Investigating the Protective Effects of Mentha Piperita Leaf Extract on Mercury-Chloride Induced Cardiorenal Toxicity in Adult Male Wistar Rats

Ogbuokiri Doris Kasarachi[1], Ezeokafor Emmanuel Nonso[2], Ugwuta Ifeoma Anastesia[3], Okafor Simeon Chiemelie[4], Ejiogu Ikedichukwu Chibueze[5], Obiesie Ifechukwu Justicia[6], Afuberoh Francis Chukwudi[7], Amasi Mitchell Onyinyechukwu[8], Anyiam Kennedy Ekenedirichukwu[9]*

[1,3,4,6]Faculty of Basic Medical Sciences, Department of Anatomy, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.

[2]Faculty of Basic Medical Sciences, Department of Human Physiology, Nnamdi Azikiwe University, Nnewi Campus.

[5]Dept.of Human Biochemistry, Nnamdi Azikiwe University, Nnewi Campus.

[7]Faculty of Basic Medical Sciences, Department of Physiology, Nnamdi Azikiwe University, Nnewi Campus.

[8]Dept.of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus.

[9]Department of Human Anatomy, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus.

***Corresponding author**

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ABSTRACT

Mercury, a potent environmental toxin, poses significant health risks to humans and animals, making it a major global health concern. This study investigates the potential protective effects of Mentha piperita (peppermint) ethanoic leaf extract on the heart of adult male Wistar rats exposed to Mercury Chloride. Thirty-five adult male rats were divided into seven groups, receiving varying doses of Mercury Chloride (5mg/kg and 0.5mg/kg) and M. piperita extract (800mg/kg and 1600mg/kg), alone or in combination, over a 4-week period after a 3-week acclimatization phase. The experiment aimed to assess changes in body weight, relative heart weight, and heart muscle integrity. Results revealed that exposure to Mercury Chloride induced weight loss in rats, with significant decreases observed in Group B (low-dose Mercury). The administration of M. piperita extract did not significantly affect body weight. Regarding heart weight, no significant differences were found between the groups, though Mercury exposure showed some alterations in cardiac function. The biochemical analysis indicated an increase in the cardiac marker CREATINE KINASE-MB in groups receiving M. piperita extract in combination with Mercury, particularly in Groups G and H, which showed significant elevation compared to the control group. There was an increase in the urea level in each group which were not statistically significant. Histological examination revealed normal cardiac myocyte arrangement in all groups. These findings suggest that M.piperita extract may offer partial protection against the cardiovascular effects of Mercury exposure and a significant protective effect on the morphology and functions of kidney, further research is needed to elucidate its mechanisms of action.

Keywords: Acclimatization, Cardiovascular, Mentha piperita, Mercury

INTRODUCTION

Mercury, a ubiquitous environmental pollutant, exists in various forms, including inorganic compounds like

Mercury Chloride, and poses significant health risks to both humans and animals (Clarkson et al 2003). Exposure to Mercury compounds can occur through industrial processes, consumption of contaminated food and water, and inhalation of Mercury vapor from dental amalgams and industrial emissions (Carocci et al., 2014). Once absorbed, Mercury accumulates in tissues, particularly in organs such as the brain, kidneys, and heart, where it exerts toxic effects (Clarkson et al., 2003).

Inorganic Mercury, well-established toxicant to human health, is found in the environment like water, food, and air (Atkinson et al., 2001, Sharma et al., 2007a, Sharma et al., 2007b). Mercuric Chloride is one of the most toxic forms of Mercury because it easily forms organoMercury complexes with proteins (Boujbiha et al., 2009). It is well known that hepatotoxic (Perottoni et al., 2004), neurotoxic (Franco et al., 2007), nephrotoxic (Sharma et al., 2007a, Sharma et al., 2007b), hematotoxic (Durak et al., 2010), genotoxic (Rozgaj et al., 2005) effects of inorganic Mercury. In addition, it is reported that mercuric Chloride has been adverse effect on reproductive system in experimental animals (Rao & Gangadharan; 2008, Boujbiha et al., 2011).

The cardiovascular system is particularly vulnerable to Mercury toxicity due to the high metabolic demands of cardiac tissue and its reliance on proper cellular function (Carocci et al., 2014). Mercury-induced oxidative stress can disrupt cellular signaling pathways, impair mitochondrial function, and lead to inflammation and cell death in the heart (Flora et al., 2012). Chronic exposure to Mercury has been associated with adverse cardiovascular outcomes, including hypertension, atherosclerosis, and myocardial dysfunction (Carocci et al., 2014).

The kidney is a key organ that is in charge of preserving the body's equilibrium. Creatinine is a by-product of muscle metabolism and is continuously produced and excreted in the urine due to its constant body content. Creatinine is slightly secreted by the kidneys so that at low plasma [creatinine] the clearance of creatinine is about 5–10% greater than the inulin clearance (Feher, 2017; Milne, 2017).

In contrast, interest in natural compounds with potential cardioprotective effects has grown substantially. Mentha piperita, commonly known as peppermint, is a medicinal plant with a long history of use in traditional medicine (McKay & Blumberg, 2006). Its leaves contain bioactive compounds, including menthol and menthone, which exhibit antioxidant, anti-inflammatory, and vasodilatory properties (Mimica-Dukić et al., 2003). Peppermint extract has shown promise in preclinical studies for its ability to mitigate oxidative stress, improve endothelial function, and reduce cardiovascular risk factors (McKay & Blumberg, 2006).

Given the potential therapeutic benefits of Mentha piperita extract, there is interest in exploring its role in mitigating Mercury-induced cardiac toxicity. Studies involving Mercury Chloride and peppermint extract in animal models provide an opportunity to investigate potential interactions between environmental toxins and natural compounds.

Overall, investigating the effects of Mercury Chloride and Mentha piperita extract on the heart and kidney of adult Wistar rats contributes to our understanding of Mercury toxicity and highlights the potential cardioprotective properties of natural compounds. This research holds promise for developing novel therapeutic strategies to combat Mercury-induced cardiovascular damage and improve public health outcomes.

MATERIALS AND METHODS

Location of the study

This study was carried out at the Animal house of the College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, Anambra State, Nigeria.

Ethical approval

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

Materials

1. 35 male Wistar rats.

2. Latex medical hand gloves.
3. Standard cages.
4. Rat feed (Vital pelleted finisher).
5. Animal weighing balance.
6. Measuring cylinder (Pyrex).
7. Beaker.
8. Absolute ethanol.
9. Oral cannula.
10. Cotton wool.
11. Methylated spirit.
12. 2ml syringes (Disposable).
13. A square box.
14. Normal saline.
15. Cardboard paper.
16. Mentha piperita extract.
17. Tripod stand.
18.Stopwatch.
19. Camera.
20. Drugs (Diazepam)

Plant Collection and Preparation

Fresh leaves of Mentha piperita were collected for identification and authentication by a botanist from the Botany Department of Nnamdi Azikiwe University, Awka. The leaves were thoroughly washed with clean running water to remove dirt and soil. The leaves were separated and air dried. The dried plant material was powdered using a heavy-duty blender.

250g of the grinded Mentha piperita leaf was mascerated in 1000mls of 98% absolute ethanol (BDH England) for 48hours under mechanical sheker (Uniscope101) The mixture was sieved after 48hours using porcelain cloth, and was further filtered using whatmann no1 filter paper into a clean glass beaker.

The filtrate was concentrated using Digital Rotary Evaporator (TT-55 Technical and Technical USA) and was further dried using thermostat oven (DHG 9023A PEC Medicals USA) into a paste- like form and stored in a Nexus refrigerator for further usage.

Extraction of Phytochemicals

0.2g of sample was weighed and transferred in a test tube and 15ml of Methanol was added. The test tube was allowed to react in a water bath at 600c 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. These extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution as 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

The experiment lasted for a period of 7weeks; three (3) weeks for acclimatization and four weeks of

administration of Mercury Chloride and ethanoic leaf extract of Mentha piperita and were done using the oral gavage method. Animals were weighed and grouped as follows:

Group A received 5mg/kg of Mercury Chloride once daily for four weeks with animal feed and distilled water ad libitum.

Group B received 0.5mg/kg of Mercury Chloride once daily with feed and distilled water ad libitum.

Group C served as the control group which received animal feed and distilled water daily ad libitum.

Group D received 800mg/kg of ethanolic leaf extract of Mentha piperita once daily for four weeks with animal feed and distilled water ad libitum.

Group E received 1600mg/kg of ethanolic leaf extract of Mentha piperita once daily for four weeks with animal feed and distilled water ad libitum.

Group G received 800mg/kg of ethanolic leaf extract of Mentha piperita and 0.5mg/kg of Mercury Chloride once daily for four weeks animal with feed and distilled water ad libitum.

Group H received 1600mg/kg of ethanolic leaf extract of Mentha piperita and 0.5mg/kg of Mercury Chloride once daily for four weeks with animal feed and distilled water ad libitum.

Model for Testing Anxiolytic Activity

In this experiment, the open field model was used.

Open Field Test:

In order to determine if the ethanolic Mentha piperita (pepper mint) extract has an effect on mercury induced toxicity in the Wistar rats, animals were tested in an open field arena after two weeks of oral administration of therapeutic doses of the extract.

A large, open square measuring about 40cm x 40cm was constructed with 20cm walls all round. A grid of 16 smaller squares was drawn in the middle of the arena, specifically, within the four inner squares.

This model was used in all behavioral assays.

Statistical Analysis

Research objectives and hypothesis of the study will be considered before analyzing data. Results obtained were expressed in mean ± standard error mean where applicable. The data were analyzed using paired samples t-test, analysis of variance (ANOVA) and post Hoc LSD multiple comparison of groups. Significant differences were obtained among means using Duncan's Multiple Range Test at $p< 0.05$.

RESULTS

Table 3.1: effect of ethanolic leaf extract of Mentha piperita on Creatine kinase-MB level following Mercury Chloride induced toxicity.

	Creatine kinase-MB level (IU/L)
	MEAN±SEM
Group A (5 mg/kg of HgCl)	1.70±0.31#
Group B (0.5 mg/kg of HgCl)	1.32±0.44#b
Group C (control)	1.07±0.25

Group D (800 mg/kg of EMPL)	2.11±0.57#b
Group E (1600 mg/kg of EMPL)	2.57±0.48#b
Group G (0.5 mg/kg of HgCl + 800 mg/kg of EMPL)	2.49±0.45#b
Group H (0.5 mg/kg of HgCl + 1600 mg/kg of EMPL)	4.34±0.40*a
P-value	0.002
F-ratio	6.342

Data was analyzed using GraphPad Prism 9.5.1 using ANOVA and post LSD comparison. *: significant, #; not significant when compared to group C. a: significant, b: not significant when compared to group A.

Table 3.1 result revealed showed an increase in the Creatine kinase-MB level in groups A, B, D, E, G, and H (p=0.322, p=0.688, p=0.109, p=0.036, p=0.027) compared to group C, which indicate significance in groups E, G, and H, groups A, B, and D had no significance. Also, there was an increase in the Creatine kinase-MB level in groups D, E, G, and H (p=0.506, p=0.173, p=0.216, p=0.001), group B had a decrease compared to A, which indicates significance in group H, groups B, D, E, and G had no significance.

Table 3.2: effect of ethanolic leaf extract of Mentha piperita on relative heart weight following Mercury Chloride induced toxicity.

	Relative heart weight (g)
	MEAN±SEM
Group A (5 mg/kg of HgCl)	0.37±0.03#
Group B (0.5 mg/kg of HgCl)	0.38±0.01#b
Group C (control)	0.41±0.01
Group D (800 mg/kg of EMPL)	0.39±0.05#
Group E (1600 mg/kg of EMPL)	0.38±3.84#b
Group G (0.5 mg/kg of HgCl + 800 mg/kg of EMPL)	0.41±0.00#b
Group H (0.5 mg/kg of HgCl + 1600 mg/kg of EMPL)	0.38±0.01#b
P-value	0.385
F-ratio	0.876

Table 3.2 revealed a decrease in the relative heart weight in groups A, B, D, E, G, and H (p=0.336, p=0.336, p=0.634, p=0.375, p=0.999, p=0.430) compared to C, which indicates no significance. Also, groups B, D, E, G, and H (p=0.951, p=0.591, p=0.938, p=0.366, p=0.856) had an increase in the mean relative heart weight compared to group A, which had no significant difference.

Table 3.3: the impact of ethanoic leaf extract of mentha piperita on the outcome of kidney function test of wistar rats exposed to mercury chloride.

Group	Creatinine Level (mg/dL) (Mean ± SEM)	Urea Level (mg/dL) (Mean ± SEM)	P-value	T-value
Group B (0.5 mg/kg of HgCl)	49.67 ± 2.73	3.37 ± 0.77	0.073	2.96
Group D (800 mg/kg of MP)	66.33 ± 2.40	5.00 ± 0.23	0.481	0.83

Group F (400 mg/kg of EMP)	70.67 ± 3.93	3.47 ± 0.27	0.824	0.24
Group G (800 mg/kg of MP + 0.5 mg/kg of HgCl)	76.67 ± 3.48	4.47 ± 0.52	0.655	-0.5
Group H (0.5 mg/kg of HgCl + 1600 mg/kg of EMP)	82.00 ± 7.55	4.60 ± 0.15	0.423	-0.89

Data was analyzed using paired t-test and values were considered significant at $p \leq 0.05$.

Table 4.3 result showed an increase in the creatinine level in groups B, D, F, G and I when the creatinine level was compared to the urea level, groups B, D, F, G, and I had no significant difference.

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

The results in Table 3.1 showed an increase in Creatine kinase-MB levels in groups A, B, D, E, G, and H (p=0.322, p=0.688, p=0.109, p=0.036, p=0.027) compared to group C, with significant increases observed in groups E, G, and H, while no significant changes were found in groups A, B, and D. Additionally, Creatine kinase-MB levels were higher in groups D, E, G, and H (p=0.506, p=0.173, p=0.216, p=0.001) This increase can be suggestive of myocardial injury or injury to the cardiac muscles, with group B showing a decrease compared to group A, and a significant difference observed only in group H, while groups B, D, E, and G showed no significance. These findings are consistent with Salimi et al. (2023), who demonstrated that Mentha piperita essential oil possesses antioxidant properties capable of reducing oxidative stress in the heart. The study found that CREATINE KINASE-MB levels were significantly elevated in diabetic rats, indicating cardiac injury. However, peppermint essential oil treatment reduced CREATINE KINASE-MB levels, suggesting a protective effect on the heart by reducing myocardial injury. This cardioprotective effect was attributed to the antioxidant and anti-inflammatory properties of peppermint essential oil.

Table 3.2 indicated a reduction in relative heart weight in groups A, B, D, E, G, and H (p-values of 0.336, 0.336, 0.634, 0.375, 0.999, and 0.430, respectively) when compared to group C, though no significant differences were observed. Additionally, groups B, D, E, G, and H (p-values of 0.951, 0.591, 0.938, 0.366, and 0.856, respectively) showed an increase in mean relative heart weight compared to group A, but again, no significant differences were noted. However, following treatment with peppermint essential oil, the heart weight of the treated rats significantly decreased, suggesting that peppermint oil may help alleviate the heart enlargement associated with diabetes and provide cardioprotective benefits.

Table 3.3 result showed an increase in the creatinine level in groups B, D, F, G and I when the creatinine level was compared to the urea level, groups B, D, F, G, and I had no significant difference.

The protective effect of Mentha piperita observed in this study can be attributed to its potential to enhance detoxification pathways and promote healing in organs affected by mercury. As noted by Rusyniak et al. (2010), the kidneys are primary targets of mercury toxicity, and any intervention that can support renal function or reduce the of toxic substances may reflect positively on overall health indicators, including body weight.

Conclusion

This study explored the effects of Mentha piperita (peppermint) on Mercury-induced changes in Wistar rats. While no significant differences were observed in relative heart weight or histological heart structure, increased Creatine kinase-MB levels in some groups indicated potential cardiac stress. Histological analysis revealed normal myocardial structure with no abnormalities. Additionally, body weight changes aligned with previous findings suggesting that Mentha piperita may positively influence body weight gain.

Recommendation

Future research should focus on exploring the underlying mechanisms of peppermints potential cardioprotective

effects against Mercury toxicity, using more sensitive biomarkers and advanced imaging techniques to detect subclinical myocardial injury and dose-response relationships.

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