ANTI-TRYPANOSOMAL PROPERTIES OF AQUEOUS SEED EXTRACT OF *MORINGA OLEIFERA* ON EXPERIMENTAL ALBINO RATS

G. A. Amuga[1]*, S. O. Inarigu[1], A. C. Igwe[2] and B. Iliysu[2],

[1]Department of Biological Sciences, Nasarawa State University, Keffi, Nigeria
[2]Nigerian Institute of Trypanosomiasis Research (NITR), Field Office Jos, Nigeria
*Corresponding author: kamuga2@yahoo.com

Received: April 19, 2012; **Accepted:** July 12, 2012

Abstract

The side effects, high cost and availability of fake drugs have necessitated the search for natural (herbal) antitrypanosomal products. Aqueous extract of seeds of Moringa oleifera was investigated for antitrypanosomal activity in 23 adult albino rats acclimatized under laboratory conditions for two weeks. Rats were infected with 1 x 10^6 Trypanosoma brucei brucei intraperitoneally in 0.25 ml of blood/normal saline solution. The plant extract was administered orally at 3 dose levels of 200, 400 and 800 mg per kg body weight at the establishment of Parasitaemia. Treatment lasted for a period of 7 days. Fluctuation in the log values of Parasitaemia was observed throughout the period of the study. The highest log value recorded was 9.0 on the 18^{th} day of the experiment while the lowest log value was 5.7 recorded on the 6^{th} day of the experiment. Mortality recorded in this study was attributed to anemia a nd parasitaemia. This did not compare favorably with the tabulated standard range (37.0 % - 50.6 %) Pack Cell Volume. Mild Antitrypanosomal activity was observed in the aqueous seed extract. This was attributed to the effect of the phytochemical constituents of the seed extract. Berenil® (the standard drug for treatment of Trypanosomiasis) was found to be more effective than the Moringa seeds. M. oleifera seeds showed a difference in the mean survival of female and male rats. The aqueous extract of M. oleifera singly or in combination with other plants should be properly studied in search of treatment options for animal trypanosomiasis.

Keywords: *Moringa, Trypanosomiasis, antitrypanosomal property, parasitemia, extract.*

INTRODUCTION

Trypanosomiasis is a disease complex transmitted by tsetse fly (*Glossina sp*), caused by protozoan flagellates belonging to the complex *Trypanosoma brucei.* Two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans. *T. brucei gambiense* causes West African sleeping sickness and *T. brucei rhodesiense* causes east African sleeping sickness. A third member of the complex, *T. brucei brucei*, under normal conditions does not infect humans. It is infective to livestock causing nagana disease in cattle (Cheesbrough, 2005). The disease is endemic in some regions of sub-Saharan Africa, covering about 36 countries and 60 million people. It is estimated that 50,000 to 70,000 people are infected (WHO, 2006), and about 48,000 people died of it in 2008 (The Guardian, 2009). The disease is a serious public health problem in some regions of sub-Saharan Africa. Currently about 10,000 new cases each year are reported to the World Health Organization; however, it is believed that many cases go undiagnosed and unreported (Centers for Disease Control (CDC), 2010

Moringa oleifera known locally in Hausa language of Nigeria as zogale and drum stick in English, is one of the thirteen species belonging to the genus *Moringa*. It is a short slender deciduous drought resistant perennial pan tropical tree of the family Moringacea which grows up to 10 m in height (Keay, 1989). The leaves are widely consumed as human and animal food in North and Central Nigeria (Lockett *et al.*, 2003), as well as in other parts of the world (Sena *et al.*, 1998; Sheshadri & Nambiar, 2003). In Indian folk medicine, it is used as an antidiabetic (Kar *et al.*, 2003); while in Nigeria it is locally used as tonic and aphrodisiac, and in treatment of intestinal worms and asthma (Antia *et al.*, 2009). Studies have indicated that the plant possesses antiplasmodial activity (Kohler & Jenett-siems, 2002), chemomodulatory effect in hepatic metabolizing enzymes (Bharali *et al.*, 2003), hepatoprotective and radio protective capacities (Rao *et al.*, 2001) and thyroid hormone regulatory properties (Tahiliani *et al.*, 2003). Other biological activities associated with *M. oleifera* include hypocholestrolemic action (Ghasi *et al.*, 2000), hypotensive (Faizi *et al.*, 1998) and antifungal effects (Nwosu & Okafor, 1995), arbortificient (Nath *et al.*, 1992) and antisplasmodic properties (Caceres *et al.*, 1992). Fahey (2005) gives the Medicinal uses of different parts *M. oleifera*. Despite the widespread medicinal importance of *M. oleifera*, limited information is known in respect to its antitrypanosomal properties. Phytochemical constituents of *M. oleifera* that are of economic and therapeutic importance includes: alkaloids, tannins,

terpenes and steroid, cardiac glycosides, saponins, anthraquinone, and flavonoids (Caceres *et al.*, 1992)

Most synthetic drugs used in the treatment of trypanosomiasis are known to have various side effects, expensive and sometimes unavailable. It is therefore necessary to try some local or indigenous plants as a substitute for synthetic drugs. *M. oleifera* is known to have antiplasmodial properties and as such since both *Plasmodium* and *Trypanosomes* are protozoa and both causes anemia, it is therefore a suitable candidate for ethno-botanical research. This study is designed to evaluate the antitrypanosomal potentials of the aqueous extract of the seed of the plant at different dose levels.

MATERIALS AND METHODS

Experimental Animals

23 adult albino rats were used for the experiment. They were obtained from the Parasitology Laboratory, Field Operation Division, Nigerian Institute of Trypanosomiasis Research, Vom, plateau State. They were acclimatized under laboratory conditions for two weeks and fed with growers mash and clean drinking water in water bottles.

Test Organism

T. brucei brucei was obtained from the Parasitology Laboratory of Field Operation Division of the Nigerian Institute of Trypanosomiasis Research, Vom.

Collection and Preparation of Plant Extract

Seeds of *M. oleifera* were obtained from the wild in Keffi Local Government area of Nasarawa State and brought to Zoology Laboratory of Nasarawa State University, Keffi for processing. The plant seeds were air-dried in the laboratory at room temperature, they were grinded to fine particles and finely sieved (Adamu *et al.*, 2009; Antia *et al.*, 2009). 100 g of the sample was weighed and dissolved in 1.5 litre of distilled water and allowed for 24 hours, and was refrigerated to avoid fermentation. It was then filtered with laboratory sieve (porosity 150 us) and allowed to stand and decant. The solution was filtered using Whatman filter paper (no 1, 24 cm size) and dried in the oven at 40^{o}C for 3 days. The extract was transferred into a small container and kept air tight to avoid moisture. The extract was administered through oral route in mg/kg body weight of the rat. Treatment commenced at the establishment of Parasitaemia. 1.5 g of the extract was dissolved in 4 ml of distilled water to give a concentration of 600 mg/ml. Serial dilution was carried out by taking 2 ml of the first solution and adding 2 ml of distilled water to give 300 mg/ml and then another 2 ml was obtained from the 300 mg/ml and 1ml of distilled water was added to make 150 mg/ml concentration. The volume required per animal was calculated and administered through oral route using 1 ml syringe.

Experimental Design

5 rats (2 male & 3 female) were kept in each of cages A-C, 4 (2 male 2 female) in cage D and 2 (1 male and 1 female) each in cages E and F.

Cage A: *T. brucei* infected rats treated with aqueous seed extract at dose 200 mg/kg.

Cage B: *T. brucei* infected rats treated with aqueous seed extract at dose 400 mg/kg.

Cage C: *T. brucei* infected rats treated with aqueous seed extract at dose 800 mg/kg.

Cage D: 4 rats, 2 treated with Berenil® at dose 3.5 mg/kg and the other 7.0 mg/kg.

Cage E: infected untreated rats (control) given distilled water daily.

Cage F: uninfected untreated rats (control) given distilled water daily.

Procedure for Inoculation

$1x10^6$ *T. brucei* were inoculated into the rats intraperitoneally in 0.25 ml blood/normal saline solution. For several passages the blood was obtained by puncture of the vein under the eye of the infected rat using a heparinized capillary tube. 0.25 ml of the blood was collected using 1 ml syringe or blood (diluted with normal saline to contain approximately 1×10^6 parasites per ml) was injected into the animals.

Examination of Parasitaemia

A drop of blood obtained from the tail of the infected rats pre-sterilized with methylated spirit was examined in wet mounts 24 hours after inoculation. The number of parasites was determined microscopically by x 10 objective at x 100 magnification using the "Rapid matching" method of Herbert & Lumsden (1976). The method involves the microscopic counting of parasites per field in pure blood or diluted with phosphate buffered saline (PSB, pH 7.2). Logarithm values of these counts were obtained by matching with the table of Herbert and Lumsden & converted to antilog to provide absolute number of trypanosomes per ml of blood (Atawodi & Shehu, 2010).

Physical Observation

In the course of infection, the feeding potential, reaction to plant extract as well as the activeness of the rats were noted. The longevity of the surviving animals was also observed in order to ascertain the dose that is suitable for administration. Mean and standard deviation were used to analyze the result.

RESULTS AND DISCUSSION

Phytochemical Properties of *M. oleifera* Seeds

Table 1 shows the phytochemical properties of *M. oleifera* seed extract used in this study. Saponins, steroids/terpenes, cardiac glycosides, alkaloid and flavonoids were present whereas anthraquinones and tannins were absent. Atawodi & Shehu (2010)

reported that different plant parts show varying levels of antitrypanosomal properties.

Table 1: Phytochemical constituents of *Moringa oleifera* seeds

Phytochemical constituents	Remark
Saponins	+
Anthraquinones	-
Steroids/terpenes	+
Cardiac glycosides	+
Alkaloid	+
Flavonoids	+
Tannins	-

+ = Present;
- = Absent

Administration of Plant Extract

Table 2 shows the volume of extract administered per body weight of the rats. In group A, the highest volume (0.27 ml) of the extract was administered to rat 4 with body weight of 200.3 g while the lowest volume (0.21 ml) was administered to rat 3 with body weight of 156.4 g. For group B, the highest volume (0.22 ml) of extract was administered to rat 8 with body weight of 161.5 g while the lowest volume (0.02 ml) was given to rat 9 with body weight of 27.2 g. Rat 11 in group C was given the highest volume of extract (0.26 ml) with body weight (192.4 g), whereas the lowest volume of 0.18 ml was administered to rat 13 with body weight (137.7 g). In group D, the highest volume (0.21 ml) was administered to rat 18 with body weight of 60.3 g while the lowest volume (0.09 ml) was given to rat 16 with body weight of 24.3 g. All the animals in group E were given 1 ml of distilled water per animal. The result of the study shows that the aqueous extract of *M. oleifera* exhibited mild antitrypanosomal activity but did not completely clear the parasite. This finding agrees with Olukunle *et al.* (2010). This is evident in group C treated at 800 mg/kg with a complete clearance on day 9 post infection and a relapse on day 10 in one member of the group. This is supported by the fluctuations in the $\log_{10}$ values of the parasite for groups A, B, and C.

Effects of Extract of *M. oleifera* on Rat

Table 3 shows the different experimental groups with their specific dosage of administration. The lowest mean of the prepatent period in this study was recorded in group B (7.4 days) and C (7.0 days) the highest record was in group D (9.5 days) and E (9.0 days). The highest standard deviations recorded were in group A (14.3), B (13.2), and C (12.5). The lowest values recorded were in group D (6.7), (6.4) and E (6.4). No measurements were taken for F as the rats showed (0) which was as a result of the parasites being cleared from the blood on day 12. Day 19 also recorded zero (0), indicating that all animals died on that day (the last surviving member of group C). The were not treated with anything. Higher mean survival periods were recorded in groups E (15.5 days), C (14.6 days) and A (14 days). The lower value of mean survival period was recorded in group B (13.6 days). The standard deviation for the survival period was higher in group C (26.1), followed by A (25.0) and B (24.3) while the lowest was in group E (10.9). Group D (Berenil drug) recorded (0.0) as a result of the parasite being completely cleared from the blood of the rats. Diminazine aceturate (Berenil®) had total clearance of Parasitaemia 3rd day post commencement of treatment. This finding is in agreement with Olukunle *et al.*, (2010) and Ezeokonkwo & Agu (2004). The animals in group A treated at 200 mg/kg died on days 3, 5 and 7 post commencement of treatment while those in group B treated at 400 mg/kg recorded days 3,4,6,7 and 8 post commencement of treatment. Group C on the other hand treated at 800 mg/kg had a survival period of 3,5,8,10 and 12 days post infection, with the last surviving member dead on day 19 post infection thereby terminating the experiment.

Table 4 shows the days, the various groups of animals and the $\log_{10}$ of mean Parasitaemia per ml of blood for each group for a period of nineteen days while the experiment lasted. The values of the Parasitaemia were compared with the table of Herbert & Lumsden (1976) using the rapid matching method of estimation of Parasitaemia. For each group, the mean Parasitaemia was calculated for each day. Day's 1-5 shows the commencement of inoculation to the establishment of parasite in the blood of the experimental animals. Parasitaemia became established from day 6 with $\log_{10}$ values, group C (7.3), A (5.7) and B (7.2). Parasitaemia for group D (treated with standard drug Berenil®)

$\log_{10}$ of mean values increased with increase in days of survival of the animals and decreased as the animals in each group reduced by mortality.

Table 2: Volume of extract Administered per body weight of the Rats

Identification No	Experimental Group	Body Weight	Dose (mg/Kg)	Amount Required (mg)	Concentration Prepared (mg/ml)	Volume Required (ml)
1		172.1		34.42		0.23
2	A	165.3	200	33.06	150	0.22
3		156.4		31.28		0.21
4		200.3		40.06		0.27
5		193.3		36.66		0.24
6		155.6		62.24		0.21
7	B	155.4	400	62.16	300	0.20
8		161.5		64.60		0.22
9		27.2		10.88		0.02
10		29.0		11.60		0.04
11		192.4		153.60		0.26
12	C	185.5	800	148.40	600	0.25
13		137.7		110.16		0.18
14		169.8		135.84		0.23
15		143.0		114.40		0.19
16		24.3		0.09		0.09
17	D	29.8	3.5	0.10	1	0.10
18		60.3		0.42		0.21
19		29.3	7	0.21	2	0.11
20	E	102.1				1
21		67.7			Distilled water	
22	F	43.9				
23		144.7				

Table 3: Mean and Standard deviation of the prepatent period and survival period of Albino Rats

Experimental Group	Dosage (mg/kg)	Prepatent period (in days) Mean (x)	Prepatent period (in days) Standard Deviation (δ)	Survival period (in days) Mean (x)	Survival period (in days) Standard Deviation (δ)
A	200	8.0	14.3	14.0	25.0
B	400	7.4	13.2	13.6	24.3
C	800	7.0	12.5	14.6	26.1
D	3.5	9.5	6.7	0.0	0.0
E	7.0	9.0	6.4	0.0	0.0
F	9.0	6.4		15.5	10.9

Table 4: Log_{10} of parasites per ml of blood examined for the period of nineteen days

Experimental Days	Log_{10} of parasites/ml of blood/Group				
	A	B	C	D	E
1 - 5	0.0	0.0	0.0	0.0	0.0
6	5.7	7.2	7.3	0.0	0.0
7	6.7	6.6	7.9	0.0	0.0
8	7.5	7.7	7.2	0.0	0.0
9	7.8	8.6	8.0	7.6	6.4
10	8.0	8.2	8.4	7.3	7.6
11	8.5	8.3	8.0	7.9	8.1
12	8.3	8.4	8.1	0.0	8.6
13	8.2	8.4	8.4	0.0	8.7
14	8.5	8.4	8.7	0.0	8.5
15	8.7	8.7	8.3	0.0	8.6
16	0.0	0.0	8.9	0.0	8.7
17	0.0	0.0	9.0	0.0	6.0
18	0.0	0.0	9.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0

Table 5 shows the sum and mean of the packed cell volume (PCV) for each group taken at three different periods; preinoculation, establishment of Parasitaemia (time taken for parasites to start showing in the blood) and the commencement of treatment, and at the period of massive infection. The positive control recorded 11 and 17 days post infection. The mean packed cell volume for all the groups at preinoculation period was highest in group E (50.5 %) while the lowest was group B (43.6 %). However at the establishment of Parasitaemia, the mean packed cell volume was highest in group C (46.3 %) and lowest in group B (41.4 %). At massive infection, the mean packed cell volume recorded highest in group C (37.7 %) whereas group A recorded lowest (30.5 %). The mean prepatent period was highest in group D (9.5) treated at dose 3.5 mg/kg while the lowest mean was (7.0) in the same group. Standard deviation of the prepatent period show highest in group A (14.3) whereas the lowest was group (6.4) treated at dose 800 mg/kg and D (6.4) with 7.0 mg/kg. The mean of the survival period was highest in group E (15.5) as a result of length of prepatent period showed. Lowest mean for survival period was group D (0) since the parasite was completely cleared from the blood on the 3rd day post commencement of treatment. However, group C (26.1) recorded the highest standard deviation while the lowest was group D (0). Table 6 shows the effect of the aqueous extract of *Moringa oleifera* in relation to sex. This was seen in the difference in mean survival period of female (28.6) to that of the male (13.6) as well as the mean PCV at massive infection with female (68.2) and male (34.0).

Table 5: Records of Packed cell volume (PCV) measured at different periods of infection

Group	Preinoculation Period		Parasitaemia/ treatment Period		Massive infection Period	
	ΣPCV (%)	χPCV (%)	ΣPCV (%)	χPCV (%)	Σf PCV (%)	χPCV (%)
A	248	49.6	215	43.0	122	30.5
B	218	43.6	207	41.4	102	34.0
C	221	44.2	185	46.3	113	37.7
D	198	49.5	_	_	_	_
E	101	50.5	91	45.5	74	37.0

Table 6: Effect of *M. oliefera* extract on mean survival rate and PCV of albino rats

Sex	χ survival	χ PCV (%)
Males	13.6	34.0
Females	28.6	68.2

CONCLUSION

Mortality recorded in this study was as a result of anemia and can also be attributed to the parasite load (Parasitaemia) or that the parasite may be a virulent strain. This was shown in the mean packed cell volume at massive infection. The PCV values can be related to the rat hematologic reference range of 37.6 % - 50.6 % (Johnson, 1996). This result shows that *M. oleifera* seeds possess mild antitrypanosomal properties and this may be as a result of the Phytochemical constituents acting together to generate a combined effect. It also shows that Berenil® is more effective than the *Moringa* seeds and that there is a difference in the mean survival of the females in relation to males. It is recommend that: Further studies can be carried out with the same plant part but at a higher doses to ascertain its effect; the number of parasite per ml of blood inoculated can be reduced for more effective result and use of a different *Trypanosoma species* may extend the survival period of the animals.

ACKNOWLEDGEMENT

Management of NVRI, Vom, Plateau State for confirming the phytochemical analysis of the seed extract.

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