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Original Article

EVALUATION OF THE ANXIOLYTIC ACTIVITIES OF AQUEOUS LEAF EXTRACT OF ANNONA MURICATA AND ITS EFFECT ON THE MICROANATOMY OF THE CEREBRUM.

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ABSTRACT

Anxiety disorder is one of the most common neurological disorders in the world. This study was carried out to investigate the anxiolytic activities of leaf extract of Annona Muricata and its effect on the microanatomy of the cerebrum. The experiment lasted for 7 days, involving 24 adult Wistar rats. Nine (9) rats were used for oral toxicity test, while 15 rats were randomly divided into three groups A, B, and C. A served as the experimental group and received 50mg/kg of aqueous leaf extract of *A. muricata*, B served as positive control and received 0.5mg/kg of diazepam, while C received served as negative control and received 1mm of distilled water. The anxiolytic activity of the extract was explored using elevated plus maze. In the elevated plus maze, the extract treated rats spent significant time in the open arm and the number of entries into the open arm increased. There was increase in rearing, head dip and grooming in the extract treated group. In the group that received the standard drug only had head dip increased while rearing and grooming decreased. The open arm activities of the extract treated group were comparable to that of positive control i.e the reference standard drug (diazepam). The extract therefore showed anxiolytic activity that is comparable to diazepam. The extract showed a minimal side effect on the microanatomy of the cerebrum when compared to the reference standard drug that caused inflammation of the dendrite cells and mild distortion of molecular layer cells. This distortion can affect cell functions. The result of this work showed that aqueous leaf extract of *Annona muricata* has anxiolytic effect. In conclusion, the results of the study showed that aqueous leaf extract of *Annona muricata* has anxiolytic effect on the animal anxiety models.

Introduction

Plants have been used in the treatment of diseases even before civilization and traditional medicare is still a major part of treatment for different maladies today. The popularity of folk medicine has grown in recent times in both developing and developed countries of the world. The ready availability and economy of the plants as direct therapeutic agents might be responsible for its increase in popularity [3]. In developing countries and Asia in particular, plants are used in the treatment of diseases such as Malaria, Obesity, anaemia, diabetes, anxiety etc.[1].

Annona muricata is widely used in the treatment of wide variety of ailments and as anti-anxiety treatment in Benin and in West Africa. *Muricata* is a deciduous tree that belongs to the *Annonaceae* family and produces a heart-shaped highly aromatic fruit. The fruit's nectar is commonly used in smoothies and yoghurts, giving this plant yet another cultural use [21]. *A. muricata* is known in the United States as "soursop," in Benin as "chap-chap," and in South America as "graviola," "guanabana," and "pawpaw" (2). *A. muricata* is believed to have its origin in South or Central America and spread across the World including West Africa and Southeast Asia, all in the tropical climate. Natural medicine exploits the bark, leaves, roots, fruit, and fruit seeds of the plant [25, 12]. *A. muricata* have been used to treat ailments such as cancer (prostate and liver), diabetes mellitus, and have elicited an anti-viral effect against Herpes simplex virus-1 [7, 2, 26]. Extracts of *A. muricata*, specifically the leaf extract have exhibited strong antioxidant properties with a high success rate in capturing free radicals and have also exhibited anti-inflammatory and antinociceptive properties [18, 13]. The anxiolytic activity of *A. muricata* has not been extensively investigated. Anxiety disorder is one of the most common neurological disorders in the world. In US about 15-26 million of its citizens suffer this disorder yearly [16]. A good example of anxiety disorder in human is generalized anxiety disorder (GAD). GAD is characterized by obsessive chronic worrying and requires long term treatment. The four main anxiety disorders are GAD, panic disorder, obsessive compulsive disorder, and post-traumatic stress disorder (PTSD), and they can be caused by a number of things, such as dietary deficiencies, hormonal changes, traumatic experiences, life stressors, aging, and genetics [6,8].

Current synthetic, short-term anxiety treatments are costly and may come with many undesired adverse effects [17]. Benzodiazepines are typically prescribed to patients of anxiety disorders, either instead of other treatments that include selective

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serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs), or in combination with these medications for quick relief that the other treatments do not provide [6]. However, their usage is often accompanied with development of a strong dependence as well as anterograde amnesia, impaired spatial and motor awareness and coordination [22]. Therefore the search for novel agents that is safe with minimal or no side effect becomes necessary. Plant medicine seems to provide the answer.

Aims and objective

1. *To evaluate the anxiolytic effect of aqueous extract of A. muricata.*
2. *To investigate the effect of the extract on the microanatomy of cerebrum.*

Materials and methods

Sample collection and Authentication

Fresh leaves of *A. muricata* were collected from Ukwuakwu Ututu in Arochukwu L.G.A, Abia State, Nigeria and authenticated by Mr. John Onyeukwu of the department of plant Science University of Nigeria Nsukka.

Samples preparation and extraction

The leaves of *A. muricata* were washed with water and cut into small pieces and dried under shed at room temperature, and the dried leaves were crushed into powdered using an electric blender. The sample was divided into two A and B. Sample A was used for phytochemical screening, B was used for the aqueous extraction. Five hundred (500g) of powdered leaves were soaked in 1500ml of distilled water and left for 48 hours. The mixture was filtered using cheese cloth and the filterate was then concentrated using rotary evaporator and kept in refrigerator until used.

Phytochemical screening

The phytochemical screening was done at the department of food science and technology, Faculty of Agriculture Ebonyi state university Abakaliki. The 500g of the dried powder of the leaves were subjected to qualitative and quantitive phytochemical screening. Qualitative test were carried out to determine the presence or absence of some pharmacologically active secondary metabolites.

Animals Procurement

Twenty one (21) adult Wistar rats of average weight of 160g were procured from the animal house of the College of Medicine University of Nigeria Enugu campus and kept in the Animal House of college of Medicine Ebonyi State University Abakaliki. The animals were housed in netted cages, fed with grower's mesh and allowed water *ad libitum.*

Animal Grouping

The animals were allowed to acclimatize for a period of two [2] weeks before treatments commenced. The animals were divided into three groups [A, B, C] of four [5] animals each. Group B served as positive control, while C served as negative control. Group A, B, C. received aqueous extract, diazepam and distilled water respectively.

Toxicity test

Modified Lorke's method was used in the LD_{50} study [20] of aqueous leaf extract of *Annona Muricata*. This test was carried out in two phases. In the first phase, Wistar rats randomized into three groups of three rats each, were given 10, 100, 1000 mg/kg of the prepared extract orally. The rats were observed at the very first four hour and subsequently daily for 7 days for any behavioural sign of toxicity. The same procedure as used in first one was adopted in phase two but with different dose levels of 1600, 2900 and 5000 mg/kg.

Drug administration

Dosages were calculated based on body weight of each animal in mg/kg body weight of the animal. The animals were weighed and the average weights of the animals were used in the calculation of the dosage. The extracts were administered by oral intubation through orogastric tube. The administration lasted for a week.

Tabel 3.1: Showing Administration Schedule

Groups	Treatment	Weight	Dosage
Groups A	Extract	120-180	50mg/kg
Groups B	Diazepam	120-180	0.5mg/kg
Groups C	Distilled water	120-180	1ml

Elevated Plus-Maze Model

The elevated plus-maze study was carried-out using the method described by Lister, 1987. The elevated plus-maze consists of two open arms (25×10cm) and two closed arms (25×10×10cm) with an open roof. All four arms were radiated from a central platform (10×10cm). The maze is elevated to a height of 50 cm in a dimly lit room. The behaviors that were typically recorded when rodents were in the elevated plus maze are the time spent and entries made on the open and closed arms. Beside spatiotemporal measures, ethological measures of risk assessment such as head dip, rearing, grooming and stretch attend. After a week of treatment of the Wistar rats with the drugs, the rats were placed in the centre of the elevated plus-maze, facing one of the open arms. During a 5 min test period the following parameters were taken: the number of entries and time spent in the open and closed arms. Entry into an arm was recorded when the rats crossed the demarcation of respective arm with its four paws, and was considered to be on the central platform whenever two paws were on it. All tests were recorded by using a video camera and every precaution was taken to ensure that no external stimuli could evoke anxiety in the rats. After each test, the maze was carefully cleaned up with a wet tissue paper (normal saline) to eliminate the interference of the olfactory cues on the next rat.

Histological study

After behavioral study, the rats were anaesthetized with chloroform. The brain were harvested and fixed in 10% formol saline for 48 hours. The tissues were thereafter processed using normal histological techniques. The photomicrographs of the slides were taken and were, subsequently read and interpreted by a Pathologist.

Data Analysis

Results of the experiments and observations were expressed as Mean ± Standard Error of Mean (SEM). The significance differences between groups were determined using one-way analysis of variance (ANOVA) followed by at least one of the following post hoc tests: t- test comparison tests $P< 0.05$ where level of significance was considered for each test.

Results

Toxicity test

The oral toxicity test result showed that the LD_{50} was found to be above 2000mg/kg.

Phytochemical Screening

The phytochemical screening of the extract revealed the presence of Alalkaloid, phenol, Saponin, Flavonoid, Tanin and Glycoside.

Table 1: Showing the effect of aqueous leaf extract of *Annona muricata* on time spent and the number of entries in open and closed arms of EPM

GROUPS	Number of Entries		Time Spent	
	Open arm	Close arm	Open arm	Close arm
A	4.50±1.66	4.00±1.23	63.75±32.62	236..25±15.86
B	3.25±0.85	4.00±0.91	50.00±22.73	250.00±22.73
C	0.25±0.25	2.25±1.03	75.00±75.00	225.00±75.00

Values expressed as mean ± SEM, n=5, *(P<0.01), *** (P<0.02)

Fig. 1: Showing the effect of aqueous leaf extract of *Annona muricata* on time spent and the number of entries in open and closed arms of EPM

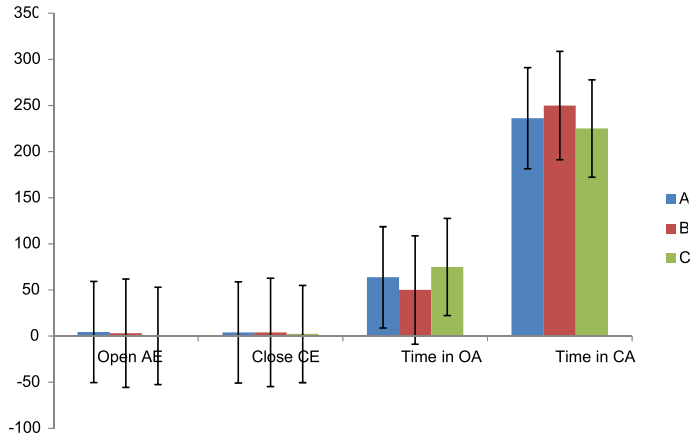


Table 2: Showing the effect of aqueous leaf extract of Annona Muricata on ethological measures for assessing anxiety.

Groups	Rearing	Head dip	Grooming
A	7.25 ±3.35	9.50±1.32	15.50 ± 2.22
B	1.00±0.577	17.75±3.038	5.75± 1.44
C	7.50 ±0.96	9.50±1.32	9.00 ± 3.34

Values expressed as Mean ± SEM, n=5, *(P<0.01), *** (P<0.02)

Fig. 2: Showing the effect of aqueous leaf extract of Annona Muricata on ethological measures for assessing anxiety Histological Studies

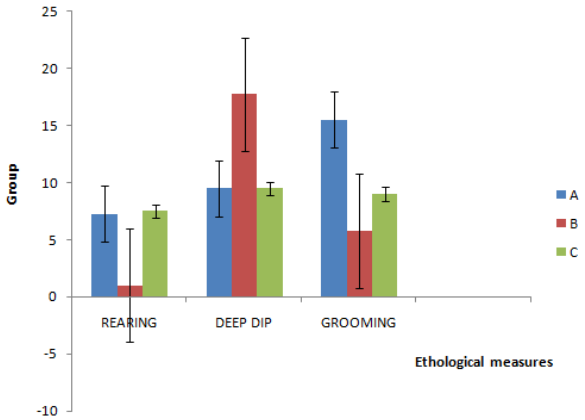


Fig. 3: Photomicrograph of cerebrum (negative control) showing normal pyramidal cells (MF), dendrite cells (DCL) and myelinated fibres (MF). Stain: Haematoxylin and Eosin. X200

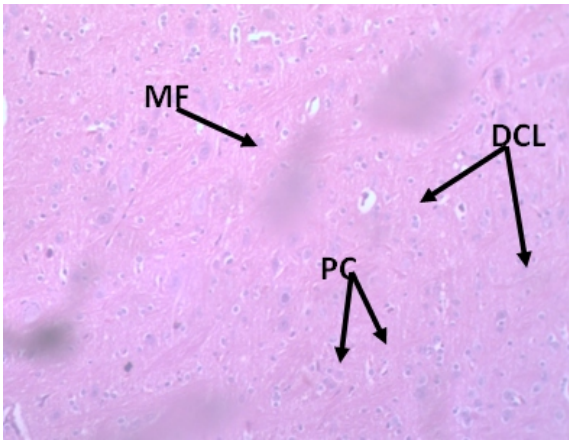


Fig. 4: Photomicrograph of cerebrum (group d) treated with 50mg/kg of aqueous leaf extract of *annona muricata* showing focal clumping of dendrite cells (FCDCL). Stain: Haematoxylin and Eosin. X200

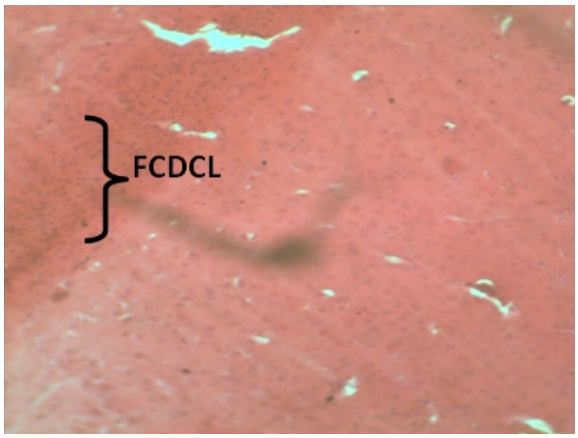
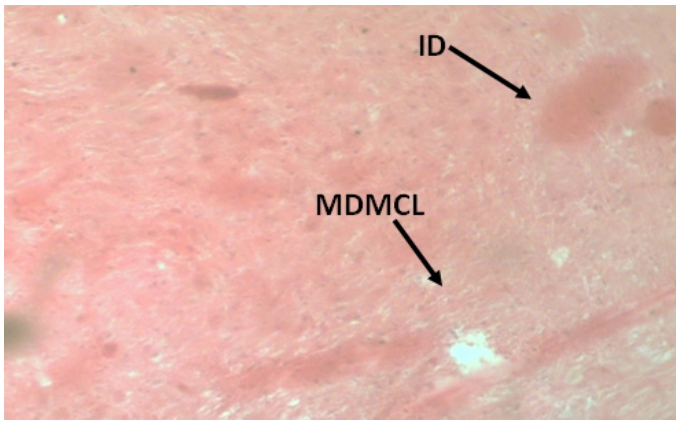


Fig. 5: Photomicrograph Of Cerebrum (Group C) Treated With 0.5mg/Kg Of Diazepam Showed Mild Distortion Of Molecular Cell Layer (MDMCL) And Inflammation Of Dendrites Cells(IDCL) Stain: Haematoxylin And Eosin. X200



DISCUSSION

Anxiety is an emotional state experienced by people, and is not readily modeled in animals [30]. The incidence of pathologic anxiety in the community is very high and is associated with lot of morbidity. Life time prevalence anxiety in women is 30.5% and male is 19.2%10. Hence, it is very important to address this problem of anxiety and find effective remedies [27]. In order to extend till now ethologically derived paradigms used in the evaluation of anxiety and fear in rodents [30] elevated plus maze was designed . Elevated plus maze is the most widely used screening method used to screen both the anxiolytic and anxiogenic effect of herbs and standard anxiolytic and anxiogenic agents.

The anxiolytic makers commonly associated with anxiolytic agents in the elevated plus maze model are increase in time spent in the open arms as well as increase in open arm entries [27]. These makers are important parameters that validate test agents with anxiolytic property. Beside the spatiotemporal measures, ethological measures are also used in the assesement of anxiety in animal models. The use of *A. muricata* as an anti-anxiety agent in traditional medicine have not been investigated scientifically, so this study was carried out to investigate the anxiolytic effect of aqueous leaf extract *A. muricata*. The extract treated animals showed increase in open arm entries and time spent in open arm that was slightly higher than that of the standard drug (diazepam). This is an anxiolytic-like effect. This agrees with the finding of Brian Lallier in his thesis sumbmitted to Graduate School-Camden Rutgers, The State University of New Jersey. The extract increased head dip, rearing and grooming, these increase in these parameters is an anxiolytic-like effect. There was decrease in all the ethological measures in the diazepam group except head dip, Cruz, and Griebel, have also reported inconsistences with these ethological measures which are dependent on species and dose which agrees with our findings [10, 15]. The phytochemical screening of the extract revealed the presence of flavonoid, alkaloid, phenol, tannin and glycoside.

Flavonoids (flavanones) have shown anti-anxiety activity in various studies. Further, the anxiolytic effect of flavonoids has been attributed to its effect on central nervous system and BZD receptors, as it was found that flavanones bind with high affinity BZD site of GABA A receptors [31, 32]. The animals that received distilled water showed normal pyramidal cells (PC), dendrites cells (DCL) and myelinated fibres. The group treated with aqueous leaf extract showed a normal tissue with focal clumping of dendrite cells, while the group that received the standard drug, diazepam showed mild distortion of molecular cell layer and inflammation of dendrite cells.

The inflammation of the dendrite cell layer, could be attributed to the damage done to the dendrite cells. Inflammation is local reaction of living tissues to injurious agents. It is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair [14]. The diazepam must have damaged some dendrites cells which elicited the inflammatory response in the cells, so as to protect the cells from injury. Besides inflammation the drug equally caused mild distortion of molecular cell layer which could have affected the neuronal circuit and cell functions. The reported side effects of benzodiazepine such as development of a strong dependence as well as anterograde amnesia, impaired spatial and motor awareness and coordination [22] could be attributed to this distortion of cells. The search for alternative medicine is propelled by these undesired side effects [9] and Annona muricata is the answer. The aqueous extract showed minimal side effect and with anxiolytic activity close to diazepam. Aqueous leaf extract of A. muricata can be used in the management of anxiety.

Conclusion

The results of the study showed that aqueous leaf extract of *Annona muricata* has anxiolytic effect on the animal anxiety models used and substantiate its use in the treatment of anxiety disorders and has minimal side effect on the microanatomy of the cerebrum.

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