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BEHAVIOURAL AND HISTOLOGICAL STUDY OF CRUDE EXTRACTS OF COSTUS AFER ON TEMPORAL LOBE OF WISTAR RATS

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ABSTRACT

Medicinal plants contain bioactive ingredients that can be used in the treatment of neurobehavioural disorder. In this study the neurobehavioural and histological effect of ethanolic extract of Costus Afer was studied. The study lasted for 14 days involving 29 adult Wistar rats, nine (9) rats were used for oral toxicity test, while 20 rats were divided into four groups of five rats each, A, B, C, D. Group A served as the negative control and received 1m of distilled water, B and C served as experimental group and received 100mg/kg body weight and 150mg/kg body weight respectively, while D served as positive control and received 2.5mg/kg of diazepam. On the fourteenth day neurobehavioural test was carried out using elevated plus maze and the animals sacrificed and the brain harvested. In the elevated plus maze the animals spent more time in the closed arm and closed arm entries increased when compared to the positive control while Head dip, grooming and rearing decreased. The extract showed an anxiogenic effect in this study. The extract distorted the histology of the temporal lobe. The distortion increased with increase in dosage. In conclusion, the result of this study showed that ethanolic extract of Costus Afer lack anxiolytic effect and altered the normal histology of the temporal lobe.

KEYWORDS: Neurobehaviour, Costus Afer, anxiogenic, diazepam, histology.

INTRODUCTION

Anxiety is a feeling of apprehension and fear characterized by physical symptoms such as palpitations, sweating and feelings of stress. Anxiety disorders are the most common mental disturbances prevalent across all ethnic populations and about 40 million adults in the United States experience some form of anxiety disorder.[1] They can have a profound negative effect on a person's general health and well-being and can acutely affect surgical outcomes if untreated. Anxiety causes particularly complex alterations within the sympathetic nervous system. The sympathetic nervous system responds to acute stress by the release of hormones by the endocrine system, neurotransmitter release via the hypothalamus, regulation of various body functions by the pituitary gland and stimulation of the adrenal medulla. Activation of the sympathetic nervous system involves release of potent catecholamines such as epinephrine, norepinephrine and dopamine into the bloodstream. The resulting activation uses a tremendous amount of energy as the cardiovascular, digestive, pulmonary, and other systems mobilize energy stores in a fight-or-flight response.[2] Because of the profound metabolic changes caused by anxiety-induced stress-related catecholamine release, healthcare providers routinely give benzodiazepines specifically midazolam as a premedicant for anxiety before initiating many invasive hospital procedures.[3] Because of the numerous side effect of this orthodox drugs, the search for novel agents with minimal or no side effect better tolerated and efficacious continues and it is thought that herbal remedies could provide the answer.

C. afer which belongs to the family *Zingiberaceae* is a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. It is commonly found in moist or shady forest of West and Tropical Africa.[4] *C. afer* is a perennial, rhizomatous herb that can attain a height up to 4m. Leaves are arranged spirally, simple and entire. Sheath is tubular, closed, green with purple blotches; ligules 4-8mm long, leathery and glabrous; petiole is 4-12mm long; blade is elliptical to obviate, 15-35cm x 3.5-9.5cm, base is rounded to subcordate, apex is acuminate, margin is sparsely hairy, usually glabrous above, sometimes shortly hairy beneath. Inflorescence is a very compact, terminal, conical spike 2.5-7.5cm long, sessile; bracts is oblong, convex, 3.5cm long, densely imbricate, upper ones often smaller, apex is truncate to rounded, green with purple markings, each subtending 2 flowers; bracteoles is boat-shaped, 2.5cm x 1cm, keel is thick and ridged, pale green with pink

markings and thin pink papery margin. Flowers are bisexual and zygomorphic. *C. afer* is found in the forest belt from Senegal to Ethiopia and in the East to Tanzania, Malawi and Angola, in the South and in West Africa. It is common plant in Nigeria, Ghana, Togo, and Cameroun. *Costus afer is* also known as ginger lily or bush cane.[5] In Nigeria, among the Ibos, it is commonly called "Okpete", "Okpoto" or "Okpete Ohia"[4], the Hausas call it "Kakizuwa", Yorubas call it "Tete-egun" and the Efik, "Mbritem".[5] The succulent stem is chewed as a remedy for cough. The root decoction is administered for the treatment of sleeping sickness and stomach ache.[5] This plant is used in the treatment of diabetes mellitus in folklore medicine[6], seizure and cold water extract is used to treat epilepsy.

This present study was designed to study its behavioural effect, whether it can be used in the treatment of anxiety and its possible effect on the histology of the temporal lobe.

MATERIALS AND METHOD

Plant Collection

Fresh leaves of *Costus afer* were collected from Oroke Onuha village in Abakaliki Ebonyi state.

Identification

The leaves were identified in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka, by Mr. Onyeukwu Chijioke John.

Plant Extraction

The leaves of Costus afer were washed with distilled water and dried in ventilated room. The dried samples were differently blended into fine powder using a Q-link electric blender Model QBL-18L40, and stored in air-tight containers. The powder was divided into two portions (A and B), portion A of the powder was used for the phytochemical analysis, while Portion B was used for the crude extractions.

All preparations were performed at the Department of food science and technology, Faculty of agriculture, Ebonyi University Abakaliki. Three hundred grams (300g) of the powder was weighed using an electronic weighing balance and soaked in 1500mL of ethanol (powder/solvent). The mixture was agitated using an electric blender (to enhance proper mixing of the solvent with the powder) and then poured into air-tight plastic container.

The mixtures were filtered with cheese cloth. The filtrates were separately concentrated *in vacuo* using Rotary Evaporator (Model RE52A, China) to 10% of their original volumes at 37◦ C - 40◦ C. These were concentrated to complete dryness in water bath. The extracts were stored in a refrigerator.[7]

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. The methods adopted have been variously reported.[8] Frothing test for saponins was used for saponins, Bromine water test for tannins, Lead acetate test for flavonoids, Wagner's reagent test for alkaloids and test for cyanogenic glycosides (using sodium picrate) was done for glycoside.

Chemicals

Routine histological reagents such as ethanol, xylene, paraffin wax etc. were purchased from chemical stores in Abakaliki, Ebonyi State, Nigeria.

Animals

Procurement

Twenty nine (29) adult Wistar rats with 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 same college. The animals were housed in netted cages fed with grower's mesh and allowed water *ad libitum.*

Methods

Animal Grouping

The animals were allowed to acclimatize for a period of two (2) weeks before treatments commenced. The animals were divided into four groups of five (5) animals each.

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 two weeks. Distilled water (1ml/kg) was given to the animals in group A, the extracts doses of (100mg/kg, 150mg/kg) were given to B and C and D diazepam of 2.5 mg/kg orally were administered to groups of 5 rats each.

Table 1: Experimental protocol of the animal treatment and dosage.

Group	Treatment	Dosage
Group A	Distilled water	1ml
Group B	Extract	100mg/kg
Group C	Extract	150mg/kg
Group D	Diazepam	2.5 mg/ kg

Acute Oral Toxicity Study

Modified Lorke's method was used in the LD_{50} study (9) of crude leaf extract of *Costus afer* This test was carried out in two phases. In the first phase, nine rats randomized into three groups of three rats each were given 10mg/kg, 100 mg/kg and 1000 mg/kg of the prepared extract orally. The rats were observed at the very first four hour and subsequently daily for 14 days for any behavioural sign of toxicity. The same procedure as used in first one was adopted in phase two to check for the toxicity of other leave extracts.

Elevated Plus-Maze Test (EPM)

The procedure used to evaluate the possible anxiolytic effect was similar to that described previously (10, 11). The wooden EPM apparatus was shaped in the form of a cross with two open arms (50 × 10 cm^2) with sides of 1 cm in height and two closed arms (50 × 10 × 15 cm). The central area of the maze measured 10 × 10 cm^2. The apparatus was elevated to a height of 50cm. During the test, each animal was placed at the center of the maze facing the open arm. All entries to the open or closed arms were scored for 5 minutes and the total time spent in each arm was recorded. An entry was defined as placing the rats four paws into an arm and no time was recorded when the animal was in the center of the maze. The test was carried out at the end of the 14^{th} day of the drug administration.

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 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

Phytochemical Screening

The phytochemical screening of the extracts revealed the presence of alkaloid, saponin, flavonoid, tannin, phenol and glycoside.

Acute toxicity test

The results of the acute toxicity study indicated that the LD_{50} of the ethanolic leaf extract of *C. afer* is more than 1600 mg/kg.

Behavioural Studies

Table 2: The effect of leaf extract of *Costus afer* on duration and the number of entries in open and closed arms.

Groups	Time spent in seconds			Number of entries	
	Open arms	Close arms	Center	Open arms	Close arms
A	4±4.2	292±4.3	3.5±1.12	0.75±0.8	7.5±1.8
B	10±10.6	285±10.4	4.75±2.17	0.75±0.83	9.25±3.27
C	6.7±4.7	320±43.96	4.3±1.9	1.3±1.25	9±2.16
D	242.3±46.9	40.75±41.1	21.25±23.3	5.75±2.05	1±0.7

Values expressed as mean±SEM, n=5, *(P<0.05), *0.004.

The close arm entries and time spent in close arm is higher than time spent in open arm and open arm entries.

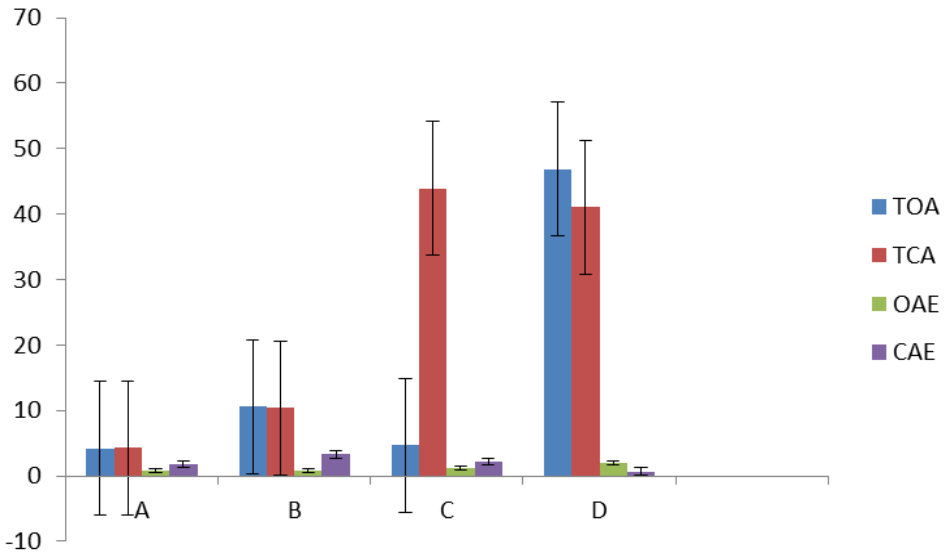

Fig. 1: A graph showing time showing the time spent in open arm (TOA), time spent in close arm (TCA), open arm entries (OAE) and close arm entries (CAE).

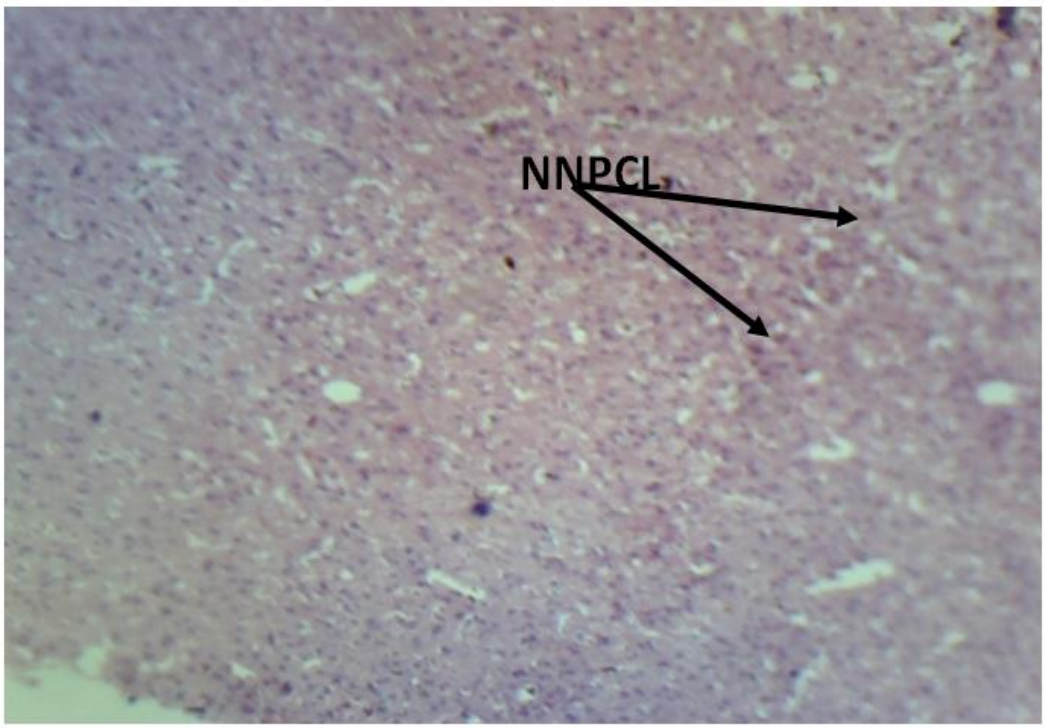

Plate 1: Photomicrograph of wistar rat temporal lobe (control) treated with distilled water showing numerous normal pyramidal cells (NNPCL); H & E stained x150.

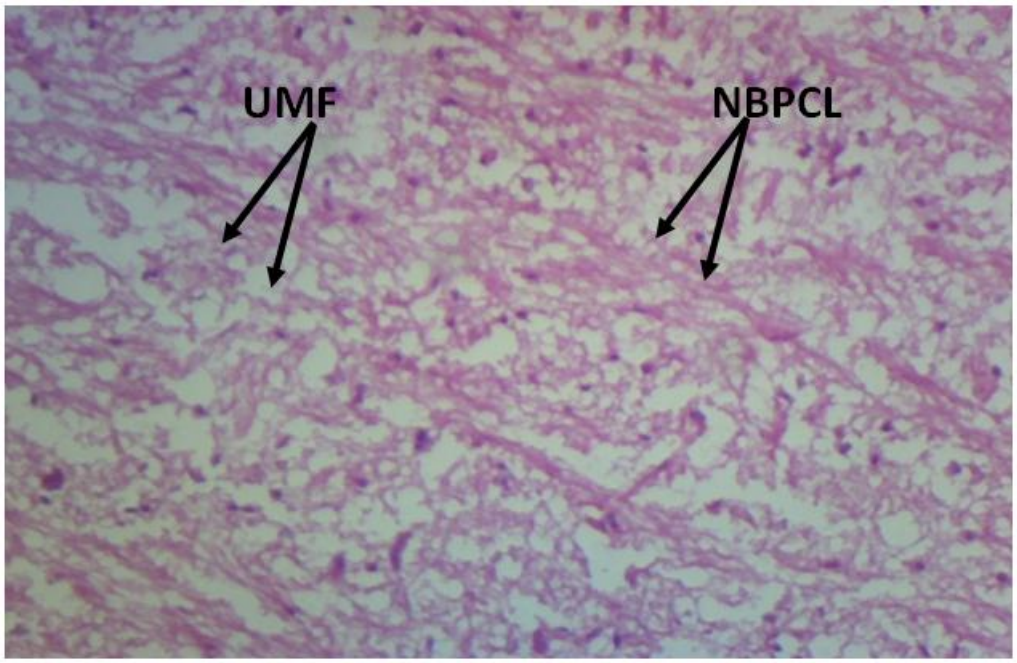

Plate 2: Photomicrograph of Wistar rat temporal lobe treated with Costus afer extract 100mg/kg) showing numerous bi-nucleated pyramidal cells (NBPCL); H & E stained x150.

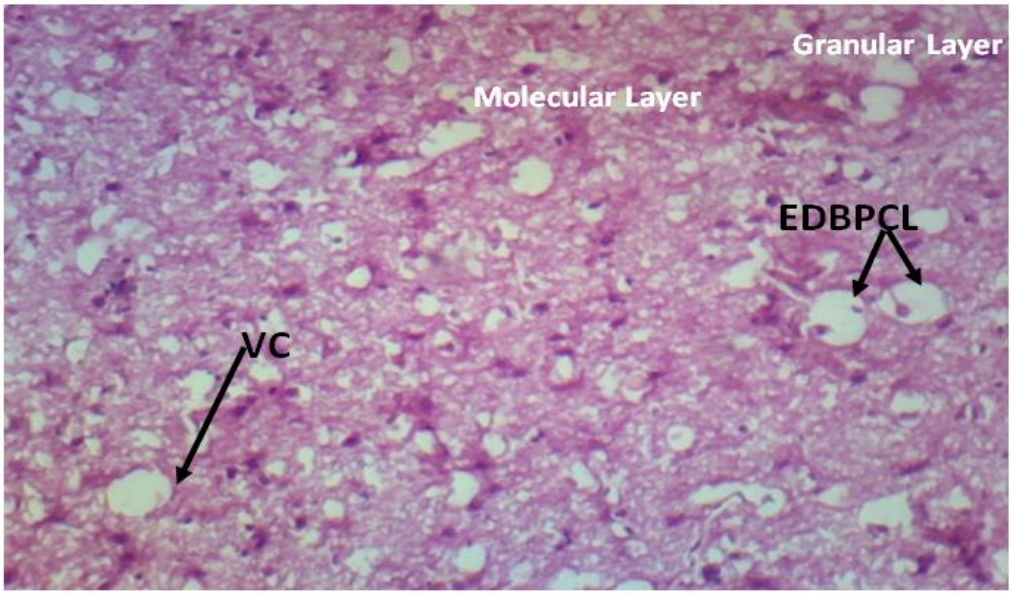

Plate 3: Photomicrograph of wistar rat temporal lobe treated with Costus Afer extract 150mg/kg) showing extensive distribution binucleate pyramidal cells (EDBPCL); H & E stained x150.

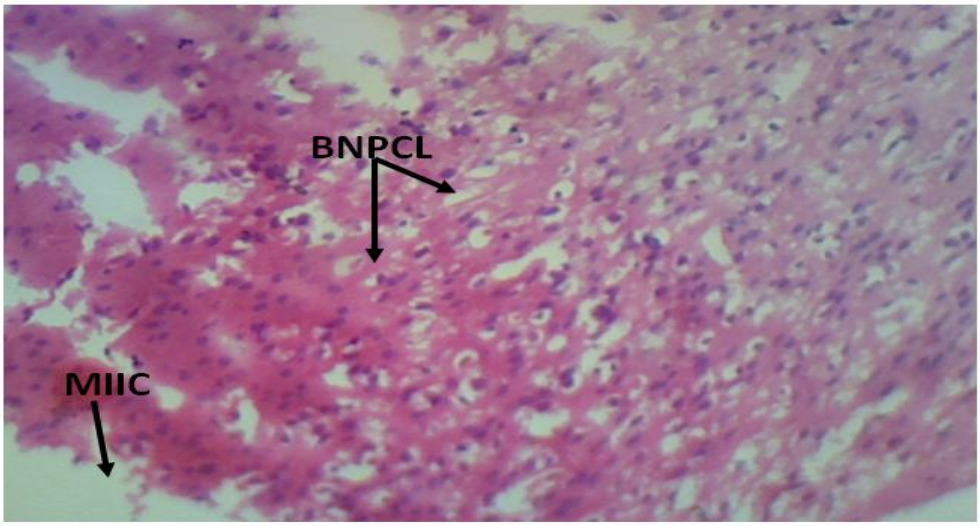

Plate 4: Photomicrograph of wistar rat temporal lobe treated with diazepam 2.5mg/kg) showing mild infiltration of inflammatory cells (MIIC), binucleate cells (BNPCL); H & E stained x150.

DISCUSSION

The Medicinal properties of *Costus afer* have been widely reported and its use in folklore medicine has been documented.[12] This plant is used as a remedy for cough, inflammation, arthritis, as laxative, aperients, purgative, diuretic, in rheumatism and treatment of several other diseases.[12] Its medicinal properties could be attributed to the presence of phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, and phenols. A lot of works have been done on the medicinal properties of the herb but no work has been done on its effect on neurobehavior i.e. anxiolytic and its anxiogenic properties. The present study was carried out to assess its effect on neurobehavior. The study revealed the absence of anxiolytic properties but anxiogenic property as the animals spent more time in close arm of the elevated plus maze and the number of entries into the close arm was equally higher than that spent in the open arm. The EPM has been frequently used to detect and evaluate the anxiolytic/anxiogenic properties of drugs.[13,14] The entries and time spent in the open arms is the main indicator of fear in the EPM, given the fact that an open area is extremely aversive to rodents.[13,15] In addition, in the open arms there is no thigmo taxis[16] which enhances the state of fear and aversion in the animals. The anxiogenic effect of the extract increased as the dosage increased though the difference was not significant. There was decrease in head dip, rearing and increase in rearing while fecal boli and stretch attend posture was not observed. The decrease in head dip and rearing is consistent with anxiogenic effect. There was decrease in all the ethological measures in the diazepam group. Cruz and Griebel also reported

inconsistences with these ethological measures which are dependent on species and dose.[17,18]

The treated groups that received the extract at dose of 100mg/kg and 150mg/kg showed numerous bi-nucleated pyramidal cells and extensive distribution of pyramidal cells and vacuolated cytoplasm (plate 1 and 2). When compared to the control, the cell population increased this increase in cell population is attributed to initiation of mitotic cell division by the extract. This is evidenced by the presence of binucleate pyramidal cells. This may lead to neoplasm, abnormal growth of cells without coordination. Precancerous neoplasms are cell masses that have the potential to become cancerous. It has been reported that bi-nucleated cells appear mostly in cancer cells and that bi-nucleation occurs at much higher rate in cancer cells.[19] The chronic consumption of this extract may result to the development of cancer. This has a devastating consequence and may finally lead to death. As the dosage increased to 150mg/kg body weight, extensive distribution of bi-nucleated cells was observed, this effect was dose dependent. Those who use it for treatment of various illnesses should not presume that it is safe since it is herb, but limit its usage to disease condition.

CONCLUSION

Crude extract of Costus afer does not possess anti-anxiety but rather showed anxiogenic properties in this particular work and altered the normal histo;ogy of the temporal lobe, of the brain.

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