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REMEDIAL POTENTIAL OF MENTHA PIPERITA METHANOLIC EXTRACT ON LEAD ACETATE INCUCED NEPHROTOXICITY

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ABSTRACT

Mentha piperita L. (Pepper mint) a medicinally important plant that is well-known and widely cultivated for its use in flavor, fragrance, medicinal and pharmaceutical application. The medicinal parts of pepper mint are the essential oil composed of menthol and menthone together with several other minor constituents extracted from the aerial parts of the flowering plants, the dried leaves, the fresh flowering plant and the whole plant (Rajinder *et al*., 2015). This study was aimed at investigating the effect of Methanolic leaf extract of Mentha piperita on lead induced nephrotoxicity, kidney function and histology, on adult male wistar rat. A total of twenty-six (26) adult male wistar rats were obtained for this experiment. 16 adults male wistar rats were used for the experiment while 10 adult male wistar rats were used for Ld_{50}. The rats were divided into 4 groups of four rats each – Group A (Control group received feed and water only), group B (400mg/kg of methanolic leaf extract *of Mentha piperita* once daily), group C (received 30mg/kg of Lead acetate at 2 days interval) and group D (received 30mg/kg of Lead acetate at 2 days interval and 400mg/kg of methanolic leaf extract *of Mentha piperita* daily). The experiment lasted for a period of 8 weeks; two (2) weeks for acclimatization and six (6) weeks for administration using the oral gavage method. The result on the body weight changes showed a significant weight gain of those in the control group, and the same was observed for rats exposed to peppermint and those that received lead acetate and peppermint, but no statistically significant difference in the body weight of rats in the lead acetate group ($p<0.05$). The results of rat serum urea and creatinine levels show no statistically significant difference in the experimental groups when compared to the control group. The findings in this study indicate that *Mentha piperita* leaf has no renal normative potential and cannot improve renal functions in animals.

KEYWORDS: *Mentha Piperita*, peppermint, methanolic, lead acetate, wistar rats, nephrotoxicity.

INTRODUCTION

Mentha piperita, commonly known as peppermint, is a hybrid plant of the Mentha genus, native to Europe and the Middle East (Adam, 2001). Peppermint is widely cultivated for its essential oil, which is extracted from its leaves and used in various applications, such as flavoring agents, aromatherapy, and medicinal purposes (Tzakou, Couladis & Verykokidou., 2001). Pepper mint contains various active compounds, including menthol, menthone, rosmarinic acid and flavonoids, which are responsible for its pharmacological properties include antispasmodic, carminative, analgesic, anti-inflammatory, and antioxidant effects (Chaudhary & Walker, 2019). Due to these properties, peppermint is used to treat various

health conditions, such as irritable bowel syndrome, nausea, headaches, and respiratory problems (Pittler & Ernst, 1998). The antispasmodic and carminative properties of peppermint make it a popular natural remedy for digestive disorders (Alammar *et al*., 2019). Its active compounds help to relax the muscles in the gastrointestinal tract, reducing cramps and bloating (Hill & Aaronson, 1991). Additionally, peppermint oil has been shown to have antimicrobial effects against several bacterial strains, including Helicobacter pylori, which is associated with stomach ulcers (Zellers *et al*., 2018).

Lead is a ubiquitous, versatile and one of the most widely scattered toxic metals in the environment (Khan *et al*., 2019). It enters into the body either by ingestion, inhalation or by dermal contact (organic lead). Once absorbed, around 99% of lead is retained in the blood for nearly 30-35 days and is subsequently disseminated and accumulated in various tissues like kidneys, brain, liver, aorta, lungs, spleen and bones (Gillis *et al*., 2007). Exposure to lead causes variety of deleterious effects in almost all the organs and organ systems of the body (Sokol & Berman 1991). Long-time exposure to lead has been reported to cause anemia, along with an increase in blood pressure. Severe damage to the brain and kidneys both in adults and children, were found to linked to exposure to heavy lead levels resulting in death. Subchronic exposure to lead acetate induces oxidative stress in renal tissues, evidenced by elevated markers of lipid peroxidation and reduced antioxidant enzyme activity. Natural antioxidants, such as those found in Mentha piperita, may mitigate these effects, though the extent of protection varies with dosage and duration of exposure (Ezeokafor *et al*., 2022).

MATERIALS AND METHODS

Ethical approval

Ethical approval was obtained from Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC).

Materials

Methanol, rotary evaporator, oven-dryer, weighing balance, porcelain cloth, distilled water, measuring cylinder, surgical gloves, heavy duty grinder, storage container.

Experimental Procurement

A total of 26 adult male wistar rats were obtained for this experiment. 16 adults male wistar rats were used for the experiment while 10 adult male wistar rats were used for Ld_{50}. They were obtained from a private animal farm in Nnewi. The rats were kept in four cages and divided into 4 groups (A-D) of 4 rats each. The animals were fed ad libitum with Vital top feed finisher and water for a period of two weeks to enable them acclimatize to their new environment before the commencement of the experiment proper. The animal care handling was conducted in compliance with the National Regulation for Animal Research.

Experimental design

After acclimatization, sixteen (16) adult male wistar rats were divided into four groups with four rats in each;
Group A was used as control group and received normal feed and water daily *ad libitum* throughout the period of experiment.

Group B received 400mg/kg of methanolic leaf extract *of Mentha piperita* once daily, feed and distilled water for 6 weeks.

Group C received 30mg/kg of Lead acetate at 2 days interval, feed and distilled water for 6 weeks.

Group D received 30mg/kg of Lead acetate at 2 days interval and 400mg/kg of methanolic leaf extract *of Mentha piperita* daily for 6 weeks.

The administration was done using an oral gavage needle daily.

Procurement and Methanolic extraction of Mentha Piperita

Fresh leaves of *Mentha piperita* were procure from Jos/Plateau and identified by a botanist from Nnamdi Azikiwe University botany department. The leaves were washed properly in a running tap water to remove dirt present, separated from the stems and placed to air dry in a cool environment for maximum yield. After proper air drying, it was ground into powder using a heavy-duty grinder. The powdered mint was infused in 2 liters of methanol and left for 48 hours according to Maceration method. After proper infusion, the extract was filtered with a porcelain cloth and its chaff discarded. The filtrate was concentrated in a rotary evaporator (Digital) TT-52 (Techmel & Techmel USA), stirred and boiled at 65 C, the equivalent boiling point with solvent. The concentrated extract was oven dry with a laboratory oven TT- 9023A at 40 C for 3 days until it turns jelly like, freeze for 48 hours and stored at -20 C.

Acute Toxicity Test (Ld_{50}) Of Methanolic Extract Of Mentha Piperita

The acute toxicity test of methanolic extract *of Mentha piperita* was carried out in the Animal House of College of Health Science Nnamdi Azikiwe University according to the method described by Enegide *et al*; (2013). This procedure was carried out in three phases with a total of ten (10) rats, after they were acclimatized for 8 days and weighed with their mean body weight calculated. A stock solution was prepared by diluting 1g of methanolic extract *of Mentha piperita* in 10ml of distilled water. In phase one, 4 adult wistar rats were used and each rat was administered a dosage of 10mg/kg, 100mg/kg, 300mg/kg, and 600mg/kg of methanolic extract *of Mentha piperita* diluted stock solution respectively. In phase two, 3 wistar rats were used and each rat was administered a dosage 1000mg/kg, 1500mg/kg, and 2000mg/kg of methanolic extract of *Mentha piperita* diluted stock solution respectively. In phase three, 3

wistar rats were used and each rat was administered a dosage of 3000mg/kg, 4000mg/kg, and 5000mg/kg respectively. Based on this, a confirmatory test was carried out according to the method described by Enegide *et al*; (2013), by administering 5,000 mg/kg of the extract to each of two groups of one rat each. The Observation was made on the rats for 1 hour after administration and 10 mins for every 2-hour interval for 24 hours and no mortality was recorded.

$$LD_{50} = [M_0+M_1]/2$$

Where M_0= Highest dose of the test substance that gave no mortality= 0

M_1 = Lowest dose of the test substance that gave mortality = 0

$$\text{Mortality ratio} = \frac{\text{Number of dead rats}}{n}$$

Where n= number of rats used.

Animal Sacrifice and Sample Collection

The experimental animals were sacrificed, after 24 hours of the last administered dose of the extract, using Jugular vein laceration method, following the protocol approval by the Institute Animal Ethics Committee. Blood samples was collected and analyzed for kidney function test. The kidney was harvested and stored in 10% formal saline as a preservative in a container before taken for histopathological analysis.

Statistical Analysis

Statistics analysis of the data was performed by analysis of variances (one way ANOVA). Following one way ANOVA post Hoc test using least significance difference (LSD) and by sample 't' test at $p<0.05$ using SPSS statistical software data for individual parameters represents average value calculated.

RESULTS

Table 3.1: Effect of Methanolic Leaf Extract of Metha Piperita And Lead Acetate on Rat Body Weight.

GROUPS	INITIAL WEIGHT (g) ± SEM	FINAL WEIGHT (g) ±SEM	P-VALUE
CONTROL(A)	141.08±7.48	176.80±20.24	0.015*
MINT(B)	143.08±7.48	198±25.57	0.038*
LEAD ACETATE(C)	185.25±6.82	192.5±12.19	0.369
LEADACETATE + PEPPERMINT (D)	211.03±3.15	275.28±23.80	0.008*

The table above shows a significant change between the initial and final body weights of the rats in groups A, B and D (P value < 0.05 denoted by *). But no significant change in group C. (SEM: standard error of mean).

Table 3.2: Rat Serum Urea Concentration Level.

KIDNEY ENZYME UREA	GROUPS	UREA LEVEL ± SEM (mg/dl)	P-VALUE
	A	6.35±2.91	
	B	8.15±1.57	0.318
	C	9.38±3.04	0.201
	D	9.57±1.31	0.089

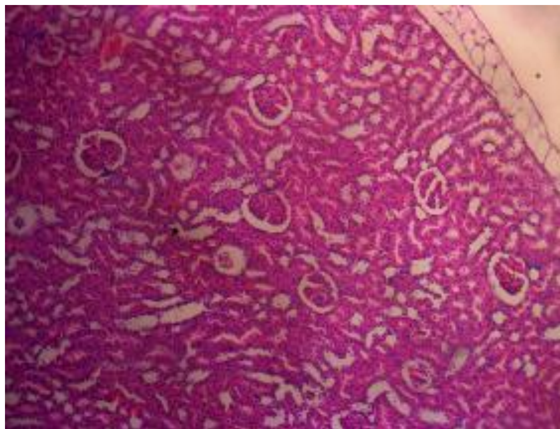
The table shows the activities of serum urea level concentration and no significant change obtained in all experimental groups when compared to A.

Table 3.3: Rat Serum Creatinine Level Concentration.

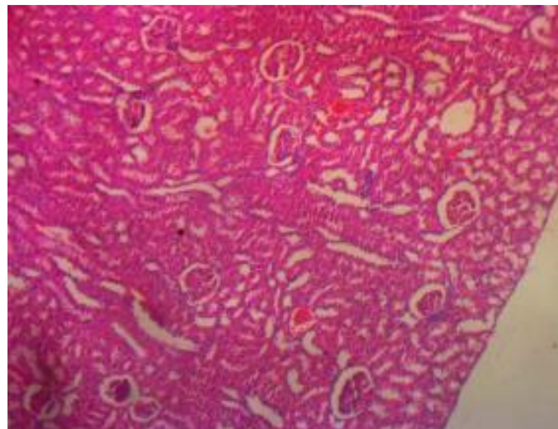
KIDNEY ENZYME CREATININE	GROUP	CREATININE LEVEL (mol/L) ± SEM	P-VALUE
	A	83.75±7.23	
	B	78.75±7.23	0.3657
	C	92.75±16.60	0.3585
	D	86.00±16.41	0.8102

This table shows the activities of serum level on creatinine level concentration with no significant change obtained in all experimental groups when compared to A.

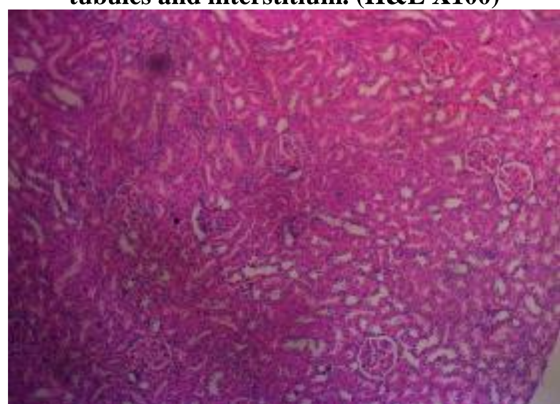
EFFECTS ON KIDNEY HISTOLOGY



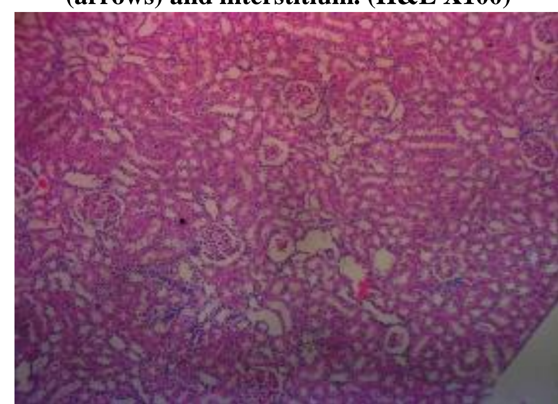
Group A photomicrograph showing normal kidney with unremarkable glomeruli (arrow), tubules and interstitium. (H&E X100)



Group B photomicrograph showing normal kidney with unremarkable glomeruli, tubules (arrows) and interstitium. (H&E X100)



Group C photomicrograph showing normal kidney with minimal lymphocytic inflammation (arrows) within the interstitium. The glomeruli and tubules are unremarkable. (H&E X100)



Group D photomicrograph showing normal kidney with minimal lymphocytic inflammation (arrows) within the interstitium. The glomeruli and tubules are unremarkable. (H&E X100)

DISCUSSION

CONCLUSION AND RECOMMENDATIONS

Urea and Creatinine Concentration Levels

Result of Urea and Creatinine concentration level after six (6) weeks of administration showed no significant difference in urea and creatinine levels in group B (Mint only), group C (Lead acetate only), and group D (lead acetate + peppermint), when compared to group A (control group). The result of the creatinine and urea level in group B agrees with the research work of Akdogan *et al.* (2003) who reported no statistically significant changes in urea levels in experimental animals administered 20mgl/kg of Mentha piperita. The result of urea and creatinine level in group C disagrees with the research work of Sri *et al*., (2017) who reported statistically significant changes in urea and creatinine 30mg/kg BW orally once a day for 60 days. Reason for difference in result may be due to duration of the experimental study, dosage of lead acetate administered and method of administration of lead acetate.

Body Weight

Result of the body weight showed significant weight gain in rat weight changes in group A(Control group), group B (Peppermint only group) and C (Lead acetate group) at the final stage of the experiment when compared to the initial stage and no significant differences in the body weight of rats in group D (lead acetate + peppermint) when compared to group A (control group). The result of group D (lead acetate + peppermint) disagrees with the research work of Djebli *et al.* (2004) who reported no significant decrease in body weight in the (lead acetate + peppermint) group when compared to group A (control group).

Kidney Histology

Histological findings of the present study indicated the normal basic architecture of the kidney with unremarkable glomeruli, renal tubules and interstitium in group A (control group). The group B animals showed normal kidney with unremarkable glomeruli, tubules and interstitium. This disagrees with Akdogan *et al.* (2003) who reported hydropic degenerations in tubular epithelial cells, and presence of picnotic nuclei and eosinophilic cytoplasm. The difference may be due to different phytochemicals present in Mentha piperita obtained from Jos/plateau State, dosage of Mentha piperita administered or the route of administration of Mentha

piperita. Group C (lead acetate group), showed normal kidney with minimal lymphocytic inflammation with interstitium. The glomeruli and tubules are unremarkable. This disagrees with Missoun *et al*. (2010), who reported nonspecific tubular atrophy and interstitial fibrosis in 8 weeks of administration of 1000 ppm lead acetate), this may be as a result of the difference in dosage of lead acetate administered, route of administration or length of the experimental period. The group D (lead acetate + peppermint) administered 30mg/kg of lead acetate and 400mg/kg of Mentha piperita only, showed normal kidney with minimal lymphocytic inflammation with interstitium. The glomeruli and tubules are unremarkable. This disagrees with Annar *et al.* (2021) who reported glomerulus deformation and congestion on administration of 20% mint against 0.046% lead and glomerulus deformation but absent of congestion on shrinkage nuclei.

CONCLUSION

The results presented in this study indicates *that Mentha piperita* leaf has no renal normative potential and cannot improve renal functions in animals.

Recommendation

Despite the lack of significant changes observed in this study, further research using different doses, exposure duration, route of administration, or other assessment methods to validate these findings and obtain a more comprehensive understanding of the effects of lead acetate and peppermint on kidney health is recommended.

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