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Research Article

IN VITRO ANTHELMINTIC ACTIVITY OF ARTESUNATE AGAINST THE CESTODE *RAILLIETINA ECHINOBOTHRIDA*

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ABSTRACT

Artesunate is an anti malarial compound discovered from the Chinese medicinal plant *Artemisia annua*. It has been proven to be the best drug not only in the treatment of malaria, but also of human schistosomiasis. It is also shown to be highly effective against human virus, trematodes and various cancer cells. This study is a further exploration on its pharmacological potential as an anti cestodal agent. The cestode, *Raillietina echinobothrida* Mégnin, were collected from fowls and treated with varying doses of artesunate, namely 1, 2, 5, 10 and 20 mg/ml. The anthelmintic activity was compared with that of the standard prescription drug albendazole. Artesunate showed higher anthelmintic activity than albendazole. Microscopic examination of the cestodes treated with drugs showed extensive structural damage, including surface erosion and destruction of the scolex. The findings suggest that artesunate is a potent drug that may be used for cestode infection.

Keywords: ALbendazole, anthelmintic, artesunate, *Raillietina echinobothrida*.

INTRODUCTION

Artesunate is a semi-synthetic derivative of artemisinin, a natural compound isolated from the *Artemesia annua*.[1,2] It is more potent and innocuous than any other drug being used as anti-malarial agent. A series of clinical trials established that it is the best drug in the treatment of severe and complicated malaria.[3] Since 2006 the World Health Organization has advocated its use in the management of malaria due to *Plasmodium falciparum*, the leading cause of human mortality through the ages.[4] Further experimentations have broadened its medical value in other diseases such as in human schistosomiasis, viral infection, cancer and asthma.[5-7] Members of *Raillietina* are the most prevalent cestode parasites in birds throughout the world. *R. echinobothrida* Mégnin, 1880, is the most important species in terms of incidence and virulence.[8] It can severely cause health problems and economic production in poultry farming. Its infection often results a symptom called nodular tapeworm disease, which is indicated by enteritis and development of granuloma in the intestine. This leads to unchecked necrosis, anemia and ultimate death.[9] Artesunate is characterized by high biological activity, safety, solubility (the most soluble among all arteminins) and molecular stability. Its adverse effects are minimal. The only known side effect is that it causes haemoglobin digestion, but this is considered in clinical trials as relatively harmless.[10] So far its anthelmintic activity is known only in trematodes. It was shown to cause severe tegumental damage in *Fasciola gigantica*. More pronounced structural changes were seen in *F. hepatica*.[11] Similar damaging effects were noted in *Schistosoma mekongi*,[12] and *S. mansoni*.[13] Small intestinal trematodes, heterophyids, were also fatally damaged.[14] It is important to investigate further on its potential use in the management of important helminth parasites. Therefore, the present study was designed to test the effect of artesunate on *R. echinobothrida*.

MATERIALS AND METHODS

Chemicals and drugs

All the chemicals and reagents used were standard analytical grades, obtained either from Hi Media or S.D. Fine Chemicals Limited, India. Artesunate (Falcigo) was a product of Zydus Cadila Healthcare Limited, India. A prescription anthelmintic, albendazole (Zentel®) was a product of Glaxo Smith Kline Pharmaceutical Limited, Mumbai, India.

Helminth parasites and *in vitro* treatments

Local fowls (*Gallus domesticus* Linnaeus) were purchased from the chicken vendor at the New Market, Aizawl, India. Using an overdose of chloroform, they were sacrificed at the Department of Zoology, Pachhunga University College. Necropsy was performed, the intestines were dissected open, and live cestodes, *Raillietina echinobothrida*, were recovered. Collection, identification and processing were done as previously described.[15] The cestodes were collected in culture dishes which contained 0.9 % neutral phosphate-buffered saline (PBS, pH 7-7.3) and were then incubated at 37 ± 1°C in a glass-chambered bacteriological incubator. One hour prior to the experimental treatment different doses of artesunate, such as 1, 2, 5, 10 and 20 mg/ml, were prepared by dissolving them in pre-incubated PBS supplemented with 1 % dimethylsulfoxide (DMSO). The different doses were kept in separate culture dishes in the incubator. Similar doses of albendazole were also prepared for standard comparison. One set of culture dishes contained only PBS with 1 % DMSO was reserved to serve as control experiment. The cestodes were evenly divided in each of the culture media. Each experimental assay was performed in triplicate.

Survival test and statistics

Motility and mortality of the worms were monitored visually through the glass chamber. Total inactivity or death was defined as complete loss of spontaneous motor activity upon physical provocation of the worms, which was done by gently agitating the culture media. The time taken for death was routinely checked and recorded. The survival duration was represented as means plus or minus standard deviation. The relative survival time of treated worms against control groups were calculated using unpaired student's *t*-test, with the level of significance considered when the *p* value is greater than 0.05.

Microscopy

Artesunate-treated and untreated cestodes were washed with fresh PBS. They were stained with borax carmine and completely dehydrated through different grades of alcohol. They were mounted on glass slides and observed under Olympus-Jenoptik image analyzer.

RESULTS

The relative survival time of *R. echinobothrida* in different test media is given in Table 1. In the control medium containing PBS with DMSO, they survived up to 51.55 ± 3.00 h. The cestodes responded to artesunate in less than 2 hours (1.73 h) at 20 mg/ml dose. They took 13.23 h to indicate complete mortality. At the different doses tested, time of death was directly proportional to the time and dose of treatment. At all doses tested the anthelmintic effect was significant at $p < 0.05$. Similar pattern of mortality was seen in those treated with albendazole. But overall survival was longer. For the highest dose death was observed at 1.85 h, and for lowest dose, it took 18.53 to cause death. The comparative efficacy of artesunate and albendazole are depicted in Figure 1, which shows that artesunate is more potent than albendazole. Under microscopic image analyzer, untreated cestode showed normal body features. Its anterior end has a knob-like scolex (Figure 2). The scolex bears four lateral suckers, which radially surround an apical rostellum. These suckers and rostellum form the organs of attachment. Each sucker is oval, almost circular in shape and lined with rows of spines. The body proper called the strobila is made up of a chain segments called proglottids, which forms a ribbon-like body. Each of the mature proglottid contains numerous eggs that are arranged side-by-side, and which occupy almost the entire body space (Figure 3). Cestode treated with 20 mg/ml artesunate showed extensive structural changes. The scolex was severely damaged with most of the suckers eroded (Figure 4). Only a tiny portion of the sucker with its spines is visible. The general smooth texture of the tegument is also lost. In the mature proglottids there are no apparent intact eggs (Figure 5). In their place are a number of irregular clumps of tissues. The regular tegumental contour seen in normal cestodes were also lost, indicating extensive erosion of the body surface.

Table 1: Survival of *R. echinobothrida* under control experiment and treatment with albendazole and artesunate

Media	Dose (mg/ml)	Survival time in h (± SD)	*t* value	*p* value
PBS + DMSO	0	51.55 ± 3.00		
Artesunate	1	13.23 ± 0.70	21.53	< 0.05†
	2	09.28 ± 0.53	24.03	< 0.05†
	5	05.52 ± 0.39	26.31	< 0.05†
	10	03.23 ± 0.63	27.46	< 0.05†
	20	01.73 ± 0.78	27.84	< 0.05†
Albendazole	1	18.53 ± 0.79	18.43	< 0.05†
	2	12.97 ± 0.89	21.36	< 0.05†
	5	05.68 ± 0.83	25.61	< 0.05†
	10	03.22 ± 0.58	27.39	< 0.05†
	20	01.85 ± 0.61	28.13	< 0.05†

† Significantly different in comparison to control (0) group; $n = 3$

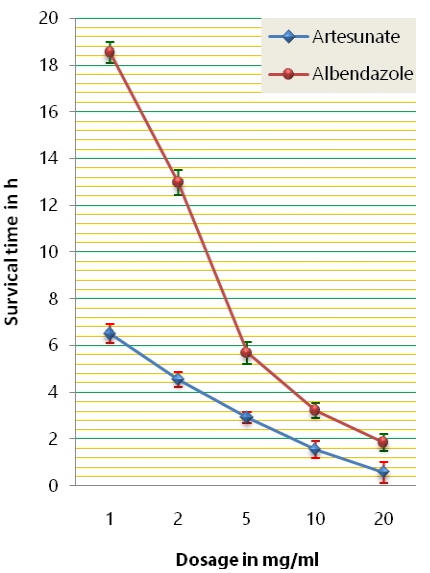

Figure 1: Comparison of the efficacy of albendazole and artesunate against *R. echinobothrida*

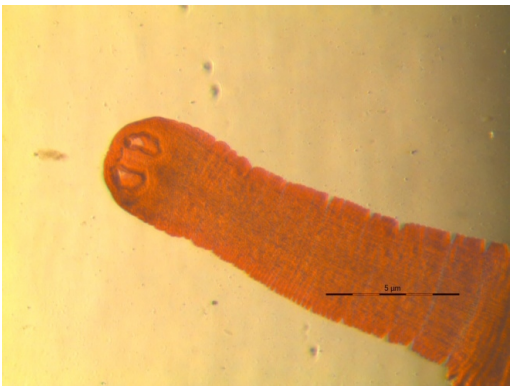

Figure 2: Scolex of an untreated *Raillietina echinobothrida* showing tow oval suckers (x200)

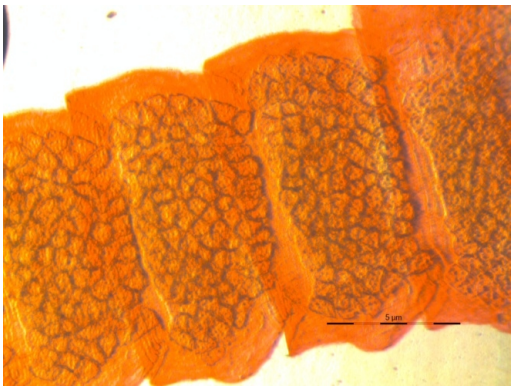

Figure 3: Proglottids of an untreated *R. echinobothrida* showing organized eggs (x200)

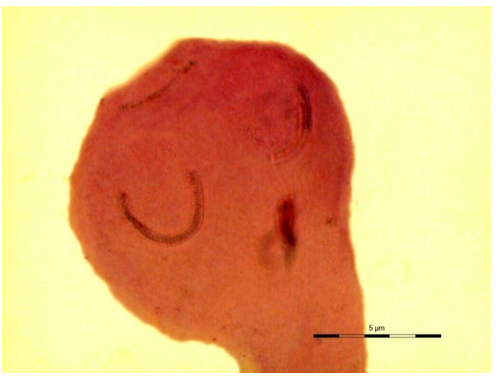

Figure 4: Scolex Damage in the suckers is visible, and one of them is almost completely removed (x200)

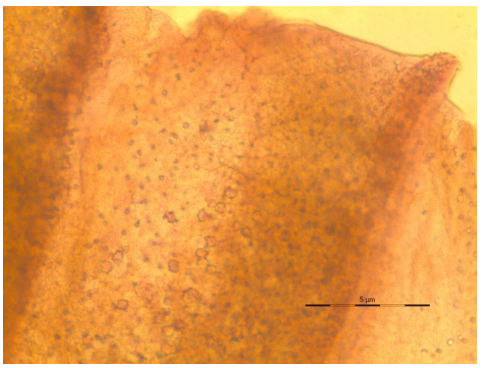

Figure 5: A mature proglottid of *R. echinobothrida* treated with artesunate showing complete damage of eggs; Only irregularly shaped debris are visible (x400)

DISCUSSION

In spite of a tremendous rigour in the search for new anthelmintics in the recent decades, little progress has been made owing to new compounds that are highly potent but are not promising due to their adverse effects. The danger of increasing drug resistance in helminth parasites remain a serious concern both in veterinary and human issues.[16,17] Cestode infections are particularly one of the most neglected parasitic diseases although they are responsible heavy losses in animal industry, as well as in human development.[18] Artesunate appears to be the most promising candidate as broad-spectrum drug for its non-toxic nature, stability and solubility.[19,20] The effectiveness of artesunate on trematodes is well established. The worm burden of small intestinal trematodes, heterophyids, in mice was reduced by 100 % after three days of routine medication with 200 mg/kg/day of artesunate. Microscopically examination of the worms revealed bleb formation, tegumental disruption, erosion and peeling.[14] Experimental infection of mice, rabbits and dogs with *Schistosoma japonicum* resulted in worm reduction by 77.50-90.66 %, 99.53 % and 97.10 % respectively after treatment with artesunate.[21] Artesunate and artemether at a dose of 400 mg/kg given to hamsters infected with *Opisthoschis viverrini* resulted in worm burden reductions of 77.6 % and 65.5 %, respectively. In *Clonorchis sinensis*-infected rats, a single dose of artesunate and artemether at 150 mg/kg caused reduction of worm burden up to 98.6-100 %.[22] Intramuscular injection of 40 mg/kg aresunate in sheep naturally infected with *F. hepatica* resulted in reduced faecal egg count and worm burden by 97.6 % and 91.9 %, respectively.[23] An *in vitro* treatment on the 3-week-old juveniles of *Fasciola gigantica* resulted in dose-dependent lethal effect. Microscopic study showed pronounced swelling of the tegumental ridges, followed by blebbing and later rupturing of the blebs, leading to erosion and lesion, and general destruction of the tegument.[24] Rats experimentally infected with *F. hepatica*; were effectively treated. Extensive tegumental damage was observed on the trematode.[11] Tegumental damages were also noted in *S. mekongi*, which were experimentally infected in rats.[12] Extensive tegumental erosions were described in *S. mansoni*.[13] These detrimental effects are quite similar to the effects seen in the present experiment. The extensive damage on the tegument is typical of anthelmintic activity described for various drugs. Teguments in cestodes and trematodes are primary absorptive and sensory sites, and hence are the target sites of drugs. Consequently, it has been profusely documented that the distinctive effect of anthelmintic drugs is irrevocable modification and impairment of the tegument.[25-27] Albendazole and its related benzimidazoles are known directly interfere with the tegument and its supporting muscle layers by binding specifically to β-tubulins, thereby, inhibiting assembly and functioning of the cellular motor proteins.[28] Benzimidazoles are known for their broad-spectrum activities including antibacterial, anthelmintic, antiprotozoal, anticoagulant, antifungal, analgesic, antidiabetic, antiviral and anticancer activities.[29,30] One of the most important benzimidazoles, albendazole was shown to cause complete shrinkage of the tegument throughout the body in *R. echinobothrida*, blebbings at various locations and erosion of the scolex.[31] Treatment of the human cestode, *Echinococcus granulosus*, with albendazole and its sulphoxide combination resulted in formation of numerous blebs on the tegument, rostellar disorganization and loss of the microtriches.[32] Albendazole and praziquantel combination caused loss of sucker concavity, separation and disintegration of the

germinal layers, loss of microtriches and destruction of the tegument in *E. granulosus* and *Mesocestoides corti*.[26] The effects of albendazole, flubendazole and nitazoxanide are quite similar and are shown to cause decreased number and length of the microtriches, degeneration of the rostellum, blebbings on the tegument, disintegration of hooks and microtriches, accompanied by vesiculation in *E. granulosus* and *E. multiloculoris*.[25,27] This study thus present an evidence that artesunate is highly effective on the cestode *R. echinobothrida*. The effects are characteristics of the anthelmintic activities of standard drugs. This is also a confirmation that the drug is generally effective on soft-bodied helminths such as trematodes and cestodes. It is also suggestive that it may be used as the treatment of cestode infection both in human and veterinary cases.

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