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Article

Characterization of Hippocampal Histomorphology Following *R. vomitoria* Root Extract Treatment

Rauwolfia vomitoria Induces Hippocampal Alteration

Caracterización de la histomorfología del hipocampo después del tratamiento con extracto de la raíz de *R. vomitoria*

Rauwolfia vomitoria induce alteración del hipocampo

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ABSTRACT

Rauwolfia vomitoria Afzel. is an antipsychotic plant used by several African communities in the management of psychiatric conditions with good outcomes. Concerns about its dosages on brain activity lead to this investigation of its action on the hippocampal microstructure.

Twenty-four adult male Wistar rats of average weight 200 g, were assigned into four groups (n = 6): control; 200, 300 and 400 mg/kg body weight of RV root bark extract, respectively. The administration was once daily, and orally for seven days. Daily observation of the animals was done till on day eight when they were sacrificed after deep anaesthesia. Each brain was processed for histology and immunohistochemical studies.

Animals in the 200, 300 and 400 mg/kg RV groups appeared generally dull and drowsy, and barely fed. Their hippocampal histology showed neuronal atrophy and karyorrhexis, with no difference in cell count, although the pyramidal cell numbers decreased in the 300 and 400 mg/kg RV groups. Neuron-specific enolase decreased in the 400 mg/kg RV group, while neurofilament decreased in all test groups. Glial fibrillary acidic protein expression and density increased in the 200 and 300 mg/kg RV groups, but not the 400 mg/kg RV group, all compared with the control group.

The given doses of RV root bark extract in adult Wistar rats showed sedative activities with hippocampal histopathological changes, which may not be reversible, thereby leading to the hippocampal functional deficit.

Keywords: *Rauwolfia vomitoria*; Histology, Immunohistochemistry; Hippocampus; Wistar rats

1. Introduction

The application of herbal medications has expanded beyond local practice (Ekor, 2013; Welz *et al.*, 2018). Adverts of different brands of herbal products stating their efficacies now floods most streets and the mass media of developing countries (Ayimey *et al.*, 2013; Chattaraj *et al.*, 2018). These products are promoted for dieting and treatments of numerous ailments, thus, exposing the unsuspecting populace to unwanted and adverse outcomes.

Researches on herbs and herbal products have also increased with reports showing their beneficial potentials. Herbs such as *Gongronema latifolium, Moringa oleifera* and *Rauwolfia vomitoria* among others, have been reported to be of immense benefit to the human race (Akpanabiatu *et al.*, 2009; Ekong *et al.*, 2015a; Ekong *et al.*, 2017a; Ekong *et al.*, 2017b). In the treatment of psychiatry conditions, *R. vomitoria* (RV) feature prominently (Obembe, 2001; Amoateng *et al.*, 2018).

This antipsychotic plant belongs to the Apocynaceae family, and is made up of several bioactive carboline and indole alkaloids (Burkill, 2002; Okereke *et al.*, 2017), which give the pharmacological activities associated with the herb. Two of the alkaloids whose properties are well characterized are reserpine and yohimbine. Others include; rauwolfine, rescinnamine, serpentine, ajmaline, serpentinine, alstonine and saponin among others (Iwu and Court, 1977).

R. vomitoria is commonly used locally in the treatment of snake and insect bites, and is reported to protect the body generally (Obembe, 2001; Amoateng *et al.*, 2018). Reported researches on RV shows that it promotes body weight loss, lowers blood pressure, sedates and detoxifies, as well as being antipyrexic among others (Amole and Onabanjo, 1999; Eluwa *et al.*, 2008; Ekong *et al.*, 2015a). Previous studies have also showed that RV ameliorated neurotoxicity caused by chlorpromazine and *Sida acuta* leaf extract (Ajao *et al.*, 2015; Okon *et al.*, 2020). However, adverse reports such as; hepatotoxicity and cardiotoxicity have also been reported (Eluwa *et al.*, 2013a; 2013b).

RV effect on the central nervous system may be detrimental, especially with its constituent, reserpine, inhibiting monoamine oxidase, vital for the normal synaptic neurotransmission necessary for the control of motor and cognitive functions (Yu *et al.*, 2011; Mirowska-Guzel, and Balkowiec-Iskra, 2014). Behaviourally, RV administration results in decreased locomotor activities and loss of olfaction among others (Eluwa *et al.*, 2008; Ekong *et al.*, 2014, 2016a; Bisong *et al.*, 2019). Microstructural reports include alterations in the cerebellum, cerebral cortex and the olfactory bulb (Ekong *et al.*, 2015a, 2015b, 2016a; Ekong and Nwakanma, 2017). Dopaminergic neurons, numerous in the hippocampus are either inhibited or depleted by the RV constituent, reserpine

(Metzger *et al*., 2002), coupled with highly limited data on RV effect on hippocampal structure, which warrants further investigation on this brain area. Thus, this study investigated the hippocampal histomorphology following treatment with RV.

2. Materials and methods

Twenty-four adult male Wistar rats of average weight 200 g were divided into four groups (n = 6) of control, 200, 300 and 400 mg of RV per kilogram body weight (kg bw). Dosages were arrived at following a previous study (Ekong *et al.,* 2016b). The animals were kept at the animal house of the Faculty of Basic Medical Sciences of the University at room temperature of 27 - 30 °C and 12 hours light and dark cycle, and were allowed access to standard rat chow (Vital Feed Company Limited, Nigeria) and clean water *ad libitum*. The animals were maintained and cared for strictly following the guidelines for the care and use of experimental animals, while the experimental protocol was approved by the Faculty of Basic Medical Sciences Ethical Committee, University of Uyo, Nigeria.

3. Preparation of *Rauwolfia vomitoria* Roots

Roots of RV were harvested in a local farm at Ekpene Obo in Esit Eket Local Government Area of Akwa Ibom State, Nigeria. The RV plant was authenticated by the botanist of the Plant Herbarium unit of the Department of Botany and Ecological Studies of the University, and specimen voucher number UUPH 6(c) assigned. The roots were washed and the bark was separated from the cambium, air-dried for one week and then pulverized into powder using a kitchen blender. The RV root bark powder was extracted using 80% ethanol and processed as previously described (Ekong *et al*., 2014, 2016a).

Briefly, 200 g of the composite sample was soaked in 80% ethanol for 24 h, and the extract was filtered and concentrated using a rotary evaporator and then dried in a Plus 11 Gallenkamp oven at 45-50 °C. The dry extracts obtained were stored in a refrigerator at 4 °C until used. Distilled water was used as the vehicle to dissolve the extract. Two grams of the RV extract was dissolved in 30 mL of distilled water, and the actual dosages were calculated based on the body weights of each rat.

The control group was administered 5.0 mL/kg of distilled water orally (p.o), while groups 2, 3 and 4 were administered oral doses of 200 mg/kg, 300 mg/kg and 400 mg/kg body weights of the extract of RV (p.o) respectively, for seven days as previously reported (Ekong *et al*., 2016a). On day 8, the animals were sacrificed after deep anaesthesia with ketamine hydrochloride (Rotex Medical, Germany; 50 mg/kg, i.p). Each animal was initially perfused with 1M phosphate-buffered saline, and subsequently by 10 % buffered formalin through cardiac puncture. The brains were removed, post-fixed in 10 % buffered formalin for 48 h, and routinely processed for paraffin wax embedding. Paraffin sections of 10 µm thickness were then processed for histology with haematoxylin and eosin stains (H&E) and immunolabelled for neuron-specific enolase (NSE), neurofilament (NF) and glial fibrillary acidic protein (GFAP).

Immunolabelling was done as previously described (Ekong *et al*., 2016a). Briefly, serial paraffin sections on slides were brought to water, and antigen retrieval was performed using citrate buffer (pH 6.0) in a microwave oven for 5 min, followed by protein block using 3% hydrogen peroxide for 10 min. Sections were thereafter pre-incubated in 2% normal goat serum for 30 min and incubated in monoclonal mouse anti-enolase-2 for NSE (Novocastra, Leica Biosystems, 22C9, 1:100),

mouse monoclonal anti neurofilament (NCL-NF 68-DA2, Novocastra, Leica, 1:100) for NF, and mouse monoclonal anti-GFAP (1:100) for GFAP (NCL-L-GFAP-GA5). These were followed by 1 h incubation in goat anti-mouse secondary antibodies (1:100), for all of them. Detection of the reaction was by means of the avidin-biotin complex with diaminobenzidine as the chromagen. Sections were then counterstained with haematoxylin, washed, dehydrated, cleared, and coverslipped with DPX. Processed slides were viewed under the light microscope, and photomicrographs obtained using a computer-assisted digital microscope camera.

Cellular density was determined manually using ImageJ® software (Ekong and Nwakanma 2017). Briefly, images of a two hundred and forty square millimetre (240 mm^2) area of the dorsal hippocampal CA1 region (three slices at 10 µm each) per animal were quantified, making a total of 18 slices per group ($n = 6 \times$ brain slices $= 3$). Each section was randomly mapped with the ImageJ® gridlines. Counting of cell nuclei was done manually, taking into consideration the nuclei on the upper and right borders of the mapped areas. One way analysis of variance was used to analyze obtained data, followed with posthoc Tukey's test. Data are presented as Mean ± Standard Error of Mean, and data with probability level $p \leq 0.05$ is regarded as significant.

4. Results

General observations

Before the experiments, each group of animals appeared healthy and agile, with normal solid dark faecal boli. During the course and termination of the experiment, the animals in the groups administered the different doses of RV appeared generally dull and drowsy, and barely fed compared with the control.

Histomorphology

The section of the dorsal hippocampal CA1 region of the control group showed normal histological features: outer molecular, pyramidal and polymorphic layers. The molecular layer contained mostly nerve processes with scanty cells. The pyramidal layer contained a dense population of large pyramidal shaped neurons with other cell types within, while the polymorphic layer contained mostly neuronal fibres with sparse cells (Figure 1a).

The dorsal hippocampal CA1 histology of the RV groups showed the same tripartite layers. However the 200 mg/kg RV group showed few pyramidal cells with atrophic appearances (Figure 1b). The 300 mg/kg RV group showed some of the pyramidal cells being karyorrhectic (Figure 1c), while the 400 mg/kg RV group showed some of the pyramidal cells also being karyorrhectic (Figure 1d).

The cellular density of the dorsal hippocampal CA1 region (240 mm^2 area) showed no significant ($p > 0.05$) difference between the RV groups and the control, and among the RV groups. Nevertheless, there were significantly ($p < 0.05$) less pyramidal cell density in the 300 and 400 mg/kg RV groups compared with the control and the 200 mg/kg RV groups., while there was no significant ($p > 0.05$) difference between the 200 mg/kg RV and control groups (Figure 2).

The density of the other cell types showed no significant ($p > 0.05$) difference between the 200 and 300 mg/kg RV groups, but a significantly ($p < 0.05$) higher density of the 400 mg/kg group compared with the control group. There was also significantly ($p < 0.05$) higher density of the 300 and 400 mg/kg RV groups compared with the 200 mg/kg RV group, but not among the 300 and 400 mg/kg RV groups (Figure 2).

Immunohistochemistry

In neuron-specific enolase (NSE) immunolabelling, the dorsal hippocampal CA1 of the control group showed NSE positive cells expressed in the neuronal cytoplasm, and especially in the pyramidal layer (Figure 3a). The 200 mg/kg RV group showed slightly increased expression of NSE compared with the control group (Figure 3b). The 300 mg/kg and 400 mg/kg RV groups showed increased expression of NSE compared with the control group (Figure 3c and d).

The CA1 NSE labelled cells were significantly ($p < 0.05$) less in the 400 mg/kg RV group, but not significantly ($p > 0.05$) different in the 200 and 300 mg/kg RV groups compared with the control group, and among the RV groups (Figure 4).

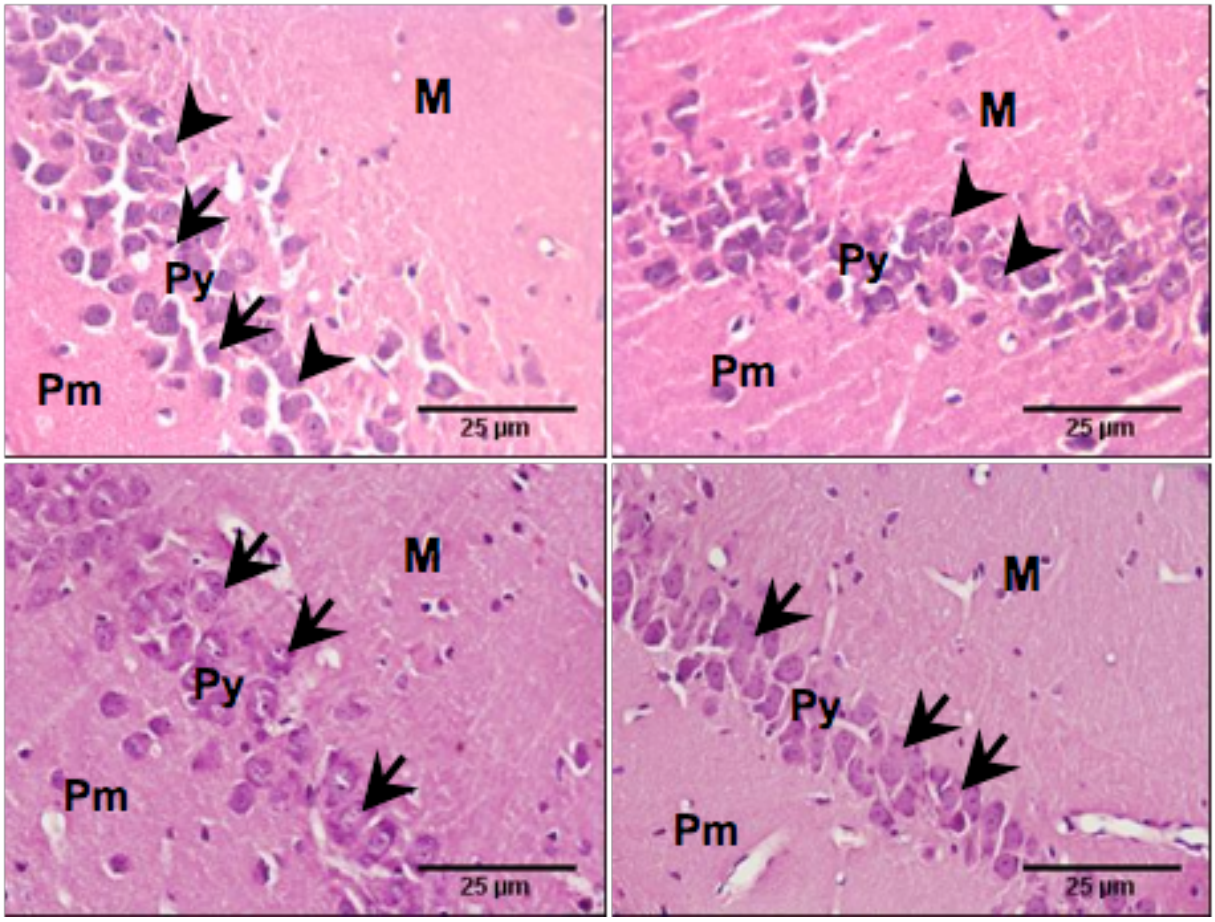
In neurofilament (NF) immunolabelling, the dorsal hippocampal CA1 of the control group showed NF positive cells throughout the cortical layers, especially in the neuronal processes (Figure 5a). The 200 mg/kg RV group showed decreased expression of NF compared with the control group (Figure 5b). The 300 mg/kg and 400 mg/kg RV groups also showed decreased expression of NF compared with the control group (Figure 5c and d).

The dorsal hippocampal CA1 NF labelled cells in the (240 mm^2 area) was significantly ($p < 0.05$) less in the 200, 300 and 400 mg/kg RV groups compared with the control. However, there was no significant ($p > 0.05$) difference among these RV groups (Figure 6).

In glial fibrillary acidic protein (GFAP) immunolabelling, the dorsal hippocampal CA1 of the control group showed GFAP positive cells throughout the cortical layers. The GFAP expression was mostly in the astrocytic processes, although some of their soma also showed expressions (Figure 7a). The 200 mg/kg RV group showed increased expression of GFAP throughout the cortical layers, in the astrocytic processes and soma compared with the control group (Figure 7b). The 300 mg/kg RV group showed increased expression of GFAP mostly in the astrocytic processes, throughout the cortical layers, while the 400 mg/kg RV group showed decreased expression of GFAP in the astrocytic processes and soma throughout the cortical layers compared with the control group (Figure 7c and d).

The dorsal hippocampal CA1 GFAP labelled cells were significantly ($p < 0.05$) high in the 200 and 300 mg/kg RV groups, with no significant ($p > 0.05$) difference in the 400 mg/kg RV group compared with the control. There was significantly ($p < 0.05$) high GFAP labelled cell density in the 200 and 300 mg/kg RV groups compared with the 400 mg/kg RV group, but not among the 200 and 300 mg/kg RV groups (Figure 8).

Figure 1:
Sections of the dorsal hippocampal CA1 region of the test and control groups: outer molecular (M), pyramidal (Py) and polymorphic (Pm) layers. H&E, ×200



a. The control group shows normal histological features. The molecular layer is mostly nerve processes and contained scanty cells. The pyramidal layer contained a dense population of large pyramidal shaped neurons (arrow head) with other cell types (arrow) within. The polymorphic layer has mostly neuronal fibres with sparse cell as well.

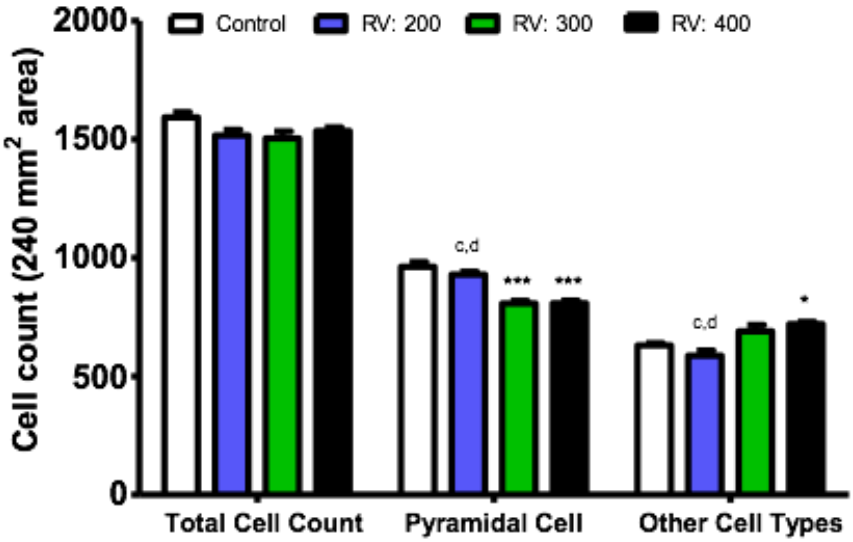
b. The 200 mg/kg RV group shows few of the pyramidal cells having atrophic appearances (arrow head).

c. The 300 mg/kg RV group shows some of the pyramidal cells being karyorrhectic (arrow).

d. The 400 mg/kg RV group shows some of the pyramidal cells being karyorrhectic (arrow).

RV – *Rauwolfia vomitoria*

Figure 2:
Dorsal hippocampal CA1 region cellular population estimate



Data are presented as mean ± standard error of mean

***Significantly different from the control group at $p < 0.001$

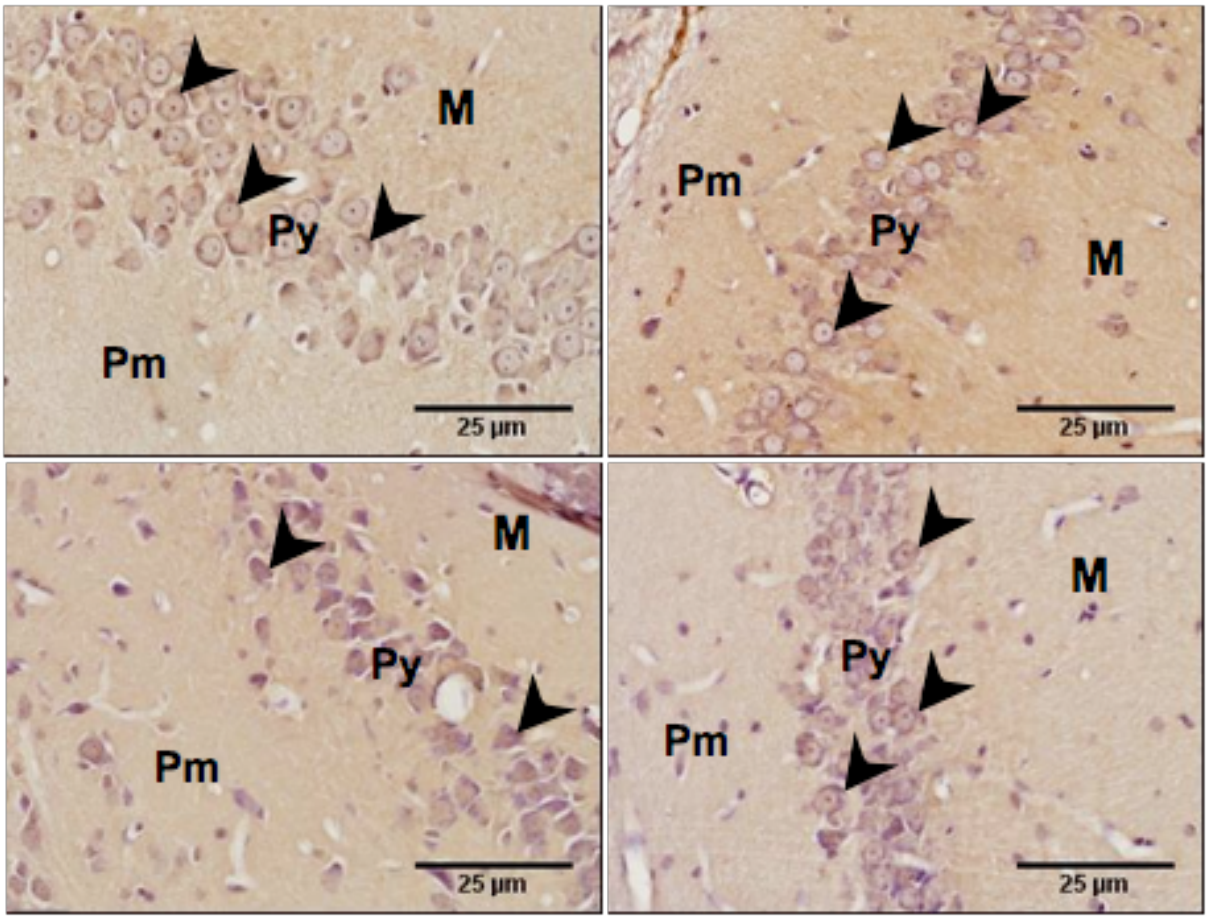
c Significantly different from the RV: 300 mg/kg group at $p < 0.05$

d Significantly different from the RV: 400 mg/kg group at $p < 0.05$

RV – *Rauwolfia vomitoria*

(n = 6, brain slices = 3)

Figure 3:
Sections of neuron specific enolase (NSE) immunolabelled dorsal hippocampal CA1 region of the test and control groups: outer molecular (M), pyramidal (Py) and polymorphic (Pm) layers. NSE, ×200



a. The control group shows NSE (arrow head) positive cells in the neuronal cytoplasmic surfaces especially in the pyramidal layer.

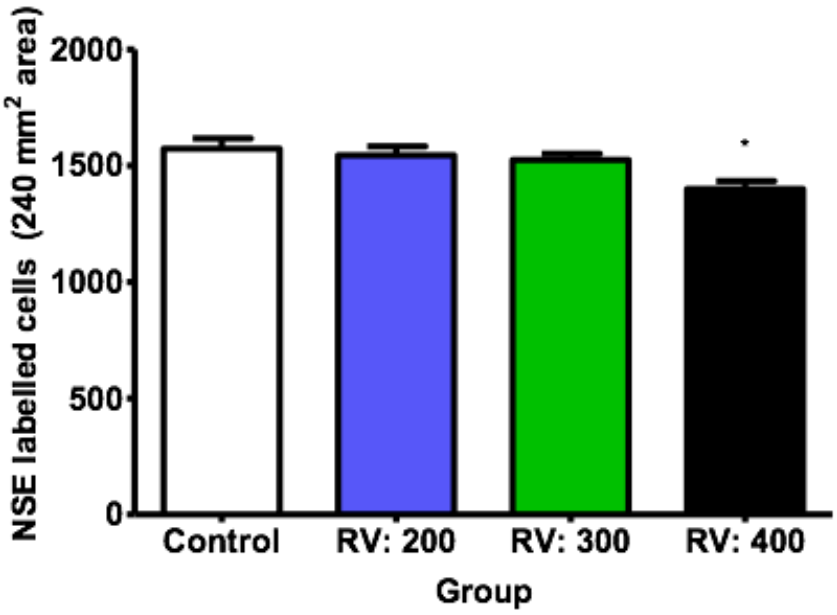
b. The 200 mg/kg RV group shows slight increased expression of NSE (arrow head) in the neuronal cytoplasm in the pyramidal layer.

c. The 300 mg/kg RV group shows increased expression of NSE (arrow head) in the neuronal cytoplasm in the pyramidal layer.

d. The 400 mg/kg RV group shows increased expression of NSE (arrow head) in the neuronal cytoplasm in the pyramidal layer.

RV – *Rauwolfia vomitoria*

Figure 4:
Dorsal hippocampal CA1 region NSE labelled cells estimate



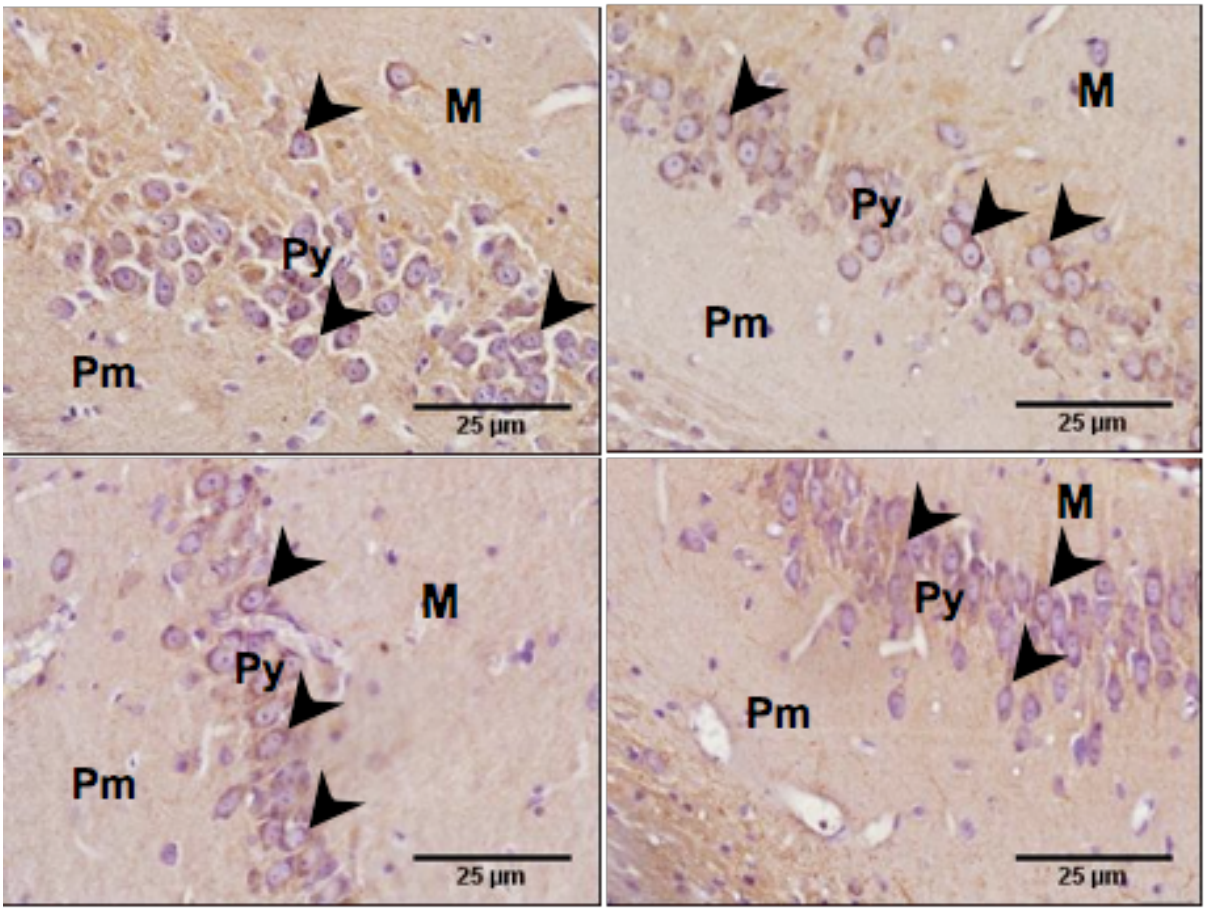
Data are presented as mean ± standard error of mean

* Significantly different from the control group at $p < 0.001$

NSE = neuron specific enolase; RV – *Rauwolfia vomitoria*

(n = 6, brain slices = 3)

Figure 5:
Sections of neurofilament (NF) immunolabelled dorsal hippocampal CA1 region of the test and control groups: outer molecular (M), pyramidal (Py) and polymorphic (Pm) layers. NF, ×200



a. The control group shows NF (arrow head) positive cells throughout the cortical layers, especially in the neuronal processes and neurolemma.

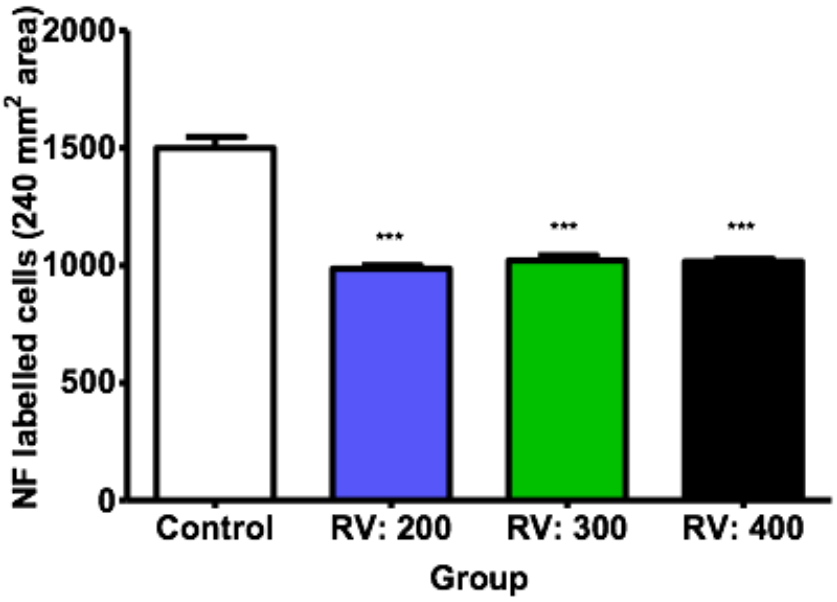
b. The 200 mg/kg RV group shows decreased expression of NF (arrow head) in the neuronal processes, although NF expression in the neurolemma was not affected.

c. The 300 mg/kg RV group shows decreased expression of NF (arrow head) in the neuronal processes, with slight decreased neurolemmal NF expression.

d. The 400 mg/kg RV group shows decreased expression of NF (arrow head) in the neuronal processes, with slight decreased NF neurolemmal expression.

RV – *Rauwolfia vomitoria*

Figure 6:
Dorsal hippocampal CA1 region NF labelled cells estimate



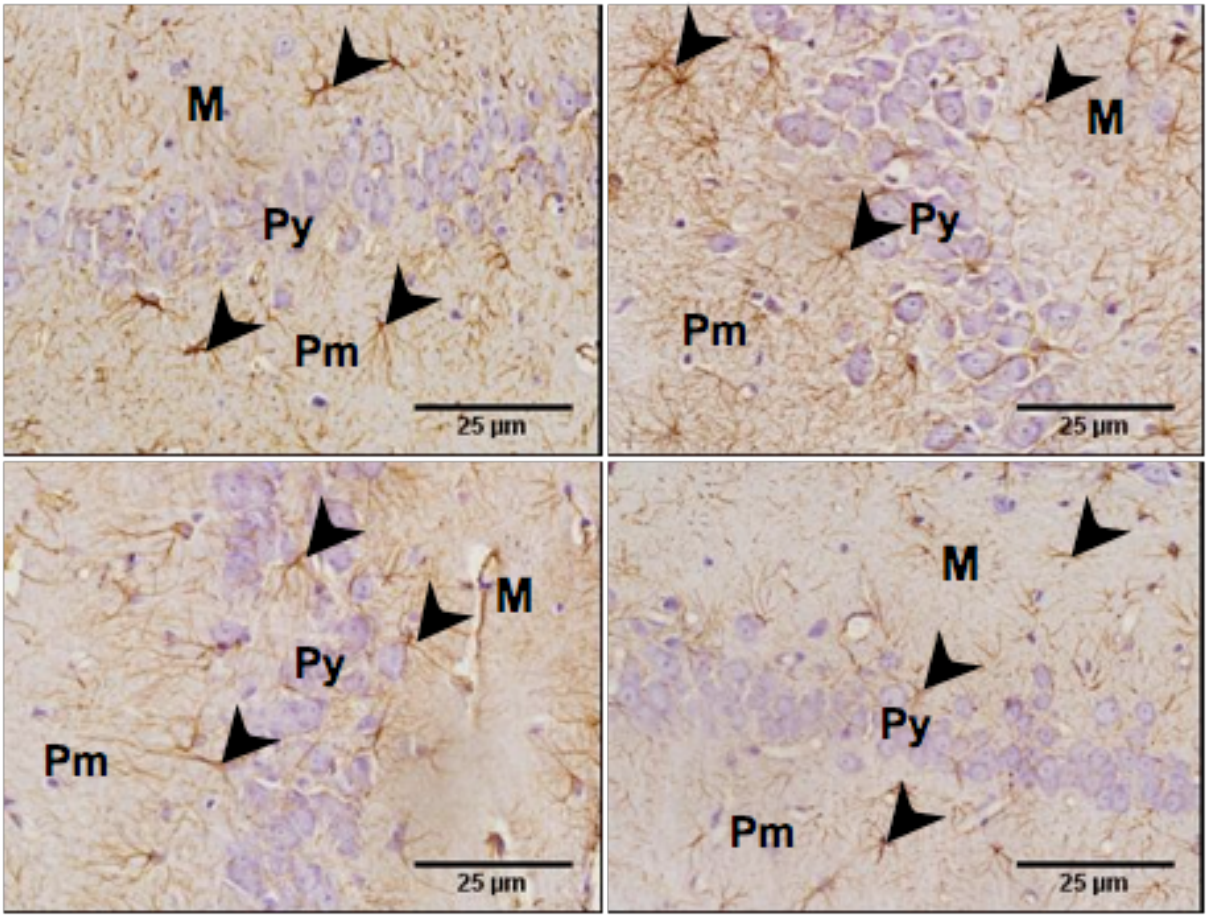
Data are presented as mean ± standard error of mean

***Significantly different from the control group at $p < 0.001$

NF = neurofilament protein; RV – *Rauwolfia vomitoria*

(n = 6, brain slices = 3)

Figure 7:
Sections of glial fibrillary acidic protein (GFAP) immunolabelled dorsal hippocampal CA1 region of the test and control groups: outer molecular (M), pyramidal (Py) and polymorphic (Pm) layers. GFAP, ×200



a. The control group shows GFAP positive cells throughout the cortical layers, and within the astrocytic processes (arrow head), although some of their soma (arrow) also expressed the protein.

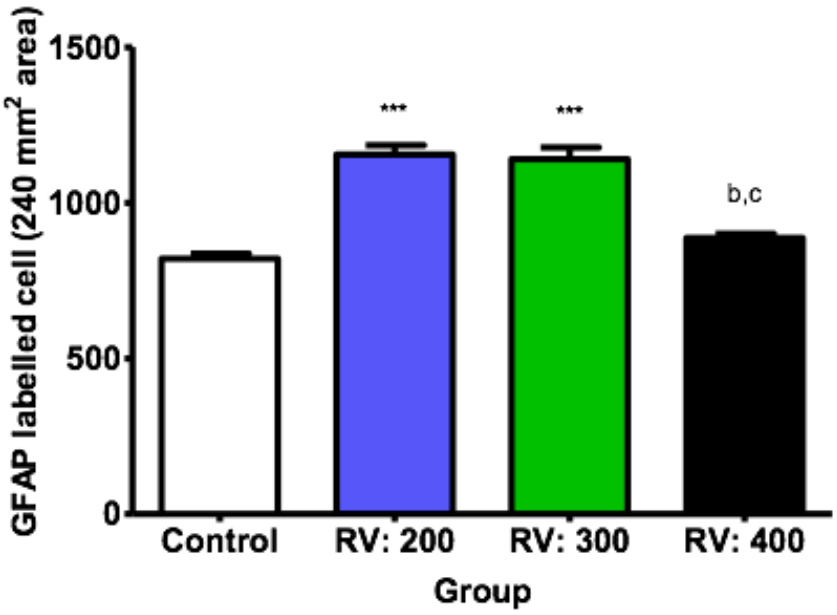
b. The 200 mg/kg RV group shows increased expression of GFAP throughout the cortical layers, in the astrocytic processes (arrow head) and soma (arrow).

c. The 300 mg/kg RV group shows increased expression of GFAP, mostly in the astrocytic processes (arrow head), throughout the cortical layers.

d. The 400 mg/kg RV group shows decreased expression of GFAP in the astrocytic processes (arrow head) and soma (arrow) throughout the cortical layers.

RV – *Rauwolfia vomitoria*

Figure 8:
Dorsal hippocampal CA1 region GFAP labelled cells estimate



Data are presented as mean ± standard error of mean

***Significantly different from the control group at $p < 0.001$

b Significantly different from the RV: 200 mg/kg group at $p < 0.05$

c Significantly different from the RV: 300 mg/kg group at $p < 0.05$

GFAP = glial fibrillary acidic protein; RV – *Rauwolfia vomitoria*

(n = 6, brain slices = 3)

5. Discussion

This study investigated the effect of *R. vomitoria* (RV) root bark extract on the microstructure and some immunohistochemical protein expression in the hippocampus of adult rats. Physically and behaviourally, the animals in the 200 mg/kg, 300 mg/kg and 400 mg/kg RV groups barely fed and appeared generally dull and drowsy compared with the control group. These observations indicate that RV may affect the satiety centre that controls feeding leading to general body weakness and behaviour. It is reported that reserpine, an active alkaloid of RV affects olfaction as well as causing sedation (Bisong *et al.*, 2010; Ekong *et al.*, 2016a), which may also be a reason for reduced feeding ability of the animals administered RV. The present result corroborates the reports of Eluwa *et al.* (2008), and Ekong *et al.* (2014, 2015a, 2016a, 2018) who reported similar observations.

Histologically, the dorsal hippocampal CA1 of the 200, 300 and 400 mg/kg RV groups showed either atrophic or karyorrhectic cells, which are at variance with Ajao *et al.* (2015) and Okon *et al.* (2020) who reported normal histology of the hippocampus at 150-300 mg/kg RV probably due to treatment duration. The population of these pyramidal cells were significantly less in the 300 and 400 mg/kg RV groups compared with the 200 mg/kg RV and control groups. However, other population

of cells were significantly ($p < 0.05$) higher in the 400 mg/kg group compared with the 200 mg/kg RV and control groups. Cellular atrophy and karyorrhexis are usually signs of trauma and degeneration respectively, to tissues (Strayer and Rubin, 2009; Garman, 2011), and these have been reported as some consequences of oral administration of RV (Ekong *et al.*, 2015a, 2016a, 2020). Atrophy of cells may be reversible if physiological but may be irreversible if pathological, and this may have been the case leading to karyorrhexis in the higher dosages of RV. Karyorrhexis, on the other hand, is an irreversible alteration of nuclei that may eventually lead to cell death, occurring when the cells cannot cope with insults. The neurons of the hippocampus are not known to regenerate *in vivo* (Ihunwo *et al.*, 2016), which may have also resulted in decreased cellular population, especially in the 300 and 400 mg/kg RV groups of the present study. These actions may cause general atrophy/shrinkage and functional disruption of this brain region. RV and its constituents have been indicted in neuronal damage (Ekong *et al.*, 2014, 2016b, 2017a, 2000), which the present study also shows.

Nevertheless, there was an increased population of other cell types, especially in the 400 mg/kg RV group, a possibility that these were small size neurons and glial cells. Neurons of the hipoocampus may not proliferate, but astrocytes and microglia are known to increase during insults (Afridi *et al.*, 2020). Thus, this population of cells may have been more of astrocytes and/or microglia.

Immunohistochemically, the dorsal hippocampal CA1 of the 200, 300 and 400 mg/kg RV groups showed increased neuronal cytoplasmic expression of NSE in the pyramidal layer, whose population was significantly ($p < 0.05$) less in the 400 mg/kg RV, but not the 200 and 300 mg/kg groups compared with the control. NSE is a cytosolic structural protein necessary for neuronal metabolic activity, and thus, serves as a marker for neuronal activities. Increased NSE expression is an indication of either increased metabolic activity or neuronal damage (Yardimoğlu *et al.*, 2008; Haque *et al.*, 2018). The present result is in line with Ekong *et al.* (2016b, 2020) who reported that RV stimulates brain expression of NSE.

The 400 mg/kg RV group showed significantly ($p < 0.05$) higher NSE-labelled cell population, and this is consistent with the highest RV dose already reported to be more deleterious in the histology results. Thus, suggesting that higher RV dose may cause more intoxication of this brain area.

The dorsal hippocampal CA1 of the 200, 300 and 400 mg/kg RV groups showed decreased expression of neuronal filament (NF), and significantly ($p < 0.05$) less NF-labelled population in these RV groups compared with the control group. NF is a neuronal cytoskeletal protein, an intermediate filament that provides structural support for axons, as well as regulating their diameter (Iwanaga *et al.*, 1989). Decreased NF expression indicates adverse protein rearrangement arising prior to alterations in axonal cytoskeleton (Mages *et al.*, 2018), and RV is reported with adverse neuronal activities (Ekong *et a*l., 2020; Nduohosewo and Ekong, 2020), which may have resulted in decreased expression and population in the present study. The present result is in line with Ekong *et al.* (2016b), who reported that RV cause decreased NF expression in the olfactory bulb.

The dorsal hippocampal CA1 of the 200 and 300 mg/kg RV groups showed increased expression of GFAP, with significantly ($p < 0.05$) more GFAP-labelled cell population compared with the control and the 400 mg/kg RV groups. The 400 mg/kg RV group showed decreased expression of GFAP-labelled cell population that was not significantly ($p > 0.05$) different from the control group. GFAP is an intermediate filament-III protein present in astrocytes and therefore serves as its marker (Yang and Wang, 2015). Increased GFAP expression indicates astrogliosis (Bondan *et al.*, 2019; Trautz *et al.*, 2019) and RV has been reported with such action on astrocytes (Ekong *et al.*, 2016b, 2020; Nduohosewo and Ekong, 2020). Decreased GFAP is indicative of detrimental and/or diseased conditions in nervous tissues (Bondan *et al.*, 2013; Molina *et al.*, 2018), which may be associated with astrocytes remodelling

after injury (Bondan *et al.*, 2013). RV has been reported with such action on astrocytes as well (Ekong *et al.*, 2016b, 2020). Thus, lower doses of RV tend to stimulate astrogliosis, while a higher dose does the opposite.

The hippocampus is vital for memory consolidation, and is easily affected by extraneous agents (Kiernan, 2009), which RV is. This brain region is reported to contain large population of dopaminergic cells (Perez and Lodge, 2017; Puighermanal *et al.*, 2017), whose stores of dopamine can easily be depleted by reserpine and yohimbine, two active constituents of RV. Reserpine irreversibly binds to the storage vesicles of dopamine, consequently depleting dopamine, while yohimbine interacts with serotonin and dopamine receptors blocking them (Millan *et al.*, 2000; Metzger et al, 2002). Eventually, catecholamine depletion occurs because of the body's inability to store these neurotransmitters, which may be responsible for some of the adverse structural effect on the hippocampus observed with RV.

It can be postulated from the present study that RV acted by usurping large stores of ATP leading to their depeletion and reduction of neuronal energy stores (increased neuron-specific enolase expression). This was further compounded by astrocytes gliosis (increased GFAP expression) that utilized the reduced energy stores resulting to degenerative processes setting in (decreased neurofilament expression, atrophic and karyorrhectic features and reduced cellular population). These structural abnormalities may lead to functional alterations, and eventually memory loss.

6. Conclusion

In conclusion, oral administration of the given doses of RV root bark extract in adult male Wistar rats showed sedative activities with hippocampal histopathological changes including neuron-specific enolase, neurofilament and glial fibrillary acidc protein, which may not be reversible, and thereby leading to hippocampal functional deficit.

7. Acknowledgements:

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8. Competing interests:

The authors declare that they have no competing interests.

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RESUMEN

Introducción: *Rauwolfia vomitoria (RV) Afzel* es una planta antipsicótica utilizada por varias comunidades africanas en el tratamiento de enfermedades psiquiátricas con buenos resultados. Las preocupaciones sobre sus efecto sobre la actividad cerebral conducen a esta investigación de su acción sobre la microestructura del hipocampo.

Materiales y métodos: Se asignaron veinticuatro ratas Wistar macho adultas de un peso medio de 200 g, en cuatro grupos (n = 6): control; 200, 300 y 400 mg / kg de peso corporal de extracto de corteza de raíz de RV, respectivamente. La administración fue una vez al día y por vía oral durante siete días. Se realizó una observación diaria de los animales hasta el día ocho, cuando fueron sacrificados después de una anestesia profunda. Cada cerebro fue procesado para estudios histológicos e inmunohistoquímicos.

Resultados: Los animales en los grupos de RV de 200, 300 y 400 mg / kg parecían generalmente apagados y somnolientos, y apenas alimentados. Su histología hipocampal mostró atrofia neuronal y cariorrexis, sin diferencia en el recuento celular, aunque el número de células piramidales disminuyó en los grupos de RV de 300 y 400 mg / kg. La enolasa específica de neuronas disminuyó en el grupo de RV de 400 mg / kg, mientras que el neurofilamento disminuyó en todos los grupos de prueba. La expresión y densidad de la proteína fibrilar ácida glial aumentó en los grupos de RV de 200 y 300 mg / kg, pero no en el grupo de RV de 400 mg / kg, todos en comparación con el grupo de control.

Conclusión: Las dosis administradas de extracto de corteza de raíz de RV en ratas Wistar adultas mostraron actividades sedantes, con cambios histopatológicos del hipocampo, que pueden no ser reversibles, lo que conduce al déficit funcional del hipocampo.

Palabras clave: *Rauwolfia vomitoria*; Histología; Inmunohistoquímica; Hipocampo; Ratas Wistar.