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Screening for Antitrypanosomal effect of *Moringa oleifera* (*Lam)* Leaf Extract on Rats experimentally infected with *Trypanosomabrucei*

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ABSTRACT

Screening for antitrypanosomal effect of *Moringa oleifera* leaf extract on rats experimentally infected with *Trypanosoma brucei* was done using methanol, ethanol and water as solvent. Phytochemical screening indicated the presence of phenolics, saponins, tannins, alkanoids and flavonoids. Fifty-five male Wistar albino rats weighing between 100 to 450g sorted into groups according to their weight. The animals were divided into five groups (A, B, C, D and E) with fifteen (15) rats each in group A, B and C, while group D and E had five (5) rats each. All rats were managed under same condition with 12hour light/dark circle and feed with rats' pellets (Grand Cereal Ltd) with water provided ad'libitum. All groups were inoculated Intra-Peritoneally (IP) with 0.2ml of blood containing about 1 x 10^6 trypanosomes. On establishment of infection, *Moringa oleifer*leaf extracts were administered to rats at graded doses of 200, 150 and 100mg/kg body weight (i.p) once to groups A, B and C while group D were administered with Diminizene Aceturate (3.5mg/kg body weight i.p), group E were left untreated which served as negative control. All infected rats developed chronic trypanosomiasis with the following clinical sign and symptoms: less fecal pellet, weakness, dull and rough coats, anaemia. Parasitaemia was monitored daily in all groups and only the 200mg/kg body weight of the extract in group A, B and C display a significant effect ($p< 0.05$) and survival period. Treatment resulted in the prolongation of life and infected but untreated group died on the third day of parasitaemia rise. The result showed chronic anaemia based on the low PCV and RBC values ($p<0.05$). In conclusion, despite the significant differences in blood parameters, and temperature, the extracts have exerted effects on the rats. We also conclude that *Moringao leifera* leaf extract at 200mg/kg is fairly effective and that aqueous extract exhibit maximum antioxidant activity compared to the organic solvent extracts.

KEYWORDS: *Moringa oleifera*, *Trypanosoma brucei*, Trypanosomiasis, Phytochemicals

Introduction

Moringa oleifera (Lam) also known as *Moringa pterygosperm (Gaerth)* is a member of the Moringaceae family of perennial- angiosperms which includes 12 other species (Olson, 2002). It is a native of the sub-Himalayan northern parts of India/Bangladesh and it is widely cultivated throughout tropical and sub-tropical areas of the world. In some parts of the world, *Moringa oleifera* is referred to as the 'drumstick tree' or the 'horseradish tree' or the 'Malunggay or 'Makonkom.

Moringa oleifera is an edible plant. A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits and seeds (Anwar *et al*., 2007; Kumar *et al*.,2010). Its phytochemical analysis has shown that the leaves are particularly rich in potassium (K), calcium (Ca), phosphorus (P), iron (Fe), Vitamins A and D, essential amino acids as well as antioxidants such as Carotene, Vitamin C and flavanoids (Aslam *et al*., 2005; Amaglo *et al*., 2010; Gowrishankar *et al.,* 2010).

Moringa is especially promising as a food source in the tropics because the plant is in full leaf at the end of the dry season when other foods are typically scarce (Fuglie, 1999). leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more potassium than bananas and that the protein quality of its leaves rivals that of milk and eggs (Fuglie, 2000).

Moringa preparations have been reported as having antibiotic, anti-trypanosomal, and antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic and hypoglycaemic activities, as well as to have considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of Schistosomecercariae titre value (Fahey, 2005).In many regions of Africa, it is widely consumed for self-medications by patients affected by diabetes, hypertension or HIV/AIDS (Kasolo *et al*., 2010).It would therefore be of value to continue to explore the potentials of this "wonder" plant as a therapeutic agent not only for metabolic diseases and other ailments but also for parasitic infections of public health significance that is Trypanosomiasis.

Depending on the trypanosomal parasites involved, Human African Trypanosomiasis HAT exist in two potentially fatal clinical forms: a chronic form caused by *T.b.gambiense* affecting countries in West and Central Africa, and an acute form caused by *T.b.rhodesiense* in East and Southern Africa (Kuzoe, 1993; Boza and Cassels, 1996; WHO, 1998; Atouguia and Costa, 1999).Similarly, cattle and dogs infected with other species, particularly *T.vivax, T.congolense* and *T.b.gambiense* are usually chronically ill, demonstrating reduction in milk production, weight gain and reproductive performance. This causes further reduction in the already limited sources of animals' protein in sub-Saharan Africa (Reichard, 2002). In general, the disease is of major health and economic concern in rural areas of sub-Saharan Africa (Atouguia and Costa, 1999; Reichard, 2002) where it is threatening about 60 million people in over 30 countries (WHO, 1998). The search for new anti-trypanocidal agents has received some considerable attention in recent studies evaluating several medicinal plants of Tanzanian and Ugandan origin and different chemical/drugs for *in -vitrotrypanocidal* activity have been documented (Freibughaus *et al.,* 1996, 1997, and 1998). Some of these reports showed that, at least under *invitro* conditions, some of these plants especially *Moringa oleifera* possess trypanocidal activity. Owing to these findings, it could be of interest to select this plant for *invivo* evaluation.

Materials and methods

Study Area

The study was carried out in the Entomology and Parasitology laboratory of the Department of Biological Sciences, Adamawa State University, Mubi between October and November, 2013. Mubi comprises of two Local

Government Areas; Mubi North and Mubi South. It is located between latitudes 10° 05' and 10° 30'N of the equator and between longitude 13° 12' and 13° 19'E of the Greenwich meridian. The two Local government areas occupy a land area of 192,307Km2 and support a total population 260,009 people (National Population Census 2006).

Mubi exhibit a tropical wet and dry type of climate, with annual rainfall of about 900mm and the highest occurrence is in July and August. The temperature regime is warm to hot throughout the year, however, there is usually a slightly cool period between November and February (Adebayo., 2004).

Collection and processing of Plant Materials:

The fresh leaves of *Moringa oleifera* were collected from the Botanical garden of the Department of Biological Sciences, ADSU. The plant materials were air-dried in the laboratory for two weeks and then grounded into powdered form using mortar and pestle and kept in air-tight bottles until required for use.

Extraction and preparation of Extracts:

50g of *Moringa oleifera* leaf powder was dissolved in 200ml of absolute methanol, ethanol and water respectively, in Ehlnmeyer flask. The mixture was vortexed at two hours' interval during the day for 3 days (72hrs), then filtered using Whatman's filter paper no.1 (125mm) and residue dried at room temperature; aqueous extract was evaporated through water bath at 60^0C. The dried residue was kept in air-tight amber bottles in a refrigerator until required (Atawodi *et al*; 2010, Kasolo *et al*., 2010). The extracts, 0.2g, 0.15g and 0.1g were dissolved in 100ml of distilled water to give a desired stock concentration of 200mg, 150mg and 100mg respectively. All the solutions were stored in the refrigerator at 4^0C till required. (Parvathy and Umamaheshwari, 2007).

Phytochemical Analysis*:*

Qualitative detection of saponins, alkanoids, tannins, phenols, flavonoids, glycosides and volatile oils was performed on the methanolic, ethanolic and aqueous leaf extracts as described by AOAC (2000) and Talukdar *et al*. (2010).

Grouping and inoculation of parasites:

A total of 55 Wister albino male rats weighing 100-450g were used. They were purchased from the Department of Biological Sciences Animals unit, Adamawa State University, Mubi. Animals were kept in rat cages and fed with commercial rat cubes twice a day (Grand Cereal Ltd) and allowed free access to clean fresh water ad libitum. The rats were kept in the animal house with a 12hr light/dark circle. The rats were divided into five (5) groups based on their PCV; (A) methanol, (B) ethanol and (C) aqueous which had fifteen (15) rats each, while the standard positive (D) and negative control (E) had five (5) rats each. Heavily infected blood samples from donor rats was collected from sacrificed rats and it was immediately diluted with physiological saline (2:2) to give 1×10^4 parasites per ml. About 0.2ml of the blood was injected intraperitonealy into apparently clean rats already acclimatized under laboratory conditions for three weeks. (Atawodi *et al.*, 2003).

Determination of Parasitaemia:

Parasitaemia was monitored daily by wet film examination of blood sample obtained from the tail snip pre-sterilized with methylated spirit. The number of parasite was determined microscopically with x 40 magnification using the "Rapid Matching method for Estimating host parasitaemia as described by Herbert and Lumsden, 1976. The parasites were counted and approximate number of the parasite was recorded.

Administration of extracts*:*

Administration of extracts commenced at establishment of infection to group A-C. Group D animals were treated with diaminezine (standard drug) at 3.5mg/kg body weight while group E were inoculated but only treated with placebo. Group A to C were treated with methanolic, ethanolic and aqueous extract at a dose level of 200,150 and 100/mg/kg body weight once using 1ml insulin syringes, with continuous monitoring of parasitaemia for 28/days for surviving animals. All extract administration was through the intra- peritoneal rout as described by Atawodi *et al*. (2003). All extract administration was done through the intra- peritoneal route.

Determination of haematological parameters of infected animals:

1ml of blood samples were collected by tail snip into bottles containing EDTA. Packed cell volume (PCV), Red blood cells (RBC) and White blood cells (WBC) count were determined by the Automated Haematologic Analyzer (Sysmex Kx-21). All these parameters were monitored up to four weeks at weekly intervals.

Results

Phytochemical composition of leaf extracts of Moringa oleifera

Phytochemical screening of Moringa oleifera have shown that the leaf of the plant contained saponins, tannins, alkanoids, phenols and flavanoids with phenols and tannins highly present.

Parasitaemia

The result is presented in Figure 1. There was fluctuation in the level of parasitaemia of all the treated groups, which were however, kept at relatively very low level. In all groups patent parasitaemia was apparent from day 3. Treatment of the infected group also resulted into the prolongation of life by 1, 9 and 2 days for groups A, B and C respectively.

Effect of different extracts of M.oleifera leaf on body weight of infected rats.

The effect of different extract on *Trypanosoma brucei* is as shown on Table 1 below. There was no significant difference between methanolic and ethanolic treated rats ($p>0.05$). The result also showed that there was weight gain in all the treated groups. The negative control group (E) suffered weight loss and died.

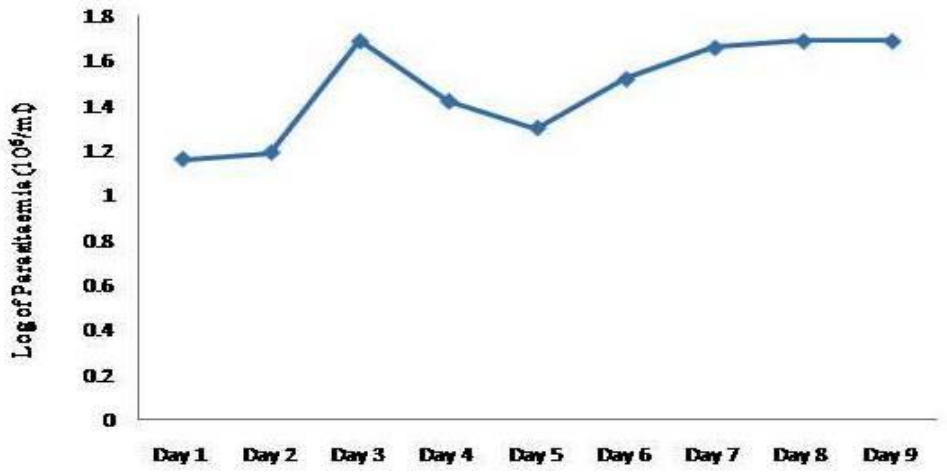

Fig. 1: Parasitaemia of *Trypanosomabrucei* infected rats and treated with *M.oleiferan* Leaf Extract

Table1: Effect of different extract of *M.oleifera* leaf on the body weight (g) of *T.brucei* infected rats.

Parameters	A	B	C	D	E
Wt (gm) before extract administration	257.3±20.4[a]	227.7±15.1[b]	234.4±24.1[b]	197±35.1[c]	253±33.1[a]
Wt (gm)after extract administration	450±50[a]	410±14.1[a]	374±42.2[ab]	303±72.7[b]	-
Difference in Wt	192.7	182.3	139.6	106	253

Means with same letters are not significantly different at ($p<0.05$)
-sign means no data collected because all the rats died.

Mean values of PCV of infected rats

Result in Table 2 below showed that there was low PCV of all groups which showed that there was significant decrease ($p<0.05$). There was no significant difference between the methanolic and ethanolic treated rats ($p>0.05$).

Table 2: Mean values of PCV (%) of rats infected with *T.brucei*

Treatments	Pre-infection	Weeks (Post-infection 1	2	3	4
A	34.29^{a}	15.68^{ab}	*	*	*
B	39.31^{a}	12.06^{ab}	*	*	*
C	39.20^{a}	1.94^{b}	*	*	*
D	36.24^{ab}	21.46^{a}	25.96^{a}	19.72^{ab}	23.08^{ab}
E	38.35^{ab}	*	*	*	*

Means with same letters are not significantly different ($p<0.05$)
*Sign means there was no data collected because all the rats dead.

Mean values of RBC of rats infected with T. Brucei

The results in Table 3 showed that RBC values of all groups' decreases ($p<0.05$). There was a significance difference ($p<0.05$) between the RBC values of pre-infection and post-infection.

Table 3: Mean values of RBC (µl x 10^{6}) of rats infected with *T.brucei*

Treatments	Pre-infection	Weeks (post-infection) 1	2	3	4
A	6.59^{ab}	2.79^{ab}	*	*	*
B	6.99^{a}	2.27^{ab}	*	*	*
C	7.17^{a}	1.93^{b}	*	*	*
D	6.11^{b}	4.35^{a}	4.99^{a}	3.96^{ab}	4.47^{a}
E	7.07^{a}	*	*	*	*

Means with same letters are not significantly different ($p<0.05$)
*Sign means no data was collected because all the rats' dead

Discussion

From the investigation carried out the phytochemical composition of leaf extracts of *Moringa oleifera,* showed that methanol, an organic solvent contains tannins and phenols which were also observed by Koruthu *et al*., (2011). The phenolic compounds in the *M.oleifera* leaves were found to have laxative effects. There was absence of alkanoids and flavanoids but was observed in the findings of Atawodi *et al*., 2010. This could be as result of geographical differences and the type of soil. Ethanolic extract was found to contained saponins, tannins and phenols which were also reported by Kasolo *et al*., (2010). Aqueous extract was found to contained saponins, alkanoids, tannins, phenols and flavanoids as was also reported by Kasolo *et al*., 2010; Kwaghe and Ambali., 2009. None of the extracts contained glycosides and volatile oils. The absence of this compound could be the reason why the animals could not survive for a longer period as was reported by Olugbemi *et al*., (2010) or because parasites here were passaged severally thereby adapting the environment of the rats, thus, getting used to the body of the rats and when

experimental rats were infected, the parasite did not require time to hide themselves in tissues. Also here the rats were treated the intraperitoneal route once, unlike that of Olugbemi *et al*., (2010) who treated the twice daily for five consecutive days.

There was prolongation of life by one, nine and two days for group A, B and C respectively when extracts were administered as was reported by Olugbemi *et al* (2010). From the increase in life span and phytochemicals in both are more or less the same content, then it is likely that the amounts of methanol could be responsible for the death of rats within a day, although more days were observed by Atawodi *et al* (2010). The study also showed that all the rats used in the study gained weight (Table 2) as was also reported in the findings of Adedapo *et al*., (2009).

There was significance difference ($P<0.05$) between the PCV of all groups during pre-infection and post infection (Table 2). Most groups died due to the infectivity of the parasites and the mean result of week 1 (post-infection) was compared with week 4 (pre-infection). The result from this investigation showed there was severe anaemia based on the low PCV and RBC values, unlike the one reported by Ezebuiro *et al*., 2012.This may be as a result of the chronic nature of the course of infection. There was no significant difference ($P>0.05$) on the mean values of RBC during post infection (Table 3). This study also showed that there was a significant decrease ($P<0.05$) in the level of WBC count (Table 4). This observation was not in agreement with the work of Adedapo *et al*., (2009) and Otitoju *et al*., (2014). The reason could be the low dosage that we use. From the investigation carried out the results showed that only rats that were treated with 200mg/kg body weight survived up to the next day after extracts administration. This shows that the effect of *Moringa oleifera* leaves are dose- dependent.

Conclusion

Although *Moringa oleifera* leaf extracts of methanol, ethanol and aqueous acted poorly, it can be concluded that *Moringa oleifera* leaf extracts is trypanostatic, it reduces anaemia and promote weight gain in experimental African trypanosomiasis. It can also be concluded that *Trypanosomabrucei* could be an important wasting disease in both cattle and human. The effects of this plant are dose- dependent.

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