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RESEARCH ARTICLE

Investigation of Antioxidant and Histopathological Effects of Aqueous and Ethanol Extracts Obtained from *Sideritis akmanii*

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ABSTRACT

In this study, it was aimed to investigate the antioxidant activities and histopathological effects of the aqueous and ethanolic extracts obtained from the endemic *Sideritis akmanii* plant, which spreads on the Şuhut-Sandıklı road in the Kumalar plateau region of Afyonkarahisar province. The number of 48 Wistar albino male rats were divided into 8 groups; Control Group (Group I), CMC Group (Group II), 0.5% CMC (carboxymethyl cellulose), Aqueous and ethanolic plant extract groups (Group III-IV-V-VI-VII-VIII). Three different doses (1%, 2%, and 4%) extracts from the determined LD50 dose were applied to 6 experimental groups for 30 days. At the end of the experiment, malondialdehyde (MDA), glutathione (GSH) levels, superoxide dismutase (SOD), and catalase (CAT) enzyme activities were measured in healthy rat's blood and tissue (liver, heart, brain, kidney, testis) samples. The whole blood MDA level decreased in all doses of the ethanolic extract group compared to the control group, while the GSH level increased in both extract groups compared to the control group. SOD and CAT enzyme activities were increased in the ethanolic groups compared to the control group, except for erythrocyte and testis tissue ($p<0.05$). On the other hand, no changes were observed in the histopathological examination results. As a result of the study, it was observed that the aqueous and ethanolic extracts of *Sideritis akmanii* had antioxidant properties and the ethanolic extract was more effective on antioxidant enzymes.

Keywords: Antioxidant enzymes, Histopathology, Lipid peroxidation, *Sideritis akmanii* extract (aqueous and ethanolic)

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Sideritis akmanii'den Elde Edilen Sulu ve Etanol Ekstraktlarının Antioksidan ve Histopatolojik Etkilerinin Araştırılması

ÖZ

Bu çalışmada, Afyonkarahisar ili Kumalar yaylası bölgesinde Şuhut-Sandıklı yolu üzerinde yayılış gösteren endemik *Sideritis akmanii* bitkisinden elde edilen sulu ve etanolik ekstraktların antioksidan aktivitelerinin ve histopatolojik etkilerinin araştırılması amaçlanmıştır. 48 adet Wistar albino erkek rat 8 gruba ayrıldı; Kontrol Grubu (Grup I), CMC Grubu (Grup II), %0,5 CMC (karboksimetil selüloz), Sulu ve etanolik bitki ekstrakt grupları (Grup III-IV-V-VI-VII-VIII). Belirlenen LD50 dozundan uygun oranlarda üç farklı doz (%1, %2 ve %4) ekstraktı 6 deney grubuna 30 gün uygulandı. Deney sonunda sağlıklı ratların kan ve doku (karaciğer, kalp, beyin, böbrek, testis) örneklerinde malondialdehit (MDA), glutatyon (GSH) düzeyleri, süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktiviteleri ölçüldü.Tam kan MDA düzeyi etanol ekstrakt grubunun tüm dozlarında kontrol grubuna göre azalırken, GSH düzeyi her iki ekstrakt grubunda da kontrol grubuna göre arttı. Etanol gruplarında eritrosit ve testis dokusu dışında SOD ve CAT enzim aktiviteleri kontrol grubuna göre arttı ($p<0.05$). Histopatolojik inceleme sonuçlarında ise herhangi bir değişiklik gözlenmedi. Çalışma sonucunda *Sideritis akmanii*'nin sulu ve etanol ekstraktlarının antioksidan özelliklere sahip olduğu ve etanol ekstraktının antioksidan enzimler üzerinde daha etkili olduğu gözlemlendi.

Anahtar kelimeler: Antioksidan enzimler, Histopatoloji, Lipid peroksidasyonu, *Sideritis akmanii* ekstraktı (sulu ve etanollü)

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INTRODUCTION

The therapeutic properties of plants have been known to humans since ancient times. As in the whole world, medicinally important plants have been used by the Anatolian people for centuries in Turkey as well. Sideritis species growing in the Mediterranean flora, especially in Spain and Turkey, are used as herbal tea due to the unique pleasant smell and aroma of their dried flowers and leaves. Sideritis species are widely used in traditional medicine in Turkey due to their properties such as nervous system stimulant - sedative, gastrointestinal disorders, rheumatism, cough caused by cold, pain reliever, and diuretic (González Burgos et al. 2011). Considering these common features among the public, the effect of extracts obtained from Sideritis species has been a matter of curiosity. In a study, it was revealed that different types of Sideritis exhibit anti-inflammatory, antinociceptive, and antioxidant activities, as well as nervous system stimulant or anti-stress activities (Ozturk et al. 1996, Guvenç et al. 2010). In another study, it was expressed that it has a good inhibition on enzymes that have an important role in Alzheimer's disease (Çarıkçı 2020). It has been determined that the extracts obtained from *S. scardica* have gastroprotective activities as well as anti-inflammatory effects in rats. It has also been demonstrated to have cytotoxic effects on tumor cell lines (Tadić et al. 2012).

There are a few studies on *Sideritis akmanii*, an endemic species belonging to Kumalar Mountain in Afyonkarahisar province. In a chemical content study, it was determined that the species contains essential oils and flavones in its structure, and the basic components are linearol, isolineraol, sideroxol, foliol, isofoliol, sideridiol (Bondi et al. 2000). Thanks to its rich components, it has an antibacterial effect and is effective on some pathogenic bacteria (Temel et al. 2014). In a recent in vitro study, its anticarcinogenic effect was demonstrated (Cigerci et al. 2023). Aksoy et al., in their in vitro study using *Sideritis akmanii* methanol and acetone extract, found that this species has antioxidant properties and can inhibit acetylcholinesterase, α-glucosidase, α-amylase enzymes. In the study, both total phenolic content and DPPH radical scavenging activity of methanol extract were higher in methanol extract compared to acetone extract, and in this context, the importance of solvent was emphasized (Aksoy et al. 2022).

In this study, it was aimed to investigate the antioxidant activity of the aqueous and ethanolic extracts obtained from the plant *Sideritis akmanii*, which is endemic to Afyonkarahisar, in rat blood and tissues, and its histopathological effects on the tissues.

MATERIAL and METHODS

Sideritis akmanii Plant Supply

The plant material of this study, *Sideritis akmanii*, was collected from above-ground parts such as leaves, flowers etc. on 20 July 2016, from the Kumalar plateau region of the Şuhut-Sandıklı highway in Afyonkarahisar. Prof. Dr. Mustafa Kargıoğlu conducted the identification of the plant. The plant sample is stored at the Afyon Kocatepe University herbarium with the registration number AKU8314. The aerial parts of *Sideritis akmanii* were dried in the shade and at room temperature and mechanically cut into small pieces. Dried plant samples were pulverized in a blender device.

Preparation of *Sideritis akmanii* Aqueous and Ethanolic Extracts

The aerial parts of *Sideritis akmanii* were ground into powder, and 50 g was taken and added to a 2-liter volumetric flask. A cooling system was set up by adding twenty times the amount of ethanol (1000 ml) of the sample for ethanolic extract and distilled water (1000 ml) twenty times the sample for water extract on the volumetric flask. In this way, it was kept in a

container filled with water at 50 °C for 24 hours. The filtered extracts were removed separately with ethanol and water at 50 °C in a rotary device (Şerbetçi 2007). Afterward, 4.9 g of greenish-black, moist, sticky ethanolic extract and 1.5 g of milky-brown, moist, sticky water extract were obtained. The obtained extracts were dissolved in 0.5% CMC (Carboxymethyl Cellulose) / distilled water solution and used for the experiment.

Determination of Lethal Dose 50 (LD50)

The lethal dose 50 (LD50) was determined with these two extracts. For this, the OECD (2001) (Organization for Economic Co-operation and Development) method was used. According to this method, 10 male Wistar albino rats weighing 250-350 g were divided into two groups.

To the rats in the 1st Group, in order of 175 mg/kg, 550 mg/kg, 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg ethanolic extract, (n: 5)

Similarly, 175 mg/kg, 550 mg/kg, 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg aqueous extract was given to the rats in the Group 2, (n: 5)

It was administered as a single dose via gastric gavage on the same day and time. After the application, the general clinical conditions and behaviors of the rats were followed for 48 hours. In the evaluation, If no animals died at the doses administered to the rats, the LD50 dose was interpreted as greater than the highest dose given to the rats (OECD, 2001). As a result of the application of ethanolic and water extracts prepared from the aerial parts of the *Sideritis akmanii* plant to the rats, the lethal dose was found to be 50 (LD50) > 5000 mg/kg. The amounts of 1% (50 mg/kg), 2% (100 mg/kg), and 4% (200 mg/kg) of the OD50 dose determined from the aqueous and ethanolic extracts were determined and administered to the rats (Çelik and Küçükkurt 2016).

Experimental Protocol

For this study, study approval was obtained from Afyon Kocatepe University Experimental Animals Ethics Committee with reference number 79-18. Wistar albino male rat weighing 200-300 g was used as animal material. Rats in their cages in the experimental animals unit were kept at a temperature of 24±1 °C, a 12 hours light/dark, and a regularly ventilated environment. In feeding the rats, standard rat food and drinking water were given fresh every day.

Rats were divided into 8 groups with 6 in each group. The Control Group (Group I) was fed only with standard rat chow. CMC Group (Group II) was fed with standard rat chow and 0.5% CMC was given by gastric gavage. In addition to the standard rat feed, the other six groups were given plant extracts (1%, 2%, and 4%) by preparing the plant extracts in accordance with the LD50 dose. The groups given the aqueous extract were divided into S1, S2, and S3 (Groups III, IV, V), and the groups given the ethanolic extract were divided into E1, E2, and E3 (Groups VI, VII, VIII). These determined doses were given to the rats by gastric gavage for 30 days. At the end of 30 days, 24 hours after the last application, blood and tissue (liver, heart, kidney, brain, testis) samples were taken from animals under xylazine and ketamine anesthesia.

Preparation of Whole Blood, Erythrocyte Lysate, and Tissue Homogenates

Blood samples taken into heparinized tubes were divided into two parts. Some of the blood was used as whole blood for the measurement of MDA and GSH, and some of it was prepared for the measurement of SOD and CAT activities. For this, erythrocyte and plasma were separated by centrifugation at 3500 g for 15 minutes at 4 C within 30 minutes. The precipitated erythrocytes were washed three times with isotonic saline and the fluffy layer was removed. Then, isotonic saline and erythrocytes were added in the same volume and stored at -20 C (Winterbourn et al. 1975).

After the animals were sacrificed, liver, kidney, heart, brain, and testis tissues were removed and thoroughly washed with cold 0.9% NaCl. Tissues were homogenized 1:40 w/v in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA. After centrifugation at 18000xg for 15 min at 4 C obtained supernatants were stored at -20 C until analysis (Kucukkurt et al. 2008).

Biochemical Analyses

Whole blood MDA levels, which are an important marker for lipid peroxidation, were determined by Draper and Hadley (Draper and Hadley, 1990), and tissue homogenates by Ohkawa et al. (1979) were determined by the method. MDA in the catabolite of lipid peroxide reacts with thiobarbituric acid (TBA) to produce a pink compound with maximum absorption at 532 nm. GSH level was measured in blood and tissue according to Beutler et al. (1993). The amount of GSH is determined by reading the optical density of this yellow compound at 412 nm in the spectrophotometer. Antioxidant enzyme activity of SOD in erythrocyte lysate and tissue homogenate Sun et al. (1988) was measured according to the method. CAT activity was determined by Luck (1955)'s method in erythrocyte lysate and by Aebi's (1974) method in tissue homogenate. CAT activity was measured by the rate of reduction in H2O2 at 240 nm for 45 seconds at 25°C. Protein content in tissue Lowry et al. (1951) and the amount of hemoglobin were measured by the Drabkin method (Drabkin and Austin 1935). All spectrophotometric measurements were performed using the Shimadzu 1601 UV-VIS spectrophotometer (Tokyo, Japan).

Preparation of Tissues for Histopathological Analysis

Histopathological examination was performed in order to see the possible damage of the extracts on healthy tissues due to oxidative stress. At the end of the study, the liver, kidney, heart, brain, and testis tissues of the sacrificed rats were placed in 10% formalin solution for histopathological analysis and fixed in 10% formalin solution for 48 hours. Tissues were dehydrated by passing through graded alcohol (70% to 100%). After cleaning the tissues in xylene, they were embedded in paraffin, cut into 5-6 μm sections, and stained with hematoxylin-eosin (H&E). As a result, each section was examined under a light microscope (Microscopic Digital Picture Analysis System with Olympus BX51 and DP20 attached, Tokyo, Japan).

Statistical Analysis

The results obtained from the research, one-way ANOVA test were applied in the SPSS 20.0 statistical package program. Duncan's test was applied to the statistically different results, and the data were expressed as "mean ± standard deviation". $P < 0.05$ was accepted for statistical significance.

RESULTS

Effect on Lipid Peroxidation and Reduced Glutathione

MDA level is widely used as a marker of free radical-mediated LPO. It was determined that the extracts obtained from *Sideritis akmanii* did not cause a statistical change ($p>0.05$) in the MDA levels in both aqueous and ethanolic groups in other tissues, except blood, compared to the control group (Table 1). Blood MDA was significantly decreased in the ethanolic extract groups compared to the control and CMC groups ($p < 0.005$), the decrease in the aqueous extract groups was not statistically significant. GSH is a non-enzymatic antioxidant substance in detoxification and reduces the toxic effect of xenobiotic metabolites. The GSH levels obtained in the study are given in Table 2. It is seen that the extracts obtained from *Sideritis akmanii* have a positive effect on GSH, but this is not statistically significant in other tissues except blood

(p>0.05). Blood GSH levels increased significantly in all extract groups (p < 0.001).

Effect on Antioxidant Enzymes

The antioxidant enzymes SOD and CAT activities were determined in the erythrocyte, liver, kidney, heart, brain, and testis tissues of the rats as shown in Tables 3 and 4. When the CMC group was compared with the control group, there was no difference in both SOD and CAT enzyme activities. A statistically significant increase was observed in SOD enzyme activity when compared to all ethanolic groups of liver, heart, and brain tissues and the E1 group of kidney tissue, control, and other groups. When the S1 and S2 groups of brain tissue were compared to the control group, an increase was observed, while there was no difference in the aqueous extracts of other tissues compared to the control group. An increase in CAT enzyme activity was observed in the whole ethanolic extract group of kidney tissue compared to the control group. The increase in E2 groups in liver tissue and E2 and E3 groups in heart and brain tissues is significant. A significant increase was observed in kidney tissue S1 and S2 groups. No statistically significant difference was observed in all groups of erythrocyte and testis tissue in both analyzes.

Histopathological Changes

Histopathological examination was performed for the following changes in each organ;

- In the brain, neuron degeneration, edema and myelin degeneration,
- In the liver, sinusoidal dilatation and hyperemia, Kupffer cell activation, degenerative changes in hepatocytes,
- Degenerative changes in the kidney, tubules, and changes in Bowman's capsule in the glomerulus,
- In the heart muscle, hyaline degeneration and Zenker's necrosis.
- Decreased spermatozoid density in the testicular tubulous seminiferous contortus (TSC) lumen vacuolization areas in the TSC lumen.

The histopathological changes in the organs of the animals in the experimental groups were examined in detail and are shown in Figure 1. No pathological changes were observed in the histopathological analysis of all groups.

Table 1. Effect of *Sideritis akmanii* aqueous and ethanolic extracts on whole blood and tissue MDA levels in rats

MDA	Blood (nmol/mL)	Liver (nmol/g tissue)	Kidney (nmol/g tissue)	Heart (nmol/g tissue)	Brain (nmol/g tissue)	Testis (nmol/g tissue)
Control	7.07±1.10^{a}	2.98±0.86	2.17±0.21	2.29±0.33	2.29±0.20	2.76±0.86
CMC	7.05±0.86^{a}	3.14±0.18	2.88±1.77	2.58±0.63	2.19±0.25	3.27±0.96
S1	6.06±0.76^{ab}	3.41±0.64	2.78±0.81	2.93±0.91	2.14±0.21	2.70±0.57
S2	6.27±0.96^{ab}	2.73±0.51	2.16±0.23	2.20±0.17	2.21±0.32	2.75±1.01
S3	6.12±0.38^{ab}	2.37±0.27	2.63±0.59	3.19±0.99	2.63±0.29	2.09±0.14
E1	5.49±0.54^{b}	3.33±1.03	3.95±1.02	3.83±1.14	2.90±0.63	3.36±0.87
E2	5.63±0.93^{b}	3.61±1.01	3.19±1.12	3.47±2.17	2.45±0.55	2.78±0.77
E3	5.37±0.90^{b}	2.87±0.35	2.58±1.07	3.26±0.14	2.52±0.71	2.71±0.61
p	0.004	0.073	0.063	0.061	0.064	0.198

Mean ± standard deviation; n=6

a,b Values with different letters in the same column are statistically significant

Table 2. Effect of *Sideritis akmanii* aqueous and ethanolic extracts on whole blood and tissue GSH levels in rats

GSH	Blood (nmol/mL)	Liver (nmol/g tissue)	Kidney (nmol/g tissue)	Heart (nmol/g tissue)	Brain (nmol/g tissue)	Testis (nmol/g tissue)
Control	13.83±1.16^{b}	7.57±0.46	8.45±0.68	7.41±0.31	8.11±0.42	8.60±0.58
CMC	14.53±1.11^{b}	8.02±0.55	7.70±0.39	7.37±0.56	8.23±0.90	8.71±1.10
S1	18.43±1.36^{a}	8.04±0.23	8.53±0.38	8.23±0.53	8.63±1.20	8.13±0.66
S2	19.73±3.90^{a}	8.02±1.39	9.01±0.63	8.53±0.87	8.35±0.34	9.23±0.63
S3	20.23±3.22^{a}	8.93±1.39	8.35±0.91	9.04±0.58	8.29±1.21	9.64±0.92
E1	21.00±2.70^{a}	7.88±1.77	8.16±1.44	8.80±1.09	8.95±1.35	8.18±3.06
E2	20.83±2.82^{a}	9.40±2.40	8.44±1.16	8.81±1.81	8.66±1.73	7.41±0.87
E3	21.36±3.61^{a}	9.03±1.46	8.75±1.39	9.43±2.71	8.78±1.01	7.98±1.83
p	0.000	0.227	0.456	0.093	0.885	0.207

Mean ± standard deviation; n=6

a,b Values with different letters in the same column are statistically significant

Table 3. Effect of aqueous and ethanolic extracts of *Sideritis akmanii* on erythrocyte and tissue SOD levels in rats

SOD	Erythrocyte U/mgHb	Liver U/µg protein	Kidney U/µg protein	Heart U/µg protein	Brain U/µg protein	Testis U/µg protein
Control	8.7±1.87	2.90±0.33^{d}	4.15±0.29^{bc}	4.46±0.97^{d}	7.21±1.19^{d}	6.40±1.22^{ab}
CMC	6.82±2.9	3.56±0.27^{d}	3.45±0.53^{c}	5.87±1.43^{cd}	8.53±1.78^{cd}	7.03±2.43^{ab}
S1	6.52±4.93	4.24±0.95^{cd}	3.71±3.70^{bc}	5.88±1.82^{cd}	10.10±2.52^{bc}	5.71±1.04^{b}
S2	6.92±1.98	5.43±3.03^{bcd}	5.01±3.34^{abc}	5.98±2.08^{cd}	10.58±1.25^{abc}	6.09±1.63^{a}
S3	6.61±1.48	5.33±2.48^{bcd}	3.65±0.73^{bc}	6.66±1.76^{bcd}	7.08±1.85^{d}	5.24±0.52^{b}
E1	9.46±5.05	7.65±3.21^{ab}	7.74±1.34^{a}	7.98±1.57^{abc}	10.86±1.68^{abc}	8.21±1.69^{a}
E2	9.02±3.61	6.52±1.96^{abc}	6.34±1.41^{abc}	8.77±3.14^{ab}	12.87±2.76^{a}	8.31±2.26^{a}
E3	9.38±1.26	8.92±2.48^{a}	6.47±3.36^{ab}	10.02±2.86^{a}	11.91±2.77^{ab}	8.18±2.39^{a}
p	0.148	0.000	0.011	0.001	0.000	0.009

Mean ± standard deviation; n=6

a,b,c,d, Values with different letters in the same column are statistically significant ($p<0.05$)

Table 4. Effect of aqueous and ethanolic extracts of *Sideritis akmanii* on erythrocyte and tissue CAT levels in rats

CAT	Erythrocyte U/mgHb	Liver U/µg protein	Kidney U/µg protein	Heart U/µg protein	Brain U/µg protein	Testis U/µg protein
Control	53.05±16.01	3.91±0.57^{bc}	2.39±1.05^{c}	3.09±0.73^{c}	5.52±0.89^{c}	4.64±0.82^{abc}
CMC	41.49±15.39	3.85±0.84^{c}	1.96±0.43^{c}	3.75±0.74^{c}	5.63±0.34^{c}	4.52±2.01^{abc}
S1	52.83±24.02	2.83±0.55^{b}	3.77±0.49^{b}	4.15±0.57^{bc}	6.82±1.01^{bc}	4.27±0.56^{bc}
S2	31.09±24.09	3.76±0.46^{c}	3.92±0.81^{b}	5.86±1.87^{abc}	6.67±1.15^{bc}	5.04±0.55^{ab}
S3	32.45±13.81	2.98±0.31^{b}	2.49±0.61^{c}	3.40±0.73^{c}	6.79±1.09^{bc}	3.96±0.29^{c}
E1	54.77±25.87	3.96±0.68^{bc}	5.28±1.01^{a}	5.22±1.39^{abc}	7.78±1.79^{abc}	4.86±1.11^{ab}
E2	43.02±30.22	5.91±0.36^{a}	4.36±1.06^{ab}	7.23±3.39^{a}	8.79±1.81^{ab}	5.67±1.27^{ab}
E3	54.51±25.25	4.80±1.41^{b}	5.17±1.28^{a}	6.06±1.67^{ab}	9.67±3.94^{a}	6.06±1.66^{a}
p	0.120	0.000	0.000	0.001	0.000	0.009

Mean ± standard deviation; n=6

a,b,c,d, Values with different letters in the same column are statistically significant

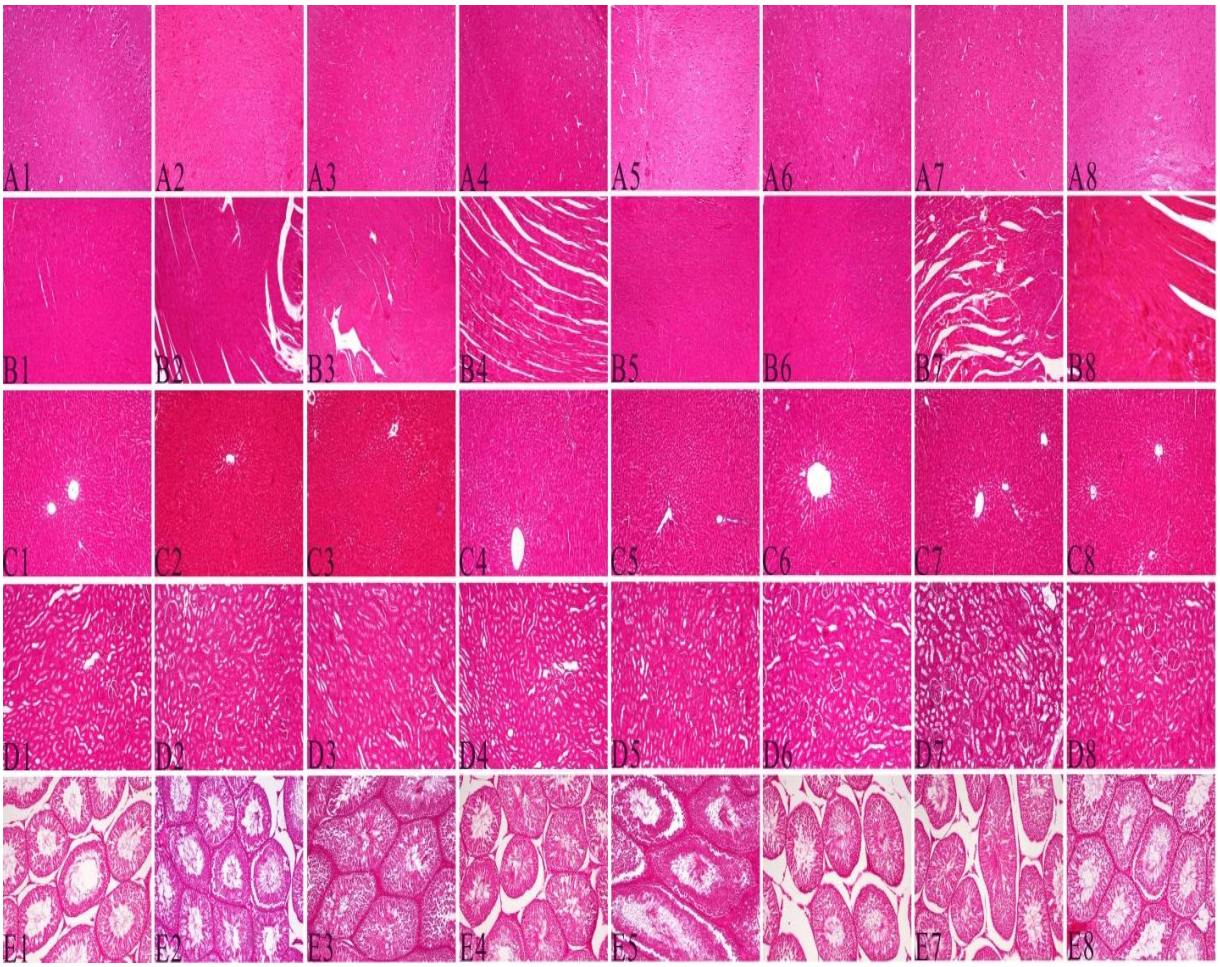

Figure 1. Effect of aqueous and ethanolic extracts from *Sideritis akmanii* on the brain (A), heart (B), liver (C), kidney (D), and testis (E) tissues of male rats. All figures are painted with H&E. 20x and 100 µm were used as the original magnification. (1) Control, (2) CMC, (3) Aqueous 1%, (4) Aqueous 2%, (5) Aqueous 4%, (6) Ethanol 1%, (7) Ethanol 2%, (8) Ethanol 4%.

DISCUSSION

Antioxidants protect the organism from harmful effects by inhibiting the formation of reactive oxygen metabolites. Cells maintain their vital functions against oxidative damage with the help of the antioxidant system (Dündar and Aslan 2000). Many chemicals found in plants, especially phenolic acids and flavonoids, contribute to this system by showing strong antioxidant activity. Phenolic substances, flavonoids, and glycosides in the chemical structure of Sideritis species have an antioxidant effect (González Burgos et al. 2011). Biological systems remove free radicals by means of enzymatic antioxidants such as CAT, and SOD (Şerbetçi 2007) and non-enzymatic antioxidants such as GSH. The increase in free oxygen radicals causes tissue damage resulting in the formation of MDA, a product of lipid peroxidation (Özden et al. 2004).

Many in vitro studies have been carried out to measure the antioxidant activity of Sideritis species. Sagdic et al. (2008) determined in their study that methanolic extracts obtained from *S. ozturkii* and *S. caesarea* have antioxidant effects. Zengin et al. (2014) reported that methanol and water extracts obtained from *Sideritis galatica* had higher phenolic content and antioxidant capacity. In a study, ethanolic and aqueous extracts of 5 different Sideritis species were compared in terms of phenolic acid and flavonoid components. As a result of this comparison, it was seen that ethanol showed higher performance in extracting phenolics (Ozkan 2009). In general, it is seen that the antioxidant effects of Sideritis species have been investigated in vitro. Few in vivo studies show the effect of its

components and antioxidant properties on living organisms. In an in vivo study, the researchers administered an infusion of the above-ground parts of *Sideritis caesarea* in rats in which a model of oxidative stress was established. As a result of the study, it was stated that the MDA level, which increased due to oxidative damage in the tissues, decreased significantly with the infusion, and the GSH level increased significantly. It has been emphasized that Sideritis caesarea provides protection against chemical-induced oxidative damage (Celik and Kaya 2011). In another in vivo study, they reported that herbal tea obtained from *S. clandestina* increased antioxidant capacity in mice, especially in brain tissue (Linardaki et al. 2008). Contrary to these studies, there was no statistically significant difference in MDA level in tissue homogenates, but a statistical decrease was observed in whole blood MDA level only in the ethanolic extract group. This situation between the ethanolic extract and the aqueous extract suggests that different solvents may have different effects. In this study, GSH level in whole blood increased in both water extract and ethanol extract compared to the control group, but no difference was observed in tissue homogenates. It is seen that two different extracts are effective on the GSH level.

Free radicals that are constantly produced in the cell are destroyed by the antioxidant defense system. Antioxidant defense systems produced during normal metabolism can also be taken into the body through food. Antioxidant defense systems have complex enzymatic and non-enzymatic systems. The first line of defense is in the class of SOD and CAT enzymatic antioxidant defense systems that suppress the formation of free radicals (Lobo et al. 2010, Surai et al. 2019). The superoxide dismutase enzyme removes the superoxide radical, but as a result, hydrogen peroxide, another highly toxic substance, is formed. One of the enzymes effective for the breakdown (detoxification) of hydrogen peroxide is CAT (Day 2009).

The effects of Sideritis species on some enzymes have recently attracted attention and various studies have been presented. In an in vivo study, the effect of aqueous and ethanolic extract obtained from the *Sideritis akmanii* plant on biochemical parameters in rats was investigated, and it was observed that ethanolic extract caused a decrease in liver enzymes. (Coğuplugil 2019). Similarly, it was stated that antioxidant enzyme activities such as SOD and CAT of Sideritis caesarea infusion increased significantly in the brain, liver, and kidneys of rats against the harmful effects of TCA, but decreased significantly in the group given Sideritis (Celik and Kaya 2011).

In another in vitro study, the total phenolic content, DPPH radical scavenging effect, total antioxidant capacities, and bio element levels were investigated by using different solvent extracts of the Sideritis akmanii plant. Researchers have emphasized that the amount of phenolic substance in the methanolic extract is higher than that of the acetone extract and that *S. akmanii* contains elements that participate in the antioxidant enzyme (SOD and CAT) structure (Güzey 2017). In this study, it was observed that the enzyme levels were affected by the ethanolic extract causing an increase in both SOD and CAT enzyme activities.

When the literature is examined, there are limited studies on the histopathological effects of Sideritis species. In a study investigating acute and repeated dose oral toxicity in mice, *Sideritis scardica* ethanolic extract reported that all animals survived and no pathological abnormalities were observed. (Feistel et al. 2018). Similarly, no pathological changes were observed in the tissues examined histopathologically in this study.

CONCLUSION

In conclusion despite the large number of studies examining the antioxidant properties of Sideritis species, especially Sideritis akmanii, in vitro, it is noteworthy that the number of in vivo studies is insufficient. Although the effect of the extracts on the MDA level in the tissue homogenate was not observe it caused a decrease in the ethanolic extract in whole blood. While no effect was observed in tissue homogenates in GSH level, both extracts increased in whole blood compared to the control group. The effect of sideritis species on enzymes has been demonstrated by some studies, albeit in limited numbers. In this study, a significant increase in antioxidant enzymes, especially ethanolic extract, is striking. In this study, the effect of Sideritis akmanii aqueous and ethanolic extracts on healthy tissues was investigated, and further studies are needed to observe the effect in case of oxidative stress.

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Ethical Statement: The experimental protocols were approved by the Animal Care and Use Committee of Afyon Kocatepe University and were by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (AKUHADYEK-79-18).

Conflict of Interest: There is no real or potential conflict of interest to declare.

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