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THE EFFECT OF ADMINISTRATION OF ETHANOLIC LEAF EXTRACT OF *MENTHA PIPERITA* ON THE SPLEEN OF ADULT MALE WISTAR RATS EXPOSED TO MERCURY CHLORIDE

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Abstract: **Mercury, a toxic environmental contaminant, poses significant health risks to humans and animals. Exposure to mercury occurs through various sources, including seafood, dental fillings, and energy-efficient light bulbs. This study aimed to evaluate the effects of Mentha piperita (peppermint) on the spleen and immune functions of adult male Wistar rats exposed to mercury chloride. Thirty rats were divided into six groups, Group B received 5mg/kg of mercury chloride, groups D and F were given Mentha piperita extract (800 mg/kg and 400 mg/kg respectively) while group G received 800mg/kg of ethanolic leaf extract of Mentha piperita and 0.5mg/kg of Mercury chloride amd Group I received 400mg/kg of ethanolic leaf extract of Mentha piperita and 0.5mg/kg of Mercury chloride. Group C served as the control. Body weight, spleen weight, and hematological parameters were assessed, along with histological analysis of spleen tissue. Results showed no significant changes in body weight between groups, although group F (800 mg/kg Mentha piperita) showed a notable increase in weight. The relative spleen weight did not differ significantly between groups, though some changes were observed. Immunoglobulin M (Ig-M) levels were elevated in groups exposed to Mentha piperita alongside mercury, particularly in groups G and I, while white blood cell (WBC) counts showed a significant decrease in group F. Red blood cell (RBC) counts and hemoglobin levels did not exhibit statistically significant differences, though some variations were noted. Platelet counts were significantly reduced in group F. Histological analysis revealed mostly normal splenic architecture, with minor changes in group F, such as smaller follicles, and group I showing more prominent histopathological alterations. Overall, while Mentha piperita demonstrated some protective effects, further studies are needed to explore its potential as a therapeutic agent against mercury-induced toxicity.**

Keywords: **Follicles, Immunoglobulin, Mentha piperita, Mercury exposure, Toxicity, Wistar rats.**

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1. INTRODUCTION

Mercury is a harmful ecological pollutant that poses extreme health dangers to people and animals, particularly influencing organs like the spleen, which assumes a crucial part in immune response. Mercury chloride is viewed as one of the most dangerous types of mercury, provoking oxidative stress, disturbance, and tissue harm in exposed individuals [7]

Mentha piperita is a helpful plant commonly used for its antioxidant, defensive, and antimicrobial properties. Taking everything into account, it has been utilized for treating stomach related issues, respiratory problems, and immune regulation[5].Given its protective potential, this study examines the impact of ethanolic leaf extract of Mentha piperita on the spleen of Wistar rats exposed to mercury chloride. The aim is to investigate its ability to relieve mercury-induced toxicity, effect on hematological parameters, and generally the spleen wellbeing.

2. MATERIALS AND METHODS

Location

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

Materials

- Male wistar mice - Saw dusts
- Laboratory coat and gloves - Ethanol
- Beakers - Syringes
- Measuring cylinders - Refrigerator (Nexus)
- Mentha piperita leaves
- Normal saline
- Cages - Canula
- Centrifuge (search tech instruments, British standard) model 80-2
- Thermostat oven DHG-90 23A, PEC medical, USA
- Neubauer Counting Chamber (England)
- Electronic weighing balance, M-methlar model M311l, China
- Mice feeds (non pelletized grower) and water

Extract Procedure

Fresh leaves of Mentha piperita were collected for identification and authentication by a botanist from a 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) 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 refridgerator for further usage.

The method followed the procedure described by (Al Attar and Abu Zaid 2012).

Experimental Design

The experiment lasted for a period of seven (7) weeks; three (3) weeks of acclimatization which was followed by four (4) weeks of administration of Mercury Chloride and Ethanolic Leaf extract of Mentha piperita. This was done using the oral gavage method. The Animals were weighed and grouped as follow;

The entire group received vital feed and water ad-libitum on a daily basis.

Group C was used as the control group and received vital feed and water for twenty-eight (28) Days.

Group B received 0.5mg/kg of Mercury Chloride once daily for twenty-eight (28) Days.

Group D received 800mg/kg of ethanolic extract of Mentha piperita once daily for twenty-eight (28) Days.

Group F received 400mg/kg of ethanolic extract of Mentha piperita once daily for twenty-eight (28) Days.

Group G received 0.5mg/kg of Mercury Chloride + 800mg/kg of ethanolic extract of Mentha piperita once daily for twenty-eight (28) Days.

Group I received 0.5mg/kg of Mercury Chloride + 400mg/kg of ethanolic extract of Mentha piperita once daily for twenty-eight (28) Days.

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 student 't' test at p=0.05 using SPSS statistical software data for individual parameters represents average value calculated.

3. RESULTS

Table I: Effect of Ethanolic Leaf Extract of *Mentha Piperita* on Body Weight in Mercury Exposed Rats

	Initial weight (g) MEAN±SEM	Final weight (g) MEAN±SEM	P-value	t-value
Group B (0.5 mg/kg of HgCl)	180.66±12.45	213.10±6.88	0.223[b]	-1.748
Group C (Control)	152.00±13.65	166.67±9.33	0.572[b]	-0.670
Group D (800 mg/kg of EMPL)	165.33±7.96	178.33±16.74	0.313[b]	-1.338
Group F (400 mg/kg of EMPL)	120.00±6.08	156.33±8.01	0.046[a]	-4.478
Group G (800 mg/kg of EMPL + 0.5 mg/kg of HgCl)	166.00±2.31	176.33±3.17	0.187[b]	-1.972
Group I (400 mg/kg of EMPL + 0.5 mg/kg of HgCl)	188.00±5.13	215.00±13.23	0.194[b]	-1.927

Data was analysed using paired t-test and values were considered significant at $p \leq 0.05$. a: significant, b: not significant. Table I result showed an increase in the body weight in groups B, C, D, F, G, and I when the initial weight was compared to the final weight, which significance in group F, and groups B, C, D, G, H, and I had no significant difference.

Table II: Effect of Ethanolic Leaf Extract of *Mentha Piperita* on Relative Spleen Weight and Immunoglobulin M Level in Mercury Exposed Rats

	Rel. Spleen weight (g) MEAN±SEM	Ig M (mg/dL) MEAN±SEM
Group B (0.5 mg/kg of HgCl)	0.44±0.12#	125.00±3.43#
Group C (Control)	0.35±0.11b	106.70±1.95b
Group D (800 mg/kg of EMPL)	0.36±0.03#b	95.36±11.37a#
Group F (400 mg/kg of EMPL)	0.39±0.01#b	120.00±7.40b#
Group G (800 mg/kg of EMPL + 0.5 mg/kg of HgCl)	0.40±0.01#b	158.40±8.09a*
Group I (400 mg/kg of EMPL + 0.5 mg/kg of HgCl)	0.42±0.02#b	141.60±3.69b*
p-value	0.944	0.001
F-ratio	0.226	11.32

Table II result showed an increase in the mean relative spleen weight in groups B, D, F, G, and H (*p=0.409, p=0.952, p=0.727, p=0.643, p=0.511*) compared to group C, which had no significant difference. Also, groups D, F, G, and I (*p=0.443, p=.628, p=0.711, p=0.863*) had a decrease in the relative spleen weight compared to group B, which had no significance.

Table III: Effect Of Ethanolic Leaf Extract Of *Mentha Piperita* On White Blood Cells And Differential White Blood Cell Count In Mercury Exposed Rats

	White blood cell (x10^9/l)	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)
	MEAN±SEM	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group B (0.5 mg/kg of HgCl)	8.26±0.49#	94.56±2.08#	4.03±1.41#	1.40±0.68#
Group C (Control)	8.47±1.12b	97.50±0.55b	2.00±0.45b	0.50±0.12b
Group D (800 mg/kg of EMPL)	7.94±2.19#b	95.56±2.79b#	3.17±1.82#b	1.27±0.97b#
Group F (400 mg/kg of EMPL)	3.91±0.91#a	96.10±2.44b#	2.83±1.58#b	1.07±0.87b#
Group G (800 mg/kg of EMPL + 0.5 mg/kg of HgCl)	6.99±1.23#b	92.06±4.41b#	5.13±2.45#b	2.80±1.96b#
Group I (400 mg/kg of EMPL + 0.5 mg/kg of HgCl)	11.62±1.01#b	96.93±0.39b#	0.52±0.30#b	0.67±0.08b#
p-value	0.026	0.701	0.726	0.658
F-ratio	3.845	0.601	0.564	0.664

Table III result revealed a decrease in the mean WBC level in groups B, D, F, and G (*p=0.908, p=0.771, p=0.026, p=0.426, p=0.105*), group I (*p=0.105*) had an increase compared to C, which indicate significance in-group F, groups B, D, G, and I had no significance. Also, groups D, F, and G (*p=0.860, p=0.032, p=0.494*), group I (*p=0.086*) had an increase compared to B, which had significance in-group F.

Table IV: Effect Of Ethanolic Leaf Extract of *Mentha Piperita* On Red Blood Cells, Haemoglobin Level, And Packed Cell Volume In Mercury Exposed Rats

	Red blood cell (x10^12/l)	Hemoglobin (g/dl)	PCV (%)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group B (0.5 mg/kg of HgCl)	6.31±0.39#	12.26±0.38#	41.26±1.96#
Group C (Control)	6.66±0.29b	12.60±0.65b	38.70±0.78b
Group D (800 mg/kg of EMPL)	6.29±0.18#b	12.40±0.32b#	30.86±10.57#b
Group F (400 mg/kg of EMPL)	4.80±1.61#b	9.93±3.31b#	39.80±1.01#b
Group G (800 mg/kg of EMPL + 0.5 mg/kg of HgCl)	6.53±0.27#b	12.93±0.32b#	40.36±1.01#b
Group I (400 mg/kg of EMPL + 0.5 mg/kg of HgCl)	6.51±0.35#b	12.90±0.95b#	41.40±2.36#b
p-value	0.496	0.700	0.726
F-ratio	0.929	0.601	0.564

Table IV result shows a decrease in RBC level in groups B, D, F, G, and I (*p=0.741, p=0.722, p=0.092, p=0.900, p=0.890*) compared to group C, which had no significant difference. Also, groups D and F (*p=0.979, p=0.162*) had a decrease and groups G and I (*p=0.837, p=0.847*) had an increase compared to B, which had no significance.

4. CONCLUSION

Findings revealed that Mentha piperita increased body weight in certain groups, aligning with studies by Behzad et al., suggesting peppermint promotes weight gain. However, mercury exposure alone (Group B) caused weight loss, consistent with [3], who linked mercury to metabolic disruption. Relative spleen weight increased in groups exposed to mercury chloride and/or Mentha piperita but lacked statistical significance. [2] noted similar non-significant spleen weight changes under environmental toxin exposure. Ig-M levels fluctuated across groups, with significant increases observed in specific groups exposed to both mercury chloride and Mentha piperita. These findings align with [8], who documented Ig-M variations due to stressors or infections. White blood cell (WBC), red blood cell (RBC), hemoglobin, and packed cell volume (PCV) levels showed minimal changes, except for a significant WBC decrease in Group F. These results match that of [1] who reported mercury's impact on immune and blood parameters.

Most spleens showed normal architecture, with subtle changes such as extravasated red blood cells and congested sinusoids in specific groups. [6] similarly reported mild histopathological effects in mercury-exposed rats. Mercury exposure led to a dose-dependent decrease in platelet count, as observed by [4].Group F showed significant reductions, while other groups remained unaffected.Overall, while Mentha piperita demonstrated some protective effects, further studies are needed to explore its potential as a therapeutic agent against mercury-induced toxicity.

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