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Histo-morphological Effects of *Carica papaya* on Cadmium Induced Prefrontal-Cortex Damage in Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HBA and ODO designed the study and wrote the final manuscript. Authors MNN and ARO performed the statistical analysis and managed the protocols. Authors EO and AAA wrote the first draft of the manuscript. Authors FEO and PKO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: This research investigated the recuperative (restorative) effect of aqueous extract of *Carica papaya* fruit on cadmium induced prefrontal-cortex damaged in adult Wistar rats (*Rattus norvegicus*). Previous research reports have confirmed that cadmium toxicity results in cellular damage which is due to an increase in production of reactive oxygen species and prevention of the activities of antioxidant enzymes. Various parts of the brain (prefrontal cortex, hippocampus and so on) are majorly affected by cadmium as its induced damage.
Methods: 30 Wistar rats (70 g-190 g) were used for this research. The rats were randomly selected into six groups of five animals each. A single dose of $3CdSO_4.8H_2O$ (Cadmium sulphate octahydrous) 3.5 mg/kg body weights was administered intraperitoneally to three of these groups against control a group that was not exposed to Cadmium. Two groups were treated with different doses of *Carica papaya* fruit extract for the period of four weeks. After four weeks, the rats were sacrificed and organs excised, weigh and fixed in fixative for histological processing. The photomicrographs of the normal control, induced control and treated groups were observed and compared for histomorphological similarities and differences.
Results: Cadmium was observed to have caused a distortion, disruption and calcification in the cells and tissue of the prefrontal cortex. There was shrinkage of nuclei of the neurons in cadmium induced rats. It was also observed that cadmium caused a loss in function of cell in the process of protein biosynthesis. The morphology of the neuronal cells of rats treated with high and low doses of *Carica papaya* extract was found to be slightly normal with increased viable neuronal cells as compared with the neurons of the normal control group 1 animals, though the restorative effects of the high dose treated rats were more pronounced. Also, it was observed that the damage to the brain section neurons treated with vitamins C and E before induction was not pronounced. Moreso, loss in body weight were observed in cadmium induced group animals and over treatment with *Carica papaya*, gain in the rats body weight was observed in the treatment animal groups as compared with the body weight of rats in normal control. Animal body weight before cadmium inoculation, after inoculation and before animal sacrifice were compared across all the groups and it was found that, there was a progressive increase in rats body weight (99±2,35≤ 150 ±3.21), (120±2.32≤189±3.21) and (135±1.35≤175±2.15) respectively which was significant at $P \leq 0.05$.
Conclusion: It can be ascertained from this present study that *Carica papaya* has ameliorative properties against deleterious effects of cadmium on the neurons and neuroglia of the prefrontal-cortex in Wistar rats which is dose dependent.

Keywords: Carica papaya; cadmium; histomorphology; prefrontal cortex; cellular damage and intraperitoneally.

1. INTRODUCTION

Exposure to cadmium (Cd) in industries and in the environment is well known to produce toxicity in multiple organs of the human body. Not much is known about the toxicity of the nervous system by cadmium despite the fact that it is one of the widest spread toxic metals in the environment [2]. In the past, the limelight has been on the storage of cadmium in soft tissues such as liver and kidneys, hardly ever in the brain [3].

The modes of action of the toxicity of cadmium are not totally clear, though it has been observed that damage of some chromosomes is responsible for some of the effects of cadmium on the cell [4]. In cadmium exposure, a number of the definite modifications that result in tissue injury and death have been linked to oxidative stress and exhaustion of thiol [5]. Severe exposures in humans are not often explained, almost entirely in the manifestation of work-related accidents among metal workers causing dizziness, dyspnoea, dysuria, and headache and chest pain.

The brain is an organ, about 1.4 kg in weight in humans that organizes all essential functions of the body. It receives and interprets (infers) information from the external environment, and represents the fundamental nature of the intellect and nature of man. Some of the numerous processes controlled by the brain include: emotion, intelligence, memories and creativity. The five sensory organs; eye (sense of sight), ear (sense of hearing), nose (sense of smell), tongue (sense of taste) and the skin (sense of touch), are the major input organs from which the brain receives information in the form of impulses/ stimuli [6].

The information from the external environment is arranged, such that it can be understood and can be stored in our memory. The brain controls our memory and speech, views, movement of the limbs, and several actions of numerous organs of the body [1]. It also determines the individual's response to situations, such as the fight or flight response during a scary occurrence. The brain makes up the central nervous system alongside the spinal cord. The cerebrum, cerebellum and brainstem are the major parts of the brain [6]. Cerebrum is the biggest portion of the brain and it is made up of the left and right hemispheres. It controls higher functions such as vision and hearing, speech, reasoning, learning, interpretation of touch, fine movements and emotions. Cerebellum is situated under the cerebrum that is it is placed inferior to the cerebrum. Coordination of muscle movements, maintenance of balance and posture are the functions of this part of the brain. *Carica papaya Linn* is a flowering plant of the family Caricaceae which originates in Central America. It has male and female parts which are situated on different trees. Papaya is a lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The fruit is not just delicious and healthy, but whole plant parts, fruit, roots, bark, peel, seeds and pulp are also known to have medicinal properties [7]. Papaya is a source of various nutrients which can be found all through the year. Papaintha is a digestive enzyme, present in papaya, which is used to successfully remedy different causes of trauma, allergies and sports injuries.

Functioning together as a unit; the nutrients present in papaya serve to protect the cardiovascular system from: heart diseases, heart attacks and strokes. It also helps to avoid cancer of the colon. Beta carotene which helps to mop up free radicals that cause various forms of cancer, thereby preventing, is found in papaya. Studies show that it also helps to prevent the occurrence of diabetic heart disease [8]. Since papaya is a good source of fiber, it helps to decrease elevated level of cholesterol in the body. Papaya is recommended to people who find it difficult to digest protein as it is known as a digestive agent. As a result of the enzyme papain being a constituent of the latex of papaya plant, it is also used after surgery to break up blood clots.

The latex from the trunk of the tree is also applied externally to speed the healing of wounds, ulcers, boils and warts [8]. The seed is used to expel worm, the flower may be taken in an infusion to induce menstruation [9,10,11]. The efficacy of treatments with *Carica papaya* L. is dependent on the quantity of the different bioactive compounds in the herbal preparation [12]. Papaya helps to improve immune system because of its vitamin A and C contents. Pawpaw fruits are rich in antioxidant nutrients like carotene, vitamin C, vitamin B, flavonoids, folate, panthotenic acids and minerals such as potassium and magnesium, the fruit is also a good source of fibre. The extract of *Carica papaya* is also known to have antioxidant properties [25]. It aids in the prevention of colon cancer. Beta carotene which helps to mop up free radicals that cause various forms of cancer, thereby preventing it from metastasizing. Studies have shown that it also helps to prevent the occurrence of diabetic heart disease [26].

2. METHODS

2.1 Plant Materials

Three *Carica papaya* were obtained and authenticated and assigned with voucher number (No. FHI. 102574) in the Department of Botany, Bingham University, Nigeria.

2.2 Preparation of Extract

A mature unripe *Carica papaya* (pawpaw) fruit weighing 1518 g was purchased from Kuchikau town, Nasarawa state, Nigeria, and taken to the Biology department of Bingham University, Nigeria for authentication. The *Carica papaya* was peeled and the seed discarded. The *Carica papaya* was cut into pieces and 1000 g was weighed. The weighed *Carica papaya* was then put in 1000 ml of distilled water. The mixture was left to soak for 72 hours (3 days) at room temperature. After the 72 hours, it was sieved and the residue was weighed. The weight of the residue was 570 g, and the supernatant was 1150 ml. The supernatant was then stored in the refrigerator till when it was needed [13].

2.3 Experimental Animals

30 Wistar rats weighting between 70 g-190 g were used for this research work. The rats were randomly selected into six groups as follows 1, 2, 3, 4, 5, and 6 each group containing five rats. They were kept in the animal house of Bingham University, Nigeria and given water and feed twice daily. Their beddings were changed routinely to maintain a conducive environment for the rats. The treatment for the various groups

Table 1. Animal groupings for administration and treatment

Animal groups	No. per animals	Duration	Induction/animal treatment
Group 1	Five (5)	4 weeks	0.5 ml, 0.9% w/v PBS
Group 2	Five (5)	4 weeks	3.0 mg/kg $CdSO_4$
Group 3	Five (5)	4 weeks	300 mg/kg Vit. C & 100 mg/kg vit. E + 3.0 mg/kg $CdSO_4$
Group 4	Five (5)	4 weeks	3.0 mg/kg $CdSO_4$ + 400 mg/kg *Carica papaya*
Group 5	Five (5)	4 weeks	3.0 mg/kg $CdSO_4$ + 250 mg/kg *Carica papaya*
Group 6	Five (5)	4 weeks	325 mg/kg *Carica papaya*

Grp1 – NC; Grp2 – -veC; Grp3 – PRL; Grp4 – HiDose; Grp5 – LoDose; Grp6 - +veC

was administered orally accordingly. All experimental investigations were done in compliance with humane animal use as stated in the "Guide to the care and use of Laboratory Animals Resources". National Research Council, DHHS, Pub. No NIH 86 – 23 (1985) and in accordance with ethical approval of the Anatomy Department, Bingham University, Karu, Nigeria.

2.4 Administration of Chemical and Animal Treatment

The cadmium stock solution was made by dissolving 10 mg of Cadmium sulphate salt in 5 ml of 0.9% w/v phosphate buffer. The cadmium stock solution was administered intraperitoneally in doses corresponding to the weight of the rats using 1 ml insulin syringes.

2.5 Procedure for Animal Sacrifice

The rats were sacrificed through cervical dislocation. The skulls were dissected and the brains were harvested. The brain tissues were fixed in 10% formal calcium.

Data were expressed as Mean ± Standard Error of Mean (S.E.M), One- Way ANOVA using Medicalc software packages to determine the level of significance $P < 0.05$ (95% CI) was taken as the significant level.

3. RESULTS

3.1 Morphological Observation

Following administration of 250 mg/kg and 400 mg/kg body weight of *Carica papaya* fruit extracts to treated rats group 4 and 5 respectively. 0.5 ml of 0.9%w/v phosphate buffer were administered to the normal control rats and a single dose of 3.0 mg/kg of $3CdSO_4.8H_2O$ were administered to group 2 (negative control). Vitamins C and E was administered to Group 3 rats for four weeks after which they were induced with a single dose of $3CdSO_4.8H_2O$, making it the prophylactic group. Positive control group 6 rats were given only *Carica papaya* for a period of four weeks. Changes in the weight of the rats and organ weight were observed as shown in the Table 1. The table reveals animal weight and organ weight variations that occurred in the four weeks of daily treatment.

3.2 Haematoxylin and Eosin Staining

Sections of the pre-freontal cortex stained with H&E revealed variations in cellular morphology according to the treatments received by the animals in each group (Figs. 2-11).

4. DISCUSSION

In a normal brain tissue, there are six basic layers; molecular layer, external granular layer, external pyramidal layer, internal granular layer, internal pyramidal (ganglionic) layer and multiform layer [14]. This normal cell arrangement and architecture is observed in the micrograph of the tissue of the control group 1. The various layers of cells in the frontal cortex are seen clearly.

The micrograph of group 2 animals induced with only cadmium and left for 28 days as negative control, was observed and it showed features of damaged cells and distortion in the histoachitecture. The tissue cytoarchitecture was distorted and the nuclei of most cells were damaged, due to this, it is expected that normal cell activities such as respiration and nutrient uptake are disrupted, which would cause problems in the body system functioning [15]. This is confirmed by previous experiments which

proved the inhibition of cell activities by cadmium [16].

Group 3, the positive induced group, which was treated with vitamin C and E for 28 days and induced with cadmium, left for 24 hours before sacrifice showed slight damage to the tissue cytoarchitecture. This suggests that vitamin C and E supplements have minimal preventive action in case of cellular damage [17].

There was an improvement in the morphology of cells in the tissue of animals treated with *Carica papaya,* when compared to the group 2 animals' cell morphology. While there was a loss of cell integrity and shrinkage of nuclei in the induced group, the treated groups showed restoration to the normal histomorphology of the animals. Many of the metabolites from medicinal plants like *Carica papaya,* especially flavonoids exhibited potent antioxidant activity *in vitro* and *in vivo* according to previous studies [18,19,20]. The activities of these antioxidants improve cell morphology [21,22]. The micrograph of group 4 animals' tissue showed an almost complete restoration to the normal. The treatment to Group 5 animals was observed to have just a slight ameliorative action on cell damage, improvement from the distorted appearance of the cells in group 2.

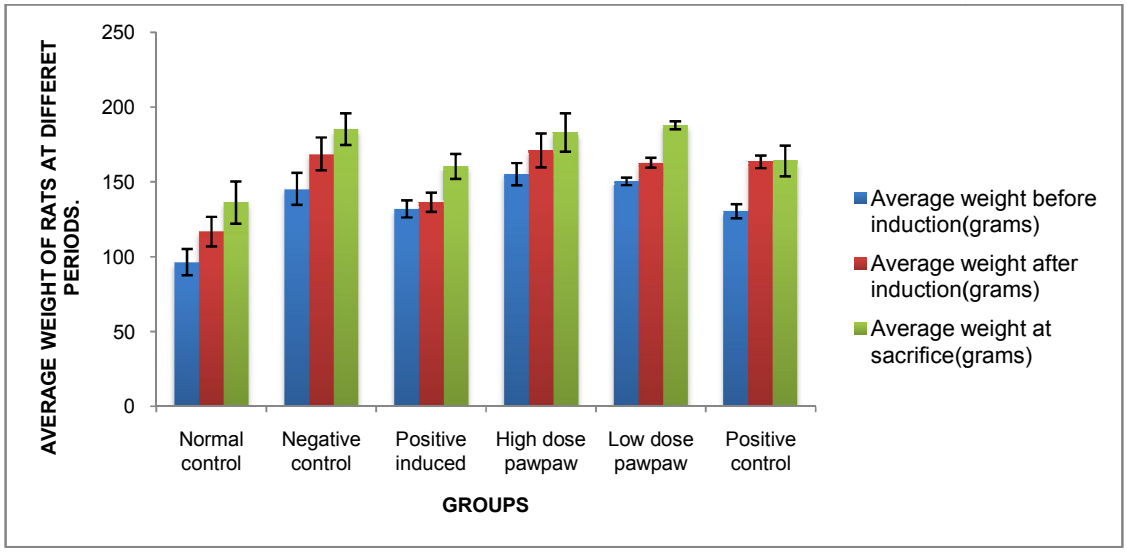

Fig. 1. Average weights of animals at different periods

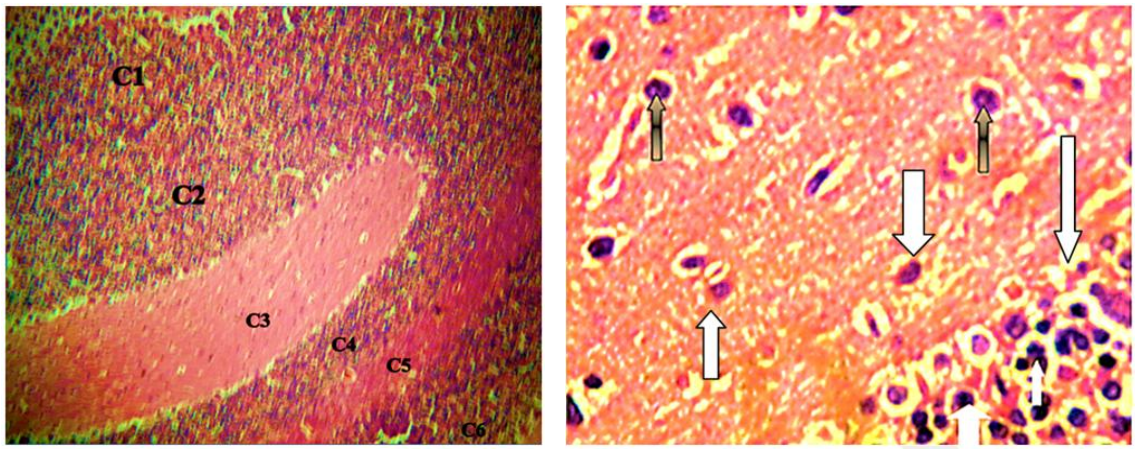

Fig. 2. Normal control group 1: Showing cerebral cell layers intact. H/E x40 and x400

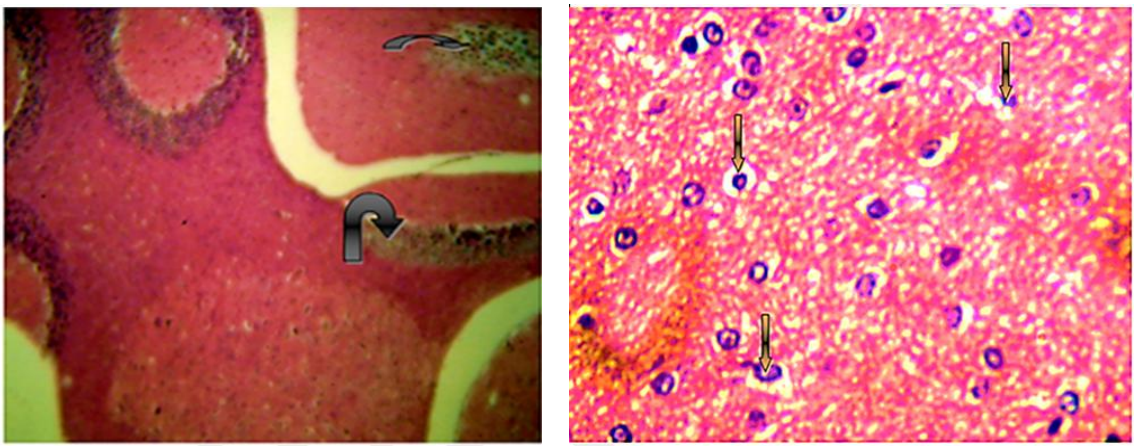

Fig. 3. Induced control group 2: Cells vacoulation and pyknotic cells has indicated by brown arrows. H/E x40 and x400

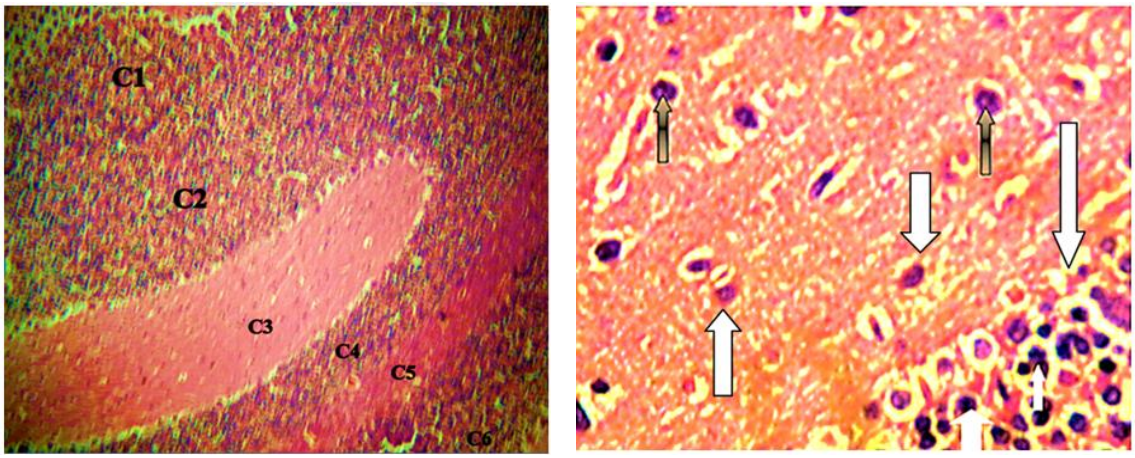

Fig. 4. Normal control group 1: Showing cerebral cell layers intact. H/E x40 and x400

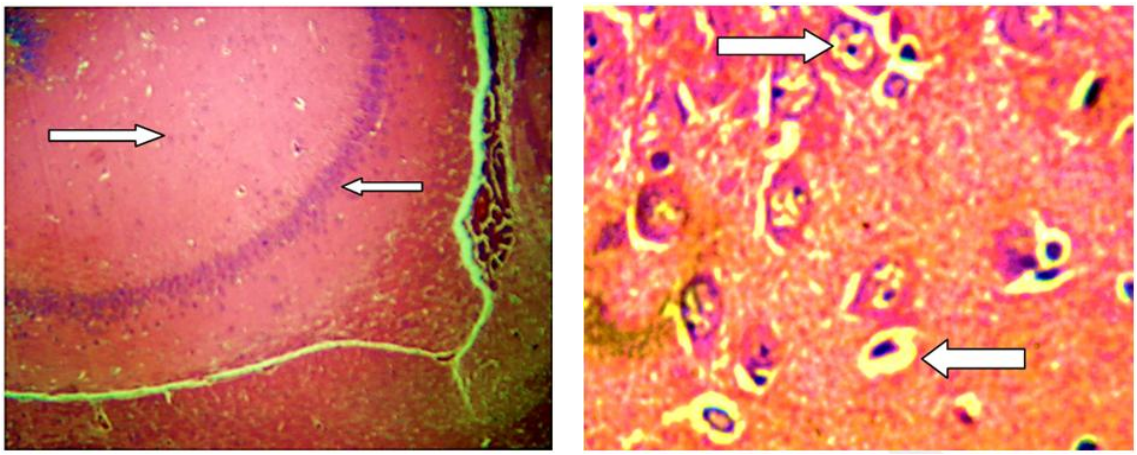

Fig. 5. Prophylactic group 3: Few cells with intact neurons, lacerations observed in tissue and as indicated by the white arrows. H/E x40 and x400

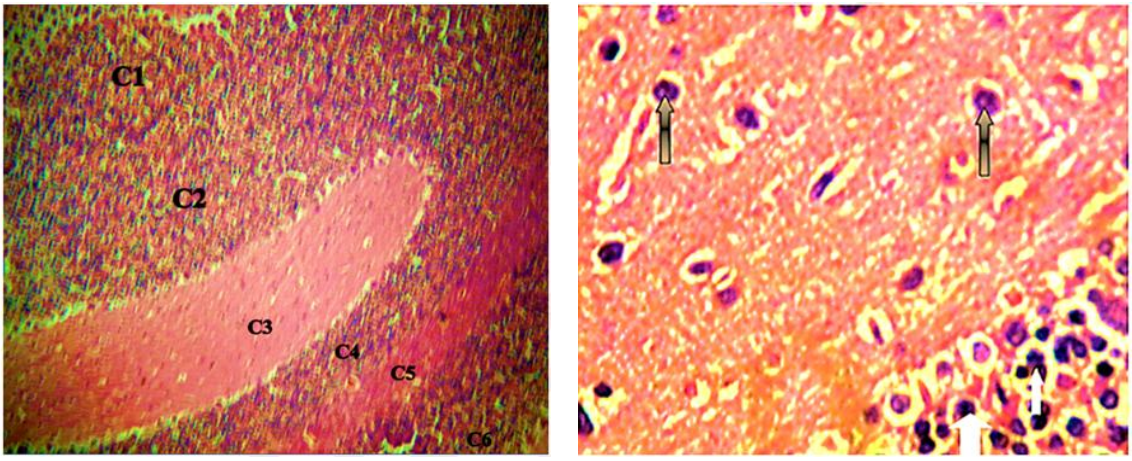

Fig. 6. Normal control group 1: Showing cerebral cell layers intact. H/E x40 and x400

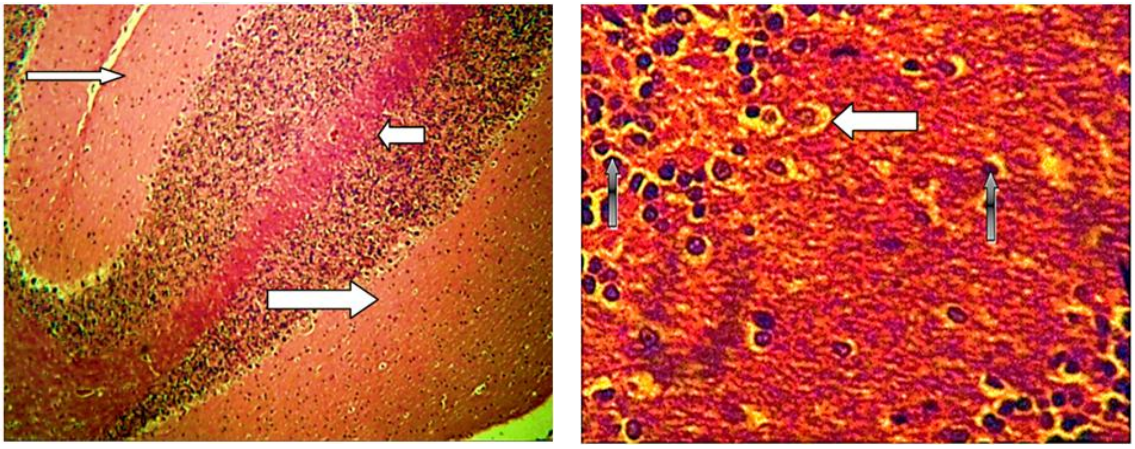

Fig. 7. *Carica papaya* treated high dose group 4: numerous cells and intact neurons observed in brain tissue as indicated by white and brown arrows. H/E x40 and x400

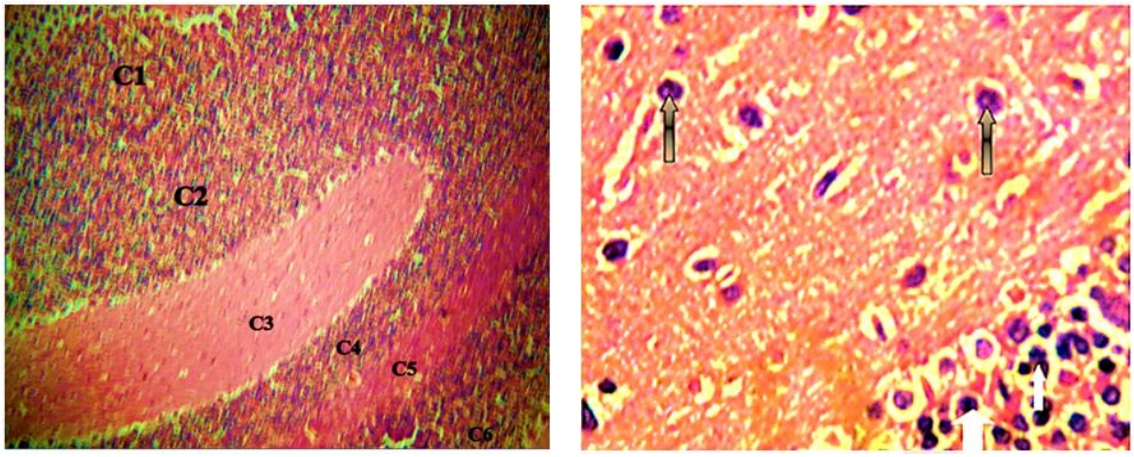

Fig. 8. Normal control group 1: Showing cerebral cell layers intact. H/E x40 and x400

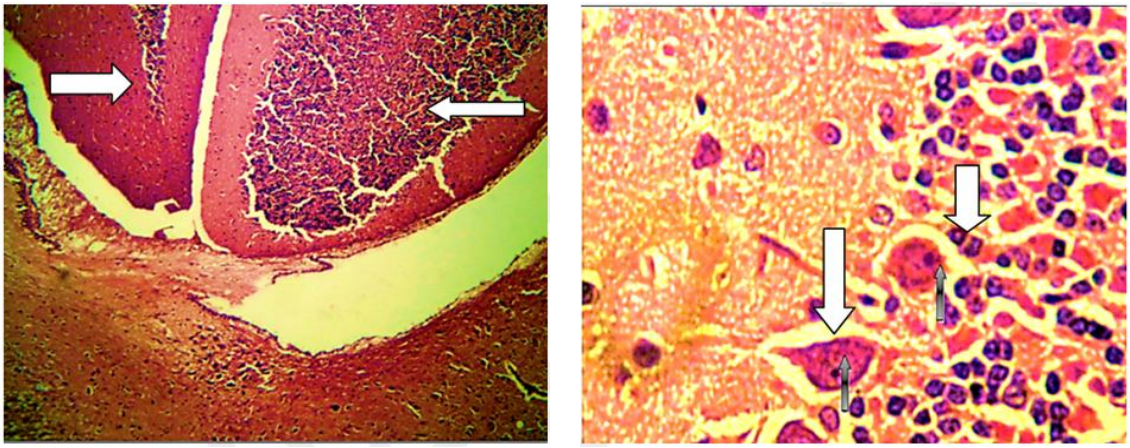

Fig. 9. Treated low dose group 5: numerous cells and intact pyramidal neurons was observed has shown by the white arrows. H/E x40 and x400

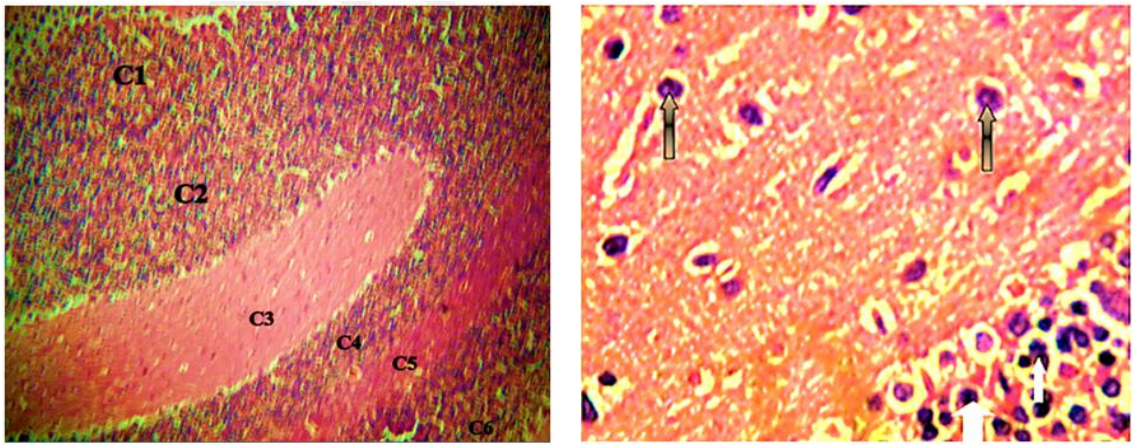

Fig. 10. Normal control group 1: Showing cerebral cell layers intact. H/E x40 and x400

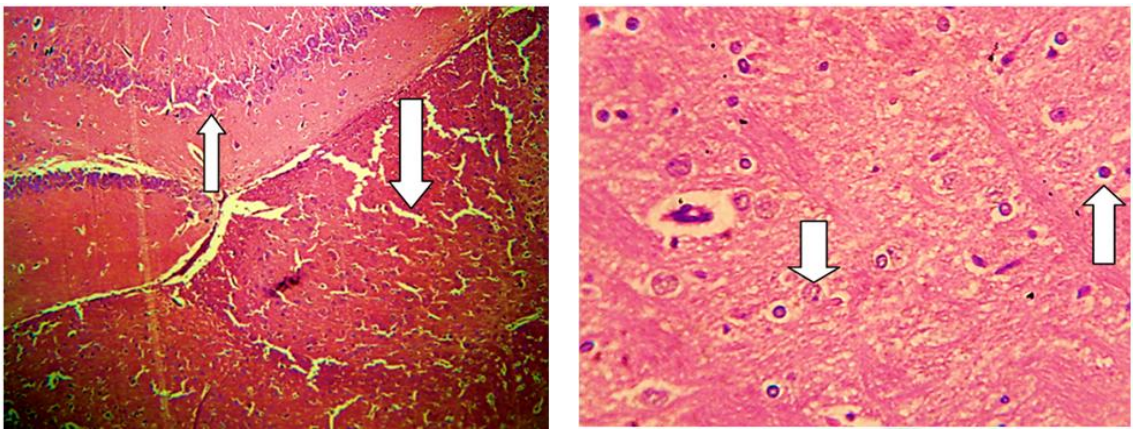

Fig. 11. Positive control group 6: Normal cerebral histology and neuronal cells were observed. H/E x40 and x400

No cellular damage was observed in group 6, treated only with *Carica papaya*, which may suggest that it has a protective effect against damage to cell morphology from activities metabolic waste products. Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced during the normal metabolism can damage the cells resulting in lipid peroxidation, alteration of protein and nucleic acid structures [23,24]. The variability in the animal body weight increases in cadmium induced groups as suggested by Filipič [1]. Inflammation is complicated in cadmium assault but, there were decreases in the body weight upon treatment with *Carica papaya*. The increase and decrease in body weight was significant at P≤ 0.05 which was in support of [18] research report.

5. CONCLUSION

The ameliorative property of *Carica papaya* on histomorphology of the prefrontal cortex of the adult Wistar rats against cadmium induced prefrontal-cortex is dose dependent and the use of *Carica papaya* should be encouraged in the developing countries because of its ready availability and medicinal properties.

CONSENT AND ETHICAL APPROVAL

It is not applicable since experiments were performed on animal models and All experimental investigations were done in compliance with humane animal use as stated in the “Guide to the care and use of Laboratory Animals Resources”. National Research Council, DHHS, Pub. No NIH 86 – 23 (1985) and in accordance with ethical approval of the Anatomy Department, Bingham University, Karu, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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