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Research Article

Neurobehavioural Changes in Wistar Rats during the Hot-Humid Season Attenuated by the Co-administration of Ascorbic acid and Zinc Gluconate

***Okey, Samuel Maduabuchi[1] and Isa, Ahmed Sherif[2]**

[1]Department of Human Physiology, Federal University Wukari, Taraba State, Nigeria.
[2]Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria
****Corresponding Author's email*: samfatuma@gmail.com; Phone: +2348081584224**

ABSTRACT
The hot-humid season produces mild heat stress, which may affect performance of man and animals. The study was aimed at investigating the effect of co-administration of ascorbic acid and zinc gluconate which are antioxidant nutrients on sensorimotor changes in Wistar rats exposed to the hot-humid season. Twenty male Wistar rats weighing 110 - 186 g were divided into two groups of ten rats each. The first group served as the control while rats in the second group served as the experimental animals. The regimens were given once daily and orally by gavage for a period of 28 days. Sensorimotor parameters of neuromuscular coordination, motor strength and excitability scores were assessed at various intervals during the experimental period. The ambient temperature was taken and relative humidity and heat index were calculated. Results showed that the heat index was significantly ($P < 0.05$) higher when compared between the hours of 7.00 h, 13.00 h and 18.00 h. The Neuromuscular coordination assessment showed no significant difference ($P > 0.05$). The motor strength assessment was higher in ascorbic acid and zinc-treated group compared to the control, although the difference was not significant ($P > 0.05$). Percentage excitability scores recorded were higher in the ascorbic acid and zinc-treated group than the control group. The thermal stress produced by the hot-humid season resulted in sensorimotor responses in Wistar rats, attenuated by the co-administration of ascorbic acid and zinc gluconate.

Keywords: Ascorbic acid; Heat stress; Hot-humid season; Motor strength; Neuromuscular co-ordination; Zinc gluconate

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INTRODUCTION

Global warming is a subject of great concern to human health. It involves the synergy of high temperature with humidity, wind speed and solar radiation that result in heat stress which could be detrimental to human comfort and productivity (Morakinyo *et al*., 2024). Heat Stress arising from different seasons in Guinea Savannah zone of Nigeria induces different behavioural and physiological changes in the body (Minka & Ayo., 2007; Sinkalu *et al*., 2009; Balogun *et al*., 2019). The changes may be partly due to the production of free radicals and other reactive oxygen species that distort the functions of macromolecules, cells and tissues of the body (Sunil Kumar *et al*., 2011; Pallares *et al*., 2020). The hot-humid season (May-October) produces mild heat stress which may have negative effects on performance of man and animals. It has a very high rainfall (155.9 ± 26.2mm), high temperature maxima (30.2 ± 0.6 0C), very high relative humidity (63.2 ± 5.6%) and low evaporation rate (154.2 ± 9.71mm) (Enander,1990; Habeeb, 2020).

The thermal environmental stressors such as relative humidity, ambient temperature, thermal radiation and air speed form a complex system that act upon human body (Ayo *et al*., 2011; Morakinyo *et al.*, 2024). Heat index is an index of comfort also known as apparent temperature. It is the apparent temperature felt by the individual, resulting in behavioural responses and biochemical changes

(Kendall and Webster 2009; Dzenda *et al*., 2011; Boonruska *et al*., 2020).

The antioxidant nutrients, especially vitamins C, A, E, and zinc and chromium, may attenuate the negative effects of environmental stress in the local tropical environment as reported in the works of Ayo *et al*. (2007), Minka & Ayo (2007), Sinkalu *et al*. (2009), Ajakaiye *et al*. (2010), Adenokola *et al*., (2011) and Hosseinabadi *et al*., (2020).

L-ascorbic acid is the most important water-soluble antioxidant in extracellular fluid as it is capable of neutralizing reactive oxygen species in the aqueous phase before lipid peroxidation is initiated (Rahma, 2007; Tripathi, 2009; Alhassan *et al*., 2016). Zinc is the second most abundant trace element in the body (Zhou *et al*., 2007). Its antioxidant effects are well documented in the works of Li *et al*. (2021) and Lokesha *et al*. (2025).

This present study was aimed to investigate the modulatory effects of co-administration of ascorbic acid and zinc gluconate on sensorimotor changes in Wistar rats during the hot-humid season.

MATERIALS AND METHODS

Site of Experiment

The experiment was carried out in the Physiology Research Laboratory of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria (11°10/ N, 07° 38/ E) located at the elevation of 650 m above the sea level located in the Northern Guinea Savannah zone of Nigeria and with an annual rainfall of 1107 mm (Okey and Ayo, 2015).

Experimental Animals

Twenty (20) adult male Wistar rats weighing 110 – 186 g were used for the experiment. They were given access to feed (Vital Feeds, Jos, Nigeria) and water *ad libitum.* The rats were pre-conditioned to the experimental procedures at least one week before the commencement of the experiment.

Drug Preparation

Ascorbic acid tablets (Mopson Pharmaceutical Limited, Lagos, Nigeria.) and zinc gluconate tablets (Good 'N Natural Bohema, USA) were each dissolved in 1 ml of distilled water to obtain 100 mg/ml and 50 mg/ml suspension, respectively prior to daily administration.

Treatment Protocol

The Animals were grouped into two groups of 10 rats each: Group I, which served as the control animals, were given distilled water at the dose rate of 1 ml/kg; and Group II, which comprised the experimental animals, were first given ascorbic acid at the dose rate of 100 mg/kg and immediately after were administered with zinc gluconate at the dose rate of 50 mg/kg.

The regimens were given once and orally by gavage for a period of 28 days.

Neurobehavioural Tests

The Inclined Plane

The effect of treatments on neuromuscular coordination was assessed using the performance on inclined plane as described by Petrich (2006). The inclined plane performance was evaluated by placing each rat on an apparatus made with an angled rough wooden plank, with thick foam pad at its bottom end. The plank was raised to 35° and, thereafter, gradually increased stepwise by 5° until the subject could no longer stay and be situated horizontally on the plank for 3 sec, without sliding down. Angles were measured and marked on the apparatus beforehand, and are obtained by propping the plank against a vertical bar with several notches. The test was performed with the head of the rat first facing left and then right. The highest angle at which each rat stayed and stood horizontally facing each direction was recorded. Two trials were performed for each testing period and their average value was taken. This assessment was conducted on days 0, 7 and 21 of the experimental periods.

Forepaw Grip Time

The forepaw grip time according to the method of Abou-Donia *et al.* (2001) was used to evaluate the effect of treatments on motor strength of the rats. This was assessed by having the rats hung from a 5 mm diameter wooden dowel, gripped with both forepaws. The time spent by each rat before releasing the grip was recorded in seconds. This assessment was conducted on days 0, 7 and 21.

Measurement of excitability score

The excitability of each rat performed using the method of Kannan *et al*. (2002) and modified by Ayo *et al.* (2006). Briefly, each rat was held at the tail and with its face facing downwards. A grade of 0 – 5 was used to assess each animal as follows

Grade 0 – Rat did not show any form of wriggling.

Grade 1 – Rat wriggling was low and also showed feeble paw movement.

Grade 2 – Rat responded through a stronger wriggling and feeble paw movement.

Grade 3 – Rat vigorously wriggled and a strong fore and hind-limb movement.

Grade 4 – In addition to observation in grade 3 above, rat made unsuccessful attempt to climb its tail.

Grade 5 – In addition to observation in grade 3 above, rat successfully climbed the tip of its tail.

Measurement of Meteorological Data

Dry- bulb temperature and wet bulb temperatures inside the laboratory were taken using the wet and dry-bulb thermometer on days 1, 2, 3, 7, 10, 14, 17, 21, 27 and 28 of the experimental periods at 7:00 h,

13:00 h and 18:00 h, and relative humidity was calculated using the manufacturer's manual attached, while the heat index was calculated according to Jackson (2009).

Data Analysis

Data were expressed as mean ± SEM. Student T-test was used to analyse the neurobehavioural parameters except for excitability scores that was expressed in percentage. The analysis was done, using the Statistical Package for Social Scientist (SPSS, Chicago, IL, USA). Values of $P < 0.05$ were considered significant.

RESULTS

Thermal Environment Data

Tables 1 and 2 show the thermal environment data and heat index, recorded during the study period. The overall heat index recorded during the experimental period was 28.3^0C ± 0.57. Minimum and maximum values of heat index recorded at 7.00 h and 18.00 h were 24°C and 34°C, respectively. The heat index obtained at 7.00 h was the lowest, and the value was lower than that recorded either at 13.00 h or 18.00 h. The difference between the values was significant ($P < 0.05$). The heat index recorded at 13.00 h when compared to the corresponding values obtained at 7.00 h and 18.00 h was significantly different ($P < 0.05$). Furthermore, the highest heat index recorded at 18.00 h when compared with either that of 7.00 h or 13.00 h was significantly different ($P < 0.05$).

Neuromuscular Coordination

Figure 1 shows the dynamics of neuromuscular co-ordination using the inclined plane test on days 0, 7 and 21. On day 0, the angles of inclination were 56.75 ± 0.65° and 55.5 ± 1.33°, for the control and experimental groups. The corresponding values recorded on day 7 are 57.25 ± 1.08° and 54.75 ± 3.99°, respectively while on day 21, the corresponding values were 54 ± 0.65° and 53.25 ± 1.67°, respectively. The differences in the values obtained on days (0, 7 and 21) for experimental and control groups were not statistically significant ($P > 0.05$).

Motor Strength

Figure 2 shows the dynamics of motor strength using the fore-grip time test. The values were higher on days 7 and 21 in the ascorbic acid and zinc group when compared to those of the control group. Values recorded on day 0 were 34.3 ± 7.88s for the control group, while ascorbic acid and zinc group had the value of 19.6 ± 6.17s. The highest values of 31.7 ± 8.2s and 38.2 ± 11.04s were recorded in the control group and ascorbic acid + zinc-treated group, on day 7. On day 21, values of 21.5 ± 9.13s and 23.6 ± 5.35s were obtained in control, and ascorbic acid + zinc treated group respectively. Differences in the values recorded on days 0, 7 and 21 were also not statistically significant ($P > 0.05$).

Excitability Scores

Table 3 shows the percentage excitability scores of wistar rats during the hot-humid season. No rats had grades 0 -2 in both groups. An excitability score of 3 was recorded in 30% of rats in the control group, 10% of rats in the experimental group. 40% of rats belonging to the control group and 30% of that experimental group had an excitability score of 4. Grade 5 was recorded in 30% of control rats and 60% of the experimental rats.

Table 1: Mean values of thermal environmental parameters recorded during the study period

Thermal environment Data	Mean ± SEM	Maximum	Minimum
Ambient temperature (°C)	26.2 ± 0.35	30	23
Relative humidity	81.2 ± 1.52	92	67
Heat Index (°C)	28.3 ±. 0.57	34	24

Table 2: Diurnal variation in heat index during the study period.

Heat Index	Mean ± SEM	Maximum	Minimum
7.00 h	25.6 ± 0.48^a	29	24
13.00 h	28.3 ± 0.79^b	33	25
18.00 h	31.0 ± 0.84^c	34	26

Values with different superscript letters are significantly difference ($P< 0.05$)

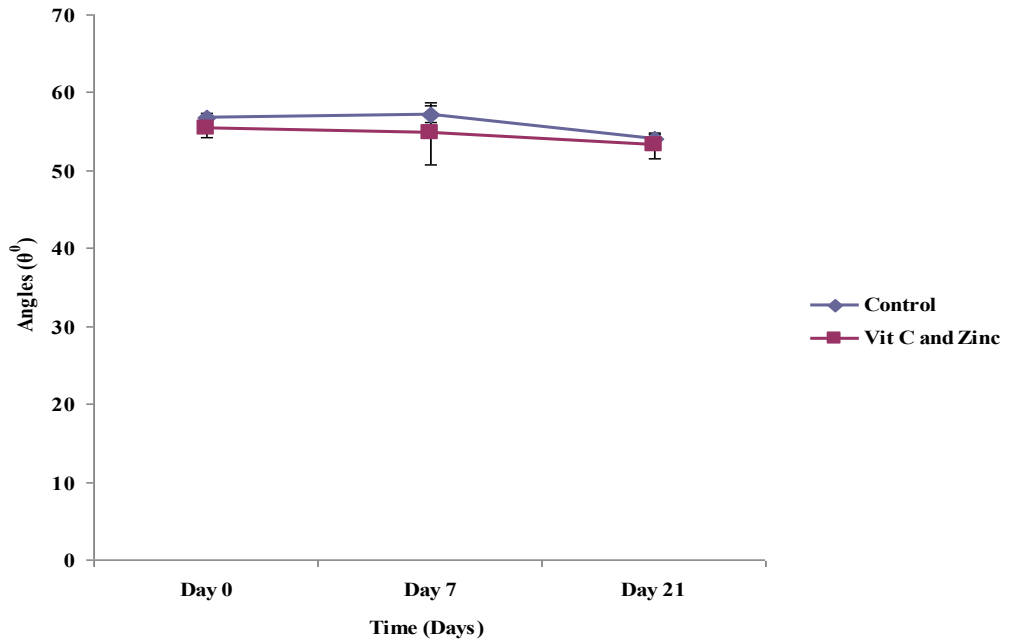

Figure 1: Effects of ascorbic acid and zinc gluconate on the dynamics of neuromuscular co-ordination using inclined plane test, in rats during the hot-humid season

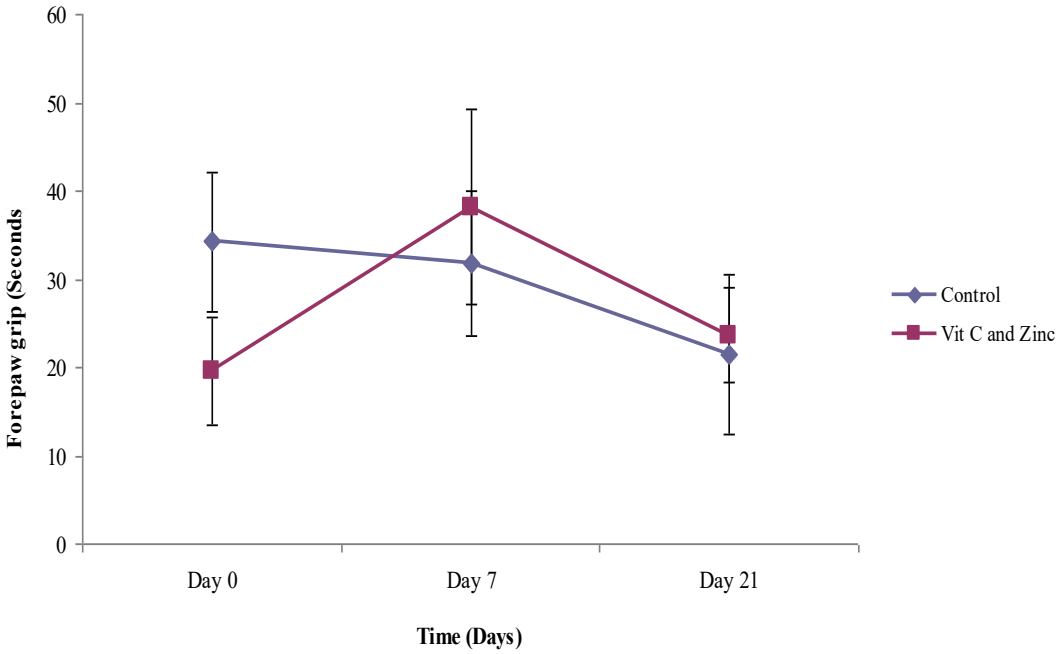

Figure 2: Effect of ascorbic acid and zinc gluconate on the dynamics of motor strength using fore-grip time test in rats during the hot-humid season

Table 3: Effects of ascorbic acid and zinc gluconate on excitability scores in Wistar rats during the hot - humid season

% Excitability Score	% Control	% Ascorbic acid +	Zinc gluconate
Grade 1	**0**	**0**	
Grade 2	**0**		**0**
Grade 3	**30**		**10**
Grade 4	**40**	**30**	
Grade 5	**30**	**60**	

DISCUSSION

The present results showed that overall mean value of heat index recorded during the experimental period, corresponding to the peak of rainfall in the study area (Zaria), was $28.3^0C \pm 0.57$. The heat index obtained during the study period showed that the experimental animals were exposed to moderate heat stress, characteristic of the hot-humid season (August – September), which was the peak of rainfall, and by increased ambient temperature and high relative humidity. The moderately high heat index obtained in the present study is consistent with the findings of Ayo *et al*. (2008) and Habeeb (2020) that the hot-humid season in the Northern Guinea Savannah zone of Nigeria is thermally stressful to animals. Heat stress occurs when there is an imbalance between heat production within the body and its dissipation, resulting in several physiological and behavioural responses in an attempt to adjust to the thermally stressful environment. The result of the present study showed that adjustments to thermal stressful environment may decrease cognitive and sensorimotor functions in man (Habeeb, 2020).

The findings of the study showed relatively higher values for neuromuscular coordination in rats in the control group than those treated with ascorbic acid + zinc. The result suggested a more efficient endogenous enzymatic defense system in the ascorbic acid + zinc-treated group than in the control group (Alhassan *et al*., 2016; Lokesha *et al*., 2025).

The results for Motor strength obtained on days 7 and 21, showed that values of ascorbic acid + zinc-treated group higher than those of the control group, although the differences between the values were not significant ($P > 0.05$). This may be due to the biological role of ascorbic acid and zinc in the body. Vitamin C neutralizes reactive oxygen species (ROS) induced by heat stress through its donation of an electron to the free radical, thereby reducing ROS activity. Consequently, zinc is a cofactor to several enzymes and it mitigates against reactive oxygen species produced during heat stress conditions. This is essential for maintaining the integrity of neuronal structure by facilitating protein folding to generate active molecules and synaptic transmission, involved in glutamate storage (Li *et al*., 2021; Hieu *et al*., 2022; Lokesha *et al*., 2025).

The results of the excitability scores of wistar rats showed that the maximal score of 60% was recorded for grade 5 in the ascorbic acid + zinc-treated group suggesting the ability of ascorbic acid to stimulate the sympathetic nervous system through the modulation of adrenaline and 5-hydroxytryptamine, involved in the control of brain function and mood. Zinc plays a vital role in adult neurogenesis and proper hippocampal functioning such as increased membrane excitability in hippocampal CA1 neurons in wistar rats (Tian *et al*., 2010; Sim *et al*., 2021; Baj *et al.* 2023).

CONCLUSION

In summary, the hot-humid season is mildly thermally stressful and its negative effects on the sensorimotor changes can be attenuated by combined administration of ascorbic acid and zinc gluconate.

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