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EFFECT OF QUEBRACHO TANNIN DRENCHING ON *HELIGMOSOMOIDES BAKERI* INFECTION IN MICE

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ABSTRACT
In order to combat the increasing problems of anthelmintic resistance, alternative methods of control of gastrointestinal (GI) nematode infections are being sought. Thirty 8-9 weeks old outbred albino mice were placed in 5 groups of six mice each. Mice in four groups were infected with 150 *Heligmosomoides bakeri* infective larvae (L3) per mouse while one was reserved as the uninfected control. The anthelmintic potential of quebracho tannin was investigated by drenching three different groups of infected mice with three different dose levels viz: 2, 4 and 6mg/kg body weight. FEC, worm burden and body weight were recorded. The quebracho extract reduced the nematode faecal egg counts (FEC) of treated mice. At the end of the experiment the mice treated with the extract at the dose rate of 4 and 6mg/kg body weight had significantly lower FEC compared to the untreated control group ((F = 3.0756, *P* = 0.015; F = 3.440, *P* = 0.008) respectively. The worm burdens of mice treated with 4 and 6mg/kg body weight was lower than those of mice treated with 2mg/kg body weight although the difference was not significant (P = 0.023). Worm burden was negatively correlated to the dose of quebracho (r = -0.9642, *P* = 0.02). The uninfected mice gained significantly more weight (*P* = 0.056) in comparison to the infected groups.

KEYWORDS: Quebracho tannin; drencing; outbred albino mice; *H. bakeri*

INTRODUCTION

The consequences of gastrointestinal nematodosis are enormous (Chiejina, 1986. Fabiyi, 1987, Gill and LeJambre, 1986). The over dependence on chemical-based drugs, frequent treatments and misuse of chemical based anthelmintics in the last 2-3decades has lead to development of anthelmintic resistance particularly in sheep and goats worldwide (Jackson, 1993, Prichard, 1994; Waller, 1997). Development of newer generation of anthelmintics is no doubt needed to combat helminth infections in farm animals. Progressive testing of candidate plant extracts is vital for the development of dependable drugs (Hammond, Fielding and Bishop, 1997). *In vitro* testing of tannin-containing plant extract indicated that these were active against larval stages of *H. contortus* and *H. polygyrus* (Ngongeh and Fakae, un published observation) and Fakae *et al*. (2000). Any attempt to develop a sustainable approach to the use of condensed tannin-containing plants may have to evaluate the effect of direct drenching of these extracts in infected animals. *Trichostrongylus colubriformis* infected sheep have been shown to have their FEC and worm burdens reduced following a drenching experiment with quebracho extract (Athanasiadou *et al*., 2000). The following experiment was conducted to evaluate the effect of direct drenching of different concentrations of Quebracho bark extract on adult *H. bakeri* infection in tropical albino mice.

MATERIALS AND METHODS

Soluble quebracho bark extract produced from the bark of the tropical dicotyledon *Schinopsis species* was used. It was kindly donated by Professor Peter Buttery of Food Biochemistry, University of Nottingham, U.K. Dissolution was done carefully with the aid of a vortex mixer (Chitern Scientific Instrumentation Ltd Wendover, Bucks. U.K). Quebracho powder was added gradually to the water to avoid sticking and partial dissolution.

Experimental animals

All mice used for the study were obtained from the Experimental Parasitology Unit, Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka where they were bred and maintained as specific-pathogen-free animals. 30 male mice, 8-9 weeks old and weighed 20-25kg each were randomly placed in 5 groups of 6 mice each and allowed to acclimatise for one week prior to the start of the experiment. Of the 5 groups, group 1 was left as naïve control while groups 2,3,4 and 5were all infected with *H. bakerei* infective larvae (L3). While group 2 remained the untreated control, groups 3, 4 and 5 mice were all drenched with graded doses (2, 4 and 6g/kg body weight) of the extract solution.

Infection of the mice

The mice were infected orally with 150 L3 *H. bakeri* suspended in not more than 200µl of distilled water. Mice were properly restrained before dosing with the larval suspension to avoid spillage of some larvae. Delivering exact volumes of the larval suspension were further assured by using an automatic micropipette (Finnpippette®; Labsystems Oy, Helsinki, Finland) adapted to take a blunt and slightly curved 19-gauge needle (Fakae *et al*., 2001) as the dosing aid.

Treatment of infected mice

The mice were drenched for two consecutive days (D16 and D17) using an automatic micropipette (FinnpipetteR) (Labsystems Oy, Helsinki,Finland) adapted to take a blunted and slightly curved 20-gauge needle as described by Fakae *et al*. (2001).

Faecal egg counts (EPG)

Faecal egg counts were carried out every two days from the establishment of patency of the infection on day 10 of the infection using both salt floatation and McMaster techniques during early counts and when counts began to rise respectively (MAFF, 1997). Individual faecal samples were collected, by placing each mouse in a large plastic cup and enough faeces for faecal egg counts (FEC) was collected within 20 minutes as described by Ngongeh (2008; 2011).

PCV

Packed cell volume (PCV) evaluation was carried out weekly during the experiment. Mice were bled from the tail directly into heparinized capillary tubes (Camlab Ltd, Cambridge).

Weight measurements

Mice were weighed weekly from the first day of the study using a desktop balance (Sartorius GMBH Gottingen Germany).

Post mortem worm counts

The mice were sacrificed on the last day of the study and the worms harvested and counted as described by Ngongeh (2008).

Statistical analysis

Results were analysed using standard statistical procedures, ANOVA and Student's t-test and difference with probability level, $P< 0.05$ was accepted as significant.

RESULTS

Changes in FEC in quebracho tannin drenching

Infections became patent 9DAI. The changes in FEC are shown on Fig 1. FEC increased gradually in all groups of animals following infection. The FEC of all treated groups stabilised following the treatment. At the termination of the experiment the FEC of 4gQT and 6gQT were significantly lower than those of the control ($F=3.0756$, $P=0.015$ $F=3.440$, $P=0.008$) at 4g/kg and 6g/kg dose levels respectively. The 2gQT group although lower did not differ statistically from the control ($P>0.05$).

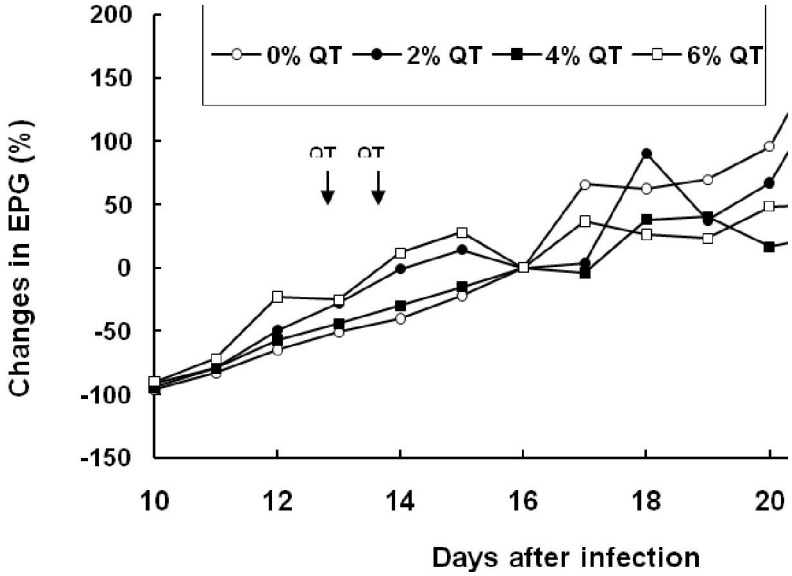

FIGURE 1. EPG in QT drenching

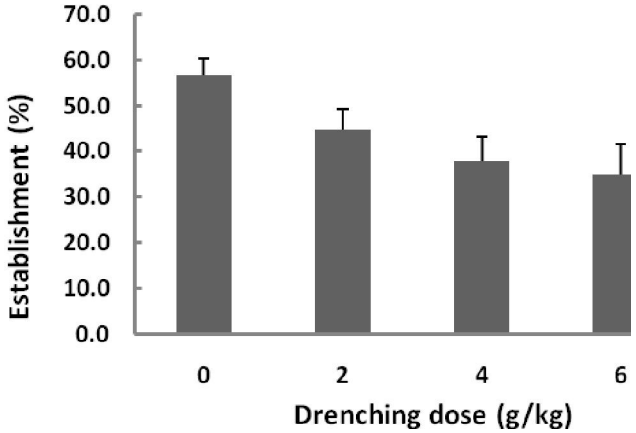

FIGURE 2. Worm count

Worm establishment

The total worm burdens of the mice drenched with 4 and 6g/kg quebracho tannin extract were lower than those of mice drenched with 2g/kg and the control mice (Fig 2). Statistical analysis however revealed that the differences were not different at 95% confidence interval (P = 0.023). Worm burden was negatively correlated to the dose of quebracho tannin (r = -0.9642, *P* = 0.023).

Weight changes

The weekly weight changes during the study are shown in Fig 3. The naïve control gained weight steadily till the end of the experiment. Although all infected animals also gained weight, this lasted only until 21DAI but dropped markedly on 28DAI. By comparison, the overall weight increase in the naïve control differed significantly (*P* = 0.056) from the infected animals. In the infected groups, there was however no difference between the non-treated group (0g/kgQT) and the treated.

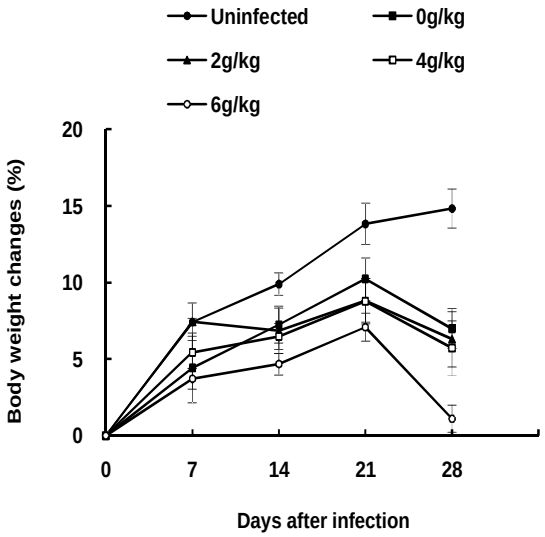

FIGURE 3. Body weight

DISCUSSION

The drop in FEC of the mice infected with *H. bakeri* following treatment with extracts of quebracho tannin does indicate its anthelmintic potential. This is in agreement with the work of Paolini *et al*, (2003), where goats infected with *H. contortus* and administered quebracho extract representing 5% of the diet dry matter (DM) for 8 days led to reduction in FEC. The reduction in FEC was associated to reduced fecundity of the worms and therefore reduced egg output. Earlier studies by Athanasiadou *et al*. (2000a; 2001) also showed drop in FEC following quebracho tannin administration conducted with sheep infected with *Trichostrongylus colubriformis* on the one hand and sheep infected with either *H. contortus* alone, *T. colubriformis* and *N. battus* conjointly or *T. colubriformis* alone. In the present study, mice that received the higher concentrations of quebracho (4 and 6g/kg) had FEC reduced more than those drenched with 2g/kg of the extract. This pattern agrees with that of Ahanasiadou *et al.* (2001), where sheep dosed with 16%w/w quebracho had their FEC reduced more compared to those dosed with 8% w/w extract.

Apart from the drop in FEC possibly being due to decreased fecundity and decreased egg production, a reduction in the absolute worm burden (Paolini *et al*., 2003) could also be a contributory factor. Worm burden may only had contributed in part to the reduced FEC because the mice given 2g/kg quebracho also had a slight drop in FEC, while its total worm burden was slightly higher than that of the control mice. Clinical observation of animals had revealed that those on high quebracho extract dose consumed less feed, which indicated a possible toxic effect of the extract which resulted in the death of three in the highest dose group. One would have thus expected a concentration effect in the FEC that is, higher FEC due to reduced volume of faeces. Although high concentration of certain types of condensed tannins have been considered to be toxic in ruminants (Reed, 1995), this can only occur when some types of condensed tannins are depolymerised and absorbed (Clausen *et al*., 1990).

Part of the observations on the live weight gain in the study, where treated animals showed reduced weight gain compares favourably with the work of Athanasiadou *et al*. (2000, 2001), where sheep drenched with 8% w/w quebracho had reduced live-weight compared to that drenched with 4% w/w and un-drenched controls. Again, it has also been shown that the digestibility of a food containing 50g/kg DM quebracho has been found to be reduced in sheep due to the presence of quebracho and consequently their growth was impaired (Dawson *et al*., 1999). In this study however, since untreated infected

animals also had reduced weight gain. Drop in weight may not have been entirely due to quebracho tannin treatment but a function of the nematode infection. Other studies have shown that sheep fed *ad libitum* with forages high in condensed tannins had increased food intake and live-weight gain compared to sheep fed on forages low in condensed tannins (Niezen *et al*., 1988a). A method that would allow lower concentrations of condensed tannin solution over a long time for the mice to get used to the product thereby limiting toxicity may achieve similar results as those in infected sheep grazed on the condensed-rich pasture.

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