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THE DEVASTING EFFECTS OF PRAZIQUANTEL AND ALBENDAZOLE, ON CERCARIAE OF THE DIGENEAN *PHANEROPSOLUS PRAOMIDIS* (BAER, 1971)

By
MOHAMMED E. ELZEINY, OLA A. SAMAK, SHEREEN A. FAHMY, and ABDEL AZIZ A. KHIDR
Department of Zoology, Faculty of Science, Damietta University, Egypt
(Correspondence: mohammedelzeiny@du.edu.eg,olaabusamak@du.edu.eg, shereenfahmy@du.edu.eg & akhidr@du.edu.eg

Abstract

Phaneropsolidae is one of the digenean intestinal parasites of reptiles, birds, and mammals. To cease its life cycle, some cercariacidal drugs were used, such as praziquantel and albendazole. The intermediate host, a freshwater snail, *Lanistes carinatus*, was collected from Al-Inaniyyah village in the north-east of Damietta Governorate, Egypt. *Phaneropsolus praomidis* cercariae (xiphidiocercaria type) were harvested by exposing snails to strong artificial illumination. Lethal effect of both drugs, they were tested on the living cercariae of *Phaneropsolus praomidis*. The emerging cercariae were divided into three groups, the first group (group 1), which is the control group, contained 40 cercariae, the second group (group 2); and the third group (group 3) were to test the effect of praziquantel and albendazole, respectively, on the vitality of cercariae. The LC50 and LC95 of praziquantel against the present cercariae of *Phaneropsolus* in group 2 were 0.03 and 0.21 ppm, respectively. While in group 3, the LC50 and LC95 of albendazole were 0.87 and 2.7 ppm, respectively. Praziquantel has a more devasting effect against the treated cercariae of *Phaneropsolus praomidis* compared with albendazole. The ultrastructural changes using SEM that occurred on the tegumental surface of the treated cercariae with the two drugs were also observed compared to the untreated cercariae. The untreated cercariae have a pentagonal-shaped body with a long tail. In comparisons, cercariae treated with both drugs lost all healthy morphological features, but in varying degrees and effects between the two drugs.

Keywords: *Phaneropsolus*, xiphidiocercaria, *Lanistes,* praziquantel, albendazole

Introduction

Family Phaneropsolidae Mehra, 1935 is a large trematoda family contained 26 digenean species (Lotz and Font, 2008). Some species infect humans, such as *Phaneropsolus bonnie* and *P. spinicirrus* (Lie, 1951; Kaewkes *et al,* 1991). *P. bonnie* intestinal fluke with size 0.5-0.8mmx0.2-0.4mm, large oral sucker, short intestinal caeca, and two large, oval testes laterally found, single oval ovary situated on right of ventral sucker, and large vitelleline follicles in groups, of 8 in each group in worm anterior part (Manning *et al,* 1970). *P. bonnei* life cycle requires first-intermediate snail host, *Bithynia goniomphalus* (Manning and Lertprasert, 1973), and *P. praomidis* first intermediate host is the apple snail *Pomacea canaliculated* (Dellagnola *et al,* 2019).

Albendazole is an FDA-approved medication for treatment of some many varieties of helminthes infections (Naguib *et al,* 2023). Also, Albendazole (=Alzental 200mg) was the topical studies as a potential anticancer agent due to limited toxicity to normal cells, but high toxicity to both tumour and parasitic cells (Movahedi *et al,* 2017). The drug is critical for parasite by bound to intracellular microtubules, preventing their growth elongation (Verrest and Dorlo, 2017).

Praziquantel (=Biltricide) belongs to family of medicines called anthelmintic (mainly trematodes) and used in treating worm infections by causing severe spasms and paralysis of their muscles, and some worms are passed in stools (CDC, 2024). Awadalla *et al.* (1992) in Egypt by the TEM examined praziquantel residual worms after the curative dose found that male worms with deep tortuous pits and sharp apically directed spines, lacked in some regions as well as disruption and vacuolization. Chai (2013) in Korea rep-

orted that the emerged problems with praziquantel treatment, such as resistance in treating *Schistosoma mansoni* and possibly *S. japonicum,* along with the allergic or hypersensitivity reactions.

Generally speaking, albendazole and praziquantel are used in treating intestinal parasites, such as *Opisthorchis viverrini*, *Taenia crassiceps*, and *Mesocestoides corti* (Markos ki *et al,* 2006; Palomares *et al,* 2006). Also, Pechdee *et al.* (2017) reported that albendazole and praziquantel have *in-vitro* anti-cercarial activity on *Opisthorchis viverrini* causing tegumental deformation, induced tail shedding and paralysis. Nevertheless, there was rare study on praziquantel and albendazole effects on *Phaneropsolus praomidis* cercariae (El-Zeiny *et al,* 2024).

This study aimed to use scanning electron microscopy to investigate the lethal effects of praziquantel and albendazole *in-vitro* on *Phaneropsolus praomidis* cercariae.

Materials and Methods

Collection and identification of *Phaneropsolus praomidis* cercariae: The freshwater snail, *Lanistes carinatus* was collected by hand netting from Al-Inaniyyah Village canal on north east of Damietta Governorate, between 31.39"N & 31.81 N".

Phaneropsolus praomidis cercariae (xiphidiocercariae type) were harvested by exposing the snails to strong artificial illumination for 4 to 6 hours, and then identified (El-Zeiny *et al,* 2021).

Drugs effect on cercariae: Praziquantel® and Albendazole® (Sigma-Aldrich Chemise GmbH) were used to determine lethal effects on the live cercariae. They were divided into three groups. G1: 40 cercariae as control, G2: 120 cercariae treated with praziquantel, and G3:120 cercariae treated with albendazole. To determine mean cercarial death, each of G2 & G3 was subdivided into 3 subgroups of 40 cercariae each. The subgroups were exposed to three tested doses of praziquantel (0.025, 0.05, & 0.1ppm) and albendazole (0.8, 1.5, & 2ppm) for two hours respectively. The mortality rate was determined by movement ceasing, and then probit analysis in toxicity using SPSS version 25 to evaluate lethal doses (LC_{50} & LC_{95}) for each drug (Finney, 1952).

SEM: Cercariae were separated into control and treated ones. Cercariae were exposed to lethal doses of praziquantel and albendazole. For examination preparation, cercariae were buffered with sodium cacodylate at pH 7.4 for 2 hours, fixed in 2.5% glutaraldehyde, osmium tetroxide (4%) was used to postfix in the same buffer and dehydrated via an ascending ethanol series (30%, 70%, 96% & absolute), and then coated with gold.

All cercariae were examined using a JEOL JSM 6510 LV SEM in Scanning Electron Microscope Unit at Mansoura University.

Results

The xiphidiocercariae type belongs to the family Phaneropsolidae and genus *Phaneropsolus*. Compared to active movement in the control, cercariae exhibited abnormal movement after 2hours of exposure to both drugs.

Abnormal manifestations include slow tail movement and body contraction, as well as separation of cercarial tail. LC_{50} & LC_{95} of praziquantel against cercariae of *P. praomidis* in were 0.03 & 0.21ppm, respectively. But with albendazole LC_{50} & LC_{95} were 0.87 & 2.7ppm, respectively. The cercarial death rate increased with high doses of both drugs. The increase was more with praziquaantel concentrations than albendazole.

SEM analysis: Control cercarial body was slightly pentagonal-shaped with 37.5x 40µm in size. Tail was longer than its body and was 105x12µm. It was wide proximally and gradually decreased in thinness until reached its distal tip. Oral sucker was terminal, circular in shape and armed with a xiphoid-shaped spine. Oral sucker posteriorly carried ciliated sensory papillae embedded in tegument. Ventral sucker was slightly larger than oral sucker. It protruded from body surface as thick, spiny muscular wall, and small dome-shaped, non-ciliated sensory papillae. Cercarial tail was cylindrical, and covered with parallel transverse rows of tegumental

processes, decorated as dome-shaped, non-ciliated sensory papillae. Tail terminal end, carried longitudinal tegumental striations converged and closely converged to one another, forming a crowded mass projection. Cercarial body tegument was covered with irregularly scattered spines, sometimes non-ciliated sensory papillae.

Cercaria treated with praziquantel showed a semi-ovate body andwas 22x25µm in size. Tail was slightly larger than cercarial body (30x6µm). Oral sucker lacked xiphoid spine and ciliated sensory papillae. Ventral sucker was retracted inside cercarial body, shrunken, with a decline in spiny muscular wall, but without sensory papillae. Treated tail became flattened and serrated. All transverse tegumental processes disappeared. At the tail end, all tegumental were destroyed without tegumental structures**.** The cercarial tegument underwent extensive peeling and erosion with neither spines nor sensory papillae.

Cercariae treated with albendazole showed a semi-circular body 20x60µm in size, and its tail was longer than swollen body (155 x10µm), with thicker transverse tegument Tegument was tiny spines and dome-shape, without ciliated sensory papillae. Oral sucker was contracted with neither spines nor ciliated sensory papillae**.** Ventral sucker was more visible, with a thick, spiny muscular wall, but without any sensory papillae.

Ethical approval: The study protocol was approved by the Ethics Committee of theFaculty of Science, Damietta University, Egypt (Du Res. No.38).

Details were given in figures (1, 2, & 3).

Discussion

El-Zeiny *et al.* (2021) in Egypt described the morphological structure of xiphidiocercaria type in Damietta Governorate.

In the present study, LC_{50} and LC_{95} were 0.036 & 0.2ppm, respectively, for praziquantel and 0.87ppm & 2.7ppm, respectively, for albendazole. Praziquantel was a more effective on *P. praomidis* cercariae than albe ndazole. This agreed with El-Zeiny *et al.* (2024), who reported that praziquantel has more efficacies against the gymnocephalus cercariae of *Echinochasmus* sp. than albendazole. Also, Ben and Useh (2017) reported that praziquantel was more effective than albendazole in treating *S. haematobium*. Alves *et al.* (2019) reported that *Colossoma macropomum* death rate was 100% with albendazole (500 to 2000ppm). But, El-Zeiny *et al.* (2024) found that cercarial vitality rate decreased with albendazole dose than with praziquantel dose.

In the present study, *P. praomidis* cercariae dead number was 21, 30, & 37 with albendazole 0.8, 1, & 1.5 ppm respectively, after 2 hours exposure. Therefore, even if it was proved that this drug has an effect on the parasites generally; it is useful to test drug every time when beginning in a strategy to combat certain parasites.

In the present SEM study, oral sucker of normal cercaria was armed with a xiphoid spine and ciliated-type sensory papillae. Its ventral sucker has a thick, spiny muscular wall and small, dome-shaped, unciliated sensory papillae. Tail contained parallel transverse rows of tegumental processes and unciliated randomly arranged sensory papillae. Body tegument was regularly decorated with overlapped spines, and small, unciliated sensory papillae were distributed irregularly and scattered on ventral sucker, body, and tail. This agreed with Min and Jong-Yil. (1995) in Korea, who studied ultrastructure of *Gymnophalloides seoi* (Digenea: Gymnophallidae) metacercarial tegumental layer. Also, Hong *et al*. (2004) in Korea by SEM of *Macroorchis spinulosus* reported that the sensory papillae acted as chemoreceptors and mechanoreceptors.

In the present study, praziquantel affected the tegument cercariae of *P. praomidis*. The shape of cercarial body changed from pentagonal-shaped in the normal one to semi-ovate in praziquantel treated. By comparing body dimensions of normal and praziquantel ones, there was a marked, reduction of body dimensions, and tail length in treated ones than normal one. Tegument lacked sensory

papillae, spines of oral sucker, and tail transverse processes, and ventral sucker spiny muscular structure were retracted in body. This agreed with Andrews *et al.* (1983) in Germany, who found that praziquantel affected the musculature and caused rupture of tegument of cestodes and trematodes worms. Also, this more or less agreed with Becker *et al.* (1980) who reported praziquantel in-vitro treated *S. mansoni*, *Dicrocoelium dendriticum* and *Fasciola hepatica*. Mehlhorn *et al.* (1981) who found praziquantel in-vitro and in-vivo affected *S. mansoni* causing tegumental vacuolization. Besides, Gonçalves *et al.* (2013) studied praziquantel action on *Echinostoma paraensei* reported that the drug caused damage and erosion of oral sucker and tegumental surface, as well as swelling of the spines collar.

In the present SEM study, the cercarial body and tail width and length treated with albendazole were larger than normal one. Cercarial body and tail appeared fleshy and swelling with deformed oral and ventral suckers were. Based on measurements of praziquantel treated cercariae and normal one, albendazole showed a complex effect depended on the tegumental function and its osmotic capacity.

Lacey (1990) in Australia reported that benzimidazoles is only broad-spectrum anthelmintic class with little or no mammalian toxicity. He added that cercarial tegument treated with benzimidazoles showed tiny spines and irregularly distributed dome-shaped, non-ciliated sensory papillae.

However, Chen *et al.* (1983) in China reported that praziquantel is extensively metabolized by the liver via the cytochrome $P4_{50}$ system and might cause hepatic injury as a result of a toxic intermediate of its metabolism. Khafagy (1999) in Egypt reported that among 2157 children treated with praziquantel, the commonest symptoms were abdominal colic (81.1%), diarrhoea (57.8%), dizziness (23.0%), nausea (18.2%), headache (14.4%), vomiting (14.1%) and allergic skin rash (1.5%). Omar *et al.* (2005) reported that praziquantel *in-vitro* proved to be hepatotoxic, genotoxic, and carcinogenic drug. Harm *et al.* (2022) in USA reported that albendazole was a widely used anthelmintic drug for specific nematodes and flukes in ruminants, but its toxicosis was reported in the pigeons, doves, alpacas, humans, dogs, and cats.

Conclusion

Albendazole and praziquantel interrupted the worm life cycle as an effective treatment control. Both drugs on *Phaneropsolus praomidis* cercariae caused aggressive erosion on tegument, tail lost ciliated sensory papillae, oral sucker's xiphoid spine, as well as destruction of oral and ventral suckers.

The cercarial mortality increased with the increase of drugs' doses.

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Data availability: All the study data were presented in the materials and manuscript were used in of the study highly quality rate.

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Explanation of figures

Fig. 1: Mortality rate of *Phaneropsolus praomidis* cercariae at various concentrations of praziquantel and albendazole, respectively, at 2hr. exposure. a- Praziquantel effects on cercariae of at lethal doses of 0.025, 0.5, & 0.1 ppm, respectively, & b- Albendazole effects on cercariae at lethal doses of 0.8, 1, &1.5 ppm, respectively. Number columns= cercarial maximum death M ±SD of each dose.

Fig. 2: SEMs of control cercariae emerged from *L. carinatus*. a- Ventral view showed a pentagonal-shaped body (B) and elongated tail (T), b-Terminal oral sucker (Os), surrounded by ciliated sensory papillae (arrow heads) and armed with a xiphoid spine (Xi), c- Ventral sucker (Vs) with thick rows of spiny muscular wall (S), d- Tail (T) transverse cytoplasmic processes (scale bar 2µm). e- Tail end (TE) with a mass of crowded projections without ciliated sensory papillae (arrow), & f- Dorsal tegumental body (TB) rough with tiny spines (S) and dome-shaped unciliated sensory papillae (arrows).

Fig. 3: SEMs of praziquantel treated cercariae. a- Ventral view showed a semi-ovated body (B) and a deformed tail (T), b- Oral sucker (Os) destroyed and devoid of sensory papillae and xiphoid spines, c- Ventral sucker (Vs) shrunken with a decline in its spiny muscular wall (S), d- Tail (T) serrate without tegumental transverse processes, e- Tail ends (TE) destructed with neither tegumental structures nor sensory papillae. & f- Body tegument (TB) deformed with tiny spines (S).

Fig. 4: SEMs of albendazole treated cercariae. a- Ventral view showed a semi-circular, swollen body & elongated tail, b- Oral sucker destroyed spine, c- Ventral sucker swollen and thick rows of spines, d- Tail swollen tegumental transverse structures, e- Tail end swollen, conical, and devoid of any sensory papillae or tegumental processes, & f- body tegument deformed with tiny spines.