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Assessment of Triclabendazole Efficacy Against *Fasciola hepatica* by Histopathological Changes Induced in Tegument and Gut of Flukes

Oksana Bibik[1], Mahmoud Abdelhamid[2*]

[1]Department of Biology and Parasitology, Kemerovo State Medical University, Kemerovo, 650029, Russia.
[2]Department of Parasitology, Faculty of Veterinary Medicine, Aswan University, Aswan, 81528, Egypt.

***Correspondence**
Mahmoud Abdelhamid
Department of Parasitology, Faculty of Veterinary Medicine, Aswan University, Aswan, 81528, Egypt.
E-mail address: mahmoud.ahmed2509@aswu.edu.eg

Abstract

Triclabendazole is the drug of choice against *Fasciola* spp. infections in humans and animals. However, parasitic resistance against triclabendazole is spreading in the veterinary field. The objective of this study was to assess of flukicide efficacy of triclabendazole through evaluation of the histopathology of *Fasciola hepatica* adult fluke's specimens. For this, the efficacy test was performed on naturally infected sheep treated with triclabendazole (Fazinex®) at the dose recommended for *F. hepatica*, in which the flukes recovered at necropsy on the 7th day post-treatment and separated for histological examination. The teguments and intestines from *F. hepatica* recovered from treated and control sheep were examined by microscopy. In the outer and inner parts of the tegument, edema and swelling of its structural components are noted. The spines in the tegument were enlarged, swollen, take on a more rounded shape and have changes in color, perceiving the eosinophilic dye in greater concentration. There was a detachment of the brush border of the intestinal epithelium and the accumulation of microvilli of the apical part of the epithelium in the intestinal lumen. The cellular structures of the intestinal epithelium were melted. The results indicated that the teguments and intestines of *F. hepatica* were severely affected by triclabendazole and demonstrated the importance of the use of histopathology for the diagnosis of therapeutic efficacy in field strains.

KEYWORDS
Fasciola hepatica, Trematodes, TCBZ, Anthelmintic, Histopathology.

INTRODUCTION

Fasciola hepatica is a parasitic trematode of one health importance due to its global distribution and ability to infect multiple host species, including humans. Several species have been described within the genus *Fasciola* (Mehmood *et al*., 2017; Arafa *et al*., 2018; Çelik and Celik, 2018; Aghayan *et al*., 2019; Nerway *et al*., 2021). Only two species, *F. hepatica* and *F. gigantica*, are commonly recognized as taxonomically valid species that occur in domestic animals and humans (Simsek *et al*., 2011; De Agüero *et al*., 2020). The definitive host range of both fasciolid species is vast, including herbivorous mammals, mainly livestock species, while freshwater snails of the family Lymnaeidae act as intermediate hosts or vectors (Dar *et al*., 2010; Nguyen *et al*., 2012; Chaudhry *et al*., 2016). Fascioliasis is a worldwide parasitic zoonosis and a significant food-borne neglected tropical disease (NTD) caused by the trematodes of the genus *Fasciola* (Alba *et al*., 2021). It has a negative impact on food security through its effect on livestock productivity. In sheep and cattle, effects of infection range from clinical disease, with high levels of mortality and morbidity, to long-standing subclinical infections, which reduce animal productivity, growth and fertility (Fürst *et al*., 2012).

Triclabendazole (TCBZ), benzimidazole derivative, is one of the major anthelminthic drugs used to control fascioliasis in domestic animals. Triclabendazole is the only effective treatment for fascioliasis infection caused by *F. hepatica* or *F. gigantica*, which is recommended by WHO and recently approved by the FDA. However, several studies have suggested that these parasites have developed resistance to triclabendazole (Brennan *et al*., 2007; Fairweather, 2009; Marcos *et al*., 2021). The process of searching for alternative and improved existing anthelmintic drugs is essential to overcome the resistance from helminthes (Galkina *et al*., 2010; Lanusse *et al*., 2018). To improve the old treatment regimens, develop alternative and most rational strategies for the treatment of helminthiasis, knowledge of the mechanisms of action of drugs on parasites and the host organism, their comparative characteristics are necessary. This information determines the direction of prospecting for their synthesis and provides a correct assessment of new compounds' advantages in relation to existing anthelmintic drugs. The study for the effect of anthelmintics on the parasitic organism is interesting for veterinary and human medicine (O'Neill *et al*., 2015; Carneiro *et al*., 2019; Fairweathera *et al*., 2020; Abdelhamid *et al*., 2021).

Numerous studies confirm that histological methods are informative when studying drug's mechanism of action on helminths. It is suitable to be used as criteria for assessing the action of anthelmintics on the organs and tissues of trematodes (Savage *et al*., 2014; Carneiro *et al*., 2019). Visual changes in tissue and

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cell structures in the body of a helminth as a reaction to a chemical drug's action enable to identify the general patterns of their damage. These methods visually complement scatological data on the effectiveness of drugs, and histological micropreparations provide an objective examination that can be repeated at any time. The chemical structure of the active substance that forms the drug's basis determines the degree of its action on the body of helminthes. It causes a process of morphologic restructuring and a change in intracellular metabolism (Lanusse *et al*., 2018). Therefore, this study aimed to evaluate the histopathological examination as an informative criterion in evaluating the efficacy of anthelmintic (Triclabendazole) against hepatic fluke (*Fasciola hepatica*) after deworming spontaneously invasive sheep.

MATERIALS AND METHODS

Animals and experimental design

Three live mature flukes of *Fasciola hepatica* were collected from the bile ducts of naturally infected sheep slaughtered at Kemerovo slaughterhouse, Russia and considered as control group. To eliminate all traces of blood and bile, the flukes were washed several times with warm normal saline solution (37ºC) and examined immediately (to avoid any disruption) in the Department of Biology, Genetics and Parasitology, Kemerovo State Medical University. Other three flukes of *Fasciola hepatica* (experimental group) taken, after slaughtering at Kemerovo slaughterhouse, from the bile ducts of naturally infected sheep that underwent deworming with triclabendazole (Fazinex®), chemically 5-chloro-6- (2,3-dichlorophenoxy) -2-methylthiobenzimidazole, on the 7th day after giving the drug in a dose of 10 mg/kg of active substance in the treatment of sheep fascioliasis, once. *Fasciola hepatica* specimens from the control and experimental groups were fixed in a solution of methyl alcohol and glacial acetic acid (3: 1) for further examination.

Histopathological examination of Fasciola hepatica

For histopathological examination, the three flukes were taken from each group. The fixed trematodes were processed according to the generally accepted histological technique (Scott *et al*., 2005). Briefly, after fixation, flukes were dehydrated in alcohols of ascending concentration (40%, 50%, 60%, 70%, 80%, 90%, and 100%) for 1-2 days; then passed through a mixture of chloroform and absolute alcohol (in a ratio of 1:1); then through pure chloroform in 2 portions for 10-15 minutes; impregnated the material with a mushy mixture of chloroform and paraffin in a thermostat at a temperature of 370C for 12-18 h; impregnated trematodes with paraffin in a thermostat at 56ºC for 30-45 minutes; embedded in paraffin wax with added wax. Prepared sections with a thickness of 5-7 microns stained by histological satins and were studied with a light microscope.

RESULTS

Micromorphological studies on histological preparations from mature flukes *Fasciola hepatica*, from the control group, confirm the presence of large conical spines in the outer part of the tegument. The base of the spines is immersed in the depth of the tegument. In the tegument, the spines are retained by a thin layer of cytoplasm that covers them along their entire length (Fig. 1).

Pathomorphological analysis after the action of triclabendazole revealed destructive changes in the spines. After the action of triclabendazole on *Fasciola hepatica*, the spines look enlarged, swollen, take on a more rounded shape and have changes in color, perceiving the eosinophilic dye in greater concentration (Fig. 2). In the outer and inner parts of the tegument, edema and

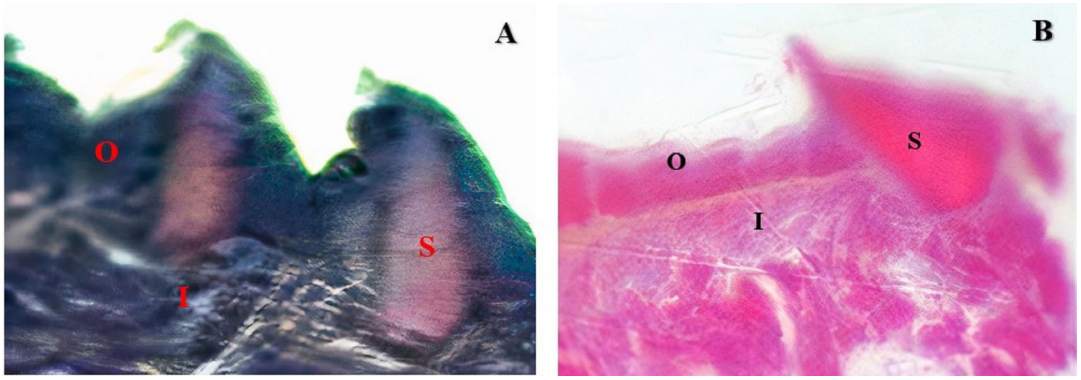

Figure 1. Fragments of the mature *Fasciola hepatica* tegument: S – spines; O – outer part of the tegument; I – the inner part of the tegument. (Microphoto, magnification 7 x 8, H&E staining (A); magnified 10 x 8, Mallory stain (B)).

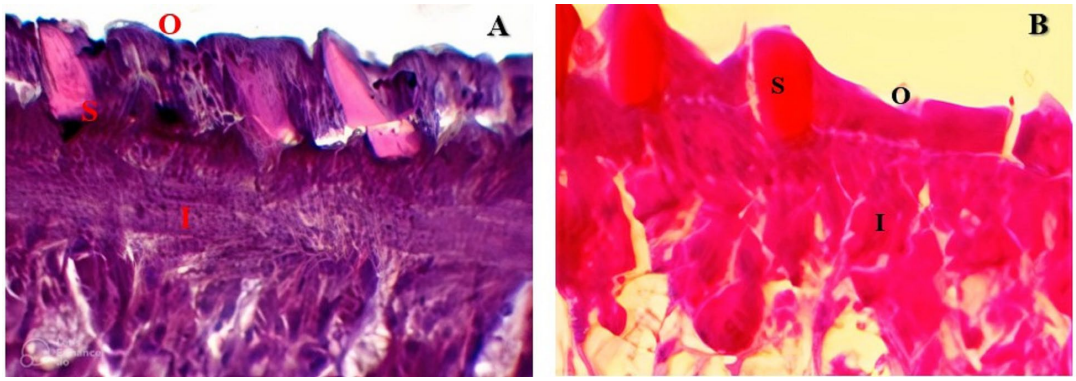

Figure 2. Fragments of the *Fasciola hepatica* tegument after the action of triclabendazole: S – spines; O – outer part of the tegument; I – inner part of the tegument (Microphoto, magnification 10 x 20, H&E staining (A); magnification 10 x 20, Mallory stain (B)).

swelling of its structural components are noted.

Intestinal morphology in *Fasciola hepatica* is represented by a single-layer cylindrical microvillous epithelium and has a general structure plan: Cytoplasmic membrane, the apical and basal parts of the epithelium, microvilli, and fibrous basement membrane. The nucleus is located in the basal part of the enterocyte cell (Fig. 3B). The apical parts of the cells protruded into the intestinal lumen and look uneven.

Changes in the intestinal structure of *Fasciola hepatica* exposed to triclabendazole (Fig. 4) showed a detachment of the brush border and accumulation of microvilli of the apical part of the epithelium in the intestinal lumen. The cellular structures of the intestinal epithelium are melted. The boundaries of the layers are blurred, it is impossible to determine the apical and basal parts of the intestinal epithelium. The cells of the basal part of the epithelium are represented by a layer consisting of stuck together cells, with the absence of clear nuclei. The nuclear envelope is destroyed. The preserved epithelial layer in the basal part is stained with Mallory's dye in a dark magenta color with a reddish tint. Normally, this dye has a uniform distribution of orange-red color in the intestinal epithelium. The fibers of the basement membrane of the intestine of *Fasciola hepatica* have a swollen appearance.

The results of this study showed that the action of triclabendazole changes the structure of the tegument and intestinal epithelium of *Fasciola hepatica* and clearly confirmed the presence of edema, and lysis of the structures of these tissues and organs. The effect of the force of action of triclabendazole on the intestine exceeds the value of the action of the anthelmintic on the tegument of *Fasciola hepatica*

The results show that the effect of triclabendazole changes the structure of the tegument and intestinal epithelium of *Fasciola hepatica* and destruction is mostly present in the intestines. Micromorphological structures of the apical and basal part of the intestinal epithelium under exposure to triclabendazole as a result of lysis have a blurry appearance (Fig. four). In the distal and proximal parts of the tegument fasciolus after the action triclabendazole marked severe edema (Fig. 4). At the distal tegument, lysis of structural components is observed. Fibrous structures of the basement membrane of the tegument and intestines have a swollen appearance (Figure 2; Figure 4). In the intestine *Fasciola hepatica*, edema of the basement membrane is noted more strongly than in the tegument.

DISCUSSION

Fasciolosis caused by *Fasciola hepatica* is a parasitic disease prevalent in ruminants raised in many regions of the world. Undoubtedly, the development of pathological processes in the body of the parasite is determined by the chemical structure of the active substance of the anthelmintic. The selective entry of anthelmintics into the body of trematodes through the tegument and intestines was discussed in the works of various authors (Alvarez *et al*., 2004; Meaney and Fairweather, 2004; O'Neill *et al*., 2015; Carneiro *et al*., 2019). Hexosaminoglycans, common proteins, and mucoproteins were discovered in the tegument, intestines, and parenchyma, which provide the basis for the functional morphology of helminths (Morales and Espino, 2012). The effect of anthelmintic drugs during chemotherapy on the breakdown of this "parasite-host" system, as well as the effect of drugs from various chemical groups on the parasite and host, as components of this system, are of particular interest (Bibik and Abdelhamid, 2021). The tegument of trematodes has several important roles, including osmoregulation, protection, secretion, or synthesis, and hence represents a primary drug target (Halton, 2004). Researchers believe that the destruction of border organs, primarily the genus *Fasciola* tegument, opens the way for the drug to all internal structures of the parasite, disrupting the structural organization of the basement membranes and parenchyma (tissue of the internal environment of the parasite), reaching the organs of the

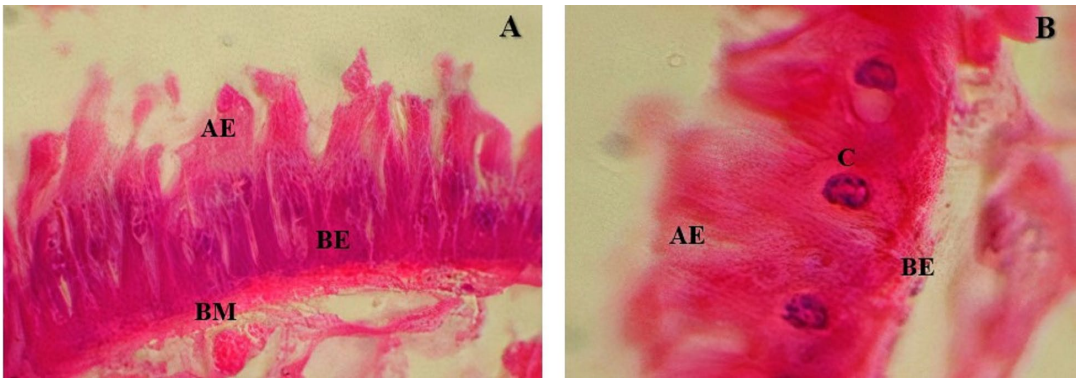

Figure 3. Fragments of the intestine of *Fasciola hepatica*: AE – the apical part of the intestinal epithelium; BE – the basal part of the intestinal epithelium; BM – basement membrane; C – nucleus (Microphoto, magnification 10 x 20 (A), 40 (B) H&E staining (A)).

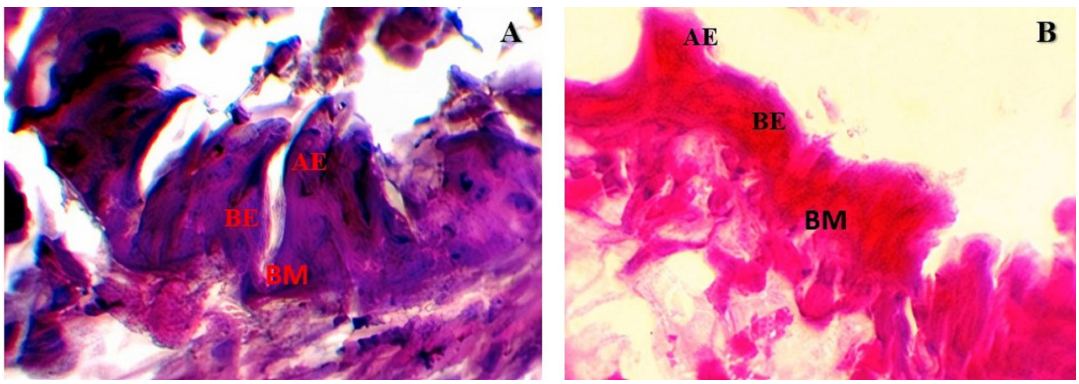

Figure 4. Fragments of the intestine of mature *Fasciola hepatica* after the action of triclabendazole: AE – the apical part of the intestinal epithelium; BE – the basal part of the intestinal epithelium; BM – basement membrane (Microphoto, magnification 10 x 40 H&E staining (A), Mallory staining (B)).

reproductive system (McKinstry *et al*., 2003; Devine *et al*., 2010; Toner *et al*., 2010).

Scientists currently pay more attention to triclabendazole's effect on genus *Fasciola*, since this drug is widely used for fascioliasis in animals and medicine to treat fascioliasis in humans at a therapeutic dose of 10 mg/kg (Gandhi *et al*., 2019). However, there are reports on the mechanism of resistance of *Fasciola* spp. to triclabendazole (Robinson *et al*., 2003; Savage *et al*., 2014; Fairweathera *et al*., 2020). TCBZ resistance to liver fluke in sheep appeared in England and Wales (Kamaludeen *et al*., 2019), The Netherlands and Spain (Fairweather, 2009). The resistance of helminths to chemical preparations requires constant updating and improvement (Wanga *et al*., 2018).

The result of this histopathological study of *Fasciola hepatica*, after the action of TCBZ, showed that the spines in the tegument are enlarged, swollen, take on a more rounded shape and have changes in color, perceiving the eosinophilic dye in greater concentration. Surface blebbing, swelling of the tegument and microvillus formation were common features of drug treated parasites. They were considered indicators of stress reaction by the fluke, to replace damaged surface membrane (McKinstry *et al*., 2003). Swollen spines due to the swelling of the tegument surrounding them. Removal of the apical plasma membrane led to their loss, the imprint of the empty spine socket being left behind in the remaining syncytium and subsequently in the basal lamina following sloughing of the entire syncytium (Meaney *et al*., 2002). Edema of the organs and tissue structures of trematodes, due to the process of swelling of biopolymers, confirms the violation of the permeability of their membranes. Damage to the membranes of the border organs – the tegument and intestines, as well as membrane-type structures inside the body of the parasite, disrupts the active transport of sodium (Na+) and potassium (K+), the concentration of which on both sides of the membrane changes (levels off) and deviates from physiological conditions (McConville *et al*., 2009; Toner *et al*., 2010; Savage *et al*., 2014; Carneiro *et al*., 2019; Bibik and Arkhipov, 2020). This causes the penetration into the cells of low molecular weight anions and then cations. The result is an increase in intracellular osmotic pressure and a violation of membrane water-electrolyte transport. Lysis, swelling of ultrastructure and tissue edema occur, followed by the dissolution of biopolymers. An excess of interstitial fluid leads to the development of water intoxication of the parasite's body. The data from the previous studies, together with the results of the present study, suggested that the tegument of *F. hepatica* had been shown to be very susceptible to the action of TCBZ (Walker *et al*., 2004; Halferty *et al*., 2008; Toner *et al*., 2009).

CONCLUSION

Histopathological studies of the organs and tissues of *Fasciola hepatica* after the action of triclabendazole (TCBZ) is highly informative criteria demonstrating the effect of the anthelmintic on the parasite organism when assessing its helminthic properties. This study has shown that triclabendazole is capable of causing substantial disruption to the tegument and intestine of adult flukes of *Fasciola hepatica*. The study of changes in the microstructure of organs and tissues of helminthes, during deworming is of paramount importance in the development of effective measures to combat helminthiasis and is promising for identifying general patterns of drug action on the body of trematodes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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