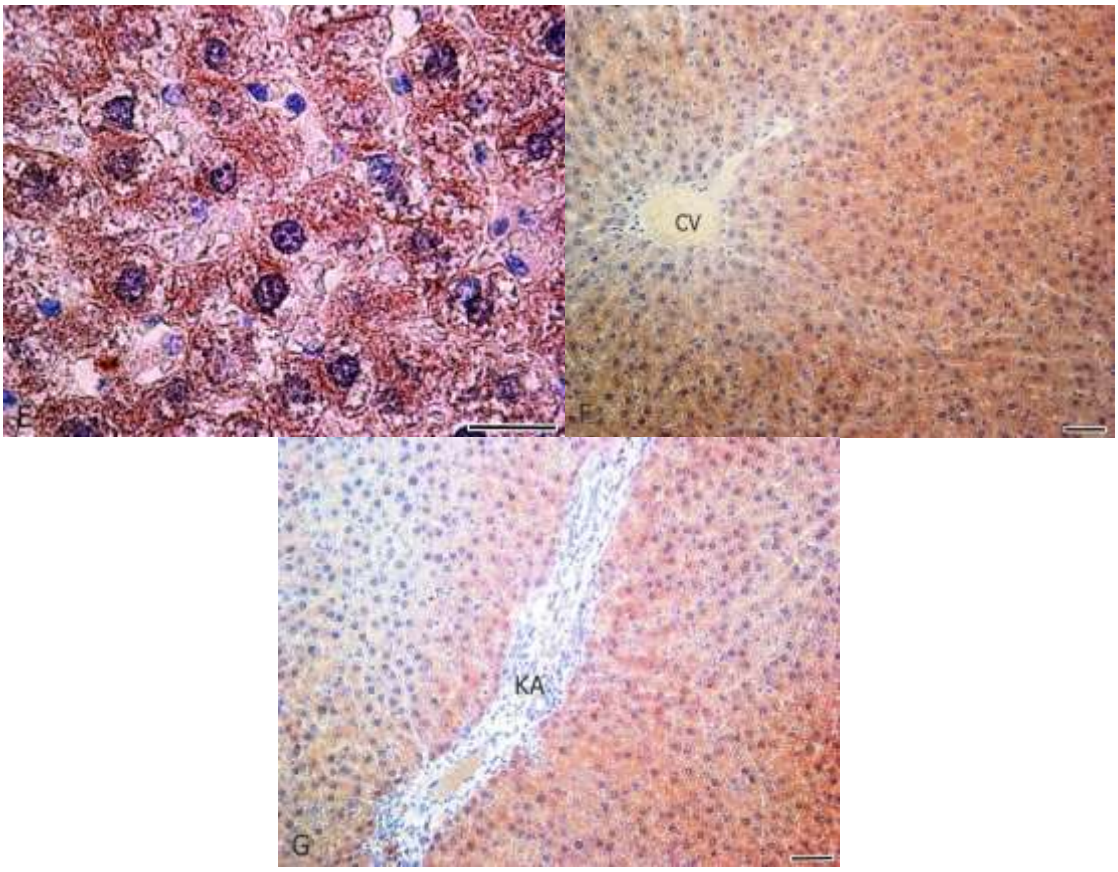


Figure 3. COX-2 immunoreactivity in the liver tissue of the rats. A, B. Control group, moderately dense immunoreactivity in hepatocyte cytoplasm and nucleus in some regions. C. *Ganoderma lucidum* group, moderately dense immunoreactivity in the hepatocyte cytoplasm around central vein. D, E. Carbon tetrachloride group, moderately dense in hepatocyte nucleus between central vena and kiernan's space, and dense immunoreactivity in the hepatocyte cytoplasm. F, G. Carbon tetrachloride+*Ganoderma lucidum* group, mildly dense around the central vein in hepatocyte cytoplasm and moderately dense immune reaction in the area up to the remaining kiernan's space. CV: central vein, KA: Kiernan's space, Bar: 50 µm.

Şekil 3. Sıçan karaciğer dokusunda COX-2 immunoreaktivitesi. A, B. Kontrol grubu, bazı bölgelerde hepatosit sitoplazması ve nükleusunda orta yoğunlukta immunoreaktivite. C. Ganoderma lucidum grubu, vena sentralis çevresindeki hepatosit sitoplazmasında orta yoğunlukta immunorektivite. D, E. Karbon tetraklorür grubu, sentral vena ve kierman aralığı arasında hepatosit nükleuslarında orta yoğunlukta, hepatosit sitoplazmasında yoğun immunoreaktivite. F, G. Karbon tetraklorür+Ganoderma lucidum grubu, hepatosit sitoplazmasında vena sentralis civarında hafif, geri kalan kierman aralığına kadarki alanda orta yoğunlukta immun reaksiyon. CV: vena sentralis, KA: kierman aralığı, Bar: 25 µm (E), 50 µm (A, B, C, D, F, G).

liver enzymes, lactic acid dehydrogenase, blood glucose, uric acid, urea, MDA, and serum kreatinine values (El-haskoury et al., 2018). In a study in which the ethanol extract of *G. lucidum* was administered, as a result of improvements in liver and kidney MDA levels, reductions were observed in the injury of these organs (Shieh et al., 2001). In another study, the curative effect of 100 mg kg^{-1} dose of *G. lucidum* administered in the liver against alcohol-induced liver cirrhosis was explained (Kwon & Kim, 2011).

Determination of alternative treatment sources against many degenerative and chronic diseases takes place among the most popular issues in the scientific world. Side effects and concerns of synthetic treatments in the society have increased the tendency to natural alternative treatment resources. The studies on the biological activities of plants and mushroom supporting this situation have increased in recent years (Ozkan et al., 2016). Liver injury after CCI_4 exposure is characterized by elevated levels of serum hepatic marker enzymes indicating cellular leak and loss of functional integrity of the hepatic membrane architecture. High levels of ALT and AST activities are sensitive indicators of liver cell injury. The increase in serum hepatic marker levels revealed that an extensive liver injury has occurred by CCI_4 due to the increased lipid peroxidation which can cause membrane injury (Pradeep et al., 2010). Many chemicals can have toxic effects on the liver. For example, liver and kidney function biomarkers of rats exposed to aluminum were adversely affected. In the group given pomegranate juice as a preservative, it was stated that serum biomarkers almost approached the values of the control group (Çiftci et al., 2022).

In recent years, *G. lucidum* has drawn attention due to its oxidative stress-reducing capability. The current results have also indicated that treatment with *G. lucidum* has decreased the effect of the CCl_4-induced hepatotoxicity as shown by the decrease in AST, ALP, and ALT liver enzymes (Lin et al., 1995; Gao et al.,

2019). Results obtained from animal experiments have shown that CCl_4 causes fibrosis and injury in the liver tissue and also increases the liver enzyme levels such as Alanine aminotransferase (ALT) and Aspartate transaminase (AST) (Gao et al., 2019). It is observed that warm water and ether extracts of orally and intraperitoneally administered *G. lucidum* have an active hepaprotective effect against CCl_4-induced liver injury and cause recovery in aspartate and alanine transaminase (AST-ALT) and lactate dehydrogenase (LDH) values that are accepted as markers for liver injury (Lin et al., 1995; Kim et al., 1999).

In the studies conducted by Koçak et al. (2019) and Atasever & Yaman (2014) for TAS, TOS, and OSI levels, they reported that a single dose of 1 ml/kg decreased the serum TAS level of CCl_4 and increased TOS and OSI levels. It is shown that the use of antioxidant legalon (silymarin) against CCI_4 reduces total oxidant and oxidative stress index levels and increases the total antioxidant level (Verma et al., 2015). In studies in which CCl_4 was administered chronically, it was noted that there was a significant increase in ALT enzyme due to the deterioration of cell membrane permeability depending on hepatocyte destruction (Abdel-Daim et al., 2016; Atasever et al., 2020). The data obtained from the current study is parallel with the findings of other researchers.

CCl_4 is widely used to cause injury in the tissues, especially in the liver (Lv et al., 2006; Lida et al., 2009; El-haskoury et al., 2018). Rats were administered with a dose of 0.2 ml/100 g of CCI_4 three times a week for eight weeks (Basu, 2003) and once a week for ten weeks (Lida et al., 2009), and then, widespread areas of necrosis were detected in the fibrous tissue in the portal region of the liver tissue. In addition, fat degeneration and inflammatory cell infiltrations have been reported in hepatocytes. In other studies, it was shown that CCI_4 caused fatty vacuoles in the hepatocyte within the liver tissue, inflammatory cell infiltrations at varying severities, and fibrosis (Atasever et al., 2020, Basu, 2003). In the present study, congestion, multifocal necrosis areas, cell infiltration, and sinusoidal dilatation around the central vein and portal region of CCI_4 were observed. This is compatible with findings of other studies in which CCI_4 was administered. Grape seed oil slightly reduced the number of fat vacuoles and partially reduced the necrosis areas, and also prevented the formation of fibrous tissues in the liver. Numerous studies have reported that grape seed oil caused histological recovery in the liver lesions caused by other toxic materials. In the studies conducted with grape seed oil against various hepatotoxins, it was thought that the hepatoprotective effects of grape seed oil were caused by antioxidant and free radical scavenger components (Khalifa et al., 2011; Al-Attar, 2015). In the current study, it is understood that *G. lucidum*, which is used against the toxic properties of CCI_4, reduced the histopathological lesions in the liver, however, it was not sufficient alone in the treatment of lesions.

Immunohistochemical findings are a current method that has been used by many researchers in order to determine and reveal the releasing tissue parts of expressed proteins (Yıldız et al., 2013; Nur et al., 2014; Nur et al., 2015). While CCl_4 increased iNOS, COX-2, TNF-α and IL-1ß mRNA, and protein expression levels in the liver tissues, it significantly decreased iNOS, COX-2, TNF-α, IL-1ß mRNA and protein expression levels that are similar to the findings in the silymarin group as a result of the inflammation inhibiting effect of the hawk tea. As a result of CCl_4 administration in the histological examination of the tissue, congestion in the central zone of the liver, haemorrhage, and necrosis in the hepatocytes were observed, and decreases in the severity of the lesions were detected in the groups to which hawk tea and silymarin were applied. Serum AST, ALT, and LDH levels that increased by the CCl_4 administration have significantly decreased as a result of hawk tea application (Zhao, 2013). In a study examining the expression of cyclooxygenases in liver tissue, COX-1 and COX-2 immunoreactivity was detected in all the groups at similar intensity and in hepatocyte cytoplasm (Nur et al., 2015). COX-2 immunoreactivity in the kidney tissue of the rats to which capsaicin was administered in puberty decreased in the macula densa, Henle's loop, distal and proximal tubules in the capsaicin group compared to the control group, while COX-1 immunoreactivity increased in the proximal tubules in the capsaicin group when compared to the control group (Yıldız et al., 2013). In a study investigating the protective effect of berberine and silymarin against CCl_4 administration, COX-2 immunoreactivity was detected in the hepatocyte cytoplasm and nucleus in the necrosis areas. Berberine and silymarin decreased the COX-2 immunoreactivity when compared to the CCl_4 group. In the histological examination of the liver, vacuole-fat formation, inflammatory cell infiltration, and extensive necrotic areas were stated in the CCl_4 group. When compared to the control group, serum AST, ALT, and alkaline phosphatase (ALP) levels increased in the CCl_4 group (Domitrovica et al., 2011). In a study that investigated the effect of L-thearinin, which is an amino acid in the tea, against CCl_4 hepatotoxicity, it was reported that L-thearinin that suppresses COX-2 and iNOX expression in the liver with increased serum TNF-α and IL-1ß in CCl_4 group, thus, inhibited the inflammatory response. L-thearinin decreased AST, ALT, and bilirubin levels and also, and there was a decrease in the severity of histopathological changes in the liver (Jiang et al., 2012).

CONCLUSION

In the present study, similar to the other studies conducted with toxic substances, it was concluded that carbon tetrachloride can cause significant biochemical and histological changes in the rats due to oxidative damage, while *G. lucidum* has a therapeutic feature by showing a reduction in the severity of the lesion. It is thought that the hepatoprotective effect of *G. lucidum* may be related to decreasing oxidative stress and inhibiting the inflammatory response in the liver due to its free radical scavenging feature. When the results of the study were assessed, *G. lucidum* showed a protective character against acute liver injury by suppressing the inflammatory response by preventing the increase of carbon tetrachloride-induced oxidant capacity and reducing the apoptotic reaction developing in hepatocytes.

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Author's Contribution

MSc. HCY, Dr. GN, Dr. HAD and Dr. IG researched literature and conceived the study. They were involved in protocol development, gaining ethical approval, experimental design. Data analysis was completed by whole authors. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Conflict of interests/Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

This study was approved by Gaziantep University Animal Experiments Local Ethics Committee (permission no. 2018/22).

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