

POTENTIALS OF METHANOLIC EXTRACT OF *N. LATIFOLIA* STEM BARK AGAINST *T. CONGOLENSE* INFECTION IN EXPERIMENTAL RATS

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ABSTRACT

Methanolic extract of *Nauclea latifolia* stem bark was investigated for antitrypanosomal activity using albino rats infected with *Trypanosoma congolense*. Fifteen albino rats placed in 5 groups of 3 each were labeled A to E. Groups A and B served as positive and negative controls, respectively. Groups C, D and E were infected with the parasites and treated with 100, 200 and 300mg/kg body weight of methanolic extract of *Nauclea latifolia* stem bark respectively. Parasitemia was monitored as well as hematological and biochemical parameters were analyzed. The results showed suppression in levels of parasites in all the infected and treated groups. Equally, the negative effects of the disease were lowered as shown in the hematological and biochemical parameters. The antitrypanosomal potential of the extracts was therefore demonstrated.

Keywords: *Trypanosoma congolense*, trypanosomiasis, *Nauclea latifolia*

INTRODUCTION

Animal trypanosomiasis in sub-Saharan Africa largely account for the low livestock productivity [1]. About 66 million people in 36 African countries are afflicted and 3 million cattle die annually [2] making the disease important [3]. The *plant N. latifolia* has demonstrated antimicrobial activity [4], antipyretic and antinociceptic effects [5] and analgesic tramadol in the bark [6]. Therefore, this study was designed to determine the effect of *Nauclea latifolia* stem bark extract in alleviating some hematological and biochemical symptoms on *Trypanosoma congolense* infection in albino rats.

MATERIALS AND METHODS

Plant Material

Fresh stem bark of *N. latifolia* was collected from Shere Hills, Jos, Plateau State, Nigeria. The plant was identified and authenticated at the Herbarium unit of Department of Biological Sciences, Federal Institute of Forestry, Jos, Plateau State, Nigeria. The Fresh stem bark of *N. latifolia* was cut into smaller sizes, air dried, pulverized and sieved. Two hundred gram were macerated in 1 litre of methanol respectively for 24 hours, after which each was filtered using a Buckner funnel and Whatman No 1 filter paper. The filtrate was concentrated and the solvent evaporated to dryness in a hot air oven set at 35°C. The extract was weighed and stored in air tight container.

Experimental Animal

Fifteen albino rats of weights (100 – 180) were purchased from the animal house of Department of Physiology, University of Jos, Plateau State, Nigeria. The albino rats were labeled weighed and placed into groups A – E as shown below, each group containing three rats.

- A. Not infected not treated
- B. Infected and not treated
- C. Infected and treated with 100mg/kg body weight methanolic extract of *N. latifolia*
- D. Infected and treated with 200mg/kg body weight methanolic extract of *N. latifolia*
- E. Infected and treated with 300mg/kg body weight methanolic extract of *N. latifolia*

Body weight of each rat was taken and recorded on days one, seven and fourteen.

Parasites

Trypanosoma congolense was obtained from stabilates maintained at the National Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. The parasite was maintained in the laboratory by continuous passage in rats. The potency of *T. congolense* in the infected donor rats was determined by wet film preparation and observed under microscope. An estimate of the average number of parasite per 100 red blood cell was determined using the 'Rapid Matching' method[7]. Donor blood

was diluted with phosphate buffered saline (PBS). 0.5ml of diluted donor blood containing about 2.0×10^3 parasites was inoculated into the rats in group for infection.

Determination of Parasitemia

Blood films were made from the caudal vein of each rat after sterilization. Trypanosome count was determined by examination of the wet mount microscopically at x 40 magnification using the "Rapid Matching" method. Briefly, this method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with PBS (pH 7.2). Logarithm values of these counts obtained by matching with the table in the method [7] then converted to antilog to provide absolute number of trypanosomes per ml of blood.

Treatments

Extracts were administered immediately the first parasite was seen per microscope field. Treatment was administered orally to the designated albino rats daily according to the experimental design that is respective dose per body weight daily.

Hematological Analysis

The surviving rats were decapitated under ether anesthesia on the 25th day post-infection and blood from the neck vessel collected into an EDTA containing bottle. Hematological parameters such as packed cell volume, hemoglobin counts,

white blood cell count, lymphocyte count, neutrophil count, platelets count, monocyte percentage, and basophil percentage were assessed using the Mindray Auto Haematological Analyzer.

Biochemical Analysis

Serum harvested from the blood of the experimental animals was used to measure some liver function parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, and total protein, total cholesterol and triglycerides using the Hitachi A and B Auto Chemistry Analyzer.

RESULTS

Percentage Yield

The percentage yield was calculated to determine the percentage of extract obtained per weight of plant materials. The percentage yield of the methanolic extract is 11.6%.

Phytochemical Screening

The phytochemical screening of the extract of *N. latifolia* stem bark was presented on Table 1. The phytochemicals abundant in these extract were cardiac glycosides, steroids, carbohydrates, and phlobatanins in the methanolic extract.

Table 1: Phytochemical Screening of Methanolic Extracts of *N. latifolia* Stem Bark

Phytochemicals	Methanolic extract
alkaloid	+
saponin	+
Tannin	+
Anthraquinone	-
Flavonoid	+
Cardiac glycoside	++
Steroid	+++
Resin	-
Phobatannin	++

Keys: +++: highly present; ++: moderately present; +: faintly present -: absent

Parasitemia

The result from monitoring parasitemia in the experimental albino revealed that from the sixth day after infection parasites began to increase in all the groups with infected rats. The negative control group showed consistent parasite growth up to the end of the study period. On the other hand all the other experimental groups (C, D and E) presented a similar pattern in parasitemia. There was steady increase in parasite level from day six up to around day 15 when a decline was recorded in all

groups. It is also noteworthy that the levels of parasitemia in the infected and treated rats were lower than in the negative control group on any particular day post infection. The result was presented on Figure 1.

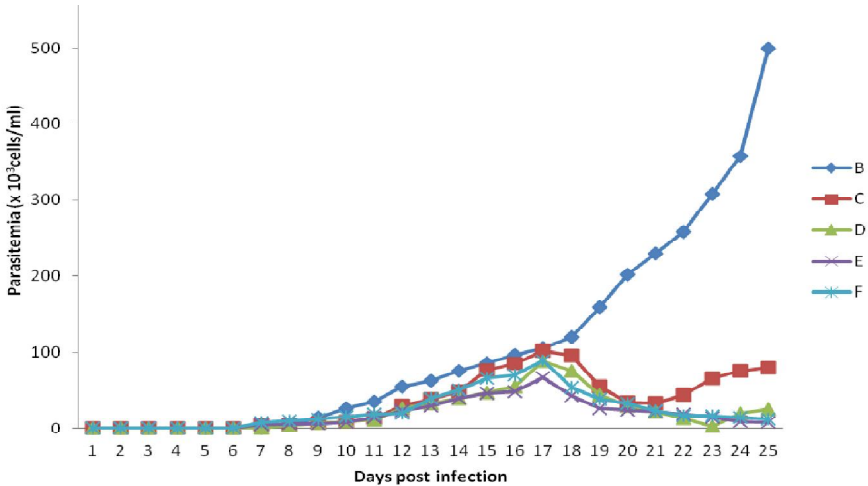


Figure 1: Effects of Methanolic Extract of *N. latifolia* Stem Bark on *in vivo* growth *T. congolense*

Table 2: Effects of Methanolic Extract of *N. latifolia* Stem Bark and *T. congolense* Infection on Rats' Biochemical Parameters

Groups	Biochemical Parameters							
	TP (x10 g/dl)	ALB (x 10g/dl)	TB(x100 mg/dl)	ALT (µl)	AST (µl)	ALP (µl)	CHOL (g/dl)	TG (g/dl)
A	94.5±1.5	50.0±0.5	20.0±0.1	87.9±0.5	353.3±0.3	91.0±1.5	180.0±1.0	160.0±0.5
B	300.1±5.0	60.0±0.5	30.0±0.3	600.2±0.3	600.1±0.1	630.0±1.0	50.5±0.5	130.0±2.0
C	83.1±2.0	46.0±0.3	26.0±1.2	328.3±0.3	345.3±0.3	374.0±1.0	220.0±0.5	234.0±1.0
D	86.3±0.5	43.5±0.2	27.0±0.1	79.4±0.5	295.2±0.2	78.5±0.7	161.0±1.4	136.5±0.4
E	81.3±0.4	44.5±0.2	17.0±0.1	49.4±0.5	195.2±0.2	68.5±0.7	261.0±1.4	236.5±0.4

Table 2 revealed that there was a reduction in the mean total protein, albumin and total bilirubin level of the other groups when compared with the negative control (infected untreated group i.e. group B). However, compared to the positive control group, the levels of these parameters were close. Also, significant drop in the ALT, AST, and ALP level $P>0.05$ was observed in the infected and extract treated groups especially the group infected and treated with 300mg/kg body weight methanolic extract.. The values are also comparable with those obtained from the positive control. Significant increase in cholesterol level $P>0.05$ was observed in the infected and extract treated groups when compared to that of the positive control.

Table 3: Effects of Methanolic Extract of *N. latifolia* Stem Bark and *T. congolense* Infection on Rats' Haematological Parameters

Groups	Haematological Parameters							
	HB (x10 ³ g/dl)	PCV (%)	WBC(x10 ³ /µl)	L (%)	N (%)	PLT (10 ³ /µl)	M (%)	E (%)
A	160.0±1.5	48.0±0.5	120.0±0.1	84.9±0.5	120.0±0.3	659.0±1.5	20.0±1.0	20.0±0.5
B	90.1±5.0	27.0±0.5	144.0±0.3	60.2±0.3	330.0±0.1	80.0±1.0	50.0±0.5	20.0±2.0
C	113.1±1.0	29.0±0.3	105.0±0.2	65.3±0.3	110.0±0.3	207.0±1.0	40.0±0.5	50.0±1.0
D	107.3±0.4	28.0±0.2	98.0±0.5	68.5±0.5	105.0±0.2	364.0±0.7	35.0±1.4	40.0±0.4
E	127.3±0.4	38.0±0.2	98.0±0.1	84.5±0.5	115.0±0.2	344.0±0.7	30.0±1.4	30.0±0.4

HB: haemoglobin; PCV: packed cell volume; WBC: white blood cell; L: lymphocyte; N: neutrophil; PLT: platelet; M: monocyte; E: eosinophil.

From Table 3 the values for HB, WBC, L, N, PLT, M, E and PCV obtained from the infected and extract treated groups compared well with the positive control that is uninfected and untreated. The untreated disease in group B gives rise to high levels of these parameters. The infection had a general negative effect on the infected rats when compared with the normal ones as recorded on Table 4.

Table 4: Effects of Methanolic Extract of *N. latifolia* Stem Bark and *T. congolense* infection on rats' weights

Groups	Weights in grams/Days post infection		
	1	7	14
A	138.0±11.5	148.0±15.5	153.0±2.1
B	150.0±5.0	138.0±2.5	120.0±5.3
C	123.0±5.0	120.7±5.3	116.7±5.2
D	144.0±14.4	141.3±1.2	128.0±7.6
E	141.0±14.4	141.0±2.2	126.0±7.6

DISCUSSION

The results of this study show that methanolic extract of *N. latifolia* stem bark possesses some potential activity against *T. congolense* infection in rats. This is evident from the reduction of levels of parasites in the blood smear of the albino rats and extension of their survival. The extracts also helped in alleviating the high drop in body weight, changes in biochemical and hematological parameters which are obvious in trypanosomiasis. Anemia is a consistent feature in the pathology of trypanosomiasis [8]; however, the anemia in the extract-treated infected rats was less severe than the one recorded for the untreated infected rats (positive control). This may be partly due to the relatively lower parasitaemia in the former groups, since the degree of anemia in trypanosomiasis has been positively correlated with the onset and level of parasitemia. Hyperproteinemia, hyperalbuminemia and elevated serum alanine aminotranferese activity have been reported in

trypanosome infections [9, 10] and were, respectively, attributed to proteinaria [11] and hepatocellular damage [12]. These disease-associate conditions were, however alleviated by consumption of the methanolic extract of *N. latifolia* as illustrated in this study. African plants species have been reported to possess trypanocidal activity [13, 14]. Aqueous and methanolic extract of stem bark of *N. latifolia* shows *in vitro* activity against *T. congolense* at 20 mg/ml, when compared to the control that had parasites still active after 2 h of observation [15]. It has also been reported that the root extract of *N. latifolia* have trypanocidal properties on *Trypanosoma brucei*[16]. Conclusively, this study provides evidence that methanolic extract of *Nauclea latifolia* stem bark possess antitrypanosomal potential and have potential to correct anaemia, hepatotoxic, and hypocholesterolemia effect induced by trypanosomiasis. It also attempts a scientific basis for its continuous use in traditional medicine for the management of African trypanosomiasis.

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