PROTECTIVE EFFECT OF ASTAXANTHIN ON LIPOPEROXIDATIVE CHANGES IN RATS INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

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ABSTRACT

The study was carried out to determine the effect of astaxanthin on lipoperoxidative changes in rats experimentally infected with *Trypanosoma brucei brucei*. Twenty male Wistar rats weighing between 110 – 170 kg were randomly divided into four groups (I-IV) of five animals each. Group I rats (negative control) were treated *per os* with distilled water (1 mL/kg) only. Groups II, III and IV were inoculated with10^2 trypanosomes/mL, intaperitoneally. Group II rats were left untreated to serve as the positive control while rats in groups III and IV were treated with astaxanthin at 25 mg/kg body weight, *per os*. Group III rats were first pretreated with astaxanthin at 25 mg/kg body weight, *per os* before inoculation with *Trypanosoma brucei brucei*. Treatment with astaxanthin continued to the end of the experiment. The level of parasitaemia, erythrocyte osmotic fragility (EOF) and malondialdehyde (MDA) concentration were evaluated. Astaxanthin administration caused relative decrease on the level of parasitaemia, EOF and MDA concentration compared to the control. This study has shown that astaxanthin possesses lipoperoxidative potentials evident by the relative decrease in EOF and MDA observed in the treated groups.

Keywords: Astaxanthin, *Trypanosoma brucei brucei*, EOF, MDA

INTRODUCTION

Trypanosomosis is a debilitating disease affecting both man and animals. Trypanosomes species affecting man and his domestic animals have been subdivided into two groups, the haematinic group (*Trypanosoma congolense, Trypanosoma vivax*) which remains in the plasma and the tissue invading group (*Trypanosoma brucei brucei, Trypanosoma evansi, Trypanosoma brucei gambiense, Trypanosoma brucei rhodensiense and Trypanosoma equiperdum*) found in extra and intravascular spaces (Ngure *et al*., 2008.). The presence of trypanosomes in blood produces numerous changes in the cellular and biochemical constituents of blood (Taiwo *et al*., 2003). *Trypanosoma brucei* infection like other trypanosome infections, precipitate increased red blood cell destruction resulting in anaemia and tissue damage (Akanji *et al*., 2009). Increasing evidence demonstrates that oxidative stress plays an important etiologic role in the pathogenesis of African trypanosomosis (Ogunsanmi & Taiwo, 2001). Lipids especially polyunsaturated 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). Trypanosomes Products of lipid peroxidation formed in various biochemical reactions are normally scavenged by antioxidants. Astaxanthin is a powerful antioxidant and have been reported to play an important role in regulating immunity and disease etiology (Chew *et al*, 2009). It is a powerful scavenger of singlet oxygen and peroxyl radicals (Goto *et al*., 2001). Thus, its use in *Trypanosoma brucei brucei* infection may help in ameliorating the lipoperoxidative changes induced by ROS production during trypanosome infection in Wistar rats.

MATERIALS AND METHODS

Twenty (20) male Wistar rats were divided at random into four groups of five animals each and treated as follows:

Group I: treated with distilled water only at 1 mL/kg *per os.*

Group II: intraperitoneally infected with 10^2 trypanosomes/mL of blood only.

Group III: pre-treated with astaxanthin at 25mg/kg *per os* once daily for one week and then intraperitoneally infected with 10^2 trypanosomes/mL of blood. Astaxanthin (25mg/kg) administration continued for 9 more days.

Group IV: infected with 10^2 trypanosomes/mL of blood intraperitoneally and treated with astaxanthin at 25mg/kg *per os* once daily for 9 days.

Parasites in the blood were estimated daily according to the rapid 'matching' method of Herbert and Lumsden (1976). At the end of the experiment, animals were euthanized after light chloroform anaesthesia and blood was collected into plain and EDTA containing sample bottles for the evaluation of MDA concentration and EOF, respectively.

***In vitro* determination of erythrocyte osmotic fragility**

Erythrocyte osmotic fragility was determined as described by Faulkner and King (1970) and as modified by Oyewale (1991). The percentage haemolysis from each sample was calculated using the following formula: Percentage heamolysis (%) = $\frac{\text{Optical density of test solution}}{\text{Optical density of standard solution}} \times 100$

Determination of malondaldehyde concentration

Lipid peroxidation was determined as thiobarbituric Acid reactive substances according to Okhawa *et al* (1979) with slight modification by Atawodi *et al* (2011) using Trichloacetic acid 15% (TCA) and thiobarbituric acid 0.67% (TBA).

Statiscal Analysis

Values obtained were expressed as mean ± SEM. Data were subjected to one-way analysis of variance (ANOVA); followed by Turkey's multiple comparison post-hoc test, using GraphPad Prism version 4.0 for windows (GraphPad Software, San Diego, California, USA). Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

There was no significant ($P < 0.05$) difference in the level of parasitaemia, MDA concentration and percent hemolysis of rats at 0.1%, 0.3%, 0.5%, 0.7% and 0.9% NaCl concentration in the positive control (Group II) when compared to astaxanthin pretreated (Group III) group. However, the level of parasitaemia, MDA concentration and percent hemolysis was relatively higher in the positive control (Group II) when compared to the astaxanthin pretreated (Group III).

Parasites were first observed in the blood of the infected rats at day 6 post inoculation. The prepatent period of 6 days observed in this study disagrees with the findings of Kobo *et al*. (2014) who reported a prepatent period of 2-4 days in rats infected with *T. brucei brucei*. The prepatent period of 6 days observed in this study may be attributed to the low infective dose of 10^2 typanosomes/mL as against the 10^6 trypanosomes/mL used in Kobo *et al*. (2014). The group pretreated with astaxanthin showed relative decrease in the level of parasitaemia compared to the positive control. This may be due to the antioxidant effects of astaxanthin that boosted the host immune system thus reducing the level of parasitaemia. Formulations or natural products with antioxidant properties have the potential to boost the host immune system and possibly reduce parasitaemia or completely remove parasites from the host system (Chibale, 2005).

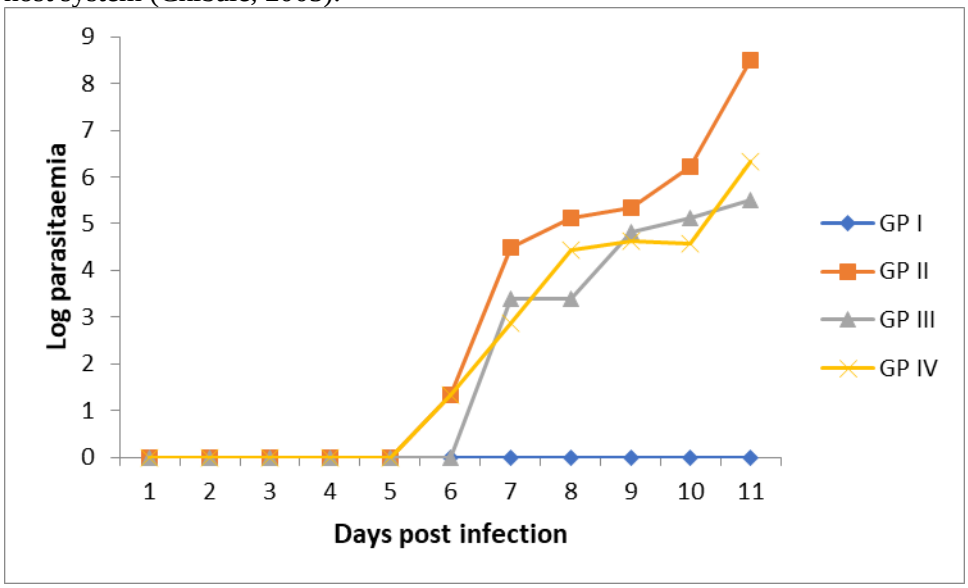

Figure 1: Effect of treatment with astaxanthin on level of parasitaemia in rats infected with *Trypanosoma brucei brucei.* **KEY**: GP = group

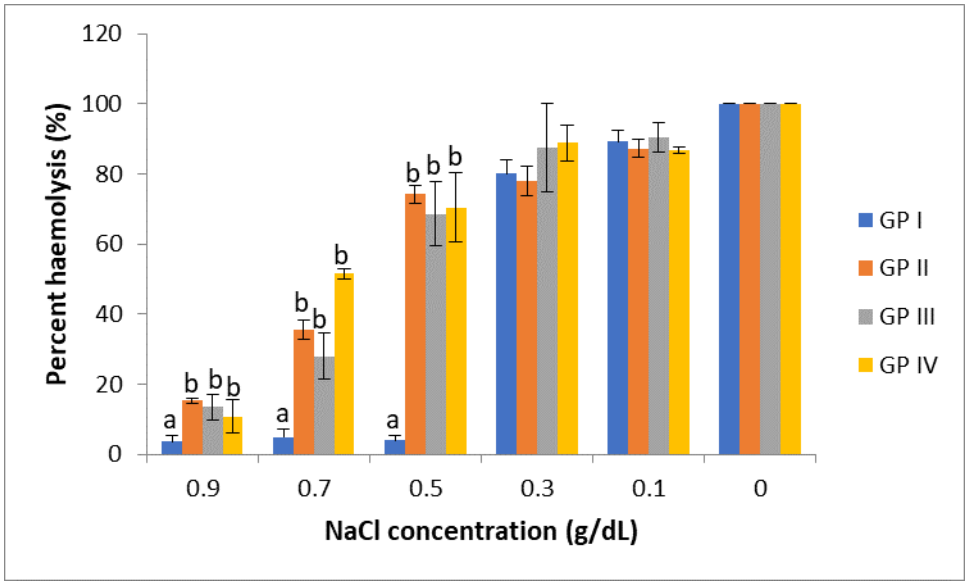

Figure 2: Effect of treatment with astaxanthin on erythrocyte osmotic fragility of rats infected with *Trypanosoma brucei brucei.*

[a, b] = Means with different superscript letters are statistically significant (P <0.05) from one another.
KEY: GP = group

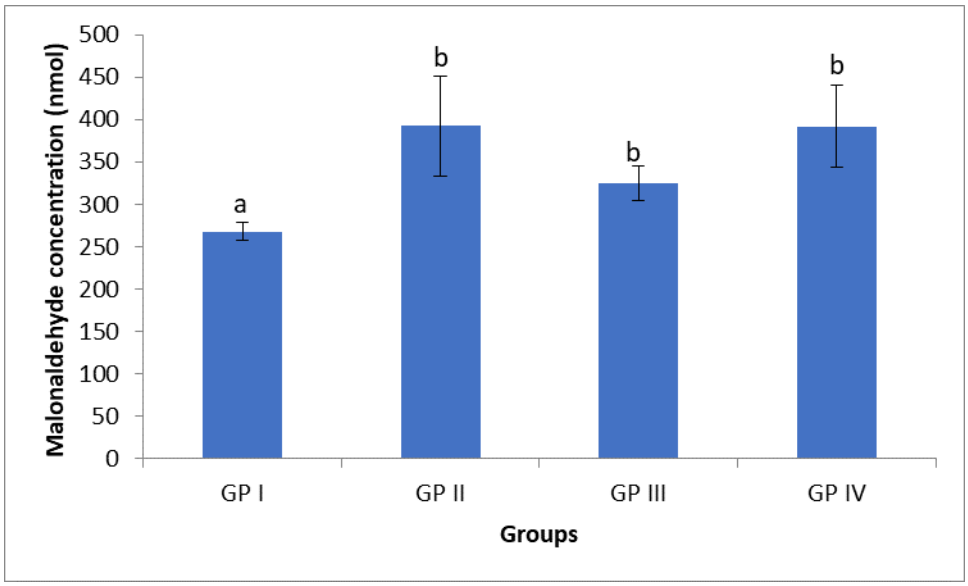

Figure 3: Effect of treatment with astaxanthin on serum malondialdehyde concentration of rats infected with *Trypanosoma brucei brucei*.
[a, b] = Means with different superscript letters are statistically significant (P <0.05) from one another.
KEY: GP = group

The increase observed in the EOF and MDA concentration of rats in the positive control group agrees with the work of Saleh *et al*. (2009) in *T. evansi* infected rats. Malondialdehyde is a by-product of lipid peroxidation, and elevated MDA concentration in serum is an indicator of lipid peroxidation

(Uchendu *et al*., 2014). By-products of lipid peroxidation have been shown to cause profound alterations in the structural organization and functions of the cell membrane (Ambali *et al*., 2010). This lipoperoxidative alteration in the structural and functional components of the erythrocyte membranes may have caused perturbations in the membrane integrity (Ambali *et al*., 2010), resulting in increased EOF observed in the positive control group. The group pretreated with astaxanthin showed relative decrease in EOF and MDA concentration when compared to the positive control group. This may be due to the ability of astaxanthin to scavenge the free radicals generated during trypanosome infection thus ameliorating the lipoperoxidative damage caused by the infection. Astaxanthin have been reported to effectively scavenge lipid radicals and destroy peroxides, thereby protecting fatty acids and biological membranes from oxidative damage (Ghlissi et al., 2014).

CONCLUTION

This study has shown that astaxanthin which possesses antioxidant properties has the potentials to protect lipid membranes from oxidative damage, evidenced by the relative decrease observed in EOF and MDA concentration in the group pretreated with astaxanthin.

RECOMMENDATION

Further study should be carried out to evaluate the effects of different doses of astaxanthin in trypanosomes infection.

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