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PSIDIUM GUAJAVA EXTRACT REDUCES TRYPANOSOMOSIS ASSOCIATED LIPID PEROXIDATION AND RAISES GLUTATHIONE CONCENTRATIONS IN INFECTED ANIMALS

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ABSTRACT

We have investigated the effects of the aqueous leaf extract of *P. guajava* on reduced glutathione (GSH) and malondialdehyde (MDA) concentrations in rats experimentally infected with *Trypanosoma brucei brucei*. The results obtained showed that the MDA concentrations in the serum and tissues of the infected animals were significantly increased ($P<0.05$) relative to the control (Table 2). However there was an insignificant difference in the GSH concentrations in the brain for the infected group and the infected but treated group relative to control ($P>0.05$) (Table 1). In contrast, a significant decrease ($P<0.05$) was observed for the GSH concentrations in the liver and kidney for the infected animals compared to the uninfected control and the infected but treated groups. The MDA concentrations in the serum and tissues of the infected but treated animals were significantly reduced when compared to the infected groups ($P<0.05$) (Table 2). In this study it was demonstrated that the aqueous extract was able to reduce the trypanosomosis associated lipid peroxidation as well as raise the level of GSH in the infected but treated animals significantly ($P<0.05$). We present evidence that the ability of the leaf extract of *P. guajava* to lower the MDA concentrations in the treatment group may be attributed to its antioxidant properties.

Keywords: MDA, GSH, Trypanosoma brucei, P. guajava, lipid peroxidation, antioxidant

INTRODUCTION

Tissue damage has been indicated in the pathophysiology of African Trypanosomosis (Igbokwe, 1994; Ogunsanmi & Taiwo, 2001; Umar et al., 2007). Increasing evidence demonstrates that oxidative stress plays an important etiologic role in the pathogenesis of African sleeping sickness (Ogunsanmi & Taiwo, 2001). Oxidant stress arises when there is an imbalance between radical-generating and radical-scavenging activity; it may therefore cause an increase in the formation of oxidation products (Saleh et al., 2009). It has been shown that infections by the *T. brucei* group of parasites may alter the host's antioxidant defence against free radicals (Igbokwe et al., 1996; Omer et al., 2007; Umar et al., 2007). Peroxides and oxygen radicals are aggressive cellular toxins that can destroy connective tissue, damage biological membranes, oxidize sulphydryl groups, inactivate enzymes and cause peroxidative damage of nucleic acids (Ogunsanmi & Taiwo, 2001). Lipids especially polyunsaturated

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fatty acids are sensitive to oxidation, leading to the term lipid peroxidation, of which, malondialdehyde (MDA) is the most abundant (Igbokwe et al., 1996). The accumulation of MDA in tissues or biological fluids is indicative of the extent of free radical generation, oxidative stress and tissue damage (Gutteridge, 1995). There are evidences in experimental studies suggesting that lipid peroxidation is implicated in cell destruction in African trypanosomosis (Igbokwe, 1994). Products of lipid peroxidation formed in various biochemical reactions are normally scavenged by antioxidants. Antioxidants are compounds that are involved in effective scavenging of free radicals and in suppressing the actions of reactive oxygen substances. Reduced glutathione (GSH) and its redox enzymes are the most important cellular antioxidants and play a major role in protecting cells against oxidative stress caused by ROS (Shan et al., 1990). In a separate report by Ogunsanmi & Taiwo (2001), decline in the concentration of serum and liver GSH was associated with oxidative haemolysis in rats experimentally infected with *T. brucei*. We had earlier reported the trypanocidal activities of *P. guajava* leaf extract (Adeyemi et al., 2009). The present study aims at accessing the activity of aqueous extract of guava leaves in ameliorating tissue lipid peroxidation in rats experimentally infected with African *T. brucei*. Malondialdehyde (MDA) values and glutathione (GSH) concentrations were used as a measure of tissue lipid peroxidation and the antioxidant status respectively.

MATERIALS & METHODS

Plant material

Fresh samples of *P. guajava* (guava) leaves, which were used for the study, were collected from a local farm in Ilorin, Kwara State, Nigeria. The plant was identified and authenticated at the Herbarium of the Department of Plant Biology University of Ilorin, Nigeria and voucher specimen has been deposited in the department for reference purpose.

Leaf extract preparation

The ethanolic extract of the leaf was prepared according to the method of Vieira et al., (2001) as described by Adeyemi et al. (2009). 200 g of fresh samples of *P. guajava* leaves were air dried and ground. The ground sample was soaked in ethanol 80 % (v/v) and left for 24 hours. The mixture was filtered and the filtrate concentrated using rotary evaporator. The concentrate was later evaporated to dryness at 40 °C to obtain dry sample matter which was re-dissolved in distilled water before intraperitoneal administration to the animals.

Parasite

Trypanosoma brucei 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

Sixty adult albino rats of either sex weighing between 180 g–220 g were obtained from the Animal Holding Unit of the Department of Chemical Sciences, Bells University of Technology, Ota, Nigeria. The rats were kept in well-ventilated house with free access to normal rat pellets and clean water. They were randomly distributed into twelve groups of five rats each. Groups A-D served as the control and were not inoculated with the parasite but rather received an intraperitoneal injection of 0.3 ml normal saline. All experiments were performed according to the “Principles of Laboratory Animal Care” (Tijani et al., 2009). Further details of distribution is as shown below:

Groups A-D: Control for days 1, 3, 5 and 7 respectively; they were not infected and did not receive administration of *P. guajava* leaf extract.

Groups E-H: Infected with an inoculum containing the parasite for days 1, 3, 5 and 7 respectively. No treatment was given to this group.

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Groups I-L: Infected with inoculum containing the parasite and treated with *P. guajava* leaf extract for days 1, 3, 5 and 7 respectively.

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 180 g – 220 g. The suspension was as described by Ekanem & Yusuf (2005) and Ekanem et al. (2006) 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 which were processed individually were allowed to stand for 10 min at room temperature and then centrifuged at 1000 rpm for 15 min on laboratory centrifuge (Uniscope SM 112, Surgifriend Medicals, U.K.) and the supernatant (serum) carefully removed with Pasteur pipette, and stored frozen until needed for analysis. The tissues (liver, kidney and brain) were excised and transferred into ice-cold 0.25 M sucrose solution. They were later blotted with clean tissue paper and weighed. The tissues were cut finely with clean sterile blade before being homogenized in ice-cold 0.25 M sucrose solution [1:5 w/v] using Teflon homogenizer. The homogenates were kept frozen overnight before analyses.

Biochemical analysis

The tissues were homogenized for MDA determination. Lipid peroxidation as evidenced by the formation of TBARS were measured by the method of Niehaus & Samuelson (1968). In brief, 0.1 ml of tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37 %, 0.25 N HCl and 15 % TCA) and placed in water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1,000 rpm. The absorbance of clear supernatant was measured against reference blank at 535 nm.

Tissue preparation and GSH concentration measurements were done according to Ellman (1959) as described by Rajagopalan et al. (2004). To the homogenate added 10 % TCA, centrifuged. 1 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5,5'-dithiobis(nitrobenzoic acid) (DNTB) in 100 ml of 0.1 % sodium nitrate) and 3 ml of phosphate buffer (0.2 M, pH 8). The absorbance was read at 412 nm.

Statistical analysis

The group mean ± S.E.M. was calculated for each analyte and significant difference between means evaluated by analysis of variance (ANOVA). Post test analysis was carried out using the Tukey multiple comparison test. Values of $P<0.05$ were considered as statistically significant.

RESULTS

Concentrations of GSH in the tissues and serum of infected, infected and treated and uninfected control are shown in Table 1. Concentrations of lipid peroxidation product in the tissues and serum are shown in Table 2. There was an insignificant difference ($P>0.05$) in the GSH concentrations in the brain for the infected group and the infected but treated group. However a significant difference was observed for GSH in the brain of infected animals relative to control ($P>0.05$). A significant decrease ($P<0.05$) also was observed for the GSH concentrations in the liver and kidney for the infected animals compared to the uninfected control. In contrast, no significant difference ($P>0.05$) was observed for GSH concentrations in the liver and kidney of the treated group relative to control. Serum GSH concentrations were higher in the infected but treated group when compared to the infected group ($P<0.05$). Results for

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lipid peroxidation indices showed that the MDA values were significantly (P<0.05) raised in the tissues and serum of all the infected animals when compared to the control (Table 2). MDA values in the tissues and serum for the infected but treated group was significantly reduced (P<0.05) relative to the infected group.

DISCUSSION

Increase in MDA concentrations have been related to the amount of stress and well correlated with lipid membrane damage and deterioration of membrane integrity (Ekmekci & Terzioglu, 2005). Increased generation of MDA in plasma, tissues and erythrocytes was also reported in murine models and humans infected with Trypanosoma cruzi (Chandrasekar et al., 2000; Malvezi et al., 2004; Wen et al., 2004).

Table 1: Effects of aqueous extract *of P. guajava* leaf on GSH concentration in *T. brucei* infected rats

Brain	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control	8.38 ± 0.11	8.44 ± 0.02	8.46 ± 0.02	8.50 ± 0.22
Infection	9.30 ± 0.12*	5.40 ± 0.12*	5.18 ± 0.13*	4.72 ± 0.11*
Infection & treatment	8.96 ± 0.02*	6.74 ± 0.01*	6.34 ± 0.01*	6.94 ± 0.09*
Kidney				
Control	6.06 ± 0.09	6.90 ± 0.01	6.88 ± 0.22	6.60 ± 0.05
Infection	5.90 ± 0.04***	4.18 ± 0.06***	3.64 ± 0.23***	3.28 ± 0.08***
Infection & treatment	5.88 ± 0.00	5.72 ± 0.12	5.94 ± 0.22	6.00 ± 0.01
Liver				
Control	8.86 ± 0.42	9.00 ± 0.32	8.90 ± 0.00	9.24 ± 0.12
Infection	9.02 ± 0.12***	8.56 ± 0.21***	6.14 ± 0.02***	5.38 ± 0.01***
Infection & treatment	8.38 ± 0.11	8.60 ± 0.06	7.99 ± 0.01	8.11 ± 0.09
Serum				
Control	2.46 ± 0.00	2.24 ± 0.02	2.40 ± 0.07	2.56 ± 0.00
Infection	2.30 ± 0.05***	1.48 ± 0.10***	1.08 ± 0.13***	0.80 ± 0.12***
Infection & treatment	2.41 ± 0.01**	2.11 ± 0.00**	2.24 ± 0.02**	2.38 ± 0.14**

*Values are mean ± S.E.M., n=5. Superscripted items differ significantly at P<0.05.

Table 2: Effects of aqueous extract of P. guajava leaf on MDA concentration in T. brucei infected rats

	Day 1	**Day 3**	**Day 5**	**Day 7**
Control	15.71 ± 0.08	15.18 ± 0.12	16.67 ± 0.01	16.38 ± 0.12
Infection	25.15 ± 0.00***	28.98 ± 0.01***	31.95 ± 0.12***	42.76 ± 0.01***
Infection & treatment	18.78 ± 0.12**	23.29 ± 0.20**	18.48 ± 0.15**	18.09 ± 0.80**
Kidney				
Control	15.42 ± 0.52	15.77 ± 0.43	15.10 ± 0.39	15.13 ± 0.44
Infection	24.99 ± 0.29***	25.58 ± 0.48***	26.41 ± 0.32***	27.33 ± 0.48***
Infection & treatment	20.64 ± 0.06**	19.39 ± 0.23**	18.13 ± 0.25**	17.56 ± 0.19**
Liver				
Control	12.36 ± 0.80	13.26 ± 0.39	13.42 ± 0.20	13.78 ± 0.37
Infection	27.65 ± 0.00***	28.86 ± 0.28***	37.18 ± 032***	38.18 ± 0.22***
Infection & treatment	25.85 ± 0.23**	24.53 ± 0.38**	20.51 ± 0.42**	19.22 ± 0.40**
Serum				
Control	4.50 ± 0.80	4.56 ± 0.00	4.35 ± 0.40	4.60 ± 0.40
Infection	7.20 ± 0.27***	9.79 ± 0.00***	12.32 ± 0.91***	13.87 ± 0.53***
Infection & treatment	5.64 ± 0.26**	7.78 ± 0.60**	6.21 ± 0.32**	5.86 ± 0.38**

*Values are mean ± S.E.M., n=5. Superscripted items differ significantly at P<0.05.

- * is significantly different from the control at P<0.05
- ** is significantly different from the control and infection at P<0.05
- *** is significantly different from the control and infection & treatment at P<0.05

The results obtained for the MDA concentrations in the infected groups agree with several other reports (Igbokwe et al., 1996; Ogunsanmi & Taiwo, 2001; Omer et al., 2007; Umar et al., 2007; Saleh et al., 2009). Previous studies showed a decrease in erythrocytic and hepatic glutathione concentrations in rats infected with *T. brucei* (Ameh, 1984; Igbokwe et al., 1998). However in the infected but treated group the MDA values were significantly lower ($P<0.05$) than obtained in the infected group (Table 2), thus suggesting the ability of the leaf extract to ameliorate the cell oxidative stress usually associated with trypanosomosis. This property may be attributed to the radical scavenging activities of *P. guajava* leaf extract (Kamath et al., 2008; Gutiérrez et al., 2008). Its radical scavenging activity could be related to its iron chelating capacity (Settheeworrarit et al., 2005), which may be due to the presence of tannins/polyphenols phytochemical in the leaf extract (Adeyemi et al., 2009). George et al., (1999) reported that tannins/polyphenols are excellent chelator of irons. In a separate study, Scalbert (1993) reported that tannins/polyphenols inhibit microorganism/pathogen proliferation through iron deprivation.

Significant decrease in GSH concentrations in the tissues and serum of infected rats compared to other groups might be attributed not only to the oxidation of GSH to GSSH by activated oxygen produced as a result of trypanosome infection (Igbokwe et al., 1996; Ogunsanmi & Taiwo, 2001; Saleh et al., 2009), but also to high increase in the glutathione peroxidise activity (Omer et al., 2007) since the reaction catalysed by this enzyme consumed GSH. Ataley et al., (2000) also reported that under condition of oxidative stress (such as obtainable during an infection with trypanosomes) activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase increase. The low levels of the GSH observed in the serum of the infected animals may also be as a result of its usage in scavenging oxidant molecules (Igbokwe et al., 1998; Omer et al., 2007). The antioxidants (GSH) are likely consumed as free radical scavengers during the oxidative process in the natural chronic *T. brucei* infection in camels (Saleh et al., 2009). The concentrations of GSH in the serum and tissues of infected but treated animals were quite higher ($P<0.05$) relative to the infected group. This is not surprising considering the fact that several studies (Chen & Yen, 2007; Kamath et al., 2008) have reported *P. guajava* leaf extract as possessing excellent antioxidant properties. In a separate study, Qian & Nihorimbere (2004) also reported the free radical scavenging capacity of the leaf extract of *P. guajava.*

The significant increase of MDA generation shown in this study reflects increased lipid peroxidation which is a pointer to increased levels of free radical in serum and tissues of *T. brucei*-infected rats. However treatment with aqueous *P. guajava* leaf extract resulted in raised concentrations of GSH as well as a significant amelioration of *T. brucei* infection associated lipid peroxidation.

CONCLUSION

In this study it was demonstrated that *T. brucei* infection resulted in high amounts of MDA and low concentrations of GSH in the *T. brucei*-infected animals however the aqueous extract of *P. guajava* leaf was able to reduce the trypanosomosis associated lipid peroxidation as well as raise the level of GSH in the treatment group. We speculate that the ability of the leaf extract of *P. guajava* to lower the MDA concentrations in the treatment group may be attributed to its antioxidant properties.

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