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ON THE COVER

Culex pipiens (Diptera: Culicidae), a globally distributed mosquito species of considerable veterinary and epidemiological significance, recognized for its capacity to transmit a broad range of zoonotic and vector-borne pathogens. Cover image: high-resolution macro photograph obtained from Pixabay (Public Domain, CC0 license.

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Molecular Epidemiology of Canine Heartworm (*Dirofilaria immitis)* in *Culex pipiens* Mosquitoes Collected from Tehran

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ABSTRACT

Dirofilaria immitis is a nematode parasite that inhabits and develop within the heart and pulmonary arteries of canids, felids, and also humans. The microfilariae of this parasite circulate in the bloodstream and are transmitted to new hosts through the bites of *Culex pipiens* mosquitoes. *Culex pipiens*, commonly known as the common house mosquito, is a native urban pest species. Since the early 20th century, various control campaigns have been implemented across European countries targeting this species. It exhibits high ecological plasticity, resulting in complex feeding behaviors and vector potentials. This study was conducted from June to September of 2024 in Tehran. A total of 100 *Culex pipiens* mosquitoes were collected from different areas of the city and examined *Dirofilaria immitis* infection using molecular methods (PCR). The results showed that 15 of the collected mosquitoes were positive for D. immitis microfilariae. Given the infection rate observed in this study, the high potential for infection in the studied areas, and the importance of D. immitis associated disease, implementing hygienic measures in mosquito prone areas and dog shelters, along with effective mosquito control, especially in humid regions during warm seasons, will be essential to prevent the spread of this zoonotic disease among dogs and humans.

Keywords

Microfilariae, Dirofilaria immitis, Culex pipiens, PCR

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Abbreviations

D. immitis: Dirofilaria immitis

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Introduction

Dirofilariasis is a zoonotic parasitic disease caused by the filarial nematode Dirofilaria immitis, commonly known as the canine heartworm. This parasite primarily infects canids, where adult worms inhabit in the pulmonary arteries and right side of the heart, leading to progressive cardiopulmonary disorders. Microfilariae produced by adult worms circulate in the host's bloodstream and, when ingested by hematophagous mosquitoes such as Aedes, Culex, and Anopheles species, develop into infective third stage larvae. During subsequent blood feeding, infected mosquitoes transmit the parasite to new hosts [1].

In dogs, clinical manifestations vary depends on infection intensity. Cases may range from asymptomatic infections to severe disease characterized by persistent coughing, exercise intolerance, anorexia, weakness, and dyspnea. Both the mechanical presence of worms and host's immunological responses contribute to disease pathogenesis. In humans, however, the larvae fail to mature into adults; instead, immature stages induce pulmonary dirofilariasis through inflammatory reactions to dying larvae [2].

Dogs serve as the main reservoir hosts, often harboring infections for years and producing large numbers of microfilariae. Because mosquitoes are not host specific and feed on a wide range of species, they play a crucial role in transmitting dirofilariasis to both canids and humans in endemic areas [3]. By 2014, more than 300 human pulmonary dirofilariasis cases had been reported worldwide, most from the United States, yet no confirmed human cases have been documented in Iran. This absence may be attributed to limited serological screening and the diagnostic challenge of distinguishing pulmonary nodules caused by D. immitis from those arising from other etiologies [4]. Given the importance of reservoir hosts and mosquito vectors in disease transmission, monitoring mosquito populations can provide valuable insights into the circulation of D. immitis. Therefore, the present study aims to investigate the molecular epidemiology of canine heartworm (D. immitis) in Culex pipiens mosquitoes collected from Tehran.

Results

Molecular Results

PCR amplification of the Cox1 gene using specific primers produced a 689 bp fragment. One sample was sequenced and used as the positive control. Agarose gel electrophoresis confirmed the presence of the expected 689 bp band. Lane M contained the 100 bp DNA ladder, lane 1 represents the positive control, lane 2 served as the negative control (no genomic DNA), and lanes 3–5 correspond to the tested samples, all of which produced the specific 689 bp amplicon (Figure 1).

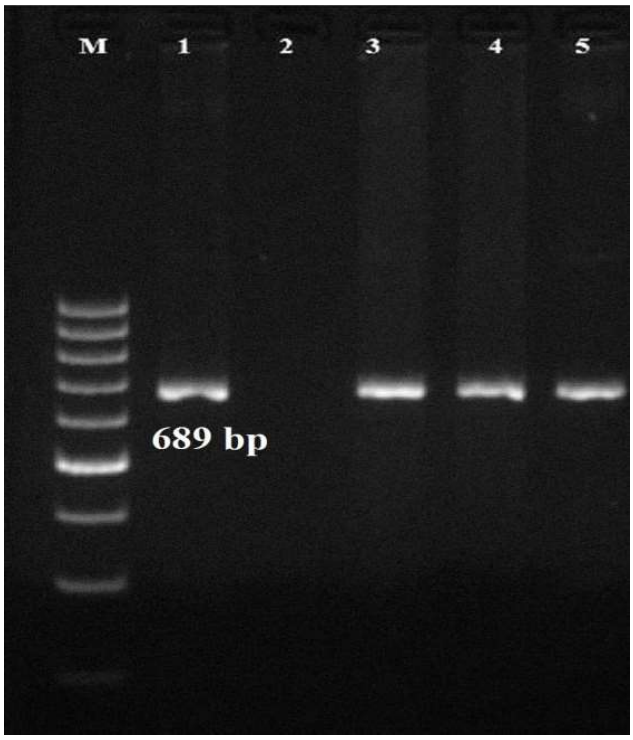

Figure 1.
Agarose gel electrophoresis of PCR amplification of the Cox1 gene (689 bp) of Dirofilaria immitis. Lane M: 100 bp DNA ladder; Lane 1: positive control; Lane 2: negative control; Lanes 3–5: tested mosquito samples showing the expected 689 bp amplicon.

Statistical Analysis

Of 100 Culex mosquitoes collected in this study, 15 were found to carry microfilariae of Dirofilaria immitis. This indicates that 15% of the Culex mosquitoes collected from the study areas between June and September of 2024 were vectors of this parasite (Table 1).

Table 1.
Distribution of *Dirofilaria immitis* infection rates in *Culex pipiens* mosquitoes across different regions of Tehran

Region	Mosquitoes	Number Infected	Infection Rate
Chitgar	20	2	10%
Niyavaran	20	3	15%
Janat abad	20	4	20%
Lavasanat	20	2	10%
Kouhsar	20	4	20%
Total	**100**	**15**	**15%**

Discussion

The aim of the present study was to investigate the molecular epidemiology of Dirofilaria immitis in Culex pipiens mosquitoes collected from different regions of Tehran.

A comparison of historical epidemiological data over recent years shows significant changes in the global prevalence of dirofilariasis. These changes may be related to climate change and shifts in the distribution of vector populations of Dirofilaria species. Microfilariae may enter peripheral blood circulation at specific times during the day or night and hide from circulation at other times [6]. Therefore, the samples in the present study were collected during nighttime hours.

This study focused on Culex pipiens because it is the predominant mosquito species in Tehran and has been consistently been reported as a potential vector of D. immitis in Iran. Its wide distribution in both urban and rural habitats, combined with its opportunistic feeding behavior on dogs and humans, highlights its importance in the local transmission cycle. Therefore, targeting Culex pipiens not only reflects the most epidemiologically relevant vector in this region but also provides reliable insights into the risk of dirofilariasis transmission in Tehran. The transmission rate of dirofilariasis depends on the presence of infected dogs as the main reservoir hosts and the availability of competent mosquito vectors. Consequently, transmission dynamics are influenced by two factors affecting both components of the worm's life cycle: first, human behavior toward pets; second, climatic conditions that enable the presence of competent vector populations and development of Dirofilaria larvae. Similar disease manifestations have been reported in humans, including pulmonary and cardiac diseases [7,9]. Although serological findings indicate that the current prevalence in Tehran is lower than in other regions of Iran, it is still considered relatively high given the city's warm and semi-arid climate. Previous studies have reported two Dirofilaria species, including D. immitis (canine heartworm) and D. repens, in several regions of Iran [8].

Azari Hamidian et al. (2009), described the COX1 sequence of third-stage larvae of D. immitis from Iranian samples.

Sharifdini et al. (2022), in a study on 334 dogs from Mazandaran and Gilan provinces, reported that 75 dogs (21.8%) were infected with D. immitis based on PCR results. In Mazandaran, infection was confirmed in 22% of dogs for D. immitis and in 4.5% Acanthocheilonema reconditum. Previous studies in this province showed infection with D. immitis in 15.2% of dogs by microscopy and 60.9% by necropsy [10].

Studies conducted by Ranjbar-Bahadori et al. (2007, 2011), reported microfilariae infection rate of 12.29% with D. immitis among stray dogs in Garmsar. Additional infection rates were recorded in Tonekabon (15%), Golestan province (18.18%), and Shiraz (9.5%). In another study, 100 herd dogs from around Mashhad were examined; microfilariae were found in 15 dogs, but none were D. immitis, and the species were identified as A. reconditum based on morphology and morphometry [11,12].

Borthakur et al. (2015) in northeastern India, conducted an study on stray, domestic, and working dogs using multiple diagnostic methods including modified Knott's test, fresh blood smear, ELISA, PCR, and gene sequencing. Their results showed positivity rates of 11.38% using Knott's test, 18.03% by ELISA, and 13.93% by PCR. No significant difference in infection was observed between males and females or different regions, however stray dogs were significantly more infected than other groups. ELISA revealed 22.69% occult infection in working dogs, and PCR detected D. repens in 1% of stray dogs. The ITS region of D. immitis showed high genetic homology with South Asian isolates [13].

Similarly, Nguyen et al. (2016) used Real-Time PCR in Australia to differentiate D. immitis from A. reconditum in 39 dogs positive by Knott's test [14].

In the present study, 15% of mosquito samples collected from areas populated by dogs were positive for of D. immitis microfilariae. This finding indicates that both humans and dogs living in these areas are at risk of infection.

Khamesipour et al. (2020) also reported an infection rate of 21.8% with Dirofilaria among 75 dogs in Mazandaran province, consistent with the present study [15].

Conversely, a study by Khodabakhsh et al. (2016) on 103 cats in Meshginshahr, Ardabil province, found only 1 cat (0.96%) was a carrier of the disease, which contrasts with the present study [16].

Manshoori et al. (2023) reported a 17.4% infection rate with Dirofilaria among stray dogs in Mazandaran, Gilan, and Qazvin provinces, consistent with the present study [17].

Malmasi et al. (2009) documented a 25.5% prevalence of D. immitis, in stray dogs from Mazandaran and Golestan provinces, also aligning with the present study [18]. Similarly, Khodari et al. (2014) studied 120 stray dogs in Sistan and Baluchestan, and Kerman provinces, with infection rates of 24.2% and 27.4%, respectively, consistent with the present study [19].

The positive rate of D. immitis in various provinces of Iran appear closely linked to climatic and ecological factors. The highest prevalence was recorded in Gilan (78.6%) and Mazandaran (50%) in the Caspian region, likely due to a Mediterranean temperate climate

year-round. Also, in Isfahan (0.9%), Lorestan (6.9%), and Qazvin (27.3%), this parasite has been reported, possibly due to increased temperature and decreased annual rainfall. Since the first report of D. immitis infection in dogs in northern Iran, several studies have reported prevalence rates as high as 62.8%. Although data remain limited for 9 out of 31 provinces, reports of human infection with D. immitis and D. repens in Alborz and Hormozgan provinces indicate possible ongoing parasite circulation in dog populations. Additionally, wild carnivores such as wolves, jackals, and foxes play an important role in the epidemiology of dirofilariasis, therefore further epidemiological studies are necessary to fill knowledge gaps regarding wild reservoir hosts [20].

Amoung parasitological diagnostic methods, serological assays such as enzyme-linked immunosorbent assay (ELISA) and immunochromatographic tests are widely used for detecting antibodies against D. immitis. Antigen tests can help identify occult infections like a microfilaremia case, but positive dogs may have cleared infection or only been exposed without actual infection [21,22].

Conclusion

The results of the present study demonstrated the presence of Dirofilaria immitis microfilariae in Culex pipiens mosquitoes collected from different areas of Tehran, with an infection rate of 15%. This finding confirms the active circulation of the parasite within mosquito populations in the studied regions and highlights the role of Culex pipiens as a competent vector in the local transmission cycle. The detection of infected mosquitoes in urban areas with close human–dog interactions indicates a potential risk for both canine and human populations. Overall, the findings emphasize the epidemiological importance of mosquito-borne transmission of D. immitis in Tehran.

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

Artificial intelligence tools were used only for language editing, improving the clarity and readability of the English text, and assisting in the organization and formatting of the reference list. All scientific interpretations, data analysis, and conclusions were entirely performed by the authors.

Study Area

This study was conducted from June to September of 2024 in Tehran. The foothill regions of the city host local and indigenous dog populations, which may become infected by Culex mosquitoes in the area. Therefore, public places and veterinary clinics in Niyavaran, Lavasanat, Jannatabad, Kouhsar, and Chitgar were selected for sampling (Figure 2).

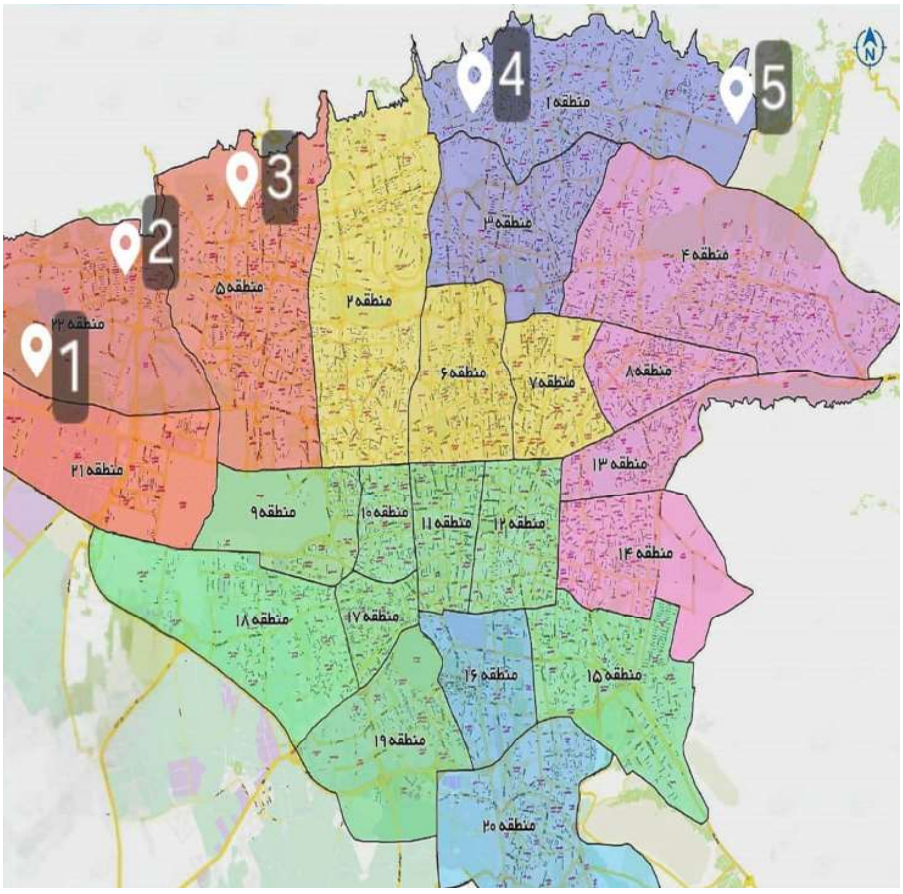

Figure 2.
Map of Tehran showing the sampling locations for Culex pipiens mosquitoes, including Niyavaran, Lavasanat, Jannatabad, Kouhsar, and Chitgar districts

Figure 3.
Morphological characteristics of Culex pipiens mosquito, including uniformly dark wings, unbanded proboscis, short female palps, and dark legs, used for species identification

Table 2.
Nucleotide Sequences of Primers Used for Amplification of Cox1 Gene Region

Reactions	Name of primer	Nucleotide sequence (5′– 3′)	PCR product
Primers	Colint-F	TGATTGGTGGTTTTGGTAA	689 bp
	Colint-R	ATAAGTAC GAGTATCAATATC	

Sample Collection

Mosquito sampling was conducted at night using light traps and aspirators. A total of 100 Culex mosquitoes were collected from the selected locations and divided into five groups of 20 individuals each. Every mosquito was placed in a separate container and assigned a unique identification code. The five groups were stored in individual vials filled with 100% ethanol and kept in a freezer at -21 °C.

Morphological characteristics

Culex pipiens is a medium sized mosquito (3–6 mm) with a brownish body covered in mixed pale and dark scales. The proboscis is uniformly dark and unbanded, and females possess short palps. Both the legs and wings are evenly dark, with the abdomen exhibiting dark tergites with pale basal bands, while the thorax lacks distinct longitudinal stripes. This species can be distinguished from Aedes mosquitoes by the absence of leg and thoracic banding, and from Anopheles mosquitoes by the presence of short female palps and a larval siphon. Within the pipiens complex, C. pipiens differs from C. quinquefasciatus by having a longer larval siphon and by being more common in temperate climates. Its wings are uniformly dark and unmarked, unlike the white banded wings Aedes or the alternately dark and pale spotted wings of Anopheles. The plain dark wings therefore represent a key diagnostic feature of Culex pipiens (Figure 3).

DNA Extraction

Mosquito samples were removed from the -21°C freezer, and DNA extraction was carried out using the MBST DNA extraction kit (Iran) following the manufacturer's protocol. The extracted DNA samples were stored at -21°C until further use.

PCR Reaction

PCR amplification was performed using Colint-F and Colint-R primers targeting the ribosomal Cox1 gene, generating a 689 bp fragment (5) (Table 2). Each PCR reaction had a total volume was 30 µL and contained One Time PCR Buffer, 3 µL genomic DNA, 1.25 U Taq polymerase (Ampliqon, Denmark), 10 pmol of each primer (Metabion, Korea), 200 µM of each dNTP (Ampliqon, Denmark), and 1.5 mM $MgCl_2$.

PCR was performed in an automatic thermocycler (SimpliAmp, USA) under the following cycling conditions: an initial denaturation at 94°C for 5 minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 45 seconds, and extension at 72°C for 60 seconds; followed by a final extension at 72°C for 7 minutes.

Negative controls without genomic DNA were included in all reactions. PCR products were resolved on a 1.5% agarose gel in 1X TBE buffer, stained with ethidium bromide, and visualized under a UV illuminator.

Statistical Analysis

The results and infection rates according to age and sex were analyzed using the Chi-square test. Data analysis was performed using SPSS software version 24 (SPSS Inc., Chicago, USA), and a significance level of $p < 0.05$ was considered statistically significant.

Authors' Contributions

Seyed Reza Hosseini and Marzieh Kefayat planned the experiments. Milad Hamzehali Tehrani. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflict of interest

The authors declare that there is no conflict of the interest.

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Hematological Parameters and Splenic Histopathology in Offspring of Wistar Rats Exposed to Prenatal Stress and *Moringa oleifera*

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ABSTRACT

This study investigates the effects of prenatal exposure to Moringa oleifera leaf extract (MoLE) and chronic unpredictable stress (CUS) on the immune development and splenic histopathology of offspring in rat model. Twenty-five pregnant Albino-Wistar rats were divided into five groups: a control group, two MoLE-treated groups (5 mg/kg and 10 mg/kg), and two CUS-exposed MoLE-treated groups (5 mg/kg and 10 mg/kg). MoLE was administered via gavage from gestation day 8 to 21. Hematological parameters and splenic histopathology were assessed in offspring at puberty. Results showed that MoLE exposure affected blood cell counts and splenic histology. Specifically, MoLE treatment resulted in decreased monocyte and granulocyte counts, while lymphocyte levels remained unchanged. The high-dose MoLE group exhibited increased mean platelet volume (MPV) and platelet distribution width (PDW). Severe histopathological changes, including fibrosis and hemorrhage, were observed in the CUS-exposed groups. These findings suggest that prenatal MoLE and CUS exposure influence immune development.

Keywords

Prenatal Stress, Hematotoxicity, Immunity, Blood, Moringa oleifera

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Abbreviations

AE-FUNAI: Alex Ekwueme Federal University Ndufu-Alike
ANOVA: Analysis of Variance
CUS: Chronic Unpredictable Stress
CV: Coefficient of Variation
EDTA: Ethylenediaminetetraacetic Acid
GC–MS: Gas Chromatography-Mass Spectrometry

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Introduction

Chronic unpredictable stress (CUS) during pregnancy represents a significant public health concern due to its potential to disrupt fetal development, and impacting both physiological and behavioral outcomes in offspring [1]. Prenatal stress, especially in the form of CUS, has been associated with alterations in hematological profiles, such as changes in white blood cell (WBC) counts and platelet function, which could have lasting effects on immune development and blood clotting processes [1-3]. These hematological alterations may increase offspring susceptibility to infections and other health complications [4]. Thus, identifying potential interventions to mitigate these adverse effects is crucial for promoting healthy offspring development.

Moringa oleifera, commonly referred to as the "drumstick tree," is renowned for its health-promoting properties. This drought-resistant tree is cultivated in tropical and subtropical regions and is particularly valued for its leaves, which are rich in essential nutrients, vitamins, minerals, and bioactive compounds, including flavonoids, glucosinolates, and phenolic acids [5, 6]. These compounds are believed to contribute to the plant's antioxidant, anti-inflammatory, and immunomodulatory effects [7].

Although the therapeutic potential of MoLE, has been increasingly documented, its effects during sensitive developmental periods, particularly during pregnancy, remain insufficiently characterized. Furthermore, the combined influence of MoLE and CUS exposure on offspring development, especially regarding hematological parameters and splenic health, has not been fully elucidated. This study aims to bridge this gap by examining the effects of MoLE on hematological markers and splenic histopathology in offspring exposed to prenatal CUS, thus contributing valuable insights into maternal health and offspring development.

Abbreviations-Cont'd

GD: Gestational Day
Gran: Granulocytes
H&E: Hematoxylin and Eosin
HPA: Hypothalamic–Pituitary–Adrenal (Axis)
i.p.: Intraperitoneal
Lym: Lymphocytes
MID: Monocytes/Intermediate Cells
MoLE: Moringa oleifera Leaf Extract
MPV: Mean Platelet Volume
PCT: Plateletcrit
PDW: Platelet Distribution Width
PDW-CV: Platelet Distribution Width Coefficient of Variation
PDW-SD: Platelet Distribution Width Standard Deviation
P-LCR: Platelet Large Cell Ratio
PLT: Platelet Count
SD: Standard Deviation; SEM: Standard Error of the Mean
BC: White Blood Cell.

Results

The majour compounds identified in the GC-MS analysis of MOLE are known for their antioxidant and anti-inflammatory activities[8](Table 1).

White Blood Cell and Differential Counts

Prenatal exposure to Moringa oleifera leaf extract (MoLE) and/or chronic unpredictable stress (CUS) resulted in significant alteration in leukocyte profiles in the offspring (Table 2). Total white blood cell (WBC) counts were significantly elevated in the High dose MoLE group ($17.42 \pm 1.78 \times 10^9$/L) and the CUS + High dose MoLE group ($15.32 \pm 3.39 \times 10^9$/L) when compared with the Control group ($5.40 \pm 0.22 \times 10^9$/L, $p < 0.05$). Lymphocyte counts exhibited a similar pattern, with significant increases observed in the High-dose MoLE and CUS + High dose MoLE groups ($p < 0.05$).

Granulocyte counts were significantly higher in the High dose MoLE ($3.45 \pm 0.15 \times 10^9$/L) and the CUS + High dose MoLE groups ($2.83 \pm 0.72 \times 10^9$/L) compared to Control ($0.46 \pm 0.15 \times 10^9$/L, $p < 0.05$). In contrast, no significant changes were observed in monocyte counts across groups ($p > 0.05$). These findings suggest a stress induced inflammatory response that was partially modulated by MoLE supplementation in a dose dependent manner.

Platelet Counts and Indices

Platelet profiles also significantly affected by prenatal treatments (Table 2). Offspring from High dose MoLE and both CUS exposed groups exhibited a sharp reduction in platelet counts compared to Control, showing a decrease of over 70% ($p < 0.05$). In contrast, CUS + Low-dose MoLE group exhibited a significant increase in platelet count ($1445.0 \pm 101.9 \times 10^9$/L, $p < 0.05$) compared to Control.

Analysis of platelet indices revealed that mean platelet volume (MPV) and platelet distribution width (PDW-SD and PDW-CV) were significantly elevated only in the High-dose MoLE group ($p < 0.05$). No significant differences in MPV or PDW values were observed in the CUS + Low-dose MoLE group compared with Control, indicating preservation of platelet morphology under low-dose supplementation.

Histopathological Findings

Representative photomicrographs of spleen sections are shown in Figure 1 (Panels A–E). Spleens from Control animals exhibited normal histological architecture, characterized by well-demarcated white pulp and red pulp regions (Figure 1A). Low-dose

Table 1.
GC-MS analysis of MOLE; An exempt from "GC–MS analysis of *Moringa oleifera* leaf extract and effects of administration on histology of reproductive organs and liver of female rats exposed to chronic unpredictable stress" by Chukwu et al., 2024 [10]

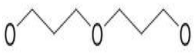
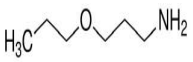
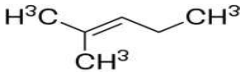
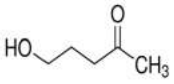
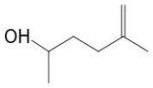
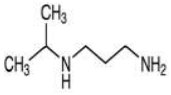
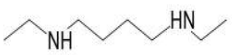
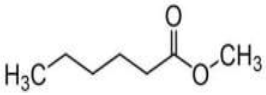
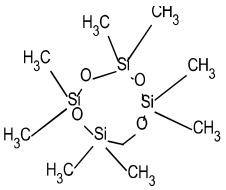
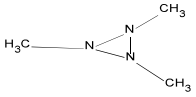
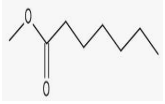
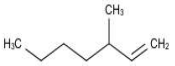
S / No	Name of Compound	Mol. formular	Mol. Wt(g)	RT (min)	% TIC	Structure	Activity
1	1-Propanol, 3,3'-oxybis-	$C_6H_{14}O_3$	134.	3.045	1.521		Humectants (Food additive/ Moisturizer)
2	1-Propanamine, 3-propoxy-	$C_5H_{12}NO$	117	3.327	0.803		Textile resins, Drugs, Pesticides
3	2-Pentene, 2-methyl-	C_6H_{12}	84	3.778	0.593		Photochemical and ozonolysis studies
4	Pyridine	C_5H_5N	79	4.285	4.763		Drugs, Vitamins, Food flavorings, Pesticides,
5	2-Pentanone, 5-hydroxy-	$C_5H_{10}O_2$	102	4.820	0.630		Anti-malarial drugs, Vitamin B1
6	5-Hexen-2-ol, 5-methyl-	$C_7H_{14}O$	114	5.102	0.887		Natural substances and Extractives
7	1,3-Propanediamine, N-(1-methylethyl)-	$C_6H_{16}N_2$	116	5.440	0.546		Useful research chemical compound
8	1,4-Butanediamine, N,N'-diethyl-	$C_8H_{20}N_2$	144	5.553	0.183		Unidentified
9	Hexanoic acid, methyl ester	$C_7H_{14}O_2$	130	5.722	0.843		Flavouring agents
10	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	6.031	0.468		Pharmaceuticals, Polymers, Hair/Skin care products, Antiperspirants and Deodorants, Lubricants, Sealants, Adhesives, Waxes and Coating.
11	1,2,3-Trimethyldiaziridine	$C_4H_{10}N_2$	86	6.285	0.515		Unidentified
12	2-Hexyn-1-ol	$C_6H_{10}O$	98	6.426	0.212		Flavour and fragrance
13	Heptanoic acid, methyl ester	$C_8H_{16}O_2$	144	6.595	1.204		Human Metabolite, Flavouring agents, Fragrance,
14	1-Heptene, 3-methyl-	C_8H_{16}	112	6.905	0.608		Hydrocarbon
15	1-Fluorononane	$C_9H_{19}F$	146	7.158	0.459		Unidentified

Table 1 Cont'd

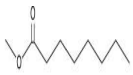
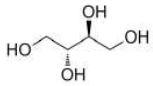
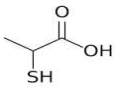
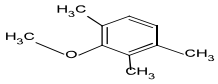
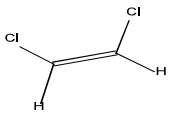
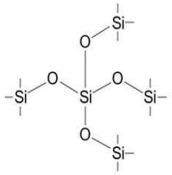
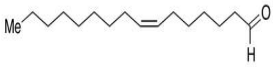
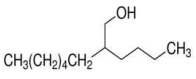
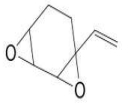
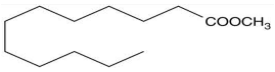
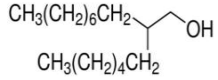
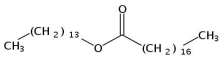
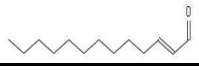
S / No	Name of Compound	Mol. formular	Mol. Wt(g)	RT (min)	% TIC	Structure	Activity
16	Octanoic acid, methyl ester	$C_9H_{18}O_2$	158	7.440	2.299		Metabolite
17	Erythritol	$C_4H_{10}O_4$	122	7.834	6.842		Food additive and Sugar substitutes
18	2-Mercaptopropanoic acid	$C_3H_6O_2S$	106	8.313	2.240		Flavour and Fragrance agents
19	Triethylene glycol	$C_6H_{14}O_4$	150	9.243	1.079		Pesticides, Fragrance, Humectant, Disinfectant, Plasticizer for vinyl polymers
20	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	9.440	1.750		Biodiesel surrogate
21	Benzene,2-methoxy-1,3,4-trimethyl	$C_{10}H_{14}O$	150	10.849	2.298		unidentified
22	Ethylene, 1,2-dichloro-, (Z)-	$C_2H_2Cl_2$	97	10.894	0.200		Pharmacology, Refrigerant, Degreaser, Adhesives, Lacquers, oils, and Resins
23	10-Undecenoic acid, methyl ester	$C_{12}H_{22}O_2$	198	11.102	0.275		Flavouring agents
24	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl) oxy]-	$C_{12}H_{36}O_4Si_5$	385	11.581	0.360		Paints, Coatings, and Cosmetics, including some Personal care products
25	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238	12.539	0.016		Derivative of essential oils with potential antibacterial activities.
26	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186	12.623	0.015		Human metabolite, Humectant
27	3,8-Dioxatricyclo[5.1.0.0(2,4)] octane, 4-ethenyl-	$C_8H_{10}O_2$	138	12.905	0.003		Undefined
28	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	13.356	6.008		Therapeutic uses, Flavouring agents
29	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242	14.342	0.497		Fungicidal properties, Inhibitor (Candida glabrata), suggesting useful for treating Skin cancer
30	Myristyl stearate	$C_{32}H_{64}O_2$	481	14.623	0.181		Skin conditioning and Deodorant
31	2-Tridecenal, (E)-	$C_{13}H_{24}O$	196	14.877	0.219		Flavour and Fragrance agents

Table 1 Cont'd

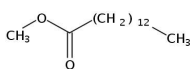
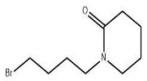
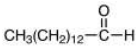
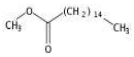
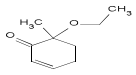
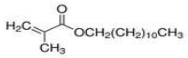
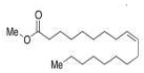
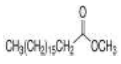
S / No	Name of Compound	Mol. formular	Mol. Wt(g)	RT (min)	% TIC	Structure	Activity
32	Cyclotetradecane	$C_{14}H_{28}$	196	15.468	1.427		Plant metabolite and a human metabolite
33	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	15.891	4.899		Plant metabolite, Flavouring agent and a Fragrance.
34	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234	16.426	1.766		Antimicrobial
35	Tetradecanal	$C_{14}H_{28}O$	212	16.736	1.853		Human metabolite, Flavouring agent and Fragrance
36	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314	16.877	1.420		Surfactant
37	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	17.384	26.182		Metabolite
38	6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{14}O_2$	154	17.778	3.234		Flavouring agent
39	n-Dodecyl methacrylate	$C_{16}H_{30}O_2$	254	18.004	3.814		Drug (Clinical trials), Metabolites, Fragrance, Pesticides etc.
40	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	18.567	4.770		Flavouring agents and Fragrance
41	Methyl stearate	$C_{19}H_{38}O_2$	298	18.736	12.113		Metabolites and Flavouring agents

Table 2.
Hematological Parameters (White Blood Cell, Differential Count, and Platelet Indices) of Offspring of Dams Exposed to CUS and MoLE

Variable (Units)	Control	Low-dose MoLE	High-dose MoLE	CUS + Low-dose MoLE	CUS + High-dose MoLE
Total & Dif-ferential WBC					
WBC ($\times 10^9$/L)	5.40 ± 0.22^a	10.87 ± 1.64^b	17.42 ± 1.78^c	7.32 ± 2.15^{ab}	15.32 ± 3.39^c
Lymphocytes ($\times 10^9$/L)	4.43 ± 0.07^a	7.93 ± 0.77^b	13.03 ± 1.54^c	6.90 ± 2.07^{ab}	11.86 ± 2.49^c
Monocytes ($\times 10^9$/L)	0.51 ± 0.10^a	0.54 ± 0.19^a	0.95 ± 0.11^{ab}	0.30 ± 0.06^a	0.63 ± 0.18^{ab}
Granulocytes ($\times 10^9$/L)	0.46 ± 0.15^a	2.40 ± 0.71^b	3.45 ± 0.15^c	0.12 ± 0.03^a	2.83 ± 0.72^c
Platelet Indices					
PLT ($\times 10^9$/L)	1087.3 ± 115.9^a	355.7 ± 51.0^b	312.3 ± 40.1^b	1445.0 ± 101.9^c	316.0 ± 17.2^b
MPV (fL)	7.07 ± 0.03^a	7.27 ± 0.12^{ab}	7.77 ± 0.23^c	6.83 ± 0.09^a	7.60 ± 0.15^{bc}
PDW-CV (%)	13.97 ± 0.13^a	14.00 ± 0.25^a	15.07 ± 0.12^b	13.83 ± 0.09^a	14.10 ± 0.17^{ab}
PDW-SD (fL)	9.77 ± 0.19^a	10.53 ± 0.45^{ab}	12.30 ± 0.44^c	9.43 ± 0.03^a	11.50 ± 0.42^{bc}
PCT (mL/L)	7.71 ± 0.86^a	2.59 ± 0.35^b	2.44 ± 0.37^b	5.59 ± 2.80^{ab}	2.41 ± 0.13^b
P-LCR (%)	14.60 ± 0.26^a	15.33 ± 0.94^a	18.77 ± 1.27^b	12.60 ± 0.65^a	16.77 ± 0.98^b

Values are expressed as Mean ± SEM. Within each row, means that do not share the same superscript letter differ significantly ($p < 0.05$). Superscripts with the same letter indicate no statistically significant difference between the corresponding groups.

MoLE-treated offspring displayed mild splenic tissue degeneration accompanied by moderate inflammatory infiltration and focal ground-glass changes (Figure 1B). In contrast, High-dose MoLE offspring showed mild to moderate fibrotic changes with occasional hemorrhage (Figure 1C). The most severe histopathological changes were observed in the CUS + Low-dose MoLE group, characterized by marked tissue degeneration, extensive fibrosis, and hemorrhagic red pulp (Figure 1D). The CUS + High-dose MoLE group exhibited moderate tissue degeneration with focal fibrosis but less extensive damage compared to the CUS + Low-dose MoLE group (Figure 1E), suggesting a partial protective effect of higher MoLE supplementation under stress exposure. Semi-quantitative lesion scoring (Table 3), confirmed these histological observations: fibrosis and hemorrhage were significantly more severe in the CUS + Low-dose MoLE group compared to Control ($p < 0.05$). In contrast, co-treatment with High-dose MoLE attenuated lesion severity under CUS conditions ($p < 0.05$).

Figure 1. Representative photomicrographs of spleen sections (H&E staining) showing:

(B) Low-dose MoLE: Mild degeneration with inflammatory infiltration;

(C) High-dose MoLE: Mild fibrosis and hemorrhage;

(D) CUS + Low-dose MoLE: Severe degeneration, fibrosis, and hemorrhagic red pulp;

(E) CUS + High-dose MoLE: Moderate degeneration with focal fibrosis.

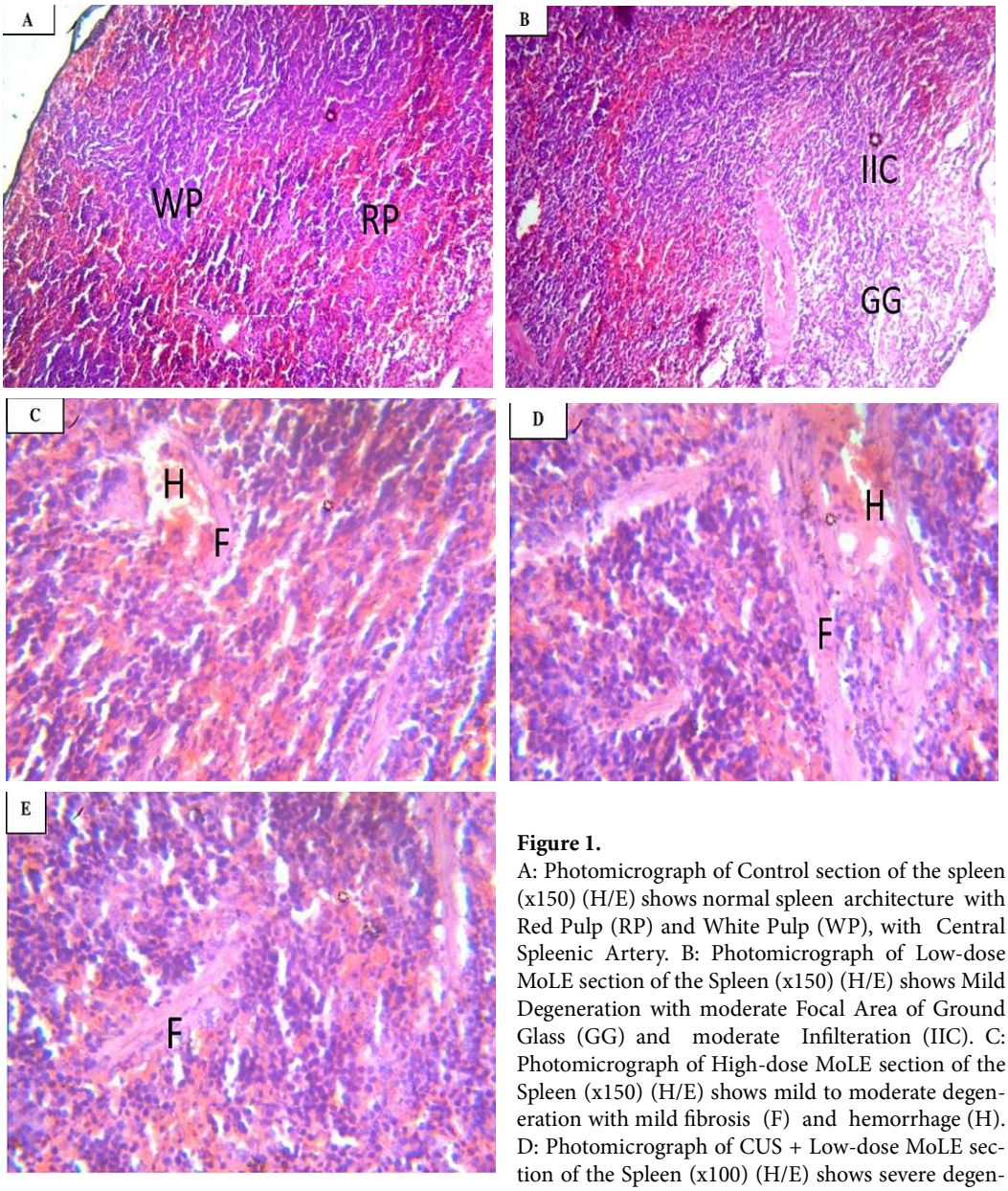


Figure 1.
A: Photomicrograph of Control section of the spleen (x150) (H/E) shows normal spleen architecture with Red Pulp (RP) and White Pulp (WP), with Central Spleenic Artery. B: Photomicrograph of Low-dose MoLE section of the Spleen (x150) (H/E) shows Mild Degeneration with moderate Focal Area of Ground Glass (GG) and moderate Infilteration (IIC). C: Photomicrograph of High-dose MoLE section of the Spleen (x150) (H/E) shows mild to moderate degeneration with mild fibrosis (F) and hemorrhage (H). D: Photomicrograph of CUS + Low-dose MoLE section of the Spleen (x100) (H/E) shows severe degeneration of the Spleenic Tissue with severe Fibrosis (F) and Hemorrhagic (H) Red Pulp. E: Photomicrograph of CUS + High-dose MoLE section of the Spleen (x100) (H/E) shows moderate Degeneration with mild Focal Area of Fibrosis (F).

Table 3.
Semi-quantitative Scoring of Splenic Lesions in Offspring

Group	Fibrosis (0–3)	Hemorrhage (0–3)	Inflammatory Infiltrate (0–3)	Ground Glass Areas (0–3)	Total Lesion Score (0-12)
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[a]
Low-dose MoLE	1.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	5.00 ± 0.00[b]
High-dose MoLE	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.00[b]
CUS + Low-dose MoLE	3.00 ± 0.00	3.00 ± 0.00	2.00 ± 0.00	0.00 ± 0.00	8.00 ± 0.00[c]
CUS + High-dose MoLE	2.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	4.00 ± 0.00[b]

Values are mean ± SEM (n = 3 animals per group). 0 = absent, 1 = mild, 2 = moderate, 3 = severe. Superscripts within the Total Lesion Score column indicate statistically distinct groups ($p < 0.05$). Groups sharing the same superscript are not significantly different from each other. [a]Control, [b]Low-dose MoLE, High-dose MoLE, and CUS + High-dose MoLE (not significantly different from one another), [c]CUS + Low-dose MoLE (significantly higher than all other groups)

Materials & Methods

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During manuscript preparation, a generative AI tool (ChatGPT, OpenAI) was used only as a supportive language aid to improve sentence structure, grammar, and clarity in selected sections of the manuscript. The tool was not used to generate scientific hypotheses, experimental design, data, statistical analyses, figures, or interpretations. All content was critically reviewed, edited, and finalized by the authors, who take full responsibility for the originality, accuracy, and integrity of the work.

Ethical Approval

All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Faculty of Basic Medical Sciences Research Ethics Committee, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria (Approval Code: FBMS/EC/AE/1983).

Plant Collection, Identification, and Extraction

Fresh Moringa oleifera leaves were collected in the early morning from a cultivated garden in Abakaliki, Ebonyi State. Botanical authentication was confirmed at the Herbarium Unit, Department of Biology, Alex Ekwueme Federal University Ndufu-Alike (AE-FUNAI). Leaves were thoroughly washed, air-dried at room temperature for seven days, and pulverized into a coarse powder using an electric blender (Model MS-233, China). Extraction was performed using methanol according to standardized procedures [5, 6]. The filtrate was concentrated at 40°C under reduced pressure to yield a dark-green paste, which was stored at 4°C until use.

Phytochemical characterization of the extract was performed using gas chromatography–mass spectrometry (GC–MS) as previously described by Chukwu et al. [8]. Analysis was performed using an Agilent GC–MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm). The oven temperature was programmed from 60°C (2 min hold) to 280°C at 10°C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Major compounds identified are known for antioxidant and anti-inflammatory activity [8] (Table 1).

Experimental Animals and Housing Conditions

Twenty-five mature, nulliparous, virgin female Albino-Wistar rats (weighing 150–180 g) were obtained from the Animal House, AE-FUNAI. Animals were housed in well-ventilated polypropylene cages under standard laboratory conditions, including 12h light/dark cycle, temperature of 23±2°C, and humidity of 50-60%. Rats had free access to standard rat chow (Vital feed®, Nigeria) and tap water. Animals were acclimatized for two weeks before mating.

Estrous Cycle Monitoring and Mating

Estrous cycle monitoring was performed by vaginal cytology using light microscopy following established protocols [9]. Females with two consecutive regular four-day cycles were considered for mating. During the proestrus phase, identified by the predominance of nucleated epithelial cells and absence of cornified cells, females were co-housed overnight with proven male breeders (1:2 ratio). The presence of spermatozoa in morning smears confirmed successful mating and was designated as gestational day (GD) 1 [10].

Experimental Design and MoLE Administration

Pregnant rats were randomly assigned (n=5 per group) to the following treatment groups:

- Control: Standard diet and water ad libitum.
- Low-dose MoLE: MoLE 5 mg/kg/day via oral gavage from GD 8–21.
- High-dose MoLE: MoLE 10 mg/kg/day via oral gavage from GD 8–21.
- CUS + Low-dose MoLE: CUS exposure plus 5 mg/kg/day MoLE from GD 8–21.
- CUS + High-dose MoLE: CUS exposure plus 10 mg/kg/day MoLE from GD 8–21.

Doses were freshly prepared in distilled water daily and administered in volumes not exceeding 1 mL/100 g body weight to avoid gastric discomfort [8]. The gestational window GD 8-21 was selected to reflect the late second to third trimester in human pregnancy, a critical period for immune and hematopoietic development [11, 12].

Chronic Unpredictable Stress (CUS) Protocol

Animals in CUS groups were subjected to a validated CUS protocol [13] consisting of the following randomly applied stressors:

- Wet bedding (300 mL water mixed with sawdust).
- Cage tilting at 45° for 6 hours.
- Overnight food deprivation.
- Psychological stress by exposure to a caged cat.
- Sleep deprivation using a pedestal in shallow water.
- Restraint stress in 50 mL plastic tubes for 2-hour intervals.
- Continuous overnight light exposure.
- Social isolation for 6-12 hours.

Stressors were rotated daily in an unpredictable order from GD 8–21 to mimic human psychosocial stress [13. 14].

Sample Collection and Litter Effect Consideration

At the onset of puberty (postnatal day 21 onward), five offspring per treatment group were sampled for hematological analysis, with at least one pup selected from each dam (total 5 dams per group). To minimize litter effect bias and maintain balanced sex representation, pups were randomly selected across litters, ensuring that no more than one male and one female pup per dam contributed to the dataset. This approach ensured equal representation of each dam while avoiding over-representation of littermates.

Although individual pup measurements were recorded, the dam (litter) was treated as the experimental unit (n = 5 dams per group) for all statistical analyses, in accordance with internationally accepted developmental toxicology guidelines [12, 16], thereby preventing pseudo-replication. Approximately 2 mL of blood was collected via retro-orbital venous puncture under light isoflurane anesthesia into EDTA-coated tubes and processed within 3 hours at 4°C to preserve cell morphology and ensure accurate hematological profiling.

Hematological Analysis

A calibrated automated hematology analyzer (Mindray BC-2800, Shenzhen, China) was used to determine:

- Total WBC counts ($\times 10^9$/L).
- Differential counts: Lymphocytes (Lym), monocytes (MID), and granulocytes (Gran), reported as absolute counts and percentages.
- Platelet parameters: Platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW-CV, PDW-SD), plateletcrit (PCT), and platelet-large cell ratio (P-LCR) [16, 17].

Histological Studies

Following blood collection, animals were humanely euthanized using sodium pentobarbital (150 mg/kg, i.p.). Spleens were excised, trimmed of fat, and fixed in 10% neutral-buffered formalin for 24-48 hours. Standard tissue processing, paraffin embedding, and hematoxylin-eosin (H&E) staining were performed [18]. Sections were examined under a light microscope (×100–150 magnification). Histopathological changes, including fibrosis, hemorrhage, inflammatory infiltration, ground-glass appearance; were semi-quantitatively graded on a scale of 0-3 by two independent blinded observers. Any scoring discrepancies were resolved by consensus [19].

Statistical Analysis

All data are presented as mean ± standard error of the mean (SEM). The dam was considered as the experimental unit (n = 5 per group), to avoid pseudo-replication. When more than one pup per dam was sampled, values were averaged to obtain a single representative data point per dam. Hematological parameters were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Non-parametric data from semi-quantitative splenic lesion scoring were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism (Version 9.0, GraphPad Software, San Diego, CA, USA).

Authors' Contributions

O.O.C., A.C.U.E, conceived, planned and carried out the experiments, O.O.C., S.N.I.and N.G.K carried out data collection and analysis. O.O.C. wrote the first draft of the manuscript, and all authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Evaluation of the clinical and histopathological findings of uterus and ovaries in ovariohysterectomized cats in Ahvaz district

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ABSTRACT

The present study aimed to evaluate clinical findings and histopathological lesions of the uterus and ovaries and emphasizes the diagnostic role of ovariohysterectomy in cats from Ahvaz region between October and February 2022. A total of Fifty-four cats, referred to the Veterinary Hospital, underwent ovariohysterectomy surgery. The Mean±SD age of the cats was 10.94 ± 0.74 months. In the macroscopic examination of the samples, except for three cases that were pregnant, all the other cases had normal structure and no specific clinical signs were observed prior to surgery. Histopathological findings identified uterine and ovarian lesions in five cats, including one case of cystic endometrial hyperplasia, one granulosa cell tumor, one uterine cyst with ovarian hemorrhage, one case of uterine and ovarian hyperemia with hemorrhage; and one case of uterine hemosiderosis. In the cat affected by cystic endometrial hyperplasia, endometrial cells were proliferated and cysts located in the endometrial mucosa; also, several large follicles and corpora lutea were seen inside the ovary. This lesion was classified as Grade I cystic hyperplasia according to the Dow classification. In the microscopic evaluation of samples with granulosa cell tumor, a mass of proliferating ovarian cells were observed. The mentioned cells were placed together in the form of a ladder. These cells were characterized by large nuclei with one to two prominent nucleoli and a small amount of cytoplasm. The present study, demonstrate that ovariohysterectomy can play an important role to diagnose pathological lesions of the uterus and ovaries in cats. The importance of diagnosis, is much greater, especially in the cases of tumors.

Keywords

Uterus, Ovary, Histopathology, Ovariohysterectomy, Cat, Ahvaz, Iran

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Abbreviations

CEH: Cystic Endometrial Hyperplasia
DLH: Domestic Long Hair
DSH: Domestic Short Hair

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Introduction

Pathological lesions of the uterus and ovaries, including the cystic endometrial/pyometrial hyperplasia complex, are most commonly reported in older cats, however, many lesions cannot be identified due to their subclinical nature. In addition to physical examination and diagnostic tests, histopathological evaluations following ovariohysterectomy plays a critical role in the accurate identification of uterine and ovarian lesions in cats. Subclinical uterine disorders such as pyometra, may turn into life-threatening conditions. Pyometra in both dogs and cats is frequently associated with cystic endometrial hyperplasia (CEH), a condition influenced by progesterone hormone imbalances. Under the hormonal effect, myometrial contractility decreases, while the growth of endometrial glands increase [1].

Histopathological evaluation, in conjunction with clinical findings, is a valuable diagnostic tool that enables the differentiation between uterine infections and other non-infections uterine disorders. Removal of the uterus and ovaries allows for histopathological examination of various diseases of the female reproductive system [2]. It is probably influenced by repeated exposure of the endometrium to high concentrations of estrogen in the luteal phase, although the cause of cystic hyperplasia of the endometrium is not completely clear. The immobility of the uterus and the secretions of the endometrial glands provide a suitable environment for the growth and proliferation of bacteria. Among intrauterine bacterium Escherichia coli is the most common isolated in pyometra [3].

Clinically, most cats with pyometra exhibit signs such as anorexia, lethargy, weight loss, or even septic shock and peritonitis. In cats with open cervix pyometra a wet perineal area and purulent vaginal secretions are commonly observed, polyuria and polydipsia may also occur [4]. A reliable method to definitively diagnose pyometra is to evaluate the uterine by ultrasonography. In healthy cats, the uterine horn diameter usually ranges from 0.4 to 0.7 cm, depending on the stage of the estrous cycle. Appropriate treatment for pyometra is ovariohysterectomy; however, fluid therapy, antibiotic administration, and appetite stimulants are required before surgery to stabilize the patient's body. Evaluation of ovarian pathology is helpful in cases suspected of ovarian cystic hyperplasia [5]. Using dopamine agonist in medical treatment may delay the recurrences of pyometra for in the short term, however ovariohysterectomy remains the most effective and definitive treatment for pyometra [3].

Despite numerous studies conducted on feline reproductive disorders in recent years, the actual prevalence of uterine infection and their underlying mechanisms remain poorly understood [6, 7]. In the USA and Europe,the proportion of privately owned cats that are neutered ranges from 27% to 93%. Regarding the frequency of histopathological lesions of the uterus and ovaries, there are relatively few reports in clinically healthy cats, with most available studies presented as isolated case reports. For instance, Pasteurella multocida was isolated from a 16-year-old Persian cat with pyometra, which presented with intermittent vomiting, anorexia and progressive abdominal distension [9]. In another survey involving 44 cats with inflammatory uterine diseases, ovarian cystic or active follicles were observed in 19 cats; while the remaining 25 cats exhibited luteal phase ovaries [10 Breed predisposition has also been reported, with a higher prevalence of pyometra in Bengal cats, accompanied by active corpora lutea on the ovaries, in connection with proliferative adenomatous changes, in the glandular and surface epithelium of uterine [11].

In another study conducted on fourty five cats with pyometra; it was showed that although dopamine agonist therapy may temporarily delay disease recurrence; ovariohysterectomy remains the most effective treatment option [5].

In Iran Considering that ovariohysterectomy is not routinely performed in privately owned cats articularly those kept individually. As a result, the prevalence of uterine infections in the domestic cats population may be relatively high. To date, there was no comprehensive study investigating uterine and ovarian pathologies in cats in Iran . The present study was conducted for the first time in cats of Ahvaz district in Khuzestan province. The current survey is practical and the obtained results will be of great help to veterinary clinicians. The purpose of the present study was to evaluate the histopathological lesions of the uterus and ovaries, including cystic hyperplasia of the uterus, pyometra, mucometra, neoplastic conditions in cats. Additionally, this study aimed to investigate the clinical signs and emphasizes the diagnostic and preventive value of ovariohysterectomy in the feline population in Ahvaz district.

Results

Clinical findings

In the present study, a total of fifty-four cats were evaluated. Among them, three cats were pregnanent, two cats had blood-filled uterus, and the remaining cats showed normal uterine and ovarian structures. The mean±SD of the studied cats were 10.94 ± 0.74 months, with an age range from six months to 2.5 years.

No significant difference association was ob-

served between access to outdoor environments and the presence of uterine or ovarian lesions ($p > 0.05$). Only five cats had access to outdoor envirements. There was no recorded history of uterine diseases and administration of different drugs, including corticosteroids. At the time of examination, clinical findings, including vulva and vagina condition, presence of vaginal discharge, mammary gland abnormalities, lactation status, polyuria, polydipsia, and skin and hair coat condition, were within normal limits in all cats.

Based on the microscopic evaluation of the uterus and ovaries, cats were classified into four groups according to the estrous cycle:

Proestrus (Group 1): twenty-one cats were in the proestrous phase, In these samples, several large follicles were observed in the ovary and corpus luteum. Also, the uterine endometrium was thin and mildly hyperemic.

Estrus (Group 2): Fourteen cats were classified as being in estrus cycle. In these samples, the uterine mucosa showed marked hyperemia and superficial hemorrhage, with a large number of abundant erythrocytes present within the endometrial layer. A very large follicle was observed in the ovary.

Diestrus (Group 3): Eight cats were in diestrous phase. A large corpus luteum was present in the ovaries, and the endometrial layer was markedly thickened due to the proliferation of endometrial glandular cells.

Anestrus (Group 4): Eleven cats classified as anestrous. In these samples, ovarian follicles exhibited the smallest diameter, and the corpora lutea was markedly regressed.

Among thirteen Persian cat, five were in proestrus phase, three in estrus, four in anestrus, and one in diestrus. Thirty-nine cats were of the DSH breed; among them, fourteen were in proestrous, ten in estrous, eight in diestrous in all cats, and seven in anestrous. One DLH cat and one Siamese cat were in the proestrus phase. They were showing irregularities in estrous cycle distribution, which may be attributed to induced ovulation in cats or differences in environmental and management conditions [3].

Histopathological examination revealed uterine or ovarian lesions in five out of fifty-four cats, Identified lesions included: one case of endometrial cystic hyperplasia, one case of granulosa cell tumor, one case of uterine cyst with ovarian hemorrhage, one case of uterine and ovarian hyperemia with hemorrhage, and one case of uterine hemosiderosis.

Out of five affected cats, involvement was observed in four cases (80%), ovarian involvement in two cases (40%), and simultaneous uterine and ovarian involvement in two cases (40%). All lesions were identified in DSH breed. The mean±SD age of affected DSH cats was 10.71±0.95 months. The relatively high standard deviation may be related to variations in age at ovariohysterectomy and the small number of affected cases[12].

Measurements of endometrial thickness, myometrial thickness, total uterine thickness, the number of follicles and number of corpora lutea were performed in all samples. The fifty-four uterine and ovarian samples were categorized into four estrous cycle groups. In the diestrous group (Group 3), a reduction was observed in endometrial thickness, myometrial thickness , uterine thickness, and the number of large corpora lutea. The greatest amount of thickness was recorded in the second group, during the estrous phase ($p < 0.01$; Table 1). The number of follicles gradually decreased from Group 2 to Group 4 ($p < 0.05$). The highest mean ± SD number of follicles was observed in the proestrous group (11.76 ± 0.95), with a maximum of 17 follicles. The lowest follicles count was noted in the cat affected by CEH. Although variations were observed in cats with uterine cysts and ovarian hemorrhages, these differencse were not sataistically significant. In the estrous group, the maximum of uterine thickness was 15.11 ± 0.78 µm, while the lowest uterine thickness (6.5 µm) was observed in the cat affected by granulosa cell tumor, possibly due to the effect of the tumor in uterus ($p < 0.05$). No significant difference were observed in the number of corpora lutea among the four groups in the involved samples.

Table 1.
Measurements of endometrium, myometrium, uterine thickness, number of follicles and ovarian corpus luteum (mean±SD) in 54 cats in Ahvaz district (W.F x 10 graduated lenses)

Mean ± SD	Cycle			
	Proestrus	Estrus	Diestrus	Anestrus
Tickness of endometrium	9.33 ± 0.64	11.14 ± 0.61	11.00 ± 1.21	9.21 ± 0.93
Tickness of myometrium	1.49 ± 0.16	1.84 ± 0.16	1.55 ± 0.07	1.43 ± 0.25
Tickness of uterine	13.79 ± 0.59	15.11 ± 0.78	14.75 ± 0.99	13.48 ± 0.94
Number of follicles	11.76 ± 0.95	11.42 ± 0.97	7.00 ± 0.90	10.66 ± 0.66
Number of corpus luteum	2.66 ± 0.68	2.87 ± 0.58	2.42 ± 0.52	1.05 ± 0.34

Among the five cats affected by uterine and ovarian disease, corpora lutea were observed in four cases. Two cats were in the proestrous cycle, one in estrous and two in diestrous. Microscopic examination revealed pathological changes in both uterus and ovaries tissues. One cat was diagnosed with cystic endometrial hyperplasia. This sample showed proliferation of endometrial cells with cysts formation within the endometrial mucosa. Additionally, several large follicles and corpora lutea were observed in the ovary. This lesion was categorized as Grade I CEH, based on the Dow classification (1962) [11, 13] (Figures 1 and 2).

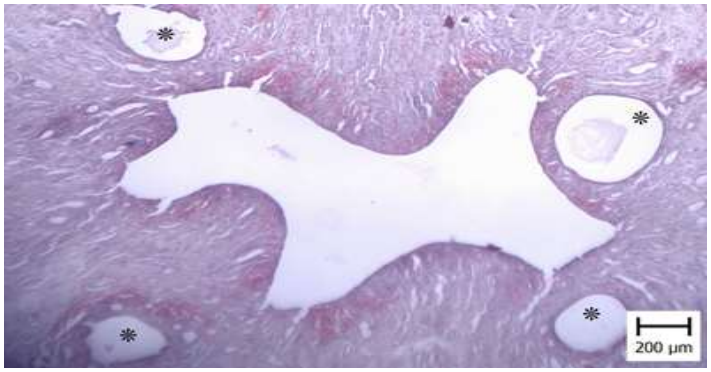

Figure 1.
Cystic hyperplasia of the uterine endometrium.
Note on cysts of varying sizes (asterisks) (Hematoxylin and Eosin).

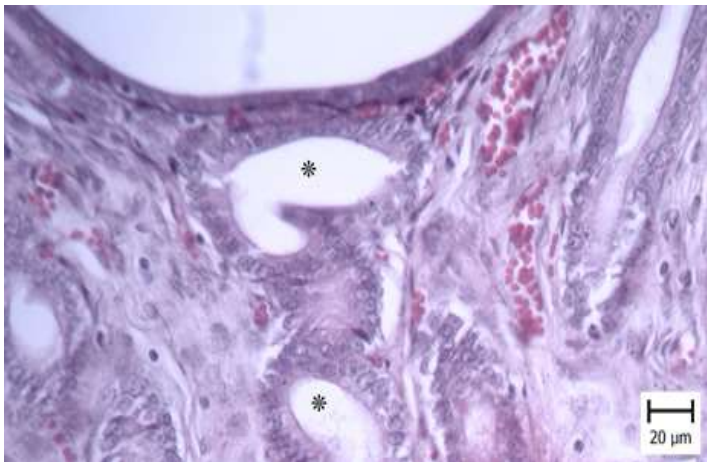

Figure 2.
Higher magnification of the previous figure. Note proliferating columnar cells of the endometrial glands (asterisks) (Hematoxylin and Eosin).

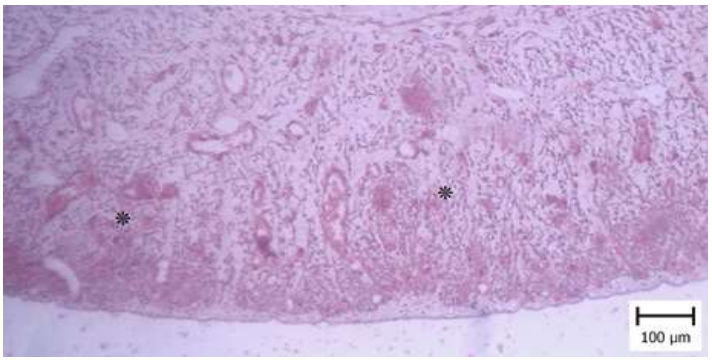

Figure 3.
Hemorrhage (asterisks) in the uterine perimetrium (Hematoxylin and Eosin).

In one case, severe hemorrhage was observed in the uterine perimetrium and ovary. A large ovarian hematoma containing numerous erythrocytes was detected in ovary (Figures 3 and 4).

In another case diagnosed with hemosiderosis, iron particles were observed within uterine cells. In the sample with uterine cyst and ovarian hyperemia, hematoma and accumulation of erythrocytes were observed, consistent with severe uterine and ovarian hemorrhage. Microscopic evaluation of the tumor affected samples, revealed a mass of proliferating cells in ovarian tissue, arranged in the form of ladder and ropes. Minimal connective tissue and blood vessels were observed at the periphery of the proliferated cells layer. Tumor cells had large nuclei with one to two distinct nucleoli and a small amount of cytoplasm around them. Based on microscopic features, the lesion was diagnosed as a granulosa cell tumor (Figures 5 and 6).

Discussion

The aim of the present study was to evaluate histopathologyical lesions of the uterus and ovaries and emphasizes the diagnostic role of ovariohysterectomy in cats from Ahvaz region.There are relatively few reports on the frequency of histopathological lesions of uterine and ovarian in cats, and most available publications are limited to case report. For example, in a study on pyometra in a 16-year-old Persian cat, from which Pasteurella multocida was isolated. The disease in that case was started from two years ago, with symptoms intermittent vomiting, anorexia and progressive abdominal distension, pallor of mucous membranes, tachycardia, tachypnea, poor hair coat condition and severe emaciation [9]. In contrast, all fifty-four cats included in the present study were clinically healthy and showed no apparent clinical signs.

In another study, conducted on sixty dogs with cystic endometrial hyperplasia and pyometra, eighteen cases were diagnosed with open pyometra and three cases with closed form. Histopathological examination revealed hyperplasia of the endometrial glandular epithelium accompanied by

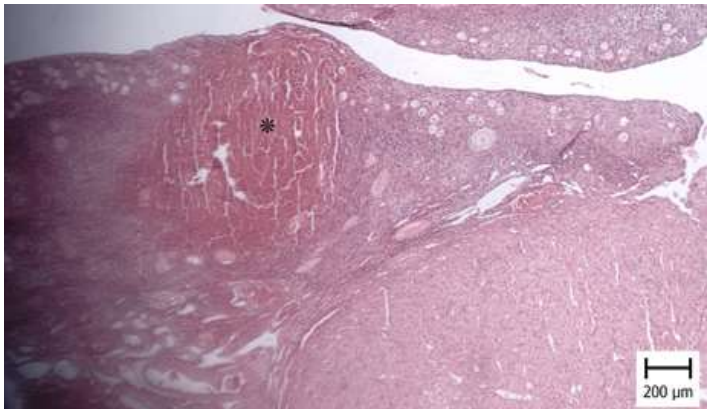

Figure 4.
Hematoma in the ovary. Note to the accumulation of erythrocytes (asterisk) at the periphery of ovary (Hematoxylin and Eosin).

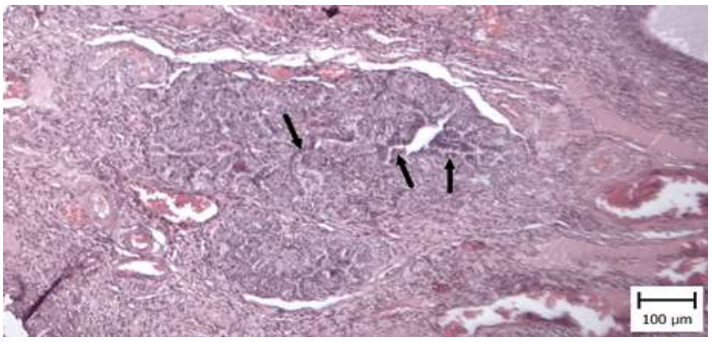

Figure 5.
Granulosa cell tumor in the ovary. Note on foci of proliferated columnar cells (arrows) within the ovarian tissue (Hematoxylin and Eosin).

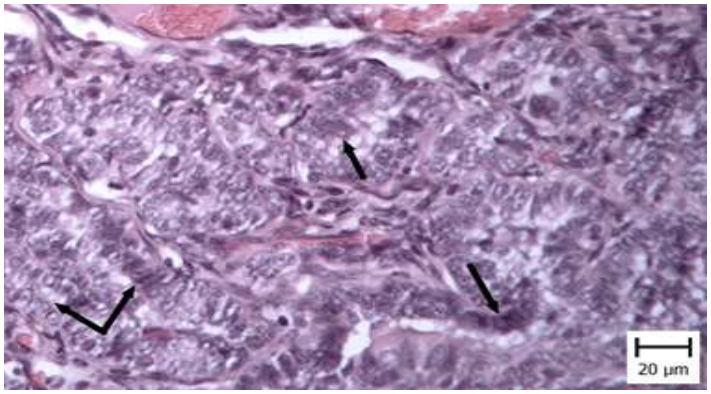

Figure 6.
Granulosa cell tumor in the ovary. A higher magnification of the previous image. Note proliferating columnar with vesicular nuclei arranged in a palisading pattern (arrows) (Hematoxylin and Eosin).

numerous cysts [14]. Similarly, in the present study, the cat diagnosed with endometrial cystic hyperplasia showed proliferated of endometrial cells and cysts formation within the endometrial mucosa. According to the Dow classification (1962), this lesion was categorized as Grade I CEH [13]. However, unlike the canine study, no cases of pyometra were identified in the present study. The difference between two studies may be attributed to the higher incidence of pyometra in dogs compared with cats[3].

CEH is not associated with any specific phase of the estrous cycle; however, uterine enlargement is usually observed in affected animals [15]. in the present study, morphometric measurements of uterine thickness showed no significant difference between healthy cats and the cat affected by CEH. This finding may be explained by the early stage or short duration of the disorder in the affected animal [3]. Additionally, the small number of affected cases and young age of of the cats limit definitive conclusions and do not allow for direct comparison with other studies.

CEH is considered relatively common in cats and is characterized by endometrial epithelial hyperplasia and cystic dilation of endometrial glands. Affected uteri may appear covered with translucent cysts. Cats with CEH usually do not show clinical signs unless the disease is complicated by infection. Vaginal bleeding is rare may occur in association with uterine or ovarian torsion, caused by polypoid changes [15]. In the present study, only one case of the grade I CEH was detected among fifty-four samples (based on the Dow grading, 1962) [13], and no vaginal bleeding and uterine torsion was observed in this sample. Except FOR two cases with uterine hyperemia, all other uteri and ovaries appeared to be in normal status. Pyometra is regarded as one of the most important uterine disease in cats, develops secondary to CEH. The incidence of CEH increases with age, possibly due to cumulative nature of the disease. One of the definitive methods in the treatment of this disease is ovariohysterectomy. The cause of pyometra is complex and probably depends on various factors; but hormonal influences play a critical role in the pathogenesis of pyometra [16]. In the present study, the affected cat by CEH was two years and three months old, indicating that CEH can occur at a relatively young age, even before three years.

In a case report, on an affected dog to pyometra, abdominal distension was observed due to the accumulation of the purulent fluids, which had led to pressure on the rectum and intestinal obstruction. In histopathological confirmation, the remaining white tissue was ovarian tissue remnants [17]. In the present study, none of the cats were referred after ovari-

ohysterectomy. Previous research has demonstrated associations between age, breed and the occurrence of pyometra in cats. Breeds such as Siberian, Osikat, Kurat, Siamese, Ragdoll, Maine Coon and Bengal are susceptible to this disease. The average age of pyometra in cats has been reported to be approximately four years, with a mortality rate of 5.7% slightly higher than existing reports in dogs (3-4%) [11]. In the present study, only uterine and ovarian lesions were observed in DSH cats, which may be related to the higher representation of this breed in the study population.

In another study involving seventy-nine female cats with endometrial hyperplasia with or without pyometra, uterine lesions were more prevalent in cats older than five years. Reproductive history and age at first pregnancy had no significant relationship with pathological symptoms of the disease. Microscopic evidence of pyometra was observed in 71% of clinically symptomatic cats, compared with only 19% of asymptomatic cats. These findings suggest an increased risk with advancing age. [18]; in the present study, the age of the affected cat was two years and three months, indicating that CEH also can develop even before age of three years.

In a study, a 12-year-old, sexually intact, female DSH cat with mild weight loss and recurring vaginal discharge, was diagnosed with CHE, accumulation of mucoid fluid in lumen of uterine corpus, uterine corpus adenocarcinoma and uterine cervix leiomyoma, following histopathological and immunohistochemical examination. [19].

Additionally, in a study examining histopathological features of endometrial lesions in six cats, one cat with papillomatous nodules and three cats with pyometra were identified. According to their results, the distribution of endometrial histopathological changes in cats were similar to humans [20]. In the present study, irregular proliferated of endometrial cells was observed in the cat affected by CEH.

A large-scale study involving 106 cats reported pathological alterations of the uterus and ovaries in twenty-nine cats. Ovarian cysts were found in 15 cats. Six cats had purulent endometritis with or without distension of the uterine lumen. Hyperplastic lesions of the endometrium were detected in two cats. In one cat, a uterine horn malformation with duplication of one uterine horn lumen was diagnosed. In their study, the prevalence of lesions increased with age, and fifty percent of female cats older than 3 years and eighty-three percent of cats over 5 years showed more or less pronounced pathological alterations of the uterus or ovaries. This may indicate a cumulative effect of repeated gonadal steroid hormone stimulation in subsequent oestrous cycles [8]. The broader range of pathological lesions in that study compared with the present study is probably attributable to the larger sample size.

In a PCR-based study, uterine samples were collected from 50 cats, and Chlamydia. felis infection was detected in 6% of the samples. Non-specific histopathological changes included varying degrees of edema, hyperemia, hemorrhage, inflammation, necrosis, fibrosis, cyst formation and endometrial hyperplasia.

These results highlighted the potential role of C. felis in uterine pathology and underline the need for surveillance of this zoonotic agent in domestic cats [21]. Other studies have reported a significant level of fungal organisms in the feline reproductive systems [22]. Gelberg and McEntee (1985) [23] identified hyperplastic endometrial polyps in fourteen cats, and ten of them were associated with diffuse or multifocal CEH. Their morphological evidence showed that endometrial polyps arise from focal areas of CEH. No breed association was observed and polyps were more common in adult cats. In the present study, considering the low age mean of the samples, no morphological evidence of polyps in the focal areas of cystic hyperplasia were observed, possibly due to the relatively young age of the affected cat.

Regarding ovarian neoplasms, a study examining reproductive disorders in 28 female cats, aging between seven months to thirteen years, reported a prevalence of 35.29% for ovarian neoplasms and 5.88% for theca granulosa cell tumor [24]. In contrast, only one case of granulosa cells tumor was identified in the present study.

Previous case reports have described granulosa cell tumors in cats presenting with systemic and reproductive signs, including weakness, anorexia, weight loss, lethargy, vomiting, and estrous abnormalities despite ovariohysterectomy, with Persian cat suggested as a susceptible breed [25, 26]. In the present study, the affected cat was seven months year old DSH cat with no clinical signs, suggesting early-stage disease, and probability of occurrence of this tumor in other breeds and young ages. The granulosa cell tumor in the present study was detected incidentally during histopathological examination.

Granulosa cell tumors are the most common type of ovarian tumor in cats, and are considered malignant, with potential for metastasis [25]. Histopathological findings in another study done by Tawfik et al., (2015) [24], have included vascular congestion, hemorrhage, and hemosiderin deposition of golden yellow to brown granules in uterine tissue. Uterine tumours are rare in cats, accounting for approximately 0.3% of all feline neoplasms, with endometrial adenocarcinoma and uterine leiomyoma being the most common tumours of the feline genital tract. Hyperplastic lesions are potentially associated with hormonal stimulation are more likely [26]. In the present study, iron particles deposition (he-

mosiderosis) was also observed in uterine cells; however, due to early ovariohysterectomy, assessment of metastatic behavior was not possible.

Ovarian hematoma is more common in maresbut are rarely reported in cats. They typicall result from excessive bleeding following ovulation in follicular cavity. Hematomas can be large (up to 10 cm) and may resolve spontaneously, and their rupture and drainage can result in affected animal's death (3). In the present study, a large hematoma with extensive hemorrhage was observed in the uterus and ovary. No comprehensive study has been conducted on hematoma in the ovary of cats. This article provides valuable information on the histopathological findings in the uterus and ovaries of cats undergoing ovariohysterectomy in the Ahvaz district. One limitations of the present study was the relatively small sample size (fourty-four samples) and limited age range of the cats examined, all of which were collected over cases referred to Ahvaz Veterinary Hospital during approximately six months.

Conclusion

Based on the findings of the present study, histopathological evaluation plays a crucial role in controlling, managing and providing appropriate diagnostic views for veterinarians, particularly when clinical and gross examinations fail to reveal abnormalities. Histopathological findings identified uterine and ovarian lesions in five cats, including one case of cystic endometrial hyperplasia, one granulosa cell tumor, one uterine cyst with ovarian hemorrhage, one case of uterine and ovarian hyperemia with hemorrhage; and another one case of uterine hemosiderosis. Overall, the present study demonstrates that ovariohysterectomy in cats can play an important role in diagnosing pathological lesions of the uterus and ovaries, prevention and treatment of genital diseases. Further studies are recommended on a larger population of cats in other regions of Iran.

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

The research and writing were done wiyhout relying on the artificial intelligenca tools.

Ethical Approval

Ethical approval of this study was obtained from the Ethics Committee of Shahid Chamran University of Ahvaz (EE/1401.2.24.173448/scu.ac.ir).

In the present study, samples were collected from fifty-four cat, referred for ovariohysterectomy. Of these cats, 39 were domestic short hair (DSH), 13 were Persian, one was domestic long hair (DLH), and one was Siamese. The mean±SE age of the cats was 10.94 ± 0.74 months, with an age range of six months to 2.5 years. Body weight ranged from 2.1 to 4.2 kg (3.1 ± 0.45). The cats were referred to the Veterinary Hospital of Shahid Chamran University of Ahvaz or different clinics in Ahvaz city between October and February 1401. All cats were in a healthy condition, with no recorded history of underlying disease or administration of specific medications. One litter had more than one birth and three litters had a history of one birth. All four litters belonged to the DSH breeds and no pregnancy history was recorded in other breeds. Most cats were kept indoors, and only five cats had access to the outdoor environments. .

Before ovariohysterectomy, a detailed clinical history was recorded for each cat, including age, breed, for each cat, including, status of uterine infections, administration of various drugs (including corticosteroids), and clinical findings. Clinical examinations assessed the condition of the vulva and vagina, presence of vaginal purulent discharge, mammary gland abnormalities, lactating state, polyuria and polydipsia, and body coat condition.

Following ovariohysterectomy, the macroscopic evaluation of the ovary and uterus was performed to assess the presence and the type of secretions, as well as the number and size of any macroscopic cysts. For histopathology evaluation, uterine and ovarian tissue samples were fixed in 10% buffered formalin. After fixation, tissues were trimmed into smaller sections and placed under running water for tissue passage. The working steps were including dehydration, clarification and impregnation. Tissue sample were slowly embedded in paraffin blocks using standard metal molds, and the blocks were cooled on ice. A microtome machine was used for cutting and sections with a thickness of 2-3 microns. Prepared slides were stained with Hematoxylin and Eosin in the following standard protocols. For dehydration, slides were placed in 80% alcohol for 10 seconds and twice in 96% alcohol in three steps. In the next step, in order to clarify of the tissues, the slides were placed in two different containers in Gezilol. Finally, the slides were mounted using Montalan glue and examined with an optical microscope. In histological examination, samples were evaluated to determine the estrus cycle stage based on uterine and ovarian histological characteristics. Measurements included uterine wall thickness, endometrium and myometrium thickness, number of mature follicles, number of corpora lutea and follicles. All measurements and counts were performed using a graduated lens.

Grouping

After examining the prepared samples with a microscope based on the histological changes in the uterus (including congestion and hyperemia) and ovary (follicular development and corpus luteum condition), samples were classified into four groups (proestrous, estrus, diestrus and anestrus).

Statistical Analysis

This study was designed as a descriptive analysis. Data were expressed as percentages, and Mean±SD. Statistical analyses were performed using SPSS software, version 22 (SPSS Inc., IL, Chicago, USA).

Authors' Contributions

B.M., A.R., and Be.M. conceived and planned the research. S.G., and Sh.G. carried out the investigation. B.M., and Sh.G. contributed to sample preparation. B.M., A.R., and S.G. contributed to the interpretation of the results.

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Conflict of interest

Authors declare that they have no conflict of interest. There were no ethical considerations to be considered in this research.

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Changes Induced by Exercise and Physical Training on Serum Biochemical Parameters in Arabian Horses after 15 kilometer running in a hot and dry climate

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ABSTRACT

This study investigated exercise-induced changes in serum biochemical parameters including calcium, phosphorus, sodium, potassium, chloride, magnesium, glucose, urea, creatinine, triglycerides, cholesterol, total protein, albumin, and total bilirubin, as well as serum enzyme activities such as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in Arabian horses subjected to physical training under hot and dry climatic conditions. A total of 22 Arabian horses, aged 3 to 7 years, participated in the study. Blood samples were collected from the jugular vein at four time points: one hour before (T0), immediately after (T1), six hours after (T2), and eighteen hours after (T3) a warm-up followed by maximum effort running over a 15-kilometer course. The obtained data were analyzed using one-way repeated measures ANOVA, and pairwise comparisons with baseline values (T0), were conducted using the Bonferroni post hoc test was used. In all statistical comparisons , a P-value < 0.05 was considered significant. The results demonstrated that serum concentrations of urea, creatinine, triglycerides, calcium, phosphorus, and total bilirubin exhibited significant changes over time following exercise ($p < 0.05$). The results show that the concentrations of glucose, triglycerides, cholesterol, urea, phosphorus, sodium, chloride, and the serum activities of ALT, AST, and LDH (higher in mares), and magnesium (higher in stallions) were significantly different between sex ($p < 0.05$). Moreover, the results indicate that the mean concentrations of total protein and the mean serum activities of ALT, AST, ALP, GGT, and CPK were significantly different between age groups ($p < 0.05$).

Keywords

Arabian Horses, Biochemical parameters, Dry climate, Performance, Physical training

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Abbreviations

CPK: Creatine phosphokinase
LDH: Lactate dehydrogenase
GGT: gamma-glutamyl transferase
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
ALP: Alkaline phosphatase

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Introduction

The Arabian horse is one of the oldest and most influential, and widely recognized horse breeds in the world [1]. Due to the considerable economic value associated with Arabian horses racing, monitoring of disorders resulting from intense exercise and physical activity, as well as diseases that impact the performance of these horses, is particularly importante in this breed [1]. When racehorses display poor performance, blood sampling is performed to assess their health status and conduct biochemical tests [2]. Since biochemical parameters are influenced by exercise and physical training, blood collection at different time points can reveal the pattern of changes in serum parameter concentrations over time [2].

Horses participate in wide range of sports s including racing, jumping, and equestrian competitions, all of which require a high level of physical fitness. Regular and well-planned training can improve their physical abilities and athletic performance. However, intensive or prolonged training may induce physiological and biochemical changes that can compromise health and performance. The ability of the horse to adapt to the physiological changes occurring during competition is an essential factor influencing its athletic success or failure [3].

Alterations in horses' performance during exercise and physical activity are often associated with changes in biochemical parameters. Therefore, measuring physiological exercise parameters in racehorses is of special importance for the optimal use of training programs [4].

The biochemical and physiological responses of horses during and after exercise are critical in equine sports medicine and veterinary practice. Blood biochemical markers, particularly those related to metabolism, muscle performance, and electrolyte balance, serve as important indicators for assessing athletic fitness, diagnosing exercise-related disorders, and optimizing training regimens [5,6,7].

Breed-specific differences can significantly influence these responses. For example, studies in Arabian horses have demonstrated distinct sweating patterns and biochemical adaptations during endurance racing compared with other breeds such as the Žemaitukai horse [8,9]. These findings highlight the importance of breed considerations when interpreting exercise-induced changes in serum parameters. In addition, variables such as sex, age, and environmental conditions have been reported to affect the dynamics of biochemical markers, including muscle enzymes (CK, LDH, AST), liver- associated metabolites, electrolytes, and serum proteins [6,7,10]. Horses competing in hot and dry climates may be particularly vulnerable to dehydration and electrolyte imbalance, which can impair thermoregulation, metabolic processes, and athletic performance.

The present study was designed to investigate the effects of time (at multiple post-exercise sampling points), sex, and age on serum biochemical and electrolyte parameters in Arabian horses subjected to a 15-kilometer run under hot and dry climatic conditions. The findings are expected to contribute to the development of more precise training protocols and health monitoring strategies for this valuable breed. It is assumed that there is a correlation between changes in serum biochemical parameters and the intensity of physical training, which could help trainers assess the health status of their horses and adjust training programs according to their physical condition. Since all the horses in this study were kept in Yazd city, an area characterized by a hot and dry climate, environmental conditions may influence thermoregulation and energy expenditure [11]. Therefore, the effects of climate and weather were also considered in the present study.

Results

The results of the present study are summarized in Tables 1 to 3. The serum concentrations of urea, creatinine, calcium, phosphorus, triglycerides, and total bilirubin showed significant changes over time as a result of physical exercise ($p < 0.05$). These temporal changes are illustrated using line graphs at different time points (Figures 1-6).

A significant increase in total bilirubin and creatinine concentrations was observed immediately after exercise (T1) compared with pre-exercise values (T0) ($p < 0.05$) (Figures 3 and 6). In contrast, although calcium concentrations decreased and phosphorus concentrations increased immediately after exercise, these changes were not statistically significant compared to the pre-exercise stage. From T1 (immediately after exercise) to T2 (six hours post-exercise) calcium concentrations demonstrated a noticeable upward trend, while phosphorus and creatinine concentrations demonstrated a downward trend during the same period (Figures 3-5).

Total bilirubin concentration did not show a significant difference between T1 and T2 (Figure 6). Creatinine levels at T2 and T3 decreased compared with T1 and approached baseline levels (T0), resulting in no significant differences from pre-exercise values (Figure 3).

Total bilirubin levels significantly increased immediately after exercise (T1) ($p < 0.05$) but then decreased again at T2 and T3, returning toward pre-exercise values (Figure 6).

Although time had a significant effect on the con-

centrations of calcium, urea and triglyceride ($p < 0.05$), no significant differences were observed between individual sampling times. Phosphorus concentrations showed a non-significant increase immediately after exercise (T1), followed by a significant decrease at T2 ($p < 0.05$), and then a non-significant increase at T3 (Table 1).

Data collected during visits to equestrian clubs including information on age, sex, weight, and administered medications. The mean and median ages of the horses included in this study was 4 years. For stallions, the mean, median, minimum, and maximum ages were 4.3, 4, 3, and 7 years, respectively, while for mares these values were 3.6, 3, 3, and 6 years, respectively.

The results demonstrated that mares had significantly higher mean concentrations of glucose, triglycerides, cholesterol, urea, phosphorus, sodium, and chloride, as well as significantly higher mean serum activities of ALT, AST, and LDH, compared with stallions ($p < 0.05$). In contrast, the mean serum magnesium concentration was significantly higher in stallions compared to mares ($p < 0.05$) (Table 2).

Moreover, the results indicate that horses younger than 4 years had significantly higher mean concentrations of total protein and significantly higher mean serum activities of ALT, AST, ALP, GGT, and CPK compared with horses aged 4 years and older ($p < 0.05$). Conversely, serum creatinine concentrations were significantly lower in horses younger than 4 years compared with older horses ($p < 0.05$). Age effect in relation to other parameters was not statistically significant (Table 3).

Table 1.
The Concentration of measured variables (Mean ± SE) in the serum of Arabian horses before and after exercise.
In each row, values with different superscripts are significantly different ($p < 0.05$).

Parameters	Unit	Before exercise (T0)	Immediately after exercise (T1)	6h after exercise (T2)	18h after exercise (T3)	P-value
Glucose	mg/dl	91.4 ± 1.7	92.2 ± 2.4	93.2 ± 1.7	92.7 ± 1.7	0.74
Triglyeride	mg/dl	38.6 ± 2.75^{a}	45.3 ± 4.4^{a}	36.5 ± 2^{a}	43.4 ± 3^{a}	0.01
Cholestrol	mg/dl	72.1 ± 2.5	73.2 ± 2.5	74 ± 2.2	74 ± 2.4	0.39
Total Protein	mg/dl	6.6 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	0.65
Albumin	mg/dl	3.1 ± 0.4	3.1 ± 0.45	3.1 ± 0.5	3.1 ± 0.3	0.27
Urea	mg/dl	34.8 ± 1.15^{a}	35.5 ± 1.2^{a}	37.9 ± 1.2^{a}	35.5 ± 1.1^{a}	0.02
Creatinine	mg/dl	1.05 ± 0.05^{a}	1.2 ± 0.05^{b}	1.05 ± 0.05^{a}	1.05 ± 0.06^{ab}	0.008
T.Bili	mg/dl	1.05 ± 0.05^{a}	1.13 ± 0.04^{b}	1.12 ± 0.05^{ab}	1.1 ± 0.04^{ab}	0.05
ALT	U/L	9.4 ± 0.95	9 ± 1.15	11.1 ± 1.9	12.2 ± 2.5	0.17
AST	U/L	279.2 ± 19.2	293.7 ± 21.5	286.9 ± 16.6	273.8 ±17.8	0.35
ALP	U/L	364.5 ± 20.1	372.7 ± 20.5	367.8 ± 20.8	369.2 ± 22.3	0.64
GGT	U/L	23.5 ± 1.1	23.1 ± 1.3	22.5 ± 0.9	23.2 ± 1.1	0.58
LDH	U/L	215.5 ± 12.7	234.3 ± 15.3	247.4 ± 19.5	245.6 ± 23.3	0.17
CPK	U/L	200.1 ± 41.9	265.3 ± 68.9	232.6 ± 52.8	187.9 ± 25.6	0.142
Calcium	mg/dl	10.9 ± 0.1^{a}	10.4 ± 0.1^{a}	11 ± 0.2^{a}	10.9 ± 0.1^{a}	0.01
Phosphorus	mg/dl	3.9 ± 0.1^{ab}	4.2 ± 0.2^{a}	3.7 ± 0.2^{b}	4 ± 0.1^{ab}	0.001
Magnesium	mg/dl	2.45 ± 0.1	2.45 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	0.57
Sodium	mEq/L	136.8 ± 1.3	138 ± 1.4	136.8 ± 1.2	137.2 ± 1.2	0.86
Potassium	mEq/L	3.8 ± 0.07	3.7 ± 0.07	3.8 ± 0.15	3.9 ± 0.15	0.62
Chloride	mEq/L	99.4 ± 0.9	99.5 ± 1	100.1 ± 0.8	99.8 ± 0.8	0.86

Table 2.
The concentration of measured variables (Mean ± SE) in the serum of Arabian racehorses for different sexes

Parameters	Unit	Stallion (n=14)			Mare(n=8)			P-value
		Mean ± SE	Min	Max	Mean ± SE	Min	max	
Glucose	mg/dl	89.6 ± 1.6	73	110	97.2 ± 2.1	82	112	0.01
Triglyeride	mg/dl	36.6 ± 3.1	21	80	48.6 ± 4.1	24.5	111	0.029
Cholestrol	mg/dl	68.6 ± 2.3	46.5	91	81.5 ± 3.1	68	100	0.003
Total Protein	mg/dl	6.7 ± 0.1	5.8	7.7	6.6 ± 0.1	5.5	7.7	0.698
Albumin	mg/dl	3.1 ± 0.04	2.55	3.56	3.1 ± 0.05	2.68	3.47	0.479
Urea	mg/dl	34.4 ± 1.2	22.5	41.5	38.6 ± 1.5	28	51	0.042
Creatinine	mg/dl	1.1 ± 0.6	0.37	1.6	1.1 ± 0.8	0.47	1.62	0.855
T.Bili	mg/dl	1.2 ± 0.05	0.76	1.79	1 ± 0.06	0.69	1.41	0.061
ALT	U/L	7.7 ± 1.6	1.26	18.1	15.2 ± 2.2	3.26	61	0.012
AST	U/L	255.5 ± 19.5	166	374	332.1 ± 25.8	173	578	0.028
ALP	U/L	354.9 ± 25.9	196	497	392.3 ± 34.3	257	604	0.395
GGT	U/L	22.2 ± 1.2	14.5	35.5	24.7 ± 1.6	12.6	38.5	0.23
LDH	U/L	208.9 ± 17.6	134	352	282.6 ± 23.2	166	658	0.02
CPK	U/L	157.5 ± 53.1	69.5	444	342.6 ± 75.1	113	1399	0.059
Calcium	mg/dl	10.8 ± 0.1	8.9	12.5	10.7 ± 0.1	9.5	12.1	0.703
Phosphorus	mg/dl	3.7 ± 0.15	2.57	5.2	4.3 ± 0.2	3.09	6.4	0.038
Magnesium	mg/dl	2.6 ± 0.12	1.59	3.62	2.15 ± 0.15	1.85	2.56	0.03
Sodium	mEq/L	135.5 ± 0.8	118	148	140.1 ± 1.1	124	149	0.003
Potassium	mEq/L	3.8 ± 0.07	2.9	5.1	3.7 ± 0.09	2.3	5.2	0.237
Chloride	mEq/L	98.4 ± 0.5	88	107	102 ± 0.7	92	108	0.00

Table 3.
The concentration of measured variables (Mean ± SE) in the serum of Arabian horses for different age groups

Parameters	Unit	< 4 years old (n=10)			≥ 4 years old (n=12)			P-value
		Mean ± SE	Min	Max	Mean ± SE	Min	max	
Glucose	mg/dl	94.3 ± 2.1	75.5	112	90.8 ± 1.9	73	111	0.246
Triglyeride	mg/dl	46.4 ± 3.8	24.5	111	36.4 ± 3.4	21	73	0.067
Cholesterol	mg/dl	75.6 ± 3.3	46	100	71.3 ± 3	52	87	0.36
Total Protein	mg/dl	6.9 ± 0.1	5.9	7.7	6.5 ± 0.1	5.5	6.8	0.008
Albumin	mg/dl	3.1 ± 0.05	2.67	3.48	3.1 ± 0.04	2.55	3.56	0.614
Urea	mg/dl	35.8 ± 1.5	22.5	48.5	35.9 ± 1.4	26	51	0.959
Creatinine	mg/dl	0.95 ± 0.06	0.37	1.38	1.2 ± 0.05	0.56	1.62	0.013
T.Bili	mg/dl	1.02 ± 0.06	0.71	1.25	1.17 ± 0.05	0.69	1.79	0.077
ALT	U/L	14 ± 2	1.33	61	7.4 ± 1.8	1.26	17.1	0.024
AST	U/L	333.3 ± 21.2	173	578	241.8 ± 19.4	166	391	0.005
ALP	U/L	428.9 ± 25.3	314	604	318.2 ± 23.1	196	483	0.004
GGT	U/L	25.4 ± 1.3	14.5	38.5	21.1 ± 1.2	12.6	28	0.025
LDH	U/L	266.6 ± 22	141	658	210 ± 20.1	134	361	0.073
CPK	U/L	332.2 ± 64.4	100	1399	134.5 ± 55.7	69.5	248	0.032
Calcium	mg/dl	10.7 ± 0.13	9.2	12.1	10.9 ± 0.12	8.9	12.5	0.183
Phosphorus	mg/dl	4.1 ± 0.2	2.82	6.4	3.8 ± 0.2	2.57	5.2	0.228
Magnesium	mg/dl	2.3 ± 0.15	1.96	3.35	2.6 ± 0.15	1.59	3.62	0.134
Sodium	mEq/L	138.6 ± 1.2	124	149	136 ± 1.1	118	146	0.113
Potassium	mEq/L	3.8 ± 0.1	2.5	5.2	3.8 ± 0.1	2.3	4.9	0.873
Chloride	mEq/L	100.6 ± 0.8	90	107	99 ± 0.7	88	108	0.127

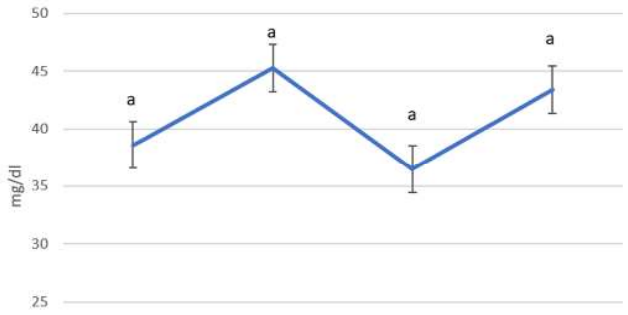

Figure 1.
Triglyceride Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

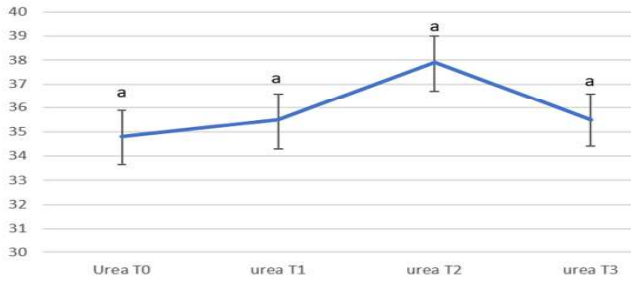

Figure 2.
Urea Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

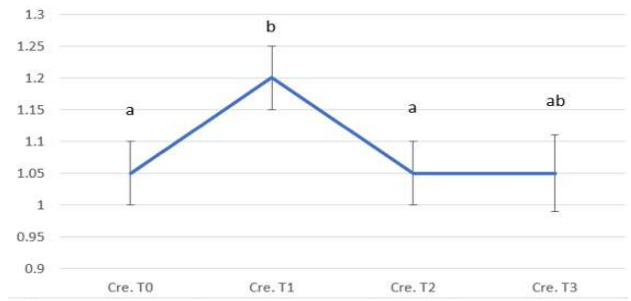

Figure 3.
Creatinine Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

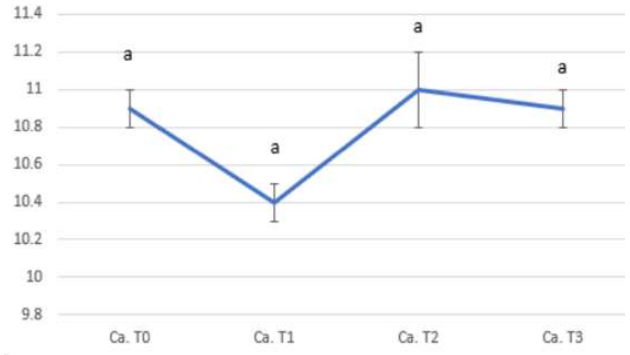

Figure 4.
Calcium Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

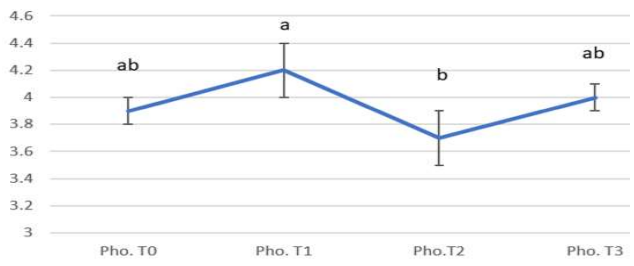

Figure 5.
Phosphorus Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

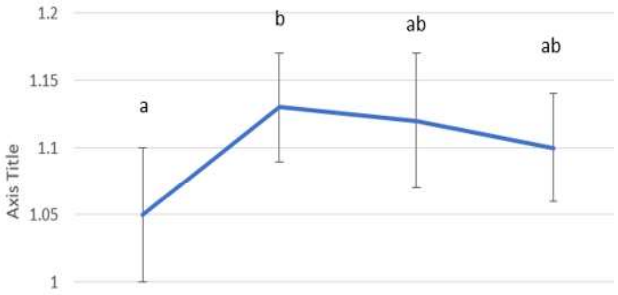

Figure 6.
Total Bilirubin Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

Discussion

Exercise and physical activity are well known to induce changes in certain serum biochemical parameters in horses, and these responses are associated with factors such as individual variability, breed, and exercise intensity [12].

In this study, a decrease in serum calcium concentration was observed immediately after exercise (T1) compared with pre-exercise values (T0); however, this change was not statistically significant (Figure 4). The binding of calcium ions (Ca^{2+}) to phosphate ions, albumin, and lactate can be considered one reason for this decreased calcium amount. In addition, ionized calcium is actively taken up by working muscles and stored in the sarcoplasmic reticulum, which may further contribute to the observed decrease in calcium levels [13]. Other studies have suggested exercise-induced hypocalcemia may also result from sustained calcitonin activity or severe sweating during physical activity [14]. Martins et al. reported a slight post-exercise decrease in calcium concentration in Brazilian Mangalarga horses, attributing this change to either calcium loss via sweating or to increased calcium influx into muscle cell, where calcium binds to troponin molecules to facilitate actin-myosin interactions for muscle contraction during exercise [15].

In the present study, although the overall effect of time on calcium concentration was significant ($p < 0.05$), no significant difference were detected between post-exercise sampling time (T1, T2, T3) and the pre-exercise time (T0). Similarly, in a study by Inoue et al. on changes in serum minerals and electrolytes during exercise in Thoroughbred horses over five days using a treadmill, the effect of time on calcium concentration was significant ($p < 0.05$), but no significant difference was found between pre-exercise and immediately post-exercise calcium levels [16].

Grimston et al. demonstrated that exercise in humans increases blood calcitonin levels, an effect more closely associated with hypercalcemia-induced stimulation than with dehydration [17]. Another human study have shown that elevated calcitonin levels may partially prevent dehydration-induced hypercalcemia [18].

In the present study, no increase in calcium concentration was observed immediately after exercise (T1); instead, a non-significant decrease compared to pre-exercise levels (T0) was noted. Inoue et al. suggested that exercise induced hypocalcemia may be attributable to sweating, which significantly ($p < 0.05$) counteracts blood concentration-induced hypercalcemia [16]. Kanungo et al. evaluated the intermittent effects of exercise on serum metabolites in racehorses under hot and humid conditions, and found no significant changes in serum calcium concentration at 30 minutes, four hours, and eight hours after exercise were not significantly different [19]. Similarly, Zobba et al. also reported a downward trend in calcium concentration following intense exercise in ponies, with values returning close to baseline within 30 minutes after exercise [20].

In this study, phosphorus concentrations showed a trend on increase immediately after physical exercise (T1) compared to pre-exercise levels (T0), although this change was not statistically significant (Figure 5). Previous research on Thoroughbred horses indicated a significant rise in phosphorus levels five minutes post-exercise [21]. This phenomenon may be linked to phosphate release from skeletal muscles during the breakdown of high-energy phosphate compounds, such as ATP [22, 23]. Studies involving Arabian horses participating in endurance races have also demonstrated significant increases in phosphorus levels immediately after the race compared with pre-race level [24]. In the current study, the non-significant increase in phosphorus concentration at T1 was followed by a significant decrease at T2 (six hours post-exercise), a pattern consistent with previous reports [24]. Additionally, other studies have noted a non-significant increase in serum phosphorus immediately after exercise, which aligns with the present findings regarding phosphorus changes from T0 to T1 [10].

In this study, serum creatinine concentrations remained within the normal range [25] at all sampling times but increased significantly ($p < 0.05$) immediately after exercise (T1) compared with pre-exercise (T0). This increase was followed by a significant decrease ($p< 0.05$) at T2, likely due to rehydration during rest, and a further decline at T3, with concentrations approaching baseline levels (Figure3). The initial increase in creatinine at T1 is consistent with pre-renal azotemia secondary to reduced blood volume caused by excessive sweating [26, 27]. The subsequent decline and return toward baseline suggest that other causes, such as renal failure or exertional rhabdomyolysis, are unlilely [3]. Similar patterns have been reported in various equine exercise studies. Poskiene et al. reported a 21-30% post-race increase in creatinine concentration in 112 endurance horses, followed by a decline [8]. In Standardbred horses competing over 1600-2000 m, creatinine rose significantly ($p < 0.05$) up to 60 min post-race, then decreased [6]. Thoroughbreds showed peak creatinine 5 min after a 1600 m race, normalizing within 60 min [21]. In endurance races ranging from 65 to 120 km, creatinine increased by about 19% post-race and decreased by 13% within 12-18 hours [24]. In contrast, a short-distance study in Standardbreds reported no significant change in creatinine concentrations 48-72 hours after exercise [2]. The present findings align with these observations,

showing a transient post-exercise creatinine rise followed by recovery within 18 h, reflecting its relations to hydration changes rather than persistent renal dysfunction.

In the present study, serum urea concentrations remained within the normal range [25] at all sampling times. Although an upward trend was observed immediately after exercise (T1) and persisted at six hours post-exercise (T2), these changes were not statistically significant, and concentrations declined toward baseline from T3 (Figure 2). Such transient, increases may be related to mild dehydration, hemoconcentration from sweating, or increased protein catabolism during exercise [4]. Similar patterns have been reported in previous studies on horses subjected to varying exercise intensities. In ponies without regular training, urea increased significantly ($p < 0.05$) post-exercise and returned rapidly to baseline, whereas trained ponies showed smaller changes; this was attributed to fluid loss and reduced renal blood flow [28]. Endurance race studies in Arabians, Zemaitukai, warmbloods, and mixed breeds consistently demonstrated post-exercise elevations in serum urea, sometimes reaching about 40%, particularly after longer distances. These increases have been linked to both dehydration-related hemoconcentration and adaptive renal mechanisms that enhance urea reabsorption to support water and sodium conservation during prolonged exertion [9]. Taken together, the literature suggests that post-exercise urea changes-whether significant or not-are generally short-term, reflecting hydration status and metabolic protein turnover rather than persistent renal dysfunction. The present findings, showing only mild, non-significant fluctuations, are consistent with these physiological responses.

In this study, serum triglyceride concentrations stayed within the normal range [25], showing a slight, non-significant increase immediately post-exercise (T1) followed by a decrease at six hours (T2) and a modest, non-significant increase at 18 hours post-exercise (T3) (Figure 1). These fluctuations likely reflect transient changes in lipid mobilization for muscle energy supply [5]. Previous equine studies reported variable findings: some report significant post-exercise increases, particularly after prolonged or high-intensity phases such as cross-country or 120-km endurance rides [5, 24], while others found no significant increases after shorter endurance events or standardized training [7, 29]. Collectively, these results suggest that exercise-related changes in triglycerides are influenced by exercise type, duration, and intensity, and in most cases remain transient without exceeding physiological limits.

In the present study, total bilirubin concentration remained within the normal range [25], but increased significantly ($p < 0.05$) immediately after exercise (T1) compared with baseline (T0), with no further significant changes at six (T2) or 18 hours (T3) post-exercise (Figure 6). Similar transient increases in bilirubin concentration are consistent with previous reports showing marked bilirubin elevations after both short-distance races and prolonged endurance events, often returning toward baseline within hours [10,21, 24]. In endurance horses, increases of 60-184% have been observed within hours after competition, followed by partial recovery within 12-18 hours [24], whereas Thoroughbreds in sprint races showed rapid normalization within 60 minutes [21], These changes are attributed to factors including hemoconcentration from fluid loss, reduced renal clearance, and increased bilirubin production from enhanced hemolysis during exercise, with hydration status inversely correlated with bilirubin concentration [24]. Collectively, evidence suggests that post-exercise hyperbilirubinemia in horses is a reversible physiological response to physical activity, influenced by exercise duration, intensity, and hydration rather than an indicator of persistent hepatic dysfunction.

Differences in serum biochemical parameters between stallions and mares after training were observed in the present study. Regarding the effect of sex on parameters measured before and after exercise, the following possible reasons can be suggested to researchers for future studies: The lower concentrations of glucose, triglycerides, cholesterol, and phosphorus in stallions during exercise may be due to their more efficient energy metabolism, such that the consumption of these variables by muscle cells occurs at a greater rate and with higher efficiency. Lower urea concentration in stallions may be related to more efficient protein anabolism. Lower activities of transaminases (ALT and AST) and lactate dehydrogenase (LDH) in stallions may indicate better muscular adaptability during exercise, resulting in reduced enzymes leakage into the bloodstream. Higher sodium and chloride concentrations in mares following exercise may suggest a greater dehydration experienced by mares during physical activity. The reason for the higher magnesium concentrations in stallions remains unknown.

There are also differences between serum biochemical parameters in horses under four years of age compared to horses four years of age and older following exercise. Regarding the effect of age on parameters measured before and after exercise, the following possible reasons can be suggested to researchers for future studies: Higher total protein concentrations in horses younger than four years compared to those aged four years or older, which may either relates to greater protein production in the body or to

greater dehydration. Lower Creatinine concentrations in horses younger than four years compared to horses older than four years, which may be attributed to smaller muscle mass or higher renal clearance in this age group. Higher serum activities of ALT, AST, and CPK in horses under four years of age are likely associated with greater muscle damage during exercise and the leakage of these enzymes into the bloodstream in younger horses. ALP activity was also higher in horses younger than four years, probably due to growing animals in this age range. The higher GGT activity observed in horses younger than four years compared to those older than four years has no clearly established explanation.

In conclusion, this study highlights the significant biochemical alterations in serum parameters of Arabian horses following a 15-kilometer run in hot and dry conditions. The observed effect of time, sex and age on serum biochemical parameters alongside electrolyte levels, provides important insights into the physiological responses induced by exercise. Our findings emphasize the importance of monitoring biochemical markers at multiple time points post-exercise to gain a comprehensive understanding of their dynamics and implications for equine health and performance. This research contributes to the broader field of equine exercise physiology, facilitating the development of tailored training regimens that optimize the well-being and athletic capabilities of Arabian horses. However, the need for further studies regarding the duration and type of training in horses of this breed is still felt.

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

Grammarly software was used to correct minor grammatical errors in the manuscript. Furthermore, chat GPT AI was employed to improve the quality of language, merge and summarize a couple of paragraphs in the discussion section.

Ethical Approval

All stages and procedures in this study followed the ethical standards of the 1975 Helsinki Declaration (revised in 2000 and 2008). The study was also approved in accordance with national regulations (Ethics Code: IR.UM.REC.1400.219).

Horses and Physical Training

This study was conducted on 22 apparently healthy Arabian horses, comprising 14 stallions and 8 mares, aged 3 to 7 years, selected from two equestrian clubs located in the Ashkezar and Zarach regions of Yazd Province, Iran. All horses were indigenous Arabian horses exhibiting typical Arabian breed markings, confirming their breed authenticity. The horses were maintained under similar conditions, including diet and training protocols. Yazd is one of the three main centers for Arabian horse breeding in Iran, and is characterized by a hot and dry climate. Prior to inclusion in the study, each horse underwent a thorough physical examination performed by a specialist in veterinary internal medicine to ensure its health status throughout the study, including history assessment and clinical examination. All horses followed a unified vaccination and deworming program at the breeding centers, and none of the mares were pregnant, during the study. Horses were housed individually in stalls, with the ambient temperature maintained between 25 and 27°C. A uniform photoperiod conditions was applied during the summer, with sunrise at 5:30 a.m. and sunset at 5:45 p.m.. The horses' diet was formulated according to the National Research Council (NRC) Nutritional Requirements table (1989). The daily ration consisted of dry alfalfa hay at approximately 1.5% of body weight and concentrate feed at approximately 1% of body weight. The horses were trained under conditions similar to racing six days a week, with one rest day. Training sessions began at sunrise (5:30 a.m.) and lasted two hours. A standardized daily training program was applied to all horses, although minor adjustments were permitted by the trainer based on individual age, physical ability, and performance. However, the overall duration and intensity of training were generally similar among all horses. Each training session consisted of 30 minutes of warm-Up, 45 minutes of walking (Pacing), 20 minutes of trotting, and 25 minutes of cantering at a moderate pace. The total distance covered during each training session was approximately 15 kilometers, with an average cantering speed of approximately 12 m/s.

Blood Sampling

All stages and procedures in this study followed the ethical standards of the 1975 Helsinki Declaration (revised in 2000 and 2008). The study was also approved in accordance with national regulations (Ethics Code: IR.UM.REC.1400.219).

Blood samples were collected from the jugular vein of each horse at four-time points:

- T0: Before the start of exercise and physical activity
- T1: Immediately after exercise and physical activity
- T2: Six hours post-exercise
- T3: Eighteen hours post-exercise

Sampling was carried out in the summer season from June 22 to August 22, 2019, in Yazd province. Blood samples were collected into tubes without anticoagulant and centrifuged at 2000 g for 20 minutes. Serum was separated within 20 minutes of blood collection and stored at −20°C. Serum samples were transported from Yazd to Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, using a nitrogen tank at −196°C, and subsequently stored at −80°C. The serum concentrations of glucose, urea, triglycerides, cholesterol, creatinine, total bilirubin, calcium, phosphorus, total protein, albumin, sodium, potassium, chloride, and magnesium, as well as the serum activities of ALT, AST, ALP, GGT, LDH, and CPK, were measured using commercial diagnostic kits produced (Pars Azmoon Company, Tehran, Iran) and an Alpha Classic refrigerated biochemical autoanalyzer (Esfahan, Iran). Serum concentrations of sodium, potassium, and chloride were measured using an ion specific electrode analyzer (STARLYTE III, Alpha Wasserman, Netherlands). The accuracy of biochemical parameter measurements was ensured using commercial control serum (Centronorm, Centronic GmbH Ltd., Wartenberg, Germany).

Statistical Analysis

Statistical analyses were performed using SPSS software (version 16.0). A One-way repeated measures ANOVA was used to compare the concentrations of parameters across consecutive time points. When significant differences were found, pairwise comparisons were conducted using the Bonferroni post hoc test. The effects of time, group (age and sex), and time × group interactions were assessed. Differences were considered statistically significant at $p < 0.05$.

Authors' Contributions

F.K., M.Z., K.S., and M.M. conceived and planned the experiments. F.K., M.Z., and K.S. carried out the experiments. F.K., and M.Z. planned and carried out the simulations. F.K., M.Z., K.S., and M.M. contributed to sample preparation. F.K., M.Z., K.S., and M.M. contributed to the interpretation of the results. M.M. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

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Comparative analysis of some hematobiochemical and electrocardiographic changes following administration of medetomidine alone and combination with selected opioid agonists in Goats

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ABSTRACT

Medetomidine is effective for sedation in small ruminants and is often combined with opioids to tailor depth and mitigate adverse effects. We conducted a randomized Latin-square crossover study in goats (n=10) comparing five intravenous regimens: medetomidine 20 µg/kg alone (MED) or combined with methadone 0.5 mg/kg (MME), morphine 0.5 mg/kg (MMO), tramadol 5 mg/kg (MTR), or pethidine 1 mg/kg (MPE). Drugs were administered via the left jugular vein over 2 min. Blood was collected at 0, 120 min, and 24 h for WBC, PCV, cardiac troponin I (cTnI), homocysteine (Hcy), and ALT/AST; bipolar-limb ECGs were recorded at 0, 5, and 120 min. Baseline hematologic, biochemical, and ECG values did not differ across periods. cTnI remained within assay reference limits with no within-regimen change, although between-regimen differences appeared at 120 min and 24 h, with MED lower than methadone/tramadol at 120 min and lower than pethidine/tramadol at 24 h. Hcy showed no intergroup differences but increased modestly only with MED at 24 h. Hematologic effects were regimen-specific: WBC decreased with MMO at 2 h ($p < 0.05$), and PCV decreased with MTR at 2–24 h ($p < 0.05$). Hepatic enzymes showed no significant treatment or time effects. On ECG, PR and QT intervals lengthened over time (notably with MED and MMO), RR intervals generally prolonged consistent with bradycardia, and MPE increased T-wave amplitude ($p < 0.05$). Overall, medetomidine–opioid combinations produced distinct hematologic and ECG signatures without detectable within-regimen cTnI elevation. These data support individualized opioid selection with routine ECG monitoring when using medetomidine in goats.

Keywords

Sedation, Medetomidine, Opioid agonists, Goats

Number of Figures: 15
Number of Tables: 0
Number of References: 49
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Abbreviations

MED:Medetomidine

MME: medetomidine/methadone

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Introduction

Anesthetic and sedative agents are essential in both surgical and non-surgical procedures for humans and animals alike [1, 2]. Alpha-2 adrenoceptor agonists' medications (such as medetomidine and xylazine) are commonly performed in veterinary medicine to induce sedation, provide analgesia, and facilitate anesthesia [3]. Ruminants have α2 adrenergic receptors, which makes them sensitive to their sedative effects [4].

Opioids such as tramadol, methadone, pethidine and morphine are routinely used to provide sedation and analgesia during surgery and also to control severe arthritis pain, as well [5]. However, the compounds are associated with several adverse side effects, including notable respiratory depression, constipation, urinary retention, and bradycardia [6].

Medetomidine has high α 2 receptor selectivity and shows fewer significant side effects compared to xylazine, detomidine and romifidine [7]. The combination of medetomidine with selected opioids has been shown to reduce adverse effects while providing effective somatic analgesia and rapid sedation onset, accompanied by minimal cardiorespiratory complications [8, 9].

There are several studies documenting changes in hematologic and biochemical parameters following anesthetic treatments in ruminants [10]. Biochemical parameters related to cardiovascular and hepatic function are the most important diagnostic tests, can be an effective step in determining the effect of drugs on the body of animals.

Hepatic enzyme evaluation remains a common component of biochemical monitoring during sedative and anesthetic protocols in veterinary medicine. Although alanine aminotransferase (ALT) is a highly specific indicator of hepatocellular injury in small animals, its diagnostic value in ruminants is limited because hepatic ALT activity is low and extrahepatic tissues contribute minimally to serum levels. Nevertheless, ALT measurement can still provide supplementary information when interpreted alongside other markers. In contrast, aspartate aminotransferase (AST) is more widely distributed in ruminant liver, cardiac, and skeletal muscle, and thus serves as a sensitive but non-specific index of hepatocellular and myocellular injury. The combined interpretation of AST and ALT can therefore offer a broad overview of hepatic and muscular enzyme activity during pharmacologic interventions. In the absence of liver-specific enzymes such as sorbitol dehydrogenase (SDH) or glutamate dehydrogenase (GLDH), simultaneous measurement of AST and ALT remains a practical approach for evaluating general hepatocellular integrity in ruminants under experimental or clinical conditions [11-14].

Homocysteine (Hcy) is a sulfur-containing amino acid and is a byproduct of methionine metabolism. It has been reported that high level of serum Hcy can be employed as a risk factor for a number of disorders including vascular and neurodegenerative and renal diseases [15]. Cardiac troponin I (cTn I) is a 23.8 kDa cardiac protein, acting to inhibit muscle contraction in the absence of calcium [16]. It is a highly specific and sensitive biomarker for a spectrum of cardiovascular disease such as acute myocardial infarction, coronary heart disease, and heart failure [17]. The effects of various anesthetics on cardiovascular and hepatic functions are not yet well demonstrated in goats.

Analysis of electrocardiographic variables is essential for evaluating health, nutritional status, disease differential diagnosis, and drug pharmacodynamics in animals [18, 19]. Electrocardiography is a noninvasive, affordable, and valuable diagnostic tool for assessing cardiac electrical activity [20]. The significance of electrocardiographic parameters following the administration of adrenergic α2-agonists in specific animal species has been highlighted [21, 22].

Our hypothesis proposed that administering medetomidine, alone and in combination with intravenous opioids, could induce changes in electrocardiographic and biochemical parameters related to cardiac and hepatic functions in goats. This would enable us to identify the most effective sedative combination for

Abbreviations-Cont'd

MMO : medetomidine/morphine
MTR : medetomidine/ tramadol
MPE : medetomidine/pethidine
ALT: alanine aminotransferase
AST: aspartate aminotransferase
GGT: gamma-glutamyl transferase
GFR : glomerular filtration rate
Hcy : Homocysteine
cTn I : Cardiac troponin I
kDa : Kilo Dalton
WBC: white blood count
PCV: packed cell volume
K-S :Kolmogorov-Smirnov
χ^2 :Chi-squared
ECG : electrocardiogram
AST: aspartate aminotransferase
ALT:alanine aminotransferase
AV:atrioventricular
HR: heart rate
RR:respiratory rate
RT:rectal temperature
CRT: capillary refill time
RM: ruminal motility
EDTA: Ethylenediaminetetraacetic acid
ELISA: enzyme-linked immunosorbent assay
ANOVA: Analysis of Variance
IACUC: Institutional Animal Care and Use Committee

livestock.

To the best of our knowledge, this is the first report that combines medetomidine, medetomidine with opioids (tramadol, methadone, pethidine, and morphine) and compares changing in hepatorenal, cardiovascular and electrocardiographic parameters in goats.

Results

Descriptive parameters for the WBC variable across the studied groups are presented in Fig 1. A significant difference was observed in the morphine group ($p < 0.05$). Furthermore, the paired samples t-test revealed that the WBC variable in the morphine group demonstrated a significant decrease at 2 hours after treatment compared to the pre-treatment time (time 0) ($p < 0.05$).

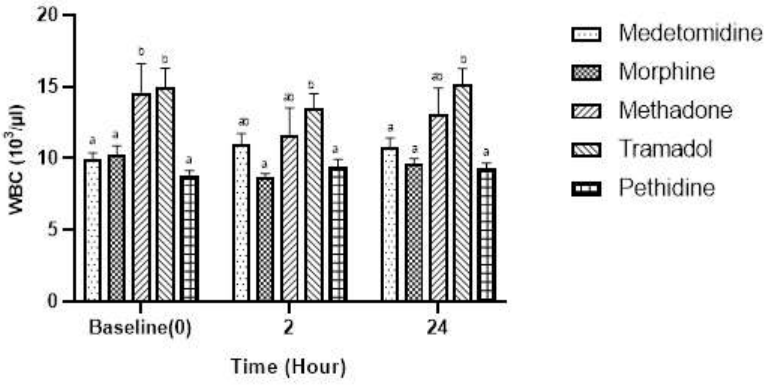

Figure 1.
The changes in white blood cells (WBC) in various treatment groups at different time points. Different letters indicate statistically significant differences ($p < 0.05$)

The PCV showed a significant time effect only in the tramadol group ($p < 0.05$), whereas no temporal changes were detected in the other groups ($p \geq 0.05$). A statistically significant decrease in the PCV variable level in the tramadol group 24 hours after treatment compared to the time before treatment. ($p < 0.05$) (Fig 2).

The results showed significant differences in the mean WBC variable among the treatment groups at the time points ($p < 0.05$). The mean WBC variable at 0 time was significantly higher in the methadone and tramadol treatments compared to the other treatments ($p < 0.05$). The mean WBC variable at 2 hours after treatment in the tramadol group was significantly higher than in both the morphine and pethidine groups ($p < 0.05$). At 24 hours after treatment, the mean WBC variable in the tramadol treatment was significantly higher than in the morphine, medetomidine, and pethidine treatments ($p < 0.05$). However, no significant differences were noted between the tramadol and methadone treatments, nor among the morphine, pethidine, methadone, and medetomidine treatments ($p > 0.05$). Additionally, no significant differences in the mean PCV variable among the studied treatments at the studied time points.

Baseline mean cTnI values were similar among all groups ($p > 0.05$), indicating comparable pre-dose conditions. Repeated-measures ANOVA showed no significant time-dependent changes in cTnI within any treatment ($p > 0.05$ for all). Mean concentrations remained within assay-appropriate reference limits throughout the 24-hour period.

However, between-group comparisons revealed significant differences in cTnI at 120 minutes ($F_{4,45} = 3.81$, $p < 0.05$) and 24 hours ($F_{4,45} = 4.20$, $p < 0.05$) post-injection. Post hoc Tukey testing demonstrated that cTnI levels at 120 minutes were significantly lower in the medetomidine group compared with the methadone and tramadol groups ($p < 0.05$). Similarly, at 24 hours, cTnI was significantly lower in medetomidine-treated animals than in those receiving pethidine or tramadol ($p < 0.05$). No other between-group differences were observed. Overall, cTnI values across all regimens remained within physiological limits, suggesting no biochemical evidence of myocardial injury (Fig 3).

A significant time effect of Hcy only in the medetomidine group ($F_{2,18} = 4.58$, $p = 0.025$, $\eta^2 = 0.34$, power = 0.70). Paired-sample t-test analysis indicated that Hcy in this group increased significantly at 24 hours compared with baseline by 0.61 µmol/L (95% CI: 0.09–1.13; $p = 0.01$). In contrast, changes in Hcy over time were not statistically significant in the morphine, methadone, tramadol, or pethidine groups ($p > 0.05$).

One-way ANOVA demonstrated no significant intergroup differences in Hcy concentrations at baseline, 120 minutes, or 24 hours (all $p > 0.05$). Thus, the transient increase observed with medetomidine likely reflects a mild, regimen-specific metabolic response rather than a pathologic effect (Fig 4).

The changes in the studied liver biomarkers among the treatment groups at the time points are illustrated in the following figures (Fig 5 and 6).

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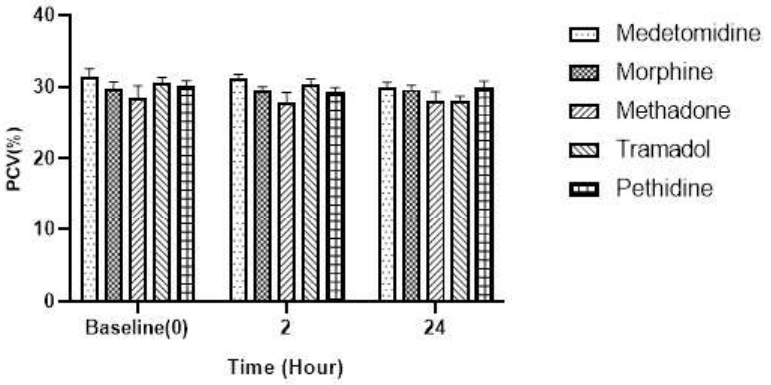

Figure 2.
The changes in packed cell volume (PCV) in various treatment groups at different time points.

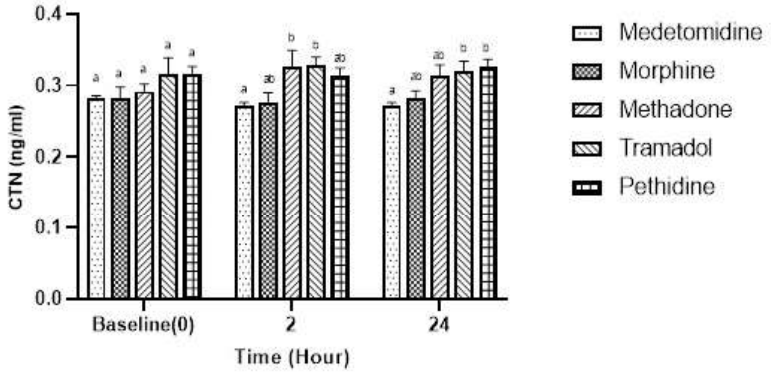

Figure 3.
The changes in cardiac troponin I (cTn I) in various treatment groups at different time points. Different letters indicate statistically significant differences ($p < 0.05$)

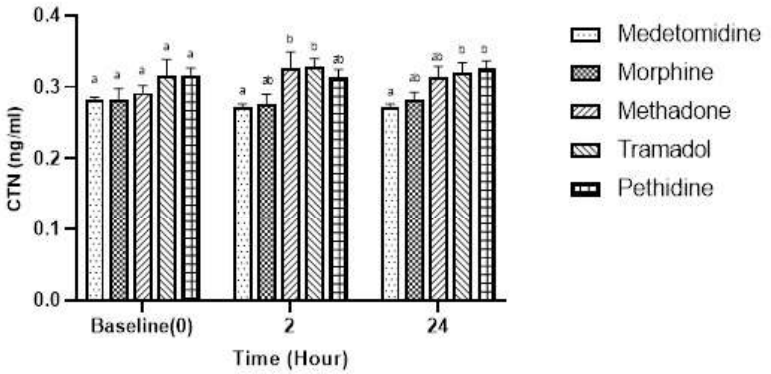

Figure 4.
The changes in homocystein (Hcy) in various treatment groups at different time points. Different letters indicate statistically significant differences ($p < 0.05$)

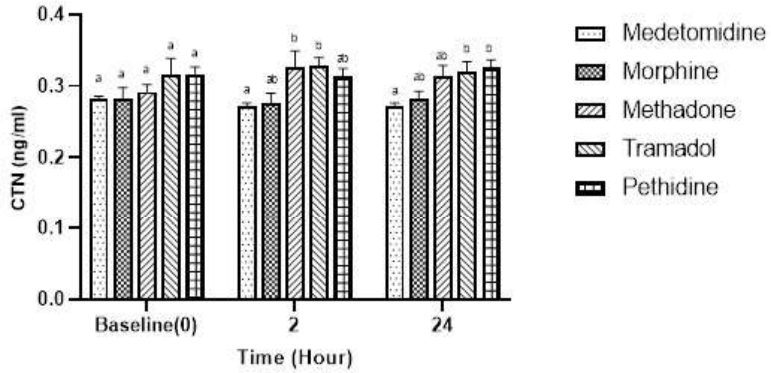

Figure 5.
The changes in aspartate aminotransferase (AST) in various treatment groups at different time points.

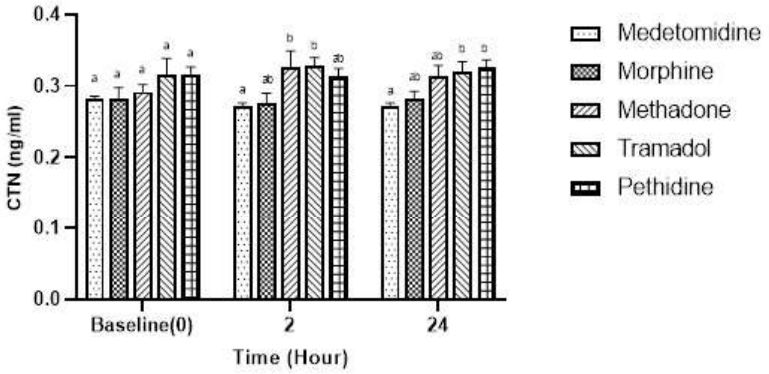

Figure 6.
The changes in alanine aminotransferase (ALT) in various treatment groups at different time points.

Results of the friedman test showed no significant effect of sampling times on the mean P amplitude component in the medetomidine (χ^2=0.333, p = 0.846), methadone (χ^2=3.938, p=0.140), and pethidine (χ^2=1.727, p = 0.422) groups. However, significant temporal differences were observed in the morphine (χ^2=8.720, p = 0.013) and tramadol (χ^2=7.00, p = 0.030) groups. Results of the Wilcoxon signed-rank tests indicated a significant decrease in mean *P* amplitude at 120 minutes post-injection compared to baseline in both morphine and tramadol groups (p < 0.05) (Fig 7). For QRS amplitude, no significant time effect was found in morphine, methadone, tramadol, and pethidine groups, but medetomidine showed a significant increase at 120 minutes post-injection compared to 5 minutes post-injection (χ^2=6.414, p = 0.040; Wilcoxon p < 0.05) (Fig 8). Regarding T amplitude, significant changes over time were only noted in the pethidine group (χ^2=13.130, p = 0.001), with increases at 5 and 120 minutes post-injection compared to baseline (Wilcoxon p < 0.05) (Fig 9).

Results of the Kruskal-Wallis test revealed no significant differences among groups in mean P amplitude before injection (p = 0.372) or at 120 minutes post-injection (p = 0.349), but a significant difference at 5 minutes post-injection (p = 0.024). Pairwise Mann-Whitney tests showed medetomidine differed significantly from morphine, tramadol, and methadone at this time point. For QRS amplitude, no group differences were found before injection or at 5 minutes, but significant differences emerged at 120 minutes post-injection (p = 0.0090, with medetomidine differing from pethidine, tramadol, and methadone. T amplitude showed no baseline differences, but significant group differences were found at 5 and 120 minutes post-injection(p < 0.05). Pairwise comparisons indicated significant differences involving medetomidine, pethidine, tramadol, and morphine groups at these times.

No significant temporal changes in *P* duration were observed in medetomidine, morphine, methadone, or tramadol groups, but pethidine showed a significant increase at 120 minutes post-injection (p = 0.009) (Fig 10). QRS duration showed significant time effects in morphine (p = 0.048) and pethidine (p = 0.028) groups, with morphine showing a decrease at 120 minutes compared to 5 minutes, and pethidine showing decreases at 5 and 120 minutes compared to baseline (Fig 11). T duration was significantly altered only in the morphine group (p = 0.025), with an increase at 120 minutes compared to 5 minutes post-injection (Fig 12).

No significant differences among groups were found for P duration at any time point. Similarly, QRS

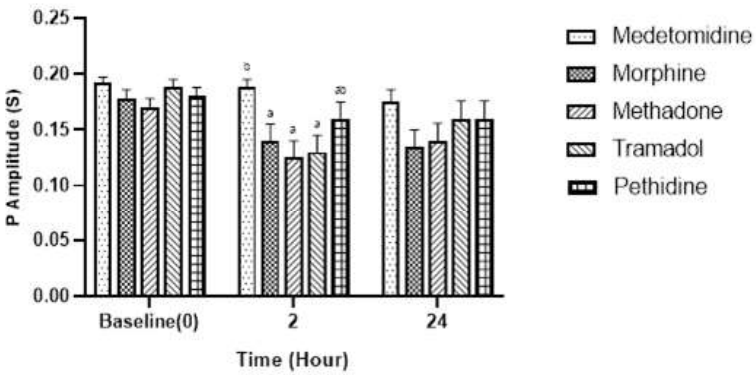

Figure 7.
Trend of changes (mean ± standard error) in the P Amplitude component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

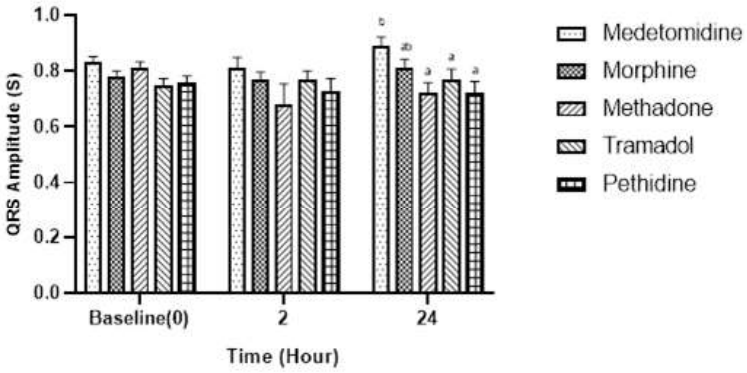

Figure 8.
Trend of changes (mean ± standard error) in the QRS Amplitude component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

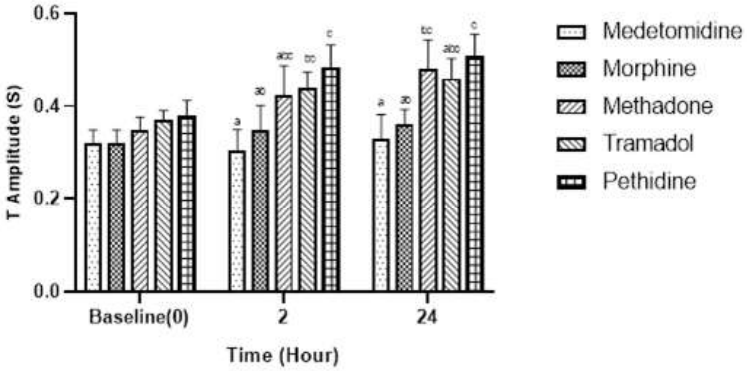

Figure 9.
Trend of changes (mean ± standard error) in the T Amplitude component across the studied groups. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

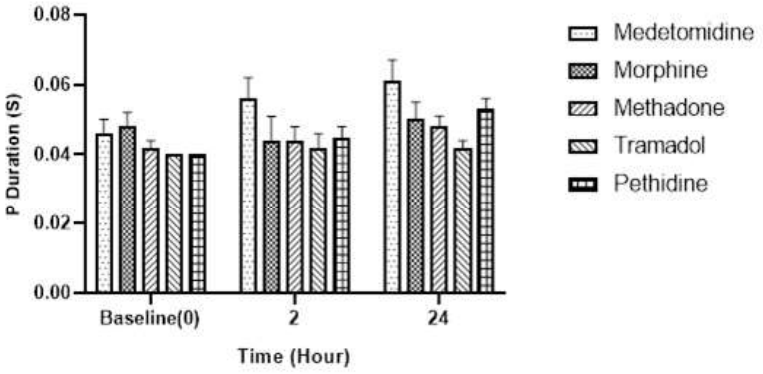

Figure 10.
Trend of changes (mean ± standard error) in the P Duration component across the studied groups. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence l

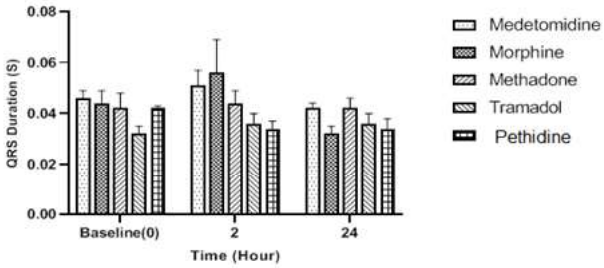

Figure 11.
Trend of changes (mean ± standard error) in the QRS Duration component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

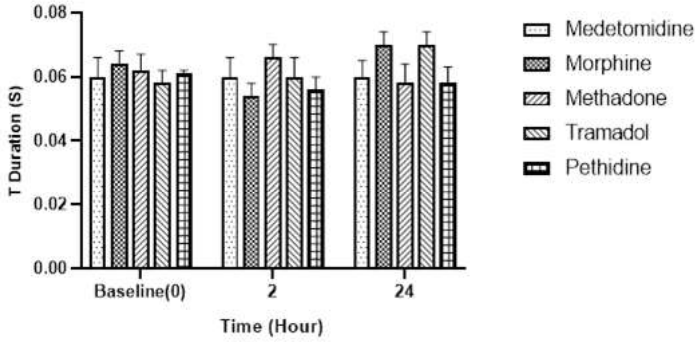

Figure 12.
Trend of changes (mean ± standard error) in the T Duration component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

duration and T duration showed no significant group differences before or after injection.

PR interval showed no significant time effect in methadone, tramadol, or pethidine groups, but significant increases over time were found in medetomidine and morphine groups ($p = 0.001$) (Fig 13). Wilcoxon tests confirmed significant increases at 5 and 120 minutes post-injection compared to baseline. QT interval showed significant temporal changes in all groups ($p < 0.05$). Wilcoxon tests indicated significant increases at 5 and 120 minutes post-injection compared to baseline in morphine, methadone, tramadol, and pethidine groups; medetomidine showed a significant increase at 5 minutes (Fig 14). RR interval was not significantly changed over time in tramadol but showed significant increases in medetomidine, morphine, methadone, and pethidine groups at various post-injection times (Fig 15).

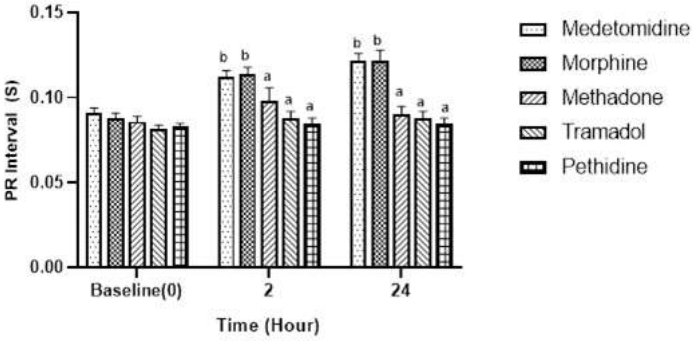

Figure 13.
Trend of changes (mean ± standard error) in the PR Interval component across the studied groups. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

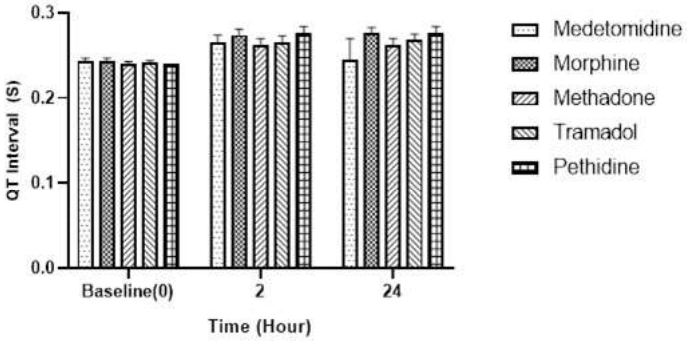

Figure 14.
Trend of changes (mean ± standard error) in the QT Interval component across the studied groups before and after drug administration. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level.

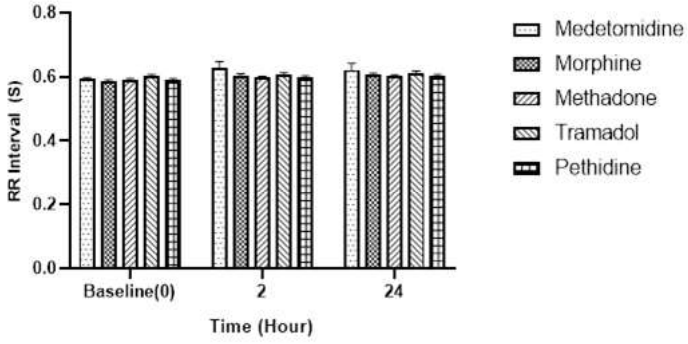

Figure 15.
Trend of changes (mean ± standard error) in the RR Interval component across the studied groups. At each time point, treatments with identical letters do not differ significantly based on the Mann-Whitney test at the 95% confidence level

Discussion

Various α2 agonist drugs and opioids are widely used to provide sedation, analgesia, and restraint in small ruminants [3, 23].

There was a synergistic effect in combining α2 agonist drugs with opioids. Alpha-2 agonist drugs act by binding to α2 receptors in the brain and spinal cord. The present study aimed to evaluate the effects of medetomidine administered alone and in combination with intravenous opioids in order to determine the most effective sedative protocol for livestock. The doses of medetomidine, tramadol, morphine, methadone, and pethidine used were based on previous studies on small ruminants [24, 25].

Alpha-2 agonist drugs can cause hypoxemia in healthy ruminants and serious complications in animals with pre-existing hypoxia. Therefore, it is important to check PCV before anesthetic drugs because animals with a PCV of less than 25% can be at risk of complications during anesthesia [26].

Our results demonstrated that the mean PCV variable in the medetomidine, morphine, methadone, and pethidine groups did not show statistically significant differences at studied time points which indicating overall stability in red blood cell volume[1]. But tramadol caused a significant reduction of PCV, 24 hours after treatment that was similar to that reported with the use of dexmedetomidine and xylazine. A previous study showed that dexmedetomidine and xylazine cause a severe drop in PCV in sheep [10]. This finding was not consistent with the report of Bani Ismail et al. In their study, they investigated the effect of diazepam, xylazine, and ketamine anesthesia on clinical pathological factors in small ruminants .Their results showed that PCV was significantly increased after 24 h in sheep and 2 h in goats [27].Clinically, all PCV values, remained within caprine reference ranges, indicating preserved oxygen-carrying capacity; the drop likely reflects transient hemodilution/splenic sequestration and merits observation only [41].

In ruminants, hypoxemia with α2-agonists mainly stems from effects in the lungs: they quickly disturb ventilation; perfusion balance and make the air–blood barrier leaky, a response amplified by resident intravascular macrophages. The accompanying fall in PCV most likely reflects temporary pooling of red cells in the spleen and mild dilution of the blood, rather than true anemia, supporting straightforward measures such as oxygen supplementation and routine hemodynamic monitoring during α2-based sedation [28, 29].

Our results indicated that the mean WBC variable for the medetomidine, methadone, tramadol, and pethidine groups did not exhibit statistically significant differences at the time points. These findings were in agreement with the previous studies after xylazine-ketamine and ketamine alone administration in sheep [30, 31].

In the present study, a significant reduction in WBC was observed in morphine group, these changes can be attributed to the stress of moving the animal and the pain caused by the jugular intravenous injection during anesthesia [32]. Because a differential was not performed, the observed WBC change is compatible with (but not diagnostic of) a stress response. Future work should include manual/automated differentials (neutrophils, lymphocytes, eosinophils, eosinophils, monocytes) and adjunct measures to confirm mechanism.

Previous studies have shown that anesthetic drugs can modulate immune responses, potentially leading to changes in WBC. Clinically, all WBC values stayed within caprine reference ranges. The morphine-associated drop is most consistent with a transient stress response, not clinically significant leukopenia.

In addition, the reason for the decrease in PCV and WBC could be due to the decrease in the accumulation of circulating blood cells in the spleen due to the decrease in sympathetic activity. Also, the decrease in PCV during the anesthesia period may be attributed to the transfer of fluid from the extravascu-

lar to the intravascular compartment of the animals to maintain normal cardiac output [33].

The importance of analyzing cardiovascular biomarkers during sedation by α2 adrenergic agonists has been emphasized for specific animal species [34]. Hcy and cTn I are known as cardiovascular biomarkers [35].

In our crossover study, baseline levels of cTnI and homocysteine (Hcy) were comparable across treatment groups, supporting reliable within-subject analyses. Throughout the 24-hour period, cTnI levels remained stable within each regimen and stayed within assay-specific reference ranges. Minor inter-regimen differences; where certain opioid combinations produced slightly higher values than medetomidine, are more likely due to pharmacodynamic effects rather than myocardial injury. This observation aligns with existing data on healthy goats, which typically show minimal cTnI levels [37], though it contrasts with reports in other species (e.g., canines),[38] where α_2-agonists or α_2–opioid combinations have been associated with slight post-sedation increases. These findings highlight the importance of species- and protocol-specific interpretations. Homocysteine showed a modest increase only in the medetomidine group, with stable levels in others. Given the lack of standardized diagnostic thresholds for Hcy in goats and its known sensitivity to metabolic and stress-related variables, this isolated elevation carries uncertain clinical weight. Overall, the biomarker pattern (cTnI remaining within normal limits and a regimen-specific but limited Hcy change) suggests no evidence of myocardial injury over 24 hours. These results support clinical monitoring over intervention in healthy animals, though isolated biomarker fluctuations should not be overinterpreted without corresponding clinical signs, echocardiography, or extended follow-up.

Liver biomarker concentrations (ALT, AST) are changed by conditions such as hepatocytes, fibrosis and cirrhosis, hepatomegaly, and inflammatory conditions such as hepatitis. The use of sedatives and narcotics (inappropriately or in high doses) can cause hepatotoxicity [39, 40]. The results of our study indicated that hepatic biomarkers did not show statistically significant differences 24 hours after administration, which is consistent with a previous study [34]. Other previous studies were not consistent with the results of the present study and reported that liver enzyme levels changed significantly 24 hours after administration [42]. Another study reported that tramadol use increased the level of the liver enzyme ALT [2]. Clinically, ALT/AST stayed within reference limits at 24 h, indicating no hepatocellular injury [11-14]. Given ALT's limited utility in ruminants, the unchanged AST profile is particularly reassuring.

Because ALT is not a reliable marker of hepatocellular injury in ruminants, normal ALT values do not exclude liver damage. In large animals, SDH and GLDH are preferred for detecting hepatocellular leakage, while AST lacks specificity and GGT reflects cholestasis; future studies should include these analytes for a definitive hepatic assessment.

This study systematically evaluated the temporal effects of various analgesic and sedative agents-medetomidine, morphine, methadone, tramadol, and pethidine on electrocardiographic (ECG) amplitude, duration, and interval parameters. The findings reveal distinct drug-specific patterns of cardiac electrophysiological response, with implications for both clinical and research settings.

The significant reduction in P wave amplitude observed at 120 minutes post-injection in the morphine and tramadol groups suggests a potential depressant effect on atrial depolarization. Previous studies have demonstrated that opioids, particularly morphine, can modulate autonomic tone and reduce sympathetic activity, which may underlie these changes [43]. In contrast, medetomidine, an α2-adrenergic agonist, induced a significant increase in QRS amplitude at 120 minutes, consistent with its known effects on cardiac conduction and contractility through enhanced vagal tone and decreased sympathetic outflow [44, 45]. The significant increases in T wave amplitude in the pethidine group at both 5 and 120 minutes post-injection may reflect alterations in ventricular repolarization, possibly mediated by direct myocardial effects or changes in electrolyte balance, as previously reported for synthetic opioids [41].

Duration parameters were less consistently affected. Notably, Pethidine significantly prolonged P wave duration at 120 minutes, while morphine and Pethidine both affected QRS duration, albeit in opposite directions. The shortening of QRS duration with morphine and pethidine may have different effects on ventricular conduction velocity and could be mediated by their different receptors and intrinsic cardiac functions [43]. The increase in T wave duration in the morphine group aligns with prior reports of opioid-induced changes in ventricular repolarization [46].

The PR interval was significantly prolonged in both Medetomidine and morphine groups, consistent with enhanced atrioventricular (AV) nodal delay. This is in agreement with the known bradycardic and AV-nodal slowing effects of α2-agonists and opioids [44, 45]. The QT interval was significantly increased in all groups, a finding that warrants clinical attention due to the risk of proarrhythmic effects, particularly torsade's de pointes, associated with QT prolongation [47]. The RR interval was also prolonged in

most groups, reflecting drug-induced bradycardia, especially with medetomidine and morphine, which is well-documented in the literature [44].

Intergroup analyses revealed that medetomidine and morphine most consistently induced significant changes in ECG parameters compared to other agents, particularly at early (5 min) and late (120 min) post-injection time points. These findings are clinically relevant, as they underscore the need for careful cardiac monitoring during and after administration of these drugs, especially in patients with pre-existing cardiac conditions.

The observed drug-specific ECG changes highlight the importance of individualized drug selection and monitoring in both veterinary and human clinical practice. While the study provides valuable insights, limitations include the lack of direct measurement of serum drug concentrations and the absence of long-term follow-up for arrhythmic events. Future studies should integrate continuous ECG monitoring and explore the underlying molecular mechanisms.

Overall, none of the opioids eliminated the characteristic cardiovascular effects of medetomidine, although the risk profile differed by agent. Morphine with medetomidine produced more marked atrioventricular nodal delay and bradycardia (PR and RR prolongation) and a transient fall in total WBC, making it less suitable in animals with conduction disease or high vagal tone. Tramadol with medetomidine caused a significant reduction in PCV at 24 hours (still within reference limits), consistent with relative hemodilution or splenic sequestration and less desirable when anemia or peri-procedural blood loss is a concern. Pethidine with medetomidine increased T-wave amplitude at early and late times, warranting caution in patients with repolarization abnormalities or concurrent QT-prolonging therapies. In contrast, methadone with medetomidine showed a neutral hematologic profile and did not exacerbate conduction delay, offering a more balanced option when an opioid adjunct is needed. Across regimens, cardiac troponin I remained within reference limits without within-regimen rises, and homocysteine was stable except for a modest 24-hour increase with medetomidine alone, supporting monitoring rather than intervention in healthy animals. Although some between-group differences reached statistical significance, their absolute magnitudes were small and largely within caprine reference intervals, reinforcing individualized opioid selection based on comorbidities (conduction disease, anemia risk, repolarization concerns) and the routine use of electrocardiographic and oxygenation monitoring.

Conclusion

In summary, among medetomidine and opioid combinations in healthy goats, methadone with medetomidine showed the most neutral overall profile, with no hematologic disturbance and no atrioventricular nodal delay beyond medetomidine's class effect. By comparison, morphine with medetomidine accentuated atrioventricular nodal delay and bradycardia, tramadol with medetomidine produced a reduction in PCV at 24 hours, and pethidine with medetomidine increased T wave amplitude. Cardiac troponin I remained within reference limits without rises within each regimen, and homocysteine rose modestly only with medetomidine alone. Clinically, opioid choice should be individualized: avoid morphine in animals with conduction disease, avoid tramadol where anemia risk or hemodilution is a concern, use pethidine cautiously in repolarization abnormalities, and consider methadone when a balanced adjunct is desired, recognizing that observed effects were modest and largely within species reference ranges under the conditions of this study.

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

For manuscript editing and writing, we used Perplexity (Open AI) solely to improve grammatical accuracy and readability; all outputs were reviewed and verified by the authors."

Ethical Approval

In accordance with international legal frameworks, the present article ensures the protection and respect for animal rights. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study involving ten goats was conducted with the approval of the Animal Welfare Committee at the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman (IR.UK.VETMED.REC.1399.023).

Animals

The selected goats had a mean age of 12±4 months and an average weight of 22±3 kg. All goats were female. These animals were chosen using a simple randomization method, specifically a sample lottery approach, ensuring unbiased selection. All goats were housed in a single group pen where they experienced uniform environmental, nutritional, and management conditions.

The goats were provided with a consistent diet that

included a mix of roughages, primarily alfalfa hay and wheat straw, and concentrates, consisting of barley grain, soybean meal, and wheat bran. This diet was formulated to meet their physiological maintenance needs, maintaining a forage-to-concentrate ratio of 90:10. Additionally, the goats had access to sufficient vitamins, minerals, and fresh water to support their overall health. To prevent potential internal and external parasitic infestations, all animals were treated with broad-spectrum antiparasitic medications two months prior to the experiment.

Routine health assessments were conducted to monitor the well-being of the goats. These assessments included clinical examinations that measured heart rate (HR), respiratory rate (RR), rectal temperature (RT), capillary refill time (CRT), and ruminal motility (RM). Furthermore, paraclinical evaluations were performed, which involved hematological tests white blood count (WBC) and packed cell volume (PCV) and fecal analysis for parasites.

Two days before the study began, the animals underwent comprehensive clinical and paraclinical evaluations to confirm their normal health status. This step is essential to ensure that any effects observed during the experiment are attributable to the treatments rather than pre-existing conditions.

To standardize physiological measurements, food was withheld from the animals for 12 hours and water for 6 hours prior to the experiment. This fasting period helps reduce variability related to digestion and hydration. This controlled setting minimizes external stressors that could impact animal behavior and physiological responses.

The animals were weighted prior to each treatment to determine the appropriate drug dosages. At any given time, two animals were studied each time separately, and they were kept from seeing one another. The animals were carefully restrained on a specialized bed, which was placed on a soft mattress, in a calm, enclosed area measuring 5×6 m^2. They rested for 20 minutes prior the initial measurements of clinical and para-clinical variables were taken.

The area over the left jugular vein was surgical prepared for intravenous administration. Clinical and para-clinical assessments were conducted again 48 hours post-experiment to assess the animal's health condition.

Treatments

The study involving goats utilized a randomized crossover Latin square design to evaluate the effects of five intravenous treatments. Each goat was assigned to receive one of the treatments at a time, with a minimum washout period of 8 days between each treatment to ensure that any effects from the previous treatment has dissipated before the next one was administered [21,22].

The study used a randomized crossover Latin-square design with five intravenous regimens:

MED: medetomidine (DorbeneVet; N-Vet AB, Sweden) 20 µg/kg IV, a dose widely used/recommended for small ruminants and supported by recent goat/sheep studies. (BioMed Central)[48].

MME: medetomidine (20 µg/kg) + methadone (Faran Shimi Pharmaceutical Company, Iran) 0.5 mg/kg IV, within the reported 0.05–0.5 mg/kg range for sheep/goats and used in goat studies combining α2-agonists with opioids [25].

MMO: medetomidine (20 µg/kg) + morphine (Dimorf; Cristália Produtos Químicos e Farmacêuticos Ltda) 0.5 mg/kg IV, consistent with small-ruminant recommendations (0.2–0.5 mg/kg), and within pharmacokinetic dose ranges evaluated in goats [49].

MTR: medetomidine (20 µg/kg) + tramadol (Tehran Chime Pharmaceutical Company, Iran) 5 mg/kg IV, a dose supported by sheep antinociception data (4–6 mg/kg IV) and used in recent goat work with α2-opioid combinations [25].

MPE: medetomidine (20 µg/kg) + pethidine (Caspian Tamin Pharmaceutical Company, Iran) 1 mg/kg IV; a conservative IV dose selected to limit histamine-mediated effects recognized for meperidine, while remaining within reported efficacious IV ranges in sheep (antincioception at 5 mg/kg IV) [25]

For all treatments, drugs were prepared in a single syringe and diluted with 0.9% saline to a final volume of 5 mL, then administered via the left jugular vein over 2 minutes using an 18-gauge needle, with animals standing, to standardize injection rate/volume across regimens.

Blood sample collection

Blood samples were taken from the jugular vein of the goats at the beginning of the study, 120 min and 24 hours after the start of the study.

Hematology: PCV and WBC measurement

Hematology in this study was limited to serial PCV and total WBC by design; RBC indices, platelet counts, and leukocyte differentials were not obtained.

Jugular venous blood (2 mL) was collected into K_2-EDTA tubes, gently inverted 8–10 times, and analyzed within 2 hours of collection; hemolyzed or clotted specimens were excluded.

Packed cell volume (PCV): Whole blood was drawn into heparinized microcapillary tubes, sealed with clay, and centrifuged in a microhematocrit centrifuge (Sigma-Aldrich, Model 5254, Germany) for 5 minutes. The hematocrit was read with a microhematocrit card-reader and recorded as a percentage. Each sample was run in duplicate and the mean reported.

White blood cell count (WBC), WBC was determined by hemocytometer using Natt–Herrick diluent. Briefly, well-mixed EDTA blood was diluted 1:200 in Natt–Herrick solution, mixed for 2–3 minutes, and the improved Neubauer chamber was charged. After a short settling period, leukocytes were counted in the four large corner squares on each grid (both sides), and the average count was used for calculation of WBC.

Biochemical parametrs

Blood samples from all animals were collected via the jugular vein and stored in tubes without anticoagulants. Subsequently, after clotting, serum was separated using a centrifuge (3000 rpm) for 15 minutes. The serum concentrations of hepatic biomarkers such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as serum cardiac biomarkers including cardiac troponin I (cTn I) enzyme and homocysteine (Hcy) were measured using a specific commercial kit with the ELISA method.

Electrocardiogram

Heart rate monitoring was performed on all animals using the bipolar limb lead derivation method and the Cardiomax FX-2111 electrocardiograph machine (Fukuda, Japan) at 0, 5, and 120 minutes. The paper speed on the electrocardiograph machine was set at 25 millimeters per second, and the voltage setting was considered as 1 millivolt equivalent to 10 millimeters. The heart rate recording was done after stabilizing the animal in a seated position on a soft mat in a quiet and soundless environment with electrodes attached to the skin. The positive electrode was placed at the apex of the heart (fifth intercostal space on the left side, on the chest wall, behind the elbow), the negative electrode at the black ridge of the neck at 1/3 lower left side, and the neutral electrode in the body junction area of the animal. The electrodes were installed on the skin after using methyl alcohol spray. The heart rate was calculated by measuring the average time interval between 6 RR intervals. The QRS complex was described as the first negative deflection below Q, the first positive deflection as R, and the negative deflection after R as S. A comparison was made between different periods of electrocardiography in terms of heart rate, the potential presence of cardiac arrhythmias, the shape and size of waves, and the measurement of intervals on the electrocardiogram

Statistical analysis

The statistical analysis of the data obtained was conducted using IBM SPSS software, version 27. Initially, descriptive findings of the studied variables were calculated and reported, including indicators such as mean, standard deviation, standard error, etc. First, the normality of the data was assessed using the Kolmogorov-Smirnov test. In cases where the significance level from the K-S test was below 0.05, statistical methods were employed to normalize the data distribution. Subsequently, research hypotheses were tested using parametric tests. In the next phase, for data with a normal distribution, the effect of measurement times on the mean indices examined before and after drug injection was analyzed using repeated measures ANOVA. For data that did not meet normal distribution criteria and for non-parametric data, the Friedman test was utilized. If significant differences were found, to determine differences between measurement times in parametric data, the student's t-test for dependent groups was applied, while for non-parametric data, the Wilcoxon signed-rank test was used. Additionally, to compare parametric data with normal distribution between treatment groups at identical measurement times, one-way ANOVA and Tukey's post-hoc test were employed. For comparing non-normally distributed residuals and non-parametric data, the Kruskal-Wallis test and Mann-Whitney post-hoc test were utilized. It is noteworthy that throughout all stages of analysis, a significance level of 5% was considered for rejecting the null hypothesis.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' Contributions

M.S: Data curation; funding acquisition; investigation; methodology; visualization; writing–original draft; formal analysis; project administration; E.S: Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; validation; visualization; A.S.S: Conceptualization; data curation; formal analysis; project administration; resources; writing–review and editing; software; supervision; visualization; writing–original draft; O.A: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; Z.S: investigation; methodology; visualization; writing–original draft and formal analysis.

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Conflict of interest

The authors declare that there is no conflict of interest.

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A comparative study on *Echinococcus granulosus* and curcumin therapeutic effect in mouse model of multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system, characterized by demyelination and neurodegeneration affecting the brain and spinal cord. Echinococcus granulosus the causative agent of Cystic echinococcosis induces profound immunomodulation effects that enable evasion of host anti-parasitic immune response. Curcumin is a natural polyphenol component with well-documented anti-inflammatory and antioxidant properties. The present study was conducted to investigate the immunomodulatory effects of *E. granulosus protoscolices* (PSCs) in experimental autoimmune encephalomyelitis (EAE), an established animal model of MS, and to compare their therapeutic effect with that of curcumin. 40 male Sprague Dawley rats were randomly divided into four groups (n = 10 per group): control, EAE, EAE treated with E. *granulosus* PSCs and EAE treated with curcumin. Levels of proinflammatory cytokines, brain nitric oxide (NO) concentration, serum myeloperoxidase (MPO) activity and serum malondialdehyde (MDA) were assessed. Based on our results, experimental autoimmune encephalomyelitis caused significant increase in proinflammatory cytokines, MPO activity, MDA levels and NO concentration as compared with controls. Treatment with either curcumin or PSCs reduced the release of inflammatory cytokines, MPO activity, MDA levels and NO concentration compared with EAE group. No difference was observed between curcumin and PSCs treated groups. The results of current study demonstrate that PSCs exert a therapeutic effect in EAE model by decreasing the production of pro-inflammatory cytokines and inhibiting oxidative stress. The similar role was also observed for curcumin. Notably, the efficacy of PSCs treatment was comparable to that of curcumin.

Keywords

Curcumin, Echinococcus granulosus, Experimental autoimmune encephalomyelitis (EAE), Multiple sclerosis, cytokine

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Abbreviations

MS: Multiple sclerosis

EAE: Experimental autoimmune encephalomy-

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Introduction

Multiple sclerosis (MS) is a chronic autoimmune neurodegenerative disease affecting approximately 2.8 million individuals worldwide. The disease is characterized by demyelination, axonal injury, the formation of lesions in the brain and spinal cord and progressive neurological dysfunction. MS represents as one of the main causes of neurobiological disability in young adults with wide psychological, financial, social, and health burdens. Certain regions around the world have experienced a notable rise in the prevalence of MS. In 2016, the global number of MS cases was estimated at 2,221,188, corresponding to a prevalence of 30.1 cases per 100,000 individuals [95% uncertainty interval (UI): 27.5–33.0]. Between 1970 and 2015, the Middle East and North Africa (MENA) region reported a high prevalence of MS. Among countries in the Eastern Mediterranean Region (EMR), Iran reported the highest prevalence rate, with 72.11 cases per 100,000 population. In Tehran, the capital and most populous city of Iran, MS prevalence increased from 79.3 cases per 100,000 people in 2006, to 162.38 cases per 100,000 by 2019 [95% confidence interval (CI): 160.27–164.52] [1].

Despite advances in the management of MS, current treatment options remain limited in their ability to halt disease progression or reverse neurological damage. Most approved treatments focus on immunomodulation to reduce the relapse frequency and severity; however, their effectiveness in preventing long-term disability, particularly in progressive forms of MS, is limited. In addition, these treatments are frequently associated with significant adverse effects, including increased susceptibility to infections, hepatotoxicity, cardiovascular complications, and reduced quality of life due to long-term treatment burden. Furthermore, the heterogeneity in patients' responses to disease-modifying therapies (DMTs) further complicates treatment strategies, underscoring the need for more personalized and targeted approaches. As the underlying mechanisms of MS continue to be elucidated, there is a growing demand for innovative therapies [2].

Although multiple environmental and genetic factors have been implicated in MS susceptibility, the exact etiology of MS remains unknown. In experimental studies, the EAE in animals is used as a model of MS for pharmacological studies and drug discovery. Accumulating evidences in animals with EAE and MS patients suggest that inflammation plays a fundamental role in MS pathogenesis. The disease progression is mediated by T cells, B cells, macrophages and activated microglial cells [3]. Cytokines produced by T-helper 1 (Th1) and T-helper 17 (Th17) cells, such as interferon-γ (IFN-γ) and tumor necrosis factor-alpha (TNF-α) were considered primarily responsible for the induction of the inflammatory process during the development of MS. Proinflammatory molecules secreted during MS development cause myelin and oligodendrocyte destruction [4].

In addition, oxidative stress has been closely linked to the progression of neuroinflammation. Previous studies found that, the CNS damage was induced by the excessive production of ROS including NO and hydrogen peroxide (H_2O_2) in MS patients [5,6].

Current pharmacological treatments for MS primarily rely on immunomodulatory or immunosuppressant drugs, aimed at attenuating immune reaction and reducing ROS production. However, these drugs are often accompanied by several undesirable side effects, such as headache and gastrointestinal disturbances [7]. Therefore, the identification of alternative treatment strategies with improved efficacy and fewer side effects has been the focus of recent studies.

Curcumin ($C_{21}H_{20}O_6$) is a polyphenolic substance extracted from turmeric (Curcuma longa) and belongs to the Zingiberaceae family. It has been shown that curcumin can play pharmacological roles in various autoimmune and neurodegenerative disorders such as dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease, and MS. the anti-inflammatory mechanism of curcumin are largely attributed to its ability to suppress proinflammatory cytokines production, inhibit TNF-α and NO release and prevent the differentiation and proliferation of Th17 cells [8].

Echinococcosis is a globally distributed zoonotic disease caused by infection with the larval stage of *Echinococcus granulosus*, a cestode belonging to

Abbreviations-Cont'd

elitis
PSCs: Protoscolices
MPO: Myeloperoxidase
NO: Nitric oxide
MDA: Malondialdehyde
SOD: Superoxide dismutase
TNF-α: Tumor necrosis factor-alpha
IL: Interleukin
iNOS: Inducible nitric oxide synthase
ROS: Reactive oxygen species
Th1: T-helper 1
Th2: T-helper 2
Th17: T-helper 17
FCA: Freund's complete adjuvant
PBS: Phosphate-buffered saline
ELISA: Enzyme-linked immunosorbent assay
CAT: Catalase
GPx: Glutathione peroxidase
Nrf2: Nuclear factor erythroid 2–related factor 2
AREs: Antioxidant response elements
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
JAK/STAT: Janus kinase/signal transducer and activator of transcription

the taeniidae family. Beyond its parasitic pathology, *E. granulosus* has garnered increasing attention for its unique immunomodulatory capabilities. The parasite employs sophisticated strategies to manipulate the host immune system, prominently through the induction of regulatory immune pathways and a skewed T-helper type 2 (Th2) response. This shift suppresses proinflammatory T-helper type 1 (Th1) and Th17 pathways, which are central to the development of autoimmune diseases such as MS. Recent experimental evidence suggests that parasitic infections, including E. granulosus, can attenuate autoimmune and inflammatory conditions by downregulating pathological immune responses and promoting immune tolerance. Accordingly, the therapeutic potential of *E. granulosus* and its derivatives has been explored in diseases such as colitis, type 1 diabetes, and EAE. Previous work demonstrated that *E. granulosus* may exert beneficial effects in MS by modulating immune responses and limiting neuroinflammation. Therefore, investigating its therapeutic effect in an MS model is not only scientifically justified but also contributes to the broader understanding of parasite-based immunotherapy [9,10].

Also, previous studies showed that the *E. granulosus* exhibits enhanced survival in pregnant mice, likely mediated through increased secretion of Th2 associated cytokines [11]. Current study was conducted to investigate he immunomodulator effect of *E. granulosus* on cytokine production and oxidative stress markers in EAE model of MS and to compare their therapeutic efficacy with curcumin, a well-established natural anti-inflammatory components.

Results

Effect on body weight

In the present study, the EAE model reproduced several characteristic with MS. All immunized animals displayed clinical signs of EAE and reached at least grade 2 of disease, confirming the successful induction of the model. The mean body weight of EAE rats was significantly reduced, compared to the normal control rats (188.2 ± 8.89 versus 340.3 ± 9.93; $p < 0.05$). In contrast, treatment with curcumin (303.3 ± 7.49 versus 340.3 ± 9.93) or PSCs (291 ± 11.46 versus 340.3 ± 9.93) significantly attenuated body weight loss, compared to EAE animals ($p < 0.05$). These findings suggest that *E. granulosus* infection may be able to protect rats against EAE.

Effects on inflammatory cytokines

The results showed a significant increase in the levels of proinflammatory cytokines in EAE group as compared to control group ($p < 0.05$). However, their levels decreased significantly in curcumin and PSCs treated groups ($p < 0:05$) compared with EAE group, indicating the notable effect of treatments on proinflammatory cytokines. The difference between the Cur and PSCs was not statistically significant ($p > 0.05$). These findings demonstrate the anti-inflammatory activities of curcumin and PSCs against EAE-induced neurodegeneration.

Effect on oxidative stress markers

In this study, EAE induced ROS generation and lipid peroxidation, as reflected by elevations in MDA, MPO activity and NO levels. The detection results of blood biochemical indicators showed that EAE induced a significant increase of the contents of serum MDA and MPO compared to control group ($p < 0.05$), as presented in Table.

Treatment with both curcumin or PSCs significantly reduced serum MPO activity and MDA levels compared to EAE group ($p < 0.05$), indicating attenuation of lipid peroxidation and neutrophil infiltration indicating attenuation of lipid peroxidation and neutrophil infiltration indicating attenuation of lipid peroxidation and neutrophil infiltration. No significant difference were observed between the curcumin and PSCs treated groups ($p > 0.05$).

Furthermore, EAE rats showed a significant elevation in brain NO level compared to controls (According yo the table). Importantly, both curcumin and *E. granulosus* significantly decreased NO production compared with the EAE group (According yo the table) ($p < 0.05$).

Discussion

Recent advances in helminth-based therapies have introduced innovative strategies for the management of immune-related disorders by exploiting the immunomodulatory properties of parasitic organisms. Several studies have highlighted the beneficial effects of helminths and their secreted products in reducing the severity and incidence of allergic reactions and other inflammatory conditions [19].

In the present study, we investigated the anti-inflammatory and anti-oxidant effects of PSCs, and to compare their efficacy with that of curcumin in EAE as an animal model of MS. The EAE model has the similar characteristic with MS to some extent. Successful induction of EAE was confirmed, as all immunized animals developed clinical disease of at least grade 2 severity. The mean body weight of EAE rats was significantly reduced, compared to the normal control rats (188.2 ± 8.89 versus 340.3 ± 9.93; p < 0.05). Importantly, treatment with either curcumin (303.3 ± 7.49 versus 340.3 ± 9.93) or PSCs (291

Table 1.
Effects of Curcumin and E. granulosus PSCs on Biochemical Parameters of EAE affected rats. Values Expressed are Mean ± SD.

Parameters	Animal treatments			EAE + PSCs
	Control	EAE	EAE + Curcumin	
IL-1β (pg/mL)	130.07 ± 0.44	148.3 ± 0.44	129.42 ± 0.44	130.07 ± 0.44
IL-6 (pg/mL)	370.43 ± 0.44	342.86 ± 0.44	342.86 ± 0.44	370.43 ± 0.44
TNF-α (pg/mL)	656 ± 0.44a	759.03 ± 0.44[b]	750 ± 0.44b	656 ± 0.44[a]
NO (µMOL/L)	785.7 ± 0.44a	1047.42 ± 0.44[b]	882.6 ± 0.44[c]	785.7 ± 0.44[a]
MPO (mU/mL)	1106.22 ± 0.44a	976.8 ± 0.44[b]	974.04 ± 0.44[b]	1106.22 ± 0.44[a]
MDA (N mol/Ml)	1.6 ± 0.2a	12.4 ± 0.1[b]	5.5 ± 0.3[b]	7.4 ± 0.1[a]
SOD (U/L)	1250.45 ± 0.44a	1346.67 ± 0.44[b]	1364.09 ± 0.44[b]	1250.45 ± 0.44[a]

MPO: Myeloperoxidase; NO: Nitric oxide; IL: Interleukin; TNF-α: Tumor necrosis factor-α; SOD: Superoxide dismutase, Cur: Curcumin, PSCs: Protoscolices, EAE: Experimental autoimmune encephalomyelitis. a denotes significant difference compared to control group ($p < 0.05$), b denotes significant difference compared to EAE group ($p < 0.05$).

± 11.46 versus 340.3 ± 9.93) significantly attenuated EAE-associated weight loss ($p < 0.05$). These findings indicate that *E. granulosus* infection may be able to protect rats against EAE.

Excessive production pro-inflammatory cytokines plays a central role in mediators cause remarkable tissue damage. In this study, the production of proinflammatory cytokines TNF-α, IL-6 and IL-1β in the supernatant of stimulated spleenocytes in spleen of individual rats were measured by sandwich ELISA. The results showed a significant increase in the levels of these cytokines in EAE group as compared with controls ($p < 0.05$). However, their levels decreased markedly in curcumin and PSCs treated groups ($p <$ 0:05) compared with EAE group, showing the notable effect of treatments on proinflammatory cytokines. The difference between the Cur and PSCs was not statistically significant ($p > 0.05$). These findings outline the anti-inflammatory activities of curcumin and PSCs against EAE-induced neurodegeneration.

The development of multiple sclerosis (MS) is influenced by various factors, including Th1 cell activation and the excessive release of pro-inflammatory mediators. During early disease stages, T cells that recognize myelin, along with peripheral immune cells like macrophages, traverse the compromised blood–brain barrier and infiltrate the central nervous system (CNS). Once inside the CNS, these activated T cells release high levels of inflammatory cytokines including IL-1β, IL-6, and TNF-α, which contribute to myelin damage and axonal degeneration. Among these cytokines, IL-6 plays a critical role by promoting Th17 differentiation in peripheral lymphoid tissues, thereby enhancing disease severity and spinal cord lesions. These Th17 cells drive ongoing neuroinflammation and demyelination, particularly in the experimental autoimmune encephalomyelitis (EAE) model. IL-1β stimulate astrocytes to produce cytokines, chemokines, adhesion molecules, and matrix-degrading enzymes, while, TNF-α sustains demyelination and promotes macrophage infiltration during EAE-associated neuroinflammation [20].

Curcumin has been previously recognized for its neuroprotective properties and its potential to enhance cognitive function within the central nervous system (CNS). Its beneficial effects in autoimmune disorders have been attributed to the regulation of key pro-inflammatory cytokines, including IL-12, TNF-α, IL-1β, IL-6, and IFN-γ. In EAE, curcumin's anti-inflammatory actions appear to be primarily mediated through its influence on cytokine and growth factor signaling pathways. Furthermore, curcumin has demonstrated the capacity to suppress astrocyte activation and reduce the secretion of inflammatory molecules by microglial cells [21]. Curcumin can downregulate IL-6 gene expression in astrocytes [8] and inhibit the differentiation and development of Th1 and Th17 cells in EAE [12].

At the molecular level, curcumin modulates cytokine expression by downregulating pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), while promoting the production of anti-inflammatory cytokines like interleukin-10 (IL-10). This immunomodulatory profile is primarily mediated through the inhibition of nuclear factor-kappa B (NF-κB) signaling pathway and modulation of the Janus kinase/signal transducers and activators of tran-

scription (JAK/STAT) pathway. In parallel, curcumin exerts antioxidant effects by enhancing the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx); while simultaneously reducing reactive oxygen species (ROS) and lipid peroxidation products, thereby mitigating oxidative damage to neuronal structures. These combined effects contribute to the restoration of redox balance and suppression of oxidative stress-driven demyelination in MS [22].

E. granulosus has been reported to exert immunomodulatory effects on host immune response and has been investigated in the treatment of allergic and autoimmune conditions including allergic airway inflammation, asthma and bacterial sepsis. Previous investigations on murine bacterial sepsis [9] demonstrated that administration of E. granulosus cyst fluid increases anti-inflammatory cytokines such as IL-10 and TGF-β, while simultaneously decreasing levels of pro-inflammatory markers including TNF-α and IFN-γ. These findings are consistent with the results obtained in our study. Infection with E. granulosus has been shown to promote a shift in the immune profile from a Th1-dominant to a Th2-dominant response, thereby suppressing Th1 and Th17 cells and reducing the associated inflammatory cytokine production—an effect that supports the modulation and downregulation of immune-mediated inflammation [23]. Earlier investigations in EAE models have shown that E. granulosus cyst fluid in an EAE mouse model of multiple sclerosis led to a marked reduction in the expression of pro-inflammatory cytokines such as IFN-γ, IL-1β, and TNF. This treatment also promoted a shift in the immune profile from a Th1-dominated response toward a Th2 phenotype, as evidenced by decreased IFN-γ and elevated IL-4 levels. Similarly, the laminated layer isolated from E. granulosus cyst wall have been reported to inhibit IL-1β, IL-6, and TNF-α production, potentially through modulation of NF-κB signaling cascade and the IRAK pathway [24].

Recent studies have also highlighted the immunoregulatory potential of E. granulosus protoscoleces (PSC), which may provide a novel helminth-based approach to modulating immune responses in autoimmune diseases such as MS. PSC components are thought to exert immunosuppressive effects through the induction of regulatory T cells (Tregs) and the shift of the immune response toward a Th2-dominant profile, thereby attenuating pathogenic Th1/Th17-driven inflammation characteristic of MS. In addition, PSC may influence oxidative stress pathways by reducing ROS production and enhancing antioxidant defenses. Although the precise molecular mechanisms remain incompletely defined, helminth-derived antigens, including those from E. granulosus, have been shown to upregulate nuclear factor erythroid 2–related factor 2 (Nrf2), a key transcription factor that regulates antioxidant response elements (AREs) [25].

Oxidative stress is a critical contributor to both the initiation and progression of EAE and MS. Immune cells infiltration into affected tissues triggers excessive ROS production, which in turn target essential cellular components. These free radicals compromise cellular integrity by damaging membrane lipids, altering protein structures, and inducing mutations or breaks in DNA molecules [26]. In the present study, EAE induced ROS formation and lipid peroxidation as indicated by elevation of MDA, MPO activity and NO levels.

The detection results of blood biochemical indicators showed that EAE induced a significant increase of the contents of serum MDA and MPO compared to control group ($p < 0.05$) (Table1).

MDA is a product of oxygen free radicals triggering lipid peroxidation of unsaturated fatty acids on cell membranes

MPO is widely recognized as a reliable indicator of neutrophil infiltration and is frequently used to assess inflammatory responses in both human and animal studies. MPO is produced not only by neutrophils but also by activated microglia, astrocytes, and neurons, may contribute to the development of neuropathological changes. Moreover, MPO activity and neutrophil accumulation have been implicated in blood–brain barrier disruption and neuroinflammation [27].

Previous studies have reported the increased levels of serum MPO and MDA as confirmed by blood–brain barrier disruption damage, infiltration of inflammatory cells into CNS and demyelination. These findings are observed in mice models of EAE , as well as patients with MS [28]. In agreement with these findings, the present study showed a significant increase MPO and MDA levels in the EAE group. On the other hand, treatment with both curcumin and PSCs ameliorated neutrophil infiltration and lipid peroxidation as evidenced by suppression of serum MPO and MDA (Table) compared to EAE group ($p < 0.05$). No significant difference was observed between curcumin and PSCs treated groups ($p > 0.05$).

It is well established that EAE is associated with elevated levels of inducible nitric oxide synthase (iNOS) and enhanced NO production. Notably, iNOS expression has been observed in astrocytes located within active demyelinating lesions, indicating that astrocyte-derived NO, generated through iNOS activity, may play a significant role in promoting neurotoxicity driven by oxidative stress. [29]. During oxidative

stress, nitric oxide (NO) interacts with superoxide anions to generate peroxynitrite (ONOO−), a highly reactive oxidant responsible for neuronal injury observed in both EAE models and multiple sclerosis patients. In the present study, brain NO levels were significantly increased in EAE mice compared to controls (Table 1). The NO produced by activated astrocytes contributes to mitochondrial dysfunction, DNA damage, and neuronal cell death [29]. In this study, brain NO levels were significantly elevated in EAE model and MS patients. Importantly, curcumin and E. granulosus significantly decreased the brain NO production compared with EAE group ($p < 0.05$).

Curcumin possesses a molecular structure characterized by a central carbon linker connecting two aromatic rings. These aryl rings bear phenolic hydroxyl and methoxy substituents, which enhance the compound's ability to neutralize reactive oxygen species (ROS), thereby contributing to its antioxidant activity. Remarkably, curcumin also demonstrates neuroprotective properties by preventing oligodendrocyte apoptosis and inhibiting axonal damage induced by nitric oxide [21].

Similarly, E. granulosus cyst fluid has been reported to suppress iNOS expression in the liver, lungs, and kidneys of septic mice [9]. Consistent with our results, E. granulosus infection has been shown to alleviate clinical manifestations in a dextran sulfate sodium-induced colitis model, accompanied by reduced production of NO and TNF-α, as well as downregulation of iNOS and nuclear factor-κB expression in the colon [30]. Given the established involvement of TNF-α and NO in oxidative stress pathways, the observed decline in MPO activity and MDA concentrations may reflect reduced ROS generation, likely mediated by the immunomodulatory properties of E. granulosus.

Conclusion

In conclusion, the present findings indicate that administration of PSCs effectively mitigates neurodegeneration in the EAE rat model by attenuating both inflammatory and oxidative processes. These results suggest that helminth-based interventions using E. granulosus may represent a promising therapeutic approach for multiple sclerosis and other autoimmune disorders characterized by chronic inflammation.

Limitations

This study has several limitations. First, therapeutic efficacy was primarily assessed using clinical scoring, without incorporating detailed histopathological analyses of neural tissues to evaluate demyelination or axonal damage. Second, the lack of immunohistochemical and molecular evaluations limited mechanistic insights into cytokine signaling and oxidative stress pathways. Finally, long-term effects and potential side effects of curcumin and PSC treatments were not evaluated.

The data that support the findings of this study are available on request from the corresponding author.

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

In the process of writing this article, no AI was used.

Ethical Approval

All animal experiments were approved by the Ethics Committee of the University of Tabriz (Ethical code number: IR.TABRIZU.REC.1398.004) and conducted in strict accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Experimental animals

A total of 40 healthy adult male Sprague-Dawley rats (weighing 250~300 g), were obtained from the Pasteur Institute (Tehran, Iran). Animals were housed in laboratory cages (10 rats per cage). Food and water were freely available. The rats were kept in a room with a 12:12 hour light-dark cycle and a constant temperature of 21±2°C. The efforts were made to minimize animal unnecessary pain and suffering within labs. After one week acclimatization, the animals were randomly divided into four groups (ten animals per group) and treated as follow:

1. Control group: Received normal saline orally once daily until the end of the study)
2. EAE group: Rats with EAE that received no treatment (positive control)
3. EAE+ curcumin group: EAE induced rats received curcumin at a dose of 100 mg/kg via oral gavage, starting from the onset of clinical symptoms and continuing for 60 days.
4. EAE + PSC group: EAE induced rats were intraperitoneally inoculated with PSCs of E. granulosus at the onset of the first clinical signs of disease.

Induction of EAE as an experimental model of MS

The procedure was conducted based on previous study protocol [12]. According to the procedure described by Chen et al. (2010), an emulsion was prepared using homogenized guinea pig spinal cord tissue (SICBD, Karaj, Iran) mixed in equal parts with Freund's complete adjuvant (FCA; Sigma Co., Burlington, MA, USA), which contained 10 mg/kg of heat-killed Mycobacterium tuberculosis. Rats were anesthetized using a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) (Alfasan, Woerden, The Netherlands). A total of 400 µL of the antigen-adjuvant emulsion was injected subcutaneously into the dorsal region, followed by an additional 100 µL injection into the footpad using a 25-gauge needle. After 48 hours, 1 ×109 colony-forming units of Brucella parapertussis (IBRC-M 10710, ATCC 15311) were injected intraperitoneally in 300 µL of phosphate-buffered saline (PBS). Disease progression was monitored daily by assessing motor deficits in each rat. Clinical scores and body weight measurements were recorded from day 0 to day 12 post-induction according to established evaluation scales[13]. Successful induction of EAE was defined as the attainment of a clinical score exceeds 2.

Preparation and Viability Assessment of PSCs:

Fresh hydatid cysts were obtained from the livers of naturally infected sheep slaughtered at the Tabriz industrial abattoir (East Azerbaijan province, Iran). These samples were transferred to the Parasitology Laboratory, Faculty of Veterinary Medicine, University of Tabriz. To begin, the surface of each cyst was disinfected twice using 70% ethanol. Following disinfection, the cyst fluid was aspirated using a 50 ml syringe, collected in glass tubes, and centrifuged at 1500 rpm for 3 minutes. The supernatant was carefully discarded, and the collected protoscoleces (PSCs) were washed several times with phosphate-buffered saline (PBS; pH 7.2).

To determine PSCs concentration, 5 µl of the suspension was placed on a microscope slide, covered with a coverslip, and examined under a light microscope at 40× magnification. This procedure was performed in triplicate, and the mean count was used to calculate PSCs density per milliliter. The suspension was then adjusted to contain 500 PSCs/mL in 0.9% normal saline, ensuring a viability of over 90% for subsequent inoculation into mice.

Viability was assessed according to the method described by Smyth and Barrett (1980)[14]. Briefly, 20 µl of the PSC suspension was mixed with an equal volume of 0.1% eosin solution (prepared by dissolving 1 g eosin powder in 1 L of distilled water) in a clean microtube. After 15 minutes of staining, the PSCs were observed under a light microscope: unstained (colorless) PSCs were considered viable, while stained (red) ones were considered non-viable. Viability was calculated as the percentage of unstained PSCs relative to total count. This assessment was also conducted in triplicate [15].

As a control, a portion of the PSC suspension was heat-inactivated by incubation at 60°C for 30 minutes in an oven (Fan Azma Gostar, Karaj, Iran), and subsequently stained with 1% eosin using the same protocol.

Induction of E. granulosus infection

Experimental infection with E. granulosus was performed according to a previously published protocol [11]. Following PSCs preparation and viability confirmation, 500 PSCs were injected intraperitoneally to each rat.

Preparation of Curcumin Suspension for Oral Gavage:

Curcumin powder (≥94% purity) was suspended in sterile olive oil to prepare a uniform suspension suitable for oral gavage. The mixture was vortexed thoroughly to ensure proper dispersion and gently warmed at 37°C to enhance solubility. The final concentration was adjusted to deliver a dose of 100 mg/kg body weight. Fresh suspensions were prepared daily under aseptic conditions, maintained at room temperature, and protected from direct light. Prior to administration, the suspension was mixed thoroughly to ensure uniformity and administered orally using a sterile gavage needle.

Tissue preparation and biochemical analysis

At the end of the study, body weights were recorded, and rats were euthanized under ether anesthesia. Spleen tissues were harvested for cytokine analysis. Spleen cells were stimulated, and levels of TNF-α, IL-1β, IL-6 were quantified using Commercially ELISA kits (Bender Med Co., Austria) according to the manufacturer's described protocol [16]. Cytokine concentrations were expressed as pg/mL. Following anesthesia, blood samples were collected directly from the heart. To obtain serum, the samples were centrifuged at 1500 rpm for 10 minutes. Serum levels of MPO were measured using a colorimetric activity assay kit (BioVision, USA), following the manufacturer's protocol. Additionally, the activity of SOD in the serum was assessed using an established method as previously described. [17]. The preparation of brain tissue and measurement of NO levels was performed following previously described protocol [18], and NO levels were expressed as µmol/L.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical comparisons among experimental groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value less than 0.05 was considered statistically significant. All analyses were carried out using Stata software version 14 (StataCorp, Texas, USA).

Authors' Contributions

Y.J. conceived and planned the experiments. Y.J., A.IB. SJ.E. carried out the experiments. Y.J. planned and carried out the simulations. Y.J., A.IB. contributed to sample preparation. Y.J., SJ.E., A.IB., S.A. contributed to the interpretation of the results. S.A. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Characterization and phylogenetic analysis of a mitochondrial targeting alpha/beta hydrolase protein in *Strongyloides ratti*

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ABSTRACT

Strongyloides ratti is widely used as an experimental model for studying and diagnosing humans strongyloidiasis. The alpha/beta hydrolase domain proteins are ubiquitously distributed across all organisms. In this study, a 780-nucleotide cDNA fragment was amplified by RT-PCR, and BLAST analysis revealed that 98.54% sequence identity with the only incomplete S. ratti mRNA sequence available in GenBank (XM_024652394.1). Using this fragment as a probe, two expressed sequence tags (ESTs), 856 bp (FC815910.1) and 526 bp (BI742498.1), corresponding to the 3'and 5' ends, respectively, were identified and assembled into a full length cDNA of 919 bp, designated SrABH. Its amino acid sequence exhibited 97.98% identity with the only available *S. ratti* sequence in GenBank (XP_024505975.1). The molecular weight (MW) and isoelectric point (pI) of the protein are 33.484 kDa and 7.18, respectively. Signal peptide analysis indicated the absence of a secretion signal, however a mitochondrial targeting cleavage site was identified between amino acids 21-22. The highly conserved motif consists of nine amino acids spanning positions 111 to 120. The 3D structural analysis revealed that SrABH consists of 9 alpha-helices (39.18%), 8 beta-sheets (18.21%), and loop regions (42.61%). Phylogenetic analysis clustered SrABH with related nematode sequences, displaying by a high bootstrap support of 98. The lowest genetic distance (0.01%) was observed between SrABH and the only sequence isolated from *S. ratti* (XP_024505975.1). Given its molecular characteristics, this parasite's data may be valuable in structure-based drug design strategies using *S. ratti* as a model system for the human pathogen *S. stercoralis*.

Keywords

Esterase, alpha/beta hydrolase domain, Strongyloides ratti, RT-PCR, mitochondria

Number of Figures: 6
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Abbreviations

S. ratti : Strongyloides ratti

S. stercoralis: Strongyloides stercoralis

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Introduction

Strongyloides is a genus of gastrointestinal nematodes of medical and veterinary importance, infecting a wide range of mammalian species, encompassing both humans and other animals. Among these species, *Strongyloides ratti* is closely related to the human parasite *S. stercoralis* and has therefore been widely adopted as a robust experimental model for investigatinFg the *S. stercoralis*. The infective third-stage larvae of *S. ratti* actively penetrate the skin of their rodent hosts and subsequently migrate to the mucosal lining of the small intestine, where they mature into adult worms. Hatched eggs and first-stage larvae are then released into the environment through fecal excretion [1].

Strongyloidiasis typically presents with mild symptoms; however, its clinical manifestations can become severe in individuals receiving immunosuppressive drugs, undergoing organ transplantation, or suffering from malignancies and malnutrition, all of which are linked to immunodeficiency. *S. stercoralis* is a human-specific parasite and is endemic in tropical and subtropical regions worldwide. Due to its close evolutionary relationship with *S. stercoralis*, *S. ratti*, has been frequently used as a laboratory model for studying *Strongyloides* treatment, diagnostics and genetic mapping [2, 3].

Alpha/beta hydrolase domain protein-encoding genes are present in nearly all sequenced genomes. This enzyme family has rapidly become one of the largest structural groups, exhibiting diverse catalytic functions in various biological processes, including parasite-host interactions. Despite their ubiquity, the substrates and metabolic pathways of these enzymes remain poorly characterized. A defining feature of this enzymes family is the presence of a conserved catalytic triad composed of Nucleophilic-Histidine-Acidic residue, which enables efficient process of substrates with diverse chemical compositions and physicochemical properties in various biological settings. Structurally, these proteins are similar and highly conserved across evolution, and play critical roles in energy metabolism, cell signaling, and growth and development [4]. This family includes a wide range of esterases, which include enzymes such as acetylcholinesterase, dienolactone hydrolase, lipase, thioesterase, serine carboxypeptidase, proline iminopeptidase, proline oligopeptidase, haloalkane dehalogenase, haloperoxidase, epoxide hydrolase, and hydroxynitrile lyase.

This protein has been identified in the excretory/secretory materials of parasitic nematodes, including *Haemonchus contortus* [5], *Heligmosomoides polygyrus* [6], and *Mesocestoides corti* [7]. Similar proteins have also been detected in free-living and parasitic organisms, such as *Caenorhabditis elegans* [8], the thioesterase type II enzyme from *Cryptosporidium parvum* [9], and the lysophospholipase from *Schistosoma japonicum* [10]. Beyond parasites, this protein and its homologues, including ABHD5 and ABHD11, have been reported in *Arabidopsis thaliana* [11, 12] and in *Saccharomyces cerevisiae* [13]. Members of this protein family are thus broadly distributed across eukaryotic species, including the Ndr1 protein in *Helianthus annuus* (common sunflower), known as Sf21.

In this study, we aimed to perform a computational characterization and phylogenetic analysis of an alpha/beta hydrolase domain-containing gene from *S. ratti*, given its close genetic relationship with the human pathogen *S. stercoralis*.

Results

Amplification of the SrABH sequence was performed by RT-PCR, using cDNA as a template and two gene specific primers. The PCR reaction yielded a single product of approximately 800 bp, as confirmed by agarose gel electrophoresis (Figure 1). Sequencing analysis demonstrated that this fragment corresponded to a portion of the central region of the alpha/beta hydrolase gene. A BLASTn search against the GenBank database revealed that the amplified product shared 98.54% sequence identity with the only incomplete *S. ratti* mRNA sequence available (XM_024652394.1), covering approximately 95% of the overlapping regions. To obtain the full-length cDNA sequence, the amplified fragment was used as a probe to search the

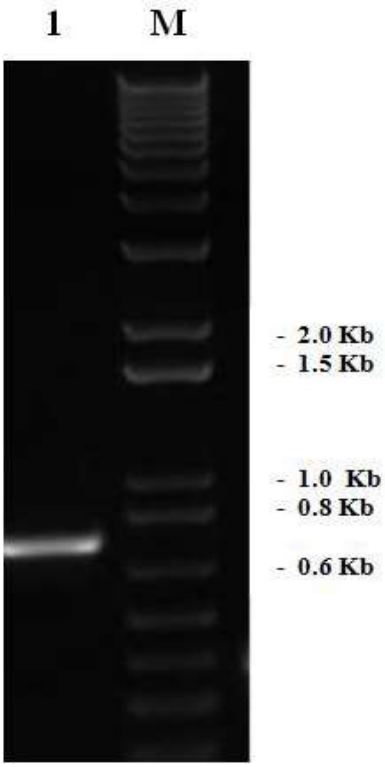

Figure 1. Agarose gel electrophoresis of RT-PCR products from infective S. ratti larvae. M: DNA size marker. Lane 1: RT-PCR amplification products.

Expressed Sequence Tags (EST) database. Two ESTs were identified: FC815910.1 (856 bp), corresponding to the 3' end, and BI742498.1 (526 bp), corresponding to the 5' ends of the gene. Assembly of these EST sequences and determining its overlapping regions, allowed reconstruction of a complete Open Reading Frame (ORF), which was designated SrABH.

The assembled cDNA sequence measured 919 bp, containing an ATG start codon at position 1 and a TAG stop codon at position 871. A 43-nucleotides 3' untranslated region (UTR) was identified downstream of the stop codon (Figure 2). The predicted

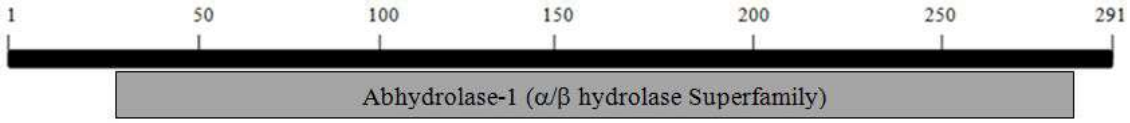

Figure 2.
Visual representation of the SrABH domain.
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protein sequence of this fragment, consists of 291 amino acids encoded by a complete open reading frame (ORF). Analysis of amino acid composition revealed that the protein contains aliphatic (22%), aromatic (11%), positively charged (15%), and negatively charged (12%) residues. Leucine was the most abundant amino acid, with 32 residues (11%), whereas tryptophan was the least abundant, with 2 residues (0.69%). The predicted molecular weight of the protein was determined to be 33.484 kDa, with the isoelectric point (pI) of 7.18. SignalP analysis indicated the absence of a signal peptide . ScanProsite analysis identified a highly conserved motif in the SrABH protein consists of nine amino acids (DLLGHSMGG) located between residues 112 and 120, with a serine residue precisely positioned at amino acid 117.

Molecular analysis and Secondary structure prediction.

Domain analysis of the SrABH protein revealed a significant similarity to the conserved alpha/beta hydrolase domain superfamily (within, pfam00561). This domain spans amino acids 38 to 278, with an E-value of 2.02e-25 (Figure 3).

Taxonomic analysis of SrABH at the protein level revealed 128 homologous sequences within the phylum Nematoda, of which 84 belonged to the order *Rhabditida*. Notably, 4 of these sequences were belonged to the *Strongyloidoidea* family and exhibited the highest similarity scors, including two hits derived from *S. ratti*, with a maximum score of 593. In contrast, nucleotide level analysis identified only a single hit. This finding further supports the phylogenetic placement of SrABH within the nematodes.

Secondary Structure and three-dimensional Modeling of SrABH.

Secondary structure prediction of the SrABH protein was performed using the PSIPRED program, which assigns structural elements, alpha, helices, beta sheets, and loops, using a seven-amino acid window. This approach allows for high-confidence predictions of amino acid positions based on structural probability calculations. Based on this analysis, both the secondary structure model (Figure 4A) and a three-dimensional (3D) structure model (Figure 4B) were generated. The 3D structure revealed a folded core characteristic of the alpha/beta hydrolase domain, containing 9 alpha helices and 8 beta sheets. Using PSIPRED, the SrABH protein was determined to consist of 39.18% alpha helices, 18.21% beta sheets, and 42.61% loop regions. The 3D structural modeling was based on 258 amino acids, representing 89% of the total coding sequence, and was generated with a confidence level of 99.99%. The modeled structure showed close similarity to the GenBank c7c4dA transcript. The protein was ultimately classified as a hydrolase enzyme.

Analysis of the N-terminal region of the SrABH protein using the TargetP-2.0 program identified the presence of a mitochondrial transfer peptide (mTP) with a confidence score of 0.9558. The predicted cleavage site was located between amino acids 21 and 22, corresponding to the motif RLF-SS (Figure 5).

Phylogenetic analysis and genetic distance

phylogenetic analysis of SrABH was conducted using amino acid sequence, from 18 parasitic nematode species. The SrABH sequence was positioned in cluster A, together with other nematode parasite sequences, supported by a high bootstrap value of 98 (Figure 6). Within this cluster, SrABH formed a distinct sub-cluster exclusively with the only available *S. ratti* protein sequence (XP_024505975). In contrast, all filarial nematode sequences grouped within cluster B, supported by a bootstrap value of 74. To ensure accurate phylogenetic placement, *Azospirillum agri-*

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ATG TTT TGT AAA TCA CTT TCT TCA TCA TTG TTA TTT CGT AAC ATC CTC TCT CAG CGC CTA  60
 M   F   C   K   S   L   S   S   S   L   L   F   R   N   I   L   S   Q   R   L
TTT TCT TCA CTT TCT TAT ACA AAA TAT GGA GAA TGT TCT AAT ACT AGT CTT CCA CCA CTC 120
 F   S   S   L   S   Y   T   K   Y   G   E   C   S   N   T   S   L   P   P   L
TTG ATT GCT CAT GGT CTT TTT GGA AAC AAA AAA AAT TGG CAC TCT GCA GCA AAA GCA CTT 180
 L   I   A   H   G   L   F   G   N   K   K   N   W   H   S   A   A   K   A   L
CAA AAA AAA TTA GAC AAT CAA ATA TAT ACT ATT GAT CTT AGA AAT CAT GGA GAT AGT CCA 240
 Q   K   K   L   D   N   Q   I   Y   T   I   D   L   R   N   H   G   D   S   P
CAT TTA GAT TCA ATG ACT TAT CAA GAA ATG GCA GAA GAT GTG AAT AAT TTT ATA ACA AAT 300
 H   L   D   S   M   T   Y   Q   E   M   A   E   D   V   N   N   F   I   T   N
ATA ATT TTT GAA GAA ACA GGA TAC GAT GAA TTA GAT CTT TTG GGA CAT TCT ATG GGT GGT 360
 I   I   F   E   E   T   G   Y   D   E   L   D   L   L   G   H   S   M   G   G
AAA GTA AGT ATG GAA TTA GCT TTT AAC AAA GAA AGT TCA AAA AAT ATA AGA AAA TTA ATT 420
 K   V   S   M   E   L   A   F   N   K   E   S   S   K   N   I   R   K   L   I
ATT GAA GAT ATT TCA CCA ATT TAT GTT AAT AAA ACT CAT TTA TTT CCT AAA TAT ATT AAA 480
 I   E   D   I   S   P   I   Y   V   N   K   T   H   L   F   P   K   Y   I   K
GCT TTA ATG AAT ATT GAT TAT TCA TTA AGT CGT ATA GAA ATT GAT AAT ATT CTT AAA AAA 540
 A   L   M   N   I   D   Y   S   L   S   R   I   E   I   D   N   I   L   K   K
GAT ATT ACT GAT AAT GCT ACA AGA GCA TTC CTT TTG ACA AAC TTA CAA AAA GAC CAA GGT 600
 D   I   T   D   N   A   T   R   A   F   L   L   T   N   L   Q   K   D   Q   G
GCA GAA AGT TTT AGA TTT AAA GCA AAT TTA AAA AGT ATT AAT GAA TAT GTA GAT CAT ATT 660
 A   E   S   F   R   F   K   A   N   L   K   S   I   N   E   Y   V   D   H   I
ATA GGA TAC ACT TTT GAA AAA GGT TGT CAA TTT GAA AAA GAA ACT CTT TTT TTA TCA GGA 720
 I   G   Y   T   F   E   K   G   C   Q   F   E   K   E   T   L   F   L   S   G
GAA CAT TCA TTA TAT GTT ACT CCT AAT GAT AGA AAT TTA ATA AGA CGA TAC TTT CCT AAT 780
 E   H   S   L   Y   V   T   P   N   D   R   N   L   I   R   R   Y   F   P   N
GTT ACT TTT AAA GTT ATT GAA AAT TCA GGA CAT TGG ATA CAT GCC GAA CAT TTT CCT GCT 840
 V   T   F   K   V   I   E   N   S   G   H   W   I   H   A   E   H   F   P   A
TTC AAA GAA GCT GTT GTT TCA TTT TTA AAA AGT TAG ataaatttttattattaagaaatttttacaataa 908
 F   K   E   A   V   V   S   F   L   K   S   .
tttacacatag                                                                    919
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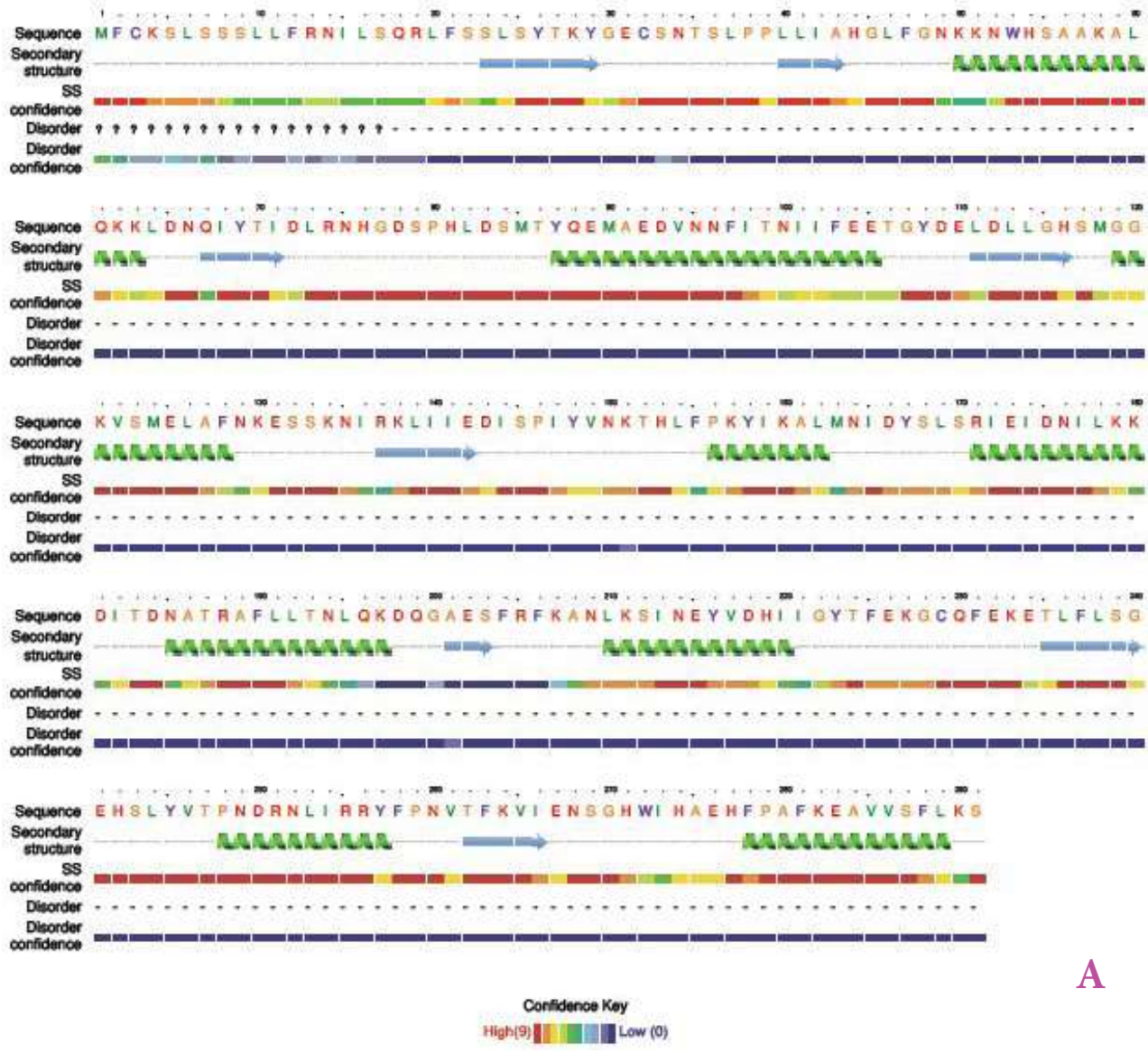
Figure 3.
Assembled Nucleotide Sequence of SrABH from S. ratti and Its Predicted Primary Structure. The coding sequence is represented in uppercase letters, whereas the 3'-untranslated regions are displayed in lowercase. Primer sites are underlined. Asn-Xaa-Ser/Thr motifs in the sequence are highlighted in gray. Asparagines predicted to undergo N-glycosylation are bolded and marked with a star. The most conserved protein region is boxed, with a serine residue active site in the center, indicated in bold.



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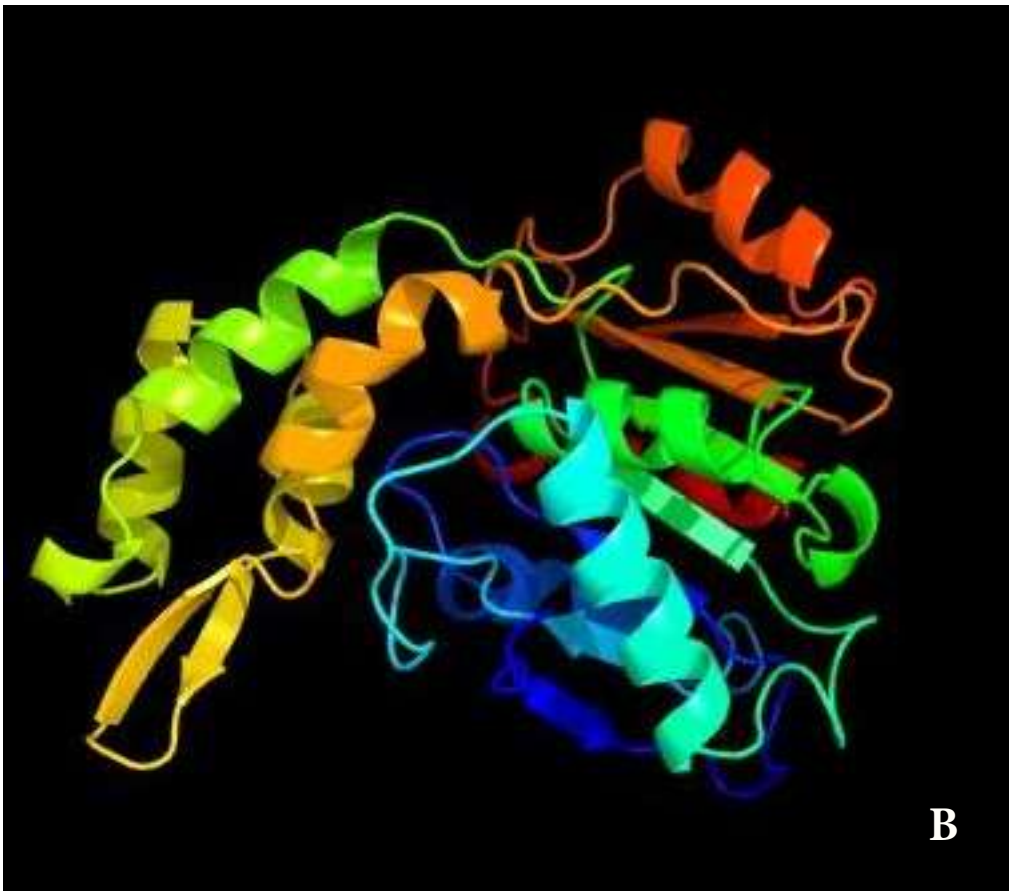

Figure 4.
Predicted secondary structure (A) and three-dimensional (3D) model (B) of the SrABH protein. Confidence levels for the secondary structure are color-coded, with red indicating high confidence and purple indicating low confidence.

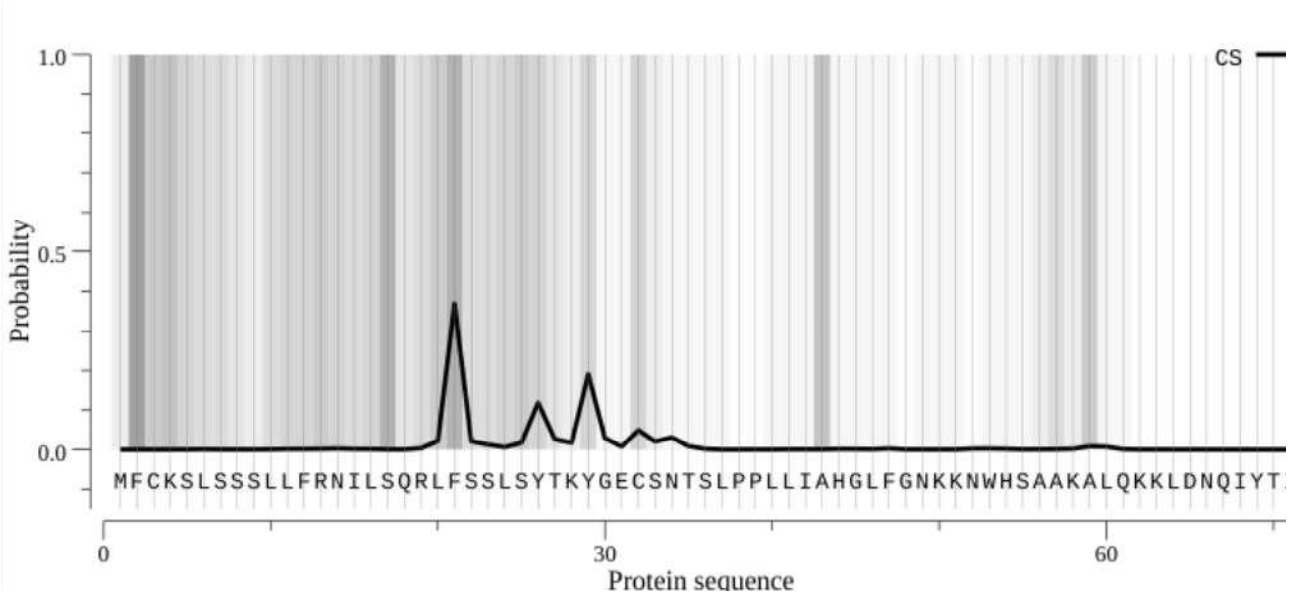

Figure 5.
Analysis of the N-terminal region of the SrABH protein, highlighting the predicted mitochondrial transfer peptide (mTP).

cola (WP_085556886.1) was used as an outgroup sequence.

Genetic analysis was performed using MEGA7 software, comparing SrABH with 12 nematode protein sequences retrieved from GenBank. SrABH sequence exhibited the lowest genetic distance (0.08%) relative to only known *S. ratti* protein sequence (XP_024505975) (Table 1). Genetic distance between SrABH and other related nematode species ranged from 5.2% to 5.7%, indicating notable sequence divergence within this family.

Discussion

Parasitic worms exhibit complex mechanisms for coexisting within their hosts across diverse environments [21]. One of the most effective survival mechanisms employed by these organisms is the secretion of excretory/secretory proteins, which play a central role in suppressing host immune response [22]. With advancements in *S. ratti* genome analysis, particularly the expansion of its Expressed Sequence Tags (ESTs) datasets, has significantly enhanced opportunities for molecular investigations. From a therapeutic perspective, a strategic and rational approach for development of anti-parasitic drugs, involves exploiting metabolic differences between parasites and their hosts to design specific enzyme inhibitors.

Among potential molecular targets, alpha/beta hydrolase proteins, which are widely conserved across all organisms [23], have been proposed as promising candidates for parasite-specific drug development. Despite this, no structural characterization of this protein has been reported in *S. ratti*. While several studies have reported alpha/beta hydrolase domain sequences in mammals, the complete cDNA sequence of this gene remained unknown in *S. ratti*.

The present study aimed to identify and characterize the protein from infected *S. ratti* larvae. Given

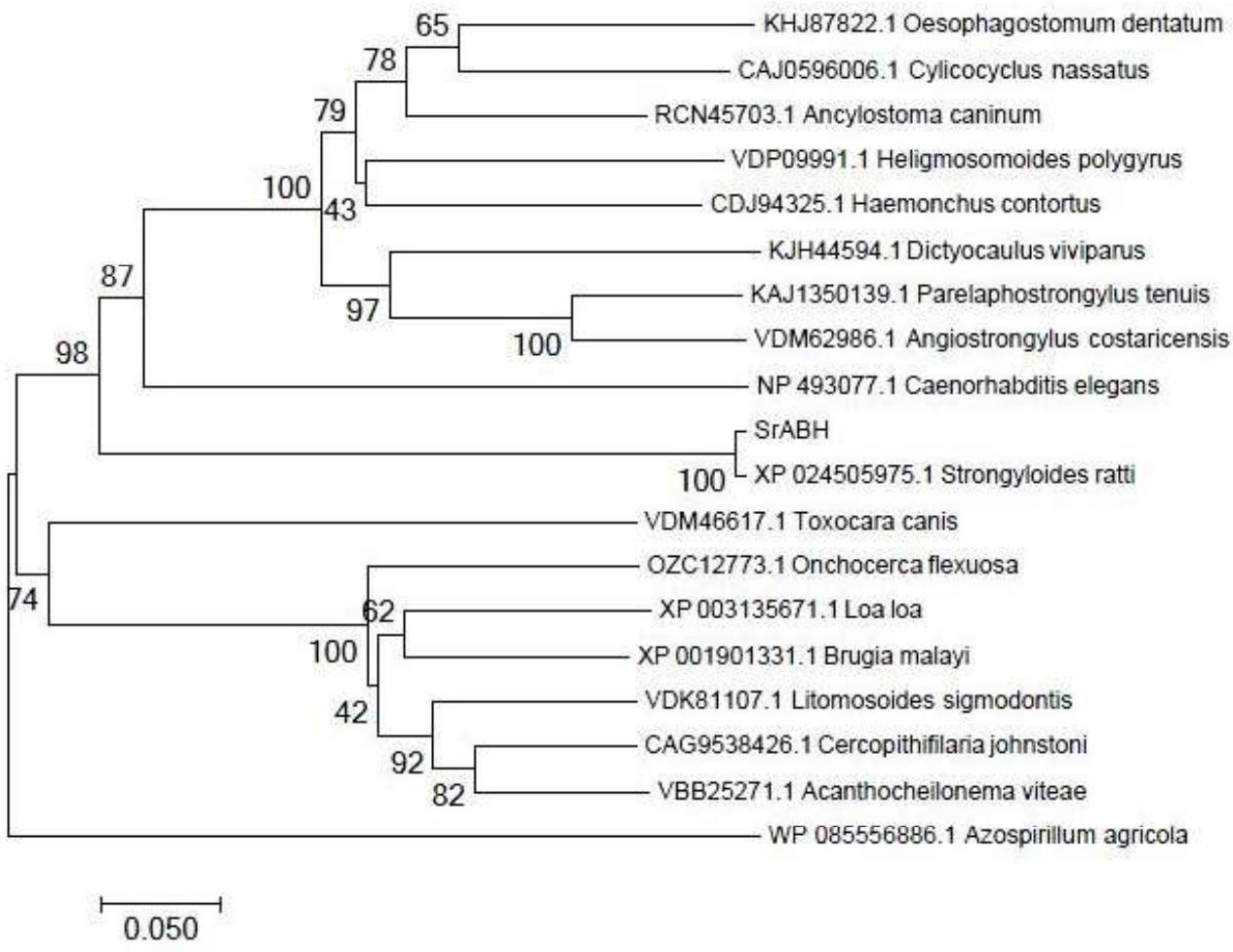

Figure 6.
Phylogenetic analysis of SrABH protein sequences and homologs using the Neighbor-Joining technique. Bootstrap values are derived from 1,000 replicates. Numbers preceding species names represent GenBank accession numbers, while numbers above the branches indicate phylogenetic relationships between groups.

Table 1.
The genetic pairwise distances of *Sr*ABH compared to the related *S. ratti* protein sequences

	1	2	3	4	5	6	7	8	9	10	11	12
1- SrABH												
2-XP_024505975.1 S. ratti	0.008											
3-RCN45703.1 A. caninum	0.528	0.524										
4-VDP09991.1_H. polygyrus	0.520	0.520	0.272									
5-NP_493077.1 C. elegans	0.537	0.537	0.480	0.504								
6-KHJ87822.1 O. dentatum	0.573	0.573	0.215	0.350	0.520							
7-CAJ0596006.1 C. nassatus	0.561	0.561	0.244	0.301	0.472	0.232						
8-CDJ94325.1 H. contortus	0.549	0.553	0.272	0.280	0.467	0.317	0.264					
9-KAJ1350139.1 P. tenuis	0.545	0.541	0.321	0.341	0.533	0.354	0.366	0.366				
10-VDM46617.1 T. canis	0.561	0.561	0.545	0.565	0.577	0.537	0.577	0.577	0.573			
11-KJH44594.1 D. viviparus	0.565	0.561	0.329	0.378	0.520	0.370	0.382	0.362	0.289	0.577		
12-VDM62986.1 A. costaricensis	0.561	0.565	0.317	0.350	0.516	0.337	0.366	0.386	0.146	0.545	0.333	
13-WP_085556886.1 A. agricola	0.630	0.630	0.602	0.593	0.638	0.614	0.638	0.622	0.630	0.581	0.630	0.622

that only a partial mRNA sequence was available in GenBank, we employed a combined strategy involving RT-PCR amplification and analysis of genomic and transcriptomic GenBank database to reconstruct the complete sequence, ensuring a more comprehensive understanding of its structural and functional characteristics. To amplify cDNA fragments encoding this gene, PCR was performed using cDNA as a template. Additionally, an Expressed Sequence Tag (EST) search strategy was employed to reconstruct the full-length cDNA by identifying overlapping regions. Subsequent multiple sequence alignment and phylogenetic analysis confirmed that SrABH possesses the alpha/beta hydrolase domain and belongs to the hydrolase enzyme family.

Although the precise cellular functions of hydrolase family proteins in parasitic nematodes remain poorly defined, accumulating evidence suggests that they play fundamental role in host-parasite interactions. For example, a member of this protein family has been identified as a key regulator of programmed cell death in *Arabidopsis thaliana* (24). Other studies have proposed that this gene may participate in cellular stress response pathways or function as a transcriptional factor (23, 25), although their molecular and cellular mechanisms are not yet fully understood. Notably, a component of the excretory/secretory material isolated from *Haemonchus contortus* has been classified as an alpha/beta hydrolase domain protein, where it has been shown to interact with host T cells [5]. By comparing the role of SrABH protein with HcABHD protein in H. contortus, particularly the HcABHD involvement in cell proliferation and apoptosis, it is likely that SrABH plays a similar function in the critical regulation of the cell cycle and host cell survival. A homologous protein, ABHD10, has been characterized in humans [26], and shown to consist of 306-amino acid [27]. This protein was identified alongside ABHD11, another mitochondrial-targeting protein isolated from human skeletal muscle mitochondria [27]. Additionally, ABHD11 has been reported to be enriched in the mitochondrial fraction of BV-2 mouse microglial cells [28]. Further analysis of the N-terminal region of SrABH revealed the absence of a signal peptide, suggesting that the protein is not secreted and instead functions intracellularly. Importantly, TargetP-2.0 analysis of SrABH predicted the presence of a mitochondrial transfer peptide (mTP) with high confidence, supporting its potential mitochondrial localization. Mitochondrial localization signal (MLS), also referred to as mitochondrial targeting sequence (MTS), are short peptides generally ranging from 15 to 70 amino acids and are enriched in positively charged residues. These sequence may play a critical role in protein trafficking to mitochondria [29]. Moreover, the three-dimensional (3D) structure of SrABH exhibits a balanced alpha/beta domain arrangement, characteristic of proteins within this family. Its core structure consists of beta sheets flanked by alpha helices, forming a stable fold.

The predicted structure, consisting of 9 alpha helices and 8 beta sheets, forms a well-defined catalytic domain that supports a diverse range of enzymatic functions, including mitochondrial targeting for intracellular protein transport, energy regulation, and protein degradation.

Consistent with these structural features, ScanProsite analysis identified the highly conserved motif centered on a serine residue (Figure 2). This residue, along with histidine and aspartic acid, as catalytic triad is known to participate in a catalyzing the substrate cleavage efficiently [30]. Similar conserved motifs have been reported in prokaryotic lipases and lecithin cholesterol acyltransferase (EC 2.3.1.43), which facilitates fatty acid transport among phosphatidylcholine and cholesterol [31].

In conclusion, SrABH plays a significant role in cellular processes regulating mitochondrial protein transport.

These findings provide valuable insights in understanding the function of this protein and pave the way for utilizing this knowledge in a structure-based drug design strategy and exploration of potential therapeutic interventions, particularly within a model system for human *S. stercoralis*.

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

Manuscript writing was done without the use of artificial intelligence tools.

S. ratti life cycle.

S. ratti were maintained by serial passage in male Wistar rats (Charles River). Infective third stage larvae (iL3) were obtained from fecal culture as previously described [14].

Total RNA isolation and cDNA Synthesis

Total RNA was isolated from 100,000 *S. ratti* infective third stage (iL3) larvae using RNX Plus solution (Cinagen, Iran), following the manufacturer's guidelines.

For cDNA synthesis, 2 µg of total RNA was incubated with 0.5 µg of oligo(dT) primer at 70°C for 10 minutes and immediately chilled on ice. Subsequently, 1 µl of RNasin (Cinagen, Iran), 1 µl of a 120 mM dNTP mixture (each nucleotide), 2.5 µl of 5× reaction buffer, and 1 µl (200 units) of Moloney Murine Leukemia Virus (M-MulV) reverse transcriptase were added. CDNA synthesis was carried out using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) in accordance with the manufacturer's protocol. The reaction was incubated at 42°C for 1 hour, and subsequently finally inactivated at 70°C.

RT-PCR amplification

RT-PCR amplification was performed using primers ABH-F (5'-AACATCCTCTCTCAGCGCCTATTT) and ABH-R (5'-AGCAGGAAAAATGTTCGGCATGTA). Primers design was carried out using Primer3Plus software, based on the only available incomplete *S. ratti* mRNA sequence (XM_024650090.1) in GenBank. Each PCR reaction contained 5 µL of cDNA template, 0.2 µM of each primer, 250 µM of each dNTP, and 0.5 units of *Taq* DNA polymerase in a standard PCR buffer. Amplification was performed under the following cycling conditions: an initial denaturation at 94°C for 3 min, followed by 30 cycle of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 min, with a final polymerization at 72°C for 7 min. PCR reactions were carried out using an Eppendorf Mastercycler Nexus Thermal Cycler (Eppendorf AG, Germany). Amplified products were analyzed by electrophoresis on a 1% agarose gel.

DNA sequence analysis

PCR products were sequenced using the original PCR primers with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and analyzed on an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems). Sequence assembly was performed by aligning overlapping fragment, and primers were designed using the Primer3 program. To evaluate evolutionary relationships, the sequences were compared with existing data in NCBI GenBank using the BLASTn algorithm.

Assembled nucleotide sequences were compared with existing sequences in the NCBI GenBank using the BLASTn algorithm to assess evolutionary relationships. Target sequences from other species were retrieved from NCBI GenBank, and signal peptide prediction was conducted using SignalP software [15] (cbs.dtu.dk/services/SignalP). Multiple sequence alignments were generated using CLUSTAL_W [16], and CDD-Search software from NCBI was employed to identify conserved domains [17]. The molecular weight (MW) and isoelectric point (pI) of the amino acid sequences were calculated using Expasy tools (ca.expasy.org/tools). Secondary structure prediction of the protein was performed using the PSIPRED protein sequence investigation (bioinf.cs.ucl.ac.uk/psipred) [18]. Mitochondrial transfer peptides at the N-terminus were predicted using TargetP-2.0 software (services.healthtech.dtu.dk/services/TargetP-2.0). Three-dimensional (3D) structure prediction was performed using Phyre2 server [19]. Phylogenetic analysis along and genetic distance calculations were performed using the neighbor-joining method with 1,000 bootstrap replicates in MEGA7 software [20]. Protein statistical parameters were analyzed using the Gene Infinity program (org/sms/sms_proteinstats.html). Protein domains were further examined using the ScanProsite tool (prosite.expasy.org/scanprosite), and N-glycosylation site was predicted using NetNGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc).

Authors' Contributions

A.J. conceived and planned the experiments. A.J. carried out the experiments. A.J. planned and carried out the simulations. A.J. Contributed to sample preparation. A.J. contributed to the interpretation of the results. A.J. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Office at the Shahid Chamran University of Ahvaz, Ahvaz- Iran.

Conflict of interest

The authors declare that there is no conflict of interest.

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Ultrastructural organization of endothelial cells of the cerebellar cortex capillaries of the domestic bull

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ABSTRACT

The aim of this study was to characterize the ultrastructural organization of endothelial cells in the cerebellar cortex capillaries of the domestic bull. Investigation of cerebellar endotheliocytes is of considerable interest for both fundamental science and applied veterinary medicine and neurobiology, as these cells play a central role in regulating microcirculation and metabolism mechanisms. A clearer understanding of their ultrastructure may contribute to the development of new methods for diagnostics and treatment. Fragments of cerebellar cortex tissues were processed using standard methods, and were studied using electron microscopy. The terminology was described in accordance with the International Histological Nomenclature. The analysis demonstrated that the endotheliocytes of the cerebellar cortex capillaries of the domestic bull represent the primary structural component of the blood-brain barrier. They form the capillary lumen, are intercontacted by well-developed tight junctions, and rest upon a continuous basal membrane. The continuity of the latter and the presence of tight junctions restricts the possibility of spontaneous transport of macromolecular compounds from the bloodstream to the brain tissue.

Keywords

brain, cerebellar cortex, histohematic barriers, blood-brain barrier, homeostasis

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Number of References:: 23
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Abbreviations

A : astrocyte;
AS : astrocyte stalk
BV : blood vessel lumen
CO : endothelial cell cytoplasmic outgrowths
EC : endothelial cell
P : pericyte
RBC :red blood cell

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Introduction

The brain coordinates the functioning of all organs and systems of the body, ensuring its integrity and harmonious interaction with the environment. Its proper functioning depends on strict adherence to homeostatic principles [1,2]. The majority of metabolic processes that ensure the constancy of the environment occur between neural tissues and the vascular system at the level of the hemomicrocirculatory bed [3,4]. Within this system, hemocapillary, are the principal structural element, and their organization directly influences the physiological and biochemical processes of the central nervous system [5-7]. Based on these considerations, the objective of this study was to characterize the ultrastructural organization of endothelial cells in the capillaries of the cerebellar cortex of a domestic bull.

The ultrastructural investigation of endotheliocytes in the cerebellar cortex capillaries of domestic bulls (Bos taurus) holds considerable importance for both fundamental science and applied veterinary medicine and neurobiology. The relevance of this topic is determined by several key factors.

First, the cerebellum plays a critical role in motor coordination and balance in mammals, including bovines [8, 9]. Understanding the ultrastructural organization of capillary endothelial cells in the cerebellar cortex provides better insight into the mechanisms of microcirculation and metabolic support in this important part of the brain. Such knowledge may ultimately contribute to the development of new methods for diagnostics and treatment.

Second, capillary endotheliocytes play a key role in maintaining the blood-brain barrier, which protects the brain from toxins and pathogens [9-11]. Studying the ultrastructural characteristics of these cells in bovines expands our understanding of blood-brain barrier function across mammalian species. These findings also have relevance in the context of comparative neuroscience, where bovine data may serve as a useful model for broader interspecies extrapolation, including humans.

A final important aspect concerns the potential application of the research results in veterinary practice. Diseases associated with cerebellar microcirculation disorders can result in severe neurological disorders in bulls, negatively impacting their productivity and well-being. [9-11] Understanding the ultrastructural organization of endothelial cells provides a basis for the development of new methods for the diagnosis and treatment strategies for vascular and neurodegenerative diseases in bovines.

Results

Endothelial cells of the cerebellar cortex capillaries in the domestic bull constitute the primary structural component of the blood-brain barrier. These cells are flat, and elongated along the blood vessel. These cells contact each other with tight junctions and rest on a continuous basal membrane, thereby forming a capillary wall. Based on the obtained electronograms, the diameter of the lumen of the latter varied from 1.0 to 5.0 μm, while the thickness of the basal membrane varied from 40 to 200 nm. The continuity of the basal membrane, together with the presence of tight junction complexes, restricts the passage of macromolecular compounds from the bloodstream through the capillary wall into the brain tissue. The nuclei have an ovoid shape and occupy most of the cell, corresponding to its maximal height, which varies from 3.5 to 4.5 μm (Figure 1).

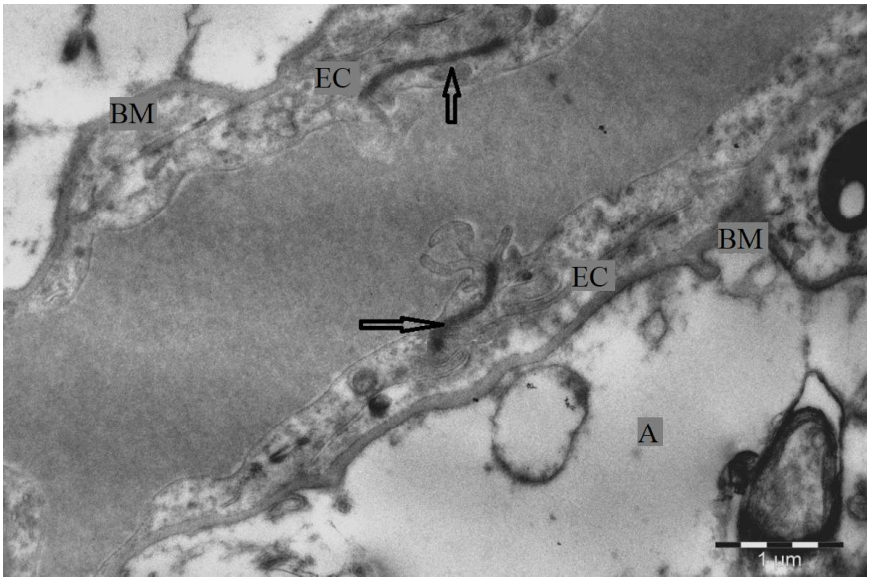

Figure 1.
Ultrastructure of the basal membrane of the cortical capillary mammalian cerebellum. Electron micrograph: EC – endothelial cell; A – astrocyte; BM – basal membrane; ↑ – tight junctions.

In some cells, the the nuclear surface has a tortuous shape due to the undulations of the karyolem-

ma. (Figure 2) The nuclear envelope consists of two lipoprotein membranes, each 7 to 8 nm thick. The perinuclear space lies between the membranes. The membrane sometimes contains pores with diameters ranging from 70 to 100 nm. The central part of the matrix is usually electron light. Along the periphery of the nucleus, electron-dark accumulations of heterochromatin are noticeable, contacting the internal karyolemma.

The cytoplasm occupies the marginal part of the cell. Its thickness is slightly lower than the central part, the region occupied by nucleus, and varies within 1.3–1.8 μm. Electron-light mitochondria are detected in the cytoplasm. They contain thin cristae formed by folds of the inner membrane. The outer membrane of the organelles forms their shape, which varies from round to oval. The diameter of the mitochondria is about 1.0 μm. Also the cytoplasm contained a significant number of ribosomes, which look like electron-dense granules with a diameter of 25 to 30 nm. In addition to individual ribosomes, polyribosomes, ribosomes clusters, as well as a small number of pinocytotic vesicles and single short channels of the granular endoplasmic reticulum were identified (figure 3).

The cytoplasmic membrane of endothelial cells formed their outer shell, and its thickness varies within 7.5–10.0 nm in the obtained electronograms. In accordance with the location of the endotheliocytes, it forms their luminal and basal surfaces. Luminal surface facing the inside of the vessel. With an increase in the functional activity of the endothelial cell, it is able to form outgrowths and protrusions. The basal surface of the cells contacts the basal membrane (Figure 4).

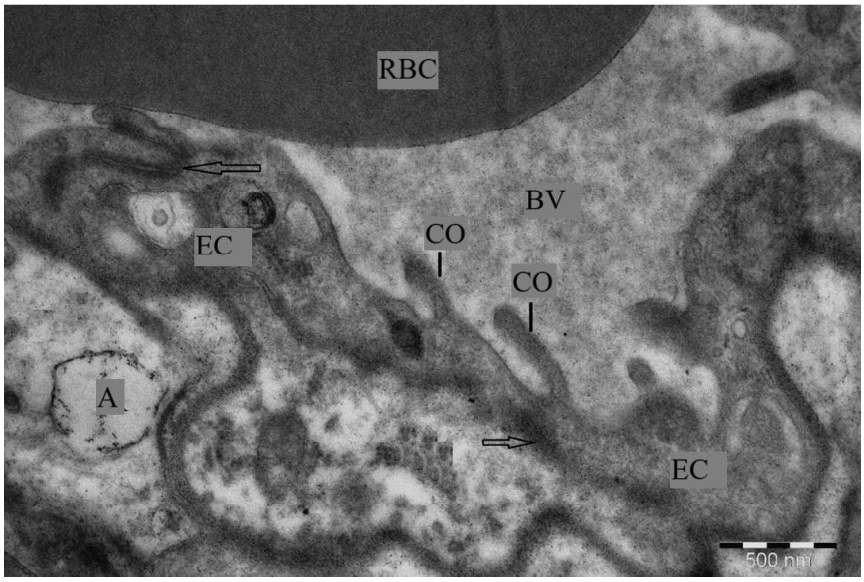

Figure 2.
Ultrastructure of the blood-brain barrier of the capillary of the cerebellar cortex of a domestic bull. Electron micrograph: EC– endothelial cell; P– pericyte; A– astrocyte; BV– blood vessel lumen; E– erythrocyte; ↑ – basal membrane.; PL– phagolysosomes; M– mitochondria.

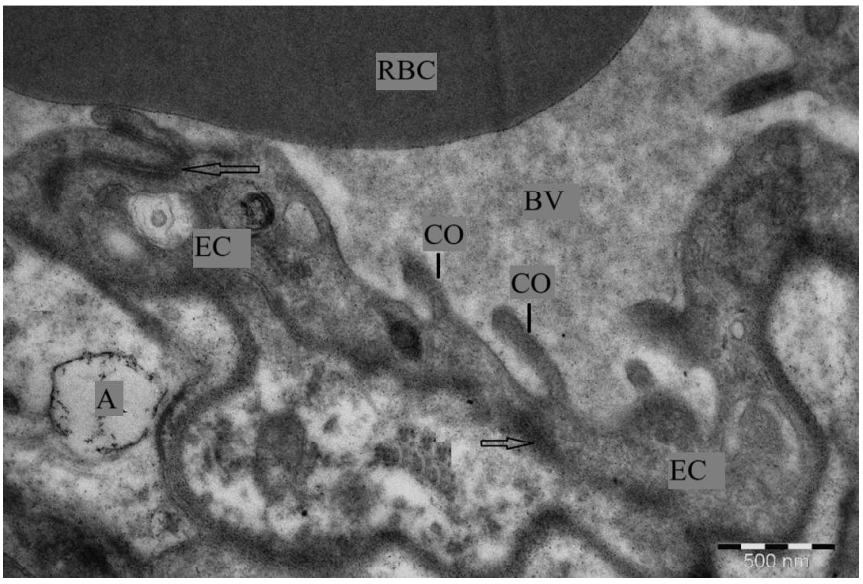

Figure 3.
Ultrastructure of the endothelial cell of the capillary of the cerebellar cortex of a domestic bull. Electron micrograph:
EC – endothelial cell; P– pericyte; A– astrocyte; BV– blood vessel lumen; GER – granular endoplasmic reticulum; ↑ – basal membrane.

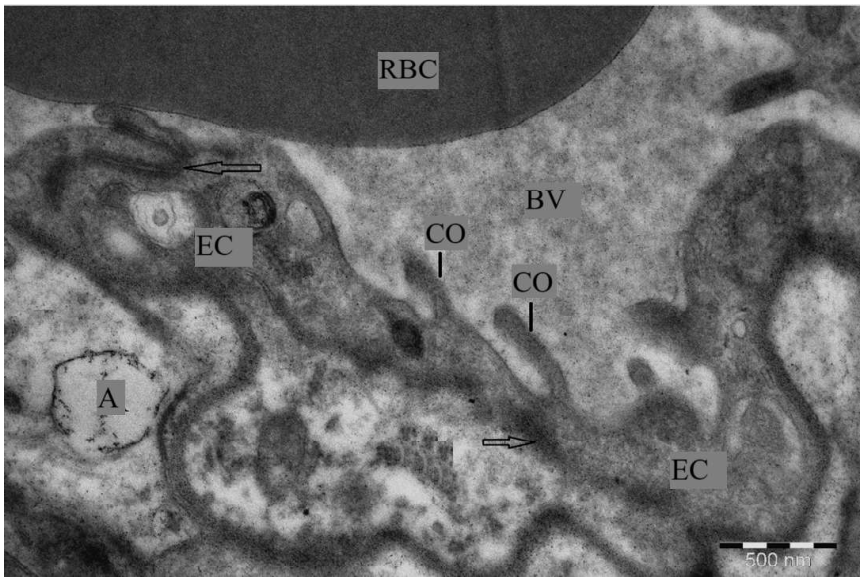

Figure 4.
Ultrastructure of a mammalian cerebellar cortex capillary endothelial cell in the active phase. Electron microphotography: EC—endothelial cell; A– astrocyte stalk; BV– blood vessel lumen; CO – endothelial cell cytoplasmic outgrowths; RBC– red blood cell; ↑ – tight junctions.

Discussion

The present study revealed several key features and ultrastructural characteristics of endothelial cells that deserve detailed discussion.

First, the endothelial cells of the bovine cerebellar cortex capillaries exhibit a high mitochondrial density. This feature indicate an intensive level of metabolic activity, likely reflecting the high energy requirements of endothelial cells associated with transport of nutrients and oxygen to cerebellar neurons. [10, 15] Similar mitochondrial abundance has been reported of other mammalian species, emphasizing the universality of this phenomenon. [16-20]

Second, the obtained data indicate the presence of numerous pinocytotic vesicles within the endothelial cytoplasm. This indicates active transport of substances through the endothelium, which plays a key role in maintaining homeostasis of the cerebellar microenvironment. The variability in the number and size of pinocytotic vesicles may be influenced by the physiological state of the animal. [9,11,18]

The third important aspect is the presence of tight junctions between endotheliocytes, which form the blood-brain barrier. These junctions play a critical role in preventing potentially harmful substances from entering the brain from the blood [8,17]. The results show that tight junctions in the capillaries of the bovine cerebellar cortex are highly organized, which may be due to the need for strict control over the composition of the cerebellar microenvironment.

In addition, the presence of pericytes, which are closely associated with endothelial cells, is observed. Pericytes play an important role in regulating blood flow and maintaining the capillaries structural integrity [17]. Their presence in the capillaries of the bovine cerebellar cortex supports their critical role in microcirculation dynamics in this part of the brain.

Thus, the results confirm that endothelial cells of the cerebellar cortex capillaries of the domestic bull are the primary element of the blood-brain barrier. Forming the lumen of the capillary, they contact each other with tight junctions and lie on a continuous basal membrane. The continuity of the latter and the presence of tight junctions block the possibility of spontaneous transport of macromolecular compounds from the bloodstream to the brain tissue.

CONCLUSION

In conclusion, this study highlights the complex ultrastructural organization of endothelial cells in the cerebellar cortical capillaries of the domestic bull. Revealed features, including high mitochondrial density, active pinocytosis, well-developed tight junctions, and the presence of pericytes, indicate the specialization and high functional activity of these cells.

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

AI-Assisted technologies were not applied at all stages of the work process.

Ethical Approval

The experiment was approved by the ethical committee Saint Petersburg State University of Veterinary Medicine.

Five fragments of the cerebellar cortex tissue from an adult domestic bull were used in the study. Each specimen measured no more than 2.0 mm^3. The tissue samples were pre-fixed in a 2.0% glutaraldehyde solution prepared in a cacodylate buffer (pH 7.2–7.4) for 2 hours. Following primary fixation, fragments were rinsed in three changes of the same buffer. Post-fixation was performed in a 1.0% solution of osmium (VIII) oxide (diluted in cacodylate buffer, pH 7.2–7.4) for 1 hour. After post-fixation, the samples were dehydrated through a

graded series of ethanol solutions, and subsequently treated with absolute acetone. The dehydrated tissue fragments were then embedded in Epon-812 resin, in accordance with the generally accepted methodology [12]. From each embedded block, three ultrathin sections were obtained using an ultramicrotome (LKB-III, Sweden). The sections were contrasted with 2.0% aqueous uranyl acetate solution and then with lead citrate solution. Imaging was performed using a Jem-1011 electron microscope (JEOL, Japan) at magnifications ranging from 2500× to 3000× [13]. All terminology used in this study follow the International Histological Nomenclature [14].

Authors' Contributions

P.A.V. planned the experiments, carried out the experiment, contributed to the interpretation of the results. V.S.P. contributed to sample preparation, contributed to the interpretation of the results, took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflict of interest

Authors declare that they have no conflict of interest. There were no ethical considerations to be considered in this research.

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اپیدمیولوژی مولکولی کرم قلب سگ (دیروفیلاریا ایمیتیس) در پشه‌های Culex pipiens جمع‌آوری‌شده از شهر تهران

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چکیده

دیروفیلاریا ایمیتیس یک انگل نماتود است که در قلب و شریان‌های ریوی سگ‌سانان، گربه‌سانان و همچنین انسان مستقر شده و رشد می‌کند. میکروفیلرهای این انگل در جریان خون میزبان گردش داشته و از طریق گزش پشه‌های *Culex pipiens* به میزبان‌های جدید منتقل می‌شوند. پشه *Culex pipiens* که با نام پشه خانگی معمولی شناخته می‌شود، گونه‌ای بومی و آفت‌زا در محیط‌های شهری است. از اوایل قرن بیستم، برنامه‌ها و کمپین‌های کنترلی متعددی در کشورهای اروپایی برای کنترل این گونه اجرا شده است. این پشه دارای انعطاف‌پذیری اکولوژیکی بالایی بوده که منجر به رفتارهای تغذیه‌ای پیچیده و توان بالای انتقال عوامل بیماری‌زا می‌شود. این مطالعه از خرداد تا شهریور سال ۱۴۰۳ در شهر تهران انجام شد. در مجموع، ۱۰۰ عدد پشه *Culex pipiens* از مناطق مختلف شهر جمع‌آوری و آلودگی آن‌ها به *Dirofilaria immitis* با استفاده از روش‌های مولکولی (PCR) بررسی گردید. نتایج نشان داد که ۱۵ عدد از پشه‌های جمع‌آوری‌شده به میکروفیلرهای *D. immitis* آلوده بودند. با توجه به میزان آلودگی مشاهده‌شده در این مطالعه، پتانسیل بالای انتقال عفونت در مناطق مورد بررسی و اهمیت بیماری‌های مرتبط با *D. immitis*، اجرای اقدامات بهداشتی در مناطق پشه‌خیز و محل نگهداری سگ‌ها، همراه با کنترل مؤثر جمعیت پشه‌ها ـ به‌ویژه در مناطق مرطوب و در فصول گرم سال ـ برای جلوگیری از گسترش این بیماری زئونوز در میان سگ‌ها و انسان‌ها ضروری است.

واژگان کلیدی

میکروفیلر، دیروفیلاریا ایمیتیس ، پشه کولکس ، پی سی آر

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Abstracts (in Persian)

ارزیابی یافته های بالینی و هیستوپاتولوژی رحم و تخمدان در گربه های اواریوهیسترکتومی شده در منطقه اهواز

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چکیده

هدف از انجام مطالعه حاضر، ارزیابی یافته های بالینی، ضایعات هیستوپاتولوژیک رحم و تخمدان و تاکید بر نقش تشخیصی اواریوهیسترکتومی، در جمعیت گربه ها در منطقه اهواز و در فاصله زمانی مهر تا اسفندماه ۱۴۰۱ بود. تعداد ۵۴ قلاده گربه، ارجاعی به بیمارستان دامپزشکی اهواز، تحت عمل اواریوهیسترکتومی قرار گرفتند. میانگین انحراف معیار سنی گربه ها، ۰/۷۴ ± ۱۰/۹۴ ماه بود. در بررسی ماکروسکوپی از نمونه های اخذ شده، بجز سه مورد که آبستن بودند، مابقی دارای ساختار طبیعی بودند و علایم بالینی خاصی قبل از جراحی دیده نشد. نتایج هیستوپاتولوژی، نشان داد که ضایعات رحم و تخمدان در ۵ مورد شامل یک قلاده مبتلا به هیپرپلازی کیستیک آندومتر، یک مورد مبتلا به تومور سلول‌های گرانولوزا، یک مورد مبتلا به کیست رحمی و خونریزی در تخمدان، یک قلاده مبتلا به پرخونی و خونریزی در رحم و تخمدان و یک قلاده دیگر مبتلا به هموسیدروز رحم بودند. در گربه مبتلا به هیپرپلازی کیستیک اندومتر، سلول‌های آندومتر تکثیر یافته و در مخاط آندومتر، کیست‌هایی وجود داشتند؛ همچنین در داخل تخمدان چند فولیکول بزرگ و جسم زرد در حال تحلیل دیده شدند که بر اساس طبقه بندی داو، ضایعات در دسته هیپرپلازی کیستیک درجه اول، طبقه بندی گردید. در ارزیابی میکروسکوپیک از نمونه مبتلا به تومور سلول‌های گرانولوزا، توده‌ای از سلول‌های تکثیر یافته در بافت تخمدان مشاهده گردید. سلول‌های مذکور به صورت نردبانی در کنار هم قرار گرفته بودند. این سلول‌ها دارای هسته درشت همراه با ۱-۲ هستک مشخص و میزان کمی سیتوپلاسم در اطراف آن‌ها بودند. مطالعه حاضر نشان می دهد که اواریوهیسترکتومی در گربه ها می تواند نقش مهمی در تشخیص ضایعات پاتولوژیک بافت رحم و تخمدان داشته باشد؛ اهمیت تشخیص، به ویژه در مورد تومورها، به مراتب بیشتر است.

واژگان کلیدی

رحم، تخمدان، هیستوپاتولوژی، اواریوهیسترکتومی، گربه، اهواز، ایران

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بررسی اثرات ناشی از ورزش و تمرین بدنی بر غلظت پارامترهای بیوشیمیایی سرم و الکترولیت‌ها در اسب‌های عرب

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چکیده

این مطالعه تغییرات القا شده توسط ورزش و تحرک بدنی را بر روی پارامترهای بیوشیمیایی سرم از جمله کلسیم، فسفر، سدیم، پتاسیم، کلر، منیزیوم، گلوکز، اوره، کراتینین، تری گلیسیرید، کلسترول، پروتئین تام، آلبومین، بیلی روبین تام و همچنین فعالیت سرمی آنزیم هایی چون کراتین فسفوکیناز(CPK) ، لاکتات دهیدروژناز(LDH) ، گاماگلوتامیل ترنسفراز(GGT) ، آسپارتات آمینوترنسفراز(AST)، آلانین آمینوترنسفراز(ALT) و آلکالین فسفاتاز(ALP) را در اسب های عرب تحت تمرین در آب و هوای گرم و خشک بررسی می کند. ۲۲ اسب عرب ۳ تا ۷ ساله در این مطالعه مشارکت داشتند. نمونه های خون از ورید وداج در چهار زمان مختلف جمع آوری شد: یک ساعت قبل(T0) ، بلافاصله بعد (T1)، شش ساعت(T2) و هجده ساعت (T3) پس از گرم کردن و دویدن با حداکثر توان در مسیر ۱۵ کیلومتری جمع آوری گردید. داده های به دست آمده با استفاده از آنالیز واریانس یک طرفه با مقادیر تکراری تحلیل شدند و برای مقایسه های دوگانه بین مقادیر اندازه گیری شده در هر زمان و حالت پایه (T0) ، از آزمون بونفرونی اصلاح شده استفاده شد. تمامی مقایسه های آماری در سطح $p < ۰.۰۵$ معنی دار در نظر گرفته شد . نتایج نشان داد که غلظت سرمی اوره، کراتینین، تری گلیسیرید، کلسیم، فسفر و بیلی روبین تام طی زمان به دنبال ورزش و تمرین بدنی تغییرات معنی داری داشت ($p < ۰.۰۵$). نتایج نشان داد که غلظت گلوکز، تری گلیسیرید، کلسترول، اوره، فسفر، سدیم، کلر و نیز فعالیت سرمی AST ، ALT و LDH (که غلظت آنها در مادیان ها بالاتر است) و همچنین منیزیوم (که غلظت آن در نریان ها بالاتر است) بین دو جنس تفاوت معنی دار ($p < ۰.۰۵$) از خود نشان می دهد. افزون بر این، تفاوت معنی داری در میانگین غلظت پروتئین تام و فعالیت های سرمی GGT ، ALP ، AST ، ALT و CPK بین گروه های سنی مشاهده شد ($p <۰.۰۵$).

واژگان کلیدی

اسب‌های عرب، پارامترهای بیوشیمیایی، آب و هوای خشک، عملکرد، تمرین بدنی

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بررسی مقایسه‌ای برخی تغییرات هماتوبیوشیمیایی و الکتروکاردیوگرافی متعاقب تجویز مدتومیدین به تنهایی و در ترکیب با آگونیست‌های اوپیوئیدی در بزها

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چکیده

مدتومیدین برای ایجاد آرامبخشی در نشخوارکنندگان کوچک کاربرد دارد و اغلب برای تنظیم عمق آرامبخشی و کاهش عوارض ناخواسته با اوپیوئیدها ترکیب می‌شود. ما یک مطالعه تصادفی متقاطع با طرح مربع لاتین بر روی تعداد ۱۰ راس بز انجام دادیم و ۵ روش تزریق داخل‌وریدی را به ترتیب زیر با هم مقایسه کردیم: مدتومیدین به‌تنهایی با دوز ۲۰ میکروگرم به ازای هر کیلوگرم (MED) یا در ترکیب با متادون نیم میلی‌گرم به ازای هر کیلوگرم (MME)، مورفین نیم میلی‌گرم به ازای هر کیلوگرم (MMO)، ترامادول ۵ میلی‌گرم به ازای هر کیلوگرم (MTR) و پتیدین ۱ میلی‌گرم به ازای هر کیلوگرم (MPE). داروها طی ۲ دقیقه از طریق ورید وداج چپ تجویز شدند. نمونه‌های خون در زمان‌های صفر، ۱۲۰ دقیقه و ۲۴ ساعت پس از تزریق برای شمارش گلبول‌های سفید (WBC)، هماتوکریت (PCV)، تروپونین قلبی (cTnI)، هموسیستئین (Hcy) و آنزیم‌های کبدی (ALT/AST) جمع‌آوری شدند. همچنین الکتروکاردیوگرام در زمان‌های صفر، ۵ و ۱۲۰ دقیقه پس از تزریق ثبت گردید. مقادیر پایه هماتولوژیک، بیوشیمیایی و الکتروکاردیوگرام بین دوره‌ها تفاوتی نداشتند. سطح تروپونین قلبی در محدوده مرجع آزمون باقی ماند و در هیچکدام از روش ها تغییر درون‌گروهی مشاهده نشد، هرچند در ۱۲۰ دقیقه و ۲۴ ساعت تفاوت‌های بین گروه‌ها دیده شد؛ به‌طوری‌که در ۱۲۰ دقیقه پس از تزریق مقدار تروپونین قلبی در گروه مدتومیدین کمتر از متادون/ ترامادول و در ۲۴ ساعت پس از تزریق کمتر از پتیدین/ ترامادول بود. هموسیستئین تفاوت معنی داری بین گروه‌ها نشان نداد و تنها در گروه مدتومیدین در ۲۴ ساعت افزایش خفیفی داشت. اثرات هماتولوژیک وابسته به روش به کار برده شده بودند. تعداد گلبول های سفید ۲ ساعت پس از تزریق مدتومیدین / مورفین کاهش یافت ($p < 0.05$) و هماتوکریت پس از تزریق مدتومیدین / ترامادول در بازه ۲ تا ۲۴ ساعت کاهش نشان داد ($p < 0.05$). آنزیم‌های کبدی اثر معنی‌داری از نظر نوع درمان و بازه زمانی نشان ندادند. در الکتروکاردیوگراف، فواصل PR و QT با گذشت زمان طولانی‌تر شدند (به‌ویژه پس از تزریق مدتومیدین و مدتومیدین / مورفین) ، فواصل RR عموماً طولانی شدند که با برادی‌کاردی سازگار بود و مدتومیدین / پتیدین باعث افزایش دامنه موج T شد ($p < 0.05$). درمجموع، ترکیبات مدتومیدین/اوپیوئید الگوهای متمایز هماتولوژیک و الکتروکاردیوگرافیک ایجاد کردند، بدون آنکه افزایش قابل تشخیص تروپونین قلبی درون هر گروه مشاهده شود. این داده‌ها از انتخاب آگونیست های شبه مخدری همراه با پایش مداوم الکتروکاردیوگرام هنگام استفاده از مدتومیدین در بزها حمایت می کند.

واژگان کلیدی

آرامبخشی، مدتومیدین، آگونیست های شبه مخدری، بز

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مطالعه مقایسه ای اثر درمانی اکینوکوکوس گرانولوزوس و کورکومین در مدل موش مولتیپل اسکلروزیس

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چکیده

مولتیپل اسکلروزیس (MS) یک بیماری مزمن خودایمنی در سیستم عصبی مرکزی است که با دمیلیناسیون و نورودژنراسیون در مغز و نخاع مشخص می‌شود. اکینوککوس گرانولوزوس، عامل بیماری کیست هیداتید، با ایجاد تعدیل ایمنی در میزبان، از پاسخ‌های ایمنی ضد انگلی فرار می‌کند. کورکومین، یک پلی‌فنول طبیعی، خواص ضدالتهابی و آنتی‌اکسیدانی اثبات‌شده‌ای دارد. این مطالعه با هدف بررسی اثرات تعدیل‌کننده ایمنی پروتواسکولکس‌های *E. granulosus* در مدل انسفالومیلیت خودایمن تجربی (EAE) از MS در رت‌ها و مقایسه آن با اثرات درمانی کورکومین انجام شد. چهل رت نر نژاد اسپراگ-داولی به‌صورت تصادفی به چهار گروه (هر گروه ۱۰ سر) تقسیم شدند: کنترل سالم، کنترل EAE، EAE + پروتواسکولکس *E. granulosus* و EAE + کورکومین. سطوح سایتوکاین‌های پیش‌التهابی (IL-6، IL-1β، TNF-α)، غلظت نیتریک‌اکسید (NO) در مغز، فعالیت سرمی مایل‌پراکسیداز (MPO) و میزان مالون‌دی‌آلدهید (MDA) در سرم اندازه‌گیری شد. القای EAE موجب افزایش معنی‌دار همه شاخص‌ها در مقایسه با گروه کنترل شد ($p < 0.05$). درمان با کورکومین یا پروتواسکولکس‌ها باعث کاهش معنی‌دار این شاخص‌ها شد ($p < 0.05$) و تفاوتی بین اثر دو درمان مشاهده نگردید. یافته‌های ما نشان می‌دهد که پروتواسکولکس‌های *E. granulosus* در مدل EAE دارای اثرات ضدالتهابی و آنتی‌اکسیدانی مشابه کورکومین هستند. هر دو درمان با کاهش استرس اکسیداتیو و سرکوب سایتوکاین‌های التهابی موجب بهبود وضعیت ایمنی شدند. این نتایج نشان می‌دهد که *E. granulosus* می‌تواند به‌عنوان یک راهکار درمانی بالقوه برای MS در نظر گرفته شو

واژگان کلیدی

کورکومین، اکینوکوکوس گرانولوزوس، آنسفالومیلیت خودایمنی تجربی، مولتیپل اسکلروزیس، سایتوکاین

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تعیین خصوصیات ملکولی و تجزیه و تحلیل فیلوژنتیکی یک پروتئین میتوکندریایی α/β-hydrolase از *Strongyloides ratti*

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چکیده

Strongyloides ratti نماتود است که به عنوان یک مدل مناسب در تشخیص استرونژیلوئیدازیس در انسان بکار می رود. پروتئین های دامنه آلفا/بتا هیدرولاز بطور وسیعی در همه موجودات یافت می شوند. یک قطعه cDNA 780 نوکلئوتیدی توسط RT-PCR افزوده سازی شد.در جستجوی بانک ژن NCBI مشاهده گردید که محصولات افزوده سازی شده با تنها توالی mRNA ناکامل (XM_024652394.1) از *S. ratti* به میزان 98.54٪ مشابهت داشت. با جستجو در پایگاه داده های Expressed Sequence Tags (EST) موجود در بانک ژن و با استفاده از این قطعه ژن افزوده سازی شده به عنوان جستجوگر، دو توالی ۸۵۶ (FC815910.1) و ۵۲۶ (BI742498.1) نوکلئوتیدی مربوط به انتهای ۳' و ۵' شناسایی شدند. قطعه cDNA بزرگتر مونتاژ شده به اندازه ۹۱۹ نوکلئوتید با استفاده از نواحی همپوشان مشخص و بنام SrABH نامگذاری گردید. توالی اسید آمینه SrABH با تنها توالی (XP_024505975.1) *S. ratti* موجود در بانک ژن 97.98٪ مشابهت داشت. قطعه ژن ۸۹۳ نوکلئوتیدی پروتئینی با اندازه ۲۹۱ اسید آمینه، با وزن ملکولی ۴۸/۳۳ کیلو دالتون و نقطه ایزوالکتریک ۱۸/۷ را کد می کرد. قابل توجه اینکه SrABH فاقد پپتید سیگنال بود، ولی یک محل برش برای هدف قرار دادن این پروتئین در میتوکندری بین اسیدهای آمینه ۲۱-۲۲ شناسایی گردید. یک ناحیه بسیار حفاظت شده شامل نه اسید آمینه در موقعیت های ۱۱۱ تا ۱۲۰ نیز شناسایی گردید. تجزیه و تحلیل ساختاری سه بعدی نشان داد که SrABH از ۹ مارپیچ آلفا (39.18٪)، ۸ صفحه بتا (18.21٪) و لوپ ها (42.61٪) تشکیل شده است. تجزیه و تحلیل فیلوژنتیک مشخص نمود که با توالی‌های نماتودی مرتبط، با بوت استرپ قوی ۹۸ در یک کلاستر قرار دارند. کمترین فاصله ژنتیکی (0.01٪) بین SrABH و تنها توالی جدا شده از (XP_024505975.1) *S. ratti* مشاهده شد. با توجه به ویژگی‌های داده های مولکولی می توان چنین نتیجه گرفت که این پروتئین در استراتژی‌های طراحی دارو مبتنی بر ساختار با استفاده از *S. ratti* به عنوان یک سیستم مدل برای پاتوژن انسانی *S. stercoralis* ممکن است ارزشمند باشد.

واژگان کلیدی

میتوکندری ، RT-PCR ،استراز، دامنه آلفا/بتا هیدرولاز، استرونژیلوئیدس رتی

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Mouse beta actin gene: Actb

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Protocol for DNA/RNA extraction, including quantification and determination of purity.

Reverse transcription (if used): amount of RNA, concentration of all reagents: primers conce ntration (either random primers or oligonucleotides), reverse transcriptase and master mix components.

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References for the above example:
1. Hull J, Forton J, Thompson A. Paediatric respiratory medicine. Oxford: Oxford University Press; 2015.
2. Eckerman AK, Dowd T, Chong E, Nixon L, Gray R, Johnson S. Binan goonj: bridging cultures in

Aboriginal health. 3rd ed. Chatswood, NSW: Elsevier Australia; 2010.
3. Johnson C, Anderson SR, Dallimore J, Winser S, Warrell D, Imray C, et al. Oxford handbook of expedition and wilderness medicine. Oxford: Oxford University Press; 2015.
4. McLatchie GR, Borley NR, Chikwe J, editors. Oxford handbook of clinical surgery. Oxford: Oxford University Press; 2013.
5. Petitti DB, Crooks VC, Buckwalter JG, Chiu V. Blood pressure levels before dementia. Arch Neurol. 2005 Jan;62(1):112-6. Doi: 10.1001/archneur.62.1.112 .
6. Liaw S, Hasan I, Wade, V, Canalese R, Kelaher M, Lau P, et al. Improving cultural respect to improve Aboriginal health in general practice: a multi-perspective pragmatic study. Aust Fam Physician. 2015;44(6):387-92.Doi: 10.1001/archneur.62.1.112 .
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-The Editor-in-Chief and members of the editorial board will not use unpublished materials disclosed in a submitted paper for their own research purposes without obtaining explicit written consent from the author.

-Editors are expected to give fair consideration to all manuscripts submitted for publication, evaluating each on its own merits and without prejudice based on the author(s)' country, race, religion, nationality, sex, seniority or institutional affiliation. Decisions about editing and publishing are made solely based on the quality and relevance of the manuscript and are not influenced by external policies of governments or other agencies beyond the scope of this journal.

-The Editor-in-Chief has complete authority over the editorial content of the journal as well as the timing of its publication.

Ethical guidelines for Publisher

"Ferdowsi University of Mashhad press (FUM)" is promising to ensure that the decision on manuscript submissions is only made based on professional judgment and will not be affected by any commercial interests.

- FUM is committed to maintain the integrity of academic and research records.

- FUM is monitoring the ethics by Editor-in-Chief, Associate Editors, Editorial Board Members, Reviewers, Authors, and Readers.

- FUM, together with the Journal's editors, shall take reasonable steps to identify and prevent the publication of manuscripts where research misconduct has occurred, and under no circumstances encourage such misconduct or knowingly allow taking place.

- FUM is always checking the plagiarism and fraudulent data issues involving in the submitted manuscripts and willing to publish corrections, clarifications and retractions involving its publications as and when needed.

-FUM as the publisher supports the Journal for each published issue by paying a defined budget according to its published annual rank in the Portal of Scientific Journals of Iranian Ministry of Science, Research and Technology for costs including those pertaining to setup and maintenance of the publication infrastructure, routine operation of the Journal, processing of manuscripts through peer-reviews, editing, publishing, maintaining the scholarly record, and archiving.

Violation of Publication Ethics

The Editorial board of IJVST acknowledges that plagiarism is unacceptable in any of its forms:

Plagiarism:

Plagiarism is intentionally using someone else's ideas or other original material as if they are one's own. Copying even one sentence from someone else's manuscript, or even one of your own that has previously been published, without proper citation is considered by the JAM as plagiarism. All manuscripts under review or published with JAM are subject to screening using plagiarism prevention software (e.g. iThenticate). Thus, plagiarism is a serious violation of publication ethics.

Simultaneous Submission:

Care should be taken to ensure that the work has not been published elsewhere, in any language and is not simultaneously submitted to other journals.

Duplicate Publication:

Duplicate publication occurs when two or more articles, without full cross referencing, share essentially the same hypotheses, data, discussion points, and conclusions.

Redundant Publications:

Redundant publications involve the inappropriate division of study outcomes into several articles, most often consequent to the desire to plump academic vitae.

Data Fabrication:

Data fabrication means the researcher did not really carry out the study, but made up data or results and had recorded or reported the fabricated information. Data falsification means the researcher

did the experiment, but manipulated, changed, or omitted data or results from the research findings.

Citation Manipulation:

Citation Manipulation implies excessive citations in the submitted manuscript that do not contribute to the scholarly content of the article and have been included solely for the purpose of increasing citations to a given author's work, or to articles published in a particular journal. This leads to misrepresenting the importance of the specific work and journal in which it appears and is thus a form of scientific misconduct.

Improper Author Contribution or Attribution:

All listed authors must have made a significant scientific contribution to the research in the manuscript and approved all its claims. Do not forget to list everyone who made a significant scientific contribution, including students and laboratory technicians.

Handling Misconduct Cases

The Editorial board of IJVST takes the necessary measures to examine the incoming papers on their originality, reliability of contained information and correct use of citations.

-If any of the unethical publishing behavior is detected by the Journal Editorial board or by one of the reviewers, the first action is to inform the Editor-in-chief by supplying copies of the relevant material and a draft letter to the corresponding author asking for an explanation in a nonjudgmental manner.

- If the infraction is less severe, the Editor, upon the advice of the Committee on Publication Ethics, sends the author a letter of reprimand and reminds the JAM publication policies; if the manuscript has been published, the Editor may request the author to publish an apology in the journal to correct the record.

- If the author's explanation is unacceptable and it seems that serious unethical conduct has taken place, the matter is referred to the Publication Committee via Editorial board. After deliberation, the Committee will decide whether the case is sufficiently serious to warrant a ban on future submissions.

Post-Publication Discussions and Corrections

This journal allows debate post publication on journal's site, through "Send comment about this article" section to the editor up to one month before final publication. Our mechanisms for correcting, revising or retracting articles after publication depends on the content of the received comment and if the sent comments are useful and applicable for readers/authors, they will be showed under reference section of the articles pages.

Complaint Policy

If the authors disagree with the editorial decision on their manuscripts, they have a right to appeal. Authors who wish to appeal an editorial decision should contact the Editor-in-Chief of the Iranian Journal of Veterinary Science and Technology. In such cases the Editor-in-Chief will review the manuscript, the editorial and peer reviewers' comments and gives his/her decision for accepting or rejecting a manuscript. Editor-in-Chief may, if so required, send the manuscript to a new handling editor for a fresh editorial review and to new reviewer for further peer reviewing. In such case, the final decision

maker will be the Editorial board of the journal.

How to Make a Complaint

The procedure to make a complaint is quite simple. The complaint can be made by writing an e-mail to: ijvst@um.ac.ir. All complaints will be acknowledged within a week.

PEER REVIEW PROCESS

Iranian Journal of Veterinary Science and Technology peer reviews all submitted manuscripts with contents within the scope of the journal.

Initial assessment

The submitted manuscript will be subjected to a primary review by the editor or a member of the editorial board for suitability and relevance of the findings to the scope of the journal and quality of the science presented in the paper (sufficient originality, having a message that is important to the general field of Veterinary Medicine, quality of data, novelty, English language, and overall manuscript quality) within two weeks. If the paper is evaluated to be relevant to the scope of the journal and having enough scientific rigor and novelty, it will be sent for the next stage. Otherwise, those manuscripts which are evaluated as not-appropriate in the initial review will be rejected at this stage.

Initial screen

The initial screen will be performed by the editorial office for the structure and format of the manuscript.

Peer review (double-blind)

The manuscripts which are found to be appropriate after the initial screen will be sent for external review by experts in the related field. We have prepared a checklist for reviewers that summarizes their evaluation of the manuscript. The items in this checklist are:

1. TITLE is clear and adequate
2. ABSTRACT clearly presents objects, methods, and results.
3. INTRODUCTION well-structured and provides a rationale for the experiments described.
4. MATERIALS AND METHODS are sufficiently explained and is detailed enough to be reproduced.
5. RESULTS are clearly presented and supported by figures and tables.
6. DISCUSSION properly interprets the results and places the results into a larger research context, and contains all important references.
7. Conclusions are logically derived from the data presented.
8. English Language/style/grammar is clear, correct, and unambiguous.
9. Figures and tables are of good quality and well-designed and clearly illustrate the results of the study.
10. References are appropriate.
11. Regarding this article are you concerned about any issues relating to author misconduct such as plagiarism and unethical behavior.

12. Comments on the importance of the article.

Final Decision

Based on the reviewers' recommendations a final decision is made by the editor and if needed the help of a member of the editorial board (depending on the field of study). Decisions will include accept, minor revision, major revision with and without re-review, and reject. We aim to reach a final decision on each manuscript as soon as their review results are available.

Iranian Journal of Veterinary Science and Technology

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