Effects of Mercury Chloride And Lead Nitrate Induced Cardiotoxicity in Male Rats

Civa Klorid ve Kurşun Nitrat'ın Erkek Ratlarda Kardiyotoksik Etkisi

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Abstract

Background: Lead nitrate and mercury chloride are widespread environmental and industrial pollutant, which induces severe alterations in the body tissues of both humans and animals. In the present study, the effects of lead and mercury induced cardiotoxicity was studied in Wistar rats.

Methods: Lead nitrate (LN, 45 mg/kg bw/day) and mercury chloride (MC, 0.02 mg/kg bw/day) and their combination were administered orally for four weeks. Four groups of rats were used in the study: control, LN, MC and LN plus MC groups. Malondialdehyde (MDA) level, antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST)] and histopathological changes in heart tissue were evaluated**.**

Results: Results showed that LN and MC exposure and also their combination resulted in an increase in the SOD, CAT, GPx, GST activities and also MDA levels, with respect to the control group. Light microscopic investigations revealed that mercury chloride and lead nitrate and also co-treatment of them induced histopathological changes in heart tissue.

Conclusion: As a result, combination of LN plus MC was more harmful than use them alone.

Key words: Lead, Mercury, Oxidative Stress, Pathology, Heart

Öz

Amaç: Kurşun nitrat ve civa klorid insan ve hayvan dokularında çeşitli değişikliklere yol açan çevresel ve endüstriyel kirleticilerdir. Bu çalışmada, kurşun nitrat ve civa klorid'in erkek sıçanlardaki kardiyotoksik etkisi araştırılmıştır.

Materyal ve metod: Kurşun nitrat (LN, 45 mg/kg vücut ağırlığı) ve civa klorid (MC, 0.02 mg/kg vücut ağırlığı) ve bunların kombinasyonları 4 hafta boyunca oral yoldan hayvanlara uygulanmıştır. Bu çalışmada 4 deney grubu bulunmaktadır: kontrol, kurşun nitrat, civa klorid, kurşun nitrat ve civa klorid. Deney bitiminde kalp dokularındaki malondialdehit seviyesi (MDA), antioksidan enzim aktiviteleri (süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx), gluıtatyon-S- transferaz (GST) değerlendirme yapılmıştır.

Bulgular: Kontrol grubu ile karşılaştırıldığında, kurşun nitrat, civa klorid ve kurşun nitrat+civa klorid uygulaması SOD, CAT, GPx ve GST aktivitelerinde artışa yol açmıştır. Aynı zamanda MDA seviyesi de artmıştır. Işık mikroskobu incelemelerinde uygulama gruplarında kalp dokularında ciddi histopatolojik değişimler gözlenmiştir.

Sonuç: Kurşun nitrat ve civa kloridin birlikte uygulanmasının tek başlarına uygulanmalarına göre daha toksik etkili olduğu tespit edilmiştir.

Anahtar kelimeler: Kurşun, Civa, Oksidatif Stres, Patoloji, Kalp

Introduction

Heavy metals are a group of environmental chemicals which have toxic effects on living organisms (1). Though metals have adverse effects emanating from their exposure are widely known, their usage and concentrations in the environment are increasing (2). Numerous studies have showed the toxicity of metals to living systems such as testicular toxicity (3), nephrotoxicity (4) and hepatotoxicity (5). Heavy metal toxicity related to heart disease in animals and it is a cause of death. Exposure to heavy metals has been linked to increased incidence of cardiovascular diseases (6). Mercury, a well-known toxicant, causes toxicity through binding with cellular thiols and the formation of reactive oxygen species (ROS). Mercury is a widespread environmental and industrial pollutant, which induces severe alterations in the body tissues of both humans and animals (7, 8). These ROS include superoxide, hydrogen peroxide as well as hydroxyl radicals. ROS induce cell dysfunction through oxidative damage in membrane lipids and proteins (9). The mercury chloride is one inorganic form of this metal. Although the most common form of mercury encountered is the inorganic one (10). Previous studies suggested that inorganic mercury can be methylated in the gut lumen prior to absorption (11) and cross the placental barrier (12) and also easily passes through the blood brain barrier reaching the central nervous system (CNS) (13).

Lead is an important environmental toxin that induces a broad range of physiological, biochemical, and behavioral dysfunctions. It can hinder biological functions by changing the molecular interactions, cell signaling processes and cellular function. It affects organs with low antioxidant defense such as the heart (14, 15). There are many studies which have indicated that LN exposure could cause biochemical and physiological dysfunctions in experimental animals and humans (16).

Recently, studies showed that oxidative stress is very important to environmental chemicals also, heavy metals generate ROS (17). Oxidative stress is defined as an imbalance between production of free radicals and their elimination by antioxidant systems. This imbalance leads to damage of important biomolecules and tissues with potential impact on the whole organism (18, 19).

The aim of this study was to assess toxicities of low dose mercury chloride and lead nitrate on rat heart tissues. For the purpose, in the present study we investigate the biochemical and pathological effects of low dose LN and MC.

Materials and methods

Chemicals

The heavy metals, lead nitrate and mercury chloride, and all the other chemicals were purchased from Sigma Aldrich. Lead nitrate and mercury chloride

were dissolved in distilled water (20, 21).

Animals and Experimental Procedure

Sexually mature male Wistar rats (310±10 g, 90 days old) were used throughout the study. They were obtained from the Gazi University Laboratory Animals Growing and Experimental Research Center. The experimental protocols were approved by the Gazi University Committee on the Ethics of Animal Experimentation (Approval number: G.Ü.ET–13.011) and experiments were performed according to the international guidelines for care and use of laboratory animals. They were allowed to get into the habit of new situation for 10 days. The animals were housed at 18-22 °C, humidity 40 % and they were given water and food ad libitum, while a 12-h on/12-h off light cycle was maintained. Animals were randomly divided into 4 groups as follows (n=6 for each group). These are: Control group (1ml/ kg body weight (b.w) distilled water), lead nitrate-treated group (45 mg/kg b.w, 1/50 LD50, LN) (Sharma et al., 2010), mercury chloride-treated group (0,02 mg/ kg bw., 1/50 LD50, MC) (Yole et al., 2007) , lead nitrate+mercury chloride-treated group (45 mg/kg b.w LN+0,02 mg/ kg b.w MC). Distilled water, LN and MC were exposed to rats orally via gavage. At the end of the 28 days, the rats were dissected using ketamin+xylazin than the hearts were removed for investigations about MDA levels, antioxidant enzyme activities and light microscopic examinations.

Biochemical Analysis

Hearts were washed with sodium phosphate buffer (pH 7.2) then they were homogenized with Heidolph Silent Crusher M. The homogenates were centrifuged for 15 min at 4°C. Activities of antioxidant enzymes and MDA levels of hearts were detected using a spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). The protein concentration was determined by the method of Lowry (22). The activity of SOD was obtained by Marklund and Marklund's study (23) at 440 nm and the enzyme activity was expressed as U/mg protein. CAT activity was estimated following the method of Aebi (24) at 240 nm and the activity is defined as mmol/mg protein. According to the study of Habig et al. (25), activity of GST was determined at 340 nm and the data was given as µmol/mg protein. Activity of GPx was measured by Paglia and Valentine's method (26). At 340 nm, the reaction was monitored and value was stated as nmol/mg protein. MDA level of heart was analysed at 532 nm by thiobarbituric acid method described by Ohkawa et al., (27) and it was defined as nmol/mg protein.

Histopathological Evaluation

Rats were dissected and the hearts were removed and placed into the fixative bouin. After, heart samples were dehydrated in ascending grades of ethanol and embedded in paraffin and paraffin block of the tissues were prepared. The specimens were cut in 6-7 µ slices and were stained with Hematoxylin-Eosin (H & E). The tissues were then evaluated under a light microscope (Olympus BX51, Tokyo, Japan) and photographed with a camera (Olympus E-330, Olympus Optical Co., Ltd., Japan). Ten slides were prepared from each heart tissue. The severity of changes was assessed for each slide by scoring using a scale of no (-), mild (+), moderate (++) and severe (+++) damage.

Statistical Analysis

The data were expressed as mean±SEM. The data of all groups were compared with each other. Groups were compared using one-way analysis of variance (ANOVA) test followed by Tukey. Significance was accepted at $p<0.05$.

Results

Biochemical Results

Treatment with LN and MC increased the activities of SOD, CAT, GPx and GST in heart tissues but MC showed more toxicity than LN. In combination with LN and MC induced more damages than use of them alone (Figures 1-4) ($P<0,05$).

Levels of MDA were determined in heart tissues of rats. At the end of the experimental period, all of the treatment groups showed increasing of MDA levels compared to control. We observed more increasing in MC group than LN group. Treated with LN+MC caused more harmful effects than use of them alone (Figure 5) ($P<0,05$).

Histopathological Results of Heart

The result of the histopathological examination is shown in Figure 6. The histological examination of the heart tissues of the control treated rats showed normal structure (Fig. A-B). There were steatozis in interstitial tissue, necrosis, and disorganization of myocardial fibers, inflammatory cell infiltration and steatozis in myocardial fibers (Fig. C-D) in the MC group. There were disorganization of myocardial fibers and inflammatory cell infiltration (Fig. E-F) shown in LN group. Also there was edema, steatozis in myocardial fibers, necrosis (Fig. G-H) shown in LN plus MC group.

Discussion

It is reported that even low doses heavy metals, they disturb the homeostasis of essential metals in various organs (brain, liver, and kidney) of experimental animals (1). In previous studies, the relationship between increased levels of mercury and risk of coronary heart disease is well documented (28). It is also reported that mercury can induce deleterious effects on the cells through ROS production and this is one of the risk factors of coronary heart diseases (29). The toxic effects of mercury and lead can be prevented to some extent either by chelating or enhancing antioxidant defense mechanisms (3). The cardiovascular effects of mercury and lead together have not been attentively evaluated until recently.

Authors reported that mercury and lead cause oxidative stress and stimulate ROS in different organs (5). In previous studies researchers reported that heavy metals caused also histopathological changes (2,30). Baş et al., reported that in diabetic rats lead caused histopathological changes such as inflammation, degeneration, vacuolization and necrosis in rat hearts treated with lead in diabetic rats (30). In this study, low doses of LN and MC also caused histopathological changes in heart tissues like steatosis, inflammation, necrosis etc. Oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases (31). It is related to be enhanced ROS and oxidative stress in heart tissue, because biochemical alterations and heart tissue damage support each other. It is known that LN and MC can cause oxidative stress in several tissues of experimental animals (5,32). Histopathological changes in the heart tissue could be due to increased ROS generation.

MDA, one of the major oxidation products of peroxidized polyunsaturated fatty acids, has been used as a marker of oxidative stress (5). Enhanced lipid peroxidation levels were also reported in lead and mercury toxicity by Apaydın et al (3) and Agarwal et al (8). Several studies have shown mercury and lead increased lipid peroxidation levels in experimental animals (4). Free radical scavenging enzymes such as SOD, CAT, GPx and GST are the first line of cellular defense against oxidative injury. It is known that xenobiotics can induce superoxide

radical production and if additionally SOD is inhibited the amount of oxygen radicals formed in cell can reach dangerous levels and detoxifies the superoxide radicals to hydrogen peroxide (H_2O_2) (33, 34). The GPx enzyme is a selenoenzyme that can prevent oxidative damage of cell membranes (35). GPx catalyzes the reduction of H_2O_2 to H_2O (36). GST is detoxifying enzyme that catalyzes the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms (37). The present study demonstrated that oxidative stress generated by lead nitrate and mercury chloride resulted in increasing of morphological alterations with the increasing in MDA levels and SOD, CAT, GPx and GST activities. Thus, present study indicates that low doses of lead nitrate and mercury chloride cause heart toxicity in male rats. It may be related to oxidative effects of mercuric chloride and lead nitrate on heart tissue membranes. The elevated level of MDA could be due to an increase in free radicals resulting from the induction of oxidative stress in the heart tissue. Previous studies indicated that MDA may be increase due to adverse effects of LN on fatty acids of cell membranes (38).

In conclusion, the present study showed that mercury chloride and lead nitrate intoxication caused ROS generation which in turn induced histopathological alterations in rat heart. We show that some parameters results were greater in MC treated group than LN. It may be due to LD_{50} value of MC which is more toxic than LN. Therefore we can say according to the data of this paper, treated with combination of lead nitrate and mercury chloride caused more harmfull effects than use of them alone.

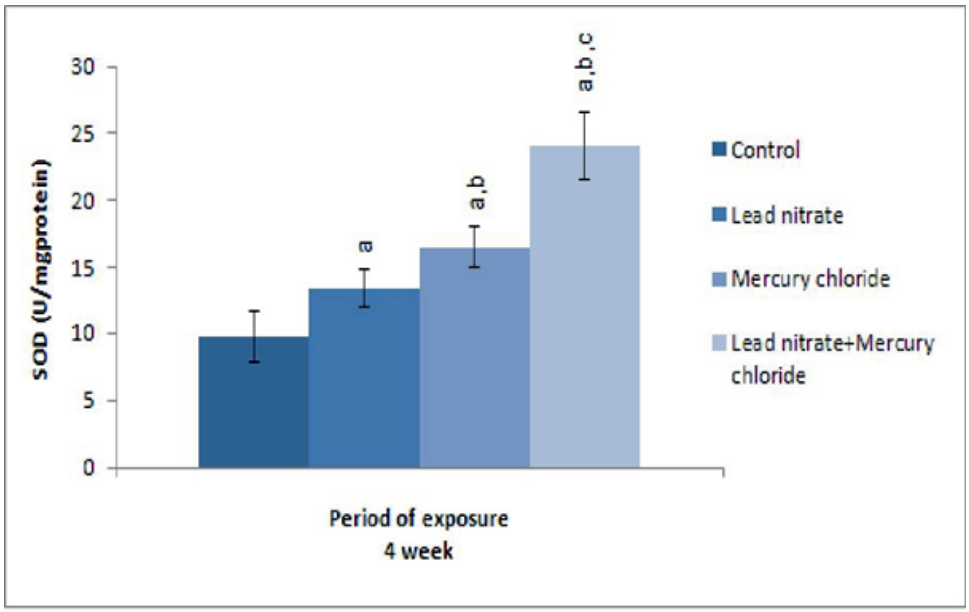

Figure 1. Effects of subacute treatment of LN and MC on SOD activities in the heart tissues of rats. Each bar represents mean±SEM of six animals in each group. Significance at $P<0.05$.[a]Comparison of controlandothergroups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups.

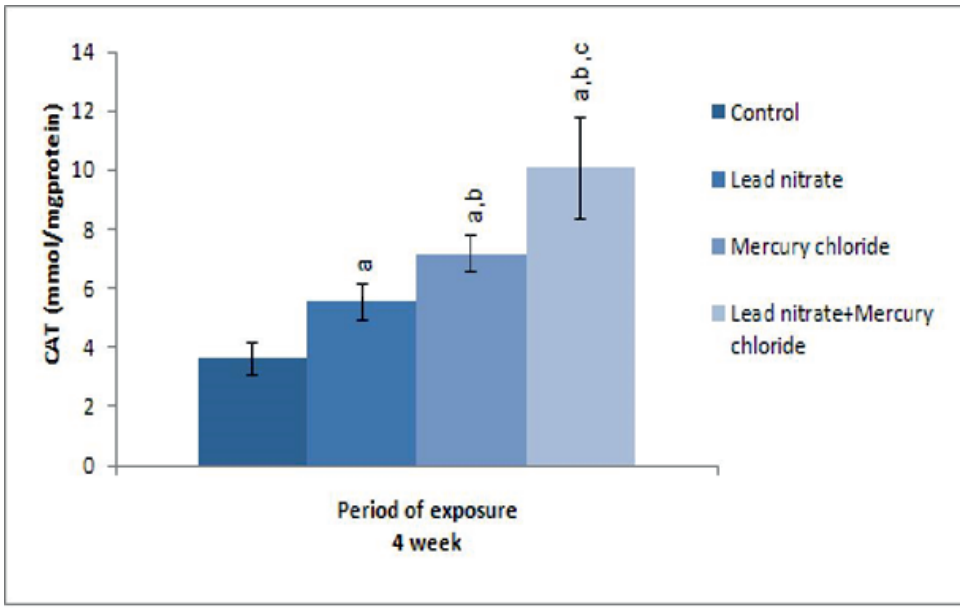

Figure 2. Effects of subacute treatment of LN and MC on CAT activities in the heart tissues of rats. Each bar represents mean ± SEM of six animals in each group. Significance at P<0.05.[a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups.

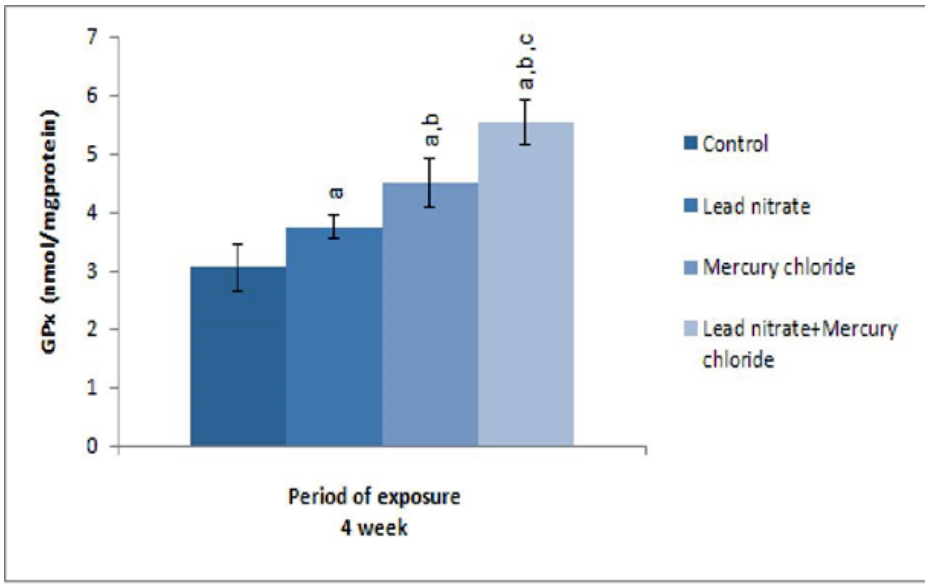

Figure 3. Effects of subacute treatment of LN and MC on GPx activities in the heart tissues of rats. Each bar represents mean±SEM of six animals in each group. Significance at P<0.05.[a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups.

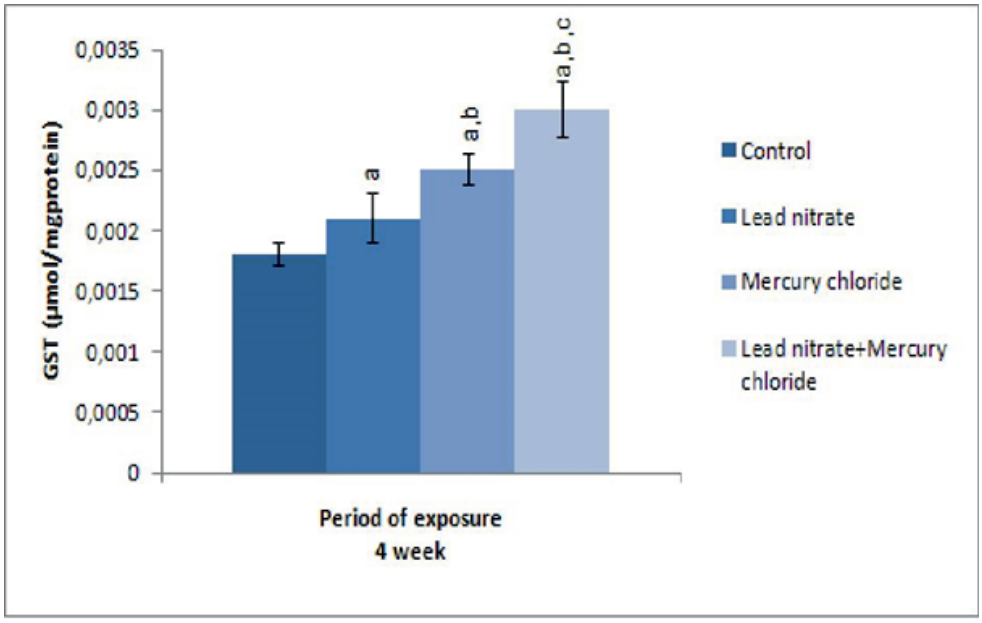

Figure 4. Effects of subacute treatment of LN and MC on GST activities in the heart tissues of rats. Each bar represents mean±SEM of six animals in each group. Significance at P<0.05.[a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups.

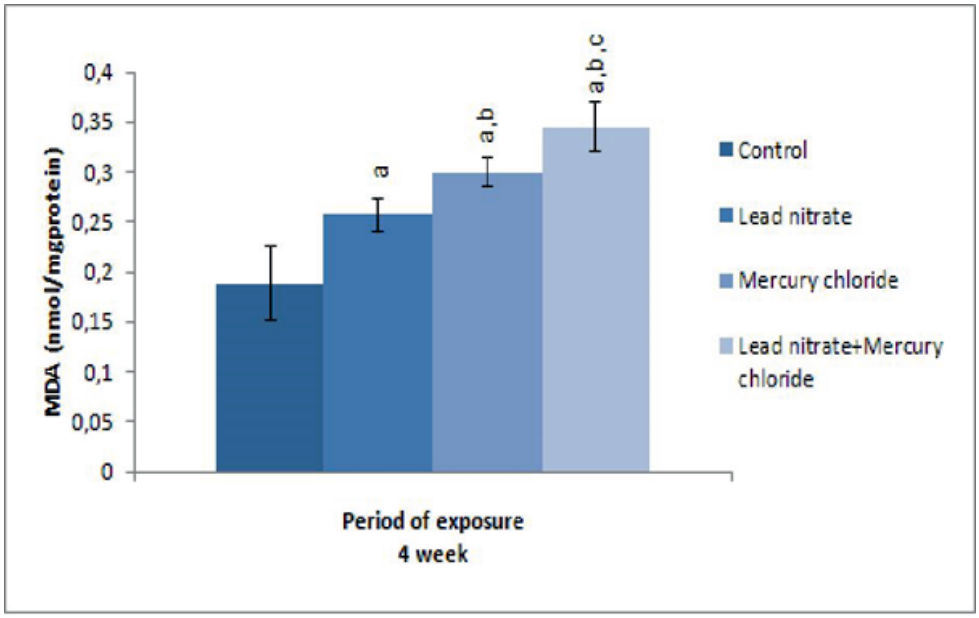

Figure 5. Effects of subacute treatment of LN and MC on MDA activities in the heart tissues of rats. Each bar represents mean±SEM of six animals in each group. Significance at P<0.05.[a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups.

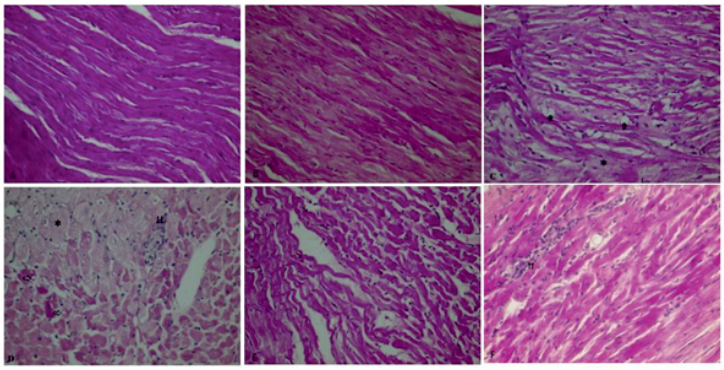

Figure 6- (A-B) Heart section of control rats, ×400. (C-D) Heart sections of mercuric chloride-treated rats showing steatozis in interstitial tissue (), necrosis (), disorganization () of myocardial fibers, inflammatory cell infiltration () and steatozis () in myocardial fibers X400.(E-F) Heart section of lead nitrate-treated rats showing disorganization () of myocardial fibers, inflammatory cell infiltration () X400. (G-H) Heart sections of mercuric chloride and lead nitrate treated rats showing edema (), steatozis () in myocardial fibers, necrosis () X400.

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