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Full Length Research Paper

Erythrocyte osmotic fragility of Wistar rats administered ascorbic acid during the hot-dry season

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The experiment was carried out with the aim of investigating the effect of an antioxidant ascorbic acid on erythrocyte osmotic fragility (EOF) of Wistar rats during the hot-dry season. Fifteen adult Wistar rats administered with AA at the dose of 100 mg/kg *per os* and individually served as experimental animals, and 15 others administered orally with sterile water were used as control animals. The animals were kept in the laboratory and the meteorological parameters within the period of study were determined using wet and dry bulb thermometer while the blood samples for EOF determination was obtained at the end of the experiment which lasted 8 weeks and this was done using standard procedure. EOF decrease significantly (P < 0.05) in experimental rats compared to the control group. The results indicated that AA protected the integrity of the erythrocyte membrane in experimental rats administered with AA as demonstrated by lower percentage haemolysis, and thus may alleviate the risk of increase in haemolysis due to heat stress in rats during the hot-dry season.

Key words: Wistar rats, ascorbic acid, hot-dry season, erythrocyte osmotic fragility.

INTRODUCTION

Environmental heat which animals are unavoidably subjected to produces heat stress by causing discomfort, irritation and some degree of psychomotor disturbances (Rakesh and Amit, 2004). The changes in thermal environment caused by fluctuations in ambient temperature (AT) and relative humidity (RH) have been demonstrated to induce a variety of physiological responses, which may adversely affect productivity and health in livestock (Ayo et al., 1998a and b; Adenkola and Ayo, 2009a) . Heat stress is one of the most important stressors in the hot regions of the world (Altan, 2003) resulting in the generation of enormous free radicals and other reactive oxygen species (ROS).

The ravaging effects of oxidative free radicals are quenched by antioxidants (Akinwande and Adebule, 2003) . Ascorbic Acid (AA) is an outstanding antioxidant found in the human blood plasma (Frei et al., 1989) and it stabilizes free radicals and terminates free radical induced lipoperoxidation of cytochromes, thereby maintaining the structural integrity of cells (Chews, 1995; Candan et al., 2002; Adenkola and Ayo 2009b). It has also been established that AA ameliorates heat stress and the adverse effects of environmental conditions (Tauler et al., 2003; Adenkola and Ayo, 2006a; Adenkola and Ayo, 2009a). Currently AA is the most widely used vitamin supplement throughout the world (Naidu, 2003).

Therefore, AA supplementation may attenuate the negative responses of Wistar rats to heat stress during hot-dry season, described as one of the most thermally stressful season in the Northern Guinea Savannah zone of Nigeria (Igono et al., 1982).

The aim of the present study was to determine the modulatory role of AA on the erythrocytes osmotic fragility in Wistar rats during the hot-dry season prevailing in the Northern Guinea Savannah zone of Nigeria.

MATERIALS AND METHODS

Experimental site

The experiment was carried out during the hot-dry season at the

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Table 1. Composition of pelletised growers feed.

	Percentage
Crude protein	14.5
Fat	7.0
Crude fibre	7.2
Calcium	0.8
Available phosphorus	0.4
Metabolisable energy	2.500 kcal/kg

Data were obtained from the manufacturer (Vital Feeds, Jos, Nigeria).

Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria (11^{o} $10^{/}$ N, 07^{o} $38^{/}$ E), at elevation of 650 m above sea level located in the Northern Guinea Savannah zone of Nigeria (Akpa et al., 2002). The area has three climatic seasons which consists of the cold dry season (November – February), hot-dry season (March – May) and the wet season (June – October) (Igono et al., 1982) with an annual rainfall of 1107 mm (Rekwot, 2002).

Meteorological data

During the study period, wet and dry-bulb temperatures (DBT) were recorded at 06:00, 13:00 and 19:00 h using dry- and wet- bulb thermometers (Brannan, England), and relative humidity (RH) was calculated using the manufacturer's standard manual attached.

Experimental animals and management

A total of 30 adult Wistar rats comprising of 14 males and 16 females weighing between 140 – 160 g. The animals were fed with pellets made from grower's mash (Table 1), maize bran and groundnut cake in the ratio 4:2:1 with wheat flour as binder . The rats were allowed to acclimatize to the environment for two weeks before the experiment commenced and they were divided into two groups. Group 1 made up of 15 rats (7 males and 8 females) which served as the control were given tap water orally for two weeks, while the second group were made up of (7 males and 8 females) and this served as the experimental animals. The experimental animals were administered with ascorbic acid (AA) at a dose of 100 mg/kg (Chervyakov et al., 1977) daily for a period of eight weeks. Each tablet was dissolved in 1 ml of distilled water to obtain 100 mg/ml suspension, just prior to its administration. At the end of the experiment, the animals were euthanized and 4 ml of blood sample was collected into bijou bottles containing the anticoagulant, disodium salt of ethylene diaminetetra-acetic acid at the rate of 2 mg/ml of blood (Oyewale, 1992). After collection, the samples were transferred to Physiology Research Laboratory, Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where erythrocyte osmotic fragility (EOF) test was carried out as described by Faulkner and King (1970).

Erythrocyte osmotic fragility determination

Sodium chloride (NaCl) solution was prepared according to Faulkner and King (1970) in volume of 500 ml for each of the samples in concentrations ranging from 0.05 to 0.85% at pH 7.4. A set of 10 test tubes, each containing 10 ml of NaCl solution of concentrations, ranging from 0.05 to 0.85%, were arranged serially in a test tube rack. One set was used to analyse each sample. The test tubes were labeled with corresponding NaCl concentration. One millilitre pipette was used to transfer exactly 0.02 ml of blood sample into each of the ten test tubes. Mixing was performed by gently inverting the test tubes for about 5 times. The test tubes were allowed to stand at room temperature (26 - 27^{o}C) for 30 min. The contents of the test tubes were maintained at pH 7.4. There-after, the contents of the test tubes were re-mixed and centrifuged at 1,500 x g for 15 min. The supernatant of each test tube was transferred into a glass cuvette. The concentration of haemoglobin in the supernatant solution was measured using a spectrophoto-meter (Spectronic- 20, Philip Harris Limited, Shenstone, UK) at 540 nm by reading the absorbance. The same procedure was repeated for every blood sample of each pig used for the study. The percent haemolysis was calculated using the formula (Faulkner and King, 1970):

$$\frac{\text{Optical density of test}}{\text{Optical density of distilled water}} \times 100 = \text{Percent haemolysis}$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis against the saline concentrations.

Statistical analysis

All data obtained were subjected to statistical analysis using Student's *t*-test using Graph Pad Prism version 4.00 for Windows (www.graphpadprism.com). Data were expressed as mean ± standard error of mean. Values of $P < 0.05$ were considered significant.

RESULTS

The dry bulb temperature recorded during the study period was highest at 13:00 h with a value of 30.00 ± 2.25°C and the lowest dry bulb (26.00 ± 2.65°C) reading was obtained at 17: 00 h which was significantly ($P < 0.05$) different. The overall reading of relative humidity was 15.00 ± 1.73% (Table 2). There was a statistically ($P < 0.05$) different in the value of male Wistar rats at NaCl concentration of 0.5, 0.6, 0.7, 0.8 and 0.9% (Figure 1). Also the erythrocyte osmotic fragility was significantly ($P < 0.05$) increased at NaCl concentration of 0.4, 0.5, 0.6, 0.7 and 0.8% in the female Wistar rats (Figure 2). At 0.5% NaCl concentration a significant increase ($P < 0.05$) exist between a male and female Wistar rats. However, the erythrocyte osmotic fragility was not significantly ($P > 0.05$) different between the two group of rats at other concentration.

DISCUSSION

The meteorological results obtained in the present study showed that the microclimatic condition prevailing during the study period were thermally stressful to the rats. Although mortality was not recorded during the study period, it was demonstrated that temperature exceeding 28.5°C could lead to thermo tolerance being overcome

Table 2. Meteorological parameters during the study period.

Hour of the day	Meteorological parameters	
	Dry bulb (°C)	Relative humidity (%)
06:00	28.00 ± 2.02	12.00 ± 1.35
13:00	30.00 ± 2.25	15.00 ± 2.00
19.00	26.00 ± 2.69	18.00 ± 2.30
Overall mean ± SEM	28.00 ± 1.56	15.00 ± 1.73

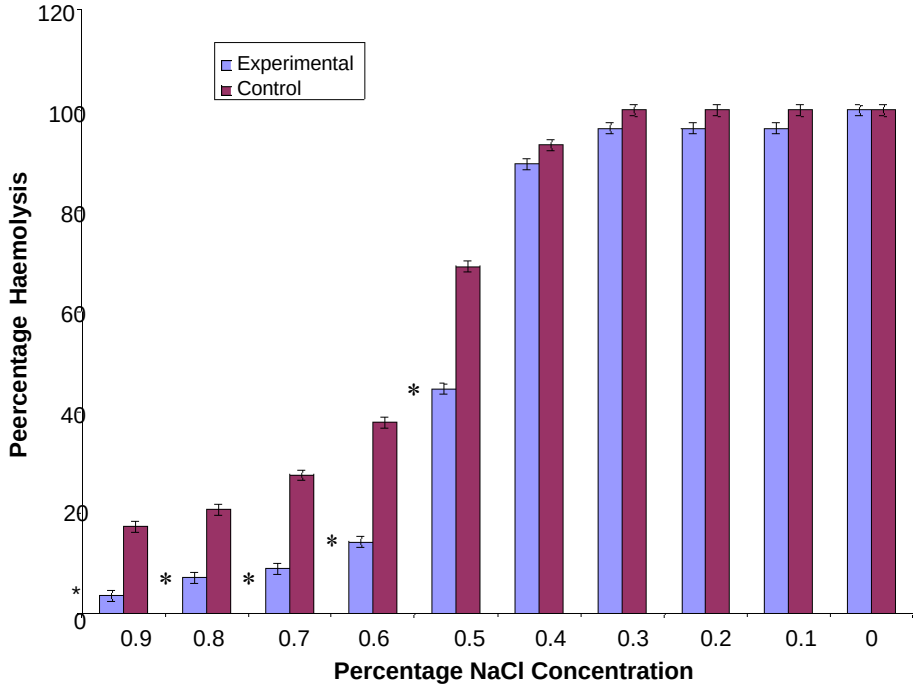

Figure 1. Erythrocyte osmotic fragility of experimental and control male Wistar rats during the hot-dry period.
* = "P < 0.05".

which could result in marked mortality (Zapata and Gernet, 1995). The meteorological results obtained in this study thus indicated a need to ameliorate the risk due to the adverse effects of hot-dry season thermal stress. It has been demonstrated that heat stress is one of the most important stressors in the hot regions of the world (Altan, 2003) resulting in the generation of enormous free radicals and other reactive oxygen species (ROS) (Chihuailaf et al., 2002) which play a vital role in cellular and tissue damage (Sudha et al., 2001; Tkaczyk and Vizek, 2007) and they have been demonstrated to have adverse effects on erythrocytes (Sumikawa et al., 1993; Avellini et al., 1995; Adenkola and Ayo, 2009b).

The significant difference observed between the experimental and control rats with a lower haemolysis in experimental rats could be attributed to the enormous ROS produced in the control animals as a result of thermal stress which play a vital role in tissue damage and have deleterious effects on erythrocyte cytomembrane (Avellini et al., 1995; Adenkola and Ayo, 2009b). Although free radicals were not measured directly in this study, it has been shown that they are generated in animals subjected to stress (Halliwell, 1996; Senturk et al., 2001; Chihuailaf et al., 2002). The observed increase in EOF in control rats further supports this fact. Drooge (2002) demonstrated that ROS and other free radicals generated contribute to protein degeneration, lipid peroxidation and DNA oxidation. The membrane of erythrocyte is rich in polyunsaturated fatty acids which is susceptible to lipid peroxidation and this result in the loss of membrane fluidity and cellular lysis (Brzezinska-Slebodziiska, 2003) hence higher haemolysis in the control rats.

However in the experimental rats that was administered AA the lower percentage of haemolysis recorded was in agreement with observations of Senturk et al. (2001),

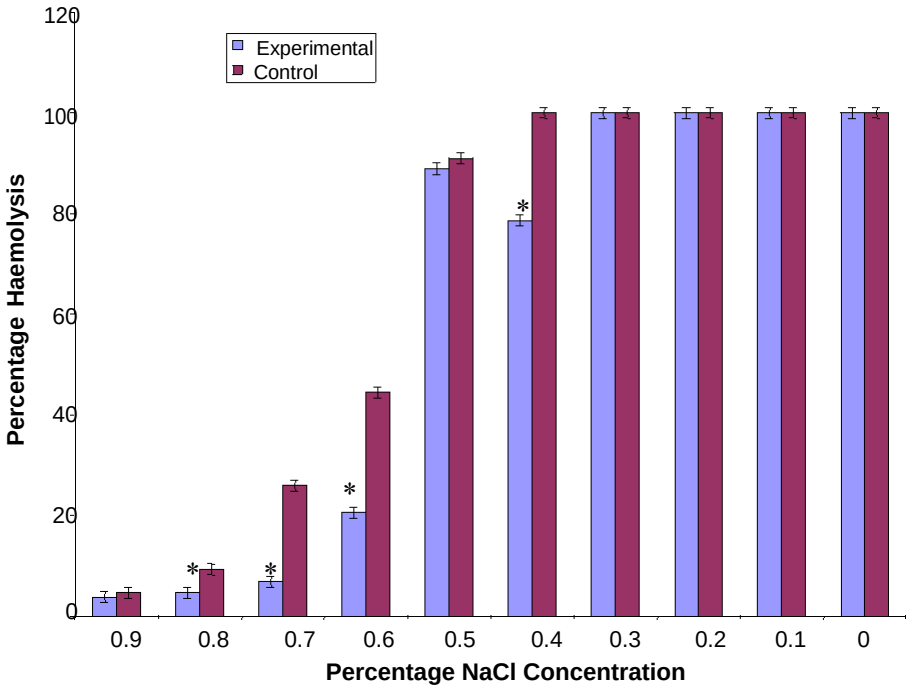

Figure 2. Erythrocyte osmotic fragility of experimental and control female Wistar rats during the hot-dry period.
* = "P < 0.05".

Candan et al. (2002) and Adenkola and Ayo (2009) that AA consolidates the integrity of erythrocyte membranes of and, therefore reduces their oxidative damage. Oxidative stress occurs when the antioxidant defence systems in the body are overwhelmed by free radicals (Williams et al., 2008). AA administration to experimental rats apparently, reduced the intensity of oxidant stress by enhancing the antioxidant defense mechanisms and suppressing the thermal stress which greatly minimized the destruction of erythrocyte. The result of this study agrees with those of Chen et al. (2000), Frei (2004) Adenkola et al. (2009a) and Adenkola and Ayo (2009b) that AA is an effective antioxidant in various biological systems. Antioxidant AA could thus be administered to animals during the hot-dry season in the region in order to prevent the unavoidable heat stress which is one of the most important stressors that animals are subjected to in the hot regions of the world.

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