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Virgin olive oil and vitamin E protect against mercury-induced hematological and immunological alterations in rabbit

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Abstract

Mercury is one of the environmental pollutants that could lead to a serious threat to the health of living organisms. This study was designed to determine the toxic effect of mercury chloride on hematological and immune cell levels, relative weight and tissue structure of the spleen and thymus, and concentration of mercury in these immune organs. The antioxidant protection of virgin olive oil and vitamin E against mercury toxicity in the local male rabbit *Oryctolagus cuniculus* was also investigated.

Twenty four adult male rabbits were divided randomly into four groups, the first group was kept as a control (C-group), and three groups (M-group, O-group, and E-group) were treated respectively with 0.25 mg/kg of HgCl₂, 0.25 mg/kg of HgCl₂ supplemented with 3 ml/kg b.w. of virgin olive oil, and 0.25 mg/kg of HgCl₂ supplemented with 3 mg/kg b.w. of vitamin E. The mercury, virgin olive oil, and vitamin E were administered orally, for 30 days-period.

The results showed a decrease in red blood cell count, hemoglobin and hematocrit levels white blood cell count, lymphocytes, monocytes, neutrophils, eosinophils, and basophils in rabbits intoxicated with mercury. In contrast, the relative weight of the spleen and thymus seemed to increase compared to the control. The measurement of the quantity of mercury in organs demonstrated a notable accumulation in the spleen and thymus. The histological examination of immune organs resulted in severe morphological changes, which seemed more severe in the spleen than in the thymus. In contrast, The combined treatment with olive oil or vitamin E led to an improvement of hematological and immunological indices, demonstrating the protective role of vitamin E and the antioxidant components of olive oil against the oxidative damage caused by mercury.

Keywords: Mercury chloride, virgin olive oil, vitamin E, hematological and immune indices, oxidative stress, antioxidant.

1. Introduction

Mercury is considered one of the environmental chemicals of major public health concern. Its toxicity causes harmful effects and has several consequences on animal and human health. Mercury, whatever its concentration in the body, can have a toxic impact on the cells, tissues, and organs (Belles *et al.*, 2002; Basu 2023, Zafar *et al.*, 2024), and can induce psychophysiological damage (Pizzarno and Murray, 1993). The toxic impact of mercury can be attributed to its solubility in the blood and hemoglobin. Whereas, once in the bloodstream, mercury is distributed and accumulated in the kidney, brain, and nervous system. It can also pass the placental barrier and pose a threat to the life of the fetus. In addition, it is excreted in breast milk, which is dangerous for newborns. Mercury is also known to have a negative effect on the immune system (Schwenk *et al.*, 2009). The direct influence of mercury compounds is obvious. Indeed, there are changes in lymphocyte levels, morphological alterations in immune tissues, a decrease in the total number of immune cells, and alterations in immune function (Luster *et al.*, 1984). It has also an immunosuppressive effect that results in part from its cytotoxic actions, which induce apoptosis and/or necrosis in immune cells, an increase in susceptibility to infections (Shenker *et al.*, 1997), and development of autoimmune diseases (ATSDR, 1993), particularly in the kidney. Several studies have been carried out on the harmful effects of inorganic mercury on the immune system. It develops glomerular lesions in the kidney (Moumen *et al.*, 2024) and autoimmune reactions. These reactions manifest as lymphoproliferation, associated with an increase in the total level of IgG (Prouvost-Danon *et al.*, 1981) and develop of proteinuria (Druet *et al.*, 1978; Bernaudin *et al.*, 1981). Furthermore, mercuric ion, one of the most potent thiol-binding chemicals (Zahir *et al.*, 2005), is known to enhance the level of cellular ROS and generate oxidative stress (Hussain *et al.*, 1999), which can lead to tissue damage (Reus *et al.*, 2003).

However, the body's endogenous defenses are reinforced by natural antioxidants, to prevent damage caused by oxidation, researchers have studied the beneficial role of various antioxidant molecules supplemented in the diet, such as the minerals and trace elements (selenium, zinc, manganese, etc.), vitamins E, vitamin C and carotenoids (Pastre, 2005). The antioxidant protection of vitamin E against heavy metal toxicity is particularly important for health (Al-Attar, 2011, Buzoeva, 2022). Studies reveal that vitamin E may reduce oxidative stress and the accumulation of heavy metals such as mercury in organisms, it decreases oxidative damage by promoting the antioxidant potential (Shi *et al.*, 2024). It has also a crucial role to play in immune function, DNA repair, and other metabolic processes (Farrell and Roberts, 1994). Moreover, Natural antioxidants can be found in medicinal plants,

vegetables, and fruits, including olives and olive oil. Numerous epidemiological, clinical, and experimental studies, conducted over the past few decades, have investigated the value of olive oil as a staple food, medicinal resource, and cosmetic ingredient for Mediterranean cultures since 4000 BC. These studies have confirmed that olive oil has protective properties due to the antioxidant capacity of its minor components. Olive oil contains triglycerides and the main fatty acids represented by monounsaturates (oleic), with a small quantity of saturates and sufficient amounts of polyunsaturates. In addition, the minor components such as carotenoids, phytosterols, α -tocopherol, squalene, phenolic compounds, and chlorophyll (Publio and Marzia, 2009) play an important role in protecting against oxidative stress and improving health.

This study highlighted the effect of mercury on hematological, and immunological parameters, the organ's relative weight, and the tissue morphology of the spleen and thymus of male rabbits *Oryctolagus cuniculus*. The role of vitamin E and antioxidant components presented in virgin olive oil in the detoxification of mercury was also investigated.

2. Materials and methods

Experimental animals

Twenty four local male rabbits, aged 5 months old, and weighing 1628.83 ± 121.64 g were used, they were obtained from a rearing center and placed in the animal house at the University of Oum El-Bouaghi under controlled conditions of humidity, temperature, and photoperiod. Food and water were supplied *ad libitum*.

Plant material and extraction procedure

Virgin olive oil, which is known as the best kind of olive oil produced in Algeria, was used in this experiment. It was obtained from nearby farmers in Algeria's northeastern, Skikda region. Instead of using chemical processes, farmers in this region extract virgin olive oil by using traditional mechanical methods. The steps involved in making virgin olive oil include generally grinding, pressing, settling, filtering, and storing.

Vitamin E

The chewable tablets 100 mg of vitamin E (API E 100MG COMP. A CROQ. B/20) produced by the API laboratory of the pharmaceutical business in Constantine, Algeria, were purchased from a local pharmacy. It is used in this study to mitigate disturbances related to mercury toxicity.

Experimental design

Before treatment, animals were acclimatized to the conditions of the animal house for 15 days. The administration of HgCl₂, vitamin E, and virgin olive oil was carried out orally, for four weeks, according to the following experimental design.

The 1st group, the control (C-group) received a standard daily diet, the 2nd group (M-group) was treated with 0.25 mg/kg of HgCl₂, the 3rd group (O-group) was treated with 0.25 mg/kg of HgCl₂ supplemented with 3 ml/kg of virgin olive oil, and the 4th group (E-group) was treated with 0.25 mg/kg of HgCl₂ supplemented with 3 mg/kg of vitamin E.

collection of organ and blood samples

After the treatment period, the rabbits were killed, and the blood was taken into EDTA-labelled polyethylene tubes. Automated blood counters (Model BC 2300, Socimed Sarl, Stains, France) were then used to determine the complete blood count (CBC).

The organs (spleen and thymus) were removed and weighed, the relative weight of each organ was estimated. For the histological study, a sample of these organs was preserved in diluted formalin (10 %).

Histological study

The realization of the histological sections visible under the light microscope was carried out according to the method of Martoja and Martoja, (1967). The procedure involved several steps: dehydration and substitution, by using increasing concentrations of alcohol, toluene, and paraffin. The next steps were paraffin embedding, blocking of the samples, and making thin sections of 3 to 5 µm, by using a microtome apparatus ((SLEE CUT 4062, TECH Inter Lab, Thoiry, France). The staining of the samples and the mounting were carried out using two stains, hematoxylin, and eosin. Finally, three drops of the mounting medium; Eukit, were placed on the sample, and the sections were covered by the coverslips.

Measurement of mercury in organs

To determine the concentration of mercury in organs, we followed the method of Peixoto et al. (2008); samples were digested in vessels containing an acid medium (HNO₃), heated in a microwave, and then diluted in water. The result of the digestive procedure was a colorless solution. Mercury levels in the samples were measured using an atomic absorption spectrophotometer, which is equipped with a deuterium background corrector, Mallow cathode lamps, and a vapour generating system. HCl (3 M) and NaBH₄ (4% m/v) were used as the acid medium and reducing agent, respectively, while argon was the purge gas. The digests analysis of certified reference material SRM NIST, and measurements of analytical standards were used to evaluate the results.

Statistical study

All data were expressed as a mean $\pm$ SD. The statistical analysis of the data was performed using Student's t-test for the comparison of two means, whereas, the comparison of all means was made by one-way analysis of variance (ANOVA). The statistical test was considered significant at $p \leq 0.05$ level (MINITAB 19 software, LLC.).

$p > 0.05$: non significant, $p \leq 0.05$: * significant difference, $p \leq 0.01$: ** highly significant difference, $p \leq 0.001$: *** very highly significant difference, a: the comparisons of all means by using test ANOVA, b: comparison between C-group and M-group, c: comparison between C-group and O-group, d: comparison between C-group and E-group

3. Results

The red blood cell, hemoglobin, and hematocrit levels

The results of hematological markers showed a non significant decrease in red blood cell count (Fig. 1) and hematocrit (Fig. 3) in the M-group compared to the control. The treatment with olive oil resulted in a significant increase in the O-group. The exposure to mercury alone induced a notable reduction in hemoglobin levels (Fig. 2) compared to the C-group. The supplementation of vitamin E induced a moderate increase in hematological parameters compared to the control.

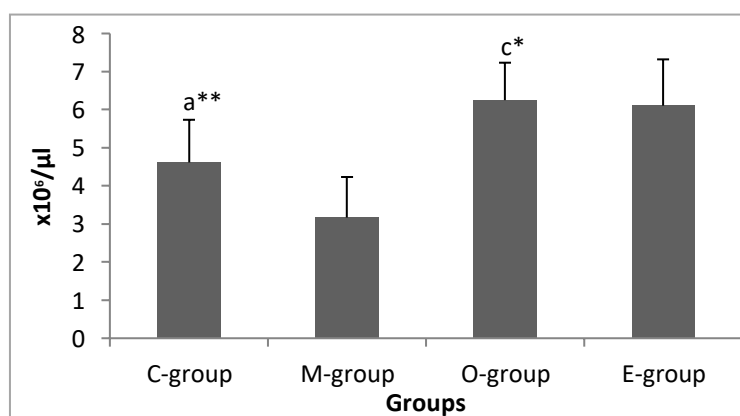


Figure 1: Variations of red blood cell count in control and treated groups after four weeks of treatment

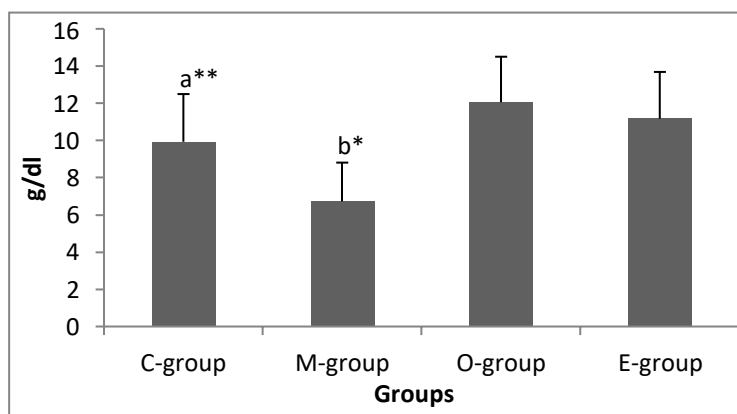


Figure 2: Variations of hemoglobin in the control and treated groups after four weeks of treatment.

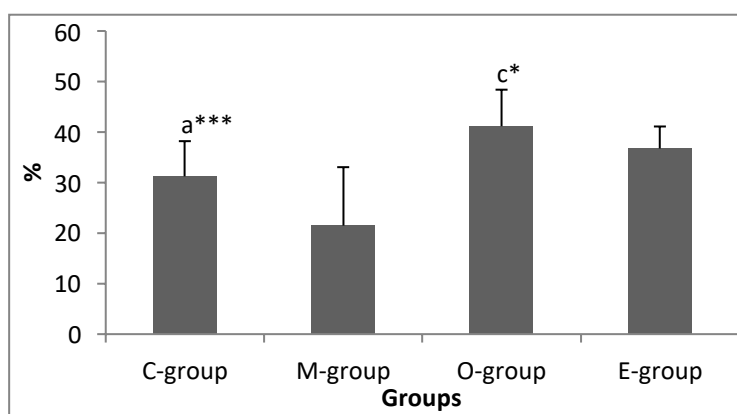


Figure 3: Variations of hematocrit in the control and treated groups after four weeks of treatment.

The immune cell levels in the blood

The results showed a significant decrease in the white blood cell count, lymphocytes, monocytes, neutrophils, and eosinophils in rabbits intoxicated with mercury (M-group) compared to the control, but the basophil levels decreased moderately. However, the group of rabbits treated with mercury combined with olive oil showed a notable increase in lymphocyte and monocyte levels. In contrast, a moderate decrease in neutrophil, eosinophil, and basophil levels was observed in the O-group compared to the control. Vitamin E supplementation caused a significant increase in the white blood cell count, lymphocyte, monocyte, and basophil levels. Neutrophil and eosinophil concentrations, on the other hand, showed a moderate decrease compared to the control (Table 1).

Table 1: Variations of white blood cell percentage in the control and treated groups after six weeks of treatment.

	Immune cells					
	white blood cells (10 ³ xµl)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
C-group	4.681.253 ^{a**}	25,329±5,24 ^{a**}	4,745±1,5 ^{a***}	53,333±6,92 ^{a**}	3,017±0,372	0,329±0,024 ^{a**}
M-group	3.63+1.422 ^{b***}	10,85±1,82 ^{b**}	2,467±1,011 ^{b*}	33,72±6,378 ^{b*}	0,701±0,021 ^{b*}	0,145±0,008
O-group	5.737+2.499	25,742±4,63 ^{c*}	6,133±2,597 ^{c*}	49,533±5,132	2,533±0,172	0,212±0,006
E-group	5.113+1.602 ^{d*}	26,111±389 ^{d**}	5,567±1,226 ^{d*}	46,783±4,626	2,35±0,057	0,441±0,008 ^{d*}

The quantities of mercury in the organs and their relative weights

The evaluation of the relative weight of the spleen and thymus in rabbits exposed to mercury alone revealed a non significant increase compared to the control. The exposure to mercury supplemented with vitamin E in the E-group resulted in a notable decrease in the relative weight of the spleen compared to the C-group. The relative weight of the thymus was also affected by the olive oil treatment, resulting in a significant increase compared to the control. The vitamin E supplementation revealed a moderate change in the relative weight of the thymus compared to the control.

The measurement of the quantity of mercury in organs demonstrated a notable accumulation in the spleen in the M-group. The administration of olive oil and vitamin E induced a reduction in mercury concentration in the spleen. However, the accumulation of mercury in the thymus was significant in rabbits exposed to mercury alone. The rabbits treated with olive oil and vitamin E showed a moderate increase in the quantity of mercury compared to the control (Table 2).

Table 2: Variations of spleen and thymus relative weights (mean ± SD) in the control and treated groups after six weeks of treatment.

	The relative weight of organs (%)		The concentration of mercury in organs (µg/g)	
	Spleen	Thymus	Spleen	Thymus
C-group	0.059±0.003 ^{a**}	0.046±0.004 ^{a**}	0.08±0.004 ^{a**}	0.01±0.002 ^{a*}
M-group	0.068±0.001	0.099±0.027	0.25±0.007 ^{b**}	0.04±0.004 ^{b*}
O-group	0.053±0.005	0.077±0.01 ^{c*}	0.11±0.004 ^{c*}	0.02±0.003
E-group	0.056±0.0018 ^{e*}	0.048±0.006	0.1±0.005 ^{d*}	0.01±0.002 ^{d*}

Histological study

The accumulation of mercury in the spleen and thymus resulted in severe morphological changes, with necrosis and thickness of interlobular septum in the thymus, congestion splenomegaly, necrosis in the red pulp, and thickness trabeculae (Fig. 4 and 5. M). It seemed that damage in the spleen was more severe than that in the thymus. In contrast, histological sections of the spleen and thymus of rabbits treated with mercury and olive oil (Fig. 4 and 5. O) or vitamin E (Fig. 4 and 5. E) revealed less pronounced morphological changes compared to rabbits exposed to mercury alone (Fig. 4 and 5. O and E).

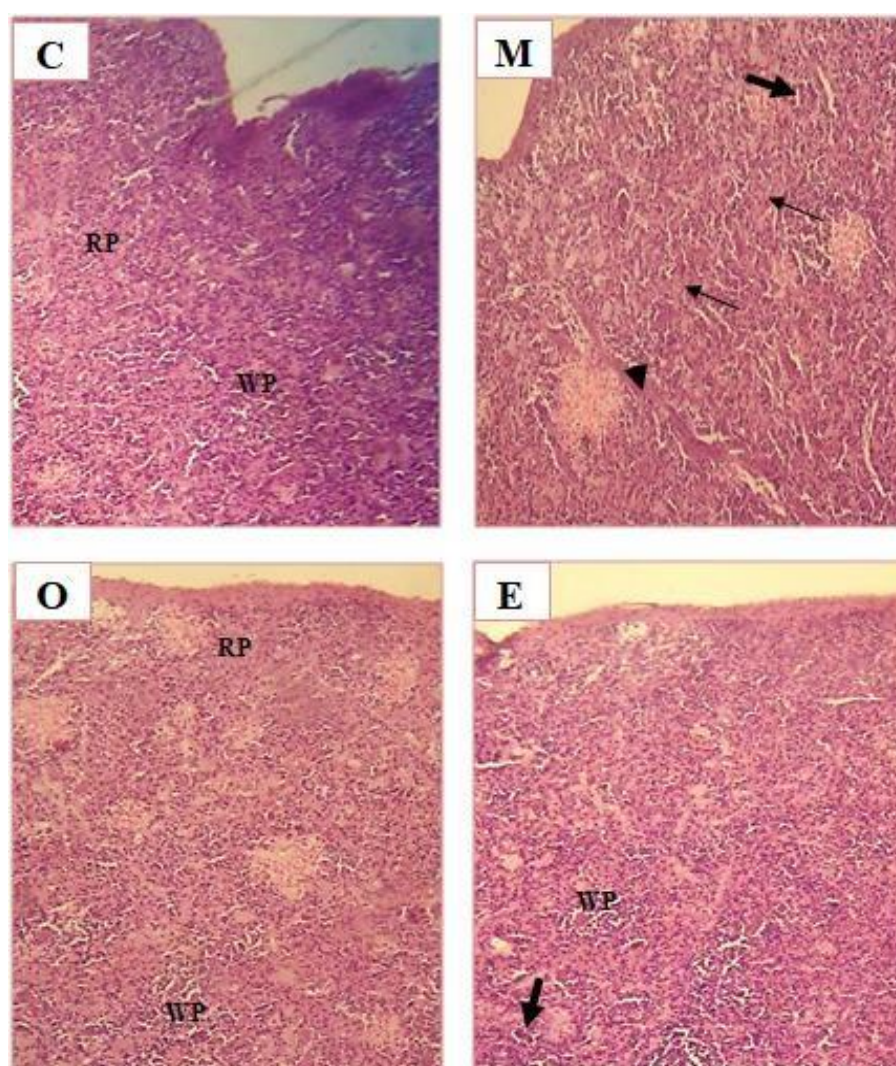


Figure 4: Histological sections of the spleen in rabbits received a standard diet (C), treated by mercury alone (M), combined with virgin olive oil (O), or vitamin E (E) (H&E staining, magnification 100x, zoom 5.0x). **RP:** red pulp. **WP:** white pulp. **Thin arrow:** congestion. **Thick arrow:** necrosis. **Arrowhead:** thickness of trabeculae.

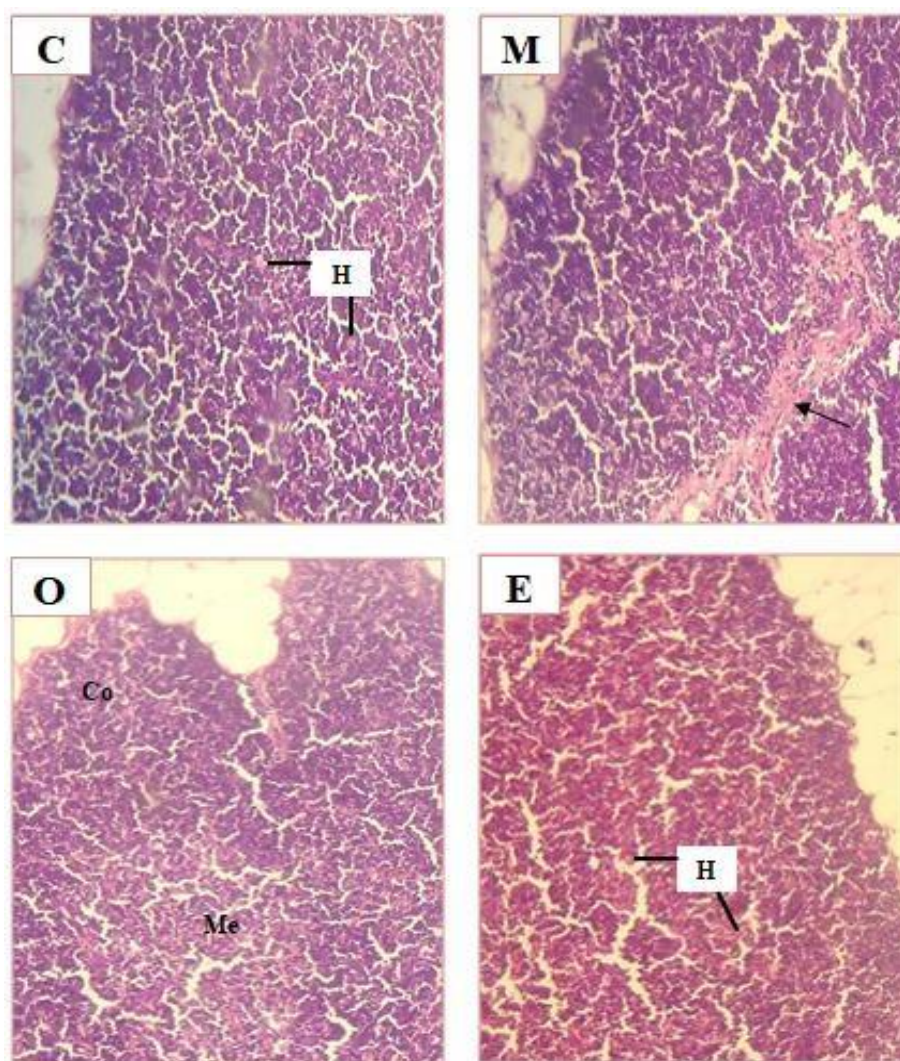


Figure 5: Histological sections of the thymus in rabbits received a standard diet (C), treated by mercury alone (M), combined with virgin olive oil (O), or vitamin E (E) (H&E staining, magnification 100x, zoom 3.2x). **Co:** cortex. **Me:** medulla. **H:** Hassall's corpuscles. **Thin arrow:** interlobular septum thickness.

4. Discussion

Exposure to chemical pollutants, including mercury, has serious consequences for public health. After penetration into the body through various pathways, mercury combines with the molecules of cells such as proteins, enzymes, etc., leading to their oxidation, and generating oxidative stress through the excessive production of free radicals. Mercury can also accumulate in tissues and organs, inducing cellular and tissular damage. Indeed, blood components are affected by the toxicity of mercury. In this regard, our results suggested a decrease in red blood cell count, hemoglobin, and hematocrit levels in rabbits exposed to

mercury. These findings are in agreement with previous investigations (Chita and Jayaprakash, 2013; Ibegbu et al., 2014; Ruyani et al., 2019). According to Ekawanti and Krisnayanti, (2015), the workers exposed to mercury in the gold mines had lower hemoglobin and hematocrit levels than non-miners. In addition, Maheswaran et al., (2008); Calisi et al., (2011) indicated that exposure to mercury could reduce hemoglobin levels, the mechanisms behind the decrease in hemoglobin through mercury exposure are not only by impairing kidney function but also by interfering with iron metabolism and causing the destruction of red blood cells (Ribarov et al., 1983, Saljooghi and Mendi, 2013, Chitra and Jayaprakash, 2013). Furthermore, Huq et al., (2008), found that exposure of mice to mercury chloride for 28 days induced a decrease in total red blood cell count (TEC), total white blood cell count (TLC), and hemoglobin levels, the reduction in hematological indices may be due to the adverse effects of mercury chloride on the hemopoietic organs in the body (Huq et al., 2008). Ekawanti and Krisnayanti, (2015) claimed that many authors hypothesized that mercury might be responsible for the hematological effect observed, due to its direct toxicity to the bone marrow, apoptosis, hemolysis, and loss of blood (Vianna et al., 2019). Other studies have considered that mercury is a free radical that affects antioxidant enzymes and binds to red blood cell components, inducing fragility and destruction of red blood cell membranes and hemolysis (Vahter et al., 2000; Grant and Lipman, 2009; Jomova and Valko, 2011). Mercury can also reduce hemoglobin synthesis by blocking the conversion of coproporphyrinogen III to protoporphyrin IX, resulting in a reduction in the life span of red blood cells (Schurz et al., 2000; Ruiz-Ramos et al., 2010).

Mercury compounds can directly or indirectly modulate the immune system function, leading to immunopathological changes (Goyer, 1986; Kowolenko et al., 1992). In our results, the exposure to mercury led to a decrease in immune cell levels compared to the control. Similar results have been suggested by several studies. The works of Shenker et al., (1992) revealed that HgCl_2 inhibits the immune response and reduces T cell proliferation. In addition, mercury chloride and methylmercury suppress various lymphocyte functions in animals and humans, such as the production of cytokines, expression of surface activation markers, and proliferation (Moszczyński, 1997). In the same order, Hu et al., (1997) showed that the administration of high doses of mercury induces a decrease in the proliferative activity of splenocytes, leading to a reduction in the lymphocyte and antibody levels. However, the findings of Queiroz et al., (1994) showed that in a work environment, exposure to mercury can obviously lead to a notable stimulation of humoral immunity. Furthermore, the accumulation of mercury in the bone marrow can lead to a decrease in immune cell levels

such as neutrophils and basophils (Moumen et al., 2022), and a disruption in monocyte production, which in turn induces a decrease in macrophage levels. These results were confirmed by other studies. Wataha et al, (2000); Shen et al, (2005) showed that exposure of monocytes to metal ions caused a significant reduction in cell proliferation. Similarly, Noda et al, (2002) showed that dental amalgams containing mercury altered the response of monocyte cells, it could block the normal stimulation of neutrophils and inhibit their function, leading to the impairment of their ability to destroy foreign substances (Zhang et al., 2020). Moreover, the exposure of neutrophils to oxidants can induce impairment of cell motility, which is thought to be caused by the oxidation of membrane lipids, leading to changes in cell membrane fluidity. On the other hand, mercury exposure caused changes in organs' relative weight and tissue structure of the spleen and thymus, due to the accumulation of mercury in these organs. According to our results, the spleen and thymus receive an important concentration of mercury, due to their affinity for the sulfhydryl group (-SH), leading to their accumulation in these organs. According to Huang and Lin, (1997); Intitoris et al, (2001), after exposure to HgCl_2 , the concentration of mercury in the spleen is significantly increased. Moreover, Hee-Young et al, (2009) reported that mercury is absorbed in the gastrointestinal tract, distributed, and accumulated in the thymus, spleen, and liver in a dose-dependent manner (Son et al., 2010), it could therefore affect the immune system. However, the study of Jadhav et al., (2007) showed that subchronic exposure to a mixture of metals containing mercury can lead to an increase in the relative weight of the spleen and thymus. Mercury can also induce a notable accumulation of macrophages in the spleen tissue (Kaewamatawong et.al., 2013; Tjahjaningsih et. al., 98 2017). In contrast, Ilbaäck, (1991) demonstrated that mercury led to a reduction of thymus weight to 20 % and thymocyte number to 50 %.

The accumulation of mercury in the spleen and thymus resulted in severe morphological changes, with necrosis and thickness of interlobular septum in the thymus, congestion splenomegaly, necrosis in the red pulp, and thickness trabeculae. Tissue damage was more pronounced in the spleen, as it has the capacity to accumulate and retain a larger quantity of mercury than the thymus, this capacity is related to its blood filtration function, its abundance of macrophages, its particular vascular structure, and other biochemical factors. The accumulation of mercury in the thymus was less important, due to its different function and structure, which is less adapted to the retention of heavy metals. A study of Ibegbu et al., (2014) demonstrated that the damage to spleen tissue caused by mercury resulted in severe central artery hemorrhage and vacuolization of the germinal center with trabeculae necrosis. Indeed, The study of Li et al., (2014) demonstrated that mercury induces cellular oxidative

damage, and affects the antioxidant system in the thymus and spleen, decreasing GSH activity, GPx, and SOD. The morphological damage induced by mercury in the thymus and spleen could have important consequences for the functions of these organs, such as reducing the body's immune response or immunosuppression (Li et al., 2014).

Supplementation of vitamin E and virgin olive oil can improve some hematological and immunological indices, by stimulating antioxidant defenses, modulating different cellular functions, and neutralizing the effects of oxidative stress (Di Meo et al. 2020). Vitamin E contributes significantly to the antioxidant system (Sies and Stahl, 1995; Stahl and Sies, 1997). It allows the extraction of a hydrogen atom from the antioxidant molecule by free radicals, thus breaking the reaction chain of free radicals, the antioxidant radicals that result being relatively unreactive species (Pascoe et al., 1987). Vitamin E is crucial for maintaining the stability of cell membranes, it scavenges free radicals (Navarro et al., 1999, Warren et al., 2000), and prevents protein and membrane-lipids oxidation produced by mercury intoxication. It has also the capacity to protect against mercury toxicity by inhibiting oxidative damage in the liver and other tissues (Rana et al., 1996). According to Rendon-Ramirez et al., (2007); Traber and Atkinson (2007), vitamin E is the most important lipid-soluble antioxidant, this property allows it to react with lipid radicals generated by the lipid peroxidation chain reaction, resulting in maintaining cell membranes from oxidation. On the other hand, the diversity of bioactive molecules and antioxidant components, such as polyphenols, flavonoids, and vitamin E in olive oil, allows it to have several antioxidant properties. Some flavonoids can bind metal ions, forming flavonoid-metal complexes, once chelated, metal ions are inactivated and unable to react with other species. Hydroxytyrosol, one of the antioxidant components in olive oil, can stimulate the synthesis of antioxidant enzymes in the body, strengthening natural defenses against oxidative stress.

Conclusion

Mercury is considered a generator of free radicals in the organism. It is also known that it induces pathophysiological changes, which particularly affect the hematological and immune cell functions, mercury compounds can also induce changes in the ratio of lymphocytes, alter tissue morphology, modulate the activity of the immune system, increase the susceptibility to infections, and develop of autoimmune diseases. Vitamin E and some components of virgin olive oil are powerful antioxidants, with antiradical activity. They play an important role in the neutralization of free radicals and the cellular protection against oxidative stress, they offer therefore interesting protection against the harmful effects of mercury on the body.

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