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Effects of Some Neem Leaf Phytochemicals On Weight and Packed Cell Volume of *Trypanosoma brucei brucei* Infected Albino Rats

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Abstract:- Trypanosomiasis is virulent, inoculable and highly fatal protozoan disease that affects both human and domestic animal. The effects of some neem leaf phytochemicals on weight and Packed Cell Volume (PCV) of *Trypanosoma brucei brucei* infected albino rats have been investigated in this study. Neem leaves preparation, phytochemicals extraction were done using standard protocols. Experimental animals and Test Organism (*Trypanosoma brucei brucei*) used in this study were obtained from Nigeria Institute for Trypanosomiasis Research, (NIFTR) Kaduna. Albino rats were inoculated with the parasites and screened for development of infection using the buffy coat technique. Thirty two rats were randomly distributed into 8 groups. Group A infected but no treatment; Group B infected and treated with Diminazine aceturate. Group C to K were infected and treated with varying concentration (100, 200 and 400 mg/kg body weight) of either alkaloids, glycoside neem extract or both. All groups were treated for 4 days, and given food and water of equal proportion. Body weights and packed cell volume (PCV) were determined using standard procedure. The study revealed that albino rats in the untreated control group and those administered with 100mg/kg/bodyweight of either Alkaloids, Glycosides, or both extract of neem leaves showed gradual decrease in body weight. While *T. b. brucei* infected albino rats on extract inoculums of 200 and 400mg/kg/bodyweight and standard drug showed increase in body weight. The results shows significant difference ($p<0.05$) in PCV values between those in the treatment groups when compared with group administered with standard drug. There is need for continuous studies in order to extract and purify more compounds responsible for the antitrypanosomal activity of the studied plant.

Keywords:- *Trypanosomiasis; Neem; Phytochemicals; Albino rats; Weight and PCV.*

I. INTRODUCTION

African animal trypanosomiasis (AAT) is among the disease of domestic animal covering about 37 sub-Saharan countries and about one-third of the total land area of Africa's (Mattioli *et al*., 2004). AAT is a virulent, inoculable and highly fatal protozoan disease. Annually, AAT is responsible for the deaths of about 55,000 people and 3 million livestock in agricultural and mixed farming areas. Therefore making it a critical factor for the agricultural sector and public agencies (Mulumba, 2003, Aliyu *et al*., 2010). Plants as sources of natural products have been the basis for medical and traditional treatment for several diseases (Abedo *et al.*, 2015). The World Health Organization (WHO) reported that 80% of the population of some African and Asian countries are using traditional medicine for the treatment of some diseases (Sherman and Hash 2001; Moquin, 2009). Trypanosomiasis is shown to cause anemia, weakening, abortions, coma, decreased fertility, loss of meat and milk production and even death in animals. Thus, investigation of the possible management mechanisms is central to addressing these losses, with the aim of finding a solution which can help combat the disease. The most commonly used herbal plants in Africa include *Azadiractha indica* (neem tree) for the treatment of fever; *Magnjfera indica* (mango tree) for the treatment of malaria and thypoid fever; *Allilum sativum* (Garlic) rheumatoid arthritis; *Acacia nilotica* (Acacia) for migraine and pile; *Khaya senegalenses* (African mahogany) for irritable bowel syndrome and cancer; *Carica papaya* (Pawpaw tree) formalaria and typhoid (Sofowora, 1993). Plants belonging to the angiosperm group have been investigated and shown to have some antitrypanosomal activity with minimal toxicity (Hoet *et al*., 2007; Shuaibu *et al.*, 2008; Abedo *et al.*, 2013 and Abedo *et al.*, 2015). According to Abedo *et al.* (2015) the antitrypanosomal activity of these plants was due to the effects of some phytochemical compound present in the plant. Plants contain compounds mainly secondary metabolites like glycosides, alkaloids, saponins, flavonoids, terpenes and tannins (Jorgensen, 2007). So this study tries to study the effects of some neem leaf phytochemicals on weight and Packed Cell Volume (PCV) of *Trypanosoma brucei brucei* infected albino rats.

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II. MATERIALS AND METHODS

A. Acquisition of Alkaloids and glycosides

Neem leaves preparation, Alkaloids and Glycosides extraction were done at Kebbi State University of Science and Technology Aliero, according to the method described by Prashant *et al.* (2011).

B. Experimental animals

The experimental materials and procedures used on animals in this were approved by the Institutional Animal Care and Use Committee (IACUC) of Nigeria Institute for Trypanosomiasis Research, (NIFTR) Kaduna, in accordance with the Good Laboratory Practice (GLP) regulation of the World Health Organization (WHO). Adult albino rats (6-8weeks old) of average weight of 110g obtained from Nigeria Institute for Trypanosomiasis Research, Kaduna were used for the study. Rats were housed in cages at room temperature and fed with commercial feed (Vital feeds, GCOM Nig. Ltd) and provided with water ad libitum.

C. Test Organism and its maintenance

Trypanosoma brucei brucei used in this study were also obtained from NIFTR, Kaduna. Parasites were reproduced and managed in clean white albino rats prior to the commencement of the experiment.

Albino rats were inoculated with the parasites and screened for development of infection as described by Murray *et al.* (1977) using the buffy coat technique. Blood were collected from tail vein, which were fixed and stained with methanol and Giemsa stain respectively and then observed using microscope with oil immersion objective lense (100×) for identification of trypanosomes. The morphology of the trypanosome in the stained field was compared with that of reference species in standard texts micrographs and literature (WHO, 1998).

D. Experimental Designs

Thirty two albino rats were randomly distributed into 8 groups, 4 rats per group. Group A (-tive control) with *Trypanosoma brucei brucei*, no treatment , + tive control (Group B) infected with *Trypanosoma brucei brucei* but with Diminazine aceturate. Group C, D and E were infected and treated with varying concentration (100, 200 and 400 mg/kg body weight) of alkaloids neem extract. Group F, G and H were infected and treated with varying concentration (100, 200 and 400 mg/kg body weight) of glycoside neem extract. All groups were treated for 4 days, and given food and water of equal proportion.

E. Determination of body weight

The Body weights were determined as described by Nweze *et al.* (2011). Body weight of each albino rats in all groups were recorded on the day of parasite inoculation challenge, day of treatment initiation and every 4days for 16days by using digital scale.

F. Determination of packed cell volume (PCV)

As described by Kobo *et al*. (2014), at the end of the experiment, the rats were sacrificed by jugular venisection. Blood (5 ml) were collected from each experimental albino rat into sample bottles, containing Ethylene diamine-tetra acetic acid (EDTA) as anticoagulant for the evaluation of haematological parameters. PCV were determined using the automated haematologic analyzer (Sysmex, KX-21, Japan).

III. RESULTS

A. Effects of Alkaloids and Glycosides of Azadirachta Indica leaves on Weight of T. b. brucei infected albino rats

Results showing the effects of Alkaloids and Glycosides of *Azadirachta Indica* leaves on Weight of *T. b. brucei* infected albino rats are showed in figure 4.1. All *T. b. brucei* infected albino rats in the untreated control group (Negative control) and those administered with 100mg/kg/bodyweight of either Alkaloids, Glycosides, or both extract of *Azadiracta indica* leaves showed gradual decrease in body weight. While *T. b. brucei* infected albino rats on extract inoculums of 200 and 400mg/kg/bodyweight and standard drug (positive control) showed increase in body weight.

B. Effects of Alkaloides and Glycosides of Azadirachta Indica leaves on PCV of T. b. brucei infected albino rats

Effects of Alkaloides and Glycosides of *Azadirachta Indica* leaves on Packed Cell Volume (PCV) of *T. b. brucei* infected albino rats are shown in figure 4.2. The results shows significant difference ($p<0.05$) in PCV values between those in the treatment groups when compared with group administered with standard drug (positive control). The significant decrease was observed in the PCV of albino rats in different treatment groups and negative control group when compared with albino rats administered with 400mg/kg/bodyweight of synergistic crude Alkaloids and Glycosides of *Azadirachta Indica* leaves. However, the decrease was dose dependent. It shows that albino rats administered with 400mg/kg/bodyweight of synergistic crude Alkaloids and Glycosides of *Azadirachta Indica* leaves had the highest PCV count of 39.71±0.01%. This was followed by group administered with standard drug (positive control) and 100mg/kg/bodyweight of Glycosides which have 37.97±0.58% and 37.57±0.58% respectively. While other administered groups and Negative Control group shows low PCV count range from 28.30±0.10% to 35.97±0.58% respectively.

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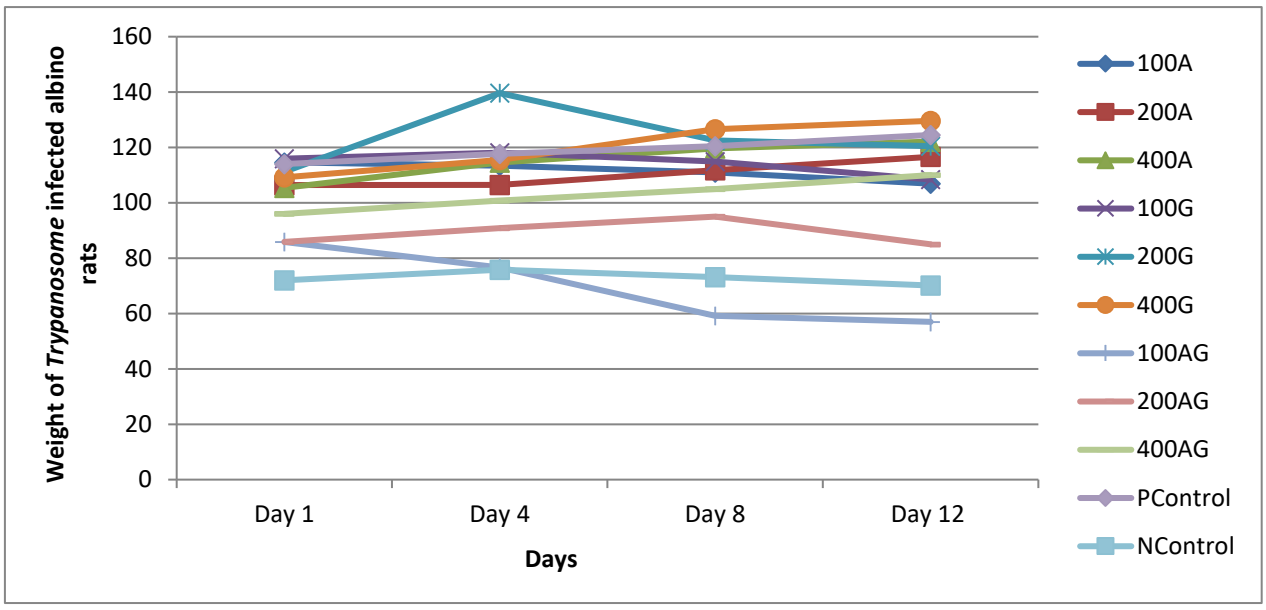

Fig. 1: Effects of Alkaloids and Glycosides of *Azadirahcta Indica* leaves on Weight of *Trypanosome* infected albino rats

Key: Dosage
100 A = Group administered with Alkaloids 100mg/Kg
200 A = Group administered with Alkaloids 200mg/Kg
400 A = Group administered with Alkaloids 400mg/Kg
100 G = Group administered with Glycosides100mg/Kg
200 G = Group administered with Glycosides200mg/Kg
400 G = Group administered with Glycosides 400mg/Kg
100 AG = Group administered with Alkaloids and Glycosides 100mg/Kg
200 AG = Group administered with Alkaloids and Glycosides 200mg/Kg
400 A = Group administered with Alkaloids and Glycosides 400mg/Kg
P Control = Positive Control
N Control = Negative Control

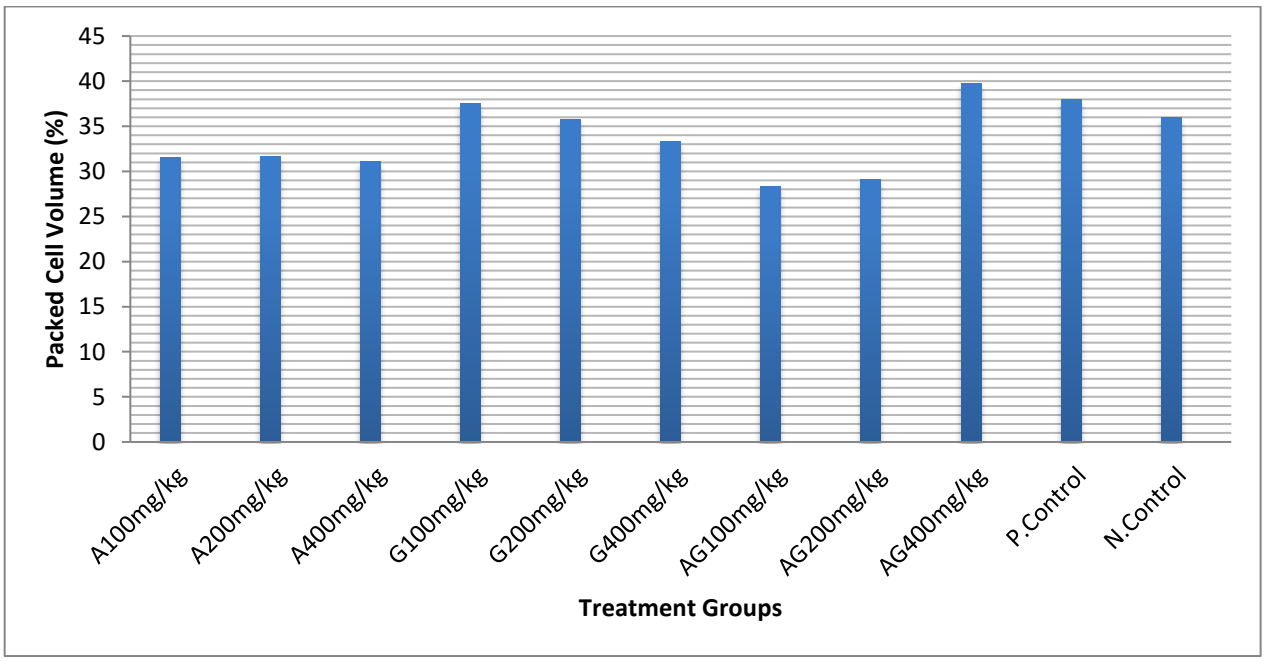

Fig. 2: Effects of Alkaloides and Glycosides of *Azadirahcta Indica* leaves on Packed Cell Volume (PCV) of *T. b. brucei* infected albino rats

Key: Dosage
100 A = Group administered with Alkaloids 100mg/Kg
200 A = Group administered with Alkaloids 200mg/Kg
400 A = Group administered with Alkaloids 400mg/Kg
100 A = Group administered with Glycosides100mg/Kg
200 A = Group administered with Glycosides200mg/Kg
400 A = Group administered with Glycosides 400mg/Kg
100 AG = Group administered with Alkaloids and Glycosides 100mg/Kg
200 AG = Group administered with Alkaloids and Glycosides 200mg/Kg
400 A = Group administered with Alkaloids and Glycosides 400mg/Kg
P.Control = Positive Control
N.Control = Negative Control

IV. DISCUSSION

The mean body weight for Alkaloids and Glycosides treated rats indicates a gradual decrease through day 0 to day 8 post treatments for all treatments groups except in a group treated with 400mg/kg/bodyweight of synergistic crude Alkaloids and Glycosides and the standard control group which shows gradual increase. The result indicates that groups treated with higher doses and the standard control group shows higher mean body weight. This corroborate with finding of Kobo *et al.* (2014), who reported slight increase in body weight when plant extract was used in *Trypanosome* infected mice and also corroborated with the findings of Feyera *et al.* (2014) who reported the treatment with crude extracts of *Zingiber officinale* prevented loss of weight associated with parasitaemia. Results in the present study also agrees with the work of Kifleyohannes *et al*. (2014) who reported that the weight in the untreated infected rats group started to decrease 12 days post infection till all the rats died by day18. While, those standard drug and extract of *Artemisia absinthium* and *Moringa stenopetala* treated rats generally showed a gradual increase in mean weight until the end of the experimental period.

The finding is also in agreement with finding of Tadesse *et al.* (2015) who reported, treatment with the crude extracts prevented loss in body weights particularly at higher doses. However the report is not in agreement with reports of Ngure *et al.* (2009) who reported that extract of *A. indica* and suramin-treated groups had significant decline in body weight. The aqueous and methanol extracts of *Verbascum sinaiticum* were capable of improving body weight of treated animals on days 8–14 as compared to the untreated control group (Mergia *et al*, 2016).

The packed cell volume PCV of the rats treated with the extracts show significant difference ($P<0.05$) among the treatment group with glycoside and control being treated with standard drug has highest PCV. The decrease in PCV in this study possible since anaemia is the most outstanding laboratory and clinical feature of trypanosomosis (Suliman and Fieldman, 1989). Anemia as shown by PCV level was reported to worsen with the increase in the level of parasitaemia (Ogbadoyi *et al.,* 1999). Though the *infected* rats treated with 400mg/kg/bodyweight of synergistic crude Alkaloids and Glycosides was able to overcome the infection after 4 days of treatment but has low PCV compared to rats treated with 100mg/kg/bodyweight of Glycosides which has high PCV. Anaemia could also be the reason for the death of other animals after treatment. This shows that Glycosides extract of *Azadirachta indica* leaf extractmight have PCV improving properties.

The extract (Alkaloids and Glycosides) significantly decreased weight loss and decline in PCV associated with parasitaemia. As reported by Kagira *et al*. (2006) PCV and weight loss are critical features in the pathogenesis of trypanosomiasis contributing to mortality. Infected albino rats treated with Alkaloids and Glycosides crude extract in this study showed significant PCV levels in some treatment groups compared to the infected untreated (negative control) group, thus can be ascribed to an enhanced resistance of erythrocyte haemolysis. These can be attributed to the presence of phytochemicals in extracts of *A. indica* (Kagira *et al*., 2006).

V. CONCLUSIONS AND RECOMMENDATIONS

The current study provides strong confirmation on the use of plant base products in the management of trypanosomiasis, in a natural, safe and cheap approach. The study also revealed that Alkaloids and Glycosides can increased the weight and PCV when used at appropriate dose. The study also confirmed that phytochemical are responsible for anti trypanosomal activity of most of the plant extracts. There is need for continuous studies in order to extract and purify more compounds responsible for the antitrypanosomal activity of the studied plant. The Alkaloids and Glycosides of different *Azadirachta indica* parts should be tested on the same and other trypanosomes. Other toxicological studies lipid profile and other parameters analysis also need to be investigated.

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