Neurohistological Study of the Interactive Influence of Ethanolic Leaf Extract of S*ida acuta* and *Rauvolfia vomitoria* on the Cerebellum of Albino Rats.

Abstract

Background: *Sida acuta* and *Rauvolfia vomitoria* are medicinal plants whose phytochemical analysis have revealed the presence of alkaloids and have also been reported to be useful for the treatment of closely related diseases. It is also efficient for the management of cold, fever and chronic catarrh. There is therefore a need to sufficiently document the interactive influence of the alkaloids and other bioactive constituents of *Sida acuta* and *Rauvolfia vomitoria*.

Objectives: To provide information on the interactive influence of *Sida acuta* and *Rauvolfia vomitoria* on the cerebellum of albino rats using neurohistological parameter.

Method: Thirty-six adult albino rats weighing between 180g and 200g were used for this research. They were randomly divided into six groups of six animals in each group as follows. Group I: The animals in control group were given feed and water *ad libitum*. Group 2: The animals were administered orally with 200mg/kg body weight of *Sida acuta* for 14 days. Group 3: The animals were administered orally with 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days. Group 4: The animals were administered orally with 200mg/kg body weight of *Sida acuta* for 14 days and 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days. Group 5: The animals were administered orally with 400mg/kg body weight of *Sida acuta* for 14 days and 425mg/kg body weight of *Rauvolfia vomitoria* for 14 days. Group 6: The animals were administered orally with 600mg/kg body weight of *Sida acuta* for 14 days and 825mg/kg body weight of *Rauvolfia vomitoria* for 14 days. After 28 days, the animals were sacrificed by chloroform inhalation and their skull opened and brain removed, fixed in 10% buffered formalin, processed and stained with Hematoxylin and Eosin staining technique.

Result: *Sida acuta* at the tested dose of 200mg/kg body weight induced degeneration of Purkinje cells when compared to the control, *Rauvolfia vomitoria* at the tested dose of 212.5kg/mg body weight induced cellular swellings in the Purkinje cell layer, administration of *Rauvolfia vomitoria* after *Sida acuta at* increasing doses significantly reversed these changes when compared with the group that received 200mg/kg body weight of *Sida acuta* for 14 days.

Conclusion: This result suggests that ethanolic leaf extract of *Sida acuta* may cause some neurological damages while *Rauvolfia vomitoria* exert some levels of amelioration possibly through its neuroprotective properties.

Keywords: *Rauvolfia vomitoria*, *Sida acuta*, cerebellum.

Introduction

The use of medicinal plants as a source of therapy and curative aids has attained an indispensable height in the health system all over the globe. Two-third of persons around the world depends on herbal medicine for the maintenance of health.[1] *Sida acuta* is a malvaceous weed and it is one of such medicinal plants that have been used for different medicinal purposes in different part of the world. It is one of the most commonly used plants in Nigeria, used in the treatment of malaria, ulcer, fever, gonorrhoea, poisoning and inflammation.[2] It has been reported to contain tannins, saponins, alkaloids, flavonoid, terpenes and phenolics.

Rauvolfia vomitoria is a large shrub and a member of the Apocynaceae family.[3] It has numerous medicinal uses and has been used across it range. In Nigeria, it is reported to be used in the treatment of hypertension and mental disorders. Phytochemical analysis reveals that *Rauvolfia vomitoria* is rich in indole alkaloids, flavonoid, tannins, saponins and tepernoid.[4] The main alkaloids present include; reserpine, ajmaline, yohimbine, sarpagine etc. Alkaloids have the potential of reducing headache associated with hypertension. It is also efficient for the management of cold, fever and chronic catarrh.[5] The two medicinal plants (*Sida acuta* and *Rauvolfia vomitoria*) are known to be used in the management of closely related illnesses ranging from malaria to mental disorder. It is therefore relevant to study the interactive influence of the two leaves extract.

Materials and Methods

Plant Material and Authentication

Fresh leaves of both *Sida acuta* and *Rauvolfia vomitoria* leaves were obtained from the city of Uyo, Nigeria. They were identified and authenticated by Mrs. E. G. Udoma of the Faculty of Botany, University of Uyo herbarium with voucher number UUPH 6(c) for *Sida acuta* and UUPH 46(e) for *Rauvolfia vomitoria*.

Experimental Animals

Thirty- six adult female albino Wistar rats, weighing between 180 and 200g were used for this study. Institutional ethical clearance for the work was obtained by the ethical clearance committee of the University. The Wistar rats were obtained from the Faculty of Basic medical sciences animal house, University of Uyo, Nigeria. They were transferred to the Faculty of Pharmacy animal house, University of Uyo, where they were acclimatized for one week before administration. They were housed in wooden cages with adequate space to enhance free movement and good ventilation. Saw dust was used as bedding and were replaced every two days. They were allowed twelve-hour light and twelve hour dark cycle at the normal room temperature obtainable in the test environment. The animals were fed with standard rat pelletized diet (Vital Feed Growers, Green Cereals Nigeria Ltd) and water *ad libitum.*

All animals were treated in accordance with the "guide for the care and use of laboratory animals" prepared by the national academy of sciences and published by the national institute of health.[6]

Extracts Preparation

The leaves (*Sida acuta* and *Rauvolfia vomitoria*) were separately washed to remove dirt, air-dried and grinded separately into powder using a manual blender. Extraction was carried out separately. They were macerated and soaked in commercial ethanol for extraction for about 48hours and were then filtered and left separately in hot water bath to concentrate. The stock concentration of the extract was determined to be 30mg/ml and preserve in the refrigerator till use.

Experimental Design

The animals were divided into 6 groups consisting of 6 animals per group.

1. Group 1: Animals in this group served as the control group, they were given food and water only.
2. Group 2: Animals in this group were administered orally with 200mg/kg body weight of *Sida acuta* for 14 days.
3. Group 3: Animals in this group were administered orally with 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days.
4. Group 4: Animals in this group were administered orally with 200mg/kg body weight of *Sida acuta* for 14 days and 212.5mg/kg body weight of *Rauvolfia vomitoria* for another 14days.
5. Group 5: Animals in this group were administered orally with 400mg/kg body weight of *Sida acuta* for 14 days

and 425mg/kg body weight of *Rauvolfia vomitoria* for another 14 days.

6. Group 6: Animals in this group were administered orally with 600mg/kg body weight of *Sida acuta* for 14 days and 850mg/kg body weight of *Rauvolfia vomitoria* for another 14days

Results

Histomorphology

The result of the section of the cerebellar cortex of rats treated with ethanolic leaf extracts of *Sida acuta* and *Rauvolfia vomitoria* on the cerebellum of rats are shown in figures 1-6.

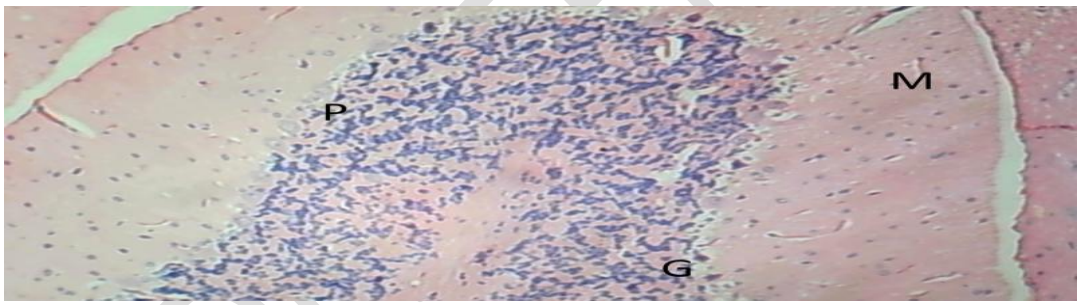

Fig. 1: Section of the cerebellar cortex of the control group showing the outer molecular layer (M), middle Purkinje layer (P), innermost granular layer (G)(H&E x100).

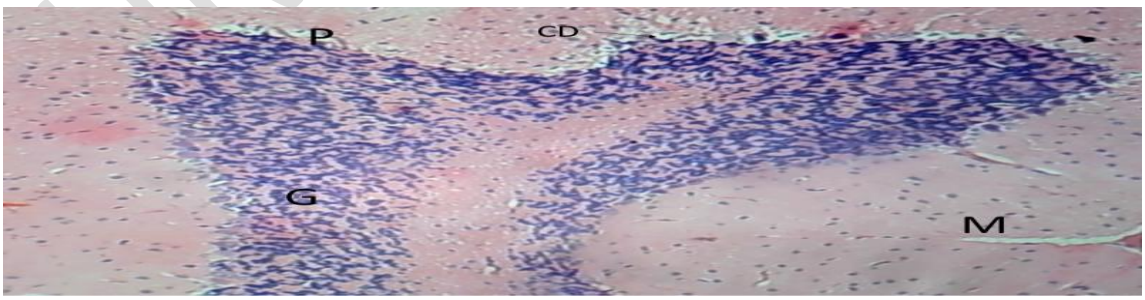

Fig. 2: Section of cerebellar cortex treated with 200mg/kg body weight of *Sida acuta* for 14days showing cellular degeneration (CD) in the Purkinje layer (P) (H&E x100).

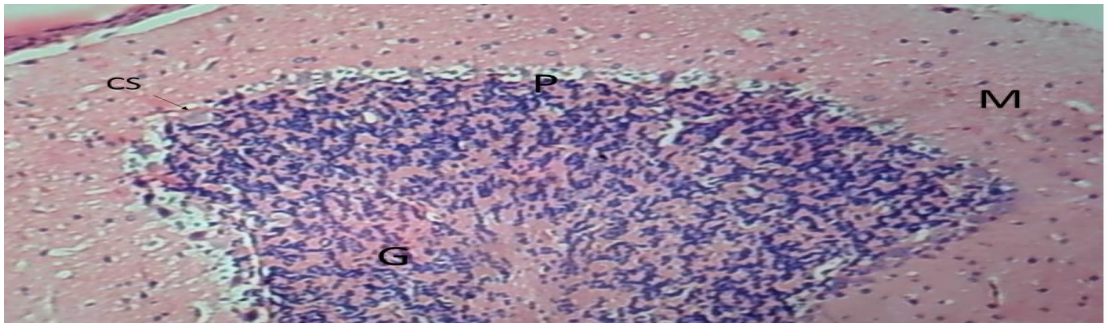

Fig. 3: Section of the cerebellar cortex treated with 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing cellular swelling (CS) in the Purkinje layer (H&E x100).

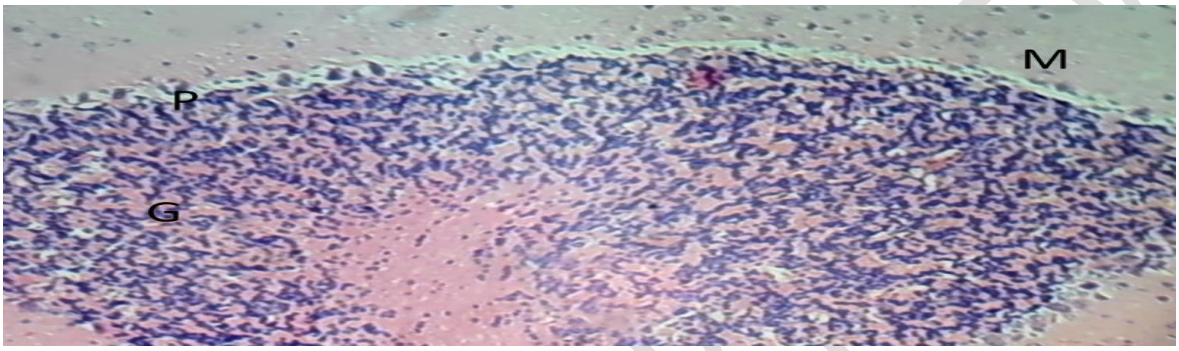

Fig. 4: Section of the cerebellar cortex treated with 200mg/kg body weight of *Sida acuta* for 14 days and 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing mild degeneration in the Purkinje layer (H&E x100).

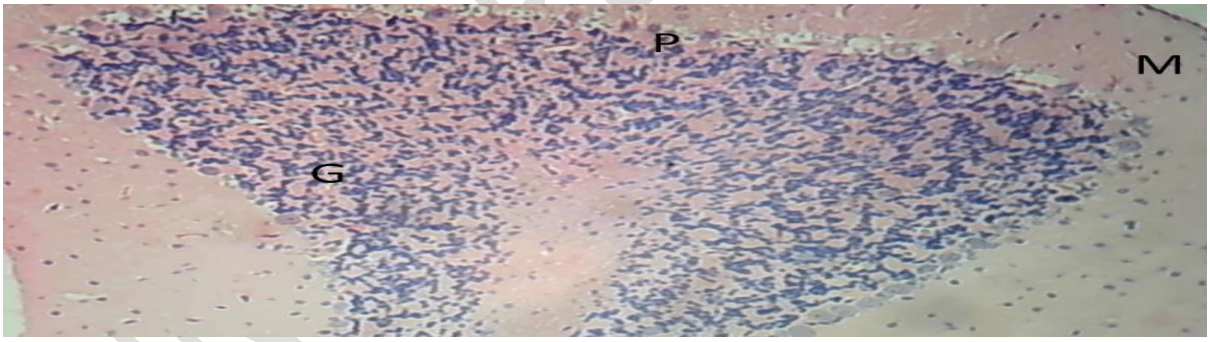

Fig. 5: Section of the cerebellar cortex treated with 400mg/kg body weight of *Sida acuta* for 14 days and 425mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing mild degeneration in the Purkinje layer (H&E x100).

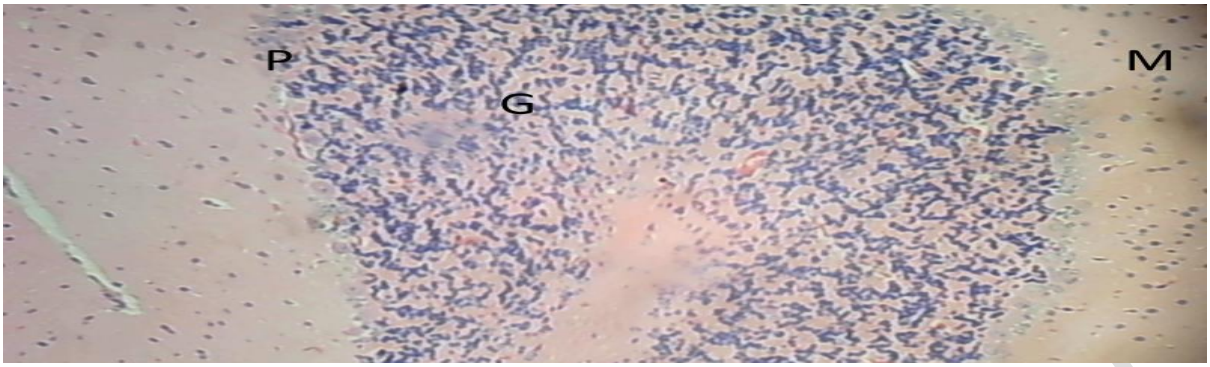

Fig. 6: Section of the cerebellar cortex treated with 600mg/kg body weight of *Sida acuta* for 14 days and 825mg/kg body weight of *Rauvolfia vomitoria* for 14 days showing the outer molecular layer (M), middle Purkinje layer (P), innermost granular layer (G) intact(H&E x100.

Discussion

The cerebellum is the part of the hindbrain in human and other vertebrates. It is responsible for coordination, maintenance of balance and control of muscle tone.[7] It is rich in neurons and about 80% of the brain's neurons are found in the cerebellum. It is made up of two hemispheres that are joined by a vermis and it is subdivided into three lobes namely; the anterior, posterior and the flocculonodular.[8] The cerebellum has an inner core of gray matter surrounded by the white matter. The gray matter is divided into the outer molecular layer, middle Purkinje layer and innermost granular layer.[9] The Purkinje layer consists of the Purkinje cells which are the major excitatory cells of the cerebellum. It receives excitatory signal from the mossy and climbing fibres. The mossy and climbing fibres are the primary inputs to the cerebellar cortex. This study reveals degeneration of the purkinje cells in the group treated with 200mg/kg body weight of *Sida acuta* in figure 2 compared to the control in fig. 1 that showed the normal cytoarchitecture of the cerebellar cortex. Similar effect was observed in rats where hyperplasia of astrocytes was evident in animals treated with 200mg/kg body weight of the ethanolic extract *Sida acuta* and hypoplasia in animals that received 400mg/kg and 600mg/kg body weight of the same extract.[10] The changes observed may be due to the effects of some alkaloids present in the leaf extract of *Sida acuta*. Gas chromatography mass spectrometric analysis of these alkaloids in *Sida acuta,* led to the isolation of two constituents; cryptolepine and quindoline.[11] These indolizidine alkaloids are known to be the toxic constituents of the *Sida* specie which are responsible for neuronal damages in animals.[12] The study also reveals cellular swelling in the Purkinje layer in the group treated with 212.5mg/kg body weight of *Rauvolfia vomitoria* for 14

days presented in figure 3. This finding is in accordance with a similar report which says that *Rauvolfia vomitoria* has adverse effects on the cerebellum with reduced locomotory and exploratory behaviours.[13] This study also witness a progressive improvement in the cytoarchitecture of the cerebellum in the groups administered with progressive dosages of *Sida acuta* for 14days and the *Rauvolfia vomitoria* for another 14 days presented in figures 4, 5 and 6. This progressive reduction in severity of cellular damage with increasing dosage is an indication of a positive interaction between the phytochemicals present in *Sida acuta* and that of *Rauvolfia vomitoria* according to.[14]

Conclusion

This study suggests that ethanolic leaf extract of *Sida acuta* has neuronal damage potentials as seen in the cellular degeneration of the cerebellum. The results of the study also further provide information on the medicinal value of *Rauvolfia vomitoria*. It contains useful alkaloids which are able to interact positively with the biochemicals present in *Sida acuta* and therefore neutralises the adverse effect associated with the use of *Sida acuta* leaf extract.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by any company rather it was funded by personal efforts of the authors.

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