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# THE IMPACT OF LONGTERM TREATMENT AND WITHDRAWING OF MORINGA OLEIFERA ON ANXIETY BEHAVIOUR IN EXPERIMENTAL MICE MODEL

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## ABSTRACT

The aim of this research was to investigate the effect of long-term administration and withdrawal of Moringa oleifera on anxiety behaviour in experimental mice induced with biological stress. Four groups 1 - 4 of five mice each were administered mash feed and normal saline, stress induction with no Moringa, Moringa oleifera without withdrawal, and Moringa plus withdrawal in that order for six weeks. They were then subjected to two models of anxiety behavior studies; Elevated plus maze and Light / Dark box test. The result suggests that continuous administration of Moringa oleifera has anxiolytic outcome, which may not be altered by its sudden withdrawal.

**KEYWORDS:** Moringa oleifera, anxiety, withdrawal, long-term, stress.

## INTRODUCTION

Moringa oleifera is widely cultivated in Central America, the Philippines, the Caribbean Islands, as well as South and North America and described by various names including Horseradish tree, Drumstick tree, Miracle tree etc (Mahato *et al.*, 2022).

Moringa is one of the most useful trees in the world, having widespread application as medicinal plant; being used as antispasmodic, expectorant, diuretic, cardiac circulatory tonic and antiseptic (Kumar *et al.*, 2010; Nadkarni, 2009). Meanwhile, research on the specific effects of Moringa oleifera on anxiety is limited, though it is believed to possess certain

properties that may contribute to its potential as a natural remedy for anxiety (Siddhuraju, & Becker, 2003).

Anxiety, the emotion trailed by pervasively displeasing outlook for looming harmful expectations, often associated with tense physiology condition and increased steady watchfulness (Barlow, 2002); may be caused by genetic, neuroanatomical, and environmental factors or early –life stress as well as inflammatory processes (Flint & Shifman, 2008; Ibi *et al.,* 2008; Nestler & Hyman, 2010; van Dijk *et al.,* 2013; Dantzer *et al*., 2008). Anxiety in mice can affect Physiological parameters such as Altered Stress Hormone Levels and Heart Rate. Also, neurological effects may include Neurotransmitter Imbalance, Neuroinflammation, and Hippocampal Changes (Esler *et al.,* 2008; Snyder, et al., 2011). In mice particularly, stresses could alter various behavioral parameters including grooming behavior, Avoidance Behavior/ reduced social interaction (Armario, Escorihuela, & Nadal, 2008; Prut, & Belzung 2003).

The body also responds to stress in various ways and researchers have developed chronic unpredictable stress (CUC) protocol widely used to study the impact of stress response in several animal models consisting of random, intermittent and unpredictable exposure to a variety of stressors during several weeks (Bondi et al., 2008) These stressors typically include social isolation, physical restraint, water and food deprivation, Noise, tilted cage, overnight illumination, inverted light, noise (Willner, 2005).

Withdrawal effect is the body's response when an individual suddenly stops using a certain substance such as alcohol, medications or other drugs; and may include combination of physical and mental effects experienced after stopping the use of a substance, essentially due to the adaptation of the body system to changes (Paikra and Gidwani, 2017).

The aim of this research was to investigate effect of chronic administration and withdrawal of M. oleifera on anxiety behaviour in experimental mice model of biological stress.

## METHODS

### Experimental Animals

Male and female Swiss white albino mice where obtained from the animal house of university of Portharcourt. The animals were housed at room temperature with 12hour dark

/light cycle and allowed free access to standard grower mash feed and water. The animals were acclimatized for 2 weeks before experimentation.

**Plant Material and Extract Preparation**

Fresh leaves of moringa oleifera obtained from cultivated sources in Delta state was air dried at room temperature. These were blended to coarse powder using a portable manual blender. The leaves were then subjected to maceration (cold extraction); in an extraction jar using 50% Ethanol for 72 hours and agitated every 6hours. The extract was filtered. The menstrum was then concentrated using a rotary evaporator. The pastry concentrate was stored in a refrigerator $40^0$c until required for use. Moringa extract was administered at a dose of 300mg/g

**Experimental Design**

Twenty mice were divided into four groups of 5 mice each. The first group served as the Normal control (fed with standard grower mash feed and normal saline water), The second group served as biological stressed mice treated with Moringa without withdrawal The third was treated with Moringa plus withdrawal and The fourth group served as No withdrawal (biologically stressed mice without withdrawal of administration of Moringa).

The mice were induced to stress using the "CUS" paradigms for 10 days as follows DAY 1: isolation, DAY 2: Inverted light cycle, DAY 3: Noise exposure, Day 4: overnight illumination, Day5: tilted cage. The process was repeated for the remaining days 6-10 in line with protocol of Bondi et al., 2008.

**Anxiety Studies**

**Elevated plus maze:** This is a standard protocol for assessing anxiety behavior in rodents by how much they explore the open and closed arms, which gives measures of their state of composure or overt alertness and anticipation (Brown et al., 1999; McFadyen et al., 2002; Podhorna and Brown, 2002; Yan *et al.,* 2004). Also behaviors scored include head dipping, rearing, grooming, urination, defecation.

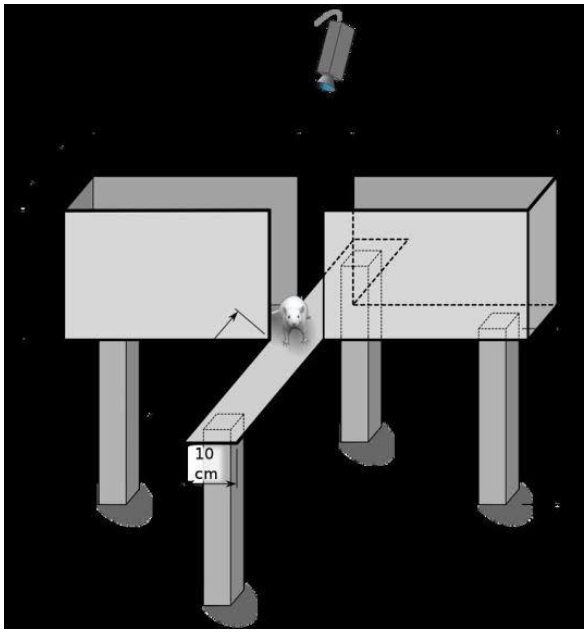


**Plate 1: Elevated plus maze.**

**Dark and light box (DLB):** This is another established variant method of anxiety studies in rodents that estimates anxiety behaviors by analyzing the times and duration mice were exploring either the dark or light (Prut,l.,& Belzung,C.2003). Some other behavior investigated are rearing, grooming, urination, defecation

**Plate 2: Dark and light box.**

## RESULTS

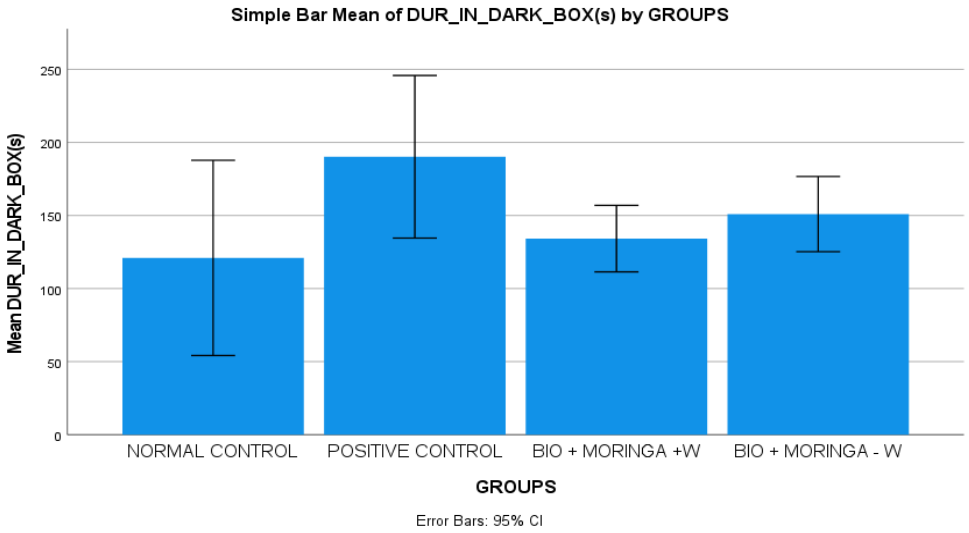


**Figure 1. Comparing dark and light box in all experimental groups.**

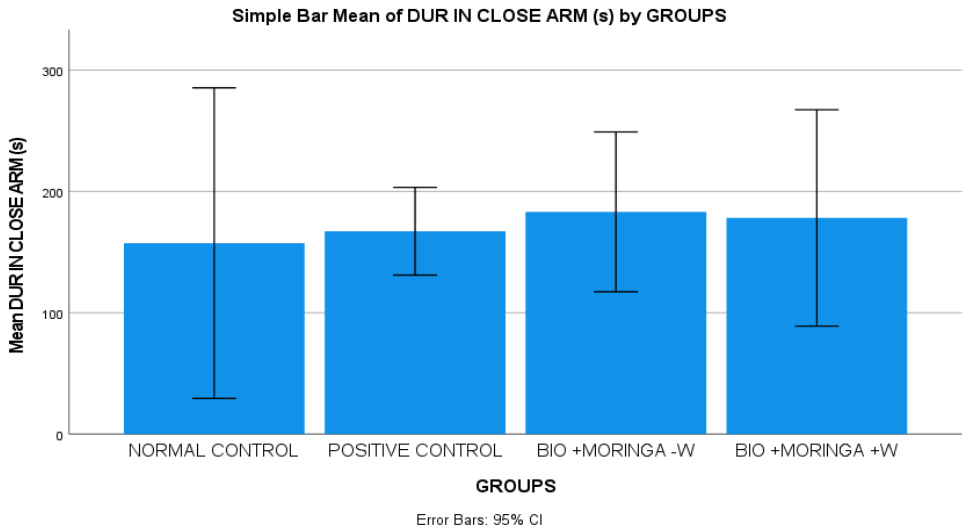


**Figure 2: Comparing time spent in open arm and close arm in elevated plus maze in all experimental groups.**

## DISCUSSION

Moringa oleifera, known as the 'Miracle Tree', has been widely consumed for its numerous therapeutic properties, including enhancing learning and memory, and improving overall health and well-being. Anxiety is a common psychiatric disorder that affects millions of people worldwide. Moringa Oleifera, a plant with potential anxiolytic properties,has been studied for its effects on anxiety While ,, the withdrawal effects of chronic Moringa oleifera use on anxiety have not been explored. This study aims to investigate the withdrawal effects of Moringa oleifera on anxiety in mice.

Previous reports states how Moringa oleifera exhibit anxiolytic signs, reducing anxiety-like behavior in mice (Kumar et al., 2016). However, withdrawal from chronic Moringa oleifera use may lead to rebound anxiety, as seen with other therapeutic drugs (Jasper L.E., 2016).

## COMPARISON OF OPEN AND CLOSE ARM DURATION IN EPM IN ALL EXPERIMENTAL GROUPS

### Normal Control vs. Positive Control

The mean difference of -9.800 seconds (p = .996) indicates duration inside close and open arm was not different for normal and positive control. This suggests that the anxiety levels between these two groups are not significantly different. Typically, increased time in the closed arms is indicative of higher anxiety.

### Normal Control vs. BIO + MORINGA - W

The mean difference of -25.800 seconds (p = .935) is also not significant, suggesting that withdrawal from Moringa did not significantly alter the anxiety-related behavior compared to the normal control. This may indicate that any anxiolytic effects of Moringa are not significantly reversed upon withdrawal.

### Normal Control vs. BIO + MORINGA + W

The mean difference of -20.800 seconds (p = .964) is not significant, indicating no substantial difference in anxiety levels between the normal control and the biologically stressed mice continuously treated with Moringa. This supports the idea that continuous Moringa treatment helps maintain anxiety levels similar to normal conditions.

### Positive Control vs. BIO + MORINGA - W

The mean difference of -16.000 seconds (p = .983) shows no significant difference in anxiety-related behavior between the positive control and mice withdrawn from Moringa. This implies that withdrawal from Moringa does not exacerbate anxiety in stressed mice.

### Positive Control vs. BIO + MORINGA + W

The mean difference of -11.000 seconds (p = .994) is also not significant, indicating no substantial difference between stressed mice and those treated continuously with Moringa. This suggests that Moringa treatment effectively mitigates stress-induced anxiety.

**BIO + MORINGA - W vs. BIO + MORINGA + W**

The mean difference of -5.000 seconds (p = .999) shows no significant difference between mice withdrawn from Moringaand those treated continuously with Moringa under stress conditions. This indicates that the anxiolytic effects of Moringamight persist even after withdrawal.

**COMPARISON OF TIME SPENT IN THE DARK AND LIGHT BOX FOR ALL EXPERIMENTAL GROUPS**

**Normal Control vs. Positive Control**

The mean difference of -69.200 seconds (p = .046) is significant, indicating that the positive control group (likely representing stressed mice without Moringa treatment) spent significantly more time in the dark box compared to the normal control group. This suggests higher anxiety levels in the positive control group, consistent with literature indicating that stress increases anxiety behaviors (Blackburn-Munro & Blackburn-Munro, 2001).

**Normal Control vs. BIO + MORINGA + W**

The mean difference of -13.200 seconds (p = .944) is not significant, indicating no substantial difference between the normal control group and the biologically stressed mice continuously treated with Moringa. This suggests that continuous Moringa treatment helps maintain anxiety levels similar to normal conditions, supporting its anxiolytic properties

**Normal Control vs. BIO + MORINGA - W**

The mean difference of -30.000 seconds (p = .600) is not significant, indicating no substantial difference between the normal control and the mice withdrawn from Moringa. This suggests that withdrawal from Moringa does not significantly increase anxiety levels compared to normal conditions, indicating potential residual anxiolytic effects.

**Positive Control vs. BIO + MORINGA + W**

The mean difference of 56.000 seconds (p = .128) is not significant, but it indicates that biologically stressed mice continuously treated with Moringa spent less time in the dark box compared to the positive control group. This suggests that Moringa treatment effectively reduces stress-induced anxiety.

**Positive Control vs. BIO + MORINGA - W**

The mean difference of 39.200 seconds (p = .382) is not significant, indicating no substantial difference between stressed mice and those withdrawn from Moringa. This implies that while there may be some reduction in anxiety, it is not as pronounced as continuous treatment.

**BIO + MORINGA + W vs. BIO + MORINGA - W**

The mean difference of -16.800 seconds (p = .893) is not significant, indicating no substantial difference between mice withdrawn from Moringa and those treated continuously under stress conditions. This suggests that the anxiolytic effects of Moringa might persist even after withdrawal, although to a lesser extent.

## COMPARISON OF ANXIETY BEHAVIOR IN ELEVATED PLUS MAZE AND DLB

Both measures indicate no significant difference between the Moringa-withdrawn and continuously treated groups, suggesting that the anxiolytic effects of Moringa might persist even after withdrawal. These results corroborate several studies reporting Moringa oleifera as possessing anxiolytic properties, meaning it may help reduce anxiety symptoms (Sulaiman *et al.* 2013).

## CONCLUSION

It appears Moringa oleifera may have benefit in effective management of anxiety in biologically stressed mice, both during continuous treatment and after its withdrawal.

**Recommendation:** If this can be extrapolated to humans**,** Moringa oleifera may be beneficial for continuous use in anxiety management, particularly in conditions involving biological stress. Gradual tapering and monitoring during withdrawal may help manage any potential residual anxiety, although current findings suggest minimal withdrawal effects and incorporating stress management.

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