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(RESEARCH ARTICLE)



## Evaluation of the *in-vivo* antitrypanosomal activity of the crude extract of *Moringa oleifera* (lam) against rats infected with *Trypanosoma brucei*

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### Abstract

The anti-trypanosomal effect of the crude extract of the leaves of *Moringa oleifera* was determined *in-vivo* in *Trypanosoma brucei*-infected rats. In the study, twenty five rats (weighing between 255-261 g) were grouped into five (A, B, C, D and E), with their parasitaemia levels and packed cell volumes (PCV) all determined before the analysis. Each rat was then injected with blood sample infected with the *Trypanosoma brucei* parasite. The infected rats were allowed to stay untreated for six days, during which the parasitaemia level for each of the rats was determined at two days intervals. The crude extract of the leaves of *Moringa oleifera* (200 mg/Kg) was orally administered ad libitum six days post-infection to rats in the first four groups (A, B, C and D), while the last group (E) was orally given distilled water (free from the extract). The effect of the extract on trypanosome-infected and treated rats was monitored for 6 days (at 2 days intervals). This was done by determining the changes in the parasitaemia levels and the packed cell volumes (PCV) of the blood of the rats. The results showed a significant decrease in the parasitaemia levels after treatment with the extract, with significant gain in the PCV of the treated rats. However, results from the untreated group (E) was not encouraging, as three of the untreated rats died before the end of the study.

**Keywords:** *Trypanosoma brucei*, *Moringa oleifera*, parasitaemia, packed cell volume.

### 1. Introduction

The African animal trypanosomiasis (AAT) found mainly in sub-Saharan Africa, is a parasitic disease that causes lots of economic losses in animals ranging, from anaemia to emaciation, with many of such cases if untreated being fatal. AAT is found mainly in the regions of Africa where the biological vector of AAT, tsetse fly, exists (CFSPH, 2009). Among the major animal diseases, bovine trypanosomiasis proves to be the major constraints of livestock production in Sub-Saharan Africa. The social, economic and agricultural development of communities within tsetsefly infested areas are greatly affected by the risk of infection in both humans and in domestic animals, with such communities roughly constituting more than a third of Africa (FAO, 2002; Radostits *et al.*, 2007).

The African animal trypanosomiasis is one of the major obstacles encountered in livestock production in sub-Saharan Africa, causing reduction in milk production, as well as causing great weight loss and hindering reproduction within the animals, and eventually, in almost all cases, death of the affected animals (Hoet *et al.*, 2004). The disease has been reported to cause the death of about 3 million cattle a year while 50-70 million animals are exposed to the infection (Ogbadoyi *et al.*, 2007). It is also reported that the disease is present in over 37 countries, causing an annual economic loss of over 4.75 billion US dollars (DFID, 2001). Human African trypanosomiasis affects more than 60 million people

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worldwide with 300,000–500,000 new cases of both East and West African trypanosomiasis reported each year (Kubata *et al.*, 2005; WHO, 2004).

There is a slim and poor chance of developing vaccines for trypanosomes, because the parasites have a glycoprotein coat encoded by genes that are antigenically distinct, and this makes the parasite able to engage an immune-evasive process of antigenic variation (Borst and Fairlamb, 1998). Thus, drugs are the only viable management options for tackling this problem. However, all the currently available drugs have serious limitations, which include high cost, serious side effects, long-course of parenteral administration, variable efficacy and emergence of drug resistant trypanosome strains (Gutteridge, 1984; Croft, 1997; McDermont *et al.*, 2002; Brun *et al.*, 2001; Maikai, *et al.*, 2008). The presence of drug resistant trypanosomes has recently risen to alarming proportions (Legros *et al.*, 1999; Brun *et al.*, 2001; Stanghellini and Josenando 2001; Maikai, *et al.*, 2008). Treatment of the late stage of sleeping sickness with Melarsoprol (Mel B), a trivalent arsenical, is hazardous, causing reactive encephalopathy in 5-10% of patients treated, with 1-5% mortality (Kennedy, 2004). The problem of drug resistance has been aggravated by lack of new drug development initiatives by major pharmaceutical firms. There is therefore, an urgent need to develop new effective and safe chemotherapeutic agents for the treatment of the African Trypanosomiasis.

In many countries where African trypanosomiasis occur, different plants have been used traditionally for centuries and are still widely used to treat this illness. It is estimated that two-third of the world population rely on traditional medical remedies due to the limited availability and affordability of pharmaceutical products (Tagboto and Townson, 2001). Some common plants known to be traditionally used in the treatment of the African Trypanosomiasis include *Adansonia digitata* (Ibrahim *et al.*, 2013), *Lawsonia inermis* and *Ficus syncomorus* (Atawodi *et al.*, 2003) *Acacia nicotida* (Ahmad *et al.*, 2008; Ogbadoyi *et al.*, 2011a), *Senna occidentalis* (Mahmout *et al.*, 2008), *Ocimum gratissimum* (Mpiana *et al.*, 2007), *Strychnos spinosa* (Lockett and Grivetti, 2000), *Vitex doniana* (Mann and Ogbadoyi 2012) and *Zingiber officinale* (Shaba *et al.*, 2011), *Moringa oleifera* (Edoga *et al.*, 2013).

In this study, the effect of oral administration of the crude extract of *Moringa oleifera* on the parasitaemia level and PCV of rats infected with *trypanosoma brucei* was investigated.

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## 2. Material and methods

### 2.1. Plant Materials

The leaves of *Moringa oleifera* were collected from the Na'ibawa Qtrs. in Tarauni Local Government, in Kano state, Nigeria, and the plant was identified by a plant taxonomist from the Department of Biological Sciences, Yusuf Maitama Sule University, Kano, with a voucher number YUHAN 0021 assigned to it, and a voucher specimen deposited at the herbarium of the department.

### 2.2. Sample Preparation

The leaves were dried at room temperature (35 to 37 °C) to a constant weight. The dried leaves were grounded to fine powder and then stored in a dry container.

### 2.3. Solvent Extraction of the Plant Samples

Hundred grams (100 g) of the powdered sample was soaked in 400 ml of absolute ethanol for forty eight hours (48 hrs) and stored away from direct light. The supernatant was decanted and filtered using filter paper. The filtrate was evaporated to dryness, and stored in sample bottles at room temperature to avoid biological degradation.

### 2.4. Experimental Animals

Twenty five rats weighing between 255 and 261 g obtained from the Laboratory animal unit of Department of Physiology, Bayero University, Kano were used for the study. The rats were housed in clean cages in fly-proof house at room temperature and fed with commercial feed (Vital feeds, GCOM Nig. Ltd) and provided with clean water ad libitum. The rats were screened for the presence of blood parasites using wet and Giemsa-stained thin films prior to commencement of the experiment. The packed-cell-volume (PCV) of the rats was also determined prior to the commencement of the trypanosomal infection. The PCV ranged between 40 to 42%, signifying that they are all within the normal PCV range for rats (39-46%) as reported by Qili *et al.*, (2017).

## 2.5. Trypanosomes and Infection

The *Trypanosoma brucei* isolate was obtained from The Nigerian Institute for Trypanosomiasis Research (NITR). The parasites were propagated and maintained in rats few days before the commencement of the research. Twenty five (25) rats were used for the research, and they were grouped into five, with each group comprising of five rats. During the trypanosomal infection group A was inoculated with 0.2 ml of the blood sample infected with *trypanosoma brucei*. Same was done for groups B, C, D and E.

## 2.6. Parasitaemia, Blood Sampling, Determination of Packed-Cell Volume and Treatment

The parasitaemia level for each rat in every group was determined at two days interval. To estimate the number of circulating parasites in each infected rat, the rapid 'matching' method by Herbert and Lumsden (1976) was adopted, where parasite were seen by direct microscopy. At two days interval, blood was taken from each rat by tail snip into 100µl microhaematocrit tubes for PCV determination in accordance with a method by Woo (1970). The average for each group was then calculated for each sampling point.

All experimental rats from groups A, B, C, D and E, showed detectable parasitaemia around day 6 post infection. Hence, treatments commenced at about day 6 post infection (taken as Day 0) at the first detection of parasitaemia. Crude *Moringa oleifera* extract was given at a dose of 200 mg/Kg orally for 6 consecutive days to rats from groups A, B, C and D, while rats from group E served as the control experiment. The infected but non-treated control group received distilled water orally for 6 days.

## 3. Results and discussion

The results for the average parasitaemia levels after inoculation and treatment are presented in Tables 1.0 and 2.0 respectively; while the PCV of the rats after infection are presented in Table 3.0.

**Table 1** Average parasitaemia level after inoculation

Day	Group A	Group B	Group C	Group D	Group E
0	0%	0%	0%	0%	0%
2	0.1%	0.2%	0.2%	0.2%	0.1%
4	0.8%	0.7%	0.7%	0.8%	0.8%
6	1.4%	1.4%	1.3%	1.4%	1.3%

**Table 2** Average parasitaemia level after treatment

Day	Group A	Group B	Group C	Group D	Group E
0	1.4%	1.4%	1.3%	1.4%	1.3%
2	0.5%	0.4%	0.4%	0.5%	1.8%
4	0.1%	0.09%	0.08%	0.1%	2.5%
6	0.05%	0.04%	0.03%	0.05%	3.0%

**Table 3** The PCV of the rats after infection

S/N	Group A	Group B	Group C	Group D	Group E
1	24%	20%	25%	25%	22%
2	25%	23%	25%	26%	24%
3	24%	26%	24%	25%	24%
4	25%	23%	25%	25%	25%
5	25%	21%	24%	24%	26%

Three of the five rats in the control group were dead by the 10th day of the experimental treatment, and this is a clear indication that the *Trypanosoma brucei* strain used was highly pathogenic to rats. The *in-vivo* antitrypanosomal activity exhibited by the crude extract of *Moringa oleifera* against the *Trypanosoma brucei* strain supports the reports from Sunday and Hassana 2010; and Ukachi *et al.*, 2015 who worked on some other trypanosomal species. But even though the activity of the crude extract of *Moringa oleifera* against the *Trypanosoma brucei* has been confirmed, there is still the need to extract, isolate and elucidate the structure of the compound responsible for such activity.

Treatment of the infected rats with the crude extract of the plant has shown extended survival of the rats in the treatment groups compared with the non-treated control group. This result agrees with previous reports by Abubakar *et al.*, (2005) and Abu and Uchendu (2011) who have applied extracts of *Momordica balsamina* and *Hymenocardia acida* on *T. brucei*.

It is also clear from the findings of this research that the crude extract of *Moringa oleifera* used against the *Trypanosoma brucei* parasite was not able to completely eliminate parasites from the blood stream of infected rat, rather it was only able to reduce the level of parasitaemia. This agrees with researches reported by Shuaibu *et al.*, (2008); Ogbadoyi *et al.*, (2011b) and Ibrahim *et al.*, (2012) that had similar observations regarding the reduction in parasitaemia, and the conclusion to that might be that presence of the high load of the parasite has masked and overshadowed the efficacy of the extract used, and this conclusion is fully in agreement with that of Ekanem *et al.*, (2008) and Abu and Uchendu (2011). Moreso, the crudeness of the extract, the route of administration and the dose might be other factors that can reduce the efficacy of the extract, and conclusion agrees with that from Tsegabirhan *et al.*, (2014).

The drastic reduction and fall in the packed-cell-volume (PCV) of the trypanosome-infected rats is a reliable indication of the disease status, as the measurement or degree of anaemia in the infected rats can be said to be directly proportional to the level of paracetaemia in the rats' blood. Reports by Ekanem *et al.*, (2008) and Saleh *et al.*, (2009) also associates high decline in PCV (acute anaemia) in trypanosomal infection to the increase in the number of the parasites. The result of this study showed that the crude extract was able to control the increase in anaemia, especially at the later stages of the infection, by minimizing drops in PCV values. In the untreated rats, the parasite count increased and the packed cell volume (PCV) decreased markedly until some of the animal died, as was also observed in previous studies by Ekanem *et al.*, (2008) and Saleh *et al.*, (2009).

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## Compliance with ethical standards

### Acknowledgments

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### Disclosure of conflict of interest

Some of the materials required for the research had to be obtained from a sister University, and this has not been as easy as it sounds, because it contradicts the policy of our University.

### Statement of ethical approval

The present research work obtained approval from the National Health Research Ethics Committee of Nigeria as the research contains some studies performed on animals (rats).

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