

Anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats

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ABSTRACT

Xylopia aethiopica A. Rich (*X. aethiopica*; Annonaceae) is used commonly in Nigeria by traditional herbalists and pastoralists to control gastrointestinal helminth parasites. The anthelmintic effect of the crude methanol extract was evaluated in rats experimentally infected with the rat hookworm *Nippostrongylus brasiliensis*. Thirty-five rats were each infected subcutaneously with 200 third stage infective larvae of the parasite and randomly grouped into seven groups. Five days after infection, rats in six of the groups were treated with the extract, the dose varying per group. Rats in the seventh (control) group were each given propylene glycol. Anthelmintic activity was assessed by comparing the number of worms recovered from treated rats to those from non-treated infected control rats. The extract at the dose of 0.8 g/kg, 1.0 g/kg, 1.2 g/kg, 1.4 g/kg, 1.7 g/kg and 2.0 g/kg produced deparasitization rates of 21%, 47%, 51%, 50%, 63% and 76% and were significant ($P > 0.05$) when compared to untreated control rats.

Key words: *Xylopia aethiopica*, *Nippostrongylus brasiliensis*, anthelmintic, medicinal plant

Introduction

Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Most diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans

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and animals than any single group of parasites. The prevalence of helminth diseases in Nigeria is very high, especially during the wet season when infection is as high as 100% in cattle. Such high infection rates prevent them from attaining optimum productivity, especially under the traditional husbandry system (FAKAE, 1990). Financial costs of internal parasitism are enormous due to increase in mortality and a reduction of growth rate and wool production (MCLEOD, 1995). The major control strategy adopted against helminth parasites in Nigeria is the use of anthelmintics (IBRAHIM et al., 1983). However, the high cost of modern anthelmintics has limited the effective control of these parasites. In some cases widespread intensive use of sometimes low quality anthelmintics (MONTEIRO et al., 1997) has led to development of resistance and hence a reduction in the usefulness of available anthelmintics (WALLER, 1997a). Although the use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of helminth parasites, and as a means of reducing the cost of controlling helminthic diseases (KELLY and HALL, 1979; OKON et al., 1980; TAYLOR and HUNT, 1989; COLES and ROUSH, 1992), the emergence of resistant strains of pathogenic helminth has stimulated the desire to search for additional chemotherapeutic agents that might allow more efficient control of helminth parasites (HAMMOND et al., 1997; WALLER, 1997b). A practical solution to this is to develop effective drugs from reasonably less expensive and available raw materials. This can rationally be approached through the study of indigenous traditional plant remedies. In Nigeria, herbal treatment of helminthiasis is widely practised by human herbalists and the nomadic Fulani's (major cattle rearers in Nigeria) (NWUDE and IBRAHIM, 1980).

Xylopia aethiopica is a tree which grows to a height of 12-24 metres. It is found mostly in forest and coastal regions in Nigeria and belongs to the family Annonaceae (DALZIEL, 1955; IRVINE, 1961). The extract of the fruit is used to treat malaria in the Hausa land of Nigeria (ETKIN, 1997), while oral infusion of the seeds is used as an antitussive agent (MAMOUDOUKANDE et al., 1994). An extract of the seeds is used as a vermifuge for roundworms (DALZIEL, 1955; AKENDENGUE, 1992). The aim of this study was to test the anthelmintic activity of the crude methanol extract of the seeds of *X. aethiopica* against experimental *Nippostrongylus brasiliensis* infection in rats.

Materials and methods

Animals. Apparently healthy Wistar rats of both sexes, weighing between 112-182 g, obtained from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria, were used. They were acclimatized for 3 weeks to adapt to laboratory conditions. During this period they were dewormed using an oral preparation of albendazole at 200 mg/kg. The rats were maintained on standard diet and provided with water *ad libitum*.

Plant collection, identification and preparation. The seeds of *X. aethiopica*, together with the leaves, were collected in the wild in October and early November from the eastern part of Nigeria. All samples were collected between 11 and 16:00 hours local time. Samples of the leaves and seeds were identified at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where voucher specimen number 547 was deposited. The dried seeds were pulverized using mortar and pestle and 350 g of the powder was macerated in 1.5 litres of methanol in a separating funnel for 48 hrs at room temperature. The liquid phase was decanted and filtered through cotton wool. Thereafter, the liquid extract was evaporated to dryness in-vacuo using a rotary evaporator coupled to a thermo-regulator. The solid extract obtained was refrigerated at 4 °C until required.

Phytochemical test. The method of BRAIN and TURNER (1975) was used to detect some of the chemicals contained in the extract. One gram of the crude extract was used to test for sterols/triterpenes, tannins, alkaloids, saponins, flavonoids and coumarins.

Evaluation of tolerated doses of the extracts. The dose that did not produce any sign of toxicity (referred to as maximum tolerated dose; MTD) was determined orally by administering serial doses (100-2600 mg/kg) of the extract to rats.

Helminth parasite. A rat-adapted strain of trichostrongylid parasite, *Nippostrongylus brasiliensis* was used as a primary screen. Infective larvae (L₃) of the parasite were obtained from the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Experimental infection. Thirty-five worm-free rats were used for the anthelmintic trial. Each of the 35 rats was infected subcutaneously in the cervical region with 200 viable L₃ (third stage infective larvae) of *N. brasiliensis* using an 18-gauge needle attached to an insulin syringe. Five days post-infection, fresh faecal samples from each infected rat were collected by squeezing them out of the rectum and were examined by simple flotation. Rats not shedding ova of *N. brasiliensis* were discarded from the experiment.

Anthelmintic activity was determined by administering various doses of the crude extract of XA orally to groups of rats. Group 1, 2, 3, 4, 5 and 6 received the extract at 0.8 g/kg, 1.0 g/kg, 1.2 g/kg, 1.4 g/kg, 1.7 g/kg and 2.0 g/kg, respectively, while group 7 served as control and received propylene glycol via the same route at 5 ml/kg (maximum volume administrable to rats at a time). All treatments were given five days post-infection and for three consecutive days. At the end of the treatments the rats were fasted for 24 hours, euthanized and autopsied. The first 15 cm of the small intestine were removed, cut longitudinally and placed between two clean glass slides. The sections were examined at ×40 magnification of a dissection microscope. Worms that were visible were counted and recorded.

Percentage deparasitization was calculated using the formula:

$$\left[\frac{N-n}{N} \right] \times 100\%$$

Where: “N” and “n” = represent number of worms found in untreated control rats and treated rats, respectively.

Statistical analysis. Worm counts were expressed as mean \pm SEM. The significance of difference between the means was determined by student t-test using a computer software package (SPSS[®] for Windows Release 6.0) and regarded as significant when $P < 0.05$.

Results

Preliminary phytochemical tests of the crude methanol extract of *Xylopi aethiopia* revealed the presence of tannins, flavonoids and terpenoids, among other constituents.

Table 1. Mean worm counts (\pm SEM) in rats infected with *Nippostrongylus brasiliensis* and treated with various doses of crude methanol extract of *Xylopi aethiopia* 5 days post-infection

Treatment	Dose*	N ^o of rats	Mean number of worms (\pm SEM)
PG (control)	5 ml/kg	5	67.6 \pm 5.26 ^a
XA	0.8 g/kg	5	54.0 \pm 4.36 ^b
XA	1.0 g/kg	5	35.8 \pm 1.98 ^c
XA	1.2 g/kg	5	33.0 \pm 4.06 ^c
XA	1.4 g/kg	5	34.0 \pm 1.82 ^c
XA	1.7 g/kg	5	24.8 \pm 1.98 ^d
XA	2.0 g/kg	5	16.0 \pm 1.05 ^e

a, b, c, d, e-data or means with different superscript are statistically different ($P < 0.05$); XA = *Xylopi aethiopia*; PG = propylene glycol; *Oral dose administered for three consecutive days beginning from day 5 post-experimental infection

The extract exhibited a significant ($P < 0.05$) anthelmintic effect; it produced a consistently high anthelmintic effect (Table 1). Anthelmintic effect produced by *X. aethiopia* was dose-dependent. At doses of 0.8 g/kg, 1.0 g/kg, 1.2 g/kg, 1.4 g/kg, 1.7 g/kg and 2.0 g/kg the extract induced 21%, 47%, 51%, 50%, 63% and 76% deparasitization, respectively (Fig. 1). When given at doses higher than 2.0 g/kg, the extract caused signs of toxicity in rats (results not shown). Therefore, a dose of 2 g/kg was considered to be the maximum tolerated dose in this study.

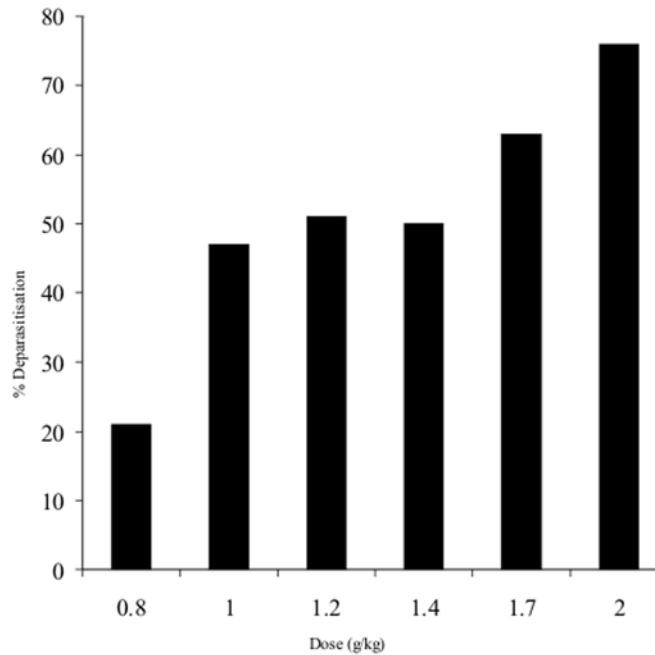


Fig. 1. Effect of various doses of crude methanol extract of *X. aethiopica* on rats infected with *N. brasiliensis*

Discussion

The results of the present study clearly indicated that the crude methanol extract of *Xylopia aethiopica* did produce anthelmintic activity against *Nippostrongylus brasiliensis* in rats. The plant possesses significant anthelmintic activity at doses between from 1.2 g/kg to 2.0 g/kg as measured by reduction in worm counts at necropsy. Although the extract produced some degree of deparasitization at doses lower than 1.2 g/kg, an anthelmintic is considered effective only when it can reduce 50% or more of worm burden in an animal. The results did not, however, exclude the possibility that doses of the extract with lower anthelmintic activity in this study might be efficacious against other species of helminths. This is because *N. brasiliensis* is known to be more resistant to anthelmintics than most other strongylidae. GORDON (1957), for instance, reported that if *N. brasiliensis* alone were used to screen phenothiazine, the anthelmintic activity of the drug would not have been discovered.

Phytochemical analysis of the crude extract has revealed tannins to be among the chemical constituent contained within it. Tannins were shown to produce anthelmintic

activities (NIEZEN et al., 1995; KAHN and DIAZ-HERNANDEZ, 1999; ATHNASIADOU et al., 2001; WALLER et al., 2001). However, the anthelmintic effect of plants containing tannins actually depends on the type and content of tannins in the plant (NIEZEN et al., 1998; ATHNASIADOU et al., 2001). 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., 1998). It is therefore reasonable to assume that food taken freely due to presence of tannins might be another way with which to control the detrimental effects of gastrointestinal parasitism.

Tannins are polyphenolic compounds (BATE-SMITH, 1962). Some synthetic phenolic anthelmintics, e.g. niclosamide, oxiclozanide, bithionol, nitroxylin, etc, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (MARTIN, 1997). It is possible that tannins contained in the extract of *X. aethiopyca* produced similar effects. In another study, polyphenols from bryophytes were shown to have anthelmintic activity against *Nippostrongylus brasiliensis* (GAMENARA et al., 2001). Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (ATHNASIADOU et al., 2001) or glycoprotein on the cuticle of the parasite (THOMPSON and GEARY, 1995), and cause death.

Several authors have reported that an increase in the supply of digestible protein (DP) does improve the resilience and resistance of sheep to gastrointestinal nematodes (COOP and HOLMES, 1996; VAN HOUTERT and SYKES, 1996; DONALDSON et al., 1997). Tannin-containing plants increase the supply and absorption of digestible protein by animals (WANG et al., 1994; WALLER et al., 2001). This is achieved by formation of protein complexes in the rumen by tannins, which later dissociate at low pH in the abomasum to release more protein for metabolism in the small intestines of ruminant animals (WALLER et al., 2001). In addition, tannins or their metabolites have a direct effect on the viability of the pre-parasitic stages of helminths (DUNCAN, 1996). Other phytochemicals reported to have an anthelmintic effect include essential oils (PESSOA et al., 2002), flavonoids and terpenoids (LAHLOU, 2002).

One problem associated with the use of this plant in traditional medicine is lack of consistency of the dose. However, this was circumvented by evaluating different doses of the plant extract within the apparently non-toxic doses as revealed during the toxicity studies (results not shown).

Factors unknown to us may influence the anthelmintic activity of this plant. For instance, the active component(s) contained in the plant may vary in relation to location, age, and stage of development of the plant and whether the plant is freshly harvested or preserved (MCCORKLE et al., 1996). It is therefore possible that seed extracts from different regions and kept under different conditions may have varying anthelmintic effects.

Since a link has been established between *Nippostrongylus brasiliensis* and trichostrongyles of sheep and hook worm of dogs and man, the results obtained in this study justify further investigation into the anthelmintic effects of the crude methanol extract of *X. aethiopica* in sheep and dogs. In conclusion, further studies to isolate and reveal the active compound(s) contained in the crude extract of *X. aethiopica* and to establish the mechanism(s) of action are required. Moreover, there is a need to conduct detailed toxicological studies of the extract in both laboratory and target animal species to justify clinical investigation in target species.

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SAŽETAK

Travari i stočari u Nigeriji tradicionalno koriste biljku *Xylopiya aethiopyca* protiv želučano crijevnih helminata. Anthelmintički učinak nepročišćenog metanolskog iscrpka istražen je na pokusno invadiranim štakorima obličem *Nippostrongylus brasiliensis*. Ukupno 35 štakora podijeljeno je u sedam skupina. Svi štakori supkutano su invadirani s 200 invazijskih ličinki trećeg stupnja. Pet dana nakon invazije, štakorima iz svih šest skupina apliciran je iscrpak u različitim dozama. Štakorima sedme skupine primijenjen je propilen glikol. Učinkovitost je određena uspoređivanjem broja parazita u liječenih i neliječenih štakora. Značajna ($P < 0,05$) anthelmintička učinkovitost dokazana je za sve primijenjene doze. Tako je rezultat primjene iscrpka u dozi od 0,8 g/kg bio smanjeni broj parazita za 21%. Veće doze (1,0 g/kg i 1,2 g/kg) smanjile su broj parazita za 47% i 51%. Za najviše doze (1,7 g/kg i 2,0 g/kg) potvrđena je i najviša učinkovitost od 63% i 76%.

Ključne riječi: *Xylopiya aethiopyca*, *Nippostrongylus brasiliensis*, anthelmintik, ljekovita biljka
