

**TRYPANOCIDAL POTENTIAL OF *CARRISA EDULIS* IN MALE WISTAR RATS INFECTED WITH *T. CONGOLENSE*****<sup>1</sup>A.U. Wurochekke\*, <sup>1</sup>N. Nuhu and <sup>2</sup>G. O. Anyanwu**

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**ABSTRACT**

Trypanocidal activity of *Carrisa edulis* against *Trypanosoma congolense* infection in rats was investigated. All extracts of the different parts of the plant had *in vitro* trypanocidal activity against the parasite at different times of incubation. The methanolic root extract, being the most active *in vitro* was used for the *in vivo* analysis. Oral treatment at different doses did not clear the parasitemia. The groups treated with 100mg/kg/day and 200mg/kg/day of extract did not significantly decrease ( $P < 0.05$ ) the parasitemia neither was there any significant increase ( $P < 0.05$ ) in PCV when compared to the infected control. However, animals treated with 100mg/kg/day survived longer than those treated with 200mg/kg/day and the infected control group. The parasitemia of all the infected animals continue to increase with decrease in pack cell volume until the death of the animals. Further work is required to substantiate on the *in vivo* activity in order to conclude on the trypanocidal effectiveness of the plant.

**Key words:** Antitrypanosomal, *Carrisa edulis*, *Trypanosoma congolense*, Parasitemia, Pack cell volume

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## INTRODUCTION

*Trypanosoma congolense* is one of the protozoan parasites that retard livestock production in the north eastern Nigeria. *Trypanosoma congolense* is a haemic trypanosome with effects mostly in the cardiovascular system. The trypanosome has the ability to sequester in small vessels and capillaries of the brain, heart, skeletal and other tissues often leading to prolonged pre-patent period (Losos and Ikede, 1972), thereby causing serious harm to its host. The trypanosome is more prominent in cattle but can cause serious losses in pigs, camels, goats and sheep (Barret, 2003).

Chemotherapeutic agents against the parasite, which were developed 30 to 50 years ago, are ineffective because of toxicity, relapse of parasitemia and resistance (Nok, 2002; Gutteridge, 1985; Anene *et al.*, 2001). New drug development is faced with lack of funds, lack of infrastructure for clinical trial sites, lack of personnel and pharmaceutical partners (WHO, 2004). These problems coupled with the high cost of existing drugs, forced most of the livestock farmers to resort to traditional treatment against the disease as alternative.

Recent investigations revealed some of the medicinal plants claimed by the traditional healers are potent trypanocides (Wurochekke and Anyanwu, 2012; Nok, 2002; Asuzu and Chinime, 1990; Igwe and Onabanjo, 1989). Some medicinal plants have been reported to have antitrypanosomal activity, such plants includes *Securidaca longependunculata* (Aderbauer *et al.*, 2008); *Aristolochia bacteolata* (Samia *et al.*, 2006); *Allium sativum* (Nok *et al.*, 1996); *Lawsonia inermis* (Atawodi *et al.*, 2003 and Wurochekke *et al.*, 2004); and *Azadirachta indica* (Mbaya *et al.*, 2010).

*Carrisa edulis* is a plant commonly called “Lemun Tsuntsu” in Hausa language within the northern part of Nigeria. The plant is used by various traditional healers to treat different types of ailments such as chest pain, rheumatism, stomach disorder and headache. Pregnant women of *suri* natives in Ethiopia use it to shorten delivery (Abink, 2001). Leaf extract of the plant also reduced significantly blood glucose level in streptozotocin diabetic rats during the first three hours of treatment (El-fiky *et al.*, 1960). Traditional healers in northern Nigeria also claim that the plant is good remedy against

trypanosomiasis. Thus, this work was aimed to investigate scientifically the effectiveness of the plant against *Trypanosoma congolense* infection in male Wistar rats.

## MATERIALS AND METHODS

### Plant material

The root, bark and leaf of *Carrisa edulis* was collected within Adamawa metropolis and identified at the Forestry Department, Moddibo Adama University of Technology, Yola, Adamawa State, Nigeria.

### Trypanosome

*Trypanosoma Congolense* (Karu strain) was provided by the National Institute of Trypanosomiasis Research, Vom, Jos, Plateau State, Nigeria. The recipient rats were inoculated with  $3.6 \times 10^3$  *T. Congolense* cells.

### Preparation of plant extract

The fresh leaf, bark and root of *Carrisa edulis* were collected, washed, cut into pieces and shade dried at room temperature. It was then ground separately into powdered form using a milling machine. Exactly 200 g each of the dried powdered form of the various parts of the plant was macerated in 400 ml of distilled water and methanol. Extracts were filtered using a Buckner funnel and Whatman No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator. All extracts were then stored in the refrigerator at 4 °C until required.

### Trypanosome infection

Infected blood collected from a previously inoculated donor rat at peak parasitemia ( $10^3$  parasites/ml of blood) was diluted with phosphate buffered saline (pH 7.4). Experimental rats were infected intraperitoneally with approximately  $10^3$  parasites/ml of blood.

### In vitro screening

Infected blood was collected by cardiac puncture from a donor rat at peak parasitemia and poured into EDTA bottle. 5% of aqueous and methanolic crude extracts were prepared separately. Aliquots of 10  $\mu$ l of the 5% crude extract preparation were incubated with 60  $\mu$ l of the infected blood in wells of microtitre plates. The crude extract was replaced with phosphate buffered saline (pH 7.4) for the control. After 5 minutes of incubation in the wells of microtitre plates, 2  $\mu$ l of test mixture was placed on microscope slides and the motility of the parasites was observed under the microscope (Mgx40) at 5 minutes interval for an hour. The procedure was carried out separately for the aqueous and methanolic extract and the screening was performed in triplicates in 96 wells micro titer plates (Flow laboratories Inc., McLean, Virginia 22101, USA). Cessation or drop in motility of the parasites in extract treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of antitrypanosomal activity.

### In vivo screening

#### Experimental animals and grouping

Twenty male Wister rats weighing between 150-200g were obtained from Department of Biochemistry, National Veterinary Research Institute (NVRI), Vom, Jos, Nigeria. The rats were kept in cages in the research laboratory of the Department of Biochemistry, Moddibo Adama University of Technology, Yola, and were allowed to acclimatize for 7 days before the commencement of the study. All rats were fed with commercial pellets (Pfizer Nigeria Plc., Ikeja, Nigeria) and watered *ad libitum* throughout the duration of the study. Thereafter, the rats were grouped into four of five rats each.

Group 1: Normal control

2: Infected control

3: Infected treated with 100mg/kg b.w/day of extract

4: Infected treated with 200mg/kg b.w/day of extract

### Administration of Extract

The methanolic root extract, being the most active *in vitro* was used for the *in vivo* analysis. The extract was administered to animals orally using a gavage for seven (7) days.

Parasitemia Determination: Wet film smears of the infected blood were made and parasites were counted under the microscope as described by Herbert and Lumsden (1976). The parasitemia was monitored for nine (9) days.

Pack cell volume (PCV) determination: Pack cell volume of the rats was determined after every two days for all animals by microheamatocrit method according to Dacie and Levis (1991).

### Statistical Analysis

The experimental results were expressed as the Mean  $\pm$  S.E.M. Statistical significance of difference in parameters amongst groups was determined by one way ANOVA followed by Least Significant Differences test at  $P < 0.05$  level of significance.

## **RESULTS**

The motility of *Trypanosoma congolense* was inhibited by all the different extracts at different times of incubation. The methanol root extract had the highest *in vitro* activity against the parasite. At minimum concentration of 8.3mg/ml of blood, parasites were immotile 25 minutes after incubation. Aqueous extract of the bark prevented motility 30 minutes after incubation, while the leaf and the root stopped motility 45 minutes after incubation. Similarly, the methanolic extracts of the leaf and the bark also had considerable *in vitro* activity (Figures 1&2).

Figure 1: Invitro trypanocidal activity of aqueous extract of *Carrisa edulis* against *T. congolense*

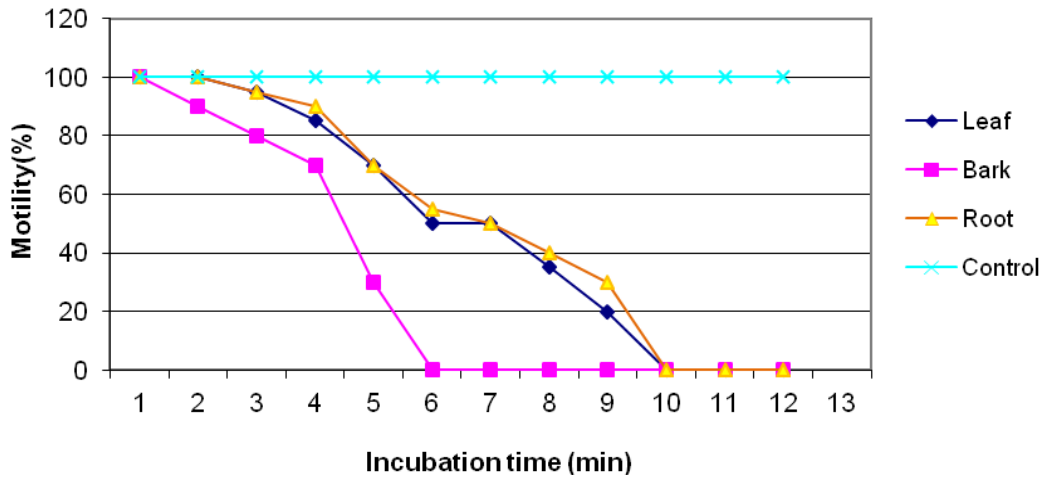
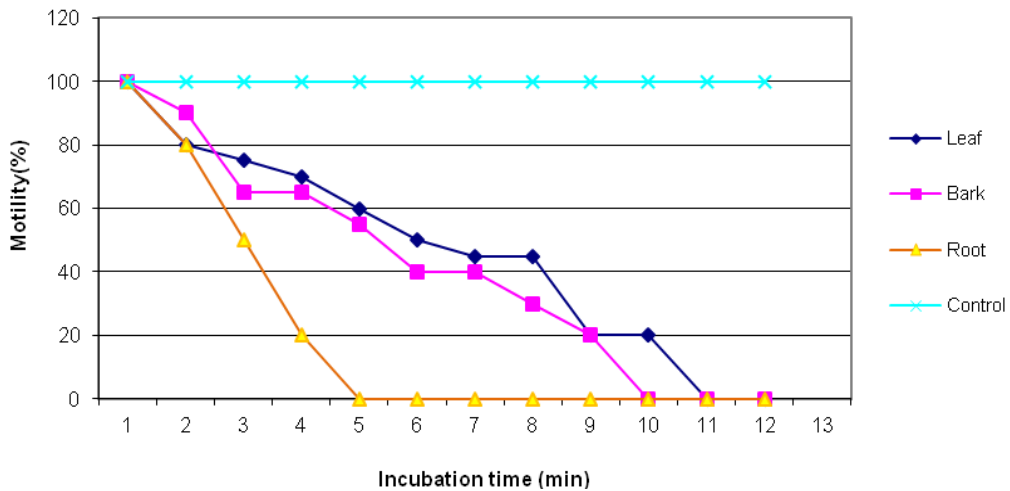


Figure 2: Invitro trypanocidal activity of methanolic extract of *Carrisa edulis* against *T. congolense*



The infected control and infected treated groups recorded progressive course of parasitemia to reach a peak on day seven post infection. Although, treatment with 100 mg/kg/day and 200 mg/kg/day of the extract prolonged the survival of the animals, it did not directly affect the course of parasitemia (Table 1). At the end of the experiment, neither the group treated with 100 mg/kg/day or 200 mg/kg/day of the extract showed any significant difference ( $P < 0.05$ ) when compared with the infected control.

**Table 1: Effect of *Carrisa edulis* extract on the Parasitemia of infected rats**

<b>GROUP</b>	Infected Control	Infected treated 100mg/kg/day	Infected treated 200mg/kg/day
<b>Day 3</b>	8.10 ± 0.02	8.11 ± 0.06	8.14 ± 0.08
<b>Day 4</b>	8.13 ± 0.04	8.10 ± 0.03	7.93 ± 0.01 <sup>#</sup>
<b>Day 5</b>	8.18 ± 0.05	7.72 ± 0.33	7.91 ± 0.06
<b>Day 6</b>	8.30 ± 0.01	8.18 ± 0.02 <sup>#</sup>	7.99 ± 0.03 <sup>#</sup>
<b>Day 7</b>	8.75 ± 0.03	8.25 ± 0.02	8.00 ± 0.06
<b>Day 8</b>	8.84 ± 0.03	8.82 ± 0.01	8.78 ± 0.03
<b>Day 9</b>	8.80 ± 0.03	8.76 ± 0.01	8.66 ± 0.02

Values are expressed as means ± SEM. (#) indicates means that are significantly different ( $p < 0.05$ ) when compared to the infected control.

The PCV of the infected groups of rats significantly decreased ( $P < 0.05$ ) when compared to the normal control through out the experiment. The PCV of the infected treated with 200 mg/kg/day of extract revealed a significant decrease ( $P < 0.05$ ) when compared to the infected control on Day 3 and 7. Although animals treated with 100 mg/kg/day of the extract did not show any significant difference compared to the infected control, they survived longer than the infected controls (Table 2).

**Table 2: Effect of *Carrisa edulis* extract on the Packed Cell Volume of infected rats**

GROUP	Normal Control	Infected Control	Infected treated 100mg/kg/day	Infected treated 200mg/kg/day
Day 1	59.5 ± 0.9	58.9 ± 1.7	59.1 ± 1.4	58.6 ± 0.3
Day 3	58.8 ± 0.7	43.5 ± 1.1*	43.0 ± 0.8*	39.0 ± 0.5*#
Day 5	53.8 ± 0.1	35.4 ± 0.2*	37.8 ± 0.3*#	39.0 ± 0.3*#
Day 7	52.6 ± 0.3	33.3 ± 0.2*	34.0 ± 0.6*	29.0 ± 0.5*#

Values are expressed as means ± SEM. (\*) indicates means that are significantly different ( $p < 0.05$ ) when compared to the normal control; (#) indicates means that are significantly different ( $p < 0.05$ ) when compared to the infected control.

## DISCUSSION

The ability of the methanolic root extract to inhibit motility of *Trypanosoma congolense* *in vitro* substantiates the claimed efficacy of the plant against trypanosomiasis. Although *in vitro* activity of crude extract alone does not confirm the trypanocidal activity, it remains one of the tools widely used in bioassay guided identification of active components in plants. The phytochemical investigation of *Carrisa edulis* showed the presence of saponins, cardiac glycoside, terpenoids and carbohydrates while tannins, alkaloids, anthraquinones and flavonoids were not detected in the extract (Ngulde, *et al.*, 2013).

The trypanocidal efficacies of plants are associated with the presence of one or more biologically active principles (Nok, 2002). Studies have shown that several flavonoids have antitrypanosomal activity (Tarus *et al.*, 2002). Atawodi *et al.* (2003) reported that plant extracts which contained either alkaloids, flavonoids, phenolics and/or terpenes showed trypanocidal activity in an *in vitro* investigation. Therefore, it's less surprising that *Carrisa edulis*, which contained one of the bioactive components showed trypanocidal activity against *T. congolense*.



The effect of the extract on parasitemia and pack cell volume of infected treated animals is not significant suggesting that the extract is not very active *in vivo*. Although animals treated with the lower dose of the extract survived longer than the infected controls, suggesting ameliorative effect, the inactivity of the plant *in vivo* was further confirmed by the insignificant effect of the extract on the course of parasitemia and pack cell volume.

The inactivity of the extract *in vivo* may be due to resistance, biochemical transformation *in vivo* or antagonistic effect of different compounds in the crude extract (Wurochekke, *et al.*, 2005; Wurochekke and Nok, 2004; Anene *et al.*, 2000). It could also be possible that the active compound did not reach the target site *in vivo* or its concentration at the site was insignificant.

Animals treated with 200mg/kg/day died 3 days earlier than the infected control. This suggests that the relatively high concentration of the crude extract have contributed to the early death of the animals. Therefore, there is need to fractionate and remove unwanted compounds in order to increase the concentration of the possible active principle. The over all result revealed that the plant is active *in vitro*, inactive *in vivo* and may be toxic at higher concentrations.

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