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Short Communication

IN VITRO ASSAY OF THE ANTHELMINTIC EFFECTS OF QUEBRACHO AND *P.THONNINGII* ON *H. CONTORTUS* AND *H. BAKERI* INFECTION LARVAE

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ABSTRACT

The anthelmintic potential of quebracho tannin and *Piliostigma thonningii* was investigated *in vitro*. Larvae of *H. bakeri and Haemonchus contortus* were introduced into wells of microtitre plates and different concentrations of the extracts added in triplicate and incubated at 4µC in the refrigerator. Readings were taken at 6,12 and 24 hours from the time of introduction of the extracts. Dead and surviving larvae were enumerated based on larval movement and activity. The bioactivity of quebracho tannin on *H. contortus* was both duration and time dependent, that of *H. bakeri* was only concentration dependent. The bioactivity of *P. thonningii* extract on *H. contortus* larvae also showed a duration and concentration dependent action while on *H. bakeri*, the effect was only duration of exposure determined.

KEY WORDS: Anthelmintic potential, sustainable control

INTRODUCTION

In order to combat the rising development of resistance of gastrointestinal nematodes to common anthelmintics (Waller *et al*., 1995), more sustainable methods for gastrointestinal helminth control are being suggested (Waller, 1997; Anthanasiadou *et al*., 2000). In some countries tanniferous plants have been advocated to reduce the use of chemical anthelmintics (Waghorn, 1982). On the other hand, extracts of some other plants have been used *in vivo* against some stages of parasitic gastrointestinal nematodes of grazing animals. Larval development/viability assays have been carried out to investigate the effect of Quebracho extract towards larvae of *H. contortus, Teladorsagia circumaineta and Trichostrongylus vitrinus* (Athanasiadou *et al*., 2001). The presence of Quebracho extract in cultures decreased the viability of infective larvae (L3) in all three species (Athanasiadou *et al*., 2001). Extracts of *Piliostigma thonningii* and Nauclea latifolia were also found to be toxic to both brine shrimp nauplii and *H. contortus* larvae (Fakae *et al*., 2000). Earlier preliminary studies on extracts of some medicinal plants from Nigeria did show some bioactivity in both *in vitro* and *in vivo* experiments against stages of gastrointestinal helminths (Asuzu and Onu, 1993; Njoku *et al*., 1996).

There is no doubt that various species of nematodes react differently to the same therapeutic substance. Since there is a report on the activity of *Piliostigma thonningii* and Quebracho against infective larvae of *H. bakeri* and *H. contortus*, the following study was undertaken as a pre-screening measure for known tanniferous plants with anthelmintic potentials.

MATERIALS AND METHODS

Larval cultures for *H. bakeri* and *H. contortus larvae* were set up with faeces from donor mice and West African Dwarf goats according to the methods.

Preparation of stock solutions

Stock solutions of 400µg/ml of both *Piliostigma* and Quebracho were made.

Decreasing concentrations

They were each diluted fourfold with distilled water to obtain different concentrations of 400, 200, 100, 50 or 25µg/ml. Levamisole hydrochloride at 0.01g/ml was used as the positive control while distilled water served as negative control.

Larval inhibition assay

The larval suspension was adjusted with distilled water such that each 50µL suspension contained approximately 18L3. 50µL of well mixed larval suspension was introduced into each well of a 96-well microtitre plate. This was left to acclimatize overnight. The appropriate concentration of Quebracho and levamisole hydrochloride (HCl) was added in triplicate. The plate was then covered properly with aluminium foil and incubated at 4ºC in the refrigerator. Readings were taken at 6,12 and 24 hours from the time of introduction of the extracts. Dead and surviving larvae were enumerated based on larval movement and activity.

RESULTS

Effect of Quebracho Tannin on *H. contortus* L3

QT had lethal effect on H. contortus as shown in Fig 6.1. Generally, this lethal activity was both concentration and duration dependent, being lowest at 6H, and highest at 24H. At 6H, 12h and 24H, 200µg/ml and 400ug/ml produced a more significant lethal effect in each case ($F = 9.786$ and $P = 0.013$ for 200µug/ml and $F = 16.153$ and $P = 0.004$ for 400µg/ml for A and B respectively) when compared to the other concentrations.

Effect of QT on *H. bakeri* L_3

QT also showed bioactivity against *H. bakeri L3, but unlike H. contortus*, its killing effect was not time dependent as shown on Fig 6.2. There was no significant difference in the number of L3 killed at the different

exposure periods (P=0.782). Lethality was however dose-dependent being significant at 25µg/ml (P = 0.001) and at 50µg/ml (F = 5.213, P = 0.049). .

Effect of *P. thoningii* on *H. contortus* L_3

The bioactivity of *P. thonningii on H. contortus* increased with the time of exposure as shown in Fig 3. The bioactivity was also concentration dependent in favour of higher concentrations. There was a significant statistical difference in the dose response after 12 hours of exposure (F = 6.010 P = 0.037 for 50µg/ml and F = 74.684, P = 0.00 for 400µg/ml respectively).

Effect of *P.thonningii* on *H. bakeri* L3

As illustrated Fig 4 indicates that the lethal activity of *P. thonningii* increased with time of exposure. Lethality was generally irrespective of the concentration once the larval suspension was exposed for up to 12 hours. However, following statistical analysis 25µg/ml and 50µg/ml had statistically significant lethality (P<0.05).

DISCUSSION

The lethal effect of QT on the infective larvae of *H. contortus and H.* polygyrus in the present study confirms the earlier results of Athanasiadou *et al*. (2001) and that of Fakae *et al*. (2000) that, condensed tannin-containing extracts are deleterious to this developmental parasdite stage. The bioactivity of *P. thonningii* has also been shown by Asuzu and Onu, (1993, 1994) and Njoku *et al*., (1996). The present study, thus show however that there are variations of activities which relates to both parasite species and the source of the condensed tannins. For instance while the bioactivity of QT on *H. contortus* was both duration and time dependent, that of *H. bakeri* was only concentration dependent. The bioactivity of *P. thonningii* extract on *H. contortus* larvae also showed a duration and concentration dependent action while on *H. polygyrus*, the effect was only duration of exposure determined.

Although more than 50% lethality was obtained with both QT and PT in both parasites within 12 hours of exposure, the results from the study suggests that activity of condensed tannins as a therapeutic agent may not be generalised. It may be recalled that while *H. contortus* is an abomasal worm, and are obvious differences in the pH at the different predilection sites. This factor together with the species of condensed tannins may affect the outcome of the tanniferous extract to control infection.

There is thus need to study the effect of condensed tannins from varied sources on the parasites in some parasite-host models in order to effectively exploit the use of tanniferous products for sustainable control of gastrointestinal helminth infection in animals.

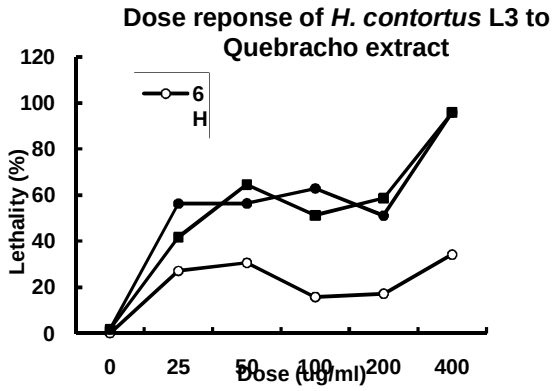

Fig 1. Effect of QT on *Heligmosomoides in vitro*

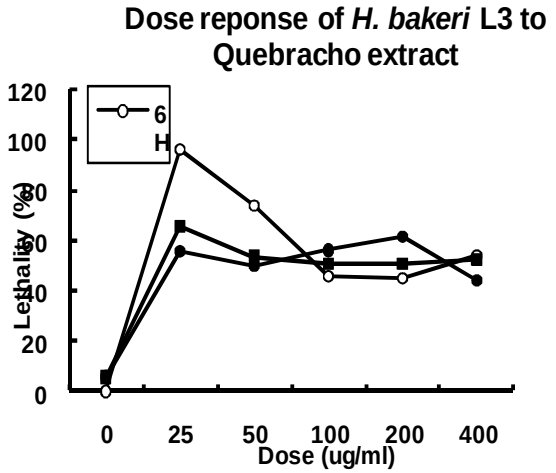

Fig 2. Effect of *Piliostigma* on *Heligmosomoides in vitro*

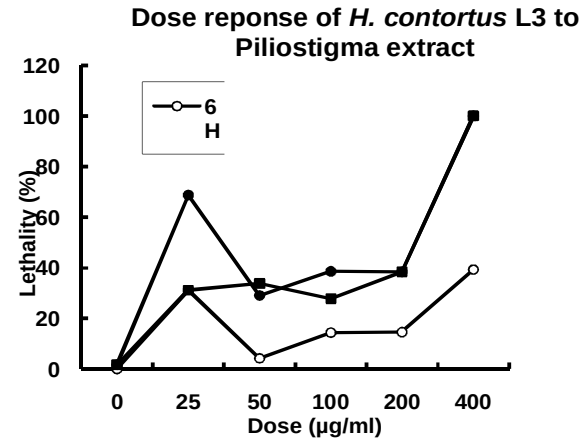

Fig 3. Effect of *Piliostigma on Haemonchus in vitro*

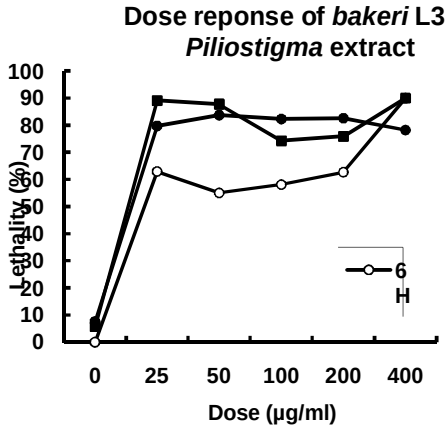

Fig 4. Effect of *Piliostigma* on *Heligmosomoides in vitro*

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