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INVESTIGATION OF THE RELATIONSHIP BETWEEN *HOMALOTHECIUM SERICEUM* (HEDW.) SCHIMP. AND CU/ZN SOD ENZYME IN RAT TISSUES WITH THE IMMUNOHISTOCHEMICAL METHOD

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ABSTRACT

In this study, the aim was to determine the production of Cu/Zn SOD (SOD1) enzyme in the heart, liver, and pancreas of rats administered *Homalothecium sericeum* extract. The effect of the use of moss extract has not been studied for Cu/Zn SOD enzyme production in different tissues of rats or for the cytotoxic effects on these tissues. In our study, 50 mg/kg (Group I, n: 6), 100 mg/kg (Group II, n: 6), 300 mg/kg (Group III, n: 6) and 500 mg/kg (Group IV, n: 6) doses of moss extract and 1 ml of distilled water (Group V, n: 6) were administered by gavage to 30 female rats for 30 days. Cu/Zn SOD enzyme immunoreactivity was investigated in liver, heart and pancreas tissues depending on the increase in moss extract dose. To determine Cu/Zn SOD immunoreactivity, after the application of Cu/Zn SOD primary antibodies using immunohistochemical staining methods, DAP was used as coloring and hematoxylin was used as inverse dye. The stained tissue samples were evaluated with an image analysis system under the research microscope and the study used the Kruskal-Wallis Test, one of the nonparametric tests, to detect the differences between the groups statistically.

KEYWORDS:
Immunohistochemistry, Cu/Zn SOD (SOD1), moss, antioxidant

INTRODUCTION

Bryophyta are more primitive than fern and flowering plants though higher in evolutionary terms than algae and fungi. Bryophytes, the "amphibians of the plant kingdom", are taxonomically placed between Thallophytes and Pteridophytes and subdivided into Bryophyta or mosses with 14,000 species, Marchantiophyta or liverworts comprise almost 6000 species and Anthocerotophyta or hornworts include 300 species [1]. Mosses are developed living tissues in the realm of plants without transmission roots, real roots, stems, and leaves. Owing to rhizoids, which are root-like structures, they attach to their environment and obtain the water they need. These properties cause these plants to be very easily and directly affected by environmental conditions. Mosses are bioindicator organisms capable of accumulating metals at a high rate. Due to these properties, they are used regularly for monitoring metal accumulation in large areas. Since ancient times, mosses have been used in China and India to make herbal medicines. Mosses are rich in oligosaccharides, polysaccharides, sugars, alcohols, amino acids, fatty acids, aliphatic components, aromatic, and phenol components. Few of these are medically effective and only certain species can be used in this sense. Mosses contain compounds with antioxidant, antimicrobial and anti-tumoral activity, and antimicrobial and antifungal studies of bryophytes include a relatively small number of anticancer studies among all anticancer studies [2, 3].

Environmental factors related to food include food contaminated with heavy metals and peptides, human consumption of frozen products or genetically modified foods, sweeteners added to foods, and so on. Cell damage may occur because of the exposure of our organs to oxidants. To prevent this damage, a lot of experimental research has been done especially in the field of alternative medicine. The first defense against free radicals in the organism occurs with the SOD (superoxide dismutase) enzyme. The physiological function of this enzyme is to protect cells metabolizing oxygen against the harmful effects of the superoxide free radical. SOD catalyzes the conversion of superoxide to less toxic H_2O_2 (hydrogen peroxide). Since the Cu/Zn SOD enzyme, which is found in cell cytoplasm and some mitochondria, produces H_2O_2, it works in collaboration with H_2O_2 removal enzymes [4].

Determining the dose-dependent increase in the Cu/Zn SOD enzyme by administering different doses of moss will reveal whether moss extract can be used in the pharmaceutical industry. As a result of this research, the aim is to determine whether moss

extract affects the release of Cu/Zn SOD enzyme with known antioxidant properties. In our study, the aim was to determine the production of Cu/Zn SOD enzyme in liver, pancreas and cardiac tissues of rats fed with extracts obtained from *Homalothecium sericeum* species and to determine the toxic effect on tissues of heavy metal accumulation in this moss species.

MATERIALS AND METHODS

Copper/zinc superoxide dismutase (SOD1) is a primary antioxidant enzyme that removes superoxide anion radicals. Superoxide dismutase (SOD), a group of metal-containing enzymes, have a vital antioxidant role in human health and scavenge superoxide anions, one of the reactive oxygen species. SOD is among the first lines of defense in the detoxification of products resulting from oxidative stress. This study includes determination of Cu/Zn SOD enzyme production in the liver, heart and pancreas tissues of rats fed with extracts from moss. In this situation, different doses of moss extracts were given to rats by gavage, then Cu/Zn SOD enzyme production in the specified organs was determined by immunohistochemical staining methods. Plant material was collected from Karabiga and Bayramic (Canakkale, Turkey) in May 2018 and identified by Özlem Tonguç Yayıntaş, Çanakkale Onsekiz Mart University. A voucher specimen was deposited at the herbarium of our department.

Preparation of the extracts. Fresh gametophytic samples of *H. sericeum* were treated with 0.8% Tween 80 aqueous solution to remove the epiphytic hosts normally found on the surface, extensively washed in tap, and distilled water, and dried on filter paper at room temperature. Extraction procedures were applied as described elsewhere [5]. The flour-form material was be treated with methanol to 10 ml/g in the dark for 24 hours. The extraction was carried out in flasks with cap, at room temperature and by shaking. The moss sample was treated again with methanol until the extract was discolored and filtered through filter paper. Methanolic moss extracts were administered to groups of rats at doses of 50 mg/kg, 100 mg/kg, 300 mg/kg and 500 mg/kg.

Animal Groups:

First group (G1, n: 6); 1 ml of distilled water for 30 days (gavage);

Second group (G2, n: 6); 50 mg/kg moss extract every day for 30 days (gavage)

Third group (G3, n: 6); 100 mg/kg moss extract every day for 30 days (gavage)

Fourth group (G4, n: 6); 300 mg/kg moss extract every day for 30 days (gavage)

Fifth group (G5, n: 6); 500 mg/kg moss extract every day for 30 days (gavage)

Histopathological Examination. Animal model in our study lasted one month. At the end of the period, rats were anesthetized with rompun and ketas and the liver, heart, and pancreas of rats were removed, trimmed, and placed in tissue transport cassettes for 24 hours. Tissue samples were passed through graduated alcohol solutions to remove water from the tissue; xylene alcohol in the tissues was cleaned, tissue samples were kept in paraffin in the oven after being blocked in base mode, blocked tissue samples were cut to microtome thickness of 3-5 microns and placed on slides in preparation boxes. Tissue samples taken from each subject and cut to a thickness of 5 microns were treated with hematoxylin-eosin (H&E) staining.

Immunohistochemical Examination. Immunohistochemical reactions were performed according to the ABC technique. First, endogenous peroxidase activity was inhibited by exposing the specimens to 3% hydrogen peroxide in distilled water for 30 minutes. After washing the sections in distilled water for 10 minutes, the binding of nonspecific antibodies was diluted by 1:4. Then, the specimens were incubated with PBS in normal goat serum (DAKO X 0907, Carpinteria, CA). Following this step, the sections were incubated with polyclonal rabbit anti-superoxide dismutase (Cu/Zn SOD1, dilution 1:50, Enzo Life Sciences) for 1 hour, and sections were washed for 3x3 minutes in phosphate-buffered saline at room temperature. Then, the sections were incubated with biotinylated anti-mouse Immunoglobulin-G (DAKO LSAB 2 Kit, İnvitrogen) and then, they were washed with phosphate-buffered saline for 3x3min. Following this step, the sections were incubated with ABC complex (DAKO LSAB 2 Kit). Then, the sections were washed for 3x3 minutes in phosphate-buffered saline. Next 3, 3-Diaminobenzidene tetrahydrochloride (DAB, Invitrogen Corporation) solution was placed on the tissues as a chromogen substance and samples were left for 5 min in the dark. At the end of this period, DAB solution was poured over the tissue samples and then they were passed through deionized water. For background staining, samples were left in Mayer's hematoxylin for 5 minutes and washed in tap water for 10 minutes. Then, they were passed through xylene and graduated alcohols and covered with a coverslip using entallan (Bio Mount, Bio-Optica) [6].

Evaluation of tissue samples and statistics. During the evaluation of the results, immunoreactivity was evaluated with the H-score method, calculating the ratio of immunopositive cells to all cells in the selected fields. Immunoreactive cell count was performed by a blinded observer and graded as follows: 0 denoted no staining; 1 denoted weak; 2 denoted moderate; and 3 denoted strong staining in a specified field. The respective score was then calculated using the following formula: H-score = (%

stained cells at 0) x 0 + (% stained cells at 1+) x 1 + (% stained cells at 2+) x 2 + (%stained cells at 3+) x 3. The H-score value varied from 0 to 300 [7]. SPSS 15 version was used for statistical evaluation of the results obtained with this formula. To determine the differences between Cu/Zn SOD immunoreactivities between groups, the Kruskal –Wallis Test, a nonparametric test, was used. Differences with $p<0.05$ between the groups was considered significant.

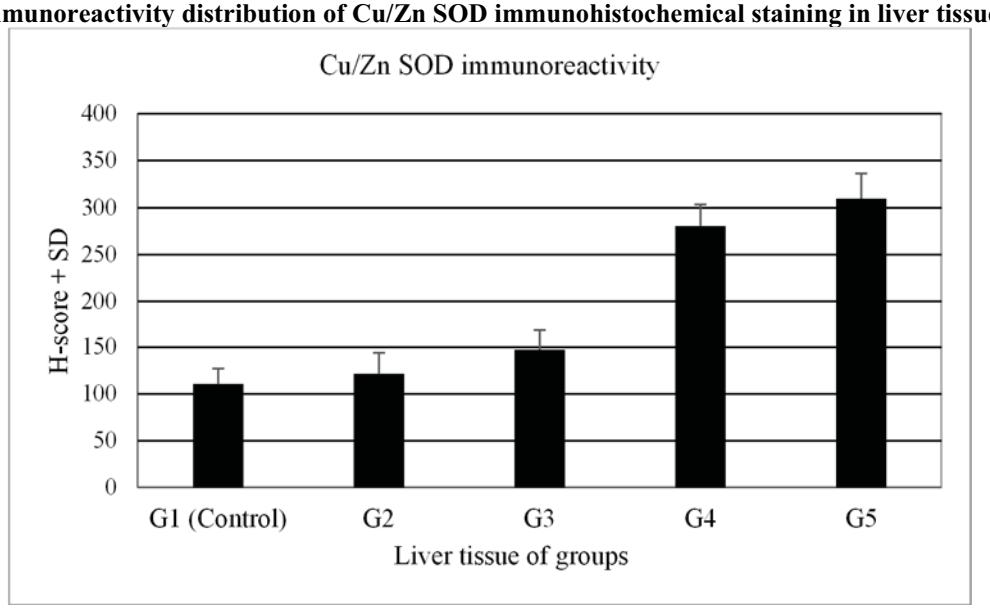
RESULTS

Liver tissue findings. Histological staining with routine hematoxylin-eosin showed no histopathological findings in the liver tissues of rats in the first group. There were no histopathologic findings except for vena centralis and portal vein dilatations in the liver tissues of the second and third groups of rats administered moss extract. The dominance of portal lobule structure was like that of the control group. There were no inflammatory cell clusters in the portal and central areas of the liver tissues in the second and third groups. Moderate mononuclear cells caused inflammation in the parenchyma of the liver and around the portal areas in groups four and five where the dose of moss extract was increased, and congestion in the central and portal vein was increased. Especially, congestion was found to be greater between the sinusoids and in the portal area in liver tissue of the fifth group (Figure 1 and Table 1). There was also a series of hepatocytes leading to necrosis. Histopathological tissue content characterized by hepatocyte vacuolization was observed. Immunohistochemical staining for Cu/Zn SOD showed positive immunoreactivity parallel to the dose increase of moss extract. In the first group, low reactivity was observed around the central vein. Immunoreactivity was found to be moderate in the central vein and portal areas in the second and third groups and Cu/Zn SOD immunoreactivity was severe in liver tissues belonging to fourth and fifth groups. Statistically, a significant difference was observed between the control group (G1) and the fifth group ($p<0.0001$). There was no significant difference between the control group and the second (G2) group with $p>0.05$. When the other groups were compared with each other, the highest significance was seen between G1 and G5, and the least significant difference was between G2/G3 and G4/G5 (Figure 2; Table 2-3).

TABLE 1
Histopathological evaluation of liver tissue samples

Groups Parameters	G1 (Control) 1 ml DW	G2 (moss extract) 50 mg/kg	G3 (moss extract) 100 mg/kg	G4 (moss extract) 300 mg/kg	G5 (moss extract) 500 mg/kg
Congestion	-	-	+	++	+++
Dilatation	-	+	+	++	++
Inflammation	-	-	-	+	++

TABLE 2
Immunoreactivity distribution of Cu/Zn SOD immunohistochemical staining in liver tissues



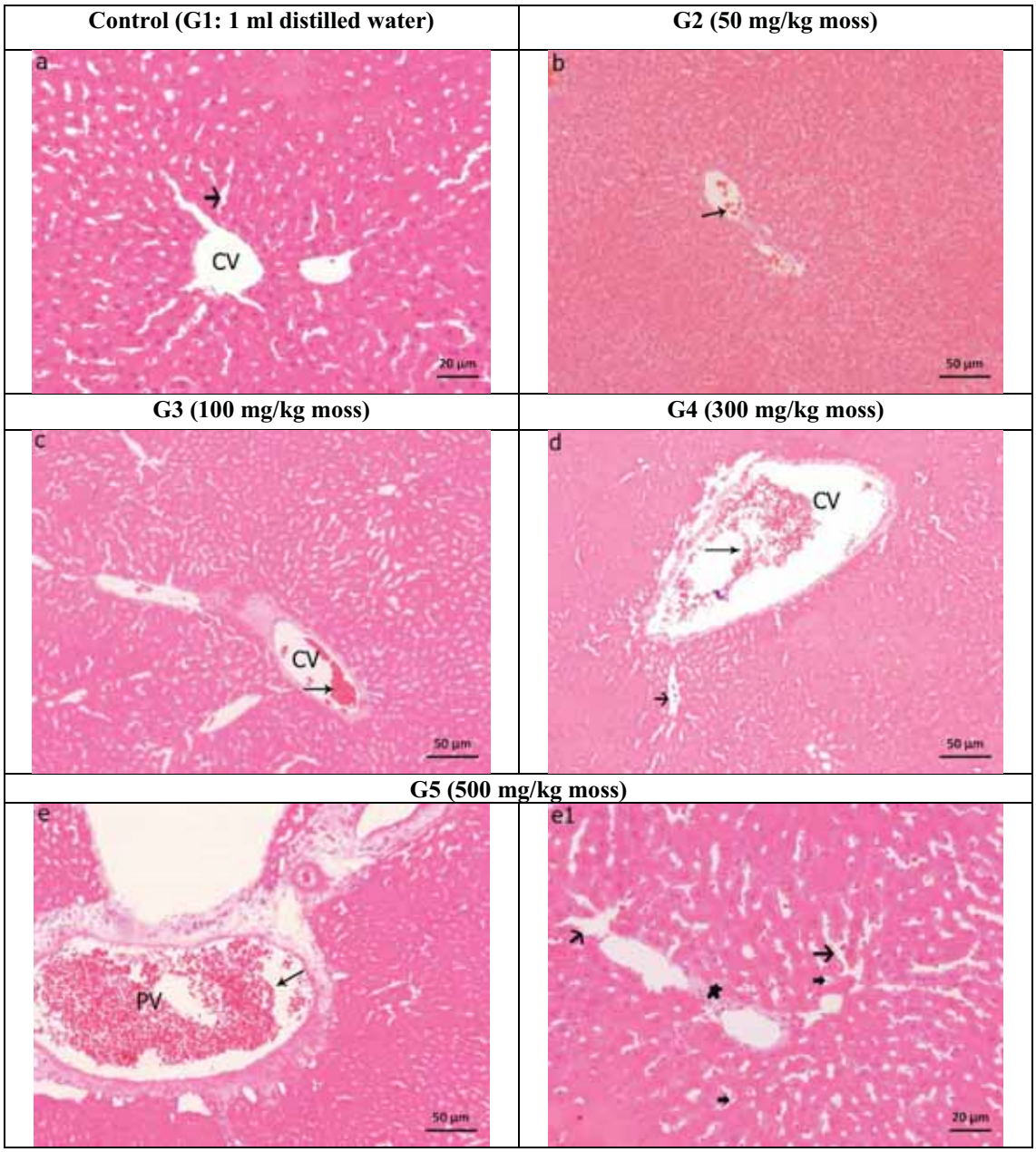


FIGURE 1

a) Control group (G1) liver tissue (CV: Central vein, short arrow: sinusoid), b) Liver tissue in G2 (long arrow: congestion), c) Liver tissue in G3, X100 (long arrow: congestion), d) Liver tissue in G4 (long arrow: congestion, short arrow: sinusoidal dilatation), e) Liver tissue in G5 (PV: Portal vein, long arrow: congestion), e1) Liver tissue in G5 (thin arrow: sinusoidal dilatation, thick arrow: hepatocyte vacuolization, star: inflammation of portal area)

Pancreatic tissue findings. Hematoxylin-eosin histological staining in the control group did not show any histopathological findings in the pancreas of rats. It was also observed that the diameter of Langerhans islets, which constitute the endocrine part of the pancreas tissues, were reduced, pyknotic cells increased, and also the exocrine tissue formed by the acinar structures and edema occurred in the fourth and fifth moss extract groups. Normal endocrine and exocrine structures were observed in the pancreas tissue of G2 and G3 groups of rats administered moss extracts. No histopathological site was found (Figure 3). Cu/Zn SOD immunohistochemical staining in the pancreas showed that immunoreactivity was significantly increased in rats treated with moss extract compared to the control group who were not given moss and statistically significant differences were found between the moss groups in terms of dose. In the control group, more positivity was detected in exocrine gland cells; in the second and third groups, immunity increased in both exocrine and endocrine islet cells. In

the fourth and fifth groups, Cu/Zn SOD immunoreactivity was also found to be severe. There was a statistically significant difference between the control group and the fifth group ($p<0.0001$). The control group and the second (G2) moss group differed, but no significant difference was observed. When the other groups were compared with each other, the most considerable significance was seen between G1 and G5 and the least significant difference was between G2 and G3 (Figure 4, Table 4 and Table 5).

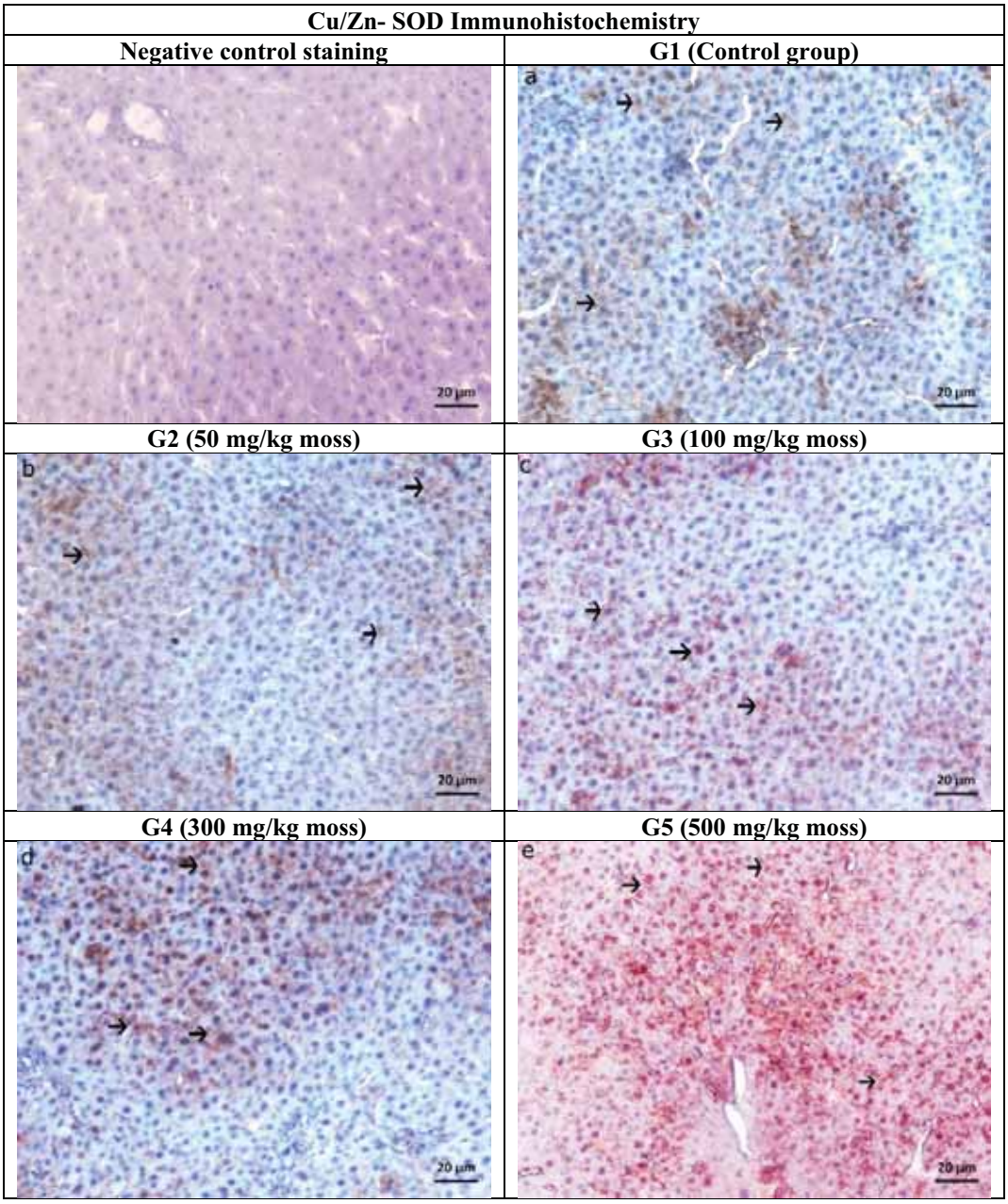


FIGURE 2

Liver tissue of control group Cu/Zn SOD immunohistochemical negative control staining, a-) Control group (G1) liver tissue Cu/Zn SOD immunohistochemistry positive staining (arrow: immunoreactivity), b-) Liver tissue in G2 Cu/Zn SOD immunohistochemistry positive staining (arrow: immunoreactivity), c) Liver tissue in G3 Cu/Zn SOD immunohistochemistry positive staining (arrow: immunoreactivity), d) Liver tissue in G4 (arrow: severe immunoreactivity), e) Liver tissue in G5 (arrow: severe immunoreactivity)

TABLE 3
Statistical comparison between groups

	G1	G2	G3	G4	G5
G1	-	p>0.05	*p<0.05	***p<0.001	****p<0.0001
G2	p>0.05	-	*p<0.05	**p<0.01	***p<0.001
G3	*p<0.05	*p<0.05	-	**p<0.01	***p<0.001
G4	***p<0.001	**p<0.01	**p<0.01	-	*p<0.05
G5	****p<0.0001	***p<0.001	***p<0.001	*p<0.05	-

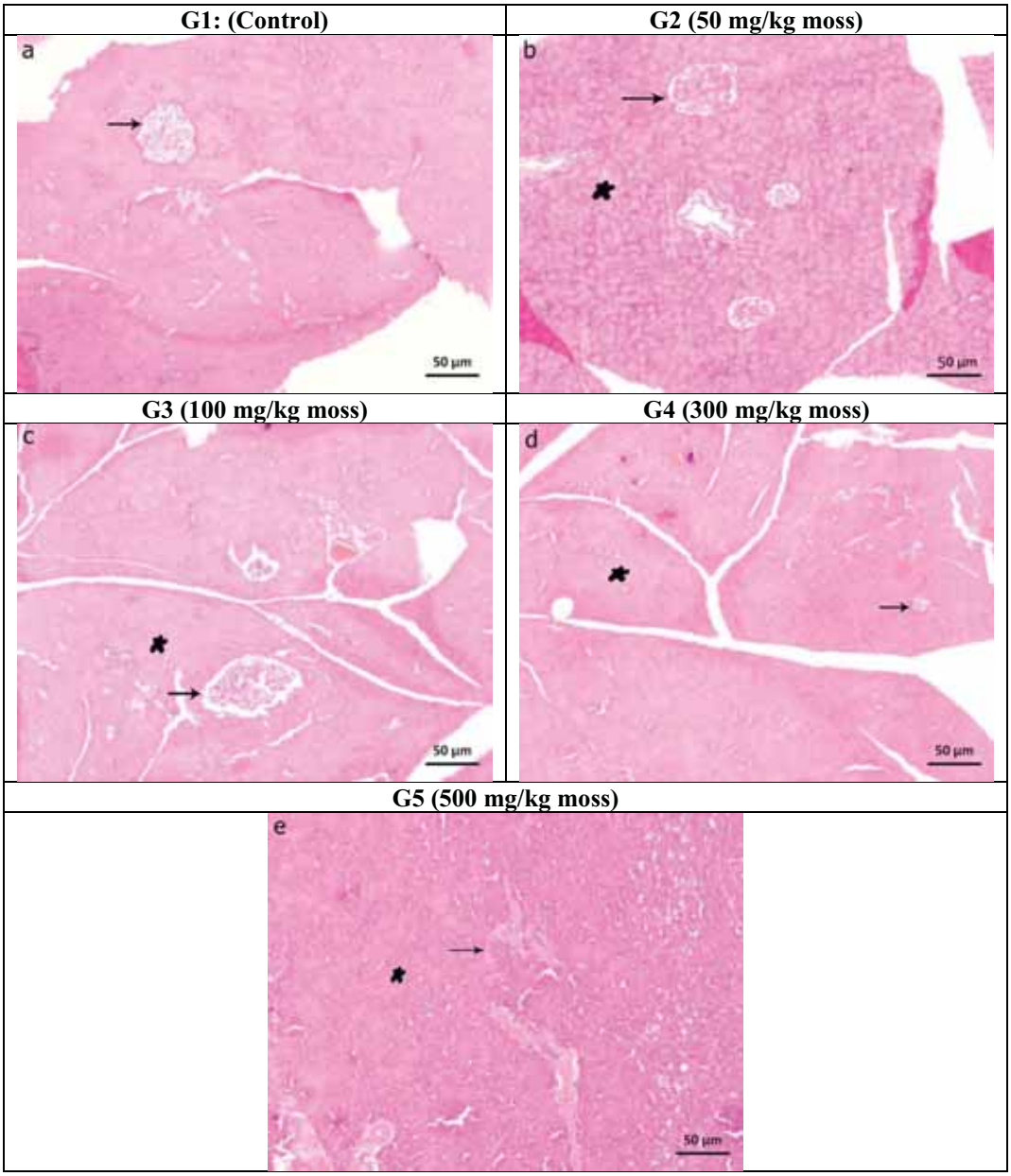


FIGURE 3
a-) Control group (G1) pancreatic tissue (arrow: Langerhans islet, star: exocrine gland), b-) Pancreatic tissue in G2, c-) Pancreatic tissue in G3, d-) Pancreatic tissue in G4 (arrow shrunken endocrine islet, star: exocrine gland e-) Pancreatic tissue in G5 (arrow: hemorrhage around the endocrine islet, star: exocrine gland)

Cardiac Tissue Findings. Histopathological staining with hematoxylin-eosin showed no histopathological myocardial tissue in the cardiac tissue of rats in the first group. Although myofibrillary deposits were observed in the myocardial layer of the fourth and fifth groups, no evidence indicating cardiomyopathy was observed. Congestion in the coronary vessels of the cardiac tissues that did not develop carditis is noteworthy. Sections from the cardiac tissue of the second and

third groups had similar histological appearance. It was observed that the myofibrillar structure began to take shape in cardiac tissue samples apart from the control group and cardiac pathology emerged in the groups with long-term moss extract administration (Figure 5).

Immunohistochemical staining of Cu/Zn SOD in cardiac tissue showed that moss extract increased myocardial immunoreactivity compared to the control group without moss extract. While mild positivity was detected in cardiomyocytes in the control group, increased immunity was observed in the second and third groups. As the dose of moss increased, the intensity of immunoreactivity increased to the highest level in the fourth and fifth groups (Figure 6). Histological scoring showed statistically significant differences between the control group and the fourth and fifth groups ($p < 0.0001$). Significance was found as $p<0.01$ between the control group and the second (G2) moss group (Table 6 and Table 7).

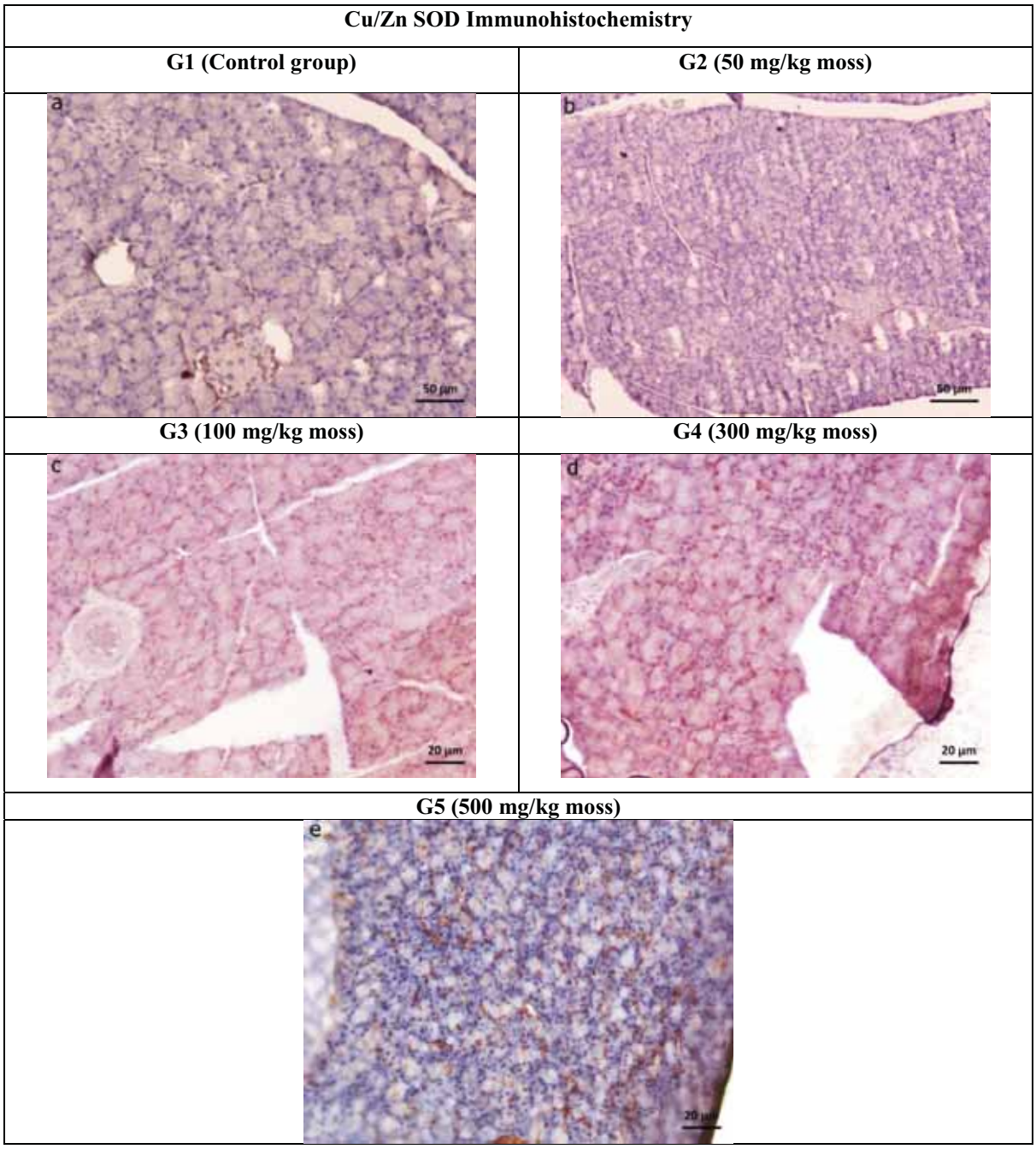


FIGURE 4
Immunohistochemical distribution of Cu/Zn SOD in pancreatic tissue

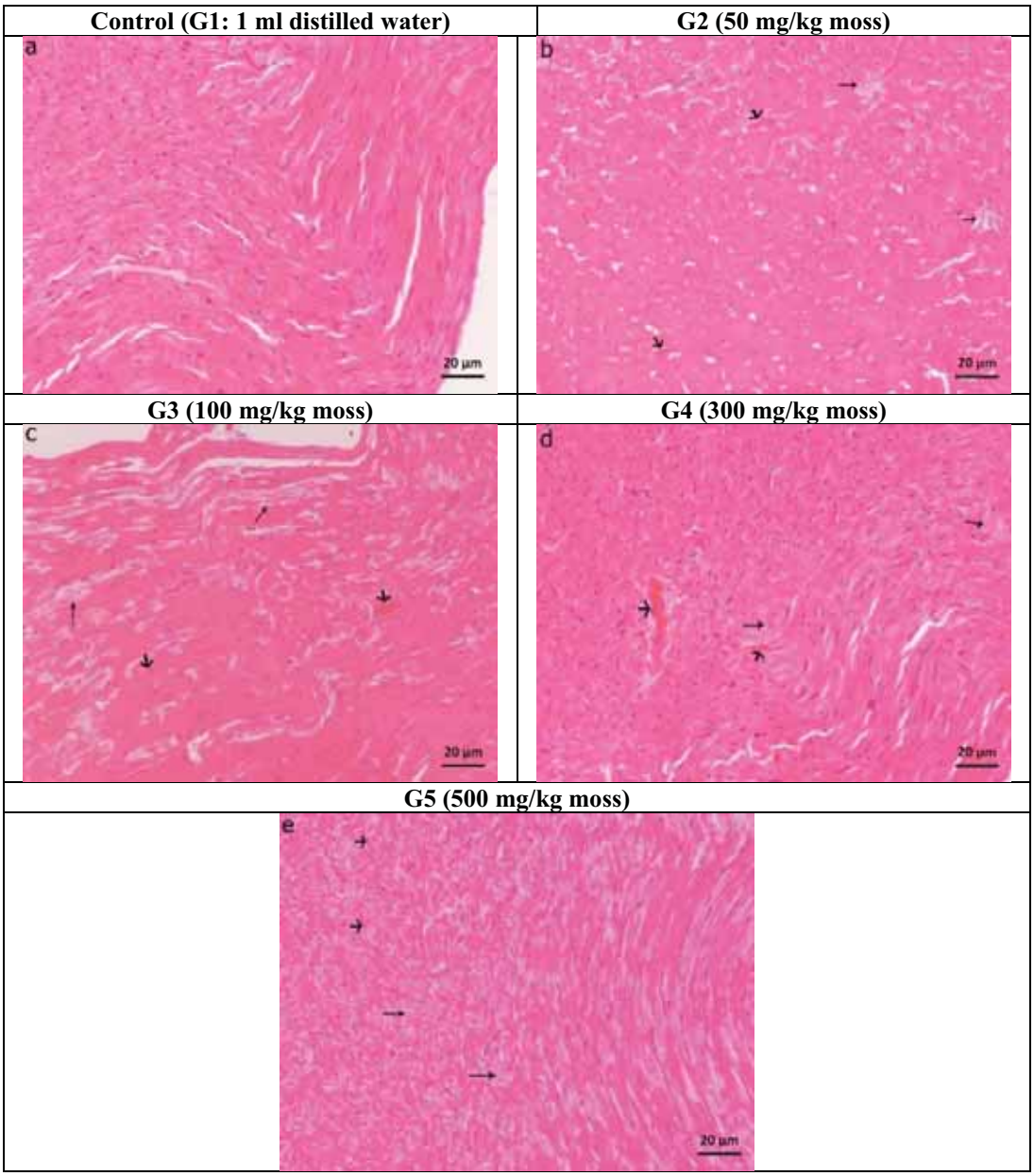


FIGURE 5

a-) Control group (G1) cardiac tissue, (Langerhans islet, star: exocrine gland), b, c, d, e) Heart tissue of the other experimental groups (long arrow: myofibrillary area, short arrow: congestion)

TABLE 4

Immunoreactivity distribution of Cu/Zn SOD immunohistochemical staining in pancreatic tissues

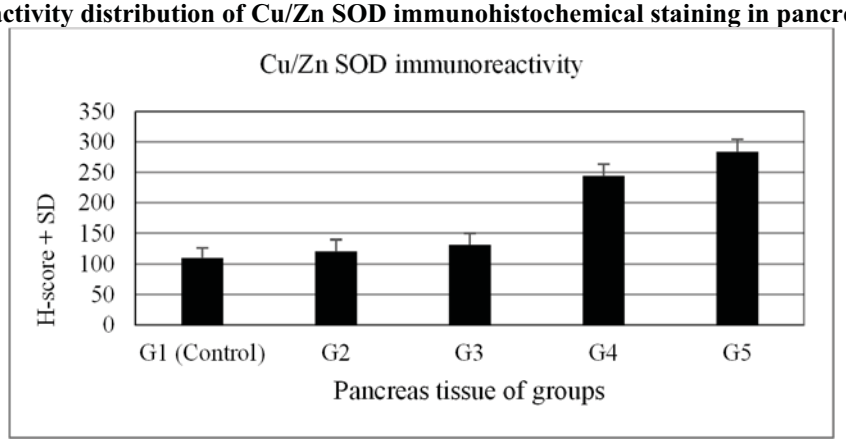


TABLE 5
Statistical comparison between groups

Groups	G1	G2	G3	G4	G5
G1	-	p>0.05	*p<0.05	***p<0.001	****p<0.0001
G2	p>0.05	-	**p<0.01	**p<0.01	***p<0.001
G3	*p<0.05	**p<0.01	-	***p<0.001	***p<0.001
G4	***p<0.001	***p<0.001	***p<0.001	-	*p<0.05
G5	****p<0.0001	***p<0.001	***p<0.001	*p<0.05	-

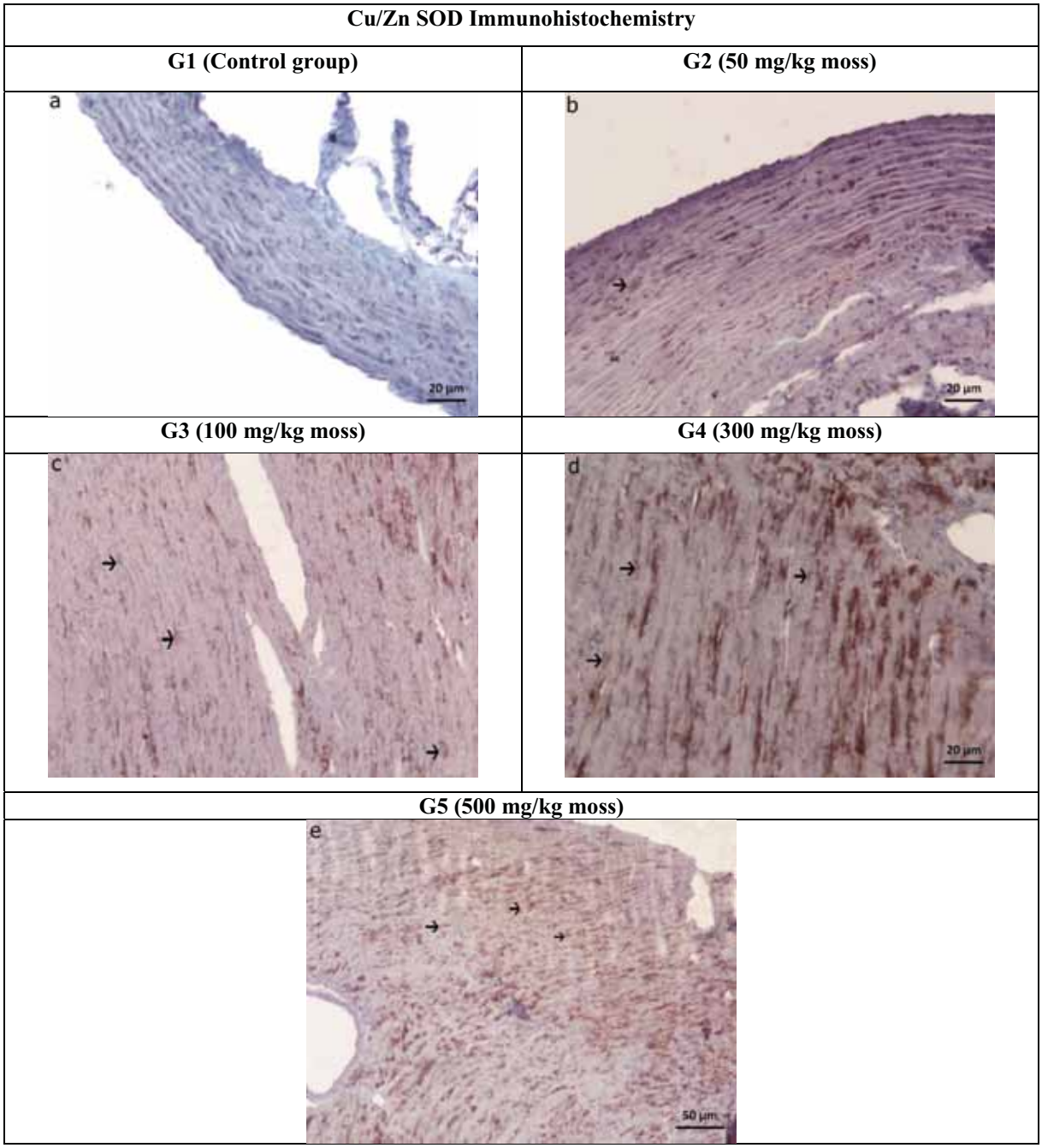


FIGURE 6
Immunohistochemical distribution of Cu/Zn SOD in cardiac tissue (arrow: immunopositive cells)

TABLE 6
Immunoreactivity distribution of Cu/Zn SOD immunohistochemical staining in cardiac tissues

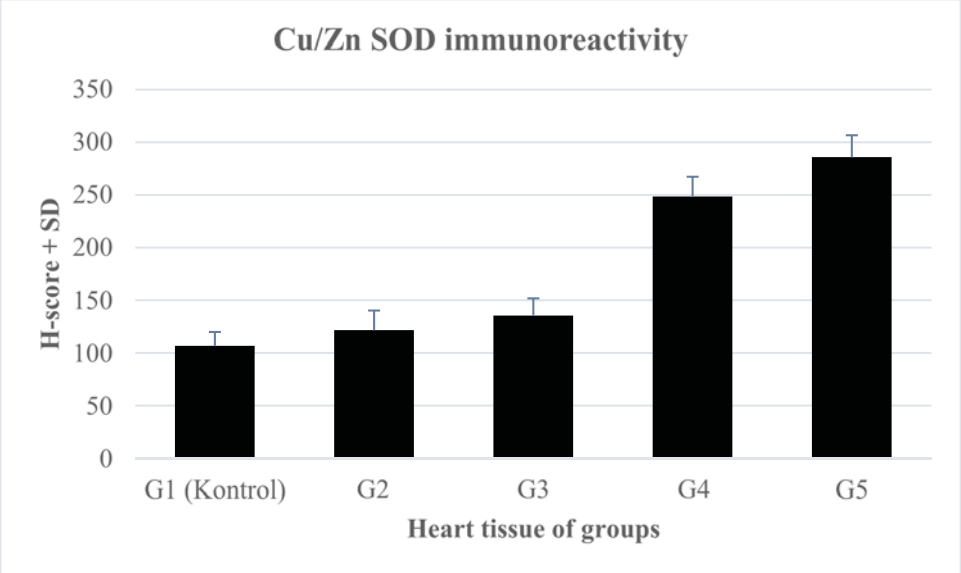


TABLE 7
Statistical comparison between groups.

Groups	G1	G2	G3	G4	G5
G1	-	**p<0.01	**p<0.01	***p<0.001	****p<0.0001
G2	**p<0.01	-	*p<0.05	***p<0.001	****p<0.0001
G3	**p<0.01	*p<0.05	-	***p<0.001	***p<0.001
G4	***p<0.001	***p<0.001	***p<0.001	-	*p<0.05
G5	****p<0.0001	****p<0.0001	***p<0.001	*p<0.05	-

DISCUSSION

Oxidative stress is defined as the disruption of the balance between free radicals and antioxidants that have a counteracting effect in biological systems. Reactive oxygen species are molecules with high reactivity to a considerable extent. They occur because of normal metabolism in cell organelles, particularly mitochondria, or due to causes such as radiation, high oxygen pressure, inflammation, ischemia-reperfusion, aging and exposure to chemical agents [8, 9]. Oxidation/reduction disorders in cells are toxic to all components of the cell including protein, lipid, DNA and peroxidase and free radical production. Also, some reactive oxygen metabolites act as intracellular messengers in oxidation/reduction events. Thus, oxidative stress may disrupt the normal intracellular signal transduction. Oxidative stress is responsible for the pathogenesis of many diseases, including cancer, atherosclerosis, inflammatory disorders, diabetes, cardiovascular and neurological diseases [10, 11].

Free radicals are very unstable and reactive molecules in biological systems, electrons easily interact with other molecules in the cell and cause oxidative stress. During normal cellular metabolism, free radicals may form. A variety of external factors can also lead to the emergence of free radicals. Disruption of the pro-oxidant and antioxidant balance in the organism is defined as oxidative stress. Radicals can cause damage to nucleic acids, lipids, and proteins, which are essential cellular components. The damage is known to occur in various diseases such liver was studied in experimental animals or damage was induced in the liver using chemicals [16]. In this study, we focused on liver histology and SOD activity in groups where we administered different doses of moss extracts without causing any damage to the liver. The results of the study showed that histopathological changes occurred in groups given increased doses of moss extract but also increased SOD immunoreactivity.

Superoxide dismutase (SODs) constitutes a very important antioxidant defense against oxidative stress in the body. The enzyme acts as a good therapeutic agent against diseases associated with reactive oxygen species. SOD has therapeutic effects in various physiological and pathological conditions such as cancer, inflammatory diseases, cystic fibrosis, ischemia, aging, rheumatoid arthritis, neurodegenerative diseases and diabetes. However, the enzyme has some limitations in clinical applications. Mn-SOD and Cu/Zn-SOD are SOD isomers in the body. Mn-SOD is a SOD free radical scavenger containing Mn^{+4} as a center of activity and found in mitochondria. Cu/Zn-SOD is another SOD free radical scavenger present in the cytoplasm with Cu^{+2} and Zn^{+2} as the activity center [17]. The liver and heart are organs with most mitochondria. After alcohol-induced liver damage, Mn-SOD activity decreases according

to the results of our study. Cu-Zn-SOD can eliminate the toxic effect and thus protect the stomach tissue [18]. Mn-SOD and Cu-Zn-SOD can help prevent liver damage by inhibiting free radicals in the body [19]. Our findings show that the moss species *H. sericeum* can be used to prevent liver damage if this plant is not given in high doses. Small-leaf Kuding tea is a traditional Chinese tea rich in polyphenols. In a study with this tea extract, small-leaf Kuding tea was found to affect D-galactose-induced oxidative aging in mice by considering changes in skin, liver, and spleen using biochemical and molecular biology techniques in experimental animals. Biochemical analysis showed that tea polyphenol extract increased the thymus, brain, heart, liver, spleen, and kidney function indices in model mice. Also, superoxide dismutase (SOD) was shown to inhibit the decrease in glutathione peroxidase (GSH-Px) levels. As a result, pathological evaluation indicated that D-galactose reduces oxidative damage in the liver and spleen. Fe-SOD, Mn-SOD, CAT, and HO, nuclear factor erythroid 2 related factors 2 (Nrf2), γ-glutamylcysteine synthetase (γ-GCS) and NAD (P) H dehydrogenase 1 (NQO1) mRNA expression was determined. SOD1 was found to increase protein levels (Cu/Zn-SOD) and SOD2 (Mn-SOD) [26].

Twenty-three liverwort and 184 moss species were analyzed in studies conducted by the US-based National Cancer Research Institute. As a result of the study, the antitumoral effect of 43 species was found to be effective at different concentrations and 75 species were not suitable for use due to toxic effects. In this research, the most intensely studied families of mosses were *Brachytheciaceae, Dicranaceae, Grimmiaceae, Hypnaceae, Mniaceae, Neckeraceae, Polytrichaceae* and *Thuidiaceae*. *Marchantia polymorpha* (T1), a liverwort, showed high extraction yield and antioxidant activity. Similarly, high activity was detected in *Hypnum cupressiforme* (T9) and *Neckera complanata* (T10) species. The antioxidant capacity of the tested species often sheds light on other activities. *Thuidium tamariscinum* was positive against *Acinetobacter haemolyticus* ATCC 19002 bactericin; *M. polymorpha* and *Isothceum myurum* were positive against *Bacillus subtilis* TCC6633 bacteria and all other species were negative. Following these results, research concentrated on the most active species and antitumoral tests were carried out. Again, fractioned polar isolate of *M. polymorpha* (T1) was the only antiproliferative species against HeLa and A549 lung cancer cells [20].

In vivo animal experiments with blueberry extract showed that blueberry regenerates the liver of mice with liver damage, reducing levels of values such as AST and ALT, and reducing serum cytokine IL-6, TNF and IFN levels in mice. Also, blueberry was shown to increase SOD activity. After treatment with the highest concentration in mice with injury induced using carbon tetrachloride, standard treatment findings were reported. mRNA expression in liver tissue was shown to regulate Cu/Zn-SOD, Mn-SOD, and chloramphenicol acetyltransferase (CAT) expression levels and low regulated cyclooxygenase (COX)-2 expression. Blueberries were shown to have a good preventive effect on liver damage in mice [21].

The protective effect of lavender oil was examined in mice with hepatic and nephrotoxic effects induced by malathion. In this study, it was shown that oxidative stress increased with depletion of MDA and hydrogen peroxide levels, sulfhydryl group content (-SH) and antioxidant enzyme activity. Increased Cu/Zn-SOD, Mn-SOD, and Fe-SOD values were reported in the kidney and liver. More importantly, it was shown that malathion-induced body weight loss, liver, and kidney relative weight increase, hemodynamic and metabolic disorders as well as hepatic and renal oxidative stress were eliminated by lavender therapy. In conclusion, lavender had potential hepato- and nephroprotective effects against oxidative stress caused by malathion in mice. This beneficial effect may be partly associated with antioxidant properties [22].

Cu/Zn SOD activity was found in tissues such as liver and kidney in studies with various plant extracts, which is like the findings in our study. The increase in histological damage due to the increase in dose in our study contradicts the findings of these and similar studies. *H. sericeum*, a type of moss, caused histopathology in many important tissues, especially the liver and kidney, with increased doses. It was also observed to provide defense by triggering stronger antioxidant activity. These results show that anti-cancer and antitumoral studies will be more effective if the dose of moss extract used is not high. Active compounds responsible for the antimicrobial effects present in many bryophyte species were identified. For example, it was stated that some liverwort extracts such as polygodial from *Porella*, and *Conocephalum conicum*, and Lunularin from *Lunularia cruciata* are not only effective fungicides and bactericides, but also have a weak biocide (stomach poison) effect against harmful insects [23]. The findings of moss studies show that the antibacterial and anticancer properties are quite high due to the flavonoid content. Experimental in vivo studies appear to be very limited. It was determined in the literature that the species we worked with have not been used in experimental animal models. In this respect, the contribution of the study to the literature is considerable. Further studies will contribute to the use of moss extracts in medical treatments with more specific findings using molecular techniques. Especially by considering the anticancer properties, it is possible to develop prodrugs which will have less cost and work in shorter duration for cancer treatments, which are rapidly increasing day by day.

Ischemia and reperfusion studies with cardiac tissue reported that myocardium produces ROS,

which plays an important role in reperfusion damage. ROS production causes Ca^{+2} entry, infiltration of inflammatory cells, platelet activation, NO production, metabolic changes, and endothelial dysfunction. The activity of antioxidant enzymes was regulated according to cellular requirements from production and can be induced or inhibited by endogenous factors. The main antioxidant enzymes include SOD isoforms. This enzyme has three isoforms, Cu/Zn-SOD located in the cytoplasm, Mn-SOD in the mitochondrial matrix and fro-SOD located in plasma. Elevation of SOD isoforms after treatment with Kerkede herb tea may contribute to the reduction of superoxide (O_2-) in the heart of rats with a metabolic disease model, which may in part help improve heart function [24]. Immunohistochemical staining methods are very important for the detection of molecules secreted from cells in the tissue. The number of histopathological investigations on these subjects is negligible.

In our study, immunohistochemical staining methods were used to determine the presence of antioxidant enzymes and cellular localization in different tissues. In our study, because of routine histological staining, the most histopathological picture occurred in liver tissues. Damage to the cardiac and pancreas tissues was determined to be less than in the liver. The moss species used in the study, *H. sericeum*, was not previously used in this type of in vivo study. Moreover, in vitro cell cultures were generally preferred in studies with mosses. Studies showed that mosses contain compounds with antibacterial and anticancer properties. From this point of view, if the histopathological picture related to the increase in dose is ignored, it is considered that the application of *H. sericeum* at 50 and 100 mg/kg dose is nontoxic and will be useful to evaluate anticancer properties. It was determined that over 300 mg/kg dose had toxic effects on the liver. Higher doses were shown to damage normal tissues. It was found that Cu/Zn SOD activity varies in different tissues and with different doses in the first defense mechanism against oxidative stress caused by the deterioration of oxidant-antioxidant balance.

In conclusion, it is a fact that new medicinal, herbal, and biochemical products are needed for alternative therapies. However, to develop these products, the path chosen is preferred to involve less cost and faster results. The importance of our study is that it is the first in vivo study with *H. sericeum* species, its effects were shown in different tissues at different doses and the dose limit that could be toxic was determined. Later studies will aim to determine the specific effects of this species through projects designed in vitro and in vivo.

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Ethics Statement. A total of 30 female Wistar albino rats, weighing 290–310 g, were used in the study. The study protocol was approved by Çanakkale Onsekiz Mart University Ethics Committee for Animal Research (Protocol number: 2018-03).

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