Effects of Goyazensolide during *in Vitro* Cultivation of *Schistosoma mansoni*

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Goyazensolide, a component extracted of Eremanthus goyazens*is showed a significant inhibitory effect on egg-laying of* Schistosoma mansoni *during* in vitro *cultivation of this parasite. Motility of the worms was also reduced under treatment with goyazensolide and 90% of mortality was reached with concentrations up to 4μg/ml. It has found that separated worms were more susceptible than worms pairing during drug exposition and female alone was significantly more susceptible than male worm in the same conditions of* in vitro *cultivation.*

Natural products isolated from plants represent potential sources for the identification of structures useful for the design of alternative molecules to be used as new drug substances against several infectious diseases.

Key words: *Schistosoma mansoni* - schistosomiasis - sesquiterpene lactones - *in vitro* cultivation

Schistosomiasis is a chronic debilitanting disease representing the second most prevalent tropical disease affecting more than 200 million people worldwilde. Chemotherapy and the reduction of transmission are presentely the two main actions in the control of schistosomiasis and this situation is unlikely to change until a suitable vaccine becomes available (Modha et al. 1990). It is essential therefore that effective anti-schistosome drugs are available and that the search continues for new chemotherapeutic agents to combat the emergence of drug resistance such as oxaminiquine (Lambertucci et al. 1980, Souza et al. 1982).

Plants have provided a number of useful clinical agents and have considerable potential as sources of new drugs, not only in their pharmacological or chemotherapeutic effects, but also in their role as template molecules with a chemical structure showing the required biological activity, and subsequent development of this lead strucutre into a safe and therapeutically effective form (Phillipson 1994).

Sesquiterpene lactones form one of the largest group of cytotoxic and anti-tumor compounds of plant origin (Picman 1986). Various studies suggested that these secondary plant metabolites play an important role in protection of plants against pathogenous organisms, herbivirous, insects, and mammals. Some purified sesquiterpene lactones were showed to have strong effects against pathogenic protozoa *Entamoeba histolytica*, *Trichomonas vaginalis*, *Plasmodium falciparum* and *P. vivax* (Picman 1986).

The schistosomicidal activity of these compounds has been demonstrated when applied on skin. They provide protection against the infection by cercariae of *Schistosoma mansoni* (Pellegrino 1967) and inhibition of egg-laying and movement by adult pairs of *S. japonicum in vitro* (Jisaka et al. 1992).

The present *in vitro* studies were undertaken to examine the antischistosomal effects of the goyazensolide on the adult *S. mansoni*.

MATERIALS AND METHODS

The LE strain of *S. mansoni* was routinely maintained in laboratory by passage through *Biomphalaria glabrata* snails and *Balb/c* mice. Infection of mice weighing 20-30 g was carried out percutaneously with aproximately 150 cercariae of *S. mansoni*.

Adult schistosomes were recovered from the mesenteric and portal veins of mice infected seven weeks previously under asseptic conditions using dissecting needles. The parasites were washed in RPMI-1640 medium (Cutilab) buffered to pH 7.5 with HEPES 20 μM and supplemented with penicilin (100 U/ml), streptomycin (100 μg/ml), and 10% fetal calf serum (Gibco). Each worm pair was transferred to 35 mm diameter (35x10

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mm) polystyrene dishes (Nunc, Denmark) with 2 ml of the same medium. Worms which became separated from their partners during the recovery procedure were discarded; incubation was carried out at 37°C in a humid atmosphere containing 5% CO_2 in the dark.

Goyazensolide powder was kindly supplied by Drs JJ Calegari Lopes and W Vichenwski (Faculty of Pharmacy, Department of Physics and Chemistry, Ribeirão Preto, State of São Paulo). For *in vitro* tests with schistosomes the goyazensolide was dissolved in dimethyl sulfoxide (DMSO) and then tapped up with distilled water to the desired concentration of stock and sterilized by passage through a millipore filter of 0.45 μm pore size. Appropriate dilutions of this stok solution were made with sterilized distilled water so that a constant 100 μl volume of drug solution was added to 2 ml of medium in each assay dish.

Control worms were treated with 10% of DMSO in sterile distilled water and the worms viability (i.e. motility) were inspected visually. Egg production was measured daily by manual counting under a inverted microscope (Leitz, Diavert).

RESULTS

The viability of *S. mansoni* adult worms was observed during *in vitro* incubation with various concentrations of goyazensolide. Fig. 1 shows that the exposure to the drug resulted in the death of the organisms and this lethal effect was dependent of drug concentration and time incubation.

In the first 24 hr of drug exposure more than 90% of the parasites were dead at concentrations of 3.5 and 4 μg/ml and with 2 and 3 μg/ml; a cumulative effect could be observed, since the mortality levels of 5% and 45% in the first 24 hr with those concentrations reached levels of 50% and 95% 24 hr later.

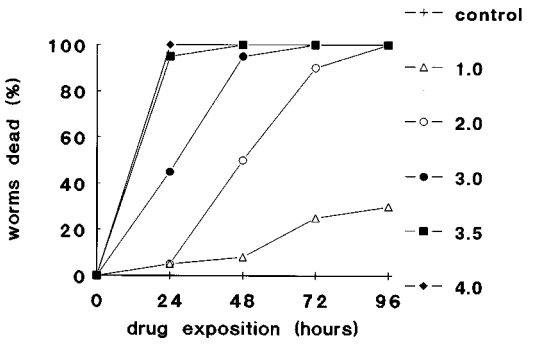

Fig. 1: viability of schistosomes following different concentrations and time exposition of goyazensolide. A pair of worms were incubated *in vitro* with goyazensolide at concentrations (μg/ml) showed in figure legends. Each point in the figure represents the mean of triplicate determinations of percentage of dead worms.

The control group containing 10% of DMSO was not affected for up to 96 hr of observation and all worms remained paired throughout the observation period and exhibited vigorous activity, especially a continous flexing of anterior regions. Light microscope observations of whole worms showed a progressive tegumental vesiculation of the parasites, analogous to the tegumental ballooning observed with Astiban (Shaw & Erasmus 1977); the amount and extent of this damage was proportional to the time exposure and drug concentration.

In order to determine the sensitivity of each sex, male and female adult worms were mechanically separated and incubated with 2 μg/ml of the drug. As shown in Fig. 2, separated worms were more susceptible than paired worms and the effect was more proeminent in the first 24 hr in which 8.3% of worms pairs were dead in contrast to 40.5% of female and 34.2% of males which dead in the same period. In this experiment, it was also clear that female worms alone were significantly more susceptible than male worms to goyazensolide killing.

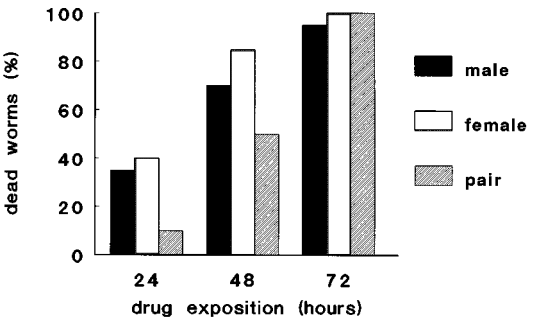

Fig. 2: effect of goyazensolide on coupled and unpaired worms *in vitro*. Worms pair, female and male were incubated with 2 μg/ml of the drug. The bars represent percentage of dead worms.

The effect of goyazensolide on daily egg production by adult worms pairs is showed in Fig. 3. There was a significant and progressive reduction in egg output by worms maintained in increasing concentrations of goyazensolide. The effect was observed after 24 hr of drug exposure where the control group showed an increase egg output from 18 eggs in the first day to 43 eggs per day by worm pair on the second and third days and this increase was significantly reduced by goyazensolide with concentrations up to 0.8 μg/ml.

Concentrations of goyazensolide higher than 0.8 μg/ml resulted in a dramatic reduction of egg production, but in this case the motility of the worms was also markedly reduced by separating adult pairs.

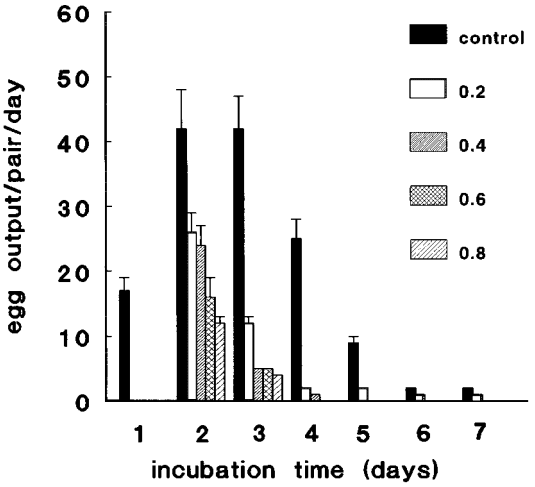

Fig. 3: effect of goyazensolide on egg output *in vitro*. Adult worms pairs were incubated with goyazensolide at the concentrations (µg/ml) showed in figure legends.The drug was added at the end of the first day cultivation The bars represent the average of sextuplicate determinations ±SD of eggs per day.

DISCUSSION

The importance of natural product molecules to medicine lies not only in their pharmacological or chemotherapeutic effects, but also in their role as template molecules for the production of new drug substances (Phillipson 1994).

The majority of sesquiterpene lactones are common constituents in members of the *Asteraceae* (Rodriguez et al. 1976). There have been several reports of anti-bacterial, anti-fungal and anti-protozoan activities of these compounds (Picman 1986). Some protection against infection by *S. mansoni* was demonstrated with extracts of certain Brazilian trees of the *Asteraceae* family (Gilbert et al. 1970, Picman 1986) and a reduction of egg-laying *in vitro* have been demonstrated on *S. japonicum* (Jisaka et al. 1992).

The present work shows that goyazensolide, a component extracted of *Eremanthus goyazensis* had a significant inhibitory effect on egg-laying of *S. mansoni in vitro* and this effect was dependent on the duration of exposure and drug concentration. Also, one complete bloking of motility of the parasites was observed with concentrations higher than those used to reduce the egg output.

Although the mechanisms responsible for oogenesis in this parasite is not known, probably some event related to this process is firstly affected by the goyazensolide without affecting the pairing and motility of the worms. Also, it was observed a significant difference in parasite susceptibility in situations where the worms were separated from their partner.

The fact that female schistosomes are more susceptible to goyazensolide raised the question of whether the observed effect on females is a direct consequence of drug action or in combination with the apparent loss of some stimulus which is essential for normal female pairing.

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