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EVALUATION OF THE NEUROBEHAVIOURAL ACTIVITIES OF CRUDE LEAF EXTRACTS OF *SARCOCEPHALUS LATIFOLIUS* AND ITS EFFECT ON MICROANATOMY OF TEMPORAL LOBE OF ADULT WISTAR RATS

Okoronkwo Samuel Okafor [*] [1], Anyanwu [2], Gabriel Emeka [3], Uchewa, Obinna Onwe [4], Okoronkwo, Akudo Christain and John E.Abraham [5]

Department of Anatomy [1], Faculty of Basic Medical Sciences, David Umahi Federal University of Health Sciences, Uburu Ebonyi State, Nigeria.
Department of Anatomy [2], Faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus, Nigeria.
Department of Anatomy [3], Alex EkwuemeFederal University, Ndufu-Alike Ikwo, Ebonyi State, Nigeria.
Department of Anatomy [4], Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.
Department of Radiography [5], Evangel University, Akaeze, Ebonyi State, Nigeria.

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Correspondence to Author:
Okoronkwo, Samuel. Okafor

Research Scholar,
Department of Anatomy,
Faculty of Basic Medical Sciences,
David Umahi Federal University of Health Sciences, Uburu Ebonyi State, Nigeria.

E-mail: okoronkwosam1@gmail.com

ABSTRACT: *Sarcocephalus latifolius* have a wide range of medicinal applications. This study evaluated the neurobehavioural activity of crude Leaf extract of *Sarcocephalus latifolius* on wistar rats and the effect on the histoarchitecture of the temporal lobe. Sixteen adult Wistar rats were divided into four groups of four rats each. Group A served as the negative control and received distilled water, B and C served as the experimental group and received 100mg/kg and 150mg/kg of the extract, while D served as the positive control and received 2.5mg/kg of diazepam. The administration lasted for two weeks and the neurobehavioural activity of the extract was explored using elevated plus maze. After the test the rats were sacrificed and the brain harvested, fixed and processed using normal histological techniques. In the elevated plus maze, the experimental group showed increased open entry, when compared to the negative control but less than the positive control, which is a measure of its anxiolytic activity. The extracts altered the microanatomy of the temporal lobe, in dose dependent manner. In conclusion the extract has anxiolytic property and modified the microanatomy of the temporal lobe.

INTRODUCTION: Plants have been used through the ages as medicine because they are important source of many biologically active product. Many drugs in use today are developed from plants. Today, medicines from medicinal plants form the basis of primary healthcare for majority of the people living in rural and urban areas.

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Herbs are used in traditional medicine for the treatment of neurobehavioural disorders such as anxiety and depression *etc*. *Sarcocephalus latifolius* commonly called African peach in English. It is Locally known as Egbesi (Yoruba), *Tafashiya* or *tuwon biri* (Hausa), *Ubuluinu* in (Igbo), *mahyann* (Fali) language, in Nigeria [1].

It is a multi-stemmed shrub with irregular and dense foliage that grows up to 12m. It is predominantly found in Africa and some parts of Asia [2]. *Sarcocephalus latifolius* have a wide range of medicinal applications which includes: cough remedy, diabetes, malaria treatment [3, 4, 5, 6] diarrhoea and central nervous system diseases such

as epilepsy [6]. Decoction root extract of *Sarcocephalus latifolius* has been reported to have anticonvulsant, anxiolytic and sedative properties. The combinbined extract of *S. latifolius*, *C. afer* and *C. scaden* have been reported to show neurobehavioural property [7]. The aim of this study was to evaluate the neurobehavioural effect of the crude leaf extract of *Sarcocephalus latifolius* on animal anxiety models.

MATERIALS AND METHODS:

Plants: Fresh leaves of *Sarcocephalus latifolius* were collected from Isieke campus of Ebonyi state university Abakaliki.

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 were washed with distilled water and dried in ventilated room. After drying, the leaves were blended into fine powder using an electric blender. 300g of the powder was soaked in 1500ml of ethanol. 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. The extracts were stored in a refrigerator [8, 9].

Phytochemical Screening: Phytochemical screening of the crude leaf extract of *Sarcocephalus latifolius* was carried out using standard procedure described by Trease and Evans modified [1].

Animals: Sixteen 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.*

Oral Acute Toxicity Study: Modified Lorke's method was used in the LD_{50} study [10, 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 10, 100, 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 but one animal in each group with different dose levels of 1600, 2000 and 2500 mg/kg.

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.

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 four (4) animals each. 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 for 14 days.

Elevated Plus-Maze Model: The elevated plus-maze study was carried-out using the method described by Razavi [8]. 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, one hour post treatment, each rat was placed in the centre of the elevated plus-maze, 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 crosses the demarcation of respective arm with its four paws and 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. The brain was 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 ± standard Error of mean (SEM). The significance of differences between groups was 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.

Oral Toxicity Study: The LD50 was found to be above 2000mg/kg body weight.

Behavioural Studies:

TABLE 1: SHOWING THE EFFECT OF CRUDE LEAF EXTRACT OF *SARCOCEPHALUS LATIFOLIUS* ON TIME SPENT 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	Open arms	Close arms
A	4±4.2	292±4.3	3.5±1.12	0.75±0.8	7.5±1.8
D1	54.8±19.8	244±21.8	3.75±2.38	3.25±1.48	6±3.08
D2	22.8±15.4	249±35.6	64±26.3	1.5±1.12	6.5±2.87
F	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=4, *(P<0.05), *** (P<0.007). The close arm entries and time spent in close arm is higher than time spent in open arm and open arm entries, however there was significant open arm activity in the extract treated groups when compared to the negative control.

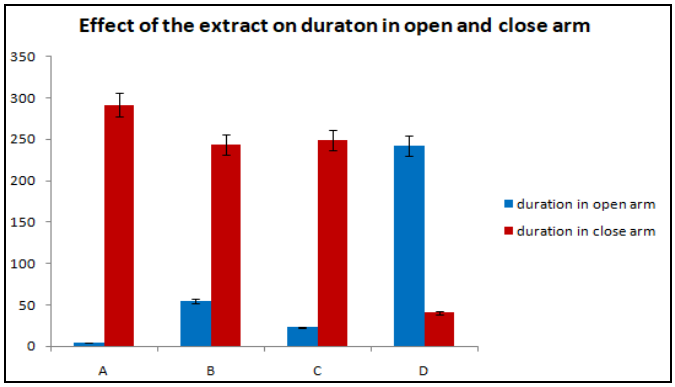

FIG. 1: SHOWING TIME SPENT IN OPEN AND CLOSE ARM

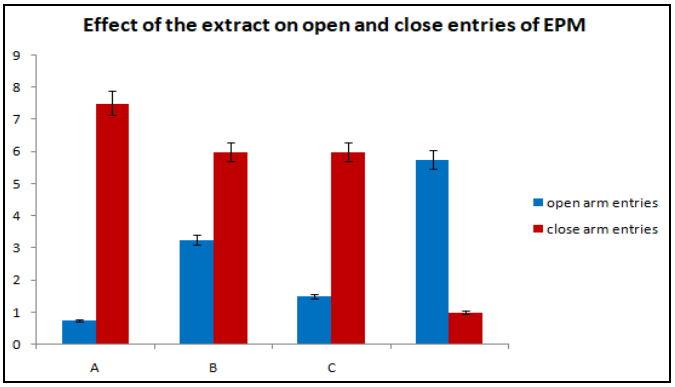

FIG 2: SHOWING TIME SPENT IN OPEN AND CLOSE ARM

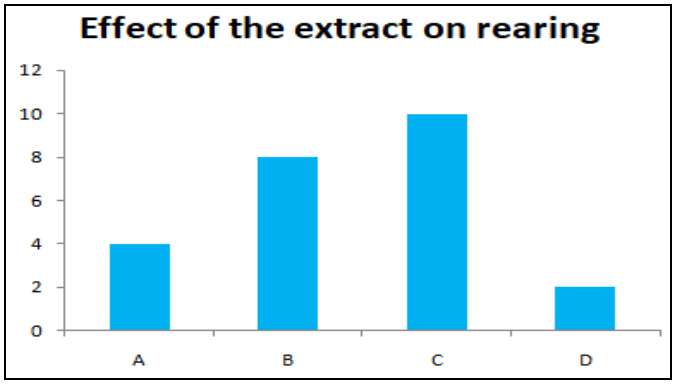

FIG. 3: SHOWING THE EFFECT OF THE EXTRACT ON REARING

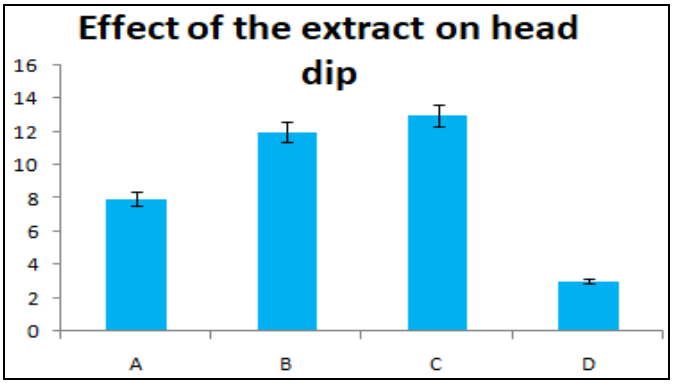

FIG. 4: SHOWING THE EFFECT OF THE EXTRACT ON HEAD DIP

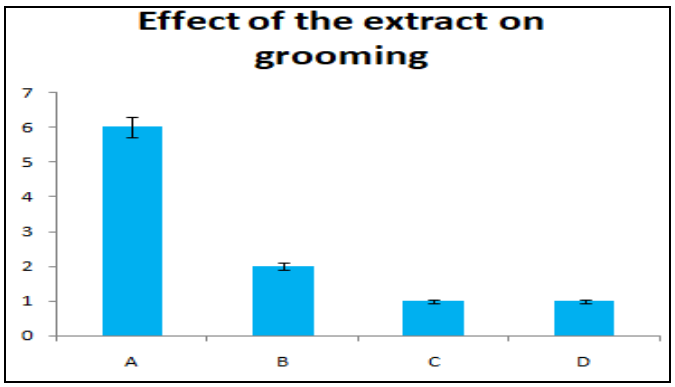

FIG. 5: SHOWING THE EFFECT OF THE EXTRACT ON GROOMIN

Histological Studies:

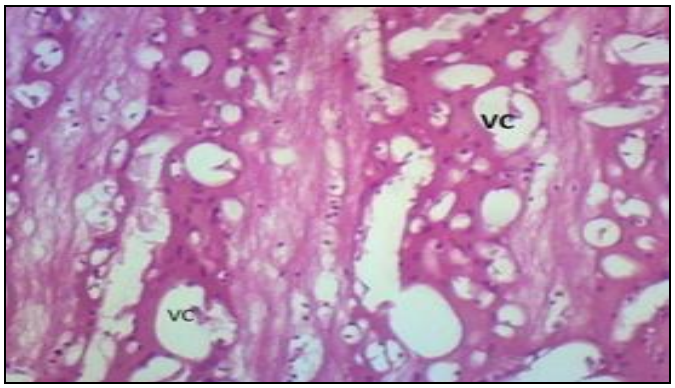

PLATE 2: PHOTOMICROGRAPH OF WISTAR RAT TEMPORAL LOBE TREATED WITH *SARCOCEPHALUS LATIFOLIUS* EXTRACT 100MG/KG) SHOWING VACUOLATED CELLS: H & E STAINED X150.

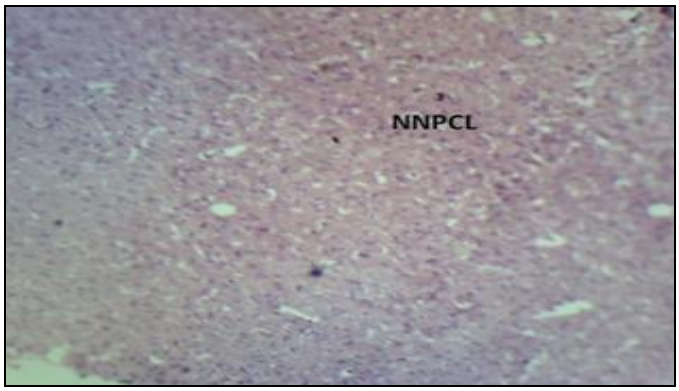

PLATE 1: PHOTOMICROGRAPH OF WISTAR RAT TEMPORAL LOBE (CONTROL) TREATED WITH DISTILLED WATER SHOWING NUMEROUS NORMAL PYRAMIDAL CELLS (NNPCL).: H & E STAINED X150.

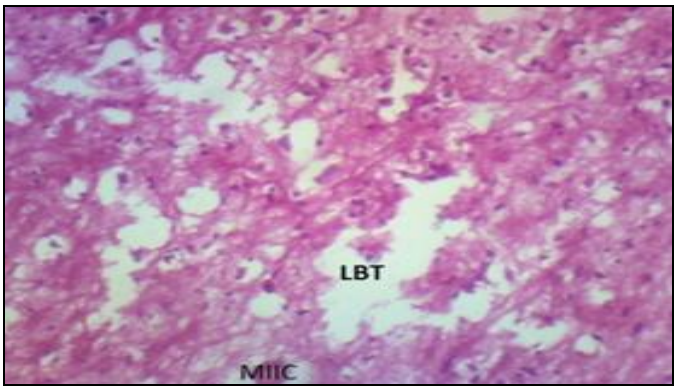

PLATE 3: PHOTOMICROGRAPH OF WISTAR RAT TEMPORAL LOBE TREATED WITH *SARCOCEPHALUS LATIFOLIUS* EXTRACT 150MG/KG) SHOWING LOSS OF BRAIN TISSUES (LBT), MILD INFILTRATION OF INFLAMMATORY CELLS: H & E STAINED X150.

DISCUSSION: The pharmacological mechanism behind the possible effect of *S. latifolius* may be traced to some phytochemical content of the plant. Qualitative tests of *S. latifolius* extract revealed the presence of alkaloids, saponins, tannin, flavanoids, phenol and Glycosides. Glycoside and phenols have been reported to possess neuroprotective property against assault particularly in ischemic stroke [1]. They are also associated with neurosensory disorders such as depression, hallucination, headache, confusion, and drowsiness. Alkaloids have been reported to have toxic effect, over 350 herbs that contain alkaloid have been reported to display wide spectrum of toxicological effect [12]. Reserpine which is the most active alkaloid have been reported to deplete stores of serotonin and nor-epinephrine in the brain and reduces the accumulation of nor-epinephrine to varying extents in different regions of the brain. However, depletion of central stores of neurotransmitter amines is responsible for the antipsychotic effects and consequently its adverse side effects such as sedation, depression, inability to perform complex tasks and Pseudo-Parkinsonism [13, 14].

Behavioural Study: The EPM test is based on two conflicting innate tendencies; the rodents drive to explore a novel environment and their aversion of height and brightly- lit open spaces [15, 16]. This test has been demonstrated to be bi-directionally sensitive to both anxiolytic drugs; in particular benzodiazepines as well as compounds that induce anxiety in man (anxiogenics e.g. Caffeine) [17, 18] Caffeine decreases the number of entries into the open arm. In contrast, diazepam causes anxiolysis in mice as earlier reported by [19], by increasing the number of entries and percentage time spent in the open arm. In addition to spatio-temporal indicators of anxiety in the EPM, ethological measures of risk assessment, such as head dipping, rearing, grooming, stretch-attend, fecal boli, are also used in the analysis of anxiety.

The group that received the extract showed higher close arm entries with significant open arm activity (which is an anxiolytic-like effect). At extract dose of 100mg/kg the open arm activity was higher, while it decreased at extract dose of 150mg/kg. Decoction preparation of the root of *Sarcocephalus latifolius* have been reported to possess anticonvulsant, Anxiolytic and sedative properties [20]. The effect may be attributed to the presence of flavanoid in the extract. Flavanoid have been reported to cross blood brain barrier and bind with high affinity to the benzodiazepine site of the GABA receptor. Their general bioavailability and particularly their presence in the brain appear to play an important role in the expression of their effects on the CNS. Diazepam increased open arm entries and time spent in open arm and decreased close arm entries. The extracts of *Sarcocephalus latifolius* increased head-dip and rearing but decreased grooming and duration of grooming. Grooming behavior is a displacement response expected to be displayed in a novel environment. Reduced grooming behaviour connotes reduced stress. Therefore, the reduction in grooming frequency and increase in the duration may indicate reduced stress consistent with anxiolytic-like effect. Increase in head dip, rearing is anxiolytic-like 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 [21]. Which agrees with our findings.

Anxiety is also associated with augmented autonomic activity resulting in increased defecation and urination [21]. Although the extracts caused varying effects on defecation and urination, the validity of these parameters as appropriate measures of emotionality remains controversial. Stretch attend posture was not observed in all the experimental groups this equally agrees with the findings of the extract has anxiolytic property.

Histological Studies: *Sarcocephalus latifolius* is reported to have a wide range of medicinal properties and it is commonly used in the treatment of malaria, hypertension, diarrhea and dysentery, dental problems [22, 23, 24]. But no investigation has been made into its effect on the histology of vital body organs especially the brain. In this study the effect of the crude extract on the histology of the temporal lobe was carried out. The study revealed that the crude extract of *S. latifolius* leaf altered the normal microanatomy of the temporal lobe. The effect was in a dose-dependent manner, as the dosage increased the extent of damage equally increased. The histolgical sections at extract dose of 100mg/kg showed vacuolated cytoplasm (plate

2). Vacuolated cytoplasm has been reported to be an adaptive mechanism to limit damage. At extract dose of 150mg/kg (plate 3) the micrograph showed mild infiltration of inflammatory cells, with the loss of temporal lobe tissues. This loss of temporal lobe tissues may affect the limbic structures; thus, memory and learning may be affected and lose of brain functions are possible. This is because temporal lobe has intimate connection with limbic structures (Hippocampus and Amydala) and dysfunction of temporal lobe is a dysfunction of these structures. Temporal lobe epilesy is also characterized pathologically by unique morphological alterations in the hippocampus. The most frequently observed alteration is massive neuronal loss in the hilus of the dentate gyrus and in the pyramidal cell layers [25, 26, 27, 28]. So, with the loss of brain tissues, emergence of temporal lobe epilepsy is possible. There is significant difference between plate 1 and plate 2 and 3. The difference is dose dependent. Thus, the use of the drug should be limited to management of a disease condition.

CONCLUSION: The results of the study showed that crude leaf extract of *Sarcocephalus latifolius* has anxiolytic property and modified the microanatomy of the temporal lobe.

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CONFLICTS OF INTEREST: The authors report no conflicts of interest in this work.

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