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Original Research Article

Effect of Combination of Three Herbs (Akaki Extract) on Frontal Lobe of the Albino Wistar Rat

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ABSTRACT

The combination of Costus afer, Sarcocephalus latifolius, and Culcasia scandens referred to Akaki is used in the management of psychiatric disorders. We investigated the effects of Akaki on spatial memory and histology of the frontal lobe. Phytochemical analysis was conducted using standard methods. Twenty rats with average weights of 200g were used and divided into 4 groups of 5 rats each (n=5). The rats in group A served as control received 0.1ml saline while rats in groups B, C & D were treated daily with 3mg/kg, 6mg/kg and 9mg/kg of the extract for seven days. Phytochemical analysis revealed presences saponins, phenol, flavonoids, tannin and glycosides. Behavioural study was performed using the T-maze to assess spatial memory and H&E for histological changes. The extract demonstrated ability to improved spatial memory of the reward arm in the T-maze neurobehavioral test. The mean difference was significant at level ($p<0.05$). The histological study showed dose dependent increase in neuronal population at low doses. The high dose group which received 9 mg/kg of the akaki extract showed focal and liquefactive necrosis in few areas. Akaki extract contain active agents, improved memory and exhibited biphasic dose dependent.

Key words: Akaki, Frontal lobe, Memory, Phytochemical, Neurobehavioral, Histology.

INTRODUCTION

Over the years, plants have been known to play important role in the sustenance of the human race. [1] Generally, plants are used by humans as food, ornaments, and medicinal purposes in the treatment of different ailments. These medicinal plants being natural products are major source of drugs in the pharmaceutical industry when properly synthesized. [2] some of these medicinal plants that possess active compounds commonly used amongst the Igbo's of Eastern Nigeria include; Oka plant also known as *Costus afer*, aki okwuru also known as Culcosiascandens and obala ilu also known as Sarcocephalus latifolius.

Costus afer is a tall perennial herb that has its leaves spirally arranged along its stem. It is a plant species of the genus – *Costus*, and a member of the family – costaceae [3] *Costus afer* is commonly used for different traditional therapeutic purposes which includes; treatment of cough, measles, malaria, eye defects and hunch back. [4] It also has socio-cultural purposes which includes; wrapping of indigenous food items, mat making, feed to small ruminants; and religious purposes like evil repellents. [4]

Sarcocephalus latifolius is commonly seen in the south east of Nigeria and is widely used as herbal remedy in the treatment of various illnesses. [5] It is a plant species belonging to the genus – *Sarcocephalus* and in the family *Rubiaceae*. The sarcocephalus is a flowering plant that can be dually described as shrubs or trees native to tropical Africa. [6] The fleshy fruits of *S.latifolius* are edible, its flowers provide nectar and pollen to bees, its wood (opepe wood) is a source of firewood and aside these, it also has its medicinal purposes. For example, it has been employed in the treatment of fever, pain, dental caries, septic mouth, malaria, dysentery, diarrhea and disease of the central nervous system such as epilepsy. [7-9] the aqueous extract of the leaf has been used as a remedy for diabetes in northern Nigeria. [10]

Culcasia scandens is a climber growing wild in the southern part of Nigeria. It belongs to the order Arales, family Araceae and subfamily Philodendroidea eof the genus *Culcasia*. [11] The leaves of *C. scandens* are popularly used in the treatment of tonsillitis, toothache and other inflammatory conditions in the southern part of Nigeria. [11] The ethno-medicinal purposes such as anti-inflammatory, anti-rheumatic actions [12] and as analgesic properties [13] have been documented.

In the south-eastern part of Nigeria, the combination of these herbs is combined in the management of central nervous systems disorders such as epilepsy and convulsion. The combination of *Costus afer*, *Sarcocephalus latifolius*, and *Culcasia scandens* is referred to as akaki. [5,7-9] This combination was used in traditional management of psychiatric disorders such as epilepsy and mild psychosis. [5] The behavior and neurobiological structure (neuron and glial) of certain areas of the brain are likely to be altered for instance, the activity on anxiety and memory which can influence the integrity of the temporal and frontal lobes. Traditional diagnoses and management of psychiatric disorders can be faulted. The herbal practitioners lack knowledge, skill and tools to make precise diagnoses on the area(s) of the brain affected based on the symptoms presented by clients. In an early study designed to mimic the traditional use of akaki extract, it exhibited antianxiety property on the elevated plus maze behavioral test apparatus and disrupts histoarchitecture of the temporal lobe. [5] It is widely noted that the temporal and frontal lobe functional are pivotal in the assessments neuropsychiatric disorders. In this study we investigated the effect of akaki extract on spatial memory and histology of the frontal lobe. This accesses the nature of effect that might be observed should there be any wrong diagnoses on the frontal lobe or treatment with akaki effect.

MATERIALS AND METHOD

Collection and preparation of the leaves

The fresh leaves of *Costus afer*, *Sarcocephalus latifolius*, and *Culcasia scandens* plants as authenticated by Finbarrs-Bello [5] were gotten from Nkannu-West in Enugu, Nigeria. The leaves were selected and washed in running tap water to avoid unwanted particles and air dried under shade. The dried leaves of the combined plants (Akaki) was then pulverized into fine powder, weighed and stored in air tight container for the experiment.

Phytochemical analysis

The akaki powder was taken to Project Development Institute (PRODA) Enugu for preliminary phytochemical analysis for the qualitative and quantitative analysis of the phytochemical agents: alkaloids, saponins, steroids, phenols, flavonoids, tannins and glycosides using their standard chemical test as demonstrated. [14]

Extraction of plant materials

The 500g of powered leaves was boiled in 1liter of water for 45 minutes from the moment the water started boiling as traditionally applied. The extract was allowed to cool and then filtered with a cheese cloth over a funnel. The residue

gotten from the extract was disposed off and the filtrate referred to as the "Akaki" extract, was evaporated using vacuum evaporator which gave the yield of 20g. This was stored in a bottle and kept in a refrigerator at low temperature.

Animal

Twenty (20) adult Wistar rats with average weight of 200g were purchased from the animal house of the Department of Physiology, College of Medicine, and University of Nigeria Enugu Campus. The animals were housed at the Animal facility of Anatomy Department, Ebonyi State University. The animals were left to acclimatize for two (2) weeks, fed with growers' mash and water *ad-libidum*. Thereafter, the animals were grouped into four, with five rats each in a group (n=5). Group A was taken as the control group, while B, C, and D represent the experimental groups.

Ethical consideration

The experimental procedures and technique used in the study were in accordance with accepted principles for laboratory animal use and care. All protocols used were approved by the Ethics Committee of Faculty of Basic Medicine, Ebonyi State University.

Administration of the Akaki extract

Akaki extract was administered to the rats based on the individual weight of the rats. The dosage was calculated and converted properly using the human dosage administered in the traditional method as a guide. The Akaki extract was administered daily for a period of seven days orally, using an orogastric tube attached to the end of a cannula. The control group received normal saline while groups B, C, and D were administered 3mg/kg/bw, 6 mg/kg/bw and 9 mg/kg/bw orally respectively for 7days.

Neurobehavioral study: T-maze

The T Maze is used to study how rodents function with memory and spatial learning on applying various stimuli. The elevated T-maze apparatus was designed wood as specified for rodents and shaped like the letter T with two turns- left and right arm and a base. The rat was placed at the base which is the starting point of the maze and reward (food) was placed in the left arm of the maze, the rat was allowed to make the choice of which arm to enter. The study was repeated after removal of the reward. The choice of the left arm was considered a right choice (memory) for two trials of 5mins each conducted on the 8th day.

Termination of treatment

At day 8 of akaki administration, the behavioral study was done and the rats were sacrificed under 50mg/kg of thiopental sodium anesthetic. The whole brain was dissected out from the skull of the rats and fixed in 10% formol saline. The frontal lobes of the rats were trimmed out and processed by routine histological technique.

Histological techniques

Fixed specimens were dehydrated in a graded alcohol, cleared in xylene and embedded in paraffin wax. The deparaffinized sections were stained using H&E stain as described by Bancroft and Gamble. [15]

Statistical analysis

The data gotten from the neurobehavioral experiments were presented as mean ± standard deviation values (SDV). The data were analyzed by one way ANOVA followed by Student-Newman–Keuls test. P values for group comparisons were considered significant at $P < 0.05$; $P < 0.01$ between control and treated groups.

RESULT

Table1: Qualitative phytochemical analysis of Akaki powder.

S/N	PARAMETER	WATER (HOT)
1.	Alkaloid	-
2.	Saponin	++
3.	Steroids	-
4.	Phenol	++
5.	Flavonoids	++
6.	Tannin	+
7.	Glycosides	+++

Table2: Quantitative phytochemical analysis of Akaki powder.

Saponin	Phenol	Flavonoids	Glycoside
1.24	1.44	1.12	3.41

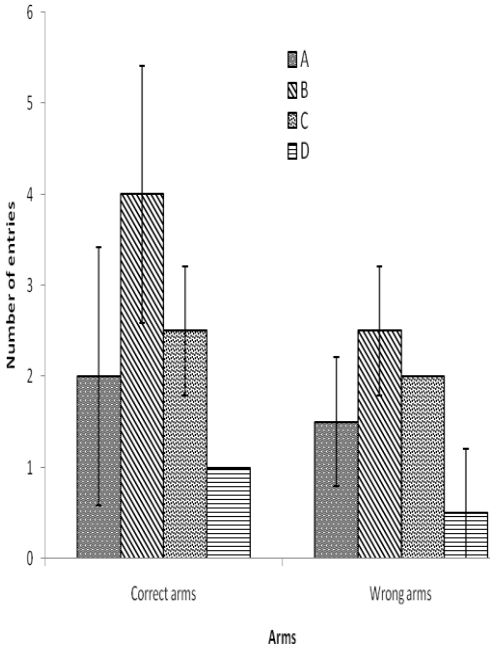

Figure 1: Effect of akaki on the numbers of entries on Elevated T-Maze. Control (A), akaki 3mg/kg (B), akaki 6mg/kg (C), and akaki 9mg/kg (D). Each bar represents mean S.E.M. *P* values for group comparisons were obtained by one way ANOVA followed by Student-Newman–Keuls test. *P* <0.05; *P* <0.01 compared to the vehicle-treated group.

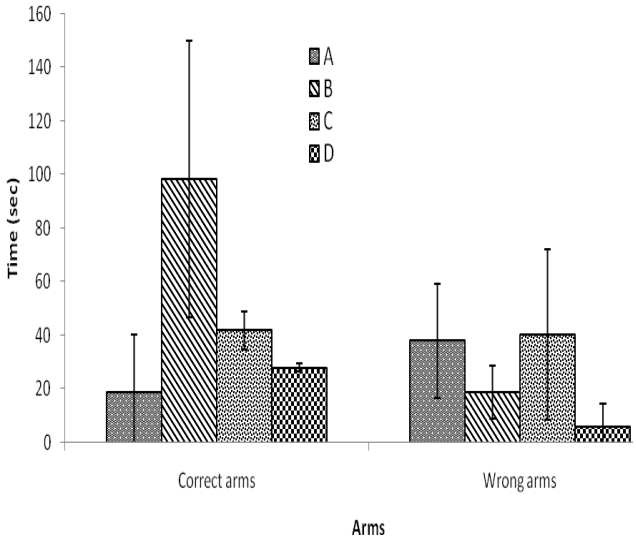

Figure 2: Effect of akaki on the percentage arm entries, and percentage time spent on the arms of Elevated T-Maze. (A) represents control while of (B, C and D) represents 3mg/kg, 6mg/kg, and 9mg/kg (D) of akaki. Each bar represents mean S.E.M. *P* values for group comparisons were obtained by one way ANOVA followed by Student-Newman–Keuls test. *P* <0.05; *P* <0.01 compared to the vehicle-treated group.

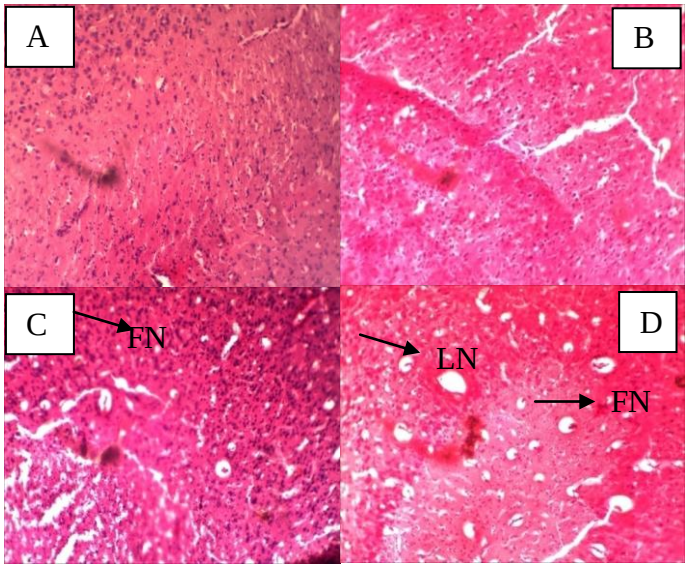

Figure 3: Photomicrographs of the frontal lobe of the akaki treated rats (b, c and d showing focal necrosis (FN), and liquefactive necrosis (LN) with distinct cell bodies of neurons compared to control (a). H& E. ×100. (A) represents control while of (B, C and D) represents 3mg/kg, 6mg/kg, and 9mg/kg (D) of akaki

DISCUSSION

The phytochemical analysis revealed the presence of saponins, phenol, flavonoids, tannin and glycosides while there was absence of alkaloid and steroid in akaki extract (Table 1). The presence of glycoside was higher than the other phytochemicals present. Phenol, flavonoids and saponins were moderate whereas tannin was in trace amount (Table 2). A study reported that glycosides possess neuroprotective property against assault particularly in ischemic stroke. [16] Glycosides are associated with disorders

such as confusion, depression, drowsiness, hallucination, headaches, has also been documented.[17] The phytochemicals present as well as their concentration or loads provide insight on the possible biological activity of this extract.

The T-maze is a neurobehavioral cognitive apparatus that assess short-term memory based on conditioned stimuli where by a reward was kept in one of the arms of the maze. The rats demonstrated they have memory of the correct arm (arm with the reward) on number of entries made and time spent in the arm on the second trial when the reward was removed (Figures 1 & 2). However, the rats in the experimental groups exhibited better spatial memory of the reward arm as compared to the control group. This could have been as a result of the treatment with Akaki extract.

In this present study, the Akaki extract administered to the rats caused changes in the histoarchitecture of the frontal lobe. The changes include increase in the number of cell bodies of neurons in the groups treated with 3mg/kg and 6mg/kg of akaki extract (Figures 3b & c). On the contrary, few areas of focal necrosis and liquefactive necrosis were observed in the group that received higher dose of 9mg/kg (Figure 3d) compared with the control (Figure 3a). In tissue necrosis, the rate is often dependent on the severity of the assault induced by an agent such as chemicals and drugs. [18, 19] Particularly, in the brain the greater the severity of assault, the more rapid the progression of the neuronal damage [20-22] since the main chemicals responsible for inducing cellular damage as observed in neurodegenerative diseases are neurotoxins. [23] In this study, Akaki extract was observed to have increased neuronal population in the lower doses. The degree of changes in the cells of the frontal lobe is dose dependent. However, the frontal lobe is associated with higher brain function such as behavior, organization and motor skills. [24] Therefore, the effect might translate into an improved function of the frontal lobe.

CONCLUSION

The Akaki extract can improve short termed memory, it also exhibited dose dependent biphasic effect that is neuroprotective when administered in low or divided dose and it high dose could be neurotoxic on the frontal lobe of the rats. These effects are possible biological activities of alkaloids, saponins tannins glycosides and flavonoids present in the extract.

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