EFFECT OF ASCORBIC ACID ON BEHAVIOUR OF WISTAR RATS EXPOSED TO ENVIRONMENTAL HEAT STRESS

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Abstract

Introduction: The hot-dry season in the Northern Guinea Savannah zone of Nigeria is characterized by high ambient temperature and high relative humidity which imposes heat stress on the body resulting in enormous generation of free radicals and behavioural changes.

Aim: The aim of the study was to determine the effect of ascorbic acid (AA) on behavior of Wistar rats exposed to environmental in heat stress.

Methods: Thirty Wistar rats weighing 140-160g were divided into 3 groups of 10 each. Group 1 served as control and was administered normal saline 1ml/kg orally, groups 2 exposed to environmental temperature of 38 ± 1 ^{0}C and group 3 were exposed to environmental temperature of 38 ± 1 ^{0}C and administered AA at 100mg/kg orally for 21 days. Behavioral activity in the open-field was assessed on days 1, 7, 14 and 21.

Results: The results showed a significant increase in pellets count, urination and immobilization in the control and heat exposed group compared to AA treated on days 1 and 7. Rearing, grooming and ambulation were significantly decreased in AA treated group on day 1, 7 and 14. There was no significant behavioral change on the 21st day indicating habituation.

Conclusion: It was concluded that ascorbic acid ameliorated some of the behavioral changes induced by environmental heat stress and therefore, individuals working in high temperature zones should be advised to be taking vitamin C supplements.

Key words: Heat stress, Behaviour, Ascorbic acid, Open-field.

Introduction

The hot-dry season in the Northern Guinea Savannah zone of Nigeria, characterized by high ambient temperature and high relative humidity, imposes heat stress on the body (Ayo *et al.,* 2011). Heat stress is the net (overall) heat burden on the body from the combination of body heat generated while working and for being subjected to environmental sources such as air temperature, humidity, air movement, radiation from the sun or hot surfaces (Threshold Limit Values 2001). In all instances, the cause of heat stress is a working environment which can potentially overwhelms the body's ability to deal with heat. Continous heat stress induces large demands on physiological and metabolic processes to compensate for changes in thermal environment which can affect the well being of animals including man (Gordon, 1993). Heat stress is one of the most important stressors in hot regions of the world leading to enormous generation of free radicals (FRs) and other reactive oxygen species (ROS) resulting in overwhelming of natural antioxidant in the tissues leading to oxidative stress (Altan *et al*., 2003).

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At the celluar level, heat stress induces the expression of heat shock proteins (HSPs), which is closely associated with adapted thermotolerancce against a sudden heat shock (Horowitz, 2002). The expression of HSPs is known to be involved in an endogenous cellular defense mechanism that enables cells to cope with stressful conditions and generation of free radicals and oxidative stress (*Molvarec et al.,*2011). Free radicals play an important role in the functioning of the brain in physiologic and pathologic conditions (Drew *et al.,* 1998). Heat stress can profoundly disturb the balance between the generation of ROS and the antioxidant defense system (*Molvarec et al.,*2011). The major outcome of oxidative stress is the irreversible damage of cell macromolecules by ROS leading to lipid peroxidation (LPO). Proteins are the major target for oxidants as a result of their abundance and their elevated reaction rate constants (Bizzozero, 2009). The deleterious effects of oxidative free radicals are ameliorated by antioxidants. Scavengers of free radicals such as ascorbic acid (AA) also known as vitamin C may play a pivotal role in preventing the cellular damage of organs and maintaining homeostasis during heat stress (Seo-Hyun *et al.,*2012) Ascorbic acid, is a potent water-soluble, naturally occurring antioxidant, a cofactor in various enzymes and a free radical scavenger by its antioxidant effect (Frei *et al.,* 1990). Currently ascorbic acid is the most widely used vitamin supplement throughout the world (Naidu, 2003) due to its variety of biological functions (Maret and Jan, 2011). It is an outstanding antioxidant in human blood plasma (Frei *et al.,*1989). Vitamin C has the ability to protect against LPO by acting as a scavenger of ROS and by one-electron reduction of lipid hydroperoxyl radicals via the vitamin E redox cycle (Halliwell, 1996). Ascorbic acid has been shown to be of value in the prevention and therapy of adverse effects of stress factors. Clinical and epidemiological studies have indicated that ascorbic acid may be beneficial in chronic diseases such as cardiovascular disease and cancer (Valpuesta and Botella, 2004; Coulter *et al.,* 2006) and the ability to fight off environmental pollutants including carbon monoxide, pesticides and heavy metals (*Iqbal et al.,* 2004). The health promoting effects of vitamin C can be attributed to its biological functions as a co-factor for a number of enzymes (Maret and Jan, 2011). Studies on the effect of heat stress and nervous activities have been studied but none has linked it to free radicals and ascorbic acid.

Materials and Methods

Experimental Design

A total of 30 adult Wistar rats weighing 140-160g were fed pellets made from growers' mash, Maize bran and Groundnut cake in the ratio of 4:2:1 with Wheat flour as binder. The rats were allowed to acclimatize to the environment for two weeks and given water *ad libitun* before the experiment commenced. Group I made of 10 rats served as the control received normal saline and exposed to normal room body temperature, group II made of 10 rats received normal saline and exposed to environmental temperature of 38 ± 1 ^{0}C and group III made up of 10 rats were exposed to environmental temperature of 38 ± 1 ^{0}C and administered vitamin C orally at a dose of 100mg/kg daily. The experiment lasted for a period of 21 days.

Drug Preparation

Ascorbic acid tablets, Med Vit C (100mg/tablet). Each tablet was dissolved in 1ml of distilled water to obtain 100mg/ml suspension, just prior to its daily administration.

Measurement of Rectal Temperature (RT)

The RT was recorded using standard digital clinical thermometer (Hartman's Company Plc, England), inserted about 5 cm into the rectum via the cloaca for 3 min until an alarm was heard, indicating that the temperature has been stabilized Rectal temperature was monitored and taken daily for three weeks.

Change in body temperature has been used as important criterion for stress reaction in animals according to Boulant (1991).

Open-field test

Behavioral activity in the open-field (OF) was assessed on days 1, 7, 14 and 21. The OF test was first described by Hall in 1934 and is currently a useful tool to assess behaviour. The open-field assessment was performed as described by Zhu *et al.*,(2001) on day 0, 7, 14 and 21 of the experiment. The open field apparatus was constructed as described by Zhu *et al.,* (2001). Briefly, it was made of a cardboard box (50 x 50 x 46cm high) with clear plexiglas on the inner surface. The floor was divided into 25 equal squares. Each animal was placed in the box and allowed to walk freely for 3 minutes, and the recordings of parameters were made in the next 2 minutes. The apparatus was used to assess activity and locomotion of each rat. It was also used to evaluate anxiety in the rats by measuring the number of stretch-attend postures (risk-assessment behaviour) which was the frequency with which each animal demonstrated forward elongation of the head and shoulders, followed by retraction to the original position and a high frequency indicates high levels of anxiety (Blanchard *et al.,* 2001) Other parameters measured using the apparatus were grooming, which was the duration of time the animal spent licking or scratching itself while stationary; grooming behaviour is a displacement response and is expected to be displayed in a novel environment (Espejo, 1997). The apparatus was first cleaned with 95% ethanol after assessing each anima in order to remove the interfering odours left by the previous animal.

Statistical Analysis

Data was expressed as mean ± standard error of mean (mean ± SEM). Analysis of variance (ANOVA) was used to assess the difference in mean values between the control and test groups using graphpad prism 4.0. Values of $p<0.05$ were considered significant.

Results

Table 1 show the body temperature distribution of heat stress rats supplemented with ascorbic acid. The ascorbic acid significantly decreased body temperature on days 1, 7 and 14 in ascorbic acid treated group as compared to stressed induced animals and no changes were observed on day 21.

Table 1: Effect of Heat Stress on Body Temperature and Ameliorating Effect of Ascorbic Acid

No. of Day(s)	Body Temperature (^{o}C)		
	Control (n = 10)	**Stress (n = 10)**	**Stress + AA (n = 10)**
Day 1	37.24	39.10*	38.02*
Day 7	37.30	38.60*	37.35*
Day 14	37.28	38.40*	37.34*
Day 21	37.44	37.46	37.45

Vales in same raw bearing the super script * are significantly different (P<0.05).
Key: AA: Ascorbic Acid

Ascorbic acid significantly increased movement and decreased pellets count on days1, 7, and 14 as compared to stressed induced animals (Table 2). A significant decrease in frequency of rearing and grooming was observed in AA treated as compared to stress induced group. There was no significant behavioral change on the 21st day indicating habituation.

Table 2: Effect of Heat Stress on Behaviour and the Ameliorating Effect of Ascorbic Acid

Parameter	DAY 1		DAY 7		DAY 14		DAY 21	
	HS	HS+AA	HS	HS+AA	HS	HS+AA	HS	HS+AA
Movement (Sec)	13.5	22.4*	16.2	21.3*	17.9	20.3*	20.1	21.6
Pellet (Number)	4.1	3.1*	3.5	2.2*	2.9	1.6*	1.4	1.6
Grooming (Number)	6.4	3.9*	8.4	5.2	7.3	4.8*	5.8	4.9
Rearing (Number)	13.2	6.3*	15.1	7.1*	8.4	7.2	6.1	5.8
Stretch Attend Posture (Number)	3.1	2.8	2.3	2.1	1.8	1.6	1.2	1.3

* Significant.

Keys: HS: Heat Stress, AA: Ascorbic Acids

Discussion

Behavioural changes are the main signs of distress and the first signs of disease in animals (Ayo *et al*., 2002) and heat stress environment is one of the main causes of distress. The open field test (OFT) is used to assay general locomotor activity levels and anxiety in rodents. Decrease anxiety leads to increased exploration behavior while increased anxiety results in less locomotor motion. Hall (1934) describes defecation and urination as indices of anxiety in rodents. He argues that the animal will have reduced locomotion in a novel environment but the autonomic nervous system will be activated which will increase defecation in this noxious arena. Repeated exposure to the open field apparatus results in time dependent changes in behavior (Choleris *et al.*,2001) and habituation as seen in this study on the 21st day. At first, when the apparatus is novel to the animals, more fear-related behaviours (such as stretch attends and activity in the corners and walls of the open field) are displayed. However, with repeated trials, more exploration and locomotor activity (such as rearing and line crosses as well as more central square activity) was observed. With repeated exposure, some strains show increased activity while others show habituation and decreased activity levels and others show no change (Bolivar *et al.,* 2000). Grooming in the open field has been associated with anxiety-related variables from several other (unconditioned and conditioned) anxiety tests (Sira *et al*., 2014). In the OFT, an interplay between freezing and stretch attend posture, at one hand, and rearings and grooming on the other hand. This interplay was in line with the notion that, once in contact with novel stimuli, the individual disengages from ongoing behavior, devotes attention to risk assessment and, if threat is ruled out, exploratory behavior takes place (Sira *et al*., 2014).

Along this sequence, arousal rises at first and falls afterwards. This notion/sequence roughly corresponds to the concept of a behavioral inhibition system proposed by (Gray and McNaughton, 2000). Thus, once placed in the OF, an animal is expected to at first show freezing and stretch attend posture (these behaviors are devoted to risk assessment) which, after some time, can be replaced by exploratory behaviors (for example: locomotion and rearing) and grooming (supposed to be related to dearousal). Therefore grooming behaviour should decrease with repeated exposure to the testing apparatus and defaecation, which was the number of faecal pellet produced and increase in defecation shows increased anxiety and rearing, which is the frequency with which the rodent stood on their hind legs in the field.

This behavior shows increased exploratory behaviour and a high frequency indicates increased exploration and locomotion. Ascorbic acid (AA) is an important dietary antioxidant which is highly concentrated in the central nervous system (Yenisey *et al.*, 2006) and therefore able to ameliorate the behavioural changes induced by environmental heat stress in this study. Ascorbic acid has a modulatory action on brain neurotransmitter like the cholinergic, serotonergic and dopaminergic systems (Dai *et al.*, 2006). These neurotransmitter systems are important in behavioural processes (Ogren *et al.*, 2008). These observations indicate its potent antioxidant effect. The mechanism of action of AA on stress factors may be caused by the fact that AA is an inhibitory vitaminergic neurotransmitter in the hypothalamus, which plays an important role in thermoregulation by inhibiting cortisol, the chief hormone of stress and the limbic region involved in the elicitation of fear and frustration (Balz 2003; Karanth *et al.,* 2000). The decrease in temperature by AA is in line with (Ayo *et al*., 2005 and 2007). The findings of the present study demonstrated that AA reduced significantly the stress on temperature by the heat stress environment. Similarly, ameliorating effects of AA on heat induced stress have been documented (Minka and Ayo 2010, 2011). The result obtained indicated that AA facilitated the physiological mechanism of thermoregulation. It has been established that AA decreases heat load through reduction in heat production or increasing heat loss by enhancing thermal exchange between the body and the environment (Minka and Ayo, 2010; Tauler *et al*., 2003). The mechanism of action of AA in reducing heat stress may also be through the detoxification of reactive oxygen species known to be in abundance in the body during stressful conditions (Minka and Ayo, 2007; Tauler *et al*., 2003). AA has been reported to be a chain-breaking antioxidant, involved in the prevention and restriction of free-radical chain formation and propagation. Ascorbic acid supplementation was found to alleviate detrimental effects of heat stress (Elsayed *et al*., 2008).

Conclusion

The results of the present study have demonstrated that AA ameliorated the behavioral changes induced by environmental heat stress, thereby establishing the relationship between thermal environment, behavioural activities and AA.

Recommendation: Individuals working in high temperature zones should be advised to be taken vitamin C supplements.

Conflicts of interest

The authors declare that there is no conflict of interests in the publication of this paper.

References

Altan, O., Pubucuogin, A., Altan, A., Konyaliogl, S. and Bayraktar. (2003**.** Effects of heat stress on oxidative stress, lipid peroxidation and some stress parameter in broilers. *British Poultry Science*, 44:Pp.545-550.

Ayo, J.O., Obidi, J.A. and Rekwot, PI. (2011). Effects of heat stress on the well-being, fertility, and hatchability of chickens in the northern Guinea savannah zone of Nigeria: A review. *ISRN Veterinary Science vol.* 2011. ID 8 3 8 6 0 6

Ayo, J.O., Oladele, S.B. and Fayomi, A. (2002). ‘Behavioural reactions of cattle to stress situations: A review’ *Journal of Agricultural Technology* 8, 15–20.

Ayo, J.O., Minka, N.S. and Fayomi, A. (2005). ‘Effects of ascorbic acid on rectal temperature of pullets transported by road during the hot-dry season’ *Tropical Journal of Animal Science* 8**,** 43–48.

Ayo, J.O., Owoyele, O.O. and Dzenda, T. (2007). 'Effects of ascorbic acid on diurnal variations in rectal temperature of Brown Nera pullets during the harmattan season', *International Journal of Poultry Science* 6, 612–616.

Balz, F. (2003). 'Vitamin C intake', *Nutritional Disease* 14, 1–18.

Bizzozero, O. A. (2009). Protein carbonylation in neurodegenerative and demyelinating CNS diseases. In: Lajtha A, Banik N, Ray S, editors. Handbook of neurochemistry and molecular neurobiology. New York: Springer; Pp. 543–62.

Blanchard, D. C., Griebel, G. and Blanchard, R. J. (2001). Mouse defensive behaviors: Pharmacological and behavioral assays for anxiety and panic. *Neuroscience and Biobehavioral Reviews*, 25, 205-218.

Bolivar, V. J., Caldarone, B. J., Reilly, A. A. and Lorraine, F. (2000). Habituation of activity in an open field: a survey of inbred strains and F1 hybrids. *Behavior Genetics*, 30, 285-293.

Boulant, J.A. (1991).Thermoregulation. In: Fever: Basic mechanism and management (Mackowlak, P. ed.), Raven Press, New York: Pp1-22.

Choleris, E., Thomas, A. W., Kavaliers, M. and Prato, F. S. (2001). A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neuroscience and Biobehavioral Reviews*, 25:235-260.

Coulter, I. D., Hardy, M. L., Morton, S. C., Hilton, L.G., Tu, W. and Valentine, D. (2006). Antioxidants vitamin C and vitamin E for the prevention and treatment of cancer. *Journal General Internal Medicine; 21:735–44*.

Dai, F., Yang, J. Y., Gu, P. F., Hou, Y. and Wu, C. F. (2006). Effect of drug-induced ascorbic acid release in the striatum and the nucleus accumbens in hippocampus-lesioned rats. *Brain Research* 1125:163–70.

Drew, G., Jak.bczyk, M. and Araszkiewicz, A. (1998). Role of free radicals in schizophrenia. *Medical Science and Monitoring*, 4(6): 1111-1115.

Elsayed, M. A., Farghaly, H. A. and Abu-Taleb, A. M. (2008). 'Effct of vitamin C supplementatin on some physiological parameters of ostrich exposed to early heat stress of temperature', *Applied Life Science* 40(4S2), 1503–1513.

Espejo, E. F. (1997). Effects of weekly or daily exposure to the elevated plus-maze in male mice. *Behavioural Brain Research*, 87, 233-238.

Frei, B., England I. and Annes, B. N. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. *Proceeding of National Academy of Science* USA 86, 6377-6381.

Frei, B., Stocker, R., England, L. and Annes, B.N. (1990). Ascorbate: The most effective antioxidant in human blood plasma. *Advanced Experimental Medical Biology* 264:155-163.

Gordon, C.J. (1993). Temperature regulation in laboratory rodents. Cambridge University Press, New York. Pp. 50-55

Gray, J. A. and McNaughton, N. (2000). The neuropsychology of anxiety: An enquiry into the functions of the septo-hippocampal system. Oxford University Press, Oxford. Pp 87-91

Halliwell, B. (1996). Vitamin C: Antioxidant or pro-oxidant *in vivo*? *Free Radical Research*, 25:439-454

Hall, C. S. (1934). Emotional behvior in the rat. 1 defecation and urination as measures of individual differences in emotionality. *Journal of Comparative Psychology,* 18, 382-403.

Horowitz, M. (2002). From molecular and cellular to integrative heat defence during exposure to chronic heat. *Comparative Biochemistry Physiology* 131:475-483

Iqbal, K; Alan k; and Muzaffar M (2004 **):** Biological significances of ascorbic acid (Vitamin C) in human health- A Review. *Pakistan Journal of Nutrition* 3 (1): 5-15.

Karanth, S., Yu, W.H., Walczewska, A., Mastronardi, C. and McCann, S.M. (2000), 'Ascorbic acid acts as an inhibitory transmitter in the hypothalamus to inhibit stimulated lutenizing hormone-releasing hormone release by scavenging nitric oxide', *Proceedings of the Academy of Sciences of the United State of America* 97, 1891– 1896.

Maret, G. T. and Jan, F.S. (2011). Vitamin C and E: Beneficial effects from a mechanistic perspective. *Free Radical Biology Medicine* 51(5):1000-1013

Minka, N. S. and Ayo, J. O. (2010). 'Behavioural and rectal temperature responses of Black Harco pullets administered vitamin C and E and transported by road during the hot-dry season', *Journal of Veterinary Behavior and Clinical Applications and Research* 5, 134–144.

Minka, N. S. and Ayo, J. O. (2011). 'Modulating role of vitamins C and E against transport-induce stress in pullets during the hot-dry conditions', *ISRN Veterinary Science*_ 497138.

Minka, N. S. and Ayo, J. O. (2007). 'Haematology and behaviour of pullets transported by road and administered with ascorbic acid during the hot-dry season', *Research in Veterinary Science* 85(2), 389–393.

Molverac, A., Szarka, A., Walentin, S., Beko, G., Karsadi, I., Prohaszka, Z. and Rigo, J. (2011). Serum heat shock protein 70 levels in relationmtocirculating cytokines, chemokines adhesion molecules and angiogenic factors in women with pre-eclampsia. *Clinical Chemistry,* 412: 1957-1962

Naidu, K. A. (2003). Vitamin C in human health and disease is still a mystery? An Overview. *Nutritional Journal* 2-7.

Ogren, S. O., Eriksson, T.M., Elvander-Tottie, E., D'Addario, C., Ekström, J.C. and Svenningsson, P. (2008). The role of 5-HT1A receptors in learning and memory. *Behaviur Brain Research 195:54–7.*

Seo-Hyun, Y., Yang-Soo, M., Sea-Hwan, S. and In-Surk, J. (2012). Effects of cyclic stress or vitamin C supplementation during cyclic heat stress on HSP70, inflammatory cytokines and the antioxidant defense system in Sprague Dawley rats. *Experimental Animal* 61 (5): 543-553.

Sira, D., Celio, E., Toni, C., Gloria, B., Andrea, R., Adolf, T. and Albert, F. (2014). Relationships of open-field behaviour with anxiety in the elevated zero-maze test: Focus on freezing and grooming. *World Journal of Neuroscience*~~,~~ 4, 1-11.

Tauler, P., Aguilo, A., Gimeno, I., Fuentespina, E., Tur, J.A. and Pons, A. (2003). 'Influence of vitamin C diet supplementation on endogenous antioxidant defense during exhaustive exercise', *European Journal of Physiology* 446, 658–664.

Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices (2001). American Conference of Government Industrial Hygienists, Cincinnati, Ohio. Page 169.

Valpuesta, V. and Botella M.A. (2004). Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant. *Trends Plant Science* 2004; 9(12):573–7.

Yenisey, C., Seyrek, K., Serter, M., Kargin, F.K. and Bardakcioglu, H.E. (2006). Effects of dietary vitamin C supplementation on glutathione, malondialdehyde and nitric oxide concentrations in brain and heart of laying Japanese quails exposed to heat stress (34.8 °C). *Review Medicine Veterinary 157:149–52.*

Zhu, H. R,, Rockhold, R. W., Rodney, C., Baker, R.C., Robert, E., Kramer, R.E. and Ho, I. (2001). Effects and single or repeated dermal exposure to methyl parathion on behavior and blood chohinestrase activity in rats. *Journal of Biomedical Science, 8: 467-478.*