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Journal Description

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Biodiversity Research Journal, Volume 3, Issue 1, June 2025

Journal Editorial

Prof. Wafa Abdullah Al-Megrin, Editor-in-Chief. Professor of Parasitology, Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, Kingdom of Saudi Arabia.

Praise be to Allah, the Lord of all worlds, and blessings and peace upon the noblest of prophets and messengers, our Prophet Mohammed.

The Biodiversity Research Journal is pleased to present to our esteemed readers the second issue of the second volume. This issue contains five distinguished and diverse scientific research from different biodiversity aspects.

The editorial board is committed to achieving scientific quality in every piece of academic output it publishes, reinforcing creative research movement in the fields of biodiversity, staying up-to-date with innovations, and encouraging global researchers to contribute their academic work to the journal.

We extend our gratitude and appreciation to everyone who contributed and assisted in producing this issue.

Biodiversity Research Journal, Volume 3, Issue 1, June 2025

Princess Nourah Bint Abdulrahman University
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كلية العلوم

Investigation of Tolerance and Avoidance Responses in Meiobenthic Copepod Species to Phenanthrene and Chrysene Exposure

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Keywords

Meiofauna; Copepods, Chrysene; Phenanthrene, Vertical migration

ABSTRACT

This study investigates the effects of two Polycyclic Aromatic Hydrocarbons (hereafter PAHs), chrysene and phenanthrene, on the vertical movement of meiobenthic copepods in open microcosm environments. PAHs are environmental contaminants of significant concern owing to their prevalence and toxicological effects on aquatic organisms. This investigation highlights the significance of PAHs as ecological stressors affecting the movement behavior of meiobenthic copepods following exposure to chrysene and phenanthrene, both individually (1.16 ng/g Dry Weight) and in combination. Indeed, it was identified that the most vulnerable species to PAHs are *Asellopsis hispida* and *Bulbamphiascus imus*. These species demonstrated a clear aversion to PAHs, probably *via* remote chemodetection. In contrast, species like *Canuella furcigera* and *Heterolaophonte stromeii stromeii* showed a high tolerance to PAHs by moving into all contaminated regions. Interestingly, the study outcomes supported that the movement patterns of meiobenthic copepods may serve as crucial indicators of the availability and nature of PAHs in benthic ecosystems.

INTRODUCTION

In recent decades, environmental pollution has emerged as one of the most pressing global challenges (Selamoglu et al., 2008, 2009; Klavins et al., 2022). Industrialization and urbanization are primary factors negatively impacting ecological health due to ongoing challenges from both traditional and emerging pollutants (Failler et al., 2015). Regardless of their sources or modes of release, aquatic ecosystems act as ultimate sinks where these substances and their degradation products accumulate. Therefore, it is crucial to evaluate the risks associated with environmental chemical pollutants. Ecotoxicology plays a crucial role in two main areas: (1) evaluating the impacts of pollutants on biota within an ecosystem before implementing preventive strategies, and (2) understanding and modeling the mechanisms of environmental contamination (Lesueur, 2014).

One significant class of organic pollutants found in marine environments is polycyclic aromatic hydrocarbons (hereafter referred to as PAHs), which can have adverse effects on aquatic life. PAHs can disrupt the natural balance of ecosystems, potentially jeopardizing human health, damaging biological resources, and interfering with various legitimate uses of the ocean (Meador, 2003).

Aquatic products and animals are essential sources of nutrients in the human diet and play a significant role in the global aquatic product industry for consumers (Selamoglu, 2021). Therefore, we need to protect our aquatic environment against several pollutants, with confirmed toxicity (Selamoglu et al., 2009; Ozdemir et al., 2006, 2007, 2010). Aquatic ecosystems and living organisms are affected by environmental impacts resulting from the emissions of volatile organic substances and water pollution caused by oil, chemicals, and various hazardous agents. Every trophic level is susceptible to hydrocarbon contamination, ranging from planktonic species to marine mammals. Mollusks and crustaceans (such as mussels, shrimp, and crabs) are particularly prone to bioaccumulating contaminants, even when the pollution level is low or the source is removed. In fact, Molds are unable to stop pollutants from infiltrating. Conversely, crustaceans that consume detritus or suspended particles will experience changes in their reproductive rate (decrease in hatch rate) or their feeding behaviors. Genotoxicity, carcinogenicity, effects on reproduction and development (Arkoosh et al., 1996; Johnson et al., 1998; Rice et al., 2000), and immunotoxicity (Reynaud and Deschaux, 2006) were emphasized to varying extents based on the molecular weight of PAHs and the metabolism processes.

Numerous studies have focused on the impact of hydrocarbons on benthic communities (Lee et al., 1981; Boucher, 1981; Beyrem and Aïssa, 2000; Mahmoudi et al., 2005). For instance, Lotufo (1997) demonstrated the substantial effects of PAHs on copepod reproduction, leading to a significant reduction in the number of eggs laid by females. Environmental monitoring of this specific type of pollutant may rely, among other factors, on its impact on both positive and negative bioindicators, which is necessary for government policymakers to consider.

The topic investigated in this research has emerged as a significant global concern. Thus, this research aims to primarily address the gap concerning PAHs, whose impacts on one of the major meiobenthic groups, copepods, have yet to be investigated based on their migratory behavior. The inquiries explored were: (1) Will copepod species exhibit tolerance or avoidance responses to phenanthrene and/or chrysene? Moreover, (2) if so, do copepods exhibit taxonomic changes after their exposure to chrysene, phenanthrene, and their combination? The applied experimental approach was original and focused on the vertical taxonomic modifications.

MATERIALS AND METHODS

Sediment collection

The initial samples were collected on March 18, 2019 (7 A.M.), at a coastal site near the city of Menzel Jemil, Tunisia, situated in the subtidal zone in Bizerte lagoon (37°13'22"N; 9°55'48"E). The sampling site was chosen for its location in a protected and low-hydrodynamic region (Boufahja et al., 2006). On the day of sampling, sediments were taken during low tide using the method proposed by Boufahja et al. (2016) to a depth of 5 cm. This layer serves as a microhabitat that exhibits the greatest taxonomic richness and abundance of meiofauna (Bin-Jumah, 2024).

Microcosm description

To investigate the potential vertical migration of meiobenthic copepods from a healthy natural environment to an azoic and contaminated medium, twelve microcosms were established. Each microcosm consisted of two sediment compartments: natural sediments on top and azoic-contaminated sediment below (refer to Fig. 1). These sediments were enriched with seawater that had been pre-filtered through a 40 μm sieve to prevent the inclusion of organisms that could skew the results. After fifteen days, all replicates were preserved in 4% neutralized formalin at hexamethylene tetramine and subsequently stained with Bengal Rose (0.2 g/l) to enhance the coloration of the specimens found in the sediment (Waweru et al., 2024). Following preservation in formalin, harpacticoids and cyclopoids were stored in 70% ethanol for subsequent taxonomic identification (Apostolov, 2016).

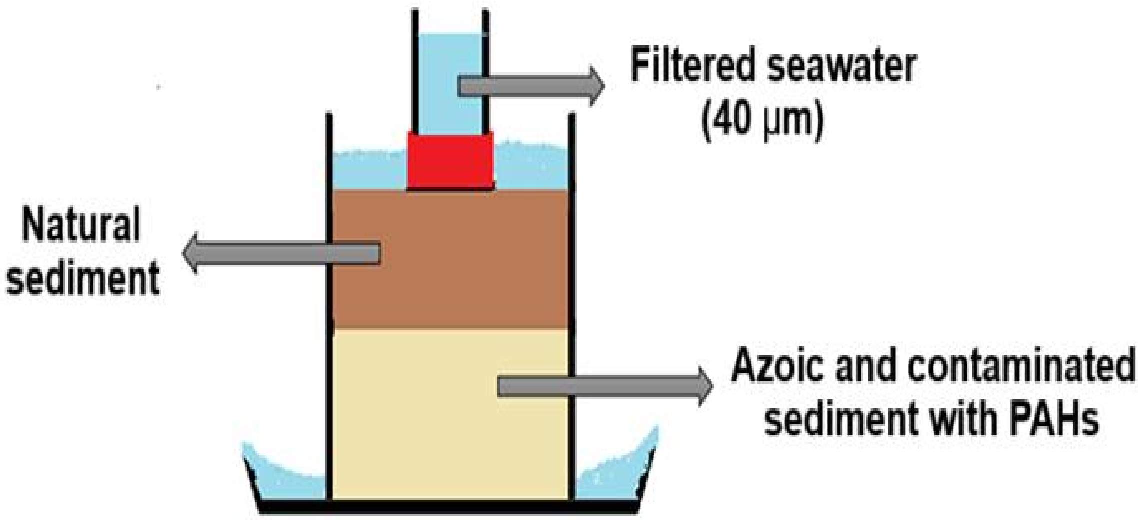

Figure 1. Experimental design used to study the vertical migration of meiobenthic nematodes after exposure to polycyclic aromatic hydrocarbons.

Sediment preparation

To render the sediment azoic, the collected samples underwent three cycles of freezing for 12 hours at -20 °C, followed by 48 hours of thawing at room temperature (Schratzberger et al., 2004). After processing, large particles were removed manually. The retained portion was then separated into two fractions: fine (for later contamination) and coarse, using a sieve with a 300 µm mesh (Boufahja et al., 2016). A 100 g fresh weight (FW) sub-sample was dried for 96 hours at 45 °C to determine sediment water content (Boufahja et al., 2016) and dry weight (DW).

Selection of PAH concentrations and sediment contamination

In this study, a single concentration was utilized for both hydrocarbons examined (chrysene and phenanthrene). This concentration was selected based on findings of Zrafi-Nouira et al. (2010), who reported levels of various PAHs in and around the discharge area of refining industries in Tunisia. The chosen concentration to initiate this bioassay is one-third of the chrysene concentration from the sedimentary layer, located 2,380 m away from the discharge area of the Bizerte refinery. Zrafi-Nouira et al. (2010) reported that at this distance, the sedimentary chrysene concentration is 347.5 ± 0.014 ng/g DW, which is approximately 348 ng/g DW. For our study, we contaminated the sediment with a concentration of 1.16 ng/g DW, a level nearly identical to that measured at a distance of 0.59 m from the Bizerte refinery rejection zone. To accomplish the sediment contamination with PAHs, it was necessary first to dissolve these hydrocarbons in acetone. Specifically, we dissolved 1 mg of phenanthrene (99% pure crystal) in 200 µl of acetone (Louati et al., 2014) and 1 mg of chrysene (99% pure crystal) in 625 µl of acetone (Lange et al., 2006).

Meiobenthic descriptors

The extraction of meiofauna from sediment was performed using the levigation-decantation-sieving method as described by Rzeznik-Orignac et al. (2017). This technique is based on the principle that the organisms to be extracted have a lower density than the sediment particles. The sediments were kept in suspension in a water-filled crystallizer through manual rotary motions. After a brief decantation period, the water containing the meiobenthic organisms was filtered twice through two sieves. The first sieve had a mesh size of 1 mm, while the second sieve had a mesh size of 40 µm. These sieves were used to separate macrobenthic organisms and large debris (≥ 1 mm) from the meiobenthos. The material collected by the 40 µm sieve was then retrieved using a gentle spray of 4% neutral formaldehyde (Guo et al., 2001) and transferred into plastic flasks containing a 70% ethanol solution (Apostolov, 2016).

The extracted material was carefully placed into a tiled Dollfus chamber, which contains 200 squares, each measuring 5 mm². The squares are bordered by raised edges that limit the movement of copepods as they were positioned on the stage of a stereomicroscope (type Leica zoom 2000) for counting. The magnifier aided in counting harpacticoids and cyclopoids, enabling their collection for both generic and species identification. Copepods were collected using a fine needle under the stereomicroscope. Each specimen was then placed on a slide and immersed in a dissection solution of glycerin (made of equal parts glycerin and distilled water). All appendages were separated and stored in a drop of glycerin. The furca and roster were additionally covered with a cover slip. To prevent the cover slip from shifting, a thin coat of nail polish was applied to its edges. The taxonomic classification of harpacticoid and cyclopoid copepods was based on two guides: Lang (1948) and Huys et al. (1996).

Data processing

Four univariate indices were considered for every microcosm: abundance, species number, Margalef's species richness, and Shannon-Wiener diversity index. Kolmogorov-Smirnov tests were utilized to assess the normality of the data, whereas the Bartlett test was applied to examine the equality of variances. Log transformations were utilized when required. A one-way analysis of variance (1-ANOVA) was conducted using Statistica version 5.1 software to identify globally significant differences among the treatments tested. If significant differences ($p < 0.05$) were found, the Tukey's HSD *post-hoc* test was applied to identify pairs of treatments that differed significantly ($p < 0.05$). All replicates related to the treatments were consistently projected from a non-parametric multidimensional scaling (nMDS) analysis. A stress factor indicated on the nMDS 2D-plot below 0.2 suggests that the resulting representation is statistically and ecotoxicologically reliable (Clarke, 1993). This author also recommended the SIMPER process (SIMilarity PERcentages) to assess the contribution of each species to the overall dissimilarity among the various treatments. These multivariate analyses were performed using the software PRIMER v. 5.0, developed by the Plymouth Marine Laboratory (Clarke and Gorley, 2001).

RESULTS

Univariate indices

Regarding the average abundance of copepods, the compartments contaminated with the different polycyclic aromatic hydrocarbons (PH, CH, and M) showed significantly lower numbers than those associated with the control treatment (see Fig. 2 and Table 1). Comparisons of these abundances revealed discernible differences ($p < 0.05$) across all contaminated compartments and controls.

Table 1. Comparisons of the average abundance of copepods from different compartments contaminated or not by polycyclic aromatic hydrocarbons using the Tukey-HSD *post-hoc* test. The values indicate the probabilities (p). Bold values indicate significant differences ($p < 0.05$). The data are *log*-transformed. C: Control; PH: phenanthrene; CH: chrysene; M: mixture of phenanthrene and chrysene.

Abundance: one-way ANOVA: F = 9.1; p < 0.05				
	C	PH	CH	M
C		**0.047**	**0.044**	**0.022**
PH			0.375	0.637
CH				0.955

M

The sediments contaminated with phenanthrene exhibited a reduced number of significant species compared to the control sediment. In contrast, the chrysene-contaminated replicates were associated with a slightly higher number of species. However, this difference was not statistically significant (see Fig. 2). The one-way ANOVA variance analysis revealed no significant differences among the Shannon indices for the various compartments, and a similar outcome was observed for Margalef's species richness.

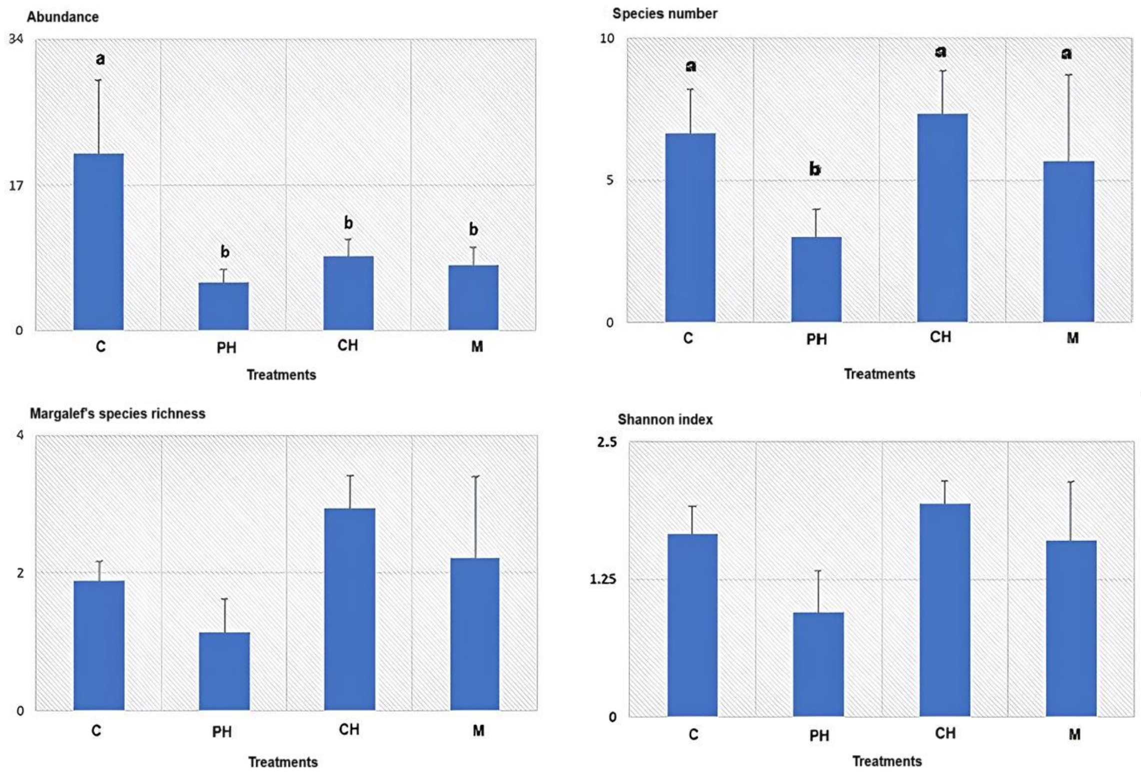

Figure 2. Changes in univariate indices related to copepod assemblages from the various sediment compartments contaminated or not with polycyclic aromatic hydrocarbons. The vertical bars represent the standard deviations. C: Control; PH: phenanthrene; CH: chrysene; M: mixture of phenanthrene and chrysene. Different letters above bars indicate significant differences (Tukey's test: $p < 0.05$).

Multivariate analyses

The results of the replicates' ordination based on the nMDS approach (Fig. 3) demonstrated a notable influence of PAHs on species vertical distribution (stress = 0.01). This effect allowed for the differentiation of replicates based on sediment quality; the controls were located on the left of the factorial plane, while contaminated sediments with phenanthrene, chrysene, and their combination appeared on the right. The results of the SIMPER analysis, detailed in Table 2, identified the cumulative species that contributed approximately 70% to the overall dissimilarity between the control copepod assemblage and those exposed to PAHs. The experiment concluded with the complete removal of two copepod species, *Enhydrosoma* sp. and *Amphiascus parvulus*, from the phenanthrene-contaminated compartment. In contrast, the compartments contaminated with chrysene and the chrysene/phenanthrene mixture experienced only a reduction in the population sizes of these species, rather than their complete elimination.

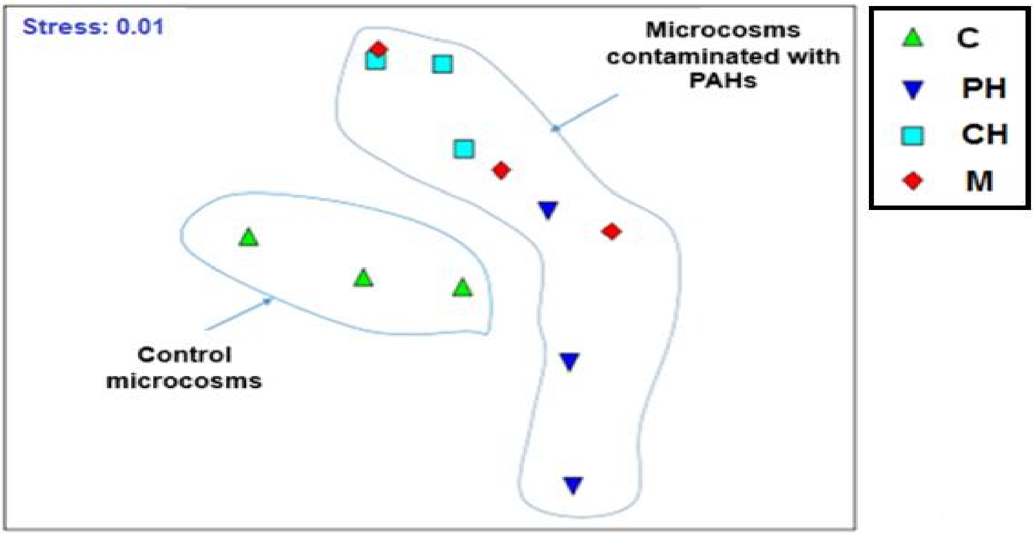

Figure 3. Ordination of the various microcosms (controls and contaminated by the HAP) according to the nMDS method based on species abundances. C: Control; PH: phenanthrene; CH: Chrysene; M: mixture of phenanthrene and chrysene.

Table 2. Relative contributions (in %) of *copepod* species participating in around 70 % of the average dissimilarity between the various treatments considered. Decrease in abundance (-); Total elimination (Elim). C: Control; PH: phenanthrene; CH: Chrysene; M: mixture of phenanthrene and chrysene.

C vs. PH	C vs. CH	C vs. M
Enhydrosoma sp.1 (32,25%) Elim	*Enhydrosoma* sp.1 (22,31%) (-)	*Enhydrosoma* sp.1 (23,86%) (-)
Canuella furcigera (46,83%) (-)	*Canuella furcigera* (43,93%) (-)	*Canuella furcigera* (44,21%) (-)
Heterolaophonte stromeii stromei (59,36%) (-)	*Heterolaophonte stromeii stromei* (55,47%) (-)	*Heterolaophonte stromeii stromei* (55,12%) (-)
Amphiascus parvulus (69,93%) Elim	*Amphiascus parvulus* (63,40%) (-)	*Asellopsis hispida* (62,19%) Elim
		Amphiascus parvulus (69,14%) (-)

DISCUSSION

This research aimed to investigate the impact of polycyclic aromatic hydrocarbons on the migratory behavior of harpacticoid and cyclopoid copepods. The bioassay was designed to connect two distinct types of sediments in open microcosms: one natural (inhabited by meiofauna and untreated) positioned at the top, and the other contaminated with PAHs—chrysene, phenanthrene, or a combination of both—located at the bottom. The city of Menzel Jemil, Tunisia, was chosen due to its abundance of copepods. At the end of the experiment, *C. furcigera* and *Enhydrosoma* sp. were predominant in the natural sediment, with these two species exhibiting distinct responses to the considered hydrocarbons. *C. furcigera* experienced a notable decline in average numbers, decreasing from 17 individuals in natural sediment to only 5 individuals in sediment contaminated by phenanthrene, and 4 individuals in sediment affected by chrysene and the combination of both hydrocarbons. This suggests that *C. furcigera* can somewhat endure pollution from PAHs. Conversely, *Enhydrosoma* sp. completely vanished from the phenanthrene-contaminated sediment but persisted in the sediment contaminated with chrysene and the phenanthrene/chrysene mixture. This suggests that *Enhydrosoma* sp. is highly responsive to phenanthrene, and an antagonistic interaction appears to regulate the relationship between the two mixed hydrocarbons. Morelis et al. (2007) examined the chemical interactions of chrysene and phenanthrene in marine sediments. They found that the combination of these PAHs was less toxic than when either chemical acted independently. These interactions may be linked to the variations in hydrosolubility of the two compounds, which are caused by their differing molecular weights: chrysene contains four aromatic rings, whereas phenanthrene has three. Additionally, two other species, present in natural sediment, displayed different behaviors toward PAHs: *Heterolalophonte stromeii stromei* and *Amphiascus parvulus*. Both copepod species, *C. furcigera* and *H. stromeii stromei*, were found in all compartments, regardless of whether they were contaminated with PAHs or not. However, there was a notable reduction in the number of individuals in the contaminated compartments compared to the control one. In contrast, *A. parvulus* exhibited behavior similar to that of *Enhydrosoma* sp.; this species was absent from compartments contaminated with phenanthrene but was present in microcosms containing sediments spiked with chrysene and in those with a mixture of phenanthrene and chrysene.

The presence of *C. furcigera* and *H. stromeii stromei* in various contaminated compartments may be attributed to several factors, including their potential tolerance to the PAHs tested or their entrapment in sediment compartments, which theoretically could be stickier, as they attempt to navigate through these altered conditions. The significance of the body surface area of copepods is heightened due to their native appendages. The absence of *Enhydrosoma* sp. and *A. parvulus* in compartments contaminated with phenanthrene—contrasting with their presence in compartments affected by chrysene and the combined PAHs—may indicate greater sensitivity of these copepod species to phenanthrene. The effects of phenanthrene and chrysene appear to be opposed. Species such as *Asellopsis hispida* and *Bulbamphiascus imus* were only found in untreated compartments, suggesting their potential sensitivity to phenanthrene and/or chrysene. These findings imply that these species might be capable of sensing stress induced by nearby PAHs and could be actively avoiding such conditions.

The results of this study are consistent with those found by Lotufo (1997), who observed high mortality rates in response to phenanthrene exposure and utilized fluoranthene alone. Conversely, Lotufo's study demonstrated that the phenanthrene/flouranthene mixture did not result in significant mortality when compared to the respective control. Our findings, however, contradict those of Pavillon et al. (2003), who reported that phenanthrene had acute toxicity at a sediment concentration of 60 mg/kg DW for copepods, with no survivors noted after 96 hours of exposure.

CONCLUSIONS

Polluted sediments in the natural environment represent a significant stressor for sensitive species of copepods, rendering their survival in such conditions unviable; these species tend to avoid habitats that are unsuitable for them. In contrast, these same polluted sediments can create ideal conditions for more tolerant species or opportunists, leading to an increase in their populations. This research aimed to assess how two hydrocarbons (phenanthrene and chrysene), used individually or in combination, influenced the distribution of mechanistic copepod species in interconnected compartment microcosms. By observing the migratory patterns of these copepods in response to PAH exposure, we aimed to understand their specific behaviors under such stressors.

The findings underscore that each compartment had a unique species composition. Sediments not exposed to stress exhibited the highest species richness, while those polluted by phenanthrene displayed the lowest diversity. Our research identified the most sensitive species to PAHs, such as *A. hispida* and *B. imus*, which were only present in control sediments. These species showed evident avoidance of PAHs, likely through distant chemo detection. Conversely, species like *C. furcigera* and *H. stromeii stromei* demonstrated a degree of tolerance to PAHs by migrating into all contaminated areas.

Notably, exposure to phenanthrene alone was found to be more detrimental to specific species in terms of their abundance than exposure to the combination of phenanthrene and chrysene, suggesting that these PAHs interact and imply potential antagonism between chrysene and phenanthrene. Ultimately, the presence of PAHs in sediments involved in ecotoxicological research significantly affects the abundance and diversity of copepods. Of the 19 species identified in the initial survey, only four displayed a relative capability to tolerate the presence of PAHs in the sediment.

From the perspective of this study, it is important to note that (1) the experiment should be replicated to track also the horizontal movement of copepods, which will allow for a more thorough confirmation of our findings, and (2) the impacts of additional vector pollutants like nanoparticles or microplastics should be analyzed using the same methodologies employed in this research.

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CONFLICT OF INTERESET STATEMENT

We declare that we have no conflict of interest.

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Vitamin C Attenuates Monosodium Glutamate-Induced Hepatotoxicity in Albino Rats

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Keywords

Vitamin C, monosodium glutamate, Inflammation, and Liver

ABSTRACT

The overconsumption of monosodium glutamate (MSG) may cause liver injury by promoting oxidative stress, along with activating inflammatory processes within the organ. This research was intended to evaluate the hepatoprotective potential of vitamin C in a murine model of MSG-triggered liver injury. Four groups of 24 adult albino male rats were arbitrarily assigned: a group for control, an MSG group (given 60 mg/kg MSG daily for 30 days), a vitamin C group (given 100 mg/kg vitamin C daily for 30 days), and a co-treatment group receiving vitamin C followed by MSG two hours later for 30 days. Biochemical assessments were performed to measure liver antioxidant markers, such as the activity of superoxide dismutase or SOD, catalase or CAT, and glutathione, or GSH, as well as lipid peroxidation markers such as malondialdehyde (MDA) and nitric oxide (NO) levels. Measurements of interleukin-1 beta (IL-1β) along with tumor necrosis factor-alpha (TNF-α) were employed to assess the inflammatory status, while serum alanine aminotransferase (ALT) along with aspartate aminotransferase (AST) activities were employed to assess liver function. Histopathological examination of liver tissues was also conducted. MSG treatment significantly impaired antioxidant defenses and increased both oxidative and inflammatory markers, leading to hepatocellular injury. Conversely, vitamin C administration effectively restored antioxidant enzyme activities, reduced oxidative and inflammatory mediators, and retained normal liver architecture. These results indicate that vitamin C represents a viable therapeutic approach for counteracting MSG-induced hepatic toxicity by attenuating oxidative stress and inflammation.

INTRODUCTION

MSG, or monosodium glutamate, has emerged as a common taste enhancer found in a lot of manufactured meals, snacks, and restaurant meals worldwide. Its popularity stems from its function as an umami taste enhancer, making it a common additive in Asian cuisines, canned soups, instant noodles, and fast food (Shi et al., 2012). Although regulatory bodies like the U.S. FDA consider MSG to be 'generally recognized as safe, growing research indicates that long-term or excessive intake could harm multiple biological systems (Ogunmokunwa and Ibitoye, 2025).
Some studies associate MSG with adverse effects such as metabolic syndrome, neurotoxicity, and hepatotoxicity, though findings remain controversial (Niaz et al., 2018, and Zanfirescu et al., 2019). Notably, in regions with high dietary MSG intake, such as East Asia, concerns have been raised about its contribution to liver dysfunction, prompting further investigation (He et al., 2021).

The liver, a primary organ for detoxification and metabolism, is particularly vulnerable to substances that promote oxidative stress and inflammation. Research indicates that high doses of MSG can contribute to oxidative stress, trigger inflammatory responses, and lead to hepatocellular damage (Eweka et al., 2011).
An important mechanism by which MSG causes injury which is attributable to a surplus of reactive oxygen species (ROS). Elevated levels of ROS can lead to the impairment of cellular integrity and peroxidation of lipids, and hepatocyte apoptosis, thereby impairing liver function (Singh and Ahluwalia, 2012). A prior study found that MSG boosted the expression of pro-inflammatory mediators as TNF-α along with IL-6 (Asejeje et al., 2023). MSG consumption promotes ROS production, which triggers lipid, protein, and DNA damage induced by reactive radical species. The peroxidation of lipids degrades polyunsaturated lipids in cell membranes, culminating in apoptotic cell death (Sahin et al., 2023).

Vitamin C (Vit. C), generally called ascorbic acid, is a vital nutrient noted for its strong antioxidant capabilities. It is essential for eliminating free radicals, strengthening the body's inherent antioxidant defense mechanisms, and also modifying inflammatory pathways (Rizvi et al., 2014). According to scientific data, vitamin C supplementation can mitigate oxidative stress, restore antioxidant enzyme activity, and support liver function in various models of toxin-induced hepatotoxicity (El-Meghawry El-Kenawy et al., 2013). Due to its protective effects, vitamin C is proposed as a potential therapeutic agent for counteracting MSG-induced liver damage. This current work aims to investigate the Vitamin C's capacity to protect the liver in counteracting MSG-triggered liver injury. By assessing oxidative stress biomarkers, inflammatory mediators, and histopathological changes, this research seeks to provide critical insights into whether vitamin C can serve as a preventive or therapeutic strategy against MSG-associated liver toxicity. Given the global prevalence of MSG consumption and the ongoing safety debates, public health can benefit greatly from this study, especially in areas where dietary exposition to MSG is substantial.

Materials and Methods

Chemicals

Morgan Pharma Co provided the pure monosodium glutamate (a concentration of 99%) in Egypt, and C-Retard (500 mg of vitamin C) was sourced from Hikma Company in Egypt. Oxidative stress indicator kits were supplied by Biodiagnostics in Cairo, while enzyme-linked immunosorbent assay kits for detecting inflammatory markers were provided by Thermo Fisher Scientific in the USA. Additionally, 10% formalin and high-purity analytical-grade stains were acquired from El-Gomhoryia Chemical Company in Cairo.

Experimental design and treatment protocol

The Medical Research Institute of Ain-Shams in Cairo, Egypt, provided twenty-four mature male rats weighing 150–180 grams per each. The work was carried out under veterinary supervision, adhering to the animal housing regulations of the Ain-Shams Research Institute. Additionally, all experimental procedures followed the guidelines of ethical research established by Ain Shams University Animal Research Unit assigned the approval number [RE (189)22]. All rats were maintained under standard laboratory conditions. After a seven-day acclimatization period, using a random number table, the rats were randomly assigned to one of four groups (n=6/group) in a randomized design. The groups were as follows:

Group 1 (Control group): Administered the 0.9% saline vehicle by daily oral gavage for 30 days. Group 2 (MSG group): Received MSG at a dose of 60 mg/kg daily via oral gavage for 30 days. This dose **reflects** established hepatotoxic levels from prior research without causing acute mortality (Hamza & Al-Harbi, 2014). Group 3 (Vit. C group): Given 100 mg/kg of vitamin C orally each day for 30 days. This dosage was chosen in accordance with previous research showing its efficacy in mitigating oxidative stress in rodent models (Wahdan & Shareef, 2016). Group 4 (Vit. C + MSG) was administered vitamin C (100 mg/kg) orally for 30 days, after this, MSG administration (60 mg/kg) two hours later each day for 30 days. The co-administration protocol was designed to assess the potential of vitamin C to mitigate toxicity triggered by MSG. Following euthanasia (xylazine-ketamine combination, i.p.), retro-orbital blood was taken and centrifuged to extract serum for liver function testing (storage at -20°C). Livers were removed and split, one portion was homogenized in Tris-HCl buffer (0.1 M, pH 7.4), centrifuged, and the supernatant was stored at -20°C for subsequent analysis of oxidative stress, antioxidant, and inflammatory parameters. The remaining piece was formalin-fixed for histopathology.

Biochemical Analysis

Oxidative stress markers: Hepatic GSH levels were quantified using a colorimetric assay with Ellman’s reagent, which serves as an indicator of nonenzymatic antioxidant capacity (Ellman, 1959). The activity of SOD was assayed by measuring the reduction inhibition of nitroblue tetrazolium (Sun et al., 1988). The activity of CAT was quantified based on the rate of H_2O_2 breakdown (Aebi, 1984). Nitric oxide or (NO) concentrations in liver tissue were evaluated using a commercial kit (Biodiagnostics, Cairo, Egypt). In this method, NO is converted to nitrous acid, measured by azo dye formation using the Griess reagents (sulfanilamide and N-(1-naphthyl) ethylenediamine), absorbance of the dye can be determined at 540 nm (Bryan and Grisham, 2007). The peroxidation of lipids was assessed via quantifying MDA levels (Ohkawa et al.,1979). Inflammatory markers: Employing particular commercial ELISA kits (Scientific Thermo Fisher, USA; Cat. No. BMS607-3 for TNF-α plus BMS6002 for IL-1β), serum concentrations of TNF-α plus IL-1β measured in accordance with the manufacturer's instructions. Liver function test: Using commercial colorimetric assay kits, the levels of AST plus ALT in sera were measured enzymatically (Biodiagnostic Co., Giza, Egypt) (Reitman and Frankel, 1957).

Histopathological examination

Tissue samples were fixed in formalin, cleared in xylene, and progressively dehydrated using ascending concentrations of ethanol before having paraffin wax infused in it. After cutting sections at 5 µm thickness, they were stained through hematoxylin and eosin to do microscopic evaluation (Slaoui & Fiette, 2011).

Statistical Analysis

The mean ± SD serves to express the data. GraphPad Prism (v.8.0) was adopted to examine group differences via a one-way ANOVA with Tukey's post-hoc test for multiple comparisons; $p < 0.05$ was deemed statistically significant.

RESULTS

Oxidative Stress plus Antioxidant Status

MSG exposure significantly (*$P<0.05$*) reduces intracellular glutathione (GSH), an essential nonenzymatic antioxidant that neutralizes reactive oxygen species, whereas vitamin C supplementation replenishes GSH levels (Figure 1a). Additionally, catalase (CAT) activity, crucial for breaking down hydrogen peroxide, is substantially impaired by MSG treatment; however, vitamin C markedly boosts CAT activity ($P < 0.05$) (Figure 1b). Similarly, MSG leads to a considerable decline in superoxide dismutase (SOD) activity, which is essential for converting superoxide radicals into hydrogen peroxide ($P < 0.05$). At the same time, vitamin C helps maintain or even enhance SOD function (Figure 1c). Furthermore, the MSG-treated group shows higher malondialdehyde (MDA) levels, a sign of peroxidation of lipid and membrane damage, but co-treatment with vitamin C significantly reduces these levels ($P < 0.05$) (Figure 1d). Finally, MSG increases nitric oxide (NO) production, suggesting heightened nitrosative stress, which is effectively *lowered ($P < 0.05$)* by vitamin C supplementation (Figure 1e). Overall, these findings suggest that vitamin C can counteract the oxidative stress induced by MSG by restoring the power of antioxidant enzymes in addition reducing markers of peroxidation of lipid and nitrosative stress (Table 1 and Figure 1

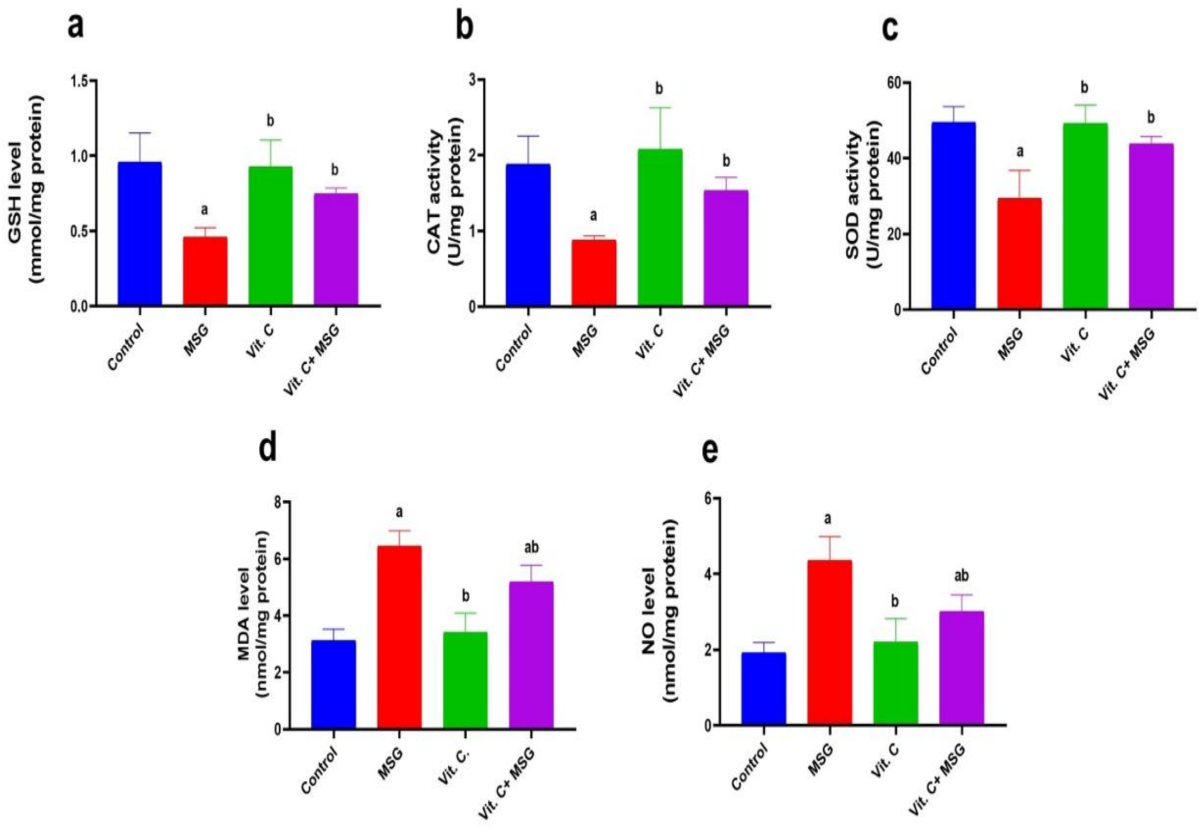

Figure 1. Vitamin C effects on hepatic oxidative stress markers in MSG-exposed rats: (a) glutathione (GSH), (b) catalase (CAT), (c) superoxide dismutase (SOD), (d) malondialdehyde (MDA), (e) nitric oxide (NO). Data shown as mean ± SD (n=6). Distinct letters (a, b) denote significant differences ($p < 0.05$) from the control and MSG groups, respectively.

Inflammatory markers:

The MSG group exhibits a substantial ($P < 0.05$) increasing in TNF-α as contrasted to the control, indicating a heightened inflammatory response. By contrast, vitamin C alone maintains TNF-α near control levels, and Supplementation with vitamin C alongside MSG substantially ($P<0.05$) lowers TNF-α compared to MSG-only treatment (Figure 2, left panel). Similar to TNF-α, MSG treatment significantly ($P < 0.05$) elevates IL-1β, indicating an intensified pro-inflammatory state. Meanwhile, vitamin C alone does not differ significantly from the control, but its combination with MSG notably ($P < 0.05$) reduces IL-1β compared to MSG alone (Figure 2, right panel). Overall, these data suggest that MSG induces an inflammatory response characterized by elevated TNF-α and IL-1β, whereas vitamin C supplementation mitigates this effect, highlighting its potential anti-inflammatory role. Different letters (a, b, ab) above the bars denote statistical significance at $P<0.05$ (Table 1, Figure 2).

Table (1): Statistical comparison of biochemical parameters across groups.

Groups / Parameters	Control	MSG	Vit. C	Vit. C + MSG
GSH: mmol/mg prot.	0.96 ± 0.19	0.46 ± 0.06 [a]	0.93 ± 0.18 [b]	0.75 ± 0.04 [b]
CAT: U/mg prot.	1.9 ± 0.37	0.88 ± 0.06 [a]	2.1 ± 0.55 [b]	1.5 ± 0.18 [b]
SOD: U/mg prot.	50 ± 4.1	29 ± 7.4 [a]	49 ± 4.9 [b]	44 ± 2.0 [b]
MDA: nmol/mg prot.	3.1 ± 0.41	6.4 ± 0.55 [a]	3.4 ± 0.69 [b]	5.2 ± 0.6 [ab]
NO: nmol/mg prot.	1.9 ± 0.28	4.4 ± 0.63 [a]	2.2 ± 0.62 [b]	3.0 ± 0.44 [ab]
TNF: Pg/mg prot.	41 ± 5.4	98 ± 9.6 [a]	42 ± 7.2 [b]	73 ± 11 [ab]
IL1B: Pg/mg prot.	38 ± 5.6	89 ± 5.9 [a]	39 ± 6.3 [b]	60 ± 5.5 [ab]
ALT: U/L	42 ± 6.4	80 ± 7.2 [a]	42 ± 6.7 [b]	65 ± 8.6 [ab]
AST: U/L	42 ± 4.9	89 ± 7.0 [a]	43 ± 5.9 [b]	66 ± 9.1 [ab]

Data shown as mean ± SD (n=6). Distinct letters (a, b) denote significant differences (*p < 0.05*) from the control and MSG groups, respectively.

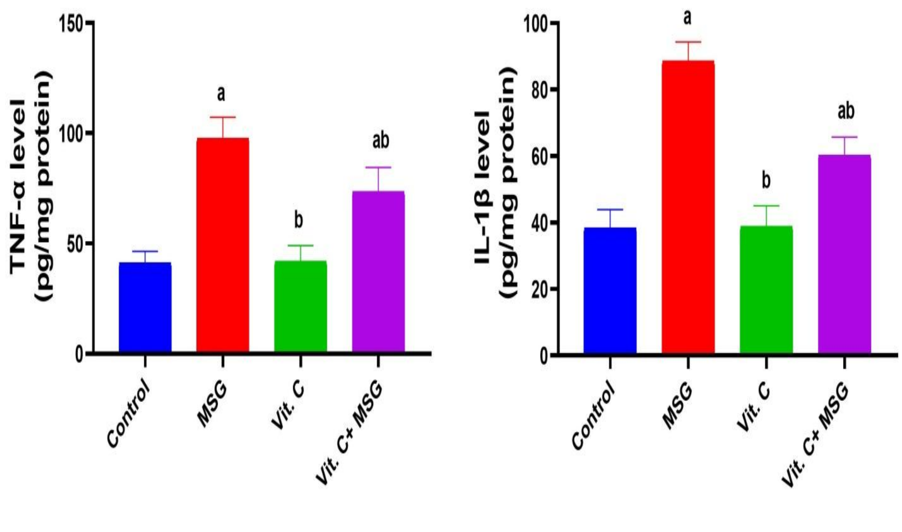

Figure 2. The effect of vitamin C on inflammatory indicators, such as TNF-*α* plus IL-1β levels, in the liver tissues of male rats received MSG. Distinct letters (a, b) denote significant differences (*p < 0.05*) from the control and MSG groups, respectively.

Liver function markers:

Figure 3 shows serum ALT and AST levels across treatment groups. The MSG-only group exhibits a pronounced (*P < 0.05*) increasing in both ALT plus AST as contrasted to the control, suggesting potential liver injury. By contrast, vitamin C alone keeps these enzymes at levels similar to those of the control, reflecting minimal hepatic stress. When vitamin C is administered alongside MSG, there is a notable ($P < 0.05$) reduction in ALT in addition to AST activities as contrasted to the MSG group, indicating that vitamin C exerts a preventive effect against MSG-triggered hepatocellular damage. Bars marked with different letters (a, b, ab) are significantly different ($p < 0.05$) (Table 1, Figure 3).

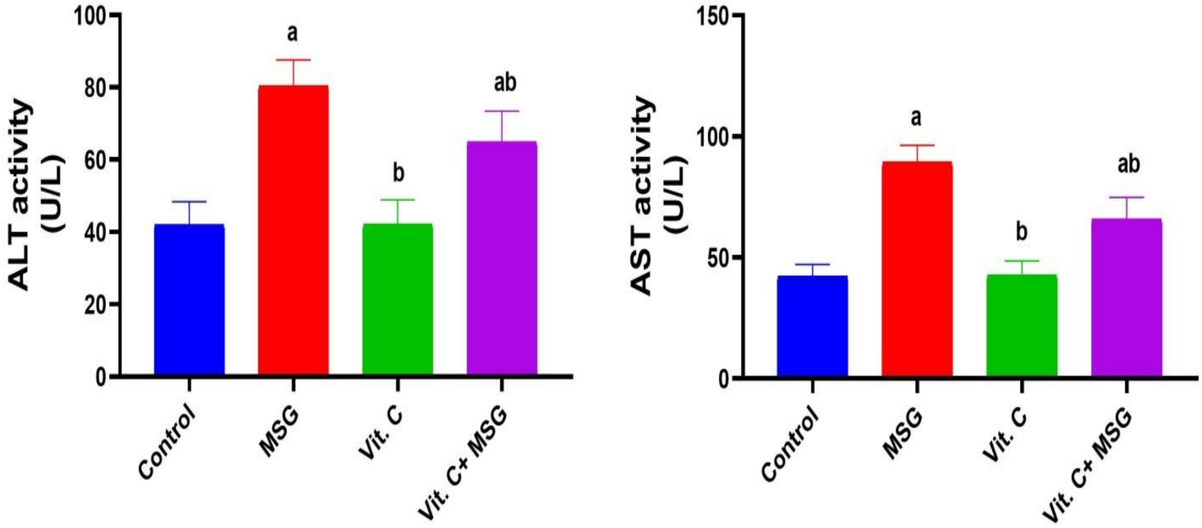

Figure 3. The effect of Vit. C on MSG-triggered abnormalities in ALT activity & AST activity in rat serum. Data shown as mean ± SD (n=6). Distinct letters (a, b) denote significant differences ($p < 0.05$) from the control and MSG groups, respectively.

Liver histopathology:

Hematoxylin and eosin (H&E) stained liver tissue photomicrographs are presented in Figure 4 at a 400× magnification (scale bar of 100 μm). The control group exhibits a normal hepatic architecture, characterized by orderly hepatocyte plates radiating from the central vein and distinct sinusoidal spaces (Figure 4a). In contrast, the MSG-treated group exhibits a marked disruption of the typical liver structure, including focal inflammatory infiltrates and numerous degenerated hepatocytes concentrated in the central region (Figure 4b). The vitamin C group maintains a histological appearance similar to that of the control, with no apparent abnormalities (Figure 4c). Finally, the co-treated group receiving vitamin C and MSG demonstrates minimal or no visible lesions, suggesting a preventive role for vitamin C toward MSG-triggered hepatic damage (Figure 4d).

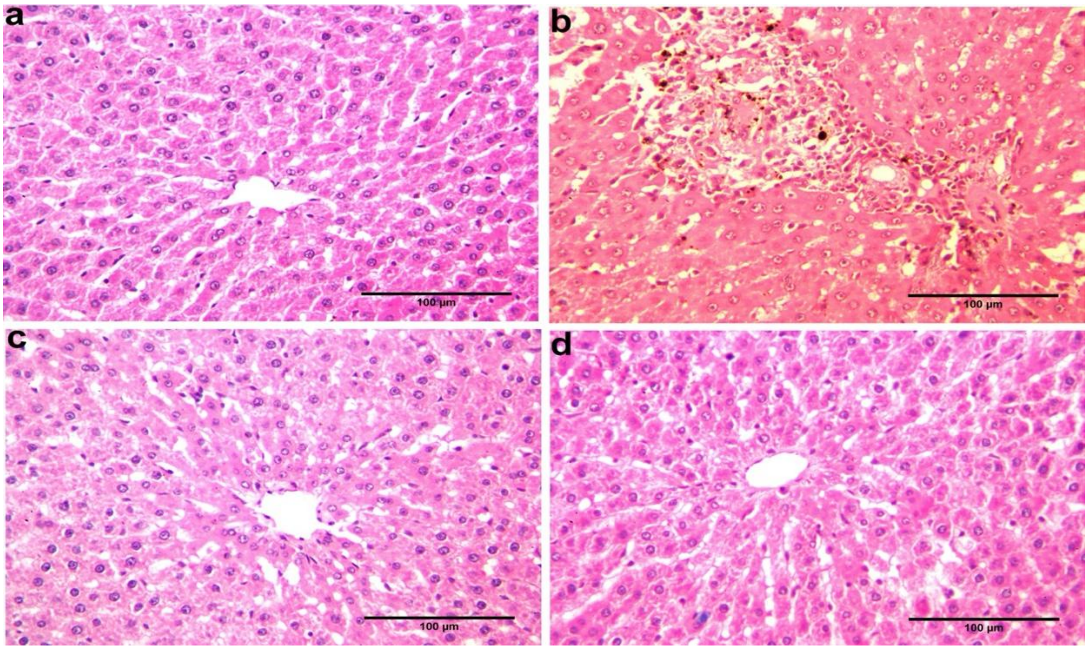

Figure 4. Representative liver histology (H&E staining). (a) Control group: typical hepatic architecture with intact hepatocyte plates emanating from the central vein and well-defined sinusoids. (b) The MSG group exhibited loss of normal histoarchitecture, characterized by marked focal inflammation and numerous degenerated hepatocytes in the center. (c) Vit. The C group showed normal liver histoarchitecture (d) Vit. C +MSG group showed no visible lesions. (Scale bar = 100µm)

DISCUSSION

The current study reveals that contact with monosodium glutamate (MSG) generates considerable oxidative stress plus inflammation in the liver, resulted in hepatocellular damage. Specifically, MSG administration triggered a substantial reduction in intracellular GSH concentration levels and impaired the potential of key antioxidant enzymes, including CAT plus SOD. This decline in antioxidant defences correlated with elevated levels of MDA, a marker of fats peroxidation, and elevated nitric oxide (NO) production, indicating heightened oxidative and nitrosative stress. These biochemical disturbances align with previous reports suggesting that MSG can promote the genesis of reactive radical species and trigger oxidative damage in liver tissues (Henry-Unaeze, 2017).

Farombi and Onyema (2006) demonstrated that vitamin C reduces oxidative damage triggered by monosodium glutamate. The antioxidant action of vitamin C in our study is indeed line with Mohammed et al. (2024) findings, vitamin C significantly reduced amoxicillin/clavulanic acid (AC)-induced hepatotoxicity. The considerable reduction of blood liver enzymes, MDA reduction, and GSH elevation in the vitamin C-treated group demonstrated that vitamin C protects against AC-induced hepatotoxicity (Mohammed et al., 2024). Our results corroborate existing evidence that vitamin C acts as a potent inhibitor of lipid peroxidation (Soylu et al., 2006). Vitamin C acts as an indirect antioxidant by inhibiting NF-κB activation, which participates in the production of ROS (Moore & Khanna, 2023). Vitamin C, as an electron donor, may diminish ROS directly, including metabolic byproducts such as superoxide plus hydroxyl radicals (Cimmino et al., 2018). Furthermore, vitamin C promotes redox balance by decreasing oxidized forms of vitamin E plus glutathione (Parker et al., 2012). Vitamin C serves as an indirect antioxidant by blocking NF-κB, which triggered the genesis of ROS (Carr & Maggini, 2017). Tan et al. (2005) mentioned that vitamin C reduces oxidative stress in human dendritic cells via decreasing NF-κB activation. Beyond its antioxidant actions, vitamin C may neutralize hazardous nitrogen-based molecules as carcinogenic N-nitrosamines and also nitrosamides (Sauberlich, 1994).

In parallel, the study found that MSG administration substantially raised TNF-α plus IL-1β levels, indicating an intensified inflammatory response. This finding supports the notion that oxidative stress and inflammation are closely interrelated processes, where increased free radical formation can activate inflammatory pathways, further exacerbating tissue injury. The reduction in these cytokines observed with vitamin C supplementation underscores the potential anti-inflammatory properties of vitamin C, which may be attributed to its capacity to stop pro-inflammatory signaling cascades and scavenge ROS (Choudhary & Tran, 2012). The impact of MSG on liver function was further evidenced by marked raising in serum ALT plus AST levels. These enzymes are well-known indicators of hepatocellular damage, and their increased levels reflect the compromised integrity of liver cells following exposure to MSG. Conversely, vitamin C alone maintained ALT and AST activities close to control levels, and its co-administration with MSG substantially reduced these enzyme levels, suggesting that vitamin C exerts a hepatoprotective effect.

Histopathological evaluation provided additional confirmation of MSG-induced liver damage. The control group exhibited normal hepatic architecture, characterized by well-organized hepatocyte plates and distinct sinusoidal spaces. In contrast, liver sections from the MSG-treated group showed pronounced disruptions in tissue organization, including focal inflammatory infiltrates and degenerative changes in hepatocytes. Notably, the liver tissues from the vitamin C-treated group were comparable to those of the controls. In contrast, co-treatment with vitamin C and MSG resulted in markedly fewer lesions, demonstrating the preventive role of vitamin C toward MSG-elicited histological damage. This finding is consistent with previous studies that have documented the deleterious actions of MSG on liver function through oxidative and inflammatory mechanisms (Henry-Unaeze, 2017; Choudhary & Tran, 2012). Furthermore, the preventive effects of vitamin C observed in the present study align with earlier research highlighting its capacity to restore antioxidant enzyme activities and reduce lipid peroxidation and inflammatory cytokine production (Carr & Maggini, 2017; Al-Ghafari, 2021). However, some discrepancies in the literature regarding the magnitude of MSG-induced damage may arise from variations in experimental protocols, including differences in MSG dosage, exposure duration, and the specific animal models used.

Limitations and Future Directions

Despite the robust findings, this study has several limitations. The short duration of MSG exposure and the exclusive utilize of rats with male gender may restrict the broader applicability of these findings to other populations, including female subjects and humans. Future studies should consider more extended treatment periods and a more diverse range of animal models or clinical populations to validate these findings. Additionally, a better comprehension of the molecular processes that underlie vitamin C's protective effects could provide valuable insights into its therapeutic potential in preventing MSG-induced hepatic injury.

CONCLUSION

In summary, the results indicate that MSG triggered significant inflammation, oxidative stress, and liver damage, as evidenced by altered biochemical and histopathological markers. Vitamin C supplementation effectively counteracts these effects by restoring antioxidant defences, reducing pro-inflammatory cytokine levels, and preserving liver tissue architecture. These results imply that vitamin C might present a viable tactic for mitigating MSG-induced hepatic toxicity; Future research is required to assess the clinical relevance of these results.

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Threats and Traditions: Citizen Science Insights into Striped hyena Conservation in Saudi Arabia

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Keywords

Human-wildlife conflict; cultural beliefs; community conservation; Aseer Region; *Hyaena hyaena*

ABSTRACT

The striped hyena (*Hyaena hyaena*) in Saudi Arabia faces mounting threats from habitat degradation, wildlife-vehicle collisions, retaliatory killings, and entrenched cultural stigmas. This study examined public perceptions, traditional beliefs, and conservation attitudes toward the species in Al Qawba, Bisha Province, a region of ecological significance in the Aseer Region. Using a cross-sectional mixed-methods approach, data were gathered through qualitative interviews and a structured questionnaire completed by 151 participants from diverse social backgrounds, including Bedouins, farmers, and urban residents. Fieldwork also documented a recent hyena road fatality. Results revealed widespread awareness of hyenas, yet dominant perceptions were largely negative, often associating the animals with personal danger or livestock loss. Despite this, most acknowledged the species' ecological role as scavengers and recyclers of nutrients. Cultural narratives were universally cited, portraying hyenas as supernatural beings or sources of traditional medicine, contributing to poaching and retaliatory killings. Major threats identified included habitat destruction, overhunting, and road infrastructure, reflecting global patterns of carnivore-human conflict amid habitat fragmentation. Encouragingly, most respondents were aware of conservation programs and supported community-led efforts. Preferred interventions included public education, local engagement, and the establishment of protected areas. Regression analysis indicated that higher education and professional background significantly predicted conservation support. This study underscores the urgency of a culturally informed conservation strategy for striped hyenas in Saudi Arabia. Key recommendations include countering harmful myths through targeted outreach, reinforcing anti-poaching laws, restoring degraded habitats, and introducing road mitigation infrastructure to reduce mortality.

INTRODUCTION

The striped hyena (*Hyaena hyaena*) is a medium-sized carnivore with a broad but fragmented distribution across parts of North Africa, the Middle East, and South Asia (Abi-Said & Dloniak, 2015). Extant populations occur in Egypt, Iraq, Palestine, Jordan, Lebanon, Oman, the Syrian Arab Republic, and Yemen (Abi-Said et al., 2007; Albaba, 2016; Al Sheikhly, 2015; Aidek et al., 2025; Eid et al., 2020). Its status remains uncertain in Kuwait and Qatar (Abi-Said & Dloniak, 2015), while in the United Arab Emirates, the species is assessed as Regionally Extinct (RE) on the UAE National Red List (assessment year 2018; published 2019). In neighboring Jordan, research using camera traps in the Dana Biosphere Reserve has estimated small population sizes, with nine individuals identified in 2010 and ten in 2012, which indicates higher visitation to waterholes during the hottest summer months (Attum et al., 2017). Other studies in eastern Jordan have documented den sites containing thousands of bone fragments, revealing scavenging behavior targeting livestock such as camels (*Camelus dromedarius*), sheep/goats (*Ovis/Capra*), and donkeys (*Equus asinus*) as well as wild fauna including gazelles (*Gazella* spp.) (Kuhn, 2005). Across the Arabian Peninsula, *H. hyaena* is largely absent from the Rub' al Khali and Nafud deserts, with most contemporary records occurring in valleys, open landscapes, and volcanic fields near agricultural areas (Khayat et al., 2024).

Within Saudi Arabia, historical and contemporary records confirm its occurrence from the northern Harrat al-Harrah Protected Area, where tracks and hairs have been found in mountainous areas (Seddon et al., 1997), to the central Najd, the western mountains, and the southwestern highlands of Asir. The species has been recorded in Madinah Province and identified as the second most persecuted carnivore in the country, with persecution including the widespread practice of hanging hyenas on trees or road signs (Aloufi & Amr, 2018; Aloufi & Amr, 2024). Such pressures reflect a broader regional pattern of human–predator conflict that has dramatically reduced populations of larger carnivores, including the striped hyena. Saudi Arabia's rich biodiversity and deep-rooted cultural traditions create a complex backdrop for its conservation (Alam & Khan, 2015; Al Ahmari et al., 2024). As natural sanitizers, striped hyenas contribute to environmental health by consuming carrion, accelerating decomposition, and reducing the spread of pathogens (Jaffa, 2020). Despite this ecological significance, the species faces mounting threats from habitat loss, retaliatory killings, and cultural stigmas (Attum et al., 2017; Bhandari et al., 2022), exacerbated by rapid urbanization, agricultural expansion, and infrastructure development that fragment habitats and intensify human–wildlife conflict (Eid & Handal, 2018; Alatawi, 2022; Al Ahmari et al., 2024; Eid & Azam, 2025). These threats, combined with traditional uses of hyena body parts in medicine and ritual practices (Aloufi & Eid, 2016; Alqahtani, 2022; Khayat et al., 2024), underscore the urgent need for culturally informed, regionally coordinated conservation strategies across the Arabian Peninsula.

Complementing these national records, beyond our focal region, the striped hyena is documented widely across Saudi Arabia, including Tabuk, the Najd/central plateau, and the southwestern highlands of the Asir–Sarawat range (Aloufi & Amr, 2018; Cunningham et al., 2009; Al Ahmari et al., 2024). Camera-trap syntheses indicate it is among the most frequently recorded carnivores, while direct killing and trade remain leading threats (Al Ahmari et al., 2024; Aloufi & Eid, 2014). In Al-Jouf, a record from the early 1980s likely represents a dispersing individual from northern populations (Mallon & Budd, 2011). Regionally, the species occurs across Jordan, with persecution and habitat loss highlighted as key pressures, and is also confirmed from Oman and Yemen, where threats broadly mirror those elsewhere in Arabia (Eid et al., 2020; Mallon & Budd, 2011).

Culturally, hyenas are frequently demonized and perceived as pests or threats to livestock, with longstanding beliefs in their supernatural powers driving their persecution and use in traditional practices (Jaffa, 2020; Osborne, 2013). A study from the Tabuk region in northwestern Saudi Arabia documented that hyena flesh, often cooked with its fat, was traditionally used to treat various ailments, including rickets in children, joint and muscle pain, physical exhaustion, and muscle fatigue, and was also consumed as a sexual tonic (Aloufi & Eid, 2016). Similar uses have been reported globally, where various hyena body parts, such as the liver and penis, are incorporated into folk medicine or employed as protective talismans (Jaffa, 2020). These culturally embedded practices highlight the multifaceted role of traditional beliefs, both as a threat to wildlife and as a potential entry point for fostering conservation awareness.

Conservation-focused surveys are practical tools for gauging public attitudes toward conservation programs and policies (Kleiven et al., 2004; McFarlane et al., 2006; Pratt et al., 2004). Examples include studies on landowners' perceptions of conservation initiatives (Winter et al., 2005), individual attitudes toward biodiversity conservation (Karanth et al., 2008), and community views on particular species or management strategies (Bandara & Tisdell, 2003; Caro et al., 2003; McFarlane et al., 2006; Soto et al., 2001). Addressing a knowledge gap, this study explores individual perceptions of the striped hyena in Al Qawba, Bisha Province, an ecologically significant region of Saudi Arabia that is affected by human activities.

MATERIALS and METHODS

Study Area

Fieldwork was conducted in Al Qawba, within Bisha Province of the Aseer Region in southwestern Saudi Arabia, focusing on localities such as Badia Al-Jamaʿa and a road junction between Al Qawba and Halba, approximately 100 km from Bisha city (Figure 1). The area comprises a heterogeneous mosaic of valleys, hills, and agricultural fields, providing diverse wildlife habitats. Bisha is notably productive agriculturally, especially for date palms, and is served by a well-developed road network that enhances connectivity with nearby urban centers. The study sites are located within the Imam Faisal bin Turki Royal Reserve and encompass several wadis and physiographic provinces, the largest being Wadi Bisha. Geologically, the region forms part of the Arabian Shield, where predominantly exposed Precambrian crystalline rocks are partly overlain by Tertiary volcanic units, themselves constituting a segment of the Afro-Arabian Shield. Climatically, Bisha is arid; recent observations (2000–2023) indicate mean monthly air temperatures from 3 °C in January to 41 °C in August (annual mean ≈ 24 °C), mean relative yearly humidity of ~30%, and annual precipitation of 105–125 mm, consistent with long-term aridity in the region (Saudi National Center for Meteorology, 2024).

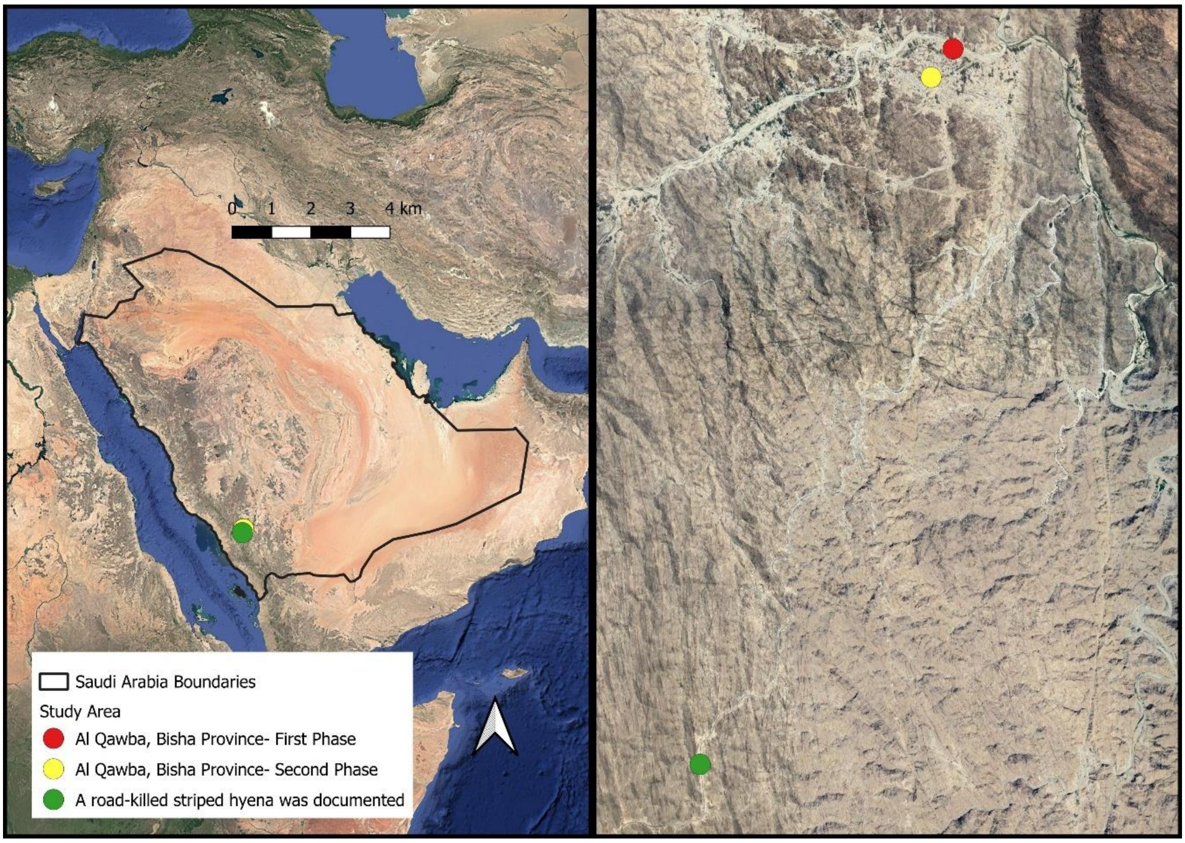

Figure 1. Study area indicating the location of the documented striped hyena road fatality

Study Design and Data Collection

Study Design

This study employed a descriptive cross-sectional mixed-methods design to investigate public perceptions, cultural beliefs, and conservation attitudes related to the striped hyena (*Hyaena hyaena*) in Al Qawba, Bisha Province. The research was implemented in two sequential phases: a qualitative exploratory phase followed by a quantitative survey phase.

Qualitative Phase

The first phase involved qualitative, exploratory interviews with local residents to document myths, traditional knowledge, and cultural narratives surrounding the species. Field observations were also conducted on two occasions between January and March 2024. These observations served as illustrative evidence complementing community-reported threats. One documented wildlife–vehicle collision (WVC) involving a striped hyena was recorded on February 21, 2024. This single event does not quantify the extent of the threat but provides visual and situational context. Fieldwork employed nonintrusive methods, such as remote photography, to minimize disturbance to wildlife.

Quantitative Phase

Insights from the qualitative phase informed the development of a structured questionnaire. The survey targeted 151 participants between January and March 2024, selected using random sampling to ensure balanced representation across farmers, researchers, Bedouins, employees, and employers from urban, rural, and nomadic settings. Data were gathered through face-to-face interviews and, where applicable, paper-based questionnaires distributed in local markets. The questionnaire comprised six sections: (1) demographic characteristics (age, gender, education, occupation, residence); (2) awareness and perceptions of predatory wildlife, particularly the striped hyena; (3) environmental threats (e.g., habitat loss, overhunting, climate change); (4) cultural and social beliefs (e.g., myths, traditional practices); (5) perceptions of conservation efforts and policy; and (6) open-ended recommendations. Question types included multiple-choice, multiple-answer, and open-ended formats to elicit structured data and nuanced personal views. The complete questionnaire is provided as Supplementary Material (Appendix S1).

Data Analysis

Quantitative data were processed using Microsoft Excel to generate descriptive statistics. SPSS Version 27 was used to analyze quantitative data (percentages, means). A binary logistic regression analysis was conducted to identify predictors of conservation support (coded as 1 for support and 0 for no support), with education level, occupation, age, and gender as independent variables. Qualitative responses underwent thematic analysis, with themes such as human–wildlife conflict and cultural myths identified through manual coding by two independent researchers to ensure inter-coder reliability.

Ethical Considerations

No formal ethics committee approval number was issued, as such approval was not required under institutional regulations for non-invasive, interview-based research. All participants provided informed consent before participation, and no personal or identifiable data were collected. The relevant Saudi authorities secured all necessary permits and approvals for field activities.

RESULTS

Demographic Profile of Respondents

The study included 151 participants: 95 males (62.91%) and 56 females (37.09%), aged 20–50 years. The largest age group was 31–40 years (82 participants; 54.30%), followed by 20–30 years (38; 25.17%) and 41–50 years (31; 20.53%). Educational attainment varied, with 67 participants (44.37%) having no formal education, 29 (19.21%) completing primary education, 13 (8.61%) completing secondary education, and 42 (27.81%) holding higher education qualifications. Occupational backgrounds included 23 researchers (15.23%), 16 farmers (10.60%), 46 employees (30.46%), five business owners (3.31%), and 61 Bedouins (40.40%). Urban residents comprised 74 participants (49.00%), rural residents 16 (10.60%), and nomadic Bedouins 61 (40.40%).

Awareness and Perceptions of Striped Hyenas

Most respondents (143; 94.70%) had seen a striped hyena in the wild, often during nocturnal encounters near water sources or agricultural fields, and typically in connection with concerns about livestock predation. Perceptions were dominated by risk framing: 94/151 (62.3%) viewed hyenas as threats to livestock or humans, 43/151 (28.5%) described them as ecologically valuable, and 14/151 (9.3%) held neutral views. Recognition depended on item format: in response to a closed-ended statement describing scavenging and nutrient-recycling services, 98/151 (64.9%) agreed that hyenas play an ecological role, whereas 53/151 (35.1%) disagreed. In open-ended responses, participants most often mentioned carcass removal and bone-breaking as benefits.

Perceived Threats to Striped Hyenas

Participants identified the main threats as habitat destruction (146; 96.70%), overhunting (144; 95.36%), road collisions (142; 94.04%), food shortages (94; 62.25%), climate change (21; 13.91%), and water scarcity (2; 1.32%). Almost all respondents (143; 94.70%) reported a decline in wildlife populations, attributing it to urbanization, desertification, and overhunting, with declines noted in hyenas, Arabian wolves, and gazelles (Figure 2).

Cultural Beliefs and Practices

Hyenas were overwhelmingly perceived as dangerous pests (143; 94.70%), with only 3 (1.99%) considering them sacred/respected and 5 (3.31%) remaining neutral. All participants (100%) acknowledged cultural myths, including beliefs in magical powers (Hyenas are connected to witchcraft), medicinal uses (Hyena eyes for improved vision), and supernatural omens (Their howl is an omen of death). Hunting for body parts (A trade exists for hyena body parts used in traditional remedies) and retaliatory killings (Farmers poison hyenas) were widely reported. A summary of qualitative themes, the proportion of participants mentioning each, and example quotes is provided in Table 1.

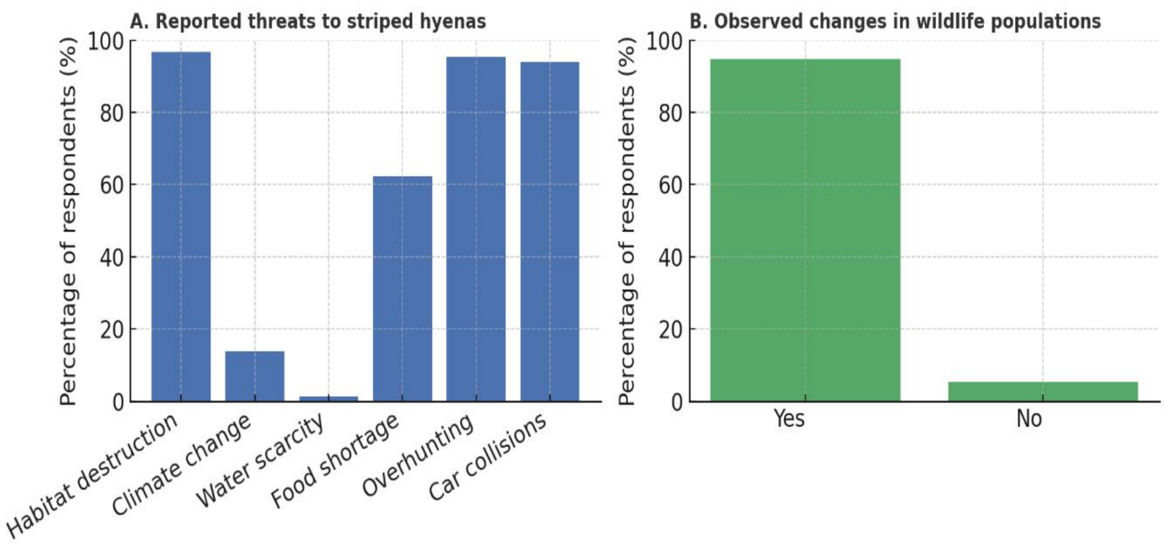

Figure 2. Reported threats to striped hyenas in Al Qawba, Saudi Arabia. (A) Most respondents cited habitat destruction (96.7%), overhunting (95.4%), and road mortality (94.0%) as major threats. (B) Nearly all (94.7%) reported declines in wildlife, primarily attributed to urbanization, desertification, and overhunting.

Table 1. Qualitative themes on striped hyena perceptions and threats in Al Qawba, Saudi Arabia, with corresponding participant frequencies and illustrative quotes

Theme	% of Participants Mentioning	Example Quote
Hyenas as livestock threats	62.25%	They kill our goats and sometimes even calves at night.
Hyenas are ecologically important.	64.90%	They clean the environment by eating dead animals.
Magical/supernatural beliefs	100%	Their howl is an omen of death.
Traditional medicine use	48.34%	The fat can be used for joint pain relief.
Road mortality concern	94.04%	I saw one dead on the road near Halba last winter.

Awareness of Conservation Programs and Preferred Interventions

Awareness of conservation programs was high (144; 95.36%), with 7 participants (4.64%) unaware. Opinions on government conservation efforts were mixed: 103 (68.21%) supported them, 39 (25.83%) did not, and 9 (5.96%) were unsure. Preferred strategies included public awareness campaigns (125; 82.78%), community involvement (106; 70.20%), establishing reserves (84; 55.63%), and stricter hunting regulations (25; 16.56%). All participants (100%) supported community-led initiatives. Open-ended suggestions included educational campaigns, stronger anti-poaching laws, increased reserves, and habitat restoration (Figure 3).

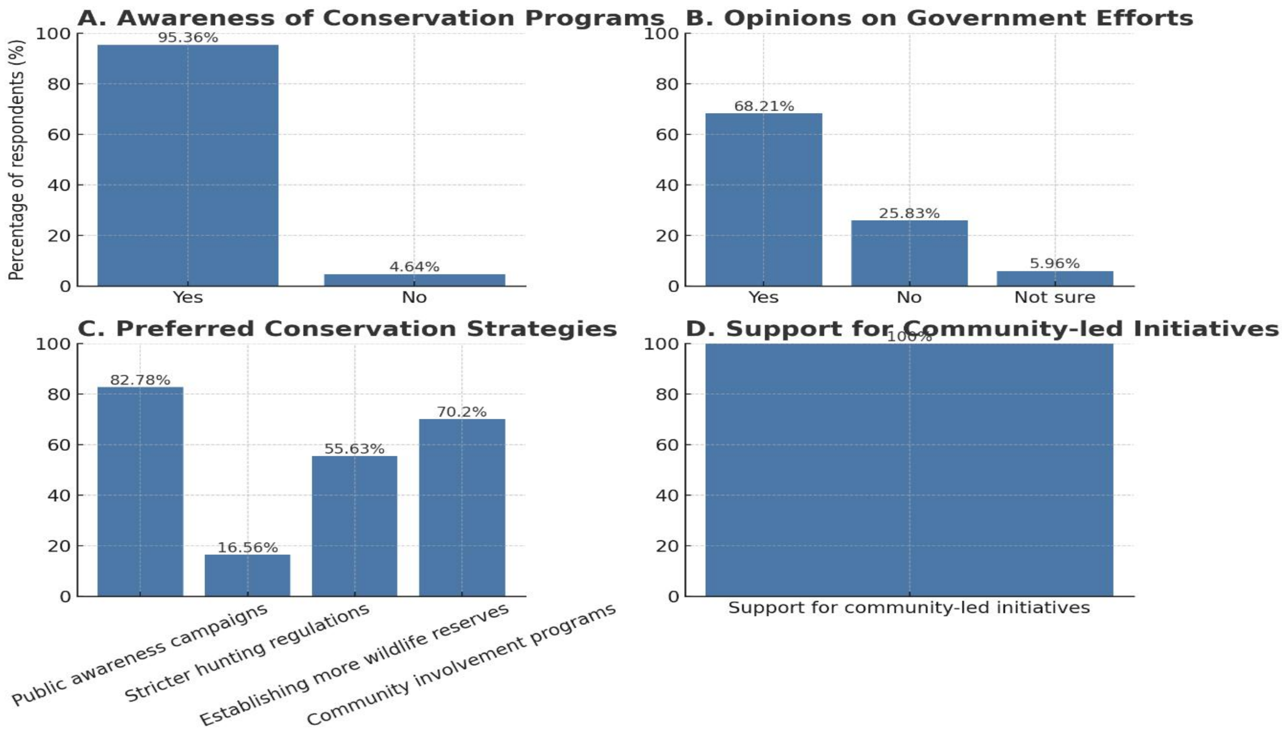

Figure 3. Community responses on striped hyena conservation in Al Qawba, Saudi Arabia. (A) Awareness of conservation programs. (B) Opinions on government efforts. (C) Preferred conservation strategies. (D) Support for community-led initiatives is presented in a single-column bar chart for improved clarity and readability.

Predictors of Conservation Support

Binary logistic regression analysis revealed that education level ($\beta = 0.41$, $p = 0.003$) and occupation ($\beta = 0.36$, $p = 0.012$) significantly predicted conservation support, while age and gender were not significant ($p > 0.05$). The model explained 38% of the variance in conservation support ($R^2 = 0.38$, $p < 0.01$). Participants with higher education and those employed in research or education were more likely to support community-led conservation initiatives compared to farmers and nomadic groups.

Field Observation

Fieldwork corroborated community-reported threats through illustrative evidence, including a single documented wildlife–vehicle collision (WVC) involving a striped hyena on February 21, 2024, at 6 a.m. in Badia Al-Jama'a. The carcass was found intact in the center of the road, suggesting a nocturnal collision (Figure 4). While this single record does not quantify the extent of road mortality, it provides visual and situational context for the threat posed by the expansion of road infrastructure.

Figure 4. Photo of a striped hyena killed by a vehicle

Climate Change and Regional Comparisons

Only 21 respondents (13.91%) cited climate change as a threat, yet its potential impacts in arid regions are significant. Projected changes in precipitation seasonality and temperature extremes could exacerbate habitat fragmentation, prey scarcity, and water shortages, leading to range contractions or shifts.

DISCUSSION

Our findings confirm the continued presence of the striped hyena (*Hyaena hyaena*) in the Bisha district (Alqahtani, 2022) and situate that persistence within a landscape of persistent human–carnivore conflict. Nearly all respondents (94.7%) reported direct encounters with hyenas. Nevertheless, perceptions were predominantly risk-oriented: most participants viewed the species as a threat to livestock or personal safety, and qualitative narratives emphasized predation incidents and nocturnal proximity to farms. These perceptions translate into concrete conflict behaviors, most notably carcass poisoning and retaliatory killing, which have already been documented elsewhere on the Arabian Peninsula and are reflected in our interview material from Al Qawba (Cunningham et al., 2009; Eid et al., 2020; Eid & Handal, 2018). As natural scavengers and nutrient recyclers, hyenas provide sanitary ecosystem services; however, that role is often discounted where immediate costs to pastoral livelihoods are salient. Consequently, the conservation challenge in Bisha is not simply biological but social, requiring attention to attitudes, incentives, and the cultural frames within which hyenas are interpreted (Treves & Karanth, 2003; Dickman, 2010).

Beyond attitudes, respondents consistently identified proximate stressors that are well known to erode carnivore viability: habitat destruction (96.7%), overhunting (95.4%), and road mortality (94.0%). These align with global evidence that land conversion and linear infrastructure fragment habitats, isolate populations, and increase mortality risk (Foley et al., 2005; Laurance et al., 2014). The hyena-wildlife vehicle collision we documented along the Al Qawba–Halba road in February 2024 provides a concrete, local illustration of these risks. In fragmented drylands, roads, farms, and water points become focal features that concentrate animal movement at predictable sites, increasing encounter probabilities with people and vehicles.

Cultural beliefs amplify these dynamics. Every participant (100%) acknowledged myths or traditional uses, ranging from witchcraft associations to medicinal applications of body parts, mirroring patterns reported from other parts of Arabia and the broader region (Aloufi & Eid, 2016). Such beliefs motivate persecution and trade, but they also offer entry points for dialogue when addressed respectfully in locally grounded outreach (Dickman, 2010). Notably, despite negative narratives, support for conservation was unexpectedly strong: 100% favored community-led initiatives, and 95.4% were aware of existing programs. Our regression results indicate that education and occupation significantly predict support for conservation, suggesting that tailored messaging toward less-engaged groups (e.g., some farmers and nomadic communities) could yield measurable gains. The unanimity of "support" should be interpreted cautiously; some fraction may reflect social desirability in interviewer-administered surveys (Krumpal, 2013). However, when combined with the qualitative data, it nonetheless represents a real foundation for co-designed action.

Climate change is likely to intensify these existing pressures. Regionally downscaled projections for Saudi Arabia indicate robust warming through mid- to late-century under high-emission pathways, with variable precipitation responses, including increases in parts of the southwest, against a background of chronically high evaporative demand (Tarawneh & Chowdhury, 2018; Saudi Arabia NC4, 2022). In arid systems, higher temperatures and elevated evapotranspiration shorten the persistence of surface water, reduce primary productivity and prey availability, and drive carnivores toward perennial or human-managed water sources, farms, and road corridors. These mechanisms are consistent with field evidence from Jordan, where hyena visits to artificial waterholes increased during the hottest months and after rainfall events (Attum et al., 2017). For Saudi Arabia specifically, species-distribution models suggest that more seasonal precipitation regimes and temperature extremes will reduce habitat suitability in the western mountains and arid steppe, prompting range shifts or contractions and further concentrating movements in human-dominated valleys (Khayat et al., 2024). In practical terms, even modest climatic shifts will likely magnify the very threats respondents already identified, poisoning, overhunting, and road kills, by increasing encounter frequency at a small number of predictable locations (roads, farms, and water points).

Taken together, these results suggest the need for an integrated, culturally sensitive program that combines conflict mitigation with targeted infrastructure and habitat measures. Priority actions emerging from our data and the broader literature include: (i) co-developed outreach that directly addresses witchcraft- and medicine-related beliefs while highlighting sanitary ecosystem services; (ii) rapid anti-poisoning response protocols and safe carcass-disposal guidance; (iii) livestock-protection support (e.g., nocturnal corralling, improved enclosures, guardian animals) co-delivered with local leaders; (iv) site-specific road-kill mitigation on the Al Qawba–Halba corridor (hotspot speed management, signage, fencing-plus-crossings at wadis and farm approaches); and (v) water-point management that minimizes risky congregation (temporal access rules, placement away from high-speed roads). Because the study area lies within the Imam Faisal bin Turki Royal Reserve (IFBTRR), these actions can be anchored institutionally: the Reserve can convene community committees, coordinate monitoring (including telemetry and camera traps at water points and road segments), and pilot conflict-mitigation micro-grants in partnership with local municipalities.

Finally, the study highlights tractable research priorities that would sharpen management. Longitudinal monitoring is necessary to track hyena abundance and the incidence of conflict. Telemetry and camera-trap arrays can quantify movement patterns around roads and water points. Before–and–after evaluations should assess the efficacy of outreach and road-mitigation pilots. Expanding geographic comparisons within Saudi Arabia and across neighboring countries will also help identify context-specific versus general solutions. Anchored in IFBTRR and aligned with national biodiversity objectives, such a program would convert strong local willingness into durable coexistence while preserving the ecological function of an often-misunderstood carnivore.

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Eco-Friendly Approach to Liver Protection: Avocado Seed Extract Against CCl_4 Toxicity

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Avocado seed, CCl4, Hepatoprotective, enzymes, antioxidants

ABSTRACT

The current study examined the potentially protective effects of aqueous avocado seed extract (ASE) against mice's liver damage brought on by carbon tetrachloride (CCl_4). Four groups of twenty male mice were created: ASE + CCl_4, ASE, CCl_4, and control. Serum levels of alanine aminotransferase (ALT) with aspartate aminotransferase (AST) were measured, and histological, Mallory's trichrome, and caspase-3 immunohistochemical analyses were performed to evaluate hepatic function. The treatment of CCl_4 resulted in collagen buildup, significant histological changes, enhanced caspase-3 expression, and noticeably elevated ALT in addition to AST values. By considerably reducing these biochemical and structural alterations, ASE treatment helped to return the liver architecture to normal. ASE by itself had no negative impacts. These results imply that ASE has hepatoprotective properties, most likely via anti-apoptotic and antioxidant pathways, and could be utilized as a natural remedy to prevent liver damage brought on by chemicals.

INTRODUCTION

The liver, a multifunctional organ, maintains physiological balance through roles in metabolism, synthesis, immunity, plus hematopoiesis. Constituting roughly 2% of adult body mass, this organ demonstrates remarkable versatility plus indispensability (Mahadevan, 2020). A notable regenerative capacity enables recovery from moderate injury; however, excessive damage often precipitates serious dysfunction. Drug-induced liver injury (DILI) presents a major global health issue, manifesting a wide clinical spectrum.

These manifestations range from mild, asymptomatic increases in liver enzymes to severe acute plus chronic hepatitis, cholestasis, or fulminant liver failure. Many pharmaceuticals, herbal remedies, plus dietary supplements demonstrate associations with DILI, with severe cases sometimes necessitating market withdrawal of the offending product (Bashir et al., 2023).

Drug-induced liver injury (DILI) presents a major global health issue, manifesting a wide clinical spectrum. These manifestations range from mild, asymptomatic increases in liver enzymes to severe acute plus chronic hepatitis, cholestasis, or fulminant liver failure. Many pharmaceuticals, herbal remedies, plus dietary supplements demonstrate associations with DILI, with severe cases sometimes necessitating market withdrawal of the offending product (Bashir et al., 2023). The worldwide burden of liver disease remains considerable, causing approximately 1.16 million deaths annually plus ranking as the 11th leading cause of mortality (Asrani et al., 2019). Diverse causes underpin liver dysfunction, including viral infections, alcohol, hepatotoxic agents, autoimmune disorders, excessive drug use, plus hereditary factors (Wang et al., 2019; Zhang et al., 2020).

Carbon tetrachloride (CCl_4), a potent hepatotoxin, also affects organs such as the lungs, kidneys, brain, plus testes. Experimental models have long employed it to induce hepatic damage in laboratory animals, including rats, mice, plus rabbits (Taamalli et al., 2020; Unsal et al., 2021). Acute exposure can cause convulsions, vertigo, weakness, plus coma, with liver injury developing within days plus kidney injury typically appearing after prolonged contact (Baig & Khan, 2023). The hepatotoxic mechanism of CCl_4 involves its conversion by cytochrome P450 enzymes into highly reactive radicals, including trichloromethyl plus trichloromethyl peroxy. These radicals attack proteins, DNA, plus membrane lipids, initiating lipid peroxidation plus leading to oxidative stress, necrosis, inflammation, fibrosis, plus eventually cirrhosis (Dai et al., 2021).

Avocado (*Persea americana* Mill.), a member of the Lauraceae family, sees wide cultivation in tropical plus subtropical regions such as Mexico, Brazil, India, plus South Africa (Alhassan et al., 2012). While people widely recognize its creamy pulp for nutritional benefits, the seed—comprising approximately 13% of the fruit—often becomes discarded waste, despite a rich content of bioactive compounds. The seed holds about 64% of the fruit's phenolics plus accounts for nearly 57% of its total antioxidant capacity (Pahua-Ramos et al., 2012). Previous studies indicate greater antioxidant activity plus procyanidin content in avocado seeds compared to the pulp (Wang et al., 2010).

The seeds show particular richness in phenolic compounds, flavonoids, plus tannins, making them a promising source of natural antioxidants (Anggraeny et al., 2017). Beyond reducing oxidative stress, their utilization also contributes to waste reduction plus environmental sustainability (Feliana et al., 2018). Antioxidants from medicinal plants function as free radical scavengers, reducing agents, metal chelators, or hydrogen donors, thereby mitigating oxidative stress plus protecting cellular integrity (Merghem et al., 2019).

Several studies confirm the strong antioxidant potential of avocado seeds. For instance, Lyu et al. (2023) reported that the Reed variety demonstrated significant hydroxyl radical scavenging activity. Similarly, aqueous seed extracts prevented oxidative brain damage induced by Fe^{2+} plus sodium nitroprusside in rats (Oboh et al., 2016). The bioactive constituents of avocado seeds have links to diverse pharmacological effects, including antioxidant, anti-inflammatory, hypocholesterolemic, antidiabetic, plus hepatoprotective activities (Lara-Márquez et al., 2020; Ogbonnaya et al., 2024).

Traditional uses of avocado seeds also include treatments for diarrhea, dysentery, dental pain, parasitic infections, plus skin conditions (Odo et al., 2013). More recently, their potential inclusion in functional foods plus nutraceuticals has attracted significant scientific interest. Given these properties, the current study evaluated the antioxidant plus hepatoprotective potential of aqueous avocado seed extract (ASE) against CCl_4-induced hepatic injury in mice.

MATERIALS AND METHODS

Preparation of Avocado Seed Extract

Ripe avocado fruits (*Persea americana*) were obtained from Cairo, Egypt. Seeds were separated, grated, dried, plus ground into a powder. An aqueous extract was prepared by steeping 100 g of powder in one liter of hot water for 12 hours, followed by filtration plus evaporation at 40°C. The extract was standardized to a concentration of 0.4 g/L (Alhassan et al., 2012).

Experimental Protocol

The study utilized twenty male mice, weighing 25–30g, sourced from the animal breeding unit of the College of Science, University of Helwan, Egypt. The mice underwent an acclimatization period under standard environmental conditions with controlled temperature and humidity, receiving food and water *ad libitum*. The study protocol received approval (HU-IACUC/Z/SR0105-45) from the institutional animal ethics committee at Helwan University, Egypt, ensuring all procedures adhered to ethical guidelines.

Experimental Design

The researchers randomly allocated the twenty adult male mice into four experimental groups (n=5 per group):

Group 1 (Control): Received no treatment.

Group 2 (CCl_4-intoxicated): Received 1 ml/kg body weight of CCl_4 twice weekly for three weeks (Singh et al., 2014).

Group 3 (ASE-treated): Received 400 mg/kg body weight of avocado seed extract (ASE) via oral gavage once daily for three weeks (Baulland et al., 2021).

Group 4 (CCl_4 + ASE): Received both 1 ml/kg body weight of CCl_4 twice weekly and 400 mg/kg body weight of ASE once daily for three weeks.

After the three-week period, mice were euthanized via chloroform anesthesia. Blood was collected directly from the heart, allowed to clot, plus centrifuged to obtain serum. Liver samples were then promptly dissected for histological analysis.

Blood Sample Collection Plus Biochemical Analysis

Serum, obtained by centrifuging blood in clot-activator tubes, was analyzed for AST plus ALT levels using commercial kits (Reflotron plus Liquicolor Analyticals), following manufacturer protocols.

Histological Examination

Liver samples from all mice in the control and experimental groups received fixation in 10% formalin for histological processing. Careful examination of liver sections from the different groups followed, with subsequent photomicrograph documentation. The application of Mallory's trichrome stain specifically demonstrated collagen fibers for fibrosis assessment.

Immunohistochemistry Examination

Liver sections underwent immunohistochemical staining for caspase-3 using the streptavidin-biotin method. The protocol involved deparaffinization, peroxidase blocking, plus incubation with a primary anti-caspase-3 antibody (1:100 dilution), followed by a hematoxylin counterstain. Negative controls replaced the primary antibody with normal mouse serum (Abdel-Wahab et al., 2015).

Morphometric plus Statistical Analysis

An image analyzer (ImageJ, v1.46) quantified the area percentage of collagen fibers plus caspase-3-positive reactions across five random fields per group (×400). Data are mean ± SD. Statistical analysis used GraphPad Prism (v8) with one-way ANOVA plus Tukey's post hoc test; a P-value less than 0.05 indicated significance.

RESULTS

Biochemical Analysis

Table 1 presents serum AST, ALT levels in mice from the control, ASE, CCl_4, CCl_4 + ASE groups. CCl_4 treatment significantly increased ($P < 0.05$) AST, ALT levels, showing 7.15-fold, 5-fold rises, respectively, against the control group. Co-administration of ASE (400 mg/kg) with CCl_4 (1 mg/kg) significantly reduced these CCl_4-elevated enzyme levels. In Group IV, the avocado seed aqueous extract demonstrated a potent inhibitory effect on AST, ALT levels versus the CCl_4-treated group, restoring these levels to values matching the control group. The ASE-treated group showed no significant ($P > 0.05$) changes in AST, ALT levels against the control group.

Table 1. Effects of Avocado seed extract (ASE) and/or CCl_4 administration on Serum activities of AST, ALT levels in mice

Parameters	GI	GII	GIII	GIV
AST(U/L)	**142 ± 22.14**	**157 ± 32.14**	**1193 ±56.34^{a}**	**308 ± 46.8^{ab}**
ALT (U/L)	33 ± 6.6	28.33 ± 3.6	212 ± 20.3^{a}	69 ± 9.7^{ab}

Data show mean (±SD). **a** Significantly different from the control group (P<0.05). **ab** Significantly different from control, CCl_4 group (P<0.05).

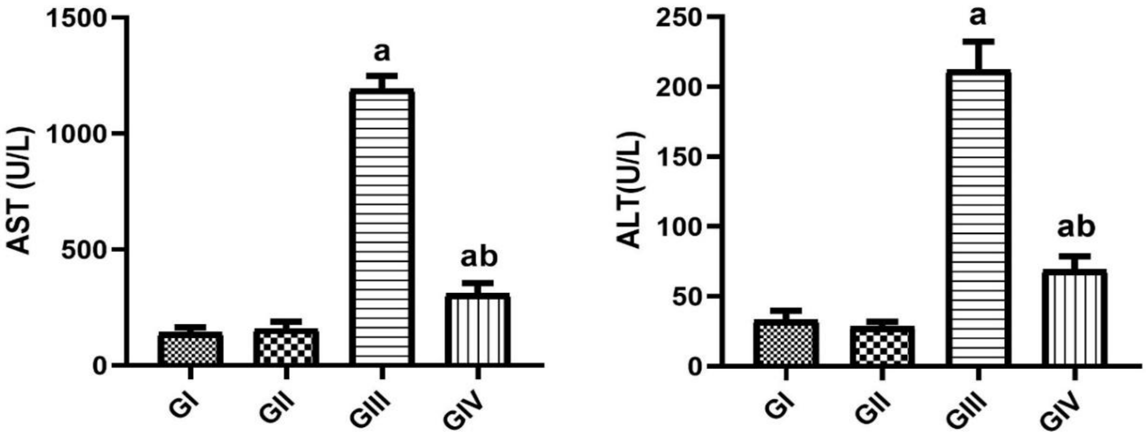

Figure 1. Effects of Avocado seed extract (ASE) and/or CCl_4 administration on AST, ALT activity in mice serum. a Significantly different from the control group (P<0.05). ab Significantly different from control, CCl_4 group (P<0.05).

Histological Results

Liver sections from control mice showed normal histology **(Fig. 2a)**, while the ASE-only group appeared nearly normal **(Fig. 2b)**. CCl_4 administration caused severe damage, including disrupted architecture, fatty degeneration, necrosis, plus inflammatory infiltration **(Fig. 2c)**. ASE treatment after CCl_4 exposure partially reversed this injury, showing a more intact structure, some normal nuclei, plus reduced degeneration **(Fig. 2d)**.

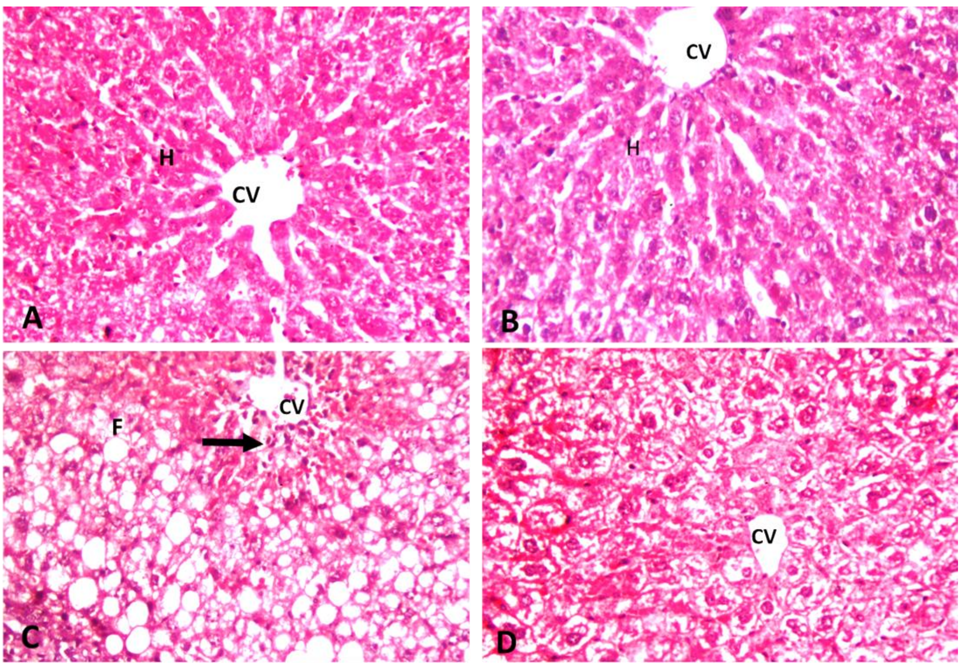

Figure 2. Liver tissue photomicrographs from control, ASE, CCl_4, and ASE+CCl_4 groups (H&E stain). **2A:** Control group showing normal central vein (CV) and hepatocytes (H)

(400X). **2B:** ASE-treated group displaying largely normal hepatocytes (H) and central vein (CV). **2C:** CCl_4-treated group exhibiting pale-stained hepatocytes, massive fatty degeneration (F), necrotic hepatocytes with severe ballooning, and extensive inflammatory cell infiltration (arrow) (400X). **2D:** ASE+CCl_4 group showing reduced fatty changes, necrosis, and ballooning degeneration versus CCl_4 alone. Pale-stained hepatocytes with cytoplasmic vacuolization and inflammatory cell infiltration (arrow) persist (400X).

Mallory's Trichrome-Stained Tissues

Mallory's trichrome staining assessed collagen deposition **(Fig. 3)**. Control **(3A)** plus ASE-only **(3B)** groups showed minimal, normal collagen. The CCl_4-treated group **(3C)** exhibited a significant collagen increase in portal areas, thick lining epithelium, plus hemorrhagic areas. The ASE + CCl_4 group **(3D)** showed reduced fiber content versus the CCl_4-only group.

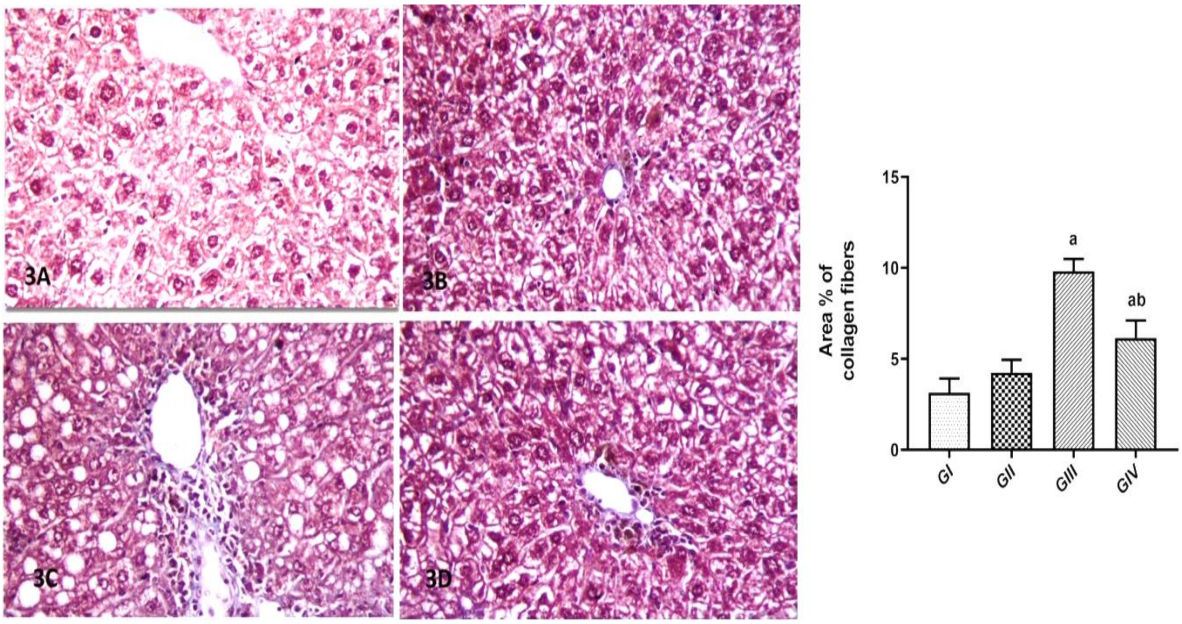

Figure 3. Liver tissue photomicrographs from control, ASE, CCl_4, and ASE+CCl_4 groups (Mallory's Trichrome Stain). **3A:** Control group showing normal collagen distribution (400X). **3B:** ASE-treated group exhibiting near-normal blue-stained collagen fiber content (400X). **3C:** CCl_4-treated mice showing extensively increased collagenous bundles (cf) throughout tissue (400X). **3D:** ASE+CCl_4 group displaying some collagenous fibers surrounding the central vein (400X).

Immunohistochemistry

CCl_4 treatment significantly increased Caspase-3, shown by intense brown staining **(Fig. 4C vs. 4A)**. The ASE-only group showed a regular appearance **(Fig. 4B)**. ASE + CCl_4 treatment slightly reduced Caspase-3, with less intense staining than the CCl_4 group, indicating incomplete resolution of damage **(Fig. 4D)**.

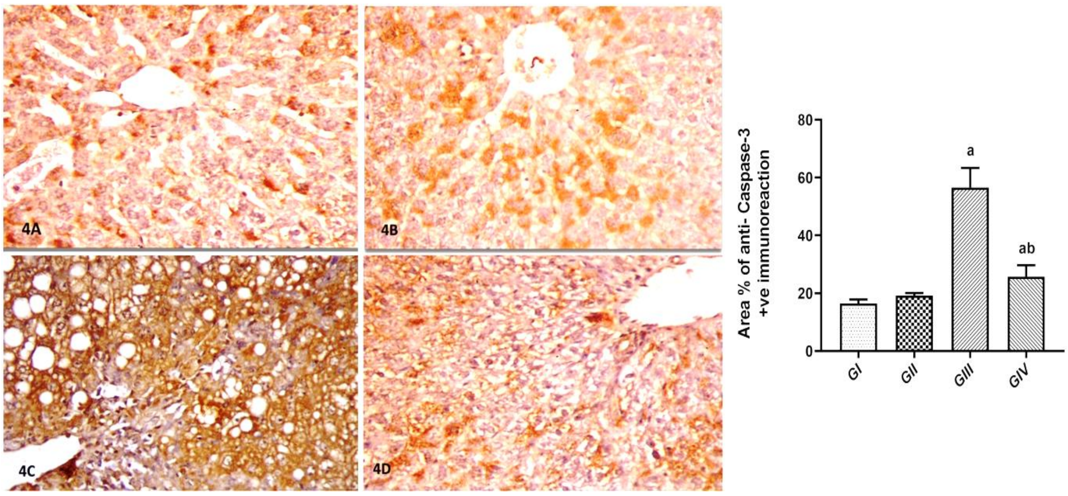

Figure 4. Photomicrographs of liver tissues from control, ASE, CCl_4, and ASE+CCl_4 groups, immunostained for Caspase-3. **4A:** Control group showing faint brown staining (400X) **4B:** ASE-treated group showing faint brown staining (400X). **4C:** CCl_4-treated group showing intensive, dense brown staining (400X). **4D:** ASE+CCl_4 group showing markedly decreased Caspase-3 immunoreactivity (400X).

DISCUSSION

Carbon tetrachloride (CCl_4) serves as a common agent for investigating hepatoprotective activity in animal models, particularly mice, rats, plus rabbits. This model induces liver damage through oxidative stress plus lipid peroxidation. The resulting pathology involves significant cellular alterations, leading to inflammation, necrosis, fibrosis, plus cirrhosis (Dai et al., 2021). Current hepatocellular carcinoma treatments, including chemotherapy, radiotherapy, plus transplantation, demonstrate poor long-term outcomes plus limited efficacy. Plants offer a resource for novel drug development, given their remarkable benefits plus reduced toxic effects (Fan et al., 2022; Yang et al., 2019).

Globally, medicinal plants serve as traditional medicines for centuries, treating various ailments. Research indicates phytochemicals hold promise as substitutes for synthetic drugs. Specific plants demonstrate therapeutic potential against liver disorders (Almatroodi et al., 2020).

This study found CCl_4 exposure caused a significant rise in serum AST plus ALT activities. Histopathological results confirmed substantial structural damage, including fatty degeneration, severe necrosis, cytoplasmic loosening of hepatic cells, plus massive inflammatory cell infiltration. These findings align with other reports noting histopathological liver alterations accompany increased serum AST plus ALT levels (Cinar et al., 2024). Elevated serum marker enzymes suggest cell membrane disintegration plus cell death (Bashandy & AlWasel, 2011).

Earlier research supports the present findings (Shahzad et al., 2021; Slaoui et al., 2017). CCl_4 exposure compromises the liver’s structural integrity in rats. Hepatic enzymes, cytoplasmic in nature, leak into the bloodstream following liver damage (Ouassou et al., 2021).

This investigation demonstrated ASE administration ameliorated the significant elevation in serum ALT plus AST activities observed in CCl_4-treated mice. Furthermore, histopathological examination evidenced the hepatoprotective effects of ASE, showing noticeable improvement (Figures 2-4). These results agree with Ahmed et al. (2022), who found avocado reduces liver carcinogenesis in mice via activated antioxidant, anti-inflammatory, plus apoptotic properties.

Elmoslemany et al. (2021) reported avocado seeds possess medical importance, reducing oxidative stress, inflammation, plus genetic effects linked to immune functions.

In the present study, Mallory's trichrome staining of the CCl_4-treated group revealed a highly increased collagen fiber content around portal vein branches. Numerous authors documented similar results using CCl_4 to induce liver fibrosis in animals. Na et al. (2015) reported CCl_4-induced liver fibrosis associates with severe lipid peroxidation plus antioxidant depletion, resulting from hepatocyte cell membrane plus organelle damage. Potent reactive oxygen species significantly induce hepatic tissue destruction, amplify inflammatory responses, stimulate profibrogenic mediator production, plus contribute to hepatic fibrogenesis (Ghiassi-Nejad & Friedman, 2008).

Reports indicate avocado seeds contain multiple active ingredients, including saponins plus flavonoids, both possessing anti-inflammatory properties. Saponins inhibit cyclooxygenase, catalyzing arachidonic acid conversion to endoperoxidase. Inhibiting cyclooxygenase plus lipoxygenase enzymes diminishes the inflammatory response plus accelerates the transforming growth factor-β (TGF-β) pathway (Okail et al., 2024). TGF-β, a protein with three isoforms (TGF-β1, TGF-β2, TGF-β3), functions importantly in renal fibrosis by inducing epithelial-to-mesenchymal transition (EMT) (Rahman et al., 2022). Consequently, TGF-β1 activation promotes profibrotic gene expression, thereby contributing to renal fibrosis development. Conversely, TGF-β1 inhibition may reduce kidney damage plus fibrosis. One study suggests flavonoids potentially compete with TGF-β1's ligand-binding site, highlighting these compounds' importance for further investigation as a kidney fibrosis treatment (Tugiyanti et al., 2019).

This study showed CCl_4-treated mice exhibited a significant caspase-3 production increase, demonstrating severe apoptosis versus the control group. These results align with a recent report showing CCl_4 causes acute hepatotoxicity, characterized by apoptotic plus necrotic hepatocellular injury, plus liver function mutilation (Algefare et al., 2024). Additionally, CCl_4 can induce oxidative stress, cell cycle arrest, plus apoptosis (Munir and Khan, 2023).

Avocado possesses abundance in tocopherols, predominantly alpha (α) plus gamma (γ), both highly bioactive antioxidants (Woolf et al., 2009). One study demonstrated γ-tocopherol exhibited superior efficacy enhancing mitochondrial functions versus α-tocopherol. This enhancement occurred through mitochondrial membrane permeability regulation, achieved by affecting VDAC1 plus CypD mRNA expression. Furthermore, the study revealed γ-tocopherol prevented apoptosis via reduced cytochrome c release plus a decreased BAX/Bcl-2 ratio. It also functioned as a potent antioxidant, preventing beta-amyloid peptide (Aβ) accumulation in an Alzheimer's disease in vitro model (Pahrudin Arrozi et al., 2020).

Sahyon et al. (2023) demonstrated avocado exhibits strong anti-cancer effects, inhibiting cancer cell proliferation. Additionally, avocados play a crucial role maintaining balance plus homeostasis across various tissues plus cells. Alkhalf et al. (2018) reported the inhibitory effect of avocado fruit plus seed on HepG2 plus colon cancer cell line (HCT116) proliferation. Avocado fruit demonstrated HepG2 plus HCT116 cell proliferation inhibition, with IC_{50} values of 58 µg/mL plus 14 µg/mL, respectively. Accordingly, the avocado seed may represent a potential source for isolating effective anti-cancer compounds, applicable as natural chemotherapy for various cancer types.

CONCLUSION

Elevated oxidative stress, overwhelming endogenous antioxidant defenses, induces liver damage. ASE demonstrates potent antioxidant activity, mitigating oxidative injury by enhancing free radical scavenging. This study confirms ASE's hepatoprotective role against CCl_4-induced damage, attributable to its antioxidant properties. While these findings establish ASE's therapeutic potential, further research is required to elucidate its precise mechanistic pathways.

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The effect of Mirazid (*Commiphora molmol*) on mitigating Brain damage caused by *Hymenolepiasis Nana: A histochemical Evaluation*

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Hymenolepis nana, immunosuppression, praziquantel, Mirazid (*Commiphora molmol*)

ABSTRACT

Hymenolepiasis is the most prevalent tapeworm infection worldwide. While praziquantel is the primary treatment for *Hymenolepis nana* (H. nana), studies on albino rats have demonstrated its hepatotoxic, genotoxic, and carcinogenic effects. Mirazid (*Commiphora molmol*) has emerged as a safer and effective alternative for treating hymenolepiasis. This study aimed to investigate the Neuropathological alterations of *H. nana* infection in immunocompromised hosts. One hundred twenty male Swiss albino mice were divided into immunocompetent and immunocompromised groups by administering a dose of 8 mg/kg body weight (BW) of dexamethasone sodium phosphate subcutaneously for seven days in order to avoid edema or unintended weight gain. At different post-infection time points (the experimental mice were sacrificed on days 15 and 21 post-infection for determination of which drug gives more effect (cure rate)), mice were sacrificed, and their brain tissues were subjected to histochemical and histological analyses. The findings revealed significant histopathological alterations in the brains of immunosuppressed mice. Immunocompetent groups showed improvements in brain histopathology when praziquantel (PZQ)-treated mice were compared to those given Mirazid oleoresin extract at a dose of 10 mg/kg/BW for six days after infection. Unlike what occurred in the immunodeficient group, where there was an increase in histological alterations. Hence, histopathological alterations in the brains of infected mice were progressive and exacerbated under immunosuppressive conditions, correlating with increased cerebral parasite burden. Histochemical staining (e.g., hematoxylin and eosin) revealed significant Neuropathological changes. However, further research is needed to determine whether these alterations are directly caused by the presence of the parasite or by host immune responses to infection and treatment.

INTRODUCTION

Neurocysticercosis (NCC) is a significant global cause of morbidity, resulting from a helminthic infection of the central nervous system (CNS) by the larval stage of Taenia solium (cysticercus), as seen in H. nana infection in immunocompromised hosts (Orozco et al., 2018). Among human cestode infections, Hymenolepis nana (dwarf tapeworm) is the most prevalent, primarily affecting children due to poor hygiene and sanitation. Estimates suggest that between 50 and 75 million individuals worldwide are infected with H. nana, with high prevalence rates in tropical regions, such as Egypt, Africa, Asia, and parts of southern and eastern Europe (El-Gammal et al., 1995 & Bagayan et al., 2015). Individuals with weakened immune systems are particularly susceptible, as the parasite can proliferate within their bodies through autoinfection (WHO, 2013). H. nana is mostly spread by the fecal-oral route and does not need an intermediary host for its life cycle, in contrast to Hymenolepis diminuta. Infection typically occurs through ingestion of contaminated eggs, leading to intestinal colonization. While Hymenolepis infections are often asymptomatic, the larval stages may trigger immunological responses. *H. nana* has also been studied for its potential immunomodulatory effects, with research suggesting it may play a role in mitigating colitis (Bhosale, 2022). Diagnosis is primarily based on microscopic detection of eggs in fecal samples. Praziquantel remains the treatment of choice, while proper hygiene practices are fundamental to preventing transmission. Despite its typically asymptomatic nature, *H. nana* infection can lead to severe CNS complications, especially in immunocompromised hosts, children, and those with ongoing illnesses (Bhosale, 2022). Rodents, especially rats and mice, serve as natural reservoirs for *H. nana*, with reported prevalence rates of 33.93% in rats and 32% in mice (Pinto et al., 1994; Gudissa et al., 2011).

Transmission occurs through the ingestion of embryonated eggs or arthropods carrying cysticercoids, This may come from tainted food, water, or direct interaction. When the eggs are consumed, hexacanth larvae are released, which penetrate the intestinal mucosa, enter the bloodstream, and migrate to various tissues, including the CNS. Once in the brain, the larvae often localize within the parenchyma, subarachnoid space, or ventricular system, developing into mature cysticercoids that provoke neuroinflammatory responses and neurological impairments (Cho et al., 2009). Astrocytes and microglia play critical roles in maintaining CNS homeostasis and responding to neural damage. Astrocytes contribute to gliosis, a reparative process that involves cytokine release and regulation of the blood-brain barrier, whereas microglia support axonal integrity and synaptic remodeling in both normal and damaged neural environments (Nimmerjahn et al., 2005; Tremblay et al., 2010; Paolicelli et al., 2011; Schafer et al., 2013). Ma et al. (2022) found that 132-hydroxy-(132-S)-pheophytin a from *Leucaena leucocephala* effectively reduced tapeworm infection in mice by lowering worm count, egg production, and enhancing immune response. Although the

brain lacks traditional lymphatic vessels, alternative pathways, such as cerebrospinal fluid drainage to cervical lymph nodes (CLNs), help regulate immune responses. The neurovascular unit (NVU) further acts as a selective barrier, limiting leukocyte infiltration into the brain parenchyma (Bechmann & Woodroofe, 2014; Dyrna et al., 2013). Due to their broad-spectrum antiparasitic properties and minimal adverse effects compared to conventional chemotherapeutics, plant-derived essential oils have garnered interest as alternative treatments for parasitic infections. The need for such alternatives is reinforced by concerns regarding the hepatotoxic, genotoxic, and potentially carcinogenic effects of widely used anthelmintics like praziquantel, particularly in immunosuppressed individuals (Omar et al., 2005). Experimental models using dexamethasone sodium phosphate to induce immunosuppression highlight the need for effective therapeutic strategies in managing H. nana infections (Medeiros et al., 2010).

Materials and Methods

Animals

Male Swiss albino mice, weighing approximately 20–25 g (±2 g), were bred and maintained at the Theodore Bilharz Research Institute (TBRI), Egypt. All experiments followed internationally recognized guidelines for animal research and ethical standards (Nessim et al., 2000). They were obtained from the Biology Supply Center of the Theodor Bilharzia Research Institute (TBRI) in Giza, Egypt. They were housed at a consistent room temperature of 22 ±2 ○C in the TBRI animal housing. The standard food and water were available to the animals without restriction. All animal care and procedures were carried out in compliance with global ethical standards after receiving approval from TBRI's institutional ethics committee. The Department of Zoology, Faculty of Science (Girls), Al-Azhar University, conducted histopathological and quantitative image analysis."

Experimental Design

A total of 120 male albino mice were divided into two main groups:

Immunocompetent Groups

•**Group 1 (Infected Untreated Control)**: Infected with *Hymenolepis nana* but untreated.

•**Group 2** According to Massoud et al. (2007), mice were infected and given oral treatment with Mirazid (Pharco, Egypt) at a dose of 10 mg/kg/BW for six days after infection. Comparing the effects of Mirazid (Commiphora molmol), a herbal remedy, to PZQ (the conventional treatment) in immunocompetent and immunocompromised patients albino mice with the infection. Mirazid is superior to PZQ in treating H. nana infections, with significant improvements in both biochemical and histopathological outcomes.

•**Group 3 (Praziquantel Treated)**: A single dosage of praziquantel (EPICO, Egypt) at a dose of 25 mg/kg/BW was administered orally to infected mice on the tenth day after infection, as per Bhat-tacharya et al. (2003).

Immunocompromised Groups

- **Group 4 (Cortisone and Infected)**: Injected with dexamethasone sodium phosphate and infected with *H. nana* (250 eggs/mouse).
- **Group 5 (Cortisone, Infected, and Mirazid Treated)**: Injected with dexamethasone sodium phosphate, infected with *H. nana* (250 eggs/mouse), and treated with Mirazid (10 mg/kg body weight) orally for six days post-infection. Mirazid showed higher cure rates than PZQ. This plant extract also showed a notable decrease in fibrotic content.
- **Group 6 (Cortisone, Infected, and Praziquantel Treated)**: Injected with dexamethasone sodium phosphate, infected with *H. nana* (250 eggs/mouse), and treated with praziquantel (25 mg/kg body weight) orally on the 10th day post-infection.

The experimental mice were sacrificed on day 21 post-infection to assess the treatment efficacy (cure rate).

Stool Sample Collection

Stool samples infected with *H. nana* were collected from patients attending TBRI. Eggs were isolated and processed according to Macnish (2001). The suspension was centrifuged, and the infective eggs were washed with phosphate-buffered saline (PBS) to remove any residual salt.

Animal Infection Protocol

Mice were infected orally with 200–250 *H. nana* eggs using a tuberculin syringe connected to a polyethylene tube, ensuring the dose was directly delivered into the esophagus (Ito et al., 1991).

Drugs Used

Praziquantel (PZQ)

Praziquantel (Discocide), manufactured by the Egyptian International Pharmaceutical Industries Company (EPICO), was administered at a dose of 25 mg/kg body weight, dissolved in 10% dimethyl sulfoxide (DMSO) (Bhattacharya et al., 2003).

Dexamethasone Sodium Phosphate

Dexamethasone sodium phosphate (Amriya Pharm. Ind., Alexandria, Egypt) was administered subcutaneously at a dose of 8 mg/kg body weight daily for seven days (Medeiros et al., 2010). Immunosuppression is assessed indirectly by monitoring immunosuppressive drug levels in the blood, observing the patient's susceptibility to infections or toxic side effects, and sometimes through assays that measure the immune system's functional response. While direct measurement of the immune state is challenging, methods such as checking blood cell counts and immunoglobulin levels can indicate a defect. Newer techniques, on the other hand, examine virus-specific T cells (Tvis) or cytokine profiles from stimulated blood to assess individual immune function and the effects of drugs (Lversen, 2013).

Mirazid

Mirazid, an oleoresin extract from *Commiphora molmol* capsules (Pharco Pharmaceuticals Company, Alexandria, Egypt), was given orally starting six days after infection at a rate of 10 mg/kg body weight each day (Massoud et al., 2007b).

Histopathological Examination

The brain tissues were fixed in 20% neutral formalin, dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax. Serial sections (5 μm) were prepared and stained using various histochemical techniques, including (H&E) General stain, Mallory's trichrome for collagen (Pearse, 1977), (PAS) for glycogen (Hotchkiss, 1948), bromophenol blue (Mazia et al., 1953), and Congo red (Sheehan & Hrapchak, 1980; Valle, 1986). Paraffin sections (4–5 μm) were stained with the Feulgen reaction, Mallory, bromophenol, Congo red, and PAS stains (for glycoproteins in neurodegeneration) (Suvarna et al., 2013). Sections that had been stained were checked for corresponding cellular, nuclear, and cytoplasmic structural reactivities.

Histopathology: Image Pro-Plus software (version 5.0) was used for image analysis. Images obtained from a digital camera were analyzed for quantitative measurements (Reedy & Kamboj, 2004).

Statistical Analysis: Data were expressed as means ± standard deviation (SD). Statistical significance was determined using one-way ANOVA, and differences were considered significant at $p < 0.05$. The SPSS software (version 10 for Windows) was used for statistical computations.

RESULTS

Brain examination showed marked histopathological changes in immunosuppressed mice. Mice infected with Hymenolepis nana showed improvements in their histological alterations**,** treated with praziquantel (PZQ), and compared with Mirazid oleoresin extract-treated groups in Immunocompetent groups; however, changes were observed in Immunocompromised groups. When comparing the two groups treated with PZQ, Mirazid, with the untreated groups, a statistically significant increase was shown (P <0.001).

Brain of mice from group G1, infected and untreated control group, showing a large focal hemorrhage area with disintegrated neurofibromas, faintly stained, and highly affected neurons. It also showed congestion of blood vessels and perivascular cuffing with mononuclear cells. Examination showed focal necrosis associated with glial cell infiltration. Most neurons are highly disorganized and faintly stained, with their nuclei significantly reduced, and are accompanied by destroyed and ruptured nanofibrous structures with highly dilated spaces in between them. Additionally, there are depressions of degenerated neurons and evidence of neuronal necrosis and neuronophagia, Fig. (1) (H&E x400).

Brains of G2 mice (infected and treated with Mirazid) showed neuronal necrosis, neuronophagia, perivascular cuffing, large, damaged areas with WBCs, hydrolyzed RBCs, and neurons with pyknotic (p) or karyolytic (k) nuclei and perineuronal spaces, Fig. (2) (H&E x400).

The brains of mice from group G3, Infected and treated with praziquantel, showed cellular edema. Most neurons contained highly atrophied and pyknotic (p) nuclei or karyolitic (k) ones. Additionally, there was necrosis of neurons and neuronophagia. Examination revealed necrosis of some neurons and neuronophagia, with no histopathological changes observed, Fig. (3) (H&E x400).

The brains of mice from group G4, Cortisone, and the infected group showed necrosis of neurons and focal hemorrhage, also showing highly ruptured and highly disintegrated neurofibrous tissue with highly congested and dilated blood vessels and focal gliosis. Examination showed necrosis of neurons and congestion of highly dilated and elongated cerebral blood vessels. It showed necrosis of neurons with dystrophic changes, including atrophied ones with highly widened pericellular spaces, completely destroyed cytoplasm and nuclei of the remnant ones and cellular organoids, with large hemorrhagic areas, containing RBCs and numerous WBCs, Fig. (4) (H&E x400).

Brain of mice from group G5 Cortisone, infected and Mirazid showing cellular oedema with highly dilated and congested of cerebral blood vessel with delaminated endothelial lining of it, also showed necrosis of neurons and neuronophagia, examination showed perivascular cuffing with mononuclear cells, with pyknotic nuclei, some degenerated neurons has karyolitic(k) with pericellular large empty spaces with dilated and elongated blood capillary (by) and showed cellular oedema, completely destructed neurons with widened pericellular spaces and disintegrated of neurofibrous with complete loss of architecture brain tissues, also showed necrosis of neurons with faintly stained neurofibrous, Fig. (5) (H&E x400).

Brain of mice from group G6 Cortisone, infected and PZQ treated group showing focal necrosis associated with glial cells infiltration, most of neurons are highly atrophic with widened pericellular spaces with increased signs of pykinosis (p) or karyolitic and highly degenerated neurofibrous, also showed perivascular cuffing with mononuclear cells, with pyknotic nuclei, some degenerated neurons have karyolitic (k) with pericellular large empty spaces with dilated and elongated blood capillary (bv), and showed necrosis of neurons, Fig. (6) (H&E x400).

Histochemical Findings

Feulgen reaction, for nuclear DNA.

Almost all of the examined sections from different experimental groups showed normal nuclear reactivities, with a more reactive hippocampus cell in groups 2 and 4. Reactive glial and round cellular aggregates are seen in the vicinity of blood vessels and peri-ventricular areas in group 1. Focal reactive gliosis with prominent nuclei is observed in the group, Figs. (1,2).

Bromophenol, for cellular protein contents.

Brain tissue from the different experimental groups stained with bromophenol showed normal cellular, nuclear, and cytoplasmic protein contents in all cerebral and cerebellar structures of the control negative group (N) and in some cerebral and cerebellar structures of all infected-treated groups (G2-G6) (yellow arrows). A moderate depletion of cellular protein is observable in all infected-treated groups (G2-G6). The depletive reaction was more potent in the infected nontreated group (G1). Figs. (3,4).

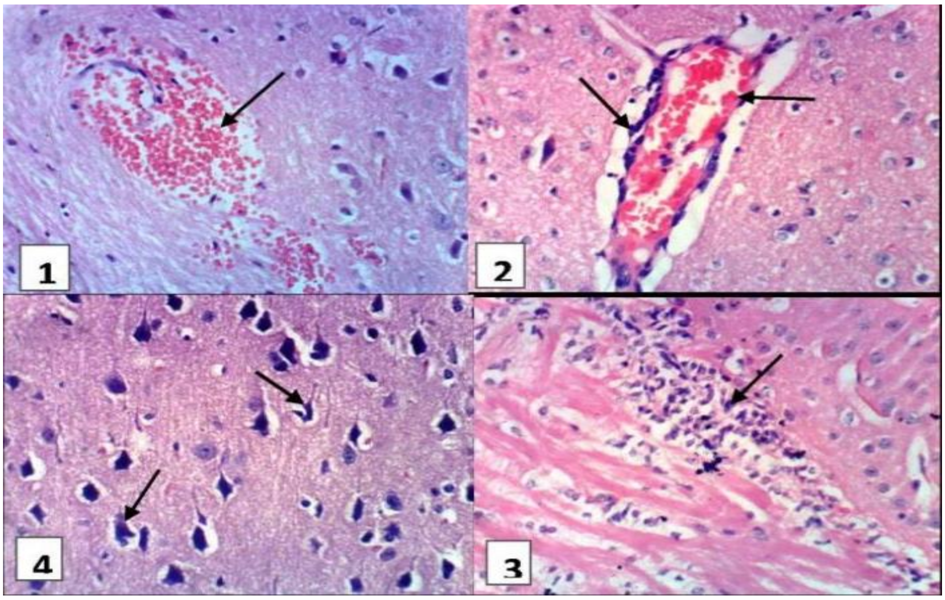


Figure 1. The brains of mice from group G1, the infected, untreated control group, show a large focal hemorrhagic area, with disintegrated neurofibromas and faintly stained, highly affected neurons (1). (2) This shows congestion of blood vessels, perivascular cuffing with mononuclear cells, and congested blood vessels. (3) This depicts focal necrosis associated with glial cell infiltration, where most neurons appear highly disturbed and faintly stained, their nuclei significantly reduced, with destroyed and ruptured nanofibers and highly dilated spaces between them. It also contains depressions from degenerated neurons. (4) This displays neuronophagia and neuronal necrosis (H & E x400).

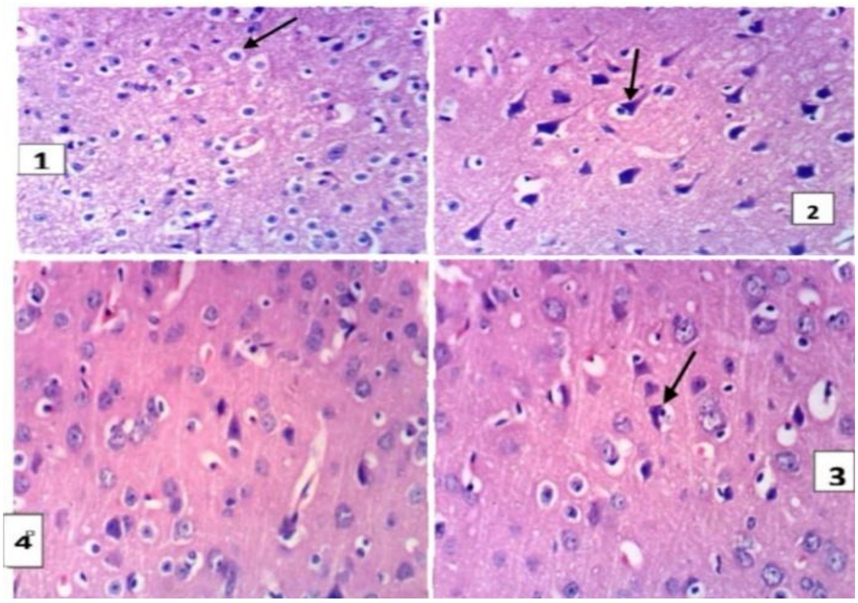
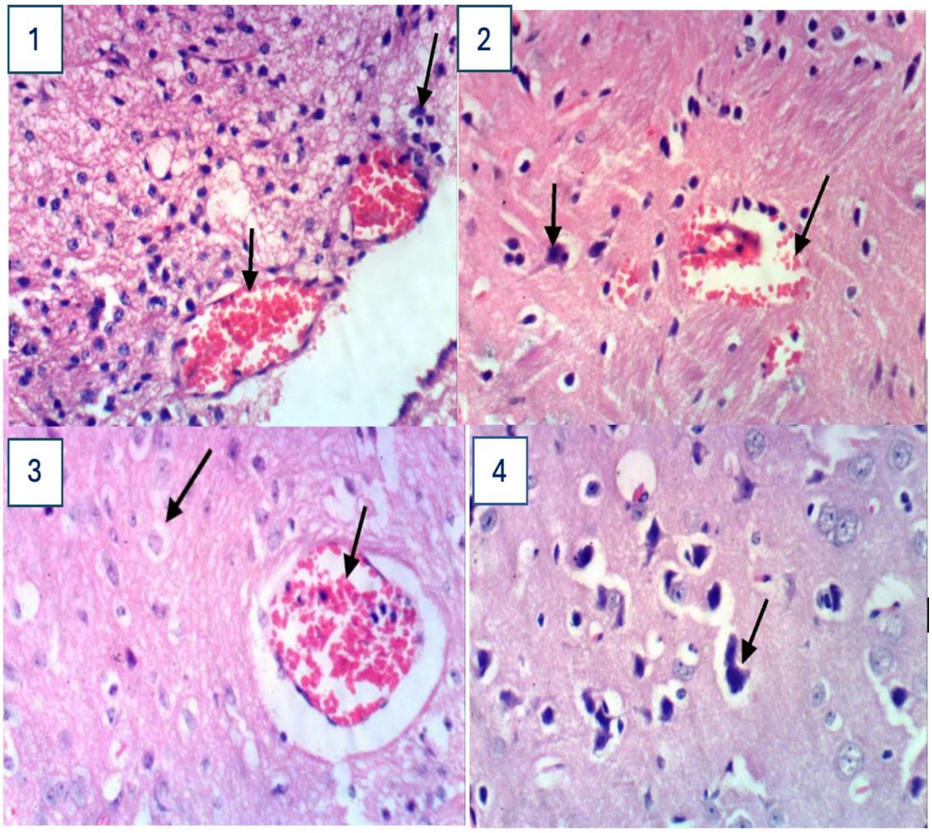
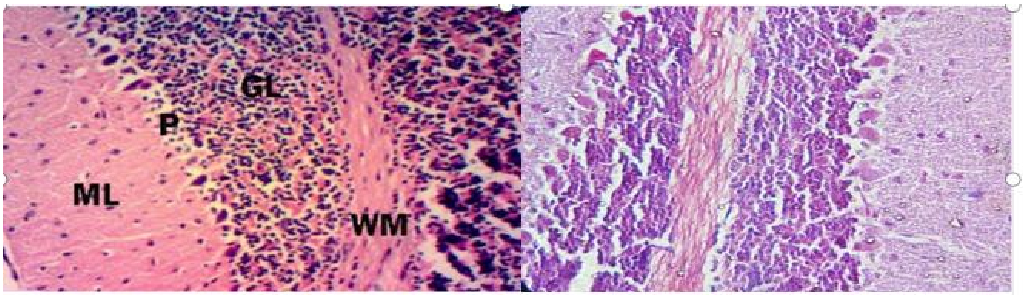


Figure 2. Brain of mice from group G3 Infected and treated with praziquantel showing cellular oedema, most of neurons contain highly atrophied and pyknotic (p) nuclei or



karyolitic(k) ones (←) (1), (2) showing necrosis of neurons and neuronophagia, (3) demonstrating neuronophagia and some neuron necrosis (4) showing no histopathological changes (H & E x 400).

Figure 3. Brain of mice from group G4, Cortisone, and infected group showing necrosis of neurons and focal hemorrhage (1), (2) showing necrosis of neurons and congestion of highly dilated and elongated cerebral blood vessels. Additionally, the brains of mice from group G5 Cortisone, infected with Mirazid, exhibited cellular edema with highly dilated and congested cerebral blood vessels, a delaminated endothelial lining (3), and neuronal necrosis (←), accompanied by faintly stained neurofibers (4) (H&E x 400).



Typical appearance of a section of brain stain with (H & E x100 and x400).

PAS Stain for polysaccharides (glycogen and glycoprotein)

PAS staining is primarily used for detecting polysaccharides, including glycogen, glycoproteins, and glycolipids. Thus, this staining has been used to evaluate liver diseases associated with glycogen deposition, lung diseases caused by mucin abnormalities, and other conditions. However, the staining is rarely used for histopathological analysis of brain tissues. Examined sections from the control negative group revealed an overall normal distribution of glycoprotein and glycolipid molecules in different cerebral and cerebellar structures. On the other hand, the treated groups (G1-G6) showed degenerative changes in neuronal, glial, and Purkinje cells, accompanied by increased deposition of glycoproteins and glycolipids, resulting in intense positive PAS staining. The reaction was more unmistakable and characteristic in group 1. Degenerated axons also intensely reacted positively. Demyelinated and vacuolated axons and neurons were negatively stained and assumed a paler color affinity. Figs. (5,6).

Congo red stain for cerebral amyloid-associated protein.

Amyloid is homogeneous and eosinophilic; the deposits are extracellular and may become sufficiently large to cause damage to surrounding tissues. When stained with Congo Red, amyloid exhibits birefringence in an apple-green color under the microscope, aided by polarizing lenses. In brain tissue, amyloid appears as extracellular plaques and intracellular neurofibrillary tangles (NFTs). Neurofibrillary tangles are abnormal accumulations of a protein called tau that collect inside neurons. Congo red-stained sections from control-negative rats were free of amyloid deposition. Large amounts of both extracellular bright red amyloid deposits and neurofibrillary tangles (abnormal protein deposition) were recorded in the larva migrants infected group (G1). On the other hand, variable amounts of amyloid deposits were seen in all treatment groups, with a minimal change in groups (5, 6). Figures 7,8.

Mallory For collagen fibers and other tissue features, use the Trichrome stain.

Mallory's trichrome is based on nuclear staining with carbol fuchsin and associated cytoplasmic staining with G-orange, as well as highly selective collagen staining with aniline blue. The selectivity of the method is based on the different degrees of chemical affinity of the dyes used for tissue macromolecules. The role of phosphomolybdic acid, in particular, is fundamental because it serves as a bridge between the tissue structures to which it selectively binds (collagen fibrils, cell membranes, etc.) and aniline blue (amphoteric dye). The other component of Mallory's trichrome, the orange G, which has no affinity for phosphomolybdic acid, stains the remaining structures (which are not linked to phosphomolybdic acid). Nuclei, neurofibrils, cartilage, and bone tissue: red. Collagen Fibers: Blue. Erythrocytes, myelin: golden yellow. Elastic fibers: pale pink-yellow or colorless. Examined tissue sections from the nervous tissue of control-free rats revealed negative deposition of collagen fibers. Sections from different infected-treatment regimens denoted axonal, neuropil, and neurofibrillary degenerative changes of variable internists with a golden yellow positive reaction to the remaining unaffected myelinated fibrils. No collagen deposition in any of the examined cases was recorded. Figs. (9,10).

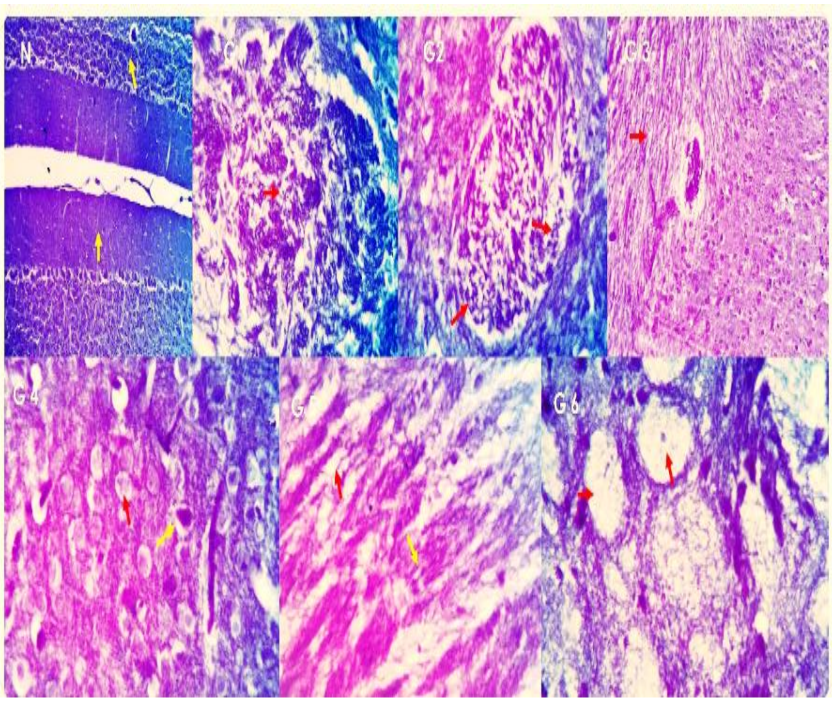


Figure 4. Photomicrographs from brain tissue of the different experimental groups stained by bromophenol stain, showing normal cellular nuclear and cytoplasmic protein contents in all cerebral and cerebellar structures of the control negative group (N) and some cerebral and cerebellar structures of all infected-treated groups (G2-G6) (yellow arrows). A moderate depletion of the cellular protein is observable in all infected-treated groups (G2-G6) (red arrows). The depletive reaction is more potent in the infected nontreated group (G1) (red arrows). (x 400).

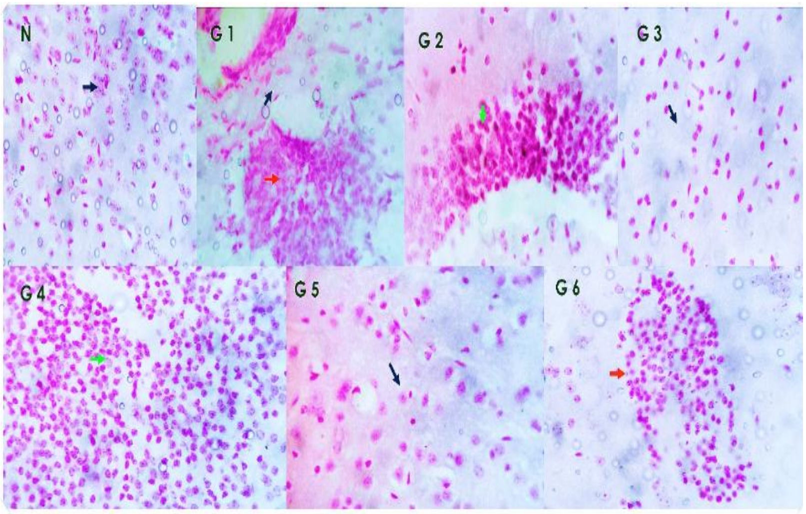


Figure 5. Photomicrographs of brain tissue from various experimental groups stained with Feulgen stain, depicting normal nuclear reactivities (dark blue arrows) alongside more reactive hippocampal cells in groups 2 and 4 (green arrows). Reactive glial cells and round cellular aggregates are seen near blood vessels and periventricular areas in group 1 (red arrow). Focal reactive gliosis with prominent nuclei is observed in group 6 (orange arrow). (x 400).

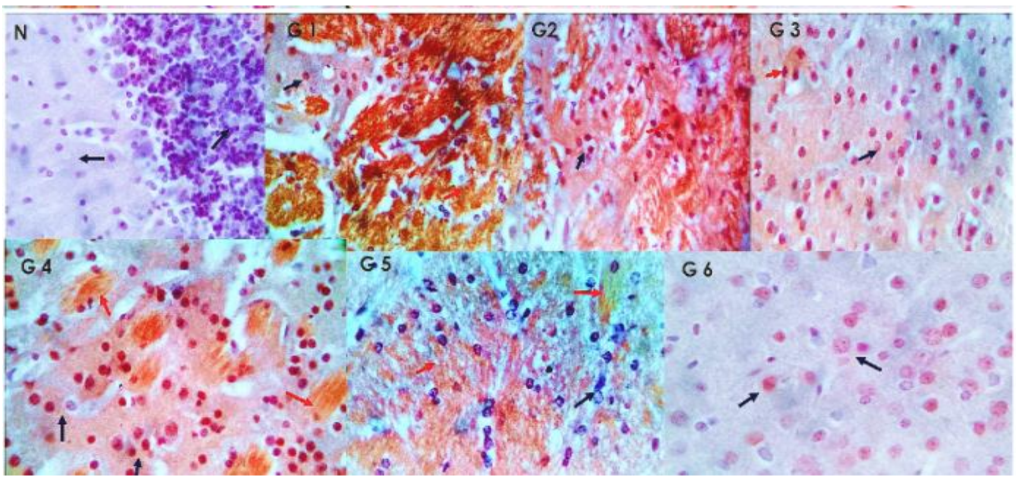


Figure 6. Photomicrographs from brain tissue of the different experimental groups stained by Congo red stain, showing amyloid-free tissue cells and neurofibrils in control negative rats (G1) (dark blue arrows). Large amounts of both extracellular bright red amyloid deposits and

neurofibrillary tangles (abnormal protein deposition) are seen in the larva migrants infected group (G 1) (red arrows). On the other hand, variable amounts of amyloid deposits are observable in all treatment groups, with minimal changes between groups (5, 6) (red arrows). Amyloid-free brain tissue is marked by dark blue arrows . (x 400).

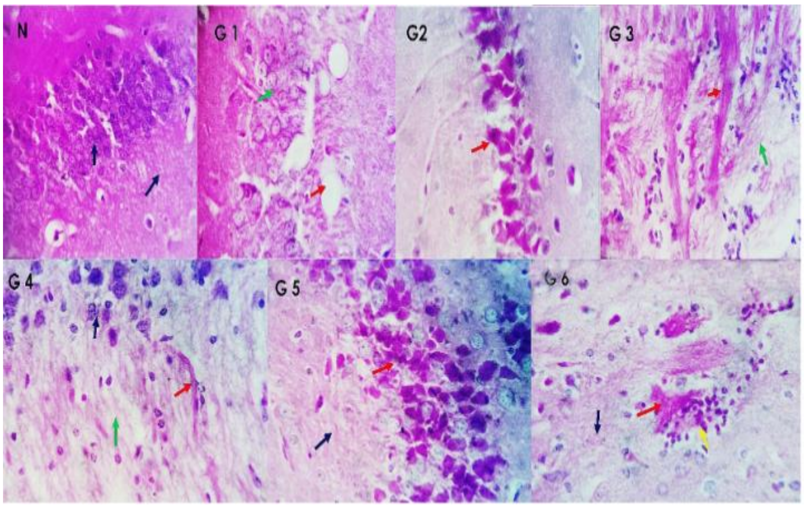


Figure 7. photomicrographs of the various experimental groups' brain tissue stained with Periodic Acid Schiff (PAS) dye, showing overall normal distribution of the glycoprotein and glycolipid molecules in different cerebral and cerebrum structures (dark blue arrows). On the other hand, the treated groups (G1-G6) exhibit degenerative changes in neuronal, glial, and Purkinje cells, accompanied by increased deposition of glycoproteins and glycolipids, resulting in intense positive PAS staining (indicated by red arrows). The reaction appears more straightforward and characteristic in group 1. Degenerated axons also appear intensely positively reacted (red arrows). Demyelinated and vacuolated axons and or neurons are negatively stained and assumed to have a paler color affinity (green arrows). (x 400).

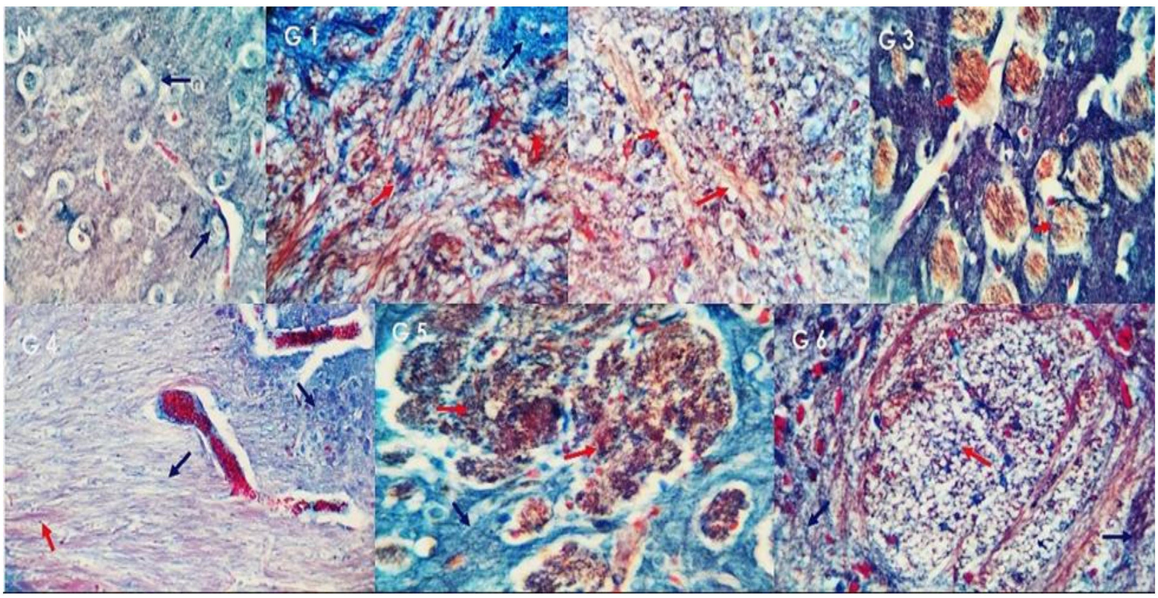
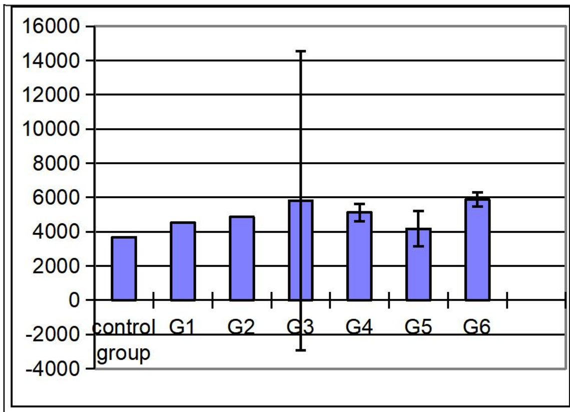
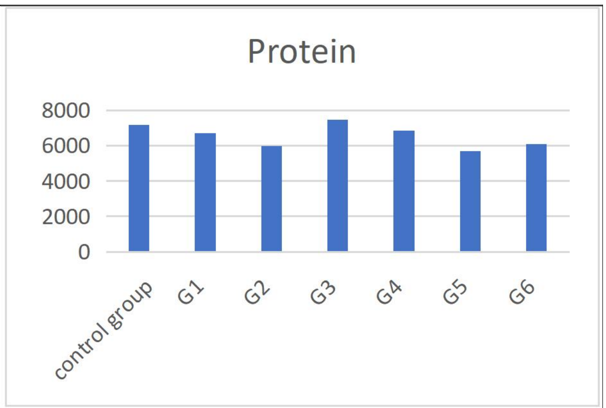


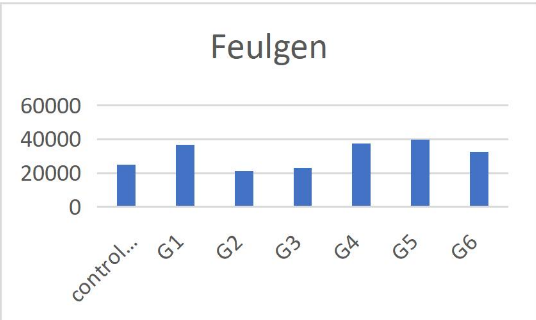
Figure 8. photomicrographs from brain tissue of the different experimental groups stained by Mallory trichrome stain, showing negative deposition of collagen fibers in control free rats (dark blue arrows). Sections from different infected treatment regimens exhibit axonal, neuropil, and neurofibrillary degenerative changes of variable intensity, accompanied by a golden yellow positive reaction in the remaining unaffected myelinated fibrils (red arrows). No collagen deposition is seen. Typical brain tissue structures marked by dark blue arrows. (x 400).



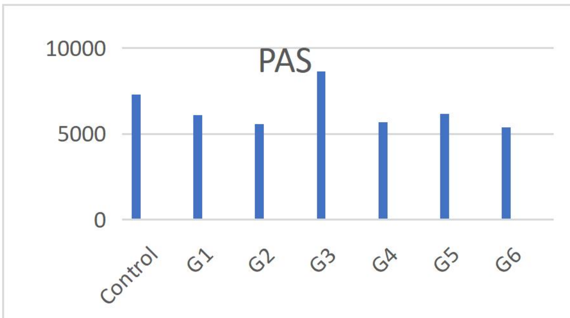
Histogram 1. Revealing the optical density values (mean±SD) of Cong red materials in the brains of mice of the different experimental groups.



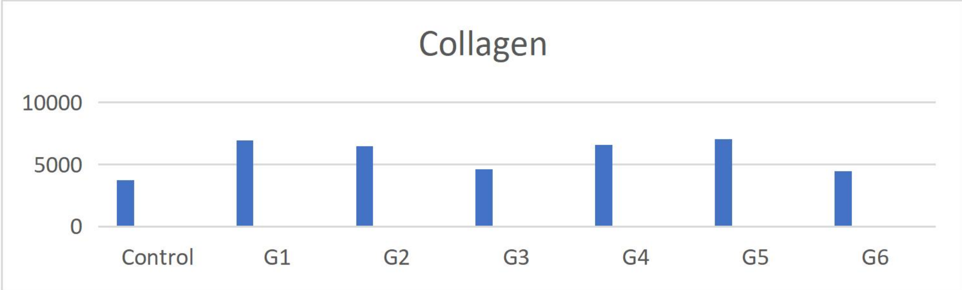
Histogram 2. Revealing the optical density values (mean±SD) of Protein materials in the brains of mice of the different experimental groups.



Histogram 3. Revealing the optical density values (mean±SD) of Feulgen materials in the brains of mice of the different experimental groups.



Histogram 4. Revealing the optical density values (mean±SD) of PAS +ve materials in the brains of mice of the different experimental groups.



Histogram 5. Revealing the optical density values (mean±SD) of Collagen materials in the brains of mice of the different experimental groups.

DISCUSSION

Hymenolepis nana (*H. nana*), commonly known as the dwarf tapeworm, is particularly prevalent in children, with high infection rates reported in Egypt and other tropical regions. Due to their compromised immune status, children infected with *H. nana* may develop neurological symptoms, including headaches, dizziness, and seizures (Cabada et al., 2016). The neurological effects of parasitic infections often arise from the direct invasion of the CNS, as seen in cases of *Toxoplasma gondii* and African trypanosomes (Carabin & Ndimubanzi, 2011). Although the CNS lacks a conventional lymphatic system, alternative immune communication pathways exist, such as cerebrospinal fluid drainage to cervical lymph nodes (CLNs). The neurovascular unit (NVU) plays a pivotal role in maintaining CNS immune privilege by limiting leukocyte infiltration and protecting neural tissues from inflammatory damage (Bechmann & Woodroofe, 2014). However, the exact contribution of the parenchymal vasculature remains unclear, particularly in the brain. While research has shown the importance of the brain's vasculature in delivering nutrients and oxygen, as well as removing waste products, many aspects of its function, particularly its role in metabolic homeostasis and cerebrospinal fluid (CSF) clearance, remain unclear. The relationship between meningeal vessels and immune responses remains inadequately studied (Dyrna et al., 2013). In *Toxocara canis* (*T. canis*) infections, the absence of inflammatory cell infiltration in brain tissue may result from antigen mimicry, enabling the parasite to evade immune detection. Alternatively, intrinsic CNS mechanisms may actively suppress inflammation to minimize tissue damage. This observation aligns with previous findings where *T. canis*-infected mice exhibited minimal inflammation despite a significant parasitic burden (Othman et al., 2010).

Furkuoka et al. (2003). Reported Neuropathological Lesions in Rabbits Infected with *Baylisascaris procyonis* Larva Migrans: Infected rabbits exhibited neurological symptoms such as circling, head tremors, and ataxia. Pathological analysis revealed extensive malacic lesions in the cerebellum, accompanied by astroglial proliferation, perivascular cuffing, and lymphatic infiltration. Additionally, the identification of ascarid larvae in brain tissue, specifically those measuring 65–75 μm in diameter, was reported in brain tissues (CDC, 2019).In our study, histopathological analysis of *H. nana*-infected mice demonstrated the accumulation of periodic acid-Schiff (PAS)-

positive material within blood vessel walls and surrounding stroma. This finding, reminiscent of immune complex-mediated reactions observed in experimental *Schistosoma* infections, highlights a potential immunopathological response (Fu CL, et al., 2012). Astrocytes, key glial cells in the CNS, respond to injury by undergoing gliosis, a process characterized by cytokine release, protein expression changes, and structural remodeling. Recent studies suggest that viral infections may induce oxidative stress by generating reactive oxygen species (ROS), which are a contributing factor to cellular damage (Janicka et al., 2024). Understanding these mechanisms in the context of *H. nana* infection could provide valuable insights into its long-term neurological impact. Bhosale (2022) confirmed that while *H. Nana* infections are often asymptomatic; larval stages may provoke symptoms. In immunocompromised individuals, chronic infections may lead to severe CNS involvement. Revealing the optical density values of protein materials in the brains of mice from the different experimental groups (Histogram 2) recorded in immunosuppressed mice, potentially due to an increased parasitic burden. Praziquantel hepatotoxic, genotoxic, and carcinogenic properties have sparked worries despite its extensive use (Omar et al., 2005).

Growing resistance to conventional anthelmintics underscores the urgent need for alternative therapies. A recent case study by Galos et al. (2022) on plant-derived essential oils reported an atypical neurological presentation of *Hymenolepis diminuta* infection in an infant who subsequently recovered without long-term neurological sequelae. These findings highlight the intricate interplay between parasitic infections and host immune responses, underscoring the need for innovative treatment strategies. Our study highlights the challenges in detecting *H. nana* larvae in brain tissue. *Hymenolepis nana* larvae do not directly invade brain tissue; they primarily infect the small intestine. The life cycle of H. nana involves the ingestion of eggs, which hatch into oncospheres (larvae) that penetrate the intestinal villi, develop into cysticercoid larvae, and eventually mature into adult worms. While *H. nana* infection can cause intestinal inflammation and potentially lead to systemic effects, brain invasion is not a typical part of its life cycle. Brain tissue invasion by H. nana larvae is a rare and unusual occurrence (Muehlenbachs et al., 2015), as their presence is rarely observed. Future studies should focus on establishing a standardized model for investigating CNS parasitic infections. Given that some parasites exhibit erratic migration patterns, they may invade ocular or other extraneural tissues.

CONCLUSION

Histopathological alterations in the brains of infected mice were progressive and exacerbated under immunosuppressive conditions, correlating with increased cerebral parasite burden. Histochemical staining (e.g., hematoxylin and eosin) revealed significant Neuropathological changes. However, further research is needed to determine whether these alterations are directly caused by the presence of the parasite or by host immune responses to infection and treatment.

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