

In vivo Anti Trypanosomal Activity of Aqueous Extract of *Azadirachta indica* Leaves on *Trypanosoma brucei brucei* Infected Mice

Okoh Martina Enyanwu*, Obadiah Happiness Igwe, Edoh Emmanuel

Department of Biological Sciences, Benue State University, Makurdi, Nigeria

Email address:

maokoh@bsum.edu.ng (O. M. Enyanwu)

*Corresponding author

To cite this article:

Okoh Martina Enyanwu, Obadiah Happiness Igwe, Edoh Emmanuel. In vivo Anti Trypanosomal Activity of Aqueous Extract of *Azadirachta indica* Leaves on *Trypanosoma brucei brucei* Infected Mice. *International Journal of Infectious Diseases and Therapy*.

Vol. 3, No. 1, 2018, pp. 13-17. doi: 10.11648/j.ijidt.20180301.13

Received: October 30, 2017; Accepted: September 17, 2017; Published: February 2, 2018

Abstract: *Azadirachta indica* commonly known as Neem is known to possess high medicinal value. This study aimed at determining the in vivo anti trypanosomal potential of aqueous extracts of *A. indica* leaves on *Trypanosoma brucei brucei* infected mice. The toxicity of *A. indica* on mice was determined after which different extract doses (100, 250 and 500mg/kg) were administered intraperitoneally on the third day after infection, administration lasted for 7 days. The effects of the extract in trypanosome infected mice were observed for 15 days by monitoring the changes in packed cell volume (PCV), Parasitemia and weight of mice. Comparison was made to the positive control group treated with Diamineazine Aceturate and negative control-infected but not treated. The leaf extract of neem plant did not have acute toxicity on the uninfected animals, there was no significant effect observed in weight (Group 3 which was given 500mg/kg had a weight of 35g by day 7 while control had a weight of 35.2g) and PCV (Group 1; 100mg/kg, Day 7 had a PCV of 44, Group 3; 500mg/kg, 45 while control had a PCV of 45) ($p>0.05$). There was however a significant difference between the different extract doses and control with respect to parasitemia, (500mg/kg extract dose showed more anti trypanosomal potential compared to other doses). PCV (mice that were given 500mg/kg of extract dose recorded a higher PCV compared to lower doses) and weight of the mice; ($p<0.05$). *Azadirachta indica* extract possess anti trypanosomal potentials. It is therefore recommended that more research on ethno botanic medicine should be encouraged and treatment options employed in the treatment of neglected diseases.

Keywords: *Azadirachta indica*, African Trypanosomiasis, Nigeria

1. Introduction

African trypanosomiasis commonly known as sleeping sickness is a vector borne parasitic disease of man and his domestic animals. Trypanosome, the causative parasite is a protozoan belonging to the genus *Trypanosoma*. The parasites are transferred to their human and animal host through insect bites which have acquired their infection from human or animal harboring the parasite [1]. There are three different subspecies of *Trypanosoma brucei* which cause different variants of trypanosomiasis. They include *T. brucei gambiense* which causes slow onset chronic trypanosomiasis in human. They are most common in central and western Africa. *T. brucei rhodesiense* which cause fast onset acute trypanosomiasis in human is common in south and eastern

Africa [2]. *T. brucei brucei* is the third subspecies. It causes animal trypanosomiasis alongside *T. vivax*, *T. evansi* etc. Although it is similar to the other two subspecies, it does not affect human due to its susceptibility to lyses by human Apo lipoprotein [1].

Trypanosomes have glycoprotein coat that is encoded by gene that are antigenically distinct thus, making the process of vaccine production difficult and this has made the control to rely principally on chemotherapy and chemoprophylaxis using salts of different compounds. However, the therapeutic and prophylactic use of trypanocides are beset by numerous limiting factor such as toxicity and drug resistant strain of the parasite [3]. The emergence of drug resistant trypanosomes is

considered a very serious problem in trypanosomiasis control especially for the population of poor farmers in Africa [4]. 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, cheap and safe chemotherapeutic agents for treatment of African Trypanosomiasis [5].

The natural world has over the years been a major source of medicinal agents and despite the recent advances in pharmacology and synthetic organic chemistry, plant biomolecules (phytochemicals) continue to provide key lead structures and therapeutic agents for the treatment of protozoan diseases with approximately 20,000 species of higher plants being used medicinally throughout the world. [6]. The use of herbal remedies in the treatment of trypanosomiasis is potentially promising with some ethnomedicinal plants used against the diseases having been demonstrated to be potent trypanocides. Pharmacologically active compounds of plant origin can provide an alternative to chemically synthesized drugs to which many infectious microorganisms have become resistant. [3].

Azadirachta indica (*A. indica*) also known as Neem, Neemtree, and Indian Lilac are trees in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta* and it is a medicinal plant used in traditional medicine for the treatment of pests, fungal, bacterial, viral and trypanosomal infections. In Nigeria, it is popularly called "Dogonyaro" [7]. The biological activities of some of the phytochemicals in the neem plant have recently been reviewed. Oil from the neem leaves, seeds and bark exhibit a wide spectrum of antibacterial action. The oil has also been reported to have some anti-protozoan properties. In spite of this knowledge, very little work has been carried out to establish the in vivo antitrypanosomal activity of Neem extracts and none with the human infective *Trypanosoma brucei rhodesiense*. [3] This study is therefore aimed at investigating the in vivo antitrypanosomal activity of crude extracts from the bark of *Azadirachta indica* in mice infected with *T. b. brucei*.

2. Methodology

2.1. Plant Material

The plant *Azadirachta indica* leaves was harvested and brought to the herbarium section of the biological sciences department. The plant parts were air dried for two weeks in the herbarium after which it was pounded into powder using a mortar and pestle.

2.2. Extraction

Forty grams (40 g) of the grounded plant parts were weighed separately into a conical flask and soaked for two hours in 400 ml of distilled water. The extracts was heated for two hours in a water bath at 60°C, allowed to cool for above ten hours after which it was filtered and refrigerated.[8].

2.3. Animals and Animal Husbandry

Fifteen albino mice which were used for the study were purchased from the animal house of college of health science, Makurdi and brought to the laboratory of biological sciences department of Benue state university, Makurdi where the study was carried out. The animal were weighed and fed.

2.4. Test Organism

Trypanosoma brucei brucei was obtained from Nigeria Institute for Trypanosomiasis Research, Kaduna. The parasite was maintained in the laboratory by continuous passage in mice and rats until required. About 0.1-0.2 ml of the blood of infected animal is diluted with phosphate buffered saline and injected into a clean mice.

2.5. Determination of Parasitemia

Parasitemia was monitored in the blood obtained from the tail vein of the infected mice. A drop of blood was placed on a grease free slide and cover with a cover slip. This was viewed under the microscope using *40 objective lens using the Rapid matching method of Herbert Lumsden. This was done from the second day of injection of parasite till the end to monitor the increasing rate of parasite and the effect of the extract.

2.6. Determination of Packed Cell Volume

This is the fraction of the whole blood volume that consists of red blood cell. The blood was obtained by bleeding the tail vein of the mice and the blood was massaged into a heparinized capillary tube. It was centrifuged at 10,000 rpm for five minute and the percentage of the blood was taken.

2.7. Measurement of Weight

The mice were spinned to make them dizzy after which their weight was taken on a weigh balance

2.8. In Vivo Toxicity Test

Eight uninfected mice were divided into four groups. Different doses (100, 250 and 500 ml/kg) of extract were administered intraperitoneally to group 1, 2 and 3 respectively while group 4 was given water only. The mice in each group were monitored closely for 15 minutes for any sign of acute toxicity. The PCV and weight of the animals were also observed for any decline [8].

2.9. In Vivo Test of Extract for Anti Trypanosomal Potential

Fifteen albino mice were divided into five groups based on the number of treatment. Different extract doses were used, diamine acetate and infected but not treated were used as positive and negative control respectively. All mice were infected with *Trypanosoma brucei brucei*. Parasites were found on the second day of infection and treatment commenced on the third day after infection. 100, 250 and 500mg/kg were administered to group 1, 2 and 3 respectively

for 7 days. Diminazine was administered to the positive control at 3.5 mg/kg while the negative control was untreated. The parasitemia, PCV and weight were monitored for 15 days as described by [8].

Statistical Analyses

All the results obtained were analyzed using analysis of variance.

3. Results

The level of packed cell volume of mice of different extract doses for one week of toxicity test is shown in figure 1. There was no significant difference ($P>0.05$) between the groups given different doses of extract and those given water. Figure 2 shows the weight of mice during toxicity test among different dose extracts and that of the control (water). There was no significant difference ($P>0.05$) in weight of the

different groups and that of water.

The level of parasitemia of different treatment groups and controls showed no significant difference ($P<0.05$) in the level of parasites among the different extract doses and amongst the controls. However, the anti trypanosomal potential of the extract was dose dependent. This is represented in table 3. Figure 4 shows weight of infected mice of different treatment groups. The weight showed a decline among the different extract dose and that of the negative control. However, the decline was dose dependent. In all, there was significant difference among the different treatment groups.

The PCV showed decline among the different dose extracts and that of the negative control. However, the decline was dose dependent significant difference was observed ($p>0.05$) Figure 4 shows the pcv chat in different treatment groups.

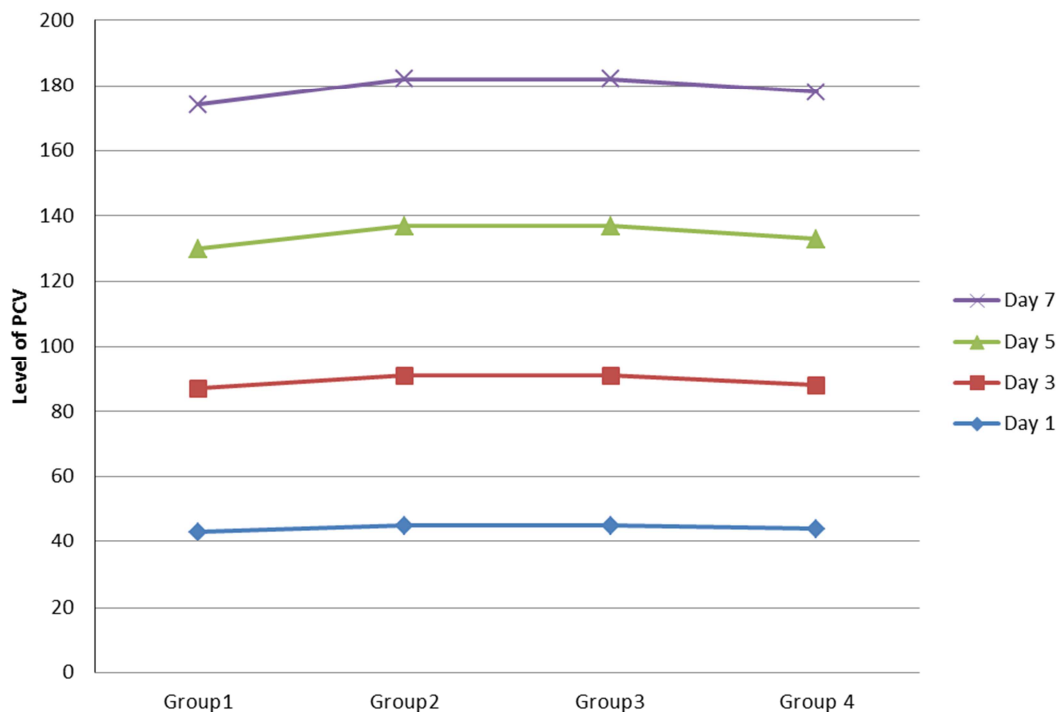


Figure 1. PCV of mice during toxicity test.

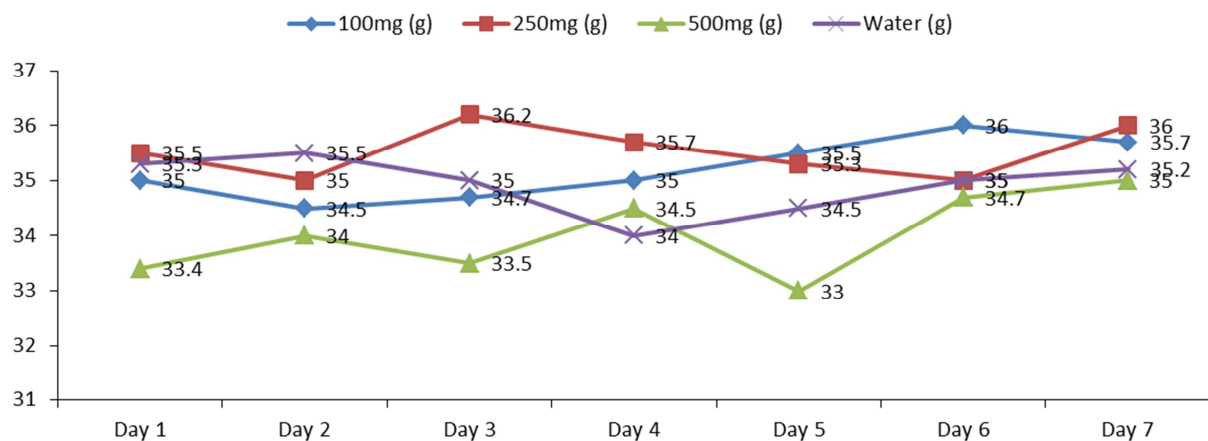
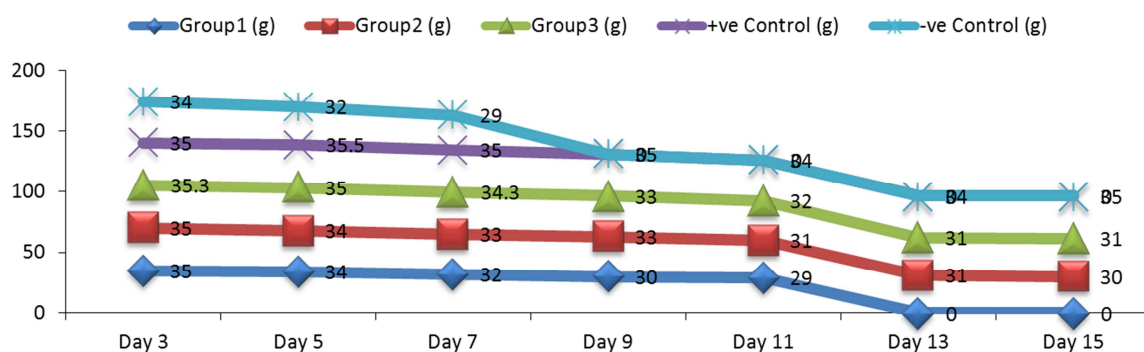
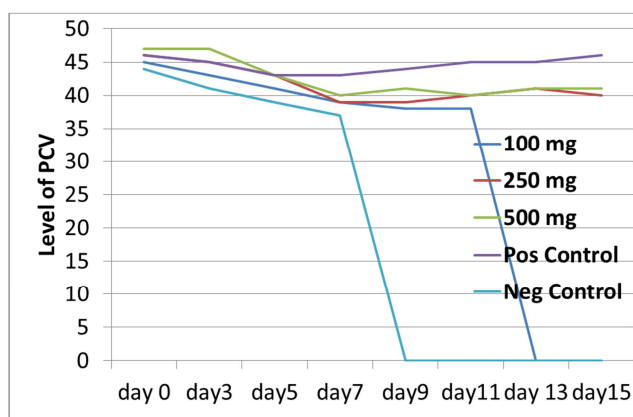


Figure 2. Weight of mice during toxicity test.

Table 1. Levels of parasitemia at different treatment groups and controls.

Days	Gp. 1	Gp. 2	Gp. 3	Positive Control	Negative Control
Day3	5	5	4	6	5
Day5	7	8	5	3	11
Day7	11	9	9	cleared	15
Day9	13	12	7	cleared	dead
Day11	11	8	8	cleared	dead
Day13	dead	10	6	cleared	dead
Day15	dead	7	5	cleared	dead

Fcal =1.07df= 4p<0.05

**Figure 3.** Weight of infected animals in different treatment groups.**Figure 4.** PCV levels of infected animals in different treatment groups.

4. Discussion

The acute toxicity test of Neem extract at different doses indicated that the extracts were not toxic as there was no significant difference in the body weight and PCV of mice given Neem and those given water. This is similar to the work of [3] where Neem extract were given to mice at 1000mg/ kg and there was no lethal case.

The level of parasitemia showed significant difference among the different extract doses and that of the controls. The negative controlled group witnessed a constant increase in parasite level till day 8 when they all died. This is similar to the work of [4]2007 in which the negative control groups all died as a result of high level of parasites on the sixth day. The positive control was able to clear the parasite at day 7 of infection. Extract dose of 100mg/kg witnessed increase in parasite level but was able to sustain the mice till day 11 when they eventually died while that of 250mg noticed

decrease in level of parasites from day 11. Extract dose of 500mg/ kg exhibited more anti trypanosomal potential compared to other extract doses. This agrees with the work of [3] who reported that anti trypanosomal potential of *Azadirachta indica* was dose dependent.

Apart from the positive control, all other treatment group witnessed decline in health status as observed in the level of PCV and weight. This shows the ability of trypanosomes to cause anemia.

The anti trypanosomal potential of *A. indica* could be attributed to the presence of secondary metabolites in the plant which is capable of generating radicals that act against the parasite's metabolism [4]. Also, the pathological effect of trypanosome is initiated by the release of cytokines and nitric oxides. Active compounds in Neem such as phenol act against toxic compounds to neutralize it, hence extending the life span of the organism [9].

The inability of the extract to fully destroy the parasite could be due to low active compounds in the aqueous extract. Also, late commencement of treatment is another factor because the earlier the treatment, the better as this prevents the parasites from fully establishing itself [10].

A. indica, oils have also been screened for potential antibacterial activity against medically important bacterial strains [11]. Natural products are known to play an important role in both drug discovery and chemical biology. Although some therapeutic benefits can be traced to specific plant compounds. There are several reports on the antimicrobial activity of different herbal extracts [12, 13].

5. Conclusion

Azadirachta indica has proved to possess anti trypanosomal potentials as can be seen in this study. The

Anti trypanosomal activities of *A. indica* could be attributed to its composition of secondary metabolites which generate radicals that act on trypanosome.

It is therefore recommended that more research on ethno botanic medicine should be encouraged and treatment options employed in the treatment of neglected tropical diseases also, early diagnosis and treatment of trypanosomiasis should be carried out to ensure it does not progress to its chronic stages.

References

- [1] Ene, A., Edeh, N., Bonny, C., Ojiako, O., Ujuwandi, C. and Igwe, C. (2013). In vivo Anti Trypanosomal Effect of Methanol and Aqueous Extract of *Picrili manitida*. *British Journal of Pharmaceutical Research* 3:200-205.
- [2] Ogoti, P., Magiri, E., Auma, J., Gabriel, M., Imbuga M. and Murilla, G. (2009). Evaluation of In vivo Anti Trypanosomal Activity of Selected Medicinal Plant Extracts. *Journal of Medicinal Plant Research*, 3(11):849-854.
- [3] Raphael, M., Bosire, O., Stephen, M., William, W., Ronald, G., Kilbagi, K. and Francis, N. (2009). Anti Trypanosomal Effect of *Azadirachta indica* on *Trypanosoma brucei rhodesiense* Infected mice. *Eastern Journal of Pharmaceutical Research*, 14-29.
- [4] Maikai, V., Nok, J., Adaadi, A and Alawa, C. (2007). In vitro Anti Trypanosomal Activity of Aqueous and Methanolic Extract of Stem Bark of *Ximenia americanai* on *Trypanosoma congolense*. *Journal of Medicinal Plant Research*. 3:2-7.
- [5] Nok, A., King, A., Ishaya, L., Samuel, A., Paul, C., Casmir, E and James, K. (1993). Trypanocidal Potential of *Azadirachta indica*: In vivo Activity of Leaf Extract against *Trypanosoma brucei brucei*. *Journal of Clinical Biochemistry Nutrition*, 15:113-118.
- [6] Tagbato, S. and Townson, S. (2001). Anti Parasitic Properties of Medicinal Plants and Other Naturally Occurring Products. *Advanced Journal of Parasitology*. 50:199-205.
- [7] Omoja, V., Anago, A., Obidik, I., Ihedioha, T., Umeakuana, P., Mhonga, L., Asuzu, U. and Anika, M. (2011). The Effect of Combination of Methanolic Leaf Extract of *Azadirachta indica* and Diameazene Diacetate in the Treatment of Experimental *brucei brucei* in rats. *Asian Pacific Journal of Medicine*, 3(4):560-563.
- [8] Luckins A. G. (2002). Surra. Office International Epizootics (OIE) Manual of Diagnostic tests and vaccines for Terrestrial Animals Pp 33.
- [9] Chem, J., Tipoe, G. and Liong, F. (2004). Green Tea Polyphenols Prevent Toxin Induced Hepatotoxicity in Mice by Down Regulating Inducible Nitric Oxide Derived Pro-Oxidants. *American Journal of Clinical Nutrition* 80:742-751.
- [10] Ibrahim, M., Njoko, G. and Salau, B. (2008). In Vivo Activity of Stem Bark of Aqueous Extract of *Khaya senegalensis* against *Trypanosoma brucei*. *African Journal of Biotechnology*. 7(5):661-663.
- [11] Mohammad, A. (2012). Antimicrobial Potential of *Azadirachta indica* Against Pathogenic Bacteria and Fungi. *Journal of Pharmacognosy and Phytochemistry*, 1(4):78-83.
- [12] Githua, M., Hassanali, A., Keriko, J., Kareru, P. Murilla, G., Ndungu, M. and Nyagah, G. (2011). Antitrypanosomal Tetra notriterpenoids from *Toonaciliata* roots. *Agriculture and Biology Journal of North America*, 2(7): 1042-1047.
- [13] Bulus, T., Ahmed, A. B., Aboi, T. Y. and Danbaki, D. A. (2016). Determination of IC50 and IC90 Values of Ethanolic Extracts of Some Medicinal Plants against *Trypanosoma brucei brucei*. *Achives of Clinical Microbiology*, 7(3):1-5.