Anti-Anaemic Properties of the Ethanolic Extracts of *Psidium guajava* in *Trypanosoma brucei brucei* Infected Rats

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**Abstract:** Anaemia has been shown to be a cardinal feature in African trypanosomosis. This study evaluated the effects of the ethanolic extract of *Psidium guajava* leaf on some haematological indices in rats experimentally infected with *Trypanosoma brucei brucei*. Observations revealed significant (p<0.05) decreases in the values for Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC) counts, Mean Corpuscular Volume (MCV), Mean Concentration Haemoglobin Count (MCHC) in infected group relative to the treated as well as the uninfected animals. There was a significant (p<0.05) increase in the White Blood Cell (WBC) counts in infected animals when compared with the infected but treated animals. Also significant changes were observed for neutrophil in the infected animals compared to control and infected but treated groups (p<0.05). However, treatment with the ethanolic extract was able to significantly (p<0.05) improve the PCV, Hb, RBC, MCV, MCHC and neutrophil levels relative to the infected but untreated animals. Results demonstrate the anti-anaemic properties of the ethanolic extract of *P. guajava* in rats infected with *T. brucei*.

**Key words:** *Psidium guajava*, trypanosoma, haematological, anti-anaemic, neutrophil, Nigeria

**INTRODUCTION**

African trypanosomosis or sleeping sickness is a disease caused by *Trypanosoma brucei* species has continued to contribute adversely to the economic and social well being of sub-Saharan Africans. This scourge which is a pressing challenge requires probable action plan that would be basic on the poor resources of affected communities. The articulation of such plan would include both preventive measures and treatment modalities (Okochi et al., 2003). Chemotherapy of sleeping sickness is unsatisfactory (Fairlamb, 2003). Current drugs used in the management of African sleeping sickness include suramin, eflornithine, melarsoprol and nifurtimox (Fairlamb, 2003; Kennedy, 2004). Trypanosomosis is further complicated by anemia, thrombocytopaenia and leucopaenia (Abubakar et al., 2005) all or some of which may be related to breakdown of the immune system and the observable pathological consequences of infection. Anaemia is a constant feature of trypanosome infections (Murray and Dexter, 1988; Ekanem et al., 1996).

The severity of anaemia usually reflects the intensity and duration of parasitaemia which also correlates with the severity of infection (Ancsa, 1988; Murray and Dexter, 1988). Formulations or natural products which boost the host immune system and possibly reduce parasitaemia or completely remove parasites from the host system could contribute extensively to the control or management of the disease (Hoet et al., 2004; Chibale, 2005). We had earlier reported that the administration of ethanolic extract of *P. guajava* to *Trypanosoma brucei* infected rats was able to reduce the parasitaemia and significantly extend the survival time of treated rats when compared with infected but untreated animals (Adeyemi et al., 2009). This study was designed to evaluate the extent to which the ethanolic extract of *P. guajava* leaf could influence the state of anaemia in *T. brucei* infected rats.

**MATERIALS AND METHODS**

**Extract preparation:** *P. guajava* leaves were harvested at a local farm in Ilorin, Kwara State, Nigeria. The leaves were identified and authenticated at the Herbarium Unit, Department of Botany, University of Ilorin, Nigeria where the specimen voucher was also deposited for reference purpose. Sample of the leaves were air dried and ground into powder form using a shear blade electric blender. About 100 g portion of the sample was soaked in 80% ethanol (v/v) for 24 h after which it was filtered and concentrated at 40°C according to the method described.
by Vieira et al. (2001). The concentrate was then evaporated to dryness at room temperature to obtain a dry sample. An aqueous preparation of the extract corresponding to the reported trypanosome parasite clearance curative dose of 150 mg kg⁻¹ body weight (Adeyemi et al., 2009) was then made in distilled water prior to intraperitoneal administration to the rats.

**Animal grouping/treatment:** Wistar rats weighing between 200-220 g were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Nigeria. The rats were kept in well-ventilated house conditions with free access to normal rat pellets (Bendel Feeds and Flour Mills, Ltd., Ewu, Nigeria) and clean water. The rats were randomly distributed into 4 groups of 20 rats each. Rats in Group A were not infected with T.b. brucei and were not administered *P. guajava* extract. Those in Group B were also not infected with *T.b. brucei* but were administered with 150 mg kg⁻¹ body weight of ethanolic extract of *P. guajava*. Rats in Group C were infected with *T.b. brucei* but were not administered with the ethanolic extract while those in Group D were infected with *T.b. brucei* and also administered with 150 mg kg⁻¹ body weight of the ethanolic extract. About 5 rats were sacrificed from each group on days 1, 3, 5 and 7, respectively (Adeyemi et al., 2009).

All experiments conform to guidelines governing the handling of laboratory animals as laid out by the University of Ilorin Committee on Ethics for Scientific and Medical Research.

**Haematological studies:** Rats were anaesthetized in glass jar containing cotton wool soaked in chloroform. Blood used for haematological analysis was collected into heparinised sample bottles and used for analyses within 24 h of collection. Blood parameters including Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Haemoglobin (Hb), neutrophil, Mean Cell Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) were determined using the Automated Haematologic Analyzer, (Sysmex, KX-21, Japan).

**Statistical analysis:** The group mean±SEM was calculated for each analyte and significant difference between means evaluated by Analysis of Variance (ANOVA). Post-hoc test analysis was done using the Tukey multiple comparison test. Values at \( p < 0.05 \) were considered as statistically significant.

**RESULTS AND DISCUSSION**

The results for the haematological studies are as shown in Table 1. There was a significant decrease (\( p < 0.05 \)) in the values for PCV, Hb and RBC counts in the infected groups (C and D) relative to the other groups (A and B) throughout the course of experiment. Lower (\( p < 0.05 \)) values were also obtained for MCV, MCHC in the infected animals when compared to the other groups. Treatment with the ethanolic extract was however able to significantly (\( p < 0.05 \)) improve the PCV, Hb, RBC, MCV and MCHC values relative to the infected but untreated animals. A significant (\( p < 0.05 \)) increase was also observed for neutrophil counts in the infected and treated groups on days 3, 5 and 7, respectively relative to control. Likewise there was a significant (\( p < 0.05 \)) increase in the WBC counts for infected animals when compared with their infected but treated counterparts.

Measurement of anaemia gives an indication of severity of the disease (Poltera, 1985; Anosa, 1988; Suliman and Feldman, 1989; Pentreath and Kennedy, 2004). The significant (\( p < 0.05 \)) decrease in the levels of PCV, RBC and Hb (Table 1) in the infected animals when compared to other groups may be attributed to the trypanosome induced disruption of red blood cell membrane (Ekanem et al., 1996). This may have resulted in subsequent haemolysis as reflected in low RBC count. Acute haemolysis has been demonstrated as a cardinal feature in African trypanosomosis (Murray and Dexter, 1988; Orhue et al., 2005; Ekanem and Yusuf, 2008; Adamu et al., 2009). The decrease in PCV may also be attributed to infection induced low reduced Glutathione (GSH) concentration on the membrane surface of the red blood cells thus making the membrane liable to oxidative lysis secondary to the metabolic activities of the proliferating trypanosomes.

Previous reports have shown that low GSH predisposes red blood cells to oxidative damage (Taiwo et al., 2003; Akanji et al., 2009). Oxidative cell damage is a prominent feature in *T.b. brucei* infections (Igbokwe 1994; Ogunsarmi and Taiwo, 2001; Omer et al., 2007; Saleh et al., 2009). There were significant (\( p < 0.05 \)) improvement in the PCV, Hb, RBC, MCV, MCHC and neutrophil levels (Table 1) of the animals administered with *P. guajava* extract.

Though, the extract did not bring the levels of these haematological indices in the extract treated animals to the levels of those of the control, results suggest less severity of anaemic condition which is usually associated with trypanosome infection.

The significant decrease (\( p < 0.05 \)) in the PCV, Hb and RBC counts observed for treatment of uninfected animals at 150 mg kg⁻¹ body weight may be as a result of the ability of the extract to chelate iron (Setheeworrarit et al., 2005).
In contrast, the administration of the ethanolic extract was able to increase the neutrophil levels of both the treated animals relative to control as well as infected but untreated animals (p<0.05). The reduced PCV in the infected animals could be due to trypanosome induced depletion of GSH on the surface of the RBC thus making the cell liable to oxidative lysis. This probably may also account for the low Hb level and RBC counts observed in this group. On the other hand increased WBC counts in the infected animals compared to the other groups could be attributed to the efforts of the defense system of the animals to eliminate invading trypanosomes. The anti-anaemic properties of the ethanolic extract of *P. guajava* may be attributed to the presence of the phytochemicals as previously demonstrated (Adeyemi et al., 2009).

**CONCLUSION**

In this study, we have been able to show that the ethanolic extract of *P. guajava* improved the anaemic states of the treated animals when compared with the trypanosome infected but untreated animals. To the knowledge, this is the first report of the anti-anaemic properties of *P. guajava* extract which supports further the traditional applications of *P. guajava* plants in the treatment of different ailments locally.

**REFERENCES**


