ORIGINAL RESEARCH ORİJİNAL ARAŞTIRMA

DOI: 10.5336/vetsci.2023-99249

Antioxidant Properties of Myrtus Extract: *in vitro* Assays and *in vivo* Experimental Animal Study

Myrtus Ekstraktının Antioksidan Özellikleri: *in vitro* Tahliller ve *in vivo* Deneysel Hayvan Çalışması

Barış DENK[a], Ümit ÖZÇINAR[b], İsmail Hakkı ÖZSANDIK[b], Syed Rizwan Ali SHAH[b], İbrahim Sadi ÇETİNGÜL[b], Ali ÇALIK[c], Mustafa MİDİLLİ[d], İsmail BAYRAM[a]

[a]Afyon Kocatepe University Faculty of Veterinary Medicine, Department of Biochemistry, Afyonkarahisar, Türkiye
[b]Afyon Kocatepe University Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Afyonkarahisar, Türkiye
[c]Ankara University Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Ankara, Türkiye
[d]Abant İzzet Baysal University Faculty of Agriculture, Department of Poultry Science, Bolu, Türkiye

ABSTRACT Objective: The study explores *Myrtus communis* L. extract (ME) as a potential antioxidant agent in drinking water. It encompassed *in vitro* assays and *in vivo* tests on Wistar albino rats. **Material and Methods:** ME's antioxidant activity (AOA) was evaluated using metal chelating activity (MCA), reducing power (RP), and superoxide radical scavenging activity (SRSA) assays. *In vivo* experiments involved exposing rat groups to various ME concentrations (0, 1.25, 2.5, 3.75 ve 5 mL/cage). Redox parameters were analyzed in blood, liver, and kidney tissues, exploring treatment, gender, and redox marker interactions. **Results:** MCA showed the highest AOA at 0.1 mg/mL and the lowest at 0.4 mg/mL, while RP exhibited the opposite ($p<0.05$). The changes in SRSA levels were not statistically significant ($p>0.05$). Pearson correlation unveiled a strong negative link between RP and SRSA ($p<0.05$). MCA and RP showed a weaker, insignificant negative correlation, while MCA and SRSA displayed a moderate, little positive correlation ($p<0.05$). *In vivo* analysis highlighted interactions among treatment, gender, and redox markers. Total oxidant status (TOS) in blood was affected by the interaction of treatment and gender. TOS exhibited dose-dependent effects in blood, while other parameters (malondialdehyde, reduced glutathione, total antioxidant status) remained unchanged primarily ($p<0.05$). **Conclusion:** ME showed concentration-dependent antioxidant effects *in vitro* and *in vivo*. Although tissue redox balance was minimally affected, kidney GSH elevation indicated potential antioxidant benefits. This underscores ME's potential as a natural health supplement, urging further investigations for optimal doses and effects. The study highlights ME's potential in the food and pharmaceutical sectors.

Keywords: Antioxidant potential; *Myrtus communis* L. extract; *in vitro* assays; redox; Wistar albino rats

ÖZET Amaç: Araştırma, *Myrtus communis* L. özütünün (ME) içme suyunda potansiyel bir antioksidan ajan olarak araştırılmasını amaçlamaktadır. *İn vitro* deneyler ve Wistar albino sıçanlarda *in vivo* testlerini içermektedir. **Gereç ve Yöntemler:** ME'nin antioksidan aktivitesi (AOA), metal kelatlayıcı aktivite (MCA), redükleyici güç (RP) ve süperoksit radikal süpürme aktivitesi (SRSA) deneyleri kullanılarak değerlendirilmiştir. *İn vivo* deneyler, sıçan gruplarının çeşitli ME konsantrasyonlarına (0, 1.25, 2.5, 3.75 ve 5 mL/kafes) maruz kalmasını içermiştir. Redoks parametreleri uygulama, cinsiyet ve redoks belirteci etkileşimlerini araştırarak kan, karaciğer ve böbrek dokularında analiz edilmiştir. **Bulgular:** MCA, en yüksek AOA'yı 0.1 mg/mL'de ve en düşük AOA'yı 0.4 mg/mL'de göstermiştir, RP ise tersini sergilemiştir ($p<0.05$). SRSA seviyelerindeki değişiklikler istatistiksel olarak anlamlı değildir ($p>0.05$). Pearson korelasyon analizi, RP ve SRSA arasında güçlü bir negatif bağlantı ortaya çıkarmıştır ($p<0.05$). MCA ve RP arasında daha zayıf, anlamsız bir negatif korelasyon bulunurken, MCA ve SRSA arasında orta derecede, küçük bir pozitif korelasyon bulunmuştur ($p<0.05$). *İn vivo* analiz, uygulama, cinsiyet ve redoks belirteci etkileşimlerini vurgulamıştır. Kanın toplam oksidan durumu (TOS), tedavi ve cinsiyet etkileşiminin etkisi altında kalmıştır. TOS, kan üzerinde doza bağlı etkiler göstermiş, diğer parametreler (malondialdehit, redükte glutatyon, toplam antioksidan durum) ise başlıca değişmemiştir ($p<0.05$). **Sonuç:** ME, *in vitro* ve *in vivo* olarak konsantrasyona bağlı antioksidan etkiler sergilemiştir. Dokuların redoks dengesi minimum düzeyde etkilenmiş olmasına rağmen, böbrek GSH yükselmesi potansiyel antioksidan faydaları işaret etmektedir. Bu, ME'nin doğal bir sağlık takviyesi olarak potansiyelini vurgulayarak, optimal dozlar ve etkiler için daha fazla araştırmayı teşvik etmektedir. Araştırma, ME'nin gıda ve farmasötik sektörlerdeki potansiyelini vurgulamaktadır.

Anahtar Kelimeler: Antioksidan potansiyel; *Myrtus communis* L. özütü; *in vitro* testler; redoks; Wistar albino sıçanlar

TO CITE THIS ARTICLE:
Denk B, Özçınar Ü, Özsandık İH, Shah SRA, Çetingül İS, Çalık A, et al. Antioxidant properties of myrtus extract: *in vitro* assays and *in vivo* experimental animal study. Turkiye Klinikleri J Vet Sci. 2024;15(1):1-8.

Correspondence: Barış DENK
Afyon Kocatepe University Faculty of Veterinary Medicine, Department of Biochemistry, Afyonkarahisar, Türkiye
E-mail: bdenk@aku.edu.tr

Peer review under responsibility of Turkiye Klinikleri Journal of Veterinary Sciences.

Received: 25 Aug 2023 ***Received in revised form:*** 03 Nov 2023 ***Accepted:*** 28 Nov 2023 ***Available online:*** 08 Jan 2024

Plants have been utilized for medicinal purposes for millennia, with their therapeutic properties subject to extensive academic investigation. *Myrtus communis* L. (MC), commonly known as *Myrtus*, is a medicinal plant traditionally used for various therapeutic purposes.[1] Myrtus is a plant that belongs to the Myrtaceae family, which comprises over 5,500 species. This plant is renowned for its diverse range of health-enhancing attributes, encompassing antioxidant, anti-inflammatory, and antimicrobial characteristics.[2-4] Its leaves are commonly used as hypoglycemic, antiseptic, and disinfectant agents.[1,5] Myrtus extract (ME) is a common natural herbal extract used in various medical treatments.[1,5,6] Researchers have found that ME contains many phenolic compounds.[7] The extract also has antioxidant properties, which help prevent oxidative damage in the body.[3,7]

In recent times, the utilization of natural substances like medicinal plants has garnered increasing attention due to their potential positive impacts on health. Oxidative stress significantly contributes to the emergence of diverse ailments, encompassing diabetes, cancer, and cardiovascular disorders.[8] Therefore, using natural antioxidants from plants such as Myrtus can be a promising approach to prevent and treat these diseases. Several studies have investigated Myrtus's antioxidant activity (AOA) and its potential health benefits.[2,3] In particular, the effect of Myrtus on rat health has been studied extensively. Rats are commonly used as animal models for studying the impact of natural products on health due to their physiological and genetic similarities to humans.[9]

The primary objective of this study is to assess the antioxidative properties of ME by conducting in vitro antioxidant assays, including metal chelating activity (MCA) assay, reducing power (RP) activity assay, and superoxide radical scavenging activity (SRSA) assay. Additionally, we aim to investigate the impact of ME on the well-being of rats by analyzing blood and tissue redox parameters. We hypothesize that ME will demonstrate significant AOA and enhance the well-being of rats. This research seeks to introduce a novel approach for preventing and treating diseases associated with oxidative stress, particularly by exploring the potential benefits of ME as a natural antioxidant source.

MATERIAL AND METHODS

PLANT EXTRACT FORMULATION

Leaves and stems were pulverized and distilled at 98°C for 22 minutes. pH adjusted to 7.2, filtered, and stored at 4°C. Stock extract diluted to 2.5, 5, 7.5, and 10% (v/v) for use. Refer to prior studies for formulation, amino acids, bioactives, and heavy metals.[2]

IN VITRO ANALYSES

In our study, AOA assays were conducted under blinded conditions. All solutions utilized in the analysis were freshly prepared and used on the same day, with measurements conducted in triplicate. Online resources, specifically the Quest Graph™ IC50 Calculator from CA, USA, and the Gain Data ELISA Calculator from Hsinchu City, Taiwan, were employed to compute EC_{50} values.

MCA ASSAY

MCA was measured using the Ferrozine assay. Five mM $FeCl_2$ and 100 µL extract/EDTA were mixed for 5 min, then 100 µL 5 mM Ferrozine and 2 mL distilled water were added. Absorbance was assessed at a wavelength of 562 nm.[2,10]

$$MCA\% = [(A_0-A_1)/A_0] \times 100$$

A_0: The absorbance measurement of the Ferrozin-Fe^{2+} complex; A_1: The absorbance value of the extract or standard.

RP ACTIVITY ASSAY

The RP measurement followed an established protocol.[11] In summary, a 2 mL aliquot of the extract was combined with 2 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2 mL of 1% (w/v) potassium ferricyanide, followed by incubation at 50°C for 20 minutes. Subsequently, 2 mL of 10% (w/v) trichloroacetic acid was introduced, and the mixture was centrifuged at 3,000 rpm for 10 minutes. The resulting supernatant (2 mL) was mixed with 2 mL of deionized water and 0.4 mL of

0.1% (w/v) ferric chloride. Following a 30-minute incubation at room temperature, the absorbance was recorded at 700 nm. A higher absorbance value indicates greater RP. The percent RP of the sample was compared with the max absorbance of α-tocopherol dilutions.

$$RP\% = (A_S/A_M) \times 100$$

A_M: Max α-tocopherol absorbance; A_S: Sample absorbance

SRSA ASSAY

The SRSA was analyzed using a previous method.[12] Nitroblue tetrazolium, NADH disodium salt, and phenazine methosulfate were adjusted to 19 mM in pH 7.4 phosphate-buffered saline (PBS) solution. Nitroblue tetrazolium (156 µM), NADH disodium salt (468 µM), and extract/control reagent (500 µL) were mixed. 500 µL of 60 µM phenazine methosulfate was added and incubated at RT for 5 min. The measurement of absorbance was conducted at a wavelength of 560 nm.

$$SRSA\% = [(A_C - A_S)/A_C] \times 100$$

A_C: Control absorbance; A_S: Sample absorbance

IN VIVO EXPERIMENTAL PROCEDURE

In accordance with ethical guidelines outlined in the Guide for the Care and Use of Laboratory Animals, humane treatment was ensured for all experimental animals, and approval was obtained from the Local Ethics Committee for Animal Experiments at Afyon Kocatepe University (date: April 14, 2021; no: 49533702/48). A total of 80 Wistar albino rats (40 male, 40 female) were utilized for the study. The experimental animal study's sample size and power calculation were meticulously performed to ensure adequate statistical power. With a total of 80 rats earmarked for distribution into 5 groups (0, 1.25, 2.5, 3.75 ve 5 mL/cage), each having a specified target sample size of 16, analysis of variance (ANOVA) was conducted with the aspiration of achieving an 80% power level at a significance threshold of 0.05. In compliance with the 3R principle and adherence to ARRIVE guidelines, the calculation factored in potential animal losses, resulting in a conservative sample size determination of n=16/per group. The animals of both genders were equally distributed into cages (n=8) using random sampling. They were acclimated for 10 days, kept under controlled environmental conditions, and provided unrestricted access to water and feed. The study lasted 35 days. The rats were administered the extract through their drinking water, with concentrations ranging from 0 to 5 mL per cage, calculated as a percentage of the total volume. On the last day, rats were euthanized. Blood samples were collected into K_3EDTA tubes with a 5-8 mL volume. The tubes were centrifuged at 2,500 rpm for 10 minutes at +4 °C. Blood samples were assayed on the collection day, and liver and kidney tissues were extracted from the animals, rinsed with PBS (pH: 7.4), and stored at -20 °C until further assays.

IN VIVO ANALYSES

Blood Redox Parameters

The malondialdehyde (MDA) levels were determined using a colorimetric method involving plasma (0.5 mL) mixed with 10% trichloroacetic acid, heated, and assessed at 532 nm.[13] Reduced glutathione (GSH) concentrations were measured through the DTNB assay, involving supernatant (0.2 mL) mixed with a solution and analyzed at 412 nm.[14] Total antioxidant status (TAS) and total oxidant status (TOS) were assessed using colorimetric test kits (Rel Assay Diagnostics LLC, Gaziantep, TR) to determine the overall redox status.

Tissue Redox Parameters

To assess the AOA of the liver and kidney, 0.5 g of tissue was homogenized in 5 mL of potassium dihydrogen phosphate buffer (50 mM, pH 7.0) at a low temperature. Homogenization involved mechanical and ultrasonic steps. After centrifugation at 5,000 rpm for 15 minutes, supernatants were used for antioxidant assays.[15]

The MDA levels were determined using an established method.[13] The composition included tissue supernatant (0.2 mL), sodium dodecyl sulfate (0.2 mL), glacial acetic acid (1.5 mL), and thiobarbituric acid (1.5 mL). Boiled at 95 °C for 1 hour, cooled, and mixed with distilled water (0.6

mL). N-butanol and pyridine mixture (15:1, v/v, 1 mL) was added, vortexed, and centrifuged at 4,000 rpm for 10 minutes. MDA levels were measured at 532 nm.

GSH levels were determined using Ellman's method, combining 0.2 mL of tissue supernatant with a precipitant solution containing metaphosphoric acid, EDTA, and NaCl.[16] After filtration, 2 mL of filtrate was mixed with phosphate buffer and 0.5 mL of DTNB solution. The absorbance was assessed at 412 nm to quantify the levels of GSH.

STATISTICAL ANALYSIS

The normality test for the data was conducted using the Shapiro-Wilk analysis. The results of all treatments for *in vitro* studies were presented as mean±standard deviation, and the student t-test was used to compare *in vitro* analyses. The t-test was performed in SPSS (v.20, IBM Corp., NC, US) software. The Pearson correlation test also evaluated the relationship between *in vitro* antioxidant parameters. The significance level for the main effects was established at $p \leq 0.05$.

RESULTS

The percent inhibition of the α-tocopherol is presented in Figure 1. The percent inhibition of the antioxidants was determined using MCA, RP, and SRSA assays, and the results are shown in Figure 2.

The percentage inhibition graph of the reducing power assay for α-tocopherol is presented. At the highest concentration (1 mg/mL), the absorbance of α-tocopherol was calculated as 100 using the RP% formula. The values in the graph represent the mean±standard deviation (n=3; $p<0.05$). ME's AOA was assessed through MCA, RP assay, and SRSA at different concentrations (data presented as mean±standard deviation; n=12; $p<0.05$). Varied letters indicated statistically significant distinctions among the compared groups at distinct concentrations.

The MCA of ME varied between 0.15% and 24.16%. At 0.1 mg/mL concentration, ME demonstrated the highest AOA, while the lowest AOA was observed at 0.4 mg/mL ($p<0.05$). The concentration curve displayed a cubic trend ($R^2=0.999$). It was determined that the EC_{50} value for the MCA assay was higher than that of EDTA (0.25±0.01; 0.02±0.001, respectively).

The RP of ME concentrations varied between 46.30% and 89.78%. ME exhibited the highest AOA at a concentration of 0.4 mg/mL, whereas the lowest AOA was evident at 0.1 mg/mL ($p<0.05$). The curve of the concentrations showed a cubic trend ($R^2=0.921$). The EC_{50} value for the RP assay was lower than that of α-tocopherol (0.38±0.12 and 0.55±0.001, respectively).

The SRSA of ME concentrations varied between 17.33% and 24.49%. ME exhibited the highest AOA at 0.1 mg/mL, while the lowest was observed at 0.4 mg/mL. Nevertheless, this variation was not statistically significant ($p>0.05$).

The findings from the Pearson correlation analysis, conducted to assess the degree to which the

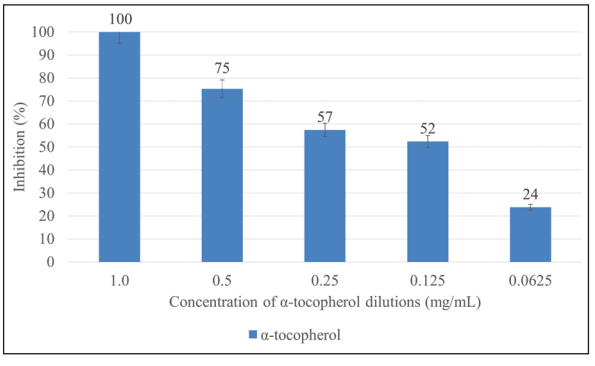

FIGURE 1: The reducing power analysis of α-tocopherol (mean±standard deviation).

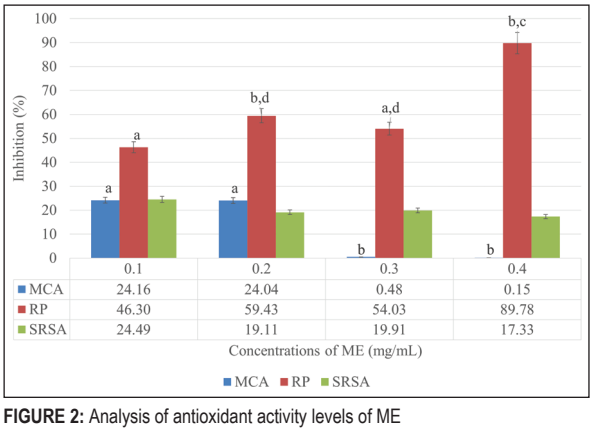

FIGURE 2: Analysis of antioxidant activity levels of ME (mean±standard deviation).
ME: Myrtus extract; MCA: Metal chelating activity; RP: Reducing power; SRSA: Superoxide radical scavenging activity.

TABLE 1: Correlation analysis of antioxidant assays for ME.

	MCA	RP	SRSA
MCA	1	-0.53	0.49
RP		1	-0.61*
SRSA			1

*The correlation holds statistical significance at the 0.05 significance level (n=12, two-tailed); ME: Myrtus communis L. extract; MCA: Metal chelating activity; RP: Reducing power; SRSA: Superoxide radical scavenging activity.

assays reflect the AOA, are summarized in Table 1. These results showed a robust and statistically significant negative correlation between RP and SRSA ($r(12)=-0.61$, $p=0.04$). There was a notable negative correlation between MCA and RP. However, it did not reach statistical significance ($r(12)=-0.53$, $p=0.08$) and a moderate and non-significant positive correlation between MCA and SRSA ($r(12)=0.49$, $p=0.11$).

In our study, we observed that the treatment x gender interaction had a statistically significant effect ($p<0.05$) on the TOS of the blood redox parameters (Table 2). The TOS was found to be elevated in the 0.3 mg/mL and 0.4 mg/mL groups in comparison to the 0.1 mg/mL group. However, no statistically significant changes were detected in the other parameters (MDA, GSH, and TAS).

The study identified a notable gender-related rise in MDA levels ($p<0.05$) within the liver redox parameters, with the 0.1 mg/mL group displaying higher levels than the 0.2 mg/mL group. Moreover, a significant gender-dependent reduction in GSH levels ($p<0.05$) was detected in the 0.1 mg/mL group compared to the control and 0.4 mg/mL groups.

Regarding kidney redox parameters, a statistically significant ($p<0.05$) treatment x gender factor was observed in the kidney MDA levels. Moreover, an increase ($p<0.05$) in GSH levels was observed in the 0.1 mg/mL and 0.3 mg/mL groups compared to the control group. The 0.4 mg/mL group exhibited significant differences compared to all other groups.

DISCUSSION

This study posited the hypothesis that ME would demonstrate significant AOA, thereby enhancing the overall well-being of the rats. To test this hypothesis, the AOA of ME was assessed through MCA, RP, and SRSA assays. The results showed that the AOA of ME varied depending on the concentration and the assay used. The MCA assay showed that ME had the highest AOA at 0.1 mg/mL and the lowest at 0.4 mg/mL. The RP assay displayed a contrasting pattern: the highest AOA at 0.4 mg/mL and the lowest at 0.1 mg/mL concentration. The SRSA assay revealed no significant variation in AOA across different concentrations. These findings indicate that

TABLE 2: Redox data.

	Treatment						p value		
	C	2.50%	5.0%	7.50%	10%	SEM	Treatment	Gender	Treatment x gender
Blood									
MDA (nmol/mL)	7.42	6.57	6.63	6.93	7.5	0.5588	0.6546	0.56	0.2849
GSH (mg/dL)	6.86	8.08	6.94	8	7.94	0.4215	0.0973	0.0212	0.1681
TAS (mmol/L)	0.85	0.86	0.87	0.73	0.92	0.0736	0.4928	0.128	0.8439
TOS (mmol/L)	1.05^{ab}	0.48^{b}	1.1^{ab}	1.4^{a}	1.25^{a}	0.2522	0.1184	0.7538	0.0148*
Liver									
MDA (nmol/g wet tissue)	118.76^{ab}	133.75^{a}	96.94^{b}	130.92^{ab}	123.92^{ab}	12.7121	0.271	0.0085*	0.1052
GSH (µmol/g wet tissue)	4.42^{a}	3.51^{b}	3.92^{ab}	4.04^{ab}	4.52^{a}	0.2992	0.1268	0.0004*	0.2623
Kidney									
MDA (nmol/g wet tissue)	358.55	295.76	352.61	325.12	295.5	47.1451	0.8035	0.4911	0.012*
GSH (µmol/g wet tissue)	4.94^{c}	5.69^{b}	5.23^{bc}	5.8^{b}	6.57^{a}	0.2521	0.0003*	0.3177	0.6017

The changes in blood, liver, and kidney redox parameters between groups were evaluated. The parameters included MDA, reduced GSH, TAS, and TOS. Statistical significance was determined with a p value of 0.05, and differences between groups were indicated with different letters (*p<0.05). SEM: Standard error mean; MDA: Malondialdehyde; GSH: Glutathione; TAS: Total antioxidant status; TOS: Total oxidant status.

the AOA of ME may depend on the specific assay used and the concentration of the extract.

The results of the Pearson correlation analysis also revealed a robust and statistically significant negative correlation between RP and SRSA, suggesting that the ability of ME to reduce metal ions and its ability to scavenge superoxide radicals may be mutually exclusive. A strong yet statistically insignificant negative correlation was observed between MCA and RP, which suggests that the ability of ME to chelate metal ions may not be related to its RP. Furthermore, a moderate, though statistically insignificant, positive correlation was noted between MCA and SRSA, which suggests that the ability of ME to chelate metal ions may be related to its ability to scavenge superoxide radicals. As previously reported in the literature, MCA appears to involve different mechanisms than antioxidant and radical scavenging activities.[3] Previous research has also reported on the MCA and RP of the methanolic extract of *Myrtus* seeds.[2]

These results suggest that the AOA of ME may vary depending on the specific assay used and the concentration of the extract. The strong correlation between RP and SRSA indicates that these two activities may be mutually exclusive, while the correlation between MCA and SRSA suggests a potential relationship between metal chelation and superoxide radical scavenging activities. In addition, the higher EC_{50} value for MCA compared to EDTA indicates that ME may not be as effective as EDTA in binding metal ions. On the other hand, the lower EC_{50} value for RP compared to α-tocopherol suggests that ME may be more effective than α-tocopherol in reducing ferric ions. These findings hold significant implications for using ME as a natural antioxidant across various industries such as food, cosmetics, and pharmaceuticals. However, further research is warranted to ascertain ME's *in vivo* AOA and explore its potential health-related advantages.

In our study, the redox parameters of the blood, liver, and kidney tissues of rat groups fed with different concentrations of ME were analyzed. Our study compared and interpreted the findings with previous studies and made some extrapolations. The observed statistically significant effect of the treatment x gender factor on TOS in the blood redox parameters suggests that gender-based differences may exist in the response to ME treatment. ME may have a dose-dependent effect on TOS in the blood. However, the other parameters lack statistically significant changes (MDA, GSH, and TAS). This suggests that the extract may not substantially influence the overall redox balance in these tissues.

It can be concluded that the treatment and gender factors can significantly impact various redox parameters in both blood and liver. The higher TOS in the 0.3 mg/mL and 0.4 mg/mL groups suggests that higher doses of the treatment may lead to increased oxidative stress in blood. The increase in MDA levels in the 0.1 mg/mL group compared to the 0.2 mg/mL group may indicate increased lipid peroxidation in the liver, while the decrease in GSH levels in the same group may suggest decreased antioxidant defense.

There was a statistically significant interaction between treatment and gender in kidney MDA levels, suggesting that the effects of the treatment may have varied depending on gender. Furthermore, significant increases in GSH levels were observed in various treatment groups compared to the control group. The substantial rise in the 0.4 mg/mL group was noteworthy, surpassing the increases observed in all other groups. This suggests that the treatment positively impacted GSH levels in the kidneys. These findings indicate that the treatment may have had differential effects on kidney MDA and GSH levels depending on gender and may be a potentially effective intervention for increasing GSH levels in the kidneys.

Previous studies have reported that the aqueous extract of MC leaves, the ethanolic extract of MC, and the MC essential oil did not cause any changes in the MDA and GSH levels of rodents' liver and kidney tissues.[4-6] In the previous study, rats were administered the aqueous-ethanolic extract of MC leaves via intragastric administration, and it was observed that there was no observable dose-dependent alteration in liver MDA and GSH levels. However, a dose-dependent rise in kidney MDA

levels was noted, particularly at 100 mg/kg of the extract.[9] Another study reported that the ethanolic extract of MC berries did not cause any changes in the MDA and GSH levels of the liver.[17] A prior investigation indicated that the introduction of intragastric aqueous-ethanolic extract from MC leaves didn't lead to noteworthy modifications in the antioxidant capacity evaluations of liver and kidney homogenates in rats, as assessed through 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) tests.[9]

In our study, similar to previous studies, no alteration in the redox system was observed in the liver and kidney (except for an increase in GSH levels in the kidney homogenate) in response to MC extract treatment. The observed increase in GSH levels in the kidney homogenate in our study indicates that the MC extract affected the intracellular redox system in favor of antioxidants in a dose-dependent manner.

CONCLUSION

The investigation involved *in vitro* assays and an experimental study on Wistar albino rats. The results indicate that ME could serve as a natural source of antioxidants that offer potential benefits on various physiological parameters without any adverse effects on the liver and the kidney. Moreover, the extract displayed a dose-dependent impact on TOS in blood and increased reduced GSH levels in the kidney, which suggests its potential for improving antioxidant defense. However, optimal dosages and the observed effects need further verification through additional investigation. In summary, this study underscores the potential of ME as a natural health supplement, and its prospective applications in the food and pharmaceutical sectors merit further investigation.

Source of Finance

Afyon Kocatepe University Scientific Research Projects Committee (AKU-BAP) funded this project as a research project.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Barış Denk, Ümit Özçınar, İsmail Bayram;* ***Design:*** *Barış Denk, Ümit Özçınar, İsmail Bayram;* ***Control/Supervision:*** *Barış Denk, Ümit Özçınar, İsmail Bayram, İbrahim Sadi Çetingül;* ***Data Collection and/or Processing:*** *Barış Denk, Ümit Özçınar, İsmail Bayram, İbrahim Sadi Çetingül;* ***Analysis and/or Interpretation:*** *Barış Denk, Ümit Özçınar, İsmail Bayram, İbrahim Sadi Çetingül, Ali Çalık, Mustafa Midilli;* ***Literature Review:*** *Barış Denk, Ümit Özçınar, İsmail Bayram, İbrahim Sadi Çetingül, Ali Çalık, Mustafa Midilli;* ***Writing the Article:*** *Barış Denk, Ümit Özçınar, İsmail Bayram, İbrahim Sadi Çetingül, İsmail Hakkı Özsandık, Syed Rizwan Ali Shah, Ali Çalık, Mustafa Midilli;* ***Critical Review:*** *Barış Denk, Ümit Özçınar, İsmail Bayram, İbrahim Sadi Çetingül, İsmail Hakkı Özsandık, Syed Rizwan Ali Shah, Ali Çalık, Mustafa Midilli;* ***References and Fundings:*** *Barış Denk, Ümit Özçınar, İsmail Bayram, İbrahim Sadi Çetingül;* ***Materials:*** *Barış Denk, Ümit Özçınar, İsmail Bayram, İbrahim Sadi Çetingül.*

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