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ANXIOGENIC AND NEURODEGENERATIVE EFFECTS OF AKAKI EXTRACT ON THE CEREBELLUM OF ADULT WISTAR RATS.

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Abstract

The combination of Costus afer, Sarcocephalus Latifolius and Culcasia Scandens (akaki extract) is used as treatment for various mental disorders in some communities in South-East Nigeria. This study was designed to investigate the effect of akaki extract on the anxiety and histology of the cerebellum of Wistar rats. Phytochemical analysis for the presences of bioactive agents was carried out using standard chemical methods. Twenty rats of average weight 200grams were randomized into four groups (n=5); control group, received feed and water, while the rats in the experimental groups were treated daily with 3mg/kg, 6mg/kg and 9mg/kg of the akaki extract for seven days. On day 8, anxiety text was carried out using the elevated plus maze (EPM) apparatus while cerebellar histoarchtecture was demonstrated by routine haematoxylin and eosin method. Phytochemical analysis revealed presences of saponins, phenols, flavonoids, tannins and glycosides. The extract exhibited anxiogenic effect in the EPM and showed dose dependent neurodegenerative changes (atrophy of neurons) in the experimental groups compared to the control. In conclusion, akaki extract induces neurotoxic and anxiogenic effects on the cerebellum.

Keywords: *Akaki Extract, Phytochemicals, Neurotoxic, Anxiogenic, Cerebellum*

Introduction

Anxiety disorder is characterized by a variety of neuroendocrine, neurotransmitter, and neuroanatomical disruptions [1]. The neurotransmitters in the brain constitute excitatory and inhibitory neuronal networks that maintain a finely tuned balance of activity critical for normal functioning. These include the glutamate and gamma-aminobutyric acid (GABA) [2]. In the healthy brain, gamma-aminobutyric acid (GABA) is regulated by neurons and glia located in different areas of the brain such as the hippocampus, amygdala and cerebellum. In the cerebellum formed the GABAnergic neuron and their receptors which are heterogeneously distributed in cerebellar cortex. Increases in anxiety are accompanied by GABAergic system deregulation which has a large impact on the brain [3]. Notably, to date no available study has examined the impact of anxiety on the cerebellum in brain diseases characterized by GABA dysregulation including anxiety disorders.

Studies have discovered anxigenic and anxiolytic properties of many interesting properties of plants based on the knowledge of their traditional applicability in the treatment of certain disease condition [4, 5]. The massive use of herbs in the treatment of diseases is encouraged by their efficiency, their availability and low cost [6]. Akaki is a combination of three medicinal plants Costus afer, Sarcocephalus latifolius and Culcasia scandens used in folklore medicine in the management of central nervous system disorders.

Costus afer is a genus of perennial tropical herbaceous plant in the family of Costaceae. Its common names are: ginger lily, gingembre spiral or bush cane. In Nigeria it has various ethnic names; Igbos calls it Opote, Okpoto or Okpeteohia; Ukhueroha in Edo, Mbritem in Efik and Ibibio, Kakii-zuwaa in Hausa, Achikku in Tiv and Atare-egun in Yoruba. It is often found in the forest belt throughout tropical Africa [7-9]. The hot water extract of its leaves and stem is widely used traditionally to treat minor epileptic attacks and insomnia [10].

Sarcocephalus latifolius belongs to genus of flowering plant in the Rubiaceae family, a shrubs or tree native of tropical Africa. Common names are African peach, Guinea peach, Negro peach [11]. In Nigeria, it has various ethnic names; Egbesi in Yoruba and Ulefum, Urevum, Ubulu-inu or Obarailu in Igbo [12]. It also has a wide range of medicinal application in the management of epilepsy and depression [13]. It also has anticonvulsant, anxiolytic and sedative properties [14].

The Culcasia scandens belongs to the order Arales, family Araceae and sub-family Philodendroideae of the genus Culcasia [15]. It is an epiphytic climbing herb with slender wiry stems, of the fringing forest and savanna in West Africa [7, 15]. The anti-rheumatic, anti-emetic and anti-abortifacient actions as well as possessing analgesic properties of C. scandens have been documented [16, 17].

In Nkanu community of Enugu State, South-east Nigeria, the decoction of the three herbs (Costus afer, Sarcocephalus latifolius and Culcasia scandens) known as Akaki in this study is used as treatment for various mental disorders. Our previous studies reported adverse effect of the three herbal combinations on the frontal and temporal lobes of the cerebral cortices while investigating the behavioural effects as well [8,9]. We opined in the present study that the alterations in GABAergic neurons which played a major role in changing the neuronal activity observed in anxiety in the areas of the cerebral cortex would likely involve the cerebellum in the holistic view. Thus providing a better understanding of the impact of anxiety on cerebellum and how it may contribute to anxiety from this scientific perspective. Hence, the study investigated the phytochemical constituents, behavioural effect on anxiety and histology of the cerebellum

Methods

Plant material and extract preparation:

The three plants (Costusafar, Sarcocephalus latifolius and Culcasia scanden) were collected from Agbani village, Nkanu, Enugu State, Nigeria (06°19′ 0″North; 07° 33′ 0″ East) during the month of July, 2017. The plants were authenticated at the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. The fresh leaves of Costus afer, Sarcocephalus latifolus and Culcasia scandens were washed thoroughly with distilled

water and room-dried for seven days and pulverised into fine powder. Two hundred (200) grams of each plant power was measured and mixed together, and then the powdered mixture was divided into two portions. A portion was taken to Project Development Institute (PRODA) Enugu for phytochemical analysis, while the rest portion was used for the preparation of the extract.

Three hundred (300) grams of the powder was submerged into 1250 ml of hot water and allowed to boil for 45 minutes as to mimic the traditional or indigenous herbal method. After which it was allowed to stand for 24 hours and filtered using muslin cloth. The filtrate was then concentrated into a green syrupy mass under reduced pressure at 60°C using a rotary evaporator. It was further dried in a hot air oven at 50°C for a week and kept in a refrigerator until further use. The yield was 30.3%. This crude extract is subsequently referred to as akaki extract in this study.

Phytochemical screening:

The presence of saponins, alkaloids, flavonoids, steroids, phenols, glycosides, and tannins in the combined leaves of Costus afer, Sarcocephalus latifolius and Culcasia scandens (Akaki) were tested by their standard methods described by Elizabeth et al. [1].

Test for alkaloids: exactly one millilitre (1ml) of 1% Hydrochloric Acid (HCL) was added to 3ml of the extract in a test tube. The mixture was heated for 20minutes in a water bath, allowed to cool, and then filtered. Two drops of Wagner's reagent were added to 1ml of the filtrate. A reddish brown precipitate observed indicated the presence of alkaloids.

Test for flavonoids: 1ml of 10% sodium hydroxide (NaOH) was added to 3ml of the extract. No yellow coloration was observed in the test indicating the absence of flavonoids.

Test for glycosides: 10ml of 50% tetraoxosulphate (VI) Acid (H_2SO_4) was added to 1 ml of the extract in a test tube. The mixture was heated in boiling water for 15minutes. About 10ml of Fehling solution was added and the mixture was boiled. A brick- red precipitate was observed in the test mixture, which indicated the presences of glycosides.

Test for phenols: a small portion (1ml) of the extract was added to 1ml of water and few drops of 5% NAOH were added. No orange colouration was observed in the test, indicating the absence of phenol.

Test for saponins: the presences of saponins were detected using frothing test. In the test 2ml of the extract was vigorously shaken for 2minutes. Frothing observed in the extract indicated the presences of saponins.

Test for steroids: presence of steroids was investigated using Salkowski test in which 5 drops of concentration (H_2SO_4) were added to 1ml of the extracts. No red coloration was observed in the mixture, indicating the absence of steroids.

Test for tannins: exactly 2 ml of the extract was boiled gently for 2 minutes and allowed to cool. Three drops of ferric chloride solution were added. No green colouration was observed, which indicated the absence of tannins.

Experimental animals:

A total of twenty rats (Wistar rats) 5 to 6 weeks old, and weighing 150g to 180g were obtained from Department of Biotechnology, Ebonyi State University, Abakaliki. The rats were housed individually in a temperature-controlled (20 - 22°C) room under 12 h : h light/dark cycles and had ad libitum access to standard rodent chow food pellets and tap water for two weeks. After this habituation pe¬riod, at the age of 8 weeks, the rats were randomized into 4 equal and weight-matched groups: a control group (controls, n = 5) and experimental groups A, B and C (N = 5 in each group) by generating random numbers using standard. All conditions and handling of the animals were ap¬proved by the Ethics Committee of Faculty of Basic Medical Sciences, Ebonyi State University Certificate Number: FBMEC 440 and conducted according to the Declara¬tion of Helsinki

Experimental design:

The animals were divided into four groups (n=5); the control group received 0.1 ml of saline, while the test groups (A, B & C) received 3 mg/kg, 6 mg/kg and 9 mg/kg of akaki extract daily for seven days as it is been locally prescribed. Behavioural study was carried out (twice) before and at the end of

administration prior to the sacrifice on the 7th day. On the 8th day the animals in all the groups were then anesthetised using 50 mg/kg of thiopental sodium and sacrificed.

Behavioural study (Elevated Plus Maze):

The behavioural test was done at the end of the administration using the elevated plus maze. A rodent anxiety screening test for putative anxiolytic or anxiogenic compound and a general research tool in neurobiological anxiety research. The text settings consist of a plus shape apparatus with two open and two close arms with a transition zone, elevated 40 – 70 cm above the floor. The model is based on rodent aversion of open spaces, which involved avoidance of open areas by limiting movement to enclose spaces or the edge of a bounded space. Anxiety reduction in the elevated plus maze is indicated by an increase in the proportion of time spend in the open arms (time in open arm/total time in open or close arm), an increase in the proportion of entries into the open arm (entries into open arms/ total entries into open or close arm). Total number of arm entries and number of closed arm entries are usually employed as measure of general activity.

Histology:

The brains were isolated from the anesthetized rats as per the study design and fixed in 10% neutral formal saline for 48 hours, and the cerebellum was dissected out. These were then processed for paraffin section (dehydration, embedding, paraffin block, section cutting at 5 µm and staining for H & E.

Results

The phytochemical analyses of the akaki extract showed the presence of relatively higher contents of glycosides followed by phenol, saponins and flavonoids. The concentration of tannins was relatively small, while alkaloids and steroids were absent.

Table 1: Shows the results of behavioural study using Elevated Plus Maze

Figure 1: Histological effect of akaki extract on the cerebellum of adult Wistar rats

Discussion

The extract revealed the presence of glycoside, flavonoids, phenol, saponins and tannin. The result concurs with our previous phytochemical screening on the akaki [8].This suggest concentrations of bioactive agent show geographical consistency because 3 herbs were collected from same location. Literatures have reported the neuroprotective effects of phytochemicals like flavonoids and saponins on the brain [18-20]. However, in this study, these phytochemicals failed to demonstrate neuroprotective effect on the histology of the cerebellum.

The standard elevated plus-maze was used to assess anxiety-like behavior in the rats. This was to investigate the effect of akaki extract on anxiety. Akaki treated rats spent more time in the close arms than in open arms though not significant ($p<0.05$). Normal rats that have not received any anti-anxiety drugs becomes moderately anxious is their new environment thus they tend to prefer the close arms over the less secure open arms [20, 21]. The outcome is in agreement with the finding of Salum et al., [23] which demonstrated that rats show strong preference for the close arms over the open arm which mimicked its natural habitat. Consequently, it suggests akaki extract might have acted as anti-anxiolytic agent or anxiogenic on the rats as they spent more time in the close arm than the control.

Several factors sometime influence the activity of a known drug or agent and the overall outcome of the influencing agent might vary depending on the region and parameter understudy. In this present study, Akaki extract acted as neurotoxin causing sparse cellular population, cellular atrophy and liquefactive necrosis in the cerebellum. Thus, the normal integrity of the neuronal cell bodies as well as the nerve processes is important for normal functioning of the central nervous system. When there is injury or trauma to the neurons, the normal structural integrity of the neurons is disrupted leading to various degenerative changes [24]. The degree of these degenerative changes on the cerebellum due to the administration of akaki extract was observed to be dose dependent. This is in agreement with our previous study which indicated that akaki extract exhibited biphasic dose

dependent effect on the frontal lobe and temporal cortices [8, 9].

Conclusion

This experiment revealed that the oral administration of aqueous akaki extract resulted in cellular adaptive changes such as sparse cellular population hypertrophy of the cells and liquefactive necrosis. Therefore, it can bring about adverse effects on the purkinje and granular cells of the cerebellum and consequently exert effect on stereotype movement behaviour such as seen with anxiogenic agents.

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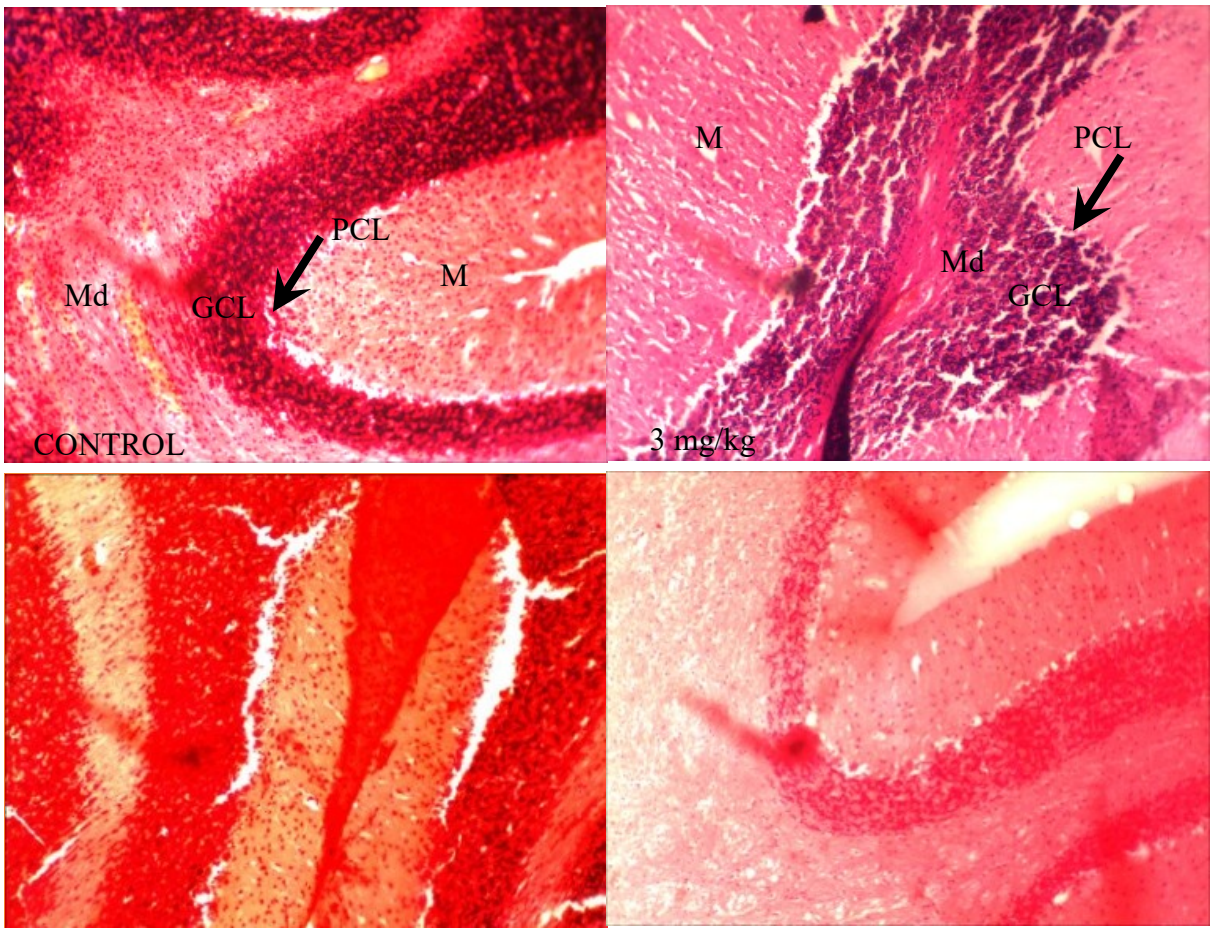
Table 1: Mean ± Std. Dev. values of head dip, arm, entries and duration

Group	Head dips	Open arms	Duration	Closed arms	Duration
control	12.50 ± 0.71	1.50 ± 0.71	6.00 ± 4.24	8.00 ± 1.41	187.50 ± 4.95
3mg/kg	14.50±0.71	0.00±0.00	0.00±0.00	6.00±2.83	219.50±92.63
6mg/kg	17.50±0.71	2.50±0.71	27.50±7.78	9.00±4.24	131.00±25.46
9mg/kg	14.50±0.71	2.00±1.41	11.00±4.24	6.50±0.71	192.00±4. 24

Figure 1.



Photomicrographs of cerebellum the control and treated rats. The control rat shows normal granular cell layer (GCL) and Purkinje cell layer (PCL). 3mg/kg of *akaki* extract showing fewer cells in the Purkinje layer and granular cell layer. 6mg/kg of *akaki* extract showing sparse population and atrophy of cells in purkinje and granular layer. 9mg/kg of *akaki* extract showing increase degeneration of cells in purkinje and granular layers. H&E.x200