The protective role of virgin olive oil and vitamin E on mercury-induced hepatic, renal, testicular and adrenal toxicity in the rabbit (*Oryctolagus cuniculus*)

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Abstract

This study aimed to strengthen the antioxidant defenses against the toxic effect of mercury, by administering a synthetic antioxidant (vitamin E) and a natural product rich in antioxidant compounds (virgin olive oil) to rabbits. Hepatic and renal biomarker levels, cortisol and testosterone synthesis, mercury concentration, relative weight of organs, and tissue architecture were studied. The results showed a significant decrease in the plasma alkaline phosphatase (ALP), plasma aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), plasma testosterone levels, and relative weight of the liver, testes, and adrenal in the mercury treated group (group M), while the other indices were significantly increased in the m-group compared to the control (group C). However, the group treated with olive oil combined with mercury (group O) showed a significant decrease in the ALP, AST, ALT, testosterone levels, and adrenal relative weight, while plasma creatinine, uric acid levels, mercury concentration in organs, and the kidney relative weight were significantly increased. Vitamin E supplementation (group E) led to a significant decrease in the ALP, testosterone levels, and adrenal relative weight, a significant increase was observed in plasma levels of triglycerides, creatinine, and uric acid. Histological sections of the liver, kidney, testis, and adrenal of group M showed severe tissue damage, while the other groups showed less important tissue alterations demonstrating that supplementation with natural or synthetic antioxidants can protect against the toxicity of heavy metals such as mercury, improving the health of rabbits.

Olea europaea fruit oil, mercury chloride, oxidative stress, antioxidant

Heavy metals are environmental pollutants that have a toxic effect on living organisms (Sardar et al. 2013), even in very small quantities; they lead to excessive production of harmful molecules known as free radicals, which generate oxidative stress (Valavanidis et al. 2008).

Mercury compounds are persistent pollutants; they have the ability to accumulate in the environment and can be transported at great distances (Pacyna 2020), leading to global contamination of ecosystems and living species in the trophic chain. The consumption of living organisms contaminated with mercury exposes the superior living organisms to high concentrations of mercury. Humans can also be intoxicated by mercury through the direct ingestion of contaminated sea animals which retain high concentrations of industrial mercury (De Almeida Rodrigues et al. 2019).

Bioaccumulation of mercury in the organs, especially in the liver, organic tissues of the lungs (Vieira et al. 2021), the kidneys (Jimmy De et al. 2019), the brain (Friberg and Mottet 1989), and the reproductive organs, may lead to disorders of body systems such as hepatic, digestive, renal, cardiovascular, nervous, and reproductive systems in the organism (Da Cunha et al. 2000; Liu et al. 2008; Bernhoft 2012; Park and Zheng 2012; Kalender et al. 2013; Omanwar et al. 2013; Trebucobich et al. 2014). Mercury

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can cross the placental barrier and accumulate in the foetus (Chehimi et al. 2012). Several studies have shown that the accumulation of mercury in fish species leads to kidney and liver diseases (Bano and Hasan 1990; Lliopoulou-Georgudaki and Kotsanis 2001). Moreover, mercury can combine with the molecules of living cells such as nucleic acids and proteins, modifying their structure and inhibiting their biological activities (Velyka et al. 2014).

Mercury is also considered an endocrine disruptor as it directly affects the function of certain target endocrine glands (Veena et al. 1997) by increasing or decreasing their activities (Rana 2014), thus inducing a hypofunction or hyperfunction of the glands. Therefore, mercury causes a potential endocrine disruption (Zhu et al. 2000), and affects the hormonal system by interfering with thyroid and steroid hormones (Rana 2014), and the function of key enzymes involved in steroid synthesis (Sanderson 2006). It can also lead to structural and functional changes in the adrenal (Rana 2014) and thyroid glands, ovaries, and testes (Knazicka et al. 2013). In addition, metallic mercury, under the action of catalase-H_2O_2, is oxidized to mercuric ions (Hg^{2+}) (Testa and Krmer 2009), and accumulates in endocrine glands, such as the adrenal cortex, the thyroid, and corpora lutea of the ovaries (Zhu et al. 2000).

In recent years, more interest has been focused on natural products, in relation to their therapeutic properties; fruits, vegetables, and medicinal plants cultivated or grown spontaneously, constitute a natural and cultural wealth for local populations in some countries, they are widely used to heal themselves or to treat various diseases (Calvet-Mir et al. 2012; Bidak et al. 2015; Caballero-Serrano et al. 2016; Hemmami et al. 2023). In fact, the biological, chemical, and pharmacological properties of medicinal plants allow them to combine several antioxidant, anti-inflammatory, antibacterial, anticancer, and analgesic properties (Andleeb et al. 2019), because they contain bioactive substances that have the ability to prevent or treat many diseases (Verma and Singh 2008).

On the other hand, several epidemiological studies and experimental works have demonstrated that the traditional Mediterranean diet is the best alternative for better health, better psychological wellbeing, and higher perceived health status (Sofi et al. 2013). Olives and olive oil, which are part of the Mediterranean culture, are considered excellent sources of fat-soluble vitamins and polyphenols. They also consist of essential polyunsaturated fatty acids (Jimenez-Lopez et al. 2020), and a large number of other components, presented in small quantities, called minor, but these components are no less important. In addition, olive oil is also rich in monounsaturated fatty acids (MUFAs) which are responsible for cardiovascular benefits (Schwingshackl et al. 2011). Oleic acid improves intestinal absorption of calcium and vitamin D (Henry 2003). Furthermore, at least 30 phenolic compounds, some of which have antioxidant properties, are considered cardioprotective key elements, as they allow a reduction in cholesterol levels and protect against lipoxidation and other risk factors for chronic degenerative diseases (El-Boustani et al. 2004).

Moreover, the diversity of antioxidant compounds in olive oil such as polyphenols and vitamin E allows it to combine several antiradical and antioxidant functions (Owen et al. 2000). These compounds prevent the initiation of radical reactions by neutralizing singlet oxygen and by scavenging free radicals such as hydroxyl ($OH^{\bullet}$) and superoxides ($O_2^{\bullet-}$) (Rice-Evans et al. 1995; Burda and Oleszek 2001; Antolovich et al. 2002; Bartosz 2003). They also reduce oxidative stress and the oxidation of cell molecules, and can protect cells against free-radical damage by delaying or preventing the oxidation of proteins, carbohydrates, lipids, and DNA (Sindhi et al. 2013).

The present study was designed to determine the capacity of mercury to accumulate in organs, and disrupt hepatic and renal biomarkers, cortisol and testosterone synthesis, relative weight and tissue architecture of organs of the rabbit (*Oryctolagus cuniculus*); and to assess the possible protective role of virgin olive oil and vitamin E against mercury toxicity.

Materials and Methods

Animals

A total of 24 adult male rabbits of a local breed weighing 1628.83 ± 121.64 g were used in the study. The animals were housed in the animal house unit at the Faculty of Exact Sciences, Life Science and Nature, University of Oum El Bouaghi, Algeria, for 15 days in order to adapt to the standard conditions of the animal house unit (temperature, humidity, and natural photoperiod). Animals were fed a pellet diet obtained from the National Livestock Feed Office ONAB (Bejaia, Algeria), and water *ad libitum*.

Mercury chloride

Mercury chloride ($HgCl_2$) was obtained from the Faculty of Exact Sciences, Life Science and Nature, University of Oum El Bouaghi, in white crystalline powder form (mercury II chloride AGR, 99.5%, Labbox, France).

Vitamin E

Vitamin E was obtained from a local pharmacy, in chewable tablet form, of 100 mg (API E 100MG COMP. A CROQ. B/20), manufactured by the API laboratory of the pharmaceutical industry, Constantine, Algeria.

Plant material

The oil used in this experiment was virgin olive oil which is considered the best type of olive oil. It was obtained from local farmers in the Skikda region, located in the northeast of Algeria. Farmers in this region rely on natural mechanical methods of virgin olive oil extraction, without resorting to chemical procedures. The general procedure for producing virgin olive oil is grinding, pressing, settling, filtering, and storage.

Experimental design

The experiment was conducted in accordance with ethical standards. The animals were divided into four groups, of six rabbits each. The control group (group C) received a standard diet. The group treated with mercury chloride alone (group M), received 0.25 mg/kg b.w. of $HgCl_2$. The group treated with $HgCl_2$ combined with virgin olive oil (group O), received 0.25 mg/kg b.w. of $HgCl_2$ and 3 ml/kg b.w. of virgin olive oil. Finally, the group treated with $HgCl_2$ combined with vitamin E (group E), received 0.25 mg/kg b.w. of $HgCl_2$ and 3 mg/kg b.w. of vitamin E.

The daily administration of mercury, virgin olive oil, and vitamin E was carried out by gavage, for 30 days.

Blood and organ sampling

Before sacrifice and dissection, the rabbits were fasted for 12 h. Blood was collected in heparinized polyethylene tubes, and centrifuged at 2,150 *g* in order to separate the components of the blood. Plasma was collected in Eppendorf tubes and used for the determination of hormones and biochemical indices. The organs were removed, weighed, and preserved in dilute formalin (10%) and then used for histological study.

Hepatic and renal biomarkers analysis

Plasma was used for the determination of biochemical indices including cholesterol (Naito 1984), triglycerides (Fossati and Prencipe 1982), plasma alkaline phosphatase (ALP) (Wenger et al. 1984), plasma aspartate aminotransferase (AST) (Murray 1984a), serum alanine aminotransferase (ALT) (Murray 1984b), urea (Searcy et al. 1967), creatinine (Murray 1984c), and uric acid (Fossati et al. 1980), by using the spectrophotometric method. All hepatic and renal biomarker assay reagents were purchased from Biolabo SAS (Maizy, France) and Spinreact S.A./S.A.U, (Ctra. Santa Coloma, Sant Esteve De Bas, Spain).

Hormone analysis

The ELISA (Enzyme Linked Immuno-Sorbent Assay) method was used for the determination of testosterone and cortisol levels in plasma, by using the TOSOH AIA System Analysers (3-8-2 Shiba, Minatoku, Tokyo, Japan) apparatus, and the commercial ELISA kits for plasma testosterone and plasma cortisol (ST AIA-Pack Tosoh Bioscience, ST AIA-PACK™ Test Cups: Testosterone, and Tosoh Bioscience AIA-PACK™ Unit Dose Test Cups: Cortisol Assay Test Cup, Tokyo, Japan). The principle of this test is based on the competition between two antigens (enzyme-labelled antigen and unlabelled antigen present in the sample) against specific antibodies covering the wells of the microplates. The level of labelled antigens bound to the antigens is inversely proportional to the concentration of the hormone to be determined.

Measurement of mercury in organs

The determination of mercury concentration in organs was carried out according to the method followed by Peixoto et al. (2008); the samples were digested in vessels containing an acid medium (HNO_3), placed in a microwave oven, and then diluted in water. The digestion step produced a clear and colourless solution.

The atomic absorption spectrophotometer apparatus was used for the measurement of mercury in the samples. The apparatus was equipped with a vapour generation system, a deuterium background corrector, and Mallow cathode lamps. The acid medium and reducing agent used in the tests were HCl (3 M), and $NaBH_4$ solutions at 4% m/v, the purge gas used was argon. Results were evaluated by measurements of analytical standards and by the digests analysis of certified reference material SRM NIST 1577.

Histological procedures

After dissection of the animals, samples of the liver, kidneys, testes, and adrenal glands were taken and preserved in diluted formalin (10%) in order to keep the tissues as close as possible to the living state. Following the method of Martoja and Martoja (1967) with moderate modifications in the method, the samples were dehydrated, using ascending concentrations of ethyl alcohol (C_2H_6O) and xylene (C_8H_{10}), and impregnated in paraffin ($C_{25}H_{52}$). Thin sections of 4 µm were confectioned by using a manual microtome (SLEE CUT 4062, TECH Inter Lab, Thoiry, France). The specimens were then stained with haematoxylin-eosin ($C_{16}H_{14}O$-$C_{20}H_6Br_4Na_2O_5$; TissuePro Technology LLC, Florida, USA), to obtain clear microscopic observations. The slides were protected against drying out by using the mounting medium (Eukitt® Classic, ORSAtec GmbH, Bobingen, Germany).

Data analysis

All data were expressed as means ± standard deviation (SD). Data were analysed using Student's *t*-test for the comparison of two means, while the comparison of the means was performed by one-way analysis of variance (ANOVA). The statistical test was considered significant at $P < 0.05$ (GraphPad Prism 9.5.1 software, LLC, USA).

Results

Hepatic biomarkers

The effect of mercury toxicity is clearly manifested in liver biomarkers. A significant increase in the cholesterol and triglyceride levels was recorded in group M compared to group C. In contrast, group O showed a significant decrease in the plasma cholesterol level and a non-significant reduction in the triglyceride level. Group E showed a moderate and a significant increase in plasma cholesterol and triglyceride levels, respectively.

On the other hand, the concentrations of ALP, AST, and ALT were decreased in the group E compared to the group C; the decrease was significant in the plasma ALP level.

Comparison between the means shows a significant difference in all hepatic biomarkers (Table 1).

Table 1. Variations in plasma levels of cholesterol, triglycerides, ALP, AST, and ALT in rabbits treated with $HgCl_2$, virgin olive oil combined with $HgCl_2$, and vitamin E combined with $HgCl_2$ (data presented as means + standard deviation).

Indicator	Group C (n = 6)	Group M (n = 6)	Group O (n = 6)	Group E (n = 6)
Cholesterol (g/l)	$0.367 \pm 0.070^{a***}$	$0.693 \pm 0.090^{b***}$	$0.285 \pm 0.050^{c*}$	0.392 ± 0.060
Triglycerides (g/l)	$2.27 \pm 0.53^{a***}$	$4.01 \pm 0.83^{b**}$	2.18 ± 0.72	$2.98 \pm 0.51^{d*}$
ALP (IU/l)	$287.50 \pm 26.85^{a***}$	$65.75 \pm 9.79^{b***}$	$232.70 \pm 43.66^{c*}$	$220.60 \pm 41.61^{d**}$
AST (IU/l)	$33.00 \pm 3.35^{a***}$	$13.00 \pm 2.61^{b***}$	$23.00 \pm 4.94^{c**}$	29.00 ± 5.48
ALT (IU/l)	$47.00 \pm 5.22^{a***}$	$26.00 \pm 3.29^{b***}$	$39.00 \pm 4.65^{c*}$	42.00 ± 6.19

Group C - control; group M - rabbits treated with $HgCl_2$; group O - rabbits treated with $HgCl_2$ and olive oil; group E - rabbits treated with $HgCl_2$ and vitamin E; ALP - plasma aspartate aminotransferase; AST - glutamic-oxaloacetic transaminase; ALT - glutamic pyruvic transaminase

[a] test ANOVA; [b, c, d] Student's *t*-test; [b] group C vs group M; [c] group C vs group O; [d] group C vs group E; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Renal biomarkers

Renal biomarkers are also affected by mercury toxicity; a significant increase in plasma levels of urea, creatinine, and uric acid was recorded in the mercury treated group M. In contrast, the groups treated with virgin olive oil or vitamin E showed a slight decrease in urea concentration compared to group C. The creatinine concentration increased moderately in group O and significantly in group E. In addition, plasma concentration of uric acid was also significantly increased in groups O and E compared to group C (Table 2).

Table 2. Variations in plasma level of urea, creatinine, and uric acid in rabbits treated with $HgCl_2$, virgin olive oil combined with $HgCl_2$, and vitamin E combined with $HgCl_2$ (data presented as means + standard deviation).

Indicator	Group C (n = 6)	Group M (n = 6)	Group O (n = 6)	Group E (n = 6)
Urea (g/l)	$0.23 \pm 0.06^{a***}$	$0.44 \pm 0.06^{b***}$	0.19 ± 0.04	0.21 ± 0.05
Creatinine (mg/l)	$10.26 \pm 2.27^{a***}$	$21.23 \pm 3.83^{b***}$	11.12 ± 2.62	$14.81 \pm 3.13^{d*}$
Uric acid (mg/l)	$28.23 \pm 4.65^{a***}$	$54.88 \pm 8.53^{b***}$	$33.92 \pm 2.96^{c*}$	$35.18 \pm 4.89^{d*}$

Group C - control; group M - rabbits treated with $HgCl_2$; group O - rabbits treated with $HgCl_2$ and olive oil; group E - rabbits treated with $HgCl_2$ and vitamin E

[a] test ANOVA; [b, c, d] Student's *t*-test; [b] group C vs group M; [c] group C vs group O; [d] group C vs group E; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Hormonal variations

Testosterone and cortisol were affected by the mercury treatment. A significant decrease in the plasma testosterone level was recorded in the treated groups M, O and E compared to group C. In contrast, plasma cortisol level was significantly increased in group M, whereas the groups treated with virgin olive oil and vitamin E showed a moderate decrease in plasma cortisol level compared to the control.

Comparison between the means revealed a significant difference in plasma cortisol level (Table 3).

Table 3. Variations in plasma testosterone and cortisol levels in rabbits treated with $HgCl_2$, virgin olive oil combined with $HgCl_2$, and vitamin E combined with $HgCl_2$ (data presented as means + standard deviation).

Indicator	Group C (n = 6)	Group M (n = 6)	Group O (n = 6)	Group E (n = 6)
Testosterone (nmol/l)	2.23 ± 0.28	$0.53 \pm 0.16^{b**}$	$1.71 \pm 0.16^{c*}$	$0.93 \pm 0.27^{d**}$
Cortisol (µg/dl)	$8.93 \pm 0.99^{a**}$	$11.38 \pm 0.69^{b***}$	9.55 ± 2.19	9.80 ± 1.20

Group C - control; group M - rabbits treated with $HgCl_2$; group O - rabbits treated with $HgCl_2$ and olive oil; group E - rabbits treated with $HgCl_2$ and vitamin E

[a] test ANOVA; [b, c, d] Student's *t*-test; [b] group C vs group M; [c] group C vs group O; [d] group C vs group E; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Mercury concentration in organs

Mercury can accumulate in organs. The concentration of $HgCl_2$ was significantly enhanced in all organs of rabbits treated with mercury compared to the control. The major target organ of mercury was the kidney, which contained the highest concentration of $HgCl_2$ accumulated, followed by the liver. However, administration of olive oil and vitamin E reduced the mercury content in organs and this reduction was particularly pronounced in the kidneys (Table 4).

Relative weight of organs

Table 4. Variations in mercury concentration in the organs of rabbits treated with $HgCl_2$, virgin olive oil combined with $HgCl_2$, and vitamin E combined with $HgCl_2$ (data presented as means + standard deviation).

Organ	Group C (n = 6)	Group M (n = 6)	Group O (n = 6)	Group E (n = 6)
Liver (µg/g)	$0.13 \pm 0.02^{a***}$	$2.23 \pm 0.98^{b**}$	$1.97 \pm 0.52^{c*}$	$1.38 \pm 0.41^{d**}$
Kidneys (µg/g)	$0.19 \pm 0.01^{a***}$	$5.41 \pm 1.35^{b**}$	$2.66 \pm 0.76^{c*}$	$1.95 \pm 0.58^{d**}$
Testes (µg/g)	$0.02 \pm 0.01^{a*}$	$0.05 \pm 0.01^{b**}$	$0.03 \pm 0.01^{c*}$	$0.02 \pm 0.01^{d*}$
Adrenal glands (µg/g)	$0.030 \pm 0.009^{a**}$	$0.090 \pm 0.003^{b*}$	0.060 ± 0.004	$0.040 \pm 0.003^{d*}$

Group C - control; group M - rabbits treated with $HgCl_2$; group O - rabbits treated with $HgCl_2$ and olive oil; group E - rabbits treated with $HgCl_2$ and vitamin E

[a] test ANOVA; [b, c, d] Student's *t*-test; [b] group C vs group M; [c] group C vs group O; [d] group C vs group E; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

The results show a significant reduction in the relative weight of the liver in the group M compared to group C. However, the groups treated with virgin olive oil (group O) and vitamin E (group E), both combined with mercury, revealed a moderate decrease compared to group C. While the relative weight of kidneys was increased in the treated groups compared to group C, this elevation was significant in group M, and moderate in groups O and E.

Regarding the relative weight of the testes, the results show a significant decrease in rabbits treated with mercury compared to group C; the decrease was less important in rabbits treated with virgin olive oil in addition to mercury. However, the group treated with the vitamin E combined with mercury showed a moderate increase compared to the control.

On the other hand, the relative weight of the adrenal glands was significantly decreased in all treated groups M, O, and E compared to the control.

The comparison between the means reveals a significant difference in the relative weight of the liver, kidney, testis, and adrenals (Table 5).

Table 5. Variations in the relative weight of organs of rabbits treated with $HgCl_2$, virgin olive oil combined with $HgCl_2$, and vitamin E combined with $HgCl_2$ (data presented as means + standard deviation).

Organ	Group C (n = 6)	Group M (n = 6)	Group O (n = 6)	Group E (n = 6)
Liver (%)	$3.48 \pm 0.57^{a**}$	$1.33 \pm 0.78^{b***}$	3.28 ± 0.49	3.15 ± 0.57
Kidneys (%)	$0.63 \pm 0.18^{a**}$	$1.09 \pm 0.30^{b*}$	0.67 ± 0.17	0.81 ± 0.21
Testes (%)	$0.305 \pm 0.036^{a***}$	$0.178 \pm 0.042^{b***}$	0.278 ± 0.048	0.330 ± 0.065
Adrenal glands (%)	$0.026 \pm 0.005^{a***}$	$0.013 \pm 0.004^{b***}$	$0.020 \pm 0.002^{c*}$	$0.019 \pm 0.002^{d**}$

Group C - control; group M - rabbits treated with $HgCl_2$; group O - rabbits treated with $HgCl_2$ and olive oil; group E - rabbits treated with $HgCl_2$ and vitamin E

[a] test ANOVA; [b, c, d] Student's *t*-test; [b] group C vs group M; [c] group C vs group O; [d] group C vs group E; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Histological study

Histological sections performed on the liver revealed significant alterations in rabbits treated with mercury compared to group C (Plate VIII, Fig. 1A), resulting in hepatocyte membrane degenerations, inflammation, and necrotic foci, covering large areas of liver tissue. In addition, fibrosis was mainly manifested in the portal spaces and interlobular regions. Some lobular areas revealed hepatocytes with pyknotic nuclei; whereas, other areas showed karyolysis (Fig. 1B). The combined treatment of mercury and virgin olive oil (Fig. 1C), or vitamin E (Fig. 1D) caused less important tissue damage, resulting in focal necrosis which covered narrow areas of tissue. Some hepatic cells had nuclear alterations, with pyknotic nuclei appearing in some cells, while karyolysis occurred in others. A particular phenomenon observed in group E was emperipolesis, the entry of a lymphocyte inside a liver cell.

Histological sections performed on the kidneys of rabbits treated with mercury showed severe changes in their histological structure compared to group C (Plate VIII, Fig. 2A). These changes resulted in focal necrosis of the renal parenchyma, especially in the renal tubules, vascular congestion due to the dilatation of the capillaries, appearing in many glomeruli. The medullar zone in which the renal tubules were concentrated was also affected by mercury toxicity; it revealed tubular necrosis in large areas of this region (Fig. 2B). Necrosis and tubular atrophy appeared also in group O (Fig. 2C). In contrast, group E (Fig. 2D) showed the presence of vascular congestion in some glomeruli.

In general, the changes observed in the kidneys of animals treated with virgin olive oil and vitamin E were less severe than those observed in the group treated by mercury only. Moreover, the kidney medullar region in both groups appeared normal and was not damaged by the mercury treatment.

The tissue architecture of the testes was modified by mercury treatment compared to the control group (Plate IX, Fig. 3A). Severe haemorrhagic necrosis appeared in the interstitial Leydig cells in some regions of the testes, with severe fibrosis in the interstitial tissue resulting from the atrophy and the decrease in the number and size of the seminiferous tubules. There was also a reduction of germ line in many tubules, as well as the disorganization of germ cells in others (Fig. 3B). A less pronounced effect of mercury was apparent in the groups treated with virgin olive oil and vitamin E. While there were necrotic areas in the interstitial tissue (Fig. 3C) and fibrosis in other areas (Fig. 3D), there was also a notable improvement in the disorganization of germ cells in the majority of the seminiferous tubules, which form the testicular tissue.

The adrenal glands were strongly affected by mercury toxicity. The tissue architecture of the gland seemed to be seriously altered; in particular, the cell layers of the adrenal cortex, and the most affected cortical region was the zona fasciculata. The latter presented with membrane degeneration, inflammation, and haemorrhagic necrosis, as well as the presence of giant cells with abundant cytoplasm, due to the storage of large quantities of steroid hormones. Furthermore, the zona reticularis and some parts of the zona fasciculata contained vacuolated cytoplasm (Plate IX, Fig. 4B). While histological sections performed on the adrenal glands of animals treated with virgin olive oil (Fig. 4C) and vitamin E (Fig. 4D) showed moderate changes compared to the mercury treated group, resulting in narrow necrotic areas, membrane degeneration, and small vacuolar areas.

Discussion

The results concerning cholesterol and triglyceride levels show an increase in group M compared to the control. These results are in agreement with those of El-Shenawy and Hassan (2008), Wadaan (2009), and Siouda and Abdennour (2015). The increase in cholesterol and triglyceride levels is a sign of hepatorenal damage and deterioration of liver activity (Strong et al. 2021). According to Mozaffarian (2009), high levels of mercury and its compounds cause metabolic changes in lipids, leading to cardiovascular disorders.

In contrast, plasma cholesterol level was reduced in group O, indicating that antioxidant components of olive oil, such as polyphenols, play an important role in the regulation of cholesterol and triglyceride synthesis (Ma et al. 2020). The study of Lockyer et al. (2016) showed that the administration of phenolic compounds lead to a reduction in plasma cholesterol levels in humans. Other studies have shown that polyphenols improve the lipid profile (Del Rio et al. 2013) and decrease low-density lipoprotein (LDL) oxidation (Bernard et al. 2003; Baba et al. 2007). Vitamin E contributes to the reduction of cholesterol levels in the blood by stimulating the synthesis of bile salts in the liver and increasing the hepatic excretion of cholesterol (Uzunhisarcikli et al. 2016). According to Guéniot (2001), treatment with a mixture of antioxidants (vitamins E and C, beta-carotene, and selenium) promotes a reduction in ‘‘bad cholesterol’’ (LDL) levels without any increase in high-density lipoprotein levels (HDL) (‘‘good cholesterol’’).

Plasma levels of liver enzymes are used as a marker of liver damage (Liz 2003; Svetlov et al. 2006). ALP is a marker of cholestasis, which is a reduction in bile formation associated with abnormal liver cell function (Onofrio and Hirschfield 2020). Our results revealed a significant reduction in plasma levels of ALP. These findings are similar to those reported by Agarwal et al. (2010). In addition, according to Lawrence and Steiner (2017),

disturbances in ALP levels in the blood indicate the presence of liver disease and duct necrosis. In addition, studies by Schulte-Wissermann et al. (1977), Gupta and Sastry (1981), and Haouem et al. (2013) have shown that mercury induces a decrease in ALP activity. This may be explained by hepatic functional disruption (Sharma et al. 2005). However, El-Demerdash (2001), Sharma et al. (2002), and Reus et al. (2003) revealed that the decline in ALP levels indicates no congestion or cholestasis.

Moreover, plasma levels of AST and ALT were decreased in group M compared to group C. These results are in agreement with the findings of Franciscato et al. (2011) and Moraes-Silva et al. (2012). Indeed, Peixoto and Pereira (2007) showed a reduction of 40% in ALT enzyme activity in young rats treated with 5 mg $HgCl_2$/kg/day. Another study conducted on lactating and non-lactating rats exposed to mercury at 5 mg/kg for 5 days revealed a decrease in ALT and AST activity (Oliveira et al. 2014). In addition, Ashour et al. (1993) found a decrease in ALT activity after incubation of hepatocytes in a high dose (100 ppm) of methylmercury, whereas low doses induced an increase in ALT activity. The results of those studies suggested that mercury at high doses induced inactivation of transaminases. This is possibly what occurred in our study; the high dose of mercury administered to rabbits caused inhibition of transaminase activities. According to Vedavathi et al. (2004), the decrease in ALT enzymatic activity is due to chemical modifications affecting the sulphhydryl group of cysteine (-SH group) involved in the inactivation of ALT. However, Devlin (1997) has suggested that the decrease in ALT levels is not related to hepatotoxicity because one of the results of liver damage is usually an increase in ALT, not a decrease.

In addition, most liver lesions, such as necrosis and apoptosis, usually enhance plasma transaminase levels. However, in rare cases, severe necrosis and cellular degeneration can lead to a decrease in transaminase concentration, especially ALT. In fact, in advanced stages of hepatic necrosis when liver function is severely impaired and membrane degeneration massively affects liver cells, it is possible that the release of ALT and AST into the blood compartment decreases, which may result from the massive destruction of functional liver cells, leading to a decrease in cell number, which in turn reduces the release of transaminases. In addition, the findings of Malota (1987) showed that changes in the catalytic activity of AST and ALT may be due to the accumulation of mercury in the liver and kidneys.

Supplementation of vitamin E and polyphenols present in olive oil can improve plasma AST and ALT levels by stimulating antioxidant defences modulation of different cellular functions and neutralization of the effects of oxidative stress (Di Meo et al. 2020).

Plasma urea and creatinine levels were increased in group M. These results are in agreement with previous studies conducted on humans and animal models exposed to organic or inorganic mercury (Agarwal et al. 2010; Gado and Aldahmash 2013; Favero et al. 2014; Othman et al. 2014). A significant positive association between blood mercury concentrations and the kidney biomarkers creatinine and urea was demonstrated by Kort et al. (2022). The plasma concentration of uric acid was also increased. According to Edelstein (2008), elevated urea and creatinine levels can be considered good markers of renal failure. These results were confirmed by increased excretion of N-acetyl-β-d-glucoaminidase (NAG), a biomarker of damage to the proximal tubules and kidney dysfunction (Gibb and O'Leary 2014). In addition, the works of Apaydın et al. (2016) and Shaukat et al. (2019) have shown that mercury induces an increase in uric acid levels. However, Mohammed et al. (2021) reported that the presence of a high concentration of mercury in the blood causes an increase in creatinine and uric acid levels and a decrease in plasma urea levels in mining gold workers. The groups treated with mercury combined with vitamin E or olive oil showed an improvement in renal function, which is in agreement with the study of Rao and Sharma (2001) who showed that vitamin E protects against the

toxic effect of mercury. Moreover, olive oil contains a variety of antioxidant compounds, which are responsible for scavenging the free radicals generated by mercury, leading to an improvement in renal function (Necib et al. 2013).

Mercury can impair the reproductive system by altering sperm characteristics (Chyb et al. 2001), spermatogenesis, fertilization capacity (Lapointe 2004), and sexual behaviour. Our results showed a significant decrease in plasma testosterone levels in rabbits intoxicated by mercury, indicating steroidogenic impairment (Vachhrajani and Chowdhury 1990; Sanderson 2006). These results are in agreement with the findings of Burton and Meikle (1980); Vachhrajani and Chowdhury (1990); Fossato da Silva et al. (2011); Moumen et al. (2011). The impairment of testosterone concentration is due to the physiopathological changes that occur along the hypothalamus-pituitary-gonadal axis, altering the follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin, oestrogen, and progesterone levels (Davis at al. 2001; Rice et al. 2014). Mercury can also alter the membrane integrity of Leydig cells, which produce the highest concentration of testosterone in the body of males (Zirkin and Papadopoulos 2018). Other studies have revealed that mercury affects testosterone synthesis by blocking the conversion of cholesterol to pregnenolone, which inhibits steroidogenesis (Burton and Meikle 1980). It also inhibits the enzymes promoting the steroidogenesis process (McVey et al. 2008), reduces the activity of 3B-hydroxysteroid dehydrogenase (3B-HSD) (Kirubagaran and Joy 1988; Vachhrajani and Chowdhury 1990), and inhibits the release of testosterone by the H295R cell line (Knazicka et al. 2013). These modifications lead to an impairment of spermatogenesis, and sexual activity in animals (Stoewsand et al. 1971; Khera 1973; Thaxton and Parkhurst 1973; Lee and Dixon 1975).

The alterations in plasma cortisol levels are an important indicator of metal stress (Cogun et al. 2012). In fact, endocrine disruptors, such as mercury, cause an increase in plasma cortisol (Cogun et al. 2012) which occurs in response to stressors that interfere in the hypothalamus-pituitary-adrenal axis (Hinds and Sanchez 2022). The corticotropin-releasing hormone (CRH) released by the hypothalamus stimulates the pituitary gland to produce the adrenocorticotropin hormone (ACTH), which in turn stimulates the synthesis and secretion of corticosteroids by the adrenocortical area (Hontela 1997; Wendelaar Bonga 1997). In addition, mercury affects the adrenocortical cells and the ACTH-producing cells of the pituitary gland (Zhu et al. 2000), causing hypertrophy of the ACTH-producing cells and an increase in the secretion of ACTH (Kirubagaran and Joy 1991). In contrast, several studies have highlighted cases of cotisolic failure caused by endocrine disruptors (Lockhart et al. 2011; Brodeur et al. 1997). Other studies have demonstrated that mercury compounds impair the plasma cortisol level (Kirubagaran and Joy 1991) due to inhibition of the adrenocortical response (Wada et al. 2009), disruption of the endocrine system, by acting on the central nervous system, steroidogenic pathways, cyclic adenosine monophosphate, adrenyl cyclase, and protein kinase (Rana 2014), reduction of adrenal steroid biosynthesis (Burton and Meikle 1980; Veltman and Maines 1986; Hontela et al. 1992), and blocking of the conversion of cholesterol to pregnenolone, which is a precursor of glucocorticoids (Burton and Meikle 1980).

Supplementation of virgin olive oil and vitamin E leads to an improvement in plasma testosterone and cortisol levels, due to the antioxidant action of the bioactive molecules present in olive oil; the latter contains a varied mixture of antioxidants which act in synergy to prevent the body from mercury toxicity (Unsal 2018). In addition, hydroxytyrosol is a component of olive oil with antioxidant properties and chelating activity, which is capable of scavenging reactive oxygen species (ROS) in human systems (Del Monaco et al. 2015). Vitamin E is a powerful free radical scavenger that protects organs against oxidative damage caused by mercury (Rana et al. 1996). In parallel, the findings of Sadeghi et al. (2020) and Saito et al. (2020), demonstrated that decreased levels

of vitamin C and E in the body cause oxidative stress and disrupt spermatogenesis and testosterone synthesis.

After mercury chloride ingestion, and due to its solubility in water but not in fat, only a small amount (7% to 15%) of $HgCl_2$ is passed from the gastrointestinal tract to the blood compartment (Liu et al. 2008; Park and Zheng 2012). It is then distributed and accumulated in the organs (Shalan 2022), where it is found in high concentrations in the kidneys, their main target organ (Park and Zheng 2012), and in lower quantities in the liver. The accumulation of mercury induces the synthesis of metallothionein, where it presents as a complex; in addition, repeated administration of mercury induces the development of tolerance to the product.

The antioxidant components of olive oil and vitamin E, which are chelating agents, have the ability to remove mercury from organs, they work effectively in the kidney (Beasley et al. 2014), inducing a reduction of mercury concentration in the kidneys and all organs.

Exposure to high concentrations of mercury can disrupt the activity and integrity of cells; this can clearly be observed in histological sections performed on the organs. The deleterious effect of mercury is noted in the relative weight and tissue structure. The liver, testes, and adrenal glands of the mercury treated animals show a significant decrease in relative weights, whereas their kidneys show a significant increase compared to the control. The disturbance in the relative weight of organs is caused by the physiological stress response, which leads to organ depletion resulting in an excessive production of antioxidant enzymes such as glutathione, glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), particularly in the liver which is considered a major organ involved in the detoxification and elimination of toxins from the organism (Petrescu and Petrescu 2020).

On the other hand, mercury accumulation in the liver and kidneys induces the synthesis of metallothionein, a sulphur-rich protein, which binds to mercury and prevents organs from mercury toxicity (Dameron and Harrison 1998; Sugawara et al. 1998). However, when metallothionein binding sites are saturated, excess mercury tends to bind to cellular components such as proteins, enzymes, lipids, etc., promoting the production of free radicals, which are responsible for structural and functional disturbances (Lobo et al. 2010).

At the molecular level, mercury has a genotoxic effect; it disrupts DNA synthesis by impairing DNA repair enzymes in the cell (Pieper et al. 2014; Ryu et al. 2014) and by acting on transcription factors (Rodgers et al. 2001), which explains the appearance of pycnotic nuclei and karyolysis in liver cells. However, according to Malota (1987), mercury can alter hepatocytes due to the presence of high mercury concentrations in serum. Other histopathological effects have been studied on target organs of mercury including the liver and kidneys (Jadhav et al. 2007; Massanyi et al. 2007; El-Shenawy and Hassan 2008; Guzzi et al. 2008; Wadaan 2009; Adams et al. 2010; Chatterjee et al. 2012; Hussein et al. 2012; Zalups and Bridges 2010).

With regard to the testes, the relative weight was reduced by mercury treatment, due to the atrophy and the decrease in the number and size of the seminiferous tubules. These findings are in agreement with those of Homma-Takeda et al. (2001); Institoris et al. (2001); Orisakwe et al. (2001). According to Ramalingam et al. (2003), mercury has a degenerative effect on the tissues, which leads to atrophy and cell death in the testes. Additionally, Bano and Hasan (1990); Moumen and Abdennour (2008); Penna et al. (2009) demonstrated that mercury induces severe histological lesions resulting in Leydig cell membrane degeneration, dilation of the lumen of certain seminiferous tubules, and absence of mature spermatozoa; these lesions are caused by oxidative stress (Boujbiha et al. 2009). Thus, the production of ROS causes damage to the various membrane and cellular components (Boujbiha et al. 2009). Tissue modifications in the testes demonstrate that mercury accumulates in the body's cells and tissues, inducing lysis of cell membranes, which in turn leads to necrosis.

The adrenal glands are also depleted due to the massive secretion of cortisol in response to stress. The study of Burton and Meikle (1980) has shown that mercury can impair the adrenal structure and function (Kirubagaran and Joy 1991). The structure impairment was observed as vacuolated areas, necrotic cells, and deposits of mercury, hyperplasia (Burton and Meikle 1980), necrosis, fibrosis, and lymphocyte infiltration (Kirubagaran and Joy 1991).

Weight variations and structural changes appear to be less severe in groups treated with olive oil and vitamin E. In fact, vitamin E inhibits lipid peroxidation by scavenging lipid peroxyl radicals and transforming them to tocopheroxyl radicals (Agarwal et al. 2010; Niki 2021), which either interact with soluble antioxidants to transform into tocopherol, or oxidize to tocopherylquinone (Agarwal et al. 2010) which has an anticoagulant effect, and antioxidant activity through its reduction to hydroquinone (Arita et al. 1998). Vitamin E can also break the chain propagation of lipid peroxidation (Niki 2021). In addition, Agarwal et al. (2010) have reported that vitamin E decreases mercury accumulation in the liver and kidneys, and controls lipid peroxidation in tissues (Lemoyne et al. 1987; Van Gossum et al. 1988). It binds to membranes and sequesters free radicals (Packer et al. 1997; Evans 2000), it also inhibits lipid peroxidation in microsomes and testicular mitochondria, suppresses the adverse effects of oxidative stress in the testes (Senthil et al. 2004; Gavazza and Català 2006), and elevates levels of antioxidant enzymes such as SOD, CAT, and GPx (Kalender et al. 2013). Phenolic compounds are the most abundant molecules in olive oil. They inhibit DNA broken by the peroxyl radical (Denny et al. 2014; De Camargo et al. 2014), leading to the protection of nuclei against karyolysis.

In conclusion, this study shows that exposure to mercury generates oxidative stress in the organism, leading to structural and functional disturbances, affecting in particular the function of the liver and kidneys which are considered the target organs. Mercury is also considered one of the most dangerous endocrine disruptors that can affect the endocrine glands and disrupt hormonal secretion.

Although the body is equipped with an endogenous antioxidant defence system which is able to create a balance between the quantity of pro-oxidants and antioxidants, this balance can be disturbed by the presence of high levels of pro-oxidants.

Supplementation of virgin olive oil, rich in natural antioxidants, and vitamin E, a powerful antioxidant, promotes balance in the body and improves organ functions. Our results demonstrate the positive effect of virgin olive oil and vitamin E in reducing mercury toxicity and contributing to the protection of cells against oxidative stress.

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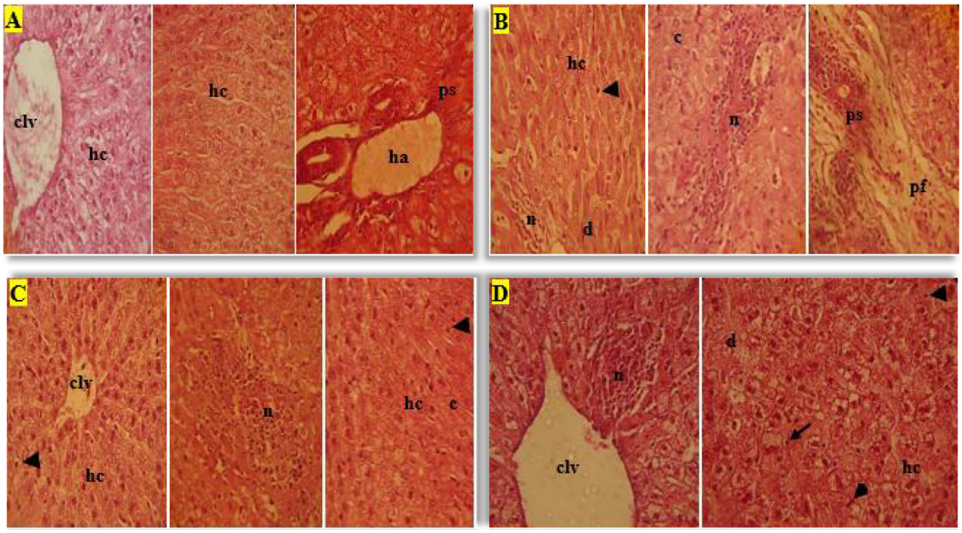

Fig. 1. Histological sections of the rabbit liver of the control (A), treated with $HgCl_2$ (B), virgin olive oil combined with $HgCl_2$ (C), and vitamin E combined with $HgCl_2$ (E) (sections were stained with H&E, magnification × 400, zoom × 1.9).

clv - centrolobular vein; hc - hepatic cells; ps - portal space; ha - hepatic artery; n - necrosis; d - membrane degeneration; c - caryolysis; pf - fibrous of portal areas; arrow - emperipolesis phenomenon; arrowheads - pycnosis

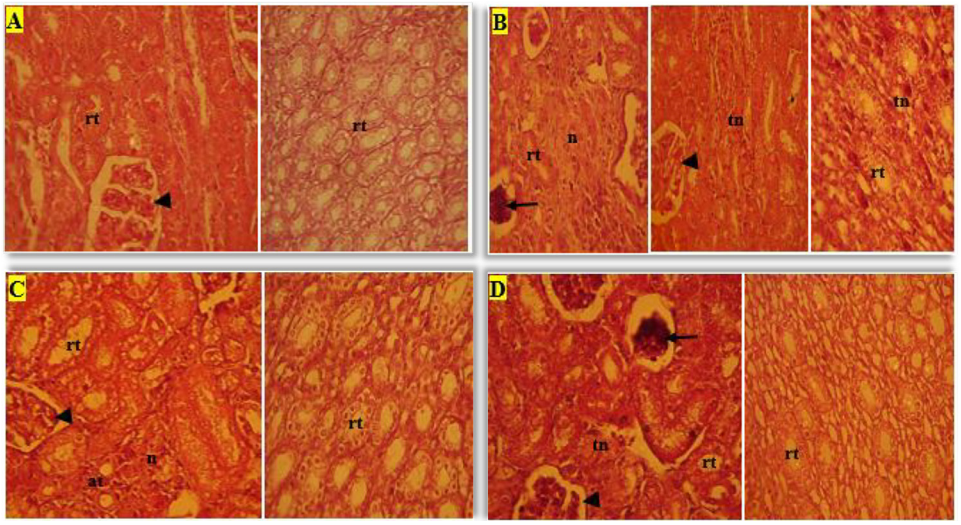

Fig. 2. Histological sections of rabbit kidneys of the control (A), treated with $HgCl_2$ (B), virgin olive oil combined with $HgCl_2$ (C), and vitamin E combined with $HgCl_2$ (E) (sections were stained with H&E, magnification × 400, zoom × 1.9).

rt - renal tubes; n - necrosis; tn - tubular necrosis; at - atrophic tubules; arrows - congestion vasculaire; arrowheads - glomerulus

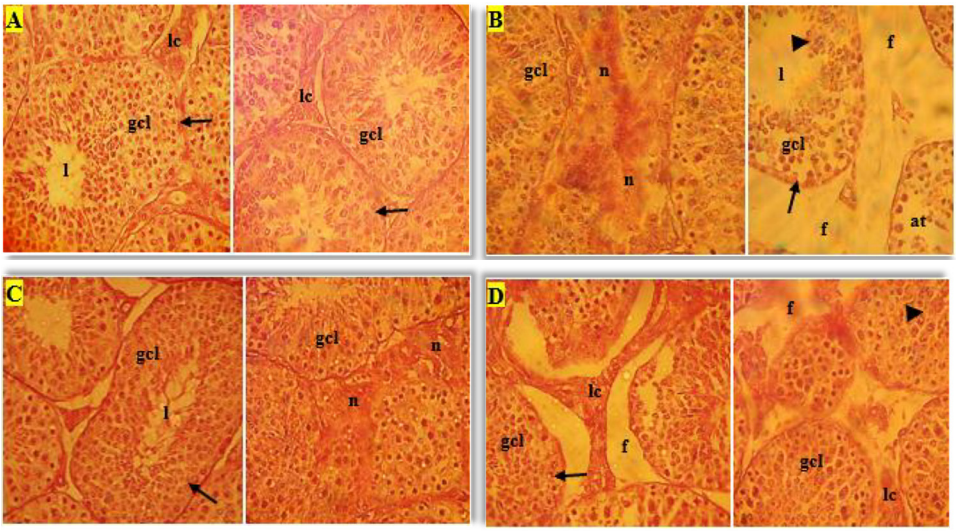

Fig. 3. Histological sections of rabbit testis of the control (A), treated with $HgCl_2$ (B), virgin olive oil combined with $HgCl_2$ (C) and vitamin E combined with $HgCl_2$ (E) (sections were stained with H&E, magnification × 400, zoom × 1.9).

lc - Leydig cells; l - lumen; gcl - germ cell layers; n - necrosis; f - fibrosis; at - atrophic tubes ; arrows - seminiferous tubules; arrowheads - disorganization of germ cells

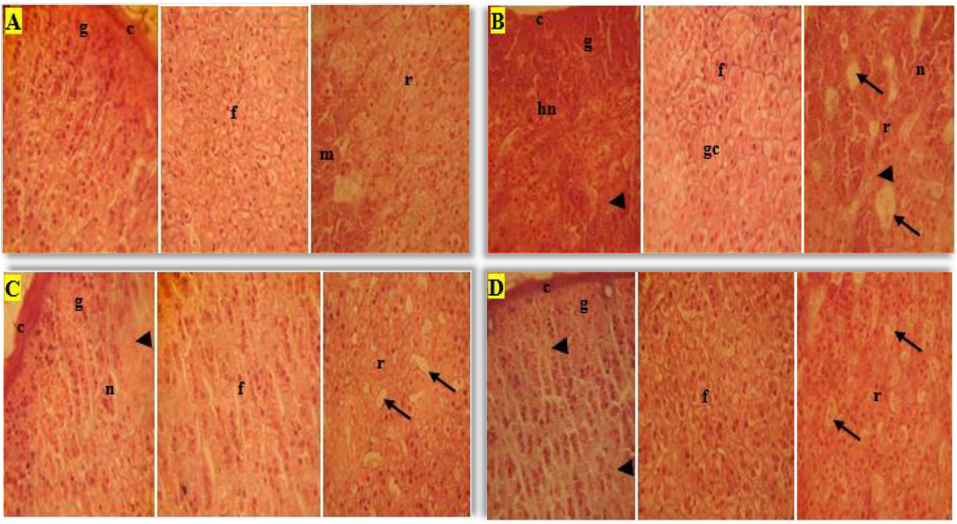

Fig. 4. Histological sections of adrenal gland of the control (A), treated with $HgCl_2$ (B), virgin olive oil combined with $HgCl_2$ (C), and vitamin E combined with $HgCl_2$ (E) (sections were stained with H&E, magnification × 400, zoom × 1.9).

c - capsula; g - zona glomerulosa; f - zona fasciculate; r - zona reticularis; m - adrenal medulla; hn - haemorraghic necrosis; n - necrosis; gc - giant cell; arrows - areas vacuolation; arrowheads - membrane degeneration