

Effect of Combined Crude Extract of *Costus afer*, *Culcasia scaden* and *Sarcocephalus latifolius* on Neurobehaviour and Histology of the Temporal Lobe of Adult Wistar Rats.

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ABSTRACT:

This combined extract is used in traditional medicine for the treatment of psychiatric disorder. This study investigated the effect of combined crude extracts of *Costus afer*, *Culcasia scaden* and *Sarcocephalus latifolius* on neurobehaviour and histology of the temporal lobe. The extract was prepared by mixing equal portions of the three extracts and dosage calculated base on the body weight of the animals. Twenty rats were divided into four groups (n=5) such as A, B, C and D. A served as negative control and was given distilled water. The D group served as positive control and received 2.5mg/kg of Diazepam, while B and C served as the experimental group and received 100mg/kg and 150mg/kg respectively for 14 days. The behavioural assay was carried out using elevated plus maze. The animals were sacrificed and the brains removed and processed using normal histological techniques. The result of the behavioural test showed an increase in close arm entry but when compared to the negative control the extract treated groups made more open arm entry and spent more time in the open arm (open arm activity) but less when compared to positive control (Diazepam). This is a measure of anxiolytic activities of the extract. Therefore the extract possesses anxiolytic property. The extract distorted the histoarchitecture of the temporal lobe showing extensive loss of brain tissues, sparsely dispersed cells, and focal loss of brain tissues at doses of 100mg/kg and 150mg/kg respectively. The extracts conclusively possess the potential to modify neurobehaviour and alter the histology of the temporal lobe.

INTRODUCTION:

Anxiety is a highly prevalent psychological and physiological state characterized by psychomotor tension, sympathetic hyperactivity and apprehension and vigilance syndromes. It affects one-eighth of the total population of the world and have become a very important area of research interest in psychopharmacology (1). The complexity of daily life in modern society leads to various degrees of anxiety and depression. Mood

depression and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries. For many years they were considered as two different mental diseases, with the benzodiazepines used as the drugs of choice for acute anxiety states and the amine uptake inhibitors and monoamine oxidase inhibitors to treat depression. However, in clinical practices, the treatment of anxiety

disorders with benzodiazepines is now being slowly replaced by antidepressants, which are not only efficacious in depression but also in the acute and chronic treatment of anxiety disorders (2).

Although benzodiazepines have well-known benefits, their side effects are prominent including muscle relaxation, sedation, physical dependence, memory disturbance, tolerance, amnesia, weakness, loss of sexual drive, gastrointestinal effects and changes in body weight and interaction with other drugs (3). In these conditions, the efficacy of such drugs is very limited so the need for newer, better-tolerated and more efficacious treatments remains high. Herbal therapies could be considered as alternative or complementary medicines in this case.

Through ages, plants have been used as medicine because they are important source of many biologically active products. *Costus afer*, *Sarcocephalus latifolius* and *Culcasia scaden* are some of the plants that have been used in traditional medicine in Nigeria for the treatment of psychiatric disorders. *Costus afer* also known as ginger lily or bush cane (4) is common in many parts of the world and also to the people of Nigeria. It is use in traditional medicine for the management of cough, stomach ache, sleeping sickness, diabetes mellitus etc. *Sarcocephalus latifolius* (African peach) is also one of the numerous plant species believed to have medicinal value. *Sarcocephalus latifolius* have been reported to have a wide range of medicinal value, common traditional uses include the treatment of fever, dental caries, malaria, hypertension, dysentery, diarrhea and diseases of the central nervous system such as epilepsy (5, 6, 7, 8). The leaves of *Culcasia scaden* are used in the treatment of tonsillitis, toothache and other inflammatory conditions (7). A combination of these three plants is used in the treatment of psychiatric disorder in some part of south-eastern Nigeria.

MATERIALS AND METHODS

Plants Collection, Identification and extraction:

The fresh leaves of *Sarcocephalus latifolius* were collected from Isieke campus of Ebonyi state university, Abakaliki, while *Culcasia scandens* from department of plant science, university of Nigeria Nsukka and *Costus afer* were collected from ukwuakwu Ututu village in Arochukwu L.G.A Abia state, Nigeria. The leaves were identified in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka by Mr John Onyeukwu. The leaves were washed with distilled water and dried in ventilated room. After drying, the leaves of each plant were blended into fine powder using an electric blender. 500g of the powder of each leave was soaked in 1500ml of ethanol in different container. 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 to 10% of their original volumes at 37° C - 40° C. These were concentrated to complete dryness in water bath to get a powder like substance. The extracts were stored in a refrigerator until required (9). Equal portions of each extract was weighed and combined to form the combined extract.

Phytochemical Screening:

Phytochemical screening of the crude leaf extracts of plants were carried out using standard procedure described by Trease and Evans, (10).

Animal Grouping and drug administration:

Twenty 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*. They 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 per group. Group A served as the negative control and received distilled water, while D received 2.5mg/kg of diazepam and served as the positive control. B and C served as the experimental group and received 100mg/kg and 150mg/kg of the extract respectively. The experiment lasted 14 days.

Oral Acute Toxicity Study:

Modified Lorke's method was used in the LD₅₀ study (11) of crude leaf extract of *Sarcocephalus latifolius*. 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.

Ethical approval:

The study complied with animal care and use ethics of the Animal Holdings protocol overseen by the head of Animal Holding unit. There was strict adherence to International guidelines for use of animal in research studies.

Elevated Plus-Maze Model:

The elevated plus-maze study was carried-out using the method described by Lister (12). The elevated plus-maze consists of two open arms (25×10cm each), and two closed arms (25×10×10cm each), with an open roof. All four arms were radiated from a central platform (10×10cm). The maze was elevated to a height of 50 cm in a dimly lit room. At the end of 14 days, each rat was placed in the centre of the elevated plus-maze after one hour of post treatment facing the open arms. During a 5 min test period, the following parameters were recorded: the

number of entries and time spent in the open and enclosed arms. Entry into an arm was recorded when the rat cross the demarcation of respective arm with its four paws was considered to be on the central platform (transition zone) whenever two paws were on it.

Beside spatiotemporal measures, ethological measures of risk assessment such as head dip, rearing, grooming and duration of grooming, stretch attend posture, fecal boli were equally taken. All tests were recorded 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 the behavioural study the rats were anaesthetized with chloroform and sacrificed. The brain were harvested and fixed in 10% formol saline for 48 hours. Thereafter the temporal lobes were removed and processed using normal histological techniques.

DATA ANALYSIS

Results of the experiments and observations were expressed as mean \pm 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.

Table 1: Photochemical constituents of the selected extracts.

Phytochemical	<i>S. latifolius</i>	<i>C. scaden</i>	<i>C. afer</i>
Alkaloids	1.4±2.0	1.1±00	59.1±0.01
Saponins	1.8±0.2	1.6±0.20	4.8±0.30
Flavonoids	5.3±0.2	2.95±1.15	20.1±0.50
Tannin	6.76±0	3.17±0.0096	750.4±0.11
Phenol	105.5±0	45.30±1.126	4.8±0.30
Glycoside	39.0±0	59.50±0	192.5±0.71

Table 2: Results of the quantitative analysis of phytochemical constituents of the extracts

Phytochemical	<i>S. latifolius</i>	<i>C. Scaden</i>	<i>C. Afer</i>
Alkaloid	+	+	++
Saponin	+	+	+
Flavanoid	+	+	+
Tanin	+	+	+++
Phenol	+++	++	+
Glycoside	++	++	+++

Oral toxicity study

The LD50 was found to be above 2000mg/kg body weight.

TABLE 3: Showing the effect of crude leaf extract of Combined extract on duration and the number of entries in open and closed arms of EPM

Group	Time spent in seconds			Number of entries	
	Open arms	close arms	Center	close arms	Open arms
A	4±4.2	292±4.3	3.5±1.12	0.75±0.8	7.5±1.8
B	40.7±12.3	244.7±18.3	28.3±17	4±1.4	10.00±0.82
C	20.5±22.5	265.5±24.5	14±9.4	1.25±1.3	4.25±2.17
D	242.3±46.9	40.75±41.1	21.25±23.3	5.75±2.05	1.00±0.7

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

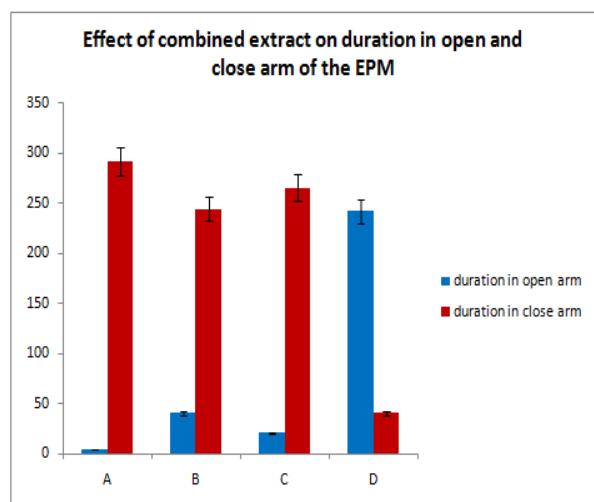


Figure 1:

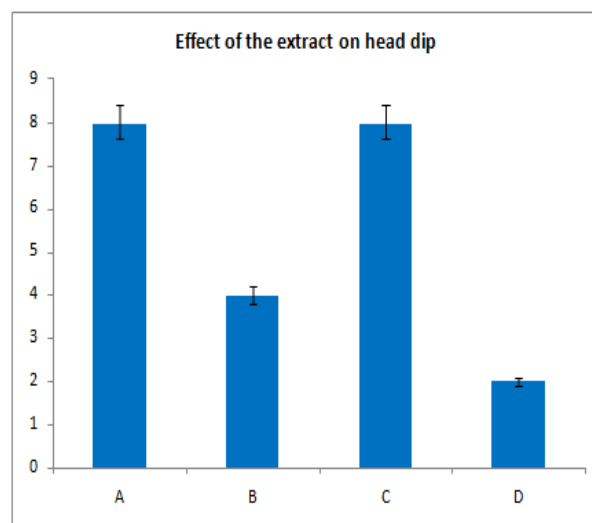


Figure 4:

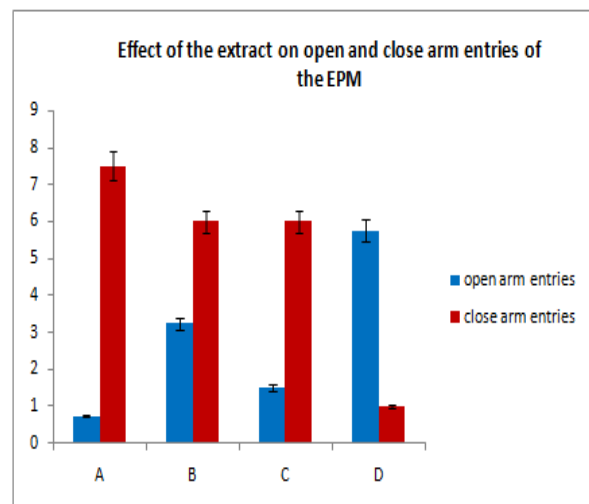


Figure 2:

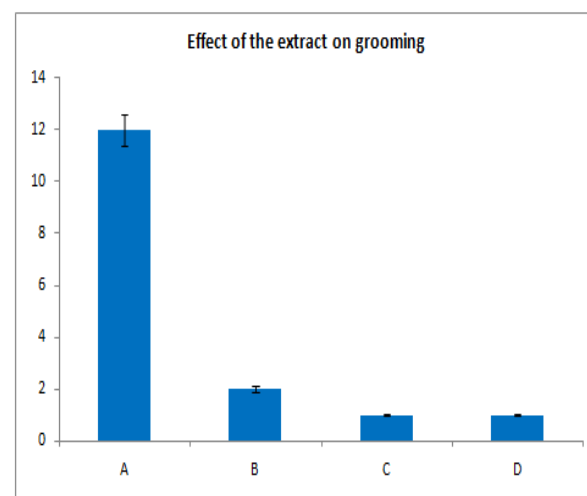


Figure 5:

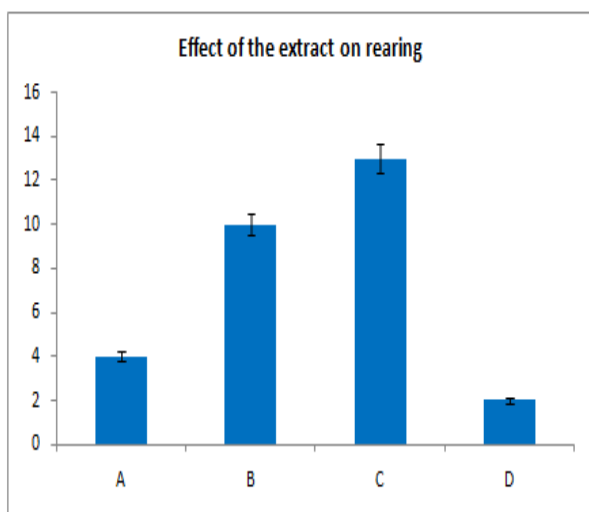


Figure 3:

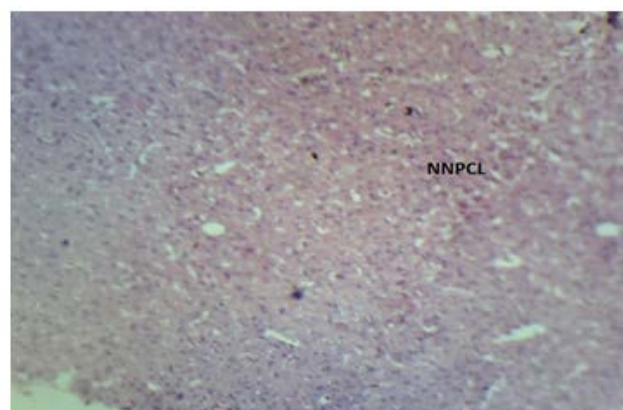


Plate 1: photomicrograph of Wistar rat temporal lobe (control) treated with distilled water numerous normal pyramidal cells (NNPCL): H&E stained ×150

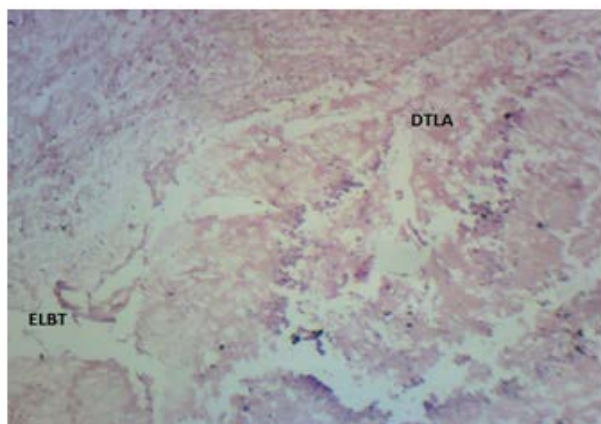


Plate 2: photomicrograph of Wistar rat temporal lobe treated with 100mg/kg showing distortion of temporal lobe architecture (DTLA), Extensive loss of brain tissues (ELBT): H & E stained x150.

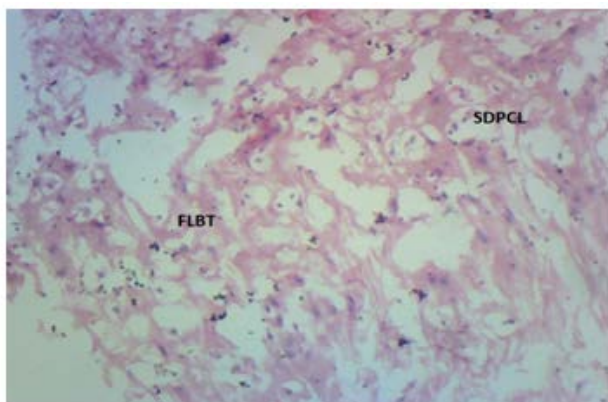


Plate 3: Photomicrograph of wistar rat temporal lobe treated with combined extract 150mg/kg) showing focal loss of brain tissues (FLBT), sparsely dispersed pyramidal cells (SDPCL): H&E stained x150

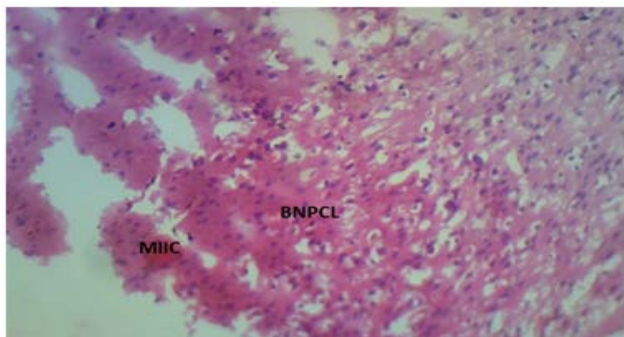


Plate 4: Photomicrograph of wistar rat temporal lobe treated with diazepam 2.5mg/kg) showing mild infiltration of inflammatory cells (MIIC), binucleate cells (BNPCL): H & E stained x150.

Discussion

In this 21st century, there are more struggles for the approval of herbal medicine more than ever, this is because in Africa especially south of the Sahara, the use of plants and their extracts for treatment and management of diseases is gaining more ground in recent times (13). The phytochemical screening of the extracts of *Sarcocephalus latifolius*, *Costus afer* and *Culcasia scaden* revealed the presence of alkaloid, saponins, flavonoids, tannin, phenol and glycoside. Glycoside and phenols have been reported to possess neuro-protective property against assault particularly in ischemic stroke. They are also associated with neuro-sensory disorders such as depression hallucination, headache, confusion, and drowsiness. Alkaloid may also affect the CNS, including nerves which control many direct body functions and behavior. Anxiety disorders present pattern responses that display two emotional states: fear and anxiety (14). The distinction between the two emotional states lies in the concept that the former is a response to actual threat while the latter is anticipatory response to potential threat (15, 16). Animal anxiety models are widely employed in the screening of anxiolytic agents and anxiogenic agents with the view of analyzing the pathological state of anxiety in assumption that some anxiety states are essential mechanism for survival and are feature of all mammals (17).

Results of this present study showed that combining the extracts produced a high level of neurobehavioral effect. Behavioral models used in this study are based on unconditioned responses to stimuli which are thought to be indicative of human generalized anxiety symptoms (18, 19, 20). The markers of its effect on neuro-behaviour commonly associated with anxiolytic agents and anxiogenic agents in the elevated plus maze model are increase in open arm entries and time spent in the open arms and closed arm entries and time spent in close arm as well as increase in the frequency of crossing the intersection (21). These indicators are important parameters that validate test

agents with neurobehavioral property as seen in this present study. The animals in the experimental group entered and spent more time in the close arm but when compared to the negative control open arm entries and time spent in the open arm was higher but less when compared to the positive control. From this finding the extract could be said to possess anxiolytic property. The animals that were given 2.5mg/kg made more entries into the open arm and spent more time in the open arm. This is a measure of the anxiolytic activities of diazepam. The extract increased head dip, rearing, decreased grooming which is an anxiolytic-like effect. Fecal boli varied and results were inconsistent. Stretch attend posture was not observed in the entire experimental group. The extract showed anxiolytic property, this finding have justified its usage in traditional medicine for the treatment of psychiatric disorder.

The combined extract was administered as it is used in traditional medicine for the treatment of psychiatric disorders and its effect on the microanatomy of the temporal lobe was studied. The study revealed extensive loss of brain tissues and distortion of temporal lobe architecture at dose of 100mg/kg. At extract dose of 150mg/kg (plate9), focal loss of brain tissues and reduction in pyramidal cells number when compared to the control. The distortion of temporal lobe architecture may be attributed to the presence of alkaloids. According to Cavanagh, chemically induced neuro-degeneration is usually characterized by different patterns of neuronal cell death, gliosis, swollen or destroyed axons, or destruction of the myelin sheath. This distortion may disturb the neuronal circuit signaling within the temporal lobe and thus affect memory and learning (22). There was possible cell death that resulted to reduction in the number of pyramidal cell number. This cell death may lead to loss of brain functions and death. Finbarr's reported that the combined extract at doses of 6mg/kg and 9mg/kg per body weight showed neuro-protection (23), the variation observed in this present study may be as a result of the method

of extraction, dosage, longer period of administration and the soil type where the herbs were grown. The diazepam group showed focal and intracellular areas of hemorrhage and mild infiltration of inflammatory cells and binucleated cytoplasm. These hemorrhages may result from the damage to blood vessels of the cerebral cortex by the drug. It is generally due to rupture of the thin-walled lenticulostriate artery, a branch of the middle cerebral artery. Cerebral hemorrhage most times involves important descending nerve fibers in the internal capsule and produces hemiplegia on the opposite side of the body. The patient immediately loses consciousness, and the paralysis is evident when consciousness is regained (24).

Conclusion

The combined extract as used in traditional medicine has anxiolytic property and distorted the normal histology of the temporal lobe. This could affect brain functions. Those who use the extract should exercise caution, because of its unwanted side effects.

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