Trop J Nat Prod Res, April 2022; 6(4):650-653 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Investigation of the Antitrypanosomal Activity of Hydromethanol Extract of *Olax subscorpioidea* Root in Rats Experimentally Infected with *Trypanosoma brucei*

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ARTICLE INFO

Article history:
Received 02 March 2022
Revised 20 April 2022
Accepted 29 April 2022
Published online 03 May 2022

ABSTRACT

The development of livestock industry in Sub-Sahara Africa has been greatly limited by the menace of African trypanosomiasis. This study investigated the antitrypanosomal effects of hydromethanol extract of *Olax subscorpioidea* in Wistar rats infected with *Trypanosoma brucei*. Thirty adult rats were randomly assigned to 6 groups (n = 5). On day zero, groups 1-5 were infected with *Trypanosoma brucei*, while group 6 was uninfected. On day 7, after establishing log of parasitaemia, groups 1-3 received extract at a dose of 100, 200 and 400 mg/kg, respectively, while group 4 received diaminazene aceturate (3.5 mg/kg), and groups 5 and 6 received distilled water. The log of parasitaemia, rectal temperature and packed cell volumes (PCV) were determined on days zero, 7, 14, 21 and 28. On days 7 and 14 post infection, the log of parasitaemia of the extract and diminazene aceturate treated groups were significantly ($p < 0.05$) reduced when compared with the negative control group. On days zero, 7, 14 and 21 post infection, there was no significant difference in the mean rectal temperature of the extract and diminazene aceturate treated group when compared with the negative control. On days 14 and 21 post infection, the PCV of extract (100 mg/kg), diminazene aceturate (3.5 mg/kg) and normal control groups were significantly ($p < 0.05$) higher when compared with the negative control group. The findings of this study suggests that hydromethanol extracts of *Olax subscorpioidea* possess antitrypanosomal activity and justified its folkloric use for this purpose.

Keywords: African trypanosomiasis, antitrypanosomal activity, diaminazene aceturate, herbal medicine, *Olax Subscorpioidea*,

Introduction

African trypanosomosis is a disease of mammals caused by an extracellular haemoprotozoan parasite that is transmitted by *Glossina sp.*[1] It is one of the major causes of morbidity and mortality in animals and humans in sub-Saharan Africa.[2] The estimated prevalence of African trypanosomosis in Nigeria is 16.1% with about 55,000 human and 3 million livestock deaths annually in sub-Saharan Africa linked to the disease.[2,3] The socio-economic losses associated with the disease is huge; over 500 million US dollars are lost in meat, milk and control program.[2,3] The development of livestock industry in Sub-Sahara Africa has been greatly limited by the menace of African trypanosomosis. The control of the disease in the region has been mainly via the use of chemotherapeutic agents and sometimes through vector control and rearing of trypanotolerant breeds of animals.[4] The clinical efficacy of these drugs have been limited by the development of resistance as well as high cost and toxicity of the medications.[1] Traditionally, farmers use some medicinal plants in the control of African trypanosomosis. According to World Health Organisation, it is imperative to explore the antitrypanosomal potential of some herbs, especially those used in the traditional management of African trypanosomosis.[5]

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Citation: Onyeabor A, Onoja SO, Uwalaka EC, Obi CF, Eze, BO. Investigation of the Antitrypanosomal Activity of Hydromethanol Extract of *Olax subscorpioidea* Root in Rats Experimentally Infected with *Trypanosoma brucei*. Trop J Nat Prod Res. 2022; 6(4):650-653. doi.org/10.26538/tjnpr/v6i4.29

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Olax subscorpioidea is a member of the family *Olacaceae* and can occur either as a shrub or tree. It is widely distributed in West African countries such as Nigeria, Zaire and Senegal.[6] It is referred to as *Ifon*, *Aziza* and *Gwano kurmi* in the Western, Eastern and Northern part of Nigeria, respectively.[7] It is used in traditional medicine for the management of asthma, cancer, infectious diseases, mental illnesses and diabetes mellitus.[7-9] The antinociceptive, anti-ulcer, antimicrobial, anti-protease and wound healing properties of *Olax subscorpioidea* have been reported.[6,7,10,11] The presence of some phytochemical constituents such as flavonoid, tannins, saponins, alkaloids, reducing sugars, steroid, phenol, terpenoid, pyrrolizidine alkaloid, etc in *O. subscorpioidea* have been documented.[11] The Fulani's uses the decoction of *O. subscorpioidea* in the folkloric management of trypanosomosis, but there is dearth of information in scientific literature on its trypanocidal potentials. This study aimed to investigate the efficacy of hydromethanolic root extract of *Olax subscorpioidea* in rats experimentally infected with *Trypanosoma brucei.*

Material and Method

Identification of plant and extract preparation

The roots of *Olax subscorpioidea* were obtained from Nsukka, Enugu State in April, 2021 and identified at the Department of Botany, University of Nigeria Nsukka by Mr A. O. Ozioko. A voucher sample (MOUAU/VPP/2021/01) was kept at the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture Umudike herbarium. The harvested plant samples were air-dried in the laboratory at room temperature, pulverized, and soaked in 80% methanol for 48 hours with intermittent shaking at three hours interval. Later, the extract was filtered with Whatmann No. 1 filter paper and concentrated in a hot air oven at 40 °C. The yield of the

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

extract was 67.4 grams and it was stored in a refrigerator at 4 °C throughout the period of the experiment.

Experimental animal
Thirty-six (36) male adult Wistar rats bred within the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU) were procured for the study. The rats were acclimatized for two weeks before commencement of the experiment and were fed with commercial pelleted feed and water *ad libitum* under environmental temperature and natural light/dark cycle. The animal handling protocol was approved by the ethical committee of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria (MOUAU/CVM/REC/202210).

Acute toxicity study
The "up and down method" of acute oral toxicity test at dose limit of 2000 mg/kg was used to evaluate the acute toxicity. Six rats divided into two groups (n = 3) were used for the study.[12]

Test organism
The test organism *Trypanosoma brucei brucei* was isolated from infected mice at the Department of Veterinary Parasitology and Entomology laboratory, University of Nigeria, Nsukka. The blood samples were collected from the tail using glass slide and a thin smear was prepared and viewed under the light microscope. Then the slide was examined for *Trypanosoma brucei brucei* based on their type of motility in the microscope field at a magnification of x40. Then 0.2 ml of the blood containing the *Trypanosoma brucei brucei* was injected intraperitoneally to two (2) laboratory mice and transported to Veterinary Parasitology and Entomology Laboratory, Michael Okpara University of Agriculture, Umudike.
After establishment of infection, the donor mice were then screened and blood samples were collected from the tail vein for thin blood smear which was prepared and viewed under the light microscope. The slide was examined for *Trypanosoma brucei* in the microscope field at x40 using rapid matching method of Herbert and Lumsden.[13]

Experimental design
Thirty (30) rats were randomly assigned to 6 groups (n = 5), infected with *Trypanosoma brucei* on day zero and extract and drug were administered once on day 7 as presented in Table 1. The rectal temperature (^{o}C), pack cell volume (PCV) and parasitaemia were determined on days zero, 7, 14, 21 and 28 post infection. The parasitaemia was evaluated via estimation of trypanosome count on the wet mount microscopically at x40 magnification using "rapid matching" method as described by Herbert and Lumsden.[13] The rectal temperature was determined with the aid of a digital clinical thermometer. An electronic weighing balance was used in the body weight determination while micro haematocrit method was used in the determination of the PCV.[14]

Table 1: Grouping and treatment of the animals

Groups	Dose	Dose level of parasite (tryps/ml)
Group 1	Extract, 100 mg/kg	$1x10^4$ in 0.2 ml blood PBS
Group 2	Extract, 200 mg/kg	$1x10^4$ in 0.2 ml blood PBS
Group 3	Extract, 400 mg/kg	$1x10^4$ in 0.2 ml blood PBS
Group 4 (positive control)	Diminazene aceturate, 3.5 mg/kg	$1x10^4$ in 0.2 ml blood PBS
Group 5 (negative control)	Distilled water, 5 ml/kg	$1x10^4$ in 0.2 ml blood PBS
Group 6 (normal control)	Distilled water, 5 ml/kg	None

Statistical analysis
Data were expressed as mean ± standard error of the mean (mean ± SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) at 95 % confidence level using statistical package for social sciences (SPSS) software version 22. Mean differences were separated using Least Significant Different.

Results and Discussion

This study investigated the antitrypanosomal potential of hydromethanol extract of *Olax Subscorpioidea* in rats. The extract was well tolerated by the rats, no signs of toxicity was observed in the treated rats, and the LD_{50} was greater than 2000 mg/kg. The extract reduced the parasitaemia in the blood of the infected and treated rats but did not clear it completely. The demonstrated antitrypanosomal activity of *Olax Subscorpioidea* could be attributed to its phytoconstituents. Previous studies have reported the presence saponins, tannins, phenol, terpenes, flavonoids, glycosides, and alkaloids.[11] These phytoconstituents have been reported to possess antitrypanosomal properties.[15]

Effects of the extract on the log of parasitaemia (%) of Trypanosoma brucei infected rats
The effects of the extract treatment on the log of parasitaemia of *Trypanosoma brucei* infected rats is presented on Figure 1. The extracts produced dose-dependent decrease in the log of parasitaemia on day 7 post infection. On days 7 and 14 post infection, the log of parasitaemia of the extract (100, 200 and 400 mg/kg) and diminazene aceturate treated groups were significantly ($p < 0.05$) reduced when compared with the negative control group. On days 21 and 28 post infection, the log of parasitaemia of the extract (100, 200 and 400 mg/kg) treated groups were significantly ($p < 0.05$) higher when compared with the negative control group. Here, the extract reduced the parasitaemia on day 7 but could not clear the parasite from the blood unlike diaminazene aceturate which cleared the parasite from the blood on day 21. A similar observation has also been made by Gabriel and co-researchers.[4] The inability of the extract to clear the parasite could be due to the route of administration and/or rate of administration. In this study, the extract was administered orally and once, which may have limited the absorption and bioavailability of the active ingredient in the bloodstream over time. The concentration of drug at the active site is affected by absorption, biotransformation and excretion.[16] The repeated administration of the extract may have sustained the plasma concentration of the active ingredient to clear the parasites from the blood.[16] The active ingredient(s) responsible for the antitrypanosomal activity was not elucidated but previous studies have reported that some of the phytoconstituents of *Olax Subscorpioidea* such as phenolics, flavonoids and alkaloids possess antitrypanosomal activity.[15] The antitrypanosomal potential of flavonoids have been demonstrated.[15] Phenolics and polyphenols have been documented to elicit antitrypanosomal activity by inhibiting the trypanosome alternative oxidase.[1,15] Alkaloids cause DNA intercalation and inhibit protein synthesis in trypanosomes.[15] The exact mechanism of the antitrypanosomal activity of *Olax Subscorpioidea* is not known but could be similar to the mechanism of action of diaminazene aceturate. Diaminazene aceturate elicit its antitrypanosomal activity via binding to trypanosomal kinetoplast DNA in a non-intercalative manner through specific interaction with the site rich in adenine-thymine base pairs.[17]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Effects extract on the mean rectal temperature (^oC) of Trypanosoma brucei infected rats

The effect of the extract treatment on the mean body temperature of *Trypanosoma brucei* infected rats is presented in Figure 2. On day 28 post infection, the rectal temperature of diminazene aceturate (3.5 mg/kg) treated group was significantly ($p < 0.05$) lower when compared with the negative control group. On days zero, 7, 14 and 21 post infection, there was no significant difference ($p < 0.05$) in the mean rectal temperature of the extract (100, 200 and 400 mg/kg) and diminazene aceturate (3.5 mg/kg) treated group when compared with the negative control. The decrease in the temperature of the rats on day 7 amid high parasitaemia is a unique observation and could not be explained in this study. The result from this study is in discordance with the reports of Mbaya and co-workers, that a direct relationship exists between undulating pyrexia and fluctuating parasitaemia in trypanosomosis.[18] Trypansoma infection is associated with pyrexia and this is one of the cardinal clinical sign of trypanosomosis.[19] Further studies would be conducted with the same strain of trypanosome to elucidate this observation.

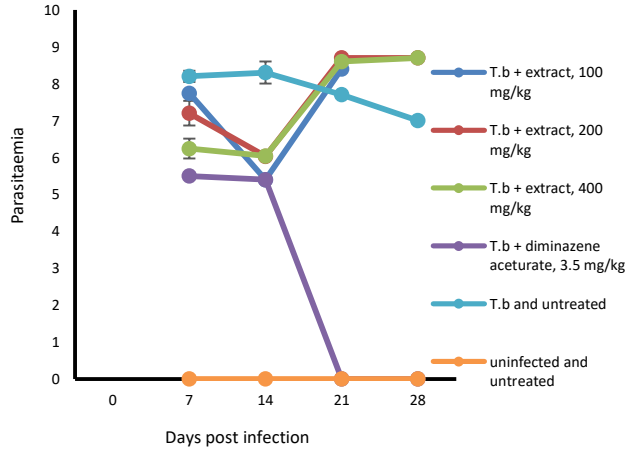

Figure 1: Effects of the extract on the log of parasitaemia (%) of *Trypanosoma brucei* infected rats

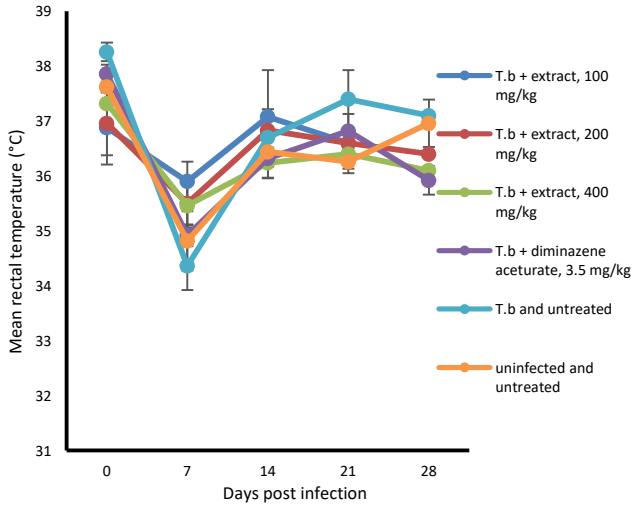

Figure 2. Effects of the extract treatment on the mean rectal temperature (^oC) of *Trypanosoma brucei* infected rats.

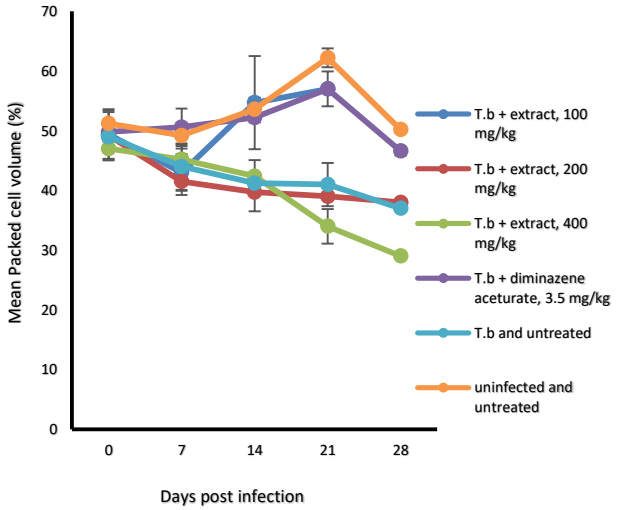

Figure 3: Effects of the extract treatment on the PCV of *Trypanosoma brucei* infected rats

Effects of the extract treatment on the PCV of Trypanosoma brucei infected rats

The effects of the extract treatment on the PCV of *Trypanosoma brucei* infected rats is presented on Figure 3. On days 14 and 21 post infection, the PCV of extract (100 mg/kg), diminazene aceturate (3.5 mg/kg) and normal control groups were significantly ($p < 0.05$) higher when compared with the negative control group. On day 28 post infection, the PCV of diminazene aceturate (3.5 mg/kg) and normal control groups were significantly ($p < 0.05$) higher, while the PCV of extract (400 mg/kg) treated group was significantly ($p < 0.05$) lower when compared with the negative control. The PCV of the infected groups were decreased on day 7 relative to day zero and it is attributed to parasitaemia. Trypanosome infection is associated with anaemia which is clinically manifested as low PCV.[20] The establishment of parasitaemia in the rats following the infection with *T. brucei* caused haemolysis of RBC and hence the reduced PCV.[21] The extract at lower dose (100 mg/kg) reversed the anaemia in the treated rats while the extract at higher dose (200 and 400 mg/kg) aggravated the anaemia further. The increased severity in the extract (200 and 400 mg/kg) treated groups could be linked to the phytoconstituents (terpene and saponins) whose concentration in the plasma would have increased with the elevated dose of the extract. Terpenes and saponins have haemolytic potential and as a result produced haemolytic anaemia.[22] Previous studies, have reported *O. Subscorpioidea* to be rich in saponins and terpenes, thus could have caused haemolytic anaemia.[11]

Conclusion

The findings of this study showed that, hydromethanolic extracts of *Olax. subscorpioidea* has a potential antitrypanosomal activity on *Trypanosoma brucei* and justified its folkloric uses for this purpose. Further studies geared toward the determination of the effects of repeated dosing and route of administration as well as the isolation, identification, characterization and purification of bioactive compounds from this plant should be conducted.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors are grateful to Mr A. O. Ozioko for the collection and identification of plant sample.

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