J Parasit Dis (Apr-June 2020) 44(2):411–419
https://doi.org/10.1007/s12639-020-01224-6

ORIGINAL ARTICLE

Study on the effect of an ion channel inhibitor "Fluralaner" on *Echinococcus granulosus* protoscolices and metacestode layers in vitro

Fatima Zahran[1] · Hayam Mohamed Ezz El-Din[1] · Mai Abdel Sameaa Shehata[1]

Received: 1 March 2020 / Accepted: 12 April 2020 / Published online: 24 April 2020

Abstract Hydatid disease has a great impact on public health, causing high morbidity and mortality. Main lines of treatment include surgery, which mostly requires the installation of a scolicidal agent into hydatid cysts to prevent dissemination. Alternatively, medical treatment involves the use of benzimidazole drugs; however, the results are not satisfactory, and new drug compounds are urgently needed. Fluralaner is a potent inhibitor of GABA-gated chloride channels and L-glutamate-gated chloride channels (GluCls) providing immediate and persistent flea, tick and mite control in dogs after a single oral dose. Researches previously identified different genes encoding ion channels in *Echinococcus granulosus*, making ion channel inhibitors a promising target for treating hydatid disease. Thus, the present study aimed to evaluate the effect of fluralaner on protoscolices and metacestode layers. Parasite materials (Protoscolices, Metacestodes layers) were exposed to different concentrations of the drug ranging from "12.5–100 ug/ml" and examined for viability after 1, 6 and 24 h. Morphological and ultrastructural alterations were recorded by both light and electron microscopies. Immunohistochemical staining confirmed caspase-3 activation as an indicator of apoptosis- induced therapy. The treated protoscolices and metacestode layers showed loss of the viability, the formation of vacuoles and lipid droplets, separation of the germinal layer, and damage in the laminated layer; apoptosis was prominent after treatment. These findings revealed that fluralaner has a potent scolicidal activity and suggested its therapeutic potential against hydatid disease. Further evaluations for animals and human use in the treatment and prevention of hydatid disease are needed.

Keywords Hydatid disease · Fluralaner · Ion channel · Echinococcus · T.E.M. · Caspase 3

Introduction

Cystic echinococcosis (C.E.) is a zoonotic disease caused by the larval stage of the dog tapeworm *Echinococcus granulosus* (Neumayr et al. 2013). Human acquires infection through ingestion of *E. granulosus* eggs excreted in the faeces of the definite host dogs and other canids. The infection is endemic in many parts of the world, including Egypt and the Middle East, and WHO listed it as one of the neglected tropical diseases (Sadjjadi 2006; El-Shazly et al. 2007; WHO 2012).

C.E. has a significant impact on public health, causing considerable morbidity and mortality as well as economic losses in communities involved (Parkoohi et al. 2018). Echinococcosis mortality rate was found to be 1.29 (per 100 patients). And the leading causes of death are either complication of hepatic and pulmonary echinococcosis or echinococcosis of the heart, which is a rare complication that occurs in 0.2–2% of cases Many complications were documented, such as inferior vena cava compression by the growing cyst, the development of fibrinous-purulent peritonitis, the rupture into the bile ducts with obstructive

✉ Fatima Zahran
Drtima@hotmail.com

Hayam Mohamed Ezz El-Din
hayamezz@hotmail.com

Mai Abdel Sameaa Shehata
mai_abdelsameaa@hotmail.com

[1] Faculty of Medicine, Ain-Shams University, Ramsis St., Abbassia, Cairo 11591, Egypt

jaundice or biliary-pulmonary bronchial fistulas development (Khachatryan 2017).

This disease is challenging to diagnose, treat, and control (McManus et al. 2012; Yang et al. 2014). The current treatment of C.E. involves surgery and the use of benzimidazole drugs, but the results are far from satisfactory, and new drug compounds are urgently needed (Brunetti et al. 2010; Salinas et al. 2011; Tawfik 2018).

Zhang et al. (2014), identified new potential drug targets for C.E. and listed that ligand-gated, voltage-gated ion channels and neuropeptides may represent possible targets. Additionally, they identified genes encoding "29" ligand-gated ion channels, "39" voltage-gated cation channels, "5" chloride channels, and "9" other types of channels in the *E. granulosus* genome (Zheng et al. 2013). Furthermore, γ-aminobutyric acid (GABA)-gated chloride channels are one of the ion channels found throughout the animal kingdom. They are distally related to the glutamate-gated chloride channels GluCls, which are the targets of a large group of anthelminthic as ivermectin and moxidectin (Vassilatis et al. 1997 and Dent 2006). They are considered the biggest-selling class of drugs in veterinary medicine (Wolstenholme 2011). The GABA gated chloride channels are present in the nervous system of nematodes (Richmond and Jorgensen 1999), trematodes (Eriksson et al. 1995), and the nervous system of some cestodes as *Diphyllobothrium dendriticum* & *Caryophylles laticeps* (Biserova et al. 2014). They are also present in ectoparasites (Gassel et al. 2014). They mediate the relaxation needed after the contraction of the body, so their drug-induced activation of them induces flaccid paralysis (Schuske et al. 2004).

Fluralaner is one of the Isoxazolines, it represents a novel class of parasiticides, that showed potency in inhibition of GABA-gated chloride channels and L-glutamate-gated chloride channels GluCls, through its binding to chloride channel leading to overexcitation of insect'' & arachnid'' nervous system leading to rapid death (Gassel et al. 2014). It has been used for dogs providing immediate and persistent flea, tick and mite control after a single oral dose (Walther et al. 2015). It is safer than ivermectin when administrated to dogs, as ivermectin showed potential neurotoxicity (Walther et al. 2015). Targeting ion channels have some apparent advantages; they tend to act quickly, which means that they will rapidly clear the infections (Wolstenholme 2011). Due to the presence of gene encoding ion channels in *E. granulosus* genome and the potent effect of fluralaner as an ion channels inhibitor, this might show a promising drug targets in case of hydatid disease.

The present study aimed to investigate the effect of the drug "Fluralaner" on *Echinococcus granulosus* protoscolices and metacestodes layers in vitro.

Materials and methods

Collection of parasitic samples

E. granulosus hydatid cysts were freshly isolated from the liver of naturally infected camels slaughtered at Cairo Abattoir. The fluid inside each cyst was aspirated by a sterile syringe then allowed to settle in a sterile falcon tube, where the protoscolices were precipitated, and the supernatant was discarded. Protoscolices were then washed by a sterile saline solution several times, according to Zou et al. (2009). Germinal and laminated layers were collected under aseptic conditions.

Viability assay

The viability of protoscolices was detected by observation of the motility of protoscolices and eosin exclusion test. An eosin solution of 0.1% was added to the protoscolices solution in a ratio of 1:1. After 15 min, the viability of protoscolices was detected by observing the change of colour under a light microscope. The numbers of viable/non-viable protoscolices were detected in ten randomly chosen fields. Dead protoscolices uptake eosin and colour red while viable protoscolices remain colourless. Viable protoscolices are mostly motile and show flame cell movement when put onto slides, covered with coverslips, and counted under a light microscope. The protoscolices suspension was considered suitable for use when $> 95\%$ of protoscolices were viable. The viable protoscolices were incubated in RPMI 1640 culture medium containing 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% calf serum in a 5% CO2 atmosphere at 37 °C. RPMI 1640 medium (Sigma-Aldrich) (Tawfik 2018).

Drug preparation, "Fluralaner"

A fluralaner stock solution (20 mg/ml) was diluted with deionized water to a fluralaner concentration of 1000 ug/ml and then further diluted to obtain the test concentration 12.5, 25, 50 and 100 ug/ml (Williams et al. 2015). A fluralaner pack size of 25 mg was purchased from TargetMol (Cat. No. T5330).

Experimental design

The viability of treated and the control parasite was assessed after 1 h, 6 h, and 24 h intervals. The effect of fluralaner on hydatid cysts was monitored by protoscolex death. The destructive effects of fluralaner on the parasite were assessed after 1 h by cellular examination (light microscopy) and ultrastructure examination (electron

microscopy). Apoptosis was determined by immunohistochemistry through measuring the activity of caspase-3. Each experiment was performed in triplicate.

Histopathological examination

Treated and control protoscolices, were fixed in 10% formalin, processed to paraffin blocks, cut, rehydrated, and stained with hematoxylin and eosin (H & E) (Alam-Eldin and Badawy 2015). Processing and examination were done at Pathology & Parasitology departments, Faculty of Medicine, Ain Shams University

Ultrastructural examination by electron microscopy

The ultrastructural changes of the parasite were studied using transmission electron microscopy (T.E.M.) 1 h after treatment. Treated and control parasitic samples fixed with 2.5% (v/v) glutaraldehyde in 0.1, cacodylate buffer, pH 7.2 for 1 h, were washed twice in PBS. Then post-fixed with 1% (v/v) osmium tetroxide in 0.1 M cacodylate buffer for 30 min, pH 7.2, at room temperature and dehydrated in increasing concentrations of ethanol, followed by a final dehydration in 100% propylene oxide. Samples were embedded in Epon 812. Ultra-thin sections were cut by an ultramicrotome, mounted on copper grids, stained with uranyl acetate and lead citrate, and examined under the JOEL1200EXII electron microscope (Tawfik 2018). Processing and examination were done at electron microscopy unit, faculty of science, Ain Shams University.

Immunohistochemistry for detection of caspase-3

Caspase-3 antibodies were diluted 1:300; all sections were subjected to high-temperature antigen retrieval and subjected to staining procedures according to the manufacturer's instructions (Dako). The slides finally went through colour development with DAB, counterstained with hematoxylin, and observed under light microscopy. Brownish staining in the cytoplasm (may include the nuclei) was considered as caspase-3 positive (Hu et al. 2011). Processing and examination were done at central laboratory, faculty of science, Ain Shams University.

Statistical analysis

Data were presented as Mean ± SD. The student's *t* test was used to detect the statistical significance and was significant when the p-value was < 0.05 and highly significant when the *p*-value was < 0.001.

Results

Effects of Fluralaner on protoscolices' viability

Eosin exclusion test done before starting the experiments showed 98% viable protoscolices with distinct movements. Different concentrations of drugs were added (12.5 ug, 25 ug, 50ug, 100 ug), and results were recorded after 1 h, 6 h, and 24 h in comparison to control (Fig. 1). The viability of protoscolices decreased to reach 40% for the lesser concentration used (12.5 ug/ml) after 1 h, to reach 9% after 6 h and complete death was shown after 24 h of incubation. With increasing drug concentrations, the lethal effect of the drug increased, reaching almost 100% death after 1 h for concentrations (50 & 100 ug/ml) (Fig. 2). There was a highly significant statistical difference ($p < 0.001$) between the percentage of viable control protoscolices compared to the treated protoscolices at all timing and all concentrations.

Morphological changes

Examination of the paraffin sections stained by H& E of the treated protoscolices after 1-h incubation with fluralaner, showed morphological alterations in comparison to control samples. Control protoscolices were clear and intact with clear hooks, and suction cups and the calcareous corpuscles were visible. Protoscolices incubated with 12.5 ug/ml of the drug (lowest concentration) showed vacuole formation and areas of discontinuation of the cuticle and multiple deformities. In contrast, those incubated with 100 ug/ml (highest concentration) showed complete loss of integrity (loss of hooks, scolex, and suction cup) (Fig. 3).

Ultrastructural changes by T.E.M

T.E.M. of *Echinococcus granulosus* treated and control protoscolices was performed after one hour of incubation with the drug. Control protoscolices showed the distinct features of the protoscolices, such as; Germinal layer (G.L.) that contain numerous cell types, internal tegumental layer (Teg), projection of intact microtriches into the laminated layer. Treated protoscolices showed different degrees of destruction with the occurrence of lipid droplets, vacuolation, and sheading of microtriches (Fig. 4). Also, T.E.M. was performed to metacestodes' wall and showed ultrastructural changes. It showed detachment of the germinal layer with only cellular debris remaining and detachment of the tegument from the laminated layer when using lower concentrations (12.5 ug/ml). The visible damage reached up to complete disappearance of the

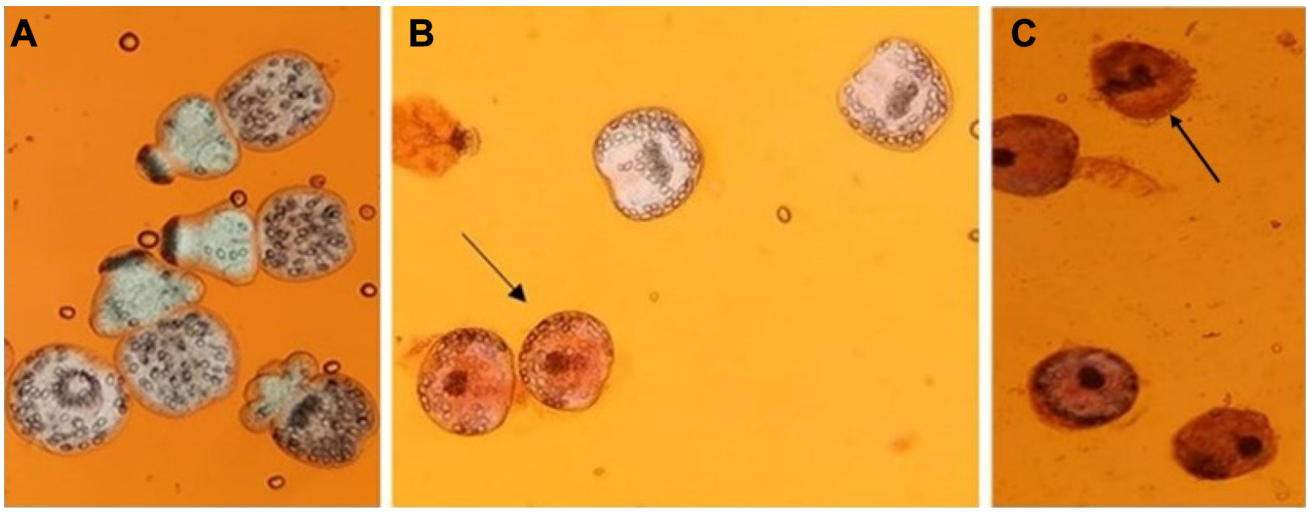

Fig. 1 Protoscolices after staining with 0.1% eosin. **a** Control viable protoscolices, **b** Treated protoscolices with 12.5 µg/ml after 1 h incubation showing viable and non-viable (black arrow) protoscolices, **c** Treated protoscolices with 100 µg/ml after 1 h incubation showing complete death of the parasite (black arrow) (× 100)

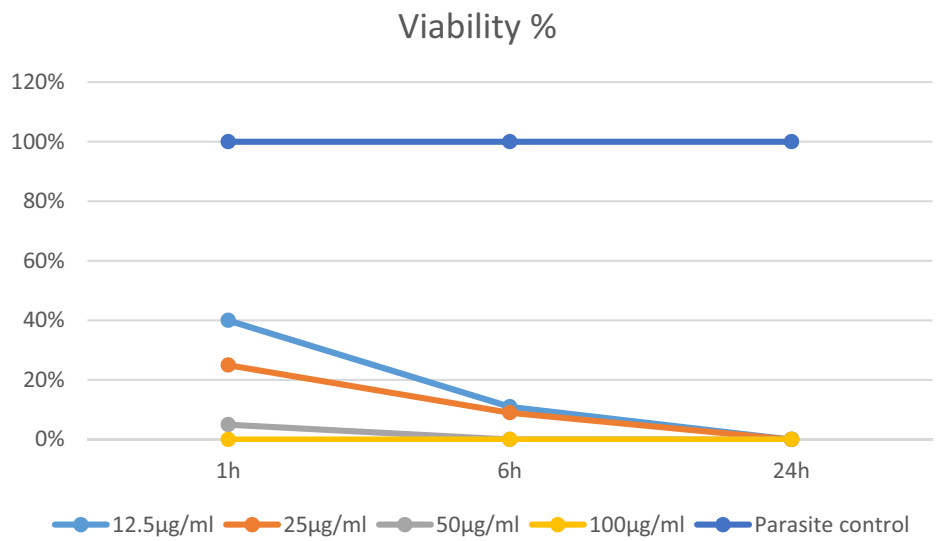

Fig. 2 Percentage of viable protoscolices after exposure to different doses of fluralaner at 1 h, 6 h and 24 h time interval

germinal layer and some destruction in the laminated layer, when using higher concentrations as 50ug/ml & 100 ug/ml (Fig. 5).

Fluralaner induced apoptosis in treated protoscolices and metacestodes layers

Caspase-3 was used as an indicator of apoptosis by immunohistochemistry. Caspase-3 activity was detected in protoscolices and metacestodes layers treated by 12.5 ug/ml for 1 h, as brownish spots. While in higher concentrations as (100 ug/ml), there was a complete brownish discolouration (Figs. 6 and 7).

Discussion

Fluralaner is a novel systemically administered isoxazoline class compound. It acts by inhibiting GABA-gated chloride channels and L-glutamate-gated chloride channels (Gassel et al. 2014). From the fact that the GABA gated chloride channels are present in the nervous system of some cestodes as *Diphyllobothrium dendriticum* & *Caryophylles laticeps* (Biserova et al. 2014). This study aimed to investigate the possible effect of fluralaner on protoscolices and metacestodes layers of *E. granulosus* in vitro, for further evaluation as a scolicidal agent for human use in hydatid disease.

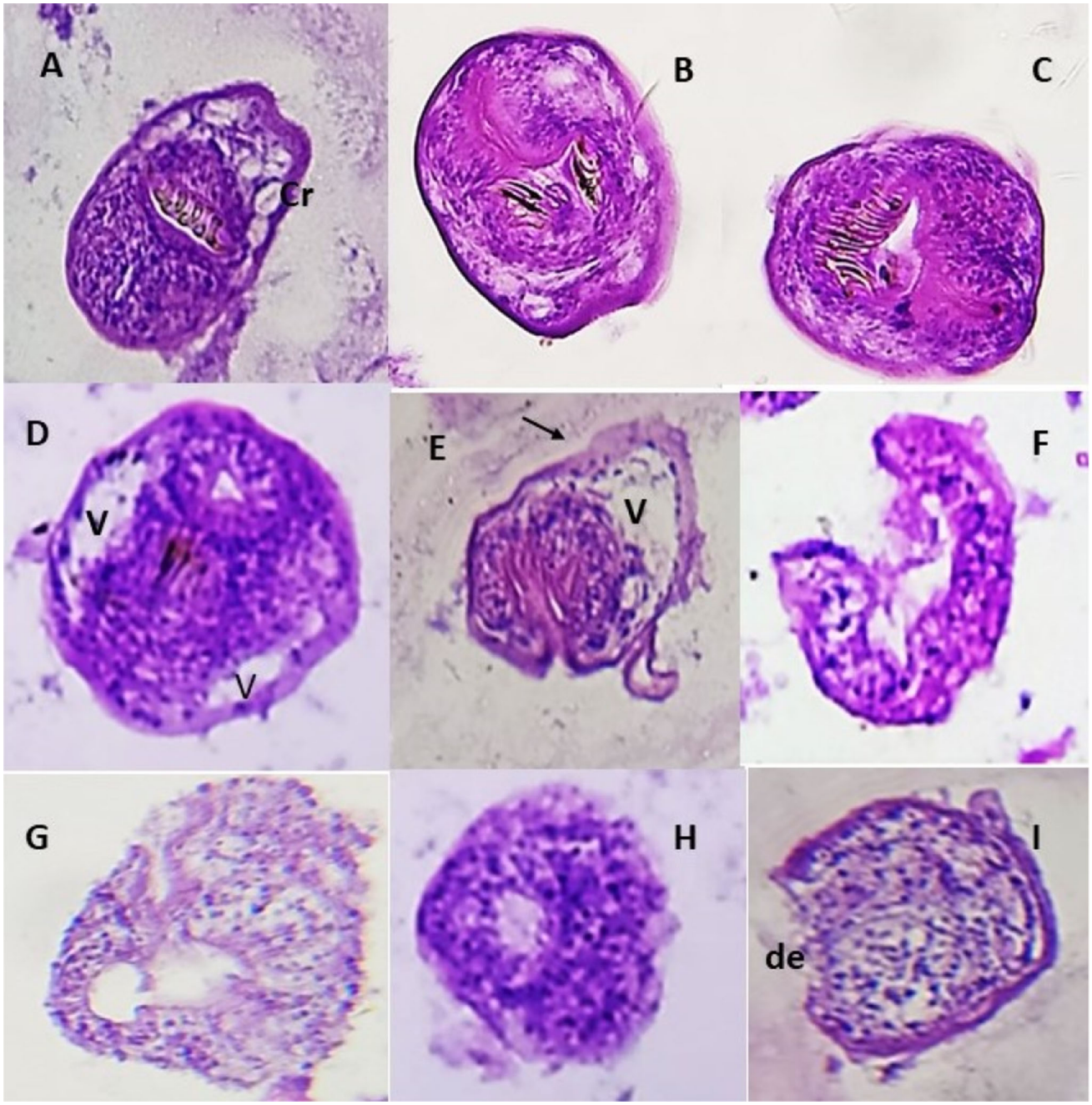

Fig. 3 Morphological alterations of *E. granulosus* protoscolices after 1 h incubation with different doses of fluralaner, stained with H & E. **a–c** Examples of control PSCs; showed normal intact PSCs, with visible hooks and calcareous corpuscles (Cr). **d–f** Treated PSCs with 12.5 ug/ml; showed vacuoles formation, multiple deformities (arrow). G-I: Treated PSCs with 100 ug/ml; showed detachment of cuticle (de) and complete loss of integrity (loss of hooks, scolex, and suction cup) (× 400)

Metacestodes layers, including the germinal layer and protoscolices, are considered remarkable targets of several therapeutic approaches to inhibit the proliferation of hydatid cysts. Germinal layer is considered the vital layer of the cyst that gives rise to brood capsules and protoscolices; within an intermediate host, protoscolices can develop into new hydatid cysts or adults within a definitive host (Zou et al. 2009).

In this study, the fluralaner showed a potent scolicidal activity, and it had a destructive effect on metacestodes layers after the administrations of different concentrations of drugs (12.5, 25, 50, and 100 ug/ml). All tested concentrations had an almost similar lethal effect on protoscolices after 6 h and 24 h, with slightly different lethal effects at 1-h incubation with the drug. The visible alteration by light microscopy and T.E.M. were similar to

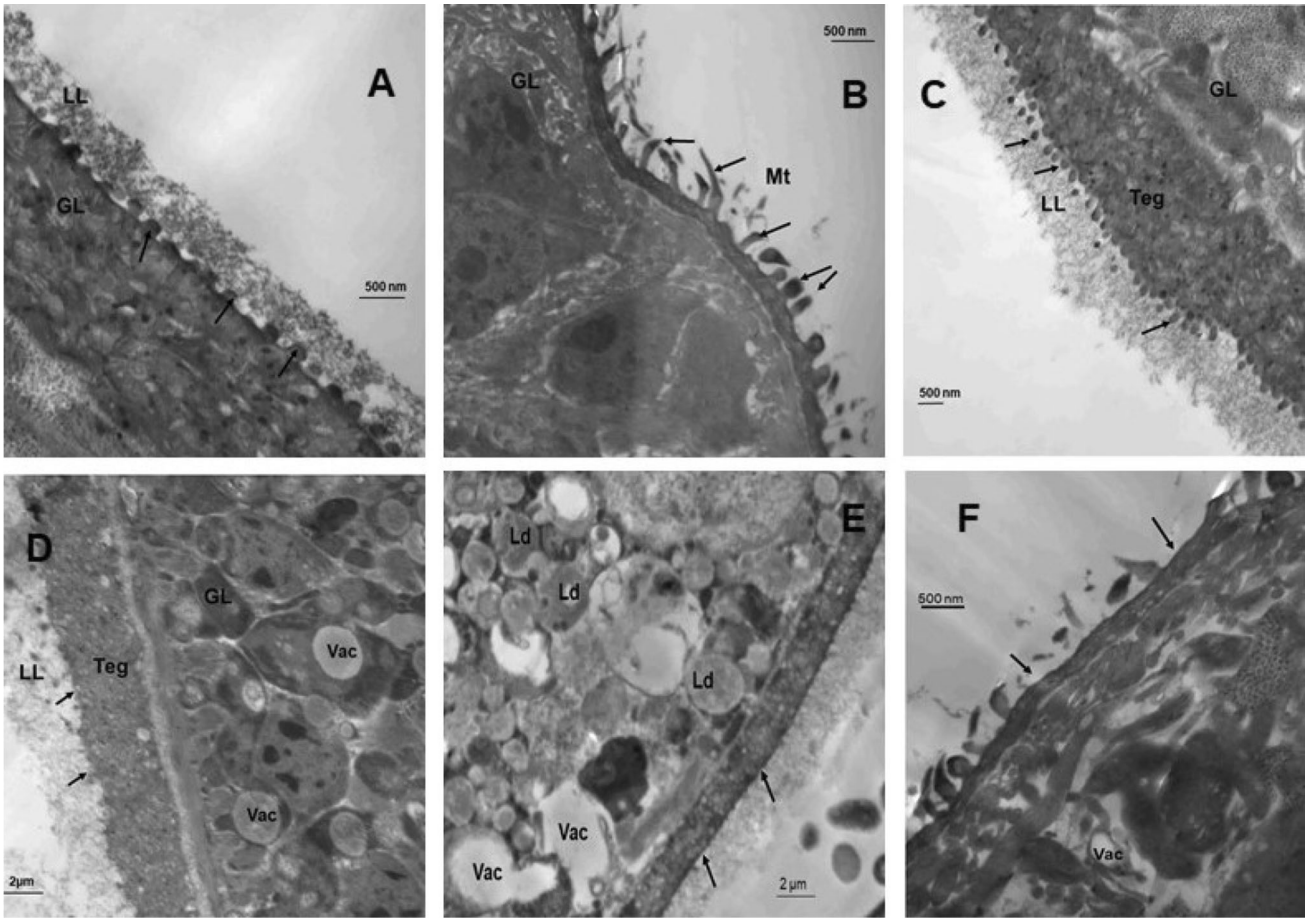

Fig. 4 TEM of *E. granulosus* protoscolices after 1-h incubation with different doses of fluralaner compared to control protoscolices. Figure **a-c** Control protoscolices showing the distinct features of the protoscolices such as; Germinal layer (GL) that contain numerous cell types, Tegumental Layer (Teg), projection of intact microtriches (Mt) (arrows) into Laminated layer (LL). Figure **d–f** Treated protoscolices showing morphological changes as sheading of microtriches (arrows), the occurrence of lipid droplets (Ld) and vacuolation (Vac). A (x7500), B&C (x4000), D (x3000), E (x2000), F (x5000)

changes reported by different drugs used in other experiments (Walker et al. 2004; Hosseini et al. 2006; Elissondo et al. 2008, 2013; Hu et al. 2011). Caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many essential cellular proteins (Porter and Janicke 1999**).** Apoptosis of fertile cysts is possibly one of the suppression mechanisms of hydatid cysts (Spotin et al. 2012) In this study, Caspase 3 immunohistochemistry detected the apoptotic effect of the drug, and it showed damage with different degrees according to the used concentration. Also, Alam-Eldin and Badawy (2015) and Tawfik (2018) discussed similar apoptotic changes. According to Walther et al. (2014), fluralaner was found to be safe for dogs following oral administration at doses much higher than the recommended treatment dose at 8-week intervals, without any significant adverse events. It has a clear preference for invertebrates in comparison to mammalian neuronal receptors, which is considered to have a good safety profile.

Fluralaner, compared to ivermectin, is another drug used in experiments and recommended as a scolicidal agent, was found to be safer and can be combined without drug interactions (Hokelek et al. 2002; Walther et al. 2015). It is interesting to take into consideration that fluralaner activity may be augmented when systemically administered as stated by (Williams et al. 2015) who tested fluralaner activity against different stages of ticks using contact and feeding exposure.

Moreover, according to them, the feeding exposure gave better results confirming that this drug is highly effective for systemic administration. Also, (Biogiorno et al. 2019), found that fluralaner can inhibit the transmission of visceral leishmaniasis in dogs through its insecticidal effect on *Phlebotomus perniciosus,* a primary visceral leishmaniasis vector. This inhibitory effect lasted for up to 84 days post

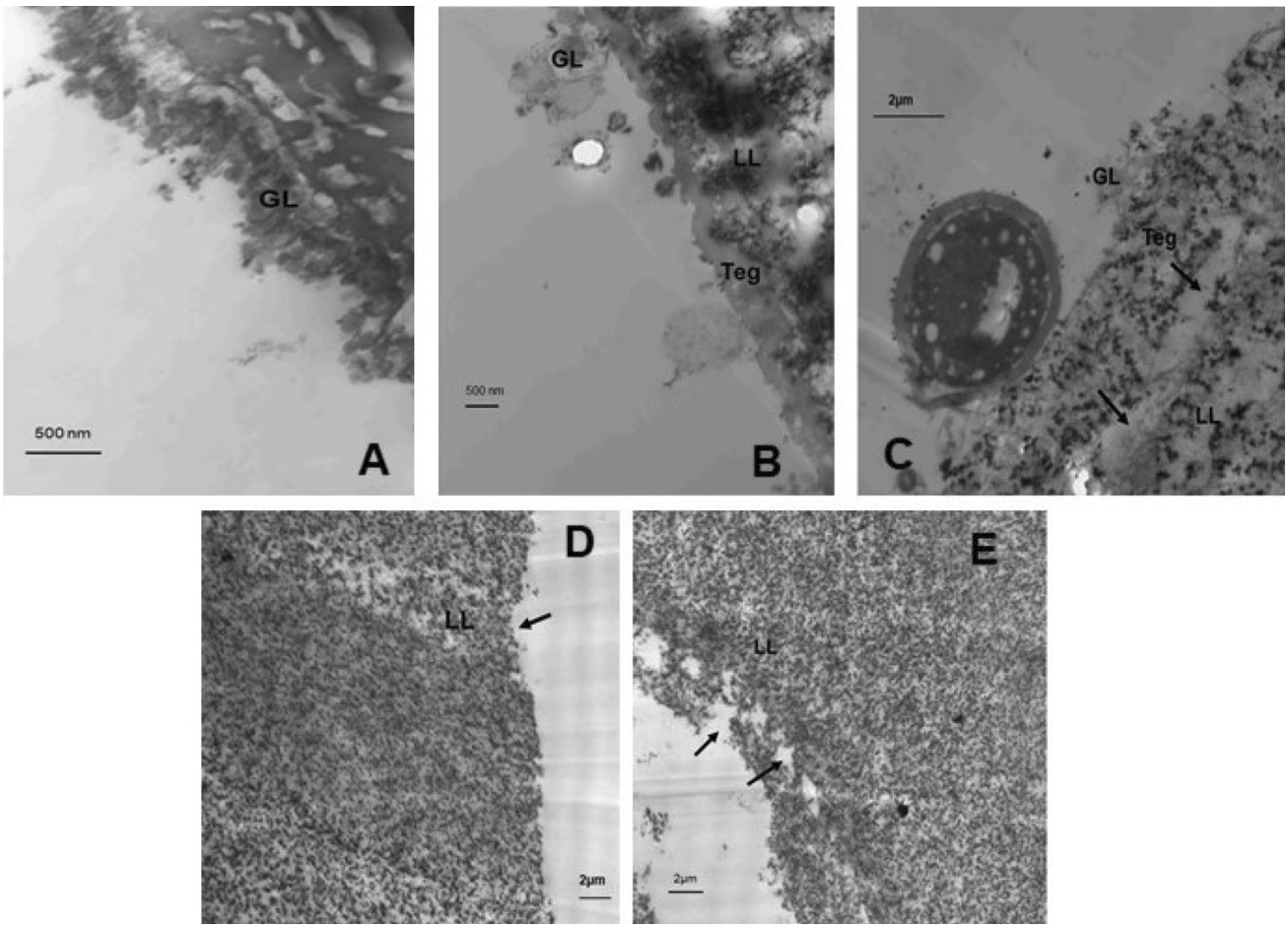

Fig. 5 TEM in *E.granulosus* camel metacestode layers after 1-h incubation with different doses of fluralaner compared to control. **a** Control sample showing intact germinal layer and the characteristic multicellular structure. **b, c** treated metacestode showing detachment of germinal layer with only cellular debris remaining and the detachment of tegument (arrow) from the laminated layer (LL). **d, e**: treated metacestode showing complete disappearance of the germinal layer and some destruction in the laminated layer (arrow). A (x7500), B (x4000), C (x3000), D&E (x1500)

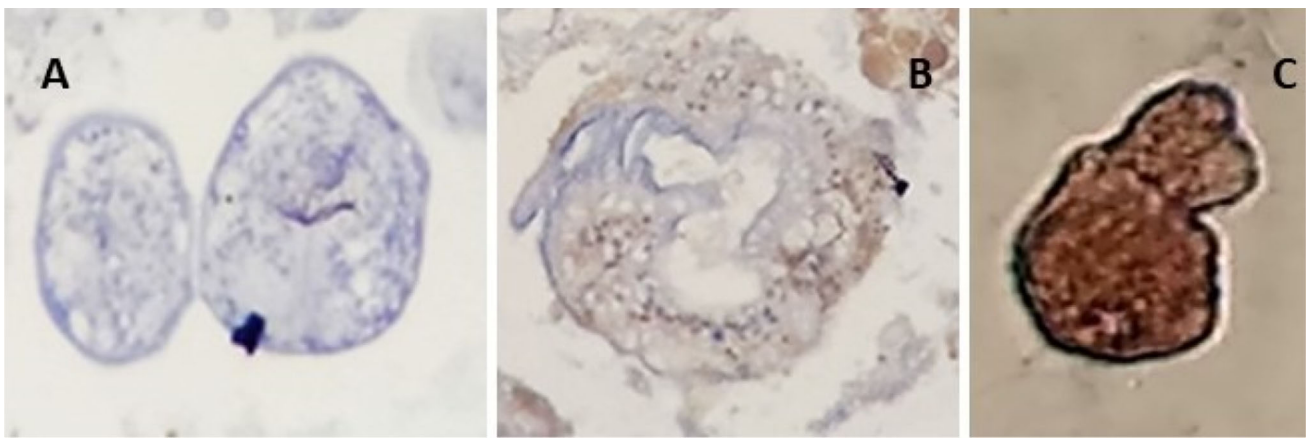

Fig. 6 Expression of caspase-3 in protoscolices by immunohistochemistry; **a** control metacestode. **b** treated protoscolices with 12.5 ug/ml for 1 h, showing few spots stained brownish. **c**: treated protoscolices with 100 ug/ml for 1 h, showing all protoscolices stained brown. (x400)

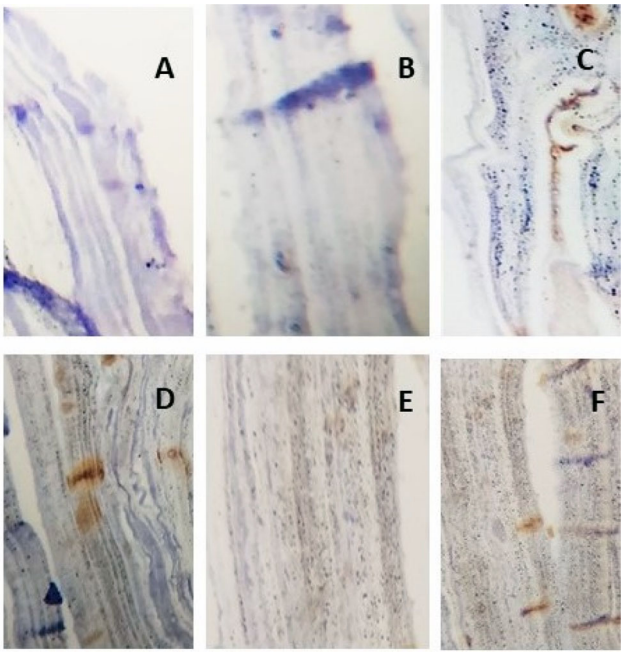

Fig. 7 Expression of caspase-3 in metacestode layers by immunohistochemistry; **a–b** control metacestode. **c-d** treated metacestodes with 12.5 ug/ml for 1 h, showing few spots stained brownish. **e–f** treated metacestodes with 100 ug/ml for 1 h, showing large areas (almost whole layers) stained brown (× 400)

blood meal. raising the concern to the stable pharmacokinetics and long half-life of the drug

Its high inhibitory effect, stable pharmacokinetics after systemic administration and its safety confirmed on dogs, raise the possibility of using this drug for treatment or prevention of hydatid disease in animals or humans after testing it on animals with hydatid cysts.

Conclusion

Fluralaner demonstrated to have a potent scolicidal effect in vitro, suggesting having therapeutic potential in hydatid disease. It should be further evaluated and developed as a promising drug for human and animal use in the treatment and prevention of hydatid disease

Author contributions All manuscript authors contributed to every aspect of it; the idea of the research, study design, collection of materials, methodology, writing the paper and revising and editing it.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval The study was approved by the Research Ethical Committee of Faculty of Medicine, Ain Shams University, Cairo, Egypt, under registration number FWA 00006644. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

References

Alam-Eldin YH, Badawy AF (2015) Destructive effect of gamma irradiation on echinococcus granulosus metacestodes. Parasitol Res 114:3145–3150

Biogiorno G, Meyer L, Evans Lekouch A, Lekouch N, Bianch R, Khoury R, Chiummo R, Thomas E, Gradoni L (2019) A single oral dose of fluralaner (Bravecto®) in dogs rapidly kills 100% of blood-fed *Phlebotomus perniciosus*, a main visceral leishmaniasis vector, for at least one month after treatment. Med Vet Entomol. https://doi.org/10.1111/mve.12420

Biserova NM, Ivan A, Kutyrev IA, Kirsten Jensen K (2014) GABA in the nervous system of the cestodes *Diphyllobothrium dendriticum* (Diphyllobothriidea) and *Caryophyllaeus laticeps* (Caryophyllidea), with comparative analysis of muscle innervation. J Parasitol 100(4):411–421

Brunetti E, Kern P, Vuitton DA (2010) Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 114:1–16

Dent JA (2006) Evidence for a diverse cys-loop ligand-gated ion channel superfamily in early bilateria. J Mol Evol 62:523–535

Elissondo MC, Albani CM, Gende L, Eguaras M, Denegri G (2008) Efficacy of thymol against *Echinococcus granulosus* protoscolices. Parasitol Int 57(2):185–190

El-Shazly AM, Awad SE, Hegazy MA, Mohammad KA, Morsy TA (2007) Echinococcosis granulosus/hydatidosis an endemic zoonotic disease in Egypt. J Egypt Soc Parasitol 37(2):609–622

Eriksson KS, Maule AG, Halton DW, Panula P, Shaw C (1995) GABA in the nervous system of parasitic flatworms. Parasitology 110 (Pt 3) (3):339–346

Gassel M, Wolf C, Noack S, Williams H, Ilg T (2014) The novel isoxazoline ectoparasiticide fluralaner: selective inhibition of arthropod γ-aminobutyric acid- and L-glutamate-gated chloride channels and insecticidal/acaricidal activity. Insect Biochem Mol Biol 45:111–124

Hokelek M, Deger BA, Deger E, Tutar E, Sunbul M (2002) Ivermectin used in percutaneous drug injection method for the treatment of liver hydatid disease in sheep. Gastroenterology 122:957–962

Hosseini SV, Ghanbarzadeh K, Barzin J, Sadjjadi SM, Tanideh N, Mehrabani D (2006) *In vitro* protoscolicidal effects of hypertonic glucose on protoscolices of hydatid cyst. Korean J Parasitol 44(3):239–242

Hu H, Kang J, Chen R, Mamuti W, Wu G, Yuan W (2011) Drug-induced apoptosis of *Echinococcus granulosus* protoscoleces. Parasitol Res 109(2):453–459

Khachatryan AS (2017) Analysis of lethality in echinococcal disease. Korean J Parasitol 55(5):549–553

McManus DP, Gray DJ, Zhang W, Yang Y (2012) Diagnosis, treatment, and management of echinococcosis. BMJ 344:e3866

Neumayr A, Tamarozzi F, Goblirsch S, Blum J, Brunetti E (2013) Spinal cystic echinococcosis–a systematic analysis and review of the literature: part 2. Treatment, follow-up, and outcome. PLoS Negl Trop Dis 7:e2458

Parkoohi PI, Hosseinzadef F, Taghians S, Rostami F, Mousavi A, Rezai MS (2018) Epidemiology and clinical features of hydatid cyst in Northern Iran from 2005 to 2015. Iran J Parasitol 13(2):310–316

Porter AG, Janicke RU (1999) Review emerging roles of caspase-3 in apoptosis. Cell Death Differ 6:99–104

Richmond JE, Jorgensen EM (1999) One GABA and two acetylcholine receptors function at the C. elegans neuromuscular junction. Nat Neurosci 2:791–797

Sadjjadi SM (2006) Present situation of echinococcosis in the middle east and Arabic North Africa. Parasitol Int 55:S197–S202

Salinas JL, Vildozola Gonzales H, Astuvilca J, Arce- Villavicencio Y, Carbajal-Gonzalez D, Talledo L, Willig JH (2011) Long-term albendazole effectiveness for hepatic cystic echinococcosis. Am J Trop Med Hyg 85:1075–1079

Schuske K, Beg AA, Jorgensen EM (2004) The GABA nervous system in C. elegans. Trends Neurosci 27(7):407–414

Spotin A, Majdi MM, Sankian M, Varasteh A (2012) The study of apoptotic bifunctional effects in the relationship between host and parasite in cystic echinococcosis: a new approach to suppression and survival of hydatid cyst. Parasitol Res 110:1979–1984

Tawfik RA (2018) *In vitro* scolicidal effect of bee venom on *Echinococcus granulosus* protoscolices. J Egypt Soc Parasitol 48(3):689–697

Vassilatis DK, Elliston KO, Paress PS, Hamelin M, Arena JP, Schaeffer JM, van der Ploeg LHT, Cully DF (1997) Evolutionary relationship of the ligand-gated ion channels and the ivermectin-sensitive, glutamate-gated chloride channels. J Mol Evol 44:501–508

Walker M, Rossignol JF, Torgerson P, Hemphill A (2004) In vitro effects of nitazoxanide on *Echinococcus granulosus* protoscoleces and metacestodes. J Antimicrob Chemother 54(3):609–616

Walther FM, Allan MJ, Nuernberger MC (2014) Safety of fluralaner chewable tablets (BravectoTM), a novel systemic antiparasitic drug, in dogs after oral administration. Parasites Vectors 7:87

Walther FM, Allan MJ, Roepke RK (2015) Plasma pharmacokinetic profile of fluralaner (Bravecto^{TM}) and ivermectin following concurrent administration to dogs. Parasites Vectors 8:508

WHO (2012) Accelerating work to overcome the global impact of neglected tropical diseases-a roadmap for implementation. WHO Press, Geneva

Williams H, Hartmut Z, Roepke RKA, Zschiesche E, Heckeroth AR (2015) Fluralaner activity against life stages of ticks using Rhipicelhalus sanguineusand *Orthodorus moubata* IN invitro contact and feeding assays. Parasites Vectors 8:90

Wolstenholme AJ (2011) Ion channels and receptors as targets for the control of parasitic nematodes. Int J Parasitol: Drugs Drug Resist 1:2–13

Yang GJ, Liu L, Zhu HR, Griffiths SM, Tanner M, Bergquist R, Utzinger J, Zhou XN (2014) China'' sustained drive to eliminate neglected tropical diseases. Lancet Infect Dis 14:881–892

Zhang W, Wang S, McManus DP (2014) *Echinococcus granulosus* genomics: a new dawn for improved diagnosis, treatment, and control of echinococcosis. Parasite 21:66

Zheng H, Zhang W, Zhang L, Zhang Z, Li J, Lu G, Zhu Y, Wang Y, Huang Y, Liu J, Kang H, Chen J, Wang L, Chen A, Yu S, Gao Z, Jin L, Gu W, Wang Z, Zhao L, Shi B, Wen H, Lin R, Jones MK, Brejova B, Vinar T, Zhao G, McManus DP, Chen Z, Zhou Y, Wang S (2013) The genome of the hydatid tapeworm *Echinococcus granulosus*. Nat Genet 45(10):1168–1175

Zou X, Wang J, Zhao H, Zhang J, Wu W, Ye B (2009) *Echinococcus granulosus*: protoscolicidal effect of high intensity focused ultrasound. Exp Parasitol 121(4):312–316