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# NOVEL INSIGHTS INTO PRO-APOPTOTIC AND ANTI-PROLIFERATIVE ANTILARVAL ACTIONS OF IVERMECTIN ON CAMEL NASAL BOTS (*Cephalopina titillator*)

**Yehia Aljasim[1], Ahmed Aljazzar[2], Mohammad Al-Sabi[3], Sameer Alhojaily[1], Ibrahim Albokhadaim[1], Ahmed Alnaeem[1] and Mahmoud Kandeel[1]**

[1]Department of Biomedical Sciences, [2]Department of Pathology, College of Veterinary Medicine, King Faisal University, 31982 Al-Ahsa, Saudi Arabia

[3]Department of Basic Veterinary Medical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan

## ABSTRACT

Camel nasal myiasis, caused by the larvae of *Cephalopina titillator*, poses significant health and economic burdens in tropical and subtropical regions. Ivermectin, a widely used antiparasitic, has shown promise, but its detailed tissue damaging effects and its mechanisms on this parasite remain underexplored. This study was aimed to elucidate the mechanism of action of Ivermectin against *C. titillator* larvae, the causative agent of camel nasal myiasis, by evaluating its histopathological and immunohistochemical effects on larval tissues, focusing on cellular structures, proliferative activity, and apoptosis. Larvae were collected from slaughtered camels in Al-Ahsa, Saudi Arabia, and exposed to Ivermectin (1 µg/ml) for 8 hours. Histopathological analysis assessed tissue damage in the cuticle, muscles, fat bodies, and gut. Immunohistochemistry was performed to detect Proliferating Cell Nuclear Antigen (PCNA) and caspase 3 expressions to evaluate cell proliferation and apoptosis, respectively. Control larvae received vehicle treatment. Ivermectin induced widespread damage across multiple larval strucutres. Structural barriers were compromised through cuticle thinning, locomotion was impaired by extensive muscle degeneration, and metabolic function was disrupted by fat body necrosis and lipid depletion. Digestive capacity was adversely affected by gut epithelial degeneration and brush border disruption. At the cellular level, ivermectin markedly suppressed PCNA expression in gut epithelium, indicating inhibition of tissue renewal, while strongly activating caspase-3-mediated apoptosis in the cuticle, fat body and gut. These combined effects suggest irreversible functional collapse rather than transient paralysis. Ivermectin impairs larval structural and cellular integrity, mobility, and metabolic functions while promoting apoptosis and inhibiting tissue repair. These findings clarify its larvicidal efficacy and support its use in managing *C. titillator* infestations in camels.

**Key words:** Antiparasitic, cephalopina, histopathology, immunohistochemistry, ivermectin

Camels are resilient creatures that can tolerate extreme temperatures and a lack of water (Bagiyal *et al,* 2024). However, they are susceptible to several diseases that might endanger their health (Khalafalla, 2023; Khalafalla and Hussein, 2021), such as "camel's nasal myiasis," which is caused by the larvae of the *C. titillator* fly, a member of the Oestridae family (Angulo-Valadez *et al,* 2010). *C. titillator* fly implants its larvae into the camel's nostrils, where they remain for eleven months. Infected camels exhibit a range of respiratory symptoms, including mucous discharge, mucous membrane inflammation, irritation, widespread petechial haemorrhaging, respiratory issues, many localised granulomas, and tissue destruction (Zayed *et al,* 2008).

The presence of these larvae in camels causes enormous economic losses. While there are several approaches to managing this, one approach is medical treatment using drugs that activate or inhibit neural channels and receptors (Basu and Charles, 2019). Given the wide variety of anti-parasitic drugs on the market, it was recommended to conduct *in vitro* tests on the larvae to determine the most effective treatment. Furthermore, a side-by-side evaluation of Ivermectin's larvicidal activities will be performed to determine its efficiency against the parasite (Aljasim *et al,* 2024; Subramanyam and Colecraft, 2015). Nasal bots are a major health concern in camels, hurting their overall well-being and productivity. This problem is especially prominent in places like Al-

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Ahsa, Saudi Arabia, where camel farming is not only a cultural tradition but also a vital part of the local economy (Fatani and Hilali, 1994).

The purpose of this study was to investigate the mechanism of action of Ivermectin on the larvae of *C. titillator*, the causative agent of camel nasal myiasis, by examining its histopathological and immunohistochemical effects. The study was aimed to determine how Ivermectin impacts larval tissues, including the cuticle, muscles, fat bodies, and gut, as well as its influence on cellular proliferation (PCNA) and apoptosis (caspase 3). By analysing these effects, the research sought to provide insights into Ivermectin's efficacy in disrupting larval integrity, mobility, and metabolic processes, ultimately contributing to a better understanding and management of nasal bot infestations in camels, which have significant economic and health implications.

## Materials and Methods

### *Animals and Collection of Larvae*

All procedures were conducted as previously described by Aljasim *et al* (2024). The study involved adult camels (aged ≥8 years) of a local Saudi breed, which were slaughtered at the Omran abattoir in Al-Ahsa, Eastern Province, approximately 328 km from Riyadh, Saudi Arabia, during 2022 and 2023.

Following slaughter, the nasal cavities of the camels were carefully examined for the presence of larvae. Detected larvae were collected using sterile techniques and placed into clean, sterile containers with tight-fitting lids to prevent contamination with blood or mucus. No more than five larvae were placed in each container. The collected specimens were then promptly transported to the laboratory for antiparasitic assays.

All procedures were approved by the King Faisal University ethics committee (approval number KFU-REC-2022-NOV-ETHICS308).

### *Antiparasitic assay*

During our previous research, the estimated IC50 of Ivermectin was estimated to be 0.0735 ± 0.016 µg/ml (Aljasim *et al,* 2024). During the current assay, the larvae were exposed to one µg/ml Ivermectin concentration, which is more than ten times higher than the estimated IC50 value. Larvae were placed in the culture media mixed with a drug to dwell in. All larvae were then placed in an incubator at 25°C for 8 hours. At the end of incubation, all dead larvae were collected for histopathology examination. A control group was kept in the same conditions without drug treatment. The control group received the same volume of the culture medium without drugs (Aljasim *et al,* 2024).

### *Histopathological examination*

Larvae specimens from both the treatment and control groups were collected for further study. After being collected, the specimens were rinsed with saline. The specimens were then put in a 10% neutral formalin solution, followed by rinsing overnight. The samples were then dehydrated using 70% ethyl alcohol and immersed in xylol. The clarified samples were then carefully placed in a covered jar containing a 50% combination of paraffin and xylol and heated to 37°C for three hours. Following the infiltration operation, the samples were immersed in molten paraffin at 48°C for another two hours. After that, the specimens were embedded in paraffin and stored in a cold environment to form a solid block that could be sectioned. Finally, 5 micron-thick sections were cut using a microtome, stained with Hematoxylin and Eosin (H & E), and mounted onto slides using DPX on a glass slide with a cover slip to be studied under a light microscope (Barbosa, 1975; Basu and Charles, 2019).

### *Immunohistochemistry examination*

The immunohistochemistry procedure was performed as previously described (Attia and Mahdy, 2022; Ibrahim *et al,* 2023). The tissue samples from the larvae were tested for PCNA and caspase 3, which are involved in cell cycle control and apoptosis, respectively.

## Results

The histological analysis of the larval body segment stained by H&E revealed the presence of significant anatomical features with an outer protective cuticle, highlighting its role in safeguarding the larva. Furthermore, well-developed striated muscles were identified, indicating the larva's capability for movement. Additionally, fat bodies, responsible for energy storage and metabolic processes, were detected. In addition, a food channel suggested its association with digestion and the absorption of nutrients within the larval body.

### *Cephalopina titillator cuticle*

The histological examination of the cuticle in the untreated larva (Fig 1A) reveals an undamaged cuticle with its epidermal layer intact. Additionally, the histological analysis of the cuticle in the

Ivermectin-treated larva at 8 hours post-treatment (Fig 1B) demonstrates mild degradation and a notable reduction in thickness.

### *Cephalopina titillator muscles*

The histological assessment of the muscular layer in an untreated larva (Fig 1C) demonstrates the presence of well-developed striated muscles with regular and dense patterns of staining by hematoxylin-eosin. Furthermore, the histological examination of the muscle in the Ivermectin-treated larva 8 hours after treatment reveals degeneration and fragmentation (Fig 1D).

### *Cephalopina titillator fat bodies*

The histological analysis of the fat body layer in untreated larvae revealed the presence of well-developed lipid droplets (Fig 1E). Moreover, the histological examination of the fat body in the Ivermectin-treated larvae 8 hours after treatment indicated evidence of necrosis and a reduction in the size of lipid droplets (Fig 1F).

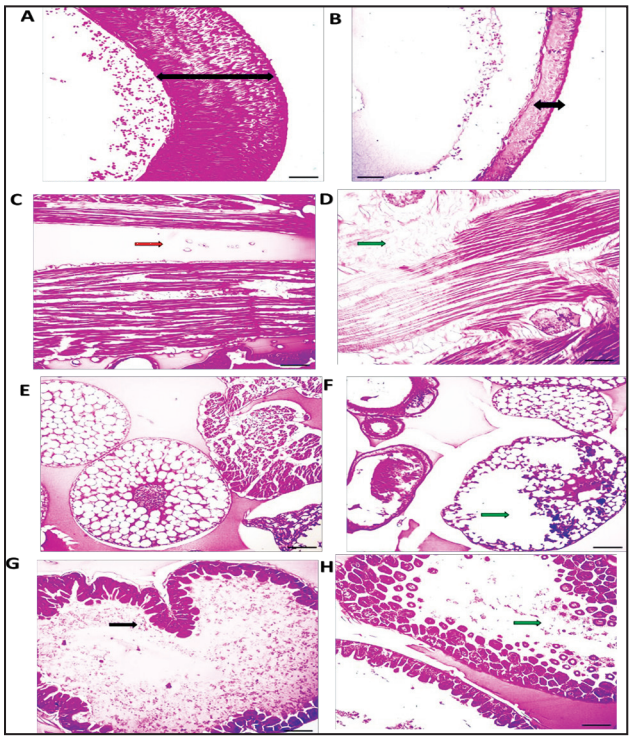


**Fig 1.** Histopathology by H&E (x400). A) *C. titillator* cuticle of untreated larva showing an undamaged cuticle with intact epidermal layer (black arrow). B) *C. titillator* cuticle of Ivermectin-treated larva showing mild degradation and reduction in thickness (black arrow). C) *C. titillator* muscle in an untreated larva showing well-developed striated muscles (red arrow). D) *C. titillator* muscle of Ivermectin-treated larva showing degeneration and fragmentation (green arrow). E) *C. titillator* fat bodies of untreated larva showing well-developed lipid droplets. F) *C. titillator* fat bodies of Ivermectin-treated larvae with necrosis and a reduction in the size of lipid droplets (green arrow). G) *C. titillator* gut of untreated group showing columnar cells with a large nucleus and a strong basophilic cytoplasm (black arrow). H) *C. titillator* gut of Ivermectin treatment with mild degeneration in the epithelial cells lining the food channel (green arrow).

### *Cephalopina titillator gut*

The gut tissue of the control showed the epithelium resting upon a basement membrane consisting of columnar cells with a relatively large nucleus and a strong basophilic cytoplasm. A detached sheath is known as a peritrophic membrane enclosing the lumen to shield the epithelium from food particles. The brush border present in the epithelial cells of the midgut exhibited a consistently tight and intact structure (Fig 1G). However, after Ivermectin treatment, mild degeneration was observed in the epithelial cells lining the food channel, characterised by the protrusion of cells and a thinning of the brush border (Fig 1H).

### *Immunohistochemical results*

### *PCNA*

The immunohistochemical analysis for PCNA in untreated larvae revealed positive nuclear staining in the gut epithelium (Fig 2A), indicating proliferative activity. In contrast, larvae treated with Ivermectin exhibited decreased PCNA immunostaining in the gut epithelium that indicate an decreased regenerative activity after Ivermectin induced insult (Fig 2B). For

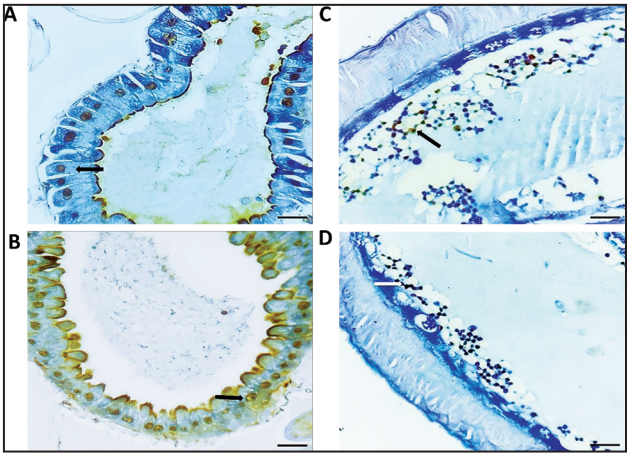


**Fig 2.** Comparative immunohistochemical analysis of PCNA expression in *C. titillator* larvae (x400). A) untreated larvae, showing positive nuclear PCNA staining in the gut epithelium (black arrow). B) larvae treated with Ivermectin revealed reduced PCNA staining in the gut epithelium (black arrow). C) untreated larvae, showing positive nuclear PCNA staining in the immune cells (white arrow). D) larvae treated with Ivermectin, revealing no change in PCNA staining in the immune cells (white arrow). Scale bar = 100 µm.

immune cells, no change in staining was detected between treated and untreated groups (Fig 2C and D).

### *Caspase 3*

The immunohistochemical examination for caspase 3 in the cuticle of untreated larvae showed no positive staining (Fig 3A). However, larvae treated with Ivermectin displayed positive caspase 3 immunoreactivity in the cuticle (Fig 3B)

The immunohistochemical examination for caspase 3 in fat body cells of *C. titillator* untreated larvae showed no positive caspase 3 staining (Fig 3C). While larvae treated with Ivermectin displayed positive caspase 3 immunoreactivity (Fig 3D).

The immunohistochemical examination for caspase 3 in Gut Cells of *C. titillator* untreated larvae showed no positive caspase 3 staining (Fig 3E). But, Gut cells of larvae treated with Ivermectin, displayed positive caspase 3 immunoreactivity, suggesting induced apoptosis in these regions (Fig 3F).

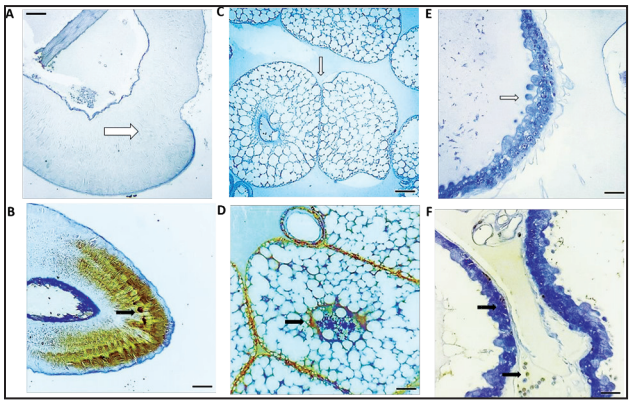


**Fig 3.** Immunohistochemical profiling of Caspase 3 expression (x400). A) Cuticle of *C. titillator* untreated larvae with no positive caspase 3 staining (white arrow). B) Cuticle of larvae treated with Ivermectin, displaying positive caspase 3 immunoreactivity (black arrow). C) fat body cells of *C. titillator* untreated larvae showing no positive caspase 3 staining (white arrow). D) fat body cells of larvae treated with Ivermectin, displaying positive caspase 3 immunoreactivity (black arrow). E) Gut Cells of *C. titillator* untreated larvae with no positive caspase 3 staining (white arrow). F) Gut cells of larvae treated with Ivermectin, displaying positive caspase 3 immunoreactivity, suggesting induced apoptosis in these regions (black arrow). Scale bar = 100 µm.

## Discussion

There have been reports of tropical botflies (*C. titillator*) infesting camels (*Camelus dromedarius*) in Iraq (Al-Ani *et al,* 1991), Libya (El-Rahman, 2010), Saudi Arabia (Banaja and Ghandour, 1994), Sudan, and Iran (Oryan *et al,* 2008). Many investigations on parasite infections in camels in Jordan have discovered infestations of *C. titillator* (Al-Ani *et al,* 1998).

Ivermectin binds to glutamate-activated chloride channels in nematode nerve and muscle cells with high specificity and affinity. This binding leads nerve or muscle cells to become hyperpolarised, increasing the chloride ion's permeability across the cell membrane. As a result, the larvae become immobile and eventually perish (Pemberton, 2001).

Ivermectin is hypothesized to be neurotoxic in larvae because it activates glutamate-gated chloride ion channels. Increased permeability to chloride ions and hyperpolarisation of nerve cells causes paralysis and death in the larvae. In addition to the enhanced activity of GABA-gated chloride channels. Because mammals lack GluCls channels and have a lower affinity for other mammalian chloride channels, they are frequently unaffected. These drugs do not alter GABA-gated channels in the mammalian CNS because they do not penetrate the blood-brain barrier (Salman *et al,* 2022).

Ivermectin showed a dose-dependent effect on *C. titillator* larvae, with higher concentrations producing more dead larvae (Aljasim *et al,* 2024). The histological investigation revealed modest degradation of the cuticle, degeneration of the muscle layer, necrosis and reduced lipid droplet size in the fat body layer, and mild epithelial cell degeneration and brush border thinning in the gut tissue. These data suggest that Ivermectin may impair larval integrity, mobility, energy storage, and food absorption, potentially leading to a general decline in larval health.

Proliferating cell nuclear antigen is a key component of nucleic acid metabolism that is involved in replication and repair. It is expressed in the nuclei of cells throughout the DNA synthesis phase of the cell cycle, as well as in the DNA damage tolerance pathway known as post-replication repair (Kelman, 1997). The immunohistochemical investigation shows a more ubiquitous PCNA staining, albeit with lower intensity staining, indicating reduction in both cell proliferation and tissue regeneration in the Ivermectin-treated group. The findings show a considerable reduction in PCNA staining, showing that cellular proliferation, which is required for tissue repair and regeneration, has been hindered. This restriction may provide long-term obstacles to larval growth and development because of a reduced ability to replace damaged or dead cells.

Caspases are cysteine-aspartic acid proteases and have recently received a lot of interest due to their critical involvement in tissue differentiation,

regeneration, and neurological development. This enzyme is a critical zymogen in cell apoptosis and is not activated until it is cleaved by initiator caspases during the apoptotic process (Nadendla *et al,* 2025; Porter and Janicke, 1999; Snigdha *et al,* 2012). They are important regulators of programmed cell death. Among these, caspase-3 is a frequently activated death protease that catalyses the particular cleavage of numerous critical cellular proteins. An increase in caspase-3 immunoreactivity in Ivermectin-treated larvae suggests increased apoptotic activity, particularly in the cuticle, certain fat body cells, and basal gut epithelial cells. These findings highlight the ability of Ivermectin to cause cellular stress, impair normal cellular functioning, and promote apoptosis in treated larvae. Concurrently, caspase-3 staining increases, indicating increased apoptotic activity in a variety of larval organs, including the cuticle, fat body cells, and basal gut epithelial cells. Although apoptosis is a typical component of development and tissue maintenance, its excessive occurrence may cause tissue degeneration and reduced functionality (Shu *et al,* 2018).

Ivermectin may cause hyperpolarisation of fat body cell membranes, inhibiting the uptake of lipids, carbohydrates, and proteins, which results in smaller cytoplasmic inclusions. The presence of these small inclusions may also indicate stress in larvae exposed to ivermectin, suggesting the mobilisation of energy reserves for detoxification. This is consistent with previous findings that attribute insecticide resistance in some mosquito larvae to the action of detoxifying enzymes (Alves *et al,* 2004).

## Conclusions

This study provides novel insights into the antiparasitic action of Ivermectin on the larvae of *C. titillator*, the causative agent of camel nasal myiasis. Histopathological and immunohistochemical evaluations revealed significant structural alterations in treated larvae, including degeneration of epithelial and muscular tissues, vacuolisation, and cellular disintegration. These findings confirm that Ivermectin disrupts key biological systems within the parasite, ultimately leading to its death. These results confirm that Ivermectin exerts its larvicidal effects through multiple pathways—disrupting protective barriers, compromising mobility and energy storage, and inducing programmed cell death. The findings underscore Ivermectin's efficacy as an antiparasitic agent against *C. titillator* larvae infestations in camels.

## Authors' contributions

Conceptualisation: Y.A., A.A., M.A., S.A., I.A., A.An. and M.K.; methodology: Y.A., A.A., M.A. and M.K.; software: M.K.; formal analysis: M.A., and M.K.; resources: A.A., M.A. and M.K.; data curation: Y.A., A.A., M.A. and M.K., project administration: S.A., and I.A., writing original draft preparation: Y.A. and M.K.; writing, review and editing: Y.A., A.A., M.A., S.A., I.A. and A.An.


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## Declarations

There is no conflict of interest.

## Ethics approval

KFU-REC-2022-NOV-ETHICS308.

## Consent for publication

Not applicable.

## Data availability and sharing policy

Data can be requested from the corresponding author upon reasonable request.

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