

## **The Effect of Methanoic Leaf Extract of *Mentha Piperita* on the Histology of the Cerebellum of adult male Wistar rats exposed to Lead acetate**

### **ABSTRACT**

Lead has been reported to produce oxidative stress by generating reactive oxygen species (ROS) which increase oxidative damage of cellular materials. Antioxidant enzymes and lipid peroxidation products such as Superoxide dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) play crucial role in counteracting the harmful effects of ROS. Peppermint leaf (*Mentha Piperita*) has anti-inflammatory, anti-spasmodic, antioxidant and anti-bacterial properties and is used in folkloric medicine in the treatment of urinary tract infections and allergies. This study is designed to investigate the protective role of peppermint leaf extract in the brain of lead exposed Wistar rats. This will be done by assessing serum levels of SOD, CAT and MDA and cerebellar histology. Twenty (20) adult male Wistar rats were divided into four (4) groups of five (5) rats in each group. Group A served as the control, Group B received 400mg/kg of mint extract only, Group C received 30mg/kg of lead acetate and Group D received both 30mg/kg of lead acetate and 400mg/kg of mint extract. They were administered orally with a syringe using an oral cannula for six (6) weeks. Rats were sacrificed by cervical dislocation. Results shows that CAT levels were significantly lower in the experimental groups B, C and D compared to the control group A, MDA levels showed no significant difference between treated groups B, C and D compared to group A, SOD levels were significantly higher in group B compared to group A but no significant difference in groups C and D compared to group A. Results of histological investigation showed no adverse effect of the peppermint extract and lead acetate on the cerebellum. We can therefore conclude that peppermint at 400mg/kg bw administered orally for 6 weeks uninterruptedly showed no adverse effect on the oxidative stress parameters and the histology of the cerebellum in male Wistar rats.

**Keywords:** *Mentha Piperita*, cerebellum, cervical dislocation, antioxidants, superoxide dismutase

### **INTRODUCTION**

“Medicinal plants and their derived compounds (phytochemicals) have been considered of pharmacological significance since ancient times. The use of plants in medicine dates back to 60,000 years ago, before the birth of civilization. Today more than 30% of all medicinal drugs (and their derivatives and analogs) derive from plants and natural products will continue to possess considerable impact in human medicine” (Nirmalet *al.*, 2013). “Among the plants with global economic and culinary importance, mint is used worldwide for perfuming sweet and savoury dishes and flavoring tea, in addition to its pharmacological importance” (Sevindiket *al.*, 2018). “It is called Ahu ogwu in Igbo, Ewe minti in Yoruba and Na’abba in Hausa”. [77, 78]

“These species are herbaceous and perennial plants, commonly cultivated for flavor and a pleasant aroma. Natural menthol has a soothing and relaxing cooling impact on the mucous membrane of the

human body and on the skin”. [79, 80]“Oil extracted from *Mentha* has cosmetic, pharmaceutical, and perfumery applications. Sometimes, it is also used for culinary purposes for food and flavors” (Ansari *et al.*, 2000). Although peppermint oil is obtained from three species namely, *M. arvensis L.* var *piperascens malinvaud*, *M. piperita L.* var *piperita* and *M. spicata L.*, oil quality of *M. piperita* and efficacy are reported to be the best.

“In addition, the use of herbal medicine for the treatment of diseases and infection is as old as mankind. World Health Organization (WHO) supports the use of traditional medicines provided they are proven to be efficacious and relatively safe. *Mentha piperita L.* (commonly known as peppermint) is a natural hybrid of a perennial herb, propagating vegetatively, spreading quickly by underground runner (raining season) and through stolon (winter). It is a well-known and important medicinal plant widely used in several indigenous system of medicines”(Gadaka *et al.*, 2021). Reed *et al.* (2008) reported that “peppermint scent can be used as an effective adjunct to decrease appetite, decrease hunger craving, and consume fewer calories, which may lead to weight reduction and greater overall health”.

“The medicinal uses of peppermint as a folk remedy include anti- inflammatory, antispasmodic, antioxidant, antibacterial, urinary tract infections, antiallergenic and sedative” (Baliga and Rao, 2010). “Peppermint being prepared in a tea form or when taken in a dose less than 270mg is usually considered as being safe” (Keifer *et al.*, 2008). “Peppermint (*Mentha piperita L.*) is among the most popular single ingredient herbal teas. It has been used for biliary disorders, dyspepsia, enteritis, flatulence, gastritis, intestinal colic and spasms of the bile duct, gallbladder and gastrointestinal tract” (Gadaka *et al.*, 2021).

“The chemical components of peppermint leaves and oil vary with plant maturity, variety, geographical region and processing conditions” (Gherman *et al.*, 2000; Blanco *et al.*, 2002; Pino *et al.*, 2002; Ruiz del Castillo *et al.*, 2003; Xu *et al.*, 2003). “The fatty acid composition of the non-polar lipid fraction of peppermint leaves is dominated by palmitic, linoleic and linolenic acids. The main volatile components identified in the essential oil of peppermint are menthol (33–60%), menthone (15–32%), isomenthone (2–8%), 1,8-cineole (eucalyptol) (5–13%), menthyl acetate (2–11%), menthofuran (1–10%), limonene (1–7%),  $\beta$ -myrcene (0.1–1.7%),  $\beta$ -caryophyllene (2–4%), pulegone (0.5– 1.6%) and carvone (1%)” (Dimandja *et al.*, 2000; Gherman *et al.*, 2000). The leaves contain 1.2– 3.9% (v/w) essential oil (0.38% yield from fresh leaves) (Kaul *et al.*, 2001), while an infusion of dried leaves is reported to contain 21% of the original oil (25 mg/L). Proportions of the individual components found in oil were both higher and lower than those found in the infused tea.

Studies regarding the mineral content of peppermint leaves are more comprehensive than those pertaining to the vitamin content. Fresh *M. piperita* leaves from Brazil were found to contain 940–1016 retinol equivalents (RE)/100 g  $\beta$ -carotene. The presence of other carotenoids and chlorophylls, as well as  $\alpha$ - and  $\gamma$ - tocopherols and ascorbic acid (Capecka *et al.*, 2005), has also been reported. “The major minerals in dried peppermint leaves (as g/kg) include K (33), Ca (15.3), Mg (5.8) and lower amounts of Na, along with smaller amounts (as mg/kg) of Fe (239), Mn (188), Zn (51) and Cu (12). Trace amounts (as  $\mu$ g/g) of Cr (941), I (325) and Se (147) are also present” (Lozak *et al.*, 2002). “Concentrations of these minerals found in an infusion of dried leaves (prepared at 95 °C, 15 min) were approximately 8–60% of the amounts present in the leaves, i.e. Ca (2.9 g/kg), Mg (2.2 g/kg), Fe (20 mg/kg), Mn (27 mg/kg), Zn (6 mg/kg), Cu (3 mg/kg), Cr (390  $\mu$ g/g), I (206  $\mu$ g/g) and Se (87

µg/g)” (Lozak et al., 2002). According to Lozak et al., (2002), “the most readily eluted elements of nutritional importance from the leaves are Se and I with Fe as the least eluted mineral”. Muller et al., (1997) reported “finding 477 µg/g Al in dried mint leaves, approximately half the amount present in black tea (899 µg/g); however, the transfer of Al into a peppermint tea infusion was very low (5%) compared with black tea (30%)”. “The total polyphenolic content of peppermint leaves is approximately 19–23% (total flavonoids 12%), which includes 59–67% eriocitrin and rosmarinic acid (combined), 7–12% luteolin 7-O-rutinoside, 6–10% hesperidin, and smaller quantities of 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone, pibrellin, gardenin B and apigenin” (Areias et al., 2001; Zheng and Wang, 2001). “About 75% of the polyphenolic compounds present in the leaves are extracted in an infusion (750 mg/L)” (Duband et al., 1992). The salicylic acid content of peppermint candies and tea was reportedly very high (7.7–75.8 mg/kg) in an early study by Swain et al., (1985); however, a more recent analysis using a more sensitive assay method by Venema et al., (1996) revealed <0.2 mg/kg.

“Phenolics belong to an important class of compounds responsible for the antioxidant activity of mint. Previous studies have found the total polyphenolic content of peppermint leaves to be approximately 19–23% with total flavonoids of 12%” (McKay and Blumberg 2006). Mata et al., (2007) reported that “the main active components in mint were eriocitrin, rosmarinic acid, luteolin 7-O-rutinoside, hesperidin, and small quantities of pibrellin, gardenin B and apigenin”. “Another study determined the major polyphenols in mint as rosmarinic acid, caffeic acid, ferulic acid and eugenol” (Tahira et al., 2011).

The antioxidant capacity of peppermint has been determined using a number of different assay methods. The oxygen radical absorbance capacity (ORAC) value for an aqueous solution of previously frozen fresh *M. piperita* leaves (supernatant of 2.0 g homogenized in 15 mL buffer) was among the highest found in an analysis of popular medicinal herbs by Zheng and Wang, (2001). At  $15.84 \pm 0.42$  µmol Trolox equivalents (TE)/g fresh weight, the ORAC value for *M. piperita* was similar to *Hypericum perforatum* ( $16.77 \pm 0.22$  µmol TE/g) and *Valerian officinalis* ( $15.69 \pm 0.37$  µmol TE/g), slightly higher than *Salvia officinalis* ( $13.28 \pm 0.40$  µmol TE/g) and lower than *Thymus vulgaris* ( $19.49 \pm 0.21$  µmol TE/g). The ORAC values for the related *M. aquatica* and *M. spicata* were  $19.80 \pm 0.43$  and  $8.10 \pm 0.26$  µmol TE/g, respectively. In a study using the ferric reducing ability of plasma (FRAP) assay, Dragland et al., (2003) found the relative antioxidant value of dried *M. piperita* (78.5 mmol/g) to be lower than *S. officinalis* (91.2 mmol/g) and higher than *T. vulgaris* (74.6 mmol/g). FRAP values >75 mmol/g are indicative of high antioxidant concentrations. As with other compounds present in *M. piperita*, seasonal variations with regard to antioxidant activity have been observed (range 59.8–96.1 mmol/g) (Dragland et al., 2003).

According to Mimica-Dukic et al., (2003), the free radical scavenging capacity of *M. piperita* oil was higher than that of either *M. aquatica* or *M. longifolia*. In their experiment, *M. piperita* reduced the radical generator 2,2-diphenyl-1-picrylhydrazyl (DPPH) by 50% ( $IC_{50} = 2.53$  µg/mL) and inhibited the generation of the OH radical in the Fenton reaction by 24%. In an assay based upon the oxidation of homovanillic acid (HVA) to its fluorescent biphenyl dimer in the presence of H<sub>2</sub>O<sub>2</sub> and peroxidase, the antioxidant capacities of aqueous solutions of peppermint (0.1, 0.5 and 1.0%), prepared with boiling water and incubated 10 min at 95 °C, were among the highest of the tea infusions tested by Pazdziuch-Czochra and Widenska, (2002). The percent of fluorescence inhibition

exhibited by a 0.5% peppermint infusion (closest approximation to an amount typically used) was ~67%; lower than comparable amounts of black (78%) and green (81%) teas, but higher than other herb teas including hibiscus (56%) and rooibos (52%). When the results were expressed as Trolox equivalents, these teas were ranked similarly, i.e. black tea ( $0.32 \pm 0.05$ ), green tea ( $0.31 \pm 0.03$ ), peppermint tea ( $0.27 \pm 0.02$ ), hibiscus tea ( $0.20 \pm 0.02$ ) and rooibos ( $0.17 \pm 0.01$ ). One limitation of this particular method includes the ability to estimate only the H<sub>2</sub>O<sub>2</sub> scavenging ability of the tested herbs, and not the scavenging of other free radicals. In other studies, ethanol extracts of dried *M. piperata* were shown to stabilize the auto-oxidation of kinetically pure triacylglycerols of sunflower oil (Yanishlieva and Marinova, 1995), and natural sunflower oil (Marinova and Yanishlieva, 1997). Essential oil derived from peppermint exhibited an even greater antioxidant effect against sunflower oil peroxidation than butylated hydroxytoluene (BHT) (Gurdipet *et al.*, 1998).

“Lead is a common industrial poison that persists in the environment and has many toxic effects on different organs & tissues especially on the central nervous system” (Bauchi *et al.*, 2016). “chronic poisoning by it is one of the major public health hazards especially in developing countries” (Flora *et al.*, 2012). “Small amount of lead is excreted in urine and the rest accumulates in various body tissues, mainly the (CNS) which may result in structural changes that can persist even after lowering of its blood level” (Sidhu, 2004; Taibet *et al.*, 2004; Flora *et al.*, 2006; Ibrahim *et al.*, 2012). “Chronic poisoning by it is one of the major public health hazards especially in developing countries” (Flora *et al.*, 2012). “Small amount of lead is excreted in urine and the rest accumulates in various body tissues, mainly the central nervous system (CNS) which may result in structural changes that can persist even after lowering of its blood level” (Sidhu and Nehru, 2004; Taib *et al.*, 2004; Flora *et al.*, 2006; Ibrahim *et al.*, 2012).

In Nigeria, exposure to occupational lead is the primary source of lead poisoning (Adeniyi *et al.*, 1999; Alasia *et al.*, 2010; Adi, 2015) with the most threatening risk emanating from welding, painting, printing, combustion, as well as copper and zinc smelting thus, posing a significant risk to workers and their families (Shaffer and Gilbert, 2017). “In the brain, lead exposure mediates neuropathologic conditions by altering cellular metabolism gene transcriptions and by inducing abnormal protein accumulation, and malfunctions in messenger systems as well as apoptosis” (Eid’ Zawia, 2016). “Several studies have discovered that lead exposure heightens the risk of Alzheimer’s disease (AD) by causing amyloid precursor protein” (Bashaet *et al.*, 2005; Zhou *et al.*, 2018; Ge *et al.*, 2018).

“Lead was reported to produce oxidative stress by generating release of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, hydrogen peroxide and lipid peroxides which increase oxidative damage of cellular materials” (El- Nekeety *et al.*, 2009). “Depending on the observation that free radicals were generated during the pathogenesis processes induced by lead, it was presumed that supplement of antioxidants will interrupt or minimize the damaging effects of lead and improve the effects of chelating agents” (Flora *et al.*, 2003).

“Oxidative stress is often described as a self-propagating phenomenon on the basis of observations that when oxidative stress-induced excessive ROS release triggers cellular damage, damaged macromolecules themselves may behave as and/or become ROS” (Salim, 2016). “Consequently, the brain, with its rich lipid content, high energy demand, and weak antioxidant capacity becomes an easy target of excessive oxidative insult” (Hulbert *et al.*, 2007). “Phospholipids in the brain are

particularly vulnerable entities for ROS-mediated peroxidation, but proteins and DNA also are targeted by ROS, which becomes particularly problematic during aging, as aged brains have been reported to exhibit high levels of oxidative stress-induced mutations in the mitochondrial DNA” (Chomyn, 2003; Kraytsberget *al.*, 2003; Trifunovic *et al.*, 2004). “Therefore, ROS accumulation is a cellular threat that, if it exceeds or bypasses counteracting mechanisms, can cause significant neuronal damage. Two kinds of protective mechanisms operate in the brain to tackle the threat posed by ROS, the antioxidant enzyme system and the low-molecular-weight antioxidants” (Kohen *et al.*, 2000). “The antioxidant enzyme system includes superoxide dismutase (SOD), glyoxalase, glutathione reductase, glutathione peroxidase, and catalase (CAT)” (Griendling *et al.*, 2000). “SOD enzymes, including Cu-Zn SOD and Mn-SOD, facilitate spontaneous dismutation of superoxide radicals to generate H<sub>2</sub>O<sub>2</sub>, which is further removed by CAT and glutathione peroxidase enzymes” (Saso, 2014).

“Oxidative stress refers to the excessive production of reactive oxygen species (ROS) in the cells and tissues and antioxidant system cannot be able to neutralize them. Imbalance in this protective mechanism can lead to the damage of cellular molecules such as DNA, proteins, and lipids” (Duračková, 2010). “Reactive oxygen species are normally produced within the body in limited quantity and are important compounds involved in the regulation of processes involving the maintaining of cell homeostasis and functions such as signal transduction, gene expression, and activation of receptors” (Kumar and Pandey, 2015). “Mitochondrial oxidative metabolism in cells produces ROS species and organic peroxides in the process of cell respiration” (Goossens *et al.*, 1999). “In addition, in hypoxic conditions, nitric oxide may also be produced during the respiratory chain reaction” (Poyton *et al.*, 2009). “This latter reactive nitrogen species (RNS) may further lead to the production of reactive species such as reactive aldehydes, malondialdehyde, and 4-hydroxynonenal” (Hussain *et al.*, 2003). “Main targets of oxidative stress are proteins, lipids, and DNA/RNA, and modifications in these molecules may increase the chances of mutagenesis. ROS/RNS overproduction notably over a prolonged period of time can cause damage of the cellular structure and functions and may induce somatic mutations and preneoplastic and neoplastic transformations. Then, excessive production of ROS in cells and tissues may be deleterious if not removed quickly” (Chang *et al.*, 2011). “Indeed, excessive ROS/RNS production may cause irreversible damage to cells resulting in cell death by the necrotic and apoptotic processes” (Wang *et al.*, 2004).

“Lead exerts its toxic potential by targeting hemesynthesising enzymes, thiol-containing antioxidants, and oxidative enzymes, including CAT, GSH, MAD, and SOD. One of the significant lead markers of oxidation is lipid peroxidation, which results in RBC hemolysis, oxidative stress, and tissue degradation” (Flora *et al.*, 2012). “In light of this, targeting this redox imbalance may be imperative in mitigating lead-induced deleterious effects. However, due to the recent trends in phytochemicals to combat oxidative damage and neurotoxicity, we employed OG to ameliorate LA-induced toxicity. Antioxidants enzymes are frequently used as markers of oxidative stress” (Gutteridge, 1995). “Among these biomarkers, superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) are important in the preservation of homeostasis for normal cell function. Enzymes control the rate of metabolic reactions by lowering the amount of activation energy needed to start a reaction. Enzymes exhibit specificity by binding to particular substrates. CAT is found in the peroxisomes of the liver and kidney and acts to metabolize hydrogen peroxide, a toxic by-

product of metabolic reactions, to water and free oxygen” (Knight, 1997). “SOD scavenges the highly toxic superoxide and converts it to hydrogen peroxide” (Fridovich, 1989). “The hydrogen peroxide that is produced by SOD is maintained at a safe concentration by the glutathione system. GPX protects the membrane lipids from oxidative damage” (Kantola *et al.*, 1988).

The cerebellum is essential for smooth, purposeful movement. Recently, human neuroimaging and animal behavior studies have implicated the cerebellum in the processing of signals for perception, cognition, and emotion (Schmahmann, 2010; Bastian, 2011; D’Angelo, 2012), particularly in circumstances involving predictions or timing. The cerebellum originates from the dorsal portion of the hind brain and its development can be summarized in four steps: organization of cerebellar territory, establishment of cerebellar progenitors (GABAergic and glutamatergic ones), migration of the granule cells, and formation of the cerebellar nuclei and circuitry (ten Donkelaar *et al.*, 2003). “The cerebellum presents an outer gray matter layer (namely the cerebellar cortex), a deeper cerebellar white matter (called arbor vitae), and within this latter, the deep cerebellar nuclei (dentate, globose, emboliform, and fastigial nuclei; Voogd, 2003). The cerebellar cortex is composed of three layers (from the deepest to the most superficial: the granular layer, the Purkinje layer, and the molecular layer), four inhibitory cell types [stellate cells, basket cells, Purkinje cells (PCs), and Golgi cells], two excitatory cell populations [granule cells and unipolar brush cells (UBCs)], and glial cells” (among which Bergman glia; Buffo and Rossi, 2013; Kandel *et al.*, 2013; Roostaei *et al.*, 2014).

“The cerebellar cortex has three layers; the molecular layer-outer cell-sparse layer that underlies the pia mater. It also contains dendritic arborizations of Purkinje cells and the parallel fibers of the granule cells. It contains stellate (outer) cells and basket (inner stellate) cells; Purkinje cell layer - found between the molecular layer and the granule cell layer; Granule cell layer -found between the Purkinje cell layer and the cerebellar white matter. It contains granule cells, Golgi cells, and cerebellar glomeruli. In general, subjects with cerebellar damage have a preserved intellectual level with poor performance with regard to definite cognitive abilities. All major cognitive domains can be affected by cerebellar damage but the cognitive scores generally result in the lower range of normal limits” (Schmahmann and Sherman, 1998; Tedesco *et al.*, 2011). “This evidence is consistent with the hypothesis that cerebellar injury does not abolish specific cognitive functions—instead rendering them less efficient” (Courchesne and Allen, 1997; Hokkanen *et al.*, 2006). It is worth noting that, among all cognitive domains, the most affected by the cerebellar damage are sequencing, linguistic, executive and visuospatial functions.

The cerebellar cognitive impairment is not linked to the motor deficits as demonstrated by the lack of correlations between cognitive and motor scores (Tedesco *et al.*, 2011) and by the evidences of better cognitive performances in patients with higher motor impairment, i.e. patients with cerebellar atrophy (Leggio *et al.*, 2000).

## **MATERIALS AND METHODS**

This study was carried out at the Animal house of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Okofia in Anambra State, Nigeria. The experimental animals were made to acclimatize for 14 days after which administration of lead acetate and

peppermint extract lasted for a period of six (6) weeks. Fresh peppermint leaves were harvested and transported from Jos in Plateau state to Nnewi in Anambra State. It was identified and authenticated at the Department of Botany, NnamdiAzikiwe University, Awka with herbarium number NAUH:-220B.

### **METHANOLIC EXTRACTION OF THE PLANT**

The plant material was thoroughly washed with clean water to remove soil and dirt. Then the leaves were separated from the stem, air dried for complete drying. The dried plant material was powdered using a blender. 400g of the powder was soaked in 1000ml of methanol and left for 48hrs. The extract was sieved with a muslin cloth. The filtrate was concentrated in a digital rotary evaporator of model TT-52, Techmel and Techmel USA at 65°C. The concentrated extract is then oven dried at 55°C for 3 days and freeze dried for 48hrs. The freeze dried extracts was stored in a refrigerator until use.

The median lethal dose (LD<sub>50</sub>) of the *Mentha piperita* methanolic leaf extract was determined using the Method described by Enegideet *al.*, (2013). 0.2g of sample was weighed and transferred in a test tube and 15ml of ethanol was added. The test tube was allowed to react in a water bath at 60°C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10% v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of petroleum ether of which 200ul was transferred to a vial for analysis.

### **EXPERIMENTAL DESIGN**

This is an experimental study involving twenty (20) adult male Wistar rats randomly divided into four groups of 5 animals each. Group I served as the control group and received rat chow and water throughout the experiment *ad libitum*. Group II, received 400mg/kg/bw of *Menthapiperita* extract once a day for 6 weeks orally. Group III, received 30mg/kg/bw of lead acetate twice weekly for 6 weeks with addition to feed and water *ad libitum*. Groups IV, which constitutes the treated group, were orally administered with 400mg/kg/bw of *Menthapiperita* extract for 6 weeks once a day and 30mg/kg/bw of lead acetate 2 times weekly for 6 weeks in addition to feed and water *ad libitum*. Both lead acetate and *Mentha piperita* methanol extract were administered to the rats orally using a 5ml syringe with an in-tube sterile cannular.

### **TERMINATION OF RESEARCH AND BRAIN COLLECTION**

After 24hrs of the last administration, animals were weighed and sacrificed by chloroform sedation and the brains harvested. Brain samples were homogenized in phosphate buffer solution and centrifuged at 1000rpm to separate the supernatant from the residue. The supernatant was used for the rest of the assay. The remaining cerebellum excised from rats from each group were fixed in 10% buffered formalin for histological studies using the H&E method. Data were analysed using one-way ANOVA followed by post- hoc turkey was carried out to determine

mean significant differences between groups with SPSS version 21. Data were presented as Mean  $\pm$  S.D and Levels of statistical significance were set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### RESULTS OF MEDIAN LETHAL DOSE DETERMINATION

After administration of 5000mg/kgbw of the extract to two groups of 4 rats each, it was confirmed that there was no mortality of the rats at 5000mg/kgbw.

Table 1. RESULTS OF QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF PEPPERMINT LEAF EXTRACT

PHYTOCHEMICAL	CONC (ug/ml)
Kaempferol	3.33
Steriod	8.29
Proanthocyanidin	10.49
Anthocyanin	2.07
Narigenin	7.43
Dihydrocytisine	5.91
Cyanogenic glycosides	20.19
Ammodendrine	22.07
Tannin	12
Flavonones	8.74
Cardiac glycoside	4.01
Flavones	5.72
Ribalinidine	0
Phytate	8.81
Sparteine	15.97
Oxalate	1.31
Aphyllidine	4.24
Epihedrine	3.94
Sapogenin	11

Table 1 showing phytochemicals present in peppermint leaf extract with its quantities in microgram per milliliter.

The mean lethal dose was carried out and determined using the method described by Eneget *et al.*, (2013). The result of mean lethal dose of the extract was investigated and showed no toxicity even at a dose of 5000mg/kg. This was useful in determining the dose to be administered in this study. A previous report by (Eickolt and Box, 1965) shows that the mean lethal dose of peppermint oil in fasted Wistar rats was 4441mg/kg after 24hrs and 2426mg/kg after 48hrs.

Table 2. RESULTS OF RAT WEIGHT

GROUPS	INITIAL WEIGHT	FINAL WEIGHT	P- VALUE
A (CONTROL)	141.08 ± 7.48	176.80 ± 20.24	
B (MINT ONLY)	143.58 ± 5.89	198.75 ± 25.57	0.038
C (LEAD ACETATE ONLY)	185.25 ± 6.82	192.95 ± 12.19	0.369
D (MINT + LEAD ACETATE)	211.03 ± 3.15	275.28 ± 23.80	0.008

Table 2 showing result of rat weight changes before and after experiment

This result of rat weight changes of table 2 shows that rats in groups A, B and D had a significant weight gain at the final stage of the experiment compared to the initial stage but no statistically significant difference in the body weight of rats in group C at the final stage compared to the initial stage.

The means of rat initial and final weights were compared using the student's t- test and the results were presented as Mean ± S.D. of 4 rats in each group.

This study also investigated the body weight of the animals before and after administration and the result showed weight gain after administration in groups B and D and no significant difference between the initial and final weight in group C but a steady weight gain in group A. Studies related to the effects of peppermint on body weight of wistar rats according to Behzad *et al.*, 2015 agrees with the weight gain of groups B and D as peppermint extracts increases the body weight of wistar rats but studies from Sidhu and Nehru, (2004) and Kabeer *et al.*, (2019) on the effects of lead acetate on body weight disagrees with the results of group C although it was not statistically significant compared to group A.

Table 3. RESULT OF LEAD ACETATE ON MALONDIALDEHYDE (MDA) LEVELS

GROUPS	MALONDIALDEHYDE (MDA) LEVELS (nmol/ml)	P- VALUE
A (CONTROL)	1.63 ± 0.30	
B (MINT ONLY)	1.42 ± 0.28	0.3496
C (LEAD ACETATE ONLY)	1.46 ± 0.21	0.3934
D (MINT + LEAD ACETATE)	2.06 ± 0.46	0.1635

This result of the rat brain MDA levels in table3 shows that MDA levels shows no significant difference in MDA levels between the treated groups B, C and D compared to group A.

Table 4. RESULT OF LEAD ACETATE ON CATALASE (CAT) LEVELS

GROUPS	CATALASE (CAT) LEVELS (Ku/l)	P- VALUE
A (CONTROL)	27.37 ± 5.12	
B (MINT ONLY)	25.49 ± 2.10	0.522
C (LEAD ACETATE ONLY)	19.52 ± 2.29	0.031
D (MINT + LEAD ACETATE)	20.42 ± 4.29	0.082

This result of the rat brain CAT levels in table 4 shows that CAT levels were significantly lower in the experimental groups B, C and D compared to group A. However, only those of group C attained statistical significance.

Table 5. RESULT OF LEAD ACETATE ON SUPEROXIDE DISMUTASE (SOD) LEVELS

GROUPS	SUPEROXIDE DISMUTASE (SOD) LEVELS (u/l)	P- VALUE
A (CONTROL)	26.63 ± 1.17	
B (MINT ONLY)	28.46 ± 0.61	0.032
C (LEAD ACETATE ONLY)	26.02 ± 1.84	0.595
D (MINT + LEAD ACETATE)	23.53 ± 2.77	0.085

This result of the rat brain SOD levels in table 5 shows statistically significant higher levels for rats in group B treated with mint only compared to rats in group A. However, values for rats in groups C and D treated with lead acetate and lead acetate + mint respectively were not significantly different from those of group A.

In this present study, lead acetate was administered at a dose level of 30mg/kg, twice a week for six weeks to groups C and D and at the end of the treatment, CAT levels were significantly lower compared to group A while SOD and MDA levels were not significantly different compared to group A. Recent studies by Udi *et al.*, (2022) and Kabeer *et al.*, (2019) disagrees with the findings reporting higher levels of MDA and reduced levels of SOD in the groups treated with lead acetate compared to the control group. Sidhu and Nehru, 2004 and Udi *et al.*, 2022 agrees with the results of the CAT levels indicating low levels of CAT.

This disagreement may be due to duration of administration of the lead acetate. In this study, lead acetate was administered twice a week for six weeks while Sidhu and Nehru, 2004 administered 50mg/kg for 8 weeks on alternate days, Udi *et al.*, 2022 administered 120mg/kg daily and other authors who administered lead acetate for longer periods reported effects of lead acetate on the oxidative stress markers and histology of the cerebellum.

Plate 1. RESULTS OF PEPPERMINT EXTRACT ON HISTOLOGY OF THE CEREBELLUM

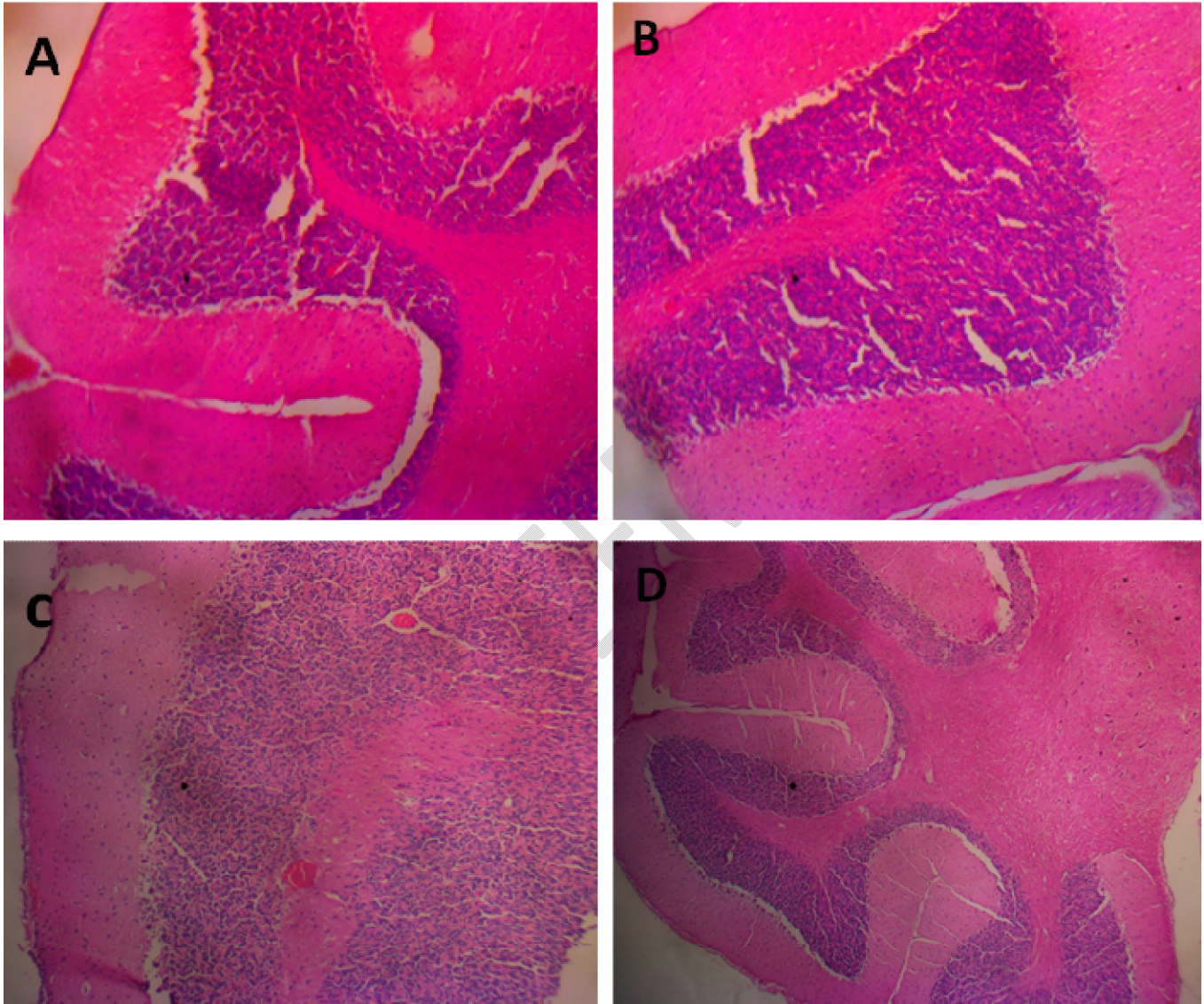


Plate A represents the normal histoarchitecture of the grey and white matter of the cerebellum in the control group (H&E  $\times 100$ ). Plate B shows no distortion in the layers of the cerebellum in the mint only group (H&E  $\times 100$ ). Plate C shows no distortion in the layers of the cerebellum in the lead acetate only group (H&E  $\times 100$ ). Plate D shows no distortion in the layers of the cerebellum in the mint and lead acetate group (H&E  $\times 100$ ).

This study also evaluated the effects of peppermint leaf extract on the histology of the cerebellum. Histological slide results (plate B) shows no effect of peppermint extract on the histology of the cerebellum and no effect of lead acetate on the histology of the cerebellum (plate C).

Studies related to this are limited but David *et al.*, (2018) reported a work that showed no effect of a mentha specie (*Menthaspicata*) on the brain histology which agrees with the result of plate B.

Studies from Saleh and Meligy, (2018), Kabeeret *al.*, (2019) and Sidhu and Nehru, (2004) disagrees with result of plate C reporting disruption in the layers of the cerebellum especially the Purkinje layer.

## **CONCLUSION**

The results of this study showed no effects of peppermint extract on the histology of the cerebellum. There was also no satisfactory result on the effects of lead acetate on the antioxidant enzymes (MDA and SOD) and lipid peroxidation (MDA) and this may be due to duration of administration of lead acetate.

## **RECOMMENDATION**

This study recommends a higher dose of peppermint leaf extract to be administered and for longer duration.

### **Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

- 1.
- 2.
- 3.

## **Ethical Approval**

Ethical approval was obtained from the ethical committee, Faculty of Basic Medical Sciences, NnamdiAzikiwe University, Nnewi Campus with the reference number NAU/CHS/NC/FMBS/499.

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