HISTOPATHOLOGICAL CHANGES ON THE HIPPOCAMPUS OF ADULT WISTAR RATS EXPOSED TO LEAD ACETATE AND AQUEOUS EXTRACT OF *Psidium Guajava* LEAVES

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**ABSTRACT**

**Aim:** The present study was aimed at evaluating the effects of aqueous leaves extract of *Psidium guajava* on histopathological changes observed in hippocampus of adult Wistar rats exposed to lead acetate.

**Methods:** Thirty-five adult Wistar rats were grouped into 7 Groups of 5 rats per Group. The rats were exposed to lead acetate at a concentration of 120mg/kg body weight (bwt.) daily for 21 days and treated with aqueous extract of *Psidium guajava* leaves at a concentration of 500mg and 1000mg/kg bwt. for 14 days. The rats were humanely sacrificed at the end of the experiment and the brain tissues were removed and fixed in Bouin’s fluid and processed for histopathological studies.

**Results:** The results of the study revealed some histopathological changes such as degeneration of neurons in the hippocampus of the adult Wistar rats exposed to lead acetate alone while the changes in the rats exposed to lead acetate and treated with aqueous leaves extract of *Psidium guajava* were ameliorated.

**Conclusion:** Thus, from the present study, the aqueous extract of *Psidium Guajava* leaves have reduced the toxicity affected by lead acetate and may likely be beneficial to the population in endemic areas that are exposed to lead poisoning.

**Key words:** *Psidium guajava*, lead, histopathological, hippocampus, cerebrum

**INTRODUCTION**

Lead poisoning is also known as plumbism, colica Pictonum, saturnism, Devon colic, or painter’s colic, which is a medical condition caused by increased levels of the heavy metal lead, in the body (Rossi, 2008). The increased in lead poisoning is a potential factor in brain damage, mental impairment and severe behavioral problems, as well as anemia, kidney insufficiency, neuromuscular weakness, and coma (Licyi *et al.*, 2001). This increased has been linked to the rapid increasing level of chemicals in the environment, particularly lead, which has well known hazardous effects (Sharma *et al.*, 2010). Lead acetate has been shown to induce cellular damage in the cerebellum of adult Wistar rats and it was also observed that ascorbic acid minimized the lead-induced cellular damages in the cerebellum of adult Wistar rats (Musa *et al.*, 2012). Several antioxidant molecules like glutathione (GSH) and glutathione disulphide (GSSG) and antioxidant enzymes like superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) are the most commonly used parameters to evaluate lead-induced oxidative damage (Ding *et al.*, 2010).
2000; Patra et al., 2001). However, the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention and treatment of diseases by increasing level of environmental chemicals such as lead and mercury, because plants have long served as a useful and natural source of therapeutic agents (Chevellier, 1996). The activities of these curative plants are evaluated by their chemical components. *Psidium guajava* have been reported to contain natural antioxidants, which neutralize free radicals and is receiving more attention from nutritionists and medical researchers for its potential effects in the prevention of chronic and degenerative changes, such as neurodegenerative diseases, cancer, cardiovascular disease and aging (Young and Woodside, 2001). The purpose of the present study was to evaluate the therapeutic effects of aqueous leaves extract of *Psidium guajava* on histopathological changes observed in hippocampus of adult Wistar rats exposed to lead acetate.

**MATERIALS AND METHODS**

**Animals**

Thirty five (35) adult male Wistar rats used in the present study were obtained from Animal House of the Department of Human Anatomy, Ahmadu Bello University, Zaria-Nigeria. The rats were acclimatized to experimental condition for a period of two weeks and feed with rat chow and water was allowed *ad libitum*.

**Psidium guajava leaves**

Fresh leaves of *P. guajava* were obtained from the Faculty of Medicine premises, Ahmadu Bello University, Zaria. The leaves were identified and authenticated with a voucher number of 3253 in Department of Biological Science Herbarium, Ahmadu Bello University, Zaria.

**Preparation of Extract**

Extraction of guava leaf was done in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Aqueous extraction of guava leaf was prepared according to the procedure of Ofodile et al. (2013). In this procedure, fresh leaves of guava were collected, washed thoroughly, shade-dried and pulverized using a mechanical grinder. The powder obtained was macerated with distilled water for 72 hours at 30±4°C. The mixture was filtered and the filtrate was concentrated in water bath at 50°C. Finally, the concentrated crude aqueous extract was subjected to drying to solid mass. The testing samples were prepared by dissolving the solid mass with distilled water to obtain a final concentration of 500mg/ml per kg body weight (kbw) and 1000mg/ml per kbw used for the experiment.

**Obtaining of lead acetate**

Laboratory grade of Lead acetate of 99% to 103% purity manufactured by BDH Chemical Ltd England was purchased from Cardinal Scientific, No.11/12 Sokoto Road, Samaru-Zaria. The chemical was identified as lead acetate at the Department of Chemistry, Ahmadu Bello University Zaria.

**Experimental Protocol**

Previous studies had established the LD$_{50}$ of *Psidium guajava* leaves aqueous extract to be 5000mg/kg body weight (Shubhangi, et al., 2013) following the OECD, (2001) guidelines. Therefore, doses of 10% (500mg/kg) and 20% (1000mg/kg) of the LD$_{50}$ were used in this study. Based on the reported oral LD$_{50}$ of Lead acetate which was 600mg/kg bw for Wistar rats (Sujatha et al., 2011), 20% of the LD$_{50}$ (120mg of lead acetate/kg body weight) was used in this study. 10mg/kg body weight of Meso-2,3-dimercaptosuccinic acid (DMSA) which is also known as Succimer which was used as a Standard drug was used according to Alan and Miller, (1998).

**Experimental Design**

Thirty five (35) adult male Wistar rats were divided into seven (7) groups of five (5) animals per group. Group I was administered with distilled water from 1$^{st}$ to 35$^{th}$ day, Group II was administered with 1000mg/kg bwt of *Psidium guajava* leaf extract only from 1$^{st}$ to 35$^{th}$ day, Groups III to VII were administered with 120mg/kg bwt of lead acetate from 1$^{st}$ to 35$^{th}$ day and Group VII was administered with 10mg/kg of Succimer from 22$^{nd}$ to 35$^{th}$ day. All the administrations were carried out orally within 35 days.
Animals Sacrifice
After the last administration, the animals were allowed to fast for 24 hours before they were anaesthetized with ketamine and humanely sacrificed. Incision was made through the skin and muscles of the skull and the skull was opened through the mid sagittal suture and the brain tissues were removed and fixed in Bouin’s fluid. The tissues were taken to the histology laboratory of Human Anatomy Department, Ahmadu Bello University, Zaria for tissue processing.

Tissue Processing
The tissues were fixed in Bouin’s fluid for 48 hours for proper fixation. The tissues were processed routinely, sectioned at 5μm thick, stained using Cresyl Echt Violet method (Freida, 2007).

Microscopic Cell Counting
Brain cells such as Pyramidal cells of the Hippocampus were counted using Digimizer image analysis software. Specific fields of the hippocampus mainly the CA3 region was studied at × 100 and × 250 magnifications with the aid of MD900 Am microscope digital camera and uploaded into the image area of the software and marker tool was used to click in the image on the cells to mark and count the number of each cell. The total of number of cells were automatically displayed in the statistics window and subjected to statistically analysis.

Data analysis
Data obtained were expressed as Mean ± SEM (Standard error of mean).

One-way analysis of variance (ANOVA) was used to compare the mean differences followed by Turkey’s post-hoc test. P-value less than to 0.05 was considered to be statistically significant. All the results were analysed using the Statistical Package for Social Scientist (SPSS version 16).

RESULTS
Histopathology of hippocampus of the rats
The hippocampus of the rats in Groups I and II showed normal appearance of pyramidal layer with normal pyramidal cells (Plates 1A and B). The hippocampus of the rats in Groups III and IV showed distortion of pyramidal layer, vacuolation, necrotic cells, lack of nerve cell bodies in some areas and some degenerated pyramidal cells (Plates 1C and D). It was observed that the hippocampus of the rats in Group V showed few degenerated pyramidal cells (Plate 1E), while the some areas in the hippocampus of rats in Groups VI and VII showed normal appearance of pyramidal layer (Plates 1F and G).

Number of Cells in Hippocampus
The results from the study show a significant decrease in Pyramidal cells of the hippocampus among rats in groups III and IV when compared to those in groups I, II, V, VI and VII. There was also a significant different in Pyramidal cells among rats in groups III and IV. There was also a significant decrease in Pyramidal cells of the cerebrum among rats in groups III, IV, V, VI and VII when compared with those in groups I and II. On the other hand, there was significant different in Pyramidal cells of among rats in III and IV when compared with those in group VII.
Plate 1 (A, B, C and D): Plate VI: Sections of the hippocampus showing; A (Control), B (AEPG<sub>1000</sub>), C and D (Pb<sup>2+</sup>). A and B: showing normal CA3 region with normal pyramidal cells (PC). C and D: showing distortion of pyramidal layer, vacuolation, necrotic cells, no nerve cell body in some areas and some degenerated pyramidal cells (Cresyl Echt Violet Stain x 250).

Plate 1 (E, F and G): Sections of the hippocampus showing: E (Pb<sup>2+</sup> + AEPG<sub>1000</sub>), F (Pb<sup>2+</sup> + AEPG<sub>500</sub>) and G (Pb<sup>2+</sup> + SUC<sub>10</sub>). E, F and G: showed normal appearance of pyramidal layer (Cresyl Echt Violet Stain x 250).

Figure 1: Bar chart showing the mean of Pyramidal cells in Hippocampus sections of the experimental rats. * and † significantly different from groups I & II and group IV respectively at p < 0.05.

**DISCUSSION**

The lead exposed rats showed degeneration of CA3 layer of the hippocampus, which manifested reduction in number of pyramidal cells. This observation could explain the impaired activities of the hippocampus including storage and retrieval of information. The present findings learn credence to previous reports. Khaled et al. (2014) had observed neuronal damage in brain cortex, hippocampus and cerebellum, with neurodegeneration of CA1 and CA3 regions. In this region of the brain, scar formation, demyelination and neuronal atrophy (Soltaninejad et al., 2003) could result from even low (0.2%) exposure to lead (Noor et al., 2012). Seddik et al. (2011) reported that chronic lead exposure in rats caused DNA fragmentation in frontal cortex, hippocampus and cerebellum, indicating an apoptotic or necrotic underlying mechanism. Loss of cellular integrity in CA3 region of the hippocampus may be responsible for the clinical features of lead poisoning such as disturbances in emotional response, memory and learning (Schneider et al., 2012). However, administration of aqueous extract of *Psidium Guajava* leaves has reduced
the impairment caused by lead acetate and this could be due to the fact that the extract has the ability to chalet lead in brain and blood. Similarly improvement has been reported that administering quercetin (Hu et al., 2008), the main active component of Psidium guajava serving to reduce the effect of lead (Lozoya et al., 2002). Quercetin chelates lead by forming a coordination bond with the lead ions through its orthophenolic groups located on the quercetin B ring (Bravo and Anacona, 2001). The hydroxyl groups of quercetin along with the carbonyl group easily donate electrons by undergoing resonance and stabilize free radicals that can initiate cellular damage (Beecher, 2003).

CONCLUSION
It could be pertinent to speculate that the ability of the aqueous extract of Psidium guajava leaves to reduce the effect of lead in hippocampus of the experimental rats may be attributed to its chelating and antioxidants properties. Therefore, Psidium guajava may likely be beneficial to the population in endemic areas that are exposed to lead poisoning.

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REFERENCES


