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INVESTIGATION OF HEPATOTOXIC EFFECT OF BRYOPHYTES (*HOMALOTHECIUM SERICEUM* (HEDW) SCHIMP.) ON RAT LIVER

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ABSTRACT

No evidence has been found to determine whether moss extract affects fibrotic, inflammatory, apoptotic and tissue homeostasis for tissue regeneration on rat's liver tissue. In our study, 1 ml of distilled water (Group I), and 50 mg/kg (Group II), 100 mg/kg (Group III), 300 mg/kg (Group IV), 500 mg/kg (Group V) for 30 days' doses of moss and by gavage of a total of 30 female rats in liver tissue due to the increase in the dose of moss histopathology was revealed. Also, immunohistochemical staining was performed to determine the immunoreactivity of transcription factor nuclear factor Kappa-B (NF-κB), tumor necrosis factor (TNF-α), which regulates inflammation and immune response, and TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) for the assessment of the apoptotic process. The stained tissue samples were evaluated with the image analysis system under the research microscope and One-way ANOVA, Tukey test, which is one of the nonparametric tests, was used to determine the differences between the groups statistically and the results were evaluated according to $p \leq 0.05$. We found that liver damage increased due to the increased dose of moss species used in the experiment. At the same time, antioxidant activity was increased, and apoptosis was increased. Considering these results, it was concluded that algae extract would have anticancer properties and could be used in the pharmaceutical industry.

KEYWORDS:
Immunohistochemistry, Apoptosis, TUNEL, TNF-α, NF-κB, Bryophytes

INTRODUCTION

Bryophyta section, which is higher in evolutionary terms than algae and fungi, is more primitive than fern and flowering plants; 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 with almost 6000 species and Anthocerotophyta or hornworts having 300 species [1]. *Homalothecium sericeum* (Hedw.) Schimp. is moderately robust, glossy, yellowish green to golden brown, occurring in dense rough mats or patches, mainly on bark of trees and on bare rocks. Sometimes it grows on man-made habitats such as walls and roofs, and it is common in Europe [2]. Bryophytes have been widely used as medicinal plants to cure cuts, burns, boils, abscesses, fractures, ringworm, convulsions, pneumonia, tuberculosis, uropathy and neurasthenia in many countries [3]. The medicinal use of bryophytes has been recorded in Chinese traditional medicine for thousands of years, and contain compounds with antioxidant, antimicrobial and anti-tumoral activity, and antimicrobial and antifungal studies on bryophytes seem to have a relatively small number of anticancer studies compared to anticancer studies [4].

Mosses are developed living tissues of the realm of plants without transmission roots, real roots, stems, and leaves. Owing to the rhizoids, which are root-like structures, they attach to the environment and provide the water they need from their environment. These properties cause these plants to be directly affected by environmental conditions very easily. Mosses are bio indicator organisms capable of accumulating metals at a high rate. Due to these properties, they can be used regularly for monitoring metal accumulation in large areas. Since ancient times, mosses have been used in China, India, and Indians to make herbal medicine. 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 on bryophytes seem to have a relatively small number of anticancer studies compared to anticancer studies [4].

Luteolin is one of the most important flavonoids found in bryophytes. This active agent can inhibit angiogenesis, triggering apoptosis, preventing carcinogenesis in animal models, reducing tumor growth in vivo, sensitizing the cytotoxic effect of certain anticancer drugs administered to tumor cells,

and chemotrophic potential [5]. [6] investigated the antimicrobial and antiproliferative properties of *H. sericeum* (Hedw.) Schimp. extract C from moss was found to be effective on C6 cells. After a period of 24 hours; A concentration of 85 µg / mL reduced the survival of cancer cells by 39% and a concentration of 170 µg / mL by 86%. In terms of biological activity, studies with mosses focus more on antimicrobial activity.

In this study, it was aimed to determine the adverse effects of the rats fed with the extract obtained from *H. sericeum* species on liver tissue. Depending on the dose of the extract obtained from *H. sericeum* species, signs of negativity in the cells of the liver tissue: inflammatory inflammation, disruption of homeostasis, oxidative stress and apoptosis have led to results that could shed light on many researchers, including us, for more comprehensive studies. Also, it will be able to speed up the studies for the use of plants such as moss containing natural antioxidant compounds in the pharmaceutical and food industry. In our study, besides many positive effects (antibacterial, antitumoral) of mosses, histopathological effects on liver tissue were revealed.

MATERIALS AND METHODS

Plant material was collected from the Karabiga and Bayramiç (Canakkale, Turkey) May 2018 and identified by Dr. O. Tonguc Yayıntas, Canakkale Onsekiz Mart University. A voucher specimen was deposited at the herbarium of our department.

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 the Canakkale Onsekiz Mart University Ethics Committee for Animal Research (Protocol number: 2018-03).

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 [7]. The flour-form material will be treated with methanol to 10 ml/g in the dark for 24 hours. The extraction will be carried out in flasks with a mouth cap, at room temperature and with shaking. The moss sample will be treated again with methanol until the extract is discolored and filtered through filter paper. Methanolic moss extracts will be administered to groups of rats designated as 50 mg/kg, 100 mg/kg, 300 mg/kg and 500 mg/kg.

Animal Group

The first group (n: 6); 1 ml of distilled water for 30 days (gavage)

The second group (n: 6); 50 mg/kg moss every day for 30 days (gavage)

The third group (n: 6); 100 mg/kg moss every day for 30 days (gavage)

The fourth group (n: 6); 300 mg/kg moss every day for 30 days (gavage)

The fifth group (n: 6); 500 mg/kg moss every day for 30 days (gavage)

Histopathological Examination. The animal model study of our study took one month. At the end of the period, the liver, heart, kidney, adrenal gland, pancreas, and ovaries of rats anesthetized with rompun and ketas were removed and 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 were cleaned, tissue samples were kept in paraffin in the oven after being blocked in base mode, blocked tissue samples with microtome thickness of 3-5 microns were cut and placed on the slide was placed on the preparation boxes. Tissue samples taken from each subject and cut to a thickness of 5 microns were treated with Haematoxylin-Eosin (H&E) staining was applied.

Immunohistochemically Examination. Immunohistochemically reactions were performed according to the ABC technique described. First, the 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 a polyclonal nuclear factor Kappa-B (NF-κB p50, Abcam, dilution 1:50), tumor necrosis factor (TNF-α, Abcam, dilution 1:50) for 1 hour, 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 placed for 5 min. period of time in the dark. At the end of this period, DAB solution was poured over the tissue samples and passed through deionized water. For background staining, it was kept in Mayer's Hematoxylin for 5 minutes and washed in tap water for 10 minutes. It was then

passed through xylene and graduated alcohols and covered with a coverslip using entallan (Bio Mount, Bio-Optica) [8, 9].

TUNEL assay. Terminal deoxynucleotide transferarasile dUTP nick end labeling (TUNEL) method detects fragmentation in the DNA nucleus, was used in situ during apoptotic cell death, the apoptosis detection kit (TdT Fragel DNA Fragmentation Kit, Cat No QIA33, Calbiochem, USA). All reagents listed below and the manufacturer's instructions below. Five-millimeter thickness testis sections were deparaffinized in xylene and rehydrated with an ethanol series rated as previously described. They were then incubated for 20 minutes with 20 mg / mL of proteinase K and rinsed in tris-buffered saline. Endogenous peroxidase activity was inhibited by incubation3% hydrogen peroxide. The sections were then incubated for 10 min to 30 min with the equilibration buffer, then the TdT enzyme in a humidified atmosphere at 37 ° C for 90 min. Then, pre-warmed working power for 10 minutes at room temperature was placed in stop/wash buffer and incubated with blocking buffer for 30 minutes. Each step was separated by extensive washings in tris-buffered saline. Labeling was elicited using diaminobenzidine tetrahydrochloride, contrast staining was carried out using methyl green and the sections were dehydrated, cleared and mounted [8]. TUNEL staining were scored semi-quantitatively to determine the number of positive staining, none (-), weak (+), moderate (++), high (+++), very high (++++). These analyses were performed in 2 sections for each animal, at a rate of X40 magnification for at least 10 different regions per section.

Evaluation of tissue samples and statistics. During the evaluation of the results, the immunoreactivity was evaluated with the H-score method, calculating the ratio of immunopositivity 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 weakly; 2 denoted moderate; 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+) x3. The H-score value varies from 0 to 300 [10]. SPSS 15 version will be applied for statistical evaluation of the results obtained with this formula. To determine the differences NF-κB and TNF-α immunoreactivities between groups, Kruskal –Wallis Test, which is one of the nonparametric tests, will be used. $p<0.05$ Difference between the groups will be 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 vena centralis and portal veins dilatations in the liver tissues of the second and third groups of rats with 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 of the second and third groups. It was observed that 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 mosses was increased, and congestion in the central and portal vein was increased. Especially the liver tissue of the fifth group, congestion was found to be greater between the sinusoids and in the portal area (Figure 1 and Table 1). There was also a series of hepatocytes leading to necrosis. Histopathological tissue content characterized by hepatocyte vacuolization was observed. Immunohistochemically staining with Cu/Zn SOD showed a positive immunoreactivity parallel to the dose increase in 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 the liver tissues belonging to IV and V. groups. Statistically, a significant difference was observed between the control group (G1) and the fifth group of subjects ($p<0.0001$). 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 (Table 2).

TABLE 1
Histopathological evaluation of liver tissue samples

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

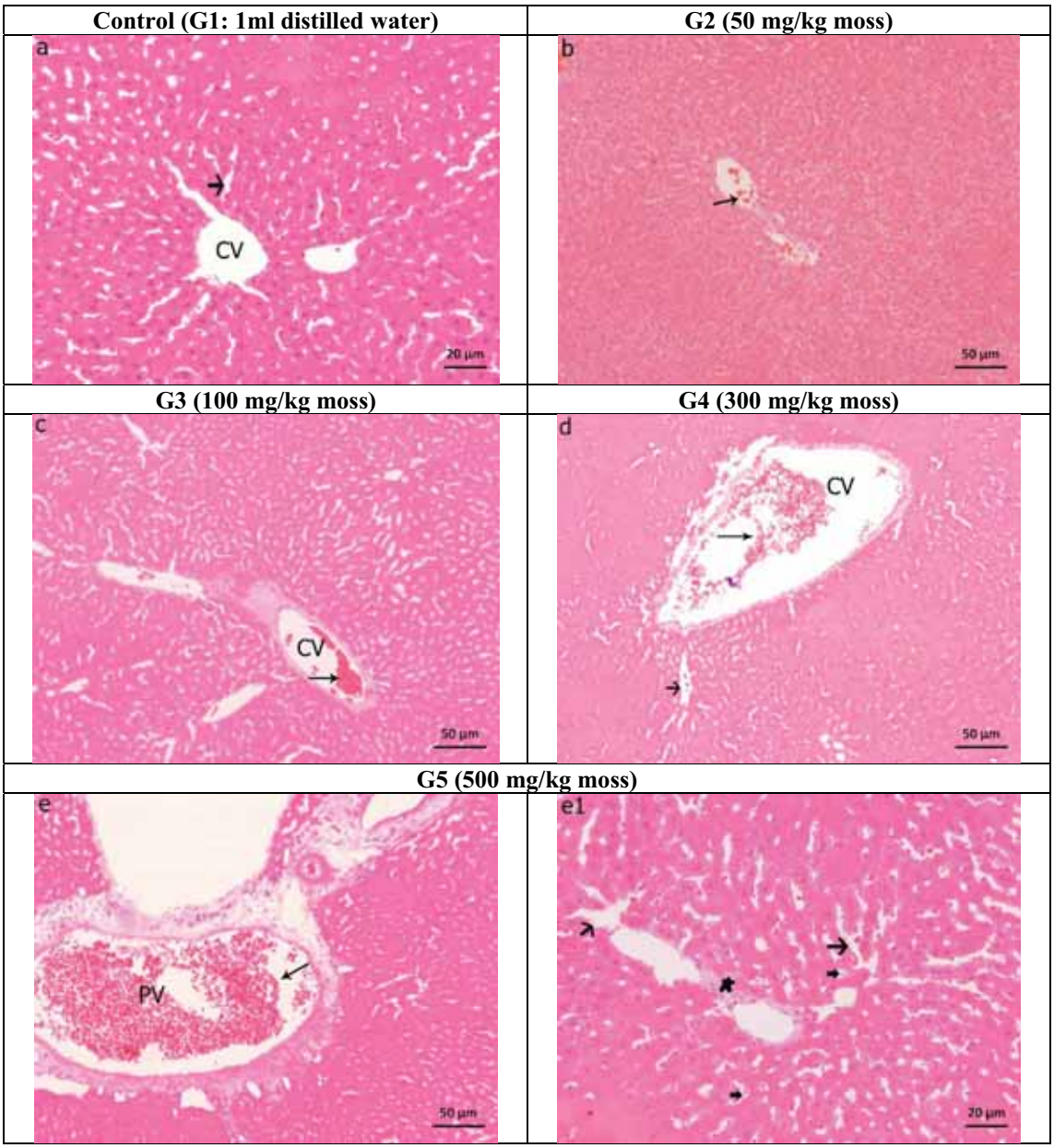


FIGURE 1

a) Control group liver tissue, magnification x20 (CV: Central vein, short arrow: sinusoid), b) Liver tissue of the second group giving moss, magnification x10 (long arrow: congestion), c) Liver tissue of the third group giving moss, magnification x10 (long arrow: congestion), d) Liver tissue of the fourth group giving moss, magnification x10 (long arrow: congestion, short arrow: sinusoidal dilatation), e) Liver tissue of the fifth group giving moss magnification x10 (PV: Portal vein, long arrow: congestion), e1) Liver tissue of the fifth group giving moss magnification x20 (thin arrow: sinusoidal dilatation, thick arrow: hepatocyte vacuolization, star: inflammation of portal area)

NF-kB and TNF-α findings. In the cell death path, activation of the nuclear factor kappa B (NF-κB) is triggered after TNF-α receptor activation. Thus, NF-κB goes to the nucleus and then NF-κB activates genes that try to block the apoptosis induced by TNF-α. In the resting cells, NF-κB is in an inactive form in the cytoplasm. TNF-α-induced NF-κB activation regulates the expression of anti-apoptotic proteins like members of the Bcl-2 family and prevents TNF-α-induced apoptosis. Also, the NF-κB pathway is a pro-inflammatory signal pathway based on NF-κB activation. NF-κB can be considered as a target for anti-inflammatory drugs. Studies have suggested that NF-κB is an inflammatory regulator and controls inflammation production, cytokines such as IL-6 and TNF-α. Immunohistochemically staining results obtained from our study, we observed that NF-κB and TNF-α expression showed higher immunoreactivity in the liver due to the increase in the dose of the mosses, and staining was largely in the cell cytoplasm. The

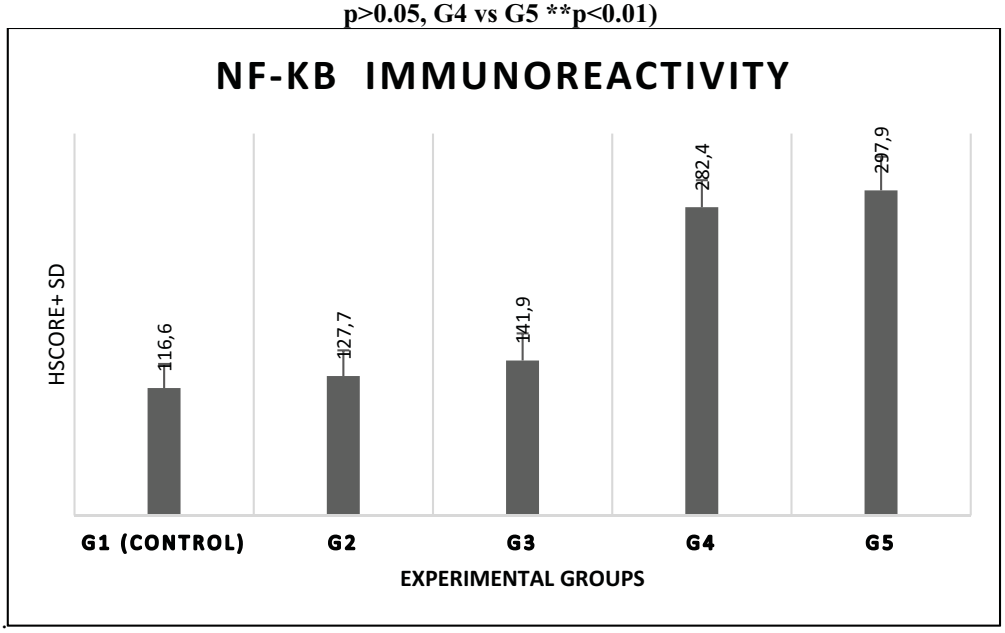
apoptotic mechanism was found to be very high in the liver tissues, especially in the G5 group.

Cells are divided into zones (regions) according to their proximity to the distributive veins in the hepatic acini. The cells in the first zone are the closest to the vessels and are most affected by blood flow changes. Cells in the second zone are less affected than these. The cells in the third zone will encounter blood modified by the cells in the first and second zones. This arrangement may explain the different degrees of damage to hepatocytes in the face of toxic agents or diseases. Therefore, the cells in the first zone are the first to take oxygen, nutrients, and toxin from sinusoidal blood and to show the first morphological change after bile duct obstruction. These cells are also the last to die and the first to regenerate when circulation breaks down. The cells in the third zone are the first to show ischemic necrosis (centrilobular necrosis) and fat accumulation in decreasing blood supply. The cells that react to toxic substances and bile obstruction are also cells in this zone. Enzyme activity, number, and size of cytoplasmic organelles and size changes of cytoplasmic glycogen stores are also observed between the first and third zones. Our findings show that apoptotic activity and inflammation increase especially in the third zone. For this reason, it is thought that NF-κB can be an important target especially in regulating inflammation and activates the inflammatory mechanism for anti-apoptotic regulation.

Immunohistochemical staining with NF-κB, positive immunoreactivity was observed in parallel with the dose increase in the moss extract. In the first group, high reactivity was observed around the central vein. In the G2 and G3 Groups, it was observed that the immunoreactivity around the central vein was moderate and in the liver tissues belonging to the G4 and G5 groups, NF-κB and TNF-α immunoreactivity was severe. A statistically significant difference was observed between the control group and the fifth group of the subjects ($p<0.0001$). There was no significant difference between the control group and the second (G2) group given the mosses. When the other groups are compared with each other, the most statistically significant was found between G1 and G5 and the least significant was between G2 and G3 and G4 and G5.

TUNEL Findings. While programmed cell death mechanism generally occurs in embryonic tissues, oxidant-antioxidant balance in adult tissue and degeneration in tissue are important processes that trigger apoptosis. In hepatocellular damage, especially oxidative stress and inflammation cause hepatocytes to be dragged into apoptosis with released cytokines. The disruption of functional processes in the tissue is one of the important conditions for apoptosis. With the release of TNF- alpha from kupffer cells, the apoptotic mechanism is activated, hepatocytes enter the apoptotic process. This event does not only result in cellular death. At the same time, the conditions for the new repair become favorable. Thus, while the liver is destroyed with apoptosis, on the one hand, the events that will rapidly create regression are shaped on the other. It was observed that apoptosis induced by dose increase and apoptotic cells increased in tissue in our study. The

TABLE 2
Immunoreactivity distribution of NF-κB immunohistochemical staining in liver tissues of groups (Control(G1) vs G2 $p>0.05$, G1 vs G3 *$p<0.05$, G1 vs G4 ***$p<0.001$, G1 vs G5 ****$p<0.0001$, G2 vs G3 $p>0.05$, G4 vs G5 **$p<0.01$)



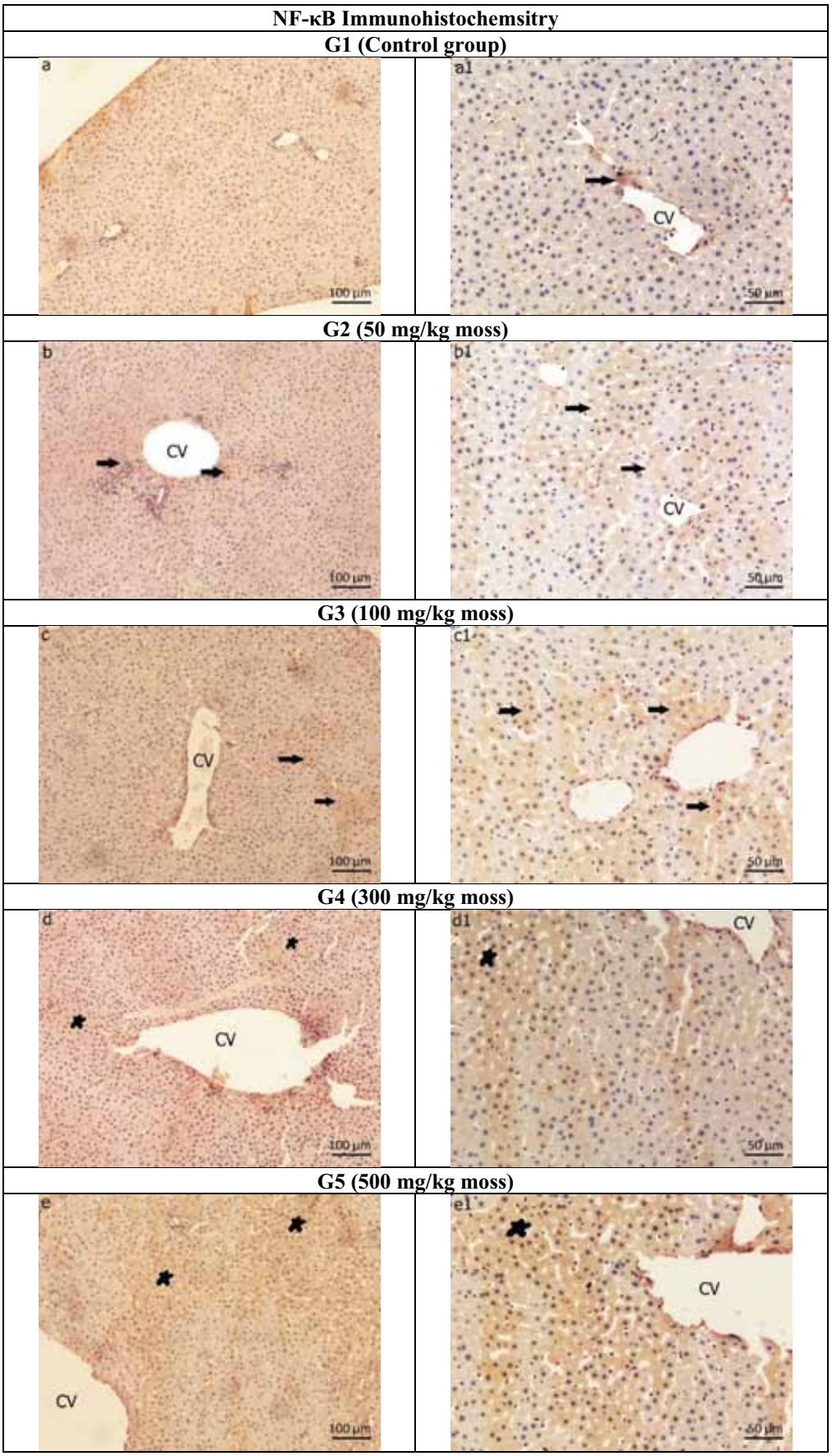


FIGURE 2
The immunohistochemical distribution of NF-κB in the liver tissue, magnification 5X and 10X

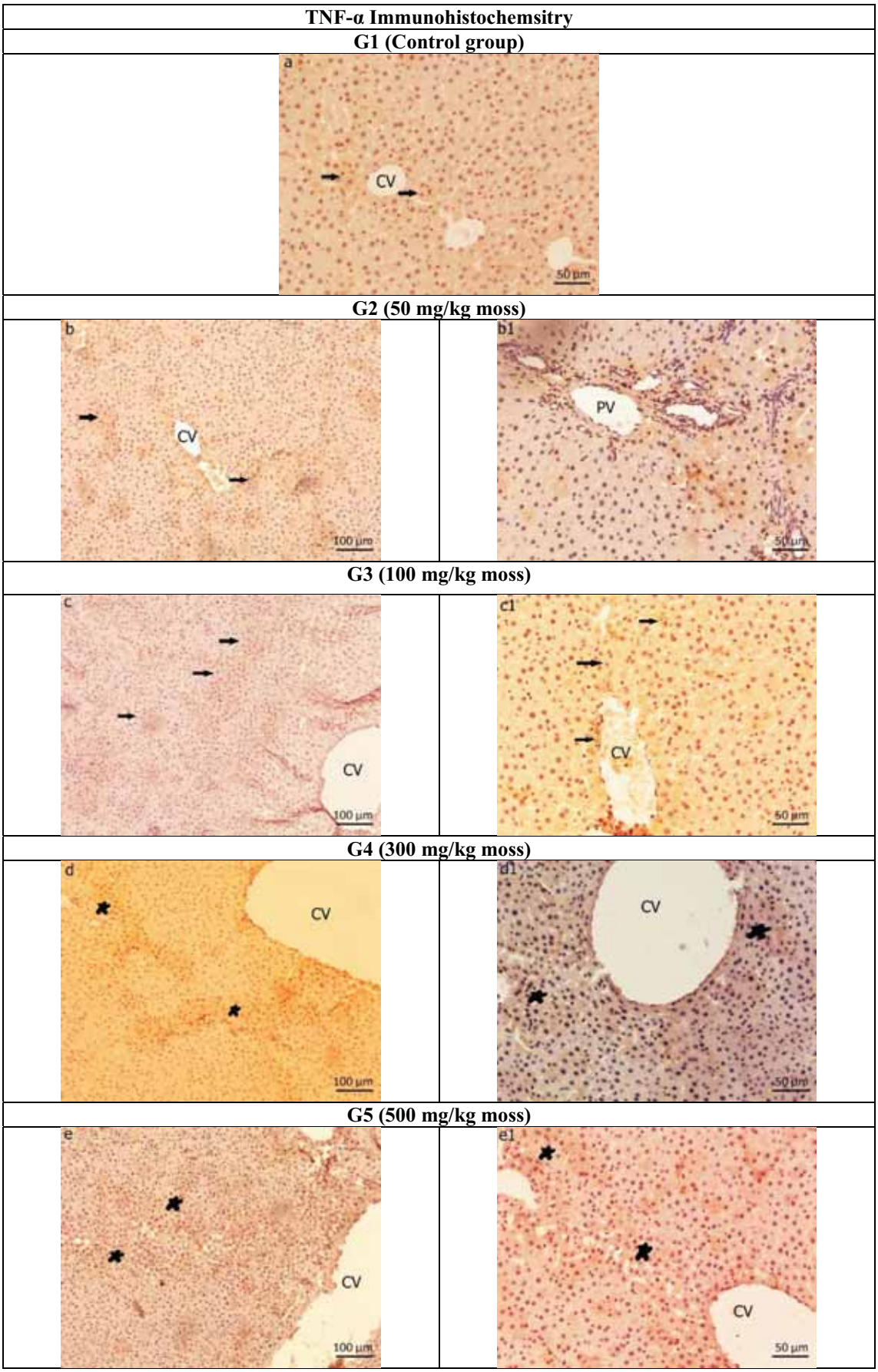


FIGURE 3
The immunohistochemical distribution of TNF-α in the liver tissue, magnification 5X and 10X

TABLE 3
Immunoreactivity distribution of TNF-α immunohistochemical staining in liver tissues of groups (Control (G1) vs G2 *p<0.05, G1 vs G3 ***p<0.001, G1 vs G4 ****p<0.0001, G1 vs G5 ****p<0.0001, G2 vs G3 ***p<0.01, G4 vs G5 **p<0.01)

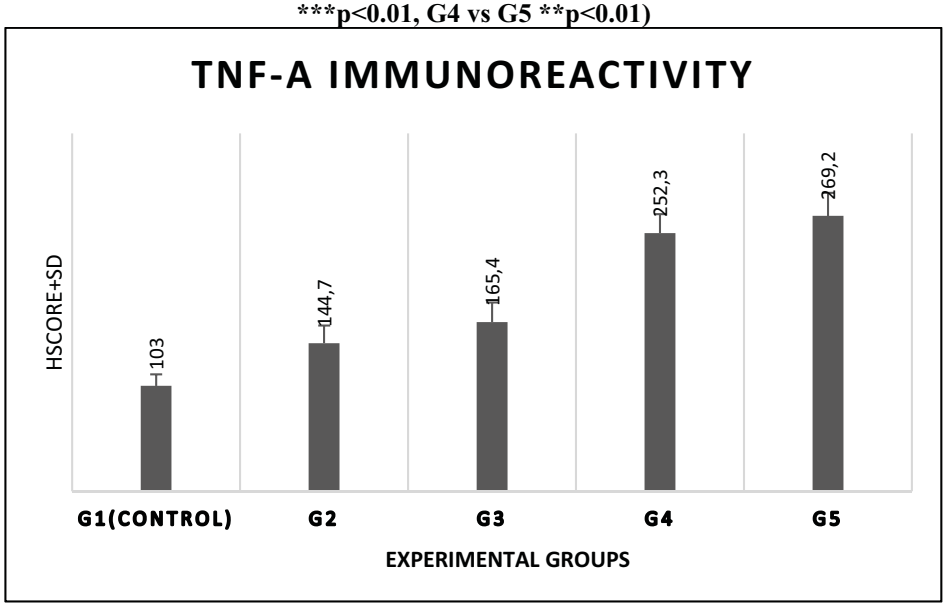
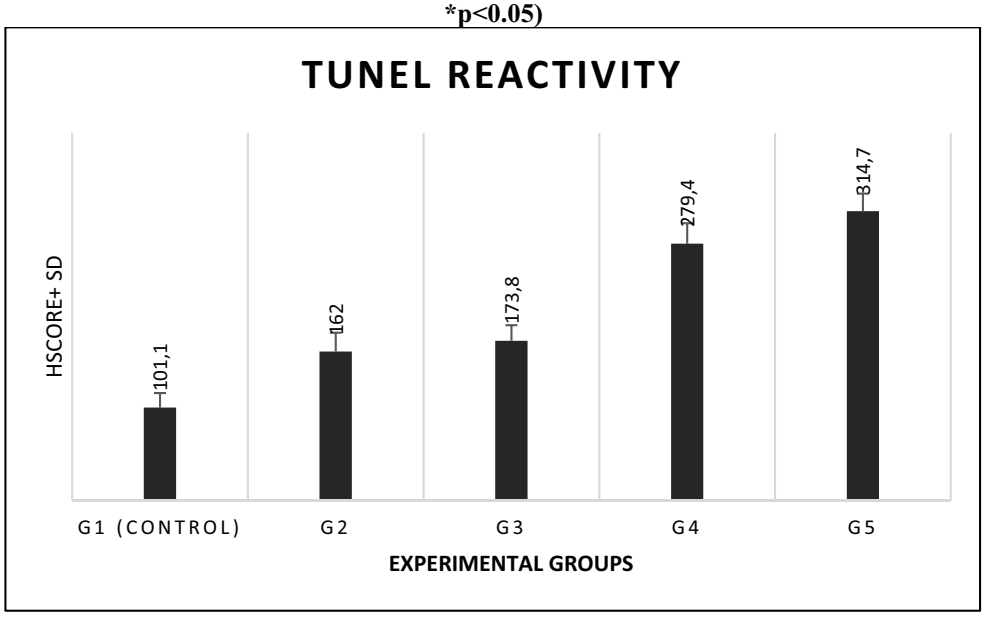


TABLE 4
Apoptotic reactivity distribution of groups in liver tissues (Control (G1) vs G2 **p<0.01 G1 vs G3 **p<0.01, G1 vs G4 ****p<0.0001, G1 vs G5 *****p<0.00001, G2 vs G3 **p<0.01, G4 vs G5 *p<0.05)



apoptotic process was observed to be at the highest level in the fourth and fifth groups of groups given increased doses of moss, while in the second and third groups it was observed at lower levels. This means that, depending on the dose increase, the moss significantly increases the apoptotic mechanism arasında (****p<0.0001) olarak belirlenmiştir. In the statistical comparisons, this positive picture was confirmed with meaningfulness. In TUNEL positivity, the highest significance among the groups was determined between the first and the fifth groups (****p<0.0001) and again between the first and the fourth group (****p<0.0001). While the least significance was observed between the second and third groups (*p<0.05), **p<0.01 was observed between the first and second groups. ***p<0.001 Significance was detected between the first and third groups. It shows that apoptotic index values increase with the dose of the mosses and that this can be used in anti-cancer treatments.

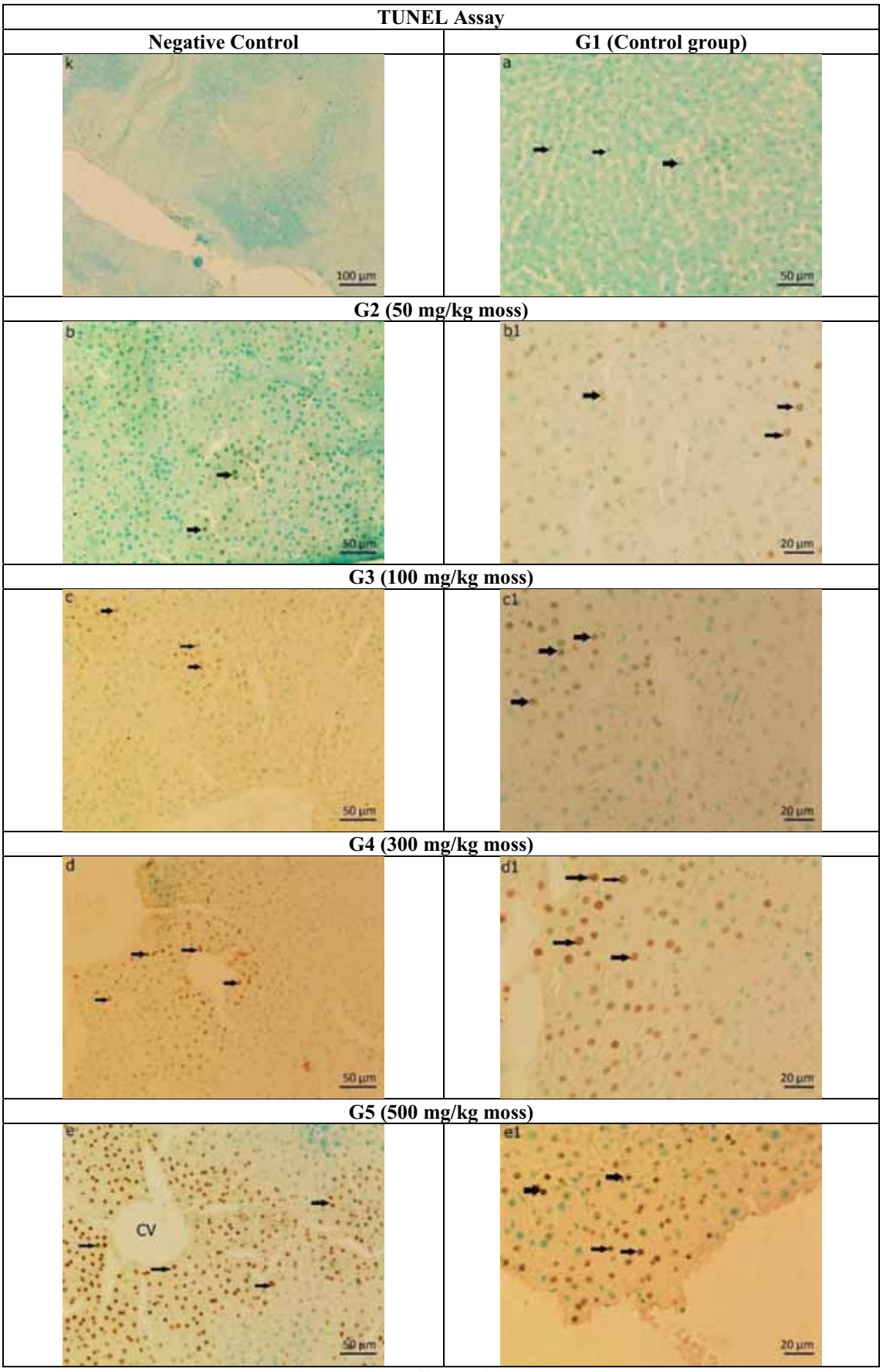


FIGURE 4
The TUNEL assay in the liver tissue, magnification negative control 5X, other groups 10X and 20X

DISCUSSION

Free radicals cause cell death by DNA damage during aerobic metabolism. Also, various studies have been presented in the literature that they cause diseases such as Alzheimer's, Parkinson, rheumatoid arthritis, diabetes mellitus due to oxidative stress in humans. Different studies have shown that dietary antioxidants are beneficial in protecting against free radicals and play an important role in preventing many diseases and cancers in humans SOD is an essential enzyme for every cell that is produced endogenously and forms the organism. The most abundant copper-zinc (CuZn) SOD in the body is in the cytoplasm. Superoxide plays an important role in cell structure and life, such as the bactericidal activity of neutrophils, apoptosis, inflammation, and regulation of vascular functions [11]. The chain of events that trigger apoptosis is NF-κB activity, which is activated by TNF-α induction and stimulates apoptotic genes, dragging the cells to programmed death *H. sericeum*, which is a moss species, is given in different doses and the apoptotic process occurring in the tissue with the increase of free oxygen radicals in the liver tissues is shown by TUNEL staining. Differences were detected with both TUNEL and immunohistochemical staining in cases where the statistical significance of $p < 0.05$ was taken in liver tissues of rats in groups given different doses of moss.

TNF-α induces cellular responses such as proliferation, inflammatory mediator production, and cell death, and is a pleiotropic cytokine that plays an important role in the pathophysiology and wasting syndrome of septic shock. It plays a role in the pathophysiology of TNF-α, viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease and, ischemia-reperfusion injury in the liver. TNF-α plays a two-cell role in the liver, where it not only functions as a means of cell death but also triggers hepatocyte proliferation and liver regeneration. TNF-α can be released mainly by macrophages, but also by other cells, including lymphoid cells, mast cells, endothelial cells, fibroblasts, and neuron cells. TNF-α is mainly produced as a type II transmembrane protein, but metalloprotease can be released in soluble trimeric form by proteolytic cleavage by the TNF-converting enzyme [12]. The molecule that plays an essential role in endotoxin-cytokine-mediated cell damage is TNF-α. TNF-α is higher in patients with nonalcoholic liver disease than the control group [13]. TNF-α is produced mainly by macrophages with oxidative stress or endotoxin stimulation. It is a molecule located at the beginning of the inflammatory cascade. Neutrophil chemotaxis, vascular endothelial cell stimulation and, the increase of adhesion molecules has the effect of increasing neutrophils secreting new cytokines and free radicals, increasing apoptosis and insulin resistance [14]. Studies have shown that when the liver enters the degenerative process, TNF-α induces increased synthesis in hepatocytes and Kupffer cells in the cytoplasms. In our study, it was determined that mosses caused degeneration of hepatocellular structure in the liver and that TNF-α immunoreactivity increased as the dose increased. TNF-α, a cytokine secreted for conservation purposes, shows that the liver's damaged structure can be cleaned by apoptosis and inflammatory events and re-trigger regeneration.

When oxidative stress in tissues and associated damage to cells increases, activation of the TNF-alpha receptor is followed by activation of the nuclear factor kappa B (NF-kB). Thus, NF-κB goes to the nucleus, and then NF-κB activates genes that try to block TNF-induced apoptosis. In resting cells, NF-B is an inactive form in the cytoplasm [15, 16]. Activation of TNF-induced NF-kB regulates the expression of anti-apoptotic proteins, such as members of the Bcl-2 family, and prevents TNF-induced apoptosis [17]. Also, the NF-κB pathway is a pro-inflammatory signal pathway based on NF-κB activation. NF-κB can be considered as a target for anti-inflammatory drugs. Cai et al. suggested that NF-κB is an inflammatory regulator and controls inflammatory production, cytokines such as IL-6 and TNF. NF-κB is activated by various stimulants in situations that present a potential hazard to the host. This results in the initiation of inflammatory, immune and, wound-healing responses and clearing of pathogens. The most potent activators of NF-kB include Toll-like receptors (TLRs), which activate against pathogen-derived molecules (lipopolysaccharide [LPs], viral and bacterial DNA and RNA), and molecules that stimulate inflammatory cytokines such as tumor necrosis factor (TNF). Activation of NF-kB leads to the transcription of hundreds of genes, most of which are involved in the regulation of inflammation, immune responses, and cell survival [18, 19].

Some research has proven the hypothesis that inflammation is not only associated with chronic liver disease but actively promotes disease progression. NF-κB has a wide range of functions in different cellular compartments, such as the survival of hepatocytes, the secretion of Kupffer cells secreting cytokines that trigger inflammation. This broad functionality means that NF-κB is an important factor in regulation at the center of the root causes of chronic liver disease and resultant wound healing responses (organ fibrosis). The critical role of NF-κB in the liver has been reported to lead to spontaneous liver damage, fibrosis and hepatocellular carcinoma in the genetic ablation of NF-κB regulators in mouse models. Therefore, NF-κB is an indispensable factor in the modeling of the most clinically important complications of chronic liver disease [20].

Stimulation of cell death in the absence of NF-κB activation is largely dependent on the long-term activation of JNK. This long-term JNK activation induced by TNF is dependent on the production of reactive oxygen species (ROS), whose production is usually inhibited by NF-κB. In NF-κB defective

cells, ROS accumulates due to decreased expression of the NF-κB target gene SOD2, which encodes the metabolizing enzyme superoxide dismutase 2 (SOD2). ROS oxidizes and inhibits JNK-inactivating phosphatase, thereby causing prolonged JNK activity upon TNF stimulation [21, 22]. In previous studies, as in many tissues, it controls the genes that regulate the NF-κB proinflammatory and anti-apoptotic mechanism against factors such as oxidative stress in the liver and other chemical stimuli and provides the dynamism necessary for cell renewal. In our study, we tried to determine whether there was an inflammatory and apoptotic effect in liver tissue due to increasing doses of moss extract. Our findings show that in previous studies, we observed that the expression of NF-κB increased in hepatocyte cytoplasms parallel to its regulatory role in liver tissue. As the hepatotoxic effect increased as the dose increased, NF-κB expression was observed to be at the highest level in the fifth group.

Yamamato et al. showed that rat livers show resistance to apoptosis during resection and they try to protect the viability of hepatocytes. They stated that the cell exposed to ischemia for any reason expresses bcl – 2 to prevent apoptosis. Gapany et al. studies have shown that the apoptosis of bile duct cells in liver allograft resections is associated with bcl–2 levels. Apoptosis of hepatocytes and bile cells is at the key point in graft resection [23]. The number of cells lost due to apoptosis is determined by the intensity of resection. In the study, which resulted in liver damage by giving copper, it was determined that apoptosis is increased copper exposure increased according to the control group [24]. In our study, we observed the apoptotic index, which is in line with the findings of these studies. It was stated that the NF-κB activated by TNF-alpha induction in the liver tissues of the fourth and fifth groups performed by increasing the dose of moss is not able to prevent the factors preventing apoptosis and as result apoptosis progresses at the highest level. In our previous study, we determined that the activity of the Cu/Zn-SOD enzyme increased in the liver tissues of which ratios were given. When this information was compared, it was found that moss raises both antioxidant activity and increased apoptosis. These results will serve as a reference for studies for active substances with new anticancer properties, which are alternative to antioxidant studies with high-plants.

In vivo animal experimental results with blueberry extract showed that blueberries regenerate the liver of mice with liver damage, reduce levels such as AST, ALT, and also reduce serum cytokine IL-6, TNF and IFN levels in mice. It has also been shown to increase SOD activity. It has been reported that after treatment with the highest concentration in mice damaged by carbon tetrachloride, it approaches its findings from standard therapy. It demonstrated that it regulates expression levels of Cu /Zn-SOD, Mn-SOD, and chloramphenicol acetyltransferase (CAT) and low regulated cyclooxygenase (COX) -2 expression by mRNA expression in liver tissue. Studies have revealed that blueberries have a good preventive effect on mice with liver damage [25].

The protective effect of Lavender oil was investigated in mice that produced hepato and nephrotoxic effects with the Malathion application. It has been shown to increase oxidative stress, which is assessed by the exhaustion of sulfhydryl group content (-SH) and antioxidant enzyme activity, as well as MDA and hydrogen peroxide levels. It has been reported that Cu Zn-SOD, Mn-SOD and, Fe-SOD increase in kidney and liver. More importantly, it has been shown that Malathion-induced whole body loss, liver, and kidney relative weight increase, hemodynamic and, metabolic disorders, as well as hepatic and renal oxidative stress, are eliminated by Lavender therapy. In conclusion, it shows that Lavender has potential hepato- and nephroprotective effects against oxidative stress caused by malathion in mice. This beneficial effect may be partially related to its antioxidant properties [26]. Inflammatory and apoptotic activities, which are similar to our findings in our study, were detected in tissues such as liver and kidney in studies with various plant extracts. The increase in histological damage due to the increase in dose in our study contradicts the findings of these and similar studies. It should be said that *H. sericeum* species have been shown to cause pathology in many important tissues, especially liver and kidney, with increasing dose, but it also provokes defense by stimulating antioxidant activity. These results show that the effect of anticancer and antitumoral studies will be more effective, provided that the dose of moss is not high.

Active compounds responsible for existing antimicrobial effects have been identified in many bryophyte species. For example; it is stated that some extract of liverwort such as Polygodial from *Porella* sp, Polygodial from *Conocephalum conicum* and Lunularin from *Lunularia cruciata* is not only an effective fungicide and bactericide but also a weak biocide (stomach poison) effect against harmful insects [3]. Alcoholic or acidic extracts of *Polytrichum juniperinum* are injected into muscle cells of CAF1 mice and show antitumor activity against carcinoma [27]. In other cases, bryophyte extracts showed a tumor-promoting activity [28]. Asakawa (1999) found that molecules such as Marchantin A, cyclopentanol fatty acids and their precursors have antimicrobial activity. Sanionin A and B are isolated from *Sanionia georgico-uncinata* collected from Antarctic Livingston Island. These compounds showed inhibitory activity against multiple resistant staphylococci, gram-positive pathogens, and vancomycin-resistant enterecocci. Inflammatory activity and low cytotoxicity have also been observed [29].

It is very important to use immunohistochemical staining methods to detect molecules secreted from cells in the tissue. Studies show that research

on antioxidants has been limited to biochemical analysis in blood. The number of histopathological studies on these issues is almost nonexistent. In this study, the distribution of mosses in liver tissue was shown by using immunohistochemical staining methods to determine TNF-alpha and NF-kB localizations, which are indicators of the apoptotic index and cellular damage in liver tissue. In routine histological staining, it was observed that the most histopathological picture occurred in the liver tissues. Moreover, in vitro cell cultures were generally preferred in studies with mosses. It has been shown in studies that they contain compounds with antibacterial and anticancer properties. Moving from this point, if the histopathological picture due to the dose increase is ignored, it is thought that the administration of *H. sericeum* species at 50 and 100 mg/kg dose is not toxic and, it will be useful in evaluating the anticancer feature. In our study, it has been shown that dosing over 300 mg/kg has a toxic effect on the liver and higher doses have been shown to damage normal tissues. It was found that apoptosis increased with the activation of TNF-alpha and NF-κB mechanisms firstly against oxidative stress especially caused by the disruption of oxidant-antioxidant balance. It was recorded that TUNEL activity in the liver was very high and this was in line with the increasing dose of the moss. As the doses of the applied moss decreased, hepatotoxic activity in the liver decreased in TUNEL activity.

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