Nigerian Veterinary Journal
Vol 34 (4) 883-889
ARTICLE

Effects of Harmattan on Erythrocyte Osmotic Fragility and Malondialdehyde Concentration in Goats Administered with Resveratrol

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SUMMARY

The aim of the study was to determine the effects of harmattan on erythrocyte osmotic fragility and malondialdehyde concentration in Red Sokoto goats administered with resveratrol. A total of 17, apparently, healthy goats of both sexes and weighing between 9-12 kg were used for the experiment. Ten goats which served as experimental animals were administered with resveratrol for two weeks while seven control goats received only diluted 95% ethanol which served as the diluent for the drug. Concentration of malondialdehyde and erythrocyte osmotic fragility test were carried out using standard methods. At first week of the experiment, there was a significant difference ($P<0.05$) between treated and control goats in percentage haemolysis recorded at NaCl concentration of 0.3. The value indicates that at 0.3 NaClconcentration, haemolysis reduced in treated goats, signifying that resveratrol can be used to ameliorate haemolysis caused by harmattan stress. There was no significant difference between the treated and control goats in the malondialdehyde concentration recorded, however, in the treated goats, the post-treatment values were higher ($P < 0.05$) than the pre-treatment values. It is concluded that resveratrol may be of value in the amelioration of stress during the harmattanseason and, consequently, reduce harmattan related-stress in goats.

KEY WORDS: Erythrocyte osmotic fragility, Goats, Harmattan, Malondialdehyde, Resveratrol.

INTRODUCTION

The harmattan season is characterized by a dry and dusty West Africantrade wind that blows from the Sahara desert southward into the Gulf of Guinea,between the end of November and the middle of March (Bamiteko, 2009). The harmattan season, which is characterized by low temperature and low humidity, is accompanied by fog or haze which conceals sun for whole days (Bannor and Ogunsan, 2003). The whole vegetation withers and the grass becomes like hay, while various respiratory diseases such as chronic bronchitis, and peste des petits ruminant are aggravated by the dusty weather (Adefolalu, 1983; Raji et al., 2000). Among all the environmental stresses, cold stress induces physiological responses which are of high priority and energy demanding in homeotherms (Johnson et al., 1992).Cold stress significantly affects the health and welfare

of animals in poor husbandry conditions and disrupts the balance in an oxidant/antioxidant system. It induces oxidative damage to several tissues by altering the enzymatic and non-enzymatic antioxidant status, protein oxidation and lipid peroxidation (Sahin and Gumulu, 2004). Acclimation at temperatures below 10oC for a period of more than three weeks alters significantly the oxidant/antioxidant system (Kennedy et al., 1977), hence an increase in the production of reactive oxygen species (ROS). The ROS including hydrogen peroxide (H2O2), hydroxyl radicals (OH-) and superoxide anion radicals (O2-), cause lipid peroxidation (Selman et al., 2000; Heiseet al., 2003). Disruption of tissue integrity caused by lipid peroxidation leads to membrane injury and increase in serum concentration of malondialdehyde (MDA) and MDA is an indicator of lipid peroxidation of cytomembranes (Bagchiet al., 1999

ROS produced during stress are known to play essential role in tissue damages as well as adverse effects on Red blood cells (RBCs) (Avelliniet al., 1995; Gumuluet al.,2002). Thus, they can lead to increase in erythrocyte haemolysis (Adenkolaet al., 2010; Minka and Ayo, 2010). The erythrocyte fragility (EOS) test indicates the ease at which erythrocytes undergo lysis when kept in a hypotonic solution (Sanjay, 2002).

Resveratrol, a nutritional supplement derived primarily from Japanese knotweed (Polygonumcuspidatum) is found widely in varying amounts in the skin of grapes, peanuts, berries of Vaccinum species, including blueberries, bilberries, and cranberries (Guet al., 1999). Resveratrol exerts a wide range of biological effects, including anti-inflammatory effects (Olas and Wachowicz, 2005). Several studies have also demonstrated that resveratrol has antioxidant properties (Frankel et al., 1993; Chanvitayapongs et al., 1997; Belguendouz et al., 1998), and that it contains highly hydrophilic and lipophilic properties, rendering it more effective in protection of biomolecules than other well-known antioxidants, such as vitamins C and E (Chanvitayapongset al.,1997) under conditions of elevated oxidative stress. The cellular antioxidant defence system of the body may be expanded by dietary supplementation of antioxidants which decrease short-term tissue damage and long-term health risks associated with oxidative stress (Jacob and Burri, 1993)

The aim of the present study was to determine the effect of harmattan on erythrocyte osmotic fragility and malondialdehyde concentration in goats administered with resveratrol.

MATERIALS AND METHODS

The study area

The experiment was carried out during the harmattan season at the animal pen of Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria (10° 10′ N, 07° 38′ E), located in the Northern Guinea Savannah zone of Nigeria.

The animals and management

Experimental animals consisted of 17, apparently, healthy goats, made up of 7 males, and 10 non-pregnant, non-lactating females aged between 8-12 months and weighing 9-12 kg. They were housed in standard goat pens, measuring 10 m x 5 m x 2.5 m. The pens were made of concrete floor, cement block wall and asbestos roof. The goats were restrained and stocked at 1 m^2/goat (Kannanet al., 2002), in well-ventilated goat research pens and maintained under proper hygienic conditions. They were given feed, consisting of groundnut hay, maize offal, beans husk and water was given ad libitum. The goats were pre-conditioned for four weeks before the commencement of the experiment. The goats were confined in an open pen throughout the day instead of locking them in an enclosed, raised wall and well ventilated pen, which could have

protected them from the harsh harmattan stress. During this period, they were screened for both endo- and haemoparasites and treated with albendazole (Unaben; Neineth, Lagos, Nigeria) administered orally at the dose of 0.8 mg/kg, and oxytetracycline (Kepro B. V®, Holland) at the dose of 20 mg/kg by deep intramuscular route.

Experimental design

The experiment was conducted for a period of eight weeks. Thermal environmental values of ambient temperature and relative humidity were recorded with wet and dry bulb thermometer (Brannan®, England) for three consecutive days at 06:00h, 13.00h and 18.00h before administration of resveratrol. The parameters were also taken on days 7 and 14 of administration of resveratrol and day 7 post-administration of resveratrol.

During the first four weeks, goats were exposed directly to the harmattan stress without any treatment with resveratrol. The animals were confined in an open pen during this period when the ambient temperature was low especially in the morning periods. Thus, the temperature recorded before the experiment ranged between 13.5-140C which was very stressful to the goats. The animals were seen huddling together and shivering. Blood samples were collected after the four-week exposure to harmattan from each goat by jugular venipuncture to determine the effect of harmattan stress on erythrocyte osmotic fragility and concentration of malondialdehye.

Administration of resveratrol

Ninety nine percent pure resveratrol powder (Candlewood Stars Incorporated Danbury, Connecticut, USA) was administered to the goats. The powder was dissolved in 95% ethanol to obtain a concentration of 50 mg/ml (Sigma, 2010). The solution obtained was diluted four times with distilled water 50mg of resveratrol was diluted in 1ml of ethanol to give 50mg/ml solution. 1ml of the solution was diluted with 4ml of distilled water making 5ml. Therefore, if 1ml of solution contains 50mg of resveratrol, 5ml of the solution will contain 10mg of resveratrol in 1ml of solution containing ethanol and distilled water, hence the 10mg/ml concentration. This was done because resveratrol does not dissolve in water but in ethanol, to reduce concentration of ethanol and to give a final concentration of 10 mg/ml. The prepared solution was administered daily for two consecutive weeks at the dose rate of 20 mg/kg per os to 10 experimental goats, while the remaining 7 goats which served as controls were given the same quantity of ethanol without resveratrol.

Blood sample collection

Blood samples were taken from treated and control groups on days 7 and 14 of administration of resveratrol and day 7 post-administration of resveratrol. On each day of blood collection, 5 ml of blood was obtained from each goat, using disposable syringes and 18 gauge x 1.5 inch sterile needles into sterile test tubes.

Determination of erythrocyte osmotic fragility

The erythrocyte osmotic fragility was determined using the method described by Oyewaleet al. (2011). Sodium chloride (NaCl) stock solution (pH 7.4) was prepared in volumes of 500 ml for each of the samples in concentration of 0.1%, 0.3%, 0.5%, 0.7%, 0.9%. Each of the 5 test tubes used contained 5 ml of the corresponding NaCl concentration from the stock solution. The test tubes were labelled with corresponding concentrations and arranged serially in a rack of 5 tubes. 1ml pipette was used to transfer exactly 0.02 ml of each blood sample into each of the 5 test tubes in a set. The contents of the test tubes were gently mixed by inverting the test tubes five times

and allowing them to stand at room temperature for 30 minutes. Thereafter, the contents of the test tubes were centrifuged at 1500 x g for 15 minutes. The supernatant was transferred into glass cuvette and measured at wavelength of 540 nm using distilled water as blank, a spectrophotometer (Spectronic-20, Philip Harris Limited®, Shenstone, UK) by reading the absorbance. The percentage haemolysis in each concentration of NaCl was determined by taking the tube with maximum haemolysis (0%) as (100%).

Determination of lipid peroxides

Standard protocol of the Northwest Life Science Specialist, Vancouver, Canada was used in the MDA assay. Briefly, the assay mixture in a microcentrifuge vial, containing 10 µl of butylatedhydroxytoluene reagent, 250 µl of serum sample, 250 µl of phosphoric acid reagent and 250 µl of 2-thiobarbituric acid, was capped and vortexed vigorously. The mixture was incubated for 60 minutes at 600C, and then centrifuged at 10,000 x g for 2-3 minutes. The reaction mixture was then transferred to cuvette and the absorbance of the test sample was read at 548 nm using a spectrophotometer (Spectronic-20, Philip Harris Limited®, Shenstone, UK). MDA concentration was calculated by multiplying the derivative analysis of the spectra for the reaction mixture by -1 x106.

Statistical analysis

Data obtained were expressed as mean ± standard error of the mean (Mean ± SEM). Malondialdehyde data were subjected to repeated measures ANOVA, followed by Tukey'spost-hoc test to analyse the effects of the sampling periods. EOF data were analysed using Student's t-test to compare the differences between the means derived from the control and experimental goats. Values of $P < 0.05$ were considered significant.

RESULTS

Meteorological conditions

During the study period, the goats shivered, not measured, but observed at 6.00 h as the ambient temperature fluctuated between 14 ± 0.290C and 19.5 ± 1.50C (Table 1). The dry-bulb temperature (DBT) measured in the study area during the experiment ranged between 13.5 - 410C while the RH fluctuated between 13 – 67% (Table 1).There was an increase in EOF in both the treated and control goats before administration of resveratrol. The EOF value was significantly higher in control goats compared to treated goats on day 7 of treatment with resveratrol at 0.3% NaCl with the values of 93 ± 2.3% and 84.5 ± 1.3% respectively.Thereafter, EOF values in control and treated goats did not differ on day 14 of treatment with resveratrol (Figure 1-3).

TABLE 1: The thermal environmental parameters before, during and after the period of study.

	Ambient Temperature (°C)			Relative humidity (%)		
	Study Period			Study Period		
Time (h)	Before	During	After	Before	During	After
06.00	14 ± 0.29 (13.5- 14)	18.5 ± 1.5 (17- 20)	19.5 ± 1.5 (18- 21)	39 ±3.0 (33- 43)	49 ± 9.0 (40- 58)	55 ± 6.0 (49- 67)
13.00	21 ± 0.17 (21.5- 22)	36.0 ± 4.0 (32- 40)	37 ± 4.0 (33- 41)	27 ± 3.8 (21- 34)	37.5 ± 2.5 (13- 63)	22.5 ± 2.5 (20- 25)
18.00	21± 0.5 (20.5- 22)	28.0 ± 1.0 (27- 29)	24.5 ± 3.5 (21- 28)	27 ± 1.5 (25- 30)	42 ± 2.3 (19- 65)	52 ± 1.5 (37- 67)
Overall Mean ± SEM	18 ± 1.3	27 ± 3.39	27± 3.6	31 ± 2.4	42.8 ± 9.0	43 ± 7.8

* Values in parenthesis are ranges

ISSN 0331 - 3026

Fig. 2: Erythrocyte Osmotic Fragility of Goats First Week of Treatment with Resveratrol.Values with different letters are significantly (P < 0.05) different.

There was no significant difference in MDA concentrations between the treated and control goats at first week of administration of resveratrol. At the second week of administration of resveratrol, values obtained in treated and control goats were also not significantly different. However, in treated goats, there was a significant (P<0.05) reduction in MDA concentration when the value recorded at the second week of administration was compared with that obtained pre-administration of resveratrol (Figure 4). Thus, the value decreased from 2.67 ± 0.1µM/l to 2.15 ± 0.2µM/l.

DISCUSSION

The DBT values of 13.5 – 41oC recorded during the study period was outside the reference range values of 20–30oC established for goats in the tropics (Richardson, 2002). This confirms the findings of Adenkolaet al. (2010) that the harmattan season is thermally stressful to livestock.

A high value of MDA was recorded in the goats before the administration of resveratrol, showing that the

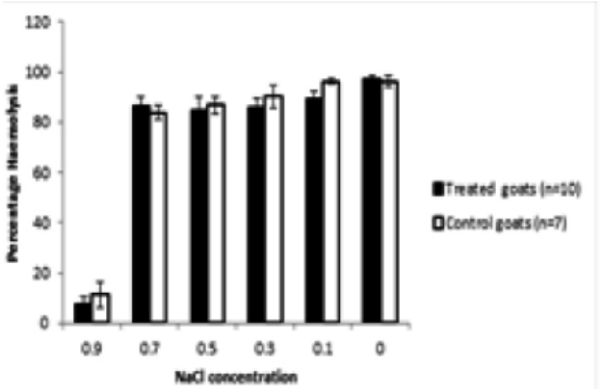

Fig. 1: Erythrocyte Osmotic Fragility of Goats before Treatment with Resveratrol.

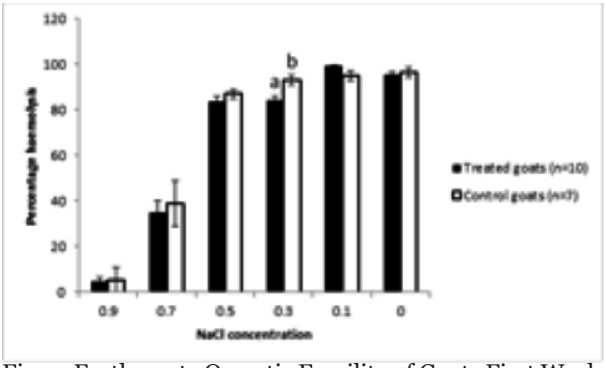

Fig. 2: Erythrocyte Osmotic Fragility of Goats First Week of Treatment with Resveratrol.Values with different letters are significantly (P < 0.05) different.

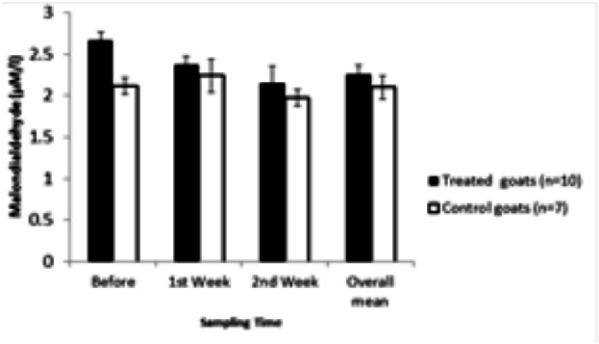

Fig.3:Fluctuation in MDA concentration in goats administered with resveratrol.

harmattanseason induced an increase in free radical production. There was no significant difference between the treated and control goats in the malondialdehyde concentration recorded, however, in the treated goats, the post-treatment values were higher (P < 0.05) than the pre-treatment values. This shows that administration of resveratrol reduced the lipid peroxidation caused by the harmattan stress. Thus, the finding agrees with the result obtained by Tadoliniet al. (2000) that resveratrol inhibits lipid peroxidation, mainly by scavenging lipid peroxyl radicals within the membrane.

The increase in haemolysis in treated and control goats before administration of resveratrol is in agreement with the finding of Adenkola and Ayo (2009) that erythrocyte osmotic fragility increases with harmattan stress. The study showed

that the haemolysis was due to increase in free radical which was observed during cold stress. This agrees with the report of Sahin and Gumulu (2004), who explained that cold stress induces oxidative stress by disrupting the balance in oxidant/antioxidant system and causing oxidative damage to several tissues.

The decrease in erythrocyte osmotic fragility value recorded in treated goats at the first week of treatment demonstrated that resveratrol ameliorated haemolysis associated with the harmattan season, apparently, by acting as an antioxidant (Tedesco, 2000). This finding is similar to that of Tadolini et al. (2000) who reported that resveratrol as a potent free radical scavenger, that prevents lipoperoxidation in cytomembranes, thus reducing damage to the membranes and cell destruction.

Indeed, the unique capacity of resveratrol to spontaneously enter the lipid environment confers on it great antioxidant potential (Frankel,et al., 1993).

CONCLUSION

The reduction in EOF in treated goats indicated that resveratrol is a potent antioxidant and may be of value in reducing haemolysis caused by the thermally stressful harmattan season.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University Zaria, Nigeria for their technical assistance.

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