

An *In Vitro* Comparative Effectiveness of Two Formulations of Praziquantel on *Mesocestoides corti* and *Taenia crassiceps* Larvae

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This work describes the effectiveness of two formulations of praziquantel namely praziquantel-polyvenylpyrrolidone solution and praziquantel-dimethylsulphoxide suspension against cystic larvae of *Taenia crassiceps* (ORF strain) and *Mesocestoides corti* (syn. *Mesocestoides vogae*) at concentrations of 5, 25, 50 and 100 µg/ml. About 15-20 larvae of *M. corti* and *T. crassiceps* were separately inoculated in duplicate into 50 ml Cannaught Medical Research Laboratories (CMRL 1066) flask-containing medium adjusted to pH 7.4±0.1 and incubated at 37 °C, exchanging the media whenever was necessary. Evaluation of the drugs effects were conducted on day 2-15 post inoculation by observation of the cysts viability under the light microscope. Praziquantel-polyvenylpyrrolidone PZQ-PVP solution was observed to be slightly more efficacious than the praziquantel-dimethylsulphoxide suspension. The findings showed that the effect of the two formulations on the cystic larvae was both time and concentration dependent.

Key words: *Taenia crassiceps*, *Mesocestoides corti*, praziquantel.

INTRODUCTION

Taenia crassiceps larvae cause chronic infections in laboratory animals such as mice [1] and rats [2]. The larvae grow in the soft tissues and pleural or peritoneal cavities, where they reproduce by budding and from there they can be harvested and individually counted. *Taenia crassiceps* larvae have been observed in other mammals such as monkey, fox and dog, including some few human cases such as in HIV/AIDS patients to whom the parasite can be an opportunistic agent have been reported [3,4].

On the other hand, the route of transmission and life cycle of *Mesocestoides corti* (syn. *Mesocestoides vogae*) a member of the family Mesocestoidae, has not yet been described satisfactorily. The putative first intermediate host is a ground-dwelling coprophagous arthropod that ingests oncospheres and accommodates the development of larvae. It is presumed that vertebrates become hosts for the larvae after ingestion of infected arthropods. This is followed by penetration of the small intestine by a postcysticercoid /cystic stage of the tapeworm,

but this has not yet been experimentally proved [5]; though also can penetrate small mammals [6, 7]. So far there are few reported cases world-wide of intestinal *Mesocestoides* tapeworm infection in humans [7, 8].

Unfortunately, to date very few studies have been conducted to evaluate the effectiveness of praziquantel (PZQ) against these cestodes. A few authors [9,10] conducted assays on *T. crassiceps*, whilst others carried out *in vivo* studies on efficacy of free and liposomized PZQ in mice and *in vitro* efficacy of albendazole and PZQ against larval stage of *M. corti*, [11, 12]. In both experimental models there was no significant difference between the two assayed formulations. *T. crassiceps* human infection has been also reported [4] and was treated with a combination therapy ABZ and PZQ for approximately two months or with single therapy of PZQ or mebendazole (MBZ) for more than two and a half month. But four months later after discontinuing treatment there was recurrence.

In addition to that, treatment of *Mesocestoides* infections with ABZ or PZQ alone in dogs

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proved to be ineffective except at high doses of fenbendazole. Oral administration of PZQ in experimentally infected mice manifested a reduction in number of *M. corti* larvae and satisfactory effectiveness against juvenile and adult stages in dogs' digestive tracts [13]. Because these drugs were particularly designed for intestinal helminthic infections [14], and that they have proved to be ineffective to most of the parasitic infections beyond the gastrointestinal tract, mainly due to their limited solubility. Thus, the value of PZQ in treatment of this parasitosis is still controversial [9, 15, 16].

Therefore, the aim of this study is to investigate the influence of complexation process with in this preliminary study, we intend to analyze whether there is any improvement on the *in vitro* PZQ bioavailability and hence its efficacy as a result of PZQ complexation process with PVP compared to the parent drug suspended in DMSO.

MATERIAL AND METHODS

Acquisition of biological materials: *T. crassiceps* (ORF strain) and larvae *Mesocestoides corti* (syn. *Mesocestoides vogae*) were aseptically removed from the peritoneal cavity of experimentally infected 2 month-old female NMRI mice, which were kept under strict laboratory conditions. Prior, the mice were killed by cervical dislocation. The larvae were briefly washed (four times for 15 minutes) under gently agitation in Hank's balanced salt solution (HBSS) adjusted to pH 7.4±0.1. Then their viability was determined by simple microscopic examination basing on general contraction movements while maintained in HBSS.

Elaboration of drug-PVP complex- solution: Drugs solubilization was performed by complexation with polyvinylpyrrolidone K12 PF (PVP) - (BASF, USA) in the proportions of 1:5 (w:w) for PQR (Sigma, USA). The solvent evaporation method was used in preparation of solid complexes as it has been previously reported [18]. Then the drug concentrations were adjusted according to the experiment conditions.

In vitro evaluation of drug efficacy: About 15-20 larvae of either *M. corti* or *T. crassiceps* were

inoculated in duplicate into a 50 ml CMRL 1066 flask-containing medium and kept at 37 °C, routinely exchanging the media after every 2-3 days or when was necessary. Effect of the drugs on the larvae was evaluated on day 2, 5, 7, 9, 11, 13 and 15 for both experimental models, and each assayed concentration for both formulations was repeated three times.

Statistical Analysis: The Data Were Entered Into A Database And Analyzed With The Statistical Package For The Social Sciences (SPSS+ 10.0) Software (SPSS Inc., Chicago, IL). Differences Among The Means Of Various Groups Were Statistically Tested By Using Analysis Of Variances. And Significance Level Was Set At P<0.05.

RESULTS

Examination of the effects of the two PZQ formulations: Typical features for *T. crassiceps* larvae viability were loss of flaccidity, tegument (larval wall) degeneration and darkening of and lack of motility of the larvae [10, 19]. On the other hand, *M. corti* drug treated larvae manifested tegument degeneration, darkening and disintegration of the tegument as well as failure of budding, this was more frequently and rapidly occurring for high drug-concentration treated groups such as 50-100 µg/ml. Effects of the two formulations of PZQ on mean percentages of surviving *T. crassiceps* and *M. corti* larvae from day 2-15 p.i. are shown on figures 1-8.

The influence of DMSO and PVP on the viability of the cystic larvae was also investigated by incorporating them in a group of positive controls (blank, PVP and DMSO) are shown on figures 1-2 and figures 5-6. There was a slight increase in number for *M. corti* larvae as result of budding process, though this was not a threat for drug treated groups as shortly stated above, since the larvae were less active compared to the control groups, this compelled us to count all larvae and then subtract a number of the dead ones.

Moreover, the effects of two identical concentrations of both formulations on the two models were compared and no significant differences were observed between the two formulations from day 2-5 p.i. for *T. crassiceps*

(figures 3-4) and from day 2-9 for *M. corti* and (figures 7-8). A number of larvae were also maintained intact and were more active in the control groups compared to the treated groups, which led to higher viability as reflected on figures 1-2 and figures 5-6.

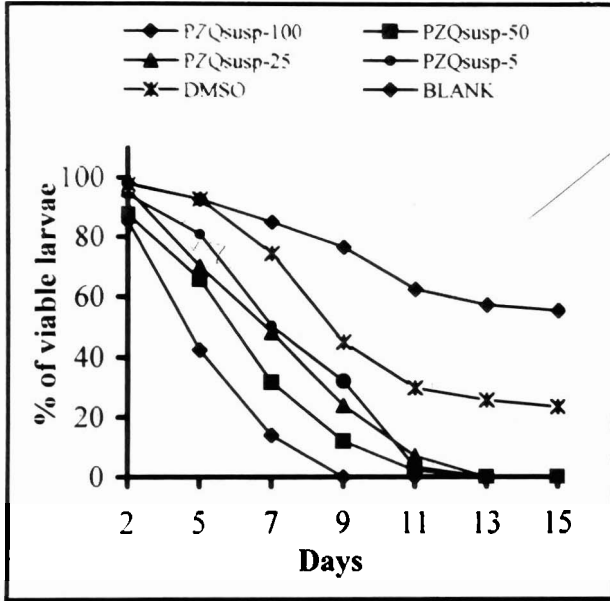


Figure 1: Comparison of effect of praziquantel-dimethylsulphoxide suspension on *Taenia crassiceps* larvae viability. Concentration: 5, 25, 50 and 100 µg/ml

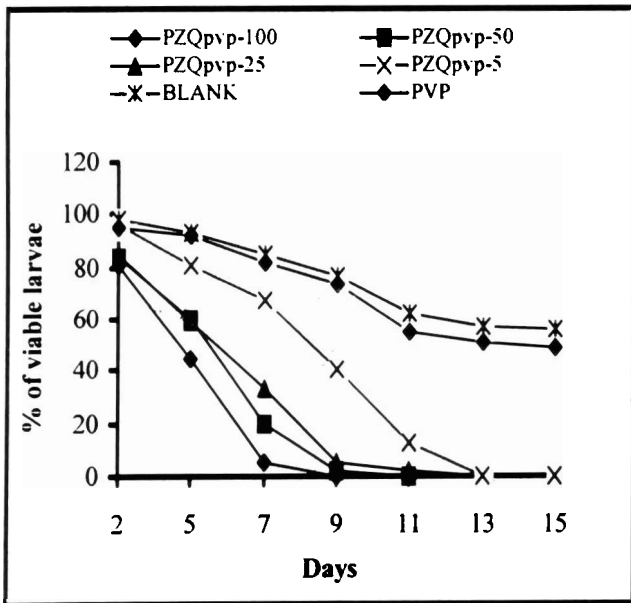


Figure 2: Comparison of praziquantel-polyvinyl-pyrrolidone on *Taenia crassiceps* larvae viability. Concentration: 25, 50 and 100 µg/ml

DISCUSSION

This study was aimed at improving the *in vitro* solubility and hence efficacy of PZQ. Nevertheless, at higher concentrations (50-100 µg/ml) for day 13-15 p.i. the drug-PVP complex manifested no significant differences with respect to the DMSO-suspended drug ($p < 0.05$), but all treated groups differed significantly from the control-groups (figures 1-2 and figures 5-6). However, some controversial results about PZQ treatment of parasitic infections have been documented [3, 4, 15, 16] that means new formulations discovery is still of top priority.

Comparative analysis on percentage of surviving larvae basing on time of exposure to PZQ revealed some significant differences between the two formulations ($p < 0.05$), and there was a significant negative correlation ($p < 0.01$) between time lapse and percentage of viable larvae (data not shown). On this aspect, the results indicate that PVP-drug solution was significantly ($p < 0.05$) more effective than the PZQ-DMSO suspensions from day 7-15 p.i. (figures 3-4, and figures 7-8 for *T. crassiceps* and *M. corti*, respectively).

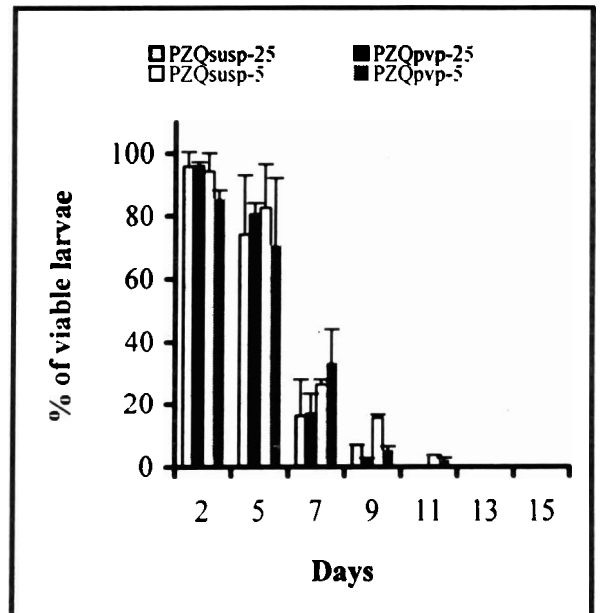


Figure 3: Comparative analysis on evolution of effect of the two formulations of Praziquantel on viability of *Taenia crassiceps* larvae. Concentration: 5 and 25 µg/ml

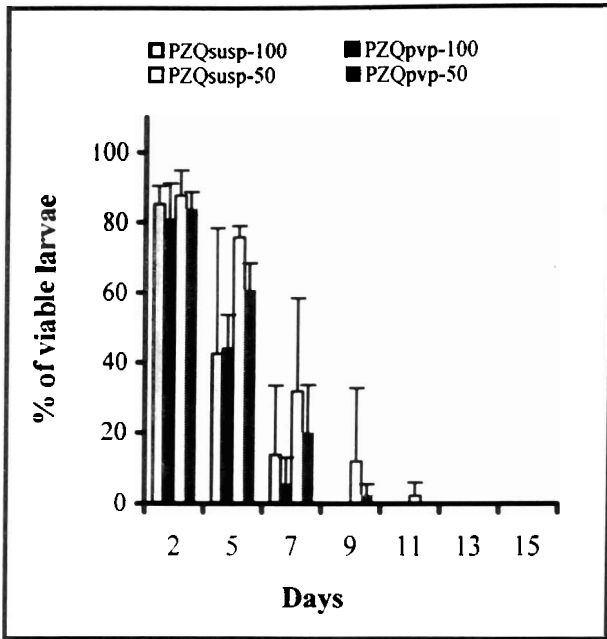


Figure 4: Comparative analysis on evolution of effects of the two formulations of praziquantel on viability of *Taenia crassiceps* larvae. Concentration: 50 and 100 µg/ml

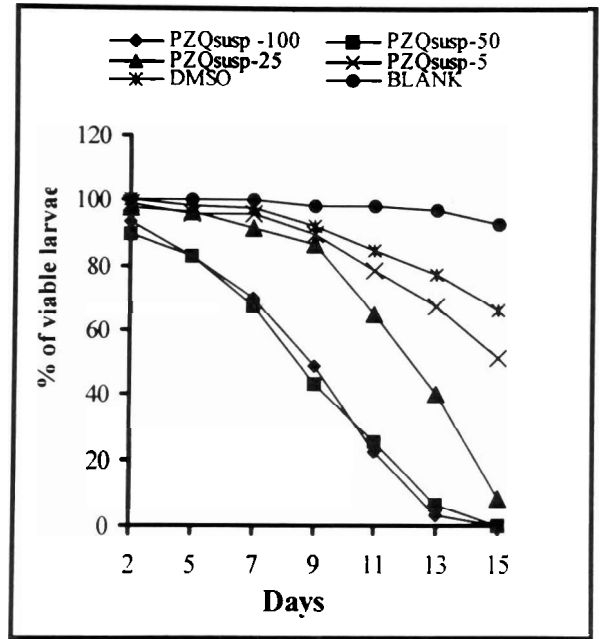


Figure 6: Comparison of effects of praziquantel-dimethyl sulphoxide suspensions on *Mesocostoides corti* larvae viability. Concentration: 5, 25, 50 and 100 µg/ml

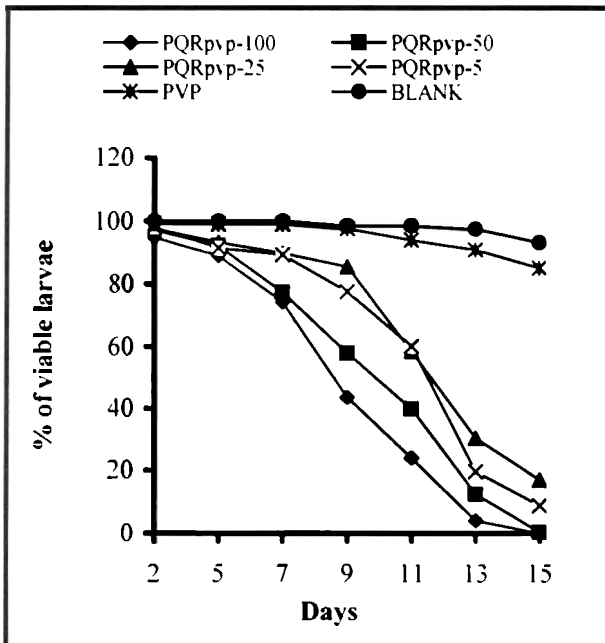


Figure 5: Comparison of effects of praziquantel – polyvenylpyrrolidone suspensions on viability of *Mesocostoides corti* larvae. Concentration: 5, 25, 50 and 100 µg/ml

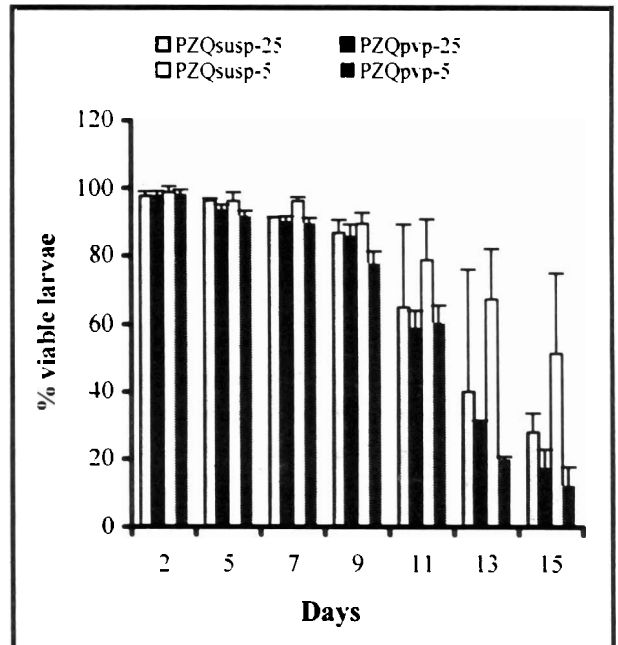


Figure 7: Comparison of effects of the two formulations praziquantel on viability of *Mesocostoides corti* larvae. Concentration: 50 and 100 µg/ml

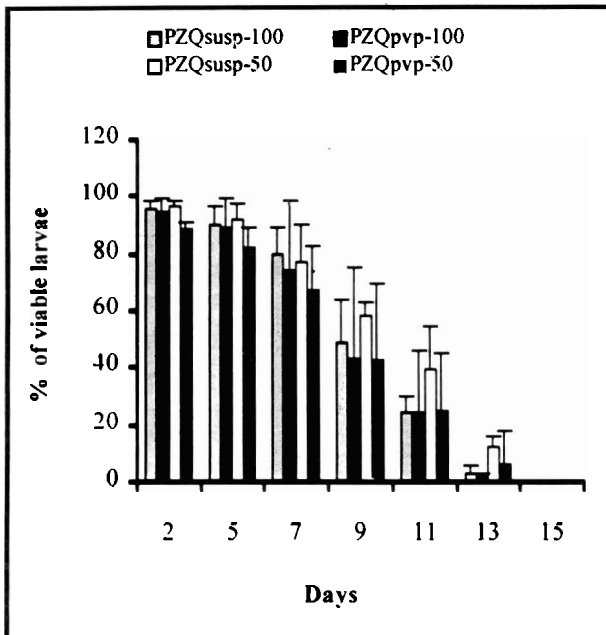


Figure 8: Comparison of effects of the two formulations of praziquantel on viability of *Mesocostoides corti* larvae. Concentration: 50 and 100 µg/ml

And the differences were more prominent at higher drug concentrations for both parasitic experimental models. These results coincide with previous studies that also showed a concentration dependent effect of PZQ [10]. The larvicidal effect of PZQ at these high concentrations could be attributed to the consequence of its effects on short-term processes that seem to affect the biochemistry and metabolism of the parasite [14], as well as the alteration of tegument integrity.

Some previous works had also demonstrated ineffectiveness of PZQ in treatment of these parasites, needing human long-term treatment regimes, [3, 4, 13, 16]. Earlier experimental studies revealed limited effects for free and liposomized PZQ and ABZ against *M. corti* larvae in mice [11]. But this could be due to continuing asexual multiplication of unaffected larvae [5], a phenomenon that was manifested in our study, which somehow interfered with efficacy evaluation at the onset of examinations.

This preliminary work intended to improve PZQ bioavailability by first conducting an *in vitro* study, showed a slight but important superiority

of the drug-PVP solution over the DMSO-drug suspension. The findings could be due to absence of some physiological barriers or conditions which normally interact and affect the *in vivo* drug solubility and hence bioavailability. However, our findings show some significant differences between the two preparations from day 7-15 p.i. for both parasitic models. In addition to that, complexation by PVP had also proved to be a very useful tool in improving bioavailability of some benzimidazole carbamates such as albendazole, ricobendazole and mebendazole, which is in line with other findings [17].

In conclusion, our results have shown that PZQ-PVP solution is more effective than PZQ-DMSO suspension and that the effectiveness observed so far is concentration and exposure time-dependent. This study also provides an alternative model for investigation of therapeutic efficacy of several non-intestinal helminthes

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