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EFFECTS OF WITHDRAWAL OF MORINGA OLEIFERA ON LEARNING AND MEMORY IN CHEMICALLY STRESSED ALBINO MICE

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ABSTRACT

Moringa oleifera has been reported for increasing rate of use among individuals as an alternative natural remedy to improve learning and memory especially in geriatrics characterized by cognitive decline. This study was aimed at assessing whether sudden cessation of its prolonged administration is associated with withdrawal effects. Mice were divided into 4 groups with five per group; 1st is normal and 2nd is positive control, while 3rd and 4th were the tests. Mice in group 2 were induced with a dosage of 3-Nitroprionic Acid (15mg/kg) likewise group 3 and 4. Mice in group 3 were further treated with a dosage of *M. oleifera* leaf extract (300mg/kg) for 6weeks without withdrawal while mice in group 4 were treated with same dosage of the extract for 6weeks and withdrawn for 3 days. Morris water maze and Novel Object Recognition Test were used to assess each group of mice for learning and memory. Statistical analysis showed no significant difference among the test groups, whereas there was difference in the test groups compared to the positive control and normal control at $p<0.05$. The order of learning and memory assessed was Group 1>group 2<group 3=4. In conclusion, *M. oleifera* improved learning and memory following chemical stress, was not associated with withdrawal effects.

KEYWORDS: Moringa oleifera, learning, memory, stress, withdrawal.

INTRODUCTION

Withdrawal effects often associated with the symptoms observed in individuals after a prolonged or chronic use of some conventional drugs has also been found to be reported in traditional and alternative medicine (Herb Caen, 1978). These may vary with kind, amount, usage period, and individual's physical and mental wellbeing. Withdrawal effects have been associated with symptoms such as changes in mood, appetite and sleep; also anxiety, depression, restlessness and cognitive decline etc. (MPME, 2019).

Stress is biological response to a perceived threat or situation that requires attention, posing the body to harm. Stress is caused by chemicals and hormones released throughout the body to help maintain optimum balance. Stress affects the body's thoughts, feelings and overall function with common symptoms such as headaches, disorientation, and general body discomfort which overtime can affect learning and memory (APA, 2018). Stress can have effects on memory and learning. It can influence performance of spatial tasks such as navigation, visuo-spatial memory and attention. In a study by Richardson and Melissa, (2022) it was reported that increase in cortisol showed improvements in visuo-spatial map memory, visual working memory for faces and sciences and visuo-spatial change detection while (Tarvenier *et al.*, 2011) reported that path-learning performance was significantly impaired after exposure to stress. Stress can inhibit the formation of short term memories and their conversion to long-term memories, making it more difficult to learn when stressed (Scott, 2021). Stress can also affect the type of memories we form. For instance, when we are stressed, we might find it difficult to remember accurately the occurrences at the event later but interestingly, if the material being learned is directly related to the stressor, memory would actually improve. Chronic stress can change the brain in a lot of ways which can affect learning and memory predisposing individuals

Memory impairment is a significant global challenge affecting both human health and quality of life. As population ages, the prevalence of memory-related disorders, such as dementia, continues to rise. Dementia is characterized by severe cognitive decline, including memory loss, and poses a substantial burden on individuals, families, and healthcare systems worldwide; which is projected to double nearly every 20 years (Afrin *et al.*, 2022). There has been a rise in the use of traditional remedies for treatment especially among elderly population. *M. oleifera* commonly known as the drumstick tree or horseradish tree has gained attention for its diverse biological functions. This plant is rich in bioactive compounds, including vitamins, minerals, and antioxidants (Jamari *et al.*, 2020). Almost all parts of the

M. oleifera tree has been found to be of use in therapy, nutrition and industrial settings (Velez-Gavilan, 2012).

In this study, researchers aimed to investigate the effect of ethanolic extract of *Moringa oleifera* leaves on learning and memory, with specific objective of assessing how withdrawal of M. Olifeira administration would affect recognition memory (using the novel object recognition task), as well as visuo-spatial learning and memory (using the Morris Water Maze test) in mice exposed to memory impairment through chemical stress induced by 3-nitroprioprionic acid.which is a known neurotoxin, (*Liu et al.*, 1992).

METHODS

Male and female albino mice were obtained from the animal house of the Department of Human Physiology, University of Port Harcourt with weights ranging from 12-33g. The animals were housed in standard plastic cages in the animal house of the Faculty of Basic Medical Sciences, Niger Delta University prior to the testing. The animals were kept under normal room temperature (22°C±3) and humidity (50-60% ±5) with 12-hour dark/light cycle and allowed free access to standard grower's mash feed and distilled water. Acclimatization of animals for two weeks was ensured before experimentation proper - induction of 3-Nitroprionic acid and further treatment of ethanolic extract of *M. oleifera* leaves. All process for housing and use of the animals were in line with the standard operating procedures as stated by the 'Institutional Animal and use committee' (IACUC, 2002).

Fresh leaves of *Moringa oleifera* were obtained from Delta state in Nigeria, and air dried at room temperature, then grinded to a nearly powder form for easy extraction using an electric blender. The powdered leaves were further subjected to maceration (cold extraction) in an extraction jar using 50% ethanol for 72 hours on standing with intermittent shaking every 6 hourly. Volume of solvent system used was four times the weight of solvent system. The crude extract was filtered using glass wool because it is inert. It was then evaporated using a rotary evaporator at 40°C until a pasty concentrate of *M. oleifera* leaves extract was gotten and the residue (marc) were discarded via clarification. The pasty concentrate was stored in a refrigerator at 12-15°C until required for use.

Calculation of Percentage Yield of Extract

$$\%\text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Powdered Plant material} \times 100/1}$$

Mice were randomly divided into 4 groups of 5 mice each. The first group served as normal control, the second group served as a positive control which was induced with 3-Nitroprionic acid and not treated with *M. oleifera*, the third group served as test group one, induced with 3-Nitroprionic acid and treated with *M. oleifera* without withdrawal. Then the fourth group (test group two) induced with 3-Nitroprionic acid and treated with *M. oleifera* which was then withdrawn. 3-Nitroprionic Acid was administered at 15mg/kg while *M. oleifera* extract was administered at 300mg/kg per body weight of experimental animal.

The positive control group was treated with a dosage of 3-Nitroprionic acid (15mg/kg). Prior to treatment with *M. oleifera* extract, the first and second test group were treated with the same dosage of 3-Nitroprionic acid and allowed a period of 3 days to mimic a state of neurodegeneration such as that experienced by geriatrics with Alzheimer's disease (Joffa, 2024). *M. oleifera* extract was administered orally to the first test group twice a day for 6weeks while the second test group was administered the extract for 6 weeks and then withdrawn for 3 days.

NOVEL OBJECTS RECOGNITION TEST (NOR)

A standard procedure of novel object recognition test (Lindsay, 2017) was adopted to test for recognition memory in mice. Its principle is based on rodent's natural ability to explore novel spaces when placed in a novel environment. Rodents that remember the familiar object would spend more time exploring the novel object in the environment. The novel object recognition test is completed within four (4) days; habituation day, training day and testing day.

Plate 1: Novel Object Recognition Box.

The Discrimination Index (DI) was implored referring to calculation of novel object exploration time - familiar object exploration time / total object exploration time.. It represents the best estimation of recognition memory as it shows object preference (Lindsay, 2017).

MORRIS WATER MAZE (MWM)

This is an established neurobehaviour studies protocol whose principle is based on the animal's inherent ability to navigate through the pool, find an escape platform and also use visual cues as guides to the escape platform for reference purposes (Charles & Michael, 2006; Morris *et al.*, 1982, McDonald & White, 1994).

Plate 2: Morris Water Maze.

STATISTICAL ANALYSIS: All results were presented in mean ± SEM, analysis was by ONE WAY and TWO WAY ANOVA, and the post/hoc Turkey's test. The computer software, SPSS 12.0 was used. Level of significance was at P <0.05.

RESULT ANALYSIS

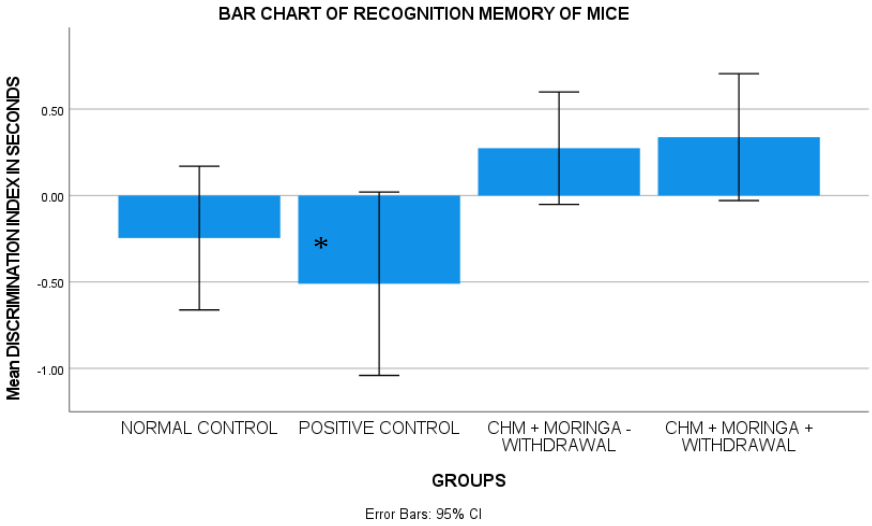

Fig. 1: Bar chart of mean Discrimination Index across all experimental groups. *Significant at P<0.05 compared to the withdrawal and non-withdrawal group.

Discrimination Index (DI) as used in the novel object recognition test is a measure of the level of recognition of difference between two things (discrimination). It is calculated as TN-TF÷TN+TF where; TN is the time spent exploring the novel object and TF is the time spent exploring the familiar object. Mice with higher DI (> or =1) have higher preference for the novel object while those with lower DI (<1) have a lower preference for the novel object. From the results, mice in the normal control group showed an almost moderate mean DI of-0.2460 ± 0.12 indicating equal preference for both objects while mice in the positive control group showed a negative mean DI of -0.5100 ± 0.11 indicating impaired memory. Mice in the test groups showed a positive DI of 0.2740 ± 0.01 and 0.2955 ± 0.02 in the non-withdrawal and withdrawal group which is higher indicating preference for the novel object.

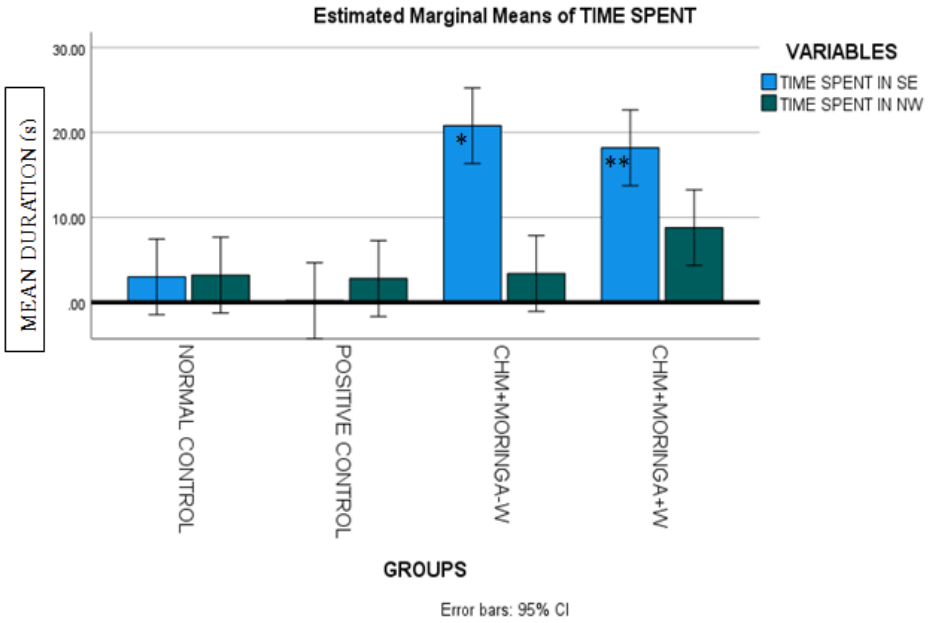

Fig. 2: Duration of Time spent in SE and NW of probe trial (day 7) across all experimental groups. *Significant at P<0.05 for withdrawal and non-withdrawal group compared to normal and positive control in SE. ** Significant at P<0.05 for withdrawal group compared to normal, positive and non-withdrawal group.

Ideologically, mice in the normal control group showed the same level of activity in both SE and NW quadrant with mean time spent as 3.00 ± 0.12 and 3.20 ± 0.01 respectively. Mice in the positive control group showed a reduced visuo-spatial learning and memory in both SE and NW quadrant with mean time of 2.80 ± 0.13 and 1.50 ± 0.11 respectively. Mice in the test groups showed a higher time spent with the non-withdrawal group at 3.40 ± 0.02 and 12.10 ± 0.01 in SE and NW respectively while the withdrawal group showed a mean time of 4.55 ± 0.03 and 7.55 ± 0.02 in both SE and NW respectively. However, there was a significant difference for time spent in SE compared to NW in both test groups which can be attributed to the association of learning to the visual cue in NW as it was higher in the withdrawal group.

DISCUSSION

COMPARISON OF DISCRIMINATION INDEX (DI) IN THE NOVEL OBJECT RECOGNITON TEST ACROSS ALL EXPERIMENTAL GROUPS

A comparison of the normal control to the positive control reveals, positive control had a lower DI with a mean difference of 0.2640, implying that 3-nitroproprionic acid at a dosage of 15mg/kg did not cause significant difference at p<0.05. Comparison of the normal control to the group without withdrawal showed no significant difference (p< 0.05) but with improved recognition memory in the group without withdrawal with a mean difference of 0.520. A comparison of the normal control to the withdrawal group also showed no significant difference but with improved recognition memory in the withdrawal group indicating that the impact of *M. oleifera* on learning and memory may be maintained even after withdrawal. Comparison of the positive control group to the group without withdrawal showed a significant difference at P<0.05 with a mean difference in Discrimination Index of -0.7840 with the positive control showing a lower discrimination index. This suggests that *M. oleifera* may have neuro-healing effects. The comparison of positive control to the withdrawal group also showed a significant difference with a mean difference in discrimination index of -0.8480 with the withdrawal group having a higher discrimination index indicating that *M. oleifera* maintains its neuro-healing properties even after withdrawal. The comparison of group without withdrawal to the withdrawal group showed almost negligible difference considered insignificant at P<0.05 indicating that *M. oleifera* had no effects of withdrawal on learning and memory following chemical stress.

COMPARISON OF TIME SPENT IN SE AND NW QUADRANT IN THE MORRIS WATER MAZE ACROSS ALL EXPERIMENTAL GROUPS

Comparing the normal control to positive control showed no significant difference in mean time spent in SE and NW quadrants, though the normal control had a considerably higher mean. A comparison of normal control to the group without withdrawal showed a significant difference at P<0.05 wherein the group without withdrawal had a higher mean (17.40 ± 0.14) in SE indicating that *M. oleifera* improved learning and memory but in the NW, there was no significant difference between the two groups with a mean difference almost negligible, indicating the ability to associate learning and memory more to the visual cue in SE than NW in both groups. The comparison of normal

control to the withdrawal group showed that the withdrawal group had a higher mean with mean difference of 15.20 ± 0.12s and 5.60 ± 0.11s in SE and NW respectively indicating that *M. oleifera* still improved learning and memory even after withdrawal. A comparison of the positive control to the group without withdrawal in SE showed a significant difference at a mean difference of 20.60 ± 0.15s with the mean of the positive control at 0.20 ± 0.02s but no significant difference in the NW quadrant showing the neuro-healing effects of *M. oleifera*. A comparison of the positive control group to the withdrawal group showed a significant in both SE and NW with the withdrawal group having a higher mean of 18.20s and 8.80s in both SE and NW with a mean difference of 18.00 ± 0.22s and 6.00 ± 0.13s in SE and NW respectively. This implies that the neuro-healing effect of *M. oleifera* persists after withdrawal. Also a comparison of the group without withdrawal to the withdrawal group showed no significant difference in the SE quadrant but however the difference was significant in the NW quadrant at $P<0.05$ with the withdrawal group having a higher mean of 8.80 ± 0.15s in NW compared to 3.40 ± 0.11s of the group without withdrawal. This indicates that *M.olifera* has no withdrawal effects on learning and memory following chemical stress. The significance in the NW could be indicative of tolerance in the group without withdrawal due to continuous administration of the extract.

Results reported by Sutalangka *et al*, (2013) showed that *M. oleifera* mitigates memory impairment and neuro-degeneration in an animal model of age related degeneration and oxidative stress even though different formulations were used however, it was not based on its withdrawal effects.

COMPARISON OF VISUO-SPATIAL ACTIVITY IN MORRIS WATER MAZE TO RECOGNITION MEMORY IN THE NOR TEST

A comparison of the two paradigms used to assess learning and memory showed the same trend in all experimental groups except normal control group compared to the non- withdrawal group in the novel object recognition test which showed significant difference. This could be attributed to the easy nature of the task which takes 3 days compared to the Morris water maze which takes about 8 days to complete.

CONCLUSION

From the findings in this study, *M. oleifera exhibits* neuro-healing properties in improving learning and memory; in a way that cessation of its administration may not be associated with withdrawal symptoms. Further research to investigate the potency as well as, dose – dependent, sex-dependent and age – dependent effects of M. Oleifera withdrawal in comparison with standard regimen is recommended.

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