

*Full Length Research Paper*

## Behavioural and haematological studies on effects of lycopene in Wistar rats subjected to psychological stress

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This study investigated the effects of prior lycopene supplementation on behaviour and haematology of Wistar rats subjected to psychological stress. Four groups of Wistar rats, each comprising seven animals were investigated: unstressed (U-Control), unstressed + lycopene (U+Lyco), stressed (S-Control) and stressed + lycopene (S+Lyco). Olive oil was given by oral gavage to each rat in the groups, either singly or mixed with 10 mg/kg lycopene daily for two weeks. S-Control and S+Lyco groups were subjected to psychological stress followed by behavioural assessment using excitability score test. Blood samples were collected after sacrificing the animals and then analyzed. Psychological stress increased ( $P < 0.05$ ) erythrocyte malondialdehyde (MDA) concentration in Wistar rats. Lycopene significantly increased excitability scores and total blood plasma proteins, and decreased ( $P > 0.05$ ) erythrocyte MDA concentration. In conclusion, antioxidant properties of lycopene may be attenuating the effects of psychological stress on excitability score and erythrocytes of Wistar rats.

**Key words:** excitability score, erythrocytes, total plasma proteins, fear stress, oxidative stress, antioxidant supplementation.

### INTRODUCTION

Contextual fear conditioning is a useful behavioural paradigm that can evoke bio-behavioural changes in response to a psychological-based stressor (Sanders and Knoepfler, 2008). It involves placing a rodent into an apparatus and receives pairings of a phasic electrical shock to its feet. Subsequently, when tested in the original training context, the rat will display a natural defensive response termed freezing (Blanchard and Blanchard, 1969). The paradigm is associated with pro-long stimulation of the body stress response mechanism (Thompson et al., 2012) and may replicate the fear responses observed

when an animal is exposed to psychological stressors such as novel task, environment or husbandry procedures. Such fear responses are capable of disrupting the body homeostatic mechanism which may lead to detrimental effects on animal well-being.

Adverse effects of psychological stressors such as fear on biological functions are well known (Thompson et al., 2012). Although physiological response to fear is beneficial when acute, it may be superimposed by excess free-radical production leading to detrimental effects on health (Shin and Liberzon, 2010). This is usually termed

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chronic stress and it occurs through persistent activation of limbic-hypothalamo-pituitary-adrenal axis and the secretion of stress hormones in the brain (Cordero et al., 2003; Grillon et al., 2007). Stress-related behavioural activities and haematological responses may be triggered for a long period by an exposure to psychological/ emotional stressor (Armario et al., 2008; Shin and Liberzon, 2010). Studies in man (Szanton et al., 2011) and domestic animals (Lay and Wilson, 2004; Minka and Ayo, 2008) have shown that psychological stress, acting singly or in combination with other factors, may induce adverse behavioural and/or haematological effects due to increased free-radical activity.

Antioxidant supplementation is useful in augmenting endogenous antioxidant capacity in animal (Tauler et al., 2006). Expanding the understanding of the biological role of antioxidant supplements is vital, particularly during stressful conditions. Lycopene is a potent dietary antioxidant supplement and previous studies have demonstrated the beneficial effects of prior lycopene supplementation (Djuric and Powell, 2001; Al-Jassabi, 2005), particularly during exposure to psychological stress (Ogundeji et al., 2012). Maintaining circulatory concentration level of lycopene may result in reduction in lipoperoxidation and other beneficial effects such as enhanced behavioural and haematological responses. The aim of the study was to investigate the protective role of prior lycopene supplementation on behavioural and haematological responses in Wistar rats subjected to psychological stress.

## MATERIALS AND METHODS

### Experimental animals, design and management

Twenty-eight adult, male, 10 to 14 week-old Wistar rats weighing 190 to 220 g were procured from the animal house of the National Institute of Trypanosomiasis Research, Kaduna, Nigeria. They were kept in the animal house of the Department of Veterinary Physiology, Ahmadu Bello University, Zaria at ambient temperature of  $25 \pm 0.8^\circ\text{C}$  and a relative humidity of  $86.14 \pm 3.44\%$ . The rats were housed seven animals per cage and on a 12 h light-dark cycle (light during 7:00 to 19:00 h). They had access to pellets made from grower's mash, maize bran and groundnut cake in the ratio of 4:2:1, and water was provided *ad libitum*. The rats were acclimatized to the experimental procedures for two weeks prior to the commencement of the experiment. They were randomly assigned to four groups, each comprising seven animals: unstressed control (U-Control), unstressed + lycopene (U+Lyc), stressed control (S-Control) and stressed + lycopene (S+Lyc). 10 mg lycopene in a gelatinous capsule (General Nutrition Corporation, Pittsburgh, U.S.A.) was reconstituted in olive oil (*Goya en espana*, S.A.U., Sevilla, Spain) to appropriate working concentration. Olive oil (1 ml/kg) was given by oral gavage to each rat in the groups, either singly or mixed with 10 mg/kg lycopene daily for two weeks (Djuric and Powell, 2001; Al-Jassabi, 2005).

U-Control and S-Control rats were administered olive oil only, while U+Lyc and S+Lyc rats were administered with lycopene

lycopene mixed with olive oil. On days 13 and 14, S-Control and S+Lyc were subjected to psychological stress using the step-down inhibitory avoidance task. This is followed by behavioural assessment using the excitability score test on day 14, which was conducted in fully wake condition between the hour of 10:00 to 11:00 h. On the 15th day, that is 24 h after the psychological stress induction, each of the overnight-fasted rat was euthanized using light ether anaesthesia. After sacrificing the rats, blood samples were collected into heparinized sample bottles. The samples were analyzed for packed cell volume (PCV), haemoglobin (Hb) concentration, erythrocyte (RBC) count, total plasma proteins (TP), total leucocyte count (WBC), and erythrocyte malondialdehyde concentration (erythrocyte MDA). The study was approved by Ahmadu Bello University Animal Research Committee and in accordance with Guidelines for the Care and Use of Animals in Neuroscience and Behavioural Research.

### Psychological stress induction

Psychological stress was induced using step-down inhibitory avoidance task that is based on contextual fear conditioning (Armario et al., 2008; Shin and Liberzon, 2010). The apparatus for the task is an acrylic chamber (40 × 25 × 25 cm), consisting of a floor made of parallel 2 mm calibre stainless steel bar, spaced 1 cm apart. An electric shock was delivered through the floor bars. Each rat was gently placed on the platform on day 13. Upon stepping down from the platform, the rat immediately received a single 80 volt foot shock. For rat that did not return to the platform, the foot shock was repeated every five seconds. Fear was considered to have been induced if the rat remained on the platform for more than two minutes. Subsequently, rats were returned to the home cage environment. Twenty-four hours later, on day 14, rats were re-exposed to this same apparatus in the absence of the foot shock, serving as a situational reminder. In this case, each rat was again placed gently on the platform until the rat voluntarily dropped down from the platform. Maximum period of staying on the platform before a rat was removed from the apparatus was two minutes.

### Behavioural assessments

The behavioural response of each rat to handling was assessed using excitability score test (Adeiza and Minka, 2010; Ambali and Ayo, 2012). The test exploits the reaction of the animal to handling and on this basis, the level of response can be graded or scored. Briefly, each rat was held by the tail upside down and kept in such position for 30 s. The level of reaction of the animal was graded and scored as follows: (i) Score 1 (calm): Rat did not show any sign of wriggling and paw movement; (ii) Score 2 (occasional shakes): Rat responded through gentle wriggling and movement of forepaw; (iii) Score 3 (repeated shakes): Rat responded through stronger wriggling and movement of forepaw; (iv) Score 4 (violent shakes): Rat responded through vigorous wriggling, strong movement of fore- and hind limb as well as successfully climbing the tip of its tail.

### Determination of haematological parameters

The PCV, Hb concentration, RBC count, WBC were determined as described by Schalm et al. (1975). The PCV was determined by microhaematocrit method and the Hb concentration by cyanomethaemoglobin method. RBC and WBC were determined by haemocytometric method.

### Determination of biochemical parameters

Erythrocyte MDA determination is a biochemical test with higher sensitivity in detection of haematological changes. To determine the MDA, heparinised blood sample (2.5 ml) obtained from each animal was centrifuged at  $4,000 \times g$  for 5 min and the plasma was separated. Washing of erythrocytes three times in cold isotonic saline (0.9 % w/v) was done to obtain erythrocyte packets for the determination of MDA concentrations using Northwest Life Science Specialties (NWLSS™) Malondialdehyde assay kits (Northwest Life Science Specialties, Vancouver, WA 98662, USA). The principle of the method of determination was based on spectrophotometric measurement of the colour developed during reaction of thiobarbituric acid with malondialdehyde as described by Lykkesfeldt (2001). The blood plasma obtained was used to assay for total protein concentration. It was performed using hand-held refractometers (Eckersall, 2008).

### Statistical analyses

Values obtained were expressed as mean  $\pm$  standard error of mean (SEM). Excitability score data were analyzed by Student's *t*-test. Haematological and biochemical parameters data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post-hoc* test. The program used for the analysis was GraphPad Prism 4.0 version from GraphPad Software, San Diego, USA. Values of  $P < 0.05$  were considered significant.

## RESULTS

### Effect of lycopene on behavioural response

Effects of lycopene on excitability score of each rat (on 14th day) are shown in Figure 1. None of the rats in the S+Lyco had excitability score of one (1), while  $14.3 \pm 0.7\%$  of those in the S-Control had excitability score of one. Excitability score of two (2) was recorded in  $57.1 \pm 2.9\%$  of the rats in S-Control, and the value was significantly ( $P < 0.001$ ) higher than that of  $42.9 \pm 2.2\%$  recorded for rats in the S+Lyco.  $42.9 \pm 2.2\%$  of rats in the S+Lyco had excitability score of three (3), and the value was significantly ( $P < 0.001$ ) higher than  $14.3 \pm 0.7\%$  recorded in the S-Control.  $14.3 \pm 0.7\%$  of rats in each of the groups had excitability score of four (4).

### Effect of lycopene on haematological parameters

There was no significant difference in PCV, Hb, RBC, WBC values, both between and within groups (Table 1).

### Effect of lycopene on biochemical parameters

The MDA concentration in the erythrocytes of S-Control

group was significantly ( $P < 0.01$ ) higher than the unstressed group (that is, U-Control and U+Lyco). However, there was no significant difference between the values of erythrocyte MDA concentration recorded for U-Control, U+Lyco and S+Lyco groups (Figure 2). TP value was significantly ( $P < 0.05$ ) increased in the S+Lyco group compared to that of S-Control. However, there was no significant difference between the TP values of U-Control and U+Lyco and when compared to the stressed group (Figure 3).

## DISCUSSION

It is well known that psychological stressors can continuously activate the sympathetic nervous system to elicit stress response, consequently leading to increase free-radical production (Armario et al., 2008). In the present study, although there are no significant differences in the haematological parameters, a further analysis reveals that psychological stress impaired erythrocyte cell membrane integrity. This is evidenced by significant increase in erythrocyte MDA concentration in S-Control compared to that of U-Control. The effects of psychological stress on erythrocytes may have been indirectly mediated through the significant overlap existing among structures involved in the fear/anxiety response and stress response in the brain, and corticotrophin releasing hormone is likely involved in the coordination of both limbic-hypothalamo-pituitary-adrenal (LHPA) axis activity and many fear/anxiety responses (Shin and Liberzon, 2010). Thus, this finding indicates that subjection to psychological stress triggered the stress response mechanism in the Wistar rats, thereby resulting in increased free-radical production.

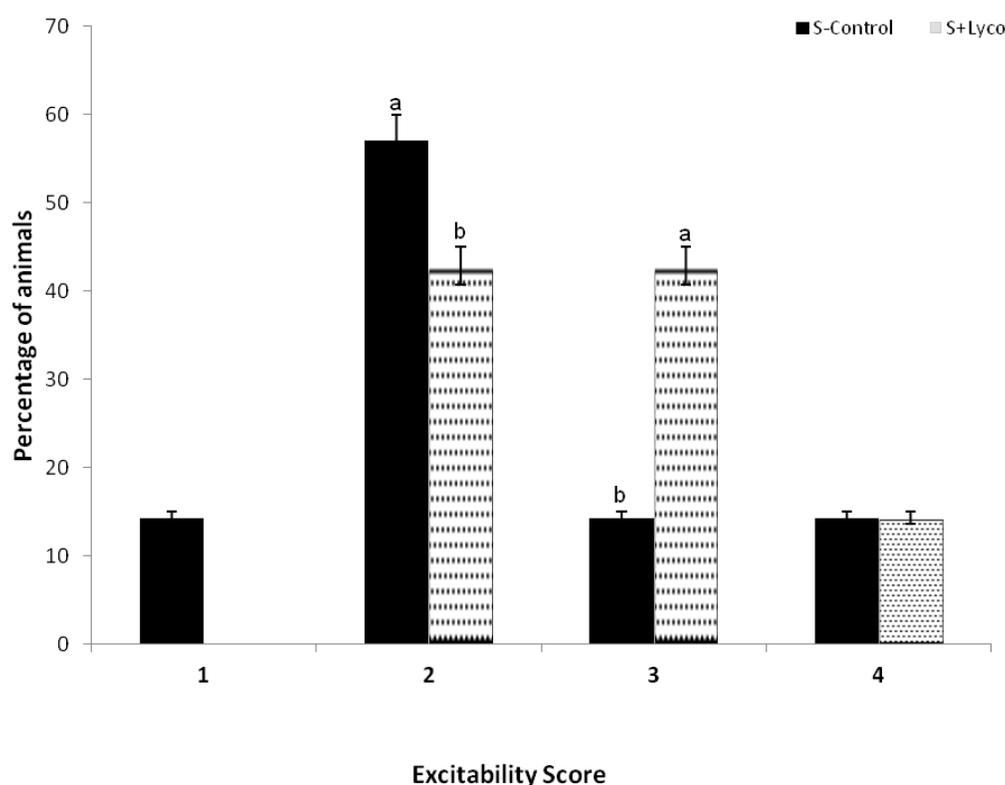
Increased free-radical activities have been generally shown to decrease viability and integrity of nerve cells (Friedman, 2010). Earlier study by Ogundeji et al. (2012) has shown that lycopene exerts antioxidant effects by enhancing behavioural responses through decreased brain free-radical activities. In the present study, none of the rats administered lycopene (that is, S+Lyco group) had excitability score of 1, instead majority obtained the score of 2 and 3, with greater percentage having score of 3. This finding indicates increased excitability. It implies that lycopene enhanced behavioural response, perhaps by activating the nervous system and facilitating transition from a state of depression to excitation through decreased free-radical activities. Ayo et al. (2006) made similar observation in goats administered ascorbic acids. Report has shown that positive behavioural response correlates with body activities leading to decreased free-radical production (Szanton et al., 2011).

The significant increase in TP value recorded for S+Lyco and the similarity in the values of erythrocyte

**Table 1.** Effects of lycopene supplementation on haematological parameters of Wistar rats (n = 5).

Parameter	U-Control	U+Lyco	S-Control	S+Lyco
PCV (%)	50.40±1.94	49.40±1.44	52.60±0.51	53.20±0.92
Hb (g/dl)	16.78±0.65	16.44±0.49	17.50±0.17	17.72±0.30
RBC ( $\times 10^{12}/\mu\text{l}$ )	8.20±0.27	8.10±0.25	8.72±0.15	8.84±0.16
WBC ( $\times 10^3/\mu\text{l}$ )	18.68±2.00	18.08±1.30	17.14±2.34	18.08±0.81
Neutrophils ( $\times 10^3/\mu\text{l}$ )	19.60±4.10	20.60±2.50	20.00±0.84	23.80±3.25
Lymphocytes ( $\times 10^3/\mu\text{l}$ )	81.80±5.52	76.20±3.28	73.80±1.32	78.20±3.26
Monocytes ( $\times 10^3/\mu\text{l}$ )	0.00±0.00	1.20±0.49	2.00±0.55	1.80±0.49
Eosinophils ( $\times 10^3/\mu\text{l}$ )	0.60±0.60	1.80±0.49	2.80±0.37	1.40±0.60
Band Neutrophils ( $\times 10^3/\mu\text{l}$ )	0.00±0.00	0.80±0.37	1.40±0.40	1.00±0.45

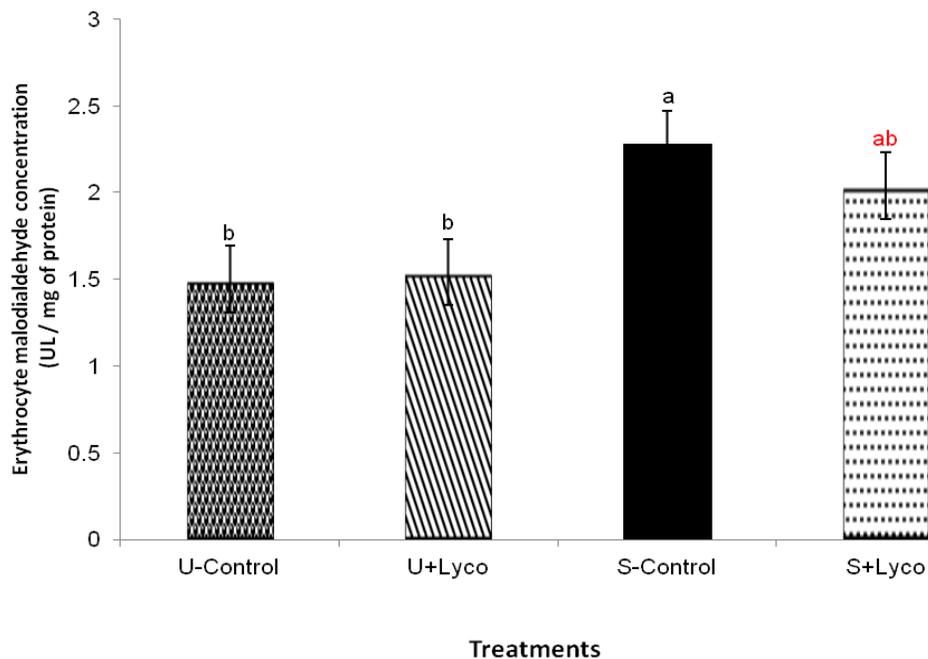
U-Control = unstressed control; U+Lyco = Unstressed + Lycopene; S-Control = stressed control; S+Lyco = Stressed + Lycopene.



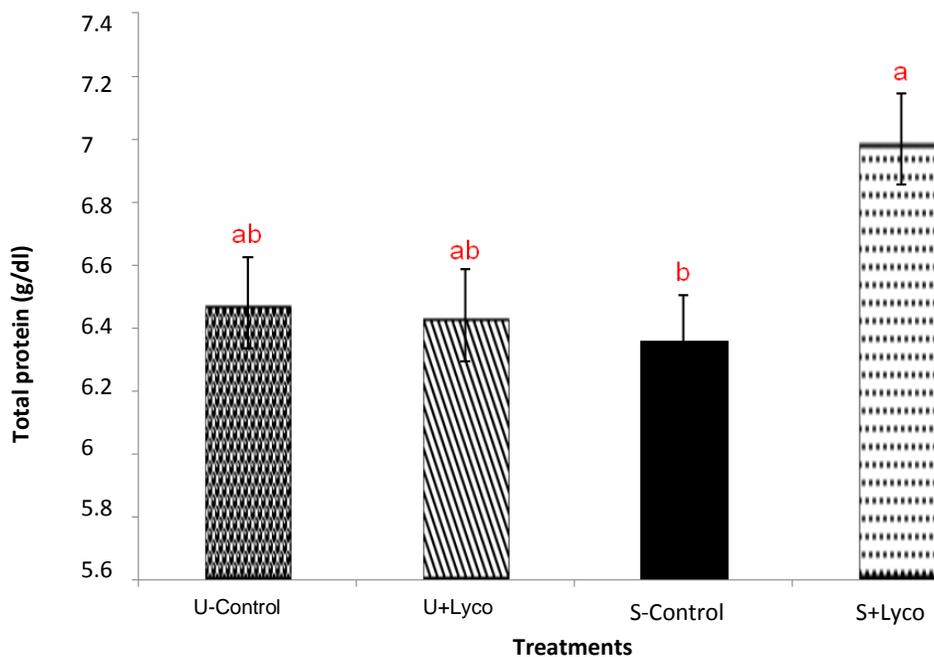
**Figure 1.** Effects of lycopene administration on excitability score in Wistar rats (n = 7). Mean values with different superscript letters are significantly ( $P < 0.001$ ) different. Score 1: Calm; Score 2: Occasional Shakes; Score 3: Repeated Shakes; Score 4: Violent Shakes

MDA concentration recorded for S+Lyco and the unstressed group suggests that lycopene protected the erythrocytes from psychological stress-induced oxidative damage by increasing the total plasma protein concentration. This agrees with the reports that proteins, particularly albumin because of its large amount in plasma, exert antioxidant property in the body (Halliwell,

1988; Bourdon and Blache, 2001; Roche et al., 2008). Although the mechanism underlying its role in increased total protein concentration was not elucidated in the present study, preferential deposition of lycopene in organs such as liver may be implicated (Bramley, 2000). Protection of biomolecules such as serum lipoproteins from oxidative injury may be another mechanism of



**Figure 2.** Effects of lycopene supplementation on erythrocyte malondialdehyde concentration of unstressed and stressed Wistar rats (n = 5). <sup>a,b</sup>P-values with different superscript letters are significantly (P < 0.05) different. U-Control = unstressed control; U+Lyco = unstressed + Lycopene; S-Control = stressed control; S+Lyco = stressed + lycopene.



**Figure 3.** Effects of lycopene supplementation on total plasma protein concentration of unstressed and stressed Wistar rats (n = 5). <sup>a,b</sup>P-values with different superscript letters are significantly (P < 0.05) different. U-Control = unstressed control; U+Lyco = unstressed + lycopene; S-Control = stressed control; S+Lyco = stressed + lycopene.

lycopene action, although conclusive studies have not been shown (Rice-Evans et al., 1997; Bramley, 2000). Serum lipoproteins transport cholesterol to and from the liver. Cholesterol is an important component of cell membrane and acts as free-radical scavenger. Relationships between serum lipid and erythrocyte membrane lipid show that the change in serum lipids may increase susceptibility of the erythrocytes to lipid peroxidation (Ulana et al., 1985; Clemens and Bursa-Zanetti, 1989). Alteration in the level of serum lipid may occur following oxidative damage to molecules such as serum lipoproteins due to increased free-radical activities.

## Conclusion

Lycopene supplementation ameliorates the effects of psychological stress on behavioural response of Wistar rats, apparently through increasing the excitability scores thereby facilitating transition from a state of depression to excitation. Increased total plasma proteins in Wistar rats administered lycopene suggest antioxidant effects which may be reducing oxidative damage to erythrocytes. In conclusion, prior lycopene supplementation is beneficial in attenuating the effects of psychological stress in animals.

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