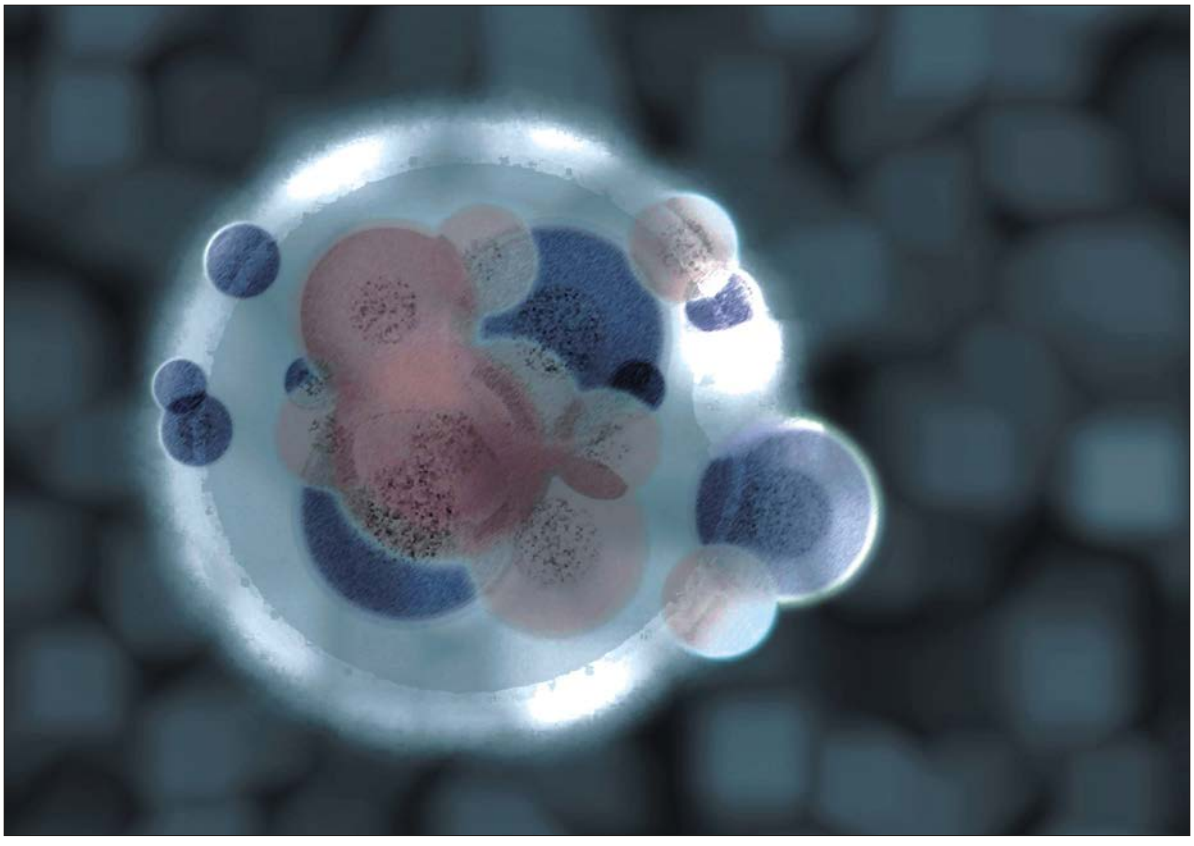

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Ethanolic Extract of *Psidium guajava* Influences Protein and Bilirubin Levels in *Trypanosoma brucei brucei* Infected Rats

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Abstract: Previous report from our laboratory demonstrated the activity of ethanolic extract of *Psidium guajava* leaf against bloodstream forms of trypanosomes in an experimental infection. The present study investigated the mitigating effects of *P. guajava* leaf extract on *Trypanosoma brucei brucei*-induced changes in rat serum and tissue total protein, albumin, globulin, unconjugated bilirubin and total bilirubin. Our data revealed that trypanosome infection caused a rise in protein and globulin concentrations but reduced albumin concentrations were observed ($p<0.05$). There was also a significant increase in the serum and tissue bilirubin concentrations of the infected animals. In contrast, however, these biochemical alteration were less severe ($p<0.05$) in the treated group relative to the untreated counterparts. The data suggest the capacity of the ethanolic extract of *P. guajava* in downplaying the severity of the conditions usually associated with trypanosome infections.

Key words: *Psidium guajava*, protein, bilirubin, trypanosomes

INTRODUCTION

African trypanosomosis is a disease caused by *Trypanosoma brucei* species for which both humans and animals stand the risk of epidemic (Chretien and Smoak, 2005). Chemotherapy is one of the major means of controlling the disease but currently the four drugs (pentamidine, suramin, melarsoprol and eflornithine) that are available for treatment of African trypanosomosis are beset with challenges of severe toxicity and increasing incidence of trypanosome resistance. This situation highlights the need to develop newer and safer trypanocides (Fairlamb, 2003; Ekanem and Yusuf, 2008) by exploring efficacious chemotherapeutic agents from locally available ethnomedicinal plants. *P. guajava* is a tropical plant belonging to the family Myrtaceae and has been used for a number of traditional medicine applications (Hawrelak, 2003; Gutierrez *et al*., 2008). Available scientific evidence revealed that *P. guajava*, plant has a long history of traditional use for several diseases as well as for consumption for its great taste and nutritional benefits (Kamath *et al*., 2008). Phytochemical screening revealed that *P. guajava* leaf extract is rich in flavonoids and tannins amongst others. Examples of phytochemicals isolated from the *P. guajava* plants include quercetin, guajaverin, flavonoids and galactose-specific lecithins which have shown promising activity in many human trials (Abdelrahim *et al*., 2002). The leaves and bark of *P. guajava* tree have a long history of medicinal uses which may still be relevant today. In a study by Nwinyi *et al*. (2008), *P. guajava* extract was implicated as a potential anti-diarrheal, antihypertensive, hepatoprotective, antioxidant, antimicrobial, hypoglycemic and antimutagenic agent.

Furtherance to evidence of anti-trypanosoma activity shown by the leaf extract of *P. guajava* (Adeyemi *et al*., 2009), this study was designed to investigate the mitigating capacity of *P. guajava* leaf extract at a therapeutic dosage of 150 mg kg^{-1} body weight (Adeyemi *et al*., 2009) against *T. b. brucei* induced biochemical alterations in rats.

MATERIALS AND METHODS

Plant materials: Fresh samples of *Psidium guajava* (guava) leaves were collected from a local farm in Ilorin, Kwara State, Nigeria. The leaves were identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Nigeria.

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Leaf extract preparation: The ethanolic extract of the plant was prepared according to the method described by Vieira *et al.* (2001) and modified by Akanji *et al.* (2009). Fresh samples of *P. guajava* leaves were air dried and ground. The ground sample weighing 200 g was soaked in 80% (v/v) ethanol and left for 24 h. The mixture was filtered using Whatman No. 1 filter paper and the filtrate concentrated *in vacuo*. The concentrate was then evaporated to dryness at 40°C to obtain a dry sample matter which was subsequently used to prepare the ethanolic extract in distilled water prior to intraperitoneal administration to rats. The percentage yield was 2.45%.

Parasite: *T. b. brucei* was obtained from the Veterinary and Livestock Studies Department, Nigerian Institute for Trypanosome Research (NITR) Vom Jos, Nigeria. The parasite was injected intraperitoneally into rats and maintained by repeated passaging into other rats.

Animal grouping/treatment: Ten week old Wistar rats weighing between 200-220 g were obtained from the 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 three 3 groups of twenty 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 infected with *T. b. brucei* but were not administered with ethanolic extract of *P. guajava*. Rats in group C were infected with *T. b. brucei* and were administered with 150 mg kg^{-1} body weight of the ethanolic extract. Five 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.

Inoculation of rats with parasite: Parasite infected blood was obtained from the tail of infected rats at high parasitaemia and used to maintain parasite suspension in 0.90% saline solution which was inoculated into the peritoneal cavity of uninfected rats weighing approximately between 200-220 g. The suspension was as described by Adeyemi *et al.* (2009) contained 3 or 4 trypanosome per view at x100 magnification.

Tissue collection and preparation: Rats were anaesthetized in slight chloroform and blood samples collected into clean, dry centrifuge tubes by cardiac puncture. Blood samples were allowed to stand for 10 min at room temperature and then centrifuged at 1000 g for 15 min using a laboratory centrifuge (SM 800B, Surgifriend Medicals, England) and the serum carefully separated and kept frozen until needed. The tissues (liver, kidney and brain) were excised, cleansed of superficial connective tissues and then transferred into ice-cold 0.25 M sucrose solution. These were blotted with clean tissue paper and weighed. The tissues were homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v) using Teflon homogenizer. The homogenates were kept frozen until required for further analyses.

Biochemical determinations: Serum and tissue total protein and albumin levels were determined using commercially available test kits, products of Randox laboratories, UK. The manufacturers instructions were strictly adhered to Serum globulin was estimated as the difference between serum total protein and albumin. Total bilirubin and direct bilirubin were determined according to the method of Jendrassik and Grof (1938) using Randox diagnostic reagent kit.

Statistical analysis: The group Mean±SEM was determined and difference between means evaluated by analysis of variance (ANOVA). Post test analysis was carried out using the Tukey's multiple comparison tests. Values at $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

This study was designed to evaluate the efficacy of the ethanolic extract of *P. guajava* leaf to modulate some trypanosome associated lesions in rats. The ethanolic extract was administered to the rats daily in an experiment that lasted for seven days. Protein, albumin, globulin and bilirubin concentrations were used as indicators for assessment. Results obtained for albumin concentrations in the brain showed no significant differences ($p>0.05$). Significant change was observed for albumin concentrations in the kidney, liver and serum of the infected animals relative to other groups ($p<0.05$) (Table 1). Globulin and protein concentrations were significantly increased in the tissues and serum of infected groups compared to the uninfected control and infected and treated groups ($p<0.05$) (Table 2 and 3, respectively). Likewise, the infection also induced an increase in the concentrations of unconjugated bilirubin in the infected groups relative to the other groups. Also a significant change was observed for total bilirubin in the infected animals relative to the other groups ($p<0.05$) (Table 4, 5).

Table 1: Effects of *Psidium guajava* leaf extract on albumin changes induced by *T. b. brucei* in rats

Rat groups	Day 1	Day 3	Day 5	Day 7
Brain				
Control	1.94±0.11	1.76±0.26	1.89±0.22	1.82±0.12
Infection	1.78±0.08	1.82±0.23	1.80±0.32	1.87±0.00
Infection and treatment	1.89±0.24	1.77±0.03	1.71±0.08	1.68±0.06
Kidney				
Control	2.04±0.05	1.95±0.31	2.00±0.18	1.99±0.00
Infection	2.01±0.01	2.17±0.37***	2.21±0.06***	2.34±0.05***
Infection and treatment	1.86±0.00**	2.05±0.02	2.05±0.03	2.12±0.21
Liver				
Control	1.89±0.04	2.08±0.04	2.09±0.04	1.76±0.20
Infection	2.00±0.10	2.05±0.05***	2.11±0.00***	2.13±0.80***
Infection and treatment	2.00±0.00	1.94±0.03	1.91±0.00	1.84±0.40
Serum				
Control	3.57±0.06	3.56±0.20	3.54±0.04	3.37±0.03
Infection	3.77±0.06	2.81±0.20***	2.89±0.00***	2.93±0.06***
Infection and treatment	3.78±0.20	3.70±0.06**	3.38±0.06**	3.08±0.43**

*Values are mean of 5 determinations±SEM, values with different superscripts differ significantly ($p<0.05$). *Significantly different from the control at $p<0.05$, **Significantly different from the control and infection at $p<0.05$, ***Significantly different from the control, infection and treatment at $p<0.05$

Table 2: Effects of *Psidium guajava* leaf extract on globulin changes induced by *T. b. brucei* in rats

Rat groups	Day 1	Day 3	Day 5	Day 7
Brain				
Control	26.96±0.00	26.12±0.02	25.84±0.54	27.65±0.29
Infection	28.54±0.21***	18.82±0.01***	16.95±0.29***	16.20±0.06***
Infection and treatment	26.27±0.33**	22.23±0.03**	23.58±0.21**	24.43±0.54**
Kidney				
Control	23.58±0.21	25.09±0.23	24.76±0.01	25.25±0.76
Infection	20.90±0.01***	22.93±0.11***	23.79±0.38***	26.98±0.65***
Infection and treatment	24.14±0.35	25.35±0.01	24.95±0.00	26.48±0.58
Liver				
Control	22.71±0.65	22.66±0.06	22.39±0.08	22.53±0.27
Infection	21.74±0.23	23.90±0.04***	24.11±0.56***	25.50±0.32***
Infection and treatment	22.38±0.03	21.28±0.63	21.26±0.09	21.75±0.29
Serum				
Control	48.73±0.23	48.47±0.48	48.94±0.03	49.57±0.45
Infection	49.57±0.32	51.17±0.43***	51.93±0.12***	52.87±0.04***
Infection and treatment	49.20±0.04	49.34±0.55	48.82±0.07	48.24±0.20

*Values are mean of 5 determinations±SEM, values with different superscripts differ significantly ($p<0.05$). *Significantly different from the control at $p<0.05$, **Significantly different from the control and infection at $p<0.05$, ***Significantly different from the control, infection and treatment at $p<0.05$

Table 3: Effects of *Psidium guajava* leaf extract on protein changes induced by *T. b. brucei* in rats

Rat groups	Day 1	Day 3	Day 5	Day 7
Brain				
Control	28.90±0.02	27.88±0.01	27.73±0.18	29.47±0.04
Infection	30.32±0.01***	20.64±0.01***	18.75±0.02***	18.07±0.01***
Infection and treatment	28.16±0.04	24.00±0.00**	25.29±0.08**	26.11±0.12**
Kidney				
Control	25.62±0.12	27.04±0.11	26.76±0.05	27.24±0.01
Infection	22.91±0.00***	25.10±0.00***	26.00±0.00***	29.32±0.01***
Infection and treatment	26.00±0.01	27.40±0.09	27.00±0.01	28.60±0.08
Liver				
Control	24.60±0.00	24.74±0.00	24.48±0.61	24.29±0.08
Infection	23.74±0.12	25.95±0.04***	26.22±0.10***	27.63±0.01***
Infection and treatment	24.38±0.01	23.22±0.01**	23.17±0.12**	23.59±0.00
Serum				
Control	52.30±0.11	52.03±0.00	52.48±0.20	52.94±0.07
Infection	53.34±0.01	53.98±0.11	54.82±0.11***	55.80±0.12***
Infection and treatment	52.90±0.21	52.12±0.20	51.65±0.16	51.17±0.20

*Values are mean of 5 determinations±SEM, values with different superscripts differ significantly ($p<0.05$). *Significantly different from the control at $p<0.05$, **Significantly different from the control and infection at $p<0.05$, ***Significantly different from the control, infection and treatment at $p<0.05$

Evaluating body fluids could give an indication of the functional state of the various body organs. According to Awobode (2006), biochemical changes in body fluids resulting from infections are dependent on the species of the parasite and its virulence. The results for total protein, albumin and globulin levels are presented in Table 1-3. Infection by trypanosomes caused significant increases in total protein and globulin ($p<0.05$) relative

Table 4: Effects of *Psidium guajava* leaf extract on direct bilirubin changes induced by *T. b. brucei* in rats

Rat groups	Day 1	Day 3	Day 5	Day 7
Brain				
Control	0.88±0.04	0.88±0.00	0.85±0.02	0.89±0.00
Infection	1.50±0.00***	1.41±0.03***	1.42±0.03***	1.55±0.06***
Infection and treatment	1.06±0.03**	1.31±0.00**	1.29±0.40**	1.26±0.40**
Kidney				
Control	1.00±0.01	1.02±0.10	1.02±0.04	0.94±0.04
Infection	1.03±0.02	1.45±0.02***	1.55±0.02***	1.64±0.00***
Infection and treatment	1.28±0.01**	1.32±0.06**	1.13±0.01**	0.88±0.06**
Liver				
Control	0.58±0.08	0.60±0.02	0.60±0.02	0.59±0.04
Infection	0.53±0.03	1.34±0.00***	1.45±0.05***	1.55±0.01***
Infection and treatment	0.57±0.02	0.55±0.00	0.64±0.00	0.57±0.01
Serum				
Control	2.23±0.00	2.14±0.06	2.13±0.02	2.12±0.01
Infection	2.50±0.02	2.66±0.04***	2.79±0.02***	2.84±0.03***
Infection and treatment	2.14±0.03	1.71±0.06**	1.79±0.01**	1.93±0.01**

*Values are mean of 5 determinations±SEM, values with different superscripts differ significantly ($p<0.05$), *Significantly different from the control at $p<0.05$, **Significantly different from the control and infection at $p<0.05$, ***Significantly different from the control, infection and treatment at $p<0.05$

Table 5: Effects of *Psidium guajava* leaf extract on total bilirubin changes induced by *T. b. brucei* in rats

Rat groups	Day 1	Day 3	Day 5	Day 7
Brain				
Control	1.49±0.08	1.54±0.04	1.44±0.03	1.48±0.00
Infection	1.41±0.01*	1.99±0.01*	1.83±0.02*	1.69±0.01*
Infection and treatment	1.03±0.12*	1.88±0.04*	1.96±0.00*	1.54±0.11*
Kidney				
Control	1.36±0.00	1.32±0.13	1.29±0.12	1.36±0.06
Infection	1.42±0.02	1.71±0.01***	1.81±0.08***	1.90±0.10***
Infection and treatment	1.32±0.11	1.26±0.01	1.25±0.21	1.29±0.00
Liver				
Control	1.32±0.02	1.26±0.12	1.21±0.00	1.25±0.02
Infection	1.26±0.0	1.93±0.20***	1.94±0.13***	1.85±0.00***
Infection and treatment	1.22±0.01	1.20±0.04**	1.32±0.12**	1.41±0.02**
Serum				
Control	2.14±0.06	2.18±0.01	2.10±0.00	2.16±0.04
Infection	2.42±0.01	3.23±0.01***	3.27±0.11***	3.74±0.02***
Infection and treatment	2.30±0.00	3.07±0.12**	3.08±0.12**	3.59±0.11**

*Values are mean of 5 determinations±SEM, values with different superscripts differ significantly ($p<0.05$), *Significantly different from the control at $p<0.05$, **Significantly different from the control and infection at $p<0.05$, ***Significantly different from the control and infection and treatment at $p<0.05$

to controls. The increase in serum total protein could be as a result of increased release of tissue specific enzymes and other intracellular proteins consequent upon parasite-induced cell membrane disruption (Kennedy, 2004; Orhue *et al*., 2005). Release of erythrocytes-derived enzymes and proteins following lysis of erythrocyte membrane by parasites may be another possible source of plasma protein. Several studies have demonstrated that the destruction of red blood cells which usually correlate with anaemia is a common presentation in *T. b. brucei* infection involving mammals (Anosa and Kaneko, 1983; Anosa, 1988; Igbokwe and Mohammed, 1992; Egbe-Nwiyi and Antia, 1993; Igbokwe *et al*., 1994, 1998; Omotainse and Anosa, 1995). Other reasons for high total protein in the infected group could be ascribed to increased mass of parasite proteins resulting from progressive infection or possibly increases in parasite derived intracellular enzymes and proteins as the parasites are lysed by the host immune system. Secretion of globulin in response to active infection would no doubt have contributed immensely to the observed total protein increases. Serum albumin was significantly decreased for infected animals relative to uninfected controls ($p<0.05$) although, there were increases in albumin levels of the liver, kidney and the brain relative to control ($p<0.05$). Decreased protein synthesis by the liver resulting from assault by infection could be responsible for drop in serum albumin level, alternatively increased protein loss through the gut or the kidney may be responsible. Also decrease in albumin levels may be due to malabsorption and increased protein need secondary to infection (Guyton and Hall, 2000). Albumin levels suggest that the tissues of the infected animals could have been damaged following infection by *T. b. brucei*. Decrease in albumin has been observed in serum of patients with tissue inflammation and damages (Gabay and Kushner, 1999). There have been reports of decreased plasma albumin concentrations in several trypanosome infections (Anosa, 1988) which demonstrated strong link to either plasma expansion, proteinuria or hepatocellular damage (Tijani *et al*., 2009).

The significant higher concentrations of direct bilirubin with growing infection suggest that there could be an acute haemolysis resulting from the activity of proliferating parasites and this agree with previous reports (Igbokwe, 1994; Orhue *et al*., 2005). Further, inconsistent changes were observed for total bilirubin concentration in the tissues and serum (Table 5) as the infection progressed. The high values of serum bilirubin could also be attributed to the gradual inability of the liver to effectively conjugate the bilirubin in circulation. Treatment with ethanolic extract of *P. guajava* resulted in significant reduction in parasite induced biochemical changes evidenced in decreased protein, globulin and bilirubin levels. Although the extract treatment did not achieve complete restoration of the selected biochemical indices to pre-infection state, results revealed significantly ($p<0.05$) reduced infection-induced alterations in the treated group relative to the untreated group. The ability of the administered extract to reduce these biochemical alterations may not be unconnected with the presence of phytochemicals like flavonoids and tannins reported elsewhere (Adeyemi *et al*., 2009; Akanji *et al*., 2009) as having diverse bioactivities including anti-trypanosomal and antioxidant properties. Infections with *T. b. brucei* may have generated reactive oxygen species with subsequent deleterious effects on cell membrane. The administered ethanolic extract acting as antioxidants could have scavenged these radicals and thus reduced damage to cellular components. Previous reports have attributed the anti-trypanosomal properties of *P. guajava* plant extract to the presence of tannins and/or flavonoids possibly because of their iron chelating properties (Adeyemi *et al*., 2009). It is probable that the extract reduced parasitaemia which subsequently led to decreased lesions observed in the treated animals. Severity of infection in trypanosomosis had been shown to correlate with parasitaemia (Ekanem *et al*., 2006; Adeyemi *et al*., 2009).

CONCLUSION

Present study showed that the ethanolic extract of *P. guajava* leaf reduced the severity of trypanosome associated alterations in the treated infected rats relative to their untreated counterparts. This suggests that extracts of *P. guajava* is a potential source of bioactive components which could be explored in the development of phytomedicine against African sleeping sickness. Our results warrant further chemical elucidation and biological profiling.

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