Bioactive Compounds in *Teucrium Polium*: A Key to Neutralizing Mercury Toxicity in the Liver

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KEYWORDS

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ABSTRACT

This study Teucrium polium (T. polium) aqueous extract was evaluated for its protective effects against mercury chloride ($HgCl_2$)-induced oxidative stress and liver damage in rats. Forty rats were divided into four groups: control, $HgCl_2$-intoxicated (2 mg/kg), $HgCl_2$-intoxicated treated with T. polium extract (125 mg/kg), and a group receiving only T. polium extract. $HgCl_2$ exposure significantly reduced body weight and increased liver injury markers, including alanine aminotransferase (ALT) by 65.89% and aspartate aminotransferase (AST) by 38.95%. Triglycerides and total protein levels dropped by 28.23% and 25.51%, respectively, while cholesterol and albumin levels remained unchanged. Histopathological analysis showed severe hepatic damage with necrotic lesions and inflammation. T. polium extract mitigated these effects by lowering ALT and AST levels, restoring biochemical parameters, reducing oxidative stress, and improving antioxidant enzyme activity. Histological analysis confirmed reduced hepatic damage in treated rats. However, no improvements were observed in body or liver weight. Despite this, T. polium demonstrated strong hepatoprotective effects against $HgCl_2$-induced toxicity, highlighting its potential as a therapeutic agent for mercury-related liver damage.

INTRODUCTION

Mercury (Hg) is one of the most toxic heavy metals, posing a significant threat to human health and the environment **[1]**. Global exposure to mercury occurs through various pathways, including inhalation of mercury vapor from industrial activities such as gold mining, forest fires, and volcanic eruptions **[2,3]**, as well as dietary consumption, particularly seafood contaminated with methylmercury **[4]**. Mercury exists in different forms—metallic, inorganic, and organic—and exerts toxic effects on multiple tissues via mechanisms such as nephrotoxicity **[5]**, hepatotoxicity **[6]**, neurotoxicity **[7]**, neurotoxicity **[8]**, immunotoxicity **[9]** , genotoxicity**[10]** , reproductive toxicity **[11]**, and cardiovascular and digestive system damage**[12,13]**.

Inorganic mercury, particularly mercuric chloride ($HgCl_2$), is one of the most toxic mercury compounds, historically used in cosmetic products, laxatives, teething powders, antiseptics, and diuretics **[14]**. The liver plays a central role in $HgCl_2$ metabolism, making it a primary target for its toxic effects. $HgCl_2$ induces oxidative stress by overproducing reactive oxygen species (ROS), which disrupts the balance between prooxidants and antioxidants, leading to cellular damage **[15]**. It also impairs systems that scavenge free radicals, exacerbating oxidative stress and contributing to hepatotoxicity **[16]**.

Given the harmful effects of mercury, there is an urgent need for effective therapeutic strategies to mitigate its toxic impact. Medicinal plants, rich in bioactive compounds with antioxidant and protective properties, have emerged as promising candidates for combating oxidative stress-related diseases **[17]**. Among these plants, *Teucrium polium* (T. polium) has gained attention due to its

diverse pharmacological activities. T. polium, widely used in traditional medicine, exhibits anti-inflammatory **[18]** , anti-hypertensive **[19]**, vasorelaxant **[20]**, hypolipidemic**[21]**, anti-diabetic**[22]**, antioxidant**[23]** , anticancer**[24]**, anti-infectious**[25]** , and antinociceptive properties**[26]**. Additionally, T. polium demonstrates cardioprotective effects **[27]**.

The plant's antioxidant potential makes it especially relevant for countering mercury-induced oxidative stress and hepatotoxicity. Antioxidants in T. polium, such as flavonoids and phenolic compounds, may neutralize ROS, restore redox balance, and protect hepatic tissue from damage **[23]**. Despite extensive research on its biological activities, the protective effect of T. polium against $HgCl_2$-induced hepatotoxicity remains underexplored.

This study aims to evaluate the ameliorative effects of T. polium aqueous extract on $HgCl_2$-induced liver damage in rats. Specifically, we assess its impact on serum biochemical markers, oxidative/antioxidant status, and histopathological alterations in the liver, highlighting its potential as a natural remedy for mercury toxicity.

MATERIALS AND METHODS

1. Plant Material and Extract Preparation

T. polium L. plants were collected from the region of Tissemsilt, in the center of north western of Algeria during June, and were identified and confirmed by Professor HADJAJ in the botany laboratory of the Faculty of Sciences, Oran1 University, Algeria.

After collection, the plant parts (leaves and flowers) were washed with distilled water than were dried for one month at room temperature.

Aqueous extract was obtained by extraction of the dried plant material (50g) was stirred in 500 mL of distilled water for 30min (two times) at 75°C. The extract was filtered then lyophilized and it was stored at -20°C before use.

2. Experimental animals and treatment

A total of 40 male Wistar rats with a weight of (104±4) g were used as experimental animals.

Each group was separately housed in two cage and fed ad libitum. They were exposed to a 10h light:14h dark cycle and the room temperature was maintained at (24 ± 2) °C.After an acclimatization period all animals were randomly divided into four groups with eight groups (n=10) were shown in Table 1. Body weight was measured weekly from the beginning of the study till the end of the experiment. At the end of the experiments (45 day), the rats were sacrificed under anesthesia.

After dissection, blood samples are collected in dry tubes and then centrifuged at 4500 rpm for 15 minutes at 4 °C. The liver was quickly removed,weighed, rinsed in ice cold saline buffer and stripped of adipose tissue and was cut into several pieces, then stored in a freezer at -20°C until biochemical assay. A part of liver from each group was fixed in formalin buffer for histological study.Hg-intoxicated rats

Table 1 : Treatment schedule

Groups	Route of administration
Experimental Group 1 (control)	Received intraperitoneal solutions of 0.9% saline solution (NaCl) for 45 days.
Experimental Group 2 ($HgCl_2$)	Were injected intraperitoneally with mercury chloride ($HgCl_2$) once a week at a dose of 2mg/kg body weight (BW)/ml dissolved in distilled water for 45 days.
Experimental Groups 3 (intoxicated treated group, $HgCl_2$ + TAE)	Were injected with mercury chloride as for group 2 and received aqueous extract of *T. polium* (125mg/kg bw /day) dissolved in distilled water by gavage, for 45 days.
Experimental Group 4 (treated group, TAE)	Received aqueous extract of T. polium (125mg/kg bw /day) dissolved in distilled water by gavage, for 45 days.

3. Preparation of liver extract

Frozen liver tissue (1 g) was cut into small pieces and homogenized at 4ºC in 10 mL of phosphate buffer solution (PBS, 0.1M; pH=7.4) using a glass. The suspension was centrifuged at 10000r/min for 15 min at 4°C. The clear supernatant was immediately used to measure of lipid peroxidation and antioxidant determination.

4. Assessment of hepatic function and lipid profile

Liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) enzyme activities, in addition total proteins and albumin concentrations, were used as biochemical markers for the hepatic damage. The serum total cholesterol, triglyceride were evaluated in the same serum, The biochemical parameters were determined by a spectrophotometer according to manufacturer's instruction in ready-made kits (Speanreact Co., Spain).

The activity of serum enzymes was expressed as IU/l (ALAT, ASAT), total proteins (g/dl), albumin (g/dl), total cholesterol (mg/dl)and triglyceride (mg/dl).

5. Assessment of oxidative stress biomarkers analyses

Antioxidant enzyme activities and Lipid peroxidation level in liver were determined by measuring the absorbance of the samples with a spectrophotometer. Protein content of supernatants was determined according to the method of Lowry et al. **[28]** using bovin serum albumin as a standard.

5.1. Lipid peroxidation level was analyzed using the thiobarbituric acid test described by Ohkawa et al. **[29]**. Briefly, 0.1ml liver homogenate was added to 0.1 ml sodium dodecyl sulfate (8.1%), then completing volume with 0.3ml distilled water. The mixture was incubated at 95°C for 60 min then cooled. After incubation, 0.5 ml distilled water and 2.5 ml butanol/pyridine (15v/1v) were added, the final solution was centrifuged at 4000r/min for 10min. The absorbance of the surnageant was measured at 535nm and the amount of malondialdehyde (MDA) formed was calculated based on the molar extinction coefficient of MDA (1.56×10^5 $M^{-1}cm^{-1}$). The results were expressed as µmol of MDA/mg protein.

5.2. The superoxide dismutase (SOD) level was determined using a method of Asada et al. **[30]** SOD activity was evaluated by measuring the inhibition of nitro-blue tetrazolium (NBT). In this assay, SOD is considered as the amount required inhibiting the photoreduction by 50% of NBT. The mixture of the reaction, containing1000 µl phosphate buffer (50mM;pH 7.8), 892.2 µl EDTA-methionine (0.1mM, 13mM),50 µl liver homogenate,85.2 µl NBT(75µM) and 22.6 µl

Riboflavin(2 µM), as to obtain a final volume, was added as the last and switching on the light started the reaction; changes in absorbance at 580nm were recorded after 20 min. Specific activity was defined as Units/mg of protein.

5.3. Catalase activity (CAT) was estimated according to the method described by Sinha. **[31]**. The mixture of the reaction, containing 0.2 ml liver homogenate, 0.2 ml phosphate buffer (1/1.5M, pH=7), and 0.2 ml H_2O_2 solution (0.2M) were agitated then incubated at room temperature for 5min, the reaction was stopped by the addition of 0.2 ml solution of acetic acid/potassium dichromate (3V/1V) and the absorbance was directly measured at 570nm. The results were presented as µmol hydrogen peroxide consumed/mg of protein.

5.4. The enzymatic activity of glutathione peroxidase (GPx) was measured by the method of Flohé and Günzler. **[32]**. This method is based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the influence of GPx. The reaction mixture consisted of 0.2 mL ml liver homogenate, 0.4 mL GSH solution (0.1 mM), 0.2ml PO_4 buffer (67mM, pH=7.8). After incubation at 25°C for 5 min, 0.2mL of H_2O_2 (1.3 mM) was added to initiate the reaction, leave to act for 10min, 1 mL de TCA (1 %),Put the mixture in ice for 30 minutes. Tubes were centrifuged at 3000×g for 10min and the supernatant was collected. 2.2 ml of Na_2HPO_4 (0.32M)solution and 0.320ml of DTNB (1 mM) was added to 0.480 ml of reaction supernatant. After mixing and 5 min incubation, absorbance was recorded at 412 nm.GPx activity was expressed as µM GSH/min/mg proteins.

5.5. Measurement of glutathione S-transferase(GST) activity was carried out using the method of Habig et al. **[33]** , this consists of providing the enzyme with a substrate, generally 1-chloro,2.4-dinitrobenzene (CDNB), which easily conjugates with glutathione under the action of numerous forms of GST. The conjugation reaction of these two products results in the formation of a new molecule which absorbs light at a wavelength of 340 nm: the method used in this study consists of causing the GSTs contained in the homogenate to act on a mixture (GSH + CDNB) at a temperature of 37°C and a pH of 6.5. The variation in optical density, due to the appearance of the GSH-CDNB complex, is measured for 1 minute for 5 minutes at a wavelength of 340 nm. The enzymatic activity of GST is obtained by the following formula: The activity of GST (nM GSH-CDNB/min/mg proteins).

6. Liver histopathology

For the anatomo-histo-pathological examination, a part of liver from each group was fixed in 10% formal in solution, dehydrated in graduated ethanol, cleared in xylene, and embedded in paraffin. Paraffin blocks were cut at a thickness of 4 µm using the rotary microtome. Finally, the sections were stained with hematoxylin and eosin (H & E) dye and examined for histopathological changes under the light microscope.

7. Statistical analysis

The results were analyzed using SPSS 26 software. The data were expressed as mean value ± standard deviation. Comparisons between the groups were performed by one-way analysis of variance ANOVA followed by Tukey's post hoc test. A level of $P \leq 0.05$was considered to be significant.

Result:

Body Weight and Liver Weight (Table 2)

The study evaluated the effects of mercuric chloride ($HgCl_2$) and *Teucrium polium* (T. polium) extract on body weight, body weight gain, and liver weight in Wistar rats.

Table 02: Effects of exposure to $HgCl_2$ and T. Polium treatment on body weight, body weight gain and liver weights of Wistar rats.

Experimental groups	Body weight			Liver weight (g)
	Initial BW (g)	Final BW (g)	Body Weight gain (g)	
Control	103.8±3.22	174.06±5.32	70.26±5.27	4.58±0.35
$HgCl_2$	104.32±2.28	139.58±5.89***	35.26±4.36***	04±0.62
$HgCl_2$ + TAE	106.22±2.80	128.84±6.58	22.62±6.22$^{\#\#}$	4.15±0.39
TAE	104.06±3.22	156.58±6.66	52.52±4.10	4.31±0.49

$HgCl_2$: mercuric chloride; bw: body weight.
Values are expressed as mean ± SD (n = 6). (*: P≤0.05; **: P≤0.01;***: P≤0.001) compared with the control group; ($^{\#}$: P≤0.05 ; $^{\#\#}$: P≤0.01, $^{\#\#\#}$: P≤0.001) compared with $HgCl_2$ group.

Exposure to mercuric chloride ($HgCl_2$) resulted in a significant reduction in both final body weight and body weight gain in Wistar rats compared to the control group. Specifically, the final body weight of $HgCl_2$-intoxicated rats was 139.58 ± 5.89 g, which was markedly lower than that of the control group (174.06 ± 5.32 g, $P \leq 0.001$). Similarly, body weight gain was substantially decreased in the $HgCl_2$ group (35.26 ± 4.36 g) compared to the control group (70.26 ± 5.27 g, $P \leq 0.001$). Treatment with *Teucrium polium* extract in intoxicated-treated rats further reduced body weight and gain, with a final body weight of 128.84 ± 6.58 g and a body weight gain of 22.62 ± 6.22 g ($P \leq 0.01$), indicating that the extract did not fully restore body weight or gain. Notably, no significant changes were observed in liver weight across all experimental groups, suggesting that *T. polium* treatment did not significantly counteract $HgCl_2$-induced weight loss in this organ. These findings highlight the profound impact of $HgCl_2$ on body weight parameters and the limited restorative effects of *T. polium* in this context.

Biochemical Parameters (Table 3)

Serum biochemical markers revealed the impact of $HgCl_2$ intoxication and T. polium treatment on hepatic function and lipid profile.

Table 03: Biochemical analyses (hepatic function and lipid profile) of rat blood treated with mercuric chloride followed by T. polium treatment.

Parameters	Control	$HgCl_2$	$HgCl_2$+ TAE	TAE
AST (U/L)	60.30±8.32	83.79±8.94***	57.95±8.74$^{\#\#\#}$	62.92±5.27
ALT (U/L)	17.46±3.47	28.97±5.23**	20.1±2.27$^{\#}$	19.22±2.51
Total protein (g/dl)	5.94±1.41	4.42±0.82*	4.68±0.53	6.34±1.73
Albumin (g/dl)	3.02±1.02	2.17±0.97	2.43±0.75	3.68±1.02
Total cholesterol (mg/dl)	50±6.08	38±7.11	64.25±7.84$^{\#\#}$	53.75±8.65
Triglyceride (mg/dl)	85±7.07	61±12.72***	83±5.65$^{\#\#\#}$	39±8.48

Values are expressed as mean ± SD (n = 6). (*: P≤0.05; **: P≤0.01;***: P≤0.001) compared with the control group; ($^{\#}$: P≤0.05 ; $^{\#\#}$: P≤0.01, $^{\#\#\#}$: P≤0.001) compared with $HgCl_2$ group.

Exposure to mercuric chloride ($HgCl_2$) induced significant hepatotoxicity, as evidenced by a marked increase in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are key markers of liver injury. Specifically, $HgCl_2$-intoxicated rats exhibited a substantial elevation in AST levels (83.79 ± 8.94 U/L, $P \leq 0.001$) and ALT levels (28.97 ± 5.23 U/L, $P \leq 0.01$) compared to the control group, indicating impaired hepatic function. Treatment with *Teucrium polium* extract significantly attenuated these increases, reducing AST levels by 30.83% (57.95 ± 8.74 U/L, $P \leq 0.001$) and ALT levels by 30.61% (20.1 ± 2.27 U/L, $P \leq 0.05$) in intoxicated-treated rats, thereby demonstrating its hepatoprotective potential.

In addition to its effects on liver enzymes, $HgCl_2$ exposure significantly decreased triglyceride (TG) levels (61 ± 12.72 mg/dL, $P \leq 0.001$) compared to controls. However, treatment with *T. polium* extract restored TG levels by 36.06% (83 ± 5.65 mg/dL, $P \leq 0.001$), suggesting a regulatory effect on lipid metabolism. Furthermore, total cholesterol levels were significantly increased following *T. polium* treatment (+78.47%, 64.25 ± 7.84 mg/dL, $P \leq 0.01$) compared to the $HgCl_2$ group, highlighting the extract's potential to modulate lipid profiles. Notably, total protein and albumin levels remained unchanged across both the $HgCl_2$-intoxicated and intoxicated-treated groups, indicating that these parameters were not significantly affected by either the toxicant or the treatment. These findings collectively underscore the ability of *T. polium* to mitigate $HgCl_2$-induced hepatic dysfunction and lipid profile alterations, positioning it as a promising therapeutic agent for mercury toxicity.

Oxidative Stress Parameters (Table 4)

The study assessed oxidative stress markers in liver homogenates to evaluate the protective effects of T. polium.

Table 04: Effects of $HgCl_2$, *T. polium*extract, their combination ($HgCl_2$+*T.polium*) on Oxidative Stress Parameters in Liver Homogenates of Rats.

Parameters	Control	$HgCl_2$	$HgCl_2$+ TAE	TAE
TBARS (nmol/mg prt)	8.10±1.38	43.64±2.34***	28.06±6.70$^{###}$	14.83±3.4
CAT (µmol/mgprt)	42.71±4.23	123.27±5.78***	54.36±8.07$^{###}$	51.37±9.53
SOD (Units/mg prt)	6.63±1.52	15.91±2.35***	5.66±0.32$^{###}$	6.83±1.02
GPx (nmolGSH/min/mg prt)	6.52±1.82	15.43±4.19*	8.9±1.26$^{#}$	10.47±0.42
GST (µM/min/mg prt)	3.03±0.50	6.55±1.43*	2.11±0.46$^{#}$	3.26±0.40

Values are expressed as mean ± SD (n = 6). (*: P≤0.05; **: P≤0.01,***: P≤0.001) compared with the control group; ($^{#}$: P≤0.05 ; $^{##}$: P≤0.01, $^{###}$: P≤0.001) compared with $HgCl_2$ group.

Mercuric chloride ($HgCl_2$) exposure induced significant oxidative stress in the liver, as evidenced by a marked increase in lipid peroxidation and altered antioxidant enzyme activities. Specifically, $HgCl_2$ treatment resulted in a substantial elevation of thiobarbituric acid reactive substances (TBARS) levels, which are indicative of lipid peroxidation, reaching 43.64 ± 2.34 nmol/mg protein ($P \leq 0.001$) compared to the control group (8.10 ± 1.38 nmol/mg protein). This finding highlights the extent of oxidative damage caused by $HgCl_2$ intoxication. Additionally, the activities of key antioxidant enzymes—catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST)—were significantly upregulated in response to $HgCl_2$ exposure, likely representing a compensatory mechanism to counteract the increased oxidative stress.

Treatment with *Teucrium polium* (T. polium) extract demonstrated a profound protective effect against $HgCl_2$-induced oxidative stress. Notably, T. polium administration significantly reduced TBARS levels by 35.68% (28.06 ± 6.70 nmol/mg protein, $P \leq 0.001$), indicating its ability to mitigate lipid peroxidation. Furthermore, T. polium restored the activities of antioxidant enzymes to near-normal levels, as follows: CAT activity decreased by 57.33% (54.36 ± 8.07 µmol/mg protein, $P \leq 0.001$), SOD activity decreased by 64.38% (5.66 ± 0.32 Units/mg protein, $P \leq 0.001$), GPx activity decreased by 42.30% (8.9 ± 1.26 nmol GSH/min/mg protein, $P \leq 0.05$), and GST activity decreased by 67.66% (2.11 ± 0.46 µM/min/mg protein, $P \leq 0.05$). These results collectively demonstrate that T. polium not only reduces oxidative damage but also restores the balance of the endogenous antioxidant defense system.

In summary, these findings underscore the potent antioxidative properties of T. polium, highlighting its ability to effectively counteract $HgCl_2$-induced oxidative stress by reducing lipid

peroxidation and normalizing antioxidant enzyme activities. This makes T. polium a promising candidate for the prevention and treatment of mercury-induced hepatic oxidative damage.

Histopathological Findings

Histopathological examination of liver tissues provided insights into structural changes induced by $HgCl_2$ and the protective effects of T. polium.

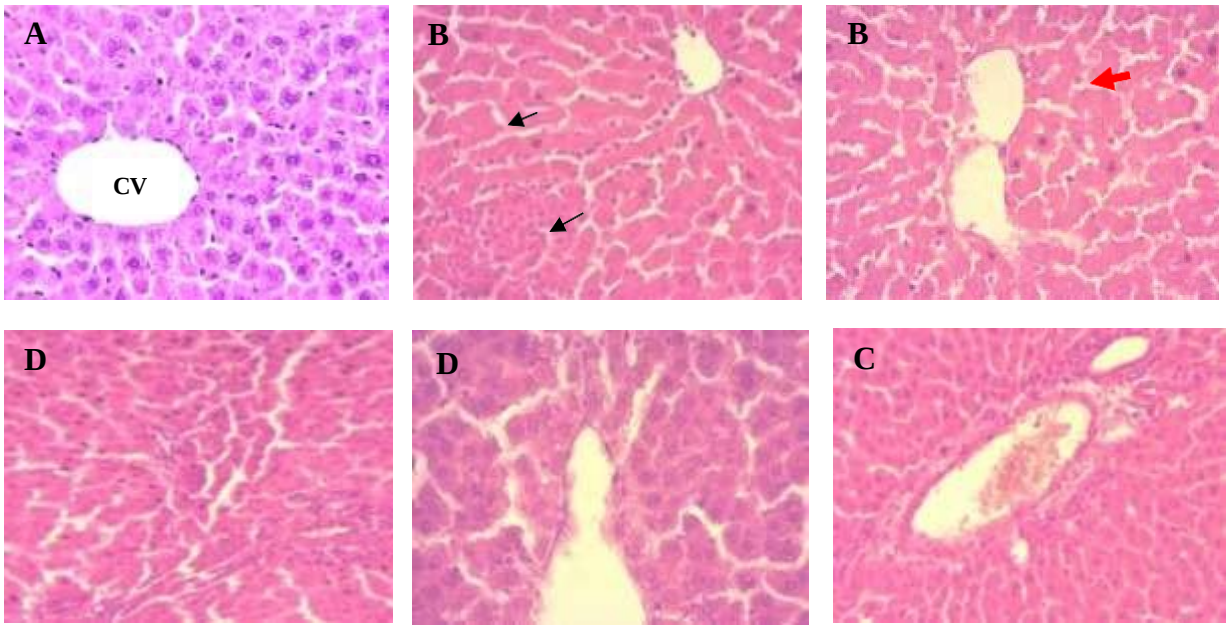

Fig. 1. Histopathological examination of rat liver tissue showing the effects of *Teucrium polium* extract on mercury chloride-induced histopathological changes (x400).

(**A**) Controls; normal histological appearance. (**B**) rats treated with $HgCl_2$; (**C**) $HgCl_2$ + *Teucrium polium* extract group; (**D**) *Teucrium* polium group

The histopathological examination of liver tissues revealed distinct alterations across the different experimental groups, providing insights into the origin and nature of the observed dysfunctions. In the control group, the liver exhibited normal architecture with no signs of damage, indicating a healthy hepatic state.

In the $HgCl_2$ group, severe histopathological changes were observed, including apoptotic and coagulative necrosis lesions, inflammatory infiltrates, and disrupted hepatic parenchyma. These alterations likely originated from the cytotoxic effects of mercuric chloride ($HgCl_2$), which induces oxidative stress by overproducing reactive oxygen species (ROS) and depleting antioxidant defenses, leading to cell death and tissue damage. The presence of inflammatory infiltrates suggests an immune response triggered by the release of damage-associated molecular patterns (DAMPs) from injured hepatocytes.

In the $HgCl_2$ + T. polium group, partial improvement in liver architecture was noted, with reduced necrosis and inflammation compared to the $HgCl_2$ group. However, some abnormalities persisted, such as edema, hemorrhage, and enlarged nuclei. These residual changes may reflect the extent of damage already inflicted by $HgCl_2$, which could not be fully reversed within the treatment period. Edema and hemorrhage are likely due to vascular dysfunction and impaired blood flow caused by $HgCl_2$-induced oxidative stress and inflammation, while enlarged nuclei may indicate ongoing cellular stress or incomplete recovery.

In the T. polium group, mild vacuolar degeneration in hepatocytes was observed, characterized by clear nuclei with peripheral chromatin condensation. The engorged blood vessels filled with red

blood cells suggest altered hepatic circulation or metabolic changes induced by the plant extract. This vacuolar degeneration could be attributed to the accumulation of lipids or other metabolites as a result of T. polium's potential effects on cellular metabolism, although it does not appear to represent significant pathological damage.

Overall, these findings highlight the specific dysfunctions associated with $HgCl_2$ exposure and the partial protective effects of T. polium, while also revealing subtle changes induced by the plant extract itself.

DISCUSSION

Mercury (Hg) toxicity is well-documented for its detrimental effects on biological tissues, particularly the liver, where it induces significant alterations affecting the health of the organism **[34,35]**. In this study, even at a low dose of $HgCl_2$ (2 mg/kg), we observed marked pathological changes in rat livers, corroborating previous findings **[36,37]**.

Body Weight and Liver Weight

A reduction in body weight is often used as an indicator of deteriorating general health in rats, while changes in organ weight are critical for evaluating organ toxicity. Our results demonstrated that $HgCl_2$ exposure significantly decreased final body weight, body weight gain, and relative liver weight compared to the control group. This aligns with earlier studies showing that mercury exposure leads to appetite suppression and disrupts essential metabolic processes, causing delays in growth and development **[38,39]**. Notably, rats treated with both $HgCl_2$ and *Teucrium polium* (T. polium) extract exhibited further reductions in body weight and gain, likely due to the combined effects of mercury toxicity and the plant extract's potential weight-lowering properties **[40]**. However, no significant changes were observed in liver weight between the $HgCl_2$-intoxicated and intoxicated-treated groups, suggesting that T. polium did not fully reverse $HgCl_2$-induced weight loss in this organ.

Serum Enzymes (AST and ALT)

Serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) are widely accepted markers of hepatic injury **[41,42]**. As expected, $HgCl_2$ treatment significantly increased AST and ALT levels, consistent with previous reports **[5,43]**. Treatment with T. polium extract markedly reduced these enzyme levels in intoxicated-treated rats, indicating its hepatoprotective effects. These findings are supported by studies demonstrating the protective role of T. polium against chemical-induced liver damage **[44,45]**. The presence of flavonoids in T. polium may contribute to its ability to decrease serum transaminase activity, as reported by Sanz et al **[46]**.

Protein and Lipid Metabolism

Hepatocyte function can be assessed through changes in protein and lipid metabolism. Our study revealed that $HgCl_2$ exposure reduced serum levels of total protein and albumin, which is consistent with findings from Uzunhisarcikli et al. **[47]** and Merzoug et al. **[48]** Mercury exposure appears to interfere with protein synthesis and metabolism in the liver. Additionally, hypocholesterolemia and hypotriglyceridemia were observed following $HgCl_2$ administration, paralleling results reported by Wadaan. **[49]**. Interestingly, T. polium treatment partially restored lipid profiles, increasing triglyceride and cholesterol levels in $HgCl_2$-treated rats, as previously documented **[50,51]**. However, no significant changes were observed in protein or albumin levels, highlighting the need for further investigation into the mechanisms underlying T. polium's effects on protein metabolism.

Oxidative Stress and Antioxidant Enzyme Activity

Oxidative stress plays a pivotal role in metal-induced toxicity. $HgCl_2$ generates free radicals, leading to increased lipid peroxidation (LPO) and subsequent hepatotoxicity **[52]**. Our study confirmed elevated thiobarbituric acid reactive substances (TBARS) levels in $HgCl_2$-intoxicated rats, indicative of LPO and membrane dysfunction. This finding is consistent with prior research **[53,54]**. Co-administration of T. polium extract significantly reduced TBARS levels, supporting its antioxidant properties **[55,56]**.

Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST) play crucial roles in combating oxidative stress. In our study, $HgCl_2$ exposure increased the activities of these enzymes, likely as a compensatory mechanism to counteract ROS production. Conversely, T. polium treatment modulated enzyme activities, reducing their induction and restoring balance. This modulation underscores the plant's ability to stabilize free radicals and mitigate oxidative damage, potentially through its rich content of polyphenols and flavonoids **[57]**.

Histopathological Findings

Histopathological examination revealed severe alterations in $HgCl_2$-intoxicated livers, including apoptotic and coagulative necrosis, inflammatory infiltrates, and disrupted parenchyma. These findings align with reports of acute liver injury caused by mercury toxicity **[6,58]** Treatment with T. polium showed partial recovery, with reduced necrosis and inflammation, but persistent signs of damage such as edema, hemorrhage, and enlarged nuclei remained. The presence of cells with dispersed chromatin suggests early regeneration of hepatocytes, indicating the plant's potential regenerative effects.

CONCLUSION

This study demonstrates that acute oral exposure to $HgCl_2$ causes significant biochemical alterations and hepatic effects in male rats. Treatment with T. polium extract (125 mg/kg bw) over 45 days exerted ameliorative effects, characterized by reduced oxidative stress, normalized antioxidant enzyme activities, and improved histopathological features. The protective effects of T. polium are likely attributed to its rich content of antioxidants, particularly polyphenols and flavonoids, which scavenge free radicals and restore cellular homeostasis. However, mild vacuolar degeneration observed in the T. polium-only group warrants further investigation into its physiological implications. While T. polium shows promise as a therapeutic agent for mercury-induced liver damage, additional studies are needed to evaluate its efficacy, safety, and optimal dosing for clinical applications.

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