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ASSESSMENT OF NEUROBEHAVIOURAL PROPERTY OF ETHANOLIC EXTRACTS OF CULCASIA SCADEN AND ITS EFFECT ON HISTOARCHITECTURE OF TEMPORAL LOBE WISTAR RATS

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ABSTRACT
Culcasia scaden is used traditional for the treatment of various disease conditions. This study was carried out to investigate the neurohavioural properties of the ethanolic leaf extract and its effect on the histoarchitecture of the temporal lobe. Twenty adult Wistar rats were used for this research and were randomly divided in four groups A, B, C and D of five animals each. Group A served as negative control and received 1ml of the distilled water, while B and C served as the experimental group and received 100mg/kg and 150mg/kg per body weight of the animal. Group D was the positive control and received 2.5mg/kg of diazepam. The experiment last for 14 days, on the fourtheenth day, behavioual test was carried out using elevated plus maze. After the behavioural test the animal was sacrificed by chloroform inhalation and the brain harvested and fixed in 10% formol saline and tissue was processed using normal histological techniques and the sections were photomicrographed read by a pathologist. The result showed that the ethonolic extract showed neurohavioural property (anxiogenic property) and altered the histoarchitecture of the temporal lobe of the brain. In conclusion the ethanolic leaf extract of Culcasia scaden affected the neurohaviour of Wistar rat and altered the microanatomy of the temporal lobe.

KEYWORDS: Neurobehaviour, Culcasia scaden, Temporal lobe, histoarhitecture Diazepam.

INTRODUCTION
Humans have been using Plants for medicinal purpose for many years ago and in modern times; they have served as a basis of many pharmaceuticals used today.[1,2] Plants make enormous secondary metabolites for defence against environment stress with other factors like pest attacks, wounds and injuries among others inclusive.[3] Similarly, the produced vast phytocompounds are used to execute vital biological functions and for defence against attack from predators like insect, fungi and herbivorous mammals.[4] These secondary metabolites demonstrate an assortment of therapeutic uses in medical services since ages.[5]

Culcasia scandens P. Beauv. is a plant that grows on trunk of other plants which is of an order; Arales, family; Araceae and subfamily; Aroidae. It is of a native of Africa and about 28 species of this plant exist.[6] *C. scandens* P. Beauv. is an epiphytic with lean and wiry stems that are up to 5 m high. It always clinges to tree trunks by means of clasping roots, and growing on forest and stream margins and savanna from Liberia, Ivory Coast, Sierra Leone, Nigeria and the Cameroun.[7,8]

Traditionally, the plant parts with preparations made from them are used to take care of diversity of infirmities and conditions namely; analgesic for earache, toothache, tonsillitis and stomach complaints.[9] In addition, the plant is used as an anti-emetic, for various skin conditions, imbibed during pregnancy as an anti-abortifacient and for venereal diseases.[8] The sap of the plant is a skin-irritant. It has been reported that the plant is rich in alkaloids.[6] The use of the plant as a fish poison and as veterinary medicine for goat ailments is due to its high alkaloid contents. The mixture of maize seeds with powdered *Culcasia* roots and seeds have been reported to improved crop performance and this could be due to *Culcasia's* insecticidal and repellent properties.[6] The combined leave extract of C. scaden, Costus afer and sarcocephalus latifolius is also used in the treatment of psychiatric disorder in South-eastern Nigeria.[10] Also, the leaves are claimed by local users to exhibit antipoisonous effects against individual bitten by wall

gecko, kocroch etc. Leaves are fragrant and are used as a source of coumarin, a perfume ingredient.[11] The extracts from *Culcasia scandens* have been reported to show antimicrobial activity against *E. coli, S. aureus, B. subtilis, P. aeruginosa* and *S. typhi.*[12]

MATERIALS AND METHOD

Plant

Plant Collection

Fresh leave of Culcasia scandens was sourced from botany Department University of Nigeria Nsukka.

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 Culcasia scaden were washed with distilled water and dried in ventilated room. The dried samples were 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. 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 were 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.[13]

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.[14]

Chemicals

Routine histological reagents such as ethanol, xylene, paraffin wax etc. were purchased from chemical stores.

Animals

Procurement

Fourty (20) 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.*

Ethical approval

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

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.

Table 1: Showing the extract and dosage administered.

Group	Treatment	Dosage
GROUP A	Distilled water	1ml
GROUP B	Extract	100mg/kg
GROUP C	Extract	150mg/kg
GROUPD	Diazepam	2.5mg/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 two weeks. Distilled water (1ml/kg) was given to the animals in group A, the extracts doses of (100mg/kg, 150mg/kg) were given B and C, while D was given (2.5 mg/kg) of diazepam orally.

Behavioural Study

Elevated Plus-Maze Model

The elevated plus-maze study was carried-out using the method described by.[15] 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 is elevated to a height of 50 cm in a dimly lit room. The behaviors that are typically recorded when rodents are in the elevated plus maze are the time spent and entries made on the open and closed arms. Behaviour in

this task (i.e., activity in the open arms) reflects a conflict between the rodent's preference for protected areas (e.g., closed arms) and their innate motivation to explore novel environments. Anti-anxiety behavior (increased open arm time and/or open arm entries) can be determined simultaneously with a measure of spontaneous motor activity (total arm/or closed arm entries), albeit the arm entries made in the maze may not be an optimal measure of motor activity. The time spent on the centre is also a measure of the anxiolytic activities and the number of crossings of the intersections. Beside spatiotemporal measures, ethological measures of risk assessment such as head dip, rearing, grooming and duration of grooming, stretch attend posture, fecal boli etc. are also used.

Behavioual test

One hour post treatment, each rat was 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 they rats cross 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 behavioural study the rats were anaesthetized with chloroform. The brain were harvested and fixed in 10% formol saline for 48 hours. Thereafter the temporal lobe were removed and processed in paraffin wax. The temporal lobe was dehydrated in graded concentration of alcohol, starting with 70% alcohol, 90% and three sets of absolute alcohol. The tissue was cleared using a clearing agent xylene. The essence of clearing was to remove alcohol and to increase the refractive index of the tissue. The tissue was impregnated with paraffin wax. This was done by placing the tissue in paraffin wax in vacuum oven set at 40◦c. The tissue was embedded in a paraffin wax in a mould and sealed to a wooden block. They tissues were sectioned using a microtome set at 5µm. Paraffin wax sections of 5µm were used for histological studies. The sections were floated on warm water and picked with a slide and dried. The tissues were stained using H&E method. The section was covered with a coverslip to prevent scratching of the sections.

Photomicrography: The photomicrographs of the slides were taken and were, subsequently read and interpreted by a pathologist in federal teaching hospital Abakaliki.

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. The data were presented as mean ± SEM.

RESULTS

Phytochemical screening

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

Table 4.2: Results of the quantitative analysis of phytochemical constituents of the extracts.

Phytochemical	Culcasia Scaden
Alkaloid	+
Saponin	+
Flavanoid	+
Tanin	+
Phenol	++
Glycoside	++

Behavioural Studies

Table 4: Showing the effect of crude leaf extract of Culcasia Scaden 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	open arms	close arms
A	4±4.2	292±4.3	3.5±1.12	0.75±0.8	7.5±1.8
B	13.±8.8	284.8±7.6	3±1.6	1±0.7	6.75±2.6
C	18±6.2	266± 21.4	2 ±0.8	1.7±0.47	9.3±1.7
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=4, *(P<0.05), *** (P<0.008)

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

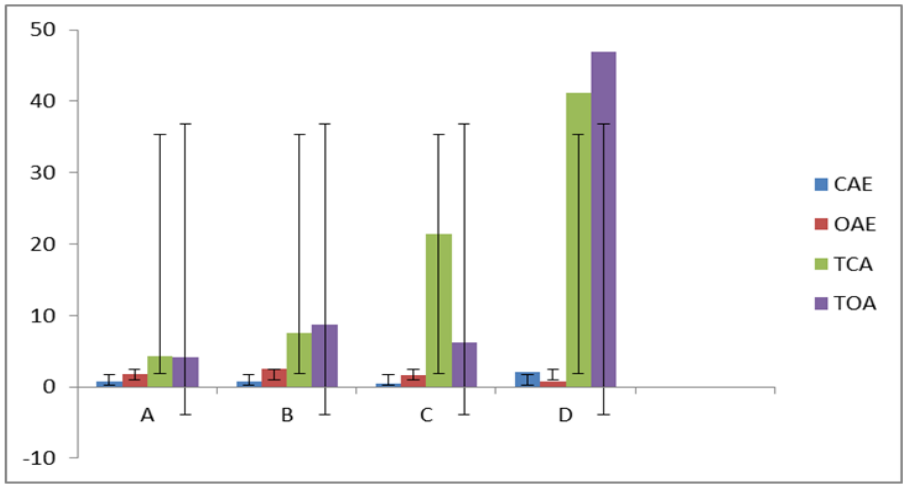

Figure 1: A table showing close arm entries(CAE), open arm entries (OAE), Time spent in close arm (TCA) and time spent in open arm (TOA).

Histology

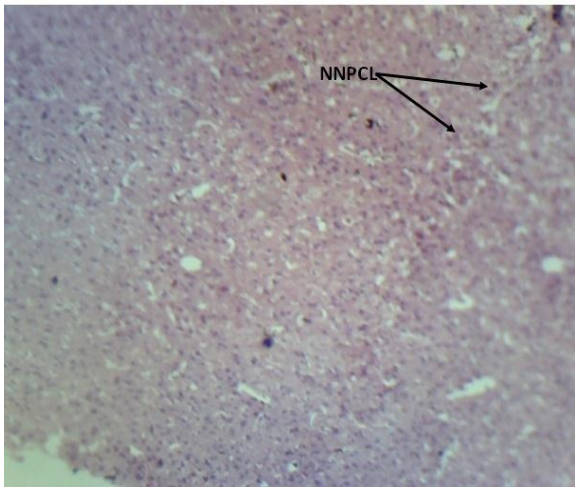

Plate 1: Photomicrograph of wistar rat temporal lobe (control) treated with distilled water showing numerous normal pyramidal cells (NNPCL).:H & E stained x150.

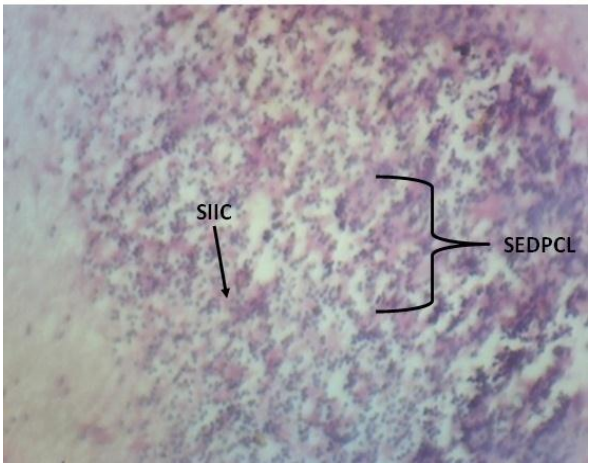

Plate 3: Photomicrograph of wistar rat temporal lobe treated with Culcasia Scaden extract 150mg/kg) showing severe infiltration of inflammatory cells, severe extensive distortion of PCL:H & E stained x150.

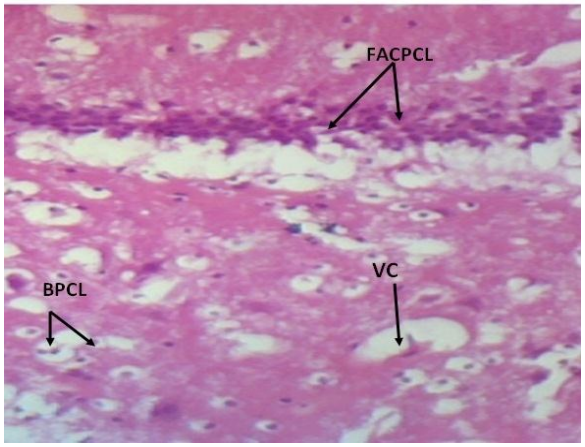

Plate 2: Photomicrograph of wistar rat temporal lobe treated with Culcasia Scaden extract 100mg/kg) showing focal area of clumping of pyramidal cells(FACPL),Vacuolated cytoplasm:H & E stained x150.

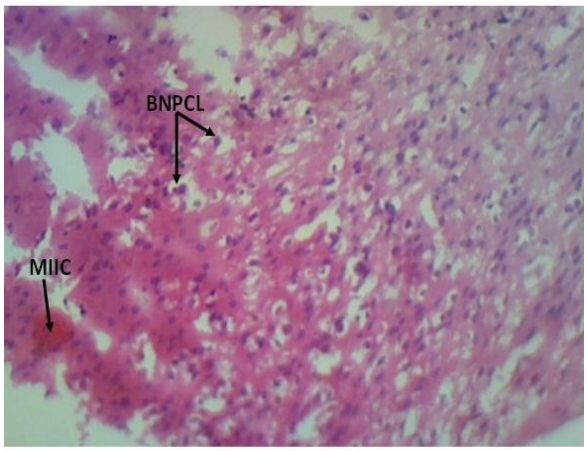

Plate 4: Photomicrograph of wistar rat temporal lobe treated with diazepam 2.5mg/kg) showing mild infiltration of inflammatory cells (MIIC), binucleate cells (BNPCL): H & E stained x150.

DISCUSSION

The pharmacological activities of Culcasia scaden have been reported by different researchers. It has equally been used in traditional medicine for the treatment of various diseases but lack scientific validation.[9] The phytochemical screening of the extracts revealed the presence of alkaloid, saponin, flavonoid, tannin, phenol and glycoside. This agrees with the findings of Uraku.[16] These phytochemicals are responsible for various pharmacological and biochemical actions of the extract when administered to animals. They are produced by as secondary metabolites in plants for defence against predators and environmental factors. These work was carried out to evaluate the neurobehavioural properties of the ethanolic leave extract of Culcasia scaden, as no work have been done to assess its neurobehavioural properties. The result of the study showed increase in closed arm entries and time spent in the closed arm. While there was a decrease in open arm entries and open arm. The markers of effects on neurohaviour 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.[17] These markers are important parameters that validate test agents with neurobehavioural property. There is no argument that the rats in all the experimental groups entered and spent more time in the close arm, a factor that shows anxiety and it can be deduced that the extracts have anxiogenic effect. The leave extract have been reported to show, antimicrobial activity against *E. coli, S. aureus, B. subtilis, P. aeruginosa* and *S. typhi, analgesic activities.*[11,10] But the current study revealed that Culcasia scaden had neurobavioural property and must have acted as an anxiogenic agent.

Despite the widespread use, not many scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. Effects of *Culcasia scaden* on the temporal lobe of the brain were investigated to highlight the possible histoarchitectural changes that could result following its consumption. The temporal lobe is one of the four main lobes of the cerebral cortex. Structures of the limbic system, including the olfactory cortex, amydala, and hippocampus are located within the temporal lobe. The temporal lobes are involved in the retention of visual memories, processing sensory input, language and speech production, storing new memories, emotion, and deriving meaning, auditory perception.[18] The temporal lobe consists of variety of areas which are particularly important in memory and learning.

Plate 1 which serves as the control showed a normal temporal lobe with numerous normal pyramidal cells and a well distributed cell body.

Plate 2&3 shows the temporal lobe of rats treated with 100mg/kg and 150mg/kg of Culcasia scaden, the micrograph revealed focal area of clumping of pyramidal cells. But at extract dose of 150mg/kg the section showed severe infiltration of the neural cells by inflammatory cells plate (3). The extracts of Culcasia scaden may have caused some damage on the temporal lobe. This is evidenced by the infiltration of inflammatory cells. Inflammation and repair are the local responses initiated to limit the damage caused by tissue injury, infection, toxins and ischaemia and to aid recovery from tissue damage. Cellular inflammation is the initiating cause of chronic disease because it disrupts neuronal signaling networks throughout the body. Chronic inflammation is the sustained activation of glial cells and recruitment of other immune cells into the brain. Chronic inflammation is associated with neurodegenerative diseases. Neurodegeneration in Alzheimer's disease is attributed to neuroinflammation.[19] and the leading hypothesis in Parkinson's disease includes neuroinflammation.[20] The effect of the extract was dose dependent, as the dosage increased the effect on the temporal lobe cells increased. The use of this extract for management of disease should be done with caution as this may lead to great damage to the brain.

CONCLUSION

The leave extract of C. scaden has neurobehavioural property, exhibited anxiogenic activities rather than anxiolytic activities and altered the histoarchitecture of the temporal lobe. The histological effect was dose dependent.

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