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Investigation of Antioxidant and Cytoprotective Effects of *Allium Schoenoprasum* L (Sirmo) Plant Ethanol Extract in Liver Damage Caused by Carbontetrachloride in Rats

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ABSTRACT

Liver diseases, morbidity and mortality rates are increasing in the world and existing drugs are insufficient for treatment. For this reason, there has been an increased interest in the discovery of new drugs against liver damage and the search for alternative therapies. This study investigated the cytoprotective and antioxidant effects of *Allium schoenoprasum* (AS) extract against carbon tetrachloride (CCl_4) toxicity in rats. The in vivo experimental procedure was established as follows; Group 1, control (C) animals; Group 2, carboxymethylcellulose (CMC); Group 3, CCl_4; Group 4, Legalon (LGL, silymarin); Groups 5, 6 and 7 were treated with AS (100, 200 and 400 mg kg^{-1}) extract only. Groups 8,9 and 10 were administered AS+CCl_4. On the 11th day of the experiment, the animals were sacrificed and analyzed. According to the histopathological and biochemical analysis results; It was found that AS extract reduced tissue damage in a dose-dependent manner against CCl_4 toxicity. When antioxidant and oxidative biomarkers were examined, positive effects of LGL and AS were observed in correcting the changed oxidant/antioxidant balance status due to oxidative stress caused by CCl_4. The results showed that AS had a dose-dependent hepatoprotective effect against CCl_4-induced liver toxicity and it was evaluated that this effect might be due to its antioxidant activity.

Keywords: *Allium Schoenoprasum* L; Antioxidant; Carbon tetrachloride; Rat; Liver.

Introduction

The liver is a vital organ in the body that regulates different functions such as metabolism, secretion, storage and detoxification (1,2). Liver damage usually begins to occur as a result of disruption of these functions. The mortality rate due to liver diseases is increasing worldwide (3). The factors that cause these diseases can be caused by alcohol abuse, obesity, diabetes and exposure of the liver to xenobiotics, and liver fibrosis that develops due to these factors has become a global health problem that has increased in prevalence in developing countries and millions of people have been affected (1,3). This situation shows that liver diseases are increasingly a serious health problem and have become one of the important causes of morbidity and mortality all over the world (3,4).

Carbon tetrachloride (CCl_4) is a non-flammable, volatile organic solvent with a clear and sweet aromatic odor (5). Today, it is used as an insecticide, disinfection of grains, cooling devices, degreaser, fire extinguishers and dry cleaning agent (6). CCl_4 is known to cause liver toxicity in living things (humans and animals) (5). CCl_4 induces oxidative stress, causing lipid peroxidation in liver cells (7). This condition can also lead to fatty liver, cirrhosis, fibrosis, and even the formation of cancer. Therefore, it is one of the toxic agents commonly used to induce liver damage in experimental animal models (7–9).

The free radicals released together with the increase in oxidative stress in the body, the understanding that they may affect the formation of various diseases, has led to an increased interest of researchers in these radicals. Oxidative stress is

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a biochemical condition that creates intermediates such as reactive oxygen and nitrogen species as a result of the reaction of nutrients with oxygen to meet the energy needs of the organism. Overgrowth of these intermediates causes oxidative damage. It is considered as one of the mechanisms that contribute to pathological diseases in organs such as the liver (10). Increasing free radical production in the organism contributes to the formation of diseases such as nervous system, circulatory system and cancer by disrupting the structure of lipids, DNA, carbohydrates and proteins (11).

Antioxidants are compounds that inhibit the damage they can cause in the organism by excessive production of free radicals. Antioxidants may not be available in sufficient amounts in the body. Therefore, it can be supplemented from herbal sources with high antioxidant potential to maintain the oxidant/antioxidant balance. The vitamins C and E, carotenoids, flavonoids and tannins in the content of these plants can be used to scavenge excess free radicals in the human body (12).

The therapeutic use of plants is as old as human history. People have understood the therapeutic power of plants and have benefited from them to maintain a healthy life (13). *A. schoenoprasum* (AS), which is one of the plants used for food and therapeutic purposes among the people, is a perennial plant species that grows in Europe, Asia and North America (14,15). In traditional folk medicine, it is used to treat infections of the upper respiratory tract, chest diseases and cancer (16–18). In addition, it has been reported that the AS plant has antibacterial (19), antioxidant (14), antifungal (17), antihypertensive (20), and anticancer (21) effects.

In this study, the antioxidant and liver-protective activity of AS plant will be tested in the CCl_4-induced liver damage model in rats, and biochemical and histopathological analyses will be attempted to elucidate it.

Materials and Methods

Chemicals; CCl_4, (Sigma-Aldrich, 289116), Ketamine hydrochloride (Ketalar, 10%, 100 mg/1ml, injectable solution Pfizer Pharma GMBH, Germany), Xylazine hydrochloride (Alfazyne®, 2%, Alfasan International, 3440 AB, Woerden, Holland), Carboxy methyl cellulose (CMC) (Sigma-Aldrich, 21902), Legalon fort (Madaus GmbH Cologne- Germany), TAS and TOS kit (Rel Assay Diagnostics, Gaziantep, Turkey), Ethanol 99% (Sigma).

Plant material and extraction process; The AS plant was collected in the mountainous parts of the Van province of Turkey. The plant material was recorded in the Van Yuzuncu Yil University (YYU) Science Faculty Biology Department Herbarium after the necessary identification procedures were performed (SM. Pınar 7357). It was then dried in the shade and stored in a suitable environment for study.

After 270 g of shade-dried AS plants were ground and powdered in an electric mill, they were kept in 4 liters of 80% ethanol for 3 days and mixed. It was then passed through Whatman filter paper and evaporated in ethanol at 50 ^{o}C in a rotary evaporator. The obtained ethanolic plant extract was placed in falcon tubes and kept at -80 ^{o}C for 5 days. Then, it was lyophilized in a lyophilizer device at -80 ^{o}C for 48 hours (Extract: 49 g, yield 18.2% w/w). The extract obtained was dissolved in 0.5% CMC solution and the plant doses (100, 200 and 400 mg kg^{-1}) to be applied to the animals were determined by revising the study of Mushtaq et al., (22).

Animals and Experimental Protocol: In the experiments, 70 Wistar albino female rats weighing 170-210 g were used. The rats were fed with standard pellet feed at room temperature adjusted to 22 ± 2 ^{o}C, which was illuminated with a rhythm of 12 hours of light and 12 hours of darkness in the research center. Rats were housed in standard plastic cages with free feed and water intake. Before the start of the study, approval was obtained from the Local Ethics Committee (2017/07).

In the pharmacological activity study, the dose of CCl_4 (1 mL kg^{-1}), CMC (10 mL kg^{-1}) and Legalon (50 mg kg^{-1}) to be administered to rats was determined by modifying liver damage models (23–26). 10 Groups were created so that there were 7 animals in the experimental groups. According to the experimental protocol, the groups were divided into; Group 1, Control (C); Group 2, CMC; CMC is used as a solvent in the food and pharmaceutical industries (27). The rats in this group were administered 0.5% CMC solution (10 mL kg^{-1}) orally by intragastric gavage for 9 days. On the 10th day, a single dose of olive oil (1 mL kg^{-1}) was administered intraperitoneally (ip). Group 3, CCl_4; The rats were mixed with CCl_4 (1 mL kg^{-1}) and olive oil at a ratio of 1:1 and administered as a single dose on the 10th day (ip). Group 4, LGL; contains 173.0-186.7 mg of thistle fruit extract, equivalent to 140 mg of Silymarin.

Based on traditional use and literature, it is used as a herbal preparation to protect the liver (23). LGL (50 mg kg-1) was suspended in 0.5% CMC solution in an ultrasonic bath and magnetic stirrer, as in the other test groups, and given to the animals by gavage. Groups 5, 6 and 7; Only different doses of 100, 200 and 400 mg kg^{-1} of AS plant were administered intragastrically, respectively. Groups 8, 9 and 10; After different doses of the AS plant were administered, CCl_4 (1 mL kg^{-1}) 10. a single dose was administered on the day. At 24 hours (day 11) after CCl_4 administration, blood and tissue samples were taken from the rats in the groups.

Biochemical analysis; Under ketamine (90 mg kg^{-1}) anesthesia, blood was taken from the heart with the most appropriate method (exsanguination) with an injector and placed in biochemistry tubes. Then, the blood samples were centrifuged at 3000 rpm (+4°C) for 10 minutes and the supernatant phases were taken. The samples were stored at -80°C until the time they were evaluated. Liver enzyme parameters, lipid profiles and other biochemical parameters were analyzed in serum samples. In addition, the levels of oxidant/antioxidant biomarkers (total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI)) were evaluated using the method developed by Erel (28–30).

Histopathological analysis; Liver tissues of mice were placed in pathology tissue dishes. Tissues were kept in formaldehyde (10%) solution. Then the tissues were embedded in paraffin blocks and 4 μm thick sections were taken. Prepared samples were stained with hematoxylin-eosin (HE). It was then examined with a light microscope. Prepared preparations were evaluated histopathologically as absent (-), mild (+), moderate (++) and severe (+++) according to their immune positivity.

Statistical analysis; Descriptive statistics for continuous variables obtained from the groups included in our study were expressed as mean and standard deviation values. Shapiro Wilk test was used for the normality distribution of the data. In terms of continuous variables, the groups were evaluated statistically using one-way ANOVA analysis of variance in comparison with each other, and then Tukey post hoc test in determining different groups. The statistical significance level (α) was taken as 0.1%, 1% or 5% in the calculations. Descriptive statistics of the semi-quantitative data obtained in the histopathological examination were given. SPSS (IBM SPSS for Windows, ver.24) statistical package program was used for these statistical analyses.

Results

Histopathological Findings; Some criteria were determined to evaluate the damage caused by toxic agent (CCl_4) administration in liver tissue. The findings are summarized in Table 1.

According to these criteria; in Group 1 (C) and Group 2 (CMC); All of the tissue samples taken from animals in these groups show a healthy appearance. A picture consistent with hemorrhage, edema and necrosis in the liver tissues was not observed (Fig.1; A and B). Group 3 (CCI_4); Edema and mononuclear infiltration progressing from serosa to parenchyma were observed in liver tissues. Severe hydropic degeneration and coagulation necrosis were detected in the parenchyma tissue. In addition, fat vacuoles in hepatocyte cells and hyperemia in vessels were detected (Fig.1; C). In Group 4 (LGL); In tissues, mild hydropic degeneration in hepatocytes in the central region, mild hyperemia in vessels, and sinusoids slightly dilated and hyperemic were observed (Fig.1; D). Serosa and parenchyma tissues were found to have a normal histological appearance in groups 5, 6 and 7 (Fig; E, F and G). In Group 8 and Group 9; Moderate serositis in the liver and serosa, moderate severity in the parenchyma, hydropic degeneration and necrosis in the central region, and hyperemia in the vessels were observed (Fig.1; H and I). In Group 10; Mild hydropic degeneration, coagulation necrosis and mild hyperemia in the vessels were detected in the liver, and central region (Fig.1;J). Histopathological appearances of the tissues are shown in Figure 1.

Biochemical Findings; A significant difference was detected in terms of ALT (Alanine aminotransferase) and AST (Aspartate Aminotransferase). According to this; There was no significant difference in ALT and AST between C, CMC and AS groups (Groups 4,5 and 6) that were applied only to the extract ($p>0.05$). However, the CCI_4 group showed an increase in ALT and AST levels compared to the C and other groups ($p<0.001$). In particular, groups 4, 8 and 9 showed a significant decrease compared to the CCl_4 group (Fig. 2). Alkaline phosphatase (ALP) levels decreased significantly in LGL and AS-400 mg kg-1 group compared to the toxic agent group. When LDH (Lactate dehydrogenase) values were examined, a significant difference was observed in groups 9 and 10 compared to group 3 ($p<0.05$).

Table 1. Histopathological Evaluation In Liver Tissue

Groups	Hydropic degeneration in hepatocytes	Coagulation necrosis	Sinusoidal dilatation and hyperemia	Mononuclear cell infiltration in the serosa
C	-	-	-	-
CMC	-	-	-	-
CCI_4	+++	+++	+++	+++
LGL+ CCI_4 (50 mg/kg,+1 ml/kg)	+	+	++	+
AS (100 mg/kg)	-	-	-	-
AS (200 mg/kg)	-	-	-	-
AS (400 mg/kg)	-	-	-	-
AS + CCI_4 (100 mg/kg +1 ml/kg)	+++	++	+++	++
AS + CCI_4 (200 mg/kg +1 ml/kg)	+++	++	++	++
AS + CCI_4 (400 mg/kg+1 ml/kg)	++	+	++	+

C; Control, AS; *Allium schoenoprasum,* CMC; Carboxymethyl Cellulose, LGL; Legalon, CCI_4; Carbon tetrachloride

When the albumin (ALB) and total protein (TP) values were examined, the CCI_4 group showed a decrease compared to the C group, and there was an increase in ALB levels only in group 10 among the CCI_4-administered groups.

In this study, the effects of AS plant against CCl_4 toxicity on lipid profiles were also examined. When total cholesterol (TC) levels were analyzed, it was observed that TC alone decreased in groups (Groups 5,6 and 7) administered at different doses compared to group C ($p<0.05$). In addition, it was determined that the TC levels of the toxic agent group (Group 3) increased. In the groups administered CCl_4 together with AS, it was determined that a high dose of AS decreased the TC level ($p<0.05$). High-density lipoprotein (HDL) levels were found to be decreased in the CCl_4 group compared to the C group ($p<0.01$). However, no significant difference was found between the other groups. In terms of low-density lipoprotein (LDL), LDL levels decreased in a dose-dependent manner compared to both AS + CCl_4 applied groups (Groups 9 and 10) and the toxic group and only AS applied groups ($p<0.05$). In addition, very-low-density lipoprotein (VLDL) and triglyceride (TG) levels were also evaluated. Our findings showed that the CCl_4 group caused an increase in both parameters compared to the C group, and the AS high dose and LGL group were more effective. The differences between the groups are summarized in figure 3.

In our study, the levels of TAS, TOS and OSI, which are oxidant/antioxidant markers, are shown in Figure 4. Compared to group C, CCl_4 (Group 3) group caused a decrease in TAS levels and an increase in TOS and OSI levels. In addition, LGL (Group 4) and AS-400 group (Group 10) exhibited an increase in TAS levels compared to group 3 (CCl_4). In terms of TOS and OSI, a decrease was detected in LGL, AS-200 (Group 9) and AS 400 (Group 10) groups.

Discussion

It is known that drugs used in liver diseases are insufficient and controversial. For this reason, people seek remedies with alternative treatment methods in the treatment of various diseases; In this sense, they mostly prefer plants. Because herbal medicines are effective in terms of treatment and have fewer side effects and are relatively low-cost treatment options, as a result of the search for alternative medicine in the last decade, researchers' interest in plant-based traditional medicines has increased (31–33).

Carbon tetrachloride initiates its damaging effect on hepatocytes by converting its toxic metabolites

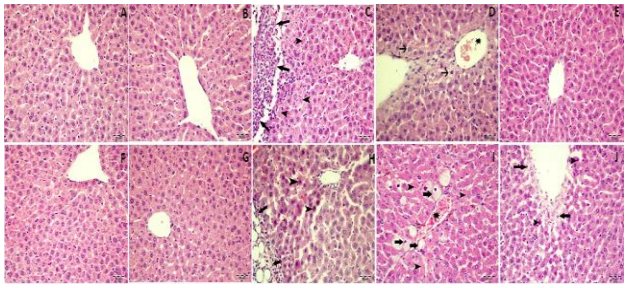

Fig. 1. Histopathological appearance of liver tissues; G: group, AS; *Allium schoenoprasum*, A; G_1-C: Control, B; G_2-CMC: carboxymethyl cellulose, C; G_3-CCl_4: Carbon tetrachloride, D; G_4-LGL: Legalon, E; G_5-AS-100, F; G_6-AS-200, G; G_7-AS-400, H; G_8- AS-100 + CCl_4, I; G_9-AS-200 + CCl_4, J; G_{10} AS-400 + CCl_4. Coagulation necrosis in hepatocytes (arrowhead), Severe mononuclear cell infiltration in serosa (arrows), severe hyperemia of the veins (stars). H&E Bar: 20µm

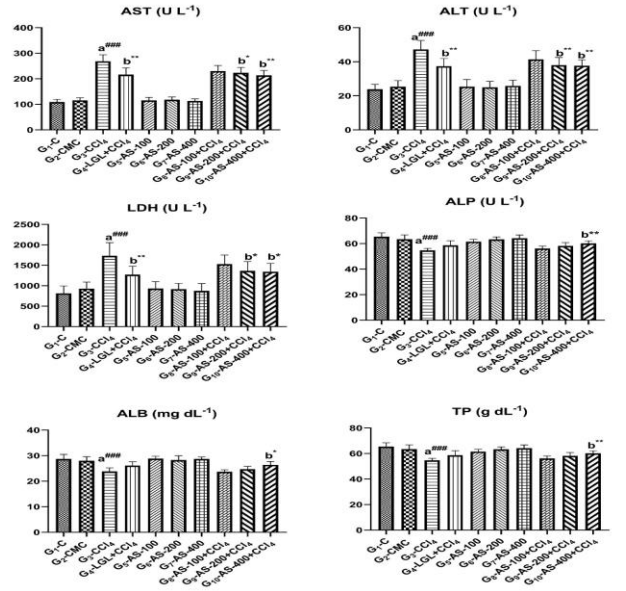

Fig. 2. $\bar{x} \pm$ SD: mean ± standard deviation, G: group, C: Control, CMC: carboxymethyl cellulose. CCl_4: Carbon tetrachloride, LGL; Legalon, AS; *Allium schoenoprasum*, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, TP: Total protein, ALB; Albumin, LDH; Lactate dehydrogenase, a: Shows the difference of the control group compared to the CMC, CCl_4 and AS groups: # p<0.05, ##p<0.01, ###p<0.001, b: Shows the difference of the CCl_4 group compared to the AS+CCl_4 groups: * p<0.05, **p<0.01, ***p<0.001.

trichloromethyl (CCl_3) and trichloromethyl peroxyl (CCl_3O_2) into free radicals that react easily with oxygen through the cytochrome P450 enzyme system in the non-granular endoplasmic reticulum. CCl_4, which reacts with unsaturated fatty acids in the cell membrane, causes lipid damage through lipid peroxidation. In addition, CCl_4 radicals directly bind to the hepatocyte cell membrane and cause cell destruction. As a result

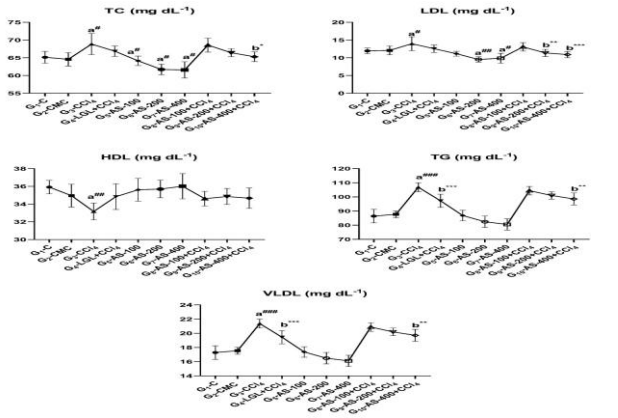

Fig. 3. $\bar{x} \pm$ SD: mean ± standard deviation, G: group, AS; *Allium schoenoprasum*, C: Control, CMC: carboxymethyl cellulose. CCl_4: Carbon tetrachloride, LGL; Legalon, AS; Allium schoenoprasum, TC: Total cholesterol, LDL: Low-density lipoprotein, HDL; High-density lipoprotein, VLDL: Very-low-density lipoprotein, TG; Triglyceride, a: Shows the difference of the control group compared to the CMC, CCl_4 and AS groups: # p<0.05, ##p<0.01, ###p<0.001 b: Shows the difference of the CCl_4 group compared to the AS+CCl_4 groups: * p<0.05, **p<0.01, ***p<0.001

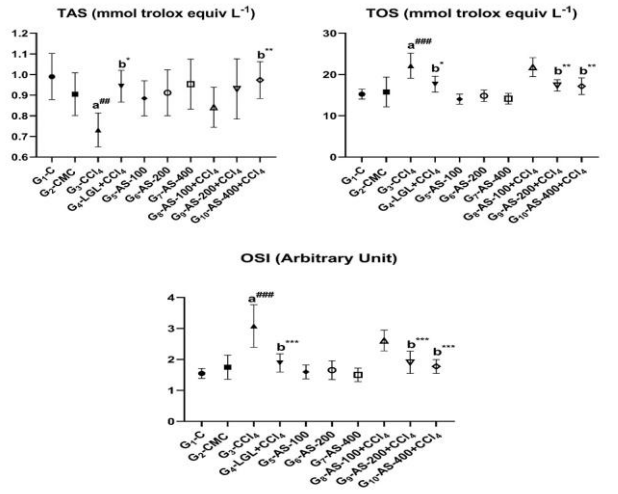

Fig. 4. $\bar{x} \pm$ SD: mean ± standard deviation, G: group, AS; *Allium schoenoprasum*, C: Control, CMC: carboxymethyl cellulose. CCl_4: Carbon tetrachloride, LGL; Legalon, AS; *Allium schoenoprasum*, TAS: Total antioxidant capacity, TOS: Total oxidant capacity, OSI; Oxidative stress index, a: Shows the difference of the control group compared to the CMC, CCl_4 and AS groups: # p<0.05, ##p<0.01, ###p<0.001 b: Shows the difference of the CCl_4 group compared to the AS+CCl_4 groups: * p<0.05, **p<0.01, ***p<0.001

of this damage, there is an increase in calcium entry into the cell and cell death occurs. Impairment of liver cell integrity by lipid peroxidation, enzymes in the cell cytoplasm pass into plasma and their levels increase (34–36).

It has been reported that CCl_4 applied at different doses causes moderate necrosis, hydropic changes

in the centrilobular area of the liver tissue, intense inflammatory cell infiltrations from macrophages and lymphocytes in the sinusoids of the central region, congestion in the central veins, cytoplasmic vacuolization in hepatocytes, and mild to severe adiposity in midzonal and peri portal hepatocytes (36,37). In previous studies, it was determined that a dose of 1 ml kg^{-1} of CCl_4 caused the aforementioned degenerations in the liver (38–41). The findings in this study support the studies mentioned above. Liver toxicity induced by the toxic agent (CCl_4) and subsequent histopathological changes, CCl_4 radicals covalently bind to the hepatocyte cell membrane and cause cell destruction (34). The resulting lipid peroxidation causes an increase in the entry of calcium ions into the cell, as well as a decrease in membrane fluidity with deterioration of liver cell integrity (36). This suggests that it may be due to the esterification of fatty acids and the accumulation of triglycerides in cells, depending on the result of mitochondrial damage in cells (hepatocytes) together with oxidative stress (42).

It was determined that the liver tissues of the groups in which only the plant extract of AS was applied (100, 200 and 400 mg kg^{-1}) had a normal histological structure. These results show that different doses of AS applied only to the plant extract did not have any toxic effects on liver tissue. LGL used in this study is a herbal preparation containing silymarin. Silymarin reduces oxidative damage due to CCl_4 toxicity with its strong antioxidant potential. These beneficial effects are shown in many studies (43–46). In the groups in which AS was administered together with CCl_4, the therapeutic efficacy of AS-400 was higher than in the low and medium-dose AS groups. It exhibited similar effects as the LGL group. The positive effect of AS on the histopathological changes in the liver, depending on the dose; may be due to its antioxidant properties due to its free radical scavenging effect (15,47). It may be related to sulfur compounds which have an antibacterial effect (14,48). In addition, it can be said that it may be related to its anti-inflammatory activity by reducing oxidative stress and inhibiting phagocytosis (17).

In liver necrosis, cell integrity is impaired and ALT and AST enzymes in the cytoplasm pass into the plasma (36). In different studies, they reported that CCl_4's ALT and AST levels increased 2 times compared to C groups (36,49,50). In this study, it was determined that serum ALT level increased approximately 2 times and serum AST level increased approximately 3 times in the CCl_4 group compared to control and only AS extract applied groups. It was determined that the ALT and AST enzyme levels in the groups of AS administered with CCl_4 and in the LGL group decreased compared to the group that was administered only CCl_4. In the literature review, no studies were found on the therapeutic effect of the AS plant in liver damage models induced by CCl_4 or other hepatotoxic agents. In the phytochemical analysis studies of the AS plant, flavonoid components of quercetin, kaempferol, anthocyanin, vitamin E and selenium were detected in the plant (14,15). These components are components with known hepatic protective activity in experimental models of liver injury and they are reported to reduce ALT and AST enzyme levels (51–53). The results of our experimental study are compatible with the literature. It was determined that AS-200 and AS-400 decreased enzyme levels. According to these results; It is thought that AS can be effective on enzyme levels by reducing oxidative stress in liver damage depending on the dose. One of the markers of liver necrosis is ALP enzyme levels. ALP is an enzyme found in the bile ducts and its level is elevated in hepatobiliary diseases (54,55). It has been shown in different studies that there is an increase in plasma ALP levels in liver damage induced by CCl_4 (56–58). In this study, it was determined that there was an increase in ALP level due to the toxic agent. The occurrence of lipid peroxidation as a result of CCl_4-induced liver damage, disruption of lysosomal balance in cell membranes or increased cell permeability may cause an increase in plasma ALP levels. It has been reported that the therapeutic effect of AS can be prevented by inhibiting the damage that may occur as a result of metabolic detoxification reaction in vulnerable target cells of organosulfide components, especially alilisulfide and flavonoids, in the content of the plant, as reported by Timite et al., (59). ALB, which is one of the biochemical markers in the determination of liver disorders, is one of the proteins synthesized in the liver. Changes in TP levels are mostly due to changes in albumin concentration (60). It has been reported in different studies that plasma TP and ALB levels decrease in liver necrosis induced by CCl_4 (38,61,62). In this study, it was determined that there was a decrease in TP and ALB levels in liver damage induced by CCl_4. In studies with Allium species, it has been reported that *Allium sativum* (garlic) increases the decreased TP levels in a lead-induced hepatotoxicity model (63). Increasing doses of AS caused an increase in TP and ALB levels. Our study is compatible with the literature. LDH is a colorimetric cytotoxicity assay indicator

that measures membrane integrity. LDH level is higher in damaged cells compared to normal cells. LDH is found in the heart, brain, kidneys, liver, and skeletal muscle (64,65). It was determined that the LDH enzyme levels, which were increased compared to the CCl_4 applied group, decreased statistically significantly. Medium and high doses of AS showed similar efficacy to LGL. These results suggest that AS may be due to its antioxidant effect, that Allium species have a protective effect against the oxidant effects of CCl_4 on liver cells, and that the liver damage preventive effect of allium species that are consumed regularly may reduce the increased serum LDH level as a result of liver cell damage (15,17,66,67). It has been reported in different studies that CCl_4 causes an increase in plasma TC and TG levels in liver necrosis caused by different doses in rats (68–70). In this study, it was determined that there was a significant increase in TC and TG levels. It is interpreted that the increase in TC and TG values may be due to the decrease in protein synthesis, esterification of fatty acids and decreased excretion of cellular lipids (71). In a previous study on AS, it was reported that the rich phenolic and flavonoid content of AS was effective in reducing TC and TG at a significant level (22). It is also known that CCl_4 raises the level of LDL, VLDL and lowers HDL (43,72,73). Our study exhibited similar effects to the study on AS by Mushtag et al. (2016). As a result of a single dose administration of CCl_4, cell damage in the liver tissue and the passage of cellular enzymes into the blood can occur. It is reported that lipid peroxidation, which occurs as a result of oxidative stress, is responsible for this situation (74,75). In this study, a limited number of studies were available on serum TAS, TOS and OSI levels in CCl_4-induced liver damage. It is known that CCl_4 decreases TAS and increases TOS and OSI levels (76,77). Our study is consistent with the findings of the researchers. In a study, conducted to evaluate the enzymatic antioxidant activity in the head, stem and leaves of AS with the DPPH Method, it was reported that AS increased the activities of antioxidant enzymes (SOD, CAT, GSH) and decreased the amounts of MDA, $O2^-$ and OH^- radicals. In addition, they determined that all parts of the plant have antioxidant effects, and the parts with the highest antioxidant capacity are the leaves (47). For this reason, the leaf part of the plant was used in our study. A high dose of AS was effective in significantly increasing TAS and reducing TOS and OSI. It shows that AS has antioxidant potential. This antioxidant effect is an antioxidant that contributes to cell repair by activating co-factor (coenzymes) enzymes due to the fact that it contains sulfur, phenol compounds, quercetin and α-tocopherol, as well as vitamins A, C, K and selenium. the feature is considered (14,17).

As a result; In the light of the histopathological and biochemical data obtained in this study, CCl_4 caused an increase in plasma enzyme levels due to the damage caused to rat livers. In addition, it was determined that CCl_4 caused increases and decreases in oxidant/antioxidant biomarkers as a result of oxidative stress caused by damage. On the other hand, it was observed that plasma enzyme levels of AS, which is an indicator of liver damage, decrease in a dose-dependent manner compared to CCl_4 and provide therapeutic effects on antioxidant defense systems. It is assumed that the protective effect of AS against liver toxicity may be due to its antioxidant properties. Detailed studies at the molecular level are needed to support the therapeutic effects of the AS plant.

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