EFFECT OF *CITRULLUS LANATUS* JUICE ON ANXIETY RELATED BEHAVIOUR AND OXIDATIVE STRESSED SPRAGUE DAWLEY RATS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY

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CERTIFICATION

This is to certify that this project work on "EFFECT OF *CITRULLUS LANATUS* JUICE ON ANXIETY RELATED BEHAVIOUR AND NEUTROPHIL TO LYMPHOCYTE RATIO IN OXIDATIVE STRESSED SPRAGUE DAWLEY RATS" was carried out by OSAGIE GIFT ESOSA with matriculation number BMS2102080; in partial fulfillment of the requirement of the Award of Bachelor of Science (B.Sc.) Degree in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State, Nigeria.

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DEDICATION

This work is Dedicated to God almighty and to my parents, whose unwavering support and encouragement have been my guiding light throughout this journey. Your belief in me has fueled my determination to pursue knowledge and excellence.

ACKNOWLEDGEMENTS

I extend my deepest gratitude to my supervisor, for her invaluable guidance, expertise, and patience throughout the process of conducting this project work. My sincere appreciation goes to the authors of the studies included in this project work, whose research has contributed significantly to the advancement of knowledge in the field.

I would also like to thank my project group members and colleagues for their support and encouragement during moments of doubt and fatigue.

TABLE OF CONTENTS

ABSTRACT

Anxiety stress, characterized by an imbalance between reactive oxygen species and antioxidant defenses, is a well-established pathophysiological factor implicated in the development and progression of anxiety disorders. The brain is particularly vulnerable to oxidative due to its high oxygen consumption and lipid rich content. Watermelon juice (Citrullus lanatus) is rich in antioxidants like Lycopene and L-Citrulline, offering a potential natural intervention. This study investigated the effects of watermelon juice on anxiety-related behavior in oxidative-stressed Sprague Dawley rats. Fifty-four rats were divided into four groups: a control, an oxidative stress-induced group, a group treated with Vitamin C, and a group receiving watermelon juice intervention. Oxidative stress was induced with phenyl hydrazine. Anxiety-like behavior was assessed using the Elevated Plus Maze (EPM). The results indicate that watermelon juice supplementation significantly reduced anxiety-like behaviors, demonstrating its potential as a functional dietary intervention for mitigating the effects of oxidative stress. Data were analyzed using Grad Pad Prism version 10, and results were expressed as mean ± standard error of mean (SEM), with $p \leq 0.05$ considered significant. The results showed that watermelon juice reduced anxiety

performance and reduced oxidative stress levels compared to the untreated induced rats.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Anxiety disorders are a major public health concern, characterized by excessive worry, fear, and physiological symptoms that significantly impair quality of life (Adwas *et al*., 2020; Ledford and Lockey, 2013). While historically considered purely neurological, the understanding of anxiety pathogenesis has evolved to incorporate strong neuro-immune and endocrine components (Barnes, 2008). A critical mechanistic link involves chronic psychological stress, which disrupts homeostasis, activating the Hypothalamic-Pituitary-Adrenal (HPA) axis and the sympathetic nervous system (SNS) (Murdoch and Lloyd, 2010). This activation leads to a state of systemic inflammation and a crucial imbalance in cellular metabolism known as oxidative stress (Holgate, 2012).

Oxidative Stress and the Central Nervous System (CNS)

Oxidative stress results when the production of Reactive Oxygen Species (ROS) overwhelms the body's intrinsic antioxidant capacity. The CNS, being lipid-rich and highly metabolically active, is exceptionally vulnerable to this damage, which causes cellular injury, alters neurotransmitter function, and contributes to the establishment of anxiety-like phenotypes (Wenzel, 2012). Therefore, interventions that can effectively restore the redox balance are highly promising for managing stress-related behavioral disorders.

WATERLEON JUICE AS A FUNCTIONAL FOOD INTERVENTION

Recent research interest has turned toward natural food sources possessing powerful antioxidant properties. Watermelon juice (Citrullus lanatus) is an ideal example, being rich in key bioactive compounds, specifically the powerful carotenoid antioxidant Lycopene and the amino acid L-Citrulline (Amelink *et al.*, 2013). Lycopene's demonstrated capacity to scavenge free radicals and prevent lipid peroxidation offers a direct mechanism to combat oxidative damage. Additionally, L-Citrulline acts as a precursor for Nitric Oxide (NO), which supports healthy vascular function and neurological signaling. Existing studies involving various natural antioxidants have demonstrated efficacy in mitigating stress-induced behavioral and biochemical alterations in rodents (Custovic, 2014).

This research employs the Sprague-Dawley rat model subjected to induced oxidative stress, providing a standardized and dependable framework for analyzing neurobehavioral and systemic pathology (Murray *et al*., 2006). The therapeutic effectiveness of watermelon juice will be determined by assessing changes in two complementary domains:

- **Behavioral Assessment:** Utilizing the Elevated Plus Maze (EPM) to measure anxiolytic activity (time spent in open arms).

While the fundamental anti-oxidant properties of watermelon components are recognized, the comprehensive, integrated effect of the whole juice on modulating anxiety-related behavior (EPM/OFT) within a targeted oxidative-stress model remains largely uninvestigated. This study is therefore critical to generating the scientific evidence needed to justify the use of watermelon juice as a functional dietary intervention for managing the dual pathological effects of stress-induced anxiety and inflammation.

1.1 JUSTIFICATION OF STUDY

Anxiety disorders are a major public health concern, often exacerbated by chronic psychological stress that triggers a state of systemic inflammation and oxidative damage. Investigating the efficacy of natural antioxidants in mitigating this dual pathology is crucial for developing non-pharmacological interventions. Watermelon juice (Citrullus lanatus) contains powerful bioactive compounds, notably Lycopene and the amino acid L-Citrulline, which are potent antioxidants and Nitric Oxide (NO) precursors shown to protect against oxidative stress. Given this evidence, it is hypothesized that watermelon juice supplementation could mitigate the adverse effects of stress-induced oxidative damage on both behavior and inflammatory status. Therefore, this study aims to investigate the anxiety-related behavioral changes induced by oxidative stress in Sprague Dawley rats, and to evaluate the potential protective role of watermelon juice against these changes.

1.2 AIM

The aim of this study is to investigate the effect of watermelon juice supplementation on mitigating anxiety-related behavior by oxidative stress in Sprague Dawley rats.

1.3RESEARCH QUESTION

1. What effect does watermelon juice consumption have on the anxiety levels of rats experiencing oxidative stress?

1.4 SPECIFIC OBJECTIVES

1. To quantify the anxiety-related behavior using the Elevated Plus Maze (EPM).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 WATER (CITRULLUS LANATUS) AND ITS BIOACTIVE PROFILE

Watermelon (Citrullus lanatus) is a highly valued global commodity, recognized for being largely composed of water (exceeding 90%), yet simultaneously containing a significant and diverse array of bioactive compounds (Sharma and Khokhar, 2020; Naz *et al*., 2020). The fruit is abundant with lycopene which is a type of carotenoid that is responsible for the red flesh color (Liu *et al*., 2010). The unique composition of watermelon, including its minerals, vitamins, and phytochemicals, reportedly has specific therapeutic and pharmacological significance (Banurek and Mahendran, 2011; Jiang *et al*., 2020; Nkoana *et al*., 2021; Ubbor and Akobundo, 2009; Zhao *et al*., 2021). Watermelon fruits are comprised of phytochemical compounds, such as cucurbitacin's and their glycoside derivatives which exhibit a peculiar medicinal significance in terms of potent biological activities, such as hepatoprotective, anti-inflammatory, anti-tumor, antimicrobial and anthelmintic effects (Biswas *et al*., 2017; Nkoana *et al*., 2021).

L-Citrulline and Cardiovascular Support

The non-essential amino acid L-citrulline is one of the most therapeutically relevant compounds found in watermelon, with the highest concentrations typically localized in the rind (Tarazona-Díaz *et al*., 2011; da Silva *et al*., 2022). Its significance stems from its role as a precursor to L-arginine in the kidneys. By raising plasma L-arginine levels, L-citrulline effectively boosts the synthesis of nitric oxide (NO), a molecule essential for regulating blood vessel dilation (Naz et al., 2020).

- **Vascular Health Benefits:** Clinical research, including a meta-analysis of randomized controlled trials, suggests that consuming L-citrulline from watermelon or supplements can lead to improved cardiovascular outcomes. Specifically, these interventions have been shown to enhance markers of vascular function, such as pulse wave velocity (PWV) and flow-mediated vasodilation (FMD) (Mulder *et al*., 2022).
- **Physical Performance:** Furthermore, L-citrulline's involvement in the ammonia detoxification cycle, along with the fruit's electrolyte content, is

thought to contribute to its traditional use in reducing muscle soreness and supporting post-exercise recovery (Ask Ayurveda, 2025).

Carotenoids: Lycopene and beta-Carotene

The vibrant red coloration typical of ripe watermelon flesh is a visual indicator of high levels of the powerful antioxidant carotenoid, lycopene (Naz *et al*., 2020).

Antioxidant Activity: Lycopene is recognized as a potent free radical scavenger, playing a crucial role in reducing oxidative stress and inflammation (Nadeem *et al*., 2022). Watermelon serves as a distinct dietary source of lycopene, comparable to tomatoes, and its intake has been associated with anti-inflammatory and potentially anti-cancer effects (Rimmaudo, 2021; Nadeem *et al*., 2022).

Vitamin A Precursor: The fruit also provides beta-carotene, a key precursor molecule that the body converts into Vitamin A, necessary for maintaining healthy vision and robust immune function.

OTHER BIOACTIVE COMPONENTS

In addition to the compounds above, the fruit contains other beneficial phytochemicals and nutrients:

Phenolic Compounds and cucurbitacin: The rind and seeds contain various polyphenolic compounds and triterpenoids like cucurbitacin, which have been studied for their mild anti-inflammatory and diuretic properties, aligning with certain traditional medicine applications (Sharma and Khokhar, 2020).

Essential Nutrients: Watermelon is an excellent source of Vitamin C (supporting the immune system) and key minerals such as Potassium and Magnesium, which are vital for nerve signaling, muscle function, and maintaining proper hydration (Sharma and Khokhar, 2020). The collective action of these diverse compounds confirms the significant therapeutic and nutritional value of Citrullus lanatus for health and wellness.

2.1.2 OXIDATIVE STRESS AND ITS ROLE IN ANXIETY

Oxidative stress, defined by an insufficient capacity to neutralize damaging reactive oxygen species (ROS) in relation to their production, is fundamentally involved in both the onset and perpetuation of anxiety states

(Salim, 2017). This biological imbalance inflicts damage directly onto brain components, mediating the progression of anxiety disorders.

ROLES IN ANXIETY PATHOLOGY

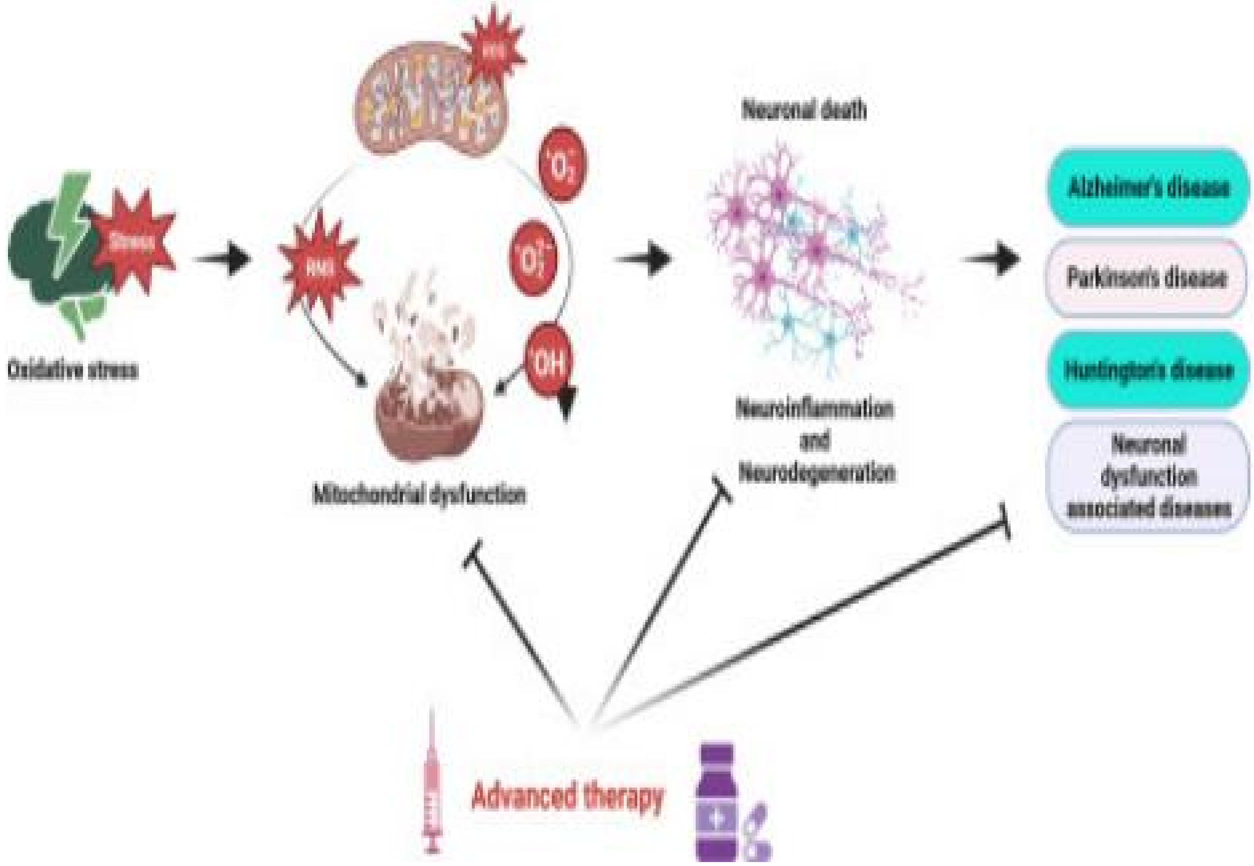
The primary ways oxidative stress contributes to anxiety are by damaging brain structures, altering neurochemical signals, and driving inflammation:

Drives Neuronal Vulnerability and Damage: Excess reactive oxygen species (ROS) compromise the integrity of cells in anxiety-related brain regions, such as the hippocampus and amygdala (Maes *et al*., 2011). This damage makes neurons more susceptible to apoptosis (cell death) and less capable of adapting to stress, thereby increasing vulnerability to anxiety.

Disrupts Emotional Regulation Circuits: The damage caused by oxidative stress impairs the function of neuronal membranes and receptors necessary for neuroplasticity and synaptic transmission (Salim, 2017). This disruption directly affects the circuits responsible for regulating fear, mood, and emotional responses, leading to an anxious state.

Perpetuates Neuroinflammation: Oxidative damage is a potent trigger for neuroinflammation, activating brain immune cells (glia) which then release pro-inflammatory molecules (Maes *et al*., 2021). This inflammation, in turn, generates more ROS, creating a vicious cycle that sustains the anxious and hyper-vigilant state characteristic of anxiety disorders.

Modulates Neurotransmitter Balance: The imbalance is implicated in the functional disruption of key neurotransmitter systems, including serotonin and GABA (gamma-aminobutyric acid). Alterations in these systems are directly correlated with symptoms of excessive worry, fear, and panic (Maes *et al*., 2011).



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2.1.4 BEHAVIOURAL ASSESSMENT OF ANXIETY-RELATED BEHAVIOUR

Behavioural assessment is an essential experimental approach for evaluating anxiety-related responses in animal models. It provides measurable indicators of emotional reactivity by exploring the balance between an animal's fear and its exploratory drive. These assessments are valuable because they mimic aspects of human anxiety, offering insights into its neurobiological and pharmacological mechanisms.

2.2 THE ELEVATED PLUS MAZE

The elevated plus-maze (EPM) test is one of the most commonly used behavioural assays to evaluate anxiety-related behavior in rodents (Carobrez and Bertoglio, 2005). The test is based on the natural aversion of rodents to open spaces and their natural spontaneous exploratory behaviour in novel environments (Lister, 1987). A novel test for the selective identification of anxiolytic and anxiogenic drug effects in the rat is described, using an elevated plus maze consisting of two open arms and two enclosed arms (Pellow et al., 1985).

Apparatus

The maze is elevated to a height of 50 cm above the floor and the arms are 30 cm long and 5 cm wide (Walf and Frye, 2007).
The open arms have a very small (0.5 cm) wall to decrease the number of falls, whereas the closed arms have a high (16 cm) wall to enclose the arm (Ohara *et al*.,).

The EPM is validated for rats (Pellow et al., 1985) and mice (Lister, 1987) (Carobrez and Bertoglio, 2005).

Diagram **Description**:
A plus shaped apparatus with two open arms (no walls) and two closed arms (with walls), joined by a central platform. The maze is elevated approximately 50 cm above the floor.

Procedure

The animal is placed on the central platform facing one of the enclosed arms and is allowed to explore the maze for a 5-minute period (Leo and Pamplona, 2014). The number of entries and the time spent in the open and closed arms are recorded as measures of anxiety-related behavior (Komada et al., 2008). The duration and frequency of entries into open and closed arms is recorded (Leo and Pamplona, 2014,).

Behavioural Parameters Measured

Anxiety is measured by the ratio of entries and time spent in the open arms compared with the enclosed arms (Lister, 1987). Anxiolytic drugs increase open arm exploration, whereas anxiogenic drugs reduce it (Pellow *et al*., 1985). The number of entries into the closed arms is used as an index of general activity (Pellow *et al*., 1985)

Interpretation

Rodents naturally avoid open arms, and a higher percentage of open arm exploration is interpreted as a reduction of anxiety (Walf and Frye, 2007, p. 323). The EPM is sensitive to the effects of both anxiolytic and anxiogenic agents (Lister, 1987).

Applications

The elevated plus-maze has been extensively used to investigate the effects of anxiolytic and anxiogenic compounds (Carobrez and Bertoglio, 2005). This model has also been used to examine the impact of stress, hormones, and genetic manipulation on anxiety-like behaviour (Carobrez and Bertoglio, 2005). The test can also be used to assess the influence of brain lesions or neurotransmitter changes on anxiety responses (Lister, 1987).

THE ELEVATED PLUS MAZE

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 RESERCH MATERIALS

a. Rat cage

b. Phenyl hydrazine (PHZ) for oxidative stress induction

c. Fruit samples: Citrullus lanatus

d. Vitamin c (Ascorbic acid)

e. Hand gloves

f. Distilled water

g. Masking tapes

h. Test tubes

i. Chloroform

j. weighing scale feed

k. Dissecting instruments

l. Syringes and needles

m. 52 male Sprague Dawley rats

3.2 STUDY DESIGN

This study is an experimental, randomized, controlled animal study designed to investigate the effects of fresh watermelon (Citrullus lanatus) in male Sprague Dawley rats under phenyl hydrazine induced oxidative stress.

3.3 EXPERIMENTAL ANIMALS

A total of 52 adult male Sprague Dawley rats, weighing 150–200 g, were obtained from a certified laboratory animal supplier. The animals were allowed to acclimatize for a period of two (2) weeks with free access to food and water before the commencement of the experiment and thereafter they were placed in plastic cages that were partitioned, having both the vaporing chamber and experimental chambers.

3.3 1 INCLUSION CRITERIA

- Healthy Sprague Dawley rats
- Body weight within the range of 150-200g

3.3 2 EXCLUSION CRITERIA

- Rats with congenital disorders or pre existing diseases
- Rats exhibiting signs of distress during acclimatization

3.4 EXPERIMENTAL GROUPS

Rats were randomly assigned to four groups. Group A served as a control group while group B, C, and D served as experimental group.

- Group B: Rats induced with oxidative stress and distilled water only
- Group C: stress induced rats receiving 1ml of vitamin c (ascorbic acid)
- Group D: stress induced rats were treated with 1ml per 100g of *Citrullus lanatus*

3.5 PHENYLHYDRAZINE (PHZ) INDUCTION

Phenyl hydrazine is a hemolytic agent that induces oxidative stress and anemia via erythrocyte membrane damage and free radical generation (Olayinka *et al*., 2021). Phenyl hydrazine was administered intraperitoneally once daily for 21days to the relevant groups.

3.6 PREPARATION AND ADMINISTRATION OF WATER MELON (CITRULLUS LANATUS) JUICE

Fresh ripe *Citrullus lanatus* were washed thoroughly with distilled water, the rind was removed, and the edible portion which is the pulp was cut into smaller pieces. The juice was obtained by squeezing the pulp using a juice extractor. The resulting extract was filtered through a clean muslin cloth to remove coarse particles and the filtrate was stored in an airtight container under refrigeration until required for administration.

3.7 BEHAVIOURAL ASSESSMENT

Behavioral assessments were performed weekly to monitor cognitive function and anxiety related behaviors. Tests included:

1. Elevated Plus Maze (EPM) – Assessed anxiety-like behavior; time spent in open vs. closed arms recorded.

3.11 STATISTICAL ANALYSIS

The data from the experiments were expressed as mean ± Standard Error of Mean (SEM). One-way analysis of variance (ANOVA) was used to analyze differences between various groups, followed by Tukey's test with GraphPad Prism 8.1 software (GraphPad, San Diego, CA). P-values ≤ 0.05 were considered statistically significant.

3.13 ETHICAL CONSIDERATIONS

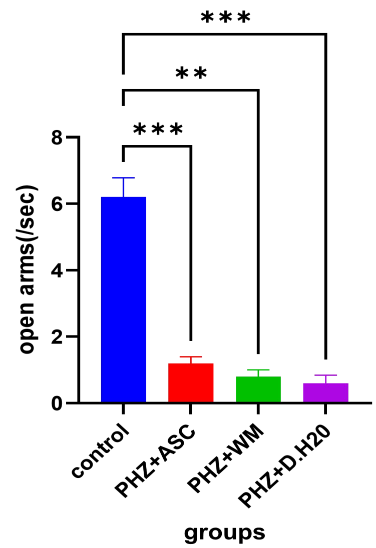
All procedures followed the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Efforts were made to minimize pain, stress, and number of animals used, and all interventions were approved by the Institutional Animal Care and Use Committee (IACUC).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 RESULT

OPEN ARMS **TIME SPENT ON OPEN**



ARM **WEEK 1**

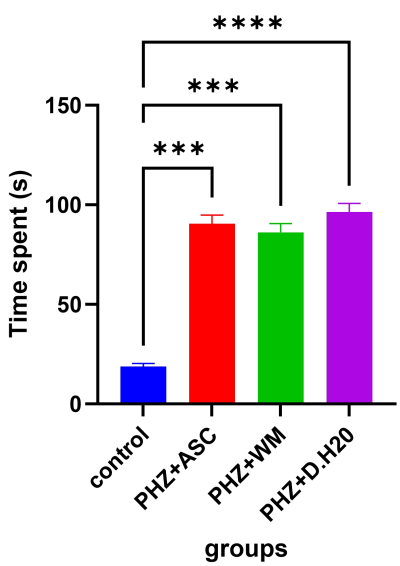
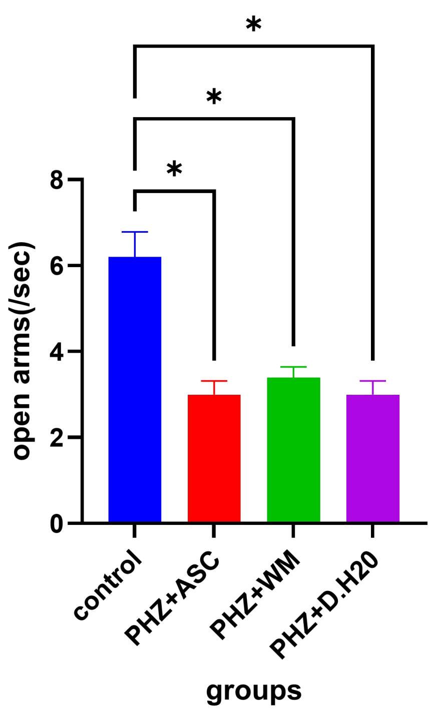
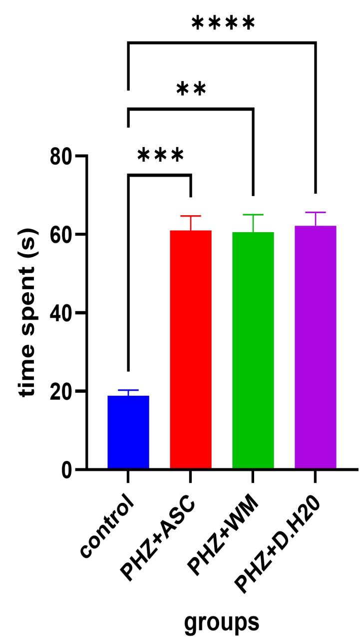


Fig. 4.1: Showing the number of entries and time spent on the open arms of the elevated plus maze by different experimental groups during Week 1.

The result shows a statistically significant difference in the number of entries and time spent on the open arms of the elevated plus maze for all oxidative-stressed and treated groups compared to the control group in Week 1 ($p>0.05$).

OPEN ARMS **TIME SPENT ON OPEN ARM**





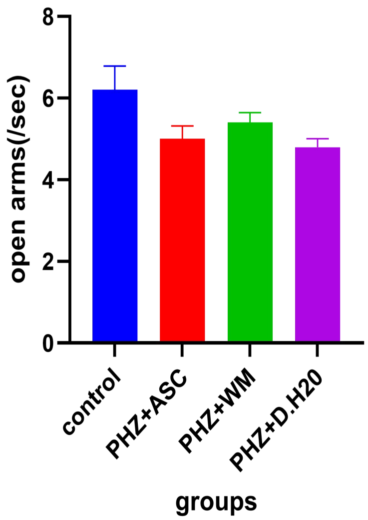
WEEK 2

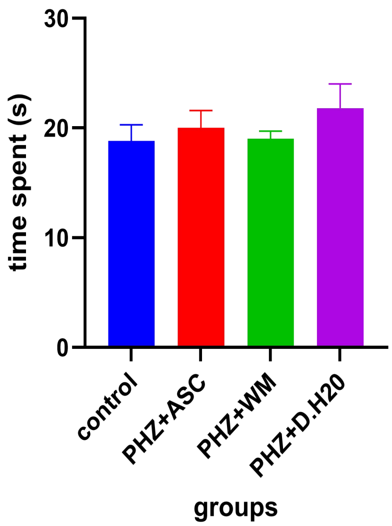
Fig. 4.2: Showing the number of entries and time spent on the open arms of the elevated plus maze by different experimental groups during Week 2.

The result shows a statistically significant increase in the number of entries and time spent on the open arms of the elevated plus maze for all treated groups compared to the control group in Week 2 ($p>0.05$).

OPEN ARMS **TIME SPENT ON OPEN**

ARM



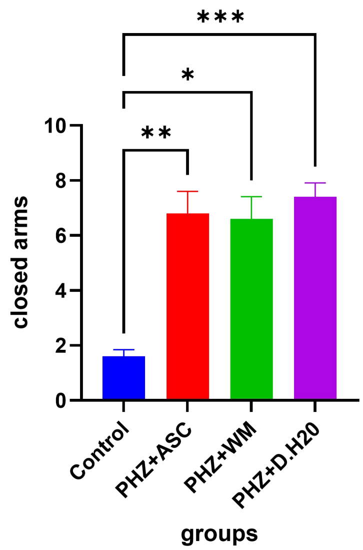


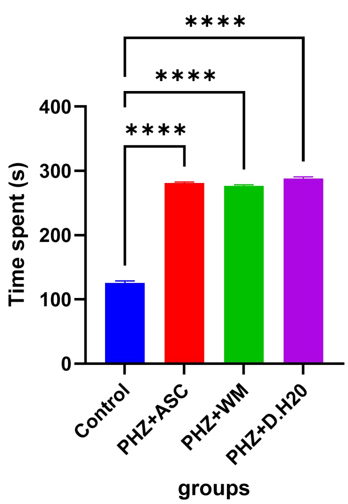
WEEK 3

Fig. 4.3: Showing the number of entries and time spent on the open arms of the elevated plus maze by different experimental groups during Week 3.

The result shows no statistically significant difference in the number of entries and time spent on the open arms of the elevated plus maze for all experimental groups compared to the control group during Week 3

CLOSED ARM **TIME SPENT ON CLOSED ARM**



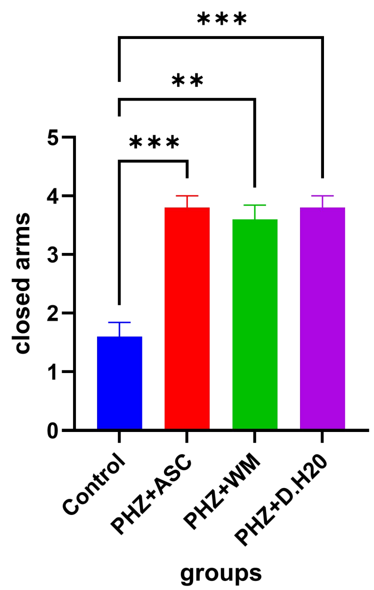


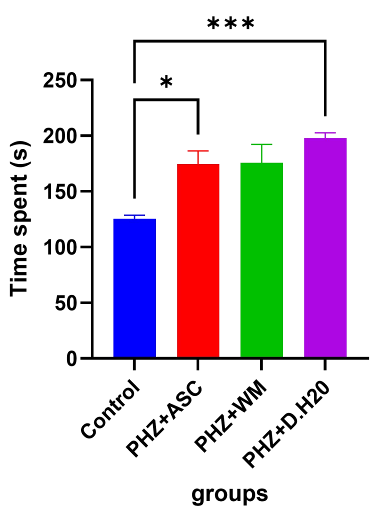
WEEK 1

Fig. 4.4: Showing the number of entries and time spent on the closed arms of the elevated plus maze by different experimental groups during Week 1.

The result shows a statistically significant increase in the number of entries and time spent on the closed arms of the elevated plus maze for all experimental groups compared to the control group during Week 1 ($p>0.05$).

CLOSED ARM **TIME SPENT ON CLOSED ARM**



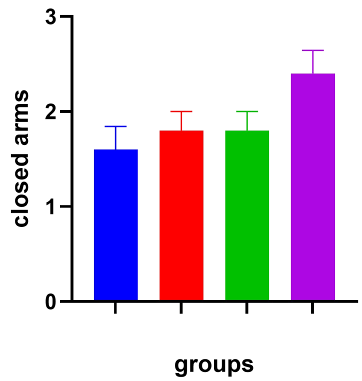


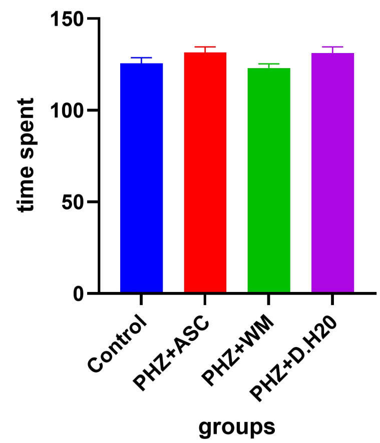
WEEK 2

Fig. 4.5: Showing the number of entries and time spent on the closed arms of the elevated plus maze by different experimental groups during Week 2.

The result shows a statistically significant decrease in the number of entries and time spent on the closed arms of the elevated plus maze for all experimental groups compared to the control group during in Week 2 ($p<0.05$).

CLOSED ARM **TIME SPENT ON CLOSED ARM**





WEEK 3

Fig. 4.6: Showing the number of entries and time spent on the closed arms of the elevated plus maze by different experimental groups during Week 3.

The result shows no statistically significant difference in the number of entries and time spent on the closed arms of the elevated plus maze for all experimental groups compared to the control group during Week 3

CHAPTER 5

DISCUSSION

The primary objective of this study was to evaluate the effect of Citrullus lanatus (watermelon) juice on anxiety-related behaviour in oxidative-stressed Sprague Dawley rats. The findings from the Elevated Plus Maze (EPM) test provide compelling evidence that watermelon juice supplementation possesses significant anxiolytic properties.

The results clearly demonstrate that induction of oxidative stress with phenyl hydrazine (PHZ) successfully produced anxiety-like behaviour in the model, as seen in the group that received no treatment post-induction. This group exhibited a strong preference for the closed arms of the maze, a classic behavioural indicator of anxiety in rodents (Pellow *et al*., 1985; Walf and Frye, 2007). This outcome aligns with established literature which posits that oxidative damage in the brain, particularly in emotion-regulating regions like the hippocampus and amygdala, disrupts neurotransmitter systems and promotes a state of hyper-vigilance and fear, manifesting as anxiety (Salim, 2017; Maes *et al*., 2011).

Crucially, the administration of watermelon juice significantly counteracted these anxiety-like effects. The watermelon-treated group showed a marked increase in the number of entries and time spent in the open arms of the EPM compared to the untreated oxidative-stressed group. This behavioural shift towards increased open-arm exploration is a validated measure of reduced anxiety and is indicative of an anxiolytic effect. The efficacy of watermelon juice was notably comparable to the positive control group treated with Vitamin C, a well-established antioxidant. This parallel suggests that the mechanism of action for watermelon juice is likely rooted in its potent antioxidant capacity.

The observed anxiolytic effect can be directly attributed to the rich bioactive profile of Citrullus lanatus. Watermelon juice is a significant source of lycopene, a powerful carotenoid antioxidant, and L-citrulline, a precursor to L-arginine and the vasodilator nitric oxide (NO) (Naz *et al*., 2020; Tarazona-Díaz *et al*., 2011). Phenyl hydrazine-induced oxidative stress generates an excess of reactive oxygen species (ROS), leading to lipid peroxidation and cellular damage in the brain.

The lycopene in watermelon juice likely functions as a potent free radical scavenger, mitigating this oxidative damage and protecting neuronal integrity. Concurrently, L-citrulline, by enhancing nitric oxide production, may improve cerebral blood flow and modulate neuroinflammation, thereby contributing to the normalization of emotional and behavioural responses (Mulder *et al*., 2022). The synergistic action of these compounds appears to restore the redox balance and interrupt the cycle of oxidative stress and neuroinflammation that underpins anxiety pathology.

CONCLUSION

In conclusion, the behavioural data from this study strongly support the hypothesis that watermelon juice supplementation can alleviate anxiety-related behaviour in a rat model of oxidative stress. Its effect is on par with a conventional antioxidant, highlighting its potential as a powerful natural intervention.

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