DOI : 10.5958/2277-8934.2024.00054.5 Vol 31 No 3, p 285-289

COMPARATIVE LARVICIDAL POTENCY OF IVERMECTIN, DORAMECTIN, MOXIDECTIN, AND EPRINOMECTIN AGAINST DROMEDARY CAMEL NASAL BOTS (*Cephalopina titillator*)

Yehia Aljasim[1], Ahmed Aljazzar[2], Mohammad Al-Sabi[3], Sameer Alhojaily[1], Ibrahim Albokhadaim[1] and Mahmoud Kandeel[1,4]

[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

[4]Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt

ABSTRACT

This study evaluates the efficacy of four commonly used antiparasitic drugs, i.e. ivermectin, doramectin, moxidectin, and eprinomectin against *C. titillator* third stage larvae (L3) in dromedary camels. In order to determine and compare the half-maximal inhibitory concentration (IC50) values of ivermectin, doramectin, moxidectin, and eprinomectin for controlling nasal myiasis in dromedary camels, present study was conducted on L3 collected from 200 naturally infested camels from the Eastern Province of Saudi Arabia. Upon slaughter, L3 were collected from the nasopharynx of the infested camels and were incubated in DMEM supplemented with 50% foetal calf serum and penicillin/streptomycin (100 IU). This media was mixed with serially diluted concentrations of ivermectin, doramectin, eprinomectin, before moxidectin from 1 mg/ml to 125 ng/ml. The larvicidal effect of the four drugs was inferred by the IC50 values that were calculated using nonlinear fitting of dose-response inhibition equations. The IC50 values indicated that ivermectin was the most potent drug with an IC50 of 0.0735 ± 0.016 µg/ml, followed by doramectin (0.249 ± 0.116 µg/ml), eprinomectin (0.46 ± 0.24 µg/ml), and moxidectin (11.96 ± 2.21 µg/ml). The efficacy of ivermectin, doramectin and eprinomectin was significantly higher than that of moxidectin. Ivermectin and doramectin could be considered more potent drugs for treating camel nasal myiasis. The results have important implications for the development of effective treatment protocols for managing parasitic infestations in camel populations.

Key words: Camel nasal myiasis, *Cephalopina titillator*, doramectin, eprinomectin, IC50, ivermectin, moxidectin

Camel's nasal myiasis is caused by the larvae of the *Cephalopina titillator* fly, which belongs to the *Oestridae* family (Angulo-Valadez *et al,* 2010). Camels that are infested with *C. titillator* exhibit a range of respiratory symptoms, including mucous discharge, inflammation of the mucous membrane, irritation, respiratory issues and tissue injury. The presence of these larvae in camels lead to significant economic setbacks (Taylor *et al,* 2007).

Prevalence of the second (L2) and third-stage larvae (L3) of the genus *C. titillator* are responsible for the infestation of dromedary camels (*Camelus dromedarius)* (Fatani and Hilali, 1994). The study indicated that the number of sick camels reached its highest point twice a year, in February and September, and that L3 are found far more frequently than L2. In addition, the research suggested that camels should be treated with larvicidals twice a year, in February and September, to eliminate the infestations (Fatani and Hilali, 1994). Another study was conducted in Sudan by removing fly larvae from the camels' nasal canals after they had been slaughtered and exposing the larvae to the following treatments: 2% pumpkin, 7.5% garlic and peppermint, 30% Lupinus, and 0.15% ivermectin (Khater, 2014). According to their findings, using the natural pumpkin oil rather than ivermectin to treat fly larvae was less hazardous to the camel. Nevertheless, ivermectin was found more effective, even though it induced undesirable side effects to the treated camels (Khater, 2014).

Avermectins are a group of 16-membered macrocyclic lactone compounds that can be naturally

SEND REPRINT REQUEST TO MAHMOUD KANDEEL email: mkandeel@kfu.edu.sa

derived or semi-synthetically produced in labs. They originate from the fermentation of the soil bacterium *Streptomyces avermitilis*. Remarkably effective against a broad range of both internal and external parasites, Avermectins have extensive uses in various sectors, such as veterinary and human medicine, farming, horticulture, and in managing pests in an assortment of agricultural goods and ornamental plants (MacNeil *et al,* 1992; Siddique *et al,* 2014). The Avermectins target the gamma-aminobutyric acid (GABA) receptors and Glutamate-gated chloride (GluCl) related to neurotransmission in parasites. These Avermectins are believed to possess neurotoxic properties (Abongwa *et al,* 2017; Crump and Omura, 2011; Parisi *et al,* 2019). The neurotransmitter GABA is responsible for the opening of the organism's chloride ion channels, which leads to an increase in the number of chloride ions in the cell.

The primary objective of this research was to evaluate and compare the larvicidal efficacy of four commonly used antiparasitic drugs—ivermectin, doramectin, moxidectin, and eprinomectin—against *C. titillator* larvae. Specifically, the study was aimed to determine the half-maximal inhibitory concentration (IC50) of each drug to identify the most potent treatment for controlling camel nasal myiasis.

Materials and Methods

The source of the larvae

The study was conducted on L3 collected from 200 naturally infested camels, all of which were slaughtered at the Omran slaughterhouse in Al-Ahsa, Eastern Province, Saudi Arabia, between October 2021 and December 2021. These camels, belonging to a local breed, were at least 8 years old. No information about prior antiparasitic treatment was available for the sampled camels. Given the presence of parasite fauna in the infected animals and the absence of dead larvae in the examined camel heads, it is highly unlikely that these camels had received any form of treatment for the prevention of *C. titillator* infestation.

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

Collection of larvae

Upon slaughter, the nasopharynx of the slaughtered camels were carefully inspected to detect the presence of *C. titillator* larvae by making incisions along the pharynx, allowing full access to the areas where larvae are typically found. Once detected, the larvae were carefully collected manually. and were immediately placed into a sterile plastic container with a tight-fitting lid to maintain a controlled environment free from external contaminants.

The containers were transported to the laboratory within one hour after collection to ensure their viability until further processing. Excess mucuos or blood on the larvae was gently removed using sterile saline solution.

Preparation of incubation media and drug solutions

The incubation medium consisted of Dubbilco's Modified Essential Medium (DMEM, Molequle-ON®, New Zealand) supplemented with 50% foetal calf serum (FCS) and 100 IU Penicillin/Streptomycin antibiotics (Molequle-ON®, New Zealand) (Khater 2014). This was termed as basic medium. In this basic medium, 1 mg of each the following drugs was dissolved to produce stock solutions of the drugs: ivermectin, doramectin, moxidectin, and eprinomectin obtained from commercial sources. From this stock solutions, serial dilutions of each of the experimented drugs were prepared to produce final concentrations of 0.5, 0.25, 0.1, 0.01, 0.001, 0.0005, 0.00025 and 0.000125 mg/ml (Khater *et al,* 2013). These media with the serially diluted drugs were later termed as experimental media.

Larvicidal assays

Five ml of the experimental media were placed in sterile petri dishes, in which 5 L3 were placed. The control group consisted of 5 larvae placed in sterile petri dishes with basic medium, free of drugs. The dishes were incubated at 37°C and 60% relative humidity until the death of the larvae in the experimental media. To protect the larvae from the toxic effect of its products, the media was changed every 6 hours. During the media changing process, the mortality of the larvae was inferred by observation of flaccid body of the larvae with complete absence of movement upon stimulation. The experiment was repeated three times.

The IC50 of the experimented drugs was determined by plotting dose-response values using GraphPad Prism software (version 8), by applying dose-response-inhibition equations. The measured IC50 indicated the concentration of the drug needed to kill half of the larvae (Aykul and Martinez-Hackert, 2016). The values of IC50 were obtained by applying nonlinear regression models using GraphPad Prism software on the results of dead larvae (Lyles *et al,* 2008; Swift, 1997).

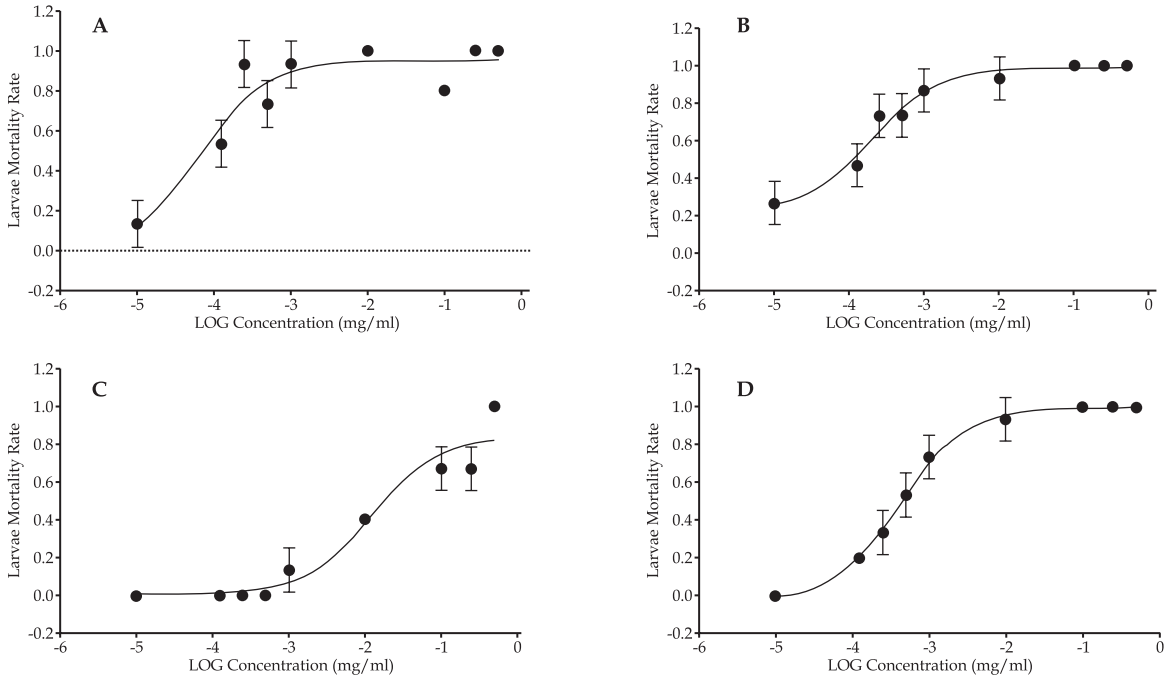

Fig 1. The dose-response curve for the treatment of *Cephalopina titillator* third stage larvae with different concentrations of ivermectin (A), doramectin (B), moxidectin (C), and eprinomectin (D). The X-axis is the log drug concentration (mg/ml). The Y-axis is the fraction of dead larvae after 12 hours after treatment.

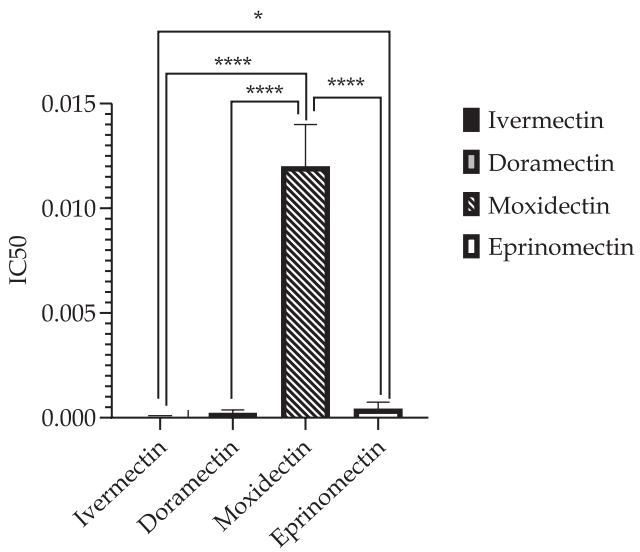

Fig 2. Bar chart showing the Mean and SD of the fraction of dead larvae after treatments. The number of asterisks (*) indicate the power of the statistically significant differences between the groups they are connected to, in which one asterisk is equivalent to ($p < 0.05$) and four asterisks is equivalent to ($p < 0.0001$).

Results and Discussion

IC50 of the experimented drugs

A gradient increase in the fraction of dead larvae was evident with increasing concentrations of all drugs tested (Fig 1). Larvae in the control group remained viable until the end of the experiment period. Additionally, the low standard deviations across different concentrations indicated that the results were consistent across the three replicates of the trials. Nonlinear regression models were applied with an IC50 of 0.0735±0.016 µg/ml for ivermectin, 0.249± 0.116 µg/ml for doramectin, 11.96±2.21 µg/ml for moxidectin, and 0.46±0.24 µg/ml for eprinomectin.

Comparison of the estimated IC50

Fig 2 depicts the fraction of dead larvae after treatment with four different drugs; ivermectin, doramectin, moxidectin, and eprinomectin. By comparing the data from all drugs, ivermectin and doramectin showed the highest efficacy, while moxidectin exhibited the lowest efficacy.

The IC50 values of ivermectin, doramectin and eprinomectin were significantly lower than that of moxidectin ($p<0.0001$), and the IC50 value of ivermectin was significantly lower than that of eprinomectin ($p<0.05$).

By enhancing GluCl ion channels in larvae, Avermectins are thought to be neurotoxic to the larvae (Salman *et al,* 2022). Increased permeability to chloride ions and hyperpolarisation of nerve cells results in the paralysis and death of the larvae. These medications also increase the activity of GABA-gated chloride channels. Due to the absence of GluCls channels in mammals and the reduced

affinity for other mammalian chloride channels, mammals are often unaffected. GABA-gated channels in the mammalian CNS are unaffected by these medications because they typically do not cross the blood-brain barrier in small concentrations (Bloomquist, 1996; Raymond-Delpech *et al,* 2005). Therefore, using minimum, yet effective Avermectin concentrations can be assessed by employing IC50 as a pharmacological metric of the drug potency (Aykul and Martinez-Hackert, 2016).

In this study, ivermectin had a IC50 value of 0.0735 µg/ml, with a standard deviation of 0.016, rendering a very small concentration of it sufficient to inhibit 50% of the *C. titillator*. Comparatively, the IC50 of doramectin was 0.249 µg/ml, with a standard deviation of 0.116. Despite this higher IC50 value than that of ivermectin, doramectin still demonstrated high effectiveness against *C. titillator* larvae. Moxidectin exhibited significantly less effectiveness than the other drugs in reducing the viability of *C. titillator* larvae, while eprinomectin provided a middle range of effectiveness between the tested drugs. Nonetheless, the results of the present study showed a dose-dependent effect of tested drugs on *C. titillator* larvae, with increasing concentrations leading to a higher number of dead larvae. The low standard deviations of the drugs' IC50 signified that the results were consistent across the replicates of the drugs' trials, adding credibility to the findings.

In a previous study which used dipping or fumigation techniques, doramectin and lavender oil showed high efficacy against the larval stages of *C. titillator*; both achieving 100% mortality within 30 hours (Khater *et al,* 2013). The later results showed that doramectin was particularly effective, outperforming essential oils like camphor and onion (Khater *et al,* 2013). In addition, using larvae immersion technique, ivermectin was effective in killing *C. titillator* larvae at low concentrations of 0.15% (Khater, 2014). In comparison, our study effectively simulated the natural habitat by permitting the larvae to reside in a nutrient medium infused with the medications. In addition, we provided detailed IC50 values, which were not reported previously. The stable viability of larvae in the control group ensured that the employed basic media and the incubation conditions supports the lives of the larvae *in vitro* for prolonged periods and that the observed lethal effects were due to the used avermectins. To the best of our knowledge, this study was unique in producing an effective *in vitro* model for evaluating the efficacy of the drugs against *C. titillator* larvae.

Camel nasal myiasis is a common disease among dromedary camels. *C. titillator* infestation was recorded in camels in Iraq (Shamsi *et al,* 2023), Libya (Abd El-Rahman, 2010), Saudi Arabia (Banaja and Ghandour, 1994), Sudan (Musa *et al,* 1989), Iran (Oryan *et al,* 2008) and Jordan (Sharrif *et al,* 1998), which shows its endemicity wherever camels are present. The results of the present study collectively provide valuable insights into the relative effectiveness of the tested drugs against *C. titillator* larvae. Given the widespread nature of *C. titillator* infestation and its significant impact on the health and well-being of affected camels, the findings of this study can be usefully integrated into broader parasitic control and management practices. This would not only aid in alleviating the immediate suffering of the infested animals but also contribute to the sustainable health and productivity of camel populations in the affected regions, with potentially positive implications for the communities that depend on them.

The study provides valuable insights into the comparative efficacy of four drugs; ivermectin, doramectin, moxidectin, and eprinomectin, against *C. titillator* larvae in dromedary camels. Among these drugs, ivermectin exhibited the highest larvicidal potency, followed closely by doramectin then eprinomectin, whereas moxidectin was significantly less effective. The dose-dependent effects of these drugs highlighted the varying degrees of effectiveness, with ivermectin requiring the lowest concentration to achieve IC50 to *C. titillator* larvae. The findings suggest that ivermectin and doramectin could be considered more potent options for treating camel nasal myiasis caused by *C. titillator* larvae. Given the widespread nature of this parasitic infestation and its impact on camel health and productivity, these results have significant implications for the development of effective treatment protocols. Further studies are recommended to validate these findings under field conditions and to explore the long-term effects of these treatments on camel health and productivity.

AUTHORS' CONTRIBUTIONS: Conceptualisation: Y.A., M.A. and M.K.; methodology: Y.A., A.A., M.A. and M.K.; software: M.K.; formal analysis: M.A. and M.K.; resources: M.A. and M.K.; data curation: Y.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: A.A., S.A., I.A. and M.K.

Funding

The authors extend their appreciation to the

Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Project# KFU241819).

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Project# KFU241819).

Declarations

There is no conflict of interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability and sharing policy

Available in manuscript. Further details are available upon request.

References

Abd El-Rahman S. Prevalence and pathology of nasal myiasis in camels slaughtered in El-Zawia Province-Western Libya: with a reference to thyroid alteration and renal lipidosis. Global Veterinaria. 2010; 4:190-197.

Abongwa M, Martin RJ and Robertson AP. A brief review on the mode of action of antinematodal drugs. Acta Veterinaria. 2017; 67:137-152.

Angulo-Valadez CE, Scholl PJ, Cepeda-Palacios R, Jacquiet P and Dorchies P. Nasal bots...a fascinating world! Veterinary Parasitology. 2010; 174:19-25.

Aykul S and Martinez-Hackert E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. Analytical Biochemistry. 2016; 508:97-103.

Banaja A and Ghandour A. A review of parasites of camels (*Camelus dromedarius*) in Saudi Arabia. Journal of King Abdulaziz University. 1994; 6:75-86.

Bloomquist JR. Ion channels as targets for insecticides. Annual Review of Entomology. 1996; 41:163-190.

Crump A and Omura S. Ivermectin,'wonder drug'from Japan: the human use perspective. Proceedings of the Japan Academy, Series B. 2011; 87:13-28.

Fatani A and Hilali M. Prevalence and monthly variations of the second and third instars of *Cephalopina titillator* (Diptera: *Oestridae*) infesting camels (*Camelus dromedarius)* in the Eastern Province of Saudi Arabia. Veterinary Parasitology. 1994; 53:145-151.

Khalafalla AI. Emerging infectious diseases in camelids. Emerging and Re-emerging Infectious Diseases of Livestock. 2017:425-441.

Khater HF. Bioactivities of some essential oils against the camel nasal botfly, *Cephalopina titillator*. Parasitology Research. 2014; 113:593-605.

Khater HF, Ramadan MY and Mageid AD. *In vitro* control of the camel nasal botfly, *Cephalopina titillator*, with doramectin, lavender, camphor, and onion oils. Parasitology Research. 2013; 112:2503-2510.

Lyles RH, Poindexter C, Evans A, Brown M and Cooper CR. Nonlinear model-based estimates of IC50 for studies involving continuous therapeutic dose–response data. Contemporary Clinical Trials. 2008; 29:878-886.

MacNeil DJ, Gewain KM, Ruby CL, Dezeny G, Gibbons PH and MacNeil T. Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilising a novel integration vector. Gene. 1992; 111:61-68.

Musa MT, Harrison M, Ibrahim AM and Taha TO. Observations on Sudanese camel nasal myiasis caused by the larvae of *Cephalopina titillator*. Revue d'élevage et de médecine vétérinaire des pays tropicaux. 1989; 42:27-31.

Oryan A, Valinezhad A and Moraveji M. Prevalence and pathology of camel nasal myiasis in eastern areas of Iran. Tropical Biomedicine. 2008; 25:30-36.

Parisi DP, Santos SAR, Cabral D, Queiroz-Hazarbassanov N, Florio JC, Bernardi MM and Kirsten TB. Therapeutical doses of ivermectin and its association with stress disrupt motor and social behaviours of juvenile rats and serotonergic and dopaminergic systems. Research in Veterinary Science. 2019; 124:149-157.

Raymond-Delpech V, Matsuda K, Sattelle BM, Rauh JJ and Sattelle DB. Ion channels: molecular targets of neuroactive insecticides. Invertebrate Neuroscience. 2005; 5:119-133.

Salman M, Abbas RZ, Mehmood K, Hussain R, Shah S, Faheem M, Zaheer T, Abbas A, Morales B, Aneva I and Martinez JL. Assessment of avermectins-induced toxicity in animals. Pharmaceuticals (Basel). 2022; 15:332.

Shamsi E, Radfar MH, Nourollahifard SR, Bamorovat M, Nasibi S, Fotoohi S, Hakimi Parizi M and Kheirandish R. Nasopharyngeal myiasis due to *Cephalopina titillator* in southeastern Iran: a prevalence, histopathological, and molecular assessment. Journal of Parasitic Diseases. 2023; 47:369-375.

Sharrif L, Al-Rawashdeh O, Al-Qudah K and Al-Ani F. Prevalence of gastrointestinal helminthes, hydatid cysts and nasal myiasis in camels in Jordan. Proceedings of the 3rd Annual Meeting for Animal Production Under Arid Condition United Arab Emirates University. 1998; 2:108-114.

Siddique S, Syed Q, Adnan A and Qureshi FA. Isolation, characterisation and selection of avermectin-producing *streptomyces avermitilis* strains from soil samples. Jundishapur Journal of Microbiology. 2014; 7:e10366.

Swift ML. GraphPad prism, data analysis, and scientific graphing. Journal of Chemical Information and Computer Sciences. 1997; 37:411-412.

Taylor M, Coop R and Wall R. Parasites of the respiratory system. In. Veterinary Parasitology 3rd ed, Blackwell Publishing Ltd, Oxford, UK. 2007:195-199.