Introduction

Percentage alternation and discrimination index are two age-long cognitive determinants used in translational memory research. DI is derived from the novel object recognition (NOR) paradigm used in the evaluation of rodents’ ability to differentiate between old and new items in an open-field box. Failure to present novelty preference indicates a rodent’s lack of discriminately ability. This assumption is because animals have the instinct to identify the newest object based on memory strength rather than unnecessary classification based on new criteria. However, since the NOR task relies entirely on the innate ability of the animal predisposition toward explaining novelty, some schools of thought suggest biases may play a role in determining its final metric. Therefore, the use of a confirmatory cognition test like the percentage alternation is vital in

Materials and Methods

Thirty-five Wistar rats were used for this study and grouped into seven (n=5); group 1 (non-pregnant), group 2 (pregnant), group 3 (pregnant + received vitamin E; 100 mg/kg/day) group 4 (pregnant + received selenium-yeast; 0.4 mg/kg/day), group 5 (pregnant + received yeast; 50 mg/kg/day) group 6 (pregnant + received selenium; 0.25 mg/kg/day) and group 7 (pregnant + received vitamin E; 100 mg/kg/day + selenium-yeast; 0.4 mg/kg/day). Discrimination index was evaluated using novel object recognition, apparatus, while percentage alternation was evaluated using Y-maze on day 19.

Results

Percentage alternation increased in group 1 and antioxidants treated groups respectively compared to group 2 (p˂0.05). Discrimination index significantly increased in group 1 and antioxidants treated groups compared to group 2 (p˂0.05).

Conclusion

The percentage alternation and discrimination index show promising results in the cognitive assessment of pregnant dams. Antioxidant molecules such as vitamin E and selenium-yeast potentiate the indices of cognitive improvement.

Keywords: Pregnant dams, discrimination index, percentage alternation, vitamin E, selenium-yeast
correcting the impression of bias and is a vital behavioral study in rodents (6). Percentage alternation is been evaluated using a Y-maze apparatus test; alternation indicates sequential entries into all the arms which are also called a triad (7). Since animals have a strong tendency toward entering a preferred side rotationally (8). This test was observed to provide higher credibility when the study involves pregnant rodents (9). Pregnancy has been observed to be a physiological stress paradigm; hence may induce oxidative damage (10). Oxidative damage has been observed to alter the physiological mechanisms, and hence result in organ damage (11). Oxidative damage has been observed to alter cognitive performance (11, 12). A study has shown the role of selenium yeast in cognitive improvement in pregnant dams (13). However, selenium yeast is a water-soluble antioxidant and may lack the penetrating ability inside the hydrophobic portion of the cells towards improving peroxisomes activity. Hence the use of dual antioxidants may play a vital role in mitigating such effects, especially vitamin-E which is a fat-soluble antioxidant; it could protect the hydrophilic portion of the neuronal cells (12). While selenium yeasts a water-soluble antioxidant; may protect the hydrophobic portion of the nerve cells (14). These may mitigate the oxidative stress that induces cognitive alteration in the behavioral test paradigm in rodents (15). However, to the best of the literature search, no study has conducted a dual behavioral cognitive study on pregnant dams using NOR and Y-maze along with the administration of antioxidants vitamin E and selenium yeast. Hence this study may correct the biases experienced using behavioral cognitive study model and possibly ameliorate pregnancy-induced oxidative stress damage associated with cognition.

The present study aims to investigate the effects of vitamin E and selenium yeast on the percentage alternation and discrimination index of pregnant dams.

Materials and Methods

Ethical Statement

Forty-five Wistar rats of both sexes were purchased from the Faculty of Veterinary Medicine, University of Abuja. The animals were housed for two weeks for acclimatization in the animal house of the Department of Physiology, Baze University, under standard temperature before the commencement of study and mating. The study protocol was approved by the ethical committee of the Department of Physiology, Baze University, with the ethical number (BUAEC0023).

Antioxidant Preparation

The selenium yeast was manufactured by Mason Vitamins, Miami Lakes. Selenium used was manufactured by Bactolae Pharmaceutical Inc, USA. Yeast tablets were manufactured by Kunimed Pharmacchem Ltd, Adelanwe Street, Valley Estate, Ikeja, Lagos, Nigeria. Vitamin E (Alpha-tocopherol) was purchased from H-Medix, Gwarimpa Estate, Abuja. Selenium, selenium yeast, and yeast were reconstituted with distilled water, while vitamin E was given in its aqueous form. All administration was by an oral gavage.

Study Design

Thirty-five female Wistar rats (30 Pregnant and five non-pregnant) were grouped into seven groups (n:5). Pregnancy was confirmed by the presence of a vaginal plug, which was checked during the early hours of each day; the first day of the appearance was tagged pregnancy day 0 (PD0). The administration of selenium, vitamin E, yeast, selenium-yeast, and vitamin E commenced on pregnancy day 3 and lasted for 15 days. Selenium and yeast were reconstituted with distilled water, while vitamin E was given in its aqueous form.

- Group I (negative control): Non-pregnant + received 1 mL/kg of distilled water
- Group II (positive control): Pregnant + received 1 mL/kg of distilled water via oral
- **Group III**: Pregnant + received vitamin E; 100 mg/kg/day
- **Group IV**: Pregnant + received selenium; 0.4 mg/kg of selenium yeast
- **Group V**: Pregnant + received 50 mg/kg of yeast
- **Group VI**: Pregnant + received 0.25 mg/Kg of selenium
- **Group VII**: Pregnant + received selenium-yeast; 0.4 mg/kg + vitamin E; 100 mg/kg/day

**Test for Cognitive Response**

Learning and memory are indices of cognition. The test for learning and memory was conducted on Day 19 of pregnancy on the dams using Y-maze and Novel object recognition test (NORT).

**Y-maze Test**

Y-Maze is a behavioral test for measuring the willingness of rodents to explore new environments (16). Rodents typically prefer to investigate a new arm of the maze rather than returning to the one they previously visited. Many parts of the brain like the hippocampus, septum, basal forebrain, and prefrontal cortex, are involved in this task (17). The Y-shaped maze has three arms at a 120° angle from each other (50x50x50 cm). In this study, the working memory was assessed based on the existing protocols from previous literature with little modification (18). The rats were placed at the center of the maze and were allowed to freely explore the three arms for 5 mins. The number of times the rats entered each arm was counted; this is when all four limbs are inside the arm. Over the course of multiple arm entries, the subject should show a tendency to enter a less recently visited arm. The number of arm entries and the number of triads or alternations (a triad when it visited all 3 arms consecutively) were used to calculate the percentage alternation (measure of working memory). The percentage alternation (%) is calculated as (number of alternation/number of arms entered-2) x 100 according to the method of (19). If a rat scored significantly above 50% alternations, these indicate a functional working memory. Note, that after each trial, the Y-maze was cleaned with 75 % ethanol which prevented olfactory cues.

**Novel Object Recognition Test**

Novel Object Recognition (NORT) is used to evaluate cognition, particularly recognition memory, in rodent models of CNS disorders. This test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. The choice to explore the novel object reflects the use of learning and recognition memory (20).

The Novel Object Recognition task was conducted based on the protocols of previous studies (21; 22) with slight modification using an open field box with a dimension (50x50x50 cm), along with two different objects. The test was divided into three (3) phases:

1) **Familiarization phase** (the animal is free to explore the space with no objects present), with 5 minutes to habituate.

2) **Training phase** (two similar objects were introduced into the open field)

3) **Testing phase** (a new object was introduced instead of the familiar object).

On the test day, the rats were placed in the test box, and after 5-min of habituation, two objects were introduced in two corners (approximately 20 cm apart from each other). The time spent exploring each object was recorded during the subsequent 5 min period (defined as the training session). After a waiting period of 15 mins, the rats were placed in the test box once again, and within an interval of 5 mins, an object was reintroduced, in which one of the familiar objects used in the previous training session was replaced with a novel object. The time spent exploring each object
was recorded during the subsequent 5 min period (defined as the test session). Exploration is when an animal sniffs, bites, or faces an object. The total exploration time of the familiar and novel objects was used to calculate the discrimination index. The discrimination index is an index of measures of discrimination between the familiar and the novel objects corrected for exploratory activity. It is calculated as (time spent on the novel object - time spent on the familiar object) / (time spent on novel object + time spent on the familiar object). The discrimination index ranges from -1 to 1, with -1 indicating a complete preference for the familiar object, 0 indicating no preference for either object, and 1 indicating a complete preference for the novel object.

**Statistical Analysis**

Data obtained from this study are expressed in mean (± SEM). And the values of p<0.05 are considered significant. The difference between the mean was obtained using a one-way analysis of variance (ANOVA) followed by a post-hoc test (Tukey's). GraphPad Prism 8 (San Diego, California, USA) is the statistical use.

**Results**

**Effects of Vitamin E and Selenium-yeast on Spatial Working Memory of Pregnant Dams on Y-maze Apparatus**

A percentage alternation above 50% is an indicator of spatial memory retention and below 50% indicates spatial memory deficit. Figure 1, shows a significant increase (p<0.05) in percentage alternation in group 1 (58.7±4.362) compared to group 2 (24.3±2.404); this may imply a deficit in spatial memory. However, there is a significant (p<0.005) increase in the percentage alternation in the antioxidants administered in groups 3 (52.1±2.42), group 4 (50.3±2.42), group 5 (51±4.93), group 6 (53±2.08) and group 7 (56.3±1.8) compared to group 2. Among antioxidant groups, group 7 (56.3±1.8) display better spatial memory retention, although not significant (p< 0.05).

**Effects of Vitamin E and Selenium-yeast on Spatial Memory on Pregnant Dams using DI.**

Discrimination index (DI), ranges from -1, 0 to 1, with -1 indicating a complete preference for a familiar object, 0 indicating no preference for either object, and 1 indicating a complete preference for the novel object.
reference for the novel object recognition (NORT), since rodents naturally want to explore new things, hence scoring 1 indicates retentive memory. Figure 2 shows a significant (p<0.05) increase in DI in group 1 compared to group 2. Also, there is an increase in DI values in the antioxidants administered groups compared to group 2 (p<0.05). Although, this increase varies among groups with group 7 showing a better improvement on NORT; despite not being significant (p<0.05), but could indicate a role of combined antioxidants compound over a single antioxidant compound.

Discussion
This study evaluated the effects of vitamin E and selenium yeast on pregnant dams using DI and PA determinants. In this study, cognition was evaluated from the values obtained from DI using NORT and PA using the Y-maze apparatus. The NORT evaluates spatial memory in rodents, it is derived from the mathematical expression: (time spent on a novel object – time spent on the familiar object)/ (time spent on novel object + time spent on the familiar object).

The discrimination index ranges from −1 to 1, with −1 indicating a complete preference for the familiar object, 0 indicating no preference for either object, and 1 indicating a complete preference for the novel object (20).

The DI decreases in the positive control compared to the negative control in PD; this decrease in DI further elucidates the postulation that prenatal stress alters cognition as described in previous studies (23, 24). However, antioxidants administered groups show an increase in mean DI, especially in group 7; the increase in DI in group 7 might be due to the synergic action of vitamin E and selenium yeast as a key protectant of lipid membrane against peroxidation that may result from imbalance from the exercitation of glutamate-calcium-ROS in the hippocampus and prefrontal cortex of the brain (24). The Y-maze test evaluates cognition by the mean percentage alternation, which is obtained from the number of arms entering and triad (sequential entering into each arm). The percentage alternation decreases in the positive control group compared to the negative control, the decrease

Figure-2. Effects of Vitamin E and Selenium-yeast on cognitive performance of pregnant dams using percentage alternation determinant (The values represent the mean±SEM. **p<0.01; ***p<0.001; #p<0.05)
in percentage alternation is a confirmation of findings that link prenatal oxidative stress with cognitive impairments (25, 26). However, in the antioxidant administered groups, the mean percentage alternation increases, improvement in the mean percentage alternation point to the protective role of antioxidants against oxidative stress-induced cognitive impairments which is along with the following findings (27, 28).

Cognitive assessment in the study is exclusively behavioral, with no use of the biochemical and molecular model in the confirmation of possible changes observed.

**Conclusion**

Pregnancy decreases cognitive performance in dams as seen by decreased values in percentage alternation and discrimination index determinant when compared with non-dams. However, pregnant dams administered with antioxidants vitamins, selenium, yeast, or selenium-yeast show improved performance in discrimination index and percentage alternation indicators of cognitive performance.

**Conflict of Interest**

All authors declare no conflict of interest

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**Reference**


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