TANG [HUMANITAS MEDICINE]

Original article

Scanning electron microscopic effects of Indian Ayurvedic drugs: Krimimudgar Ras, Kriminol and Vidangasava on a tapeworm, *Raillietina* sp.

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ABSTRACT

Ayurveda is one of the ancient systems of traditional medicine in India, however, there is a lack of proper scientific research and controlled studies on efficacy and toxicity of several Ayurvedic formulations using the methods of modern science, which hinders the proper development of Ayurveda, both in India and abroad. The present study was undertaken to further corroborate the efficacy of some popular anthelmintic Ayurvedic medicines, as evident by the pharmacological effects of Ayurvedic formulations on body surface of a cestode parasite. This study employed a common intestinal cestode of domestic fowl, *Raillietina* sp., as a model parasite, that was exposed *in vitro* to different concentrations of three common Indian Ayurvedic anthelmintic drugs, namely Krimimudgar Ras, Kriminol and Vidangasava, and also to a modern anthelmintic drug, praziquantel (PZQ). After the paralysis of parasites, the specimens from the highest drug-treated concentration (50 mg/ml), praziquantel (1 mg/ml), and control groups, were collected and processed for scanning electron microscopic studies to document the effects of drugs on the body surface of parasite. In this study, maximum degree of alterations on parasite body surface was observed in the worms exposed to Krimimudgar Ras, indicating this drug to be highly efficacious against intestinal worms and brings out a transtegumental mode of action on parasite. In conclusion, the findings of present study suggest that of the three Ayurvedic formulations tested, Krimimudgar Ras possesses significant anthelmintic effects, which are mediated through parasite's body surface. Therefore, Krimimudgar Ras may be considered as a reliable anthelmintic Ayurvedic formulation, as validated through this study.

Keywords Ayurvedic drugs, Electron microscopy, Helminthic disease, Krimi roga, Krimimudgar Ras, Kriminol, Vidangasava

INTRODUCTION

Parasitic worm infections or helminthiasis remain as one of the common health problems in developing countries. Consequences of such infections are manifested physically as well as mentally, with economic problems adding to burden of disease (Idris et al., 2019). It is estimated that more than one billion people worldwide are currently infected with intestinal helminth parasites (WHO, 2019). The global control of intestinal-helminthic diseases is based on periodic mass drug administration of populations by two synthetic anthelmintics, albendazole and mebendazole (Zhan et al., 2014). However, since these drugs have remained in use for much longer periods, there is also a threat of emergence of resistance to these drugs (Geerts and Gryseels, 2000). Presently, the traditional medicines are being trusted by a large section of people for curing various ailments throughout the world (WHO, 2012). Ayurveda is a prominent system of traditional medicine, which is widely practiced in India and several other developing countries. Besides being economical and easily available, Ayurvedic drugs and formulations are believed to have no adverse effects. However, only a limited number of systematic controlled studies or clinical trials have been done on Ayurvedic medicines, using the methods of modern science.

Some common anthelmintics used in Ayurveda for the treatment of intestinal-worm infections (Krimi Rogas) include, Vidangadi churna, Trikatu churana (Reddy and Seetharam, 2009; Mohandas et al., 2013). Another formulation, Krimighna is also considered as a broad-spectrum Ayurvedic anthelmintic (Singh et al., 2014). However, the anthelmintic activity and mode of action of these formulations have not yet been evaluated using methods of modern science.

In our previous communication, we investigated the in vitro efficacy of three Indian Ayurvedic anthelmintic drugs, namely Krimimudgar Ras, Kriminol and Vidangasava in relation to the synthetic broad-spectrum anthelmintic drugs, praziquantel and albendazole, using a cestode *Raillietina* sp. and a nematode, *Syphacia obvelata* as model test parasites (Chen and Yadav, 2018). Based on paralysis and mortality time of worms, after exposure of these worms to these Ayurvedic drugs, it was revealed that Krimimudgar Ras possesses most prominent efficacy against both the group of parasites. To further corroborate the efficacy of these anthelmintic Ayurvedic medicines, the aim of the present study was to document the pharmacological effects of Ayurvedic formulations on the body surface of a cestode parasite, *Raillietina* sp., with the help of scanning electron microscopy, so as to throw ample light on their

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mode of action.

MATERIALS AND METHODS

Ayurvedic and synthetic reference drugs

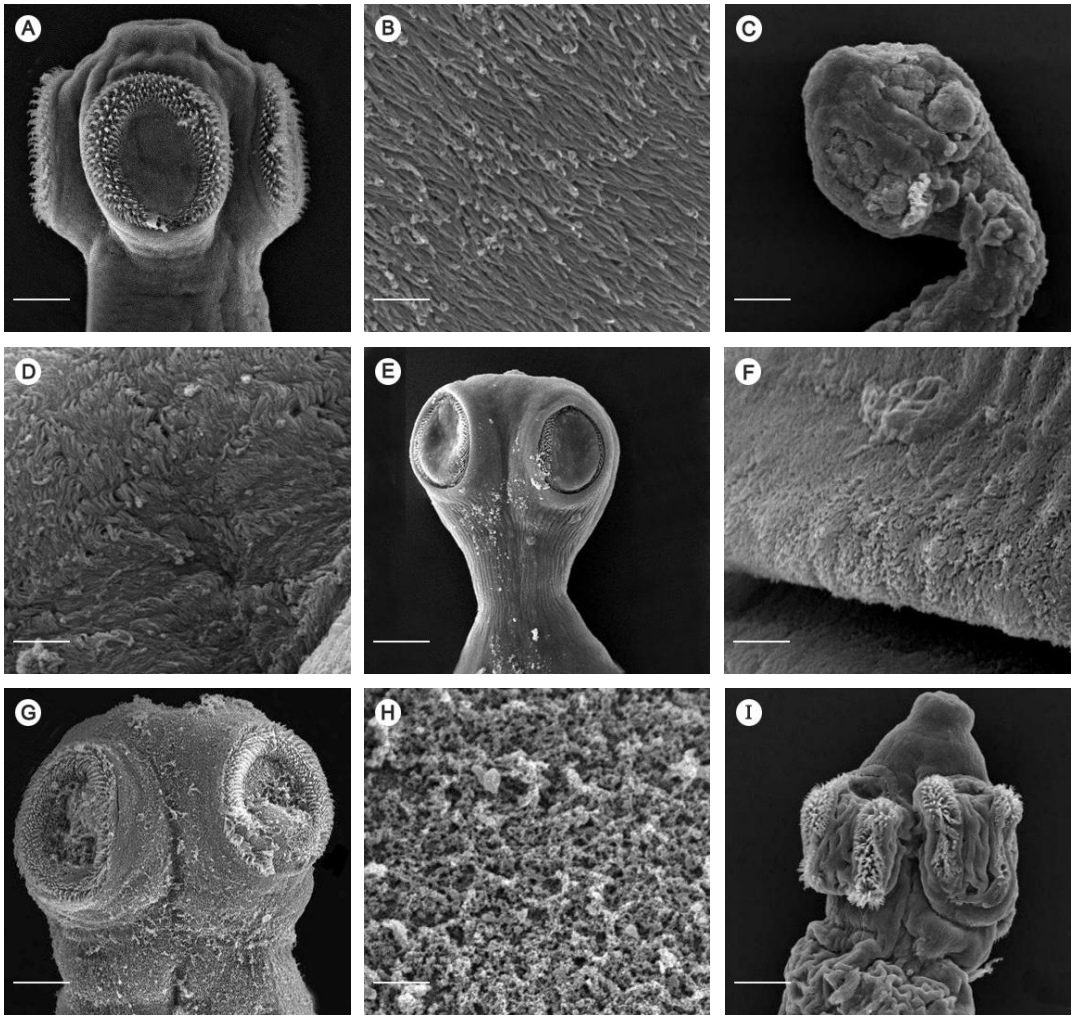
For the present study, the three Ayurvedic formulations, i.e. Krimimudgar Ras, Kriminol and Vidangasava were selected based on Ayurvedic practitioners' common prescription in Shillong and Guwahati cities of India (Chen and Yadav, 2018). Praziquantel (PZQ) was used as a modern synthetic reference drug to compare the efficacy of Ayurvedic formulations.

Parasites and electron microscopic study

Raillietina sp. tapeworms for this study were collected from the intestine of locally slaughtered domestic fowl in Shillong, India. The details about in vitro maintenance of parasites and concentrations of anthelmintic formulations used have been reported in a previous communication (Chen and Yadav, 2018). Immediately after observation of paralysis in parasites in the culture medium, the specimens from the highest concentration of each formulation, reference drug and control group were collected and washed in phosphate buffered saline. The specimens were then fixed in 10% cold neutral buffered saline and post fixed in osmium tetra oxide. The parasites were then dehydrated in an ascending grade of acetone and air-dried, using tetramethylsilane (Roy and Tandon, 1991). The dried specimens were coated with gold in a Fine coat ion sputter JFC 1100, and observed under JEOL JSM-6360 Scanning Electron Microscope.

RESULTS

The present SEM study revealed a normal contour of body of *Raillietina* sp. worms from belonging to control group. The suckers were intact and appeared as semi-circular. Each sucker was noticed to possess a row of radially arranged small hooks (Fig. 1A, 1B). The general body surface, i.e. tegument, was observed to be densely covered with a layer of fine hair-like filaments, called microtriches. Of all three Ayurvedic formulations tested, the worms exposed to Krimimudgar Ras showed the maximum degree of alterations in the body surface of parasites. In this case, the damages were evident on entire topography of scolex in the anterior region. The scolex appeared shrunken, with no traces of rostellum or small hooklets. The tegument of the worms was also observed to be damaged, with irregular microtriches throughout its surface (Fig. 1C, 1D).



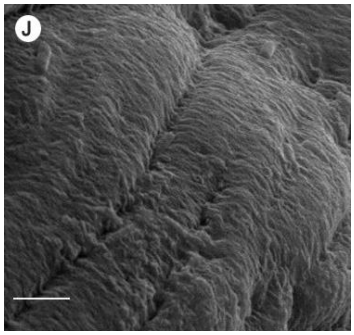


Fig 1. Effects of Ayurvedic formulations on body surface of *Raillietina* sp., as observed by scanning electron micrography: A-B: Control group. C-D: Krimimudgar-Ras treated group. E-F: Kriminol-treated group. G-H: Vidangasava-treated group. I-J: Praziquantel-treated group. (scale bars: A, C, E, G, I = 50 µm; B, D, J = 2 µm; F, H = 5 µm).

The second most noticeable effects on parasite body surface were observed in the worms exposed to Vidangasava. Herein, both the suckers and hooklets revealed quite apparent damages. Also, the tegument of worms was found to severely affected by the action of Vidangasava (Fig. 1G, 1H). On the other hand, the effects of Kriminol on parasite body surface were not much evident (Fig. 1E, 1F). In the reference drug praziquantel-exposed worms, the suckers of worms were observed to be distorted. Also, some damages were noticeable at the base of hooks on scolex. The tegument in this case was noticed to show prominent protuberances and blebs at many locations of body surface (Fig. 1I, 1J).

DISCUSSION

The body surface of cestode parasites has been implicated as one of several target sites through which natural anthelmintic products or synthetic drugs bring out their actions (Mehlhorn et al., 1983; Martin et al., 1997; Alvarez et al., 2006). In many studies, researchers have investigated the potential anthelmintic effects of anthelmintics on parasites's body surface, using scanning electron microscopy (SEM) (Challam et al., 2010; Kundu et al., 2012; Abou-Shady et al., 2014). The body of cestode parasites is comprised of a scolex, neck and a chain of segments, called proglottids. The soft body surface, called tegument, remains covered with numerous hair-like structures, called microtriches. The microtriches serve multiple functions, such as external protection, movement, absorption and digestion in cestode parasite (Halton, 2004; Levron et al., 2008).

In the present study, the worms exposed to Krimimudgar Ras showed the maximum degree of alterations in the body surface of cestode parasites. The damages were seen on the entire topography of scolex, which appeared shrunken, with no traces of rostellum or small hooklets. Also, the tegument of the worms recorded to be damaged, with microtriches appearing irregular throughout body surface. These findings suggest that Krimimudgar Ras is not only highly efficacious against the intestinal cestode, but also possesses a transtegumental mode of action on parasite. So far there is no study carried out yet to document the effects of Ayurvedic anthelmintics on helminth parasite body surface. However, the findings of present study are in agreement with Challam et al. (2010) who also observed that praziquantel brings out a destructive effect on *Raillietina* sp., causing the parasite to shrink, forming bleb-like structures on its tegument. The disorganizations and distortions in parasite tegument may be due to the tegumental enzymes which are the targets of such features (Kundu et al., 2017).

In our previous in vitro study, based on paralysis and mortality of worms, Kriminol showed the second-best anthelmintic efficacy. However, in the present study, the effects of Kriminol on parasite body surface were not much evident, as revealed by electron microscopy. This finding suggest that although Kriminol possesses anthelmintic effects, however, it does not bring out its mode of action through parasite body surface.

CONCLUSION

To sum up, the findings of the present study suggest that of the three Ayurvedic formulations tested, Krimimudgar Ras possesses significant anthelmintic effects, which is mediated through parasite general body surface. Therefore, Krimimudgar Ras may be considered a reliable Ayurvedic formulation against intestinal helminth parasites. Proper scientific validation of Ayurvedic drugs can not only add to a fast growth of Ayurvedic market but also substitute the current synthetic medicines which are believed to possess adverse effects.

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CONFLICT OF INTEREST

The authors report no potential conflict of interest.

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