Comparing the adverse impact of Ethanolic Root Bark and Leaf Extracts of *Rauwofia vomitoria* (apocynaceae) on Cerebellar Glycogen in Adult Wistar Rats

Akaninyene M. Okon[1], Eluwa, MA1 Clementina F. Iniodu[2], Gabriel D. Edem[2]*, Ekaette P. Akpan[1]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, University of Calabar
[2]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo

Abstract: The use of herbal medicine in Nigeria is on the increase but unknowingly to consumers, these herbal products may pose severe and devastating health hazards. This study was aimed at studying the adverse impacts posed by either the ethanolic extract ofroot back or leaf extract of *Rauwofia vomitoria* on cerebellar glycogen. In this study, 30 adult Wistar rats were used and were randormly divided into 6 groups (A, B, C, D and E; n =5). Identification of Glycogen was determined using Periodic Acid Schiff method (PAS). From this investigation, there was a marked increase in the staining intensities of the experimental groups. The staining intensity of PAS was higher in the groups C and D which were given 200mg/kg and 300mg/kg of ethanolic extract of *Rauwofia vomitoria* root-bark when compared to groups E and F which received 200mg/kg and 300mg/kg of ethanolic leaf extract of *Rauwofia vomitoria*. Findings in this study suggest a dose-dependent accumulation of glycogen in the neurons of cerebellum, especially in the Purkinje cells. This could be due to the effects of indole alkaloid constituents (reserpine) of *Rauwolfia vomitoria* on glycogen synthesis and utilization.

Keywords: PAS, Rauwofia vomitoria, cerebellum, glycogen, root bark, leaf extract

INTRODUCTION

A number of herbal medicines have been found to be beneficial in the treatment of disorders of the nervous system. An example is *Rauwolfia vomitoria* which have been found to be effective in the treatment of psychosis, stroke, insomnia and convulsion [1], and is used traditionally for the psychiatric management [2]. It is also used for the treatment of snake bites, insect bites, and high blood pressure and has been shown to possess analgesic properties [3]. Crude extracts of *Rauwolfia vomitoria* have been shown to have anti-inflammatory, antipyretic and anti-diabetic effects [4-6] and it has been reported to be relatively safe with a LD50 of 17.5 g/kg [7].

Despite the widespread knowledge and awareness of orthodox medicine, the use of traditional medicine is still very popular in certain parts of the world. Plants have been used for medicinal purposes for as long as history has been recorded. Today, in spite of the availability of pharmaceutical drugs for almost any ailments and diseases imaginable, there seems to be increased patronage for herbal medicine. In most parts of Africa especially in Nigeria, and Asia, certain plants are used to treat ailments.

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses [8]. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients. In some Asian and African countries, 80% of the populations depend on traditional medicine for primary health care [8] but it has been adopted by other populations outside its indigenous culture, and in these cases, is often termed Alternative or Complementary Medicine.

The users of herbal medicines believe that being from natural sources these extracts would be less toxic. However, the use of traditional medicine is fraught with many dangers. For instance, unlike the pharmaceutical drugs which are well-studied, tested and standardized, the use of herbal extracts tends to be arbitrary and crude. There are no standard procedures for extraction. The dosages are arbitrary and there are no standardized procedures of administration. Moreover, their side effects have not been systematically and scientifically evaluated and properly documented [8]. Interestingly, the use of herbal

medicine is characterized by self-prescription and self-medication.

Herbal medicine use without chronic illnesses is quite high in Lagos. In a survey, many of the respondents found herbal medicines to be safe, effective and beneficial [9]. Despite the belief of many of the respondents that herbal medicines rarely produce adverse effects, a few experienced them mildly and moderately. Considering the magnitude of popularity of herbal medicines among the respondents and their levels of ignorance of the potential toxicities, it is necessary to evaluate the safety, efficacy and quality of these preparations and products to minimizing the potential adverse effects [9]. Numerous laboratory and clinical studies have shown that herbal extracts, as well as drugs, can have adverse effects on the histology of the spinal cord and the cerebellum.

MATERIALS AND METHODS
Animal Care
Thirty (30) adult Wistar rats weighing 160g – 230g were obtained from the Department of Biochemistry, University of Calabar. They were housed in the animal house of the Department of Anatomy under standard conditions. The animals were fed with standard diet and allowed access to drinking water *ad libitum*. They were randomly divided into 6 groups (n=5).

Preparation of Extracts
The root-bark and leaves of *Rauwolfia vomitoria* were obtained from the University of Calabar farm, Calabar. They were identified and authenticated by a botanist in the Department of Botany, University of Calabar. The roots and the leaves were washed in water and the root-bark was defoliated and dried. The dried root-bark and leaves were blended into powdered form using a Binatone kitchen blender. The blended sample was soaked in ethanol for 48 hours and the extract was filtered and evaporated to obtain the crude extract.

Experimental procedure
The animals were randomly divided into 6 groups of 5 animals each labelled A, B, C, D, E, F. Groups A and B were the normal control and olive oil control respectively. Groups C, D, E, and F served as the experimental. Group A animals received 0.5ml/200g of normal saline while group B animals received 0.5ml/200g for 7 days respectively. The ethanolic extracts of *Rauwolfia vomitoria* root-bark and leaf were administered orally to the animals with the aid of orogastric tube. After the last dose schedule, the animals were sacrificed using chloroform. The cerebellum was removed and fixed in 10% formol saline solution. The cerebellar sections were stained using periodic acid Schiff method (PAS) for the identification of glycogen.

RESULTS
The glycogen content of the cerebellum was demonstrated using periodic acid Schiff. Sections of the cerebellar cortex of rats in the control groups A and B showed normal glycogen contents in the cerebellum.

There was a marked increase in the staining intensities of the experimental groups. The staining intensity of PAS was higher in the groups C and D which were given 200mg/kg and 300mg/kg of ethanolic extract of *Rauwofia vomitoria* root-bark when compared to groups E and F which received 200mg/kg and 300mg/kg of ethanolic leaf extract of *Rauwofia vomitoria*
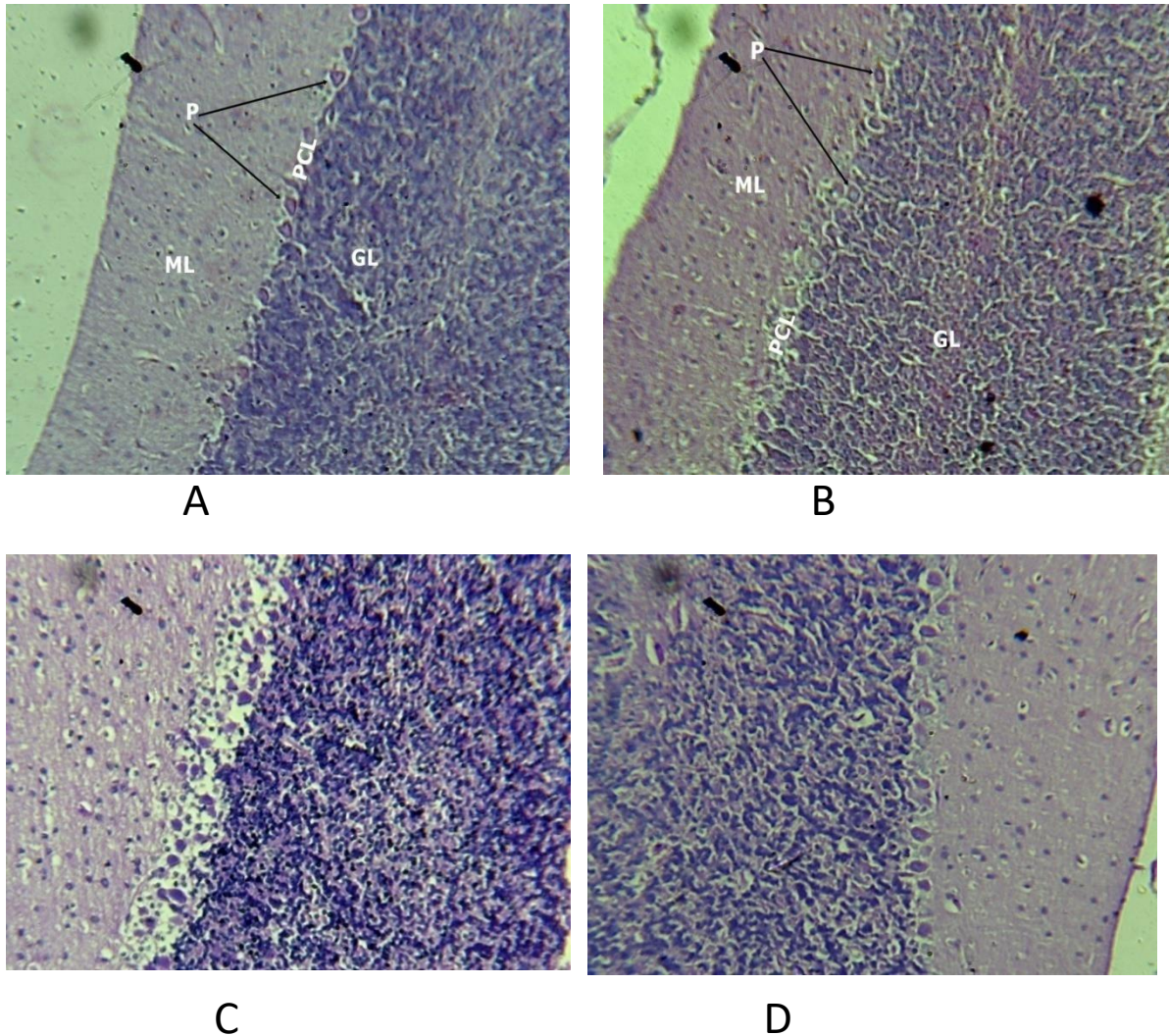
Fig-1: Photomicrographs of cerebellum of normal control, olive oil control and groups that received 200mg/kg root bark and leaf extract (PAS, Mag. X 100)

A: A section of cerebellar cortex of rats of the normal control group showing normal PAS reaction; Molecular layer (ML);Purkinje cell layer (PCL); Granular layer (GL)

B: Cerebellar cortex of olive oil control group rats showing normal PAS reaction; Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL).

C: Cerebellar cortex - 200mg/kg root-bark extract showing a high accumulation of glycogen; Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P).

D: Cerebellar cortex - 200mg/kg leaf extract showing a mild accumulation of glycogen; Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P).
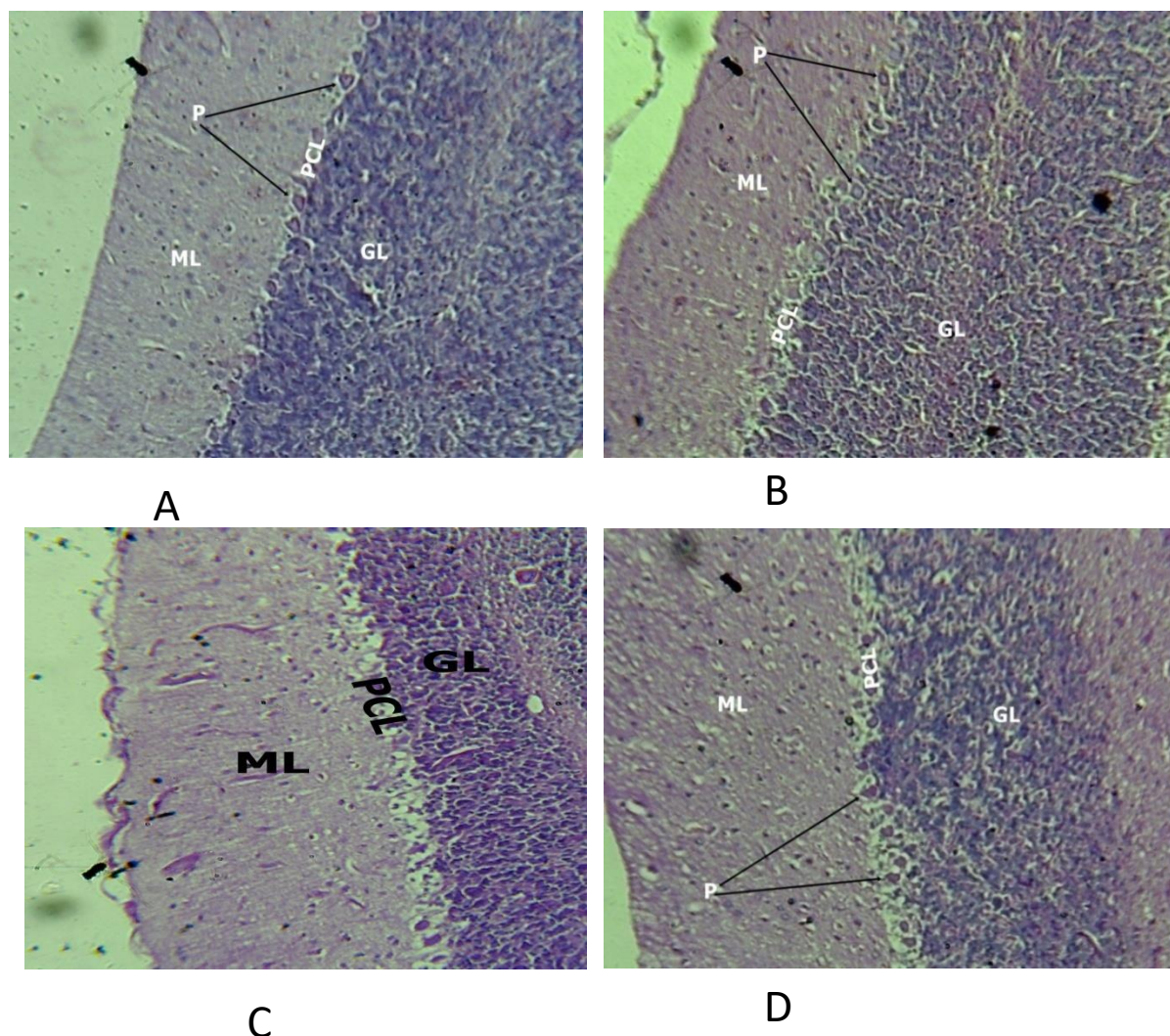
Fig-2: Photomicrographs of cerebellum of normal control, olive oil control and groups that received 300mg/kg root bark and leaf extract (PAS; Mag. X 100)
A: A section of cerebellar cortex of rats of the normal control group showing normal PAS reaction; Molecular layer (ML);Purkinje cell layer (PCL); Granular layer (GL)
B: Cerebellar cortex of olive oil control group rats showing normal PAS reaction; Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL)
C: Cerebellar cortex - 300mg/kg root-bark extract a high accumulation of glycogen; Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P)
D: Cerebellar - 300mg/kg leaf extract showing a mild accumulation of glycogen; Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P)

DISCUSSION

Glycogen is the only significant energy store in brain, and along with its mobilizing enzyme glycogen phosphorylase, is localized almost exclusively to astrocytes [10,11,12]. Utilization of astrocyte glycogen is accelerated both by neuronal activity [13] and lack of energy substrate [14, 15]. Studies using cell culture and optic nerve preparations demonstrate that elevated astrocyte glycogen can improve neuron survival and axon function during glucose deprivation [16, 17]. In vitro studies have further suggested that lactate or pyruvate derived from astrocyte glycogen can be shuttled to neurons for oxidative metabolism [17]. Astrocyte glycogen may also serve to fuel energy-demanding functions of astrocytes themselves, such as glutamate uptake, that influence neuronal survival and function [13].

It has been documented that short-term energy storage in animal cells is usually achieved through the accumulation of glucose, in the form of long and branched chains, known as glycogen. But when this accumulation happens in neurons it is fatal, causing them to degenerate. This neuronal deterioration and

death associated with glycogen accumulation is the hallmark of an extremely rare and progressive type of epilepsy known as Lafora disease (LD). Collaborative research by groups headed by scientists Joan J. Guinovart and Marco Milán at the Institute for Research in Biomedicine (IRB Barcelona) has revealed conclusive evidence about the harmful effects of the accumulation of glucose chains (glycogen) in fly and mouse neurons. Their data clearly indicate that glycogen accumulation alone kills neurons and thus dramatically reduces lifespan of flies and mice as they manipulated the neurons to produce glycogen."

In this study, there was a marked increase in the staining intensities of the experimental groups. The staining intensity of PAS was higher in the groups C and D which were given 200mg/kg and 300mg/kg of ethanolic extract of *Rauwofia vomitoria* root-bark when compared to groups E and F which received 200mg/kg and 300mg/kg of ethanolic leaf extract of *Rauwofia vomitoria*.

Findings in this study suggest a dose-dependent accumulation of glycogen in the neurons, especially in the Purkinje cells. This could be due to the effects of indole alkaloid constituents (reserpine) of Rauwolfia vomitoria on glycogen synthesis and utilization. Glycogen is broken down into glucose-1-phosphate through glycogenolysis in which glycogen phosphorylase catalyse the breakdown of glycogen into glucose-1-phosphate. Utilization of astrocyte glycogen is accelerated by lack of energy substrate [14, 15]. Findings from this study also suggest that reserpine may have inhibited glycogen phosphorylase, thereby causing glycogen to accumulate in the neurons and astrocytes. Previous studies have established that glycogen phosphorylase inhibitors affect glycogen stores and utilization, and can cause glycogen to accumulate, thus, limit the ability of glycogen to be used when needed. Suh *et al.* (2007) [18] reported that an indole carboximide inhibitor of glycogen phosphorylase, CP-316,819, can be used to elevate brain glycogen in normal, awake rats. Other compounds such as MOR-14 [19] and DAB [20] bind to the catalytic sites of the enzymes of glyconeogenesis and cause glycogen accumulation, while Methionine sulfoximine (MSO) increase astrocyte glycogen content through an indirect inhibition of glycogenolysis [21].

Catecholamines stimulate glycogenolysis during the fight and flight response as reported by Hornephrook and Brody (1963) [22] who posited that epinephrine (500 µg/kg, s.c.) caused an increase in skeletal muscle phosphorylase *a* and a decrease in glycogen content in the rat. The mechanism of action through which reserpine exerts its antihypertensive and sedative effects involves the depletion of these neurotransmitters. By depleting catecholamines,

reserpine causes the accumulation of glycogen as obtained in this study.

The present study is in line with findings made by Taksir *et al.* (2007) [23] who reported a duration-dependent glycogen accumulation in the Purkinje cells and the granular layer in Pompe Mouse model. Glycogen has been found to accumulate in large motor neurons and astroglia in the peripheral anterior horns due to abdominal aortic ligation-induced partial ischemia of the spinal cord in adult cats [24]. This study is also in line with findings by Iriye and Simmonds (1971) [25] who reported that tranquilizers consisting of reserpine and four phenothiazines caused the depression of glycogen phosphorylase activity in rat brain. Gey *et al.* (1965) [26] also reported that reserpine, chlorpromazine and phenobarbital decreased glucose-6-phosphate and fructose-6-phosphate at the time of maximal depression of the motor activity. According them, reserpine and phenobarbital, but not chlorpromazine, caused an accumulation of glycogen following the time of maximal sedation, and this supports the hypothesis that central depressant drugs suppress glycolysis in the central nervous system in vivo possibly by a diminution of glucose phosphorylation.The present study is also in agreement with the findings made by Suzuki and Ito (1974) [27] who reported that a large dose of reserpine induced a prominent transient accumulation of glycogen in the choroidal epithelial cells.

CONCLUSION
It may be concluded that the root bark extract of *Rauwofia vomitoria* is more toxic than the leaf extract. This is evident in the accumulation of glycogen in the cerebellum which may cause neuronal deterioration and death.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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