



## An in vitro trial of artesunate on intestinal helminth parasites

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### ABSTRACT

Artesunate is a prescription drug in the treatment of severe malaria due to *Plasmodium falciparum*. It has surpassed all other antimalarial drugs in terms of efficacy and safety. Serendipitous experiments in the 1980s began to unveil its true and broad-spectrum medical potential. It has been successfully shown to be highly effective antiviral, antitrepatodal, antischistosomal, and anti-cancer molecule. Although the anthelmintic activity is now established beyond doubt, it has never been tested on parasitic tapeworms (cestodes) and roundworms (nematodes). We treated the cestode *Raillietina tetragona* and the nematode *Ascaridia galli* with different doses, ranging from 0.7, 1.5, 3, 6 to 12 mg ml<sup>-1</sup>. The cestodes were highly susceptible to the various doses of the drug. But the nematodes were not at all responsive. The findings suggest that artesunate is a good candidate in the treatment of cestode infections.

**Key words:** Artesunate; anthelmintic; tapeworm; roundworm.

### INTRODUCTION

The identification of artemisinin in 1972 from the Chinese medicinal plant *Artemisia annua* was a profound medical triumph.<sup>1</sup> It became a landmark discovery in medical malariology. Though the plant itself is a weak antimalarial source, its active ingredient was found to be an extremely potent compound on *Plasmodium*, especially on *P. falciparum*, the unrivalled cham-

panion in slaying of humans through the entire history. This immediately kindled a plethora of chemical syntheses and biological tests. The compound and its horde of semi-synthetic derivatives proved to be tremendous advances in clinical medicine,<sup>2</sup> as well as in pharmaceutical science.<sup>3</sup>

Artesunate is one of the semi-synthetic derivatives of artemisinin. Having high activity, safety, solubility and molecular stability, its surplus of merits far outweigh any lingering downside, such as haemaglobin digestion (which is but clinically inconsequential).<sup>4</sup> Rigorous clini-

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cal trials in the past two decades proclaim that it is the ultimate drug of choice for falciparum malaria. It eventually snubs the glory of quinine from the medical arena. In fact the four-century-champion in antimalarial therapy, quinine was officially ousted by artesunate when the World Health Organization decreed in 2006 as no longer suitable for first line medication as a consequence of rampant drug resistance.<sup>5</sup> In addition, its adverse effects make it out-match by the relatively safe artesunate. Artesunate is now crowned as the best drug in the treatment of severe and complicated malaria.<sup>6</sup>

Further pharmacological explorations revealed that artemisinins are potent broad-spectrum drugs with robust effects on all major pathogens. It has been profusely demonstrated that they are effective against viruses (human cytomegalovirus), protozoans (*Toxoplasma gondii*), helminths (different flukes including schistosomes) and fungi (*Cryptococcus neoformans*).<sup>7</sup> Furthermore, artesunate has a strong anti-allergic effect,<sup>8</sup> and high cytotoxicity on different cancer cells.<sup>9</sup> Successful clinical trials have been conducted among patients with schistosomiasis.<sup>10</sup> It is regarded as one of the most promising drugs in the management of helminthiasis.<sup>11,12</sup> Unfortunately, there is poor scientific development in anthelmintics for tapeworm.<sup>13</sup> There is also no report on the effect of artesunate on roundworms. This study is therefore an attempt to elucidate the activity of artesunate on both tapeworms and roundworms parasitising the poultry.

## 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 (brand name Falcigo) was manufactured by Zydus Cadila Healthcare Limited, India, and procured from a local pharmacy at Upper Bazar, Aizawl, India.

### *Helminth parasites and in vitro treatments*

Live fowls (*Gallus domesticus* Linnaeus) were purchased from a poultry vendor at New Market, Aizawl, India. They were sacrificed with an overdose of chloroform at the Department of Zoology, Pachhunga University College. Upon autopsy, the intestines were dissected open, and live helminths were recovered. Both tapeworms (*Raillietina tetragona*) and roundworms (*Ascaridia galli*) were collected. Collection, identification and processing were done as previously described.<sup>14,15</sup> All the worms were recovered in culture dishes containing 0.9% neutral phosphate-buffered saline (PBS, composed of NaCl, KCl, Na<sub>2</sub>HPO<sub>4</sub>, and KH<sub>2</sub>PO<sub>4</sub> at 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<sup>-1</sup>, 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. Due to insufficiency of roundworms treatments were given only at higher doses. Each experimental assay was conducted 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. The time elapsed for death was recorded.

The survival duration was represented as means plus or minus standard deviation in tabular form, and (±) standard error of the means in bar graph. The relative survival time of treated worms against control groups were calculated

using student's *t*-test, with the level of significance considered when the *p*-value is greater than 0.05.

## RESULTS

The tapeworms *R. tetragona* survived very well for  $51.55 \pm 3.00$  h in a medium containing PBS + 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 and Figure 1. At the highest dose tested, viz  $12 \text{ mg ml}^{-1}$ , tapeworms could survive only up to  $0.71 \pm 0.12$  h, while at the lowest dose, viz  $0.7 \text{ mg ml}^{-1}$ , they survived for  $6.50 \pm 0.33$  h. At all doses tested the anthelmintic effect was significant at  $p < 0.05$ .

The roundworms *A. galli* persisted up to  $68.53 \pm 1.25$  h in control medium. As shown in Table 1, there was no significant difference in survival between control and treated worms. Thus the drug is not effective on these worms.

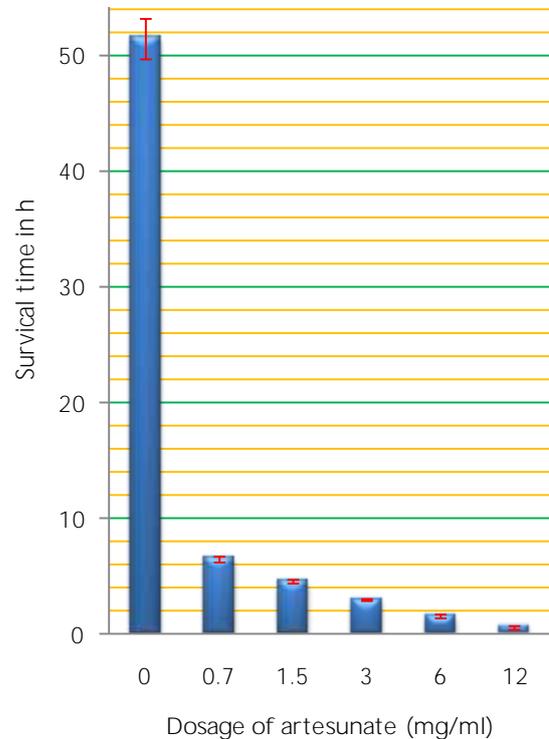


Figure 1. Relative survival of *R. tetragona*.

Table 1. Survival of the tapeworm *R. tetragona*.

Media	Dose ( $\text{mg ml}^{-1}$ )	Survival time in h ( $\pm$ SD)	Standard error of means	Degree of freedom	<i>t</i> value	<i>p</i> -value
PBS + DMSO	0	$51.55 \pm 3.00$	1.73			
Artesunate	0.7	$6.50 \pm 0.33$	0.19	2	25.84	$< 0.05^*$
	1.5	$4.54 \pm 0.24$	0.14	2	27.04	$< 0.05^*$
	3	$2.92 \pm 0.10$	0.10	2	28.02	$< 0.05^*$
	6	$1.55 \pm 0.19$	0.19	2	28.68	$< 0.05^*$
	12	$0.71 \pm 0.12$	0.12	2	29.27	$< 0.05^*$

\* Student's *t*-test, significantly different in comparison to control (0) group.  $n = 3$ .

Table 2. Survival of the roundworm *A. galli*.

Media	Dose ( $\text{mg ml}^{-1}$ )	Survival time in h ( $\pm$ SD)	Standard error of means	Degree of freedom	<i>t</i> value	<i>p</i> -value
PBS + DMSO	0	$68.53 \pm 1.25$	0.72			
Artesunate	6	$67.50 \pm 1.67$	0.96	3	28.68	$< 0.05^+$
	12	$66.40 \pm 2.20$	1.27	3	0.86	$< 0.05^+$

† Not significantly different from those of control (0) group.  $n = 3$ .

## DISCUSSION

The anthelmintic effects of artesunate have been best described in different flukes. An in vitro treatment of artesunate on the 3-week-old juveniles of *Fasciola gigantica* showed dose- and time-dependent motility effect. After 12 h of treatment swelling of tegumental ridges, followed by blebbing and later rupturing of the blebs, leading to erosion and lesion, and disruption of the tegument were observed.<sup>16</sup> An in vivo treatment of the liver fluke *Fasciola hepatica* in experimental rats resulted in extensive tegumental damage after 24 h of exposure, and the intensity of destruction exacerbate with time.<sup>17</sup> Observations from the present study show that the anthelmintic effects are very similar on *Raillietina tetragona*. The cestocidal efficacy increases with increased dosage, and lower dosage required longer exposure for the full effect to take.

Small intestinal trematodes, heterophyids, were reduced by 100% upon exposure to 200 mg/kg/day of artesunate for 3 successive days in mice. Bleb formation, disruption, erosion and peeling were noted on the tegument.<sup>18</sup> Elaborate experiments in mice, rabbits and dogs infected with *Schistosoma japonicum* showed worm reduction by 77.50-90.66%, 99.53% and 97.10% respectively after treatment with artesunate.<sup>19</sup> Administration of artesunate and artemether at a dose of 400 mg/kg to *Opisthorchis viverrini*-infected hamsters resulted in worm burden reductions of 77.6% and 65.5%, respectively. Worm burden reductions of 98.6-100% were noted in *Clonorchis sinensis*-infected rats after a single dose of artesunate and artemether at 150 mg/kg.<sup>20</sup>

The relative ineffectiveness of artesunate on *Ascaridia galli* may be explained on the basis that roundworms are hard-bodied worms with significantly different structural features. The thick body covering, the cuticle likely provides an efficient barrier for the drug action as it is known that roundworms often require unique drugs to exert anthelmintic effect through the cuticle.<sup>21</sup> The drug is most probably effective only on soft-bodied helminths which have delicate tegument

as body covering.

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