

Testicular Morphologies of Two Sympatric *Podarcis* Species

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Abstract—The reproductive strategies of reptiles vary according to many factors. In lizards, intraspecific and interspecific competition, and environmental and seasonal factors affect their reproductive biology. This study aimed to conduct a comparative analysis of testis sizes in the sympatrically coexisting species, *Podarcis muralis* and *Podarcis tauricus*, located in Kofçaz, Kırklareli, Turkey, considering both intra-species and inter-species variations. Changes in body sizes and testis sizes according to months were monitored. As a result, it was determined that in both species, there was a positive correlation between body size and testis size, and that in *P. tauricus*, both body and testis sizes were higher compared to *P. muralis*, showing a significant difference. It was also observed that in the *P. muralis* species, the testis size reached its peak value in April (mean daily temperature 9.9°C), while in the *P. tauricus* species, it reached the highest value in May (mean daily temperature 15.5°C). It was found that the testis size in *P. muralis* reached its lowest value in August (mean daily temperature 23.8°C), while in *P. tauricus*, it was lowest in September (mean daily temperature 20.2°C). The fact that testis size is high in different months in these two sympatric species suggests that their active breeding periods differ.

Keywords: testis size, body size, *Podarcis*, sympatry

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INTRODUCTION

Reproductive biology and reproductive behaviors can significantly differ over temporal and spatial scales in response to various ecological and environmental conditions (Castilla and Bauwens, 2000; Lukanov and Tzankov, 2016). Studies on lizard ecology and evolution require a fundamental understanding of their reproductive and life history traits, which is also essential for their conservation status (Cree and Hare, 2016). The diversity in reproductive and life history traits among lizard species, which are components of reproductive biology, is partially explained by the physical environment (Benabib, 1994). There have been numerous studies on various aspects of testis in relation to lizard species, including their size, volume, sperm production, histological structure, seasonal changes, and relationships with body sizes (Bostic, 1966; Boretto and Ibarguengoytía, 2006; Cardone et al., 2008; Todd, 2008; Kahrl and Cox, 2015; Sagonas et al., 2019; Pizarro et al., 2022).

Sperm morphology and testis size exhibit significant variability within species (Kahrl and Cox, 2017) and among species of lizards (Uller et al., 2010), but the extent of variation among populations remains unknown (Kahrl and Cox, 2017). Increased testis mass can be associated with elevated sperm production and

may be indicative of a mating strategy involving multiple matings with one or many females (Naretto et al., 2016). Body size serves as an important signal of mate quality in many species (Andersson, 1994) and can provide advantages to animals involved in agonistic interactions (Keogh et al., 2013). To better understand the evolutionary and environmental determinants of reproductive diversity, it is essential to investigate closely related taxa, sympatric taxa, and allopatric ones (Rheubert et al., 2020). Sympatric conspecifics can differ in terms of breeding timing, size, age of maturity, or clutch size (Ballinger, 1973; Barbault, 1976; Tinkle and Dunham, 1986; James, 1991). Moreover, sympatry can lead to differences in inter-species interactions, pre- and post-mating competition traits, or reproductive strategies (Naretto et al., 2016). Studies on the reproductive biology of sympatric lizards have been conducted (Lin and Nelson, 1981; Taylor, 2004; Carretero et al., 2006; Ramirez-Pinilla et al., 2009; Naretto et al., 2016; Kahrl and Cox, 2017). Differences in secondary sexual characteristics, relative testis mass, and sperm component length have been reported between allopatric and sympatric *Salvator* sp. lizards (Naretto et al., 2016). Despite numerous studies examining the morphology of various components of the reproductive system, there are few studies that

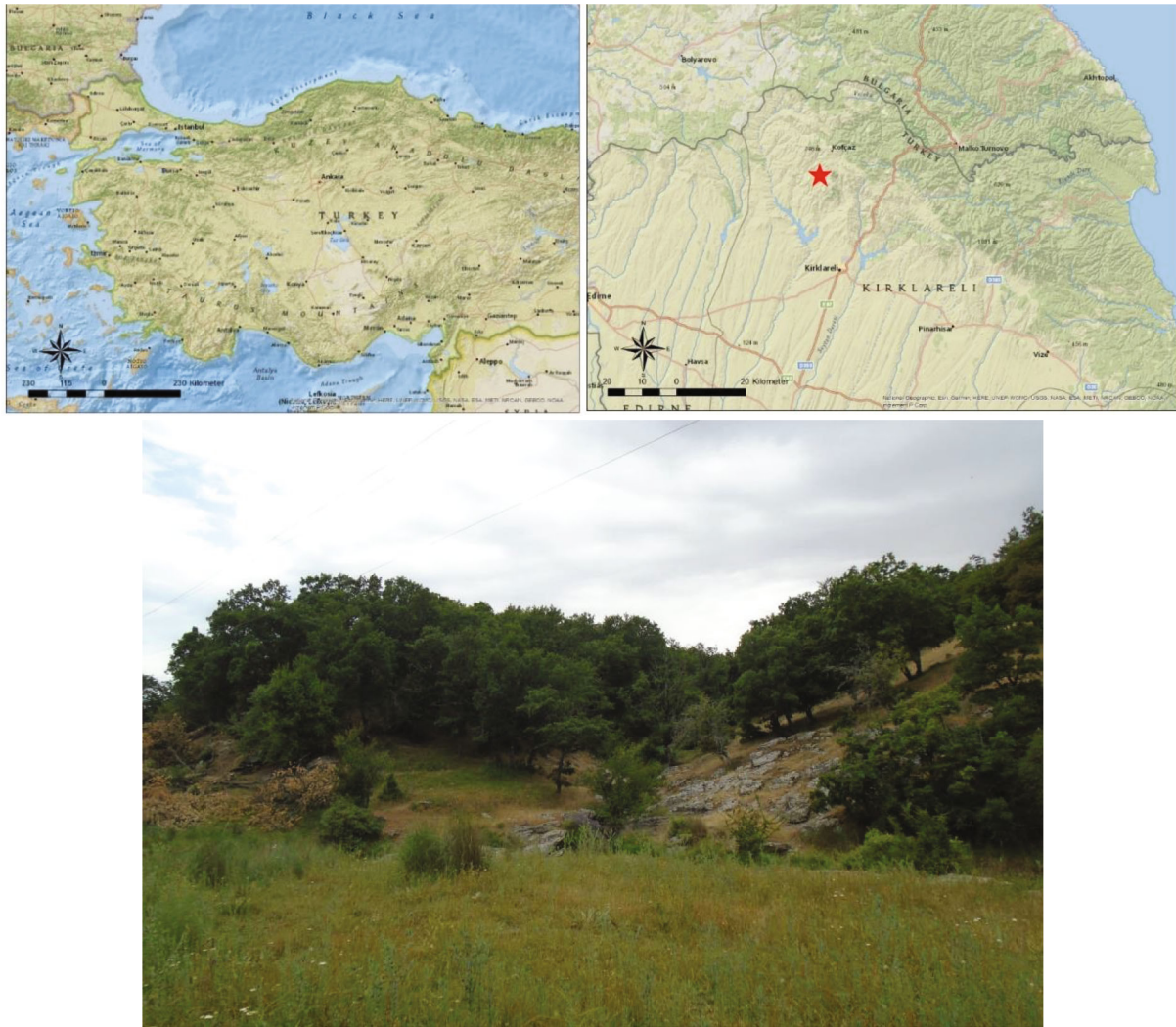


Fig. 1. Study area. Kofçaz (Kırklareli-Türkiye).

have investigated sympatric squamates (Rheubert et al., 2020). Determining the extrinsic and intrinsic interactions in the reproductive biology of sympatric species is important for monitoring and conserving these species. Therefore, this study focused on *Podarcis muralis* (Laurenti, 1768) and *Podarcis tauricus* (Pallas, 1814), which are sympatrically distributed in Kofçaz, Kırklareli, Thrace region, Türkiye.

This study aimed to conduct a comparative examination of testis sizes (testis weight, length, width, volume) and body sizes (body weight and snout-vent length) of the sympatrically living species *Podarcis muralis* and *Podarcis tauricus*, both within and between the species, across different months.

MATERIALS AND METHODS

Study Areas and Data Collection

We collected 44 male lizards of *P. muralis* and 35 male *P. tauricus* from Kırklareli/Kofçaz (Fig. 1). The

lizards were captured by hand between April and September 2023. The mean daily temperatures for the months (April–September) are as follows: 9.9, 15.5, 19.0, 27.0, 23.8, 20.2°C, respectively (Kofçaz, Meteoroloji Genel Müdürlüğü [General Directorate of Meteorology]). The samples obtained from previous studies related to these species were also used.

Morphological Measurements and Indexes

We conducted measurements on all specimens, including snout-vent length (SVL), body weight (BW), and the month of capture. The body measurements were taken with a digital caliper accurate to 0.01 mm, and weights were recorded using a digital scale with a precision of 0.01 g. For the male individuals, measurements were focused on the right testicle, including testicular width, testicular length, and testicular weight, which was measured using a sensitive scale accurate to 0.1 mg. The testicle's volume was approximated as an ellipsoid using the formula: $V =$

Table 1. Descriptive statistics of all measurements

	<i>Podarcis muralis</i> (N: 44)					<i>Podarcis tauricus</i> (N: 37)				
	min	max	mean	SE	SD	min	max	mean	SE	SD
BW, g	2.81	7.34	5.18	0.164	1.089	3.45	8.68	5.96	0.247	1.508
SVL, mm	50.36	69.44	59.84	0.629	4.172	53.10	71.18	62.97	0.865	5.266
Testicular weight, g	0.00	0.03	0.01	0.001	0.009	0.00	0.06	0.02	0.002	0.012
Testicular length, mm	2.23	6.63	4.58	0.135	0.901	2.62	6.92	5.00	0.180	1.19
Testicular width, mm	1.71	4.36	2.94	0.099	0.660	1.64	5.07	3.39	0.127	0.776
Testicular volume, mm ³	3.41	65.96	23.34	2.034	13.494	4.62	72.36	33.51	2.916	17.738

(4/3) πab^2 , where “a” represents half of the testicle’s length, and “b” represents half of the testicle’s width, as described by Mayhew in 1963.

Statistical Analyses

All the data collected from the specimens were analyzed using the SPSS software (version 26). To assess the normality of the data, the Kolmogorov–Smirnov Test was employed. For comparisons between species, the Student’s *t*-test was utilized for parametric data, while the Mann–Whitney U Test was applied for non-parametric data. Scatterplots and boxplots were generated using R Studio.

RESULTS

All the descriptive statistics for the measurements taken from a total of 44 *P. muralis* and 37 *P. tauricus* samples are provided in detail in Table 1.

When the individuals of the two species were compared, a statistically significant difference was observed in BW (Mann–Whitney test; *Z*: –2.190, *p*: 0.029) and SVL (Mann–Whitney test; *Z*: –2.944, *p*: 0.003) measurements. It was determined that *Podarcis tauricus* was both heavier and larger than *Podarcis muralis*.

Statistically significant differences were found in testicular weight (*t*-test; *t*: 2.469, *p*: 0.016), testicular width (Mann–Whitney test; *Z*: –2.257, *p*: 0.024), and testicular volume (*t*-test; *t*: 2.929, *p*: 0.004) measurements, while there was no significant difference in testicular length (*t*-test; *t*: 1.895, *p*: 0.062). Similar to morphological measurements, individuals of the *Podarcis tauricus* species had larger testicular measurements compared to those of the *Podarcis muralis* species.

While in the *Podarcis muralis* species, SVL and testicular volume did not show a significant correlation (*r*: 0.207, *p*: 0.177), in the *Podarcis tauricus* species, a significant positive correlation was observed (*r*: 0.479, *p*: 0.003). It was determined that as SVL increased in *Podarcis tauricus*, testicular volume also increased (Fig. 2).

When examining BW and testicular volume, both *Podarcis muralis* (*r*: 0.300, *p*: 0.048) and *Podarcis tauricus* (*r*: 0.497, *p*: 0.002) exhibited positive correlations. It was observed that in both species, as body weight increased, testicular volume also increased (Fig. 3).

When examining BW and testicular weight, both *Podarcis muralis* (*r*: 0.417, *p*: 0.005) and *Podarcis tauricus* (*r*: 0.485, *p*: 0.002) exhibited positive correlations (Fig. 4).

In both species, there were significant differences in testis volumes between the months of June and July (*P. muralis* *p*: 0.008; *P. tauricus* *p*: 0.003). It was observed that in both *Podarcis muralis* and *Podarcis tauricus*, testis volumes decreased after the end of the breeding season in June (Fig. 5). In *Podarcis muralis*, a gradual decline was observed from April, while in *Podarcis tauricus*, there was a decrease after reaching its highest level in May following a lower point in April.

DISCUSSION

The snout-vent length of mature individuals of the *P. muralis* species was reported to be 54 cm (Sacchi et al., 2012). However, the data we obtained indicates that the reproductive activity and sexual maturity of this species start from 50 cm. For the *P. tauricus* species, it was reported that mature individuals were larger than 53 cm (Altunışık et al., 2016). Significant differences were found in the SVL and BW values between the two populations showing sympatric distribution, with *P. tauricus* being larger than *P. muralis*.

Previous studies reported a positive correlation between body size and testis size (Aleksic and Ljubisavljevic, 2001; Taylor, 2004; Todd, 2008; Ramírez-Pinilla et al., 2009; Sagonas et al., 2019). In our study, we also found positive correlations between body size and testis size in both species. Testicular weight, testicular width, and testicular volume values showed significant differences between the two sympatric species, with *P. tauricus* having higher values. In sympatry, interspecific interactions can lead to convergence in traits related to pre- and post-copula

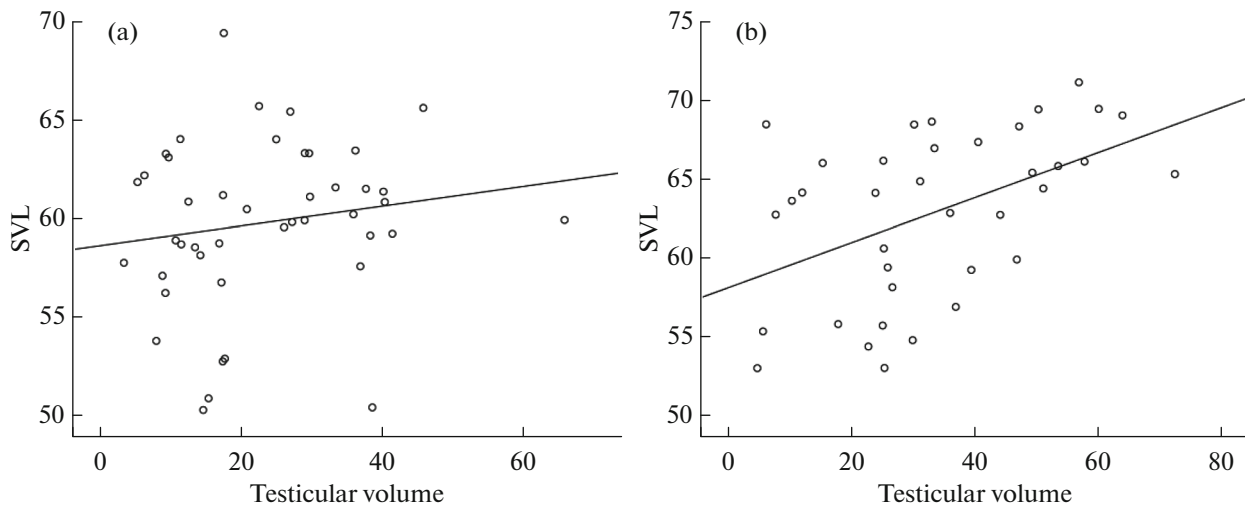


Fig. 2. Correlations between SVL and testicular volume ((a) *Podarcis muralis*, (b) *Podarcis tauricus*).

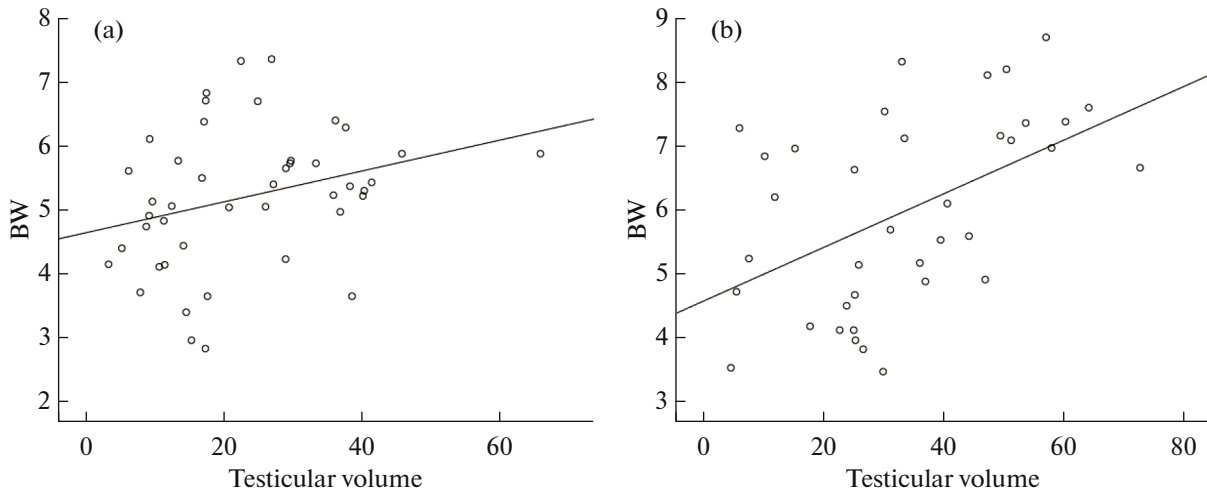


Fig. 3. Correlations between BW and testicular volume ((a) *Podarcis muralis*, (b) *Podarcis tauricus*).

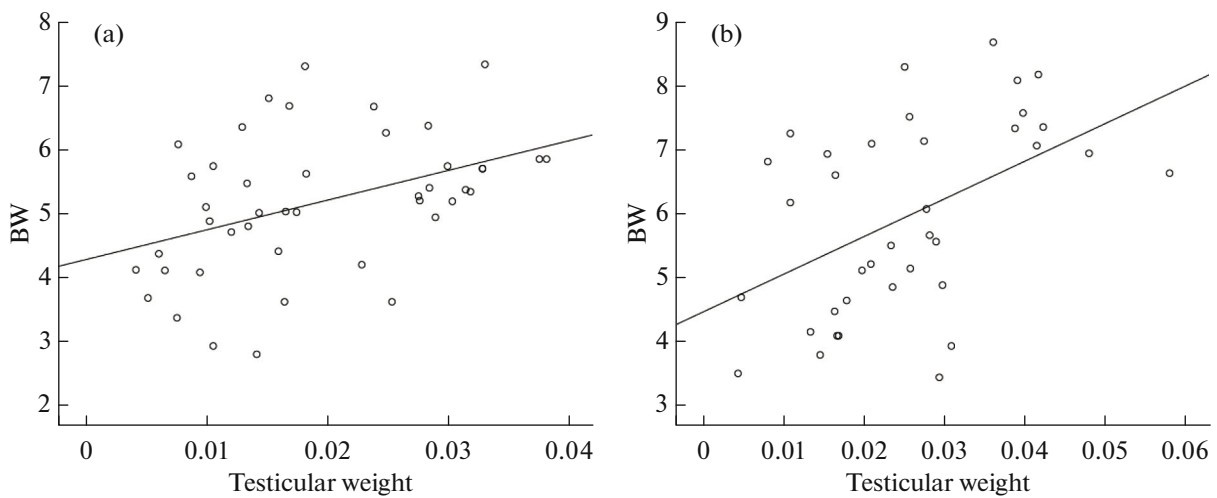


Fig. 4. Correlations between BW and testicular weight ((a) *Podarcis muralis*, (b) *Podarcis tauricus*).

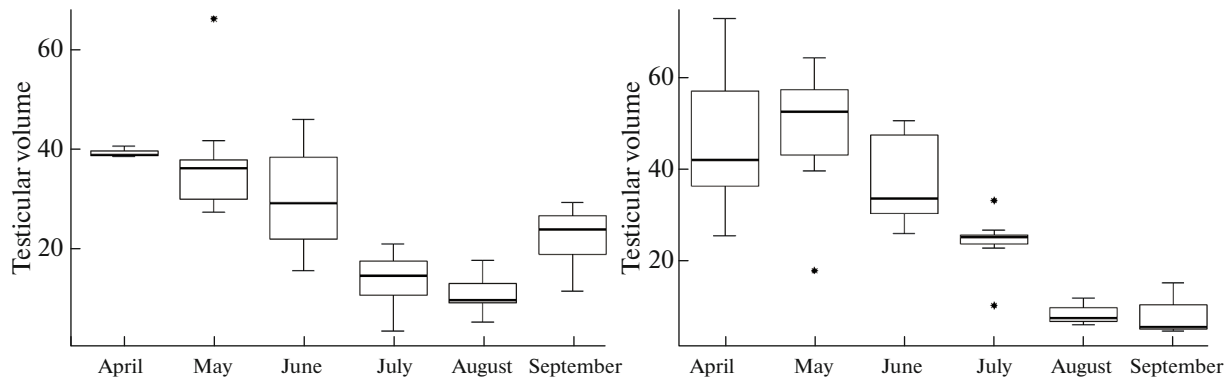


Fig. 5. Monthly variations of testicular volume between species ((a) *Podarcis muralis*, (b) *Podarcis tauricus*).

competition or divergence in reproductive strategies (Naretto et al., 2016).

It was noted that testis mass and testis volume exhibited significant variations between months (Sagonas et al., 2019) and were related to precipitation and temperature (Ramírez-Pinilla et al., 2009). In both species emerging from hibernation, *P. muralis* had the highest testis volumes in April (mean daily temperature 9.9°C), while *P. tauricus* reached its peak in May (mean daily temperature 15.5°C). The lowest testis volumes were observed in *P. muralis* in August (mean daily temperature 23.8°C) and in *P. tauricus* in September (mean daily temperature 20.2°C). It was reported that in *P. muralis*, the heaviest testis mass was observed in April, while the lightest was in July (Aleksic and Ljubisavljevic, 2001). Additionally, in the case of *Phoenicolacerta kulzeri*, it was reported that in the hottest months, July and August, testis volume decreased in 40% of males (Rizk and Nassar, 2015). After reaching peak testis volume, both species show a decline until the month of August. The males were found to be reproductive from August to December, with the maximum testis volume in both *Ctenotus robustus* and *C. taeniolatus* species occurring in early spring, while the minimum testis volume was recorded in early autumn (Taylor, 2004). Previous studies reported a decrease in testis size in lizard species after reaching peak levels (Pianka and Parker, 1975; Ortiz et al., 2001). According to our data, there was a slight increase in *P. muralis* during the September period. In *Anolis trinitatis*, it was reported that the fat body data increased after August, following a decrease in testis, indicating an increase in fat levels a few months after the decrease (Licht and Gorman, 1970). Given the correlation between body size and testis volume, it can be suggested that the increase in fat before hibernation in September results in an increase in testis volume.

Reproductive activity in lizards may be controlled by complex interactions involving phylogenetic, physiological, and environmental factors (Angelini et al., 1976, 1978; Saint Girons, 1984; Carretero, 2006). It was reported that *P. tauricus* was active during the

reproductive period at a temperature of 18°C (Fischer et al., 2019), while *P. muralis* was active at 18.1°C (Eroğlu et al., 2018). According to the testis volume, it can be suggested that *P. tauricus* is in the reproductive period at 15.5°C, and *P. muralis* at 9.9°C, indicating potential differences in sympatric settings. Differences in some reproductive characteristics among populations of *Salvator merianae* and *S. rufescens* were reported based on social contexts (sympatry, allopatry, social contexts), suggesting that they may be subjected to different selective pressures caused not only by the presence of competing species but also by the presence of mates and rivals (Naretto et al., 2016).

The gradual decline in the testis volume of the *Podarcis muralis* species, as opposed to the fluctuating decline in the *Podarcis tauricus* species, may reflect differences in reproductive strategies between the two sympatric species. Additionally, the peak in testis size occurring in April for *P. muralis* and in May for *P. tauricus* under different seasonal conditions suggests that their active breeding periods differ.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

For the conducted studies, the necessary permissions were obtained from the Ethics Committee of Animal Experiments of Çanakkale Onsekiz Mart University (decision no. 2023/05-01).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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