Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26 (1): 491 – 503 (2022)
www.ejabf.journals.ekb.eg

In vitro evaluation of antihelminthic activity of Praziquantel, Flubendazole and some plant extracts against the adult eye fluke (*Philophthalamus palpebrarum*) transmitted by the freshwater snail *Melanoides tuberculata*.

***Asmaa Abdel-Motleb[1]; Magda Ayoub[1]; Menerva Tadros[1]; Fatem Ramzy[2], Samia El-Bardicy[1]**

1- Environmental Research and Medical Malacology Division, Theodor Bilharz Research Institute
2- Parasitology Division, Theodor Bilharz Research Institute, Egypt.

*** Corresponding author: a_abdelmotlb@yahoo.com**

ARTICLE INFO

Article History:
Received: Feb. 4, 2022
Accepted: Feb. 15, 2022
Online: Feb. 19, 2022

Keywords:
Philophthalamus palpebrarum;
Praziquantel;
Flubendazole;
Jatropha curcas;
Moringa oleifera.

ABSTRACT

Philophthalmus species (the eye flukes) are digenetic trematodes that cause damage to the ocular tissues of birds and humans. Diagnosis of subclinical philophthalmosis and effective therapeutic regimens are lacking, despite the increasing number of cases reported in birds and humans. So, the present study aimed to evaluate in vitro the antihelminthic activity of Praziquantel (PZQ), Flubendazole, and plant leaves extracts (*Jatropha curcas* and *Moringa oleifera*) against the adult eye fluke *Philophthalamus palpebrarum* (*Philophthalmidae*) which extracted from the conjunctival sac of the experimentally infected chickens eyes by excysted metacercariae The cercariae obtained from the naturally infected freshwater snails *Melanoides tuberculata* collected from watercourses in Giza Governorate, Egypt. The efficacy was determined based on the dead worms percentage after treatment and morphological changes recorded by scanning electron microscopy. The worms were incubated in the culture media with different concentrations tested materials up to 72 h. The calculated LC_{50}were 7.857, 9.170, 65.534 &44.509µg/mL and LC_{90}were 10.149, 13.489, 91.474 & 53.004µg/mL, respectively. Worms incubated for 48h with LC_{50} of PZQ and *Moringa* revealed deformity, swelling of oral and ventral suckers, erosion, and disorganization of the tegument when examined with scanning electron microscopy. It can be concluded that Praziquantel, Flubendazole, *Jatropha curcas*, and *Moringa oleifera* plant leaves extracts possess in vitro antiphilophthalamus activities; however, studies in vivo should be conducted to examine their antiphilophthalamus effects.

INTRODUCTION

Philophathalamus palpebrarum are small trematodes that have the freshwater snail *Melanoides tuberculata* (**Müller, 1774**) as intermediate host and parasitize primarily the eyes (conjunctival and orbital tissues) of wild and domestic birds, commonly associated with freshwater birds and wading birds (**Kingston, 1984; Sapp *et al*., 2019**). Transmission to birds occurs by ingestion of encysted metacercariae on substrates (crustacean shells, vegetation, etc) or direct ocular contact with cercariae or metacercariae in water (**Alicata and Ching, 1960)**. The freshwater snail *M. tuberculata* is known to be the intermediate host of several species of the eye fluke. Eye flukes originating in *M. tuberculata* have been identified as *Philophathalamus grallli* in the Middle East, specifically the United Arab Emirates **(Ismail and Arif, 1993),** Jordan **(Ismail and Issa 1987)**, Saudi Arabia **(Kalantan *et al*., 1998).** Also **Radev *et al*. (1999)** recorded *Philophathalamus lucipetus* in Israel that was considered an European species in the past. While, in Egypt *Philophathalamus distomatosa* was first known by its larval stage, Cercaria *distomatosa* and its adult worm was experimentally grown and described by **Radev *et al*. (2000).** Also, has been found Venezuela (**Diaz *et al*., 2002**), Brazil (**Verocai *et al*., 2009**) and in Zimbabwe (**Mukaratirwa *et al*., 2005**). All these mentioned species have the freshwater snails of the family Thiaridae (*Melanopsis praemosa*, *M. tuberculata*, *Cleopatra bulinoides*, and *Tarebria granifora*) as intermediate hosts. *Philophathalamus hegeneri* is the only representative of *Philophthalmus* in Arabia developing in a marine snail (*Cerithium scabridum*) which was found in Kuwait (**Abdul-Salam *et al*., 2004**). Moreover, only the adult worm of *P. palpebrarum* recorded in Egypt by **Looss (1907 and 1899)** from the eyes of Yellow Billed Kite and Little Owl. But, **Ayoub *et al*. (2020)** completed and described life cycle of *P. palpebrarum* experimentally and identifying the larval stages originating in *Melanoides tuberculate* snails for the first time in Egypt.

In general a variety of mammals, including humans, occasionally serve as the intermediate as well as the definitive hosts for the *genus Philophathalamus*. Moreover, the zoonotic philophthalmiasis in human cases has been also documented in various parts of the world (**Sapp *et al*., 2019 and Prompetch *et al*., 2021**). **Lang *et al*., 1993; Kanev *et al*., 1993** & **Nollen and Kanev, 1995** reported cases of human infection and conjunctivitis in eyes due to infection with *P. palpebrarum*; *P. lucipetus* and *P. lachrymosus*, respectively. Recently, a unique pattern of granulomatous anterior uveitis in rural children swimming in El-Fayoum, Egypt, was attributed to a waterborne helminthic infection (**Nadar *et al*., 2017**). It is usually minimally pathogenic in natural hosts; heavy burdens and/or infections inaberrant hosts may be associated with conjunctivitis, epiphora, hemorrhage, and itching/swelling of the eyelids (**Pinto *et al*., 2005 and Heneberg *et al*., 2018**). Despite the increasing number of cases reported in birds and human, studies related to the diagnosis of subclinical philophthalmosis are lacking, and there are no effective therapeutic regimens available (**Assis *et al*., 2018**).

Praziquantel (PZQ), an acetylated quinoline-pyrazine, is a medication used to treat a number of types of parasitic worm infections in mammals, birds, amphibians, reptiles, and fish (**WHO, 2009**). In humans specifically, it is used totreat schistosomiasis, clonorchiasis, opisthorchiasis, tapeworminfections, cysticercosis, hydatid disease, and other fluke infections (**WHO, 2009 and Heneberg *et al*., 2014**). Also, Flubendazole has wide-spectrum activity against various nematodes and cestodes affecting human. It is a member of the class of mebendazole in which the benzoyl group is replaced by a p-fluorobenzoyl group (**William *et al*., 2003**).

Plants represent an important source for drug discovery and have produced some very effective chemotherapeutic treatments for parasites, eg. *Jatropha curcas* (*Euphorbiace*) and *Moringa oleifera* (*Moringaceae*) (**Eguale & Giday, 2009**). *J. curcas* is a species of flowering plant in the genus *Jatropha*, family *Euphorbiaceae*, originates in Central America and Mexico, and has been naturalized in a number of other tropical and sub-tropical countries in Asia, Africa and Latin America. Its roots, stems, leaves seeds and fruits of the plant have been widely used in traditional folk medicine in many parts of West Africa. The seeds have been used as purgative, anthelmintic and abortifacient, for treating ascites, gout and skin diseases. Its seeds have also been reported to be effective against *Strongyloides papillosus* infection in goats (**Vollesen *et al*., 1995**).

M. oleifera belongs to the family of *Moringaceae*, originates in the Himalayas in north western India, but this species has been planted in tropical and subtropical climates throughout the tropics (**Tilman *et al*., 2006**). *M. oleifera* is one of the important plants which have bioactive compounds with nutritive and medicinal values present in the leaves, seeds, roots, barks, and flowers (**Fahey, 2005**). In addition, this plant was utilized in the treatment of malaria, leishmaniasis, trypanosomiasis, schistosomiasis, dracunculiasis, and filariasis thus suggesting its inherent antiparasitic property (**Fahey, 2005 and Wang *et al*., 2016**). Therefore, the main aim of the present study is an attempt to evaluate the antihelminthic activity of Praziquantel, Flubendazole and some plant extracts (*J.curcas* and *M. oleifera*) against the adult eye fluke *P. palpebrarum* (*Philophthalmidae*).

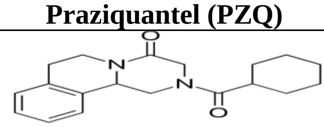
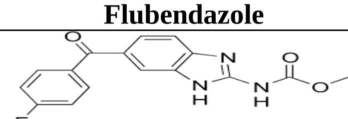
MATERIALS AND METHODS

1.Experimental materials:

Praziquantel (PZQ)**($C_{19}H_{24}N_2O_2$)**, an acetylated quinoline-pyrazine,sold under the brandname **Biltricide**

Flubendazole, sold under the brandname **Fluver**, It is a member of benzimidazoles, a carbamate ester, an organofluorine compound and an aromatic ketone(**C16H12FN3O3).**
The Physical and chemicalproperties of the two compounds are shown in **(Table 1).**

Table (1): Some physical and chemical properties of Praziquantel (PZQ) and Flubendazole

	Praziquantel (PZQ)	Flubendazole
Structure		
Molecular formula	**$C_{19}H_{24}N_2O_2$**	**C16H12FN3O3**
Synonyms	**Biltricide** **Droncit** **Pyquiton**	**Flubendazol** **Flumoxane** **Fluvermal**
Molecular weight(g)	**312.4**	**313.28**

Preparation of plant extracts: 2 species of plants used in this study consists of *J. curcas* and *M. oleifera* (Family: *Euphorbiace* and *Moringaceae*, respectively). The scientific classification of the two species are shown in **Table 2**. The leaves of each plant, was collected for preliminary bio-screening They were collected from EL-Orman Garden, Giza during full growing season (March, 2020). Plants identification was carried according to **Boulos (1999 and 2000).** At 40°C the leaves were dried in a solar oven and extracted with ethanol at ambient temperature by percolation. Extracts were filtered and methanol was evaporated to dryness under reduced pressure and totally freed from water by freeze drying, and stored under freezing at −20°C until used.The main bioactive compounds of ethanol leaf extract of *J. curcas* and *M. oleifera* were shown in **Table 3**.

Table (2): Scientific classification of *Jatropha curcas* and *Moringa oleifera*

Kingdom: Plantae	
Order: Malpighiales	**Order: Brassicales**
Family: Euphorbiaceae	**Family: Morinaceae**
Genus: *Jatropha*	**Genus: *Moringa***
Species: *J. curcas*	**Species: *M. oleifera***

Table 3. The main active compounds of ethanolic leaves extract of *Jatropha curcas* and *Moringa oleifera*.

Bioactive compounds	*Jatropha curcas*	*Moringa oleifera*
flavonoids	+	+
alkaloids	+	+
Tannins	+	+
Saponnins	+	+
Cyanogenic glycosides	+	+

2. Parasite material

Cercariae of *P. palpebrarum* (Trematoda: *Philophthalmidae* occurring in the birds-eye) emerged from naturally infected freshwater snails *M. tuberculata* collected from water courses in Giza Governorate, Egypt **(Ayoub *et al*., 2020).** Chicks (1-3 days old) are experimentally infected directly by pipetting 10-15 excysted metacercariae into each eye orbit (**Alicata and Ching, 1960; Howell and Bearup, 1967**). Infected chicks were housed in animal house of TBRI (Theodor Bilharz Research Institute, Imbaba, Giza, Egypt) according to the internationally valid guidelines regularly followed in the institute, where they were provided feed and water and libitum for 40 days. The chickens were slaughtered after 35-40 days of infection and the adult flukes were carefully tested out with fine forceps from conjuctival sac of eyes and rapidly placed in culture medium Roswell Park Memorial Institute medium (RPMI) 1640 containing 300 mg streptomycin, 300 units penicillin and 160 µg gentamycin/100 ml medium **(Ayoub *et al*., 2020)**.

3. Philophathalmusidal bioassay and determination of LC_{50} and LC_{90}

A stock solution (10 mg/ml) of each tested materials was prepared in dimethyl sulfoxide (DMSO) and diluted with RPMI to produce 3 ml test solution of 100 µg/ml final concentration for the screening (**Yousif *et al*., 2007**). The screening were bioassayed at descending concentrations, e.g. 15, 12, 9, 6 & 3µg/ml for **Praziquantel** and **Flubendazole**, while 100, 80, 60, 40 &20 µg/ml for *J. curcas* and *M. oleifera* plant leaves extracts**.** For each concentration three replicates were used and three pairs of worms were placed in each vial using sterilized tissue forceps and the incubation was maintained at 37°C. Negative control exposed only to media were similarly used. The mortality of worms was recorded at each concentration, then, treated and control worms were examined every 24 h under a dissecting microscope to record motility, integument damageand dead worms. Treated worms that did not exhibit motility for two minutes were considered dead. Based on the observations, mortality rates, and the LC_{50} and LC_{90} of each compound against adult *Philophathalmus* were calculated after 72 h using SPSS computer program version 16.

4. Scanning electron microscope examination(SEM)

To report the possible morphological changes, adult worms were treated in triplicate with LC_{50} value of the most effective anthelmintic materials against *P. palpebrarum* for 48h and another untreated group was maintained as control under the same experimental conditions. 10 living flukes were fixed in 2.5% glutaraldehyde for the examination by SEM, washed with PBS, treated with 1% osmium tetroxide (OsO4) and dehydrated in ascending alcohol concentrations. Then the samples were dried, mounted on SEM stubs, and coated with a thin layer of platinum before examination with a SEM.

5. Statistical analysis of LC_{50} and LC_{90} of tested materials were carried out using one-way ANOVA on SPSS program version 16.

RESULTS

In vitro Philophathalmusidal activity

The adult worm was extracted from the conjunctival sac of the chicken eyes after 35-40 days post experimental infection by pipetting 10-15 excysted metacercariae into each eye orbit of (1-3 days old chicks). The flukes were exposed in triplicate at 15, 12, 9, 6 & 3µg/ml for Praziquantel (PZQ) and Flubendazole, while 100, 80, 60, 40 &20 µg/ml for *J. curcas* and *M. oleifera* plant leaves extracts. The efficacy was assessed as the mortality rate based on the number of live and dead flukes after 24, 48 and 72 h post-exposure, then the LC_{50} and LC_{90} of each tested material against adult *P. palpebrarum* were calculated after 72 h using SPSS computer program version 16. The results in **Table (4)** claimed that the LC_{50} and LC_{90} values for these tested materials were 7.857&10.149µg/mL for PZQ, 9.170&13.489 µg/mL for Flubendazole ,65.534 &91.474µg/mL for *J. curcas*, 44.509&53.004 for *M. oleifera*, respectively.

Table 4. LC_{50} and LC_{90} of the the tested materials against adult eye fluke *Philophthalamus palpebrarum* (*Philophthalmidae*).

Tested materials	LC_{50} (µg/ ml)	LC_{90} (µg/ ml)
Praziquantel (PZQ)	**7.857**	**10.149**
Flubendazole	**9.170**	**13.489**
Jatropha curcas **(Leaves Methyl Extract)**	**65.534**	**91.474**
Moringa oleifera **(Leaves Methyl Extract)**	**44.509**	**53.004**
D DMSO	**No effect**	

Control worms incubated in media for 72h survived; showed normal tegumental appearance and motor activity characterized by wavy movement along the body axis. Treatment of adult *P. palpebrarum* with different concentrations of the tested materials for 24h resulted in changingthe worm behavior in the form of slow locomotion. After48 h exposure to 9 µg/mL of Praziquantel (PZQ), 66.70% of the worms died (**Fig. 1A**), with increasing the incubation time for additional 24 h, 83.33% ofworms died. At 12µg/mL, 66.70%of worms died after 24 h of incubation and the remaining wormsdied over the next 24 h (**Fig. 1A**).

The results presented in Fig. (**1B**) revealed that 6 µg/mL of Flubendazole excerted only 16.70% mortality of the worms after 72h of exposure, while increasing the concentration to 12 µg/mL resulted in 33.33 % death among the treated worms, with increasing the incubation time for 48h, the death rate reached 83.33% in comparison to the control group. 100% death of worms resulted after 72h of exposure to 15 µg/mL.

The results in Figs. (**1C and 1D**), indicated no apparent effect on worm survival was seen after 72h exposure to 20 µg/mL of *J. curcas* and *M. oleifera*, while increasing the concentrations to 60, 80and 100 µg/mL excerted (16.70, 33.33& 66.70%) and (16.70, 83.33 & 100%) mortalities after 48h of exposure, respectively. Increasing the incubation time for 72h, lead to 50, 83.33 & 100% death among worms exposed to *J. curcas* and 66.70, 100 &100% among worms exposed to *M. oleifera,* respectively.

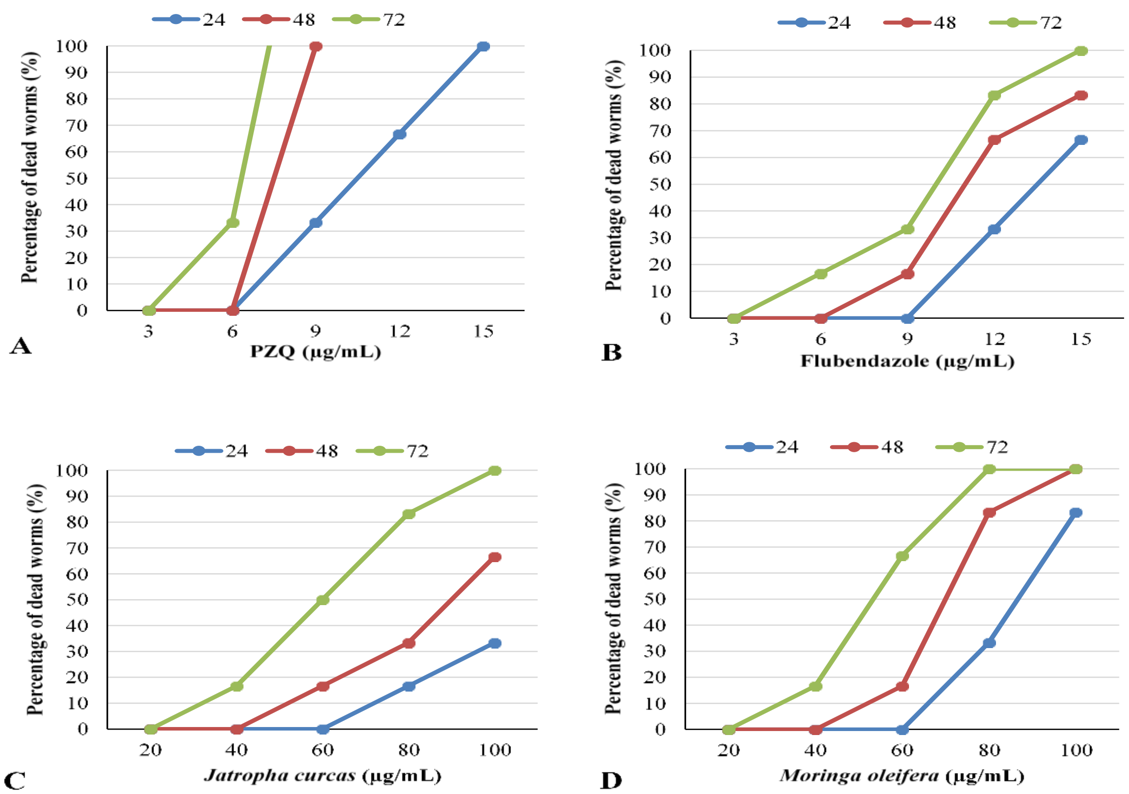


Fig.1. Mortality percent of the adulteye fluke ***Philophthalamuspal pebrarum*** treated with Praziquantel (PZQ) **(A)**, Flubendazole **(B),** *Jatropha curcas* **(C)** and *Moringa oleifera* **(D)**plant Leaves extracts for 72 h.

Scanning electron microscopy results

The characteristics of the tegument of untreated worms were compared with those exposed to LC_{50} (7.857 and 44.509 µg/mL) of PZQ and leaf extract of *M. oleifera*for 48h, respectively. Control worms exposed to culture media only has normal surface membrane with no apparent destruction and normal body structure **(Figs. 2a and b**). Generally, all exposed worms to tested materials showed tegumental or surface membrane ultrastructural damage, worms treated with PZQ and *Moringa* revealed deformity of the oral sucker and anterior part of worms, shrunken body with swellings and furrows (**Figs. 2C& E**). Moreover, disorganization, deformity and shrinkageof the dorsal aspect of the worm with presence oferosion of the tegument in several areas of the bodyand extreme distortion of the tegumental folds (**Figs. 2C & E**).Disfigurement of the ventral sucker was also noticed in the form of marked contraction and deformity, with erosion, shrinkage and wrinkling of the area between the oral and ventral suckers (**Figs. 2D & F**). Moreover, in vitro incubated worms with tested materials showed longitudinal muscle contraction, and the worms were bent, shortened.

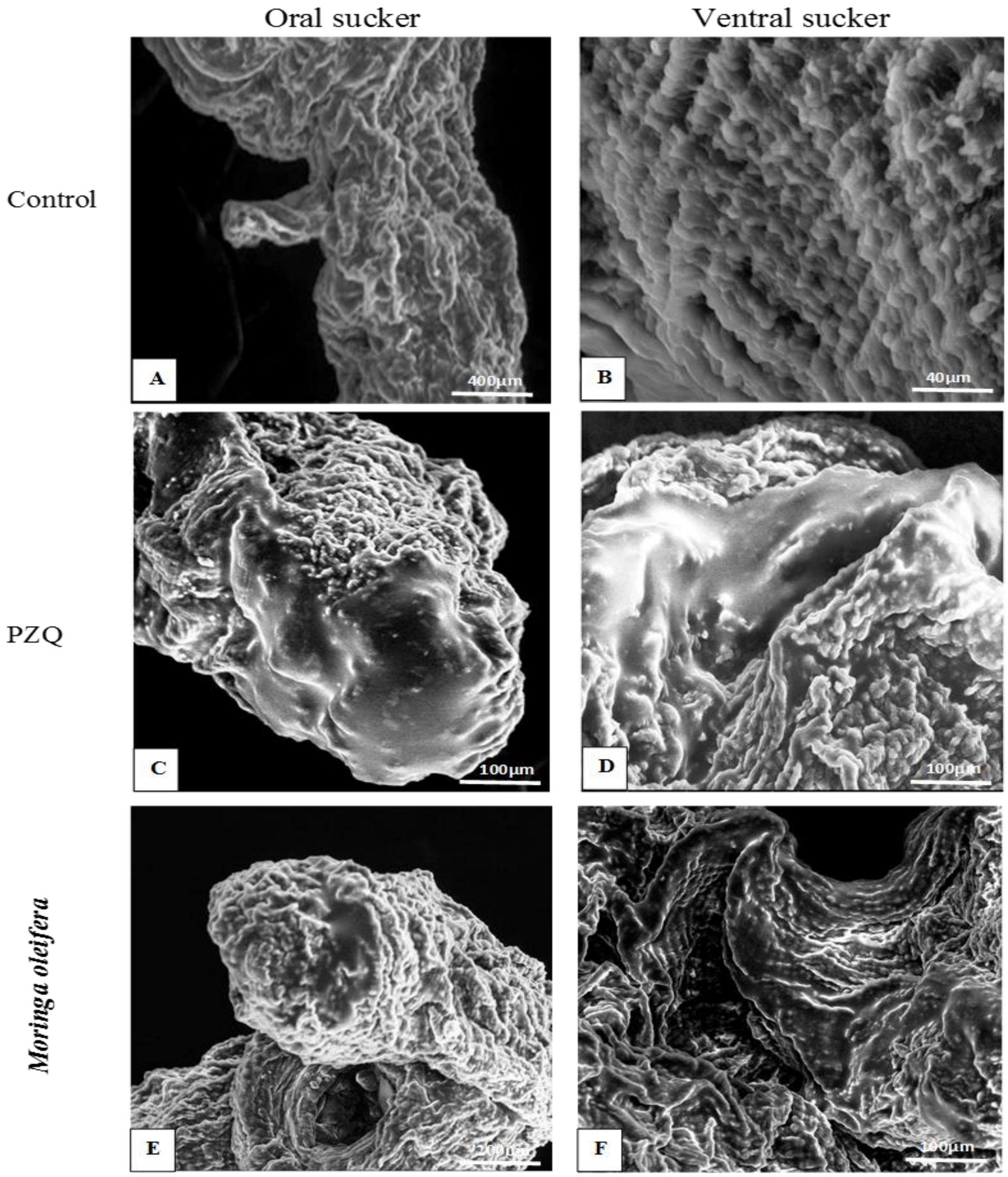


Fig. 2. Oral **(A**) and ventral (**B**) suckers of untreated eye flukes ***Philophthalamus palpebrarum*** were compared with those exposed to LC_{50} (7.857 and 44.509 µg/mL) of PZQ andleaf extract of *M. oleifera* for 48h, respectively. Deformity of the oral sucker and anterior part of worms, shrunken body with swellings and erosion (**C & E**). Disfigurement of the ventral sucker was also noticed in the form of marked contraction and deformity, with erosion and shrinkage (**D &F**).

DISCUSSION

The trematodes of the genus *Philophthalmus* are eye flukes that cause damage to ocular structures of animals and humans (**Assis *et al*., 2018**). Globally, many studies have scientifically assessed numerous natural and synthetic compounds on the *Philophthalmus* adult worm, however, a lot of them still remain unused, pending their efficacy on the parasite biology is confirmed. It is realized that the study of parasite biology is fundamental to potential drug discovery and development. In the present investigation, Praziquantel (PZQ), Flubendazole and plant leaves extracts (*J. curcas* and *M. oleifera*) showed in vitro antihelminthic activity against the adult eye fluke *P. palpebrarum* and the mortality percent of the worm is directly proportional to the time and the tested concentrations. The considerable toxic effect of the tested materials might be due to the main constituents of active compounds (**Tables 1 & 3**), that are known to possesss anthelminthic activities (**Vollesen *et al*., 1995; William *et al*., 2003; WHO, 2009; Wang *et al*., 2016**). Also, the tested materials can deeply penetrate into the internal structures to bind muscles of the parasite or causing disturbance in the normal biochemical and physiological process of the worm **(Taman *et al*., 2020).** Similar results have been previously reported by many researchers when testing the in vitro effect, eg. PZQ (**Kato *et al*., 2013; Prompetch *et al*., 2021)**, seeds of *J. curcas* (**Kandil *et al*., 2018**), seeds of *M. oleifera* (**Eguale & Giday, 2009)** on *Schistosoma mansoni*, *Philophthalmus gralli*, *Haemonchus contortus*, *Fasciola hepatica*, respectively.

On the basis of LC_{90} values, PZQ (LC_{90}= 10.149 µg/mL) and *M. oleifera* (LC_{90} = 53.004 µg/mL) were the most toxic ones. So, the two compounds were used for detailed studies in this work. The results of electron microscope of the present study demonstrated the LC_{50} of PZQ and leaf extract of *M. oleifera* caused tegumental or surface membrane ultrastructural damage if compared control worms. The tegument has a pivotal role in sensation, protection, immunomodulation, nutrient ingestion, osmoregulation, synthesis of some proteins, and excretion (**Skelly & Wilson, 2014**). It is noteworthy that alterations in the tegument structure deteriorate its functions and lead to worm death (**Shaw & Erasmus, 1987**). Also, the main changes induced by treatment are related to deformity and damage in oral and ventral suckers. Suckers are muscular organs essential for fixing worms in the conjunctival and orbital tissues. So, if this effect is the same in vivo, both compounds will affect worm nutrition and subsequent interference of worm and worm oviposition (**Halton and Maule, 2004**). The obtained results can also be supported by those obtained by **Chienwichai *et al*. (2020) who** studied the parasiticidal activity of 40 µg/mL concentration of PZQ against *Schistosoma mekongi*. The parasiticidal activity of prazquantel against csetodes, including *Hymenolepis nana* (1 g/mL) and *Diphyllobothrium latum* (0.1 g/mL), was previously reported (**Bylund *et al*., 1977; Becker *et al*., 1980**). Interestingly, **Heneberg *et al*. (2014**) studied the effective therapeutic intramuscular medication of PZQ in patient chicken with *P. gralli* infestation.

Also, the same findings was reported by **Prompetch *et al*. (2021)** who evaluated the efficacy of a novel drug delivery system of the modified rice hydrogel containing praziquantel (PZQ) against *P. gralli* isolated from ostrich eyes and determine the toxicity of the preparation on chicken eye model.

CONCLUSION

Finally it can be concluded that Praziquantel, Flubendazole and plant leaves extracts (*J. curcas* and *M. oleifera*) possess in vitro antihelminthic activities against adult eye fluke *P. palpebrarum* (*Philophthalmidae*); however, studies should be conducted in vivo to examine their antiphilophthalamus effects. As the evaluation of new protocols, routes of administration and anthelmintic drugs are needed for successful pharmacological treatment of Philophthalmosis.

REFERENCES

Abdul-Salam, J.; Sreelatha, B.S. and Ashkanani, H. (2004). The eye fluke *Philophthalmus hegeneri* (Digenea: Philophthalmidae) in Kuwait Bay. Kuwait J. Sci. Eng., 31:119–133.

Alicata, J.E. and Ching, H.L. (1960). On the infection of birds and mammals with the cecaria and metacercaria of the eye fluke, *Philophthalmus*. J. Parasitol., 46: 16.

Assis, J. C.A.; Martins, N. R. S. and Pinto, H. A. (2018). Experimental avian philophthalmosis: Evaluation of diagnosis and treatment of chickens infected with *Philophthalmus gralli* (Trematoda: *Philophthalmidae*). Vetrinary parasitology, 256: 24-28.

Ayoub, M.; Abdel-Motleb, A. and Tadros, M. (2020). First Egyptian record of the eye fluke (*Philophthalamus palpebrarum*) transmitted by *Melanoides tuberculata* snail and its experimental life cycle**.** Egyptian Journal of Aquatic Biology & Fisheries, 24(2): 147 – 162.

Becker, B.; Mehlhorn, H.; Andrews, P. and Thomas, H. (1980). Scanning and transmission electron microscope studies on the efficacy of praziquantel on *Hymenolepis nana* (Cestoda) in vitro. Z. Parasitenkd. 61 :121–133.

Bylund, G.; Bång, B. and Wikgren, K. (1977). Tests with a new compound (Praziquantel) against *Diphyllobothrium latum*. J. Helminthol., 51:115–119.

Boulos, L. (1999). Flora of Egypt 1: Azollaceae–Oxalidaceae.Cairo, Egypt, Al Hadara Publ., pp. 1–419.

Boulos, L. (2000). Flora of Egypt 2: Geraniaceae–Boraginaceae. Cairo, Egypt, Al Hadara Publ., pp. 1–352.

Chienwichai, P.; Ampawong, S.; Adisakwattana, P.; Thiangtrongjit, T.; Limpanont, Y.; Chusongsang, P.; Chusongsang, Y. and Reamtong, O. (2020). Effect of

Praziquantel on Schistosoma mekongi Proteome and Phosphoproteome. Pathogens, 9, 417.

Diaz, M.T.; Hernandez, L.E. and Bashirullah, A.K. (2002). Experimental life cycle of *Philphthalmus gralli* (Trematoda: Philopthalmidae) in Venezuela. Rev. Biol. Trop., 50:629–641.

Eguale, T. and Giday, M. (2009). In vitro anthelmintic activity of three medicinal plants against *Haemonchus contortus*. Int. J. Green Pharm., 3 (1): 29-34.

Fahey, J.W. (2005). Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Trees Life J., 1(5):1–15.

Halton, D.W. and Maule, A,G. (2004). Flatworm nerve-muscle: Structural and functional analysis. Can. J. Zool., 82(2): 316-333.

Heneberg, P.; Rojas, A.; Bizos, J.; Kocková, L.; Malá, M. and Rojas, D. (2014). Focal *Philophthalmus gralli* infection possibly persists in *Melanoides tuberculata* over two years following the definitive hosts' removal. Parasitol. Int., 63: 802–807.

Heneberg, P.; Casero, M.; Waap, H.; *et al.* (2018). An outbreak of philophthalmosis in *Larus michahellis* and *Larus fuscus* gulls in Iberian Peninsula. Parasitol. Int., 67:253–61.

Howell, M.J. and Bearup, A.J. (1967). The life histories of two bird trematodes of the family Philophthalmidae. Proc. Linn. Soc. N S W., 92:184-192.

Ismail, N.S. and Arif, A.M.S. (1993). Population dynamics of *Melanoides tuberculata* (Thiaridae) snails in a desert spring, and infection with larval trematodes in the United Arab Emirates. Hydrobiologia, 257: 57–64.

Ismail, N.S. and Issa, I.M. (1987). Development, growth and migration of *Philophthalmus gralli* (Trematoda) from Jordan in the eyes of chick. Journal of Helminthology, 61: 163–168.

Kanev, I.; Nollen, P.; Vassilev, I.; Radev, V. and Dimitrov, V. (1993). Redescription of *Philophthalmus lucipetus* (Rudolphi, 1819) (Trematoda: Philophthalmidae) with a discussion of its identity and characteristics. Ann Naturhist Mus. Wien., 94/95 B:11–34.

Kalantan, A.M.N.; Arif, M. and AL-Arefi, H.A. (1998). Larval digenea of *Melanoides tuberculatus* (Mu¨ller, 1774) (Gastropoda: Prosobranchia) from Al-Hassa Province, Saudi Arabia. J. Parasit. and App. Bio., 7: 103–113.

Kato, K.; Miura, M. and Mitsui, Y. (2013). In vitro effects of amodiaquine on paired Schistosoma mansoni adult worms at concentrations of less than 5 µg/mL. Mem. Inst. Oswaldo Cruz, 108(2): 192-196.

Kandil , O.M.; Hassan, N.M.F.; Sedky, D.; Ata, E. B.;. Nassar,S.A.; Shalaby, H.A.; Nanev, V.; Tsocheva-Gaytandzhieva, N. and Gabrashanska, M. (2018). Anthelmintic efficacy of *Moringa oleifera* seed methanolic extract against *Fasciola hepatica*. J. Parasit. Dis., 42(3): 391–401.

Kingston, N. (1984). Trematodes, in *Diseases of poultry*, edited by M.S. Hofstad. Ames: Iowa University Press.

Lang, Y; Weiss, Y.; Garzozi, H.; Gold, D. and Lengy, J. (1993). A first instance of human philophthalmosis in Israel. Journal of Helminthology, 67: 107-111.

Looss, A. (1899). Weitere Beiträge zur Kenntnis der Trematoden-Fauna Aegyptens, zugleich Versucheiner natürlichen Gliederung des Genus *Distomum* RETZIUS. – Zool. Jahrb. Syst., 12: 521-784.

Looss, A. (1907). *Philophthalmus nocturnus* nov. sp. Centralbl Bakteriol Parasitenkd Infektionskr, 43:479–480.

Mukaratirwa, S.; Hove, T.; Cindzi, Z. M.; Maononga, D. B.; Taruvinga, M. and Matenga, E. (2005). First report of a field outbreak of the oriental eye-fluke, *Philophthalmus gralli*, in commercially reared ostriches (Struthio camelus) in Zimbabwe. Ondestepoort J. Vet. Res., 72:203–206.

Müller (1774). (Gastropoda: Prosobranchia) from Al- Hassa province, Saudi Arabia. J. Parasitol. Applied Biol.,7: 103-113.

Nadar, A.H.; Kamal, M.A.; Abu Eleinen, K. G.; Kotb, A.A. and Ezzat, G.M. (2017). Granulomatous anterior uveitis in children in fayoum, Egypt a laboratory and cytological study. J. MSCR, 5(6): 23444-23450.

Nollen, P.M. and Kanev, I. (1995). The taxonomy and biology of philophthalmid eye flukes. Adv. Parasitol., 36:205–269

Pinto, R.M.; Dos Santos, L.C.; Tortelly, R. et al (2005). Pathology and first report of natural infections of the eye trematode *Philophthalmus lachrymosus* Braun, 1902 (Digenea, Philophthalmidae) in a non-human mammalian host. Mem. Inst. Oswaldo Cruz, 100:579–83.

Prompetch, T; Chailorm A.; Tiwananthagorn S.; Buranapim, N.; Okonogi, S.; Kato, H.; Katip, W. and Mektrirat, R. (2021). Preclinical Evaluations of Modified Rice Hydrogel for Topical Ophthalmic Drug Delivery of Praziquantel on Avian Philophalmiasis. Pharmaceutics, 13, 952. https://doi.org/10.3390/pharmaceutics13070952.

Radev, V.; Kane, I.; Nollen, P.M. and Gold, D. (1999). Life history and identification of *Philophthalmus lucipetus* from Israel. J. Parasitol., 85:291–294.

Radev, V.; Kanev, I. and Gold, D. (2000). Life cycle and identification of an eyefluke from Israel transmitted by *Melanoides tuberculata* (Müller, 1774). J. Parasitol., 86:773–776.

Sapp, S. G. H.; Alhabshan, R. N.; Bishop, H.S.; Fox, M.; Ndubuisi, M.; Snider, C. E. and Bradbury (2019). Ocular Trematodiasis caused by the avian eye fluke *Philophthalmus* in southern Texas. Open Forum Infect. Dis., 6 (7): ofz 265.

Shaw, M.K. and Erasmus, D.A. (1987). Schistosoma mansoni: structural damage and tegumental repair after in vivo treatment with praziquantel. Parasitology 94, 243–254.

Skelly, P.J. and Wilson, R.A. (2014). Making sense of the schistosome surface. Advances in Parasitology, 65: 185–284.

Taman, A.; El-Bardicy, S. ; Tadros, M.; Ayoub, M.; Mansour, B.; El-Shehabi, F. and El-Beshbishi, S.N. (2020). In vitro efficacy of new synthetic benzimidazole-related compounds against *Schistosoma mansoni* adult worms. Asian Pacific Journal of Tropical Medicine, 13(12): 566-572

Tilman, D.; Reich, P. B. and Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature, 441: 629632.

Verocai, G.G.; Lopes; L.G.; Burlini, L.; Correia, T.R.; de Souza, C.P. and Coumendourus, K. (2009). Ocurrence of Philophthalmus gralli (Trematoda: Philophthalmidae) in farmed ostriches in Brazil. Trop. Anim. Health Prod., 41:1241–1242.

Vollesen, K.; Edwards, S.; Mesfin, T. and Hedberg, I. (1995). *Euphorbaceae*. In: Flora of Ethiopia and Eritrea; Canellaceae to *Euphorbaceae*. Vol. 2 Addis Ababa, Ethiopia, p. 324.

Wang, L.; Chen, X. and Wu, A. (2016). Mini review on Antimicrobial Activity and Bioactive Compounds of *Moringa oleifera*. Med. Chem. (Los Angeles), 6 (9):578–82.

William, S.; Guirguis, F. and Nessim, N.G. (2003). Effect of simultaneous and/or consecutive administration of the broad spectrum anthelmintic flubendazole together with praziquantel in experimental *Schistosoma mansoni* infection. Arzneim Forsch, 53(7): 532-537.

WHO (2009). Stuart MC, Kouimtzi M, Hill SR (eds.). WHO Model Formulary 2008. World Health Organization, pp. 88, 593. hdl:10665/44053. ISBN 9789241547659.

Yousif, F.; Hifnaw, y. M.S.; Soliman, G.; Boulos, L.; Labib, T. and Mahmoud, S. (2007). Large-scale in vitro screening of Egyptian native and cultivated plants for schistosomicidal activity. Pharm. Biol.; 45(6): 501-510.