In vitro Toxicity of Ethanolic Plant Extracts from Adamawa Province, Cameroon to Infective Larvae of Strongyloides papillosus

G. Musongong, E.N. Nukenine, M. Ngassoum, T. Gangu, O. Messine,
C.N. Fokunang, F.G. Zalom, L.M. Njongmeta and V. Tanya

Parasitology Laboratory, Institute of Agricultural Research for Development,
Wakwa Regional Centre, P.O. Box 65, Ngaoundere, Cameroon
Department of Biological Sciences, University of Ngaoundere,
P.O. Box 454, Ngaoundere, Cameroon
Department of Applied Chemistry, National Advanced School of Agro-Industrial Sciences,
University of Ngaoundere, P.O. Box 455, Ngaoundere, Cameroon
Bioserve Ltd, Toxicology Unit, Athlone Institute of Technology, Co. Westmeath, Ireland
College of Agricultural and Environmental Sciences, University of California, Davis, CA 95616, USA

Abstract: Ethanolic leaf extracts from five herbal plants (Annona senegalensis, Lantana camara, Steganotaenia araliacea, Vernonia amygdalina and Vernonia tonoreana), collected in the Adamawa Province of Cameroon, were assessed for toxicity against the infective larval stage (third-instar) of Strongyloides papillosus (Weedl.), for post-exposure periods ranging from 1-48 h. Acute toxicity at 24 and 48 h post-exposure were also determined. Larval mortality increased over time for all of the herbal extracts at all concentration levels. A maximum 100% mortality at 48 h post-exposure was recorded only for A. senegalensis. LC50 values recorded at 48 h post exposure were A. senegalensis (0.107%), V. tonoreana (0.138%), V. amygdalina (0.203) and L. camara (0.496%). High acute toxicity was recorded for A. senegalensis, V. tonoreana and S. araliacea, suggesting these plants could yield natural alternative treatment products for S. papillosus.

Key words: Toxicity, Strongyloides papillosus, crude extracts, Cameroon

INTRODUCTION

Strongyloides papillosus, a highly pathogenic parasite of ruminants world wide, is one of the most important nematodes prevalent in sub-Saharan Africa. Symptoms produced by the nematode in parasitized animals include diarrhea, fever, loss of appetite, tachycardia, retarded growth and emaciation. The infection is fatal in most cases.

Anthelmintic chemotherapy is a priority method for controlling S. papillosus and other nematodiasis in ruminants. Disadvantages of this method include the possibility of drug residues in meat and milk, environmental impact through drug residues in the faeces and the imminent development of resistance in parasites. Selection pressure for resistance in nematodes such as S. papillosus is greater with one or a few drugs as compared with many drugs. Therefore, it is important to develop new classes of drugs and to investigate alternative treatments for strongyloiddiasis and other nematodiasis using less toxic but more biodegradable and environmentally friendly plant products.

Recently, research has focused on screening herbal plant products of pharmaceutical importance for nematocidal activities, in order to develop new and safer drugs. Anthelmintic plants are likely to be sustainable and environmentally acceptable. Moreover, locally available nematocidal plant materials could be a cheaper means for the peasant farmers to control infection in their animals.

The herds men in Adamawa province, Cameroon employ such plants in the treatment of many common diseases and ailments affecting their livestock, including nematodiasis (Personal Communication). However, experimental evidence for toxicity of such plants to different gastrointestinal nematodes of ruminants and effective dosages, are lacking. Based on information from the herds men, five plants Annona senegalensis Linn. (Annonaceae), Lantana camara Linn. (Verbenaceae); Steganotaenia araliacea Hochst. (Araliaceae); Vernonia
amysdolina Del. (Asteraceae). Vernonia tonoreana Linn. (Asteraceae) from Adamawa Province were selected to determine the acute toxicity in vitro of their crude ethanolic leaf extracts on the infective (third-instar) larvae of *S. papillosus*.

**MATERIALS AND METHODS**

**Plants materials:** Leaves from the five plant species were collected around Ngaoundere, Adamawa Province and dried at room temperature for seven days. A 200 g sample of each plant was weighed and ground in a milling machine until they passed through a 0.5 mm mesh screen. The milled samples were mixed into 1 L 95% ethanol (EtOH) at room temperature, agitated for 24 h and filtered. The filtrate was evaporated in a rotavapour at 50°C yielding a brownish, viscous liquid. The extracts were stored in a refrigerator at 5°C and were removed only when needed for assay.

**Strongyloides papillosus infective larvae**

**Collection of eggs and culture system:** Calves were screened for gastrointestinal nematodes infection from 2-3 months[9]. Eggs were collected from the rectum of the calves found to have only *S. papillosus* eggs in their faeces according to the methods of Hansen and Perry[7]. Thienpont et al.[10]. They were next cultured for 7 days in sterile petri dishes. Baermens apparatus was then used to recover the larvae which were placed in sterile test tubes and stored at 7°C for 12 h to immobilize the nematodes[19]. The larvae were concentrated by extracting the supernatant with the aid of a pipette. This process was repeated until all the larvae were recovered.

**Parasites and culture system:** Bioassay was performed under laboratory conditions. Little quantities from the concentrated culture were diluted in distilled water until a concentration of approximately 25 larvae/0.1 mL water was obtained. Aqueous solutions of the extract from each plant emulsified with 0.1% Tween 80 to give concentrations of 0.1, 0.2, 0.4 and 0.8% were prepared. Tween 80 plus distilled water was used as the control. Larvae were diluted in each treatment and control solution to give concentrations of approximately 25 larvae/0.1 mL of test solution. A total volume of 2.0 mL of test solution plus larvae in a test tube replicated three times was used for each run. Larval mortalities were recorded 1, 6, 12, 24 and 48 h post-incubation. During each recording time, 0.1 mL solution was removed from the 2.0 mL in the test tube after agitation and placed on a microscopic slide. The numbers of dead and live larvae were then counted under a stereoscope microscope. Mortality was determined using standard methods of Institute of Agricultural Research, Wakwa, Ngaoundere, Cameroon. Larvae which were fully straight and not motile were considered dead. Those which were curved but immobile were considered as paralyzed. Paralyzed larvae which straightened up and stayed immotile when a drop of iodine was added in their medium were considered dead. Otherwise, were considered alive. The percent mortality was calculated from an average of the three replicates.

**Statistical analysis:** The data were subjected to the analysis of variance procedure to check for differences in mortality among plant extracts, concentrations and time post-exposure[30]. Mortality data at the 24 and 48 h post-exposure time-points were analyzed using probit analysis[22,21]. L_{50} were considered significantly different if the fiducial limits did not overlap. R^2 values were estimated using the regression of mortality on Log 10 (concentration+0.1)[30].

**RESULTS**

**Mortality over time and concentration:** As expected, there were significant differences (p<0.01) in mortality among plants, concentrations and exposure time. Control mortality was generally significantly (p<0.05) lower than that recorded for any of the test concentrations. Mortality increased with ascending concentrations, irrespective of plant and exposure time (Fig. 1). Mortality also increased with time, regardless of plant and concentration. The increase in mortality over time and concentration was greatest with *A. senegalensis* and least with *L. camara*. The highest percentage mortality recorded was at the 48 h and for the concentration 0.8% They were 100.0, 64.0, 86.7, 64.0, 84.3 and 7.3 for *A. senegalensis*, *L. camara*, *S. araliacea*, *V. amygdalina*, *V. tonoreana* and the control, respectively. Overall, the rate of increase in mortality was higher during the first 6-12 h than during the last 36 h. However, *L. camara* (0.4 and 0.8%), *S. araliacea* (0.8%) and *V. tonoreana* (0.2, 0.4 and 0.8%) showed marked increases in mortality at higher concentrations. *V. amygdalina* presented a more or less linear time-mortality relationship.

**Evaluation of toxicity:** At 24 h post-exposure more than 50% mortality was recorded for all the extracts apart from that of *L. camara* which gave only 33.3% mortality (Fig. 1). By the 48 h, all the plants gave mortalities above 50%. Thus, in order to evaluate the toxicities of the plants, the L_{50} at the 24 and 48 h were determined for all extracts (Table 1).
Fig. 1: Relations between mortality and time for *S. papillosus* incubation in different concentrations of plant extracts. Vertical bars indicate 2x SE.

*LC*₅₀ values were generally higher at the 48 than at the 24 h (Table 1). *R*² values for the regression of mortality on concentration were on the whole high (0.81-0.96). Significant *χ²* values were not recorded with any of the plants at the 24 and 48 h post-exposure. For the 24 h post-exposure time, *A. senegalensis* had the highest *LC*₅₀ value which was similar to that of *V. tonoreana*, but higher and different from those of the other three plants. By extrapolation, *L. camara* recorded an *LC*₅₀ value lower than those of the rest of the plants. *A. senegalensis*
also had the steepest slope, while V. tonoreaana showed the least slope value. Slopes were comparable for V. amygdalina with V. tonoreaana and L. camara with V. amygdalina. At 48 h post-exposure time, the plants could be ranked in order of increasing LC_{50} values thus: A. senegalensis, V. tonoreaana, S. araliacea, V. amygdalina and L. camara. However, the values were not different between A. senegalensis and V. tonoreaana and among V. tonoreaana, S. araliacea and V. amygdalina. The steepest slope was again recorded by A. senegalensis while V. amygdalina had the least slope. Slopes were comparable between L. camara and S. araliacea and V. tonoreaana and S. araliacea.

**DISCUSSION**

Investigations on the in vitro toxicities of plant extracts from Adamawa, Cameroon to S. papillosus showed that the extracts possess some toxic components which could cause significant mortalities of the nematode. The death of S. papillosus could be reasonably attributed to the toxic effects of the plant extracts on the physiology of the nematode because the control recorded significantly lower mortalities than the extracts. This contention is also supported by the high R^2 values (>0.80) of the regression of mortality on concentration, which indicate that the extracts accounted for over 80% of the mortalities of S. papillosus. The non significant chi-squares show that the observed mortality did not deviate significantly from the expected and that the regression models were well fitted.

The characteristic median lethal concentration obtained for each plant extract was calculated according to the method of Finney[21]. The 24 and 48 h LC_{50} data represent the concentrations inducing 50% mortality in a population exposed for 24 and 48 h, respectively, to the treated medium. The results show that the highest toxicity to S. papillosus was attributable to A. senegalensis and V. tonoreaana. L. camara with the highest LC_{50} values represents the least toxic plant. A. senegalensis was 75% effective against another nematode, *Nippostrongylus brasiliensis*, in white rats[22]. Biological activities of *A. senegalensis* against other biotic stresses are known[23-25]. Although little is known about the biological activity of *V. tonoreaana*, this study confirms the high toxicity of the extract of this plant against *S. papillosus*. The relatively low toxicity of *L. camara* in this study is in accordance with the results from other studies. Leaf extracts of *L. camara* showed the lowest nematicidal activity among six plants[26]. By contrast, very active nematicidal components, such as lantanocide, lantanone, linaroside and camarinic acid were isolated from the aerial parts of *L. camara*[27]. One could speculate that extracts from this plant are toxic but slow acting, showing the need of carrying out acute toxicity studies for longer durations. The extracts of *S. araliacea* and *V. amygdalina* showed intermediate toxicity to *S. papillosus*. Nematicidal activities of *S. araliacea* have not been reported, but this study shows that extracts from this plant could be of value in the control of *S. papillosus*. Although studies of the biological activity of *V. amygdalina* against *S. papillosus* are not in the literature, the plant is well known for its nematicidal and other biological activities[26-29].

This study has shown that substances from the leaves of *A. senegalensis*, *V. tonoreaana* and *S. araliacea* could be of value in the development of anthelmintic drugs against *S. papillosus*. More studies with other life stages of the nematodes *in vitro* and *in vivo* may be needed to support these findings.

**REFERENCES**