PHYTOCHEMICAL COMPOSITION AND ANTITRYPANOSOMAL ACTIVITIES OF AQUEOUS LEAF EXTRACTS OF *LORANTHUS MICRANTHUS* LINN. (*LORANTHACEAE*) IN RATS INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

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ABSTRACTS

Phytochemical composition and antitrypanosomal activities of aqueous leaf extracts of Loranthus micranthus in rats infected with Trypanosoma brucei brucei was investigated for 28 days using 72 adult male albino rats weighing between 150 – 250 g. The rats were divided into six groups (A – F), replicated thrice with each replicate having four rats. Group A, B and C were given 400 mg/kg, 800 mg/kg and 1200 mg/kg of the extracts respectively, according to their body weights, while group D, E and F served as the negative, positive and normal control groups, respectively. LD_{50}, phytochemical screening, parasitaemia and body weight were determined using standard methods. It was observed that tannins and flavonoids were highly present, alkaloids, resins, steroids and saponins were moderately present in the plant extract, while terpenes and glycosides were present in trace amounts. LD_{50} of the crude leaf extract of L. micranthus showed no mortality at dose levels of up to 5,000 mg/kg after 24 hours. The level of parasitaemia in all the tested groups and negative control were significantly high ($p<0.05$) when compared with the positive and normal control groups throughout the duration of the experiment. Similarly, a significant decrease ($p<0.05$) was observed in the mean values of body weights of the infected and treated animals throughout the duration of the experiment. The results showed that all the tested rats and negative control groups died from the overwhelming parasitaemia unlike the case of those administered the standard drug. The aqueous leaf extract of L. micranthus may not be used as an antitrypanosomal agent.

Keywords: Phytochemical composition, Parasitaemia, Trypanosomiasis, Albino rats, *Loranthus micranthus,* Non antitrypanosomal agent

INTRODUCTION

The incidence of trypanosomiasis remains a source of concern in the tropics and other parts of the world. Trypanosomiasis is a lethal disease which affects both man and animals; and caused by a parasitic protozoa of the Genus *Trypanosoma* (Adeiza *et al.*, 2010). This parasite is transmitted by a vector, tsetse fly, most of which belong to the *Glossina palpalis* species complex. The distribution of trypanosomiasis corresponds roughly with that of tsetse flies. Trypanosomiasis, which is also known as sleeping sickness is caused by *Trypanosoma brucei gambiense* and or *Trypanosoma brucei rhodesiense* following an infective bite from tsetse fly (Welburn *et al.*, 2001; Haydon *et al.*, 2002; WHO, 2006).

Transmission of *Trypanosoma brucei* is mostly through the bite of an infected tsetse fly. Other transmission routes include mother to child transmission through the placenta, mechanical transmission through other blood sucking insects (though it is difficult to assess the epidemiological impact of this mode of transmission) and accidental transmissions / infection due to pricks from contaminated needles in the laboratory (Seed, 1998; Kennedy, 2006; WHO, 2006).

Trypanosomiasis and its vectors occur in vast areas of the sub-Saharan Africa with devastating impact on livestock productivity as well as posing a serious threat to the lives and livelihood of entire communities (Doua and Yapo, 1993; Engels and Savioli, 2006). Trypanosomiasis infestation constitutes the greatest single constraint to livestock and crop production thereby directly contributing to hunger, poverty, protein malnutrition and suffering of entire communities in Africa. This is as its name suggest, infected people tend to sleep a lot, leading to loss of man hours that could have been applied to productive farm work (Murray, 1994; Aroke *et al*., 1998).

Treatment of trypanosomiasis infection is dependent on the stage of infection (that is whether it is chronic and or acute infection). Normally the drugs used in the initial stage of the disease infection are of lower toxicity and easier to administer (Burri *et al*., 2001; Chappius *et al.,* 2005). The earlier the disease is identified the better the prospect of cure. Treatment success in the second stage of infection (that is the advanced stage) depends on a drug that can cross the blood brain barrier to reach the parasite. Some of the drugs used include; Pentamidine, Suramin and Eflornithine (Burri *et al.,* 2001; Chappius *et al.,* 2005; WHO, 2008). Despite the efficacious nature of these drugs, researchers have directed their energies to screen local medicinal plants as potential trypanocides. Although some herbal formula are already in circulation (such as Jubi herbal formula and African herbal formula, among others) efforts are made to develop effective drugs from medicinal plants for both the management and treatment of trypanosomiasis (Rates, 2001; Erah *et al*., 2003; Okochi *et al.,* 2003; Jodi *et al*., 2011).

The African mistletoe, *Loranthus micranthus* Linn is an ubiquitous hemi parasitic plant that thrives well in tropical climates (Obatomi, 1996). It depends on its host for mineral salts and water but can photosynthesize its own carbohydrates. Mistletoe grows on a wide range of evergreen and deciduous trees. Host plants of mistletoe include *Persea americana, Kola accuminata, Baphia nitida* and *Treculia africana* (Nzekwe *et al.,* 2009).

Mistletoe has a long history of traditional use for a wide range of diseases such as diabetes, diarrhea, epilepsy etc. Due to its medicinal values and pharmacological activities, mistletoe has been revealed to have a great potential for use in various systemic and non-systemic infections due to bacteria and fungi (Osadebe and Ukwueze, 2004). This study is therefore designed to investigate phytochemical composition and antitrypanosomal activities of aqueous leaf extracts of *Loranthus micranthus* in rats infected with *Trypanosoma brucei brucei.*

MATERIALS AND METHODS

***Loranthus micranthus* Leaf Extract:** Fresh leaves of mistletoe plant were procured from the forests around Obukpa, a community in Nsukka from the host tree *Kola accuminata*. The plant was identified (Sofowara, 1993) and authenticated by Mr. Alfred O. Ozioko of the Bioresources Development and Conservation Programme Centre (BDCP), Nsukka, Enugu State, Nigeria, where the voucher specimen (LM-KA, 2013-1) was kept. The leaves were collected, weighed and shade dried to a constant weight. The weight of the leaves during drying was monitored using a Mettler electronic weighing balance (PC 2000). After shade drying, the leaves were pulverized into fine powder using a laboratory milling machine (Honda: Model 622, China). About 500 grams of the powdered materials was soaked in 1000 ml of distilled water and allowed to stand for 24 hours at room temperature, with occasional shaking to increase the extraction capacity. The decoction was filtered using a muslin cloth (60 µm mesh sieve), and then concentrated to dryness in an oven. Weighed samples of the extract was re-dissolved in normal saline and used to prepare the stock solution for oral

administration to the animals according to their body weights.

Management of Experimental Animals: Seventy two adult male albino rats weighing between 150 – 250 g were obtained from Genetic and Animal Breeding Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were kept in stainless wire rat cages equipped with drinkers and fecal collecting trays, in a clean experimental animal house. The rats were fed *ad libitum* with commercial growers chick mash (18 % crude protein) (Vital Feeds Nigeria Limited) and allowed unlimited access to portable water. The fecal droppings in the tray were removed daily. The experimental rats were handled in strict compliance with international guidelines as prescribed by the guiding principles for research involving animals as recommended by the Committee and the International Guidelines for Handling of Laboratory Animals (Derrell, 1996).

Phytochemical Screening of *Loranthus micranthus* Aqueous Extracts: The qualitative evaluation of the phytochemical components of the extract such as tannins, alkaloids, flavonoids, terpenes, glycosides, saponins and resins were ascertained using the standard methods (Harborne, 1973; Trease and Evans, 1989).

Acute Toxicity of *Loranthus micranthus* Aqueous Extract: The LD_{50} was carried out using the method described by Lorke (1983). In phase A, rats were divided into 3 groups (A, B and C) of three (3) rats each and were treated with the plant extract at doses of 200 mg, 400 mg and 800 mg/kg body weight orally. The animals were observed for 24 hours for signs of toxicity and death. Based on the results of phase A, Phase B were divided into 3 groups of two rats each and were treated with 1,000, 3,000 and 5,000 mg/kg body weight and signs of toxicity were also recorded. The lethal dose was calculated as the arithmetic mean of the dose that killed the least number of animals and the one next lower dose that did not kill any animal (Lorke, 1983).

Experimental Design: Seventy two rats were assigned into six groups (A, B, C, D, E and F) of 12 rats per group with each group comprising three replicates of four rats per replicate. Groups A, B and C served as the treatment groups, while groups D, E and F were the negative, positive and normal control groups, respectively. Three different concentrations of the aqueous extract were administered to the different treatment groups according to their body weight. Group A was given 400 mg/kg body weight of the leaf extract, while groups B and C were administered 800 mg/kg and 1200 mg/kg, respectively. The negative control (group D) also called infected and untreated group and normal control groups (Group F) were given 1 ml/kg body weight of normal saline according to their body weights. The positive control groups (group E) were inoculated and treated with a standard drug - dimenazene aceturate (Berenil,) according to their body weight. All the doses were administered once daily orally for 28 days for all the groups using gastric gavage.

***Trypanosoma brucei brucei* Parasitaemia in Infected Rats:** Parasitaemia was determined by counting the number of trypanosomes per view under the light microscope at x100 magnification from thin blood smear obtained from the tip of the tail of the infected rats. Parasitaemia was checked every day prior to the treatment.

Body Weights: The body weights of the individual rats in a group were determined using electronics balance (Mettler 2000, China). This was done before the animals were infected and on weekly basis during treatment.

Statistical Analysis: Data accumulated was analyzed using the GENSTAT (VSN International, Hemel Hempstead, Herts, UK). Whereas a One-way ANOVA was used to test the effect of treatment, a Two-way ANOVA was used to determine the interactive effects of treatment and duration. Duncan multiple range test was used in the separation of means of the different treatment groups (Duncan, 1955).

All results were expressed as mean ± standard error of mean (SEM), while values were considered significant at $p<0.05$.

RESULTS

The qualitative phytochemical composition of *L. micranthus* leaf aqueous extracts indicated that tannins and flavonoids were highly present, alkaloids resins, steroids and saponins were moderately present, while terpenes and glycosides were present in trace amounts (Table 1).

Table 1: Phytochemical compounds in crude extract of *Loranthus micranthus* leaf

Parameter	Concentration
Resins	++
Flavonoids	+++
Tannins	+++
Terpenes	+
Alkaloids	++
Saponins	++
Glycosides	+
Steroids	++

+++ = High concentration, ++ = Moderate concentration, + = Trace concentration

In the toxicity study, there was no rat mortality at different doses (200, 400 and 800 mg/kg) of the aqueous extract of *L. micranthus* after 24 hours (Table 2).

Table 2: Acute toxicity of rats treated with aqueous extract of *Loranthus micranthus* leaf

Doses (mg/kg)	Mortality/Group
Phase A	
200	0/3
400	0/3
800	0/3
Phase B	
1000	0/2
3000	0/2
5000	0/2

Similarly, at dose levels of 1,000 mg/kg, 3,000 mg/kg and 5,000 mg/kg, there was no dead rat recorded, although behaviours such as shivering, bulging of eyes and dullness were observed in the animals. Signs of parasitemia such as intermittent pyrexia, lethargy, isolation, reduced feed intake, rapid weight loss, and rough hair coat were observed in the infected animals following a progressive and significant increase in the level of parasitaemia throughout the period of the research (Table 3). However, there was an overall time dependent and significant decreases ($p<0.05$) from week 1 to 4 for groups A – D when compared with the normal control with group D having the highest rate of increase in weight (Table 4). The increase in weight among group E animals was not time dependent. Moreover, on the effects of treatment, there was an overall dose independent and significant increase ($p<0.05$) at week 1 and 2 in all the groups when compared with the control except group A which showed a significant decrease ($p<0.05$). At week 3, a significant decrease ($p<0.05$) was observed in groups B, C and D when compared with the control.

DISCUSSION

The results of the phytochemical screening of the leaf of African mistletoe indicated the presence of important compounds such as alkaloids, tannins, flavonoids, steroids, saponins, glycosides and terpenes. Tannins and flavonoids were in abundance compared to other phytochemical components of the plant. Tannins have biological properties that may favour the prevention and management of many ailments (James *et al*., 2007). However, tannins may decrease protein quality by reducing palatability and digestibility (Hertog *et al*., 1997). Excess tannins may be toxic because tannins as metal ion chelators can decrease the bioavailability of iron which often leads to anaemia (Ukoha *et al*., 2011). The presence of flavonoids in the extract shows that the plant have antioxidant properties; this could enhance the body's defense systems against pathologically induced free-radical generation as well as modify the body's reactions to allergens and viruses (Al-Humid *et al.,* 2010).

Table 3: Level of parasitaemia in infected albino rats (*Trypanosoma* /per field) treated with varied concentrations of the aqueous leaf extract of *Loranthus micranthus*

Days (P.I)	Group A	Group B	Group C	Group D	Group E	Group F
1	0	0	0	0	0	0
3	1×10^3	1×10^3	1×10^3	1×10^3	1×10^3	0
5	4×10^4	3×10^4	3×10^4	7×10^5	5×10^5	0
7	5×10^5	3×10^4	4×10^4	6×10^5	4×10^4	0
9	7×10^5	5×10^5	7×10^5	10×10^5	5×10^5	0
11	9×10^5	9×10^5	11×10^5	13×10^5	7×10^5	0
13	11×10^5	$13x\ 10^5$	$11x\ 10^5$	13×10^5	6×10^5	0
15	19×10^5	17×10^5	23×10^5	31×10^5	9×10^5	0
17	17×10^5	17×10^5	21×10^5	33×10^5	7×10^5	0
19	20×10^5	21×10^5	20×10^5	33×10^5	4×10^3	0
21	0	25×10^5	27×10^5	37×10^5	4×10^3	0
23	0	30×10^5	35×10^5	0	3×10^3	0
25	0	0	0	0	2×10^3	0
27	0	0	0	0	3×10^3	0

Each value is the average number of Trypanosoma per microscopic field in each group; P.I = post infection

Table 4: Effects of different treatments of the aqueous leaf extract of *Loranthus micranthus* on the body weights (BW) of albino rats on weekly basis

Groups/ Treatment (mg/kg)	Week 0	Week 1	Week 2	Week 3	Week 4
A (400mg/kg)	139.5 ± 6.92^{2a}	135.0 ± 16.21^{2a}	136.7 ± 14.2^{1a}	*	*
B (800mg/kg)	149.2 ± 1.59^{3a}	147.3 ± 9.66^{2a}	142.0 ± 12.36^{1a}	137.7 ± 8.88^{2a}	*
C(1200mg/kg)	$141.4. \pm 6.31^{3a}$	140.9 ± 12.9^{2a}	138.5 ± 13.30^{1a}	136.0 ± 9.20^{2a}	*
D(infected and untreated)	147.7 ± 9.80^{3a}	144.0 ± 8.33^{2a}	140.1 ± 12.06^{1a}	137.4 ± 4.02^{2a}	*
E (standard drug)	144.2 ± 9.80^{2a}	143.7 ± 1.99^{1a}	141.3 ± 8.11^{1b}	142.3 ± 5.87^{1b}	140.5 ± 8.63^{1a}
F (Normal control)	138.1 ± 4.12^{1a}	137.4 ± 4.61^{1b}	138.8 ± 11.54^{1c}	140.5 ± 6.32^{1b}	139.3 ± 2.95^{1a}

** Represents the animals that have undergone mortality. Values with different alphabetic (lower case) superscripts differ significantly (P<0.05) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly (P<0.05) between different exposure periods within the same concentration. Results are expressed as Mean ± SEM of triplicate determination*

Steroids to some extent maintain hormonal balance by serving as a precursor or potent material in the synthesis of sex hormone (Okwu, 2001). Saponins consumption often times causes deleterious effects such as haemolysis and permeability of the intestines (Cheeke, 1996). Alkaloids, as a component, have therapeutic activity as it is a primary natural phytocomponent of plants. The extracts having moderate concentration of alkaloids indicates that it has important physiological properties and may be used as a sedative, pain-relieving drug, anaesthetic, analgesic etc (Osadebe and Uzochukwu, 2006; Malu *et al.,* 2009). The phytochemical screening agreed with the works of Uzochukwu and Osadebe (2007), Osadebe and Omeje (2009) and Egbuonu and Nwankwo (2012) where they reported that the abundant phytochemicals present in *L. micranthus* include tannins, resins, flavoniods and steroids, while other bioactive components such as flavonoids, resins, steroids alkaloids, glycosides, saponins, and terpenes were present in moderate concentrations.

Acute toxicity test (LD_{50}) of aqueous leaf extract of *L. micranthus* showed no mortality in mice after 24 hours of administration at dose level of 5000 mg/kg, although behaviours such as, shivering, bulging of the eyes, clustering together and dullness were observed. This result therefore suggested that the aqueous extracts of *L. micranthus* leaf had no lethal effects on the tested laboratory animals, thus may be save for human consumption.

Upon invasion of the mammalian system, trypanosomes proliferate rapidly to establish its population in infected host (Poltera, 1985; Pentreath and Kennedy, 2004). Toxins are released into the mammalian system (Nwagwu *et al.,* 1987; Ekanem 1989; Boutignon *et al.,* 1990; Ekanem *et al*., 1994; 1996). The antibodies produced by the host against the parasite are not effective because of the ability of the parasite to produce a large repertoire of antigens. The host defense mechanism is only partially specific and often lagging behind the progress of the disease in terms of antigen-antibody interaction (Sternberg, 2004). Eventually there is a breakdown of the host immune system coupled with parasite invasion of the central nervous system leading to coma and death. All the animals that were infected with *Trypansosoma* and treated with *L. micranthus* in this study died from overwhelming parasitaemia at week 4. The occurrence of death in the groups treated with the plant extracts when compared with the standard control groups was an indication that the aqueous extract of *L. micranthus* lacked antitrypanosomal properties. This research agreed with the findings of Jodi *et al. (*2011) who observed chronic and fluctuating parasitaema that is typical of *Trypanosoma* infections. Contrarily, the study did not agree with a similar work by Adeyemi *et al.* (2009) who observed an appreciable decline in the level of parasitaema in rat infected with trypanosome and treated with ethanolic extracts of *Psidium guajava* leaves.

The effects of the treatment of the aqueous extracts of *L. micranthus* on the body weight of the animals showed a steady decrease in the body weights of animals in the treatment groups when compared with the positive (or normal) control groups from the start to the end of the experiment. This may be due to severe anaemia arising from the parasite infection. There was gradual decrease in the body weight of the animals at week 1, which then continued at weeks 2 and 3 for groups A (400 mg/kg), B (800 mg/kg), C (1200 mg/kg) and D (infected and untreated) animals. Group E (standard drug control) did not significantly differ ($p>0.05$) with group F (normal control), although there was a minimal increase in weight gain, which later stabilized at week 4.

Conclusively, the results of the present study had clearly demonstrated that the aqueous leaf extract of *L. micranthus* appeared to have no therapeutic potential in animals infected with *T. brucei brucei*. The findings of this study showed that all infected and treated rats as well as the infected and untreated control group died from the resultant overwhelming parasitaemia, unlike the animals administered the standard drug. This is an indication that the aqueous leaf extract of *L. micranthus* lacked antitryopanosomal activity. Thus, the aqueous leaf extract of *L. micranthus* inhabiting the host plant *Kola acuminata* should not be used as an antitrypanosomal agent. The available documented evidence of African mistletoe, *L. micranthus,* being a good medicinal plant with good medicinal potentials may probably not have been exhausted. It is therefore advocated that other ways of utilizing this important medicinal plant should be explored. Hence more studies are recommended into the molecular constituents of its bioactive ingredients to ascertain its suitability in the management of diseases other than trypanosomiasis (Monthana *et al*., 2000; Monthana *et al*., 2003).

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