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Ameliorating effect of alligator pepper (*Aframomum melegueta*) on the histology of cerebellum of levonorgestrel induced female rats

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Abstract

Objective: This research study was carried out to investigate the ameliorating effect of alligator pepper (AP) on the histology of cerebellum of levonorgestrel-induced female rats.

Methodology: Twenty-five female rats weighing 110 g to 150 g were procured and acclimatized for two weeks, after which, they were divided into five groups of five rats each, and were housed in cages. The groups were designated as groups A - E. Group A served as the control group and was not induced with levonorgestrel, while groups B – E were induced. Group A and B received distilled water and levonorgestrel only, while groups C – E received Vitamin C, 100 mg/kg of body weight and 300 mg/kg of body weight of extracts of alligator pepper respectively for 14 days orally through oro-gastric tube. On the 15th day, the animals were weighed, sacrificed via chloroform inhalation, and cerebellums were harvested for histological study.

Results: Histopathological findings showed normal cerebellar histological tissues in group A, severe degeneration with severe vacoulation (V), necrosis (N) and severe pyknotic pyramidal cell (PPC) for animals in group B, mild healing with mild fatty change (FC), moderate vacoulation within the pyramidal cell layer in group C, moderate regeneration with moderate vacoulation (V) and pyknotic pyramidal cell (PPC) in group D, and moderate healing with mild vacoulation (V) of the pyramidal cells in group E.

Conclusion: The extract of AP has ameliorating effect on the histology on the cerebellums of levonorgestrel-induced female rats, and the ameliorating effect improves with increase in the dosages of the extract.

Keywords: Cerebellum; Levonorgestrel; Alligator pepper; Ameliorating

1. Introduction

The cerebellum often referred to as "little brain" is a structure of the central nervous system that is located at the back of the brain, immediately inferior to the occipital and temporal lobes, and within the posterior cranial fossa [1], and is responsible for coordinating movement, balance, and posture. It is separated from the lobes by the tentorium cerebelli, a tough layer of dura mater and lies at the same level of and posterior to the pons, from which it is separated by the fourth ventricle [1]. It is relatively small, neuron-rich, and contains over 50% of the brain's neurons [2] in a dense cellular layer, called the cerebellar cortex. Its traditionally known functions are motor movement regulation,

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including gait coordination and maintenance of posture; balance control; control of muscle tone and voluntary muscle activity and motor learning [3].

It consists of two hemispheres which are connected by the vermis - a narrow midline area. Like other structures in the central nervous system, the cerebellum consists of grey matter which is located on the surface of the cerebellum, and is tightly folded, forming the cerebellar cortex; and white matter which is located underneath the cerebellar cortex and are embedded with four cerebellar nuclei (the dentate, emboliform, globose, and fastigi nuclei) [1]. The grey matter is also referred to as the cortex and may be split into three layers; the outer molecular layer, the middle layer of Purkinje cells and the inner granular layer. There are many neurons, glial cells and fibers located in the cortex which all contribute to the motor functions of the cerebellum [4].

The cerebellum can be subdivided into anatomical lobes, zones and functional divisions. Three anatomical lobes that can be distinguished in the cerebellum; the anterior lobe, the posterior lobe and the flocculonodular lobe, and the lobes are divided by two fissures – the primary fissure and posterolateral fissure [1]. Also, it has three cerebellar zones with the vermis at the midline, and the intermediate zone at either side of the vermis. Lateral to the intermediate zone are the lateral hemispheres. There is no difference in gross structure between the lateral hemispheres and intermediate zones. The three functional areas of the cerebellum are the cerebrocerebellum, the spinocerebellum and the vestibulocerebellum. The cerebrocerebellum is the largest division, formed by the lateral hemispheres, and is involved in planning movements and motor learning [1]. It receives inputs from the cerebral cortex and pontine nuclei, and sends outputs to the thalamus and red nucleus. It also regulates coordination of muscle activation and is important in visually guided movements. The spinocerebellum is comprised of the vermis and intermediate zone of the cerebellar hemispheres, and is involved in regulating body movements by allowing for error correction. It also receives proprioceptive information. The vestibulocerebellum is the functional equivalent to the flocculonodular lobe, and is involved in controlling balance and ocular reflexes, mainly fixation on a target. It receives inputs from the vestibular system, and sends outputs back to the vestibular nuclei [1].

Microscopically, two types of neuron play dominant roles in the cerebellar circuit - Purkinje cells and granule cells. Also, revealed are three types of axons that play dominant roles: mossy fibers and climbing fibers which enter the cerebellum from outside, and parallel fibers which are the axons of granule cells. There are two main pathways through the cerebellar circuit, originating from mossy fibers and climbing fibers, both eventually terminating in the deep cerebellar nuclei [4]. Mossy fibers project directly to the deep nuclei, but also give rise to the following pathway: mossy fibers → granule cells → parallel fibers → Purkinje cells → deep nuclei. Climbing fibers project to Purkinje cells and also send collaterals directly to the deep nuclei [5]. The mossy fiber and climbing fiber inputs each carry fiber-specific information; the cerebellum also receives dopaminergic, serotonergic, noradrenergic, and cholinergic inputs that presumably perform global modulation [6]. The grey matter of the cerebellum is also referred to as the cortex, and may be split into three layers; the outer molecular layer, the middle layer of Purkinje cells and the inner granular layer [4]. There are many neurons, glial cells and fibers in the cortex which all contribute to the motor functions of the cerebellum [4].

Blood supply to the cerebellum is from three paired arteries -superior cerebellar artery (SCA), anterior inferior cerebellar artery (AICA) and posterior inferior cerebellar artery (PICA). The SCA and AICA are branches of the basilar artery, which wraps around the anterior aspect of the pons before reaching the cerebellum, while PICA is a branch of the vertebral artery [1]. Its venous drainage is by the superior and inferior cerebellar veins, and they drain into the superior petrosal, transverse and straight dural venous sinuses [1].

Cerebellar dysfunction can produce a wide range of symptoms and signs. Its aetiology varies with causes including stroke, physical trauma, tumours and chronic alcohol excess. Its clinical picture is dependent on the functional area of the cerebellum that is affected. Damage to the cerebrocerebellum and spinocerebellum presents with problems in carrying out skilled and planned movements and in motor learning [1]. A wide variety of manifestations are possible. These include dysdiadochokinesia (difficulty in carrying out rapid, alternating movements), ataxia, nystagmus (coarse), intention tremor, scanning speech and hypotonia [1]. Damage to the vestibulocerebellum can be manifested with loss of balance, abnormal gait with a wide stance [1].

Levonorgestrel is a hormonal medication used in a number of birth control methods [7]. It is combined with an estrogen to make combination birth control pills [8]. As an emergency birth control, sold under the brand names Plan B One-Step and Julie, among others, it is useful within 72 hours of unprotected sex [7]. The more time that has passed since sex, the less effective the medication becomes [8]. Levonorgestrel works by preventing or delaying ovulation so an egg cannot be released [9]. The dosage used for emergency contraception is ineffective when ovulation has already occurred, and has been found to have no effect on implantation [10]. It decreases the chances of pregnancy by 57–93 % [11]. In an

intrauterine device (IUD), such as Mirena among others, it is effective for the long-term prevention of pregnancy [8]. A levonorgestrel-releasing implant is also available in some countries [12].

Common side effects include nausea, breast tenderness, headaches, and increased, decreased, or irregular menstrual bleeding [8]. When used as an emergency contraceptive, if pregnancy occurs, there is no evidence that its use harms the fetus [8]. It is safe to use during breastfeeding [8]. Birth control that contains levonorgestrel will not change the risk of sexually transmitted infections [8]. It is a progestin and has effects similar to those of the hormone progesterone [8]. It works primarily by preventing ovulation and closing off the cervix to prevent the passage of sperm [8].

Levonorgestrel was patented in 1960 and introduced for medical use together with ethinylestradiol in 1970 [13]. It is on the World Health Organization's List of Essential Medicines [14]. It is available as a generic medication [15]. In the United States, levonorgestrel-containing emergency contraceptives are available over the counter (OTC) for all ages [16]. In 2020, it was the 323rd most commonly prescribed medication in the United States, with more than 800 thousand prescriptions [17].

Alligator pepper (also known as Ishitɛ, Ata Ire, Ose Oji, mbongo spice, or hepper pepper) is a West African spice made from the seeds and seed pods of *Aframomum daniellii*, *A. citratum*, or *A. exscapum* [18]. It is a close relative of grains obtained from the closely related species, *A. melegueta* or "grains of paradise" [19]. Unlike grains of paradise, which are generally sold as only the seeds of the plant; alligator pepper is sold as the entire pod containing the seeds [18]. The plants that provide alligator pepper are herbaceous, perennial, flowering plants of the ginger family (*Zingiberaceae*), native to swampy habitats along the West African coast [18]. Once the pod is open and the seeds are revealed, the reason for this spice's common English name becomes apparent as the seeds have a papery skin enclosing them and the bumps of the seeds within this skin is reminiscent of an alligator's back [18].

In Igboland, alligator pepper and ósè ọjị́ with kola nuts are used in naming ceremonies, as presentation to visiting guests, and for other social events with the kola nut rite [18]. Also, in the Igboland, alligator pepper is presented and ate together with kola nuts. Virtually in every Igbo ceremony, alligator peppers and kola nuts are presented to guests at the top of the agenda and prior to any other food or entertainment [18]. Prayers and libations are made together with alligator pepper and kola nuts [20].

It contains a variety of phytonutrients and antioxidants like cardiac glycosides, flavonoids, and alkaloids which are beneficial to the heart as these antioxidants assist in lowering the chance of developing cardiovascular illnesses thereby shielding vital organs from harm [21]. It provides defense against free radicals, which release and restore normal blood pressure, helps in the treatment of Asthma, and its crushed seeds can be applied topically as a counterirritant [21]. It has hypocholesterolemic, antioxidant, hepatoprotective properties, and also assist in reduction of Angiotensin-1 converting enzyme (ACE) activities [22]. It is used to control blood sugar levels by lowering blood sugar through the amino acids it contains and is also linked to increased metabolism and weight loss; helpful in treating a variety of pains, such as joint, tooth, rheumatoid, arthritis pain and during their periods [21]. Due to high tannin content in alligator pepper, it reduces inflammation by curing burns, healing wounds, and relieving pain associated with inflamed mucous membranes [21]. It increases libido in women and increases their sensitivity to touch during sex by stimulating more nerve endings, and helps to inhibit enzymes that contribute to erectile dysfunction [21]. During the Covid-19 pandemic, it was used in medicine [23].

Thus, this research work will help to educate the public on the ameliorating effect of extracts of alligator pepper on the histology of cerebella of levonorgestrel-induced female rats thereby encouraging its consumption especially by women who are taking levonorgestrel as one of birth control methods.

2. Material and methods

2.1. Animal procurement, care and treatment

Twenty-five (25) female wistar rats weighing between 110 g to 150 g were procured and housed at the Animal house of Anatomy Department, Abia State University; Uturu with wire gauze cages in a well-ventilated area, were maintained under standard laboratory conditions of temperature (22+2 ℃), relative humidity (55-65 %) and 12 hours light/dark cycle. They were fed with standard commercial pellet diet and water ad libitum and were also acclimatized for two weeks before the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

2.2. Collection, identification and preparation of plant material

Fresh alligator pepper pods (*Aframomum melegueta*) were purchased from a local market in Uturu in Abia State, and were authenticated at Herbarium unit, Botany Department, Abia State University, Uturu, Abia State with the Herbarium number ABSU/ANA/HERB/25/003. The pods were cleaned; the seeds de-hulled, and were dried thoroughly under the sun for 48 hours and later grinded into powder [24]. The dried seeds were grounded into a fine powder using a laboratory blender to obtain the powder. Later the powdered seeds were homogenized with distilled water and kept for 12hrs. The mixtures were filtered with Whatman No .1 filter paper. The filtrates were concentrated in one tenth (1/10) of the original volume at 38 - 40 ^{0}C using a rotating evaporator [24]. At the time of use, the filtrate extract of alligator pepper were filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the water. 250 mg of these extracts /kg body weights were dissolved in 10 mls of distilled water and were administered to the animals.

2.3. Induction of Levonorgestrel

Levonorgestrel was purchased at pharmaceutical shop at Ariaria Market Aba, Abia State, Nigeria. The rats were administered with 2 mg of Levonorgestrel dissolved in normal saline, at 10 mg/kg body weight, orally using oral gastric tube for 14 days since the LD_{50} of levonorgestrel (oral) is 5010 mg/kg in rats and mice [25].

2.4. Experimental protocol

The animals were grouped into five (5) groups of five (5) rats each. Different doses of the extracts of alligator pepper were administered via oral route with the aid of oral gastric tube as shown below:

- Group A: The control group + distilled water.
- Group B: Levonorgestrel only.
- Group C: Levonorgestrel + Vitamin C.
- Group D: Levonorgestrel + 100 mg/kg of body weight of extracts of alligator pepper.
- Group E: Levonorgestrel + 300 mg/kg of body weight of extracts of alligator pepper.

2.5. Sample collection and analysis

The extracts were administered for fourteen (14) days. On the 15th day, the animals were sacrificed by anaestethizing under chloroform vapour and dissected. Cerebellum were harvested from the rats, weighed, and fixed in Bouin's fluid for histological analyses.

3. Results

3.1. Histopathological findings

The histopathological findings of this research work reveals as follows: -

Micrograph 1 is the result of is the result of the histology of the cerebellums (x400) (H/E) of the animals of group A (GPA) control section showing normal cerebellar cortex with molecular layer (ML), granular layer (GL) and well outlined Purkinje cell within the Purkinje layer.

Micrograph 2 is the result of the histology of the cerebellums (x400) (H/E) of the animals in group B (GPB) induced with levonorgestrel and without treatment showing severe degeneration with, severe vacuolation (V) and necrosis (N) and severe pyknotic pyramidal cell (PPC).

Micrograph 3 is a photomicrograph of group C (GPC) section of cerebellums (x400) (H/E) induced with levonorgestrel and treated with vitamin C showing mild healing with mild fatty change (FC) (steatosis), moderate vacuolation within the pyramidal cell layer.

Micrograph 4 is a photomicrograph of group D (GPD) section of cerebellums (x400) (H/E) induced with levonorgestrel and treated with 100 mg/kg extract showing moderate regeneration with moderate vacuolation (V) and pyknotic pyramidal cell (PPC).

Micrograph 5 is a photomicrograph of group E (GPE) section of cerebellums (x400) (H/E) induced with levonorgestrel and treated with 300 mg/kg extract showing moderate healing with mild vacuolation (V) of the pyramidal cell layers.

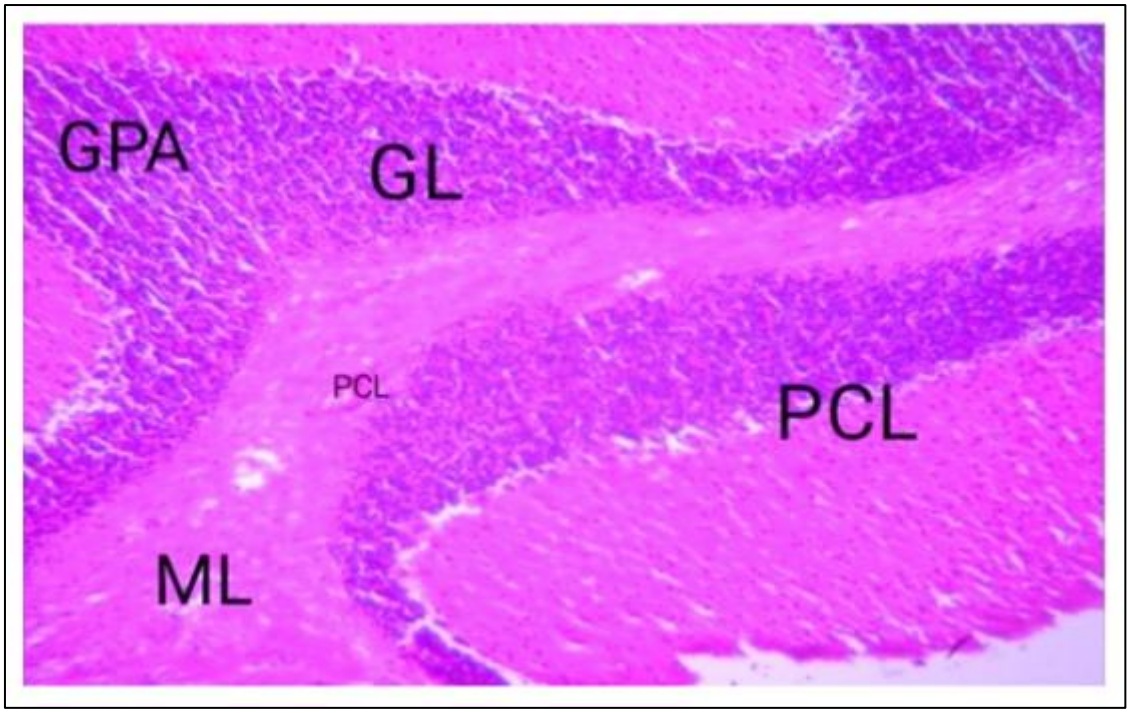

Figure 1 Micrograph 1 (x400) (H/E) is showing normal cerebellar cortex with molecular layer (ML), granular layer (GL) and well outlined Purkinje cell within the Purkinje layer

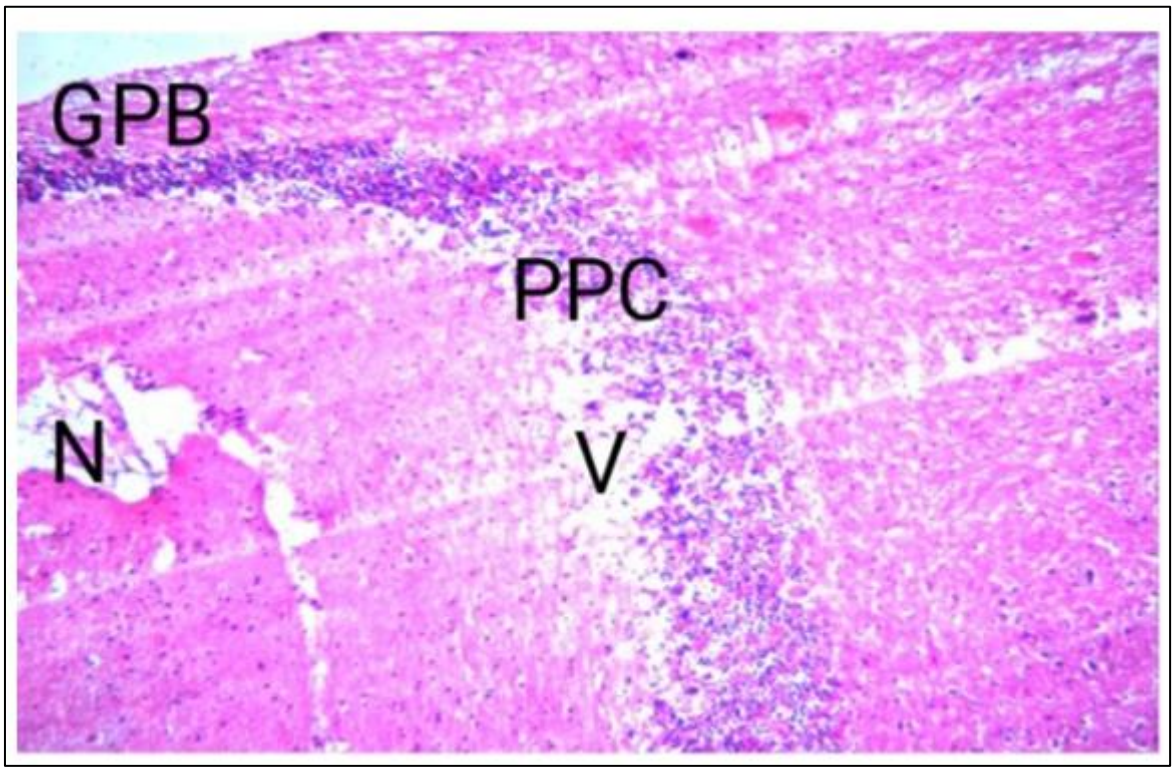

Figure 2 Micrograph 2 (x400) (H/E) is showing severe degeneration with, severe vacuolation (V) and necrosis (N) and severe pyknotic pyramidal cell (PPC)

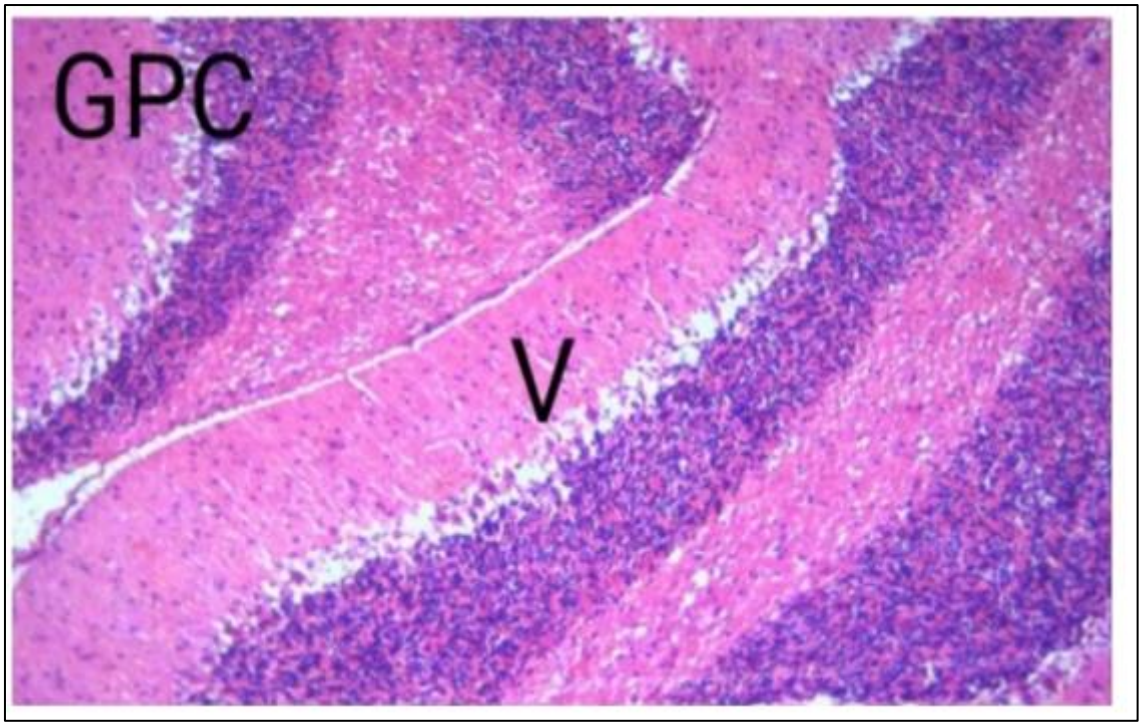

Figure 3 Micrograph 3 (x400) (H/E) is showing mild healing with mild fatty change (FC) (steatosis), moderate vacuolation within the pyramidal cell layer

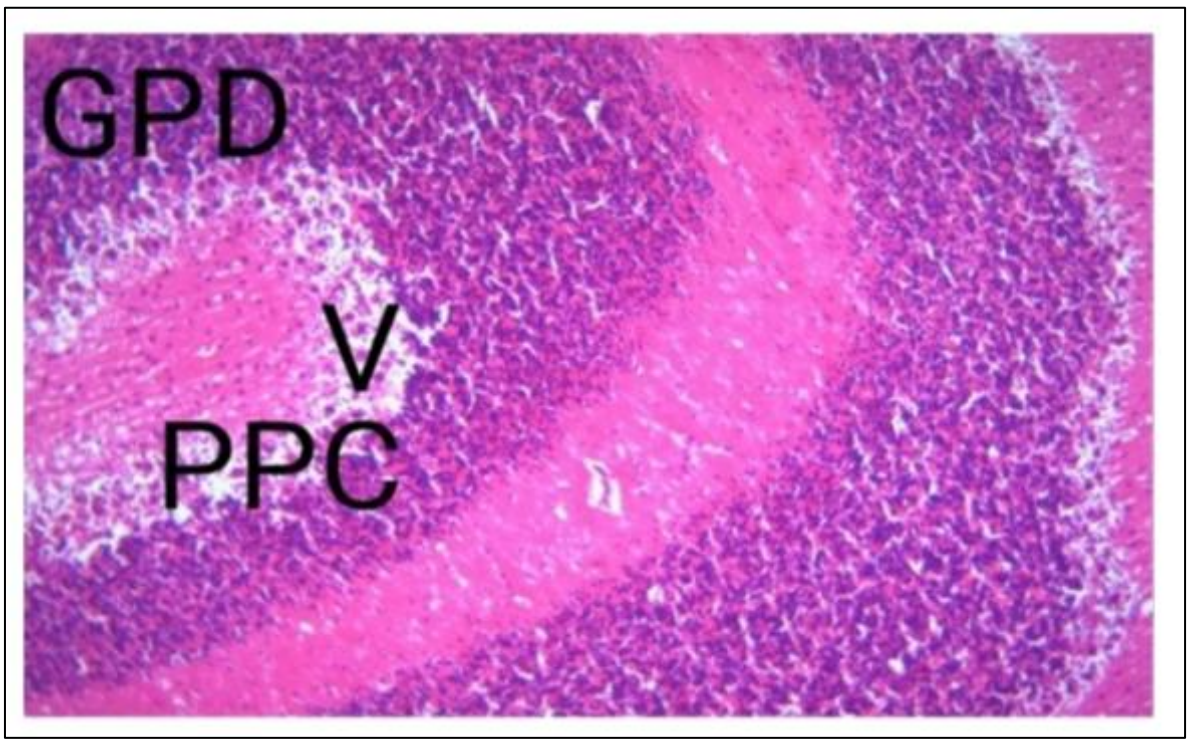

Figure 4 Micrograph 4 (x400) (H/E) is showing moderate regeneration with moderate vacuolation (V) and pyknotic pyramidal cell (PPC)

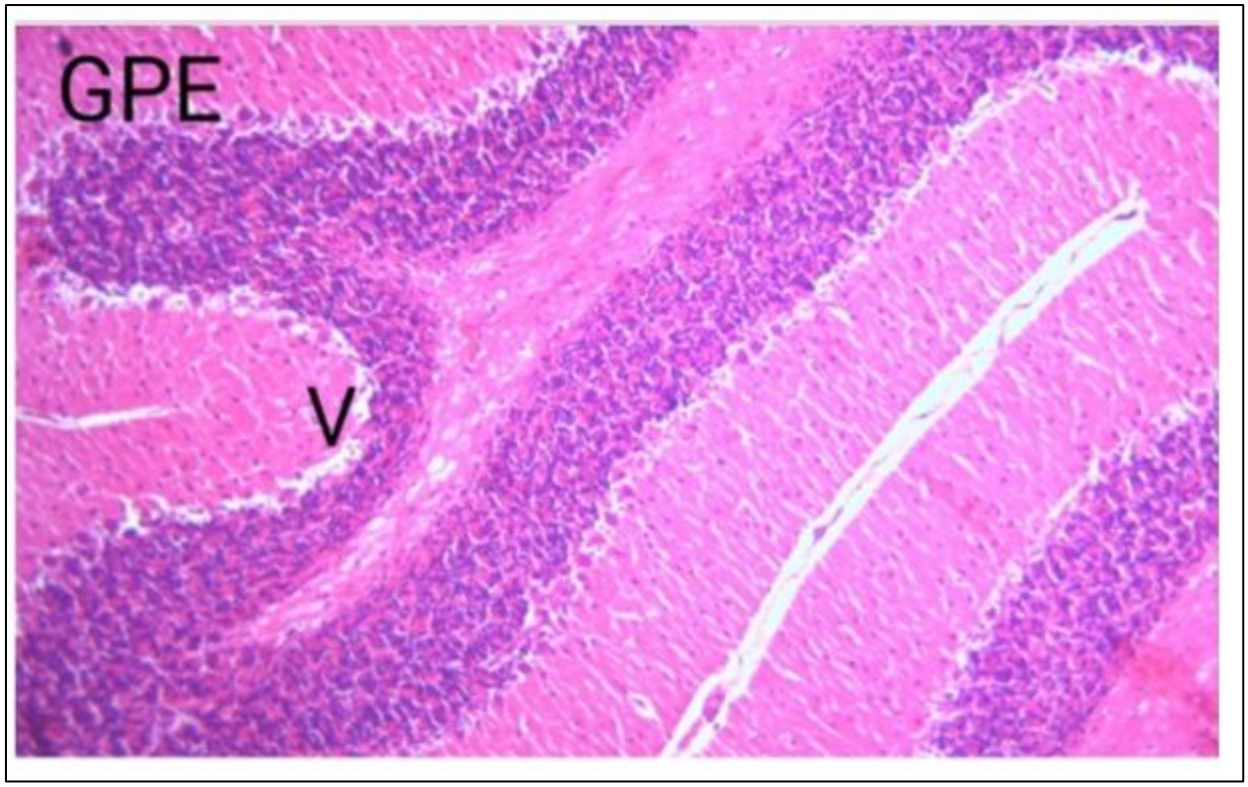

Figure 5 Micrograph 5(x400) (H/E) is showing moderate healing with mild vacuolation (V) of the pyramidal cell layers

4. Discussion

Levonorgestrel is an emergency contraception used to stop pregnancy following unprotected intercourse or when conventional birth control methods have failed. Its use as a regular method of birth control is not advised because it is an emergency contraceptive [26]. Its most commonly reported adverse effects include alterations of menstrual bleeding patterns, nausea, abdominal/pelvic pain, headache/migraine, dizziness, fatigue, amenorrhea, ovarian cysts, genital discharge, acne/seborrhea, breast tenderness, and vulvovaginitis [9]. Thus, the aim of this research study is to investigate the effect of alligator pepper extract on the histology of cerebellums of levonorgestrel-induced female rats.

The histopathological finding of this present study of the cerebellums of the animals in group A (GPA) (x400) (H/E) of Micrograph 1 (figure 1) showed normal cerebellar architecture with molecular layer (ML), granular layer (GL) and well outlined Purkinje cell within the Purkinje layer. This is in line with the normal structure of cerebellar cortex which according to research study may be split into three layers; the outer molecular layer, the middle layer of Purkinje cells and the inner granular layer [4].

While, the histopathological result of the histology of the cerebellums of the animals in group B (GPB) induced with levonorgestrel only (x400) (H/E) of Micrograph 2 (figure 2) showed severe degeneration with, severe vacuolation (V) and necrosis (N) and severe pyknotic pyramidal cell (PPC). This could be because of toxins present in levonorgestrel which increases markers of oxidative stress that may have caused the damage as it was administered orally. Research has shown that the use of oral contraceptives is associated with increased risks of myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease. The most common adverse effects seen during clinical use of oral contraceptives are menstrual irregularities [28].

The result of the histology of the cerebellums of the animals in group C (GPC) induced with levonorgestrel (x400) (H/E) and treated with vitamin C of micrograph 3 (figure 3) showed mild healing with mild fatty change (FC) (steatosis), moderate vacuolation within the pyramidal cell layer; in micrograph 4 (figure 4) the result of the histology of the cerebellums of the animals in group D (GPD) induced with levonorgestrel (x400) (H/E) and treated with 100 mg/kg of body weight of extract of alligator pepper showed moderate regeneration with moderate vacuolation (V) and pyknotic pyramidal cell (PPC); while that of micrograph 5 (figure 5) induced with levonorgestrel (x400) (H/E) and treated with 300 mg/kg of body weight of extract of alligator pepper showed moderate healing with mild vacuolation (V) of the

pyramidal cell layers. These positive results could be due to the ameliorating effect of alligator pepper which increases with the increase in the extract dosages. This could be due to the ability of the extract of alligator pepper to detoxify the toxins present in levonorgestrel thereby reducing markers of oxidative stress that could have caused the damages in micrograph 2 (figure 2) that received not the extract of the alligator pepper at all.

5. Conclusion

Therefore, the extracts of alligator pepper have ameliorating effect on the histology of cerebellums of levonorgestrel-induced female rats, and the ameliorating effect is dose-dependent, and improves better with increase in dosages of the extract.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have no conflict of interest to declare.

Statement of ethical approval

This research work was approved by the Ethical Approval Committee, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria.

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