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The Effect of Ethanolic Soursop Leaf Extract on Caffeine-Induced Anxiety in Female Wistar Rats.

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ABSTRACT

Soursop is a tropical fruit tree that has gained attention for its potential health benefits, which are attributed to its rich phytochemical composition, including flavonoids.

The present study investigates the effects of ethanolic extracts of soursop (Annona muricata) leaves on caffeine-induced anxiety in female Wistar rats. Caffeine, a widely consumed psychoactive stimulant, is known to induce anxiety-like behaviors, which can negatively impact overall well-being (Hughes & Hancock, 2017). The anxiolytic potential of soursop leaves, traditionally used in herbal medicine, is explored in this context due to their reported calming properties and ability to modulate neurotransmitter levels (Akomolafe et al., 2015). In this study, female Wistar rats were administered caffeine to induce anxiety, followed by treatment with varying doses of ethanolic soursop leaf extract. Behavioral assessments were conducted using the elevated plus maze and open field tests to evaluate anxiety levels. Preliminary results indicate that the ethanolic extract significantly reduced anxiety-like behaviors in caffeine-treated rats, suggesting a protective effect against caffeine-induced anxiety. These findings support the potential use of soursop leaf extract as a natural anxiolytic agent and highlight the importance of dietary choices in managing anxiety disorders.

Keywords: Soursop, Caffeine, Anxiety, Anxiolytic, Female Wistar rats.

INTRODUCTION

The relationship between humans and plants or how people use plants in their daily lives is the subject of ethnobotany. Species used in ethnobotany are the source of food and medicinal plants, plants are used worldwide for natural treatment. The best source of physiologically active chemicals has also been demonstrated to be medicinal plants. Science has proven that a wide variety of recognized medicinal plants have therapeutic advantages (Faustina et al., 2010).

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Soursop (***Annona muricata***) is a deciduous tree belonging to the ***Annonaceae*** family, known for producing a heart-shaped, highly aromatic fruit. Its nectar is commonly used in smoothies and yogurts, providing an additional culinary application for this plant (Coria-Téllez et al., 2016). Soursop has gained popularity worldwide for its potential health benefits and is traditionally employed in the treatment of various ailments, including cancer, diabetes, and hypertension (Coria-Téllez et al., 2016).

Soursop, scientifically known as *Annona muricata*, is recognized by various common names across the globe, reflecting its widespread cultivation and use. In English-speaking regions, it is commonly referred to as ***soursop*** or ***Graviola***. In Latin America, the fruit is primarily known as ***guanábana*** (or guanabano for the tree) (Coria-Téllez et al., 2016). In the Philippines, it is called ***guyabano***, derived from the Spanish term. Other regional names include ***sirsak*** in Indonesia, ***durian belanda*** in Malaysia, and ***tearb barung*** in Cambodia (PlantwisePlus Knowledge Bank, 2022). These diverse names highlight the fruit's cultural significance and its various culinary applications worldwide.

In Nigeria, soursop is commonly known by several names that reflect its cultural significance and usage in local cuisine and traditional medicine. The fruit is referred to as ***ebo*** or ***apekan*** in Yoruba, ***fasadarur*** or ***tuwon biri*** in Hausa, and ***sawansop*** in Igbo (Pharmanews, 2024). These names highlight the fruit's integration into various regional diets and its importance in traditional practices.

Soursop is widely appreciated for its nutritional benefits and medicinal properties, often used in folk remedies to treat ailments such as fever, hypertension, and infections (Coria-Téllez et al., 2016). Extracts of soursop, specifically the leaf extract, have exhibited strong antioxidant properties, with a high success rate in capturing free radicals, and also shown anti-inflammatory and antinociceptive properties (Lim, 2012). One of the potentially important uses of soursop that has not been investigated yet is its treatment for anxiety.

Its popularity has led to increased cultivation across tropical regions of Nigeria, where it thrives in the warm climate. The fruit is typically consumed fresh or used to make beverages, desserts, and smoothies due to its sweet and tangy flavor profile.

Soursop is a slender, small, and cold-intolerant tree reaching a height of about 4-6 meters. The soursop is adapted to areas of high humidity and relatively warm winters therefore temperatures of about 5°C (41°F) will cause damage to leaves and small branches and temperatures below 3°C (37°F) can be fatal which causes the fruits to dry and no longer good for concentration. Soursop is believed to have originated in Central or South America and spread across the world into major tropical climates, including Western Africa and Southeast Asia.

Fig 2.0- Image of Soursop (Google, 2024).

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Anxiety disorders are one of the most common and disabling psychiatric disorders all over the world (Vincenzi et al., 2016). Generalized anxiety disorder (GAD), is an example of an anxiety disorder that plagues humanity. GAD is characterized by obsessive chronic worrying and typically requires long-term treatments. Anxiety disorders are not only a problem because of chronic worrying, but also because there is a high correlation between anxiety disorders and increased rates of alcohol abuse, marital problems, and suicide attempts (Iosifescu, 2010).

The four main anxiety disorders are GAD, panic disorder, obsessive-compulsive disorder, and post-traumatic stress disorder (PTSD), and they can be caused by many things.

Therefore, the purpose of this research work is to determine the effect of ethanoic soursop leaf extract on caffeine-induced anxiety in female Wistar rats.

MATERIAL AND METHOD

Location of the Study

This study was carried out in the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

Materials

This research was carried out using the following materials:

1. 25 female Wistar rats.
2. Latex medical hand gloves.
3. Standard cages.
4. Rat feed (Vital pelleted finisher).
5. Animal weighing balance.
6. Measuring cylinder (Pyrex).
7. Beaker.
8. Absolute ethanol.
9. Oral cannula.
10. Cotton wool.
11. Methylated spirit.
12. 2ml syringes (Disposable).
13. A square box.
14. Normal saline.
15. Cardboard paper.
16. Soursop leaf extract.
17. Tripod stand.

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18. Caffeine powder.

19. Stopwatch.

20. Camera.

21. Drugs (Diazepam).

Plant Collection and Preparation

The leaves of soursop were harvested from a local farm within Okofia, close to the Collge of Health Sciences, Nnamdi Azikiwe University, Nnewi and were plucked out from the stem and identified by a botanist. The leaves were placed on a fenestrated mat to airdry for about 10 days. After drying, the leaves were ground into powdered form and was stored in an airtight container and kept in a store at normal room temperature until they were ready to be used.

Extraction Procedure

The extraction of soursop leaves (*Annona muricata*) was conducted using a maceration method. Specifically, 250 grams of dried soursop leaves were macerated in 1000 mL of 95% absolute ethanol (JHD Chemicals, Guangdong, China) for a duration of 48 hours. Following the maceration, the mixture was filtered through a clean porcelain cloth and subsequently filtered again using Whatman Qualitative Filter Paper No. 1 (Sigma Aldrich; WHA1001042, USA) to obtain a clear filtrate. The resulting filtrate was concentrated using a rotary evaporator (Digital TT-52; Techmel & Techmel, USA) and further dried in a laboratory oven (DGH-9023A, PEC Medical, USA) at a temperature of 45°C until it reached a gel-like consistency. The final extract was stored in a refrigerator (Haier Thermocool) for future use. The extraction procedure was adapted from the method described by Attar and Abu-Zeid (2013), ensuring the preservation of the bioactive compounds present in the soursop leaves.

Experimental Design

The 25 female rats were divided into five groups of five animals each. They were grouped as follows:

Group 1: Negative control, they received feed and water *ad libitum.*

Group 2: Caffeine was administered for seven days to induce anxiety.

Group 3: Caffeine was administered to induce anxiety after which diazepam (a standard drug) was administered.

Group 4: Caffeine was administered for seven days to induce anxiety, then a low dose (200mg/kg) of soursop leaf extract was administered for two weeks (14 days).

Group 5: Caffeine was administered for seven days to induce anxiety, then a high dose (400mg/kg) of soursop leaf extract was administered for two weeks (14 days).

Models for Testing Anxiolytic Activity

In this experiment, the models used includes:

Open Field Test:

In order to determine if the ethanolic soursop leaf (*Annona muricata*) extract has an effect on caffeine induced anxiety in rats, animals were tested in an open field arena after two weeks of oral administration of therapeutic doses of the plant extract.

A large, open square (40cm x 40cm) was constructed with 20cm walls all around. A grid of 16 smaller squares

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was drawn in the middle of the arena, that is, within the four inner squares.

This model was utilized in all behavioral assays.

The Novel Object Recognition Test.

Caffeine is a widely consumed psychoactive stimulant that can significantly affect anxiety levels and cognitive functions, including memory retrieval. Research indicates that while moderate caffeine intake may enhance alertness and cognitive performance (Hughes & Hancock, 2021), excessive consumption can lead to increased anxiety symptoms, particularly in predisposed individuals (Caffeine-induced Anxiety Disorder, 2021). High doses of caffeine (over 400 mg) have been shown to elevate the risk of anxiety and panic attacks, as it stimulates the central nervous system and can mimic anxiety symptoms such as nervousness and rapid heartbeat (Healthline, 2022).

The **novel object recognition (NOR)** test is a valuable behavioral assay used in studies involving Wistar rats to assess memory retrieval. This test capitalizes on the natural tendency of rodents to explore novel objects, allowing researchers to evaluate the impact of caffeine on memory function under conditions of induced anxiety. By measuring the time spent exploring familiar versus novel objects, researchers can determine whether caffeine affects the rats' ability to retrieve memories in an anxious state (PMC, 2022). Thus, while caffeine may enhance certain cognitive functions, its potential to induce anxiety could impair memory retrieval, highlighting the need for careful consideration of caffeine consumption in both human and animal studies.

Behavioural Assessment.

To assess recognition memory, the experimental procedure involved habituating the rats in an empty box (40 x 50 x 50 cm) one day prior to testing. On the first day, during the familiarization phase, each rat was allowed 5 minutes to explore two identical objects placed within the recognition box. After a 30-minute interval, the rats were returned to the recognition box, which now contained one of the familiar objects and a newly introduced object.

During this testing phase, each rat was given another 5 minutes to explore both objects freely. The exploration behavior was recorded using a camera, allowing for precise measurement of the time spent interacting with each object. Exploration was defined as the rat placing its nose within 2 cm of an object. The underlying assumption of this test is that rats will spend more time exploring the novel object compared to the familiar one, reflecting their memory of previous encounters.

If a rat spends an equal amount of time exploring both objects, it suggests that it does not remember the familiar object, indicating a potential impairment in memory retrieval (Hammond et al., 2021; Ennaceur & Delacour, 2022). This method effectively evaluates cognitive function and can provide insights into how factors such as caffeine consumption may influence memory performance in Wistar rats.

Statistical Analysis.

Data obtained from the open field test and novel object recognition test was analyzed using one way Analysis of Variance followed with Turkey’s post hoc multiple and paired samples T-test.

The values were expressed as Mean ± Standard Error of Mean (Mean ± SEM) and the difference was considered statistically significant at $p<0.05$.

RESULTS

This table below shows the effect of ethanolic soursop leaf extract on anxiety and explorative-like behaviour in caffeine induced rats:

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Group(S)	Grid Crossing	Rearing	Defecation	Urination	Center Square Entries	Stretch Attend Posture	Latency Period (Sec)	Freezing (Sec)	Grooming (Sec)
Normal Control	34.67±8.145	11.67±5.13	1.33±2.30	0.67±1.15	0.67±0.57	3.33±1.53	0.67±0.57	155.00±19.98	31.67±8.50
Negative Control	26.75±15.94^{b}	5.25±5.852^{b}	0.00±0.00^{b}	2.00±2.45^{b}	0.00±0.00^{b}	3.75±1.71^{b}	0.00±0.00^{b}	228.33±5.033^{b}	41.75±8.50^{b}
Caffeine & Diazepam	27.67±8.96^{b}	9.33±0.577^{b}	0.33±0.57^{b}	0.67±1.15^{b}	0.33±0.57^{b}	0.67±1.15^{b}	0.67±1.15^{b}	176.50±48.84^{b}	11.67±2.88^{a}
Caffeine + Soursop (Low dose)	33.67±15.63^{b}	33.67±15.63^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.33±0.57^{b}	0.67±1.15^{b}	1.67±2.88^{b}	183.33±49.33^{b}	13.33±5.77^{a}
Caffeine + Soursop (High dose)	12.00±10.80^{b}	3.50±2.380^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.25±0.50^{b}	0.25±0.50^{b}	0.50±1.00^{b}	240.00±53.54^{b}	27.50±5.00^{b}

Table 3.1.

Table 3.1 shows results which are expressed in their Mean ± SD. Results are statistically significant at p<0.05 where [a] and [b] represents; [b]= non-significant values and [a]= significant values. Analyzed by an ANOVA test followed by Turkey's post hoc multiple comparison test to show significance

In Table 3.1; all results show a non-significant effect of Soursop in locomotor and explorative-like activities when compared with group A except in grooming behavior which showed a significant effect in groups C and D.

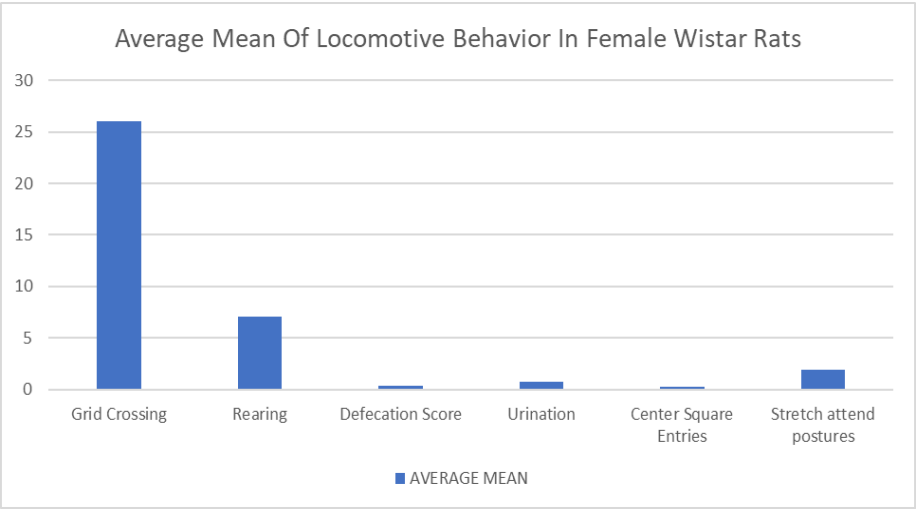

Fig 3.1.

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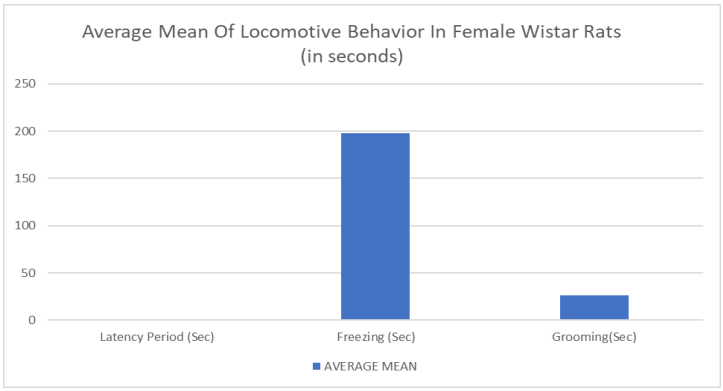

Fig 3.1.

Figure 3.1 above: Bar Chart showing the average mean of all locomotive behaviors affected by anxiety.

Table 3.2 below shows the effects of ethanolic soursop leaf extract on memory performance in Wistar rats under caffeine-induced anxiety.

GROUPS	NOVEL OBJECT	FAMILIAR OBJECT
GROUP A	8.380±2.404	4.033±0.602
GROUP B	8.380±2.404	8.460±10.10[b]
GROUP C	1.750±3.500[b]	1.3675±1.296[b]
GROUP D	6.650±6.997[b]	3.376±1.598[b]
GROUP E	10.038±10.234[b]	1.536±1.388[b]

Table 3.2.

Table 3.2 shows results which are expressed in their Mean±SD. Results are statistically significant at *p<0.05* where [a] and [b] represents; [b]= non-significant values and [a]= significant values. Analyzed by an ANOVA test followed by Turkey's post hoc multiple comparison test to show significance.

All results from Novel and Familiar object show a non-significant difference when compared to the normal control. There was a non-significant decrease in Novel object values except in groups B (Caffeine only) and E (Caffeine and high dose of Soursop) which showed a non-significant increase.

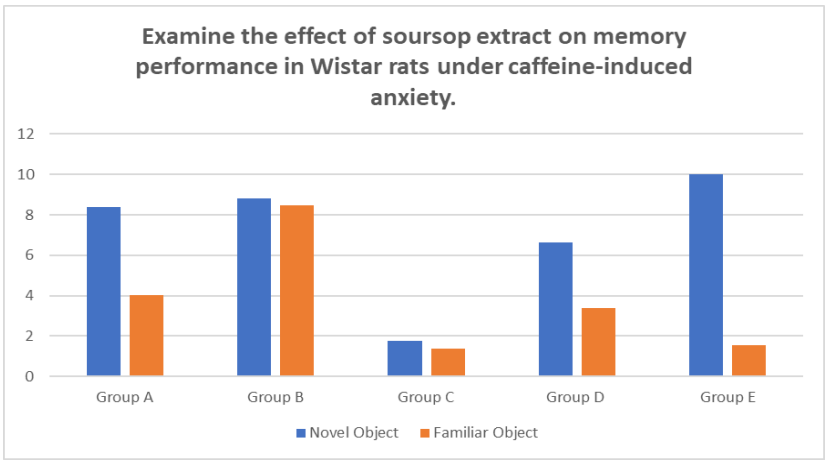

Fig 3.2.

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Figure 3.2 above: Bar chart exploring the Novel object and familiar object test to determine the effects of Soursop leaf extract on memory.

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

Generalized anxiety disorder (GAD) is a prominent anxiety disorder that affects many individuals globally (Iosifescu, 2010). This study focuses on the effects of ethanolic soursop leaf extract (*Annona muricata*) on caffeine-induced anxiety in rats.

The results indicate a non-significant difference in locomotor and exploratory activities, except for freezing behavior, which showed a significant effect in Groups C and D. All groups exhibited a non-significant decrease in Grid Crossings compared to Group A, although Group E demonstrated a more pronounced decrease than the other groups. This may be attributed to the increased dosage of soursop extract, which may contribute to locomotive disorders. These findings align with recent studies indicating that excessive consumption of soursop can lead to neurotoxic effects and movement disorders similar to Parkinson's disease (Quílez et al., 2018).

Additionally, all groups showed a non-significant decrease in rearing behavior compared to Group A. According to Deacon et al. (2022), rats utilize exploratory rearing to build and update cognitive maps based on environmental cues. Thus, decreased rearing may affect hippocampal theta activity, which is crucial for memory processing.

There was also a non-significant decrease in defecation and urination behaviors compared to Group A, with Groups B, C, and E showing no fecal deposits during or after the experiments. This observation contradicts findings by Heinz and Peters (2021), who noted that social factors significantly influence defecation and locomotion.

Group B exhibited a non-significant decrease in central square entry behavior compared to Group A, indicating increased anxiety levels among experimental animals. This supports the findings of Ennaceur (2014), which state that lower anxiety levels are associated with increased exploratory behavior. Increased anxiety typically results in reduced locomotion and a preference for staying close to the walls of the testing area.

Groups showed a non-significant decrease in stretch posture behavior compared to Group A, except for Group B, which showed a non-significant increase. This behavior is indicative of risk assessment due to an internal conflict between exploration and anxiety.

Groups B and E displayed a non-significant decrease in latency period behavior compared to Group A, while Groups C and D showed a non-significant increase. These results may be attributed to the limited space and complexity of conventional housing, which restricts natural behaviors such as running, climbing, and burrowing.

All groups exhibited a non-significant decrease in freezing behavior compared to Group A, except for Group E, which demonstrated a non-significant increase. Prolonged freezing indicates a passive behavioral strategy during defensive situations, while shorter freezing episodes suggest an active strategy with higher impulsivity (Pavlova et al., 2020).

A non-significant decrease in grooming behavior was observed when compared with Group A, except for Group B, which showed a non-significant increase. Groups C and D exhibited significant decreases compared to Group B.

In the novel object recognition test, increases in Groups B and E suggest enhanced associative recognition memory. This may result from the presence of caffeine combined with high doses of soursop extract, potentially leading to cognitive-enhancing effects that strengthen memory retention. In the familiar test, all groups showed a non-significant decrease except for Group B, which demonstrated an insignificant increase,

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indicating resilience against forgetting. Furthermore, a significant increase in body weight suggests that soursop leaf extracts are calorie-dense.

Conclusion

While both caffeine and soursop influenced anxiety and exploratory behavior in rats, their effects varied depending on the dosage and interaction between substances. The low dose of soursop may have anxiolytic and calming effects, as evidenced by reduced freezing and stretch attend postures, decreased grooming, and maintenance of exploratory behavior in caffeine-induced rats

These findings highlight the importance of dosage and potential interactions when exploring the therapeutic effects of natural compounds like soursop in managing anxiety-like behaviors

Recommendation

These findings provide preliminary evidence for the potential therapeutic use of soursop in alleviating anxiety-like behaviors induced by caffeine. Soursop, known for its antioxidant and neuroprotective properties, might offer a natural alternative for managing anxiety-related symptoms.

Further research is needed to elucidate the underlying mechanisms of soursop's effects on anxiety and exploratory behavior. This could involve investigating its interaction with neurotransmitter systems implicated in anxiety, such as the GABAergic or serotonergic pathways.

Additionally, studies exploring the long-term effects, optimal dosage, and safety profile of soursop supplementation are warranted.

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