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ANTICESTODAL ACTIVITY OF ARTESUNATE AGAINST *Raillietina tetragona*

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Abstract- Pervasive drug resistance in malarial and helminth parasites is the mainstay in veterinary and clinical medicines. These parasites remain the leading cause of death and morbidity, with associated societal and economic incapacity. Artesunate has become a prescription drug in the treatment of severe malaria due to *Plasmodium falciparum* and schistosomiasis. It has been profusely demonstrated to be highly effective against human virus, trematodes, and various cancer cells. However, nothing is known about its anthelmintic activity on parasitic cestodes. Thus, avian cestodes *Raillietina tetragona* were collected from fowls and treated with incremental doses of artesunate, ranging from 0.7, 1.5, 3, 6 to 12 mg/ml. The cestodes were highly susceptible to the various doses of the drug, and the anthelmintic activity was dose- and time-dependent. Microscopic examination on the tegument of the treated cestodes showed extensive alteration, indicated by surface erosion and obliteration of the attachment organs, including suckers and rostellum. The result advocates that artesunate is a good candidate in the management of cestode infections.

Keywords- Artesunate, anthelmintic, cestode, *Raillietina tetragona*

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Introduction

The discovery of artemisinin in the early 1970s from the Chinese medicinal plant *Artemisia annua* was a major breakthrough in modern medical and pharmaceutical research [1]. The natural compound was proven to be an exceptionally potent agent for the treatment of severe malaria due to *Plasmodium falciparum*, the leading cause of human mortality through the ages [2]. Further chemical and biological analyses have revealed that the compound could be synthesized into more efficient and safe derivatives. The artemisinins are now established through a series of clinical trials as the best drugs in the treatment of severe and complicated malaria [3]. These elaborate investigations are arguably the most important development in medical parasitology [4], as well as in pharmaceutical science [5].

Malaria and helminthiasis remain the most prevalent and debilitating parasitic diseases. Evolution of drug resistance in malarial and helminth parasites heightens the global concern of their management and effective treatments. As a matter of fact, these formidable parasites have developed pervasive resistance to all the best of drugs available, rendering most prescription drugs practically useless in the treatment of malaria [6], and helminthiasis [7,8]. The single most important antimalarial drug quinine and its analogues were declared by the World Health Organization in 2006 as no longer safe and suitable for the treatment of severe malaria [2]. Virtually all commercially available anthelmintic drugs are reported as having resistance in veterinary or human parasites [7]. Development of new innocuous and effective drugs is a matter of medical urgency.

Among the many derivatives of artemisinin, artesunate is a semi-synthetic compound known to have high biological activity, safety, solubility and molecular stability. Its adverse effects are minimal, and its major side effect on haemoglobin digestion is considered clinically insignificant [9]. Though legal approval is underway, the World Health Organization advocates it for the first line medication in severe malaria [2]. Further explorations on the pharmacological potential show it as an extraordinarily robust broad-spectrum drug with significant effects on all major pathogens. Rigorous experiments have established that it is effective against viruses (human cytomegalovirus), protozoans (*Toxoplasma gondii*), helminths (different flukes including schistosomes) and fungi (*Cryptococcus neoformans*) [10]. Furthermore, it was found to have a strong anti-allergic activity [11], as well as remarkable cytotoxicity against different cancer cells [12]. Successful clinical trials have been conducted among patients infected with different species of human schistosomes [13]. It has been advocated as the drug of choice in schistosomiasis [14,15]. Unfortunately, drug development for cestodes, one of the most prevalent parasites both in animals and hu-

mans, remains largely ignored. Therefore, in the light of new information on the pharmacology of artesunate, it was thought worthwhile to investigate its possible effect on the common poultry cestode, *Raillietina tetragona* Molin, 1858.

Materials and Methods

Chemicals and Drug

All the chemicals and reagents used were standard analytical grades, obtained either from HiMedia or S.D. Fine Chemicals Limited, India. Artesunate (Falcigo) was a product of Zydus Cadila Healthcare Limited, India.

Helminth Parasites and in Vitro Treatments

Live fowls (*Gallus domesticus* Linnaeus) were procured from a poultry vendor at the New Market, Aizawl, India. They were sacrificed with an overdose of chloroform at the Department of Zoology, Pachhunga University College. Upon necropsy, the intestines were dissected open, and live tapeworms, *Raillietina tetragona*, were recovered. Collection, identification and processing were done as previously described [16,17]. All the worms were collected in culture dishes containing 0.9% neutral phosphate-buffered saline (PBS, pH 7-7.3) and then incubated at 37 ± 1°C in a glass-chambered bacteriological incubator. One hour before the experimental treatment, 60 mg (the manufactured dosage) of artesunate was dissolved in 5 ml of PBS supplemented with 1% dimethylsulfoxide (DMSO). Incremental concentrations of the drug, such as 0.7, 1.5, 3, 6, and 12 mg/ml, were prepared by serial dilution with PBS, and maintained in separate culture dishes in the incubator. One set of culture dishes contained only PBS with 1% DMSO was set aside to serve as control. The worms were divided into even batches for each of the culture media and were incubated in them. Each experimental assay was performed in triplicate.

Survival Test and Statistics

Motility and mortality of the worms were monitored by visual observation through the glass chamber. Total incapacity or death was defined as irreversible loss of spontaneous physical movement upon subtle stimulation of the worms, which was done by gently agitating the culture media. The time taken for the worms to show complete death was routinely checked and recorded.

The data were represented as means plus or minus standard deviation. The comparison of survival time of treated worms against those of the control groups were analysed using student's *t*-test, and the level of significance was considered when the *p* value is greater than 0.05.

Microscopy

Artesunate-treated and untreated cestodes were thoroughly washed with fresh PBS. After staining with borax carmine, they were completely dehydrated through serial grades of alcohol. After treating with xylene, they were mounted on glass slides and observed under Olympus-Jenoptik image analyser.

Results

The cestode *R. tetragona* survived very well up to 51.55 ± 3.00 h in a control medium containing PBS and DMSO. Those treated with artesunate expired shortly after exposure, and time of death was directly proportional to the time and dose of incubation. The effects of the drug at various dosages are presented in [Table-1]. At the highest dose tested, *viz* 12 mg/ml, tapeworms could survive only up to 0.71 ± 0.12 h, while at the lowest dose, viz 0.7 mg/ml, they survived for 6.50 ± 0.33 h. At all doses tested the anthelmintic effect was significant at $p < 0.05$.

Table 1 - Efficacy of artesunate on the survival of *Raillietina tetragona*.

Media	Dose (mg/ml)	Survival time in h (± SD)	t value	p value
PBS + DMSO	0	51.55 ± 3.00	-	-
	0.7	6.50 ± 0.33	25.84	< 0.05*
	1.5	4.54 ± 0.24	27.04	< 0.05*
Artesunate	3	2.92 ± 0.10	28.02	< 0.05*
	6	1.55 ± 0.19	28.68	< 0.05*
	12	0.71 ± 0.12	29.27	< 0.05*

*Significantly different in comparison to control (0) group. n = 3.

Normal, untreated cestode has a knob-like anterior end called the scolex [Fig-1]. The scolex bears four suckers surrounding an apical rostellum as organs of attachment. Each sucker is oval in shape and lined with rows of spines. The body proper called the strobila is composed of a series of ribbon-like segments or proglottids [Fig-2].

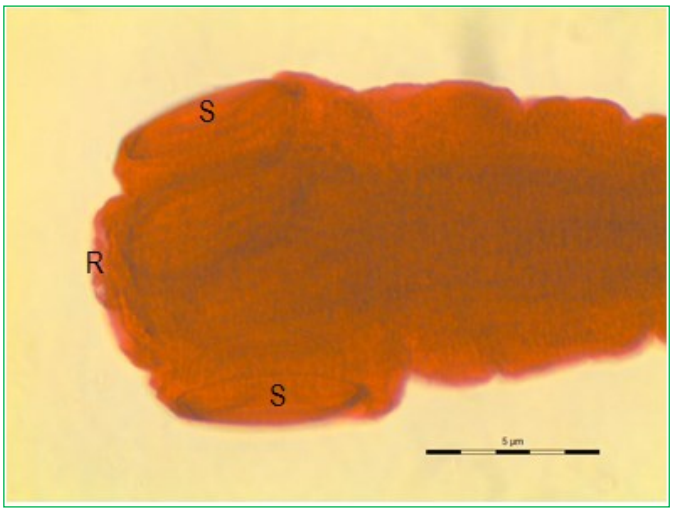

Fig. 1- The scolex of normal *Raillietina tetragona* (x 200). (S = sucker, R = rostellum)

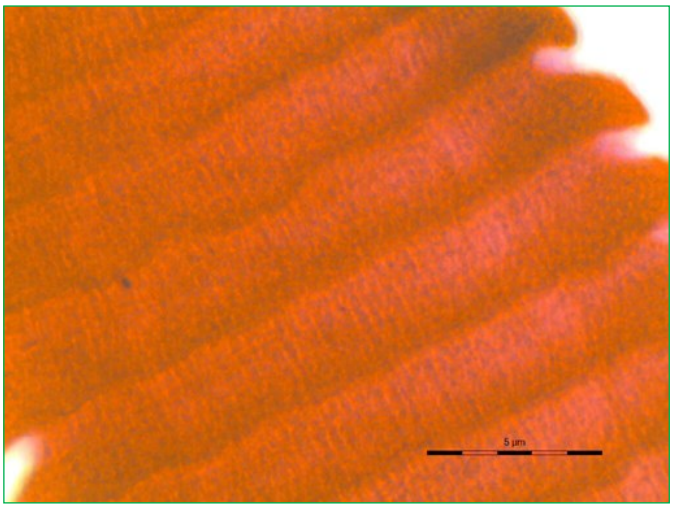

Fig. 2- The tegument of normal *R. tetragona* showing young proglottids (x200).

In each mature proglottid, numerous eggs are arranged in clusters [Fig-3]. Cestodes treated with 12 mg/ml artesunate show severe morphological alterations. The scolex is massively damaged with most of the suckers eroded. Only a tiny portion of the sucker with its spines is visible. The rostellar region is completely defaced [Fig-4]. The general smooth texture of the tegument is also lost. A mature proglottid has no apparent intact eggs. In their place are a number of irregular clumps of tissues [Fig-5].

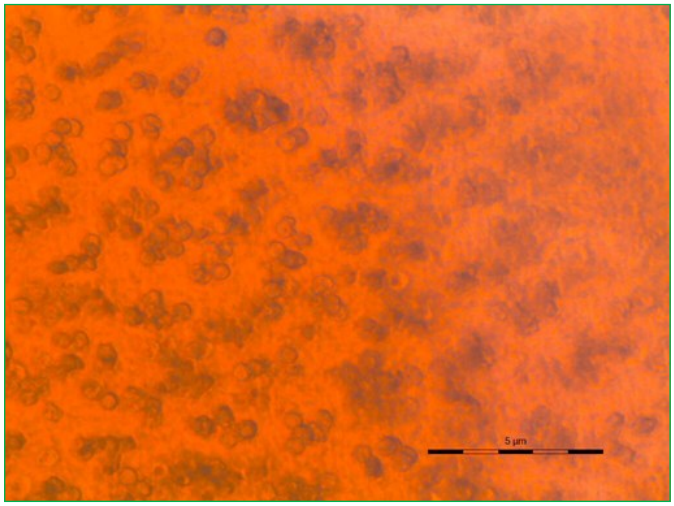

Fig. 3- A portion of gravid proglottid of R. tetragona showing clusters of eggs (x400).

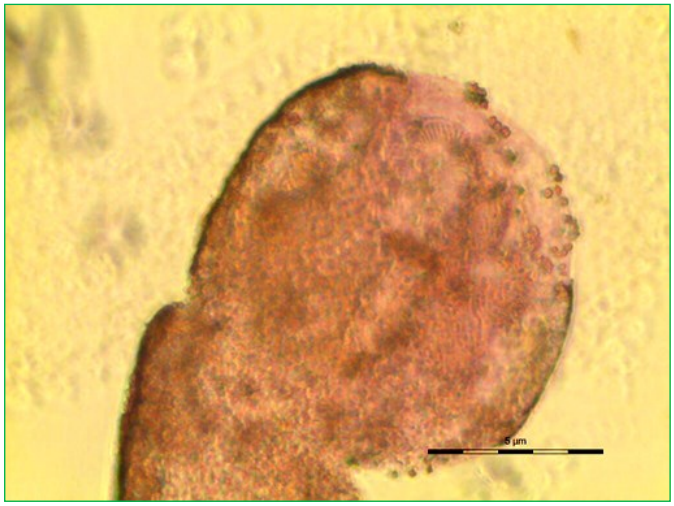

Fig. 4- Scolex of R. tetragona showing severe damage after treatment with artesunate. Note the loss of the smooth tegument, suckers and rostellum (x200).

Discussion

Anthelmintic activity is fundamentally studied by the deworming (vermifuge) activity, killing (vermicide) activity, and the structural damages caused by the drugs. In this experiment, it is demonstrated that artesunate has a significant vermicidal activity on *R. tetragona*. Its efficacy increases with increased concentration. A number of experimental evidences have shown that artesunate is highly effective on trematodes. It was treated in vitro with the 3-week-old juveniles of human trematode, *Fasciola gigantica*, and was found to effectively kill them in dose-dependent manner. Structural analyses showed that the 12 h-treated worms indicated pronounced swelling of tegumental ridges, followed by blebbing and later rupturing of the blebs, leading to erosion and lesion, and general destruction of the tegument [18]. Rats, experimentally infected with the liver fluke *Fasciola hepatica*, were effectively treated. Extensive tegumental damage was again noted after 24 h of exposure [19]. Structural damages were also noted in the human schistosome *Schistosoma mekongi*, which were experimentally infected in rats [20]. Extensive tegumental erosions were described in *S. mansoni* [21].

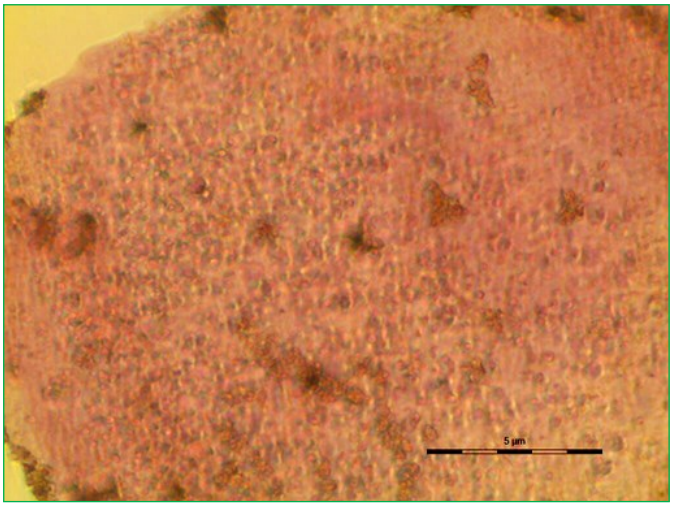

Fig. 5- Portion of gravid proglottid of R. tetragona showing degenerated structure of the tegument and numerous damaged eggs (x400).

Small intestinal trematodes, heterophyids, in mice were treated with 200 mg/kg/day of artesunate and the worm burden was reduced by 100% after three days of routine medication. Microscopical examination of the worms revealed bleb formation, tegumental disruption, erosion and peeling [22].

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 detrimental alterations and destruction of the cestodes's surface [23,24]. 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 [25].

Formation of numerous blebs on the tegument, rostellar disorganization and loss of the microtriches were described for the effects of pure albendazole and its sulphoxide combination therapy on the human cestode, *Echinococcus granulosus* [26]. Combination of Albendazole and praziquantel given to *E. granulosus* and *Mesocestoides corti* resulted in the loss of sucker concavity, separation and disintegration of the germinal layers, loss of microtriches and destruction of the tegument [23]. Damaging effects described for albendazole, flubendazole and nitazoxanide are highly comparable and typified by reductions in number and length of the microtriches, rostellar degeneration, formation of blebs on the tegument, loss of hooks and destruction of microtriches and vesiculation in *E. granulosus* and *E. multiloculoris* [24]. Albendazole also caused complete shrinkage of the tegument throughout the body, blebbing, and erosion of the scolex [27]. *R. tetragona* in the present study also showed considerable structural damages. The fine tegumental surface was disrupted, the scolex was severely eroded, and the clus-

ters of eggs were destroyed. This indicates that, similar to other anthelmintics, artesunate exerts its effect on the tegumantal organization of the cestode.

The anthelmintic effects observed in the present study are therefore a further support that artesunate is highly effective on soft-bodied helminths, trematodes and cestodes, which have delicate tegument as body coverings. It is also indicative that the drug is a good candidate for use in both veterinary and human cestode infections.

Author's Contribution: All authors contributed equally to the work. KLC designed the experiment and manuscript, performed microscopy and interpret the data. LLC and HLR collected samples and the drug, and initiate preservation of the samples. CLM, PBL, CVL, and MGCC performed the *in vitro* test. All took turn in processing the samples for microscopy as it was a laborious task. All consented to the write-up of the manuscript.

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Conflicts of Interest: The authors have declared that they have no competing interest.

References

[1] Tu Y. (2011) *Nature Medicine*, 17(10), 1217-1220.

[2] World Health Organization (Ed.) (2006) *Guidelines for the treatment of malaria*, World Health Organization. 12-36.

[3] Li Q. & Weina P. (2010) *Pharmaceuticals,* 3(7), 2322-2332.

[4] Goodrich S.K., Schlegel C.R., Wang G. & Belinson J. L. (2014) *Future Oncology,* 10(4), 647-654.

[5] Paddon C.J. and Keasling J.D. (2014) *Nat. Rev. Microbiol.*, 12, 355-367.

[6] Visser B.J., van Vugt M. & Grobusch M.P. (2014) *Expert opinion on pharmacotherapy,* 15(15), 2219-2254.

[7] Lalchhandama K. (2010) *Sci. Vis.*, 10, 111-122.

[8] Wolstenholme A.J. & Martin R.J. (2014) *International Journal for Parasitology: Drugs and Drug Resistance,* 4(3), 218-219.

[9] Miller L.H. & Su X. (2011) *Cell,* 146(6), 855-858.

[10] Ho W.E., Peh H.Y., Chan T.K. & Wong W.F. (2014) *Pharmacology & therapeutics,* 142(1), 126-139.

[11] Cheng C., Ng D.S., Chan T.K., Guan S.P., Ho W.E., Koh A.H., Bian J.S., Lau H.Y. and Wong W.S. (2013) *Allergy*, 68, 195-203.

[12] Efferth T., Sauerbrey A., Olbrich A., Gebhart E., Rauch P., Weber H.O., Hengstler J.G., Halatsch M.E., Volm M., Tew K.D., Ross D.D. and Funk J.O. (2003) *Molecular Pharmacology,* 64 (2), 382-394.

[13] Utzinger J., Xiao, S.H., Tanner M., & Keiser J. (2007) *Current opinion in investigational drugs, (London, England: 2000)*, 8(2), 105-116.

[14] Xiao S.H., Keiser J., Chen M.G., Tanner M. & Utzinger J. (2010) *Advances in parasitology,* 73, 231-295.

[15] Keiser J. & Utzinger J. (2012) *Current pharmaceutical design,* 18(24), 3531-3538.

[16] Lalchhandama K. (2009) *Sci. Vis.*, 9, 174-182.

[17] Lalchhandama K. (2014) *Int. J. Pharmacogn.*, 1, 394-398.

[18] Tansatit T., Sahaphong S., Riengrojpitak S., Viyanant V. & Sobhon P. (2012) *Experimental parasitology,* 131(1), 8-19.

[19] Keiser J. and Morson G. (2008) *Exp. Parasitol*., 118, 228-237.

[20] Jiraungkoorskul W., Sahaphong S., Sobhon P., Riengrojpitak S. & Kangwanrangsan N. (2005) *Parasitology international,* 54(3), 177-183.

[21] Utzinger J,, Chollet J., Tu Z., Xiao S. and Tanner M. (2002) *Trans. R. Soc. Trop. Med. Hyg.*, 96, 319-323.

[22] Fathy F.M. (2011) *J. Egypt Soc. Parasitol.*, 41, 469-483.

[23] Elissondo M., Dopchiz M., Ceballos L., Alvarez L., Bruni S.S., Lanusse C. & Denegri G. (2006) *Parasitology research,* 98(4), 317-323.

[24] Markoski M.M., Trindade E.S., Cabrera G., Laschuk A., Galanti N., Zaha A., Nader H.B. and Ferreira H.B. (2006) *Parasitology international,* 55(1), 51-61.

[25] Alvarez L.I., Mottier, M. L. & Lanusse C.E. (2007) *Trends in parasitology,* 23(3), 97-104.

[26] Pérez-Serrano J., Casado N., Denegri G. & Rodriguez-Caabeiro F (1994). *Int. J. Parasitol.*, 24, 219-224.

[27] Lalchhandama K. (2010) *J. Young Pharm*., 2, 374-378.