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Full Length Article

Methanolic leaf extract of *Moringa oleifera* improves the survivability rate, weight gain and histopathological changes of Wister rats infected with *Trypanosoma brucei*

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ABSTRACT

Trypanosomosis is a major disease of Man and animals. This study investigated the effect of *Moringa oleifera* leaf extract on the survivability rate, weight gain and histopathological changes of Wister rats experimentally infected with *Trypanosoma brucei*. A total of thirty (30) rats randomly divided into six groups (A-F). Rats in group A remain untreated and uninfected while rates in group F were infected and untreated. Rats in groups B and C were treated with *Moringa oleifera* leave extract orally at 200 mg/kg for 14 days pre-infection and the treatment continued in B but not in C. Rats in groups D and E were treated with the extract orally for ninety days at 200 mg/kg (pre-infection) and the treatment continued in D but not in E. The weight changes in all rats were monitored weekly. Rats in B-F groups were infected with 3×10^6 of *Trypanosoma brucei* per mL of blood. The results showed that all the infected rats died but the treated group survived extra two days when compared with the untreated group. The percentage weight gain of rats in groups B and C was high (23.9% and 21.1%) respectively as against negative control (17.2%). The groups with chronic administration of the extract (D and E) had a lower percentage weight gains (64.3% and 60.3% respectively) when compared with negative control (71.8%). The histopathology results showed that the extract was a potent ameliorative agent that reduced neuronal degeneration and congestion in the brain and the spleen of the infected rats respectively. In conclusion, *Moringa Oleifera* leave extract has mitigative effects on the pathogenesis of trypanosomosis.

1. Introduction

African trypanosomosis is a major disease that has ravaged the livestock industry in tropical Africa where disease is endemic [1,2]. *Trypanosoma congolense, T. vivax* and *T. brucei* remain the major pathogenic tsetse transmitted trypanosome species responsible for the disease in tropical regions of Africa, where the vector *(Glossina spp*) is prevalent [3]. The disease is an haemoparasitic disease of domestic animals with manifestations such as; severe anaemia, weight loss, reduced productivity, infertility and abortion, with death occurring in some animals during the acute and chronic phase [4].

Drugs of therapy and prophylaxis (Chemotherapy and chemoprophylaxis) are the main methods of controlling trypanosomosis [5]. These methods of control are still the most reliable in spite of problems of drug resistance and toxicity [6] especially since the development of an effective vaccine against the disease has been unsuccessful despite intensive effort in research [2]. Diminazene aceturate is one of the few drugs available for both treatment and prophylaxis of African trypanosomosis in livestock especially in cattle infected with *T. congolense, T. vivax* and *T. brucei* [7]. Conventional chemotherapeutic treatment of trypanosomosis is no longer befitting as a result of side effects associated with the drugs and the development of many resistance trypanosome in many part of the world. Research now focuses on new compounds for the treatment of trypanosome infection [8]. One possible source for such affordable treatment lies in the use of natural products which have served as an important source of drugs since ancient times. About half of useful drugs have been derived from natural source [9]. Antiparasitic plant-derived molecules have been used as

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lead compounds to develop semi-synthetic drugs with better efficacy and safety [10]. Due to the aforementioned reasons and implications this study was aimed at assessing the effect of *Moringa oleifera* on the survivability rate, weight gain and histopathological changes observed during the course of trypanosomosis.

2. Materials and methods

2.1. Ethics, experimental animals and study design

Ethical approval was sought from Animal Care, Use and Review Ethical Committee (ACUREC), University of Ibadan, Nigeria with approval code number as thus, UI-ACUREC/APP/2016/011

Thirty adult male and female Wistar rats between the ages of 8–12 weeks, weighing from 90 to 105 g were used for this study. The rats were housed in the animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. The animals were kept in cages under normal environmental temperature (20–22 °C) and fed with standard pelleted feed (Livestock® Lagos, Nigeria) and water given *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for two weeks before the experiment commenced. Rats were grouped into six groups of five rats each and the extracts were administered through the oral route using oral cannula.

The rats were divided into six groups, each comprising five rats and each group was treated as follows: Group A (Negative controls), treated with distil water (uninfected and untreated). Group B: *Moringa oleifera* was administered orally at 200 mg/kg daily for 14 days before infection and supplement continued after infection for 16 days. Group C: *Moringa oleifera* was administered orally at 200 mg/kg daily for 14 days before infection and treatment stopped after infection. Group D: *Moringa oleifera* was administered (daily) orally at 200 mg/kg for 90 days pre-infection and supplement continued after infection for 16 days. Group E: *Moringa oleifera* was administered orally at 200 mg/kg daily for 90 days pre-infection and treatment stopped post-infection. Group F: Infected and untreated rats (positive control).

2.2. Plant authentication and preparation

The leaves of *Moringa oleifera* were procured at Ajibode, Akinyele Local Government area of Oyo State, located in the South Western part of Nigeria, which is about 2 Km from University of Ibadan, Ibadan Nigeria. It was taxonomically identified and authenticated in the herbarium of the Department of the Forest Research Institute of Nigeria (FRIN), with voucher number UIH-10847. The leaves of *Moringa oleifera* were washed thoroughly, dried in shade and pulverized to powder. The methalonic extraction was done at the Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, University of Ibadan using the method of Adeyemi et al. [4]. The extract was concentrated to the final concentration of 7.5% stock solution using rotary evaporator and the yield was diluted in soya oil as a vehicle.

2.3. Moringa oleifera phytochemical screening

Phytochemical screening of methanol extract of *Moringa oleifera* leaves was subjected to qualitative chemical screening for identification of the various classes of the chemical constituents as previously described by Sofowora [11].

2.4. Trypanosoma stock and inoculation

Trypanosoma brucei used in this study was secured from Nigeria institute for Trypanosomiasis and Onchocerciasis research institute, Vom, Plateau state, Nigeria. The dose of inoculum was judged to be $3x10^6$ of *Trypanosoma brucei* per mL of blood using the "Rapid Matching method" described by Herbert and Lumsden [12]. The parasites were maintained by serial passage in rats. To infect the rat, 1 ml of blood was collected from heavily infected rat and then melded with 2 ml of normal saline. The mixture was viewed under microscope at X40 magnification. The blood containing the parasites was then injected into rats intraperitoneally

2.5. Weight gain determination

The weights of the rats were monitored from first day and later on weekly basis using automated electronic scale (Sensor Disc Technology, London). To weigh a rat, a round plastic container was placed on the scale and tarred to zero following which the rat was dropped inside the container and subsequently weighed.

2.6. Histological procedures

After the death of the rats, the brain and spleen were extracted from the rats and were adequately treated with 10% formaldehyde (fixation) in order to preserve both the structure and molecular composition [13]. The organs were dehydrated by bathing it successively in rated mixture of ethanol and water (70–100%). The ethanol was replaced with a solvent mixable with the embedding medium. The tissues were penetrated with xylene and it became transparent (clearing). The instilled tissue with xylene was placed in melted paraffin in an oven and was maintained at 58–60 °C (embedding). The heat allowed the solvent to evaporate and the spaces within the tissues became filled with paraffin. The tissue together with its impregnating paraffin hardened following removal from the oven. The sections (5 μm) were then floated on water and transferred to a glass slide and stained with haematoxylin and eosin stains. The slides were viewed under light microscope with magnification X40 [13].

2.7. Data analysis

All data generated were expressed as mean ± SD. The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's post hoc multiple comparison test using Graphpad Prism statistical package, San Diego, Califonia, U.S.A (www.Graphpad.Com). Statistical estimates were made at confidence interval of 95%. Probability Values less or equal to 0.05 ($P \leq 0.05$) were considered significant.

3. Results

3.1. Findings of phytochemical analysis

Phytochemical analysis of methanolic extract of *Moringa oleifera* revealed the presence of flavonoid, tannin, saponin, steroid, phenol carbohydrate and glycoside as stated in Table 1.

3.2. Survivability rate

All the rats infected with *T. brucei* died but the group treated with *moringa oleifera* leaves died at day 17 when compared with the

Table 1
The phytochemical constituents of *M. oleifera* leaves.

Component	Test	Observation	Scoring
Alkaloid	Dragendorffs	Brownish-red colour	+
Tannins	Ferric chloride	Blue ink colour	+
Flavonoid	Pew's	Red colour	+
Saponin	Frothing	Persistence foam	+
Phenol	Buchard	Violet colour	+
Carbohydrate	Molisch's	Red colour	+
Glycosides	Salkowski's	Reddish brown	+