Object Recognition Memory and Anti-Anxiety Potentials of Stem-Bark Extract of *Nauclea latifolia* (African peach), Taurine and Vitamin E on Albino Rats Exposed to Water Immobilisation Stress

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Abstract
Memory loss is a typical symptom of generalized anxiety disorder, a condition that affects millions of individuals. Memory loss with anxiety can be quite distressing, but if you can find strategies to address the anxiety, your memory may improve as well. In this study, the effects of *Nauclea latifolia* extract, taurine and vitamin E supplementation on object identification and stress-induced anxiety in Albino rats exposed to water immobilisation stress were investigated. 24 rats weighing between 100 and 120 grams were employed. The control group (A), received 1ml/kg pure water. Vitamin E was given to Group B at a dosage of 0.2 ml/kg. Group C received 200mg/kg taurine, while Group D received 200mg/kg *Nauclea latifolia* extract. For three weeks, the administration was completed. The rats were famished for 24 hours before the stress process began. The rats were sedated with chloroform vapour, and then restrained using a hardwood board (25 by 18 cm) with the four limbs fixed sideways, then dipped for two hours in water up to the xiphoid level. During the training phase, the taurine group examined the novel object the most (64.9 percent), followed by the Vitamin E group (63.90 percent), when compared to the *Nauclea latifolia* (52.75 percent) and the control groups (54.76 percent). The consolidation phase similarly, revealed that, taurine and Vitamin E groups explored the novel object the most (71.52 percent) and (70.05 percent) accordingly. In the anxiety model, *Nauclea latifolia*, taurine and vitamin E groups showed significant increase in time spent on closed arms. This study has shown that, when rats are treated to water immersion restraint stress, administration of *Nauclea latifolia* extract, taurine, and vitamin E, may decrease stress-induced anxiety-like behaviours.

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The significance of this study is that, administration of taurine, vitamin E and Nauclea latifolia extract may prevent stress-induced memory impairment.

**Keywords:** Taurine, Nauclea latifolia, Anxiety, Vitamin E, Stress.

**INTRODUCTION**

When people are exposed to some acute stressors, they have an immediate behavioural and physiological response that lasts for days (Chaoui et al., 2022). Stress has been shown to influence learning and recall depending on the learning paradigm, the animal’s sex, and their stress sensitivity (Schaack et al., 2021). It can also impair cognitive performance in both humans and animals. Nevertheless, the literature on how severe stress impacts the distinct phases of memory: acquisition, consolidation, and retrieval tend to be inconsistent (Nelissen et al., 2018). In humans and rodents, stress can alter sensory perception and cause analgesia (Aghajani et al., 2012). Acute restraint stress (ARS) is a well-known model that has been linked to a number of behavioural and neurochemical changes. Acute restraint stress affects catecholamine activity in the brain, like norepinephrine and dopamine, as well as stimulating the hypothalamic-pituitary-adrenocortical axis (HPA) (de Kloet et al., 2005).

Stress-induced reflexes, according to study, boost signaling molecules like ERK1/2, notably in the cerebral cortex (PFC) and hippocampus (Shen et al., 2004). Stress alters gene transcription and neural activity, which influences neuronal plasticity (Buyntsky and Mostofsky, 2009; Joels et al., 2013). These cumulative changes have the potential to influence brain functioning, particularly cognitive processes including novel object recognition, retrieval of long-term memory, as well as reversal learning (Tai et al., 2013). According to another study, stress before acquisition and recollection alters these mechanism by relying mostly on the learning approach, the animal's gender, and their stress responsiveness (Schaack et al., 2021). Anxiety is a feeling of increased alertness accompanied by an increase in average sensory sensitivity as a result of ambiguity or disagreement (Grupe and Nitschke, 2013). Stress has already been proven to impair learning ability, particularly working memory (Maloney et al., 2014). Anxiety and cognitive function are linked in both directions, and as a result of cognitive impairment; there is an increased risk of stress (Petkus et al., 2017). Aside from the direct effects of stress hormones on nervous system function, stress-related changes such as anxiety affect motor performance and efficiency (Lepicard et al., 2000). Anxiety disorders are the most prevalent psychiatric disease conditions, according to studies. As a result, developing animal models of anxiety is critical for detecting possible anxiolytic medicines.

The information available, as well as the object shape and/or perspectives play a big role in recognition. According to research, the rat's spontaneous exploratory activity can be used to get a reliable assessment of memory performance (Ennaceur and Aggleton, 1994). For measuring exploratory activity in rodents, the “Object Recognition Task” (ORT) has long been the measure of choice. The recognition memory of mice and rats is measured by testing the animal's capacity to remember an object that has been previously presented to it. Prior stress history can reduce or raise the amount of a number of physiological reactions elicited by stress. Physiological reactions to stresses are typically non-specific, despite the fact that stressors differ extensively. The degree of stimulation is determined by the nature of the disruption to homeostasis, as well as the animal's assessment of the stressor and its capacity to deal with it (Goldstein, 2001). To evaluate an animal's stressor reaction rates, assessing metrics that indicate the activity of the hypothalamo-pituitary axis (HPA) is often used, together with observation of behavioural changes, particularly in the context of emotionality.
When fear is intense, the physiological reaction is good, but it can be overridden by excessive free-radical generation, which can have negative health consequences. Prolonged stress can lead to the activation of the limbic-hypothalamic-pituitary-adrenal axis as well as the releasing of stress hormones in the brain over time. Exposure to a psychological/emotional stressor might activate stress-related behavioural actions and haematological reactions for a long time. Psychological stress, operating alone or in conjunction with other factors, has been proven in human and domestic animal experiments to have deleterious behavioural and/or haematological consequences due to higher free-radical activation. The stress reaction also has an effect on the brain, influencing specific cognitive functions and predisposing certain types of behaviour, resulting in anxiety and physiological responses. Stress has been linked to an increased risk of cardiovascular disease, metabolic irregularities, and mental disorders, among other ailments (Abraham et al., 2007).

Taurine (2-aminoethane sulphonic acid) is a cysteine-derived amino acid that is found in the brain, eye, heart, and muscles, as well as the cerebral cortex, basal ganglia, hippocampus, hypothalamus, cerebellum, and the caudate-putamen (El-idrissi, 2006). It is found in a lot of animal tissues and is a prevalent amino acid in mammals. It has various important functions in the body, including calcium signalling, modulation, osmoregulation, membrane stabilization, and anti-stress (Ezekiel et al., 2016).

Vitamin E is a fat-soluble vitamin that prevents superoxide radicals from forming during the oxidation of fats. Tocopherols, which have a 6-chromanol ring as well as an isoprenoid subunit, make up the majority of vitamin E. Members from each family are labelled alpha, beta, gamma, and delta based on the number of methyl covalently bonded to the nucleus. By interacting with lipid radicals generated in the lipid peroxidation chain reaction via the glutathione peroxidase route, it shields cell membranes against oxidation. Tocopherol is necessary for the removal of free radical intermediates and the interruption of the oxidation cycle (Traber and Atkinson, 2007). Vitamin E appears to have an important role as a non-specific chain-breaking scavenger that prevents the spread of free radicals. As a peroxyl radical scavenger, it maintains polyunsaturated fatty acids (PUFAs) inside membrane phospholipids and plasma lipoproteins.

The plant species Nauclea latifolia is a member of the Rubiaceae family. It's a multi-stemmed tree that can grow up to 200 meters tall. In English, it's reportedly known as the pin cushion tree or African peach, and in Nigeria, it's known as opepe (Traore-Keita et al., 2000; Udobi and Umoh 2017). The Itsekiris of Edo state, Nigeria's south-central region, call this plant itu. Among the Ibibios and Effik, it is called mbomibong, while in Hausa; the plant is referred to as tafashiya. This shrub can be found in abundance in tropical Africa and Asia, and it is commonly used in traditional medicine to treat diabetes mellitus.

The plant’s use in the majority of the circumstances below has been scientifically examined and validated in studies involving various portions of the plant. For example, investigations using diverse laboratory models have revealed cardiovascular, spasmylytic, anti-plasmodial, and anti-parasitic benefits (Morah, 1995; Marshall et al., 2000). In the roots, phytochemical analysis revealed the presence of monoterpene, triterpene, indole alkaloid, saponins, and traces of inorganic substances (Hussain and Deeni, 1991; Abreu and Pereira, 2001). Previous research has suggested that Nauclea latifolia may have sedative properties (Amos et al., 2005). The reported use of plant extracts for the treatment of malaria raises the essential...
question as to whether the extract's therapeutic benefits are attributable to its anti-plasmodial action, or their ability to alleviate malaria symptoms, or both. However, to the best of our knowledge, the plant has not been studied for memory enhancement and anti-stress activities. This study was therefore carried out to investigate the probable activities of the plant compared with taurine and vitamin E in the treatment of memory deficit due to stress and, in addition, contribute to the diverse medicinal applications of *Nauclea latifolia*.

**MATERIALS AND METHOD**

**Extract Preparation**
The extract was made by collecting fresh *Nauclea latifolia* (Stem-Bark) and air drying it at room temperature before pulverizing using a pestle and mortar. 500 mL distilled water was used to macerate 100 g of *Nauclea latifolia* powder. The extract was concentrated and evaporated to dryness in a water bath at a temperature of 60 °C. A brownish mass of 30 g was obtained as the final yield. The percentage yield of the extract was 30 %.

\[
\text{Percentage Yield} = \frac{\text{weight of final extract}}{\text{Soaked sample material}} \times 100
\]

**Chemicals and Reagents**
Three different treatments were administered to the rats as follows:
*Nauclea latifolia* (200mg/kg), Taurine (200mg/kg) and vitamin E (0.2ml/kg) were administered to the rats. Vitamin E: 1000IU (667mg) and Taurine (CAS No. 107-35-7; purity > 99 percent) analytical grade (100-Sigma-Aldrich USA) preparations were reconstituted to a 40 percent stock solution in distilled water.

**Experimental Animals**
The National Veterinary Research Institute (NVRI) in Jos, Plateau State, Nigeria, provided 24 Wistar rats weighing 100 to 120 grams for this study, which were housed in the Animal House at the Department of Biochemistry at Federal University Wukari in Taraba State, Nigeria. The rats were provided rat chow and water ad libitum for 14 days as they acclimatized.

**Experimental Design**
In this investigation, twenty-four (24) Wistar rats were employed. The rats were divided into four groups, of six animals each. Group A was given 1 ml/kg distilled water and served as control, Group B was administered 0.2 ml/kg of vitamin E, Group C was administered 200 mg/kg of taurine while Group D was administered 200 mg/kg of *Nauclea latifolia* (stem-bark) extract for three weeks via oral gavage.

**Stress Test**
The animals were stressed after the three weeks treatment. The rats were fasted for 24 hours before the stress test. The rats were minimally anesthetized with chloroform inhalation before having their four limbs immobilized on a wooden plate (25 cm by 18cm) with the forelimbs fixed horizontally and the hind limbs stretched downward. The rats were placed on wooden plates and then submerged vertically (head up) in water to the level of the xiphoid process in a 35.5 °C water bath (Shu *et al.*, 2012). After being restrained for two hours, the animals were examined for anxiety-like behaviours and their ability to distinguish novel items.
Novel Object Recognition Task (NORT)
There are two components to this test: a training phase (acquisition) and a technique test phase (consolidation). The animal was re-placed in the arena during the test phase and provided with two objects in just the same positions: one (A1) that was utilized during the training phase and the other a unique object (B). The animals were placed in the centre of the arena, facing the opposing walls, and were presented with two similar objects (A1 and A2) at the edge, separated by a defined distance (15 cm from each neighbouring wall), for a set period of 5 minutes. The length of time the animal spent investigating each object was recorded and the rat then restored to its original location. To examine long-term memory, the test phase is repeated 24 hours following the training phase. The placements of the test objects and the objects used as novel or familiar were counterbalanced between the animals. The time spent in the training phase investigating each object A1 and A2, as well as the time spent in the testing phase exploring each object B and A2, were both analysed (Ennaceur and Delacour, 1988).

Elevated Plus Maze
Elevated plus maze (EPM) is a technique used in laboratories to measure anxiety in animals. It's a frequent screening test for prospective anxiolytic or anxiogenic medicines in rats, as well as a major research tool in neurological anxiety research. The model is based on the ability to test an animal's intolerance of open spaces and tendency to be thigmotaxis. The animal trapped inside the closed arm conveys its dissatisfaction by spending less time in the open arm (File et al., 1984). This animal model of anxiety is arguably the most commonly used in modern practice. The raised plus-maze was constructed with two opposite open arms (50 X 10 cm) intersected by two closed arms of the same dimension with walls 40 cm high. To construct the shape of a plus sign, the arms were linked with 10 x 10 centimetre middle square. The apparatus was elevated to a height of 50 cm above the ground. After naïve rats were presented separately in the maze's centre, facing enclosed arms, the time spent on the open and closed arms was observed for the next 5 minutes. For an arm entrance, all four feet must be in the arm. The number of entries as well as time spent in the open versus closed arms was tallied. The gadget was cleaned after each trial.

Statistical Analysis
Data were represented as mean for tables 1 and 2 while for tables 3 and 4 as Mean ± SEM and analysed using one-way analysis of variance (ANOVA) with multiple comparisons using the Statistical Package for Social Science program (version 23). To establish the difference between groups, the Turkey's post-hoc test was used. Values of P<0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Results
When compared to the control (56.40 percent) and Nauclea latifolia (54.80 percent) groups, the taurine group explored the object provided to them better (66.30%), followed by the Vitamin E group (64.20%) throughout the object recognition model's training phase. The Nauclea latifolia group had the lowest percentage of animals exploring a new object (Table 1).
Object Recognition Memory and Anti-Anxiety Potentials of Stem-Bark Extract of *Nauclea latifolia* (African peach), Taurine and Vitamin E on Albino Rats Exposed to Water Immobilisation Stress

Table 1: Time Spent in Seconds by the Rats in Exploring Objects A1 and A2 (Trial Phase, Day 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>58.79</td>
<td>57.01</td>
<td>69.21</td>
<td>61.72</td>
</tr>
<tr>
<td></td>
<td>56.23</td>
<td>68.20</td>
<td>64.17</td>
<td>31.10</td>
</tr>
<tr>
<td></td>
<td>58.67</td>
<td>69.12</td>
<td>55.00</td>
<td>63.22</td>
</tr>
<tr>
<td></td>
<td>66.49</td>
<td>60.04</td>
<td>67.15</td>
<td>50.00</td>
</tr>
<tr>
<td></td>
<td>23.57</td>
<td>65.12</td>
<td>69.11</td>
<td>57.73</td>
</tr>
<tr>
<td>Mean</td>
<td>54.76</td>
<td>63.90</td>
<td>64.92</td>
<td>52.75</td>
</tr>
</tbody>
</table>

Note: n = 6. Group A (control 1 ml/kg distilled water), Group B (0.2ml/kg vitamin E), Group C (200mg/kg taurine), Group D (200mg/kg *Nauclea latifolia*)

During the consolidation phase, the taurine + WIS and vitamin E groups examined the novel object provided to them more (74.50 seconds) and (71.00 seconds) respectively when compared to the control and *Nauclea latifolia* groups. The *Nauclea latifolia* group had the lowest proportion (62.00 seconds) of the animals exploring the novel object (Table 2).

Table 2: Time Spent in seconds by the Rats in Exploring Objects A1 and B (Consolidation Phase, Day 2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>52.32</td>
<td>74.00</td>
<td>60.15</td>
<td>60.15</td>
</tr>
<tr>
<td></td>
<td>59.41</td>
<td>75.32</td>
<td>79.24</td>
<td>69.10</td>
</tr>
<tr>
<td></td>
<td>67.03</td>
<td>66.10</td>
<td>77.00</td>
<td>67.12</td>
</tr>
<tr>
<td></td>
<td>73.40</td>
<td>72.45</td>
<td>75.20</td>
<td>59.22</td>
</tr>
<tr>
<td></td>
<td>68.10</td>
<td>62.40</td>
<td>66.00</td>
<td>56.15</td>
</tr>
<tr>
<td>Mean</td>
<td>64.05</td>
<td>70.05</td>
<td>71.52</td>
<td>63.94</td>
</tr>
</tbody>
</table>

Note: n = 6. Group A (control 1 ml/kg distilled water), Group B (0.2ml/kg vitamin E), Group C (200mg/kg taurine), Group D (200mg/kg *Nauclea latifolia*)

In the anxiety model, vitamin E, taurine, and *Nauclea latifolia* significantly increased time spent on closed arms while decreased time spent on open arms. The taurine and Vitamin E groups significantly enhanced the amount of entries on the open arms compared to the control group (Table 3). The number of entry and duration spent in the open versus closed arms in the Vitamin E, taurine, and *Nauclea latifolia* groups was considered significant when comparing to the control group (Table 4).

Table 3: Number of Entries and Duration (seconds) Recorded by Rats on both Open and Closed Arms

<table>
<thead>
<tr>
<th>Treatments</th>
<th>The Number of Entries</th>
<th>The Time Recorded (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open arms</td>
<td>Closed Arms</td>
</tr>
<tr>
<td>Group A</td>
<td>4.66 ± 0.24</td>
<td>8.00 ± 0.30</td>
</tr>
<tr>
<td>Group B</td>
<td>7.22 ± 0.31</td>
<td>6.33 ± 0.21</td>
</tr>
<tr>
<td>Group C</td>
<td>4.00 ± 0.42</td>
<td>4.00 ± 1.05</td>
</tr>
<tr>
<td>Group D</td>
<td>4.60 ± 0.31</td>
<td>6.80 ± 0.11</td>
</tr>
</tbody>
</table>

Note: values are mean ± SEM, n = 6. Group A (control 1 ml/kg distilled water), Group B (0.2ml/kg vitamin E), Group C (200mg/kg taurine), Group D (200mg/kg *Nauclea latifolia*) a, b = differences between values with different superscripts are significant (P 0.05).
Table 4: Ratio of the Number of Entries in the Open and Closed Arms with the Ratio of Time (seconds) Spent in Open versus Closed Arms

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ratio of Number of Entries in the Open Arms VS closed Arms</th>
<th>Ratio of Time Spent in the Open Arms VS Closed Arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>0.58 ± 23</td>
<td>0.18 ± 30</td>
</tr>
<tr>
<td>Group B</td>
<td>1.14 ± 04</td>
<td>0.41 ± 27</td>
</tr>
<tr>
<td>Group C</td>
<td>1.0 ± 43</td>
<td>0.44 ± 01</td>
</tr>
<tr>
<td>Group D</td>
<td>0.68 ± 19</td>
<td>0.39 ± 20</td>
</tr>
</tbody>
</table>

Note: values are mean ± SEM, n = 6. Group A (control 1 ml/kg distilled water), Group B (0.2ml/kg vitamin E), Group C (200mg/kg taurine), Group D (200mg/kg *Nauclea latifolia*) a, b = differences between values with different superscripts are significant (P < 0.05).

DISCUSSION

This study looked at the effects of *Nauclea latifolia* (stem back) extract, taurine, and Vitamin E on object recognition and stress-induced anxiety in Wistar rats exposed to water immobilisation stress. Object identification has been described as one of the primary qualities used to test for cognitive processes in rats. According to the findings of this study, taurine and vitamin E improved memory retention on object recognition model. Results obtained from this research backed up Baydas *et al.* (2003), Kucukatay *et al.* (2007), and Shahidi and Zhong (2015) who reported that taurine plus vitamin E increased memory recall. Another study discovered that *Nauclea latifolia* stem bark extract had substantial antioxidative effects against valproate acid-induced neurotoxicity. Their findings imply that *Nauclea latifolia* stem bark extract may play a neuroprotective role by reversing sodium valproate neurotoxicity (Legbosi and Ibor, 2019). The aforementioned conclusion is comparable to ours, however ours is solely a protective study in which we discovered that rats administered *Nauclea latifolia* stem bark extract retained more than 60% of their memories when compared to the control group. During the object recognition model’s training phase, the taurine group spent more time studying the object supplied to them (66.30 percent). This is similar to another study that was carried out by Ezekiel *et al.* (2018), who discovered that, rats that were administered taurine explored the object introduced to them more throughout the training phase of acetaminophen-induced oxidative stress in male Wistar rats. Rats' greater exploration during the consolidation phase suggests that they have learned and retained what they have learned. When compared to control rats, taurine, Vitamin E, and *Nauclea latifolia* administration significantly increased the time spent by the rats on the closed arms while reducing the time spent on the open arms in the anxiety model. The number of entries on the open arms increased significantly in the taurine and Vitamin E groups. In the taurine, Vitamin E, and *Nauclea latifolia* groups, the ratio of entrances and time spent in the open arm versus the closed arm was significant high. This finding is similar to the findings by Kong *et al.* (2006), who discovered that taurine reduced anxiety-like behaviours in Wistar rats. Anxiety-prone mouse strains also show more impairment in a skilled motor task than mice that were less emotional, according to another study (Lepicard *et al.*, 2000). The rats spent considerably more time in open arms than in closed arms in this study, demonstrating that taurine, Vitamin E, and *Nauclea latifolia* administration reduced anxiety in the rats. Another study found that dietary vitamin E insufficiency tends to increase anxiety-related behaviour in rats under the stress of isolation and loneliness. The elevated plus maze test revealed that vitamin E deficient rats spent less time in open arms and had more stretch out posture than control rats, implying that dietary vitamin E deficiency increased anxiety (Okura *et al.*, 2009). The plant *Nauclea latifolia* was found to lessen fear and anxiety in mice while simultaneously increasing their motility in another study (Edagha *et al.*, 2015). A decoction of *Nauclea latifolia* roots was found to have
antidepressant and anti-anxiety properties in another study. They theorized that the extracts work in the brain by activating GABAergic receptors and/or modulating serotonergic levels (Taiwe et al., 2010).

CONCLUSION
This study has demonstrated that administration of taurine, vitamin E and Nauclea Latifolia may increase cognitive function in Wistar rat. However, further investigation is needed to ascertain these claims.

REFERENCES


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