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An evaluation of the effects of ascorbic acid on the endothelium of coronary and aorta arteries in lead-intoxicated rabbits

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Abstract

Objectives: Lead exposure has destructive effects on some organs. It may produce a variety of toxic effects on endothelial cells of the vascular system. Any changes or damages to endothelial cells may lead to cardiovascular diseases, particularly the formation of atherosclerotic plaques. The aim of this study was to determine the ameliorative effects of ascorbic acid on the endothelium of coronary and aorta arteries in lead-exposed rabbits.

Methods: In this study, 30 white male rabbits of New Zealand race (weighing about 1.6–2 kg and 5 months old) were used and divided randomly into three groups: Group 1 ($N = 10$) that served as the control and received water and normal diet, Group 2 ($N = 10$) was exposed to lead acetate 547 ppm (5 mg/L) daily for 40 days, and Group 3 ($N = 10$) received vitamin C (500 mg/kg) and underwent the same duration of lead exposure (5 mg/L) daily for 40 days. The levels of cholesterol, triglyceride, low-density lipoprotein, and high-density lipoprotein were measured using spectrophotometry, and the level of blood lead was calculated using a lead analyzer (Magellan Diagnostics, USA). The animals were anesthetized by pentobarbital (50 mg/kg). Subsequently, they were sacrificed, and their thoracic aortas and coronary arteries were removed. Then fixation, tissue processing, histological sectioning, and H & E staining were carried out. Finally, the sections were studied using light microscopy. The results were analyzed using the Mann–Whitney test.

Results: The results indicated that ascorbic acid could reduce the destructive effects of lead on vascular endothelial cells and prevent the formation of atherosclerotic plaques in coronary and aorta arteries.

Conclusion: The results of this study confirm the beneficial effects of ascorbic acid against the destructive effects of lead on vascular endothelial cells. Hence, it could be proposed as a potential prophylactic treatment for the amelioration of lead toxicity, prevention of atherosclerosis, and improvement of endothelial cells dysfunction.

Keywords

Aorta artery, endothelium, lead acetate, coronary artery, ascorbic acid, *atherosclerosis*

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Introduction

According to the World Health Organization (WHO), cardiovascular disease (CVD) is one of the major causes of death in the world, and it is predicted that by 2030, about 32 million people will die due to CVD. Lead is available everywhere and its wide distribution pollutes the environment.[1]

Lead is one of the heavy metals whose toxic effects have been known as a major environmental health hazard worldwide affecting both humans and animals at all ages, particularly young human offsprings.[2,3]

Available elements surrounding human beings have different effects on his health. Some of these elements are useful for human health and some are harmful. Lead has no beneficial role in the human body and the presence of any amount of it is considered a risk factor.[4]

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Many studies have provided noticeable amounts of data about free-radical-mediated injury in the wall of the vasculature. Several mechanisms cause oxidative stress. One of the important mechanisms is related to lead that is associated with the lack of balance between antioxidants level and the generation of free radicals. Oxidative stress-induced lipid peroxidation involved atherosclerosis. Recent studies have shown that increased oxidative stress is an important mediator of endothelial damage associated with increased oxidant protein production, such as hydrogen peroxide superoxidation, decreased nitric oxide synthesis, and decreased biological antioxidant capacity. Oxidative stress is associated with endothelial dysfunction, inflammation, hypertrophy, fibrosis, and vascular deformity.[5,6]

CVDs have been referred to in some studies as the major cause of mortality and diseases around the world. Different environmental risk factors cause the CVDs. However, air pollution is currently known as the most common environmental risk factor for CVDs.[7]

Previous studies have demonstrated that lead exposure could damage some body systems. It may effect on endothelial cells lining luminal surface of the vascular system. Damages of endothelial cells may lead to CVDs, particularly the formation of atherosclerotic plaques.[8,9]

There are many factors with antioxidant properties and scavenger of reactive oxygen species (ROS). Ascorbic acid is one of the antioxidants which are easily accessible for humans.[10]

Vitamin C has an important role in scavenging free radicals and atherosclerosis. It inhibits the development and progression of atherosclerosis, a vascular disease caused by antioxidants.[11]

Certain studies have shown that oral administration of ascorbic acid may result in lead chelation and reduce the risk of heavy metal poisoning. According to the results of some studies conducted on mice, ascorbic acid reduces intestinal absorption of lead and increases renal filtration. This vitamin is a Lactone with an Enediol group that can be combined with lead to form a very low ionic but water-soluble compound.[12,13]

Hence, it was presumed that the use of ascorbic acid could be an alternative method for lead poisoning therapy. Specifically, ascorbic acid is known as an antioxidant that reduces the effects of lead in the body. It has been widely reported that it can protect the cells from the toxic effects of lead.[14] A study has reported that vitamin C chelates lead ions. However, some studies have shown a negative relationship between the level of vitamin C in the blood and lead concentration. Moreover, previous studies have indicated that vitamin C cannot scavenge the lead ions.[12]

It has been shown that lead decreases nitric oxide production in endothelial cells, which is one of the major factors in the regulation of the vascular tone. Moreover, nitric oxide can regulate attachment of leukocytes to endothelium, duplication of smooth muscle cells in vessels' wall and platelet aggregation.[15]

It has been shown in a study that vitamin C cannot scavenge the lead ions.[11] Furthermore, previous studies have demonstrated that vitamin C has an essential role in a number of applications related to the pathogenesis of CVD. Patra et al. reported that vitamin C may not significantly reduce blood lead level in accordance with similar previous findings. They suggested that treatment by vitamin C may not be effective on the reduction of the lead level in vessels and other tissues.[16]

Since, there are several challenges with regard to treatment by vitamin C, and the studies conducted on the therapeutic efficiency of vitamin C are insufficient to determine its effect on the treatment process in the animal models.

To our knowledge, no study has been accomplished on the vitamin C effects on coronary and aorta artery endothelial cells after lead exposure in male rabbits. So this study was conducted to investigate the probable therapeutic role of ascorbic acid against injury induced by lead in vascular endothelial cells.

Methods

This experimental study was carried out in Maragheh University of Medical Sciences and was approved by the Research Ethics Committee of the Faculty of Medicine, Maragheh University of Medical Sciences (IR.MARAG HEHPHC.REC.1399.019). In this study, 30 white male rabbits of New Zealand race (weighing about 1.6–2 kg and 5 months old) were used and divided randomly into three groups. The animals were kept under standard conditions at a temperature of 22°C, a humidity of 55%, and a light cycle of 12 h of light and 12 h of darkness to adapt to the environment and food for about a month. Group 1 ($N=10$) served as the control and received water and normal diet. In Group 2, ($N=10$) the rabbits received lead acetate 547 ppm (Merck Co, Germany) orally in a dose of 5 mg/L by stomach tube once daily (5 mg Pb dissolved in 1 L water).[12,17]

In Group 3, ($N=10$) the rabbits received vitamin C (Sigma Co, USA) at a dose of 500 mg vitamin C/kg body weight, by stomach tube once and underwent the same duration of lead exposure(5 mg/L) daily for 40 days.[17,18] Vitamin C powder was obtained from Sigma company. A 500 mg/kg vitamin C was dissolved in 100 cc water and any rabbit was gavage daily 10 mL distilled water.[12] At the end of the experiment, blood samples were drawn and the level of blood lead was calculated using blood lead analyzer (Magellan Diagnostics, USA). Plasma level of lead was calculated in µg/dL. Subsequently, cholesterol levels, triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined using spectrophotometry. However, 10 cc of the blood samples was drawn and dumped in heparin-sealed plastic tubes and sent immediately to the laboratory to be measured using Method 8003 American National Institute of Occupational Safety and Health (NIOSH).[12]

After the treatment, the animals were anesthetized by the intravenous injection of pentobarbital (50 mg/kg) into an

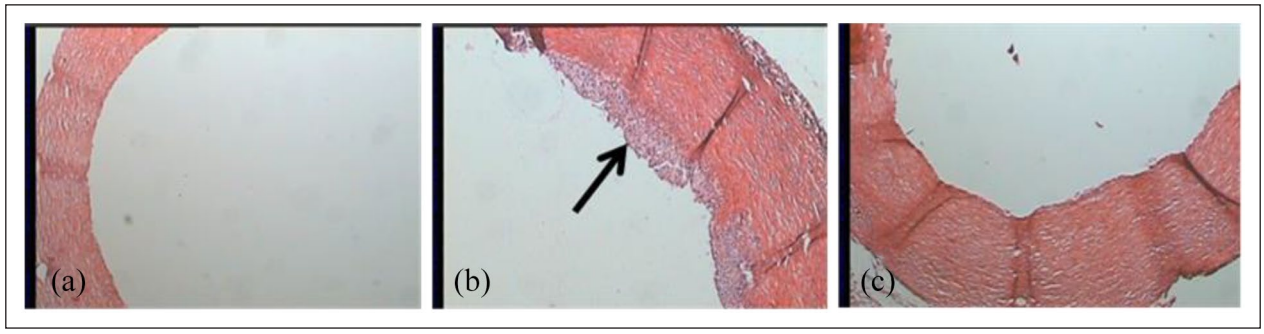

Figure 1. H & E staining of aorta artery shows the destruction of endothelium and atherosclerotic plaque in the aorta artery of Group 2 which received lead acetate (b), while, no development of atherosclerotic plaque was seen in Group 3 which received ascorbic acid (c) and Group 1(a). Magnification, 40x.

external ear vein. Subsequently, they were sacrificed and their thoracic aorta and coronary arteries were removed and then the samples were soaked in 10% formalin solution for 48 h. Then, they were fixed, and after these preparation steps, tissue processing and passage of tissues were performed according to the usual methods of tissue laboratories, and then the samples were embedded in paraffin to prepare them for section cutting on the microtome and were cut with a thickness of 5 µm. The sections were stained by H & E staining and were studied by light microscopy of Olympus model of BX51.

Compliance with ethics guidelines

All the procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Research Ethics Committee of the Faculty of Medicine, Maragheh University (IR.MARAGHEHPHC.REC.1399.019).

Statistical analyses

The results were analyzed using the Mann–Whitney test. The data were expressed as mean ± SD and analyzed by Student's *t*-test, and *p*-values less than 0.05 were considered as significant. The analysis was performed using SPSS statistical software (version 15, SPSS Inc., Chicago, IL, USA). A *p*-value less than 0.05 was considered significant.

Results

No mortality was observed in any of the rabbits throughout the study. The results of this study revealed that administration of lead acetate in rabbits had harmful effects on the endothelium of aorta arteries. These effects worsened the rupture of the endothelial layer and the formation of atherosclerosis plaque that was improved by the administration of vitamin C. (Figure 1 (a)–(c)).

The findings of this study showed that lead acetate could cause lesion and atherosclerotic plaque in the left coronary artery (Figure 2(b)) that was relatively improved by the administration of vitamin C, but the endothelium wall was discontinuous and few blood cells were observed on it compared to the control groups (Figure 2(a) and (c)).

The results of this study showed the existence of atherosclerotic plaque in the right coronary artery which received lead acetate (Figure 3(b)), while the atherosclerotic plaque was not observed in the control group and the group which had received ascorbic acid (Figure 3(a) and (c)).

There was a significant difference between the two groups ($p < 0.05$).

The results showed an increase in lead levels in Group 2, while a decrease in lead levels was observed in Group 3 that was fed the vitamin C, and there was a significant difference between Group 2 and Group 3 (Figure 4).

Moreover, it was shown that lead exposure could change the level of blood lipids, LDL, TG, and HDL. Vitamin C could increase HDL and decrease LDL, and TG. Furthermore, there were significant differences between these groups (Figure 5).

Discussion

The aim of this study was to evaluate the effects of ascorbic acid on the endothelium of coronary and aorta arteries in lead-exposed rabbits.[19] Heavy metals, including cadmium (Cd), arsenic (As), and lead (Pb), could target the vascular system and damage vascular endothelium in a variety of ways.[20] If the vascular endothelial cells were exposed to any toxic metal, the endothelial cells could be injured or killed.[21]

In this study, the results of morphological analysis revealed the formation of atherosclerotic plaque and destruction of endothelium in the aorta and coronary arteries that received lead acetate, while, no lesions in endothelial cells were observed in the control group and the ascorbic acid-fed group. It was confirmed in this study that exposure to lead acetate causes histological lesions in endothelium. With respect to the mechanisms of the harmful effects of heavy

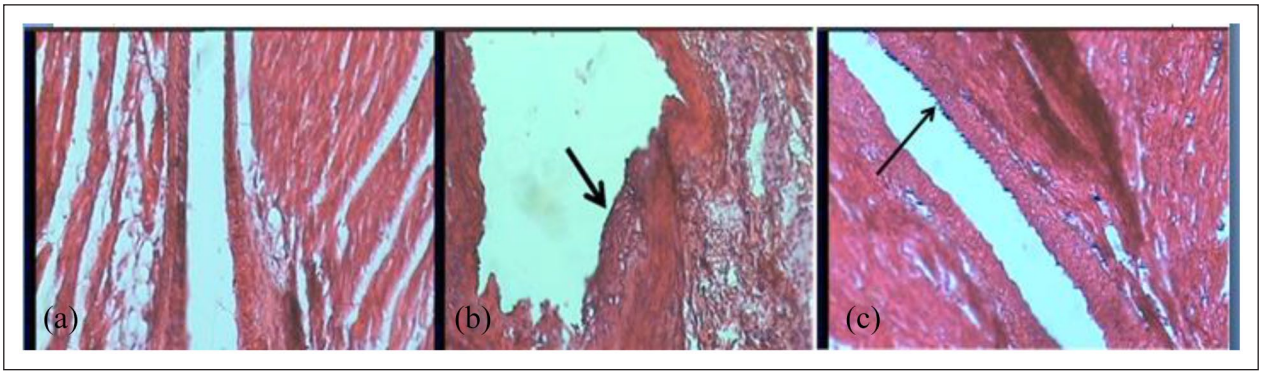

Figure 2. H & E staining of left coronary artery shows atherosclerotic plaque in the coronary artery of Group 2 which received lead acetate (b), and no development of atherosclerotic plaque was observed in Group 3 which received ascorbic acid, but few blood cells aggregation was observed (c), and the control group without any endothelial defect (a). Magnification, 40x.

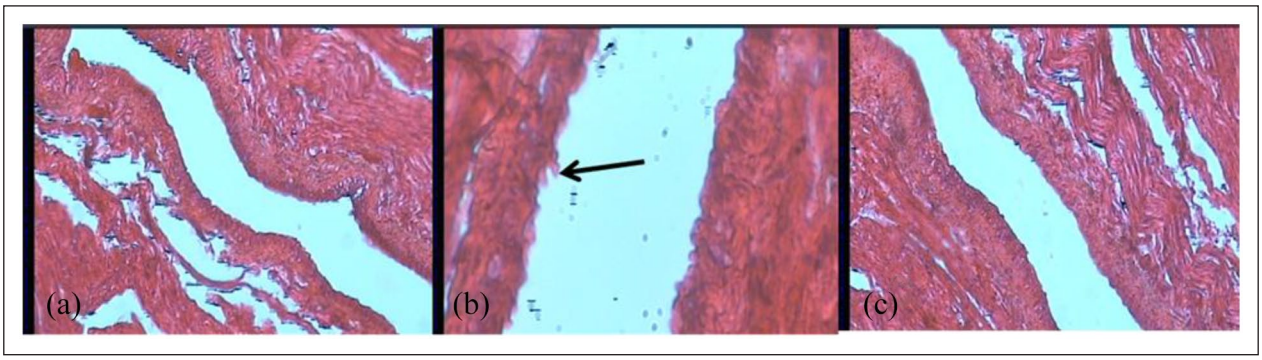

Figure 3. H & E staining of right coronary artery shows the destruction of endothelial cells by lead acetate and attachment of fatty streak on endothelium in Group 2 (b), while no lesions in endothelial cells were observed in Group 1 (a) and the ascorbic acid-fed group (c). Magnification, 40x.

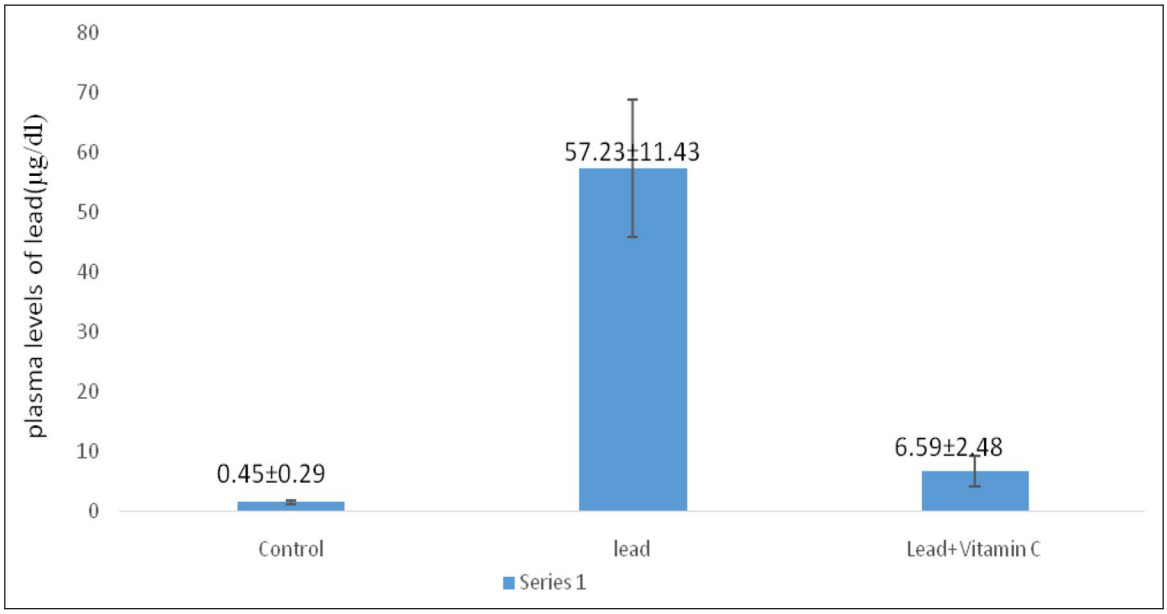

Figure 4. Plasma levels of lead (µg/dL) in the control group and experimental groups. Significant differences were observed between the groups ($p < 0.0001$).

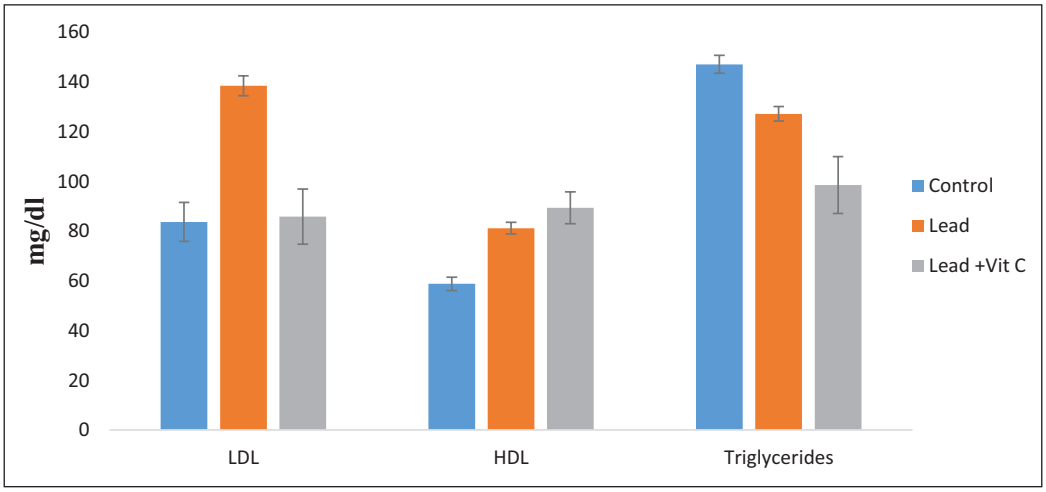

Figure 5. Plasma levels of TG, LDL, and HDL (mg/dL) in experimental groups ($p < 0.0001$).

metals on the cells, it is noteworthy to mention that they cause the release of ROS, and consequently, the defensive line of the cells is weakened or even lost and they cannot eliminate the ROS. Therefore, the lack of balance between the antioxidants level and the generation of free radicals can cause oxidative stress on cells.[22]

Previous studies have indicated that heavy metals, including cadmium, arsenic, and lead, could damage the vascular system in a variety of ways. Evidently, if vascular endothelial cells were exposed to any toxic metals existing in the blood stream, the endothelial cells could be injured or killed.[23]

Moreover, it has been indicated that lead disrupts the production of nitric oxide in endothelial cells, and damage to endothelial cells is the first and most important cause of atherosclerosis in blood vessels.[24]

The findings of this research confirm the results of previous studies. It has already been shown that any damages to endothelial cells may lead to CVDs, particularly the formation of atherosclerotic plaques. Lead causes the oxidation of LDL, HDL, and various proteins in the vessel wall through the production of oxygen-free radicals. Atherosclerosis process is associated with the peroxidation of lipoproteins.[25]

Several factors are related with the lead effects, such as blood pressure elevation, limitation of the Na+, K+-ATPase, enhanced peripheral resistance, because of endothelial dysfunction with increasing of oxidative stress, stimulating of the renin–angiotensin system, among others. The previous studies reported that exposure to lead acetate-induced hepatotoxicity associated to cause lesion, necrosis, inflammation, and hemorrhage in the liver and spleen. Alike to other toxic elements, the lead-induced damages to the cardiovascular system depends on dose/level and duration of exposure.[15,26]

It was found that vitamin C, as an antioxidant, can decrease the level of blood lead and lipid peroxidation. Hence, it prevents the formation of atherosclerosis in the arteries in the group that fed lead acetate.

Vitamin C can prevent LDL oxidation and lipid peroxidation that may cause damages to endothelial cells.[27]

Moreover, researchers have shown that some substances, including Beta vulgaris juice, are rich in antioxidants and can scavenge free radicals and decrease the level of blood lead.[28]

Furthermore, it collects essential metals, including lead acetate, calcium, and iron, and improves blood picture which changed by lead toxicity.[29]

Others studies have shown that, vitamin C has chelating capacity for lead, and it has also been proposed that it can quench the ROS and effects of lead acetate and has a prophylactic role against lead.[30,31]

Vitamin C protects the cells in the body and the cardiovascular system by facilitating fat metabolism.[32] It has a significant role in the making the connective tissue, and protection against the development and progression of atherosclerosis.[33] Deficiency of vitamin C in blood stream increased the risk factors of hypertension, myocardial infarction, and CVD.[34]

It has been indicated that vitamin C may not scavenge the lead ions.[11] Studies have reported that vitamin C could be used to treat CVD. Moreover, it has been indicated that vitamin C may not significantly reduce blood lead levels in accordance with similar previous findings. It was shown that treatment by vitamin C may not have any effect on the reduction of the lead level in vessels and other tissues.[26]

This study was conducted to evaluate the effects of ascorbic acid on the reduction of lead sedimentation in blood vessels and protection of vessels of endothelium from lead toxic effect. Therefore, the findings of this study indicated that ascorbic acid could protect the endothelial vessels and reduce the atherosclerosis induced by lead acetate.

Limitations: The major limitation of this research was the dissection and preservation of the coronary arteries. It is necessary to provide more samples in the experimental group because some of the samples were omitted during the dissection of the coronary arteries and tissue processing that was

able to cause statistical problems. Moreover, it was not possible to measure certain oxidative stress parameters due to their high costs. Another limitation was the lack of electron microscopy and high cost. We could not use electron microscopy for studying endothelium. Sample size/power analysis was not performed for this study.

Conclusion

The results of this study revealed that the administration of lead acetate in rabbits could have harmful effects on the endothelium of aorta and coronary arteries. These effects were relatively improved by the administration of vitamin C. Hence, according to the results of this study, it could be concluded that ascorbic acid improves the destruction and endothelial dysfunction in aorta and coronary arteries and prevents the formation of atherosclerotic plaque.

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Authorship

All the named authors attempted to meet the criteria determined by the International Committee of Medical Journal Editors (ICMJE) criteria for the authorship of this article. They take responsibility for the integrity of the work as a whole and have given their approval for this version to be published.

Compliance with ethics guidelines

This study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation. All the procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Research Ethics Committee of the Faculty of Medicine, Maragheh University of Medical Sciences, with the code (IR.MARAGHEHPHC.REC.1399.019). https://ethics.research.ac.ir/IR.MARAGHEHPHC.REC.1399.019

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval for this study was obtained from *Ethics Committee of Maragheh University of Medical Sciences, IRAN (IR.MARAGHEHPHC.REC.1399.019) *. Animal welfare Sciences (IR.MARAGHEHPHC.REC.1399.019).

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