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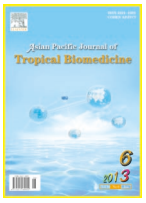

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Tegumental histological effects of Mirazid® and myrrh volatile oil on adult *Fasciola gigantica*

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PEER REVIEW

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Comments

This paper described the comparative *in vitro* effects of Mirazid®, myrrh volatile oil and TCBZ-SO (reference drug) on the tegumental structure of adult *F. gigantica* using light microscopy. The results are interesting and suggested that it might be possible to reinforce Mirazid® fasciocidal activity by increasing its content of myrrh volatile oil.

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ABSTRACT

Objective: To evaluated the histological changes within the tegument of adult *Fasciola gigantica* (*F. gigantica*) that led to the gross changes that were visible externally. **Methods:** The effects of oleoresin extract of myrrh (Mirazid®), myrrh volatile oil and triclabendazole sulphoxide (reference drug) on the tegumental structure of adult *F. gigantica* following treatment *in vitro* had been determined by light microscopy. **Results:** The internal changes in the tegument observed in this study were compatible with surface changes seen in the previous scanning electron microscopy study, using the same drugs. The swelling of tegumental syncytium was a particular feature of their action, but its level was much greater with myrrh volatile oil, in which vacuolization of the tegument and loss of spines were observed. **Conclusions:** The present study demonstrated the fasciocidal properties of Mirazid® oleoresin extract, and it might be possible to reinforce its fasciocidal activity by increasing its content of myrrh volatile oil.

KEYWORDS

Fasciola gigantica; Mirazid®, Myrrh volatile oil, Histological effect

1. Introduction

Tropical fasciolosis caused by *Fasciola gigantica* (*F. gigantica*) remains one of the most important helminth parasites of livestock and a relentless constraint on their growth and production[1]. It is now also recognized as a human disease of large public health importance[2]. Future control of fasciolosis is faced with two major challenges; a likely increase in disease and the spread of resistance to, the most potent flukicide, triclabendazole (TCBZ)[3]. This resistance is increasing since 1995[1], thus, new drugs that can kill liver flukes are urgently needed. Mirazid®, a drug containing 300 mg purified resin extract of *Commiphora molmol* (*C. molmol*), is sold in the market as an antiparasitic drug. The resin extract of *C. molmol* as schistosomicide, fasciolicide, heterophycide and molluscicide have been reviewed in detail and provided sufficient evidence for its uses as antiparasitic agent[4]. Subsequently, it was found that the resin of *C. molmol* and Mirazid® displayed therapeutic effect on hepatic coccidiosis induced by the parasite *Eimeria stiedae* in domestic rabbits[5]. In addition, Mirazid® showed therapeutic effect for *Giardia lamblia* infected rats, and produced a 100% reduction of

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intestinal and fecal parasitic counts[6].

Not only dose the resin of this genus displays antiparasitic potential, but also its volatile oil is active against parasites. Indeed, a previous scanning electron microscopy study has shown that the surface changes of adult *F. gigantica* induced by Mirazid® oleoresin extract are less severe than those observed after exposure to myrrh volatile oil[7]. This light microscopy study will evaluate the histological changes within the tegument of adult *F. gigantica* that lead to the gross changes that are visible externally.

2. Materials and methods

2.1. Parasites

Adult worms of *F. gigantica* were collected from the bile ducts and gall bladder of buffalo slaughtered in a local abattoir in Cairo province, Egypt. They were washed several times with warm (37 °C) sterile complete RPMI 1640 culture medium. Only intact and actively mobile worms were used in this study.

2.2. Drugs and myrrh volatile oil

Oleoresin extract in Mirazid® capsules were obtained from Pharco Pharmaceuticals Company, Alexandria, Egypt. Triclabendazole "Fasinex® 10%" was previously known drug for treatment of fasciolosis. It was purchased from Ciba-Geigy Company. Myrrh volatile oil was obtained from the previous study[7].

2.3. In vitro assays

Under sterile conditions in a laminar flow cabinet, flukes were transferred to fresh culture medium containing antibiotics (penicillin, 50 IU/mL; streptomycin, 50 mg/mL), 50% (v/v) heat denatured rabbit serum and 2% (v/v) rabbit red blood cells; as recommended by Hegazi *et al*[8]. Stock solutions of Mirazid® oleoresin and myrrh volatile oil at 10 mg/mL were prepared in Cremiphor EL 10%. The flukes were incubated with 60 μg/mL of Mirazid® oleoresin and myrrh volatile oil for 24 h at 37 °C in an atmosphere of 5% CO_2. The concentration used in this study was based on the findings of the previous study[7], which demonstrated that the best concentration of Mirazid® oleoresin and myrrh volatile oil that could affect on the tegument of adult flukes *in vitro* was 60 μg/mL. A positive control group was prepared by incubating whole flukes for 24 h in RPMI 1640 culture medium containing 15 μg/mL TCBZ-SO. This level corresponded to maximum blood levels *in vivo*[9]. Solvent control flukes were incubated for 24 h in RPMI 1640 culture medium containing 0.06% (v/v) Cremiphor EL. Normal control flukes were fixed immediately following the initial washing. Six flukes were examined for each tested drug.

2.4. Light microscopy

Following incubation, the adult flukes were cut into small, 5-mm pieces before being fixed in 10% buffered formol saline (6.5 g/L of disodium phosphate and 4 g/L monosodium phosphate solution). After dehydration, samples were embedded in paraffin and sectioned at 4-6 μm. Sections were stained with hematoxylin and eosin[10]. The tegument of adult flukes was studied and photographed using an Olympus CX41 microscope.

3. Results

The tegument of normal adult flukes showed even cytoplasmic

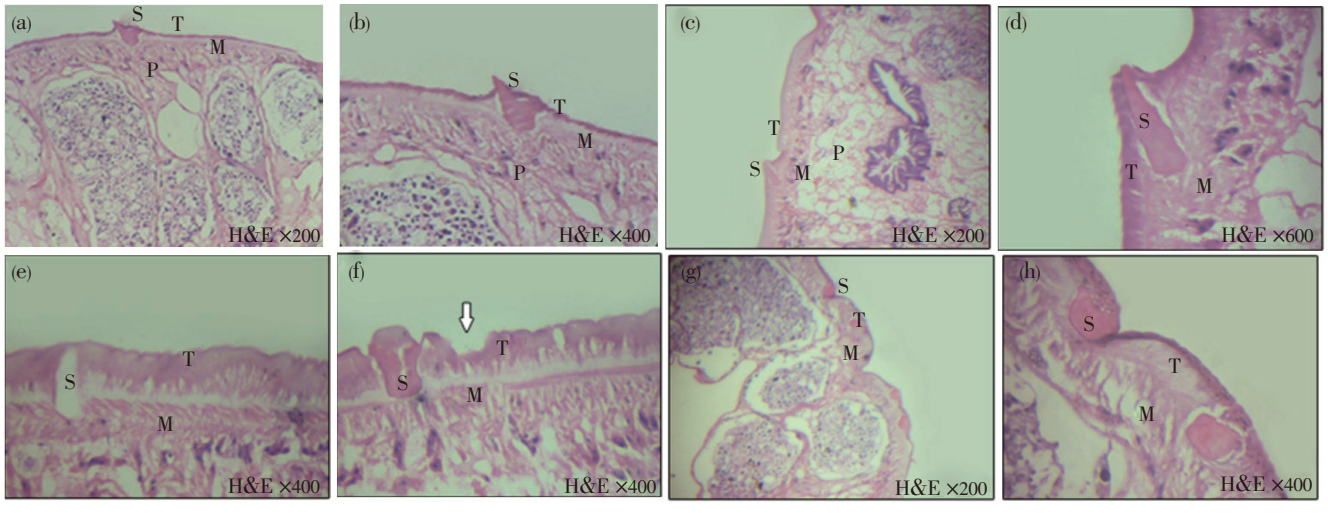

Figure 1. Light micrographs of the tegument cross section of adult *F. gigantica*.
(a) and (b): Normal fresh flukes. Note intact tegument (T), spine (S), muscular layer (M) and parenchyma (P); (c) and (d): 24 h incubation *in vitro* with 60 μg/mL Mirazid® oleoresin. Note tegumental swelling and the spines appears to be surrounded by the tegument. (e) and (f): 24 h incubation *in vitro* with 60 μg/mL myrrh volatile oil. Note spines dislodges from their sockets, appearance of numerous vacuoles in the tegument syncytium and the tegument itself is partly sloughed off (arrow). (g) and (h): 24 h incubation *in vitro* with 15 μg/mL TCBZ-SO. In this specimen, the spines have enlarged and appeared sunken as the tegument have swollen to engulf them.

syncytial layer with numerous spines embedded throughout its matrix. The outer rim of the tegument and tips of the spines were intensely stained. The tegument rested on layer of connective tissue called reticular lamina, which connected the former to the underlying and deeply stained two muscular layers. Tegumental cells locates underneath the muscles and sent their processes between the muscle cells outwardly to join up with the tegument (Figures 1a and 1b). No significant differences were observed between fresh fluke and control fluke incubated for 24 h in solvent; 0.06% (v/v) Cremiphor EL. On the other hand, after 24 h incubation *in vitro* with 60 μg/mL Mirazid® oleoresin, tegumental swelling and furrows were seen, as well as the spines appeared to be surrounded by the tegument (Figures 1c and 1d), while the underlying structures still appeared normal. These changes became more severe with spines dislodged from their sockets and others showed extensive cracking towards the base following 24 h incubation with 60 μg/mL myrrh volatile oil. Besides, appearance of numerous vacuoles in the tegument syncytium and the tegument itself was partly sloughed off (Figures 1e and 1f), but muscle underlying the tegument still exhibited normal appearance. Severe swelling of the tegument between the spines was apparent after 24 h incubation with 15 μg/mL TCBZ-SO. In these specimens, the spines had enlarged and appeared sunken as the tegument had swollen to engulf them. Flooding was observed in the intercellular spaces between muscle bundles which appeared to be entirely separated from the surrounding tissue (Figures 1g and 1h).

4. Discussion

The tegument of trematodes has a number of important roles, including osmoregulation, protection, secretion or synthesis, hence represents a primary drug target[11]. This study confirmed previous results that both Mirazid® oleoresin and myrrh volatile oil displayed fasciocidal activity *in vitro*[7], and that the tegument of *F. gigantica* was a primary drug target of them. The tegument of *Fasciola* spp. had many of the characteristics of a transporting epithelium that was involved in ion and water controls. The swelling of tegumental syncytium indicated that disruption of the apical membrane and its associated ion pumps had led to perturbation of the fluke's osmoregulatory system and the influx of water[3]. The swelling of the basal infolds would facilitate the sloughing of the tegument as a whole, by causing the detachment of the basal plasma membrane from the underlying basal lamina. This swelling was a particular feature of drug action with a number of flukicides as recently summarized[3]. The tegumental changes induced by Mirazid® oleoresin were less severe than those observed after exposure to either myrrh volatile oil or TCBZ-SO. Flukes showed tegumental swelling after these treatments, but its level was much greater with myrrh volatile oil, in which vacuolization of the tegument and loss of spines were observed. Vacuolization of the tegument in this study raised from dilation of the basal infolds as a result of a water and ion imbalance as mentioned earlier. Similar vacuolization of the tegument had been described in a number of anthelmintic-treated schistosomes, *Opisthorchis viverrini* and also in *F. hepatica*[1]. The internal changes in the tegument observed in this study were compatible with surface changes seen in the previous scanning electron microscopy study, using the same drugs. The tegumental swelling and furrowing observed on the surface can be linked to the swelling of the basal infolds. The presence of blebs and microvillus-like projections indicated that the fluke was having problems repairing and replacing surface membrane damaged by drug action.

Essential oil of myrrh was rich in furanosesquiterpenoids, and around 20 different compounds of this type had been isolated and identified. Phytochemicals present in this plant had been investigated previously and resulted in a series of metabolites including terpenoids, steroids, flavonoids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives isolated and identified from *Commiphora* species. These secondary metabolites of the *Commiphora* species exhibited diverse biological activities, such as cytotoxic effects[12]. The present study demonstrated the fasciocidal properties of Mirazid® oleoresin extract and myrrh volatile oil which caused disruption to the tegument of adult *F. gigantic* after *in vitro* incubation. This disruption was comparatively less severe after exposure to Mirazid® oleoresin extract. Taking into consideration that Mirazid® oleoresin extract contained 7%-17% volatile oil[13], it might be possible to reinforce its fasciocidal activity by increasing its content of myrrh volatile oil.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

F. gigantica is an important helminth parasite of

livestock in almost all tropical and subtropical regions of the world. Its control is based largely on chemotherapy. Triclabendazole is the drug of choice, however recent studies confirmed the occurrence of its resistance in sheep and cattle. There are no drugs of comparable activity currently available for the treatment and control of fasciolosis, hence there is a pressing need for new trematocidal drugs. Mirazid® proved to be a promising alternative fasciolicidal drug. Yet, its mode of action is still unclear. A previous scanning electron microscopy study demonstrated the surface changes of adult *F. gigantica* following treatment *in vitro* with Mirazid® and myrrh volatile oil.

Research frontiers

This light microscopy study evaluated the histological changes within the tegument of adult *F. gigantica* that led to the gross changes that were visible externally, in the previous scanning electron microscopy study. The swelling of tegumental syncytium was a particular feature of action of Mirazid® and myrrh volatile oil, but its level was much greater with the later, in which vacuolization of the tegument and loss of spines were observed.

Related Reports

The internal changes in the tegument observed in this study were compatible with surface changes seen in the previous scanning electron microscopy study, using the same drugs. The previous study suggested that the anthelmintic activity of Mirazid® was attributed to its content of volatile oil.

Innovations and breakthroughs

This study is the first to demonstrate the comparative effects of Mirazid®, myrrh volatile oil and TCBZ-SO (reference drug) on the adult *F. gigantica* using light microscopy.

Applications

The present study demonstrated more tegumental disruption in myrrh volatile oil exposed specimens, compared to that exposed to Mirazid®. Hence, it might be possible to reinforce Mirazid® fasciocidal activity by increasing its content of myrrh volatile oil.

Peer review

This paper described the comparative in vitro effects of Mirazid®, myrrh volatile oil and TCBZ-SO (reference drug) on the tegumental structure of adult *F. gigantica* using light microscopy. It evaluated the histological changes within the tegument of adult *F. gigantica* that led to the gross changes that were visible externally, in a previous scanning electron microscopy study. The fluke's tegument seems to be the main target of Mirazid® and myrrh volatile oil. The results are interesting and suggested that it might be possible to reinforce Mirazid® fasciocidal activity by increasing its content of myrrh volatile oil.

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