Tissue Lipid Peroxidation in *Trypanosoma brucei* Infection: Testing the Anti-oxidant Property of *Scoparia dulcis*

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Abstract
The ability of aqueous extract of Scoparia dulcis to protect tissues of rabbits against Trypanosoma brucei-induced oxidative damage was investigated in this study. Fifteen New Zealand White rabbits divided into 3 groups of 5 animals each were used for the study. Animals in group 1 served as controls while those in groups 2 and 3 were inoculated with Trypanosoma brucei. In addition, animals in group 3 received aqueous extract of Scoparia dulcis at a daily dose of 200mg/Kg administered orally. Results obtained show that infection of rabbits with T. brucei resulted in a significant increase in oxidative stress as measured by tissue Thiobarbituric Acid Reactive Substances (TBARS) in the liver and kidney, but not in the heart of infected animals. Whereas both superoxide dismutase (SOD) and catalase were significantly reduced in the liver and kidney 28 days post-inoculation; the decrease in SOD activity in the heart was accompanied by an increase in catalase activity in that tissue. Treatment with S. dulcis at a daily oral dose of 200mg/Kg body weight resulted in a significant protection against the trypanosome-induced oxidative stress, with a significant reduction in the level of TBARS and an improvement in both SOD and catalase levels.

Key words: *Trypanosoma brucei*, *Scoparia dulcis*, Lipid peroxidation, Catalase, Superoxide dismutase

Introduction

African Trypanosomiasis has for several decades remained a major challenge to both human and animal health especially in sub-Saharan Africa, where transmission of the parasite by the insect vector tsetse fly is still very rampant particularly in rural communities (WHO, 2006). The disease is characterised by a myriad of clinical and laboratory findings (Orhue and Nwanze 2004, 2009; Umar *et al.*, 2007; Ekanem and Yusuf ,2008; Ngure *et al.*, 2008; Yusuf *et al.*, 2012), the vast majority of which are thought to be precipitated by impaired organ function secondary to tissue damage. Thus, the progression of the disease complex is linked to a widespread degenerative damage to tissues and organs occasioned either by direct parasite attack (Banks, 1979, 1980; Anosa and Kaneko, 1983) or via agents secreted by the parasite (Knowles *et al.*, 1989;Olaniyi *et al.*,2001). It is now known that tissue damage occasioned by *T. brucei* infection can also be mediated by parasite or macrophage-derived free radicals (Knowles *et al.*, 1989, Igbokwe *et al.*, 1994). These free radicals are capable of initiating and promoting membrane lipid peroxidation. The result is a compromise in the structural and functional integrity of the membrane. Damage to the membrane subsequently results in uncontrolled/unregulated movement of materials across the membrane, thus distorting the critical intracellular balance necessary for optimal cell function. This, together with the physical damage to the cell membrane, results ultimately in the loss of cell function.

Since progression of *T. brucei* infection is free radical related, agents that possess significant antioxidant activity may prove helpful in protecting organs against *T. brucei-i*nduced organ damage (Umar *et al.*, 2008). *Scoparia dulcis* is an erect annual plant that has enjoyed significant attention both in ethnomedicine and in scientific research. Amongst the documented therapeutic properties of *S. dulcis* are its anti-diabetic (Pari and Venkateswaran, 2002), hypolipidemic (Orhue and Nwanze, 2006), anti-cancer (Nishino *et al.*, 1993) and anti-oxidant properties (Pari and Latha, 2004; Adaikpoh *et al.*, 2007). The plant is known to be rich in a number of bioactive compounds that are believed to be responsible for the observed pharmacological activities (Ahmed, 1990).

In this study, the possible role of *S. dulcis* in protecting against *Trypanosoma brucei*-induced oxidation stress and attendant membrane lipid peroxidation was investigated.

Materials and Methods

Preparation of plant material: Five hundred grams of air-dried and pulverized shoot portions of the plant, *S. dulcis* was soaked in 1.5L of distilled water overnight. This was subsequently filtered into a beaker using filter paper and funnel. The filtrate was concentrated to constant weight, first the aid of a rotary evaporator and then an oven set at 40°C. The residue was collected and stored at 4°C. The concentrate was then reconstituted into a stock solution of 200mg/ml in distilled water. The required volume of this solution (calculated on the basis of animal weight) was administered daily by gavage.

Experimental design and Animal Treatment: A total of 15 New Zealand white rabbits (average weight= 1.65kg) obtained from a private farm in Benin City were used for the experiment. These

were randomly divided into 3 groups of 5 animals each, with each group allowed 14 days acclimatization prior to the commencement of the experiment. The rabbits were fed on growers mash (Bendel Feeds and flour Mill Ewu, Edo State, Nigeria) and water *ad libitum.* Group 1 served as un-infected control while groups II and III were inoculated with *T. brucei brucei*. Inoculation was by intraperitoneal injection of 0.5ml of a 1:1 (infected whole blood: normal saline) preparation, and with each inoculum containing about 2 x 10^6 of the parasite. Parasite estimation was by the rapid "matching" method of Herbert and Lumbsden (1976). The original stock of *T. brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR) in Vom, Plateau State, Nigeria. The control animals (Group 1) were given intra peritoneal injection of 0.5ml of normal saline instead of parasite. In addition, the inoculated and treated animals (groups III) were given aqueous extract of *S. dulcis* at a daily oral dose of 200mg/kg body weight, with treatment commencing on the day of inoculation. The dose of 200mg/Kg body weight was determined from the data obtained from a preliminary pilot study

Animal sacrifice and preparation of tissue homogenate: At the end of the study, all animals were sacrificed on day 28. The liver, kidney and heart of each animal was carefully removed and freed of blood. Approximately 1 gram portion was homogenized in normal saline and the homogenate centrifuged at 3500rpm for 10 minutes to obtain a clear supernatant which was used for the biochemical assay. All assays were carried out within 48hrs of sample collection.

Biochemical Analysis: All reagents and chemicals used in this work were of analytical grade. The concentration of malondialdehyde (MDA) was assayed as thiobarbituric acid reactive substances (TBARS) in the tissues. TBARS is widely accepted as an indicator of lipid peroxidation. The method used in this determination was that described by Burege and Aust (1978). Catalase activity was determined according to the method of Cohen *et al.* (1970). Each catalase unit (CAT) specifies the relative logarithmic disappearance of hydrogen peroxide per minute and is expressed as K m^{-1} while superoxide dismutase (SOD) was assayed according to the method of Misra and Fridovich (1972) and calculated as described by Baum and Scandalios (1981).

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 done using the Tukey-Kramer multiple comparison test. P values < 0.05 were considered as statistically significant.

Results and Discussion

The results obtained from this study show that infection with *T. brucei* was associated with significantly increased ($p<0.05$) levels of thiobarbituric acid reactive substances (TBARS) in both the liver (Table 1) and kidney (Table 2) but not in the heart (Table 3). SOD and Catalase activities were also significantly reduced in the liver and kidney of *T. brucei* infected rabbits relative to controls. However, the decrease in SOD activity in the heart of infected animals was accompanied with a significant increase in catalase activity in that tissue. Treatment with *S. dulcis* provided a significant measure of protection ($p<0.05$) against the *T. brucei*-induced membrane lipid peroxidation. Superoxide dismutase (SOD) and catalase levels were significantly higher ($p<0.05$) in the liver of *S dulcis*-treated infected animals when compared with their infected but untreated counterparts (see Table 1). The degree of lipid peroxidation as measured by TBARS was also significantly less severe ($p<0.05$) in the *S. dulcis*-treated infected animals when compared with the untreated *T. brucei* positive animals (see Tables 1 and 2).

Table 1: Effect of *S.* dulcis on *T. brucei*-induced oxidative stress in the liver

	Liver		
Group	**TBARS**	**SOD**	**Catalase**
Control	3.12 ± 0.18^a	18.74 ± 1.34^a	3.45 ± 0.22^a
Inoculated untreated	7.08 ± 0.21^b	10.27 ± 0.86^b	1.14 ± 0.16^b
Inoculated treated	$5.62\pm0,37^c$	14.76 ± 0.64^c	2.06 ± 0.19^c

Values are Mean ± SEM. Values on the same column with different superscripts differ significantly ($p<0.05$). There was a significant increase in TBARS and a decrease in both SOD and catalase activities following infection. These changes appeared to have been significantly suppressed by treatment with *S. dulcis*.

Table 2: Effect of *S.* dulcis on *T. brucei*-induced oxidative stress in the kidney

	Kidney		
Group	**TBARS**	**SOD**	**Catalase**
Control	2.68 ± 0.13^a	12.73 ± 1.19^a	2.01 ± 0.12^a
Inoculated untreated	5.76 ± 0.41^b	6.63 ± 1.06^b	1.07 ± 0.18^b
Inoculated treated	$3.44\pm0,15^c$	8.91 ± 0.41^b	1.52 ± 0.25^b

Values are Mean ± SEM. Values on the same column with different superscripts differ significantly ($p<0.05$). There was a significant increase in TBARS and a decrease in both SOD and catalase activities following infection. The protection offered by S. dulcis was not statistically significant.

Table 3: Effect of *S. dulcis* on *T. brucei*-induced oxidative stress in the heart

	Heart		
Group	**TBARS**	**SOD**	**Catalase**
Control	2.84 ± 0.37^a	15.46 ± 0.38^a	3.32 ± 0.32^a
Inoculated untreated	4.09 ± 0.61^a	11.42 ± 0.56^b	4.28 ± 0.62^b
Inoculated treated	$3.26\pm0,45^a$	13.18 ± 0.81^b	3.07 ± 0.49^a

Values are Mean ± SEM. Values on the same column with different superscripts differ significantly ($p<0.05$). There was no significant change in TBARS and the decrease in SOD activity due to infection appears to have been compensated for by an increase in catalase activity.

The data obtained from this study confirm earlier reports that significant changes in the antioxidant status of the host occur during *T. brucei* infection. Meshnick *et al.* (1977) had reported that copious amounts of hydrogen peroxide (H_2O_2) are produced by blood stream forms of *T. brucei*. The generation of this molecule and subsequent generation of free radicals impose on the host a significant degree of oxidative stress. It has been shown that the level of reduced glutathione in the liver is significantly reduced during infection with *T. brucei* (Igbokwe *et al.*, 1998). The implication of this on the peroxidation of membrane lipids and subsequent tissue injury is readily conceivable. Free radicals generation and oxidative damage underlie most of the clinical and pathophysiological changes accompanying infection with trypanosomes. One of the cardinal features of *T. brucei* infection is progressive anaemia. Available evidences are that the pathogenesis of *T. brucei* anaemia is at least in part, driven by the generation of free radicals (Knowles *et al.*, 1989, Igbokwe *et al.*, 1994). Similarly, tissue invasion and damage in *T. brucei* infection is promoted by excessive free radicals generation. Supplementation of infected animals with anti-oxidant vitamins reduces the degree of oxidative stress and attendant degenerative tissue and organ damage in *T. brucei*-infected rats (Umar *et al.*, 2008). Explanations are that, administration of anti-oxidant vitamins boosts the host endogenous anti-oxidant capacity, thus enabling the host to better handle the deleterious effect of free radicals.

It should be mentioned however that free radical generation is a normal and continuous feature of all biological systems. However under normal conditions, the natural antioxidant defence system of the body sufficiently and effectively quenches the deleterious effect of free radicals. Danger ensues, and damage occurs when the natural antioxidant defence system is overwhelmed by excessive production of free radicals such as accompanies infection or the metabolism of some xenobiotics.

Under such conditions, natural or synthetic antioxidant agents can be employed to boost the body's anti-oxidant system. *S. dulcis* has been shown to possess very significant antioxidant activity in a number of studies. These include its effect against cadmium induced oxidative stress in rats (Adaikpoh *et al.*, 2007) as well as its anti-oxidant activity demonstrated in diabetes –induced oxidative stress (Pari and Latha, 2004). The ability of *S. dulcis* to quench free radicals or boost the body's antioxidant potential no doubt contributes to the conservation of both the structural and functional integrity of tissues and organs. Our earlier reports that *S. dulcis* reduces the severity of anaemia (Orhue and Nwanze, 2009) and improves both liver and kidney function (Orhue and Nwanze, 2004, 2005) in *T. brucei*- infected rabbits may not be unconnected with its ability to act as a powerful free radical quencher, and thus, slowing down both the progression and severity of infection. On account of this study and the reports of previous workers, it stands to reason that regular and adequate intake of substances rich in anti-oxidants is capable of slowing down the progression of *T. brucei* infection.

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