Evaluation of Acute Toxicity and *In vitro* Antitrypanosomal Activity of the Methanolic Leaves Extract of *Ficus exasperata*

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ABSTRACT

Trypanosomosis is one of the tropical diseases in which new and better drugs are needed. Effective treatment for African trypanosomosis is beset with problems of drug resistance and toxicity. In this study acute toxicity and *in vitro* antitrypanosomal activity of the methanolic leaves extract of *Ficus exasperata* were investigated. The acute toxicity study was conducted in two phases. Doses of 10, 100 and 1000 mg/kg were administered to 3 groups of 3 mice each at the initial phase, while doses of 600, 1,000, 1,600 and 2,900 were administered to 4 groups of 1 mouse each at the final phase. The median lethal dose (LD₅₀) was determined to be 775 mg/kg intraperitoneally in mice. The antitrypanosomal activity of the extract was evaluated *in vitro* using a micro titre plate. Blood obtained from infected rat with *T. brucei brucei* (8.4×10^6) was incubated at room temperature (27 °C) for 4 hours with methanolic leaves extract at 40 and 80 mg/ml and 3.5 mg/ml of diminazene aceturate concentrations respectively. The parasites were inactivated and became uninfective to mice at both the extract and the standard drug concentrations used, while the parasites remained active and infective to mice in the negative control. The current study revealed that the methanolic leaves extract of *Ficus exasperata* could possess trypanocidal principle that may require further scientific elucidations.

Key words: Acute toxicity, Antitrypanosomal activity, Methanolic leaves extract, *Ficus exasperata*

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INTRODUCTION

All over the world plants belonging to *Ficus* species are well known as “fig plants”. [1] The plant belongs to genus *Ficus* of the Mulberry family (Moraceae) [1]. *Ficus* comprises up of nearly 1000 species throughout tropical and warm temperate regions with greatest diversity in South East Asia, tropical South America and Australia [2]. The genus *Ficus* is readily distinguished by the highly characteristic fruits and has often been recognized by the milky juice, the prominent stipule that leaves a scar on falling and the minute unisexual flowers often arranged on variously shaped receptacles [2]. In traditional medicine, different parts of *Ficus exasperata* Vahl. (Moraceae) are used as analgesic, antiarthritic, diuretic, wound healing, antiparasitic, vermifuge, abortifacient, ecbolics and for treating hemorrhoids and venereal diseases. The plant parts are also used as animal fodder [3]. In Nigeria, young leaves of *Ficus exasperata* are prescribed as a common anti-ulcer remedy, and for various pharmacological actions such as anti-diabetic, lipid lowering and antifungal activities [4]. Some of the industrial uses of sand paper leaves include woods polishing [5], vegetable oils stabilization, foaming suppression, antimicrobial and food stock supplement [6].

African trypanosomosis is caused by haemoprotozoan *Trypanosoma* species, it is a wasting disease of animals and man. The most important *Trypanosoma* species are transmitted by the tsetse fly of the genus *Glossina specie* in Africa [7]. It occurs across more than a third of Africa, and almost all animal species, except poultry, are affected. Approximately 20% of Africa's 173 million cattle are at risk of infection [8]. In addition, 36 out of 52 African countries are endemic for sleeping sickness, with 55 million people at risk of contracting the infection [9]. The search for vaccination against African

trypanosomosis remains elusive and effective treatment is beset with problems of drug resistance and toxicity [10].

New and better drugs are needed for treatment of tropical African trypanosomosis. The current methods of controlling the disease include the use of trypanotolerant cattle, vector control and drug therapy. Suramin, pentamidine, melarsoprol and eflornithine are the four major available drugs for the treatment of trypanosomosis [11], with only melarsoprol and eflornithine being effective against the meningoencephalitis that develops in the late stages of the disease. In addition to emergency cases of drug resistance, all the four drugs require lengthy, parenteral administration and all but eflornithine have severe toxic side effects [10]. The current study aims at evaluating the acute toxicity and antitrypanosomal activity of methanolic leaves extract of *Ficus exasperata*.

MATERIALS AND METHODS

Plant material

The fresh plant was collected and identified by US Gallah of National Research Institute of Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria and a voucher specimen numbered 0516 was deposited at the Departmental herbarium.

Processing and Extraction

The fresh leaves of *Ficus exasperata* were carefully separated from the other morphological parts of the plant and washed with clean water, air dried under shade for two weeks, pounded with pestle and mortar mechanically into fine particles. Five hundred grams (500 g) of the pounded dried plants materials were weighed and extracted by maceration for 72 h in 100% methanol. The methanolic extracts were filtered and evaporated to dryness *in vacuo* and stored in capped bottles inside the refrigerator at 4°C until required.

Figure 1: *F. exasperata* tree (fig or sand paper tree), leaves and fruits; adapted from [3]

Acute toxicity study (determination of LD₅₀)

This was conducted in two phases by using the method of [12]. In the initial phase, mice were divided into 3 groups of three mice each and treated at doses of 10, 100 and 1000 mg/kg respectively. The plant extract was administered intraperitoneally (*i.p.*) to the mice and were then observed for 24 h for signs of toxicity, including death. In the final phase, mice were divided into 4 groups of one mouse each and treated with the extract at doses of 600, 1000, 1600 and 2900 mg/kg respectively. The LD₅₀ was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose i.e. the geometric mean of the consecutive doses with 0 and 100% survival respectively.

Trypanosome stock

Trypanosoma brucei brucei obtained from protozoology laboratory of Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria was used for

this study. The organisms were maintained by serial passages in rats. The parasitic load was estimated according to the method of [13].

In vitro anti-trypanosomal activity

A concentration of 40 and 80 mg/ml solutions were prepared by dissolving the plant extract into 40 and 80 ml of 5% dextrose respectively. A concentration of 3.5 mg/ml solution of diminazene aceturate was also prepared by dissolving 7 mg of the standard drug into 2 ml of 5% dextrose. The antitrypanosomal activity of the extract was evaluated in vitro using a ninety-six micro titre plate. Blood obtained from infected rat with *T. brucei brucei* (8.4×10^6) was incubated at room temperature (27 °C) for 4 hours with methanolic leaves extract at 40 and 80 mg/ml and 3.5 mg/ml of diminazene acetate concentrations respectively. Five percent (5%) dextrose was used as diluent for the extracts, diminazene acetate and blood sample. A volume of 100 µl of the extracts and drug solutions were pipetted into different wells of the micro titre plate containing 100 µl of the diluted blood sample. Another well containing only 100 µl of the diluted blood sample served as the negative control.

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Infectivity test

The infectivity of *Trypanosoma brucei brucei* incubated in a liquid medium of 5% dextrose for 240 min at 27°C in the presence of 40 and 80 mg/ml concentration of the plant extract, and 3.5 mg/ml concentration of the standard drug was determined. All the tested samples were assayed for infectivity in mice by passaging 0.3 ml of the tested solutions into a mouse. The mice were tested for presence of *T. brucei brucei* parasite using light microscopy (x 40 objective lens) on daily basis for six days.

RESULTS

At the initial phase of acute toxicity determination, a death of one mouse was recorded in the 1000 mg/kg group (Table I).

Table I: Initial investigation (Phase I LD₅₀ determination)

Dose mg/kg	Group
10	0/3
100	0/3
1000	1/3

*Number of animals which died/
number of animals used

The median lethal dose determination revealed the plant extract to possess an LD₅₀ of 775 mg/kg (Table II).

Table II: Final investigation (Phase II LD₅₀ determination)

Dose chosen for the second test	Group
600	0/1
1000	1/1
1600	1/1
2900	1/1

*Number of animals which died/
number of animals used

Therefore, the LD₅₀ was calculated as the geometric mean of 1000 and 600 using this formula

$$LD_{50} = \sqrt{(D_o \times D_{100})}$$

Where LD50 = Median lethal dose

D^o = Highest dose that gave no mortality
(600 mg/kg body weight 0/1)

D100 = Lowest dose that produced mortality
(1000 mg/kg body weight 1/1)

$$= \sqrt{600 \times 1000}$$

$$= \sqrt{600000}$$

Ld50 = 775 mg/kg body weight

The plant extract possesses a dose dependent antitrypanosomal activity (Table III).

Table III: In vitro antitrypanosomal efficacy of different concentrations of methanolic leaves extract of *Ficus exasperata* against *T. brucei brucei*

Different conc. of test solutions	Survival of trypanosomes in minutes							
	10-30	40-60	70-90	100-120	130-150	160-180	190-210	220-240
<i>Ficus exasperata</i> 40mg/ml	+++	+++	+++	++	++	+	-ve	-ve
<i>Ficus exasperata</i> 80mg/ml	+++	+++	++	+	+	-ve	-ve	-ve
<i>Diminazene aceturate</i> 3.5mg/ml	+++	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Negative Control	+++	+++	+++	+++	+++	+++	+++	+++

+++ Active and strong parasite presence,
++ Sluggish and moderate parasite presence,
-ve Inactive/ no parasite presence,

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There was a negative infectivity to mice of all trypanosomes incubated at the two different concentrations of the plant extract (Table IV).

Table IV: Infectivity test of *Trypanosoma brucei brucei* incubated at different concentrations of methanolic leaves extract of *Ficus exasperata*

Days post mice inoculation/infectivity testing

Different concentrations of tested samples 240 min post incubation	1	2	3	4	5	6	7
<i>Ficus exasperata</i> 40 mg/ml	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>Ficus exasperata</i> 80 mg/ml	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>Diminazene aceturate</i> 3.5 mg/ml	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Negative control	+ve	+ve	+ve	+ve	+ve	+ve	+ve

-ve No parasite presence
+ve Parasite presence

DISCUSSION

Acute toxicity is a term that describes the effect of a single dose or multiple doses during a 24-hour period [14]. All chemicals elicit acute toxicity at a sufficiently high dose, mortality occurring within two days of a single dose of a chemical would be a prime example of acute toxicity. However, chronic toxicity may not occur since dosage elevation may simply lead to acute toxicity. In contrast to acute toxicity, chronic toxicity is characterized by prolonged exposure and sublethal effects elicited through mechanisms that are distinct from those that cause acute toxicity [15].

Several toxicity studies have been conducted on various extracts of *F. exasperata* leaves. Few have shown potential toxic effects, while others have rendered the extracts to be relatively safe [3]. In this study behavioural signs like inactiveness, dullness, and depression were induced by the extract at the doses of 100 and 600 mg/kg. So also, death was recorded at the doses of 1000, 1600, and 2900

mg/kg of the extract. According to this study the minimum lethal dose of the extract was found to be 775 mg/kg (0.775 g/kg) and the maximum non-lethal dose of the extract was found to be 600 mg/kg. A substance with toxicity range of 0.5-5 g/kg is considered to be slightly toxic [14].

The LD₅₀ of *Ficus exasperata* was reported to be 0.54g kg⁻¹ determined by intraperitoneal administration of the extract at doses of 0.1, 0.2, 0.4, 0.8 and 1.0mg kg⁻¹[3]. Our current finding revealed the LD₅₀ of *Ficus exasperate* to be 775 mg kg⁻¹ intraperitoneally, which is close to the finding of [3]. Thus, based on our finding the methanolic leaves extract of *Ficus exasperata* is considered to be slightly toxic as categorised by [14].

Natural products with trypanocidal activity and belonging to a variety of phytochemical classes have been identified [16]. Although not comparable to the effect of the standard drug diminazene aceturate and the plant extract at different concentrations used in this study

showed considerable dose dependent antitrypanosomal activity. This finding is in line with earlier reports by [17], [18], [19] and [20] which clearly indicated that plants of different families could possess potent trypanocidal activity.

The infectivity to mice of all trypanosomes incubated at the two extract concentrations (40 and 80 mg/ml) and 3.5 mg/ml diminazene aceturate were negative six days' post inoculation, but the infectivity of the negative control incubated for 240 min was positive with parasitic load of 6.6×10^6 four days' post inoculation.

This investigation did not involve structural elucidation. Nevertheless, bioactive screening *in vitro* remains a useful method for preselection of plant for anti-trypanosomal activity [21]. However, the plant found to be active in this report must be tested *in vivo* before a definite statement can be made on its trypanocidal potentials.

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