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Original research

Impact of Melatonin on Mercury Chloride-induced Hepatorenal Histopathological Alterations in Rats

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Abstract

Mercury is considered a risk factor for the development of hypertension and other cardiovascular diseases. Heavy metal mercury chloride ($HgCl_2$) is poisonous and has been linked to significant liver and kidney damage in both humans and animals. Melatonin (MEL) has been discovered to have strong antioxidant qualities that may reduce $HgCl_2$-induced oxidative stress and related tissue damage. This investigation aims to find out whether MEL may have protective effects on $HgCl_2$-induced oxidative stress, malondialdehyde (MDA) generation, kidney, liver, heart, thyroid gland, and brain histopathological changes in rats. The experimental animals were divided at random into three groups. Group 1 (control), Group 2 ($HgCl_2$ treated), and Group 3 ($HgCl_2$ + MEL treated) for 28 days, and ALP, ALT, and AST were examined as liver function test indicators. Serum MDA levels in $HgCl_2$-treated rats were considerably (P0.05) higher than in the control group. While MEL administration for 5 weeks significantly reduced it when compared to $HgCl_2$-treated rats. The kidney, liver, heart, thyroid gland, and brain sections from the control group exhibited a normal arrangement. Conversely, rats treated with $HgCl_2$ displayed distinct histopathological changes in these tissues. Intriguingly, these histopathological degenerative changes didn't appear in the $HgCl_2$ and MEL-treated group. Conclusion: This study demonstrated that $HgCl_2$ exposure induces oxidative stress, leading to organ damage and histopathological changes in rats. Melatonin supplementation showed promise in decreasing oxidative stress-induced damage by removing free radicals, enhancing antioxidant enzymes, and decreasing MDA levels. While MEL exhibited potential protective effects in mitigating inflammatory responses, edema, and degeneration in various organs, further research is needed to fully understand its mechanisms and optimize treatment strategies for combating mercury-induced toxicity.

Keywords: melatonin, mercury chloride, oxidative stress, histopathological alterations, rats

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Introduction

Mercury can enter the environment through natural sources such as volcanic activity or through human activities such as industrial pollution [1]. Its exposure is a global concern because it is widespread in the environment due to its multiple industrial, domestic, agricultural, and medical usage. Among its various chemical forms, both humans and animals are mainly exposed to mercury chloride ($HgCl_2$), methylmercury, and elemental mercury. $HgCl_2$ is metabolized primarily in the liver [2]. Mercury pollution in water is a serious threat to natural ecosystems. Various methods and technologies are used to remove mercury from the environment. They include phytoremediation, bioremediation, activated carbon adsorption, extractions, and others [3]. Mercury exposure shows symptoms like nausea, irritability, tremors, headache, hypertension, hallucinations, and even death in certain cases [4]. Mercury chloride is a hazardous heavy metal that has been linked to severe liver and kidney damage in both humans and animals [5]. Mercury chloride toxicity is primarily attributed to the production of reactive oxygen species (ROS) and the consequent development of oxidative stress, which results in lipid peroxidation and disruption of cellular antioxidants [6]. Inhaled $HgCl_2$ vapor primarily affects the brain, while mercurous and mercuric salts primarily damage the gastrointestinal lining and kidneys. Methyl mercury, on the other hand, is distributed throughout the body [7]. The mercury concentrations of agricultural soils ranged from 0.42 to 155.00 mg/kg, determined by atomic fluorescence spectrometry of 146 soil samples, and mercury concentrations in local crops (rice, maize, pepper, eggplant, tomato, and bean) all exceeded the Chinese food safety limits [8]. The model predicted that the major emission pathway of mercury to the environment is landfill leachate, which accounted for 99.8% of the total emissions. 83% of mercury in the leachate was estimated to be inorganic form, and the rest 17% was methylmercury [9]. Organisms of aquatic biota are easily exposed to and ingest mercury deposits in the tissues, especially in the gonads, liver, kidney, and gills. Long-term mercury exposure damages the nervous system and causes tremors, spasms, memory loss, hallucinations, severe sadness, increased excitability, delirium, and personality alterations [10]. Furthermore, MDA, which is a crucial byproduct of lipid peroxidation, has frequently been used as a hallmark of oxidative stress in several tissues [11]. Aside from its negative effects on oxidative stress and MDA generation, $HgCl_2$ has also been linked to renal function impairments such as urinary protein excretion, electrolyte imbalance, and renal tubular necrosis [12]. Additionally, $HgCl_2$ has also been demonstrated to cause hepatotoxicity, which is characterized by liver damage such as hepatocyte death, steatosis, and increased liver enzymes [13]. It has been indicated that during mercury therapy, the aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) levels in the serum rose [14]. Mercury's potential biological effects are being investigated, particularly the relationship between cardiovascular conditions like hypertension, coronary heart disease, and myocardial infarction and mercury toxicity [15]. Likewise, $HgCl_2$-induced brain tissue damage [16]. Furthermore, the high polyunsaturated fatty acid concentration of brain tissues makes them more vulnerable to oxidative damage, which is crucial to necrosis and cell death [17]. On the other hand, mercury is regularly found in the thyroid, particularly in the follicular cells, and with aging, more people have mercury in their follicular cells. Cadmium is another hazardous element that is frequently found in the thyroid. Numerous hazardous metals have corrosive properties that might result in hyperthyroidism, thyroid cancer, and autoimmune thyroiditis. The majority of the potential adverse effects that hazardous metals could cause on the human thyroid are currently speculative [18]. It is capable of causing a wide variety of toxic effects, such as corrosive damage, severe gastrointestinal problems, acute renal failure, circulatory collapse, and ultimately death. Melatonin, a tryptophan metabolite primarily produced by the pineal gland, is incredibly potent and efficient at eliminating oxidative stress [19]. Melatonin has been shown to protect against oxidative stress in a variety of organs, besides the thyroid gland [20] but also, as previously revealed, in many other tissues and organs, such as the kidney [21], heart [22], liver [23], or brain [24]. It has been demonstrated that MEL has been shown to confer cardio protection in several cardiac diseases [25]. On the other hand, MEL has been discovered to be protective against epithelial cell damage, hepatocyte apoptosis, autophagy flux, oxidative stress, and inflammatory signaling [26]. In recent years, there has been an increase in interest in the molecular mechanism behind MEL-induced neuroprotection, which has led to an increase in studies. Studies reveal that anti-inflammatory, antioxidant, and anti-apoptotic mechanisms have positive effects on the recovery of intracerebral hemorrhage, cerebral ischemia-reperfusion injury, spinal cord injury, Alzheimer's disease, Parkinson's disease, and meningitis [27]. Given the aforementioned, a detailed investigation into MEL's impact on $HgCl_2$-induced liver, heart, thyroid, and brain damage in rats is highly recommended. Investigating melatonin's possible impact on $HgCl_2$-induced oxidative stress, MDA generation, and histological abnormalities in rat kidney, liver, heart, thyroid, and brain dysfunctions is the aim of this study.

Experimental

Animals

Eighteen female albino rats (Rattus norvegicus) (120–180 g), 5 weeks old, were procured from the Salahaddin University-Erbil, Animal House. Rats were employed in the in vivo trial, which required many administrations and handling for 28 days. They were housed in stainless steel cages with good ventilation and a 12-hour light/dark cycle. All rats received standard commercial pelleted food and had unlimited access to tap water.

Experimental design

The experimental animals were put into three groups of six rats each. The following was the experimental design for the in vivo investigation after a 2-week acclimatization period:

For 28 days, rats in Group 1 (control) were provided with unrestricted access to tap water and rat food.

Group 2 ($HgCl_2$): For 28 days, rats were given 4 mg/Kg b.w, (40 mg/L) of $HgCl_2$ in drinking water and a normal diet.

Group 3 ($HgCl_2$ + MEL) rats got Mercury chloride 4 mg/kg body weight (40 mg/L) in ad libitum tap water + MEL (30 mg/kg body weight rats via diet) for 28 days.

Histological Processing

After the withdrawal of blood samples, animals were dissected and the liver, right kidney, heart, cerebrum, and thyroid gland were removed. Their weights were recorded by High-Precision Electronic Balances, then put in formalin 10% fluid for fixation. Preserved tissue samples from fixative solution (formalin 10%) exposed to serial processes began with dehydration, clearing, and impregnation using a series of graded ethanol (Scharlau, Spain) in ascending concentrations and then immersed in xylene (Scharlau, Spain). Finally embedded in paraffin wax (Scharlau, Spain) and cooled. The paraffin sections were cut by a rotary microtome, and after that, samples were stained with hematoxylin and eosin. Finally, photos were taken with a novel digital microscope, and they were analyzed.

Blood Sample Collection

At the end of the experiment, the rats were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg). Through heart puncture, blood samples were collected and then transferred to a test tube containing gel and clot activator, where they were centrifuged at 1000 g for 15 minutes before being held at -20 C. Then, the extracted liver and kidney were weighed by electronic balance.

Biochemical Analyses

Blood samples were centrifuged at 3000g for 15 minutes to separate serum. The Cobas c411 fully-automated biochemical analyzer was used to measure serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferases (AST).

Serum Malondialdehyde Determination

Serum MDA was evaluated using a simple and sensitive spectrophotometric approach (Spectronic GENESYS 6 UV – Visible spectrometer, Thermo Electron Corporation) and the thiobarbituric acid (TBA) reagent from Merck KGaA, Germany. One ml of 17.5% trichloroacetic acid (TCA) and 1 ml of 0.66% TBA were mixed, and 1ml of 0.66% TBA was well mixed in a 150 µl serum sample, boiled in boiling water for 15 minutes, followed by 15 minutes of room temperature cooling. The remaining serum protein was precipitated using 1 ml of 70% TCA, which was added, vortexed well, and allowed to stand for 20 minutes at room temperature. After that, the mixture was centrifuged for 15 minutes at 2000 rpm to separate the supernatant, which was then used for spectrophotometric scanning at (532 nm).

Statistical Analysis

The data in Table 1 were analyzed to confirm their distribution according to normality tests (Shapiro-Wilk and Kolmogrove-Smirnove tests). The data are normally distributed, so they were analyzed parametrically by using One-way ANOVA and Tukey tests as a post hoc test using GraphPad Prism Version 8.0.2. The results were presented as mean ± standard error of the mean (SEM). $P < 0.05$ represents statistically significant.

Table 1. Effect of melatonin on serum malondialdehyde, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase of rats treated with $HgCl_2$.

Groups	Control	$HgCl_2$	$HgCl_2$+MEL
malondialdehyde (µmole/L)	6.734±0.2042	9.807±1.060 *	6.535±0.5944 #
alkaline phosphatase (IU/L)	107.6±23.25	196.5±16.32 *	116.7±14.77 #
alanine aminotransferase (IU/L)	32.00±3.317	42.67±4.022	25.60±1.965 ##
aspartate aminotransferase (IU/L)	108.6±4.885	171.8±23.47	109.2±18.68

Data are presented as mean ±standard error of mean. The results were analyzed with One-way ANOVA test. MDA; malondialdehyde, ALP; alkaline phosphatase, ALT; alanine aminotransferase, AST; aspartate aminotransferase, *; p>0.05, #; p>0.05, ##; p>0.001, *; indicates the comparison between $HgCl_2$ group and control group, while, #; indicates the comparison between $HgCl_2$+MEL group and $HgCl_2$ group. µmole/L; micromole per liter, IU/L; International Units Per Liter.

Results

Biochemical Analysis

The results of the study showed that rats treated with $HgCl_2$ had significantly ($P < 0.05$) greater serum MDA levels than the control group, as indicated in Table 1. However, MEL treatment (30 mg/kg body weight rats) for 5 weeks significantly decreased it in comparison to the $HgCl_2$-treated rats. Statistical analysis revealed that liver function test biomarkers were measured like ALP, ALT, and AST. Here, they elevated after $HgCl_2$ treatment, while MEL could ameliorate the result significantly. On the other hand, serum creatinine and uric acid did not change by $HgCl_2$ administration, whereas serum urea was markedly elevated in $HgCl_2$-treated rats and MEL reduced it significantly ($p < 0.01$).

Microscopic Examination

Liver

The liver sections of the control group had normal arrangements of liver hepatocytes, the central hepatic venule, and the portal tract (Fig. 1A). While, rats treated with $HgCl_2$ showed moderate mixed inflammatory cell infiltration predominantly lymphocytes in the portal tract with interface hepatitis associated with moderate vascular congestion, mild edema, mild focal fibrosis, and mild focal fatty changes (Fig. 1B, C, D, and E). On the other hand, both $HgCl_2$ and MEL treatment showed mild mixed inflammatory cell infiltration predominantly lymphocytes in the portal tract with interface hepatitis associated with mild vascular congestion, mild focal fibrosis, and mild edema (Fig. 1F and G).

Kidney

The kidney section of the control group showed a normal arrangement of glomeruli and kidney tubules (Fig. 2A). Meanwhile, the administration of rats with $HgCl_2$ showed a mild infiltration of mixed inflammatory cells, primarily lymphocytes, together with mild vascular congestion, rare focal fibrosis, and mild focal tubular degenerative alterations (Fig. 2B, C, D, E, F, and G). In contrast, the combination of $HgCl_2$ and MEL administration resulted in a modest infiltration of mixed inflammatory cells, predominately lymphocytes, along with mild vascular congestion, mild edema, and a rare focal mild fibrosis (Fig. 2H).

Heart

The observed sections of the heart in the control group showed a normal arrangement of the heart endocardium and myocardium (Fig. 3A). In the $HgCl_2$ group, the sections showed mild mixed inflammatory cell infiltration predominantly lymphocytes associated with mild vascular congestion, mild edema, rare focal mild fibrosis, and mild focal myocardial degenerative changes (Fig. 3B, C, and D). In contrast, in the $HgCl_2$ and MEL groups, the microscopic examination showed mild mixed inflammatory cells, primarily lymphocytes, infiltration with mild edema, minor vascular congestion, and rare focal mild fibrosis (Fig. 3E).

Cerebrum

The cerebral cortex of the brain of the control group showed normal morphology of cerebral cortex neurons (Fig. 4A and B). However, rats treated with $HgCl_2$ showed mild focal neuronal cell loss (death), mild cerebral edema, mild focal gliosis with reactive changes, and mild focal vascular congestion (Fig. 4C, D and E). In contrast, rare focal neuronal cell loss (death), mild cerebral edema, rare focal gliosis with reactive changes, and mild focal vascular congestion were observed in $HgCl_2$ and MEL groups (Fig. 4F).

Thyroid Gland

Thyroid gland sections of the control group showed a normal arrangement of thyroid follicles (Fig. 5A). These sections of rats treated with $HgCl_2$ showed rare mixed inflammatory cell infiltration predominantly lymphocytes associated with mild focal vascular congestion, mild edema, rare focal mild fibrosis, rare focal degenerative changes, and focal follicular cellular hyperplasia with cystic dilatation (Fig. 5B, C, D and E). In contrast, rare mixed inflammatory cell infiltration predominantly lymphocytes associated with mild focal vascular congestion, mild edema, and rare focal mild fibrosis were observed in rats treated with $HgCl_2$ and MEL as well (Fig. 5F and G).

Discussion

Free radicals are produced by mercury chloride, which raises oxidative stress, results in nephrotoxicity [5, 28], and accelerates hepatotoxicity [28]. In addition to promoting oxidative stress, $HgCl_2$ treatment starts the synthesis of highly reactive molecules such as ROS [6]. In this investigation, we determined that when, compared to the control group, $HgCl_2$ considerably increased the lipid peroxidation's end byproduct, such as MDA. Numerous experiments have shown parallel results [29]. Mercury chloride resulted in increased oxidative stress and lipid peroxidation in rat liver tissue, as indicated by increased MDA levels and reduced levels of antioxidants such as catalase and glutathione peroxidase [30]. As a result, the level of lipid peroxidation rises and the activities of antioxidant enzymes decrease. Overall, it is suggested that $HgCl_2$ induces oxidative stress and lipid peroxidation, which increases MDA levels [13]. It has been reported that exposure to mercury can cause a range of health problems, such as neurological disorders, developmental delays, and cardiovascular disease [1]. Our results agree with previous studies indicating that the observed histoarchitectural distortion of the liver in the $HgCl_2$-treated

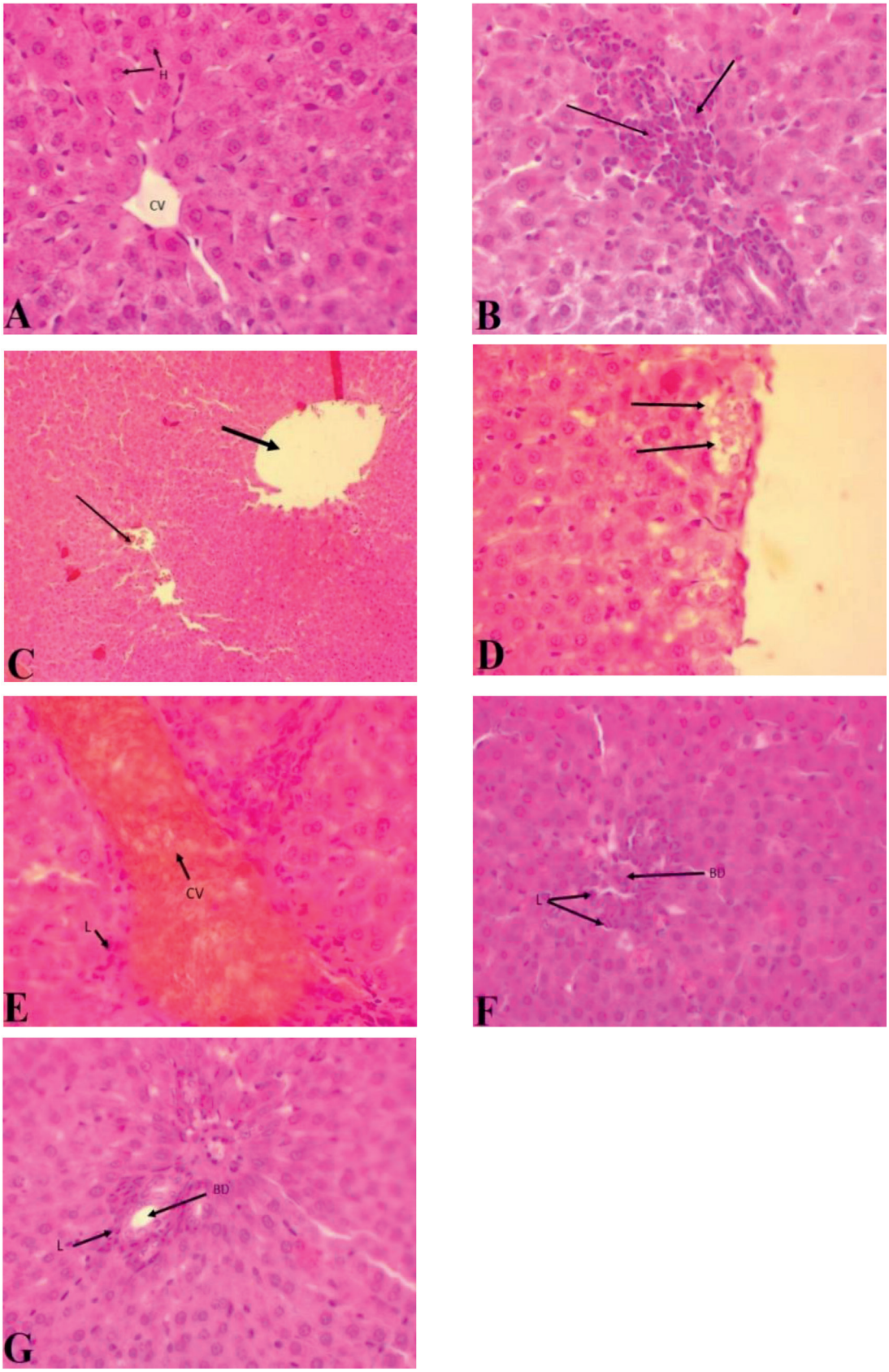

Fig. 1. The histological effect of MEL on the liver of rats treated with $HgCl_2$. The control group (A) showed normal arrangement of hepatocytes (black arrow; H) and central hepatic vein (CV). The $HgCl_2$ group (B, C, D, E) showed an increase in the number of lymphocytes (black arrow), central vein congestion (black arrow), and vacuolation inside hepatocytes (black arrow; degeneration) near the central vein and dilated, congestion of central vein (black arrow; CV) and an increased number of lymphocytes (black arrow; L). $HgCl_2$ treated with MEL group (F, G) indicated a reduction in the number of lymphocytes (black arrow; L) surrounding the bile duct (black arrow; BD) and a reduction in the number of lymphocytes (black arrow; L) surrounding the bile duct (black arrow; BD). [Note: A, B, D, E, F, and G (400X), D (100X), and Stain (H and E)].

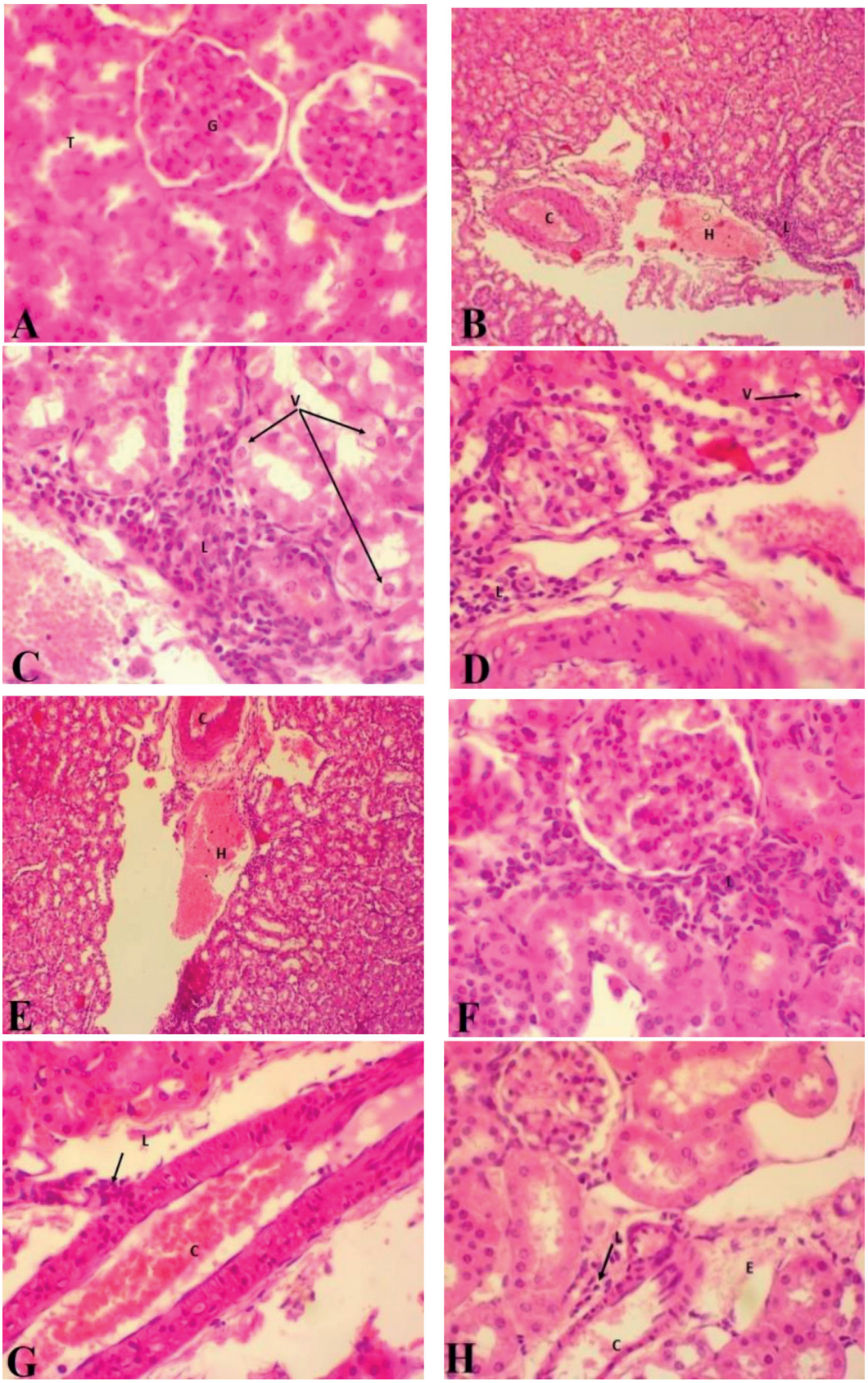

Fig. 2. The histological effect of MEL on kidneys of rats treated with $HgCl_2$. The control group (A) showed normal arrangement of glomeruli (G) and kidney tubules (T). The $HgCl_2$ group (B, C, D, E, F, G) showed congestion of blood vessels (C), hemorrhage (H), increased number of lymphocytes (black arrow; L), vacuolar degeneration inside tubules (black arrow; V), increased number of lymphocytes (black arrow; L), vacuolar degeneration inside tubules (black arrow; V), increase number of lymphocytes (black arrow; L), congestion of blood vessels (black arrow; C), hemorrhage (H), increase number of lymphocytes (black arrow; L) around glomerulus, congestion of blood vessel (black arrow; C) and mild lymphocytes (black arrow; L). The HgCl2 treated with MEL group (H), showed blood vessel congestion (C), edema found (black arrow; E), and an increased number of lymphocytes (black arrow; L). [Note; A, B, C, D, E, F, G and H (400x), Stain (H and E)].

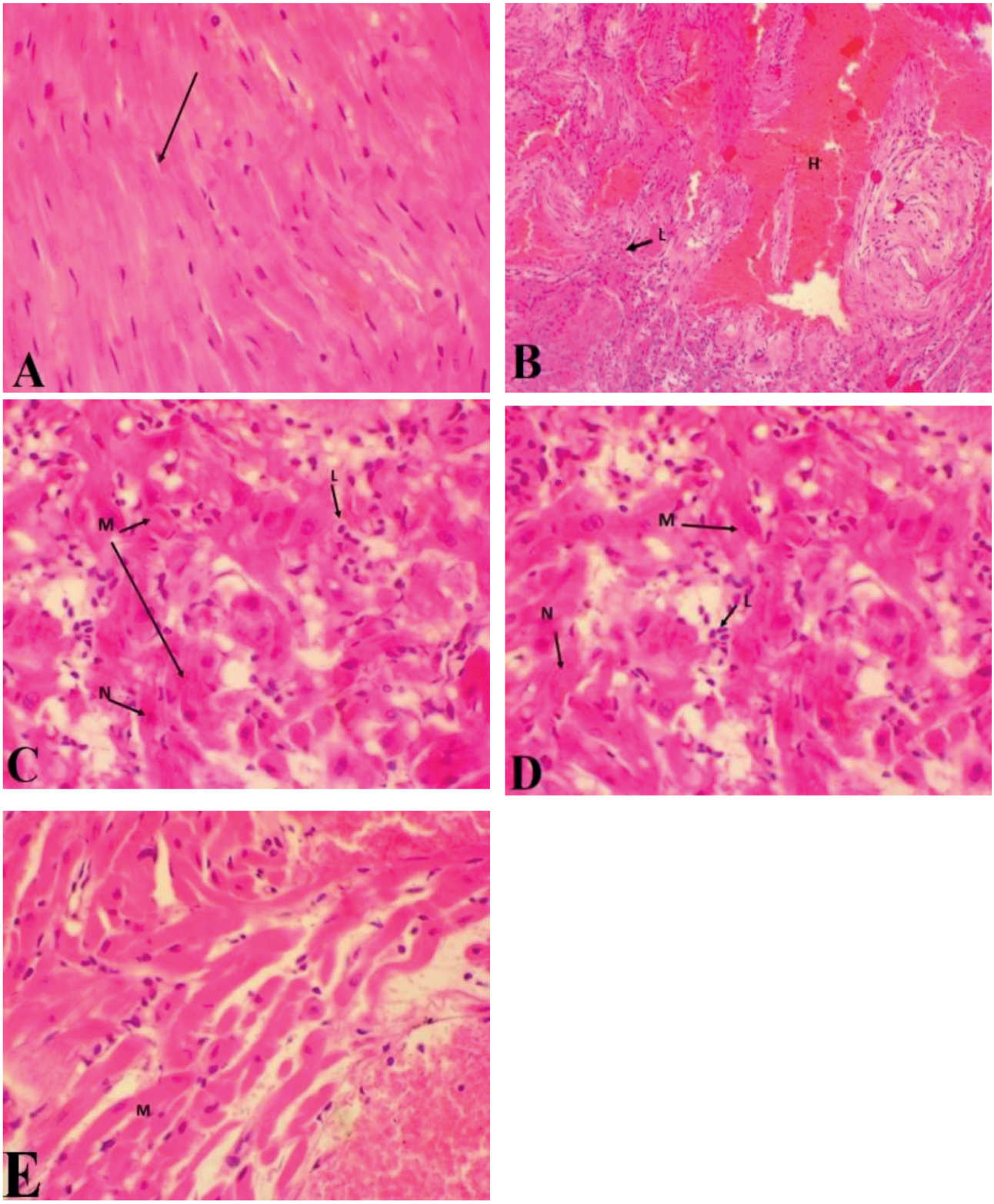

Fig. 3. The histological effect of MEL on the heart of rats treated with $HgCl_2$. The control group (A) showed a normal arrangement of the myocardium (black arrow). The $HgCl_2$ group (B, C, D) showed hemorrhage (H) lymphocytes (black arrow; L), loss of normal orientation of muscles (black arrow; M), necrosis found (black arrow; N), lymphocytes (black arrow; L) found, loss of normal orientation of muscles (black arrow; M), necrosis found (black arrow; N) and lymphocytes (black arrow; L) found. The $HgCl_2$ treated with MEL group (E) showed a better arrangement of muscle fibers found (M). [Note; A, C, D, E (400X), and B (100X) Stain (H and E)].

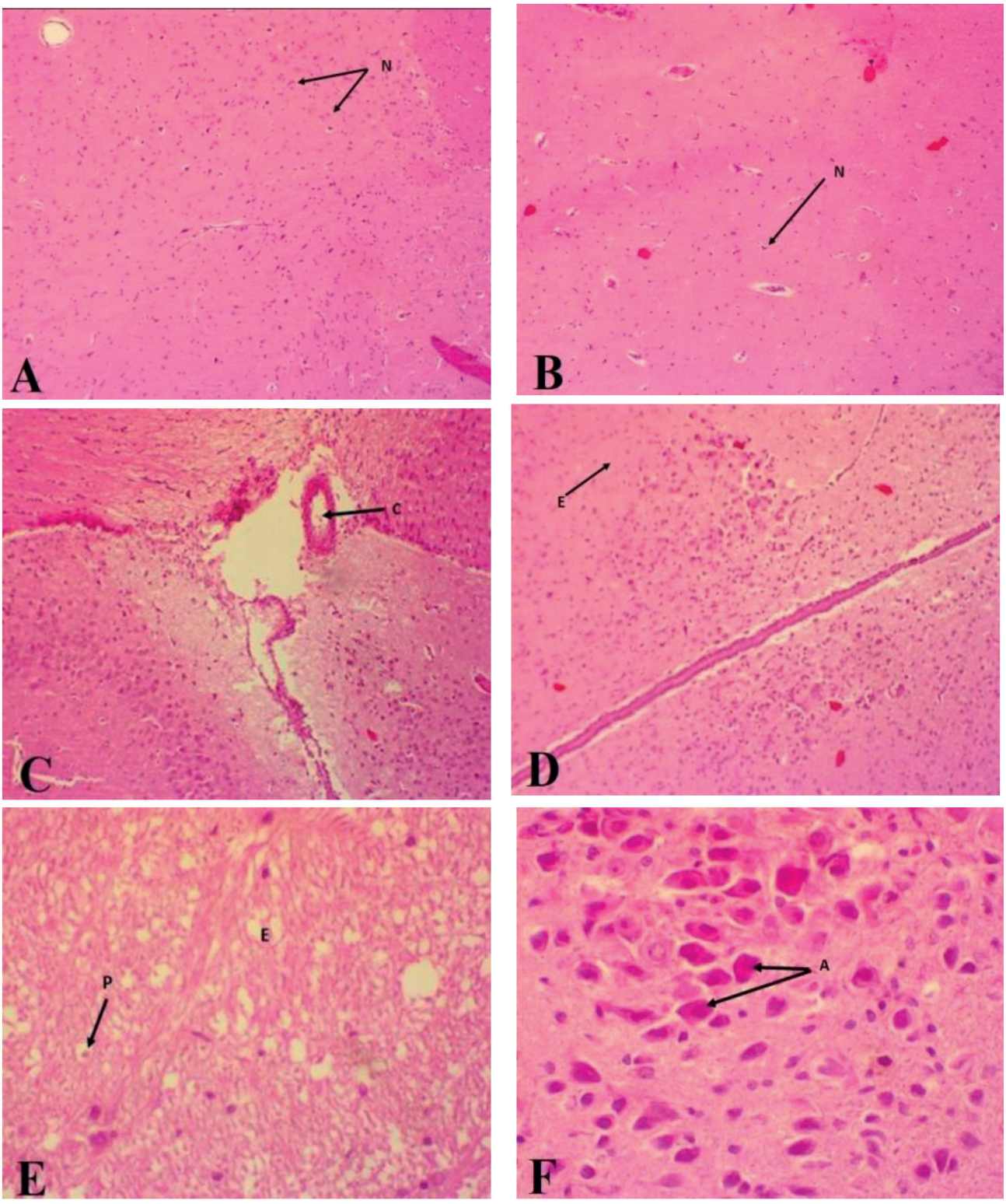

Fig. 4. The histological effect of MEL on the cerebrum of rats treated with $HgCl_2$. The control group (A, B) showed normal morphology of cerebral cortex neurons (black arow; N) and normal morphology of cerebral cortex neurons (black arow; N). The $HgCl_2$ group (C, D, E) showed mild focal neuronal cell loss, mild focal vascular congestion (black arow; C), mild focal neuronal cell loss, mild cerebral edema (black arow; E), pycnotic cells (black arow; P), necrosis, edema (black arow; E), astrogliosis (black arow; A), congestion of blood vessels and red blood cells found outside blood vessels (hemorrhage) (RBC) with necrosis arrow. The $HgCl_2$ treated with MEL group (F) showed less astrogliosis (black arow; A). [Note: A, B, C, D(100X), and E, F, G, H, I, (400X) Stain (H and E)].

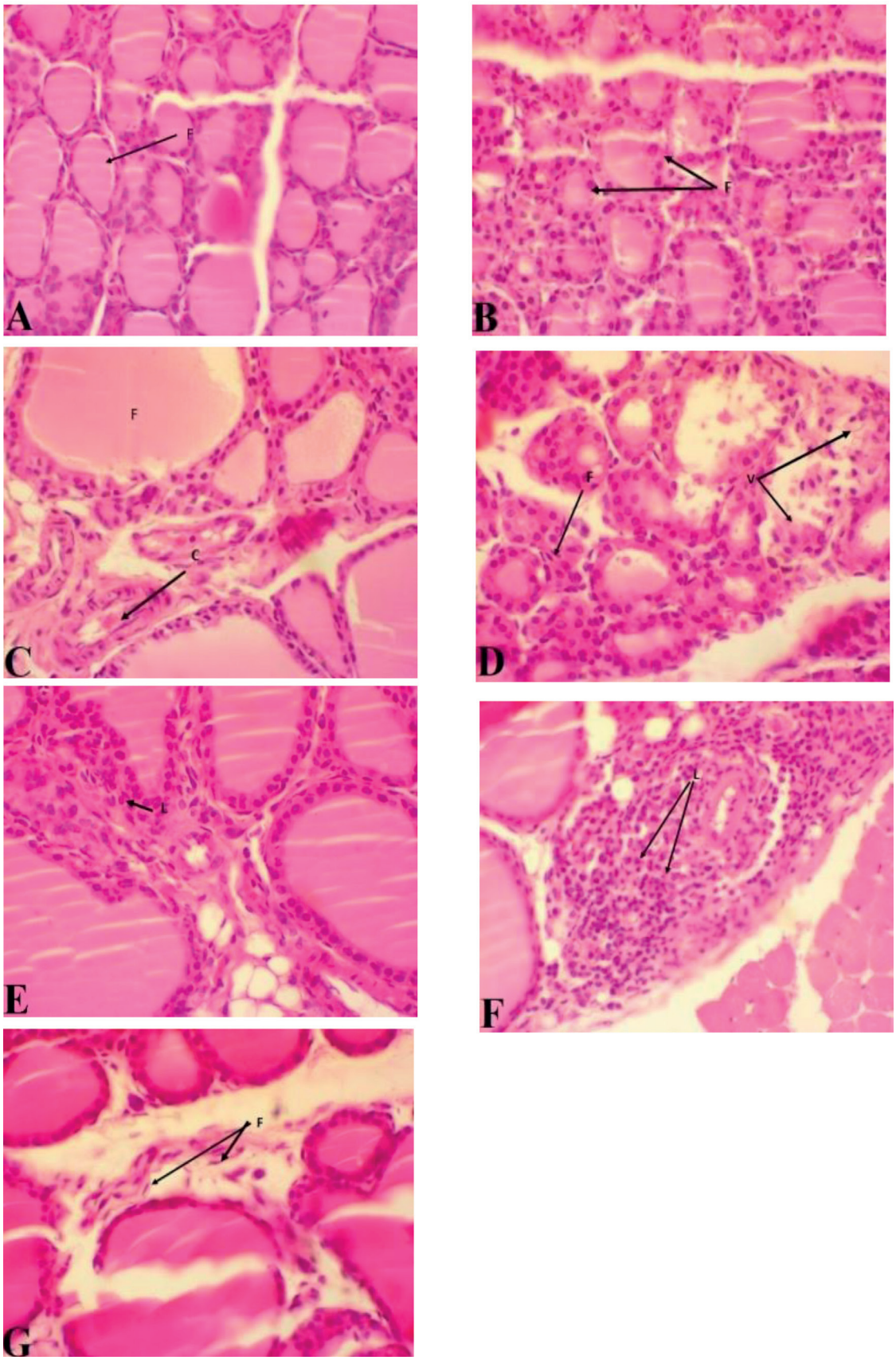

Fig. 5. The histological effect of MEL on the thyroid gland of rats treated with $HgCl_2$. The control group (A) showed normal arrangement of thyroid follicles (black arow; F). The $HgCl_2$ group (B, C, D, E) showed mild thyroid follicle hyperplasia, mild increase in size (black arow; F), edema, mild blood vessel congestion (black arow; C), mild increase in size (black arow; F), cellular degeneration with vacuolation inside follicular cells degeneration (V), mild increase fibroblast (black arow; F) and mild increase lymphocytes (black arow; L).The $HgCl_2$ treated with MEL group (F, G), showed an increase in the number of lymphocytes (black arow; L) between follicles, edema, and a small amount of fibroblast cells. [Note; A, B, C, D, E, F, G (400X), Stain (H and E)].

group, such as hepatocellular vacuolation, congestion of the central vein, and sinusoidal dilatation, could be attributed to $HgCl_2$-induced hepatotoxicity [31]. Melatonin is the hormone that is produced in the pineal gland [32], and it has been shown to have various functions in the body [33]. On the other hand, MEL supplementation (30 mg/kg body weight rats) for 5 weeks markedly reduced it as compared with the $HgCl_2$-treated rats. One of the functions of MEL is its ability to decrease MDA, which is a biomarker of oxidative stress [34]. The mechanism of action by which MEL decreases MDA can be explained in three ways [35, 36]. First, tissue injury can lead to an increase in MDA levels in the body [37]. It has been demonstrated that MEL decreases tissue damage by inhibiting the production of free radicals and lowering inflammation [38]. Its antioxidant properties, which assist in scavenging free radicals and inhibit them from causing tissue damage, mediate these effects [39]. For instance, it has been found that MEL causes a reduction in oxidative stress in the liver by eliminating free radicals and lowering lipid peroxidation [40, 41]. Second, free radicals are scavenged by MEL [42]. Free radicals are unstable molecules that can harm tissues and cells [43]. By removing free radicals from the human body and counteracting their effects, melatonin can defend against this damage [44]. Studies have shown that MEL can minimize oxidative stress by scavenging free radicals and decreasing lipid peroxidation in a variety of tissues [45]. Finally, MEL enhances the activity of endogenous antioxidant enzymes [39]. By scavenging free radicals and preventing lipid peroxidation, these enzymes aid in protecting against oxidative stress [46]. Melatonin has been found to boost the activity of vital antioxidant enzymes like catalase, glutathione peroxidase, and superoxide dismutase [47]. The current study explored the impact of $HgCl_2$ treatment on liver function test biomarkers, including ALP, ALT, and AST, as well as MEL's potential therapeutic effect. Statistical analysis revealed significant elevations in the levels of ALP, ALT, and AST following $HgCl_2$ treatment, indicating potential liver toxicity induced by mercury chloride exposure [30]. Remarkably, MEL administration exhibited a significant ability to mitigate the adverse effects of $HgCl_2$ on liver function. Rats treated with MEL displayed significantly reduced levels of ALP, ALT, and AST compared to those treated solely with $HgCl_2$ [48]. The histopathological examination of liver sections provided valuable insights into the effects of $HgCl_2$ exposure and the potential ameliorating impact of MEL. Hepatocytes, central hepatic venules, and the portal tract were all arranged normally in the liver section of the control group, indicating physiological liver morphology. Conversely, rats treated with $HgCl_2$ displayed a distinct pattern of histological alterations. These alterations included moderate mixed inflammatory cell infiltration, primarily lymphocytes, within the portal tract, accompanied by interface hepatitis. Additionally, moderate vascular congestion, mild edema, mild focal fibrosis, and hepatic tissues showed mild focal fatty alteration. All of these results point to the fact that hepatic inflammation is significantly triggered by $HgCl_2$ exposure, along with vascular congestion and degenerative changes [49]. Interestingly, the histopathological analysis of liver sections from rats treated with both $HgCl_2$ and MEL revealed a distinct pattern. Similar to the $HgCl_2$-treated group, there was mild mixed inflammatory cell infiltration, primarily lymphocytes, in the portal tract, accompanied by interface hepatitis. However, the severity of the observed effects was notably attenuated compared to the group treated solely with $HgCl_2$. Mild vascular congestion, mild focal fibrosis, and mild edema were also evident in this group, indicating that MEL administration had a mitigating effect on the histological alterations induced by $HgCl_2$ exposure [50]. The microscopic examination of kidney sections provided significant insights into the impact of $HgCl_2$ exposure and the potential reparative effect of MEL. The glomeruli and kidney tubules in the kidney sections from the control group were arranged normally, indicating unaltered renal morphology. Conversely, rats treated with $HgCl_2$ displayed distinct histopathological changes in their kidney tissues. These alterations included mild mixed inflammatory cell infiltration, primarily lymphocytes, within the renal tissue. Furthermore, mild vascular congestion and mild edema were observed, along with rare focal instances of mild fibrosis and mild focal tubular degenerative changes. These findings collectively suggest that $HgCl_2$ administration induces mild inflammatory responses in the kidney, along with vascular congestion and degenerative changes [51]. Of particular interest, the histological examination of kidney sections from rats given both $HgCl_2$ and melatonin treatment revealed a distinct pattern. Similar to the group treated with $HgCl_2$ alone, mild mixed inflammatory cell infiltration, primarily lymphocytes, was seen in the renal tissue. However, compared to the group receiving only $HgCl_2$, the intensity of inflammatory reactions was reduced in the combined therapy group. Additionally, mild vascular congestion, mild edema, and rare focal instances of mild fibrosis were observed. Notably, the presence of focal tubular degenerative changes observed in the $HgCl_2$-treated group was absent in the $HgCl_2$ and MEL-treated groups, suggesting a potential reparative effect of MEL [21]. They showed that melatonin reduces kidney damage by inducing mitophagy in diabetic nephropathy. It has been shown that MEL regulation of the kidney's renin-angiotensin system plays a critical function in reducing the symptoms of chronic renal disease [52]. The microscopic examination of heart sections provided valuable insights into the effects of $HgCl_2$ exposure and the potential mitigating impact of melatonin. Heart sections from the control group displayed a normal arrangement of heart endocardium and myocardium, indicating preserved cardiac morphology. Conversely, heart sections from rats treated with $HgCl_2$ exhibited discernible histopathological alterations. These alterations included mild mixed inflammatory cell infiltration, predominantly lymphocytes, within the cardiac tissue. Additionally, mild vascular congestion, mild edema, rare focal instances of mild fibrosis, and mild focal myocardial degenerative changes were observed. These findings collectively suggest that exposure to $HgCl_2$ results in mild inflammatory

responses in the heart, along with vascular congestion, edema, and degenerative changes in the myocardium [53]. They demonstrated that the oxidative tissue damage produced by $HgCl_2$ was manifested in tissue samples by decreased GSH and elevated MDA levels. The thromboplastic activity significantly increased after Hg injection, confirming the cardiotoxic effects of $HgCl_2$. It has been suggested that myofibrils, low dosages of $HgCl_2$, and the energy-producing mitochondria of cardiomyocytes were shown to be negatively affected also [54]. It has been observed that long-term exposure to low doses of mercuric chloride leads to non-specific qualitative and quantitative changes in all structural components of the heart, including ongoing damage to the tissue barrier and dynamic and resorptive insufficiency of the hemo-microcirculatory bed, which causes chronic swelling, diffuse fibrosis, and an increase in cardiac decompensation activities. Surprisingly, a unique pattern was observed upon microscopic analysis of heart sections from rats that had received treatment with both melatonin and $HgCl_2$. Similar to the $HgCl_2$-treated group, mild mixed inflammatory cell infiltration, primarily lymphocytes, was observed in the cardiac tissue. However, the severity of inflammatory responses was notably ameliorated compared to the group treated solely with $HgCl_2$. Additionally, mild vascular congestion and mild edema were observed, along with rare focal instances of mild fibrosis. The presence of focal myocardial degenerative changes observed in the $HgCl_2$-treated group was absent in the $HgCl_2$ and MEL-treated groups, suggesting a potential protective effect of MEL. It has been revealed that melatonin has been proven to have considerable benefits in lowering cardiac pathology and avoiding cardiac muscle death in mouse species in response to ischemia-reperfusion [55]. Furthermore, under some conditions, MEL may prevent cardiac muscle hypertrophy, hence reducing the development of heart failure [56]. Cardiotoxicity is a side effect of several commonly used conventional medicines [57]. Recently, research has revealed that MEL serves as an antioxidant and is efficient in preventing cardiac damage caused by pharmaceutical medicines [58]. However, it has been found that MEL, due to its pleiotropic effects, is a prospective therapeutic candidate [59]. This indoleamine is involved in endocrine physiology and chronobiology regulation [60]. Melatonin is a chemical that has antiaging, antioxidant, antiapoptotic, antiarrhythmic, immunomodulatory, and antiproliferative properties [61]. Melatonin's antioxidative and anti-inflammatory effects may be responsible for its reported protective effect on heart histology [62]. The ability of MEL to reduce the negative effects of $HgCl_2$ on cardiac tissues may be attributed to its capacity to scavenge reactive oxygen species and modify inflammatory pathways [63]. The microscopic examination of the cerebral cortex provided crucial insights into the effects of $HgCl_2$ exposure and the potential mitigating influence of MEL. Brain sections from the control group displayed normal cerebral cortex neuron morphology, indicative of preserved neural structure. Conversely, rats treated with $HgCl_2$ exhibited distinct histopathological alterations in the cerebral cortex. These alterations encompassed mild focal neuronal cell loss (death), mild cerebral edema, mild focal gliosis characterized by reactive changes, and mild focal vascular congestion. These findings collectively suggest that $HgCl_2$ exposure induces mild neuronal damage, gliosis, cerebral edema, and vascular congestion in the cerebral cortex [64]. They also showed that $HgCl_2$-induced impairment of memory, oxidative stress, and brain damage. Likewise, it has been shown that $HgCl_2$ poisoning impaired mice learning and memory, raised blood and brain mercury levels and elevated interleukin-6, interleukin -1, and tumor necrosis factor levels in serum [49]. Intriguingly, the microscopic examination of brain sections from rats treated with both $HgCl_2$ and MEL unveiled a distinct pattern. Similar to the group treated with $HgCl_2$ alone, rare focal instances of neuronal cell loss (death), mild cerebral edema, and rare focal gliosis with reactive changes were observed in the cerebral cortex. Additionally, mild focal vascular congestion was present. While the exact mechanisms need further investigation, the presence of MEL seemed to confer a protective effect against some of the observed alterations induced by $HgCl_2$ [65]. It has been shown that experimental stroke models have demonstrated the neuroprotective effects of melatonin [66]. The microscopic examination of thyroid gland sections provided valuable insights into the impact of $HgCl_2$ exposure and the potential attenuating effect of MEL. Thyroid gland sections from the control group displayed a normal arrangement of thyroid follicles, indicative of preserved thyroid morphology. Conversely, rats treated with $HgCl_2$ exhibited distinct histopathological changes in the thyroid gland. These changes included rare instances of mixed inflammatory cell infiltration, predominantly lymphocytes, within the thyroid tissue. Additionally, mild focal vascular congestion, mild edema, and rare focal instances of mild fibrosis were observed. Notably, focal follicular cellular hyperplasia with cystic dilatation was also present, suggesting potential thyroid gland dysfunction induced by $HgCl_2$ exposure. Studies have shown that additional hazardous metals, such as cadmium, are frequently found in the thyroid [18]. Hypothyroidism, autoimmune thyroiditis, and thyroid cancer may occur as a result of the corrosive effects of many hazardous metals [67]. Intriguingly, a different pattern was seen when thyroid gland sections from rats treated with both MEL and $HgCl_2$ were examined under a microscope. Similar to the $HgCl_2$-treated group, rare instances of mixed inflammatory cell infiltration, primarily lymphocytes, were observed within the thyroid tissue. Additionally, mild focal vascular congestion, mild edema, and rare instances of mild fibrosis were noted. Importantly, the presence of MEL seemed to have minimal impact on the observed histopathological changes induced by $HgCl_2$, suggesting limited mitigating effects in this context (Iwan et al., 2021). According to this study, MEL may have had a limited impact on thyroid histopathology due to several factors, including the duration of treatment, dose of MEL, and specific mechanisms underlying thyroid responses to mercury exposure.

Additional investigations are warranted to elucidate the mechanisms and conditions under which MEL might exert more substantial protective effects on thyroid tissues. Furthermore, it has been found that potassium iodate-induced elevated levels of lipid peroxidation in pig thyroid are decreased by MEL [20].

Conclusions

In conclusion, the findings of this investigation provide compelling evidence for the adverse effects of $HgCl_2$ exposure on multiple organ systems in rats. $HgCl_2$-induced oxidative stress, marked by elevated oxidative damage markers like MDA, was evident across various organs. These findings align with previous research highlighting $HgCl_2$'s role in generating ROS and initiating oxidative stress. The remarkable potential of MEL as a mitigating agent against $HgCl_2$-induced oxidative damage was underscored. Melatonin's multifaceted mechanisms contributed to its protective effects. Firstly, its antioxidant properties were evident through the reduction in MDA levels. Melatonin's ability to neutralize free radicals and reduce inflammation plays a crucial role. Moreover, MEL acted as a scavenger for free radicals, countering their damaging effects on cells and tissues. Furthermore, MEL augmented endogenous antioxidant enzymes, reinforcing the defense against oxidative stress. Regarding specific organ responses, the liver exhibited notable alterations in function, with elevated ALP, ALT, and AST levels post-$HgCl_2$ exposure. Melatonin administration demonstrated its potential to ameliorate these impacts, indicative of hepatoprotective effects. In the kidney, melatonin's mitigating influence was evident through attenuation of inflammatory responses and fibrosis. Histopathological changes in the heart, cerebral cortex, and thyroid gland following $HgCl_2$ exposure highlighted the damaging effects on these organs. Melatonin supplementation showcased its potential to reduce inflammation, edema, and degeneration in these tissues. The role of MEL in protecting against cardiac damage and its pleiotropic actions in various physiological processes were evident.

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Conflict of Interest

The authors declare no conflict of interest.

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