Research Journal of Pharmacology 4 (3): 74-77, 2010
ISSN: 1815-9362

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

[1]O.S. Adeyemi, [2]M.A. Akanji and [3]J.T. Ekanem
[1]Department of Chemical Sciences, Redeemer's University, P.M.B. 3005, 121001 Mowe, Nigeria
[2]Department of Biochemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria
[3]Department of Biochemistry, University of Uyo, P.M.B. 1017, Uyo, Nigeria

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.b. brucei*.

Key words: *Psidium guajava*, trypanosome, haematological, anti-anaemic, nevtrophil, 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 anaemia, 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 (Anosa, 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.b. 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

Corresponding Author: O.S. Adeyemi, Department of Chemical Sciences, Redeemer's University, P.M.B. 3005, 121001 Mowe, Nigeria

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; Ogunsanmi 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 (Settheeworrarit *et al*., 2005).

Table 1: Effect of administration of ethanolic extract of *Psidium guajava* leaf on hematological parameters in rats

Rat group days	PCV (%)	RBC Counts ($x10^{12}$ L^{-1})	WBC ($x10^{9}$ L^{-1})	Hb Concentration (g dL^{-1}l)	MCV (fL)	MCHC (g dL^{-1})	Neutrophil 10^{3} mL^{-1}
A (Control)							
1	37.79±0.25^{g}	5.65±0.13^{g}	19.04±2.20^{b}	12.46±0.06^{ef}	67.46±0.35^{f}	32.97±0.25^{bc}	24.85±0.08^{a}
3	37.26±0.13^{f}	5.73±0.06^{g}	18.54±1.11^{a}	12.52±0.03^{d}	65.21±0.02^{e}	33.60±0.14^{c}	25.50±0.13^{a}
5	37.23±0.13^{g}	5.46±0.06^{f}	18.33±1.11^{a}	12.36±0.03^{d}	68.28±0.02^{f}	33.20±0.14^{c}	25.50±0.13^{a}
7	37.32±0.22^{f}	5.61±0.07^{g}	18.48±2.87^{a}	12.41±0.09^{ef}	66.49±0.09^{ef}	33.25±0.10^{bc}	25.50±0.02^{a}
B (Extract only)							
1	37.30±0.07^{f}	5.79±0.07^{g}	18.63±1.55^{ab}	12.37±0.10^{f}	64.32±0.08^{d}	33.16±0.14^{bc}	26.00±0.01^{a}
3	34.63±0.06^{cd}	5.41±0.03^{e}	18.51±2.03^{ab}	11.52±0.08^{c}	63.38±0.21^{c}	33.22±0.04^{c}	27.62±0.99^{b}
5	31.17±0.06^{b}	4.81±0.06^{d}	19.87±0.87^{ab}	10.14±0.06^{b}	63.78±0.08^{c}	29.82±0.20^{ab}	29.37±0.05^{c}
7	29.80±0.18^{b}	4.65±0.07^{c}	18.34±1.33^{ab}	9.85±0.03^{a}	62.92±0.39^{b}	26.41±0.07^{a}	28.49±0.04^{c}
C (Infection only)							
1	37.91±0.23^{h}	5.88±0.08^{h}	18.22±1.34^{ab}	12.59±0.13^{g}	64.38±0.17^{d}	33.21±0.06^{c}	25.28±0.08^{a}
3	30.47±0.08^{b}	5.02±0.03^{e}	21.18±1.26^{bc}	8.12±0.01^{b}	60.78±0.07^{b}	27.01±0.03^{a}	25.67±0.06^{a}
5	28.53±0.03^{a}	4.46±0.02^{b}	23.32±1.06^{cd}	7.48±0.02^{a}	58.52±0.08^{a}	25.29±0.03^{a}	27.06±0.11^{b}
7	28.57±0.05^{a}	4.09±0.03^{a}	23.22±1.74^{cd}	6.45±0.02^{a}	53.62±0.04^{a}	21.36±0.20^{a}	28.87±0.08^{c}
D (Infection and extract)							
1	37.01±0.31^{f}	5.49±0.03^{f}	18.51±1.90^{ab}	12.27±0.03^{ef}	67.28±0.26^{f}	33.15±0.12^{bc}	25.56±0.03^{a}
3	33.43±0.13^{c}	5.14±0.06^{e}	19.46±1.75^{ab}	9.75±0.03^{b}	63.30±0.10^{c}	29.14±0.16^{ab}	28.72±0.07^{b}
5	34.95±0.05^{e}	4.72±0.05^{c}	18.50±1.00^{a}	10.89±0.06^{c}	64.39±0.06^{d}	31.72±0.10^{b}	27.61±0.04^{b}
7	34.39±0.25^{cd}	4.95±0.03^{cd}	19.44±2.90^{b}	10.68±0.05^{c}	64.32±0.01^{d}	31.39±0.10^{b}	27.30±0.10^{b}

*Values are mean±SEM; n = 5. Values in the same column with different superscripts are significantly different $p<0.05$

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

Abubakar, A., B. Iliyasu, A.B. Yusuf, A.C. Igweh and N.A. Onyekwelu *et al*., 2005. Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in Wistar rats. Biokemistri, 17: 95-99.

Adamu, S., N. Barde, J.N. Abenga, N.M. Useh, N.D.G. Ibrahim and K.A.N. Esievo, 2009. Experimental *Trypanosoma brucei* infection-induced changes in the serum profiles of lipids and cholesterol and the clinical implications in pigs. J. Cell Anim. Biol., 3: 015-020.

Adeyemi, O.S., M.A. Akanji and S.A. Oguntoye, 2009. Ethanolic leaf extract of *Psidium guajava*: Phytochemical and trypanocidal activity in rats infected with *Trypanosoma brucei* brucei. J. Med. Plant Res., 3: 420-423.

Akanji, M.A., O.S. Adeyemi, S.O. Oguntoye and F. Sulyman, 2009. *Psidium guajava extract* reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. EXCLI J., 8: 148-154.

Anosa, V.O., 1988. Haematological and biochemical changes in human and animal trypanosomiasis Part 1. Rev. Elev. Med. Vet. Pays Trop., 41: 65-78.

Chibale, K., 2005. Economic drug discovery and rational medicinal chemistry for tropical diseases. Pure Applied Chem., 77: 1957-1964.

Ekanem, J.T. and O.K. Yusuf, 2008. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei*-infected rats. Afr. J. Biomed. Res., 11: 79-85.

Ekanem, J.T., M.A. Akanji and A.A. Odutuga, 1996. Extracellular proteins of *Trypanosomal brucei* origin lyse erythrocytes in rat *in vitro*. Biokemistri, 6: 21-29.

Fairlamb, A.H., 2003. Chemotherapy of human African trypanosomiasis: current and future prospects. Trends Parasitol., 19: 488-494.

Hoet, S., Opperdoes, B. Brun and J. Quetin-Leclereq, 2004. Natural products active against African trypanosomes: A step towards new drugs. Nat. Prod. Rep., 21: 353-364.

Igbokwe, I.O., 1994. Mechanisms of cellular injury in African trypanosomiasis. Vet. Bull., 64: 611-620.

Kennedy, P.G.E., 2004. Human African trypanosomosis of the CNS: Current issues and challenges. J. Clin. Invest., 113: 496-504.

Murray, M. and T.M. Dexter, 1988. Anaemia in African trypanosomiasis: A review. Acta Trop., 95: 389-432.

Ogunsanmi, A.O. and V.O. Taiwo, 2001. Pathobiochemical mechanisms involved in the control of the disease caused by *Trypanosoma congolense* in African grey duiker (*Sylvicapra grimmia*). Vet. Parasitol., 96: 51-63.

Okochi, V.I., J. Okpuzor, M.O. Okubena and A.K. Awoyemi, 2003. The influence of African Herbal Formula on the haematological parameters of trypanosome infected rats. Afr. J. Biotechnol., 2: 312-316.

Omer, O.H., H.M. Mousa and N. Al-Wabel, 2007. Study on the antioxidant status of rats experimentally infected with Trypanosoma evansi. Vet. Parasitol., 145: 142-145.

Orhue, N.E.J., E.A.C. Nwanze and A. Okafor, 2005. Serum total protein, albumin and globulin levels in Trypanosoma brucei-infected rabbits: Effect of orally administered *Scoparia dulcis*. Afr. J. Biotechnol., 4: 1152-1155.

Pentreath, V.W. and G.E. Kennedy, 2004. Pathogenesis of Human African Trypanosomiasis. In: The Trypanosomiasis, Maudin, I., P.H. Holmes and Miles (Eds.). CABI Publishing, UK., 283-301.

Poltera, A.A., 1985. Pathology of human african trypanosomiasis with reference to experimental African trypanosomiasis and infections of the central nervous system. Br. Med. Bull., 41: 169-174.

Saleh, M.A., M.A. Bassam and S.A. Sanousi, 2009. Oxidative stress in blood of camels (Camelus dromedaries) naturally infected with Trypanosoma evansi. Vet. Parasitol., 162: 192-199.

Settheeworrarit, T., S.K. Hartwell, S. Lapanatnoppakhun, J. Jakmunee, G.D. Christian and K. Grudpan, 2005. Exploiting guava leaf extract as an alternative natural reagent for flow injection determination of iron. Talanta, 68: 262-267.

Suliman, H.B. and B.F. Feldman, 1989. Pathogenesis and aetiology of anaemia in trypanosomiasis with special reference to *T. brucei* and *T. evansi*. Vet. Bull., 59: 99-107.

Taiwo, V.O., M.O. Olaniyi and A.O. Ogunsanmi, 2003. Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental *Trypanosome congolense* and *T. brucei* infections in sheep. J. Isreal Vet Med. Assoc., 58: 112-117.

Vieira, R.H.S.F., D.P. Rodrigues, F.A. Gonçalves, F.G.R. Menezes, J.S. Aragao and O.V. Sousa, 2001. Microbicidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carica papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. Rev. Inst. Med. Trop. S. Paulo, 43: 145-148.