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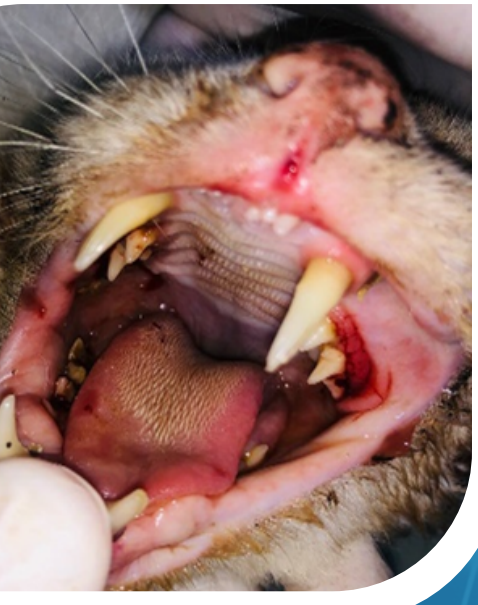

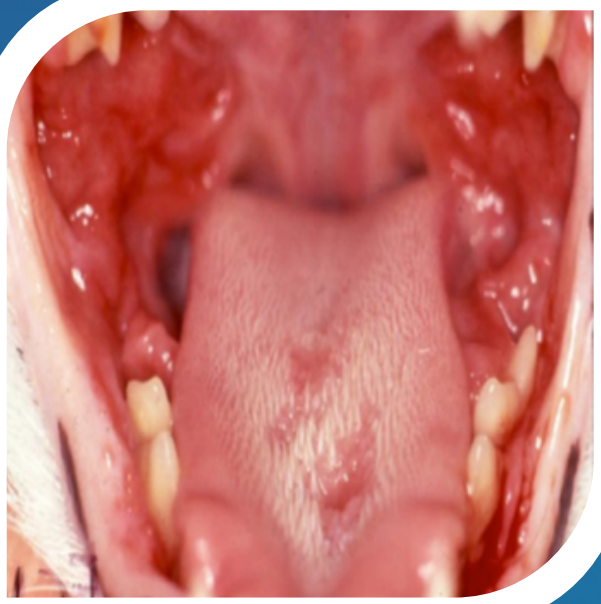

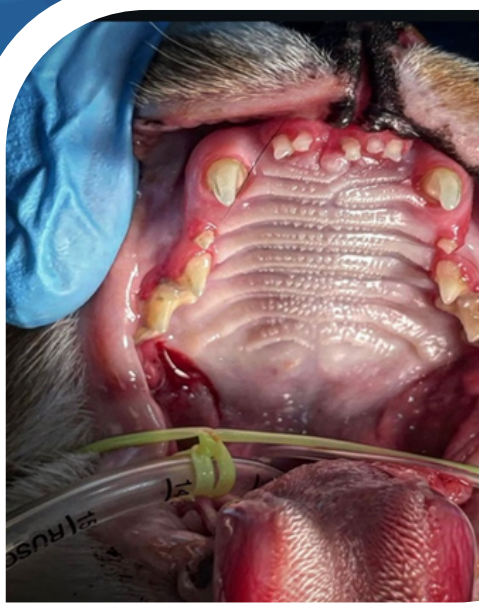

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ON THE COVER

Feline Chronic Gingivostomatitis (FCGS) is a painful, severe, immune-mediated inflammatory disease of the oral mucosa in cats. It typically presents with ulcerative-proliferative lesions lateral to the palatoglossal folds, along with maxillary gingivitis and bilateral alveolar mucositis. The disease is mainly characterized by bilateral inflammation of the caudal oral mucosa, which helps distinguish it from other oral conditions (See page 60).

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RESEARCH ARTICLE

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Investigation of *Chlamydia Abortus* Infection in Aborted Fetuses Using Molecular and Pathological Studies in East Azerbaijan Province, Northwest Iran

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ABSTRACT

Chlamydophila is an important cause of in-utero infections in sheep and goats, resulting in abortion, stillbirth, and the birth of weak offspring. The disease in sheep caused by *Chlamydia abortus* (*C. abortus*) has been known as "ovine enzootic abortion". Notably, it is recognized as a zoonotic disease, which can lead to abortion in humans. Therefore, the present study was carried out to recognize *Chlamydia* infection in aborted fetuses of domestic small ruminants, including sheep and goats in East Azerbaijan province, northwest Iran. For this purpose, a total of 62 aborted fetuses were obtained from sheep and goat flocks. At necropsy, the fetus was usually well preserved with few gross lesions. The tissue samples were collected for histopathology and molecular studies. The conventional PCR method using specific primers was performed to detect the *Chlamydia* genome. Additionally, the formalin-fixed tissue samples were routinely processed for histopathological studies. The genome of *C. abortus* was detected in 33.87% (95%CI: 0.32 ± 0.11) of the examined fetuses. Histopathological examinations presented multifocal hepatitis, pneumonia, and nonsuppurative meningoencephalitis associated with focal hemorrhage in the muscles. In conclusion, the investigation of the *C. abortus* genome in aborted fetuses with high prevalence rates indicates that this infection can play a notable role in the abortion of sheep and goats in East Azerbaijan. To prevent potential abortions in women who are in close contact with aborting ruminants, effective management and control measures for public health in the region are crucial.

Keywords

Sheep, Goat, Abortion, Zoonotic infection, Public health

Number of Figures: 2
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Abbreviations

OEA: Ovine Enzootic Abortion

PCR: polymerase chain reaction

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Introduction

Abortion is a significant problem in small ruminants, particularly in sheep and goats, and it is responsible for major economic losses in the livestock industry due to the loss of fetuses. The cause of abortion can be infectious, which is the most common or non-infectious [1]. Infectious causes can be bacterial, viral, fungal, and parasitic [2, 3]. Bacterial causative agents can be a more serious issue in small ruminants such as Chlamydia abortus [4]. One of the four families that make up the order Chlamydiales is the Chlamydiaceae family. At first, the family was divided into two genera, Chlamydia and Chlamydophila, but recent genomic and phylogenetic studies have resulted in the unification of all species under the genus Chlamydia [5, 6]. Besides the species currently known for the genus Chlamydia, which includes 21 species in various range hosts [7-11]. Some of these species, such as C. psittaci and C. abortus, are known to cause zoonotic infections. C. abortus is a major cause of chlamydiosis in small ruminants, which can lead to reproductive problems such as abortions, stillbirths, and infertility [12, 13]. C. abortus in sheep is also known as Ovine Enzootic Abortion (OEA) or Enzootic Abortion of Ewes (EAE) [12, 13].

Chlamydia is transmitted through multiple routes, including aerosol transmission, horizontal, ingestion, and fecal-oral. Fomites and environmental agents can also be important in spreading the infection. These multiple transmission pathways are involved in making this zoonotic infection more serious [14]. Oronasal route has been considered as the primary way of transmission, and it can happen from direct contact between animals or with abortion remnants. Dissemination of C. abortus to the placenta happens during pregnancy triggers inflammation and placental insufficiency, which leads to abortion or stillbirth [15]. The pathological lesions are detectable after day 90 of gestation, and the first lesion can be seen as cytoplasmic chlamydial inclusions produced in the cotyledon cells [16]. The lesions in the fetus can include primarily focal necrosis in the liver and additionally, small necrotic areas in the lungs and spleen, and less commonly in the brain and lymph nodes, and sometimes a mild form of semipurulent focal interstitial nephritis in fetal kidneys [16].

The PCR methods are frequently used for detecting chlamydia species due to their high specificity and sensitivity, quick results, and ability to handle large volumes [17]. Due to the presence of the organism in different organs, such as the abomasum and jejunum, as well as the possibility of the fetus swallowing the infected amniotic fluid, chlamydia can be diagnosed using the abomasal content of aborted fetuses [18]. This study was conducted to detect Chlamydia infection in the aborted fetuses of sheep and goat flocks in east Azerbaijan province (Iran) using the conventional PCR method due to the high prevalence of infectious abortion in the region. Also, it investigated the main histopathological changes associated with this infection to improve diagnostic accuracy.

Abbreviations-Cont'd

H&E: hematoxylin and eosin
CI: confidence interval.

Results

Molecular findings

The molecular study results associated with the age groups are presented in Figure 1 and Table 1. Briefly, the genome of C. abortus was detected in 21 out of 62 samples (33.87%%, 95%CI: 0.32 ± 0.11) from the examined fetuses. Of note, the higher infection rates were found in fetuses aged 4 to 5 months. However, the four age groups had no significant difference in infection rates.

Pathological findings

There were focal areas of cellular necrosis in the liver and spleen, which were variably surrounded by mononuclear cells. An increase in mononuclear cells was observed throughout the liver and concentrated in portal areas. In the lung, alveolar septa were thickened by mononuclear cells associated with hyperemia

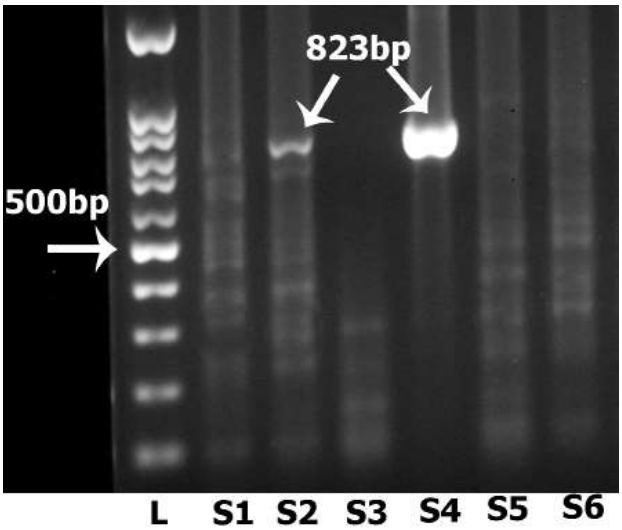

Figure 1.
Molecular findings of the present study for detection of the Chlamydia genome in aborted fetuses. The PCR products with an 83 bp band were detected through electrophoresis on agarose gels stained with a safe DNA stain. L: ladder 100 bp; S1, S3, S5: the samples with negative results; S2 and S4; positive samples with an 823 bp band.

Table 1.
PCR results for detection of Chlamydia in the aborted fetuses (N = 62).

Age groups	No. of the positive samples (%)	95% CI
60-90 days old (about 2-3 m) 5/62 (8.06%)	0/5 (0%)	0.0±0.0
90-120 days old (about 3-4 m) 14/62 (22.58%)	2/14 (14.28%)	0.1428±0.1813
120-150 days old (about 4-5 m) 36/62 (58.06%)	17/36 (47.22%)	0.472±0.162
150-155 days old (Over 5 m) 7/62 (11.29%)	2/7 (28.57%)	0.2857±0.3242
Total	21/62 (33.87%)	0.3387±0.114

of the pulmonary vessels. Additionally, there were mild to moderate hemorrhages in the muscles and spleen. In the brain, mild meningoencephalitis accompanied by hyperemia and focal hemorrhage were found. (figure 2)

Discussion

In this study, out of the 62 samples, 21 (33.87%) tested positive for Chlamydia infection using Chlamydia genus-specific primers for conventional PCR. In pathologic studies, the main lesions of Chlamydia infection were observed in the examined aborted fetuses. Due to the importance of Chlamydia in the discussion of abortion in small ruminants and also zoonotic aspects, various studies have been conducted within Iran to estimate the prevalence of this infection. Based on some studies conducted in Iran from 2015 to 2023, the prevalence of Chlamydia infection in individual animals varied between 5.71% and 56.41%. This variation was attributed to the differences in geographical areas, the detection methods used, and the types of samples taken from the animals. For example, a previous study found a seroprevalence of 25.6% using the ELISA test in individual animals across seven provinces in Iran [19]. In contrast, another study reported a higher prevalence of 56.41% in samples of aborted material from sheep and cattle in Shahr-e-Kord and Bagh-e-Malek, using Chlamydiales order-specific primers in PCR testing [20]. A recent study used cell culture for chlamydia detection, which differs from methods used in other studies. Chlamydial inclusion bodies were seen with an optical microscope in 14.28% of samples [21]. In contrast, other researchers reported a significantly lower prevalence of 5.71% as evaluated by an ELISA assay among 16 non-vaccinated goat flocks in Khuzestan province [22]. In another ELISA assay investigation, 9.7% of the samples were positively reported for sheep and goats in Khorasan Razavi province [23]. Moreover,

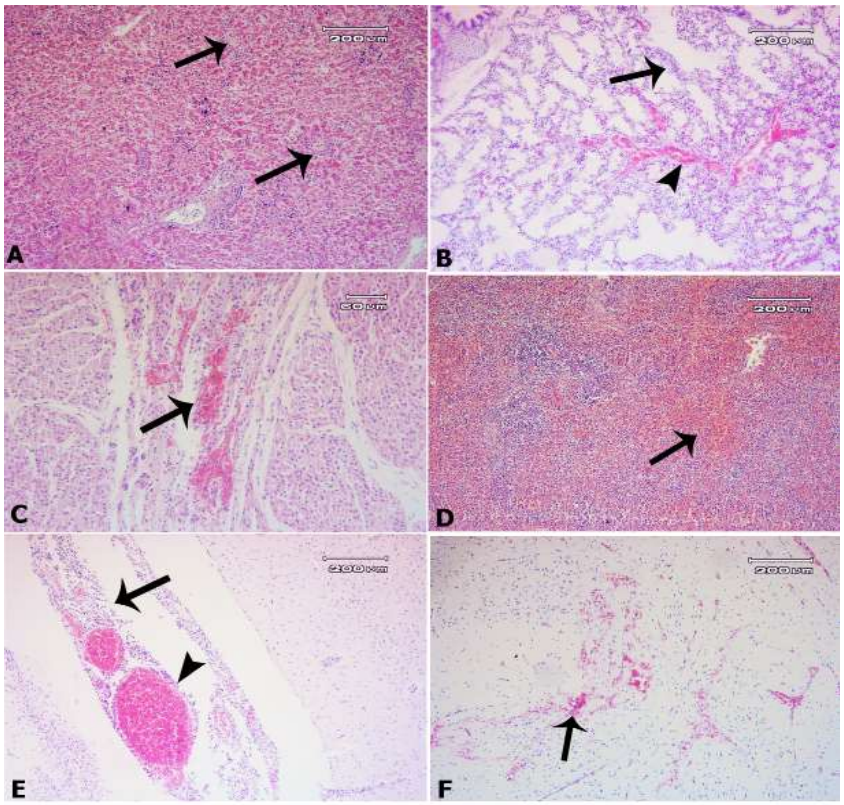

Figure 2.
A, liver: there are focal areas of cellular necrosis, surrounded by mononuclear cells (arrows). B, lung: The alveolar septa are thickened by mononuclear cells, which are associated with hyperemia of the pulmonary vessels. C, focal hemorrhage (arrow) in the cortex of the kidney. C, muscles: there are focal hemorrhages. D, spleen: there is lymphatic depletion with moderate hemorrhage E, brain: there are inflammatory cells in the meninges associated with notable hyperemia (arrowhead). F, brain: there is a focal hemorrhage (arrow). H&E.

the prevalence of Chlamydia infection in vaginal and ocular swab samples of small ruminants was 24.1% by real-time PCR [24]. Besides, the results of the nested PCR of the milk samples showed that 8.61% of milk samples were infected [25]. The seroprevalence of chlamydiosis in sera samples from sheep and goats with a history of abortion was 4.3% in sheep and 21.7% in goats, respectively, as determined by the indirect ELISA assay in Fars province [26]. Also, it was reported a prevalence of 21.6% in vaginal swab samples of sheep and goats by PCR method in Kerman province [27]. In studies in which samples were from aborted fetuses, similar variability in prevalence was reported. In this regard, evidence indicates infection rates of 0%, 11%, and 23.5% in aborted fetuses of small ruminants from southeast [28], south [29], and northwest [30] Iran, respectively, as determined by the PCR method. Studies in other countries have also reported different prevalences. In Sulaimani province, Iraq, a low prevalence of 3.33% was detected in aborted fetus samples of sheep [31]. While in north-western Italy, a higher seroprevalence was observed, with 56.6% of sheep and goat flocks testing positive for C. abortus antibodies. Sheep flocks had a higher prevalence of 71.4%, compared to 44.8% in goat flocks [32]. A study conducted in Africa found a much lower prevalence of 2% for C. abortus in small ruminants using an indirect ELISA assay [33]. This shows the correlation between prevalence and geographical differences. Emerging evidence shows that the global prevalence of C. abortus is 20.1% in sheep and 14.4% in goats, with notable regional differences. The highest prevalence has been reported in South Asia, at 30.6%, while East Asia and Oceania have the lowest rate at 14%. European countries like Romania, Hungary, and Germany reported high prevalences, ranging from 53.3% to 87%. In contrast, lower rates were found in Costa Rica, Australia, and Zimbabwe, ranging between 4.7% and 5.2% [34]. In Chlamydia infection, abortion is likely the result of several factors, including tissue destruction by Chlamydia, vasculitis, thrombosis, and a fetal inflammatory response, such as the production of TNF-α by fetal macrophages [16], In the present study, the main pathological lesions included focal necrotic hepatitis, mild interstitial pneumonia, and mild meningoencephalitis associated with focal hemorrhage in the muscles, spleen, and brain. Similar pathological findings previously described by others [16], are in agreement with our results.

Conclusion

In conclusion, the prevalence of Chlamydia infection in small ruminants shows significant variation across different regions, affected by other factors such as management practices and environmental conditions. Studies have reported prevalence rates ranging from as low as 2% in Africa to over 70% in some flocks in Italy, with significant regional differences existing globally. PCR and ELISA are common methods used for detecting infections in most studies, each with its benefits. The type of samples collected, like sera, aborted fetuses, and milk, also affects detection. Our study showed that 33.87% of the samples tested positive for Chlamydia using genus-specific primers in PCR. This significant prevalence highlights the need for more effective strategies to manage this infection and emphasizes the importance of increasing efforts toward prevention in the region for public health.

Materials & Methods

Ethical approval

All relevant international, national, and institutional guidelines for the care and use of animals, including the protocol approved by the University of Tabriz's Animal Research Ethics Committee (ID: IR.TABRIZU.REC.1403.049), were followed.

Study area

The present study was performed in seven cities in the East-Azerbaijan province in northwest Iran, including Tabriz, Charuymaq, Khoda Afrin, Jolfa, Heris, Bostan Abad, and Mianeh. This study presents findings on Chlamydia infection as part of a larger investigation into the infectious and non-infectious causes of abortion in small ruminants in East Azerbaijan province, northwest Iran. For this purpose, from November 2023 to February 2024, a total of 62 aborted fetuses were collected from sheep and goat flocks in the mentioned regions, as the owners had contacted for abortions on their farms. We studied a total of 43 sheep flocks, documenting the history of the sampled herds, which included details such as herd size and abortion rate. The sheep flocks studied were categorized by size: 7 small flocks (1–100 sheep), 19 medium flocks (101–300 sheep), and 17 large flocks (over 300 sheep).

Sampling

All samples of dead fetuses belonged to the herds with the traditional conditions. At first, the age of the aborted fetuses was estimated using the formula $(X + 17) \times 1/2$, where X is the size of the fetus in centimeters, which were measured from forehead to tail. Then, a systematic necropsy was performed, and the pathological lesions were recorded. Next, 50 mg of the abomasal contents was placed in a 2 mL microtube and stored in a freezer at -70 °C for further molecular studies. Additionally, tissue samples from various organs, including the liver, kidneys, lungs, muscles, spleen, and brain, were collected and transferred to a 10% formalin solution for histopathology purposes.

Pathological study

The tissue samples were kept in a 10% neutral buffered formalin solution for at least 48 hours. After that, they were routinely embedded in paraffin using a DS2080/H tissue processor (Didsabz, Iran) and cut into 5 µm thick sections. These sections were stained with common hematoxylin and eosin (H&E) and studied under a light microscope (Olympus, CH-30, Japan), with the observed lesions recorded.

Molecular studies (DNA extraction and PCR assay)

The genomic DNA (gDNA) of the abomasal contents was extracted using a DNA extraction kit® (Pishgam Sanjesh, Tehran, Iran) based on the manufacturer's instructions. The genome's quality and quantity were analyzed using NanoPhotometer® NP80 (IMPLEN, Germany). All PCR assays were performed in a final volume of 25 µL with Taq DNA Polymerase Master Mix RED® (Ampliqon, Denmark) and 3 µL DNA/cDNA, using the T100 Thermal Cycler (Bio-Rad, USA). The amplified products were detected through electrophoresis on 1.5% agarose gels stained with a safe DNA stain (SinaClon, Iran). To perform a PCR test, specific primers for Chlamydia abortus were used: CHLAMA1 (Forward: 5'-CTCACCATTGTCTCAGGTGGA- 3') and CHLAMA2 (Reverse: 5'-ACCGTAATGGGTAGGAGGGGT-3'), targeting an 823 base pair (bp) sequence. Moreover, C. abortus ATCC VR656 was used as the positive control. The reaction conditions included 35 cycles and an annealing temperature of 59°C.

Statistical analyses

The Chi-Square test was used to determine the correlations between infections and age groups (four groups, including 2-3, 3-4, 4-5, and over 5 months old) of the fetuses. Differences were considered significant at $p < 0.05$. The analyses were performed with IBM SPSS Statistics v.22 software. Also, the data was assessed using a 95% confidence interval (CI).

Authors' Contributions

Conceptualization: MKh, HS, JSh; Methodology: MKh, HS, JSh, SB, FJA, KN, FM, and AH; Software: MKh, FJA, FM; Writing/preparation of original draft: MKh, HS, FJA, FM; Writing, review and editing: MKh, HS, JSH, SB, FJA, FM, KN, and AH; Supervision, project administration and funding acquisition: MKh; All authors have read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of the interest

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RESEARCH ARTICLE

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Assessing the Reducing Effect of Coenzyme Q10 on Carbendazim-Induced Testicular Tissue Dysfunction Through Modulation of miR-202-5p/Apoptosis Signaling in Rats: A Histological and Immunohistochemical Study

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ABSTRACT

Widespread application of carbendazim (Carb) in agriculture and veterinary is a major environmental concern because of its residues that disrupt spermatogenesis. Recently, coenzyme Q10 (CoQ10) supplementation has demonstrated various health benefits due to its anti-apoptosis and anti-inflammatory nature. Thus, the present study aimed to investigate the possible mechanistic pathway of CoQ10 in Carb-induced reproductive dysfunction in male rats. Adult male Wistar rats were orally exposed to Carb (150 mg/kg) singly or in combination with CoQ10 (200 mg/kg). The rats received their treatments daily for 9 weeks. At the end of the work, the testis specimens were excised for histological (H & E staining), immunohistochemical, hormonal, and molecular (real-time quantitative PCR) assessments. The Carb group showed adverse testicular alterations confirmed by immunostaining and demonstrated a significant upregulation of Bax and Caspase-3 expression, while exhibiting a notable reduction in the immunopositivity of Bcl-2 protein within the testes of rats. Real-time PCR analysis revealed that Carb treatment decreased the expression of miR-202-5p with a concomitant decline in concentrations of testosterone and LH hormones. Conversely, in Carb-treated rats, co-treatment with CoQ10 restored the tissue architecture, hormonal disturbance, and declined apoptotic index to near control level. In addition, high expression of miR-202-5p was observed in the Carb + CoQ10 group, and testicular tissues returned to nearly normal histological architecture. We concluded that Carb causes adverse testicular alterations via miR-202-5p/apoptosis pathway, and CoQ10 may prove useful in combating Carb-induced adverse effects via its anti-apoptotic and gene regulatory effects.

Keywords

Histology, Testis, Carbendazim, Rat, Coenzyme Q10

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Abbreviations

Carb: Carbendazim

DNA: Deoxyribonucleic acid

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Introduction

Environmental pollutants are considered the most hazardous problems worldwide. The excessive use of agricultural fungicides is one of the principal causes of environmental pollution. Generally, fungicides are widely used in agriculture to enhance crop growth and yield by controlling fungal diseases]1[. Carbendazim (Carb: methyl-2-benzimidazole carbamate) is a highly effective benzimidazole fungicide against a wide range of fungi and is widely used throughout the world]1-3[. Meanwhile, the Carb could enter into human or animal bodies through skin absorption, as well as food or drinking water contamination]1[. Also, this substance is often transported through rain to food and water sources and seriously harms human and animal health]4, 5[. Therefore, Carb is one of the most widespread environmental contaminants of major concern to human and animal health]6[. Currently, the Carb has been recognized as a testicular toxicant in male rats]3[. Also, the Carb could induce male reproductive toxicity in different model animals]1,3, 7[. Carb has also been confirmed to interfere with hematopoiesis and metabolism, and induce chromosomal abnormalities by damaging DNA in various tissues]8[. Accordingly, exposure to Carb leads to testicular tissue abnormality and, consequently, impaired spermatogenesis. Consequently, these effects lead to testicular tissue disorders and reduced fertility in male rats]3,9-12[. An increase in the incidence of testicular germ cell apoptosis is a commonly reported occurrence after Carb exposure]13[. Also, according to previous studies, exposure to Carb adversely affects the testes, resulting in suppression of steroidogenesis, induction of oxidative stress, and apoptosis in rats' testes]14-15[, which consequently leads to degeneration of germinal tubules and loss of spermatogenic cells.

Coenzyme CoQ10 (CoQ10), also known as ubiquinone, is a useful compound naturally found in some substances. This molecule also functions as a crucial cofactor within the electron transport chain, playing an integral role in oxidative phosphorylation and adenosine triphosphate (ATP) production within the mitochondria]16[. Furthermore, CoQ10 supplementation has been found to be advantageous in both the treatment and prevention of several health issues, including testicular dysfunctions]17[. Due to its anti-apoptosis, antioxidant, and anti-inflammatory properties]18,19[; a lot of research has been done recently on the protective effects of CoQ10 against male infertility and sperm abnormality caused by different chemical compounds. For example, the ameliorative effect of CoQ10 against the toxicity caused by bisphenol-A has been investigated in the testicles of rats. Based on this, oral treatment of animals with CoQ10 at the rate of 10 mg/kg of body weight per day for 14 consecutive days combats cellular stress, improves the testicular structure, and consequently enhances the quality and viability of sperm cells]20[. In another study, supplemental dietary CoQ10 was reported to enhance testicular functions by inhibiting lead accumulation, oxidative stress, inflammation, cell apoptosis, and restoring the adverse histological changes in rats treated with lead acetate. Therefore, this substance has been considered a natural therapeutic agent to protect against reproductive disorders associated with exposure to lead acetate]21[. However, the role of CoQ10 in Carb-caused testicular dysfunction is still unclear.

Spermatogenesis is a distinct biological process that occurs within the seminiferous tubules of the testes. Sertoli cells inside the seminiferous tubules and Leydig cells in the interstitial space of testicular tissue play an essential role in starting and maintaining sperm growth and also in regulating male hormone production]22[. The growth and function of the testis are strictly regulated by microRNAs (miRNAs), which regulate the expression of numerous protein-coding genes associated with cellular differentiation in the male reproductive system. Also, different types of male infertility, such as asthenospermia, oligospermia, azoospermia, and teratozoospermia, have been evaluated using miRNAs as molecular biomarkers]23-24[. Additionally, the processes of spermatogonial differentiation and the initiation of meiosis during spermatogenesis are subject to stringent regulation by various genes, including those that encode enzymes involved in the biogenesis of miRNAs]25[. Moreover, there is a large set of miRNAs within the male reproductive system that play vital roles in the mammalian spermatogenesis by modulating the expression of protein-coding genes involved in different cell maintenance, predominantly Sertoli cells and Leydig cells]23,26-27[. As reported, miR-202 plays a crucial role in inhibiting premature spermatogonial differentiation and the initiation of meiosis during spermatogenesis in mice, and its silencing is accompanied by the age-dependent decline of fertility]25]. However,

Abbreviations-Cont'd

CoQ10: Coenzyme CoQ10
ATP: Adenosine triphosphate
miRNAs: microRNAs
Carb + CoQ10: Carbendazim and Coenzyme CoQ10
FSH: Follicular Stimulating Hormone
LH: Luteinizing Hormone
H&E: Hematoxylin and Eosin stain
ELISA: Enzyme-linked immunosorbent assay
IHC: Immunohistochemistry
RT-PCR: Real-time polymerase chain reaction
ROS: Reactive oxygen species

whether and how miR-202-5p may affect the toxicity of Carb remains unclear.

Despite the information mentioned above, the current literature on the possible protective role and the mechanisms of CoQ10 supplementation against adverse reproductive effects caused by Carb is limited. Therefore, our objective was to examine the possible effect and mechanism of CoQ10 on Carb-induced testicular apoptosis, hormonal dysfunction, and the expression patterns of miR-202-5p.

Results

Animals of different groups did not have any macroscopic anatomical changes. Histological examinations of the control and CoQ10 groups revealed a normal histoarchitecture of the interstitial Leydig cells and seminiferous tubules, which exhibited a sequential arrangement of germ cells at various stages of differentiation, with the lumens filled with spermatozoa. In contrast, the Carb-treated group displayed irregular degenerative changes in the epithelium of the seminiferous tubules, accompanied by hyperemia of the blood vessels, interstitial edema with eosinophilic secretions, and infiltration of inflammatory cells (Figure 1b). The Carb-evoked changes in the histological aspect of the testes were partially restored by CoQ10 therapy, but there were still bubbly areas in the thickness of the seminiferous tubules that lacked germ cells (Figure 1c).

These above-mentioned findings were also confirmed by quantitative histomorphometric examination. The histomorphometric evaluation of the testes revealed a significant reduction ($p < 0.05$) in quantitative parameters, such as epithelial thickness, tubular diameter, and Leydig cell count within the interstitial tissue of rats administered with Carb (Figure 2). In contrast, the measurements of the epithelial thickness, tubular diameter, and Leydig cell count of the Carb + CoQ10 group were found to be substantially higher than those recorded in the Carb-treated group (Figure 2).

To determine whether Carb-induced abnormalities occur via the apoptosis process, the expression levels of the pro-apoptotic protein Bax, caspase-3, and the anti-apoptotic protein Bcl-2 were evaluated using immunohistochemistry techniques. Compared with the control group, Carb exposure significantly increased the immunoreactive activity of Bax and Caspase 3, as well as decreased the immunoreactivity of bcl-2 protein in tissue sections of the testis. Otherwise, the declined immunoreactive activity of Bax

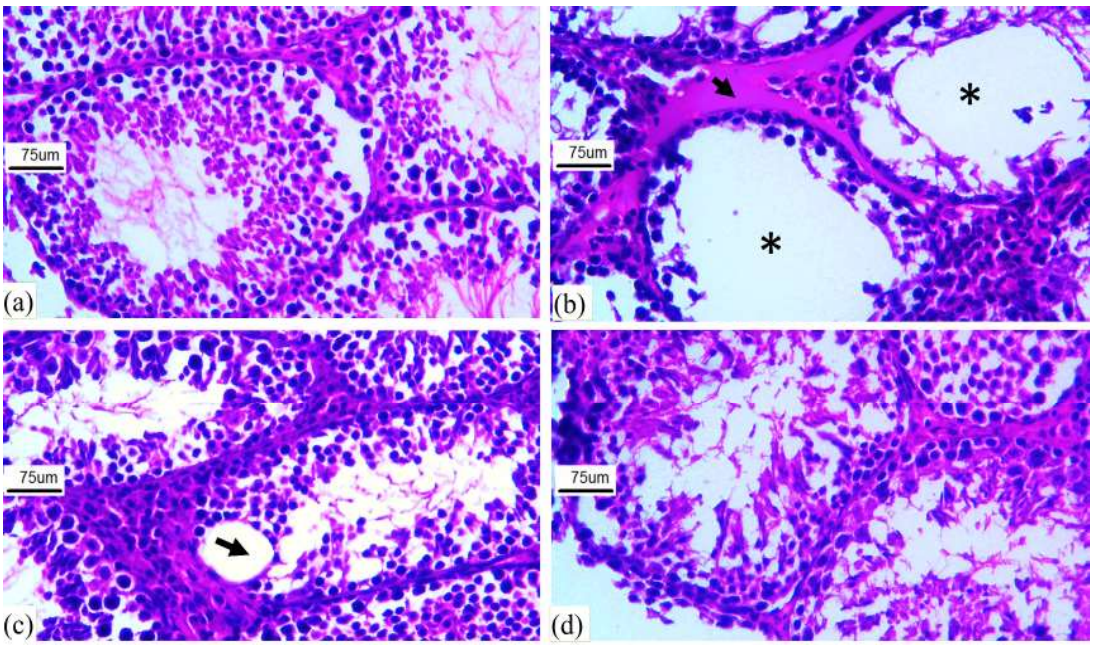

Figure 1.
Photomicrograph of Tissue Sections of Testicular Parenchyma in the Control and Carb-treated Groups, Stained with the Hematoxylin-Eosin Method. A, Control group: the regular morphology of the seminiferous tubules with the accumulated lumen of spermatozoa is seen. B, Damaged testicular tissue in the Carb-treated rats with decreased cell density (stars), especially spermatozoa, detachment of germinal epithelium, and depletion of the interstitial tissue edema (black arrows). C, Testis structure in the Carb + CoQ10 group shows partial improvement of testicular tissue toward normal appearance, although there are still bubble-like areas in the thickness of the seminiferous tubules that lack germ cells (black arrows). D, testicular tissue in the CoQ10 group shows normal tissue architecture similar to the control group. (H&E staining, 100×, scale bar = 75 µm).

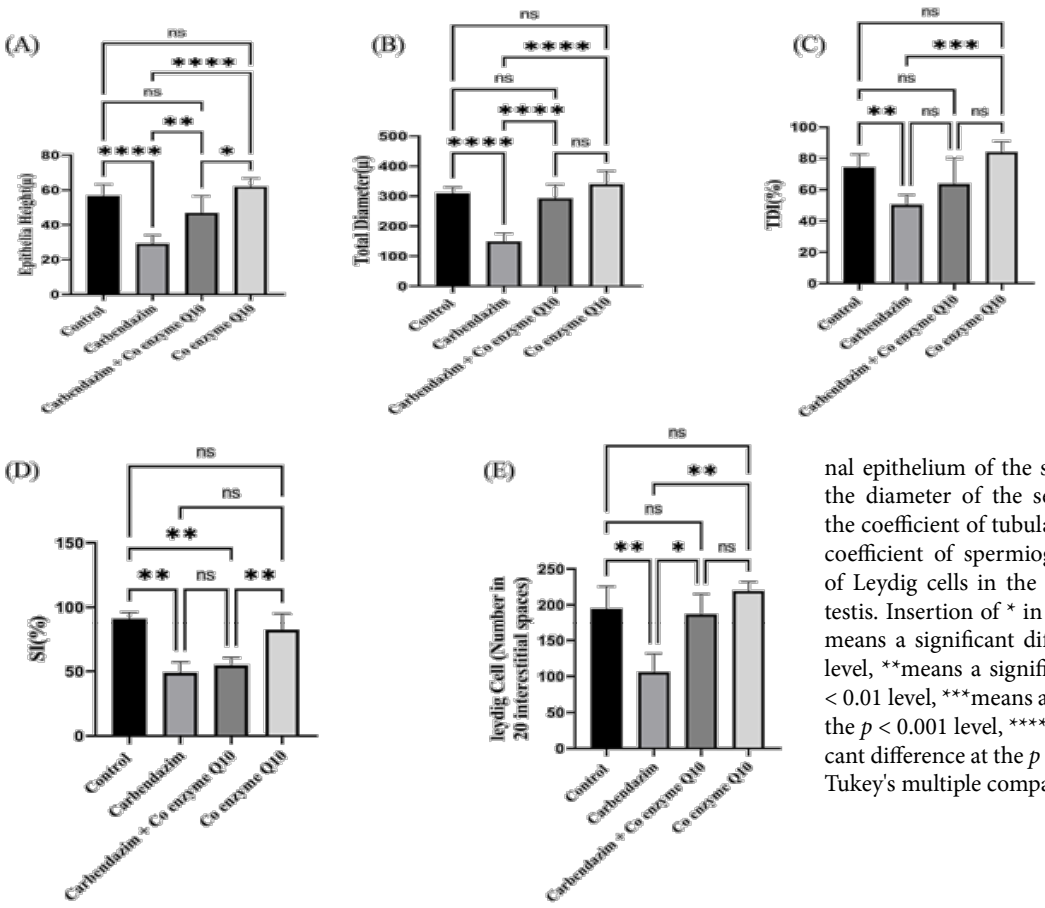

Figure 2.
The Protective Effect of CoQ10 on the Histomorphometric Criteria of Testicular Tissue Sections in the Different Groups of Study. A, The thickness of the germinal epithelium of the seminiferous tubules. B, the diameter of the seminiferous tubules. C, the coefficient of tubular differentiation. D, the coefficient of spermiogenesis. E, the number of Leydig cells in the interstitial tissue of the testis. Insertion of * in the top of each column means a significant difference at the $p < 0.05$ level, **means a significant difference at the $p < 0.01$ level, ***means a significant difference at the $p < 0.001$ level, ****means there is a significant difference at the $p < 0.0001$ level. ANOVA, Tukey's multiple comparisons test.

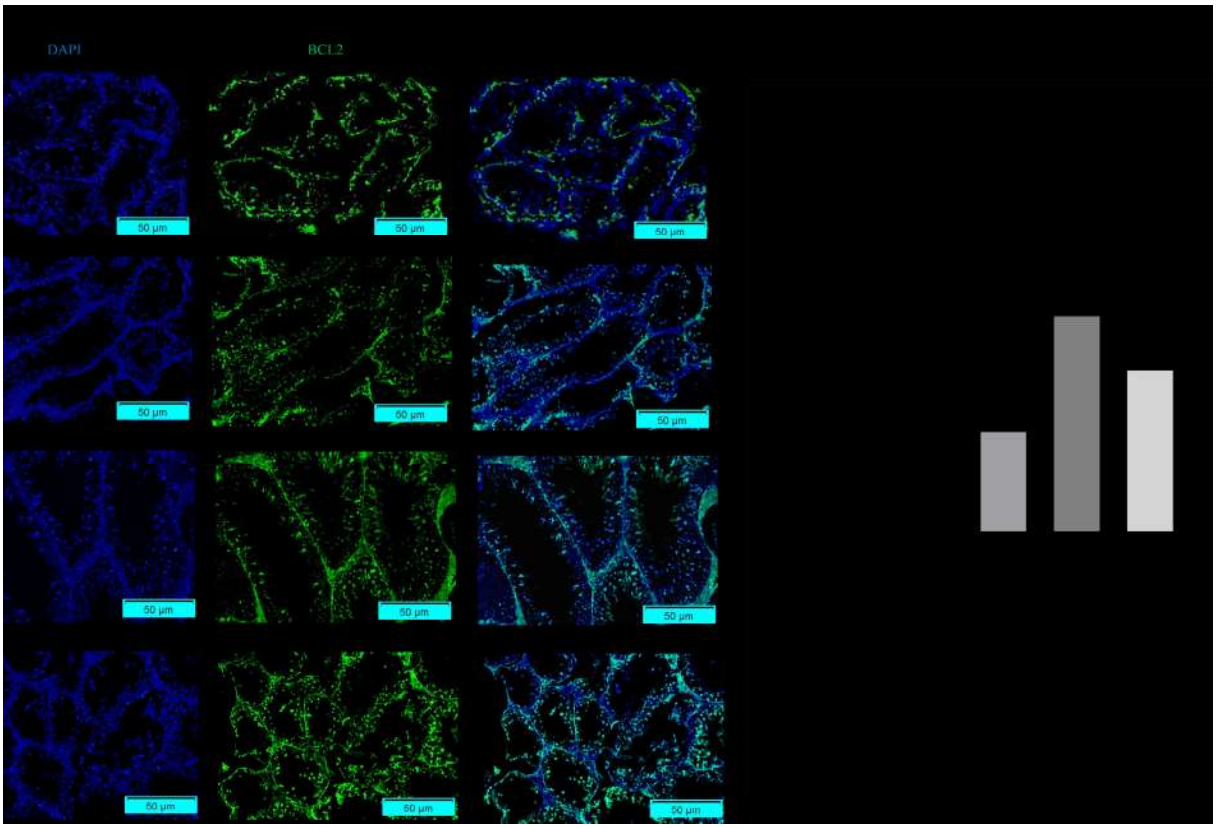

Figure 3.
Photomicrograph of Tissue Sections of Testicular Parenchyma in Different Studied Groups, Stained by IHC Method to Check Bcl-2 Protein Expression Level. A, Bcl-2 protein expression values in testicular tissue. B, Bcl-2 protein expression quantification chart compared to the control group.

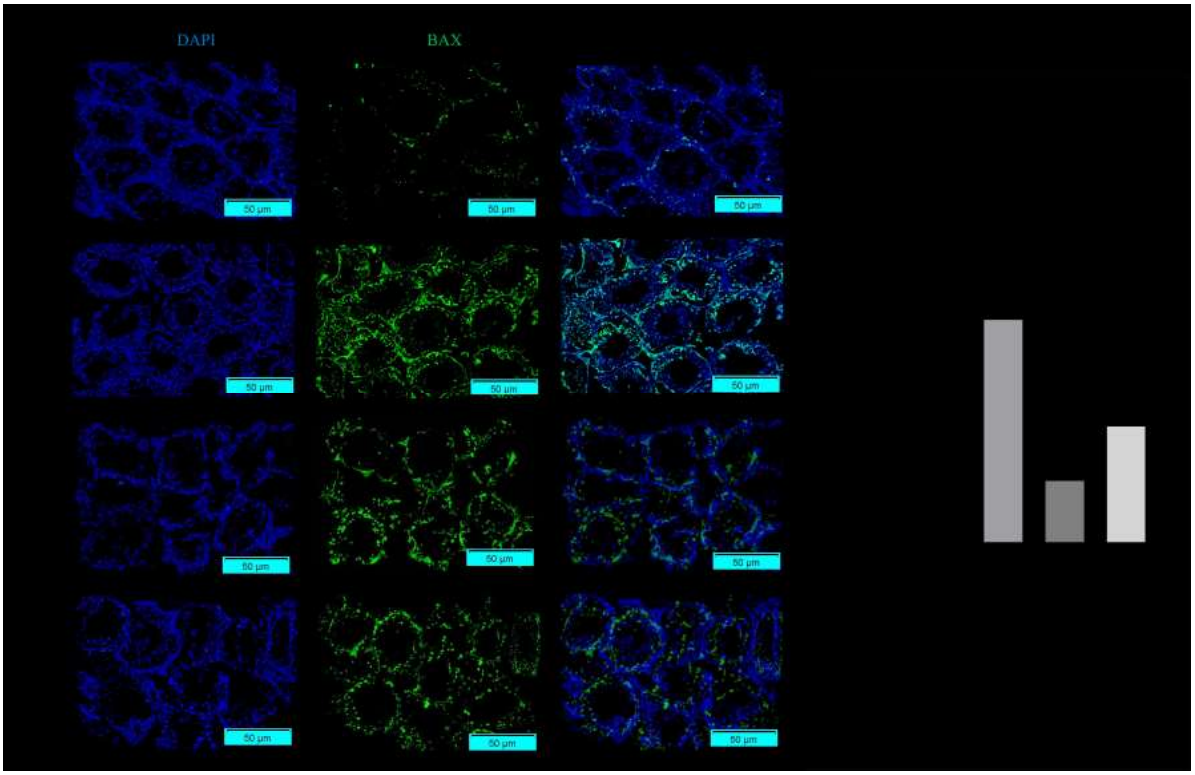

Figure 4.
Photomicrograph of Tissue Sections of Testicular Parenchyma in Different Studied Groups, Stained by IHC Method to Check Bax Protein Expression. A, Bax protein expression values in testicular tissue. B, Bax protein expression quantification chart compared to the control group.

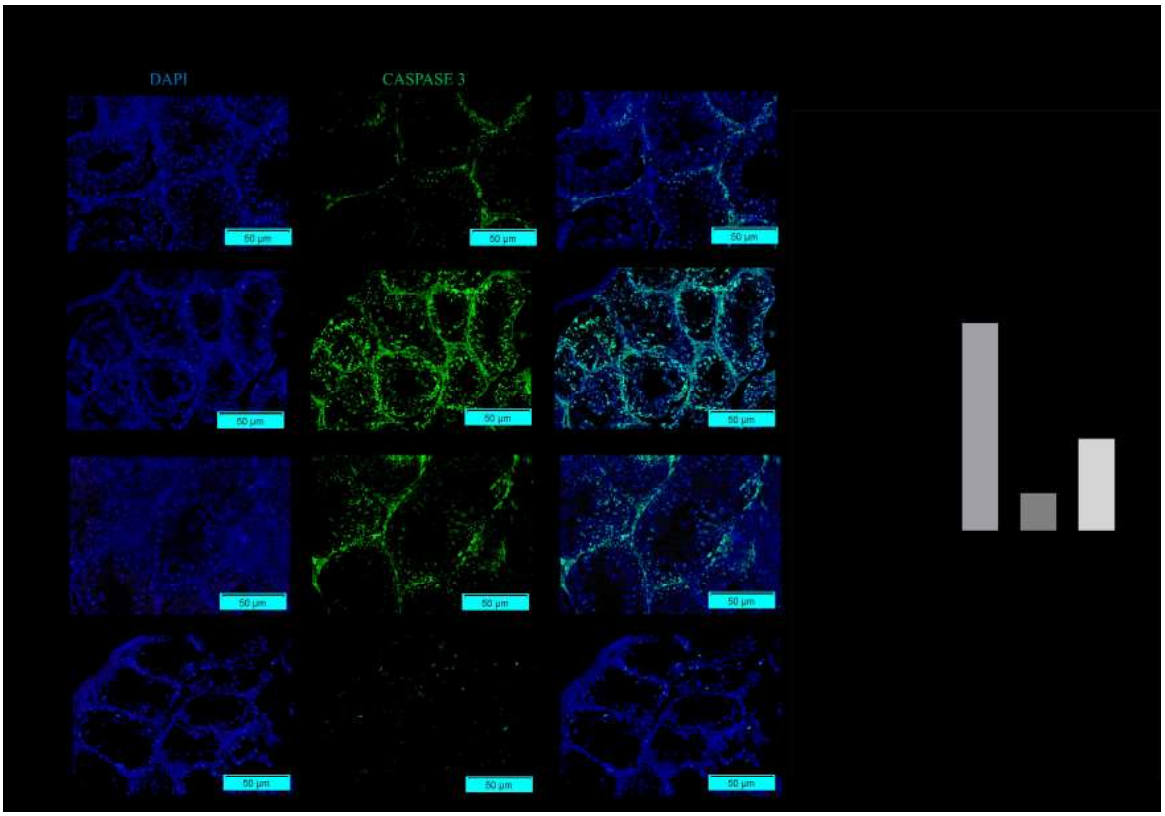

Figure 5.
Photomicrograph of Tissue Sections of Testicular Parenchyma in Different Studied Groups, Stained by IHC Method to Check the Level of caspase 3 Protein Expression. A, Caspase 3 protein expression values in testicular tissue. B, Quantification diagram of caspase 3 protein expression compared to the control group.

and caspase 3, as well as the enhanced immunoreactivity of Bcl-2, were seen in the testis sections of the Carb + CoQ10 rats, compared to the Carb group. These results mean a significant modulation in the apoptosis-related proteins when CoQ10 was combined with Carb administration (Figures 3a to 5a). In this regard, the quantification assessments of immunoreactivity of Bax, Caspase 3, and Bcl-2 confirmed the histological results. Consequently, it can be inferred that CoQ10 mitigates Carb-caused testicular apoptosis by elevating the levels of pro-apoptotic proteins while concurrently reducing the levels of anti-apoptotic protein (Figure 3b to 5b).

Figure 6 depicts how Carb and CoQ10 therapy altered testosterone and LH hormones across various groups. Specifically, the levels of testosterone and LH in the Carb-treated group were significantly reduced in comparison to the control and Carb + CoQ10 groups. By contrast, compared to the Carb alone group; co-administration of CoQ10 with Carb could modulates the serum testosterone and LH levels significantly. FSH levels did not show significant between group differences (Figure 6).

The basal amount of miR-202-5p in the testicular tissue of different groups was assessed by the real-time

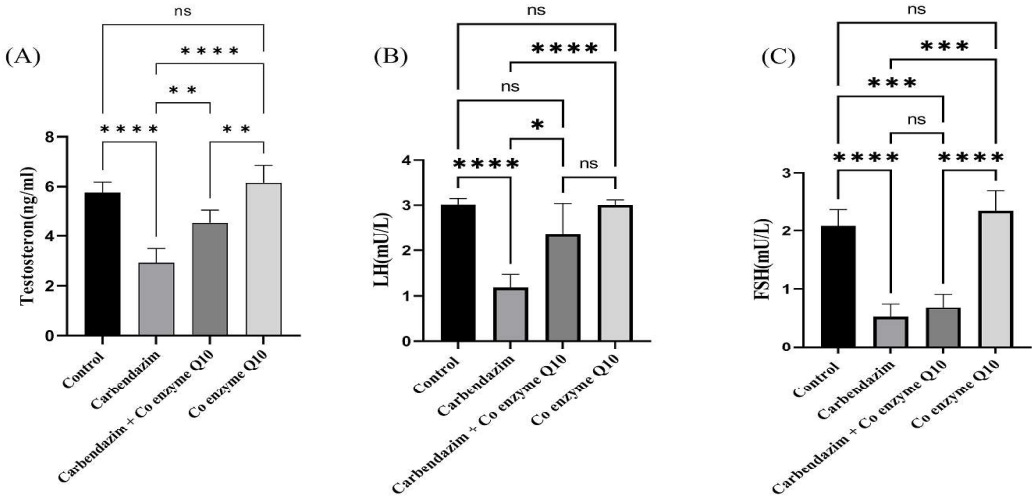

Figure 6.
The Protective Effect of CoQ10 on the Hormonal Function Criteria of the Hypothalamus-Pituitary-Testis Axis in all Groups. Values are presented as mean ± standard error of the mean (n=7). A, serum testosterone concentration. B, serum luteinizing hormone (LH) concentration. C, serum follicle-stimulating hormone (FSH) concentration. Insertion of * in the top of each column means a significant difference at the $p < 0.05$ level, ** means a significant difference at the $p < 0.01$ level, *** means a significant difference at the $p < 0.001$ level, **** means there is a significant difference at the $p < 0.0001$ level. ANOVA, Tukey's multiple comparisons test.

PCR analysis. To check the specificity, sensitivity and confirmation of the accuracy of real-time PCR products, the standard diagram of efficiency, amplification, and drawing of the melting curve was used. The highest expression of miR-202-5p was seen in the testicular tissue in the control and CoQ10-receiving groups. However, compared with the normal control rats, the Carb-administered group expressed a low level of miR-202-5p. By contrast, the expression level of miR-202-5p was significantly upregulated in Carb + CoQ10 compared with Carb-exposed rats (Figure 7).

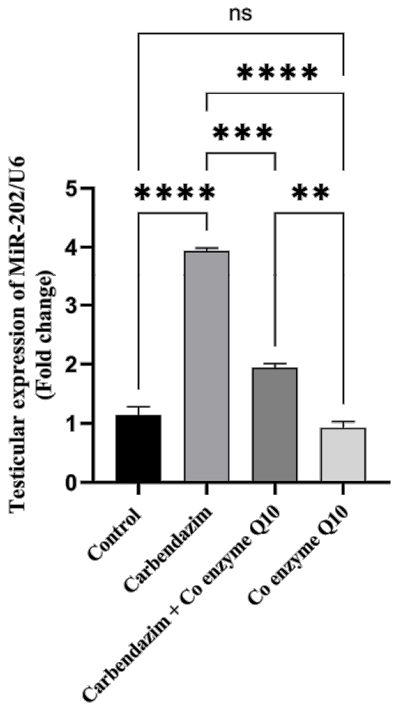

Figure 7.
The Real-time qPCR Results Regarding Regulatory Effect of CoQ10 on the Expression of miR-202-5p in Testicular Tissues of All Groups. Insertion of * in the top of each column means a significant difference at the $p < 0.05$ level, ** means a significant difference at the $p < 0.01$ level, *** means a significant difference at the $p < 0.001$ level, ****means there is a significant difference at the $p < 0.0001$ level. ANOVA, Tukey's multiple comparisons test.

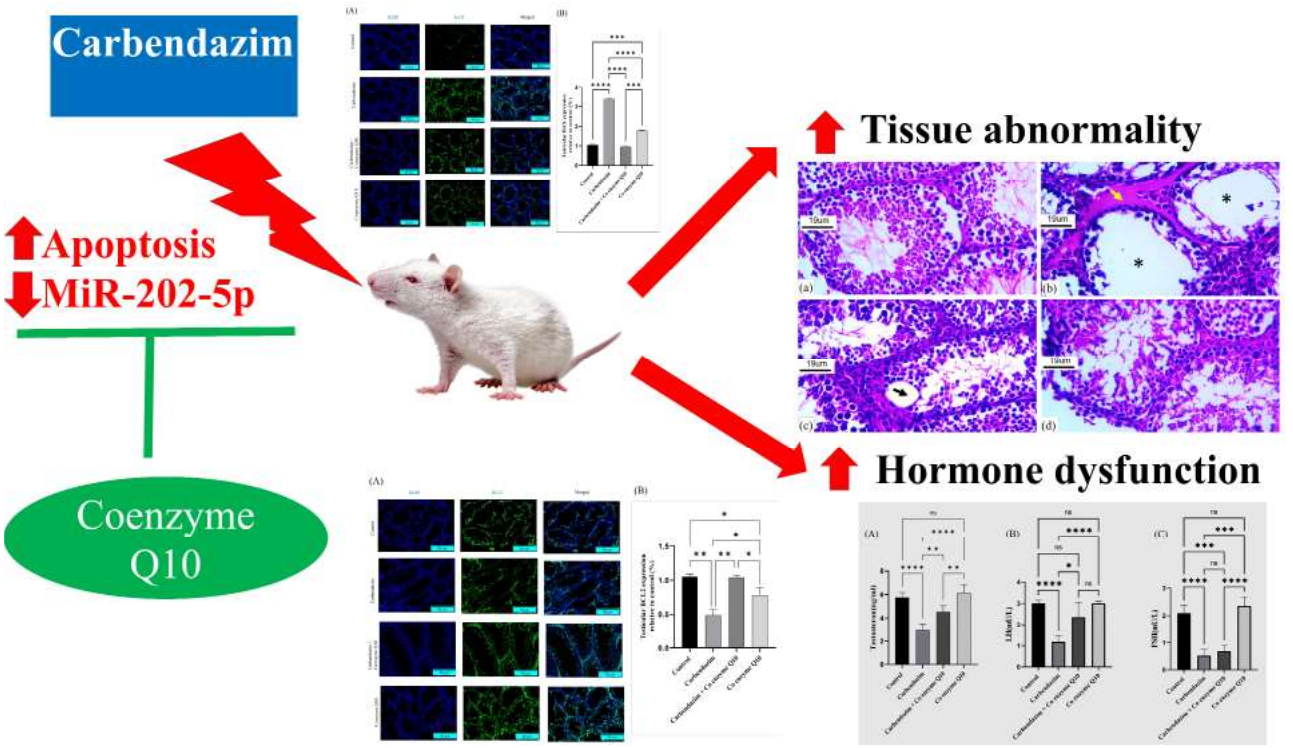

Figure 8.
Representative Summary of the Protective Role of CoQ10 Against Carb-Induced Testicular Alterations in Rats

Discussion

In the past few decades, male reproductive toxicology has attracted increasing concern because of frequent contact with chemicals due to lifestyle hypothesis and contamination of soil, drinking water sources, and agricultural products. Also, these toxicants significantly worsen sperm quality]34-35[. Carb is widely used in the control of various agricultural pathogens, and its chronic usage has hazardous impacts on the male genital system, which appears as a critical health issue]36,7,37[. Our findings indicated that Carb administration causes testicular toxicity via induction of apoptosis and downregulation of miR-202-5p. In this regard, abnormal testicular histoarchitecture induced by Carb exposure via induction of apoptosis has been reported by the previous researcher]15,37[. Conversely, treatment with CoQ10 in this study was associated with the improvement of miR-202-5p level, which coincided with the suppression of apoptosis (Figure 8). It was demonstrated that CoQ10 has gonado-protective effects against bisophenol A-induced toxicity]38[and is regarded as a clinical agent mainly for the medical treatment of asthenospermia-caused male infertility] 39[. Carb intoxication adversely led to changes in the hypothalamus-pituitary-testis axis's functionality along with a significant increase in experimental tissue injury scores, which was significantly mitigated by CoQ10. Previous research has reported similar results, including the disruptive effect of Carb on the endocrine glands]7[.

In the group treated with Carb + CoQ10, a significant improvement was observed in the histological and histomorphometric criteria of the testis compared to the Carb group. On the other hand, administration of CoQ10 in animals treated with Carb increased the immunoreactive activity of bcl-2 protein and decreased the immunoreactive activity of bax and caspase 3 proteins compared to the Carb group. These findings may imply the anti-apoptosis characteristic of CoQ10. Currently, CoQ10 has unique properties that make it potentially beneficial in a variety of testicular injury situations. First, endogenous CoQ10 is concentrated in the inner mitochondrial membrane in the middle part of sperm and regulates the bioenergetics of sperm mitochondria]40[. The development of testicular cells requires the active presence of bioenergy, and CoQ10 serves as a cofactor for the mitochondrial electron transport chain during the production of ATP. Consequently, CoQ10 is integral to cellular bioenergetics, which has facilitated its clinical use in addressing tissue-related disorders]41]. Second, in addition to its function in ATP production, CoQ10 exhibits properties as a lipophilic antioxidant, functioning as an effective scavenger of ROS and possessing anti-apoptotic effects within the testicular environment]38[. Third, CoQ10 is naturally found in semen, and its amount in the seminal fluid of men correlates with their sperm count and sperm motility]39[. Therefore, its positive effect on

the structure and function of the testis can be due to its involvement in the cellular processes of the testis. In the present study, in confirmation of CoQ10-induced structural improvement of testicular histology, lower testosterone and LH levels were reversed by CoQ10 supplementation, which implies amelioration of Carb-induced spermatogenic damage. These results are supported by those of El-Khadragy et al., who demonstrated a stronger inhibition of apoptosis and testicular histopathological changes in mice co-administered with CoQ10 and lead acetate]42[. Moreover, it has been stated that adding CoQ10 and L-carnitine to semen in vitro can improve sperm motility]39[. In addition, in another study, CoQ10 could protect the testes against methotrexate-caused gonad toxicity in mice. This ameliorative effect of CoQ10 was attributed to its antioxidant and anti-apoptotic properties and modification of the Bax/Bcl2 ratio]43[.

Many researchers have tried to discover the mechanisms underlying Carb's disruption of sperm production and infertility. They have found that Carb directly adversely affects testicular function by changing histone related to estrogen receptor, DNA methylation, and epigenetic pathways, causing testicular failure and infertility]7,37[. Also, a decrease in the concentration of LH hormone has been reported in animals exposed to Carb]36[. However, the mechanisms by which Carb impairs spermatogenesis are not fully understood. In the present study, in search of the underlying mechanisms, we investigated for the first time the relationship between miR-202-5p expression and Carb intoxication. We have chosen to investigate miR-202-5p further due to its significantly elevated expression levels in the testis relative to other tissues]24,44[. The results of this research determined that the relative expression of miR-202-5p in the Carb group diminished significantly compared to no treatment. Hence, it can be concluded that Carb accelerated the process of apoptosis and adverse histological changes by decreasing miR-202-5p expression in the rat testis. The high expression of miR-202-5p in somatic and testicular germ cells during men spermatogenesis has been reported, its expression is significantly higher in fertile compared to infertile men]45[. Also, miR-202-5p has been identified as one of the four testis-specific miRNAs in bull and testis of monkey in healthy conditions and hyperthermia-induced injuries]46,47[. Furthermore, there is evidence that miR-202-3p could regulate the biological functions of human Sertoli cells]48[. Accordingly, as a regulator of cell cycle in murine germ cells, miR-202 could maintain the differentiation of male stem cells by modulating RNA-binding proteins]44[.

In the current study, CoQ10 modulated the level of miR-202-5p, decreased the immunoreactivity of pro-apoptotic and apoptotic proteins, and increased the levels of anti-apoptotic proteins. This led to the improvement of the quality of the tissue structure of the testis and, as a result, the efficiency of its function in spermatogenesis and hormone secretion. This implies the possible association between miR-202-5p expression, suppression of apoptosis, and improvement of histological structure and function of the testis in the case of CoQ10 administration. MiR-202 is localized in human and murine testicular Sertoli cells]45,49[, and its expression mediates some of the determinant effects of SOX9 in early gonadal development and gonadal differentiation of mouse embryos]49[. In the current work, the expression level of miR-202-5p was significantly upregulated in Carb + CoQ10 compared with Carb-exposed rats. So far, no study has investigated the effect of CoQ10 on the expression of miR-202-5p in the testis. This issue highlights the novelty and innovation of the current research.

The limitation of this study was that markers related to oxidative stress were not measured. We could measure the total antioxidant capacity and levels of other free radicals in the testes to find the possible mechanism of the negative effect of Carb and the positive effect of CoQ10 on the structure and function of the testis. Another limitation was not testing the spermatozoa in the epididymis of easily euthanized animals to investigate the possible negative effect of Carb and the possible positive effect of CoQ10 on sperm morphology, number, and motility. A third limitation was not testing the testicular structure to look for specific organelles, especially mitochondria, in Carb damage and the possible protective properties of CoQ10.

In summary, we showed that 1) Carb impaired testicular histoarchitecture and consequently testosterone production by damaged Leydig cells. 2) CoQ10 improves testicular damage caused by Carb due to its gene regulation and anti-apoptotic properties. However, only enhanced miR-202-5p expression and reduced immunoreactivity of apoptotic proteins cannot solely explain the strong protective activity of CoQ10 against Carb-induced testis damage. Consequently, in subsequent studies, it is suggested that the modulation of the signaling pathways of downstream and upstream genes of miR-202-5p be considered a significant effect of this beneficial compound. The aforementioned assertion should be the primary hypothesis.

Materials & Methods

Ethical approval

All procedures in this experiment were approved by the Institutional Animal Care and Use Committee of Ilam University with Code of

Ethics ID (IR.ILAM.REC.1402.017).

In order to carry out this study, 28 male Wistar rats were purchased. The rats were allocated into 4 groups consisting of control, Carb (150 mg/kg, gavage), Carb + CoQ10 (150 mg/kg + 200 mg/kg, gavage), and CoQ10. The rats received their treatments daily for 9 weeks]28-31 [.

At the end of the trial period, the animals were anesthetized, and histological samples were collected. The removed samples were fixed in Bouin's solution for three days. The fixed testis underwent a trimming process followed by a 4-hour rinse with running tap water. Subsequently, the tissue samples were prepared using common microscopy methods, including dehydrating by gradually increasing concentrations of ethanol, clearing with xylene, and embedding with paraffin. Then, five micron slices were prepared using a rotary microtome, and testicular sections were subjected to H&E staining. Images were obtained and recorded using a microscope equipped with a high-resolution digital camera. A histomorphometric assessment of testicular tissues was undertaken in six different fields of each section. The quantitative parameters were luminal diameter (µm), epithelium height (µm), number of Leydig cells (number in twenty interstitial spaces), tubular differentiation coefficient (%), and spermiogenesis coefficient (%)]32-33[.

The concentrations of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in the collected serum samples were quantified utilizing rat enzyme-linked immunosorbent assay (ELISA) kits (ZellBio GmbH, Germany), employing the ELISA methodology.

Localization of pro- and anti-apoptotic biomarkers, including Bax, Bcl-2, and Caspase 3 in testicular tissue was done by immunohistochemistry (IHC) according to the manufacturer´s instructions. Antigen retrieval was performed after deparaffinization and rehydration of the testis sections, followed by washing with PBS. In the subsequent step, to inhibit endogenous peroxidase activity, the slides were immersed in a 5% bovine serum albumin (BSA) solution and incubated in 3% H2O2. Following this, anti-Bcl-2 (dilution, 1:100; Santa Cruz Biotechnology, Inc.), anti-Bax (dilution, 1:100; Santa Cruz Biotechnology, Inc.), and anti-Caspase3 (dilution, 1:100; Santa Cruz Biotechnology, Inc.) antibodies were used as the primary antibody. On the next step, Goat Anti-Rabbit IgG (dilution, 1:100) (FITC conjugated; Elabscience Biotechnology Inc.) as the secondary antibody (60 min incubation at ambient temperature). Subsequently, the tissue slices were developed utilizing diaminobenzidine and counterstained with hematoxylin. They underwent a series of processes, including dehydration, clarification, and mounting, before examination with a light microscope (KoreaTek). Following the application of Antifade Mounting Medium containing DAPI (Beyotime, China) at 4°C for a duration of 10 minutes, the positive signals were visualized using a fluorescent microscope (Olympus BX50, Japan). Image J software and Microbin Z5 camera (Media Cybernetics, Inc.) were used to take and analyze immunohistochemistry images. For each tissue section, ten representative microscopic fields were selected randomly. A semi-quantitative analysis on apoptosis-related proteins immunoreactivity score (green for FITC-conjugated antibodies) was then assigned based on the percentage of the protein-positive area compared to the normal control rats.

To examine the expression level of miR-202-5p, the quantitative real-time polymerase chain reaction (PCR) test was used. First, according to the miRcute™ miRNA Isolation (TianGene, China) extraction kit protocol, 50 mg of the tissue was lysed, and after RNA extraction and purification, its quality was measured by a nanodrop device (USA, Scientific Thermo) at a wavelength of 260/280. The miRcute miRNA First-strand cDNA Synthesis Kit (TianGen, China) was used to elongate miRNAs and synthesize cDNA by the polyadenylation system. For detection and amplify of miR-202-5p genes in the testis tissue, SYBR® Green Real Time PCR Master Mix (ParsTous, Iran) was used. Real-time qPCR was performed in 12.5-µl reactions using an Applied Biosystems 7900 HT TaqMan Real-Time PCR System. The comparative expression levels of the examined miR-202-5p were quantified utilizing the 2-ΔΔCt method. U6 snRNA was used as the endogenous control to normalize the expression levels of miRNAs. The primers used were as follows: miR-202-5p-Rat-Forward: CATATACTTCTTTGTGGAT; cDNA adapter reverse: GAACATGTCTGCGTATCTC; U6-Forward: TGCTTCGGCAGCACATATAC; U6-Reverse: AGGGGCCATGCTAATCTTCT.

The data from this research were analyzed by SPSS 23.0 (SPSS Inc., IBM, USA) using descriptive and analytical statistics. To asses the differences, a one-way ANOVA was conducted, followed by Tukey's supplementary test and complemented by the Kolmogorov-Smirnov test to evaluate the data's normality. The non-parametric Kruskal-Wallis test was used to assess the differences between groups and determine if there were statistically non-normal distributions. $P<0.05$ was considered statistically significant, and $p < 0.01$ was considered highly statistically significant.

Authors' Contributions

SK and HS conceived the study, conducted the work, and performed statistical analysis; ALM and HAS did the experiments and wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of the interest

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Effects of Industrial Wastewater on Gross and Histopathological Changes of Vital Organs of Swiss Albino Mice

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ABSTRACT

Industrial wastewater contaminates the land, water, and air, causing serious environmental damage. Industrial wastewater can be combustible, reactive, poisonous, or carcinogenic. Therefore, this study aimed to investigate the effects of industrial wastewater on the growth and gross and histoarchitecture of vital organs of male Swiss albino mice. Thirty-two mice of four weeks of age were divided into four groups. Normal drinking water was supplied to the control group of mice. Mice from groups 1, 2, and 3 were provided with normal drinking water mixed with the garment industry's wastewater at concentrations of 5%, 10%, and 20%, respectively, orally up to experimental week 24. Subsequently, the body weights of mice, as well as the weights of their liver, heart, and kidney, were measured after the completion of 24 weeks of treatment of mice with different concentrations of industrial wastewater. Moreover, histopathological changes in the liver, heart, and kidney were investigated. Body weight was decreased in wastewater-treated mice in comparison to control mice. An increase in the weight of the livers of mice treated with wastewater was observed. Nevertheless, the weights of hearts and kidneys were decreased in wastewater-treated mice. Congestion, hepatocellular necrosis, and infiltration of inflammatory cells were observed in the liver. Disruption of connective tissue was evident in the myocardium of the heart of wastewater-treated mice with necrosis and infiltration of inflammatory cells. Moreover, congestion, cellular necrosis, hypertrophied glomerulus, degeneration in renal tubular epithelial cells, and dilated tubules were evident in the kidney. From these findings, it was concluded that industrial wastewater has detrimental effects on the vital organs of mice.

Keywords

industrial wastewater, histopathology, heart, kidney, liver

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Abbreviations

BOD: biological oxygen demand

COD: chemical oxygen demand

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Introduction

The industrial sector of Bangladesh is growing nowadays. Bangladesh is one of the emerging nations whose economies greatly benefit from industrialization. However, it is a question of opinion that environmental contamination, including air, water, and soil pollution, is invariably associated with industrial development. The main industries that create pollution include those that deal with textiles, tanneries, fertilizer plants, medicines, cement, pulp and paper, etc. Alkaline, acidic pollutants such as benzidine, beta-naphthylamine, sulfide, lime, cadmium, copper, chromium, and numerous hazardous colors, among others, are produced by these industries. Industrial pollutants have a significant impact on the physical and mental well-being of workers [1].

Due to untreated effluent from industries and municipalities being dumped into natural water sources, the Gazipur areas are notorious for being extremely polluted [2]. From the polluted water sources, this water is dispersed into the crop field, which is a major source of food for the people of Bangladesh, which is a highly populated country. Moreover, people and animals get exposed to this wastewater and suffer from various carcinogenic problems, acute and chronic diseases.

The vital organs are considered the most important for survival. It is important to take care of the vital organs for a healthy life. The vital organs of mice are the liver, lungs, brain, heart, and kidneys. Any abnormality of these organs will reflect on the general health condition, which causes great economic losses in animal production [3]. Any kind of pathological condition of organs indicates the presence of disease in organs and systems with all its resident microorganisms [4]. Industrial wastage is very toxic as it contains dyes, alkalis, heavy metals, etc., in high concentration. It has been reported that industrial waste products contain many metals or toxic organic compounds that cause damage to the central nervous system of animals, including humans [5,6]. Some of the metals have a carcinogenic effect as well [5,6]. Several chemicals and dyes derived from the textile industry lead to bladder cancer [7].

Industrial activity is very important from a socioeconomic standpoint, but it also poses a serious environmental risk due to pollution [8]. The massive volume of solid or liquid waste products from these industries is frequently dumped in watercourses without any treatment or with insufficient or poor treatment. It has a detrimental effect on environmental ecosystems [9]. In Bangladesh, industrial waste contamination is a concerning problem. However, no comprehensive study has been performed yet in Bangladesh to know the effects of industrial wastewater on living animals. There is a scarcity of reports about the effect of these industrial wastes on the vital organs of the living body. Therefore, this research work was undertaken to investigate the effects of industrial wastewater on the vital organs of male Swiss albino mice.

Abbreviations-Cont'd

SPF: specific pathogen-free
ETP: effluent treatment plant

Results

Analysis of industrial wastewater

Wastewater collected from the garment industry was greyish and had a foul odor. The pH value of the water sample was 8.0 (Table 1), which indicated that the water was slightly alkaline. The characteristics of the wastewater used during the present study were as follows, which were compared with normal water (Table 1). The biological oxygen demand (BOD) and chemical oxygen demand (COD) levels exceeded the limitations established by the Department of Environment, Bangladesh [10]. The value of the BOD of the collected water was 78 mg/L. The value of the COD of collected water was 147 mg/L (Table 1). Amongst the heavy metals, the concentrations of lead (Pb), copper (Cu), sulphates, and nickel (Ni) ranged from 0.61, 0.33, 0.22, and 524 mg/L (Table 1) in the collected wastewater. These metals in wastewater surpassed the permissible limit in drinking water [10].

Effects of industrial wastewater on Body weight of mice

At the start of the experiment, there was no significant difference ($p > 0.05$) in the body weight of mice. However, after completing 24 experimental weeks, the body weight of mice was decreased in all wastewater-treated mice in comparison to the control group (Figure 1). Significant reduction of body weight of mice was observed in mice of groups 2 (46.42±1.35gm) and 3 (44.16 ± 0.88gm) ($p < 0.05$) compared to the mice of the control group (50.19 ± 3.03gm) (Figure 1).

Effects of industrial wastewater on the weight of liver

Weights of different vital organs, including the liver of the wastewater-treated mice, were evaluated, and the obtained results showed a significant change in the relative weight of these organs of the wastewater-treated group in comparison to the control group.

Table 1.
Characteristics of industrial wastewater in comparison to normal water (control)

Characteristics	Characteristics	Garments industrial wastewater	Normal water (control)
pH		8.0	7.0
BOD (mg/L)		78	Nil
COD (mg/L)		147	Nil
Chemicals/ Metal (mg/L)	Permissible limit in drinking water		
Lead (Pb)	0.5	0.61	Nil
Copper (Cu)	0.2	0.33	Nil
Nickel (Ni)	0.2	0.22	Nil
Sulphates	200	524	Nil
Color		**Grayish**	**Transparent**

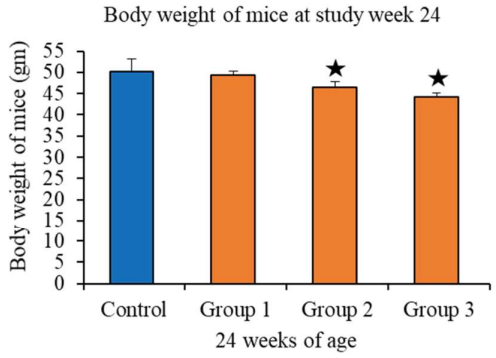

Figure 1.
Effects of watering of mice with industrial wastewater on the growth of mice. After completing 24 study weeks, a significant reduction in the body weight of mice of groups 2 and 3 and a moderate reduction in mice of group 1 compared to the control were observed. The values expressed were group average weight ± SD. Significant differences are indicated with one asterisk ($p < 0.05$).

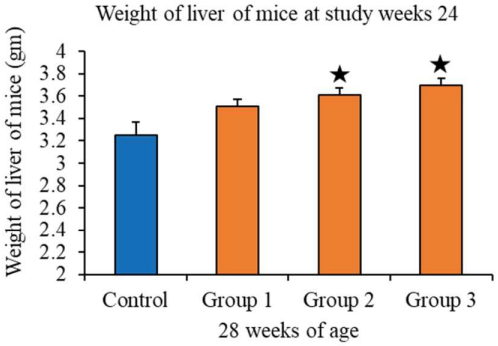

Figure 2.
Effects of watering mice with industrial wastewater on the weight of the liver of mice. After completing 24 study weeks, a significant increase in liver weight of mice in groups 2 and 3, and an insignificant increase in liver weight in those of group 1 compared to control were observed. The values expressed were group average weight ± SD. Significant differences are indicated with one asterisk ($p < 0.05$).

After completing 24 study weeks, liver weights were increased in all wastewater-treated mice in comparison to the control group (Figure 2). Significantly increased weight of liver was recorded in mice of group 2 (3.61 ± 0.06gm) and group 3 (3.7 ± 0.06gm) ($p < 0.05$) compared to control group (3.25 ± 0.12gm). The highest increased weight of the liver was observed in mice of group 3 (Figure 2).

Effects of industrial wastewater on the weight of the heart

The weights of heart were decreased in the wastewater-treated group of mice in comparison to the control group after completing 24 study weeks (Figure 3). Significant reduction of heart weight was observed in mice of group 2 (0.29 ± 0.02gm) and group 3 (0.29 ± 0.02gm) ($p < 0.05$) compared to the control group (0.34 ± 0.01gm) (Figure 3).

Effects of industrial wastewater on the weight of the kidney

Weights of kidneys were decreased in all wastewater-treated mice in comparison to the control group after completing 24 study weeks (Figure 4). Significant reduction of weight of the kidney of mice was observed in mice of groups 2(0.38 ± 0.02gm) and 3 (0.36 ± 0.01gm) ($p < 0.05$) compared to the mice of the control group (0.41±0.01gm) (Figure 4).

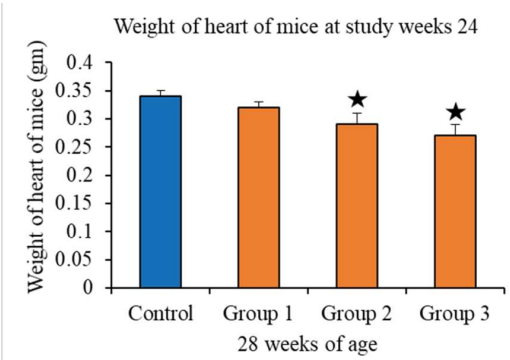

Figure 3.
Effects of watering of mice with industrial wastewater on the weight of the heart of mice. After completing 24 study weeks, a significant reduction in heart weight of mice in groups 2 and 3, and an insignificant reduction in mice in group 1 compared to the control were observed. The values expressed were group average weight ± SD. Significant differences are indicated with one asterisk ($p < 0.05$).

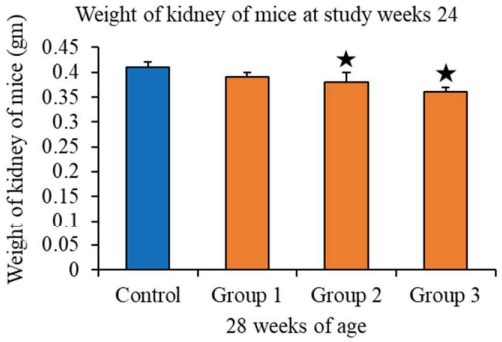

Figure 4.
Effects of watering of mice with industrial wastewater on the weight of the kidney of mice. After completing 24 study weeks, a significant reduction in kidney weight of mice in groups 2 and 3, and an insignificant reduction in mice in group 1 compared to the control group were observed. The values expressed were group average weight ± SD. Significant differences are indicated with one asterisk ($p < 0.05$).

Gross and histopathological assessment

Liver

Grossly, hemorrhagic spots and necrotic foci were observed in the livers of wastewater-treated mice. Moreover, the size of the livers was also increased in all experimental groups of mice in comparison to the control group of mice. A detailed histopathological investigation was conducted on the liver, heart, and kidney of wastewater-treated mice and the control group of mice. Histological alterations were noted in the various groups that received treatment. Hepatocellular necrosis, dilation of sinusoids, and congestion in the central vein were observed in liver sections (Figure 5A).

Heart

Grossly, the size of the hearts was decreased in all experimental groups of mice in comparison to the control group of mice. Histologically, infiltration of inflammatory cells, necrosis, and disruption of connective tissue have been recorded (Figure 5B). Intramyocardial spaces were increased in the sections of the heart in the wastewater-treated group of mice (Figure 5B).

Kidney

Grossly, the size of the kidneys was decreased in all experimental groups of mice in comparison to the control group of mice. Histologically, there was infiltration of inflammatory cells. Congestion, cell necrosis, degeneration of renal tubular epithelial cells, hypertrophied glomerulus, and tubular dilatation were observed in kidney sections (Figure 5C). In the case of all three organs, the histopathologic changes were severe in the highest concentrated effluent wastewater-treated mice (Figure 5C).

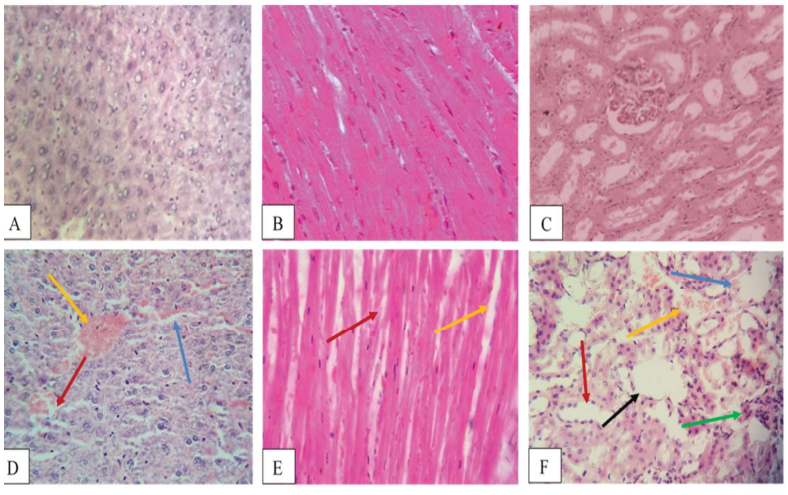

Figure 5.
Histopathology of liver, heart, and kidney of industrial wastewater-treated mice. (A-C) No significant change was observed in the liver, heart, and kidney of the mice in the control group. (D) Congestion in the central vein of the liver (yellow arrow), necrosis of hepatocytes (red arrow), and hepatic sinusoidal dilatation (blue arrow) were observed. (E) Necrosis of muscle fibers of the heart (red arrow), increased intra-myocardial spaces (yellow arrow) were evident. (F) congestion (yellow arrow), cellular necrosis (blue arrow), hypertrophied glomerulus (green arrow), degeneration of renal tubular epithelial cells (black arrow), and dilated tubules (red arrow) were reported in the kidney (H&E: ×40).

Discussion

The environment of Bangladesh suffers from the intermittent dumping of industrial waste, a consequence of insufficient regulated disposal and industry negligence. Waste streams typically contain a complex variety of toxic substances along with any metals, chemicals, and trace elements as well as pathogens that settle in lakes, streams, rivers, the ocean, and other bodies of water [11]. It has an adverse effect on environmental ecosystems [9]. In Bangladesh, textile waste contamination poses a serious threat to the environment. The high quantity of dyes, alkalis, heavy metals, and other contaminants in these wastes makes it extremely dangerous. These wastewaters also contain many dyes, organic solvents, and fixatives, which can cause damage to vital systems of the animal body, including humans. Some of the metals have a carcinogenic effect as well [5,6].

In the present study, the effects of industrial wastewater on the vital organs, namely the liver, heart, and kidney of male Swiss albino mice were investigated. We first collected wastewater from the ETP of a garment industry in the Gazipur district. The colors of the collected wastewater were greyish in color. This investigation focused on the unpleasant odor that water emits. Following their release into a river, industrial wastewaters have a significant detrimental impact on the river's water quality, as well as the quality of other nearby water sources [12,13].

The pH value of the collected wastewater was 8.0 (Table 1), which indicated that the water was alkaline. The pH value of different industrial wastewaters in Bangladesh ranged from 7.2 to 11.9 [14,15]. BOD is defined as the amount of dissolved oxygen needed by aerobic biological organisms to break down biodegradable organic material present in a given water sample at a certain temperature over a specific time period [16]. BOD values have been widely adopted as a measure of the pollution effect. High BOD content is an indicator of polluted water, while a low BOD indicates good quality water [16]. In the present study, the found BOD value was 78 mg/L, which is far above the standard permissible limits. On the other hand, COD is defined as the amount of dissolved oxygen that must be present in water to oxidize chemical organic materials by a strong chemical oxidant [16]. Both BOD and COD values are broadly accepted as the measure of the relative oxygen-depletion effect of a waste contaminant. In this study, COD value was 147 mg/L, which is extremely higher than the standard permissible limits. It has been reported that BOD and COD values were higher in industrial effluents in this study area [17]. Several heavy metals, including Pb, Cu, Ni, and sulphates, were also found at higher concentrations in these collected effluents.

In this study, the body weight of the treated mice declined after completing the study period in comparison to the control mice. This may have happened due to the dissolved heavy metals in the wastewater. Similar results were reported in rats and mice after being treated with industrial effluent [18, 19]. It has been reported that the body weights of mice drop when exposed to lead acetate. Because lead affects the satiety setup, less food was consumed, which resulted in less growth [20].

The weights of the liver were increased, and the weights of heart and kidneys were decreased in all wastewater-treated mice in comparison to the control group. The alteration rate was the highest in the mice treated with the highest concentration of industrial wastewater. Moreover, in comparison to the control group, a number of pathologic lesions have been found in the livers, kidneys, and hearts of wastewater-treated mice. Our findings are consistent with earlier research on the effects of industrial wastewaters on mammals, which documented several changes in mice and rats following exposure [21-24]. In order to gain a deeper understanding of the impacts of industrial wastewater on several organs of exposed mice, a histopathology examination was carried out. The histopathological changes in the liver, kidney, and heart of mice treated with wastewater were examined. After treatment with industrial wastewater, several histopathological lesions were observed in the liver and kidney. In the liver, congestion in the central vein, hepatic necrosis, and hepatic sinusoidal dilatation were observed. Moreover, congestion, cellular necrosis, hypertrophied glomerulus, degeneration of renal tubular epithelial cells, and dilated tubules were reported in the kidney.

In vertebrates, the liver serves as the primary organ for detoxification activities [21]. It has a number of metabolizing enzymes that are crucial to the biotransformation process of xenobiotics. Fewer or more harmful metabolites are produced as a result of this mechanism [25]. Due to the adaptive response against harmful metabolites, weight of the liver might be increased after treatment with industrial effluent. However, it has been noted that a number of metals, such as arsenic, sulphates, cadmium, lead, nickel, mercury, and hydroxyl, can increase the generation of reactive oxidant species (ROS), such as superoxide radicals, hydroxyl, and hydrogen peroxide [26]. Lead and arsenic have been linked to oxidative stress and cell damage because they change the enzymatic activity of antioxidant system [21].

Wastewater contains a variety of pollutants that might disrupt the antioxidant system and metabolic processes of an animal (such as the metabolism of fats and carbohydrates), which could account for the

toxicity of the wastewater. The damage shown in the kidneys and liver of the mice may be linked to oxidative stress in their tissue, which is most likely caused by the pollutants in the examined wastewater. The kidneys and liver have a strong correlation with histological tests and are the most sensitive markers of chemical toxicity [21].

Lead has been linked to oxidative stress and cell damage by modifying the enzymatic activity of the antioxidant system. For instance, rats exposed to lead have shown to exhibit decreased levels of both catalase (CAT) and superoxide dismutase (SOD) activity [27]. The oxidative damage in the liver and kidneys of exposed animals was linked to the inhibitory effects of heavy metals [26, 27]. Again, exposure to lead causes a substantial increase in lipid peroxidation, which oxidatively degrades membrane polyunsaturated fatty acids, resulting in the loss of membrane phospholipids and, ultimately, membrane integrity [28]. Furthermore, lead causes damage to the proximal tubular epithelium of the kidney by altering cell membrane permeability [20]. On the other hand, Ni is well known for being an immunotoxic, neurotoxic, genotoxic, aspiratory poisonous, nephrotoxic, hepatotoxic, hematotoxic, and carcinogenic agent [29]. It has been reported that Ni caused oxidative stress to regulate reactive radicals, which in turn caused necrotic and other inflammatory reactions in the livers of rats [30, 31,22]. Rats given dietary nickel acetate for a few weeks were shown to exhibit renal tubular degeneration [32]. Moreover, Ni has severe detrimental effects on the heart of rats [29]. Nickel exposure causes myocardial fibrosis in rats [32]. Nevertheless, an increased level of Cu causes dysfunctions of the liver and kidney [33].

Histopathological study of the heart of the wastewater-treated mice showed necrosis of the connective tissues of the heart and infiltration of inflammatory cells. Increased intra-myocardial spaces were evident. These findings imply that the structure and function of cardiac tissue may be impacted by industrial wastewater. As an adaptive response to improve the body's ability to clear itself of invasion, the heart increases blood flow while under stress [34]. Thus, the heart is vital to the body's defense, but several environmental pollutants may potentially disrupt or impair cardiac function. As a result, this organ might be a helpful indicator that provides information about the level of toxicity.

Since elevated cholesterol is associated with an increased risk of cardiovascular disorders [22], the disruption of lipid metabolism is most likely the cause of the observed damage to heart tissue. Furthermore, fish heart tissues (e.g., catfish) treated with effluents showed histological abnormalities such as necrosis and cellular infiltration [35]. It has been reported that endocardial inflammation and cellular disintegration have been observed in animals exposed to pharmaceutical effluent [25]. The findings of this study collectively indicated that the vital organs of mice, especially the liver, kidney, and heart, are negatively impacted by industrial wastewater.

Conclusion

The environmental pollution problem in Bangladesh is being complicated due to very fast industrialization without proper attention to the environment. In this study, the garment wastewater among the industrial wastes was used to treat the mice. In this study, the weight of livers was increased in the wastewater-treated group of mice. There was congestion in the central vein in the liver, hepatic sinusoidal dilatation, and hepatocellular necrosis. The weights of the heart and kidneys were decreased in all wastewater-treated mice in comparison to the control group. The reduction rate was the highest in the mice treated with the highest concentration of industrial wastewater. Leucocytic infiltration, cardiac necrosis, and connective tissue enlargement and disruption have all been reported. The kidney showed signs of hypertrophied glomerulus, tubular dilatation, deteriorated renal tubule epithelia, cell necrosis, and leucocytic infiltration. From these findings, it can be concluded that industrial wastewater has detrimental effects on the vital organs of mice. The findings of this study identify the effects of industrial wastewater on vital organs, which could be used for the improvement of human health and animal health by increasing awareness about the harmful and fatal effects of industrial effluent.

Materials & Methods

Ethical statement

The study on mouse experimentation was performed under the recommendation and guidance of the Animal Research Ethics Committee, Faculty of Veterinary Medicine and Animal Science, Gazipur Rahman Agricultural University, Bangladesh (FVMAS/AREC/2023/12).

Collection of industrial wastewater

Industrial wastewater was collected from the Effluent Treatment Plant (ETP) of the garment industry located in Gazipur district and was stored at 4°C during the study period.

Analysis of the chemical properties of wastewater

Samples of wastewater were collected, and their chemical composition was examined. The pH, COD, and BOD levels of the products were measured. After passing wastewater samples through a Whatman No. 42 paper filter, a drop of HNO3 (65%) was added to reduce

their pH to below 2 for preservation. The samples were then diluted to a predetermined volume. Following the manufacturer's instructions, the amounts of heavy metals in diluted samples were measured using an Atomic Absorption Spectrophotometer (AAS, Perkin Elmer, the PinAAcleTM 900H, USA).

Collection of experimental animals

Swiss albino male mice (Mus musculus) that were three weeks old and specific pathogen-free (SPF) were acquired from the International Centre for Diarrheal Disease Research, Bangladesh (icddr, b). To allow the mice to acclimate to their new surroundings, mice were housed for one week prior to their use in this study. Rectangular plastic cages with wire mesh coverings served as the mice's living quarters. Under natural daylight and well-ventilated conditions, the cages were maintained at 26 ± 2˚C with a relative humidity of 70–80%. Adequate hygienic conditions were provided for the mice.

Experimental design

Thirty-two mice at the age of week four were randomly divided into four groups, where each group comprises eight mice. All the mice were identified by ear coding. Normal drinking water was supplied to the mice in the control group. Mice of groups 1, 2, and 3 were supplied with normal drinking water mixed with industrial wastewater at 5%, 10%, and 20% concentrations, respectively, orally up to study week 24. Equal amounts of food and water were consumed by the mice in the experimental groups. The initial body weight of every mouse was determined by using a digital balance. Both at the beginning and the end of the trials, body weight was measured.

Sample collection

After completing study week 24 of treatment of mice with different concentrations of wastewater, mice were euthanized with a high dose of ketamine hydrochloride. During necropsy, different important organs (liver, heart, and kidney) were collected from the mice of both control and wastewater-treated groups to investigate the effect of wastewater. The vital organs were collected with the help of the sterile scalpel and scissors, avoiding any destruction of the organs. The weights of these organs were measured with the help of a digital balance.

Histological study

After measuring the weight of these organs, slices of these organs were collected and fixed in a 10% formalin solution. According to usual protocol, fixed tissue slices were prepared, paraffin-embedded, sectioned, and regularly stained with hematoxylin and eosin (H&E) stain [36, 37]. Photomicrography was taken using a photomicrographic camera (ZEISS AxioCam ERc5s).

Data Interpretation

At the end of the study, all the data were compiled, compared, and analyzed for constructive interpretation. The statistical analysis was carried out using SPSS (IBM@ Version 21.0, USA). The Means ± S.D. were used to depict each outcome. One-way analysis of variance (ANOVA) followed by Duncan's multiple range post-hoc test was used for the comparison. When the p-value was less than 0.05, differences were deemed statistically significant.

Data availability statement

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

Authors' Contributions

Md. Taimur Islam conceived the idea, designed the experiments and drafted the first version of the manuscript; Mohosina Mou, Nusrat Binte Rafique, Minhaz Ahmed and Md. Selim Jahangir Saurov performed the sample collection and laboratory experiment; Robius Sani Sadi edited the manuscript; Anup Kumar Talukder participated in the data analysis and edited the manuscript; Ziban Chandra Das and Md. Golam Haider reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest

There are no declared conflicts of interest involving the authors. The content of the paper has been read and approved by each co-author, and there are no financial conflicts of interest to report. We certify that the material is wholly original with no current considerations from other publishers.

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Intra-Hippocampal Injection of Abscisic Acid Attenuates Learning and Memory Deficits, and Changes Oxidative Stress Indices in REM Sleep Deprived Rats

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ABSTRACT

This study evaluated whether intra-hippocampal administration of ABA can modulate learning and memory performance and oxidative stress biomarker activities in the cerebral cortex of rats exposed to rapid eye movement (REM) sleep deprivation. Adult male Wistar rats were cannulated in the CA1 area of the hippocampus. After recovery, the rats were subjected to REM sleep deprivation for 4 days. Then, the groups of REM sleep-deprived (SD) rats were treated with ABA (5, 10, and 15 µg) and ABA (10 µg) + bicuculline (Bic), a competitive GABAA receptor antagonist. Memory and learning were evaluated with the Morris water maze (MWM) and shuttle box tests. Moreover, alterations in catalase levels as an antioxidant enzyme, MDA, and H2O2 as oxidant biomarkers were determined in rat brain cortex. REM SD rats indicated noteworthy learning and memory deficits in both the MWM and shuttle box tests when compared to control rats. However, intra-CA1 injection of ABA (10 µg) decreased cognitive impairment in REM SD rats. Bic (1 µg/rat) could not change ABA (10 µg) effects. In addition, an increase in catalase activity and a decrease in MDA and H2O2 were indicated in the brain cortex of ABA (10 µg) and ABA+ Bic treated groups. Overall, the data showed ABA's aptitude to attenuate REM sleep deprivation-induced learning and memory disruption and oxidative damage in rats. Manipulation of the GABAA receptor failed to inhibit ABA effects in REM SD rats.

Keywords

sleep deprivation , abscisic acid , bicuculline, learning and memory , Rat

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Number of References:: 45
Number of Pages: 9

Abbreviations

CAT: Catalase
REM: Rapid eye movement
STZ: Streptozotocin
Bic: Bicuculline
MWM: Morris water maze
MDA: Malondialdehyde
H2O2: Hydrogen peroxide
SD: Sleep deprivation

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Introduction

Sleep deprivation (SD) is a condition of inadequate sleep that can be considered a physiological disorder or a result of an inappropriate lifestyle [1, 2]. Sleep quality has a significant impact on the regulation of other physiological processes, including learning and memory [3, 4]. It has been shown that SD disrupts memory retrieval and consolidation by changing hippocampus structural constancy [5]. The patterns of rhythmic brain waves in non-rapid eye movement sleep also show a relationship with hippocampal activities [6]. Hippocampal-mediated learning and memory, as well as neurotransmitters, are affected by sleep quality [7, 8]. REM sleep deprivation could decline motor and sensory learning experiences in animals [9, 10].

Abscisic acid (ABA) is produced in all parts of plants and plays notable roles in their physiological functions, especially the regulation of stress responses [11, 12]. ABA is synthesized from pro-vitamin A carotenoids [13], which are found in high concentrations in plants [12]. Moreover, in animals, ABA is found in various brain areas including the hippocampus, cerebral cortex, and cerebellum [14, 15]. ABA receptors are peroxisome proliferator-activated receptors (PPARs) and lanthionine synthetase C-like protein 2 [16, 17]. ABA signalling shows variation, but changes in calcium concentration and activation of cyclic ADP-ribose are the most mutual pathways [18-20].

ABA exerts modulatory effects on a variety of physiological functions including nociception, anxiety and depression like behavior, sleep and learning and memory performances in rats [15, 21]. Central administration of ABA exhibited analgesic effect which is facilitated by the PPAR β/δ and opioid signalling [22]. Moreover, ABA meaningfully improved the pentobarbital-related sub hypnotic effects and also endorsed sleep induction. Such effects showed dependency with GABAA receptors and PPARβ/PPARγ signalling [23].

The main goal of the present study was to evaluate if intra-hippocampal treatment of ABA can alter learning and memory performance in rats exposed to REM-SD. Moreover, bicuculline was used to assess the possible association of ABA with the GABA A receptor. In a previous study, pretreatment with bicuculline was found to block ABA's ability to extend sleep duration in a rat model of pentobarbital-induced sleep. Bicuculline is a competitive GABAA receptor antagonist that blocks GABA's inhibitory effects by preventing chloride ion influx, leading to increased neuronal excitability and potential seizure activity[24]. The alteration of pro-oxidant/antioxidant biomarkers was also assessed in the cerebral cortex of SD rats.

Abbreviations-Cont'd

PPARs: Peroxisome proliferator-activated receptors
ABA: Abscisic acid
ROS: Reactive oxygen species
CGRP: Calcitonin gene-related peptide

Results

PA test

The SD group showed an increase in the number of acquisition trials when compared with the control group ($p < 0.001$) (Fig. 1A). However, the number of acquisition trials was significantly decreased in SD groups post-treated with ABA (10 µg and 15 µg) ($p < 0.001$). No major alteration in acquisition trials was found in SD rats post-treated with Bic +ABA (10 µg) as compared to SD+ABA (10 µg) group. In addition, an increase in the step-through latency and a decline in time spent in the dark cavity were determined in the SD group ($p < .001$). ABA (10 µg) was able to increase the step-through latency and decrease time spent in a dark chamber in SD rats ($p < 0.001$). In addition, no significant alteration was found in the SD rats' response infused with Bic + ABA (10 µg) as compared with the ABA (10 µg) group (Fig. 1B and 1C).

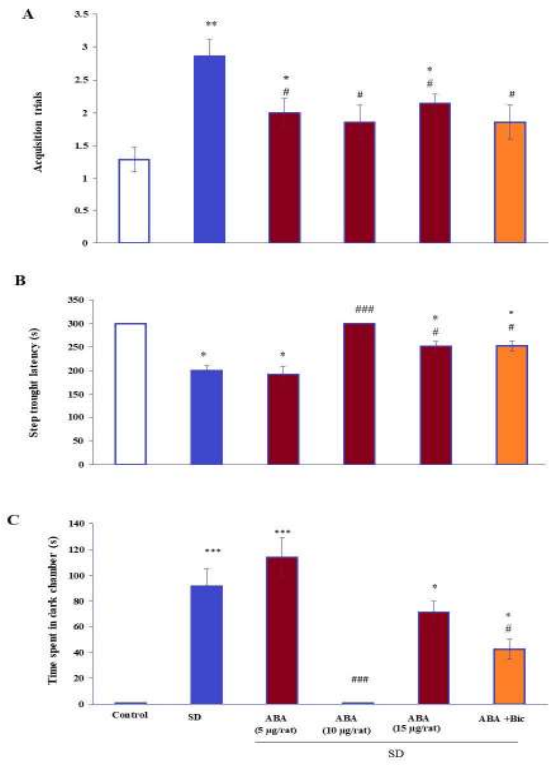

Figure 1.
The effect of intra-hippocampal administration of ABA (5, 10, and 15 µg/rat) or Bic+ABA (10 µg/rat) on the number of acquisition trials (A), step through latency (B), and time spent in a dark chamber (C) in passive avoidance test in SD rats. Values are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus control groups, # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ versus SD group

MWM test

In acquisition trials, the latency time to catch the concealed platform was pointedly increased in the SD group in comparison to the control group ($p < 0.001$). Intra-hippocampal infusion of ABA (10 µg / rat) expressively decreased the latency time to catch the concealed stage in the SD rats ($p < 0.01$) (Fig. 2A). Moreover, SD+Bic+ABA (10 µg /rat) and SD+ ABA (10 µg /rat) treated groups show no change the latency to discover the hidden platform. Moreover, the groups showed major differences in space moved to touch the concealed platform on the acquisition test. As shown in Fig. 2B, the distance trekked to touch the hidden stage was meaningfully increased in the SD group ($p < 0.001$). Besides, the SD group treated with ABA (10 µg/rat) traveled a lower distance to reach the hidden platform as compared to the SD group ($p < 0.001$). In the SD group injected with Bic+ ABA (10 µg/rat) the distance traveled to find the platform showed no difference as compared to the ABA (10 µg/rat) group (Fig. 2B).

Fig.3 indicates the results of the probe trial. The figure indicated that time spent and the traveled distance in the object zone significantly decreased in the SD group than the control group ($p <0.001$) (Fig. 3A). Moreover, ABA weakened the effects of SD on the time spent in the object area ($p <0.05$) (Fig. 3A). Further, ABA meaningfully improved distance traveled in the

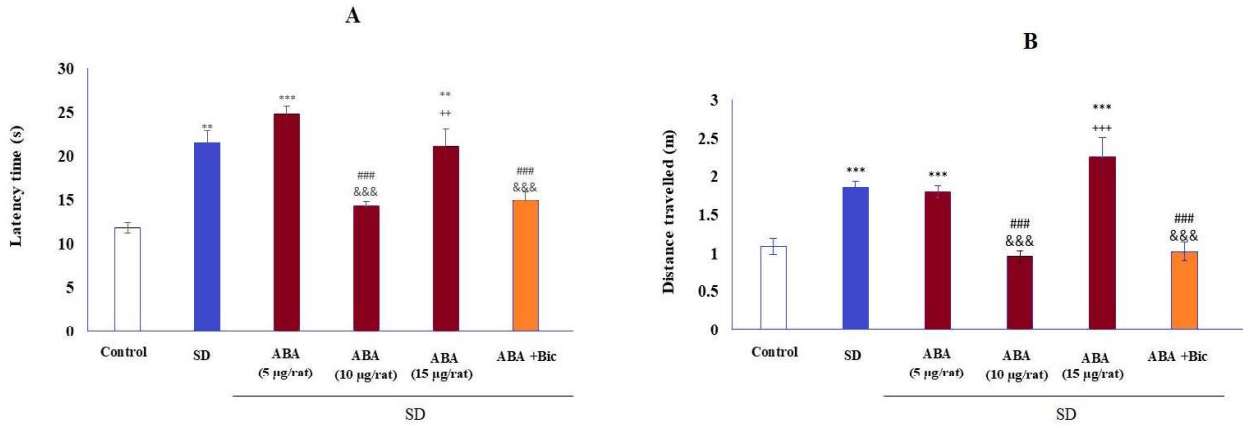

Figure 2.
The effect of intra-hippocampal administration of ABA (5, 10, and 15 µg/rat) or Bic+ABA (10 µg/rat) on the escape latency time (A) and distance travelled to find the hidden platform in the MWM test in SD rats. Values are expressed as mean ± SEM. ** $p < 0.01$ and *** $p < 0.001$ versus control groups, ### $p < 0.001$ versus SD group, &&& $p < 0.001$ versus SD + ABA (5 µg/rat) group, ++ $p < 0.01$, +++$p < 0.001$ versus SD + ABA (10 µg/rat) group

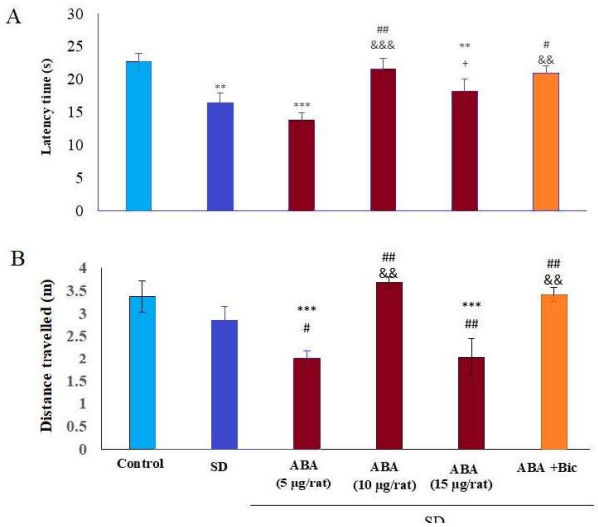

Figure 3
The effect of intra-hippocampal administration of ABA (5, 10, and 15 µg/rat) or Bic+ABA (10 µg/rat) on the duration time (A), and distance travelled in target zone in SD rats in probe trial of MWM test. Values are expressed as mean ± SEM. ** $p < 0.01$ and *** $p < 0.001$ versus control groups, # $p < 0.05$, ## $p < 0.01$ versus SD group, && $p < 0.05$, &&& $p < 0.001$ versus SD + ABA (5 µg/rat) group, + $p < 0.05$ versus SD + ABA (10 µg/rat) group

object area in SD-treated rats ($p < 0.01$) (Fig. 3B). As notated in Fig. 3, SD rats infused with Bic + ABA show no significant difference in spent time and distance traveled in the target quadrat in comparison to ABA (10 µg/rat) group ($p < 0.05$).

Biochemical assay

The activity of the antioxidant enzyme CAT was significantly decreased in the SD group when compared with control rats. As shown in Fig. 4A, ABA at 10 µg/rat and Bic+ABA (10 µg/rat) were able to increase CAT activity in the SD group. Moreover, there were significant increases in the activity of pro-oxidant biomarker H2O2 and MDA concentration in the cerebral cortex of the SD group as compared to the control group. However, post-treatment of SD rats with ABA (10 µg/rat) or Bic+ABA significantly attenuated H2O2 activity and MDA level in the cerebral cortex (Fig. 4B and 4C).

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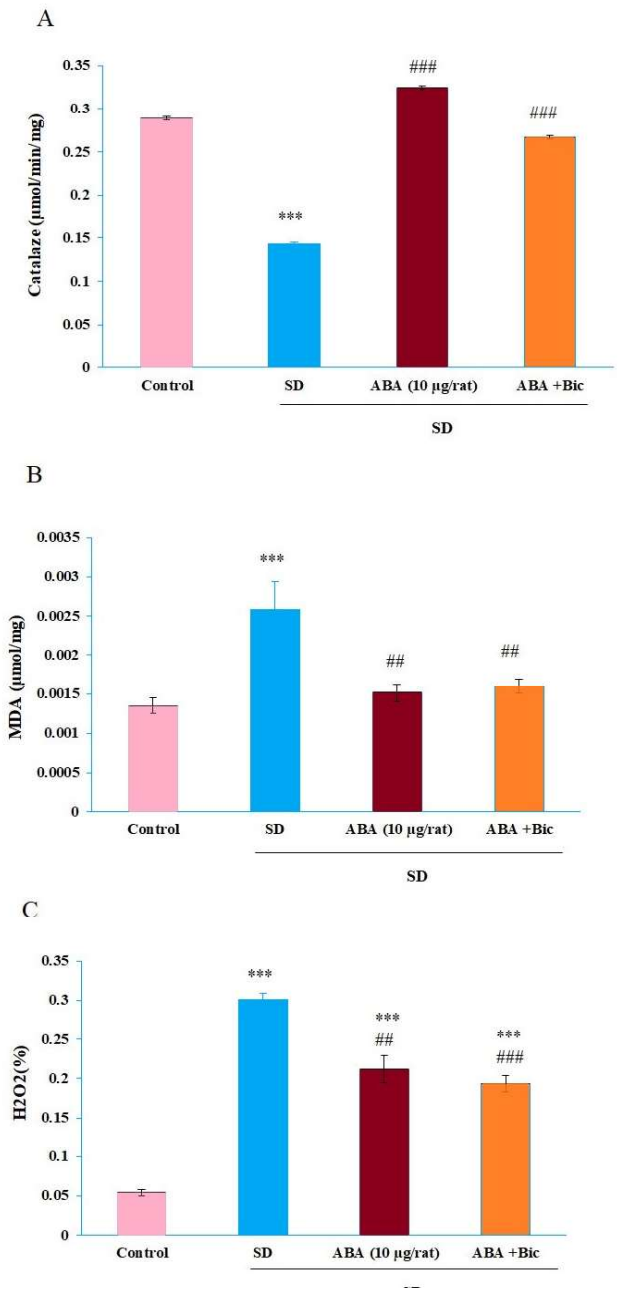

Figure 4.
The effect of intra-hippocampal administration of ABA (10 µg/rat) or Bic+ABA (10 µg/rat) on the activity of CAT enzyme (A), MDA concentration (B) and H2O2 activity in the cerebral cortex of rats. Values are expressed as mean ± SEM. ** p <0.01 and *** p <0.001 versus control groups, # p <0.05, ## p <0.01 versus SD group, && p <0.05, &&& p <0.001 versus SD + ABA (5 µg/rat) group, + p <0.05 versus SD + ABA (10 µg/rat) group

Discussion

The present study showed the deteriorating effects of REM sleep deprivation on the memory and learning performance of rats assessed in the MWM and shuttle box tests. However, intra-CA1 microinjection of ABA decreased SD-induced learning and memory deficiency in rats. Moreover, the sleep-deprived rats indicated a disruption in oxidant/antioxidant biomarkers verified by a decrease in CAT activity and increases in lipid peroxidation and H2O2 production in the cerebral cortex, which was prevented by ABA (10 µg) treatment. The ABA effects in behavioral and biochemical experiments did not diminish with the GABA receptors antagonist bicuculline.

The importance of sleep quality on cognitive performance, especially hippocampal-depended learning and memory has been strongly supported by evidence from clinical and experimental studies [25]. In this study, the rats' learning and memory performances were assessed after a continuous 72 h period of REM SD. The 72-hour REM sleep deprivation period in rats reflects severe sleep loss but is not directly equivalent to 72 hours in humans due to differences in metabolism and sleep architecture. In humans, this timeframe would likely correspond to several days of significant sleep restriction or chronic sleep disruption rather than total sleep deprivation. Rodent models typically involve more intense and compressed sleep deprivation protocols compared to human studies. To bridge the gap between rodent and human studies, future research could explore the effects of varying durations of REM sleep deprivation in animal models and attempt to correlate these findings with human studies involving partial sleep restriction or chronic sleep fragmentation. Learning and memory changes following SD are highly dependent on the lasting duration of the SD. In line with our result, most studies showed the highest detrimental effects of SD on memory performance when it lasted for 72 h. Nevertheless, in some cases, shorter terms of SD lasting for 24 or 48 hours have been associated with no alteration or even increases in hippocampal synaptic plasticity and memory impairment [26-28]. The mechanism(s) underlying different effects of SD lasting on learning and memory function are complex and still not well understood.

For the first time, this study shows ABA's ability to increase learning and memory performance in SD rats. The efficacy of ABA interventions on sleep, learning, and memory has been demonstrated in previous studies conducted on rodents. It has been indicated that ABA decreases onset time and prolongs sleep duration in a rat model of pentobarbital-induced sleep [23]. Moreover, ABA treatment reduced learning and memory deficits in rat models of STZ-induced Alzheimer's disease [29]. In addition, ABA infusion decreased learning and memory deficits in MWM and shuttle box tasks in STZ diabetic rats[30]. The mechanism(s) of ABA involvement to attenuate sleep deprivation weakening effects on learning and memory is not understood. It is postulated the effects might be intended by manipulation of related neurotransmit-

ters and distinct neural networks within the brain.

The data showed pharmacological blockage of the GABAA receptor with bicuculline did not inhibit ABA efficiency on learning and memory performances in sleep-deprived rats. In a related study, pretreatment with bicuculline could obstruct ABA impending to prolong sleep duration in a rat model of pentobarbital-induced sleep [23]. This duality suggests that ABA could engage different pathways—supporting both sleep recovery and neurocognitive resilience—depending on the physiological or experimental conditions.

GABAergic synapses are profoundly founded on hippocampus CA1 pyramidal neurons [31, 32]. While the baseline GABA levels in the hippocampus improve learning and memory performance, an increase in GABAA receptor activity has been shown to decline network excitability and reduce synaptic plasticity in the CA1 area [33, 34]. Indeed, memory retrieval is impeded by the glutamate and GABA concentration balance in the brain [35]. In the rats subjected to SD impairment of memory performance has been associated with imbalances in Glu/GABA ratio [36]. Although this study did not find ABA interfering with the GABAA receptor, however, more data are still required to describe the details of ABA's impact on the GABAergic system to modulate the learning and memory of SD-exposed rats.

In the present study, REM sleep deprivation increased oxidative stress damage defined by increases in lipid peroxidation and H2O2 levels, and a decrease in CAT activity in the cerebral cortex of rats. However, post-treatment with ABA (10 μg/rat), which was the most effective dose to increase learning and memory behaviors, could inhibit oxidative stress imbalances in SD rats. This data is supported by many previous studies that display ABA antioxidant capacity in rodents. Oral treatment with ABA in drinking water increased antioxidant defence systems indices and decreased MDA levels in many tissues of rats [37]. Moreover, intra-lateral ventricles infusion of ABA increased feeding behavior and increased the antioxidant enzymes activity, while attenuated stress oxidative enzymes [38]. In a mouse model of thioacetamide-induced hepatic fibrosis ABA treatment decreased oxidative stress enlargements and inflammation by induction of NF-κB signaling path [39]. Indeed, this study data support an association between ABA antioxidant properties and reduction of REM-SD induced learning and memory deficits.

It has been shown that as a isoprenoid plant hormone compound, ABA binds to PPARs and activates several intracellular signaling molecules essential in the regulation of learning and memory performance [16]. Pretreatment with PPAR β/δ antagonist was able to suppress ABA anti-nociceptive effects in rats [16]. Moreover, ABA decreased diabetes-induced learning and memory deficit in rats via intonation of PPARγ receptors [30]. In addition, PPARγ receptors antagonist prevented the ability of ABA to increase sleep duration in a rat model of pentobarbital-induced sleep [23]. On the other hand, motivation of PPARγ receptors with ABA modifies calcium channel activity and induces PI3K/PKC pathway in rat's brain to modulate learning and memory and anxiety-like behavior [40]. Possibly ABA efficiency on learning and memory responses in SD rats is at least partially mediated by manipulations of the PPARs system and induction of the downstream signaling molecules involved in learning and memory performance.

Our study primarily focused on learning and memory performance using specific behavioral tests (e.g., acquisition trials). While these tests provide valuable insights, they may not fully capture the broader spectrum of cognitive functions affected by sleep deprivation or ABA treatment. While oxidative stress biomarkers (catalase, MDA, H2O2) were evaluated, other potential mechanisms (e.g., neuroinflammation, synaptic plasticity) were not explored, leaving gaps in understanding ABA's comprehensive effects.

Conclusions

Overall, the data of this study showed the potential of intra-hippocampal administration of ABA to increase antioxidant indices in the brain and attenuate learning and memory deficits in RAM-SD rats. Pretreatment infusion with GABAA receptors antagonist did not change ABA-induced responses.

Materials & Methods

Animals

Adult male Wistar rats (2 months) weighing 230–270 grams were used in this study. The animals were contained four per cage in a room with a temperature of 23 ± 2 °C under a 12-h light/dark cycle with limitless entrance to food and water. All trial procedures were permitted by the Animal Research Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran.

Surgery and microinjection

Rats were profoundly anesthetized with a mixture of ketamine (100 mg) and xylazine (5 mg) and placed in a stereotaxic apparatus (Estoelting CO, USA). Guide cannulae were bilaterally inserted in the CA1 region (3.8 mm posterior to the bregma, 2.2 mm lateral from the midline, and 3.2 mm depth to the cortical surface). Afterward, rats were kept separately and endorsed for 1 week to recover from surgery before treatments [41]. The drugs (1 μL each side) were delivered using a 27-gauge stainless steel needle devoted to a Hamilton micro-syringe.

Experimental design

The animals were randomly separated into six experimental groups

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(n=7) as follows: control (untreated rats); sleep deprivation (SD): located in small platform; SD + ABA groups: treated intra-CA1 with ABA (5, 10, and 15 µg/ rat) and then located on small platform; SD + ABA (10 µg/ rat) + bicuculline (1 µg/rat): treated intra-CA1 with ABA and bicuculline and then located on small platform. The groups were exposed to SD procedures for 72 h and then injected with specific treatments. Ten minutes after intra-hippocampal injection, the rats were verified in MWM and Shuttle box tests, respectively.

Sleep deprivation (SD)

In the first tests, the single small platform method of SD was used. Animals were sited on a single stage in the center of a water cistern. The water reached up to 2 cm under the shallow of the stage. Based on the multiple small platform method, five stages (each 5 cm diameter) were used. In this method, the stages were spread out (8–10 cm apart) so that animals were able to simply travel amongst them but could not lie through any two. The control group was tested using the single large stage method, where the size of the stage was enlarged to 15.2 cm to to ensure sleep. All the treatments lasted 72 hours [42].

Learning and memory assessment

1. MWM

In this study, all the experimental groups were subjected to four days of training trials in the MWM, as defined previously [43]. A video camera was attached straight overhead the water maze pool, and the tracking system of Any maze was provided to assess the time to reach the concealed platform (the escape latency) and the length of the swim (traveled distance) of each rat in training time. Twenty-four hours later, the rats were evaluated in the probe trial, in which the escape platform was detached from the pool, and the animal was permitted to swim for 60 sec. The total time spent and the number of visits across the past position of the platform were measured to appraise spatial memory.

2. Shuttle box test

The apparatus encompassed identical-sized light and dark partitions that were separated by a sliding guillotine door. The floor of the dark and light partitions consisted of a stainless-steel shock grid. This test was divided into training and memory stages. In the instruction phase, each animal was positioned in the lightened partition, and after 5 seconds, the gate was unlocked and the rats were indorsed to transfer freely into the dark space. Upon entry into the dark chamber, the door was barred and the rat was assumed 1 mA electrical shock in 1 second. The instruction trial was completed when the rat endured in the light hall for 5 continuous min. Twenty-four hours later, in stage 2 (retrieval session), each rat was positioned on the light side of the box. Ensuing 30 s acclimatization, the door was raised. The number of electrical shock trial, latency to enter the dark chamber initial time spent to wholly enter the dark room (STL), as well as whole time consumed in the dark box were important in the passive avoidance test [44].

Biochemical assay

The rats were euthanized with CO2. The brains were detached, and the separated brain regions, hippocampus and prefrontal lobe, were kept in liquid nitrogen for assessment of biochemical parameters. Brain malondialdehyde (MDA) and hydrogen peroxide (H2O2) assay were evaluated as lipid peroxidation products and oxidative stress index [26, 45]. Moreover, the measurement of catalase enzyme activity was done as an index of antioxidant activity [46].

Statistical analysis

The results are expressed as the mean ± SEM. The statistical analyses were performed using SPSS (version 22) software. One-way analysis of variance (ANOVA) was used to evaluate significant variations among groups. Tukey's Post hoc assessment was conducted to explore the differences between the groups. A significance level of $p < 0.05$ was adopted for all tests.

Authors' Contributions

Md. Taimur Islam conceived the idea, designed the experiments and drafted the first version of the manuscript; Mohosina Mou, Nusrat Binte Rafique, Minhaz Ahmed and Md. Selim Jahangir Saurov performed the sample collection and laboratory experiment; Robius Sani Sadi edited the manuscript; Anup Kumar Talukder participated in the data analysis and edited the manuscript; Ziban Chandra Das and Md. Golam Haider reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors have no competing interests to declare.

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An Assessment of Livelihood Status, Profitability, and Obstacles Faced in Native Chicken Farming in Some Selected Areas of Bangladesh

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ABSTRACT

The present research aimed to assess the livelihood status of native chicken farmers in Bangladesh, their rate of profitability, constraints, and their suggestions for addressing these issues. Primary data were collected from a random sample of 260 native chicken-rearing farmers across six divisions in Bangladesh. The majority of farms (36.9%) fell into the small category (1–10 chickens), followed by medium (11–15 chickens) and large (>15 chickens) farms (31.9%). Common deshi hens were present in nearly 95% of the farms. In 2023, the market prices for different categories of chicken were as follows: roasters at 329.68 ± 7.20 BDT, hens at 302.22 ± 2.66 BDT, and chicks at 68.23 ± 2.28 BDT. The market prices for duck eggs, native chicken eggs, brown-shelled eggs, and white-shelled eggs ranged from 63.91 ± 0.52 to 61.07±0.58 BDT per hali. The Patuakhali district had the highest benefit-cost ratio of 2.61, while Rangpur had the lowest at 1.57. Native chicken farming contributed 7.79% to household income. A multiple regression analysis revealed that almost all variables were influenced by income from native chicken farming, except for rearing costs. The major constraints reported were disease outbreaks and predatory animal attacks, mentioned by 80.4% of the farmers. In conclusion, native chicken farming in Bangladesh is profitable despite some challenges that need to be addressed.

Keywords

Native chicken, Market demand, Benefit Cost Ratio, Constraints, Profitability

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Abbreviations

GR: Gross return
GC: Gross cost
BCR: Benefit Cost Ratio
SE: Standard Error of Mean
SSC: Secondary School Certificate
HSC: Higher Secondary Certificate
NC: Native chicken
BDT: Bangladeshi Taka

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Introduction

Bangladesh, a densely populated nation, has a strong agricultural foundation, with rural areas accounting for 68.49% of the total population [1]. The average per capita income is only $2824, and the majority of individuals are engaged in crop cultivation, fisheries, and livestock rearing. Both domestic and commercial poultry farming are becoming increasingly common. Poultry plays a crucial role in the agricultural sector of Bangladesh, offering economic benefits and allowing birds to reproduce freely. In rural areas, backyard poultry farming is a traditional method of raising chickens that supports family economies and provides food for subsistence [2]. Poultry is also raised for commercial purposes, assisting farmers in creating jobs, earn income, and contribute to building a poverty-free and healthy society. In Bangladesh, the poultry sector is crucial in creating employment, contributing to national income, improving human nutrition, and generating revenue. Increasingly, people are recognizing the value of poultry farming as a source of income for marginal and landless farmers, especially women [3]. In developing countries, poultry meat and eggs contribute approximately 20% of dietary protein [4]. In Bangladesh, native chickens are raised by rural farmers. However, some obstacles impact domestic chicken production. One major obstacle is disease prevalence, which is influenced by climate change, farm management, vaccination and deworming routines, and societal awareness. Despite these challenges, native chicken farming remains a profitable industry in Bangladesh, particularly for rural women, providing them with a source of income. The purpose of this study is to learn more about the financial conditions, profitability, and challenges faced by local chicken farmers. We also aimed to identify the support needed by farmers to expand local poultry farming. The current study provides data on the production costs and returns associated with raising chickens. The findings of this study might be useful to the authorities and rural poultry producers in making informed decisions and other districts had no significant difference. The specific objectives of the study were:

1. To evaluate the profitability of native chicken farming and the farmers' standard of living
2. To understand the limitations against farmers' perspectives on chicken farming.
3. To offer a potential way out of guidelines to enhance indigenous chicken farming.

Results

1.1 Socioeconomic Status of Farmers

The socioeconomic status of farmers in the selected regions is shown in Table 1. The mean age of the farmers in the surveyed region ranged from 38.30±0.98 to 46.86 ± 2.29 years. The average family sizes in the Rangpur, Sherpur, Feni, Pirojpur, Patuakhali, Sunamgonj, Pabna, and Joypurhat areas were 4.03, 4.36, 4.30, 4.50, 4.44, 5.36, 4.23, and 4.06

1.2. Level of Education

Approximately 13% of the farmers lacked the basic education needed for everyday tasks, while 37% were completely illiterate. The level of education farmers in the chosen region up to Class 5, up to Class 8, and had passed their SSC, HSC, and degree were 23.1%, 15.8%, 9.2%, 3.5%, and 2.7% respectively (Table 2).

1.3 Farmer Occupations

In agriculture, 34.6% of household heads were engaged, making it the major occupation among the se-

Table 1.
Socio-economic status of the native chicken rearing farmers

District	Age (Mean±SE)	Family size (Mean ± SE)	Earning member (Mean±SE)	Dependency ratio	Farming Experience in year (Mean±SE)
Pabna	40.40 ± 1.15	4.23 ± 0.29	1.26 ± 0.12	3.35	5.65 ± 0.33
Rangpur	38.30 ± 0.98	4.03 ± 0.14	1.00 ± 0.00	4.03	6.44 ± 0.29
Joypurhat	39.23 ± 1.11	4.06 ± 0.20	1.20 ± 0.08	3.38	9.70 ± 0.68
Sherpur	46.86 ± 2.29	4.36 ± 0.26	1.33 ± 0.13	3.27	15.46 ± 1.50
Pirojpur	42.36 ± 1.98	4.50 ± 0.17	1.23 ± 0.07	3.65	16.92 ± 1.16
Feni	42.60 ± 2.05	4.30 ± 0.17	1.16 ± 0.06	3.70	13.18 ± 1.42
Sunamgonj	44.33 ± 1.60	5.36 ± 0.20	1.30 ± 0.08	4.12	10.32 ± 1.36
Patuakhali	39.62 ± 0.99	4.44 ± 0.14	1.18 ± 0.05	3.76	18.38 ± 0.70
Overall	41.55 ± 0.56	4.41 ± 0.07	1.20 ± 0.03	3.67	12.50 ± 0.45

Standard Errors (SE)

Table 2.
Educational level of selected farmers

Education level	Percentage (N)	Education level	Percentage (N)
Illiterate	13.1 (34)	SSC	9.2 (24)
Slightly educated	32.7 (85)	HSC	3.5 (9)
Up to class 5	23.1 (60)	Degree	2.7 (7)
Up to class 8	15.8 (41)	-	-

SSC: Secondary School Certificate, HSC: Higher Secondary Certificate

lected farmers. According to this survey, the primary occupation of household heads was 21.5% day laborers, 18.1% business, 10% service jobs, and 15.8% were engaged in other occupations (Table 3).

1.4 Farm Size and Native Chicken Raising Type

Three categories were used to classify the native chicken farms: small (<10), medium (>10), and large (>15). According to the survey, 36.9% of farmers raised less than ten chickens per family, 31.9% raised 10-15 chickens per household, and 31.2% raised more than 15 chickens per household (Table 4).

Table 3.
Occupations of native chicken farmers in the chosen regions

Occupation % (N)	Agriculture	Day laborer	Service	Business	Others
Primary	34.6 (90)	21.5 (56)	10.0 (26)	18.1 (47)	15.8 (41)
Secondary	33.8 (88)	10.8 (28)	-	1.5 (4)	35.4 (92)

%: Percentage, N: Number

2. Households' Role in Native Chicken Production

All rural women in the research area reared native chickens with additional support by 21.9% of girls, 8.8% of men, and 7.7% of boys (Table 5). Regarding food purchasing, the majority were men (52%), followed by women (20%) and both genders in 13.5% of cases. About egg sales, 40.4% was handled by women and 23.5% by men. Chicken sellers were 36.2% women. The majority of women (approximately 91.2%) saved money from selling eggs and chickens, while 4.6% of both genders jointly managed savings and 4.2% of men saved money from native chicken farming. In terms of household spending, 41.5% of both genders participated in spending. The average weekly egg consumption per family was found to be 4.59 ± 0.17 eggs.

a. Purpose of Native Chicken Rearing

Approximately 76.5% of farmers raised native chickens for both personal use and additional revenue, while 19.2% of farmers raised chickens for their own needs and 4.2% for income (Table 6).

3. Analysis of the demand and market value for native chicken

The highest average market value recorded in the current year in the Patuakhali district was 424.76 ± 12.65 BDT for roasters and 95.80 ± 3.19 BDT for chickens (Table 7). The Patuakhali district likewise had the highest average market value of roasters the previous year which was 390.18 ± 12.72 BDT for chicken and 80.50 ± 3.27 BDT for roasters. However, the Rangpur district had the highest average market value of hens, 346.50±3.93 BDT in the current year and 313.63 ± 4.19 BDT in the previous year. Conversely, Su-

Table 5.
Household's role in native chicken production

Contribution in NC Rearing	% (N)	Category	Man % (N)	Women % (N)	Both % (N)
Woman	100 (260)	Feed buyer	52 (173)	20 (52)	13.5 (35)
Man	8.80 (23)	Egg and NC seller	23.5 (61)	36.2 (94)	40.4 (105)
Boy	7.70 (20)	Keep money	4.20 (11)	91.2 (237)	4.60 (12)
Girl	21.9 (57)	Spent money	32.7 (85)	25.8 (67)	41.5 (108)

SE: Standard Errors, %: Percentage, N: Number, NC: Native Chicken.

Table 4.
Farm size and Native Chicken type

Farm Size	Percent (n)	Native Chicken type farm	Percent (n)
Small range (1-10)	36.9 (96)	Common deshi	95.0 (247)
Medium (11-15)	31.9 (83)	Hilly	2.3 (6)
Large (>15)	31.2 (81)	Naked neck and Common deshi	1.5 (4)
Total	100.0	Naked neck	1.2 (3)

N: Number

namgonj had the lowest average market value for roasted chicken 266.83 ± 22.49 BDT, while the market value of hens was 264.33 ±3.66 BDT in Sherpur and chickens was 47.33 ± 3.79 BDT in the Joypurhat district in the current year. The lowest market values for roasters (210.50 ± 27.74 BDT), hens (193.83±25.7 BDT), and chickens (31.33 ± 4.25) were recorded in the Pirojpur district the year before. In 2023, the market prices for chickens were as follows: roaster 329.68 ± 7.20 BDT, hen 302.22 ± 2.66, and chicken 68.23 ± 2.28 BDT.

Table 6.
Purpose of native chicken rearing and data recorded by farmers

Purpose of rearing Native chicken	Percent (N)
Own need	19.2 (50)
Extra income	4.2 (11)
Both (family need + extra income)	76.5 (199)
Data record on DOC weight, weight gain, and egg production (%)	1.5 (4)

DOC: Day Old Chick, N: Number

Table 7.
Average market value of native chicken in the chosen regions

Location	Average market value of native chicken (Mean±SE) (BDT)					
	Previous year			Present year		
	Roaster	Hen	Chicken	Roaster	Hen	Chicken
Pabna	306.89 ± 7.03	282.16 ± 5.97	46.66 ± 1.99	341.00 ± 7.82	325.50 ± 5.01	58.00 ± 3.90
Rangpur	317.16 ± 4.43	313.63 ± 4.19	68.90 ± 1.21	339.50 ± 12.44	346.50 ± 3.93	76.00 ± 2.77
Joypurhat	327.33 ± 12.34	263.33 ± 9.87	43.33 ± 2.85	333.33 ± 25.07	303.63 ± 3.73	47.33 ± 3.79
Sherpur	235.83 ± 20.10	239.66 ± 3.10	41.33 ± 4.71	293.16 ± 18.70	264.33 ± 3.66	53.00 ± 4.77
Pirojpur	210.50 ± 27.74	193.83 ± 25.7	31.33 ± 4.25	283.83 ± 23.69	312.83 ± 5.85	74.33 ± 14.89
Feni	301.16 ± 11.68	267.66 ± 5.56	52.33 ± 2.28	291.66 ± 21.82	291.00 ± 6.07	63.33 ± 2.59
Sunamgonj	276.96 ± 14.34	243.13 ± 1.62	45.33 ± 3.06	266.83 ± 22.49	271.83 ± 11.48	59.66 ± 3.26
Patuakhali	390.18 ± 12.72	282.40 ± 6.00	80.50 ± 3.27	424.76 ± 12.65	302.18 ± 5.99	95.80 ± 3.19
Overall	**303.01 ± 6.36**	**262.39 ± 4.35**	**53.46 ± 1.51**	**329.68 ± 7.20**	**302.22 ± 2.66**	**68.23 ± 2.28**

SE: Standard Errors, BDT: Bangladeshi Taka

a. Demand Analysis of Native Chicken

The primary source of native chicken purchases is from farms or the home of a native chicken rearing farmer (58.8%), followed by neighbors (26.7%) and wholesalers (14.2%) shown in Table 8. Due to the fair market price, 40% of farmers favoured broiler chicken, while 30.8% preferred native chicken. In the studied locations, 1.9% of farmers favoured Layer chicken and 27.3% desired Sonali. We found that 64.4% of farmers chose native hens with an average marketable weight of about 1 kg or more. Of them, 18.8% wanted 900 g and 16.2% chose 750 g. The value chain of native chicken is influenced by different stakeholders related to the direct decision of this business. According to the value chain, 36.5% of farmers eat native chicken largely for its flavour, with 21.9% and 41.5% preferring it for roasting and health reasons, respectively (Table 8).

b. Demand Analysis of Eggs in the Market

In the study areas, most of the consumers (46.9%) preferred brown-shelled eggs, followed by native chicken eggs (28.1%), white-shelled eggs (18.1%), and duck eggs (6.9%) as presented in Table 9. The exorbitant cost of native breeds and their eggs was the cause. For duck eggs, the highest market price was recorded at 63.91 ± 0.52 BDT/hali, while it was 61.07 ±0.58 BDT/hali for native chicken eggs. However, the market price for brown-shelled eggs was 46.82 ± 0.31 BDT/hali, whereas the price for white-shelled eggs was 41.12 ± 0.26 BDT/hali. Most subjects (53.8%) who favoured eating native chicken eggs were pregnant women (22.3%) and children (20%). In addition, 3.8% of elderly individuals favoured native chicken eggs. We observed that 53.8% of patients said they would rather eat native chicken eggs, compared to 22.3% of

Table 8.
Sources of Native chicken and their demand in the selected areas

Source of buying	Percent (N)	Demanded chicken type	Percent (N)	Avg. marketable weight	Percent (N)	Value chain	Percent (N)
Farm	58.8 (150)	Native chicken	30.8 (80)	750 g	16.2 (42)	Roast	21.9 (57)
Neighbor	26.9 (70)	Broiler	40.0 (104)	900 g	18.8 (49)	Healthy	41.5 (108)
Wholesaler	14.2 (37)	Sonali	27.3 (71)	1 kg/Above	64.6 (168)	Tasty	36.5 (95)
-	-	Layer	1.90 (5)	-	-	-	-

N: Number

pregnant women and 20% of toddlers. Furthermore, 3.8% of elderly individuals said that they desired native chicken eggs because they were organic, high in nutrients, and could be considered a healthful diet.

4.Cost of managing and rearing native chickens

Rangpur district had the greatest total costs for raising and managing native chickens at 9742.67 BDT/year, while Joypurhat district recorded the lowest total expenses at 5018.47 BDT/year. The district of Pirojpur had the most cost participation (1620.47 BDT/year) for purchasing chicks, while in the Joypurhat district was 842.5 BDT. Patuakhali had the lowest cost involvement of 138 BDT/year for vaccination and medication, while farmers in Rangpur spent the most for vaccine and medication (1206.66 BDT/year).

In the Pirojpur district, individual farmers spent

Table 9.
Market demand and consumer preferences for egg in the selected areas

Demanded egg type	Percent (N)	Demanded egg price	BDT/hali Mean ± SE	Consumer type of NC egg	Percent (N)
White Egg	18.1 (47)	White Egg	41.12±0.26	Patient	53.8 (140)
Duck Egg	6.9 (18)	Duck Egg	63.91±0.52	Pregnant	22.3 (58)
NC egg	28.1 (73)	NC egg	61.07±0.58	Children	20.0 (52)
Brown Egg	46.9 (122)	Brown Egg	46.82±0.31	Old	3.8 (10)

N: Number, SE: Standard Errors, NC: Native Chicken

Table 10.
Principal costs associated with raising and managing native chickens in the chosen regions

Average income generation (BDT/year) of farmers from native chicken rearing and production (mean)									
Parameters	Pabna	Rangpur	Joypurhat	Sherpur	Pirojpur	Feni	Sunamgonj	Patuakhali	Overall
Chick price	916.4	1102	842.5	1007	1620.47	1121	1097.87	1586.24	1194.34
Vaccine and Medicine cost	1107.33	1206.67	706.67	190.00	515.00	395.00	373.33	138.00	545.08
Veterinary Service Fee	40.00	0.00	23.33	6.67	160.00	116.67	16.67	0.00	41.92
Disinfectant cost	0.00	0.00	19.33	0.00	16.67	13.33	10.00	94.40	25.00
Feed cost	4018.00	5106.67	3183.33	5746.67	4886.67	4766.67	3980.00	4523.60	4526.23
Litter cost	15.00	0.00	0.00	0.00	0.00	0.00	33.33	6.00	6.73
Labor cost	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Housing cost with 10% Depreciation	405.93	430.67	226.67	362.07	323.07	287.67	303.83	277.79	323.41
Miscellaneous cost	1875.00	1896.67	16.67	33.33	0.00	0.00	0.00	0.00	440.96
Gross Cost	8377.67	9742.67	5018.47	7345.73	7521.79	6700.27	5814.91	6625.94	7103.62

BDT: Bangladeshi Taka

a maximum of 160 BDT for veterinary services per year, while farmers in the Feni and Pabna districts paid 116.67 BDT and 40.00 BDT, respectively, for the same services. One of the main challenges was the high feed cost. For example, in the Sherpur district, annual feed cost reached 5746.67 BDT, whereas in the Joypurhat district comparatively lower feed cost of 3183.33 BDT/year was found.

Furthermore, there were no expenses associated with labor, transportation, or electricity for rearing and managing native chickens in the research areas. The Rangpur district recorded the highest housing cost of 430.67BDT/year with 10% depreciation, while the Joypuhat district recorded the lowest cost of 226.67BDT/year for a native chicken house. Additional expenses associated with raising native chickens were discovered to be 1896.67 BDT per year in the Rangpur and 1875.00 BDT per year in the Pabna district. The principal costs associated with rearing and managing native chickens in the chosen regions are shown in Table 10. Expenses related to veterinary service, veterinarian fees, litter, and disinfectant costs were minimal because only a small number of farmers invested on these items.

5. Income Generation via the Production and Raising of Native Chickens

The main sources of revenue from native chicken production are presented in Table 11. The Patuakhali district recorded the highest overall income at 17,308.20 BDT per year, while the Joypurhat district reported the lowest at 9,200.67 BDT per year. In terms of income from the sale of native chickens specifically, farmers in Patuakhali earned a maximum of 6,492.20 BDT annually, whereas the lowest income from chicken sales was recorded in Pabna, at 3,583.33 BDT per year.

Farmers in Pirojpur earned the highest income from selling native chicken eggs, with an annual average of 3,378.67 BDT, while farmers in Joypurhat earned the least, at only 216.67 BDT per year. In Patuakhali, individual farmers earned the highest income from selling chicks, at 40.00 BDT annually. The highest household consumption of native chickens was valued at 4,184.00 BDT per year in Patuakhali, which also recorded the lowest values for gifts (58.00 BDT) and closing stock (1,466.00 BDT). In contrast, Joypurhat had the lowest household chicken consumption, valued at 1,760.00 BDT annually.

Regarding egg consumption, the highest annual household value was observed in Feni (2,070.00 BDT), while the lowest was recorded in Sherpur (640.00 BDT). Pabna reported the highest value for gifted native chickens at 2,052.67 BDT per year, whereas Sherpur had the highest value from closing stock at 2,686.67 BDT per year. Across all the surveyed regions, there was no significant revenue generated from the sale of native chicken litter

6. Net Benefit and Benefit-Cost Ratio (BCR)

The Patuakhali district recorded the highest net income at 10,682.30 BDT and the highest Benefit-Cost Ratio (BCR) of 2.61. In contrast, the Rangpur district had the lowest BCR, at 1.56. Across all selected locations, the average net benefit was 7,586.70 BDT, with an overall BCR of 2.07. Two key indicators used

Table 11.
Income generation via the production and

Category	Average income generation (BDT/year) of farmers from Native chicken rearing and production (Mean)								
	Pabna	Rangpur	Joypurhat	Sherpur	Pirojpur	Feni	Sunamgonj	Patuakhali	Overall
Chicken sell	3583.33	4580.00	4733.33	4666.67	5680.67	5863.33	5920.00	6492.20	5290.12
Egg sells	1882.00	1966.67	216.67	423.33	3378.67	3233.33	2246.67	3324.40	2179.38
Chick sell	0.00	0.00	0.00	0.00	10.00	23.33	10.33	40.00	12.73
Family consumed Chicken value	2273.33	2790.00	1760.00	3740.00	3416.67	3470.00	3040.00	4184.00	3168.85
Family consumed Egg value	1647.60	1406.67	640.00	743.33	1943.33	2070.00	1651.67	1743.60	1500.99
Gift value	2052.67	2020.00	0.00	226.67	133.33	480.00	475.00	58.00	632.81
Selling Liter	0.00	0.00	33.33	0.00	66.67	0.00	0.00	0.00	11.54
Closing stock value	2119.33	2503.33	1850.67	2686.67	1596.67	1644.00	1670.00	1466.00	1905.46
Gross Income	13558.27	15266.67	9200.67	12486.67	16159.33	16784.00	15013.67	17308.20	14690.34

Bangladeshi Taka (BDT)

to assess the profitability of native chicken production are gross revenue (benefit, B) and total expenditure (cost, C). Among all regions, the Joypurhat district had the lowest net income, at 4,182.20 BDT. Detailed figures on net benefits and Benefit-Cost Ratios for native chicken farmers in the study areas are presented in Table 12.

7. Contribution of Native Chicken Farming to Family Income

As shown in Table 13, the highest contribution of native chicken farming to annual household income was observed in Pabna, at 13.83%, followed by Patuakhali (10.87%) and Rangpur (10.32%). The lowest contribution was recorded in the Feni district, at 4.84%. On average, the total annual family income across all regions was 188,623.07 BDT, of which 14,690.34 BDT came from native chicken farming.

8. Production function analysis

A multiple regression model was employed to estimate the factors influencing income or profit generated from rearing native chickens in selected areas. A total of ten (10) independent variables were considered in the analysis. Among them, seven (7) variables were identified as key contributors significantly affecting the production process, while three (3) variables were statistically non-significant based on t-statistics. The results of the multiple regression analysis on native chicken rearing are presented in Table 14.

(A) Interpretation of the estimated model

From the production function analysis, it was found that the family size, chick price, vaccine and medicine expenses, veterinary service fees, disinfectants, feed expenses, and litter significantly affected the gross returns and profit of the native chicken production.

Family size (X1): The estimated value for the coefficient of family size was 0.112 for native chicken-rearing farmers which was significant at a 5% level probability level. There was a positive relationship between family size and the gross return and indicating a 5% increase in family size on average led to 11.2% rise in the gross return and profit of native chicken farmers.

Chick price (X2): It is evident from Table 14 that the regression coefficient of the chick price was estimated as 0.25 for native chicken which was significant at 1% probability level. Therefore, there was a positive relationship between the chick price and gross return. With other variables being constant, 1% increase in the chick price on average led to a rise of 25% in gross return for native chicken rearing farmers,.

Vaccine and medicine expenses (X3): In the case of vaccine and medicine expenses, the coefficient was 0.149 for the sampled farmers which was significant at 5% probability level. Consequently, vaccine and medicine expenses had a positive relationship with gross return.

Table 12.
Net benefits and benefit-cost ratio of native chicken growers in the chosen areas

parameters/ Variables	Pabna	Rangpur	Joypurhat	Sherpur	Pirojpur	Feni	Sunamgonj	Patuakhali	Overall
Gross Income (GI) BDT/year	13558.27	15266.67	9200.67	12486.67	16159.33	16784.00	15013.67	17308.20	14690.34
Gross Cost (GC) BDT/year	8377.67	9742.67	5018.47	7345.73	7521.79	6700.27	5814.91	6625.94	7103.62
Net Income BDT/year	5180.60	5524.00	4182.20	5140.90	8637.50	10083.73	9198.80	10682.30	7586.70
BCR	1.62	1.57	1.83	1.70	2.15	2.50	2.58	2.61	2.07

Bangladeshi Taka (BDT), Benefit Cost Ratio (BCR

Table 13.
Contribution of Native chicken farming in Family income

Parameters	Income/year from NC	Total Family Income (BDT/year)	Income (%) from NC in total family income
Pabna	(BDT)	98000	13.83
Rangpur	13558.27	147900.00	10.32
Joypurhat	15266.67	157566.67	5.84
Sherpur	9200.67	196633.33	6.35
Pirojpur	12486.67	222866.67	7.25
Feni	16159.33	346766.67	4.84
Sunamgonj	16784	199600	7.52
Patuakhali	15013.67	159240	10.87

Bangladeshi Taka (BDT), Native Chicken (NC), Percentage (%)

That showed a 5% increase in vaccine and medicine expenses on average led to 14.9% rise in gross return from native chicken farming with other variables being constant. This specified that the farmers who used vaccination and medicine for their native chicken got 16.4% more profit than the farmers who did not use vaccine and medicine.

Veterinary service fee (X4): The estimated value of the coefficient of veterinary service fee was 0.220 for native chicken-rearing farmers, which was significant at 1% probability level. This value implied that the respondents who received veterinary services got 22% more profit than the respondents who did not receive any veterinary services.

Feed cost (X6): It is evident from Table 13 that the coefficient of the feed cost was estimated as 0.443 for native chicken rearing farmers which was significant at a 1% probability level. Therefore, there was a positive relationship between feed cost and gross return, showing that 1% increase in the feed cost of the farmers, on average, led to 44.3% rise in gross return, remaining other variables constant.

Litter cost (X7): In the case of litter cost, the coefficient was 0.067 for the sampled farmers, which was significant at a 10% probability level. Therefore, litter cost and gross return had a positive relationship, indicating that 10% increase in litter cost, on average, led to 6.7% rise in gross return for native chicken rearing farmers, holding other variables unchanged.

Value of R2: The estimated value of the coefficient of multiple determinations, the R2 value of the adjusted model was 0.462, which indicated that about 46.2% of the total variation in gross return under native chicken rearing farmers could be explained by the variables included in the model. In other words, 53.8% of the total variation in the gross return was unexplained due to the variables that were not included in the model.

Value of adjusted R2: The estimated value of the adjusted R2 of the model was 0.441 for native chicken rearing farmers (Table 14). Here, adjustment is for the degrees of freedom (Gujarati, 2003). This value indicated that about 44.1% of the total variation in the gross return under native chicken farming was explained by the variables included in the model considering the degrees of freedom.

F-count: The F-statistic was estimated for the overall significance of the estimated model. The F-count of the derived model was 21.407. This value was highly significant at 1% probability level implying that all the explanatory variables included in the model were important for explaining the variation in gross return and profit for native chicken rearing.

Table 14.
Multiple regression analysis

Independent Variables	Regression Coefficients	t-count	Sig.
(Constant)	4379.239	2.624	0.009***
Farmer's age	-0.049	-1.018	0.310
Family size	0.112	2.335	0.020**
Chick price	0.250	5.053	0.000***
Vaccine and Medicine cost	0.149	2.131	0.034**
Veterinary Service Fee	0.220	4.570	0.000***
Disinfectant cost	0.118	2.315	0.021**
Feed cost	0.443	8.316	0.000***
Litter cost	0.067	1.421	0.156*
Housing cost with 10% Depreciation	-0.014	-0.275	0.784
Miscellaneous cost	-0.019	-0.258	0.796
F-count	21.407		0.000***
Adjusted R Square	0.441		
R-Square	0.462		
Y=Profit			

Figures in the parentheses indicate the significance level; ***, p<0.01; **, p<0.05; *, p<0.1.

(B) Multi-Collinearity Analysis

The multi-collinearity test aimed to test whether the regression model found a correlation between the independent variables or not. For this test, the value of the correlation coefficient (r) between the independent variables was considered. According to Gujarati (1999), multi-collinearity occurs if the value of the correlation coefficient between independent variables is greater than 0.85. The value of the correlation coefficient between the independent variables is presented in Table 15. The analysis results of the multi-collinearity in Table 15 showed that the value of the correlation coefficient between the independent variables was less than 0.85. Conequetly, the data did not show multi-collinearly

Table 15.
Multi-Collinearity Analysis

Multi-Collinearity Analysis									
	X1Logs	X2logs	X3Logs	X4Logs	X5Logs	X7Logs	X8Logs	X9Logs	X10Logs
X1Logs	0.00	0.00	0.00	0.02	0.00	0.25	0.00	0.11	0.00
X2Logs	0.00	0.00	0.00	0.01	0.40	0.02	0.00	0.27	0.00
X3Logs	0.00	0.00	0.00	0.00	0.28	0.12	0.00	0.57	0.00
X4logs	0.00	0.00	0.01	0.03	0.26	0.46	0.00	0.00	0.00
X5Logs	0.00	0.00	0.00	0.35	0.02	0.06	0.09	0.01	0.26
X6Logs	0.00	0.01	0.30	0.00	0.03	0.00	0.33	0.01	0.32
X7Logs	0.00	0.05	0.10	0.38	0.00	0.07	0.51	0.00	0.08
X8Logs	0.07	0.16	0.43	0.18	0.00	0.01	0.01	0.02	0.28
X9Logs	0.39	0.60	0.01	0.01	0.00	0.01	0.05	0.00	0.00
X10Logs	0.54	0.17	0.15	0.02	0.01	0.00	0.01	0.00	0.05

or there was no relationship between the independent variables. Hence, the classical assumptions were satisfied.

9. Restrictions on the production and rearing of native chickens

Farmers face several obstacles when rearing and producing native chickens. According to field survey data, the majority of farmers (80.4%) had to deal with disease outbreaks and predator attacks, and 60.8% experienced a shortage of vaccines in the research areas. Among the surveyed producers, 51.2% reported high chick mortality, while 48.8% mentioned the very high cost of feed as constraints. Furthermore, 39.6% reported that government-provided vaccines were unavailable for native chicken. According to the results, 28.5% of farmers cited the lack of access to high-quality chicks for raising, 22.3% theft, and 6.2% the high price of DOC. A smaller proportion of farmers (3.5%) stated that DOCs were unavailable, and 3.1% reported that native chicken farming often did not ensure profit.

Table 16.
Principal constraints regarding native chicken rearing

Category	Income/year from NC
Lack of good quality chicks	28.5 (74)
Outbreak of diseases	80.4 (209)
Chicks' death rates are high	51.2 (133)
High price of one-day chicks	6.2 (16)
Unavailability of native chick	3.50 (9)
Unavailability of Govt. Vaccines	39.6 (103)
Higher price of poultry feed	48.8 (127)
predatory animals attack	80.4 (209)
Lack of vaccine	60.8 (158)
Profit not guaranteed	3.10 (8)
Problem of thief	22.3 (58)

Number (N)

Table 17.
Perspectives of farmers on how to address issues and limitations about the management and rearing of native chickens

Category	Percent (N)
Needs to make a trap to save chicken from predator animals	32.7 (85)
The authorities should arrange training programs for poultry farmer	52.7 (137)
Govt. / Bank officials should provide loans for small farmers/entrepreneurs.	37.3 (97)
Govt. vaccine supply should be available and free of cost	53.5 (139)
The authorities should encourage farmers in poultry farming	55.8 (145)
Good quality chick supply should be available to the farmers	71.5 (186)
Need sufficient knowledge about poultry disease and poultry rearing method	22.7 (59)

Number (N)

10. Perspectives of farmers on resolving issues and limitations

To address the limitations and difficulties, about 71.5% of farmers requested a supply of high-quality chicks, 55.8% suggested that the authorities should encourage farmers to engage in poultry farming,

52.7% demanded a training program on native chicken rearing and management, 53.5% vaccines provision by government, 37.3% nedded loans or other incentives for native chicken rearing, and 32.7% opined to protect their chickens from predator animals. From survey findings, 22.7% of farmers stated that they did not know about managing chicken diseases and rearing chicks.

Discussion

These findings are quite comparable to those of [5], who reported that the mean age of chicken farmers was 37.95±0.77 years. The largest household size is 5.36 in Sunamgonj, which was in line with the [6] report. The lowest household size is 4.03 in Rangpur, which differs slightly from the findings of BBS as the lowest household size is 4.00 in the Rajshahi division. This discrepancy may be attributed to the limited number of survey locations and variations in sample size. In the surveyed regions, the average number of earning members per household was 1.20 ± 0.03, with a dependency ratio of 3.67.

In the case of education, the findings are consistent with those of [7], who reported that 31.25% of individuals have an education that helps them manage their farms, 16.35% have completed SSC or above, 6.25% have completed higher education, and 33.75% are illiterate. These results were marginally lower than that of the BBS 2022 report, which indicated that nationally, 74% of people were literate and 26% were illiterate. According to [5], 9% of farmers did not go to school. This was due to the limited sample size and the random data collection from local households engaged in native chicken farming.

In agriculture, the head of the household works 34.6%, which is comparable to the 36.50% reported in the study [5]. This figure differed from the report of [5] who stated that the predominant occupation was day labor (19.50%) and others for 4.50%. According to their findings, the majority of family poultry farmers (43%) worked primarily in the agriculture sector, with the remainder in business (20%), services (10%), and other occupations (27%).

The research conducted by [8] stated that 58.33% of farmers in Sylhet raised 0-15 checkens per family, while 41.67% of farmers raised more than 15 chickens. Meanwhile, [9] reported that 98.75% of rural women reared small flocks (5-13 chickens) and 1.25% raised large flocks (21-29 chickens) because mothers had little children. About 95% of farmers reared common deshi chicken and the rest reared hilly (2.3%), naked neck (1.2%), and both naked neck and common deshi chicken (1.5%). [10] stated that the mean monthly intake of chicken for a family was 1.15±0.03, which was more than 6.02±1.61 chickens where a household consumes annually.

These data on women's contribution was very similar to that of [9], who reported that the majority of rural women (88.75%) raised backyard chickens as a source of income, followed by both (11.25%) a source of income and own consumption. A report by [11] stated that households kept poultry primarily for income generation (55%) and home consumption (22%). Halima *et al.* reported that the objectives of rearing village chicken in Ethiopia is income generation and household consumption [12]. In contrast to the current study, [13] said that the primary purpose of hens for farmers is to provide meat and eggs for domestic use.

Furthermore, the research areas found higher market value for roaster, hen, and chick than in the previous year. This scenario was comparable to that of [14] who found that the price of an adult chicken ranged from BDT 320 to BDT 370. This study was comparable to [10] who discovered that selling eggs and chicks was directly from households in 72.4% cases and via the village market in 27.6%. According to [15], approximately 48.96% of participants sold their chicken goods in the village market, 5.21% at nearby retailers, 22.92% at their doorstep, 3.13% as entire sellers, and 19.79% at home. According to [16], 50% of farmers incubate chicken eggs for newborn chicks. In addition, 18% and 32% of farmers travel to the market and neighbours. According to [17], bird sources possess 77.65%, sell 55.88%, and have a neighbouring in 1.18%. These results contrasted with those of [15], who found that consumers preferred exotic (17.71%), local (55.21%), and equal breeds of meat and eggs (27.08%). [18] stated that the producer-level egg price was found to be BDT 8.13 for local hens, BDT 9.65 for ducks, and BDT 7.69 for layers, which was in line with the findings of the current investigation.

Results of Rajsahi relate by [19], who indicated a net income of 3207; [14] reported an annual net return of BDT 3705.95, which was less than the current study. The BCR values were 1.25 relevant to [19], which was 1.24. The family poultry produced 1.90 BCR according to [14]. The BCR illustrates the financial viability of farm. A high BCR shows that rearing chickens as a family is a lucrative endeavor. According to [20], BCR was 5.57, which was greater than the present study. The difference in income results from the time frame because the paper was published ten years ago by [21]. The contribution of native chicken farming to family income was 7.79%. Native chickens play a great role for family income in different areas of Bangladesh.

This result was connected to the findings of [15],

who found that 34.38% of the respondents indicated the prevalence of illness, 18.75% reported predator assaults, and 17.71% claimed the lack of instruction regarding poultry management techniques. The remaining respondents brought up the following issues: theft (3.13%), lack of marketing, lack of money for beginning (9.38%), and the unavailability of veterinary services (12.50%). In 2023, Chowdhury *et al.* discovered that 22.5% of farms had lower egg prices, 10% had lower meat prices, 15% lacked training facilities, 25% had technical issues, 10% had housing issues, 32.5% had marketing issues, and 65% had economic issues [7]. According to [22], the majority of families (88.79% in Chapai Nawabganj and 83.80% in the Sylhet region) identified several significant challenges, such as the death of baby chicks by predators, the lack of vaccination, and the damage caused by chickens to cultivated crops. [17] reported that the death rate from predator attacks was 8.82%, the death rate from disease was 54.12%, and the death rate was 37.06%. In this regard, [16] reported the main issues with backyard chicken keeping the use of backdated techniques, the lack of feed, improper housing facilities, a high frequency of illness, a shortage of vaccines and medications, and predator attacks. The main obstacles to backyard poultry production, according to [23], were disease (38.1%) and predators (23.1%). Moreover, [24] reported 33.1% disease incidence and 12% predators in East Shewa, Ethiopia. A report by [25] showed 100% higher disease incidence and 89.17% predator attack in the Bhandara district of Maharashtra, India. [26] showed that poor housing (44.86%), unreliable and disorganized marketing system (12.78%), the lack of capital (41.86%), institutional credit facilities (47.89%), disease outbreak (16.02%), feed scarcity (8.86%), the lack of training and extension services (6.07%), and the lack of sufficient vaccines and medications (5.56%) were the main causes of chicken rearing in the native environment.

These results were also in line with [16], who suggested that high-yielding deshi bird varieties needed to be available, village women should participate in training programs on managing and rearing poultry, farmers should be able to afford feed, medicine, and vaccinations, and extension and motivational work should be practiced. According to [26], the development of poultry enterprises depended on the improvement of breeds through appropriate breeding methods (33.4%), proper vaccination programs (25.03%), proper management and veterinary training for farmers (16.05%), organized markets for buying and selling (7.50%), assurance of an easy bank loan system (7.90%), and low-cost processed feed (8.50%).

Materials & Methods

Study areas and duration

Pabna, Rangpur, Feni, Sherpur, Pirojpur, Patuakhali, Joypurhat, and Sunamgonj were eight districts from six divisions of Bangladesh, which were chosen for data collection from June 2023 to December 2023.

Data collection

A baseline survey was conducted to learn more about the issues faced by local chicken producers in the chosen regions of Bangladesh, as well as their gross production cost and revenue using a pre-designed questionnaire. Interviewers personally questioned the chosen farmers to collect primary data. Thirty different types of questions regarding poultry farming in the households of 260 farmers, 50 from Patuakhali and the remaining from other districts, were gathered through a field study that involved the first-hand observation and interviews of farmers. Secondary data might be found in several places, such as books, theses, papers, journals, government documents, and Bangladesh's statistics yearbooks. Details included the BCR, issues, native chicken marketing status, production and consumption of poultry meat and eggs, and farmer demographics.

Statistical analysis

Collected data were entered, sorted, compiled, tabulated, and organized into a Microsoft Excel sheet. Next, data were statistically analyzed by Statistical Package for the Social Sciences (SPSS) version 25. All data were then tabulated using descriptive statistics, such as frequency distribution, percentage, mean, and standard error value for further interpretation.

For calculating net return, we used the following formula:

Net return=GR-GC (Where, GR is gross return and GC is gross cost)

To calculate the BCR, we used the following formula:

Benefit-Cost Ratio = (Gross return (GR))/(Gross cost (GC))..... ..... (1)

The gross return includes the average return from the main product and by-products of native chicken. Gross cost entails the total cost of native chicken rearing. The BCR was a relative measure used to compare benefit per cost. It helped to analyze the financial efficiency of the farms. The multiple regression model was used to determine the effects of key variables. The completion of the relationship between Y and X was by regression, such as the variation of Y that was affected by the variation of X with an estimation model using the simple multiple regression method, which can be written as follows:

Y = a + b1X1 + b2X2+ b3X3 + b4X4+ b5X5 + b6X6 + ei..... ..... (2)

Where, Y=Profit of native chicken farmers (BDT/year)

a=Constant

b=Regression coefficient

X1=Age of Farmer

X2=Family Size

X3=Cost of chicken purchasing

X4=Cost of Vaccine and Medicine

X5=Cost of Veterinary Service

X6=Cost of Disinfectant

X7=Cost of Feed

X8=Cost of Litter

X9=Cost of Housing

X10=Miscellaneous Cost

Hey=Disturbance factors

The equation is converted into a multiple linear form by the logarithm of the equation to make it easier to estimate the equation above. The logarithmic form of the equation is:

Log Y = Log a + b1 log X1 + b2 log X2 +...b6 log X 6+ u..... ..... (3)

The multi-collinearity test was applied to analyze multiple regression consisting of two or more independent variables (X1, X2, X3, X4,…, Xn), in which the degree of association of the relationship or influence between the independent variables would be measured through the magnitude of the correlation coefficient (r). Multi-collinearity occurred if the coefficients between the independent variables (X1 and X2, X2 and X3, X3, and X4) were greater than 0.60 (other opinions were 0.50 and 0.90). Multi-collinearity did not occur if the correlation coefficient between independent variables was less than or equal to 0.60 (R2 depicted that there were no symptoms of multi-linearity, but if r2 < R2, it showed the model contained multi-clinical issues [27].

Authors' Contributions

This research was carried out with the collaboration of all authors. Syidul Islam designed the study, wrote the protocol, collected the data, and wrote the manuscript. Sharmin Sultana and Md. Ashraful Islam helped with data collection and manuscript writing. Dr. Shamim Ahmed helped in writing the manuscript. Dr. Razia Khatun provided support and guidelines for writing this article. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no competing interests to declare.

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Ergothioneeine Modulates Interleukin-6 Serum Concentration in Arabian Stallions Following a Two Thousand Meterm Race at Maximum Speed in a Hot-Dry Environment

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ABSTRACT

This experiment was performed to determine the effect of ergothioneine's effect on serum interleukin-6 (IL-6) concentrations after a race of 2000 m in Arabian stallions at the highest possible speed in a hot-dry environment. Twenty-four apparently healthy stallions of the Arabian breed were used. The average weight and age of the horses were 401 ± 32.11 kg and 5.7 ± 0.54 years, respectively. The experimental subjects were split assigned into three units groups of eight stallions each. Group 1 (EXEN) was neither treated with ergothioneine nor exercised. Group II (EXEC) was not treated with ergothioneine but was exercised. The third group (EXEE) was treated with ergothioneine per os at a dose rate of 0.02 mg/kg daily for one month prior to the experiment. Meteorological parameters of the study site were recorded using a dry and wet-bulb thermometer. The stallions in the EXEC and EXEE groups were made to run a race of 2000 m at the highest possible speed (60 Km/h) by trained riders. Samples of Blood samples of 10 ml were collected from all stallions before the experiment and immediately after the experiment and two hours post-experiment. The hematological parameters and concentration of IL-6 were determined in all samples. The concentration of IL-6 was found to be higher significantly higher in the EXEE group than in others, suggesting a modulatory role of ergothioneine. Therefore, it was concluded that ergothioneine enhanced IL-6 following exercise and would be beneficial to for stallions during exercise.

Keywords

Ergothioneine, Interleukin 6, Hematological parameters, Exercise, Arabian stallions

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Abbreviations

IL-6: Interleukin 6
ERG: Ergothioneine
EXEN: Not treated, not exercised
EXEC: Not treated but exercised
EXEE: Treated and exercised

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Introduction

The body's homeostatic response of the body is tremendously affected by rigorous exercise and is negatively influenced by high ambient temperatures and relative humidity [1].

Although several sources of IL-6 have been identified such as (e.g., T cells, B cells, macrophages, neutrophils, monocytes, keratinocytes, fibroblasts, endothelial cells, epithelial cells, osteoblasts, chondrocytes, adipocytes, and mesangial cells), the majority of the IL-6 that is produced during exercise and is seenfound in the peripheral blood comes from contracting muscles [2]. Exercise intensity and duration determine how much of the IL-6 response is produced [3]. Muscle contractions and IL-6 synthesis may be connected by several mechanisms and theories;: transcriptional factors that control the production of IL-6 can be activated by alterations in the homeostasis of calcium, decreased availability of glucose, and enhanced free radicals generation [4-6]. Leukocytes, the hepatic tissue, fatty tissue, and the interaction of the hypothalamus, pituitary, and adrenal glands, are all modulated by IL-6 and may all affect how the body responds to exercise in terms of metabolism and immunity. However, for a marked systemic IL-6 response to occur, a significant amount of muscle tissue must be exercised [7]. Intrleukin-6The IL-6, also known as a myokine, possesses a significant anti-inflammatory prpertyability in its effects as a myokine. It significantly increases rises during exercise and is present in the blood before other cytokines. It is believed that it acts as a hormone to increase extracellular substrates during exercise [8].

Animals cannot synthesize ergothioneine (ERG), an extremely stable, naturally occurring nutraceutical that can only be obtained through their food. It is produced by Actinomycetales bacteria and non-yeast fungi. The greatest amounts of L-ergothioneine are found in mushrooms, especially in species like such as Boletus edulis and Pleurotus ostreatus. L-ergothioneine being a potent antioxidant may enhance the synthesis of IL-6 thereby quickly promoting the repair of damaged tissues. It is eliminated from the bloodstream and accumulates in cells and tissues following damage and oxidative stress [9]. It is swiftly absorbed by tissues by an exceptional transporter known as organic cation transporter new type 1 (OCTN-1), which plays distinct and significant functions in cellular processes [10-11]. The rate at which cells collect it suggests that ERG serves a critical physiological role [12]. Ergothioneine has two major functions: it regulates energy and shields tissue from oxidative and inflammatory harm. Ergothioneine It does not oxidize at the usual body pH, making it a highly stable antioxidant. Moreover, it prevents the generation of hydroxyl radicals from ferrous (Fe2+) ions and hydrogen peroxide [13]. Ergothioneine combats reactive oxygen species produced during physical activity, such as O2–, H2O2, ·OH-, and O2, functioning both as an selfless antioxidant and as a modulator of the cellular antioxidant defense and also modulating cytokine such as IL-6, thereby influencing cellular responses and functions [11].

The diseases rheumatoid arthritis and Crohn's disease are two long-standing inflammatory disorders that have been linked to changes in the gene coding for ergothioneine ERG transporter (OCTN1) [14-15]. Higher erythrocyte and monocyte concentrations of EGT ERG were discovered in individuals with less inflammation, and these levels were strongly linked with the expression of ergothioneine ERG transporter mRNA in specific body cells [16].

Consequently, this experiment aimeds to ascertain the effect of ergothioneine ERG on the myokine, interleukinIL-6, after an exercise of 2000 m at the highest possible speed (60 Km/h) in Arabian stallions.

Results

The meteorological indices of the site of the study in the hot-dry season are shown in Table 1. The dry-bulb temperature (DBT) rose ($p < 0.05$) from 22.6 ˚C±1.23 ˚C at 6 a.m. in the morning to 38.6 ˚C ± 6.53 ˚C at mid-day. The humidity index rose ($p < 0.05$) from 64.4% ± 2.34 % at 6 a.m. to 74.3% ± 6.73 % at mid-day (12.00 h). The temperature-humidity index increased from 76.41± 0.56 at 06.00 h a.m. in the morning tto 83.36 ± 4.53 at mid-day (12.00 h).

The findings of hematological indices are shown in Table 2. A higher leukcocyte count (8.73 ± 0.94 × 109/µl)) was obtained in the EXEC group compared to the count of 4.03 ± 0.14 × 109 /µl recorded in the EXEE group. The neutrophil count of 4.04 ± 3.09 × 109 /µl recorded in the EXEE group was higher ($p < 0.05$) than the value (2.14 ± 0.63 × 109/µl)) obtained in the EXEE stallions after the race.

The value of stress index recorded in the EXEC group (4.17 ± 0.69) was higher than the value of 2.97 ± 0.13 recorded in the EXEE horses.

The IL-6 concentrations recorded in this study are shown in Figure 1. Concentrations of IL-6 obtained

Abbreviations-Cont'd

ROS: reactive oxygen species
RH: relative humidity
THI: temperature humidity index
DBT: dry bulb temperature

Table 1.
Meteorological Parameters of the Experimental Site during the Hot-Dry Season

Time of Day	Dry-Bulb Temperature (°C)	Relative Humidity (%)	Temperature-Humidity Index
06.00	22.6 ± 1.23[a]	64.4 ± 2.34[a]	76.41 ± 0.56[a]
	(22 - 24)	(63 – 68)	(68.71 - 83.65)
12.00	38.6 ± 6.53[b]	74.3± 6.73[b]	83.36 ± 4.53b
	(37-39)	(72 - 78)	(81.32 - 89.01)
18.00	36.5 ± 0.17	78.8 ± 5.98	83.24 ± 3.49
	(36-37)	(76 - 81)	(83.21 - 84.95)
Overall Mean ± SEM	37.22 ± 4.17	75.19 ± 5.98	81.45 ± 5.18
	(36.11 - 37.33)	(73.65 – 81.11)	(78.11-84.76)

[a,b]Means for the same column having different superscript letters are significantly ($p < 0.05$) different .Values in Parentheses are Minimum - Maximum.

Table 2.
Haematological Parameters of Stallions in the Hot-Dry Season

Parameters	Time	NEXE	EXEC	EXEE
Leucocytes (×10^9/L)	Pre-exercise	3.21 ± 0.13	4.41 ± 0.18	4.82 ± 0.86
	Post-exercise	3.33 ± 0.24	8.73 ± 0.94[a]	4.03 ± 0.14[b]
Neutrophil (×10^9/L)	Pre-exercise	2.53 ± 0.11	2.83 ± 0.46	2.62 ± 0.76
	Post-exercise	2.42 ± 0.13	4.04 ± 3.09[a]	2.14 ± 0.63[b]
Lymphocytes (×10^9/L)	Pre-exercise	1.32 ± 0.21	1.72 ± 0.21	1.80 ± 0.19
	Post-exercise	1.48 ± 0.42	1.69 ± 0.42	1.63 ± 0.57
Monocytes (×10^9/L)	Pre-exercise	0.11 ± 0.05	0.17 ± 0.02	0.17 ± 0.03
	Post-exercise	0.17 ± 0.03	0.18 ± 0.04	0.21 ± 0.02
Erythrocytes (×10^{12}/L)	Pre-exercise	6.12 ± 0.13	7.42 ± 0.23	6.82 ± 0.37
	Post-exercise	15.22 ± 3.74	11.63 ± 1.54	12.22 ± 0.68
Total Protein (g/dl)	Pre-exercise	4.4 ± 0.13	3.9 ± 0.16	4.35 ± 0.21
	Post-exercise	5.1 ± 0.17	6.77 ± 0.56	7.11 ± 0.58
Packed Cell Volume (%)	Pre-exercise	32.13 ± 7.25	29.07 ± 0.26	29.15 ± 1.21
	Post-exercise	35.42 ± 0.14	45.42 ± 0.24[a]	54.42 ± 3.87[b]
Haemoglobin (g/dl)	Pre-exercise	9.71 ± 0.36	8.71 ± 0.36	7.65 ± 0.31
	Post-exercise	9.95 ± 3.11	10.27 ± 2.77	8.93 ± 2.83
Neutrophil/Lymphocyte ratio	Pre-exercise	1.91 ± 0.88	1.65 ± 0.32	1.46 ± 0.64
	Post-exercise	2.02 ± 0.03	4.17 ± 0.69[a]	2.97 ± 0.23[b]

[a,b]Means for the same row having different superscript letters are significantly ($p < 0.05$) different.
Key: NEXE = Not administered with ergothioneine, not exercised
EXEC = Not administered with ergothioneine but exercise
EXEE = Administered with ergothioneine before exercise

post-exercise were significantly higher in the EXEE stallions than in the EXEC stallions.

Discussion

The environmental temperature, humidity index, and temperature-humidity index of the experimental site in the hot-dry season were all found to be relatively high. The study's overall mean DBT of 37.22˚C ± 4.17 ˚C was higher than the 20˚C–25 ˚C range considered to beas the thermoneutral range zone for horses [17-19]. Horses experience heat stress when the ambient temperature is higher than the thermoneutral zone because of increased metabolism, enhanced heat losses via skin evaporation, and lowered thermal insulation. The RH value (75.19% ± 5.98%) recorded was also higher than the 70% level recommended for horses [20]. Elevated DBT and RH values in this the current experiment suggest that the horses experienced thermal stress. High DBT and RH make it challenging for sweat to evaporate from the skin's surface because the air is already moist. The horse is therefore put under heat stress by this occurrence. According to dos Santos et al. [21], the THI, a measure of heat load, was extremely high in this study, indicating that the stallions were under stress due to the hot temperatures in the study area. Elevated THI levels found in this study indicated the need for interventions, including the installation of fans in the stallions' stables and the administration of supplements to the horses, to lessen the deleterious effects of heat stress. Exercise causes a horse to gradually lose heat from its muscles and internal organs into the blood, which worsens the effects of heat stress on the stallions' locomotor system and lowers performance

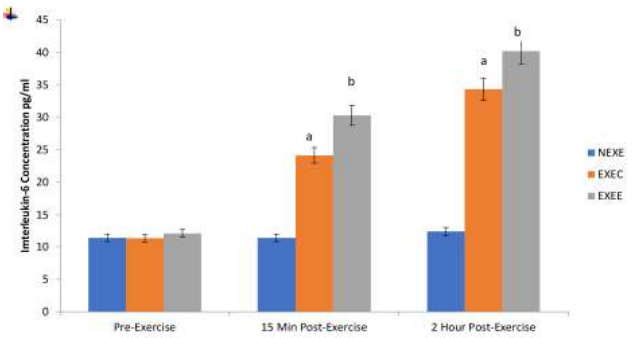

Figure 1.
Cincentration of interleukinIL-6 in the Stalions.
Key:
NEXE= not administrated with ergothioneine, not exercised
EXEC= not administrated with ergothioneine, but exercise
EXEE= Administrated with ergothioneine before exercise

[22].

According to Faria et al. [23], the stress index (neutrophil-lymphocyte ratio), which is an indicator of inflammation and stress, was higher in the EXEC stallions than in the EXEE stallions, suggesting that the treatment with ergothioneine ERG before exercise reduced the stress of exercise and lowered inflammatory response. Better stress management and a lowered inflammatory response are indicated by a lower stress index (neutrophil-lymphocyte ratio).

It is unclear how the skeletal muscle responds to exercise-induced inflammation and injury by coordinating an adaptation mechanism that protects the muscle from future harm, known as the repeated bout effect (RBE) [24]. although IL-6, which has both pro- and anti-inflammatory actions, plays a crucial role. IL-6It is a myokine and is produced and expressed by the muscles more than other cytokines, such as TNFα and IL1β. It sends out an alert signal when there is tissue damage due to exercise [8]. The anti-inflammatory impact typically occurs after a brief but intense exercise, as demonstrated in this study [25]. IL6'sThe anti-inflammatory effects of IL-6 are brought on by the classical signaling mechanism [8]. More than any other cytokine, the blood concentration of IL-6 rises after intense exercise [25]. It has been proven that IL-6 has a role in regulating the early inflammatory response brought on by exercise in horses [26]. After exercise, interleukin-6 (IL-6) is released from the contracting skeletal muscles into the blood stream and metabolism is characterizsed by a rapid increases in plasma levels, with a peak few hours after exercise, and a gradual return to basal levels within 48 hours. IL-6 plays a role in regulating energy intake during exercise, acting as a "smart meter" to signal the release of energy substrates from liver and fatty tissue [25]. Although having a low affinity for IL-6, soluble glycoprotein 130 (sgp130) possesses a marked affinity for the IL-6/sIL-6 complex, inhibiting the inflammatory action of IL-6 after brief, intense exercise as observed in this study (21). According to a study, myocytes and macrophages have quite different signaling pathways for interleukinIL-6 in the exercising skeletal muscle after a period of intensive exercise [27]. It appears that muscular interleukinIL-6 expression is controlled by a connection of signaling events, including the Ca^{2+}/NFAT and glycogen/p38 MAPK pathways, in contrast to macrophages IL-6 signaling, which depends on eliciting the nuclear factor kappa (BNF-B) signaling pathway [28]. Hence, IL-6 signaling in macrophages or monocytes results in inflammation, whereas the activation of IL-6 and its signaling in skeletal muscle after exercise is completely different from an earlier TNF response or NF-B activation and reduces inflammation [29]. Muscles are known to produce and release IL-6, which has been linked to the regulation of metabolism and the acceleration of satellite cell regeneration and proliferative activities [30]. InterleukinIL-6 is a short-term energy allocator that is also released from muscles as a result of reduced energy content. Interleukin-6 improves muscular energy uptake during exercise and releases cellular energy content through lipolysis [31]. We concluded that the greater levels of IL-6 after intense exercise may inhibit the excessive production of type 1 pro-inflammatory cytokines, hence lowering inflammation. An important enzyme involved in inflammation called myeloperoxidase (MPO) has been demonstrated to be inhibited by ergothioneine ERG. Moreover, it is widely known for terminating MPO-based reactive substances more quickly than glutathione and vitamin C. This includes HOCl [9].

Many investigations on this special molecule have revealed that it may lessen underlying disorders and tissue damage in various tissues, including the skeletal muscles [9]. Divalent metal ions, such as like Fe^{2+} and Cu^{2+} , can be bounnd to ergothioneine ERG with high stability constants [12]. By creating redox-inactive compounds with ergothioneineERG, this metal ion chelation stops the deterioration of biomolecules. Ergothioneine ERG could prevent future oxidative stress, which would reduce harm to muscles as well as, other tissues, and organs (especially the brain) [32]. While the expression of proinflammatory cytokines' expression is influenced by ergothioneineERG, it is possible that this compound could help break the cycle of hyperinflammation that is caused by macrophage activation and hyperferritinemia [33-34]. The findings of this study agree are in line with the study of Steensberg et al. [35] who reported that exercise can cause an increaseraise in interleukinIL-6, which can be explained by the synthesis of interleukinIL-6 in contracting human skeletal muscles. Ergothioneine ERG

effectively targeted reactive oxygen species produced during the exercise, such as O^{2-}, H_2O_2, ·hydroxyl ion, and oxygen, functioning both as a self-sacrificing antioxidant and as a modulator of the cellular antioxidant defense and immune systems, thereby influencing the cellular redox state. Once the cellular redox state was established, it enhanced the optimal activity of S-Adenosyl-methionine (SAM) synthase. SAM plays a crucial role in maintaining normal cellular function and survival, being involved in three essential metabolic pathways: transmethylation, trans-sulfuration, and polyamine synthesis, all of which are vital for normal cellular function and stability.

Conclusion

It can Therefore, it can be concluded that ergothioneine ERG administration to stallions before exercise will enhance some hematological parameters and the synthesis of IL-6.

Materials & Methods

Experimental Animals

We used 24 untrained, clinically healthy Arabian stallions aged 5.7 ± 0.54 (range: 5-6 years) years, with an average weight of 401 ± 32.11 kilograms (range: 395 to -404 kg). The stallions were obtained from a royal stable and were only used for pleasure riding. The stallions were divided into three groups of eight each. Group I (NEXE), did was not engaged in exercise and was not administered ergothioneineERG, the second group (EXEC), was engaged in exercise but was not administered ergothioneineERG, and the third group (EXEE), received ergothioneine ERG before exercise.

The housing for the stallions was constructed using cement bricks, a concrete floor, corrugated iron roofing sheets, and a wooden ceiling. The horses underwent two weeks of preconditioning. The horses were fed with hay and their meal was augmented with groundnut bran. They had unlimited access to water. The stallions were checked for intestinal helminths, and all the infected ones were treated using albendazole (5 mg/kg body weight; Jubaili Agrotec, Kano, Nigeria).

Determination of Thermal Environmental Parameters

The relative humidity (RH) and dry-bulb temperature (DBT) values were ascertained by deploying a dry and wet-bulb thermometer manufactured by Mark, England. The formula postulated by Hartmann et al. [17], stated below was used to calculate the Temperature Humidity Index (THI).

$$THI = (DBT \times 0.8) + \{(RH/100 \times (DBT-14.4) + 46.4\}$$

Blood Sampling

Before the experiment, the site of the sample collection was cleaned with a clean piece of cotton dipped in an alcohol solution. Samples of blood (10 mL) were obtained from each stallion 15 minutes after the exercise and 2 hours after the exercise using 18-gauge needles. Jugular venipuncture was used to get the blood samples, and they were subsequently dispensed into both plain vacuum containers and vacuum containers with the anticoagulant potassium ethylenediaminetetraacetic acid (EDTA). The hematological parameters and IL-6 levels of the samples of blood were determined in the physiological research laboratory after the samples were conveyed to the laboratory using a box packed with ice. The stallions were fasted for about three 3 hours and were fed only after the experiment.

Administration of Ergothioneine to Stallions

Ergothioneine The ERG manufactured by Oxis International, (Los Angeles, California, USA) was given orally to each horse in the EXEE group four weeks before the experiment at a dose of 0.2 mg/kg. In the morning, ergothioneine ERG was given administered before eating. Ergothioneine The ERG was placed in warm water and aspirated using a correct-sized syringe. The solution was given orally to the restrained horse by putting the syringe into the mouth's corner and depressing the plunger to dispense the medication into the animal's mouth. The head of the horse's head was then slightly raised to ensure that the entire solution had been swallowed.

Exercise Protocol

Following the treatment of the stallions in the EXEE group, each of them was mounted by an appropriately kitted and trained rider with an average weight of 70.56 ± 4.23 kg and exercised at 2000 m at the highest possible speed using a standard race track.

Determination of Haematological Parameters

Using a standardized automatic analyzer for veterinary use (KT-6610 VET) manufactured in Jiangsu, China,) the following parameters were determined: count of erythrocytes, count of leukocytes, haematocrit, and concentration of haemoglobin.

Determination of Serum Interleukin-6 Concentration

The Cloud-Clone Corporation (Houston, USA) horse IL-6 ELISA kit, a highly sensitive kit that shows detectable values in serum, was deployed to evaluate the serum content of IL-6. To obtain the serum, the whole blood was drawn, permitted to clot, and subsequently centrifuged to isolate the serum from blood cells and other elements. The clear, yellowish liquid that remains is the serum. An anti-interleukinIL-6 antibody which has been coated on the microplate was enclosed with this kit. The antibody conjugated with biotin unique for IL-6 was then placed in the appropriate microplate wells with the standards. ThenNext, each microplate well was added with horseradish peroxidase conjugated with avidin and placed in an incubator. Only wells that had interleukinIL-6, antibody conjugated with biotin, and Avidin- conjugated enzyme had altered its colour after the inclusion of Tetramethylbenzidine (TMB) solution subtrate. A solution of sulphuric acid was used to terminate the substrate-enzyme reaction, and the alteration in colour was evaluated spectrophotometrically at 450 nm wavelength. The optical density of the samples was then compared to the standard plot to ascertain the IL-6 content of the samples.

Data Analyses

The data obtained from this experiment was were expressed as mean ± standard error of the mean. The statistical test Shapiro-Wilk was used to determine whether the data was were normally distributed. The data was found to be normally distributed. The statistical test of one-way analysis of variance (ANOVA) was deployed applied to analyze the data after which it was subjected to Tukey's post- hoc test. Only values of $p \leq 0.05$ were considered significant. The Graph Pad Prism (version 5.3) manufactured by (GraphPad Software, Inc.) was used.

Authors' Contributions

ASA and JOA designed the experiment. ASA, JOA, PIR, and TA developed and edited the manuscript. DAA and ASA performed the research and analysis of the data.

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Conflict of interest

The authors hereby declare that they have no competing interests

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An update on epidemiological features, etiopathogenesis and therapeutic approaches of feline chronic gingivostomatitis

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ABSTRACT

Feline chronic gingivostomatitis (FCGS) is a severe, immune-mediated, inflammatory disease affecting the oral mucosal of cats. It is characterized by ulcerative and/or proliferative lesions, most commonly located lateral to the palatoglossal folds. Clinically, FCGS can lead to severe malnutrition and dehydration in critical cases. The pathogenesis of FCGS is poorly understood but it is considered a multifactorial disease, likely involving infectious agents and other parameters. FCGS seems to be a manifestation of an aberrant immune response to chronic antigenic stimulation. Disturbance and im balance of the oral microbiota also may play a role in the development of FCGS. Because of its unknown pathogenesis and long disease course, it is difficult to treat and has a high recurrence rate. The current standard of care involves dental extractions of at least all premolar and molar teeth, often in combination medical therapy. Standalone medical management has shown limited long-term efficacy. Emerging regenerative therapies, such as mesenchymal stem cell treatment, offer promising alternatives for management of FCGS.

Keywords

FCGS, clinical features, epidemyological features, treatment, oral inflammation.

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Abbreviations

FCV : Feline Calicivirus
FeLV : Feline Leukaemia Virus
FHV-1 : Feline Herpesvirus Type 1
FIV : Feline Immunodeficiency Virus
CPV : Canine Parvovirus Virus
FME full-mouth extractions
IFNs : Interferons
MSCs : Mesenchymal stem cells

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Introduction

FCGS is a chronic inflammatory disease marked by ulcerative and/or proliferative lesions effecting the gingiva and oral mucosa, particularly the palatoglossal folds [1, 2]. It may be referred to by other names, such as plasma cell stomatitis-pharyngitis, chronic faucitis, lymphocytic plasmacytic gingivitis-stomatitis and others [3]. FCGS is a painful and debilitating feline oral disease characterized by chronic severe bilateral inflammation of the gingiva, alveolar mucosa, labiobuccal mucosa, and caudal oral mucosa [4, 5]. Ulcerative or ulceroproliferative lesions are often observed in FCGS cases. In addition, FCGS has been shown to be associated with more widely distributed and severe periodontitis, as well as higher prevalence of external inflammatory root resorption and retained roots compared to other oral diseases [6]. This article reviews the current knowledge on the etiopathogenesis and epidemio-clinical features of FCGS and describes the leading treatment modalities. Cats affected by FCGS are often presented with dysorexia/anorexia, oral pain, weight loss, ptyalism, halitosis, and lack of grooming [7,8]. Although FCGS is a familiar condition encountered in veterinary practice [13,14], there is much confusion regarding the cause and subsequent treatment of the disease [14,15]. This article reviews the current knowledge on the etiopathogenesis and epidemio-clinical features of FCGS and descibes the leading treatment modalities.

Clinical features

Etiology

The etiology of FCGS is currently unknown [16], probably multiple etiologies may exist that, either alone or combined, can contribute to the presence of the inflammation [9]. Possible causative factors include viral infections particularly feline upper respiratory viruses such as FCV, FHV-1, bacterial infection like *Bartonella henselae* and altered immune status associated with FIV, FeLV [10, 11,12], as well as non infectious factors such as dental disease, environmental stress, and hypersensitivity [18, 19]. It has been proposed that the disease is an immune reaction to plaque and the tooth structure itself or the periodontal tissues [3]. According to Thomas et al. [16] FCGS is initiated from gingival inflammation and is perpetuated to the mucosa of oral cavity (**Table 1**).

Clinical signs

FCGS is a severe inflammatory syndrome involving the immune system that affects [17]. The disease varies in severity and may include faucitis, pharyngitis, or palatitis [3]. Clinical signs include severe oral pain,ptyalism, mandibular lymphadenopathy [10], poor grooming habits and unkempt appearance [7, 8], dysphagia, pawing at the mouth, anorexia, crying out in pain when eating or yawning[24, 25] halitosis, loss of appetite, depression, weight loss [2] and (in severe cases) even dehydration [26]. Affected cats can become severely debilitated and because of the unclear pathogenesis and relapsing course of the disease, FCGS remains one of the most challenging oral conditions to manage in feline practice [2]. Therefore, euthanasia may be considered as last resort, when quality of life is significantly declined [27].

The hallmark lesions include caudal stomatitis and alveolar mucositis, both of which are commonly assessed using a standardized five- grade scoring system [27].

-Grade 0 : No visible lesion.

-Grade 1 : Mild, non-ulcerative, non-proliferative inflammation. Lesions do not bleed spontaneously or under slight pressure.

-Grade 2 : Moderate, non-ulcerative inflammation with mild proliferative changes. Lesions do not have spontaneous bleeding even with slight pressure.

-Grade 3 : Moderate, ulcerative or ulceroproliferative inflammation, without spontaneous bleeding, but with bleeding when slight pressure is applied.

-Grade 4 : Severe, ulcerative or ulceroproliferative inflammation with spontaneous bleeding.

In addition to clinical grading, histological examination of the the oral mucosa tissues affected by FCGS, shows a diffuse and dense cell infiltration, containing lymphocytes and plasma cells which are predominantly observed. In contrast, relatively few neutrophils, mast cells have been observed, thus showing the characteristics of chronic inflammation [28, 29]. These histological features are the basis for the disease's alternative nomenclature, such as plasma cell gingivitis (-stomatitis)-pharyngitis or lymphoplasmacytic gingivitis [30]. Figure 1. shows Ulcero-proliferative lesions of FCGS in tissues lateral to palatoglossal folds plus maxillary gingivitis and alveolar mucositis both sides.

Diagnostic

Diagnosis of FCGS is primarily clinical and relies on the identification of characteristics oral lesion [3, 27] through thorough visual inspection of the oral cavity [10, 31]. FCGS is mostly characterised by bilateral inflammation of the mucosa in the caudal oral cavity, a hallmark feature that helps distinguish FCGS

Abbreviations-Cont'd

OR : Odds ratio
PME : partial-mouth extractions
RFeIFN - ω : Recombinant feline interferon omega
LPS : lipopolysaccharide
MHC- II : major histocompatibility complex class II

Table 1.
Microorganismes (bacteria and virus) associated with FCGS recorded in some studies.

References	Frequency of cats with FCGS	Bacteria or virus explored	Microorganisms associated with FCGS (P value when reported)
Thompson et al. [31]	50% (10/20)	FCV (by culture) ; FeLV (by immunochromatography)	None reported
Lommer and Verstraete [12]	51% (25/49)	FCV (by culture) ; FHV-1 (by culture)	FCV ; FHV-1
Dowers et al. [11]	53.4% (70/131)	Bartonella (by ELISA, culture and PCR); FCV (by PCR); FHV-1 (by PCR)	FCV ($p = 0.0006$)
Sykes et al. [32]	3% (9/298)	Bartonella (by culture and immunofluorescence)	Bartonella isolation ($p = 0.001$)
Dolieslager et al. [33]	62.5% (5/8)	Bacterial flora (by culture and PCR)	Pasteurella multocida subspecies multocida
Kornya et al. [34]	3.9% (203/5179)	FeLV (by ELISA) ; FIV (by ELISA)	FIV
Fernández et al. [35]	43% (154/358)	FHV 1 (by PCR); FCV (by PCR); Chlamydophila felis (by PCR); Mycoplasma felis (by PCR)	FCV (p <0.001) ; C felis (p = 0.025); M felis ($p = 0.003$)
Rolim et al. [36]	60.5% (26/43)	FCV (by immunohistochemistry); FeLV (by PCR); FIV (by PCR)	None
Thomas et al. [20]	27.7% (25/90)	FCV (by culture)	FCV ($p = 0.010$)
Whyte et al. [37]	11.8% (4/34)	FCV (by immunofluorescence); microbacteriome (by phenotype and conventional biochemical methods)	None
Nakanishi et al. [38]	30.7% (32/104)	FHV-1 (by PCR); Chlamydia felis (by PCR); M felis (by PCR); Bordetella bronchiseptica (by PCR)	FCV ($p = 0.018$)
Fried et al. [39]	54.7% (23/42)	FCV (by genomic sequencing)	FCV ($p = 6.0\times10–42$)
Krumbeck et al. [40].	50% (14/28)	Bacteriome and mycobiome (by DNA sequencing)	None

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Figure 1.
Ulcero-proliferative lesions of FCGS in tissues lateral to palatoglossal folds plus maxillary gingivitis and alveolar mucositis both sides.

from other oral diseases [4]. The affected gingiva and oral mucosa in FCGS exibit variable degrees of inflammation, proliferation, and ulceration [31]. The mucosal surfaces is typically appear bright red, with friable tissues that bleed easily [24]. Additional diagnostic tests are essential to fully evaluate the patient health, these include [31] dental radiographs, complete blood count and serum biochemical profile and evaluation of FeLV/FIV status. In cases inflammation is asymmetrical, appears atypical, or radiographic findings raise suspicion for neoplasia, a biopsy should be submitted for histopathological evaluation [32]. As previously mentioned, FCGS lesions may occur in multiple areas, from the gums in the oral cavity to the pharynx [27]. The inflammatory process often extends beyond the mucogingival junction, encompassing the alveolar mucosa

and other soft tissues including the lingual mucosa, glossopalatine folds, caudal oral mucosa and, in some cases, the fauces [33]. According to Healey et al. [13], the most frequently affected sites include gingival mucosa (ie, visible gingiva extending from the teeth to the mucogingival junction), the periodontal area (ie, the part of the visible marginal gingiva immediately adjacent to the teeth), and the glossopalatine folds, commonly referred to as the fauces..

However, the clinician must be cautious in diagnosing FCGS. The presence of severe gingivitis in a patient, even in conjunction with the detection of FCV via PCR, does not automatically provide a diagnosis of FCGS [33]. The clinical sign that differentiates caudal stomatitis from periodontal disease is the presence of caudal inflammation (distal to the teeth) referred to as caudal stomatitis. This presentation was previously called faucitis, but is now known as caudal mucositis contrast, in cases of typical periodontal disease, inflammation is associated with the gingiva tissues adjacent to the teeth, and rarely extends into the caudal oral mucosa [1]. Also, many cases of juvenile gingivitis may be mistaken for FCGS and if inflammation is restricted to gingival tissues, a diagnosis of FCGS should not be made [33].

Epidemiological features

FCGS is considered multifactorial [13]. Some studies suggest that nutritional factors, physiological or environmental stresses, dental disease and genetic predisposition may be the cause of FGS [10]. Viral infections, including FeLV, FCV, FIV, FLV and FHV-1, might be implicated in the development of FCGS [22]. However, these infectious agents have been isolated not only from affected cats, but also from control animals [34], making it difficult to establish a definitive causal relationship in each individual case of FCGS [35]. In addition to viral pathogens, certain anaerobic bacterial species have also been proposed as potential contributors [36]. Immunological studies have found alterations in cytokine expression patterns and immunoglobulin profiles in FCGS-affected cases compared to controls [37]. Furthermore, it has been suggested that immunosuppression due to an unrelated health conditions may play a role [38].

Prevalence

The reported FCGS varies considerably across studies, ranging from 0.7% to as high as 45.76% [17, 27,41]. In 2004, Verhaert and Van Wetter [39] reported a prevalence rate of 12%. Later, in 2007, Healey et al. [13] targeted domestic cats that visited a primary hospital, in his study, and reported a significantly lower prevalence of 0.7%. In 2009, Girard et al [14] studied colony cats that had no contact with the external environment and recorded a prevalence of 5.5%. More recently, in 2024, Dai et al. [2] reported a prevalence of 1.96% in cats admitted to three animal hospitals in Xi'an, China. On the other hand, high prevalences have been found by Da Silva et al. [40] who recorded a prevalence of 34.88% of stomatitis and Öztürk Gürgen et al. [41] who recorded 45.76%.

Potential viral causes

Several viruses with global distribution have been associated with the pathogenesis of FCGS, including FCV [13, 42], FHV [22], FeLV and FIV [43]. Many of the epidemiological and clinical features of these pathogens have been documented (17, 18, 44). Among these, FCV seems to has the most consistent evidence of being associated with FCGS [11, 22, 29, 45]. Nakanishi et al. [46], by using PCR assay, reported that 63% of cats diagnosed with FCGS tested positive for FCV, compared to 36% in the control group. Their findings also suggested that the microflora of the oral cavity of cats with FCGS might be disrupted. In contrast, no statistically significant difference was found in the prevalence of FHV-1 between affected and control groups. Supporting this, Martijn [30], detected FCV in 95.5% of cats with FCGS, while only 4.1% of control cats tested positive. Also FHV was detected in 2.3% in FCGS cases and and was absent in controls . Similarly, Thomas et al. [17] found the incidence of FCV to be significantly higher in cats with FCGS (60%) compared with control cats (24%). However, not all studies have been able to consistently prove that chronic infection by FCV is directly implicated in the pathogenesis of FCGS [28, 43, 47]. Also, the association of FIV and FeLV with FCGS is still not completely elucidated [47, 48, 49].

Regardless of the precise role of individual pathogens, well-known risk factors for these viruses include free-roaming behavior and residence in multi-cat environments such as shelters, shared households, and breeding catteries [17]. Notably, some studies showed that the prevalence of FCV, FeLV and FHV is higher in multi-cat environments [44, 50]. Moreover Radford et al. [51] noted that the prevalence of FCV infection is proportional to the number of cohabiting cats. There is consistent evidence that FCV is associated with the disease, and an etiologic role is suspected [11, 42]. Free-roaming behavior is a known co-factor for FeLV, FIV, FHV and FCV infection [50, 52]. Thus, it could be suggested that infection alone is not sufficient to initiate FCGS and that additional conditions related to environments may also play a critical role. Multi-cat conditions also facilitate permanent exposure to viral particles shed by chronic carriers, favor high rates of

viral evolution and facilitate cyclic reinfection of susceptible animals [53].

Although there is strong evidence supporting the involvement of FCV in FCGS, the inability to recreate the disease in naïve population and the effectiveness of treatments such as full-mouth dental extractions in many cases, have cast doubts on a singular role for FCV and raised suggestions that this disease may be influenced by the nature of the host's immune response and derangements (dysbiosis) of the oral microbiological flora [54].

Bacterial burden in FCGS

In addition to viral and host immune factors, bacterial organisms are thought to play a role in the pathogenesis of FCGS [17]. Some studies reported that bacteria, especially gram negative anaerobe bacteria, play a certain role in the pathogenesis of FCGS. Especially gram negative anaerobe bacteria [36]. In relevant studies on the oral bacteria associated with FCGS, different experimental results have been reported. One study reported that the oral microbiota diversity of cats with FCGSs was greater compared to healthy controls [55]. Notably, some studies have also reported that the detection rate of anaerobic bacteria in the oral microbiota of cats with FCGSs was significantly greater than that of healthy controls [2,46]. According to Rodrigues et al. [55] higher abundance of gram-negative and anaerobic bacteria was found in FCGS and periodontitis, suggesting a possible role of bacterial biofilms in the pathophysiology of both diseases. Among the bacteria most commonly identified in FCGS-affected cats are Porphyromonas app., Treponemas app., and Fusobacterium app., [2]. The cell membrane of gram negative anaerobe bacteria contains LPS and this component plays an important role in the initiation of the infecetion [56]. Moreover, these bacteria are also an important aetiopathologic factor in oral infections in humans [36]. The success of full mouth extractions can lighten and even remove the inflammation [57]. This suggests that dental plaque and calculus with all their residential bacteria play an important role in maintaining the inflammatory oral condition [30].

External environmemt and lifestyle

Factors relating to multicat environments as well as the stress of living in such environments may be necessary in addition to an infectious cause to trigger the development of FCGS [58]. A recent studies investigated the association of multicat environments and outdoor access with the prevalence of FCGS and showed that the prevalence of FCGS was higher in multicat than single-cat households, and that each additional cat in the household increased the odds of FCGS by more than 70% [58].

Age

FCGS can occurs in cats of all ages, after tooth replacement [14]. Although based on multiple studies, the condition is most frequently diagnosed in adult cats [13, 41] and the mean age for cats with FCGS was found to be between 5 and 8 years [13, 30, 41, 61]. Nakanishi et al. [46] showed that cats may be affected at an early age.

Sex

Many studies showed that there was no significat correlation between FCGS and sex [2,13, 27, 41]. However, Martijn [30] reported a significant positive associated between male sex and FCGS, noting that male cats were four time more infected than female (odds ratio : OR=4.1). Similarly, some other studies found high rates of FCGS in neutered males [13, 39]. A higher prevalence of FCGS was also identified in males than in females in a study done by Kim et al. [27], though the observed results were not statistically significant. Perhaps male cats, particularly those with outdoor access, are more exposed to infectious diseases, which might play a role in developing FCGS, because in general male cats have a greater territory outside and are more aggressive towards other males [30].

Breed

According to the breed, studies showed differents results Healey et al. [13] and Dai et al. [2] found that there is no significants correlation between breed and FCGS. However in other studies, some breeds like ; Siamese, Abyssinian, Persian, Himalayan and Burmese breeds, which have all been cited in the literature as potentially predisposed [59, 60]. Martijn [30] revealed that 47.7% of the FCGS cats were purebreds, while 4.5% were crossbreds and that the purebreds significantly associated with FCGS (OR=25.2). This study also found that 61.9% of purebreds were Main-Coons. Conversely, others studies noted that mixed breeds were more predisposed to FCGS. For example, in a study of Hennet [61], in a case series involving 30 cases of FCGS-effected cats, the majority were mixed breed, with only three Siamese, three Persian and one Foreign breed represented. Similarly, Healey et al. [13] found that 91% of cats with FCGS were mixed breed ; with only 2 purebreds (1 Persian and 1 Siamese), and 1 unclassified individual. In general, some authors noted that Purebreds cat may be predisposed in developing oral diseases, but in the case of FCGS specifically, a percentage of purebreds mostly ranges from 10% to 25% [13, 61].

Leading treatment modalities

In general, there are 2 approaches to the treatment of FCGS : surgical and medical, often combined. However, on its own, medical treatment typically does not have favorable long-term outcomes [17] and has been shown to only provide temporary improvement [5, 8]. Surgical treatment, particularly FME or PME involving the premolar and molar teeth, has demonstrated the best long-term outcome [23, 47]. Clinical studies report that approximately 80% of the cats submitted to dental extractions, FME or PME, obtained significant improvement, with some achieving complete remission of the clinical signs, with or without the need for combined medical treatment [8, 61] (Fig.2).

Surgical treatment

Extraction therapy is the preferred treatment for FCGS and should be performed as soon as possible [1]. Bellei et al. [7] showed that the extraction of teeth has shown better results compared to drug therapy, with clinical cure achieved in up to 57% of treated cases. According Hennet [61] approximately 60% of cats had significant improvement following dental extractions, while 20% had partial improvement, and the remaining 20% had little or no improvement. Based on the findings of Druet and Hennet [23] PME (along with other teeth that independently have indications for extraction, such as severe periodontitis, retained roots, or resorptive lesions) as the first stage of treat-

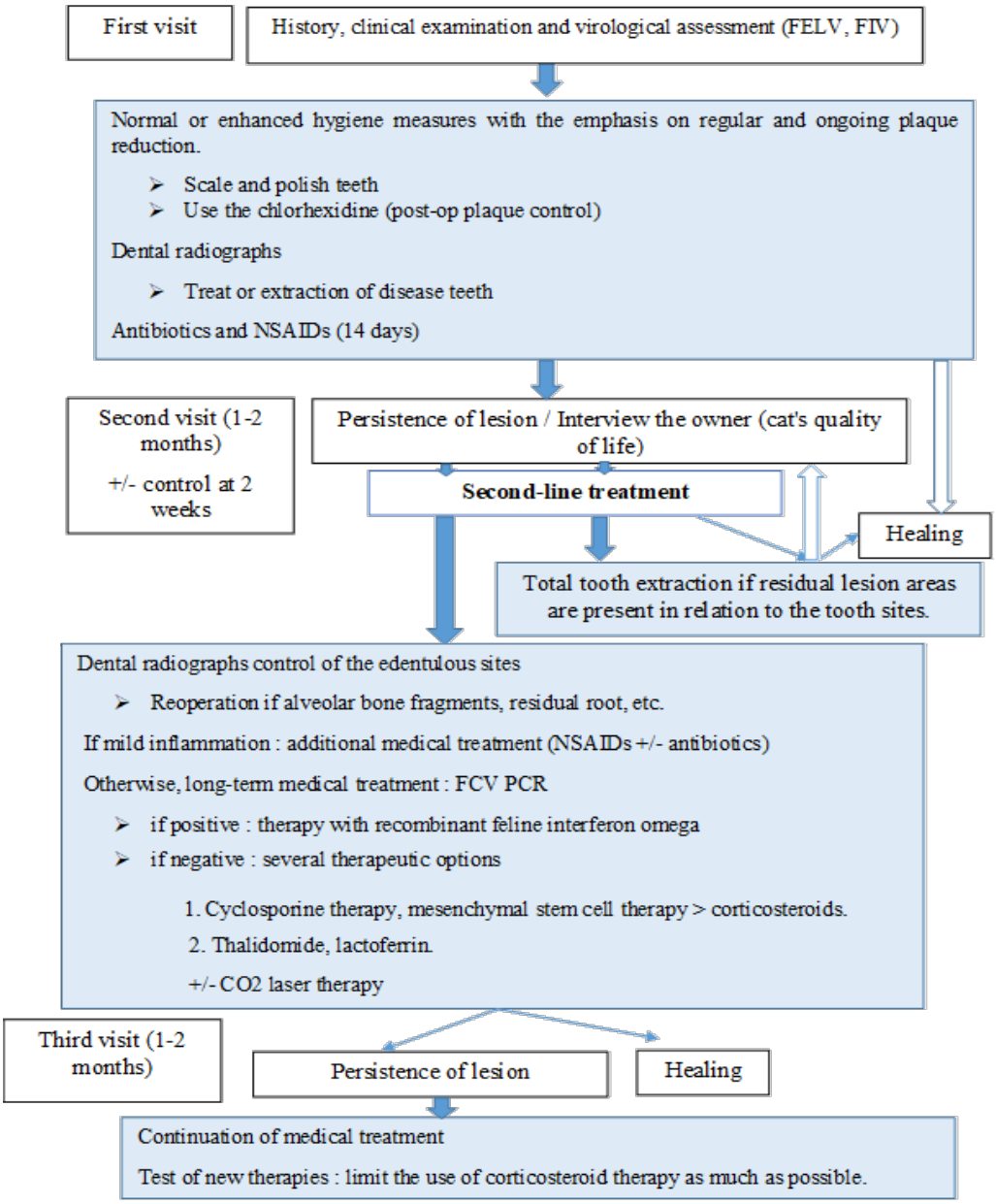

Figure 2.
Proposed therapeutic approach for a cat with FCGS.

ment is the highest evidence-based recommendation. PME also has other advantages such as reduced anesthetic time, less surgeon fatigue, and minimized surgical trauma. If there is no positive response within 1 to 4 months, FME may be pursued as the second stage of treatment. The most successful long-term treatment for FCGS is extraction of all premolars and molars, along with careful smoothing of the alveolar bone [10, 31]. Extraction of the rostral teeth is indicated when inflammation involves their gingiva [32]. While some practitioners perform FME when significant oral inflammation is present [1], others prefer to leave the canines and incisors intact, if possible [25, 32]. The vast majority of cats have an excellent response to extractions , requiring no additional therapy [4, 31, 32]. If extraction therapy is not effective, it is usually due to the presence of retained roots [31, 32]. For this reason, postoperative dental radiographs must be exposed to document complete extraction of all tooth roots [31, 32, 62].

Medical management

When owners are reluctant to have multiple extractions performed, medical management may be attempted as an alternative, however this approach has several disadvantages :

- Many products used are oral medications, which require once or twice daily administration.
- Medical therapy is almost invariably a life-long process, and many products have significant side effects.
- No medical protocol has shown to be completely effective ; usually they only reduce the clinical signs temporarily [25, 62].

Medical management consists of palliative measures, including systemic analgesics to treat associated pain, anti-inflammatories to treat the oral inflammation, and antibiotics to treat secondary infections [58]. Other available treatments are described mostly for cases that fail to respond to surgical intervention and offer variable response rates. These include: systemic ciclosporine [63], topical or systemic rFeIFN-ω [4] and MSCs therapy [20, 64].

Antibiotics

Systemic antibiotics may decrease some oral inflammation. However, this effect is generally temporary at best, and most cats will experience relapse, often even during the course of antibiotic therapy [32, 62].

Pain management

Regardless of modality, all treatment options require adequate pain management. Appropriate therapy depends on factors including comorbidities (eg, renal or hepatic insufficiency), concurrent medications being administered (eg, corticosteroids), patient compliance, and the owner's ability to assess and manage oral pain. Typically, long-term pain management includes administration of opioids (eg, buprenorphine) in combination with gabapentin. A recent randomized, prospective, blinded, controlled, crossover study showed that buccal administration of buprenorphine had a significant effect on reductions in pain scores, while maintaining low interindividual variations in plasma drug concentration in cats with FCGS [65].

Corticosteroids

Corticosteroids are, by far, the most commonly used and effective drugs for immune modulation, resulting far more reliable clinical improvement than antibiotic therapy [32]. Prednisolone, a short-acting corticosteroid, is often used to reduce inflammation [17]. However, long-term use may have side effects, such as induction of diabetes mellitus and opportunistic infections [62, 63]. Chronic corticosteroid therapy should only be used as a last resort option, typically only when surgical treatment is declined [1].

Recombinant feline interferon omega (rFeIFN-ω)

Feline interferon is reported to provide both antiviral and immunomodulatory effects, resulting in restoration of the normal local immune system [1]. IFNs are a group of signaling proteins that have the ability to interfere with viral replication. rFeIFN- ω is marketed for use against viruses like CPV, FeLV, FIV, FHV-1 and FCV [66].Oromucosal absorption of IFN has been shown to stimulate local immunomodulation via oropharyngeal lymphoid tissues, whereas gas trointestinal absorption leads to degradation of the glycoprotein [67]. In a controlled, randomized, double-blinded study evaluating oromucosal administration of rFeIFN- ω over 3 months in 19 cats, substantial improvement was seen in 45% of the cats, of which 10% achieved clinical remission. Another recent controlled study showed that subcutaneous administration of rFeIFN- ω may be effective for the treatment of FCGS in FCV-positive cats, as it appears to inhibit FCV replication [68]. Several studies have shown efficacy in resistant cases but, current evidence does not demonstrates its efficacy as a primary treatment [1].

Cyclosporine A

Cyclosporine A provides immunosuppressive effects primarily via inhibition of T-cell activation through downregulation interleukin-2 expression, a proinflammatory cytokine involved in a positive feedback loop that increases T-cell numbers [69]. It may

also have inhibitory effects on B-cell reproduction. Cyclosporine A has been proposed as an immunosuppressive drug for cats with caudal stomatitis [32] and some have promoted it as an alternative to extractions in order to avoid glucocorticoid use. While there is no published information that supports the use of cyclosporine A prior to extractions, it has been shown to be effective in cases refractory to extraction therapy [63] and may provide an alternative to long-term steroid therapy. Therefore, Niemiec [1] noted that cyclosporine should be reserve only for use in patients in which medical management is necessary post extraction [25]. Cyclosporine A must be used with caution in cats with hepatic or renal disease, and there are reports of fatal opportunistic infections associated with its use [70]. The bioavailability of the 3 available forms of cyclosporine is quite variable, and dosing depends on which form is used [62]. A veterinary specific formulation, Atopica (Novartis), is approved for use in cats with feline atopy and may be considered a suitable option for FCGS management [1].

Mesenchymal stem cells (MSCs)

MSCs are fibroblast-like, multipotent stem cells that have immunomodulatory effects through inhibition of T-cell proliferation, alteration of B-cell function, downregulation of MHC-II on antigen presenting cells, and inhibition of dendritic cell maturation [71, 72]. The efficacy of both autologous and allogeneic, fresh, adipose-derived MSCs administered intravenously has been studied in cats with refractory FCGS [20, 71]. Treatment with autologous adipose-derived MSCs in 7 cats resulted in a positive response rate of 71.4% (reflected by clinical remission in 42.8%, substantial improvement in 28.6%, and no response in 28.6% of cats), over a follow-up period of 6 to 24 months [71].

Conclusion

The etiology of FCGS is often unknown and a multifactorial disease, with potential contributions from bacteria and viral pathogens , genetic predisposition and environmental stressors. Epidemiological studies of the disease are rare, and many features have yet to be documented. Successful managment of this complex requires a logical diagnostic approach and to understand the possible etiopathogenic mechanisms, it is essential to understand the epidemiological characteristics of the disease in order to propose available treatments and preventive approaches.

Authors' Contributions

The author contributed alone to the realization of the work.

Conflict of interest

The authors declare that there is no conflict of interest.

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Concentration of blood heavy metals in terrier dogs with some common behavior problems

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ABSTRACT

Behavioral disorders in companion animals, especially dogs, are a great concern. Due to the relationship between oxidative stress and behavior problems and also heavy metals' capability of creating oxidative stress, in the current study, the effects of lead, mercury, arsenic, and cadmium on 13 common canine behavior problems (fearfulness, excessive barking, destructiveness, house soiling, inappropriate sexual behavior, coprophagia, wandering, shyness, aggression toward the owner, aggression toward familiar people, aggression toward strangers, aggression toward other dogs, and excessive activity) were evaluated. According to owners' answers to the questionnaire, 43 terrier dogs were chosen. Of these 7 dogs showed no behavior problem and 36 dogs displayed at least one behavior problem. The blood concentrations of lead, mercury, arsenic, and cadmium were measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). No significant differences in heavy metal concentrations were observed between the case and control groups. However, the cadmium concentration was significantly elevated in dogs displayed aggressive behavior toward their owners ($p < 0.048$, n=5). while arsenic level was significantly lower in dogs displaying fearfulness ($p = 0.048$, n= 25). Results of the study reported here do not support the hypothesis that "blood concentration of heavy metals may influence the occurrence or prevention of common behavioral problems in dogs". Our results suggest that there may be a direct relationship between higher levels of cadmium and aggression toward the owner and arsenic with reduced fearfulness in dogs. However, we have to consider that the behavioral effects of heavy metals are likely very complex.

Keywords

Behavior problem; Terrier dog; Cadmium; Lead; Mercury; Arsenic

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Abbreviations

ATP: Adenosine triphosphate
DNA: Deoxyribonucleic acid
IQ: Intelligence Quotient
ADHD: Hyperactivity/attention deficit disorder
ECG: Electrocardiogram
ICP-OESL: Inductively Coupled Plasma Optical

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Introduction

The definition of the term "behavior problems" totally depends on the owner's personal opinion. A behavior considered as problematic by one owner, can be seen as a normal behavior by another owner. In fact, any kind of behavior which is unpleasant or annoying for owners falls under the category of a behavior problem [1]. There are different criteria on which the definition and categorization of problematic behaviors are based. For example, behaviors can be categorize as either "normal" and "abnormal" or "physiological" and "pathological". Concerning the former context, "abnormal behaviors" are defined as any behavior that varies from the norm expected for a species. According to the latter type of categorization, most of the problematic behaviors root from the normal processes.Given the physiological origins of so-called "behavior problems", such behaviors may not be problematic for animals itself, but can become problematic when they conflict with owners expectations [2]. Domestic dogs are highly prone to display behaviors that owners may find inappropriate. It is estimated that over 90% of pet dogs may show at least one behavior that is not pleasant in their owners' eyes. There is a wide spectrum of such behaviors, ranging from minor issues, such as tail chasing or pulling on the lead, and major ones, such as aggression or destructive behavior. The most reported prevalent behavior disorders in typically include: fearfulness, hyperactivity, destructiveness, inappropriate elimination, Straying, Coprophagy, Excessive barking, aggression toward other dogs and humans, and sexual behavior problems [3]. Another study reported the most common behavioral problems in dogs in a different order: attention seeking, dog aggression, noise reactivity, aggression toward strangers, pica, destructiveness, aversion to strangers, excessive barking, compulsive body licking, aggression toward owners, possessiveness over toys or food, house soiling, and fearfulness during walks and tail chasing [2]. These behavioral problems can have serious consequences, including dog abandonment or relinquishment to kennels and shelters, property damage, welfare deterioration, erosion of the human-dog bond, and euthanasia [4, 5]. Possible risk factors of behavioral disorders are breed, age, time and source of acquisition, sex, and reproductive status [6]. Heavy metals can be a risk factor for behavior problems, especially when organisms are exposed to toxic metals chronically [7]. Dogs may be exposed to heavy metals through environmental pollutants such as man-made waste, and diet especially commercial foods [8]. Heavy metals can implicate variety of dangerous intracellular damages. These include oxidative stress and lipid peroxidation, disruption of cellular enzymatic system, decrease in ATP production, dysregulation and inhibition of some proteins and enzymes, DNA repair suppression, genotoxic effects, damage to cell membrane integrity, destruction of microtubules, ribosomes and endoplasmic reticulum, and mitochondria. . Furthermore, heavy metals can disturb intracellular-calcium homeostasis, inhibit cellular respiration and interference with mitosis. These negative changes can adversely affect nerval development and electric conductivity, ultimately leading to behavioral abnormalities. Consequently, neurological disorders such as learning disabilities, memory loss, decrease in the IQ, ADHD, and behavioral disorders may be developed [9-12].

An important hypothesis that has been proposed in the recent related literature is whether heavy metals have the ability to impact behavioral problems. Therefore, the current study was carried out to investigate the correlation between amounts of four toxic metals (cadmium, arsenic, lead, and mercury) in whole blood and 13 behavioral disorders in Terrier dogs. These behavioral disorders included: Fearfulness, excessive barking, destructiveness, house soiling, inappropriate sexual behavior, coprophagy, wandering, shyness, aggression toward the owner, aggression toward familiar people, aggression toward strangers, aggression toward other dogs and excessive activity.

Results

Demographic characteristics

The sex and neuter status of the dogs in the control and case groups are provided in Table 1. Out of

Table 1.
The number and the percentage of dogs based on their sex and neuter status in the control and test groups.

			Number	Percent
Sex	Control	Male	5	71.42
		Female	2	28.58
	case	Male	21	58.33
		Female	15	41.67
Neuter status	Control	Neutered	5	71.42
		None- neutered	2	28.58
	case	Neutered	13	36.11
		None- neutered	23	63.89

Abbreviations-Cont'd

Emission Spectroscopy
SPSS: Statistical Package for the Social Science
CBCL: Child Behavior Checklist

the 43 dogs evaluated, the case group consisted of 21 males and 15 females (dog with at least one behaviour problem). The control group included 5 males and 2 females.

Distribution of behavior problems

Among the 36 dogs with behavioral problems that are listed in Table 2, the most prevalent behaviour problems were fearfulness (n = 25), house soiling (n = 18) and hyperactivity (n = 14).

Table 2.
The number and the percentage of dogs in the case group that had each of 13 behavior problems.

Behavior problems	Case Group	Number	Percent
Fearfulness	Present	25	69.44
	Absent	11	30.56
Excessive barking	Present	5	13.88
	Absent	31	86.12
Destructiveness	Present	11	30.56
	Absent	25	69.44
House Soiling	Present	18	50
	Absent	18	50
Inappropriate sexual behavior	Present	9	25
	Absent	27	75
Coprophagy	Present	4	11.11
	Absent	32	88.89
Wandering	Present	4	11.11
	Absent	32	88.89
Shyness	Present	8	22.22
	Absent	28	77.78
Aggression toward owner	Present	5	13.88
	Absent	31	86.12
Aggression toward familiar people	Present	6	16.66
	Absent	30	83.34
Aggression toward stranger people	Present	5	13.88
	Absent	31	86.12
Aggression toward dogs	Present	5	13.88
	Absent	31	86.12
Hyper activity	Present	14	38.88
	Absent	22	61.12

Heavy metals levels

Blood concentrations of four metals (lead, mercury, arsenic and cadmium) in the case and control group are present in Table 3. The median concentrations of these heavy metals showed no significant difference between the control group and the group with behavioral disorders. However, subgroups analysis that are present in Table 4, revealed two significant findings. Dogs exhibiting aggression toward their owner had significantly higher blood cadmium levels than the controls ($p < 0.05$). Also, dogs classified as fearful had lower arsenic levels compared to the control group ($p < 0.05$).

Discussion

Trace elements and toxic metals can be measured from various loci such as serum, blood, urine, or hair. Whole blood has been considered to give a better reflection of long-term dietary intake (for example selenium) or environmental exposure. In addition, toxic metals such as lead are commonly measured from whole blood, as more than 90% of lead is bound to red blood cells after absorption [13]. In this study, we aimed to explore potential associations between behavior problems and blood concentrations of heavy metals in pet dogs.

Cadmium

Our findings illustrated that cadmium level were elevated in dogs displaying aggression toward their owners compared to those without any behavior disorders. In line with our results, Terçariol *et al.* (2011) reported that rats exposed to cadmium and immobilization stress were more frequent in exhibiting several aggressive behaviors, namely total number of attacks and total duration of attack manifestations. Also, these rats had a higher composite aggression score [14]. Similarly, Godinho *et al.* (2017) experimentally poisoned mice with cadmium and caffeine. It became evident that co-exposure to cadmium and caffeine (and not just cadmium alone) caused mice to be more aggressive [15]. It seems that cadmium increases aggressiveness, both directly, possibly through interfering with serotonin function or decreasing its level in various ways [13], and indirectly, by aggravating anxiety [16,17]. Heavy metals, including cadmium, act as catalysts for biochemical reactions, regulators of gene expression, second messengers in signalling pathways and cofactors for many vital enzymes, such pathways implicated in regulating physiological, pathological and behavioral functions. Animal studies suggests it is plausible that chronic exposure to cadmium may lead to motor hyperactivity, increase in aggressive be-

Table 3.
Amounts of median, Q1 and Q3 of metals' blood concentration in dogs with or without behavior problems.

	Lead (mg/L)	Mercury (mg/L)	Arsenic (mg/L)	Cadmium (mg/L)
Without any behavior problems (control) (n=7)	0.309 (0.182-0.391)	0.393 (0.001-0.932)	0.212 (0.175-0.335)	0.015 (0.001-0.023)
With at least one behavior problem (case) (n=36)	0.323 (0.249-0.468)	0.808 (0.710-0.888)	0.185 (0.144-0.214)	0.021 (0.016-0.025)
Blood element (ng/g) reference values from literature*	3.5±2.75 (0.28-12.37)	0.53±0.35 (0.12-1.61)	1.15±1.09 (0.04-4.30)	0.03±0.01 (0.01-0.06)

* Rosendahl S, Anturaniemi J, Vuori KA, Moore R, Hemida M, Hielm-Björkman A. Diet and dog characteristics affect major and trace elements in hair and blood of healthy dogs. Vet Res Commun. (2022) 46:261–75. doi: 10.1007/s11259-021-09854-8

Table 4.
Amounts of median, Q1 and Q3 of metals' blood concentration in the subgroups and controls of this study. Numbers marked with * have a significant difference with controls.

	Lead (mg/L)	Mercury (mg/L)	Arsenic (mg/L)	Cadmium (mg/L)
Without any behavior problems (control) (n=7)	0.309 (0.182-0.391)	0.393 (0.001-0.932)	0.212 (0.175-0.335)	0.015 (0.001-0.023)
Fearfulness (n=25)	0.322 (0.224-0.461)	0.855 (0.670-0.886)	0.178 (0.145-0.212)*	0.021 (0.014-0.025)
Excessive barking (n=5)	0.301 (0.224-0.381)	0.837 (0.699-0.867)	0.194 (0.136-0.237)	0.024 (0.017-0.027)
Destructiveness (n=11)	0.322 (0.255-0.673)	0.802 (0.766-0.894)	0.191 (0.144-0.215)	0.021 (0.017-0.024)
House soiling (n=18)	0.322 (0.267-0.456)	0.779 (0.714-0.869)	0.172 (0.126-0.215)	0.019 (0.013-0.026)
Inappropriate sexual behavior (n=9)	0.249 (0.195 -0.321)	0.837 (0.726-0.894)	0.837 (0.726-0.894)	0.020 (0.015-0.027)
Coprophagia (n=4)	0.322 (0.218-0.359)	0.759 (0.704-0.847)	0.196 (0.144-0.227)	0.017 (0.014-0.022)
Wandering (n=4)	0.328 (0.308-0.475)	0.726 (0.527-0.882)	0.184 (0.128-0.221)	0.019 (0.013-0.027)
Shyness (n=8)	0.286 (0.218-0.438)	0.822 (0.547-0.875)	0.212 (0.160-0.224)	0.021 (0.019-0.024)
Aggression toward owner (n=5)	0.328 (0.270-0.490)	0.855 (0.627-0.919)	0.221 (0.210-0.239)	0.025 (0.022-0.036)*
Aggression toward familiar people (n=6)	0.398 (0.280-0.521)	0.858 (0.744-0.964)	0.228 (0.199-0.240)	0.025 (0.019-0.035)
Aggression toward stranger people (n=5)	0.325 (0.278-0.446)	0.776 (0.703-0.855)	0.192 (0.137-0.228)	0.022 (0.014-0.026)
Aggression toward dogs (n=5)	0.345 (0.218-0.466)	0.741 (0.561-0.867)	0.189 (0.122-0.214)	0.020 (0.016-0.021)
excessive activity (n=14)	0.331 (0.252-0.429)	0.776 (0.660-0.864)	0.182 (0.150-0.203)	0.021 (0.013-0.024)

havior, impairing social memory processes, and also may alter drinking behaviour [18].

Arsenic

In the present study, no significant difference in blood arsenic concentrations was observed between the case group and the control group. This finding aligns with the study by Tolins *et al.* (2014), which also found no significant correlation between arsenic concentrations and various behavioral parameters, including ADHD prevalence, classroom behavior outcomes, behavioral scores from a validated system, answers of a self-reported behavioral test in children and behavior test in newborns [19]. Recent studies have shown that even low concentrations of arsenic exposure may impair neurological function, particularly in children [20]. Interestingly, in the present study, dogs diagnosed with fearfulness disorder had lower arsenic levels compared to the dogs without any behavioral problems. However, the importance of

this finding in the present study remains unclear, as behavioral problems may have many causes beyond heavy metals.

Regarding the potential relationship between arsenic and fear-related behavior, there are some studies in which a contextual fear conditioning test was performed on arsenic-exposed mice and rats. These studies found that freezing time decreased after arsenic exposure, showing that arsenic may be able to reduce fear responses [6, 21, 22]. Freezing behavior, defined as voluntary immobile behavior except for respiration is a well-established fear-related behavior in mice and rats and was assessed to measure fear in the previous literature [23]. A possible explanation for this mechanism of such correlation can be explained through arsenic's impact on neurological processes. Arsenic can alter DNA methylation and gene expression, which play roles in memory formation, and secondly, and impair synaptic plasticity. These changes could disrupt the consolidation of fear memory, thereby decreasing freezing time. Even in some cases, freezing time remained unaltered after arsenic exposure, whereas it was normally expected to decline over time [6].

Mercury

Our results indicated that mercury levels were not significantly related to behavioral problems. This result is consistent with the findings of Bratel *et al.* (1997), who also reported no significant correlation between mercury concentrations in blood, hair, and urine and behavior problems like depression and anxiety [24]. Contrary to our results, Lozano *et al.* (2021) reported that children with elevated hair mercury concentrations scored lower on two subscales of the Child Behavior Checklist (CBCL) and ADHD index of the Conners Parents Rating Scale-Revised: Short Form. Also, mercury concentrations in cord blood have been linked to attention problems and ADHD inattentive and hyperactive-impulsive types [25]. Further supporting this, being prenatally exposed to mercury, rats displayed hyperactivity, spatial learning impairments and adaptive behavior. Mercury poisoning is thought to cause a wide spectrum of psychological problems, such as irritability, nervousness, excessive shyness, low self-confidence, insomnia, deficits in cognitive function, attention and memory, irritability, fretfulness, aggression, anxiety, psychasthenia, alexithymia, and poor social functioning [26]. Azevedo *et al.* (2012) believe that chronic low doses of mercury have a harmful effect on vascular function by reducing Nitric Oxide bioavailability. They argue that the current mercury exposure reference values, once considered safe, should be re-evaluated and reduced [27]. One possible explanations for insignificance of our results might be the short course of study, both behavioural symptoms and heavy metal exposure.

Lead

Although, no statistically significant relationship was detected between lead levels and behavior problems in our study, a considerable number of previous researches reported plausible lead's role in developing behavioral disorders. For instance, prenatally lead-exposed children, has been associated with more intensive emotional reactions, and difficulty in emotional regulations. Moreover, lead intoxication has been correlated with aggression and depression in 7–8 year [28]. other studies have found that lead exposed children show intellectual deficits, increased risk of violent and aggressive behavior, drug abuse, criminal activity, attention deficit, and social withdrawal [9,29]. Al-Osman *et al.* (2019) stated that acute lead intoxication in children can decrease attention span and increase irritability and dullness [30]. In adults, lead exposure has been linked to major depression, panic disorders, anxiety and hostility [31]. Research on birds has also revealed that individuals from regions with high lead soil concentrations exhibit more aggressive behavior [32]. Additionally, lead poisoning is thought to be associated with ADHD particularly the Hyperactive-Impulsive subtype (ADHD-H/I) [33]. Different study conditions, such as species, age, hormonal status, neutering conditions , and sample size can contribute to the inconsistency between our data about mercury and lead impacts on behavior problems and existing literature which contradict our results Additionally, while dietary intake can also be directly related to heavy metal exposure in pets, we attempted to control this variable by selecting dogs with similar diets which can reduce the direct dietary influence on blood heavy metal concentrations.

Conclusion

The current research is the first to examine the potential relationship between behavioral problems and heavy metal concentration in pet dogs.

Materials & Methods

Ethical approval

This cross-sectional study was conducted at the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. The study protocol was ethically approved by Research Office of Ferdowsi University of Mashhad.

Dog selection

The study involved adult terrier dogs aged between 1 and 10 years, all of whom were confirmed to be in good health based on physical examination, complete blood count and serum biochemistry.

Dietary histories revealed that the dogs were fed a combination of simple home-made diet including chicken, rice, potatoes, carrots, and cheese and/or commercial dog foods. No management recommendations, behavioral modifications, or training advice were given during the study. Additionally, none of the dogs had received any drugs or supplements during 30 days before the onset of the study. All dog owners were informed about study's objectives and procedures, and informed consent was obtained from each participant.

Questionnaire

Data were obtained using a previously validated survey questionnaire [2,3], which was completed by dog owners during veterinary consultation. The questionnaire approach to collection of behavioral data, is based on the assumption that the owner usually have close and consistent interaction with their dogs and knows more about it's typical behavior. The questionnaire was used to obtain basic data about the dog's demographic information, and the dog's environment. In addition, owners were asked whether their dog had exhibited any of the 13 most common behavior problems typically seen at the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. Responses were recorded on a binary (yes/no) scale. To minimize subjectivity perception among owners of behavior problems, each individual behavior was described as objectively as possible (e.g., "Does your dog move constantly, move fast, run and jump?", for excessive activity and "Does your dog show fearful behavior such as fleeing, trembling, and panic in response to unknown noises?", for fearfulness). Owners' reports were accepted as reliable evidence of behavior problems, since owner perceptions determines whether a dog have a behavioral problem [3]. The 13 most common types of undesirable behaviors assessed were: fearfulness, excessive activity, aggression towards people (owner, familiar and unfamiliar), aggression toward other dogs, excessive barking, destructiveness, inappropriate elimination (house soiling), sexual behavioral problems, coprophagy ("eats faeces"), straying and shyness (Table 5). Participants were required to indicate whether their dog had exhibited any of these behavior problems. An open-ended "other" category was also included and allowed participants to state whether their dog had exhibited any other problem behaviors besides those mentioned.

In total, 43 healthy terrier dogs living in household settings were included in this research. Of these, 7 dogs without any reported behavioral problems served as the control group, while 36 dogs exhibiting at least one behavior disorder formed the case group. In addition, controls were compared with dogs displaying only one certain behavior problem as well. The distribution of the 13 behavioral problems evaluated in this research was as follows: fearfulness (n = 25), excessive barking (n = 5), destructiveness (n = 11), house soiling (n = 18), inappropriate sexual behavior (n = 9), coprophagia (n = 4), wandering (n = 4), shyness (n=8), aggression toward owner (n = 5), aggression toward familiar people (n = 6), aggression toward strangers (n = 5), aggression toward other dogs (n = 5) and excessive activity (n = 14).

Sample collection

Venous blood samples were obtained from the Cephalic, saphenous, and jugular veins. Between 3 to 5 ml of blood was collected from each dog using heparinized tubes.

Measurement of whole blood heavy metals

The initial volume of each blood sample was measured using graduated tubes. Samples were then diluted with 65% nitric acid and Hydrogen peroxide (Merck), in a ratio of 1:2, , to reach a final volume of 9 ml. These diluted samples were kept at room temperature

Table 5.
Binary questionnaire used in the current study.

undesired behavior	Definition
Fearfulness	Does your dog tremble, panic, and/or flee when hearing sudden noises?
Excessive activity	Does your dog move constantly, walk fast, run, jump, and rarely settle down?
Destructiveness	Does your dog damage or destroy furniture, clothes, and other objects found in your house?
House soiling	Does your dog defecate and/or urinate inside your house?
Inappropriate sexual behavior	Does your dog hump on your foot, people, or objects?
Coprophagy	Does your dog eat feces of its own, other dogs, and/or other species?
Wandering	Does your dog tend to aimlessly walk and leave your house frequently?
Shyness	Is your dog too quiet? Does your dog refuse to make contact or familiarize with others?
Aggression toward owner	Does your dog growl, bark, raise its hackles, lunge, and bite you?
Aggression toward familiar people	Does your dog growl, bark, raise its hackles, lunge, and bite when encountering familiar people (family members, relatives, and those who are being met frequently)?
Aggression toward unfamiliar people	Does your dog growl, bark, raise its hackles, lunge, and bite when encountering unfamiliar people?
Aggression toward dogs	Does your dog growl, bark, raise its hackles, lunge, and bite when encountering other dogs?

for a couple of hours. followed by incubation in a Bain-marie at 80-100°C for two hours and then, were filtered with Whatman filter paper. Heavy metal concentrations were analyzed using the SPECTRO ARCOS instrument, (model 76004555, SPECTRO, Germany) which has a detection limit of 0.001 mg/l, and the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method.

Statistical analysis

Statistical analysis was performed using SPSS software, 16th edition. For all calculations, a p-value of $p < 0.05$ was considered significant. Due to abnormal distribution of the parameters, namely lead, cadmium, arsenic, and mercury, the non-parametric Mann-Witney U test was used to analyse and compare the data between the two groups. Results were reported as the first quarter, median, and third quarter values.

Authors' Contributions

Mohammadd Heidarpour and Javad khoshnegah conceived and planned the experiments. Mohammadd Heidarpour and Javad khoshnegah and Raha Bayazi carried out the experiments. Mohammadd Heidarpour and Javad khoshnegah planned and carried out the simulations. Mohammadd Heidarpour and Javad khoshnegah and Mohammad Azizzadeh contributed to the interpretation of the results. Javad Khoshnegah 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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Abstracts (in Persian)

مقایسهٔ مقادیر برخی فلزات سنگین در خون سگ‌های تریر مبتلا به مشکلات رفتاری و فاقد هر نوع مشکل رفتاری

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چکیده

«مشکلات رفتاری» در حیوانات خانگی، به ویژه سگ‌ها، بسیار نگران کننده است. چنین رفتارهایی ممکن است برای حیوانات و نیز انسان‌ها درد سرساز باشد. به دلیل وجود رابطه بین استرس اکسیداتیو و مشکلات رفتاری و همچنین توانایی فلزات سنگین در ایجاد استرس اکسیداتیو، مطالعهٔ حاضر با هدف مقایسهٔ مقادیر فلزات سنگین در خون کامل سگ‌های دارای مشکلات رفتاری و فاقد مشکلات رفتاری صورت گرفت. بدین منظور، تأثیر فلزات سنگین سرب، جیوه، آرسنیک و کادمیوم، روی سیزده مشکل رفتاری شایع سگ‌ها شامل ترسو بودن، سر و صدای زیاد و آزار دهنده، خرابکاری، ادرار یا مدفوع در مکان نامناسب، رفتار جنسی نامناسب، مدفوع خواری، ولگردی، خجالتی بودن، تهاجم به صاحب حیوان، تهاجم به آشنایان و اعضای خانواده، تهاجم به غریبه‌ها، تهاجم به سایر سگ‌ها و فعالیت بیش از حد، بررسی شد. براساس پرسش نامه های تکمیل شده توسط صاحبان سگ‌ها، ۴۳ قلاده سگ نژاد تریر (شامل ۷ سگ بدون مشکل رفتاری و ۳۶ سگ دارای حداقل یک مشکل رفتاری)، ۱ تا ۱۰ ساله، انتخاب شدند. سپس، مقادیر فلزات سنگین خون به روش Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) اندازه‌گیری شد. تفاوت معنی داری بین مقدار غلظت فلزات سنگین در خون کامل سگ‌های فاقد هرگونه مشکل رفتاری و سگ‌های دارای حداقل یک مشکل رفتاری، مشاهده نشد. با این حال، مقدار غلظت کادمیوم در خون کامل سگ‌هایی از گروه آزمایش که فقط مشکل رفتاری «خشونت علیه صاحب» را داشتند به صورت معنی داری بیشتر از گروه کنترل بود. ($P < 0.148$ ، $n = 5$). همچنین، مقدار غلظت آرسنیک در خون کامل سگ‌هایی از گروه آزمایش که فقط مشکل رفتاری «ترسو بودن» را داشتند، به صورت معنی داری کمتر از کنترل بود ($P < 0.05$ ، $n = 25$). نتایج مطالعه گزارش‌شده در اینجا نمی‌تواند این فرضیه را که «غلظت فلزات سنگین خون ممکن است بر بروز یا پیشگیری از مشکلات رفتاری رایج در سگ‌ها تأثیر بگذارد» تأیید کند. با این حال، می‌توان نتیجه گرفت که اولاً کادمیوم پتانسیل افزایش مشکل رفتاری پرخاشگری نسبت به مالک را دارد. ثانیاً، می‌توان مشکل رفتاری «ترسو بودن» را با تجویز آرسنیک، کاهش داد.

واژگان کلیدی

مشکلات رفتاری، سگ، فلزات سنگین، کادمیوم، سرب، جیوه، آرسنیک

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Abstracts (in Persian)

بررسی اثر کاهندگی کوآنزیم Q10 بر روی اختلال عملکرد بافت بیضه ناشی از کاربندازیم از طریق مدولاسیون سیگنال دهی آپوپتوز miR-202-5p/در موش: مطالعه بافت شناسی و ایمونوهیستوشیمی

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چکیده

کاربرد گسترده کاربندازیم (Carb: methyl-2-benzimidazole carbamate) در زمینه های کشاورزی و دامپزشکی به دلیل باقیمانده های آن، که با اسپرم زایی تداخل می کند، مسائل زیست محیطی مهمی را ایجاد می کند. مطالعات اخیر مزایای سلامت مکمل کوآنزیم (CoQ10) را برجسته کرده اند که در درجه اول با اثرات ضد آپوپتوتیک و ضد التهابی آن مرتبط است. در نتیجه، این مطالعه با هدف کشف مسیرهای مکانیکی بالقوه که از طریق آن CoQ10 ممکن است اختلال عملکرد تولیدمثلی ناشی از کاربندازیم را در موش ها کاهش دهد، انجام شد. موش های صحرایی نر بالغ ویستار به مدت ۹ هفته تحت کاربندازیم (۱۵۰ میلی گرم بر کیلوگرم) به تنهایی یا همراه با CoQ10 (۲۰۰ میلی گرم بر کیلوگرم) به صورت خوراکی قرار گرفتند. در پایان دوره، بافت های بیضه برای تجزیه و تحلیل بافت شناسی، ارزیابی ایمونوهیستوشیمی، اندازه گیری هورمونی و آنالیز مولکولی جمع آوری شد.گروه تحت درمان با کاربندازیم آسیب قابل توجهی به بیضه نشان دادند، همانطور که نتایج رنگ آمیزی ایمنی نشان داد که نشان دهنده افزایش قابل توجه در بیان Bax و کاسپاز۳-، در کنار کاهش قابل توجه در ایمنی پروتئین Bcl-2 در بیضه بود. یافته ها نشان داد که قرار گرفتن در معرض کاربندازیم منجر به کاهش بیان miR-202-5p می شود که همزمان با کاهش سطح تستوسترون و هورمون لوتئینه کننده بود. در مقابل، تجویز CoQ10 در کنار درمان کاربندازیم منجر به بازسازی ساختار بیضه، تعادل هورمونی و کاهش شاخص آپوپتوز شد و این پارامترها را به پارامترهای مشاهده شده در گروه کنترل نزدیک کرد. علاوه بر این، گروه کاربندازیم + CoQ10 سطوح بالایی از miR-202-5p را نشان دادند، به علاوه بافت بیضه ساختار تقریباً طبیعی نشان داد.Carb از طریق مسیر miR-202-5p/apoptosis باعث تغییرات نامطلوب بیضه می شود و CoQ10 ممکن است در مبارزه با اثرات مخاطره آمیز ناشی از Carb از طریق اثرات ضد آپوپتوز و تنظیم ژن مفید باشد.

واژگان کلیدی

بافت شناسی، بیضه، کاربندازیم، موش صحرایی،کوانزیمQ10

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Abstracts (in Persian)

تزریق داخل هیپوکمپی آبسیزیک اسید اختلال حافظه و یادگیری و تغییر در عوامل استرس اکسیداتیو را در موش های صحرایی محروم از خواب REM کاهش می دهد

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۳گروه زیست شناسی، دانشکده علوم، دانشگاه لرستان، خرم آباد، ایران.

چکیده

در این مطالعه اثرات تزریق داخل هیپوکامپی آبسیزیک اسید (ABA) بر تعدیل عملکرد یادگیری و حافظه و تغییر عوامل استرس اکسیداتیو در قشر مغز موش‌هایی صحرایی محروم از خواب با حرکت سریع چشم (REM) مورد ارزیابی قرار گرفت. موش های صحرایی نر بالغ نژاد ویستار در ناحیه CA1 هیپوکامپ کانول گذاری شدند. پس از بهبودی، موش‌ها به مدت ۴ روز در معرض محرومیت از خواب REM قرار گرفتند. سپس گروه‌های حیوانات با ABA در دوزهای ۱۰و ۱۵ میکروگرم و ABA + بیکوکولین تحت درمان قرار گرفتند. یادگیری اجتنابی غیرفعال و حافظه فضایی به ترتیب با آزمون های شاتل باکس و ماز آبی موریس (MWM) ارزیابی شد. علاوه بر این، تغییرات در سطوح کاتالاز به عنوان آنزیم آنتی اکسیدان و MDA و H2O2 به عنوان بیومارکرهای استرس اکسیداتیو در قشر مغز موش های صحرایی تعین شد. نتایج نشان داد در حیوانات محروم از خواب REM در مقایسه با کنترل، اختلال قابل‌توجهی در یادگیری و حافظه در آزمون‌های MWM و شاتل باکس ایجاد شده است. تزریق داخل هیپوکمپی ABA در دوز ۱۰ میکروگرم باعث کاهش اختلال حافظه و یادگیری در موش‌های محروم از خواب REM گردید. پیش تیمار حیوانات با بیکوکولین قادر به تغییر اثرات ناشی از ABA بر حافظه و یادگیری نبود. علاوه بر این، افزایش فعالیت کاتالاز و کاهش MDA و H2O2 در قشر مغز گروه‌های تحت درمان با ABA در دوز ۱۰ میکروگرم و ABA+ Bic مشاهده شد. به طور کلی، داده ها نشان دهنده توانایی ABA برای کاهش اختلال یادگیری و حافظه و آسیب‌های اکسیداتیو ناشی از محرومیت از خواب REM در موش های صحرایی بود. بلوک کردن گیرنده GABA نتوانست اثرات ABA را مهار کند.

واژگان کلیدی

محرومیت از خواب، آبسیزیک اسید، بیکوکولین، حافظه و یادگیری، موش صحرایی

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Abstracts (in Persian)

مقایسهٔ مقادیر برخی فلزات سنگین در خون سگ‌های تریر مبتلا به مشکلات رفتاری و فاقد هر نوع مشکل رفتاری

رها بیاضی، محمد حیدرپور، محمد عزیز زاده، جواد خوش نگاه*

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چکیده

«مشکلات رفتاری» در حیوانات خانگی، به ویژه سگ‌ها، بسیار نگران کننده است. به دلیل وجود رابطه بین استرس اکسیداتیو و مشکلات رفتاری و همچنین توانایی فلزات سنگین در ایجاد استرس اکسیداتیو، مطالعهٔ حاضر با هدف مقایسهٔ مقادیر فلزات سنگین در خون کامل سگ‌های دارای مشکلات رفتاری و فاقد مشکلات رفتاری صورت گرفت. بدین منظور، تأثیر فلزات سنگین سرب، جیوه، آرسنیک و کادمیوم، روی سیزده مشکل رفتاری شایع سگ‌ها شامل ترسو بودن، سر و صدای زیاد و آزار دهنده، خرابکاری، ادرار یا مدفوع در مکان نامناسب، رفتار جنسی نامناسب، مدفوع خواری، ولگردی، خجالتی بودن، تهاجم به صاحب حیوان، تهاجم به آشنایان و اعضای خانواده، تهاجم به غریبه‌ها، تهاجم به سایر سگ‌ها و فعالیت بیش از حد، بررسی شد. براساس پرسش نامه های تکمیل شده توسط صاحبان سگ‌ها، ۴۳ قلاده سگ نژاد تریر (شامل ۷ سگ بدون مشکل رفتاری و ۳۶ سگ دارای حداقل یک مشکل رفتاری)، انتخاب شدند. سپس، مقادیر فلزات سنگین خون به روش Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) اندازه‌گیری شد. تفاوت معنی داری بین مقدار غلظت فلزات سنگین در خون کامل سگ‌های فاقد هرگونه مشکل رفتاری و سگ‌های دارای حداقل یک مشکل رفتاری، مشاهده نشد. با این حال، مقدار غلظت کادمیوم در خون کامل سگ‌هایی از گروه آزمایش که فقط مشکل رفتاری «خشونت علیه صاحب» را داشتند به صورت معنی داری بیشتر از گروه کنترل بود. ($P < 0.148$ ، n= 5). همچنین، مقدار غلظت آرسنیک در خون کامل سگ‌هایی از گروه آزمایش که فقط مشکل رفتاری «ترسو بودن» را داشتند، به صورت معنی داری کمتر از کنترل بود ($P < 0.05$ ، n= 25). نتایج مطالعه گزارش‌شده در اینجا نمی‌تواند این فرضیه را که «غلظت فلزات سنگین خون ممکن است بر بروز یا پیشگیری از مشکلات رفتاری رایج در سگ‌ها تأثیر بگذارد» تأیید کند. با این حال، می‌توان نتیجه گرفت که اولاً کادمیوم پتانسیل افزایش مشکل رفتاری پرخاشگری نسبت به مالک را دارد. ثانیاً، می‌توان مشکل رفتاری «ترسو بودن» را با تجویز آرسنیک، کاهش داد.

واژگان کلیدی

مشکلات رفتاری، سگ، فلزات سنگین، کادمیوم، سرب، جیوه، آرسنیک

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Guide for authors

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References: Must be up-to-dated and limited to those that are necessary. Lists of references should be given in numerical order in the text, and in the reference list. Please use Vancouver style. To download the Vancouver Style follow the link in the IJVST website which could be used in the Endnote software.

Example piece of text and reference list :
An unhealthy diet, obesity and physical inactivity play a role in the onset of type 2 diabetes, but it has been shown that increased physical activity substantially reduces the risk [1], and participation in regular physical activity is one of the major recommendation of the evidence based guidelines for the primary prevention of diseases [2]. According to the 2004-05 National Health Survey, more than half a million Australians (3.5% of the population) have diabetes mellitus which had been medically diagnosed and most of these people have the Type 2 condition [3]. Gestational diabetes is also on the increase, rising steadily between 2000-01 and 2005-06 [4]. Approximately two thirds of those with diabetes have been prescribed medication [3], but it is of concern that a recent review of the literature found that many people do not take their medication as prescribed [5]. Many patients also self monitor the disease by measuring their blood glucose levels with a glucose meter but Song and Lipman [6] have concerns about how well this is managed.

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 .
Use of Italics

Gene symbols, Latin terms (i.e. in vivo, in vitro, ex vivo, in utero, in situ, and etc.) and species scientific names (using the binomial nomenclature), should be typed in italics, while the first letter of the genus name must be capitalized (i.e. Homo sapiens).

Copyright

PUBLICATION ETHICS

Iranian Journal of Veterinary Science and Technology is a member of the Committee on Publication Ethics (COPE), best practice guidelines for dealing with ethical issues in journal publishing and adopts the COPE guidelines. The journal members (editor, editorial board and the journal manager) have agreed to meet the purposes and objectives of the Journal.

Ethical guidelines for authors:

- Manuscripts must be submitted with the understanding that they have not been previously published and are not currently under consideration by another journal.
- Authors are expected to submit manuscripts with enough detail and references to enable others to replicate the work.
-Authors may be requested to provide the original data from their study for editorial review and should be ready to make the data publicly available if feasible.
-The corresponding author is responsible for ensuring that all co-authors have approved the manuscript prior to submission.
- Only individuals who meet the authorship criteria should be listed as authors in the manuscript, as they are expected to take public responsibility for the content. The "Conflict of interest declaration and author agreement form" must be signed and completed by all authors. This statement and sig-

natures certifies that all authors have seen and approved the manuscript being submitted. Also, the authors by signing this form warrant that the article is the Authors' original work, that the article has not received prior publication and is not under consideration for publication elsewhere, and that the corresponding author shall bear full responsibility for the submission.

Before submission, all authors are required to review the Article Submission Checklist.

- Authors should disclose any conflicts of interest that might be perceived as influencing the results or their interpretation in the manuscript at the earliest stage possible. This can be done by uploading the Conflicts of Interest Form along with the manuscript submission.

-The authors are responsible for ensuring that the submitted manuscript is a complete and original work, free from any form of plagiarism. All authors are advised to use plagiarism prevention software to check for similarities.

-Authors are required to identify in their manuscript if their work involves chemicals, procedures, or equipment that have any inherent unusual hazards.

- All researchers should have a written and signed informed consent form from whom voluntarily participate in their researches. This signed form shows obviously the consent of the subject to participate. All steps of the experiment were carried out based on the Guidelines for Animal Care at Ferdowsi University of Mashhad in Iran that are approved by the Committee of Biological Ethics, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran (https://ethics.research.ac.ir/docs/pages/Guideline-En.pdf). The experiments that are carried outside the university should have a written and signed informed consent form related to ethic protocols of university or institute that they are carried.

- If the decision is 'Needs Revision,' authors are expected to respond systematically and promptly to the reviewers' comments, addressing point by point, and revising their manuscript accordingly. The revised manuscript should then be submitted to the journal within the given deadline.

- Authors are requested to clearly identify who financially supported the research and/or preparation of the manuscript and briefly describe the role of the founder/ sponsor in any part of the work at the end of their manuscript under "Acknowledgements" section.

-It is a condition for submission of a manuscript that the authors permit editing of the paper for readability.

- All authors agree to allow the corresponding author to serve as the correspondent with the Journal's editorial office, to review the edited manuscript and proof.

-Under open access license, authors retain ownership of the copyright for their content, but allow anyone to download, reuse, re-print, modify, distribute, and/or copy the content as long as the original authors and source are cited properly.

- When author(s) discover(s) a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the Journal editor or publisher to retract or correct the manuscript.

- All authors must know that the submitted manuscripts under review with the IJVST are subject to screening, using Plagiarism Prevention Software. Plagiarism is a serious violation of publication ethics and in all its forms constitutes unethical publishing behavior and is unacceptable.

- Editors and members of editorial board as authors should be excluded from publication decisions

when they are authors or have contributed to a manuscript.

-The artificial intelligence (AI) tools such as ChatGPT or Large Language Models cannot meet the requirements for authorship. Authors who use AI tools in the writing of a manuscript, production of images or graphical elements of the paper, or in the collection and analysis of data, must be transparent in disclosing in the Materials and Methods (or similar section) of the paper how the AI tool was used and which tool was used. Authors are fully responsible for the content of their manuscript, even those parts produced by an AI tool, and are thus liable for any breach of publication ethics(Authorship and AI tools).

Ethical guidelines for Peer reviewers

- Reviewers are expected to provide insightful comments that assist the editors in making a decision about whether or not to publish the submitted manuscript.

- Reviewers are expected to maintain the confidentiality of the manuscripts they are invited to review.

-Reviewers are expected to disclose any conflicts of interest they have with the authors, companies, or institutions associated with the manuscripts they are invited to review. If a conflict of interest exists, reviewers should immediately notify the Editor-in-Chief, decline the invitation to review, and suggest alternative reviewers.

- If reviewers feel unqualified to review an assigned manuscript or are unable to provide a timely review, they should inform the Editor-in-Chief and excuse themselves from the review process. If they know of any other expert reviewers, they may suggest them to the Editor-in-Chief through the dedicated email/comments section in the Reviewer Dashboard.

- Reviewers are expected to maintain the confidentiality of the manuscripts they review and not discuss any information from the manuscript with anyone other than the Editor-in-Chief, unless they have obtained explicit permission to do so. This also applies to invited reviewers who decline the review invitation.

- Reviewers are obligated to treat the manuscripts they receive for peer review as confidential and must not use any information obtained through this process for personal gain.

-Reviewers are expected to provide technical, professional, and objective comments on the manuscripts they are invited to review.

-Reviewers are expected to avoid personal biases in their comments and judgments, and express their views clearly with supporting arguments that assist the author in improving the manuscript.

-Reviewers are expected to identify any relevant published work that has not been cited by the authors. If a statement has been previously reported elsewhere, it should be accompanied by the appropriate citation.

-Reviewers should also bring to the attention of the Editor-in-Chief any significant similarity or overlap between the manuscript under consideration and any other publications of which they are personally aware.

The process has been explained in the section “Peer Review Process”.

Publication Ethics

Ethical guidelines for Editor

-The editors should evaluate submitted manuscripts to determine if they fall within the scope of the journal. Additionally, the editors should recommend expert reviewers based on their integrated recognition of specialized reviewers.

-The Editor-in-Chief is responsible for deciding whether to accept or reject submitted manuscripts for the journal. This decision takes into consideration several factors, such as the judgment of the editorial board members, the validation of the work in question, its significance to researchers and readers, as well as any feedback from reviewers. Furthermore, the decision must also comply with legal requirements regarding libel, copyright infringement, and plagiarism, which are currently in force. The Editor-in-Chief works closely with other editors and reviewers to ensure that all submissions are fairly evaluated.

-The editors ought to uphold the anonymity of both reviewers and authors.

-The editors should disclose any potential conflicts of interest and make efforts to avoid them. If such circumstances arise, they are expected to delegate the handling of the manuscript to another member of the editorial board.

-The editors, particularly the Editor-in-Chief, should demonstrate a willingness to investigate cases of plagiarism and fraudulent data. When ethical concerns are raised about a submitted manuscript or published paper, the editors will take appropriate measures in response. Any reported incidents of unethical publishing behavior will be thoroughly examined, even if they come to light years after publication.

-When dealing with cases of suspected misconduct, the Editor-in-Chief follows the COPE Flowcharts. If an investigation supports the ethical concern, the journal will publish a correction, retraction, expression of concern, or any other relevant note.

-The editors must not share any information about submitted manuscripts with anyone until they are published, as appropriate.

-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.

PUBLICATION ETHICS

Iranian Journal of Veterinary Science and Technology is aligned with COPE's (Committee on Publication Ethics) best practice guidelines for dealing with ethical issues in journal publishing and adopts the COPE guidelines. The journal members (editor, editorial board and the journal manager) have agreed to meet the purposes and objectives of the Journal.

Ethical guidelines for authors:

Authorship Criteria

IJVST requires authors to confirm that they and their co-authors meet all four criteria for authorship based on the guidelines of The International Committee of Medical Journal Editors (ICMJE) (verbatim as follows):

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

The section "Author Contributions" in the manuscript should illustrate and clarify who contributed to the work and how. If a contributor does not meet all four above criteria should be acknowledged in the "Acknowledgements" section of the article.

Author agreements and conflict of interest

Written authorization from all authors for publication of the article is mandatory for IJVST to start the review process. This form entitled "Conflict of interest declaration and author agreement form" must be signed and completed by all authors. This statement and signatures certifies that all authors have seen and approved the manuscript being submitted. Also, the authors by signing this form warrant that the article is the Authors' original work, that the article has not received prior publication and is not under consideration for publication elsewhere, and that the corresponding author shall bear full responsibility for the submission.

Artificial intelligence (AI)

Publication Ethics

The artificial intelligence (AI) tools such as ChatGPT or Large Language Models cannot meet the requirements for authorship. Authors who use AI tools in the writing of a manuscript, production of images or graphical elements of the paper, or in the collection and analysis of data, must be transparent in disclosing in the Materials and Methods (or similar section) of the paper how the AI tool was used and which tool was used. Authors are fully responsible for the content of their manuscript, even those parts produced by an AI tool, and are thus liable for any breach of publication ethics (Authorship and AI tools).

Editors and members of editorial board as authors

Editor and members of editorial board are excluded from publication decisions when they are authors or have contributed to a manuscript.

Ethical guidelines for Peer reviewers

Iranian Journal of Veterinary Science and Technology (IJVST) follows and adheres to COPE Ethical Guidelines for Peer Reviewers. IJVST peer reviews all submitted manuscripts with contents in the scope of the journal. The process has been explained in the section "Peer Review Process".

Ethical guidelines for Editor

Iranian Journal of Veterinary Science and Technology regarding the responsibilities of the editors follows and adheres to COPE Ethical Guidelines for editors. The main guidelines are summarized in the guide to ethical editing from COPE.

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.

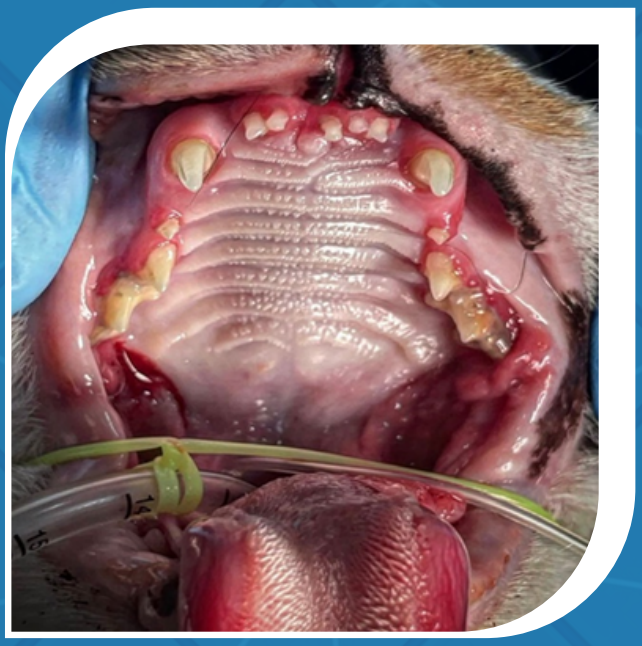
Peer review (double-blind)

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.



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