

# Effects of selenium yeast on anxiety like behaviours and oxidative stress biomarkers of restraint male Wistar rats

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## Introduction

Stress is regarded as the perception of threat, which results in discomfort, emotional tension and anxiety. From an endocrinology perspective, it is any stimulus that provokes the release of adrenocorticotrophic hormone and glucocorticoid [1]. Stress is caused by psychological, physiological and environmental extraneous factors called stressors [2]. It may be induced in laboratory animals by cold water immersion, electrical foot-shock, food deprivation, noise and restraint stress [3]. Restraint or immobilisation stress occurs due to impermissible movement. It is a convenient and reliable model to mimic psychological stress, resulting to ill-effect on various systems of the body [4]. Studies have shown that restraint stress induces oxidative stress, decreasing glutathione peroxidase activity and impairs behaviour [5-6]. Behavioural alterations include impaired feed-back mechanism and altered neuroplasticity [7]. It has been shown that prenatal restraint stress could be translated into anxiety-like behaviour during early life [8]. During restraint stress, there is excess production of reactive oxygen species (ROS) which coincide with a decrease in antioxidant concentration in experimental animals [9].

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## ABSTRACT

**Objective:** This experiment is to determine if selenium yeast modulates behavioural changes in male Wistar rats subjected to restraint stress.

**Methods:** The experiment was carried out with 15 male Wistar rats, randomly divided into three groups; Group I-normal control, Group II-stress control group, Group III- selenium yeast (0.1mg/kg) + restrained stress. Restraint stress was induced using restraint meshes for 6 hours (between 9.00-15.00 h) for 15 days. Behavioural changes were assessed, using open-field apparatus and elevated plus maze.

**Results:** In open-field test; stress control group showed increased value of line cross, neck stretch and rearing, administrations of selenium yeast shows decrease activity in open field. In elevated plus maze, time spent in open arms, number of entries in open arms and number of entries in closed arms were significantly higher in stress control group when compared to normal control group. Selenium yeast group shows a significant decrease in entries and time spent in open arms but increase time spent and entries in close arms. Stress control shows increase catalase and malondialdehyde activities when compared to normal control and selenium yeast groups. That of super oxidase and glutathione peroxidase concentration in selenium yeast group was significant ( $P < 0.05$ ) higher when compared to stress control.

**Conclusion:** restraint stress decreased anxiety-like behaviour and induces oxidative stress. Antioxidants selenium yeast ameliorates oxidative stress, but did not exert modulatory effect on anxiety-like behaviours in restraint Wistar rats.

## KEY WORDS:

Anxiety-Behaviour  
Oxidative Stress Makers  
Restraint Stress  
Selenium-Yeast

Hence, the excess ROS can be scavenged through the use of antioxidants [10]. Studies have shown that antioxidants-enriched diets slow down the progression of behavioural decline [11]. Antioxidant mineral, selenium is a component of endogenous antioxidant enzymes glutathione peroxidase

and thioredoxin [12]. However, selenium can be enriched with yeast, which induces fast restoration of antioxidants systems after oxidative stress [13]. Though yeast minimizes oxidative damage due to its ability to induce high activities of glutathione peroxidases and catalase in cells, studies on chicks suggest that selenium-yeast supplementation increased the body resistance to oxidative stress, associated with enteric bacterial infection and high temperature exposure. The increased resistance to stressors, induced by the supplementation was apparently due to an improved redox status [14]. It also exhibits a high level of nitric oxide-scavenging activity [15]. Selenium yeast is potent in protecting the brain cells from oxidative stress [16]. It abolishes pathological changes, associated with the brain, and appears to be the primary antioxidant involved in the prevention and management of pro-oxidants, inducing brain damage [17]. Selenium status was observed to decrease with ageing and may contribute to decline in neuropsychological functions among older people. The preferential retention of selenium yeast in brain suggests that it plays important functions in the regulation of mood and prevention of tardive dyskinesia [18]. Its supplementation improves mood in aged individuals, making them happier, thus affecting their life quality by its ability to protect entire brain cells from oxidative stress [19]. The aim of the present study was to investigate the effect of selenium yeast on anxiety-like behavioural responses and biomarkers of oxidative stress in restraint male Wistar rats.

## Materials and methods

### Chemicals

All reagents were of analytical grade. Selenium yeast was purchased from Sigma Aldrich (St. Louis, USA), except for phosphate buffer which was prepared on the day of decapitation.

### Animals

Fifteen young adult male Wistar rats, weighing between 140-145 g of two-month old were used for the experiment. They were obtained from a breeding stock and maintained in the animal house of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. They were given access to grower mesh and water

*ad libitum*, and allowed to acclimatize in their home cages for two weeks prior to the commencement of the experiment in a 12-h light and dark cycle. All procedures pertaining to the use of the animals were prepared in accordance to the Animal Ethical Committee and ethical clearance was issued.

### Experimental design

Rats were divided into three groups, each comprising of 5 animals (n=5), and were treated as follows: Group I rats were given rat feed and distilled water. These groups served as normal control group. Group II were fed, and subjected to restraint stress; serving as stress control group. Group III rats were fed and administered with selenium yeast at dose of 0.1 mg/kg, orally using a cannula, 8:30 h daily for 15 days and subjected to restraint stress one rat per restraint wire mesh cage restrainer; having dimensions of 8 cm (length) x 4 cm (width) x 4 cm (height) [20]. A pad lock and latch was used to secure the rat in the restrainer. The study was carried out between 9:00 – 15:00 h and adequately monitored to ensure all rats were unharmed. The rats were deprived of food and water, within 6-h of restraint induction. The anxiety-like behaviour was evaluated using an elevated plus maze test and an open-field test. At the end of the behavioural test, rats were anaesthetized prior to being decapitated, brain tissue was harvested and homogenized with phosphate buffered saline (pH =7.4). Supernatant was used to assay for biomarker of oxidative stress.

### Elevated plus maze test

The elevated plus maze test can be used to evaluate anxiety-related behaviour [19]. This was constructed from plywood, having a height of 55 cm from the floor and consists of two opposite close arms and two opposite open arms of (50 x 11 cm) with 40 cm high walls of thin wood, painted black. Rats were placed in the middle of the plus maze facing towards the open arms for 5 minutes. Behaviour measured included; total time spent in both close and open arms and total entries in both close and open arms.

After each trial the maze was wiped with a cloth dipped in 70% ethyl alcohol, which was allowed to dry up before,

cleaned with bleached.

### Open-field test

Open-field allows evaluation of animal basal activity, in response to a novelty or anxiogenic environment. It was carried out, as describe by [21]. The apparatus consists of square white Plexiglas of 1.22 m size and height of 45 cm. The floor was divided into 16 equal squares 30.5 cm size which allowed, the assessment of locomotion. The rat was placed in the centre of open field for a period of 5 minutes and the following parameters were being recorded; Line crossing: When the rat crossed one of the grid lines, which separated the squares in the open field with all four limbs, centre square entries: The duration and the number of times the rat entered one of the center four squares with all four paws, rearing: Standing on hind-legs or leaning against the walls, grooming: When the rat, while stationed, licked, or scratched using front or back paws, freezing: when the rat is stationed, urination: Number of urine spots on the floor, defecation: Number of faecal boli excreted.

### Assessment of Oxidative Stress Makers in Brain Tissue

The level of thiobarbituric-acid reactive substance, MDA, as an index of lipid peroxidation was evaluated. Quantitative measurement of lipid peroxidation of malondialdehyde (MDA) was determined using NWLSS™ MDA assay kit (Northwest Life Sciences Specialties, Product NWK-MDA01, Vancouver WA, specificity: Malondialdehyde, Sensitivity: 0.08  $\mu$ M). The principle was based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm [22].

### Superoxide dismutase (SOD) activity

Activity of superoxide dismutase (SOD) in brain tissue was determined using NWLSS SOD assay kit (product NWK-SOD02, Specificity: copper (Cu)/ zinc (Zn), manganese (Mn) and iron (Fe) superoxide dismutase, Sensitivity: 5 U/mL) the assay kit is based on the principle of superoxide inhibition of autooxidation of hematoxylin as described by [23].

### Catalase activity

Catalase (CAT) activity was assessed using NWLSS CAT

activity assay kit (Product NWK-CAT01, Specificity: Catalase, Sensitivity: 6.0 Catalase/mL). Activities of catalase were assayed based on the principle of catalase consumption of H<sub>2</sub>O<sub>2</sub> substrate at 240 nm [24].

### Glutathione peroxidase activity

Glutathione peroxidase (GPx) activity was assessed using NWLSS™ GPx, enzyme-linked immunosorbent assay (ELISA) assay kit (Product NWK-GPX02, specificity: Glutathione peroxidase, Sensitivity: 12.5 pg/ml). The NWLSS™ cGPx assay is based on a sandwich ELISA, where sample GPx concentration is determined by comparing the 450 nm absorbance, of sample to the absorbance of known standards [25].

### Statistical analysis

Data collected from this study are expressed in mean ( $\pm$  SEM). Values of  $P < 0.05$  is considered to be statistical significant. One-way analysis of variance (ANOVA) and tuck-ys *post-hoc* test was used to differentiate between the means. Statics packaged used is Graph pad prism 5.0 (San-Diego, California USA).

## Results

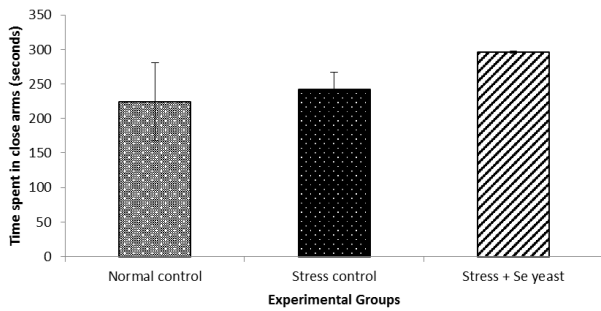
The data of anxiety like behavioral analysis following immobilization stress with respect to control group was analyzed below. Exposure to immobilization stress showed significant changes in animal behaviour in open field and elevated plus maze.

### Effect of selenium yeast on anxiety like behaviour in elevated plus Maze Test

#### Close arms

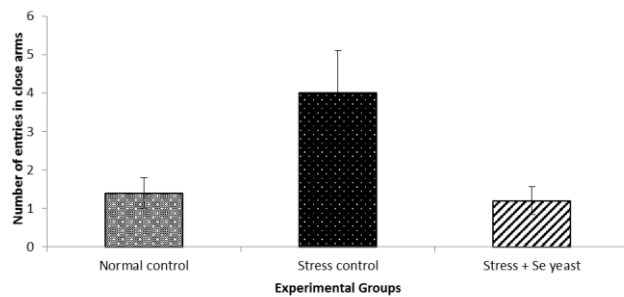
No significant difference ( $P < 0.05$ ) was observed in time spent at close arms in the normal control groups when compared with the stress control groups and the selenium yeast groups in (Fig 1). In the number of entries into close arms the stress control groups ( $4.2 \pm 0.8$ ) were significantly ( $p < 0.05$ ) higher when compared to the normal control ( $1.5 \pm 0.08$ ) and the selenium yeast groups ( $1.4 \pm 0.06$ ) in (Fig 2).

**Figure 1.** Effects of selenium yeast on time spent in close arm of elevated plus maze in Wistar rats subjected to restraint stress



Data are expressed in mean ± SEM. a, b = Means with different superscript letters are significantly ( $P < 0.05$ ) different; Group I = Control, Group II = Stress Control, Group III = Selenium-yeast Administration, n=5.

**Figure 2.** Effect of selenium yeast on entries into close arm of elevated plus maze in Wistar rats subjected to restrained stress



Data express in mean ± SEM. <sup>a, b</sup> = Means with different superscript letters within rows are significantly ( $P < 0.05$ ) different; Group I = Control, Group II = Stress Control, Group III = Selenium-yeast Administration, n = 5.

**Open arms**

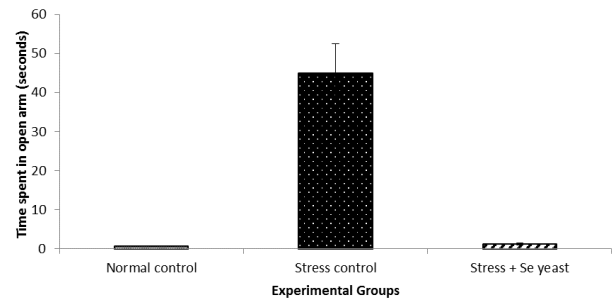
In (Figure 1) the stress control spent more time in open arms and was statistically ( $p < 0.05$ ) higher when compared to the normal control and the selenium yeast groups. A significant ( $p < 0.05$ ) increase (Figure 2) in entries into open arm was observed in the stress control ( $2.8 \pm 0.96$ ) when compared to the normal control ( $0.05 \pm 0.09$ ) and the selenium yeast groups ( $0.9 \pm 0.1$ ). Hence both event in open and close arms indicate locomotion, and the time spent in open arms by Wistar rats indicates calmness in animal using elevated plus maze model

**Effect of selenium yeast on anxiety-Like behaviour in open-field of Wistar rats subjected to restraint stress**

In the open field (Table, 1) the rats showed a significant ( $P < 0.05$ ) increase in line crossed, rearing and neck stretch in stress control group when compared with the normal control and the selenium yeast group. However, a significant

( $p < 0.05$ ), decrease in freezing was observed in stress control when compared with the selenium yeast group.

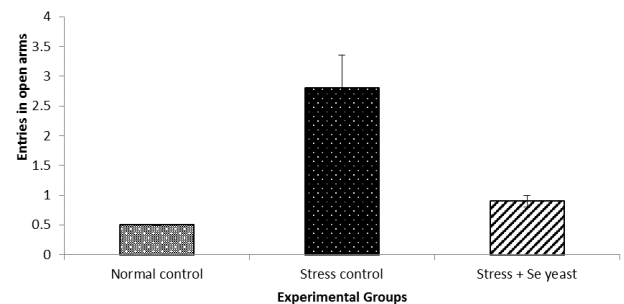
**Figure 3.** Effect of selenium yeast on time spent in open arm of elevated plus maze in Wistar rats subjected to restraint stress



Data are expressed in mean ± SEM. <sup>a, b</sup> = Means with different superscript letters are significantly ( $P < 0.05$ ) different; Group I = Control, Group II = Stress Control, Group III = Selenium-yeast, n = 5.

Though no significant difference was observed in grooming and defecation, however the stress control shows decrease in number of faecal presence during open field test when compared to the rest of the groups. However, in grooming, normal control group groomed higher when compared to both selenium yeast and stress control group.

**Figure 4.** Effects of selenium yeast on Wistar rats subjected to restrained stress in open arms of elevated plus maze.



Data are expressed, in mean ± SEM. <sup>a, b</sup> = Means with different superscript letters are significantly ( $P < 0.05$ ) different; Group I = Control, Group II = Stress Control, Group III = Selenium-yeast administration, n = 5.

**Table 1.** Effect of Selenium yeast on Anxiety-like Behaviour in Open-Field of Wistar Rats subjected to Restraint Stress

PARAMETERS	Group I	Group II	Group III
Line Crossed	6.8±3.1 <sup>b</sup>	15.0±5.9 <sup>a</sup>	0.00±0.00 <sup>c</sup>
Neck Stretch	3.4±1.5 <sup>a</sup>	5.0±1.4 <sup>a</sup>	2.6±0.24 <sup>b</sup>
Grooming	6.2±2.5	5.0±1.8	3.6±1.4
Rearing	1.2±0.49 <sup>a</sup>	5.4±1.5 <sup>b</sup>	0.80±0.40 <sup>a</sup>
Defaecation	2.8±1.0	1.8±1.2	3.0±0.95
Freezing	0.20±0.20 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.60±0.40 <sup>a</sup>

Data are expressed in mean ± SEM. <sup>a, b</sup> = Means with different superscript letters within rows are significantly ( $P < 0.05$ ) different; Group I = Control, Group II = Stress Control, Group III = Selenium yeast Administration, n= 5.

### Effect of selenium yeast on biomarkers of oxidative stress in restrained Wistar rats

In (Table 2) no significant difference ( $P < 0.05$ ) was observed in SOD, CAT and MDA among groups, although the stress control shows higher level of MDA ( $1.7 \pm 0.08$ ) and CAT ( $1.3 \pm 0.22$ ), and decreased level of SOD ( $63 \pm 4.1$ ), when compared to the normal control ( $1.6 \pm 0.13$ ), while the selenium yeasts group shows lesser levels of MDA ( $1.6 \pm 0.17$ ) and CAT ( $0.85 \pm 0.15$ ), and increased level of SOD ( $104 \pm 32$ ). However, a significant ( $P < 0.05$ ) increase was observed in reduce glutathione in the selenium yeast group ( $2.3 \pm 0.52$ ) when compared to the stress control ( $0.66 \pm 0.33$ ).

**Table 2.** Effect of the selenium yeast on oxidative stress parameters in restrained Wistar rats

	Group I	Group II	Group III
Total protein			
Superoxide Dismutase (U/g protein)	$74 \pm 4.8$	$63 \pm 4.1$	$104 \pm 32$
Catalase (k/g protein)	$1.2 \pm 0.26$	$1.3 \pm 0.22$	$0.85 \pm 0.15$
Reduce Glutathione (mmol/mg protein)	$1.1 \pm 0.26$	$0.66 \pm 0.33^b$	$2.3 \pm 0.52^a$
Malondialdehyde (mmol/mg protein)	$1.6 \pm 0.13$	$1.7 \pm 0.08$	$1.6 \pm 0.17$

Data are expressed, in mean  $\pm$  SEM. <sup>a, b</sup> = Means with different superscript letters within rows are significantly ( $P < 0.05$ ) different; Group I = Normal Control, Group II = Stress Control, Group III = Selenium-yeast Administration,  $n = 5$ .

### Discussion

Exposure of humans and animals to stress is absolutely unavoidable, restraint or immobilisation stress is a laboratory model to mimic psychological stress, leading to various ill-effects on body systems [4]. Studies have linked restraint stress to excess production of ROS and RNS [5]. ROS and RNS have been implicated in the pathology of various neurological disorders that alter behaviour [25].

In the present study behavioural performance was evaluated using open-field and elevated plus maze apparatus both were used to assess anxiety-like behaviours. In the open-field test (Table 1), the stress control group showed increased value of line cross, neck stretch and rearing, with rearing ( $5.4 \pm 1.5$ ) being significantly ( $P < 0.05$ ) higher when compared with that of the normal control group of values ( $1.2 \pm 0.49$ ), this result disagreed with the previous

finding [32]. That showed a decrease in line cross and rearing in open field, when exposed to immobilisation stress. However, administration of selenium yeast decreases locomotive activities in open field.

In the Elevated plus maze (Figure 1-4), there was a significant ( $P < 0.05$ ) increase in time spent (Fig 3) and entries (Fig 4) in open arm, in the stress control group, when compared with the normal control group. This result disagreed with the finding of [9], which showed that restraint stress suppressed entries and time spent in open arm. In close arms there was a significant ( $P < 0.05$ ) increase in entries (Fig 1) and time spent (Fig 2) by the stress control, when compared with the normal control. However, the selenium yeast group showed a significant ( $P < 0.05$ ) decrease in entries and time in open arms, and also an increase in time spent and entries in close arms.

Biomarkers of oxidative stress assayed (Table 2) in this study include superoxide, catalase, glutathione reductases and malondialdehyde. In this study, malondialdehyde level was higher in the stress control group, when compared to the normal control group. The decrease MDA level in selenium yeast group agrees with the study obtained by [34], which showed that administration of selenium yeast was not different when compared to the normal control MDA concentration. In this study SOD activity was lowered compared with that obtained in the stress control group or the normal control. This finding agrees with the study of [36], who showed that immobilisation stress decreases activity of SOD. In antioxidant administered groups, SOD activity was higher. The increase in SOD of selenium yeast group agreed with the finding of [16], indicating the role of selenium yeast in activating enzymes. The result obtained for catalase showed an increase in catalase activity in the stress control group, when compared to the normal control group. This finding corresponded to that of [37], who showed that 3 h of restraint stress increased catalase activity. However, there was a decrease in catalase activity in selenium yeast. Results obtained for reduced glutathione showed lesser value in the stress control group, when compared with the normal control, and this agreed with the finding of the study of [38]. Administration of selenium yeast showed an increase in reduced glutathione concentration when compared to that of the stress control group. The result is in agreement with the finding of [2], which showed that pre-treatment with selenium restored glutathione content, prior to 4 h of restraint stress in Rats.

The results obtained from the present study demonstrated that restraint impairs anxiety-like behaviour and induced oxidative stress. However, administration of antioxidants

selenium yeast ameliorates oxidative stress, but did not exert effect on anxiety-like behaviours in rats.

### Conflict of Interest

We declare that we have no conflict of interest.

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