

Full length Research Article

# Vitamin C Supplementation Promotes Locomotor and Exploratory Behaviours in Male Wistar Rats Exposed to Varying Stress Models

\*Olumide, O.M.<sup>1</sup>, Aderemi, T.D.<sup>1</sup>, Arikawe, A.P.<sup>2</sup>, Idowu, A.J.<sup>1</sup>, Olatunji-Bello, I.I.<sup>1</sup> and Umoren, A.G.<sup>1</sup>

<sup>1</sup>Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Lagos State University, Ikeja, Lagos.

<sup>2</sup>Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine University of Lagos, Idi Araba, Lagos, Nigeria.

**Summary:** Constant exposure to environmental stress has negative behavioural outcomes. Considering the inverse relationship between stress and Vitamin C intake, this study was aimed at investigating variable stress techniques and Vitamin C supplementation on exploratory/locomotor behaviors in male Wistar rats. Twenty-eight male Sprague-Dawley rats (100g-120g) were allotted into four groups (n=7). Control received 10ml/kg distilled water, group two received 100 mg/kg vitamin C, group three was exposed to different models of stress while group four was stressed alongside 100 mg/kg vitamin C. Vitamin C treatments were given orally for 2 weeks. Animals in groups 3 and 4 were stressed every other day with models such as multiple cage changes, exposure to noise, overnight strange objects, overnight wetting of beddings, and immobility. Exploratory and locomotor activities were assessed with the open field test, novel object recognition test, and Y maze test using a Logitech camera and ANY-maze software to track the movement of the rats. Cortisol was assayed in the serum using Enzyme-linked Immunoassay (ELISA) kit. Superoxide Dismutase, catalase, and lipid peroxidase; malondialdehyde (MDA) were also assayed in the serum. Results were analyzed using graphed prism 5.0, analysis of variance (ANOVA) was used to compare between groups while the mean with  $P < 0.05$  were considered significant. The results show that locomotor activities such as distance traveled, average speed, and time spent in the center square were significantly reduced by stress. These activities were improved with the intake of vitamin C compared with stress. Exploratory activities such as locomoting around the environment, orientating towards novelty, and touching or sniffing novel objects were significantly increased in the rats on Vitamin C supplements and reduced in the stressed group. In the serum, cortisol level was significantly increased in rats exposed to stress and decreased with Vitamin C intake. Stress also significantly increased MDA and decreased SOD and CAT while vitamin C supplement decreased MDA and increased SOD and CAT. In conclusion, oral intake of vitamin C enhanced exploratory/locomotor behavior and increased oxidative stress in rats exposed to different models of stress.

**Keywords:** Stress, Vitamin C, Explorative Behavior, Locomotor Behavior, Oxidative stress

\*Authors for correspondence: [moyosolao@yahoo.com](mailto:moyosolao@yahoo.com), Tel: +2347064817737

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## INTRODUCTION

Stress is the body's response to an environmental demand of conditions that outweighs an individual's psychological and physical ability to cope effectively (Crosswell & Lockwood 2020). The change caused by stress may be due to an individual's environmental sources or internal acuties. Other than being an emotion, stress is a physical response that travels throughout the entire body. It has an advantage when it is for a short term but when activated too often it increases the risk of biological, social, and psychological problems (Tucker *et al.*, 2008). These problems, therefore, increase the risk of developing cardiovascular disease, depression, anxiety symptoms, migraine, sleep, and appetite

alterations among others (Marik 2020). In addition, the stress response is necessary for preserved evolutionary adaptation which is instrumental in the enhancement of human complexity (Edwards *et al.*, 2008). This complexity contributes to survival (Tucker *et al.*, 2008) by evoking lasting behavioral and body changes (Blossom *et al.*, 2020). Rodents mimic the same pathophysiological and behavioral changes during stressful situations as humans (Jaggi *et al.*, 2011). A rodents-based behavioral test can detect changes in behavioral patterns in animals. When faced with an unfamiliar environment or object in an open-field test (OFT), these animals often exhibit behavioral patterns that can be termed exploration, such as locomoting around the environment, orientating towards novelty, and touching or

sniffing novel objects. This procedure provides an animal model of anxiety-like behavior that permits the evaluation of different aspects of animal behavior (Zimcikova *et al.*, 2017). The number of lines crossed, the frequency of rearing and grooming are measures of locomotive behaviors and index for the rodent's emotion. Aside from these, distance covered, speed, the number, duration, and time spent in Central Square are also regarded as measures of exploratory behavior and anxiety (Zimcikova *et al.*, 2017). In addition, grooming in rodents is a complex and ethologically rich behavior that is sensitive to stress and various genetic and pharmacological manipulations which may alter gross activity as well as patterning (Smolinsky *et al.*, 2009). Rearing also shows exploratory capacity which has consequences of neuronal dysfunction following manipulation (Idowu *et al.*, 2019). Therefore, observational analysis of these activities serves as a useful measure of stress and anxiety in both wild and laboratory animals (Smolinsky *et al.*, 2009).

According to Sharma *et al.* (2022), oxidative stress is a major cellular burden that triggers reactive oxygen species (ROS) and deteriorates antioxidants. The high oxygen consumption in the by-products of increased metabolic rate cause toxicity of transition metals that catalyze the production of reactive oxygen species. Thereby causing the accumulation of ROS to exceed the ability of antioxidants that neutralizes them (Moussa *et al.*, 2019). The outcomes of oxidative stress (OS) include lipid peroxidation, oxidative damage to DNA and proteins as well as alteration of the antioxidant enzymes response (Aboul-Ela *et al.*, 2011). Lipid peroxidation has been used successfully as a measure of oxidative stress because of the capability of free radicals in generating lipid peroxidation processes in organisms. However, malondialdehyde (MDA) is a final product of polyunsaturated fatty acids peroxidation in the cells and a marker generally acceptable for lipid peroxidation (Gawel *et al.*, 2004).

The toxic cause OS and polyunsaturated fatty acids which are free radicals seen in oxidative stress are linked with a high rate of oxygen consumption alongside low levels of endogenous antioxidants (Moussa *et al.*, 2019). These endogenous antioxidants can be enzymatic and non-enzymatic. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH) are enzymatic antioxidants which serve as the first line of defense against oxidative stress (Aguilar *et al.*, 2016). The non-enzymatic antioxidants are vitamins E, A, and C, flavonoids, carotenoids, glutathione, plant polyphenols, uric acid, theaflavin, allyl sulfides, curcumin, melatonin, bilirubin, and polyamines (Mironczuk-Chodakowska *et al.*, 2018).

The animals were supplemented with vitamin C (L-ascorbic acid) which is an optically-active hydro-soluble free radical scavenger and an essential nutrient usually obtained through diets (Moussa *et al.*, 2019). However, it is an important vitamin that participates in numerous cell functions. Our body is known to produce antioxidants but during stress, the antioxidants produced become inadequate to scavenge free radicals (Salami *et al.*, 2020). According to Blossom *et al.* (2020), stress increases the chances of neurological disorders in animals and humans (Marik 2020) However, there is still a need to explore the combined effects of the common antioxidant; vitamin C on behavioral activities during stress. This study was therefore designed to

investigate oral intake of vitamin C on behavioral outcomes in rats exposed to stress.

## MATERIALS AND METHODS

**Animals:** Twenty-eight male Wistar rats (100-120g) were obtained from the animal house at Lagos State University College Medicine, Ikeja. The rats were kept in the animal house at room temperature and a natural light rhythm of 12 hours of daylight and 12 hours of darkness. They were acclimatized for 2 weeks and provided with rat chow and water ad libitum. The research protocol was approved by the Lagos State University College of Medicine Ethics and Research Committee (Ref. No; AREC/2012/016).

**Drugs:** The Vitamin C used in this study was purchased from Sigma Chemical Co. St. U.S.A. Normal saline purchased from Unique Pharmaceuticals, Sango-Otta, Nigeria.

**Experimental Design:** The rats were randomly divided into four groups (n=7), control received 10ml/kg BW/day distilled, group II received 100mg/kg BW/day Vitamin C (Adeneye and Olagunju 2008) group III were subjected to stress regimen alongside 10ml/kg BW/day distilled water while group 4 were also stressed and received 10 mg/kg BW/day Vitamin C. Both treatment and stress were done for 2 weeks and the following parameters were carried out across the group.

**Stress models:** Rats were exposed to different stress regimens of the light and dark cycle according to Borrow *et al.*, (2018) every other day for 2 weeks. The beddings were wet overnight (dark cycle) by filling the cages with 700 ml of water. Strange objects (marbles) were kept in the cages overnight and removed in the morning (dark cycle). Multiple cage change during the light cycle was done by transferring the rats from one cage to another at an interval of ten minutes for 2 hours. The rats were restrained by keeping the animals in a cylindrical or semi-cylindrical tube with ventilation holes for 30 min. Immobilization models produce inescapable physical and mental stress with a low rate of adaptation. A noise disturbance was induced by using loudspeakers (15W) connected to a white noise generator (0-26 kHz) which is located 30 cm from the cage. The noise was set at 100 decibels. The animals were exposed to this noise protocol for 4 hours/per day.

### Open Field – (Assessment of Locomotor Activity and Exploratory Behavior):

Locomotor activity and explorative behaviors were conducted in an Open Field (OF) box (40cm x 40 cm) according to Idowu *et al.* (2019) A Logitech camera (C270, UK) was connected to a computer with ANY-maze behavioral tracking software (Stoelting Co., USA). The camera was placed on the box for easy viewing of the boundaries and dimensions of the box. In line with Idowu *et al.* (2019) the floor of the box was divided into sixteen dimensions of 10cm X 10cm squares using the ANY-maze protocol. These lines were used to track the animals based on the amount of time spent in the corners and the number of times spent in the Centre zone of the box.

For the locomotor behavior, the total distance traveled, average speed in the box, as well as the number of lines crossed during the test, was assessed. In explorative behavior, the amount of time spent in the corner square was considered.

**Novel Object Recognition Test:** The object recognition test (ORT), also known as the novel object recognition test (NORT) is used to evaluate cognition as well as learning and memory because it is less stressful for rodents (Sik *et al.*, 2003). The test relies on three sessions: habituation, training, and test session.

The habituation was done for 5 minutes in the apparatus which is the same box used for the open-field experiment prior to the NORT. This is followed by two stages (training and test session) for the novel object recognition task after 24 hours. During habituation, the rat was placed in the NORT box and allowed to freely explore the arena for 5 min. Training sessions were done after 24 hours of the habituation. Two identical objects were placed in opposite quadrants of the arena (i.e., NE corner and SW corner) equidistant from one another. The rat was then placed in the center of the arena, equidistant from the 2 identical objects, and was allowed free exploration for 5 min. At the end of the trial, the rat was removed and placed in the holding cage. After 15 minutes of the training session, the test session was done. Each rat was placed into the NORT box which has a familiar object (one of the objects from the training session) and a novel object, which was not presented during the training session. If the animal remembers the familiar object from the training session, it should spend more time investigating the novel object during stage 2. The novel object was the same for all the animals used for the study. Logitech camera (C270, UK) was also connected to a computer with ANY-maze behavioral tracking software. The behaviors were viewed, scored, and analyzed by the ANY-maze Behavioural Tracking System (Stoelting Company, USA) software. The camera was connected to a computer where behavior was viewed, scored, and recorded.

The rats were carried to the test room in their home cages and tested for the behavioral tasks individually. Each rat was moved from their home cage to the testing apparatus using a small platform that the rats can comfortably rest on. After every 5 minutes test, each rat was returned to the home cage and the OF apparatus was cleaned with 70% ethanol (ethyl alcohol) and allowed to dry between trials to remove any olfactory clues in the test box. This procedure was used for all the rats tested. The behavioral measures scored during the NORT (Podhorna *et al.*, 2002) include:

**Line Crossing:** The frequency with which the mouse crossed from the square divisions in the open field box

**Rearing:** The frequency with which the mouse stood on their hind feet or against the wall in any part of the box

**Grooming:** The frequency and duration of time each mouse spent licking or scratching itself while stationary.

**Serum sample collection:** Blood samples were taken through a cardiac puncture technique following an injection of 30 mg/kg phento barbital (Salami *et al.*, 2020). The samples were collected into a plain bottle using a 5 ml syringe with a needle. The blood was allowed to stand at room temperature for 15–30 min. Thereafter, it was

centrifuged using a cold centrifuge. The serum was pipette into a plain bottle and stored at -40°C

#### Determination of serum superoxide dismutase, catalase, and malondialdehyde activity

Superoxide dismutase activity in the serum was determined according to methods by Sun and Zigman (1978). This activity is determined by the ability of the enzyme to inhibit the autoxidation of epinephrine while the enzyme activity is monitored at an absorbance level of 480nm. The concentration was expressed as SOD unit/mg protein such that one unit is defined as the amount of enzyme needed to inhibit 50% epinephrine reduction per minute and per milligram of protein at 25°C and pH 7.8

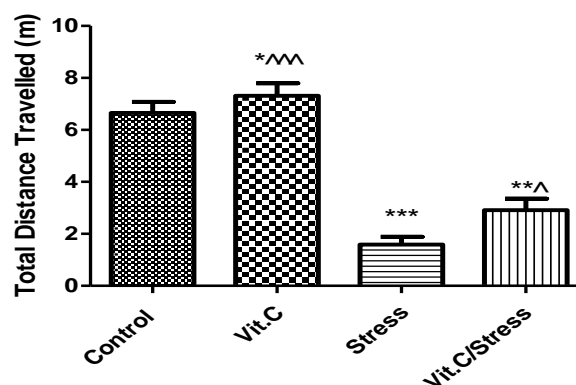
Catalase activity was determined according to the method of Aebi *et al.* (1984). This is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H<sub>2</sub>O<sub>2</sub> with the formation of perchromic acid as an unstable intermediate.

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method described by Buege and Aust (1978).

**Statistical analysis:** The results were expressed as mean ± SEM (standard error of mean). Statistical analysis was done using GraphPad Prism (version 5.0) software. One-way analysis of variance (ANOVA) was used to compare between groups while Newman keuls was used as post hoc test. Values with P<0.05 were considered significant.

## RESULTS

**Total distance traveled in the open field (OF) box for a period of 300 seconds (5 minutes):** The distance traveled in the open field box shows that the stressed rats (P≤0.001) and rats on Vitamin alongside stressed (P≤0.01) traveled less distance when compared with the control. Distance traveled was increased in rats on vitamin C (P≤0.05), (P≤0.001) when compared with the control and stressed group (Figure 1).

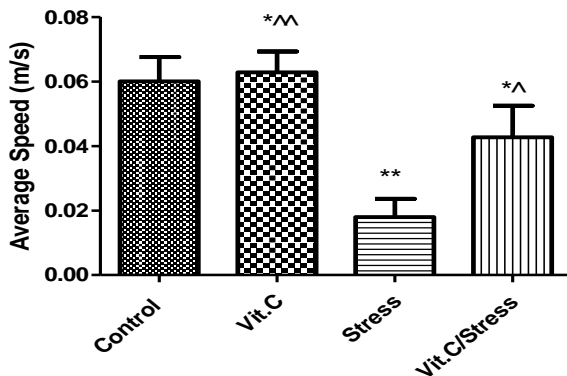


**Fig. 1:** Total distance travelled in the open field (OF) box for a period of 300 seconds (5 minutes)

Key: \*\*\*, \*\* = P<0.0001, P<0.001, P≤0.05 when compared with control, ^ = P≤0.05 when compared with stress.

**Average speed in the open field (OF) box for a period of 300 seconds (5 minutes):** Average speed in the open box was reduced in the stressed animals (P≤0.01) as well as

animals in the Vitamin C/stressed group ( $P \leq 0.05$ ) when compared with the control. The average speed was higher in Vitamin C ( $P \leq 0.05$ ) than in the control. It was also higher in Vitamin C/stress ( $P \leq 0.05$ ) as well as in the Vitamin C ( $P \leq 0.01$ ) group when compared with the stress group (Figure 2).

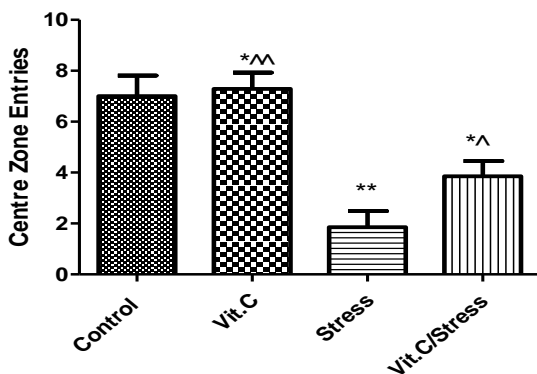


**Fig. 2:**

Average speed in the open field (OF) box for a period of 300 seconds (5 minutes)

Key: \*= $P \leq 0.05$ , \*\*= $P \leq 0.01$  when compared with control, ^= $P \leq 0.05$ , ^^= $P \leq 0.01$  when compared with stress.

**Number of entries into the center zone in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:** Centre zone entry by Stress ( $P \leq 0.01$ ) and Vit.C/Stress ( $P \leq 0.05$ ) group was reduced when compared with the control. There was an increase in the rearing episodes in the Vit. C ( $P \leq 0.01$ ) and stress/Vit. C ( $P \leq 0.05$ ) when compared with stress (Figure 3).



**Fig. 3:**

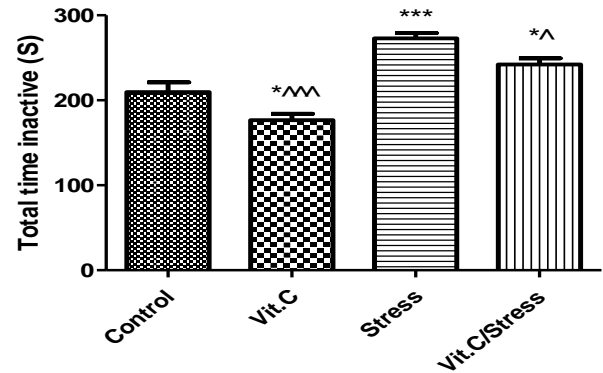
Number of entry into the center zone in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats

Key: \*= $P \leq 0.05$ , \*\*= $P \leq 0.01$  when compared with control, ^= $P \leq 0.05$ , ^^= $P \leq 0.01$  when compared with stress

**Total time inactive in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:** The total time inactive in the stress ( $P \leq 0.001$ ) and Vit.C/Stress ( $P \leq 0.01$ ) group was reduced than in the control. It was also lower in stress/Vit. C ( $P \leq 0.001$ ) and Vit. C. ( $P \leq 0.05$ ) compared with the stress group (Figure 4).

**Total inactive Episodes in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:** The episode of inactiveness was higher in stress ( $P < 0.05$ ) when

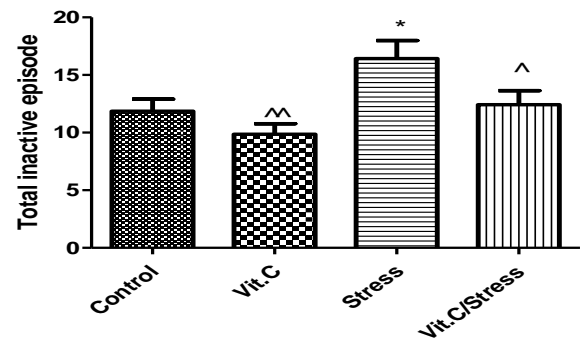
compared with the control. These episodes were lower in Vit. C ( $P < 0.05$ ) as well as Vit.C/Stress ( $P < 0.01$ ) groups than in control. (Fig. 5).



**Fig. 4:**

Total time inactive in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats

Key: \*= $P \leq 0.05$ , \*\*\*= $P \leq 0.001$  when compared with control, ^= $P \leq 0.05$ , ^^= $P \leq 0.01$  when compared with Stress



**Fig. 5:**

Total inactive episodes in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats

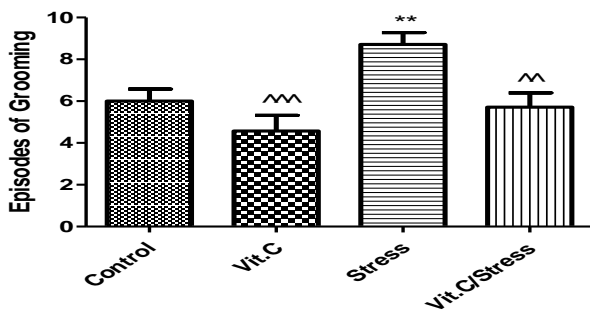
Key: \*= $P < 0.05$  when compared with control, ^= $P < 0.05$ , ^^= $P < 0.01$  when compared with Stress,

**Episodes of Grooming in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:** Grooming episodes were significantly increased in the stress ( $P < 0.05$ ) when compared with the control. There was a significant decrease in grooming episodes in Vit C ( $P < 0.05$ ) and Vit. C/Stress ( $P < 0.05$ ) group when compared with stress. (Figure 6)

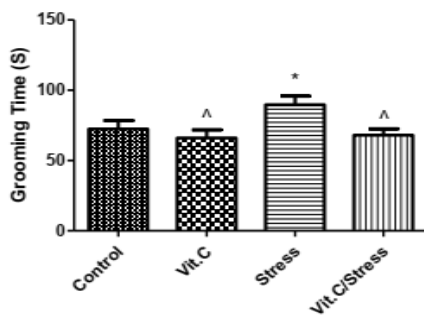
**Grooming time in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:** Grooming episodes were significantly increased in the stress ( $P < 0.05$ ) when compared with the control. There was a significant decrease in grooming episodes in Vit C ( $P < 0.05$ ) and Vit. C/Stress ( $P < 0.05$ ) group when compared with stress. (Figure 7).

**Episodes of Rearing on the wall in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:** The episodes of rearing on the wall in the stress group ( $P < 0.05$ ) were lower and higher in Vit. C ( $P < 0.05$ ) group than in the control. There was an increase in rearing

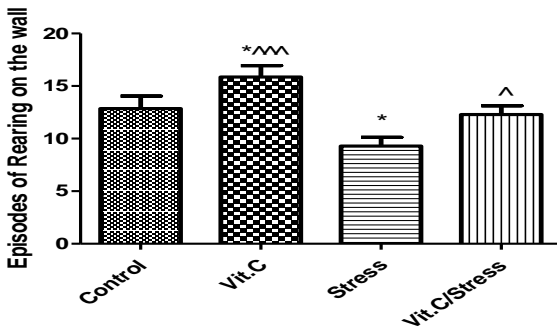
episodes in Vit. C ( $P < 0.001$ ) and Vit. C/Stress ( $P < 0.05$ ) group when compared with stress. (Figure 8)



**Fig. 6:** Episodes of Grooming in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats  
Key: \*\*= $P < 0.05$  when compared with control, ^= $P < 0.01$ , ^^= $P < 0.001$  when compared with Stress.



**Fig. 7:** Grooming time in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats  
Key: \*= $P < 0.05$  when compared with control, ^= $P < 0.05$  when compared with Stress.



**Fig. 8:** Episodes of Rearing on the wall in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats  
Key: \*= $P < 0.05$  when compared with control, ^= $P < 0.05$ , ^^= $P < 0.001$  when compared with Stress.

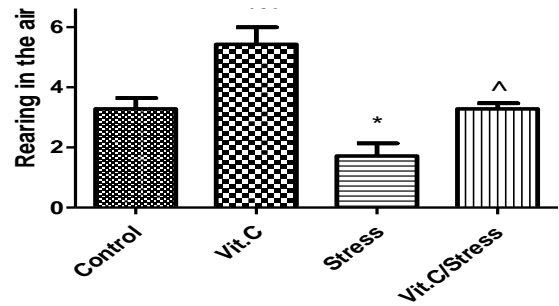
**Episodes of Rearing in the air in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:**

The episodes of rearing in the air in the stress group ( $P < 0.05$ ) were lower and higher in Vit. C ( $P < 0.05$ ) group than in control. There was an increase in rearing episodes in Vit. C ( $P < 0.01$ ) and Vit. C/Stress ( $P < 0.05$ ) group when compared with stress. (Fig. 9).

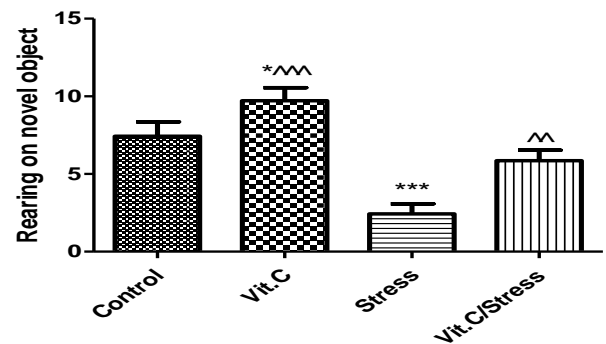
**Episodes of Rearing on familiar object in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:**

The episodes of rearing on the novel object

were lower in the stress group ( $P < 0.05$ ) and higher in Vit. C ( $P < 0.05$ ) group than in the control. Rearing was also higher in Vit. C ( $P < 0.001$ ) and Vit. C/Stress ( $P < 0.01$ ) group when compared with stress. (Fig. 10)



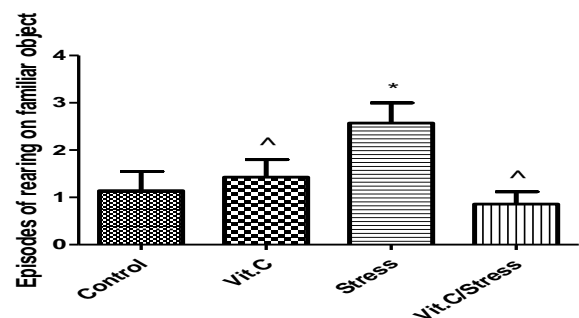
**Fig. 9:** Episodes of Rearing in the air in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats  
Key: \*= $P < 0.05$  when compared with control, ^= $P < 0.05$ , ^^= $P < 0.01$  when compared with Stress



**Fig. 10:** Episodes of Rearing on novel object in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats  
Key: \*= $P < 0.05$ , \*\*\*= $P < 0.05$  when compared with control, ^^, ^^= $P < 0.01$ ,  $P < 0.001$  when compared with Stress.

**Episodes of Rearing on familiar object in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats:**

The episodes of rearing on the familiar object were higher in the stress group ( $P < 0.05$ ) than in the control. Rearing on the familiar objects was also lower in Vit. C ( $P < 0.05$ ) as well as Vit. C/Stress ( $P < 0.01$ ) group when compared with stress. (Fig. 11).



**Fig. 11:** Episodes of Rearing on familiar object in the open field (OF) box for a period of 300 seconds (5 minutes) in Wistar rats  
Key: \*= $P < 0.05$  when compared with control, ^= $P < 0.05$  when compared with Stress.

**Cortisol, MDA, and SOD on Vitamin C and stress in Wistar rats:** Serum level of cortisol was high in the stress group ( $P<0.05$ ) when compared with the control. It was lower in Vit. C and Stress/Vit.C, ( $P<0.05$ ) than the stress group while stress/Vit. C was significantly higher ( $P<0.05$ ) than Vit. C (table 1)

In serum Malondialdehyde there was a decrease in stress ( $P<0.01$ ) and no significant difference in Vit. C and stress/Vit. C when compared with control. There was a significant decrease in the MDA level in Vit. C ( $P<0.001$ ) and Stress/Vit. C ( $P<0.05$ ) when compared with the stress group. It was increased in the stress/Vit. C when compared with Vit. C

There was a decrease in the serum SOD in stress ( $P<0.01$ ) and an increase in Vit. C when compared with control. In Vit. C ( $P<0.001$ ) and stress/Vit. C ( $P<0.001$ ) group, SOD was increased when compared with stress. It was also increased in stress/Vit. C ( $P<0.001$ ) when compared with stress (table 1).

**Table 1:**  
Cortisol, MDA and SOD on Vitamin C and stress in Wistar rats

ASSAY/ Group	Control	Vit.C	Stress	Stress/ Vit.C
Cortisol ( $\mu\text{g/dl}$ )	1.20 $\pm 0.33$	0.88 $\pm 0.24^{\wedge\wedge}$	2.58 $\pm 0.22^*$	2.10 $\pm 0.40^{\text{ns}}$ $\wedge$
MDA ( $\text{nmol/ml}$ )	1.34 $\pm 0.18$	0.83 $\pm 0.33^{*^{\wedge\wedge\wedge}}$	2.15 $\pm 0.19^{**}$	1.75 $\pm 0.07^{\wedge}$
SOD ( $\text{mg/ml}$ )	2.08 $\pm 0.22$	3.08 $\pm 0.22^{*^{\wedge\wedge\wedge}}$	0.99 $\pm 0.16^{**}$	2.03 $\pm 0.20^{\wedge\wedge}$

## DISCUSSION

In this present study, animal exposure to varying stress showed reduced locomotor activities which were identified by the distance travelled, average speed and time spent in the center square. According to Sgroi *et al.* (2014) locomotor activity assessed by the open field test (OPF), accounts for the spontaneous exploratory behavior of animals in defining a precise time course of their motor activities. The decreased locomotor activity recorded in this study is in line with previous studies in rats and mice exposed to an acute or chronic stressor (Meerlo *et al.*, 2002) Locomotion is one of the essential functions for animal survival located within the cervical and lumbar regions of the spinal cord. According to Oueghlani *et al.* (2018), the central pattern generators (CPGs) for locomotion are under the control of the supraspinal center. Usually, the CPGs arrange the rhythmical activation of motor neuronal populations which innervates the axial and limb muscles for specific gait patterns involved in locomotor behavior. The increased inactive time and episodes in the stressed rats is a sign that the animals have a decreased ability to explore.

Evidently, the decreased exploratory behaviors show that stress has a significant influence on behavioral patterns in rats. In an open field, the level of locomotion and time spent in the center of the arena are measures of exploratory behavior (Zimcikova *et al.*, 2017). The reduction in locomotive activity shows a lack of motivation in the stressed rats. This was accompanied by a decrease in exploration which also shows the lack of interest of the

animals in exploring their environment. According to Mällo *et al.* (2007), exploratory behavior by novel stimuli is supported by behavioral as well as postures which allow information about the environment. This information is highly essential for survival due to the possibilities it provides to be able to find food, water, mating partner, shelter, etc.

Furthermore, exploratory behavior is also influenced by the animal's ability to explore a potentially dangerous novel environment or to stay within secure and familiar surroundings. These are indications that an animal's behavior in a novel environment is influenced by curiosity or the motivation to explore (Mällo *et al.*, 2007)

Grooming and rearing are also common behaviors in rats (Kozler *et al.*, 2017) these behaviors and the motor activity involved are used in analyzing impaired behavioral functions in the central nervous system (Kozler *et al.*, 2017). Importantly, grooming is similar across species as well as human thus, it is an innate behavior and a way animal maintains the cleanliness of their body surfaces (Rojas-Carvajal *et al.*, 2022). It has physiological importance such as thermoregulation, social communication, and de-arousal (Kalueff *et al.*, 2016) However, excessive grooming become pathological in some behavioral disorders (Kalueff *et al.*, 2016). The increased grooming episodes observed in our study show the importance of grooming as an adaptive response to stress management (Mu *et al.*, 2020). Grooming is reported in animals with increased corticotropin-releasing hormone (CRH) (Matisz *et al.*, 2021). Thus, corticotropin-releasing hormone stimulates the secretion of pituitary adrenocorticotropin (ACTH) which increases the secretion of cortisol from the adrenal gland (Lightman *et al.*, 2021) Rearing on the hand is another novel exploratory behavior that assesses the mental and emotional impairment in rats. Generally, the hippocampus is a major control of exploratory behaviors in animals and the key target in stress response. Some component areas in the brain such as basal ganglia, brain stem and cerebellum, limbic system, including the hypothalamus, amygdala and orbitofrontal cortex are also involved in explorative behavior (Kalueff *et al.*, 2016).

Furthermore, rearing is also one of the ways animal behaviors are explored and according to Borta and Schwarting (2005), low rearing activities in rodents are considered as signs of neurologic disorders. In this study, the episodes of rearing were decreased in the stressed animals, however, this has also been reported to be a sign of disturbed motor activity (Kalueff *et al.*, 2016). The low exploratory and locomotor activities observed in the stressed rats are attributed to the high cortisol level in the serum. However, high level of cortisol is mediated by stimulation of the adrenal cortex which is regulated by the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system (SNS) (Matisz *et al.*, 2021). Although stress is an important factor in the etiology of behavioral disorders, it also increases the availability of energy for motor activities by raising blood glucose levels (Nelson 2005) In this present study, oral intake of vitamin C (Ascorbic Acid) increased locomotor activities and also improved the exploratory activities in the rats. In 2020, Moritz *et al.* (2020) reported that ascorbic acid has neuroprotective effects whose mechanism of action is poorly understood. The major route of Vitamin C to the CNS is from the plasma

to the CSF across the epithelium of the choroid plexus (Angelow *et al.*, 2003) and it enters the brain interstitium by diffusion (Harrison & May 2009). According to Moritz *et al.* (2020), ascorbic acid also modulates neurotransmitter systems such as aminergic (dopamine), norepinephrine, serotonin (5-HT), glutamatergic and cholinergic systems in the central nervous system. It serves as cofactors in several enzymes (e.g., dopamine B-monooxygenase or prolyl 4-hydroxylase and lysyl hydroxylase (Padayatty & Levine 2016). Evidently, vitamin C is an essential antioxidant in the brain that protects the components of the cells against free radicals formed during metabolism (Padayatty & Levine 2016). The constant use of oxygen in burning metabolic fuel for energy during normal cellular metabolism increases the formation of free radicals (Srivastava & Kumar 2015). Therefore, incessant exposure to stress will further increase metabolic rate and ultimately generates more free radicals that becomes toxic to the system.

According to Padayatty & Levine (2016), the inverse relationship between Vitamin C and stress in most animals provides an evidence of the significance of Vitamin C in the outcomes of stress. In most mammals, Vitamin C is synthesized in the liver from glucose-6-phosphate and from fructose-6-phosphate in plants (Marik 2020). In contrast, human, rodents and animals such as teleost fish, guinea pigs, and a few species of bats do not synthesize vitamin C due to evolutionary loss of function mutation in the L-gluconolactone oxidase (Gulo). The enzyme L-gluconolactone oxidase (Gulo) catalyzes the last rate-limiting step in the biosynthesis of vitamin C. Therefore, vitamin C must be supplied through dietary sources and supplements in humans and rodents (Mustafi & Wang 2020).

According to Khassaf *et al.* (2003) Vitamin C directly reduces oxidative stress by reducing the free radical species generated from the H<sub>2</sub>O<sub>2</sub>. However, the increased SOD and CAT are evidence of the stimulation of SOD and CAT transcription which is highly sensitive to the redox state of cells. Therefore, oral intake of Vitamin C was able to maintain the compromised cellular environment which is the likely cause of the neurogenic disorders caused by stress.

In conclusion, exposure of rats to a variety of stress increased serum cortisol levels and oxidative stress. Locomotor and explorative activities in the rats are altered with exposure to varying stress. However, with oral intake of Vitamin C, these neurogenic activities altered by stress were improved in the rats.

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