Original Article

Therapeutic Effects of the Grape Seed Extract on Lead-Induced Hypertension and Aortic Responsiveness in Rats

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ABSTRACT

Background: Several studies in the past have shown that lead causes elevated blood pressure in humans and animals and exerts devastating effects on various organs of the body, including the cardiovascular system. This study was typically conducted to investigate the effects of the grape seed extract on the treatment of lead-induced hypertension and the correction of the aortic response to isolated vascular factors.

Methods: Experiments were carried out from January to March 2009 in the Physiology Research Center of Ahvaz Jundishapur University of Medical Sciences. In total, 50 experiments were carried out on 5 groups of Wistar rats in 5 groups, each group receiving water containing lead acetate and the grape seed extract in different patterns for 8 weeks, in accordance with the groups listed in the original text. Blood pressure was measured weekly through the tail-cuff. The response of the isolated aorta to the vasoconstrictor and vasorelaxant was evaluated in the groups. Statistical analysis was performed using the SPAS software, version 22, via one-way ANOVA followed by the LSD test. A P value < 0.05 was considered significant.

Results: Discontinuation of lead and administration of the extract caused a faster drop in blood pressure. Increased contractile responses to phenylephrine were observed in the rats that continued to consume lead and did not receive the extract. Additionally, the response to acetylcholine in the extract group was higher than that in the continued lead group.

Conclusions: The current study showed that the use of the grape seed extract, even after the occurrence of lead-induced hypertension, could be a useful treatment. Considerably, the grape seed extract failed to have an effect on vascular responsiveness to vasodilator and vasoconstrictor drugs. ***(Iranian Heart Journal 2019; 20(3): 36-46)***

KEYWORDS: Lead, Grape seed extract, Hypertension

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Hypertension is one of the most common diseases in the world today. If not treated, hypertension will have serious medical consequences. Among the factors contributing to hypertension are environmental agents such as industrial life and the unwanted entrance of some heavy metals such as lead into the body, which leaves undesirable physiological effects. [1] Lead is a bluish-white color metal that exists in combination with organic and inorganic compounds. A bivalent metal, lead affects the enzymatic and metabolic activity of cells due to its ability to imitate the role of calcium. Studies have revealed that exposure to lead causes an increase in reactive oxygen species (ROS) and the depletion of antioxidants such as glutathione. [2] A large number of studies have proven a relationship between chronic and sub-chronic lead exposure and hypertension. [3, 4] Evidence shows that ROS can interfere in the pathogenesis of hypertension. [5] A great deal of research on cardiovascular disease has focused on oxidative stress and the generation of ROS and revealed that chronically lead-exposed animals exhibit a considerable increase in plasma and tissue lipid peroxidation. [6] Various antioxidants, including vitamin E and vitamin C, improve the plasma concentration of malondialdehyde and improve lead-induced hypertension. [7, 8]

Grape seed is a waste harvest of winery and grape juice industries. Polyphenol components like proanthocyanidins in the grape seed extract (GSE) have free radical-scavenging properties greater than those of vitamin E and vitamin C. [9, 10] Previous studies have shown that the use of the GSE and low levels of lead simultaneously can prevent hypertension development. [11] The main objective of the present study was to evaluate the treatment potency of the antioxidant-rich GSE and its therapeutic effects on lead-induced hypertension in rats.

METHODS

Animals and Groups

In this study, 3-month-old Wistar male rats (the Laboratory Research, Reproduction, and Maintenance Center, Jundishapur University of Medical Sciences, Ahwaz) were used. The rats were kept in the optimal condition in terms of temperature and light without restrictions on food and water. The animals received compressed food (concentrate) (the Food and Animal Husbandry Company, Shahreza, Isfahan). The weight of the rats at the start of the experiment was between 180 and 200 g and at the time of the final test (detachment of the aorta ring) between 250 and 300 g. The experiments were carried out from January to March 2009, and the study protocol was approved by the Physiology Research Center's Ethics Committee for Animals in accordance with the guidelines on experiments on animals. [12] The animals had free access to a regular rat chow and were randomly assigned (simple randomized) to one of 5 following groups (10 animals in each group): 1) control group, which received tap water; 2) lead and discontinued lead group: which received water and 100 ppm of lead acetate for 4 weeks and tap water for the next 4 weeks; 3) lead and discontinued lead+GSE group, which received water containing 100 ppm of lead acetate for 4 weeks and tap water and then the GSE for the next 4 weeks; 4) lead+GSE group, which received water containing 100 ppm of lead acetate for 4 weeks and the GSE and subsequently water containing 100 ppm of lead in the next 4 weeks; and 5) lead group, which received water containing 100 ppm of lead acetate for 8 weeks (Table 1). The GSE was administered 100 mg/kg orally once a day. The selected doses of lead acetate and the GSE were based on a previous experiment. [11]

Preparation of hydro-alcoholic GSE (*Vitis vinifera lin*)
Red grape seeds were isolated and dried after confirmation by a botanist. The seeds were then ground with an electric mill. Next, 100 g of grape seed powder was placed in 1000 mL of 70% ethanol alcohol. The solution was stored at room temperature for 3 days, during which the mixture was stirred several times daily. Thereafter, the mixture was sifted and the extract was dried at room temperature. The extract was dissolved daily with distilled water and gavage with a syringe.

In vivo blood pressure
Blood pressure was recorded at the beginning and weekly for 8 weeks and determined by tail plethysmography coupled to a computer system (PowerLab, AD Instrument, Australia). Three consecutive recordings (5 min apart) were performed, and the average of the recordings was calculated for each rat.

In vitro contraction-relaxation responses
After 8 weeks, the rats were euthanized by diethyl ether. Next, the thoracic aorta was isolated, placed in dishes containing the oxygenated Krebs–Henseleit solution buffer, and divided into the 3 to 4 rings. Each ring was mounted vertically between 2 wires and suspended in 10 mL of a tissue bath, containing a 37° C Krebs–Henseleit solution buffer with the continuous bubbling of 95% O_2 and 5% CO_2. The tension in the aorta rings was monitored with an isometric transducer (Panlab, Spain) connected to a PowerLab system. The viability and contractility evaluation of the aorta rings, in terms of kcl (60 mM), was performed in a tissue bath. After maximum contraction, acetylcholine (1nM) was established in the bath. If the aorta ring relaxed more than 50%, the tissue was considered to be in a healthy condition. [13].

Responsiveness to phenylephrine
After having been washed out 3 times, increasing concentrations of phenylephrine (10^{-9}M-5 × 10^{-6} M) were applied in the tissue bath. In another condition, the responsiveness of the aorta rings to phenylephrine along with NG-nitro L-arginine methyl ester (L-NAME) (100 µM) as an NO synthases inhibitor was examined. Additionally, the contraction response to the aforementioned amounts of phenylephrine on a removed endothelium aorta ring was examined, and the concentration curve was plotted in these conditions.

Responses to acetylcholine and sodium nitroprusside (SNP)
After the maximum contraction of the intact aortic rings, the relaxation response to the increasing concentrations of acetylcholine (10^{-9}M-5 × 10^{-5}M) in the different groups was examined. Further, the relaxation responses to SNP after the contraction of the damaged endothelium aorta rings in the different groups were examined.

Statistical Analysis
All the statistical analyses were performed using the SPSS software, version 22. The variables in the current study were continuous, and they were presented as the mean ± the standard error of the mean (SEM). Comparisons in vivo and in vitro were performed using ANOVA, followed by the least significant difference (LSD) test. A *P* value < 0.05 was considered to indicate statistical significance. Normality was evaluated using the Quantile-Quantile plot (Q-Q plot).

Table 1. Experimental groups and the design of the intervention program

	Group	Weeks 1-4	Weeks 5-8
1	Control	Tap water	Tap water
2	Lead and discontinued lead	Water containing lead acetate	Tap water
3	Lead and discontinued lead+GSE	Water containing lead acetate	Tap water+ GSE
4	Lead+GSE	Water containing lead acetate	Water containing lead acetate + GSE
5	Lead	Water containing lead acetate	Water containing lead acetate

GSE, Grape seed extract

RESULTS

Blood pressure

As is shown in Figure 1, a sustained elevation in systolic blood pressure occurred after 2 weeks in all the groups that received lead acetate. In the fourth week, this increase was significant compared with the controls (P = 0.032 lead group, P = 0.040 lead and dis-lead, P = 0.049 lead and lead+GSE, and P = 0.043 lead and dis-lead +GSE). As was expected, all the study groups were similar in the fourth week in that they had an almost identical increase in blood pressure. Because the groups had the same intervention, by the end of the fourth week and after 4 weeks different treatments were applied. In the fifth week, the lead and lead-dis lead groups still had a significant difference with the control group (P = 0.026 and P = 0.048, respectively); nonetheless, the other 2 groups showed a decrease in blood pressure, which had no significant difference with the control group. During the sixth, seventh, and eighth weeks, the drop in systolic blood pressure was observed in the groups in which lead was discontinued or the extract and lead were administered at the same time. In the sixth to eighth weeks, only the lead group had a significant difference with the control group (P = 0.0005, P =0.003, and P =0.003), and the other groups were similar to the control group. The temporal process indicated a decrease in systolic blood pressure.

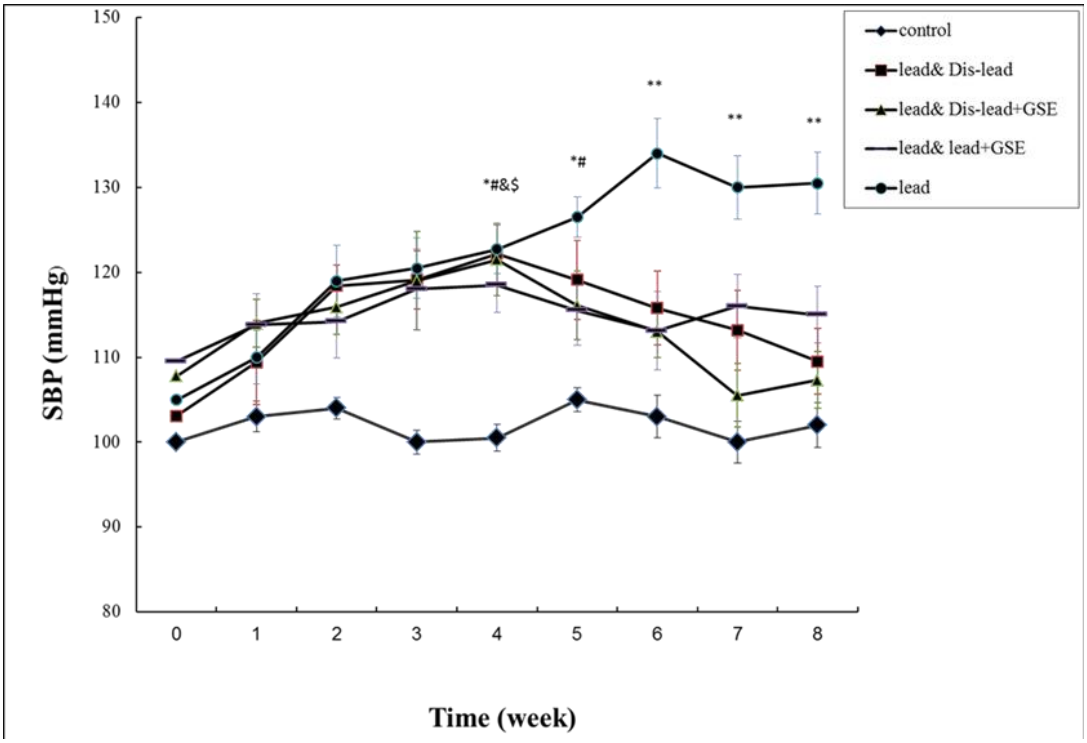

Figure 1. Effects of lead acetate and the grape seed extract (GSE) consumption, alone and in combination, on the systolic blood pressure of rats as measured by tail plethysmography

* P < 0.05 ** P < 0.01 lead group vs control, # P < 0.05 lead and dis-lead group vs control, and P < 0.05 lead and dis-lead + GSE group vs control, $ P < 0.05 lead and lead+ GSE group vs control

Data are expressed as mean ± SEM, N = 10.

Contraction response

The lead-treated groups for which lead was discontinued or the GSE was administered (groups 2 and 3) had no difference in the contraction pattern in response to phenylephrine compared with the control group. The group that received lead continually (group 5) in doses > 50×10^{-9} had significant contractions by comparison with the control group (P =0.049, P = 0.033, P = 0.018, and P = 0.021) (Fig. 2). The addition of L-NAME, as an NO blocker, to the tissue bath caused no obvious difference in contraction between the groups in response to phenylephrine (Fig. 3). In the next experiment, the endothelium of the aortic rings was removed and the contraction response to phenylephrine was examined. As is shown in Figure 4, there were no significances differences in the contractile responses between the examined groups. Another evaluation between 3 different states in the same group revealed that the use of L-NAME in the tissue bath and the removal of the endothelium from the aorta rings led to similar reactions to phenylephrine and caused a significant increase in the contraction response compared with the intact aortic rings in the same group. (Given the large number of graphs in the current section, we only revealed the final evaluation results.)

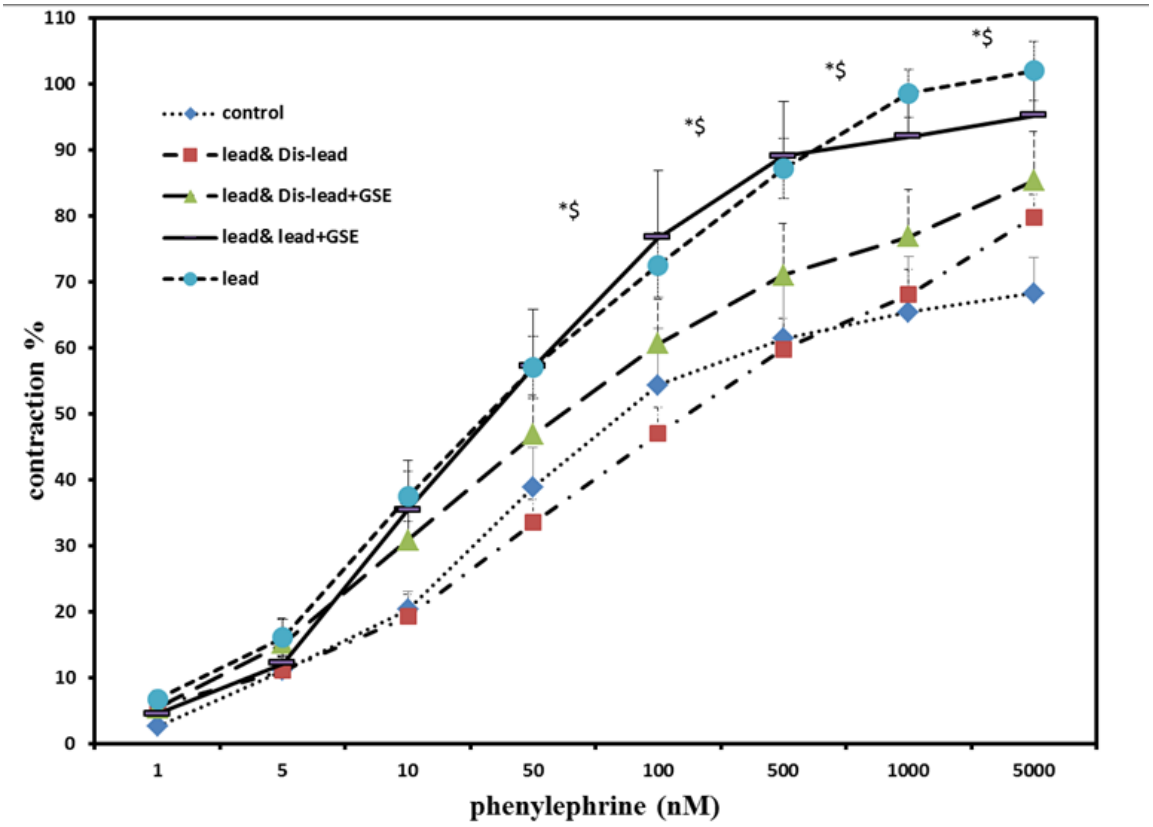

Figure 2. Effects of lead acetate and the grape seed extraction (GSE) consumption, alone and in combination, on the contraction response of the isolated aortic ring of the rats to phenylephrine at in vitro condition
* P < 0.05 lead group vs control, $ P < 0.05 lead and lead+ GSE group vs control
Data are expressed as mean ± SEM, N = 7.

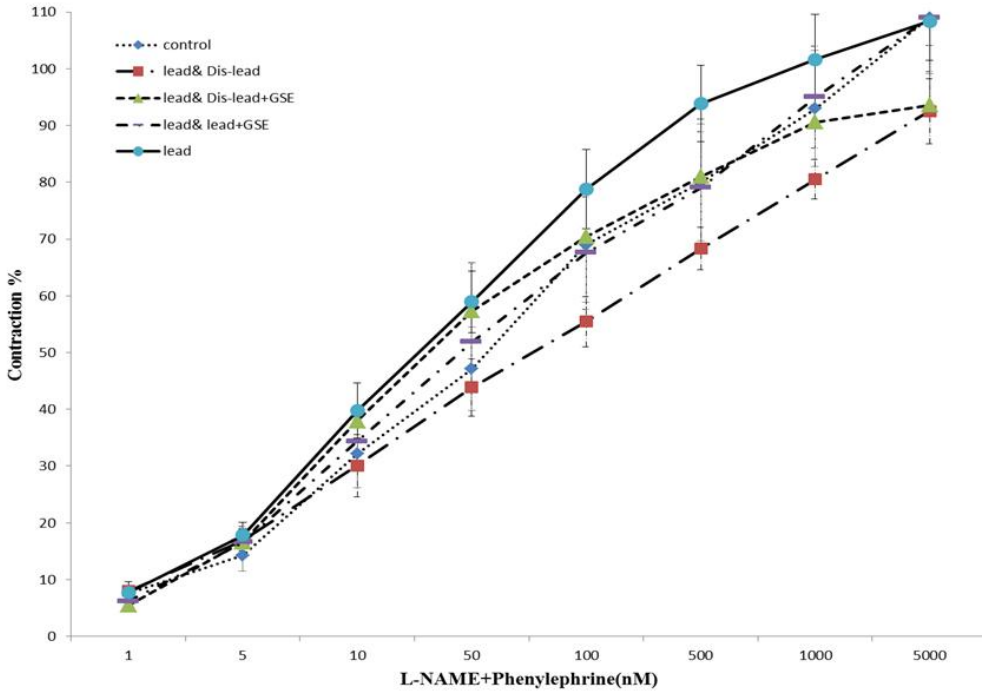

Figure 3. Effects of lead acetate and the grape seed extraction (GSE) consumption, alone and in combination, on the contraction response of the isolated aortic rings of the rats to phenylephrine along with NG-nitro L-arginine methyl ester (L-NAME), at in vitro condition
Data are expressed as mean ± SEM, N = 7.

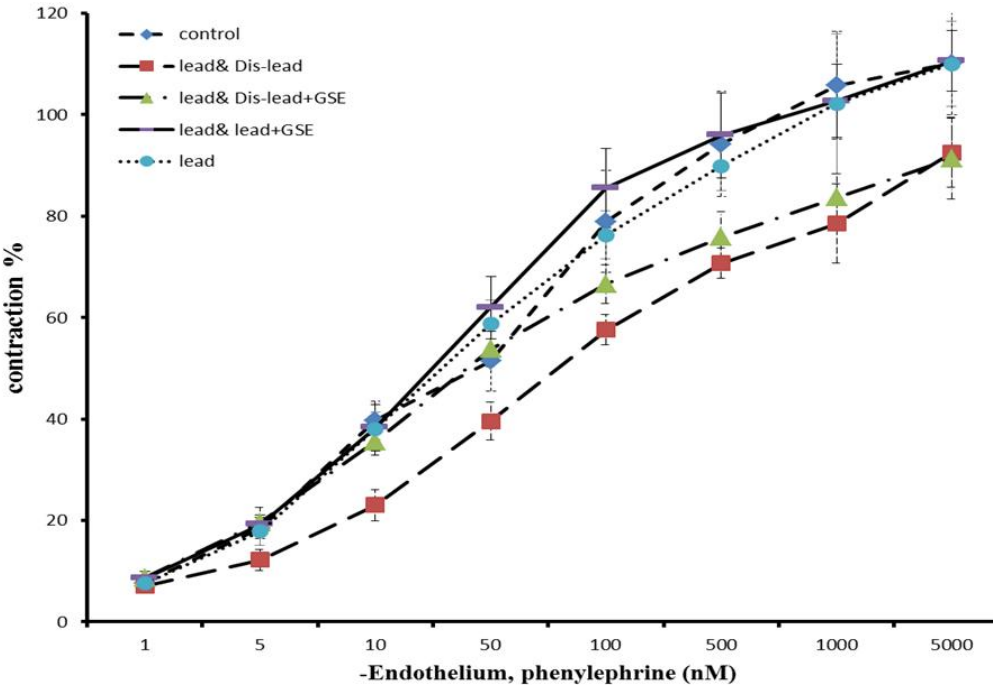

Figure 4. Effects of lead acetate and the grape seed extraction (GSE) consumption, alone and in combination, on the contraction response of the isolated aortic rings of the rats to phenylephrine at endothelium-less condition, in vitro
Data are expressed as mean ± SEM, N = 7.

Relaxation response

Acetylcholine induced relaxation in the different groups in a dose-response manner, and there was a significant decrease in relaxation between the continued lead group (group 5) compared with the other 4 groups in doses > 5×10^{-6} M (P = 0.49 and P = 0.022) (Fig. 5). In the condition where the endothelium was removed, the relaxation response to SNP revealed no difference between the various groups (Fig. 6).

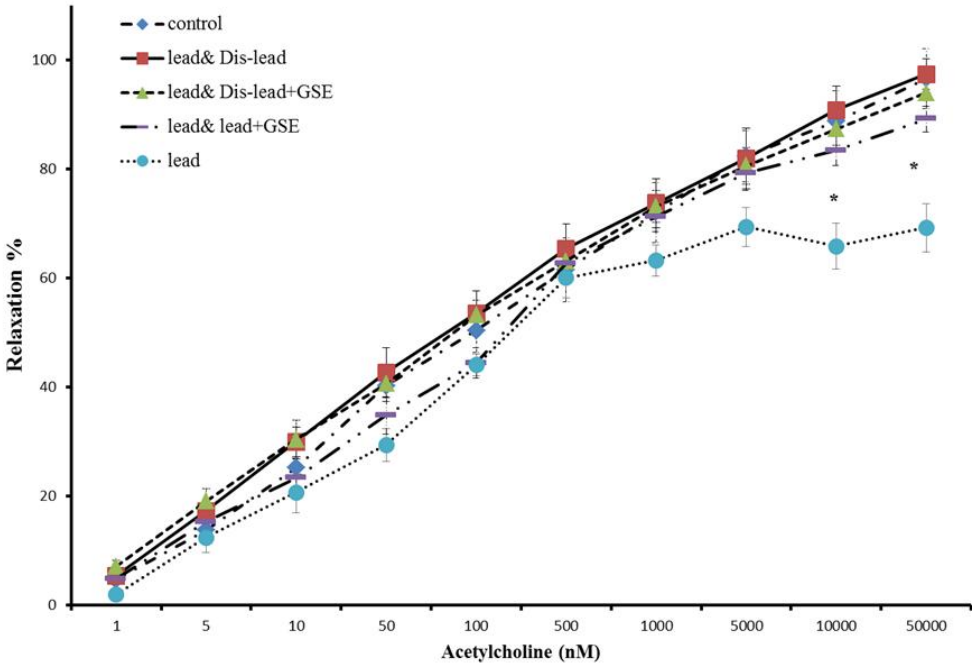

Figure 5. Effects of lead acetate and the grape seed extract (GSE) consumption, alone and in combination, on the relaxation responses of the isolated aortic rings of the rats to acetylcholine at in vitro condition
*P < 0.05 lead group vs control
Data are expressed as mean ± SEM, N = 7.

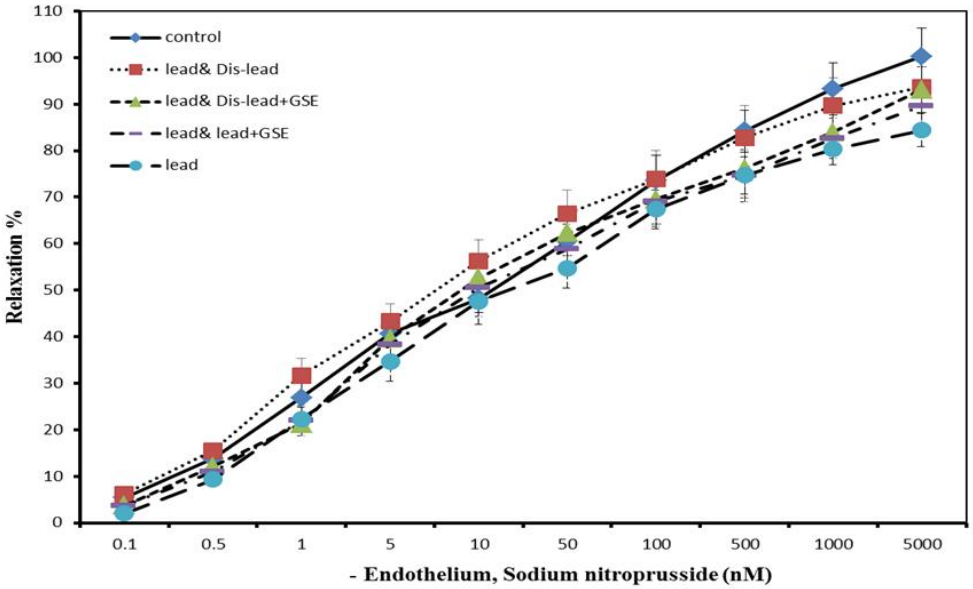

Figure 6. Effects of lead acetate and the grape seed extraction (GSE) consumption, alone and in combination, on the relaxation responses of the isolated aortic rings of the rats to sodium nitroprusside at endothelium-less condition, in vitro
Data are expressed as mean ± SEM, N = 7.

DISCUSSION

Blood pressure measurement is one of the basic parameters in the evaluation of the cardiovascular function. In the current study, our results revealed that the use of lead led to an increase in the risk of systolic blood pressure elevation and the discontinuation of lead acetate led to a gradual decrease in blood pressure. We also observed that if the discontinuation of lead acetate was simultaneously associated with the use of the GSE, the rate of blood pressure lowering to the normal levels accelerated. The amount of lead used in the current experiment was low and similar to the level seen in the environment. [14] In addition, this experiment demonstrated that the discontinuation of lead (groups 2–4) caused blood pressure to reach the normal value. Nevertheless, the GSE administration accelerated the rehabilitation process and conferred some therapeutic effects. Previous studies have shown that exposure for longer durations (3 or more months) to low levels of lead results in hypertension in rats. [15] Our previous experiment revealed that sub-chronic (4 wk) exposure to lead acetate also increased systolic blood pressure and heart rate, while the GSE administration alone had no effect on blood pressure and heart rate in rats. [11] Previous research has shown that lead through central mechanisms intensifies sympathetic activity and also reduces the sensitivity of baroreflex. [22] Polyphenols, including proanthocyanidins in grape seed, are potent antioxidants and play a very important role in the cardiovascular system; for instance, they interfere with the biochemical pathways associated with inflammation such thromboxane A2, reducing the production of harmful oxidants and ultimately inflammation. [23] Another biochemical pathway associated with procyanidins is calcium-dependent mechanisms that produce NO and increase prostaglandins as vasodilators. [24]

Another issue that was examined in the current study was the aortic response to vasopressors and vasodilators factors at the in vitro condition. We used phenylephrine as a vasoconstrictor agent, acetylcholine as an endothelium-dependent vasorelaxation agent, and sodium nitroprusside as an endothelial non-dependent vasorelaxation agent. Additionally, to study the role of the endothelium, we used L-NAME as an NO system inhibitor to evaluate the role of NO in vascular responses. In this trial, the groups receiving lead acetate for 8 weeks (groups 4 and 5) had the highest contraction response to phenylephrine, and the groups that received lead acetate only for 4 weeks (regardless of receiving or not receiving the extract) had a contractile response similar to that of the control group. It can, thus, be concluded that in this study, the groups receiving the extract did not show a unique response to vascular stimulating factors.

At in vitro condition, we removed the effects of the hormonal and nervous systems and investigated only smooth muscles and vascular endothelia. Since lead poisoning was chronic in this study, it can be assumed that no significant structural changes occurred in the vessels, and the discontinuation of lead or the administration of the extract reduced the contractile response to the values of the control group.

Nunes et al [16] stated that, under in vivo conditions, lead increased the plasma levels of noradrenaline and adrenaline, followed by systolic blood pressure; and at the in vitro experiment, under the influence of lead, the contractile responses decreased. These results chime in with those of the current study at in vivo condition but are discordant with those at in vitro condition. Nunes and colleagues attributed the reduction of contractile responses (at the in vitro part of the experiment) to the activation of calcium-dependent potassium channels. Calcium from the protein kinase C pathway can cause the phosphorylation of smooth muscle contraction proteins and increase contractile responses. [25] It can be argued that under different laboratory conditions, different biochemical pathways may

be facilitated, resulting in reduced or increased contractile responses. The important point, however, is that in all studies, there is agreement on the effects of lead exposure on blood pressure.

After the removal of the endothelium, the contraction response to phenylephrine was similar in all our study groups. In addition, the use of L-NAME as an NO blocker did not significantly increase the aortic contractile response to phenylephrine (compared with healthy endothelial conditions). In fact, in the current study, endothelium-dependent relaxation was disrupted in the groups that received lead until the end of the trial period and the groups that did not receive lead acetate at the middle of the trial, regardless of the consumption of the extract, had a more relaxation response that was similar to that of the control group. Since the relaxation responses to SNP in the aorta rings with damaged endothelia in all the groups were similar, it can be concluded that the smooth muscles of the vessels were not structurally altered and after the use of SNP, as an external NO, the relaxation response in all the groups was similar.

Previous investigations have reported the beneficial effects of the GSE at in vivo condition, so that by activating the free radical scavenging system, [26] the effects on neurotransmitters [27] and hormones have beneficial effects on the cardiovascular system. [19, 28] Further, research on the GSE indicates that permanent tissue changes more clearly occur due to the prolonged use of the extract, and there are transient changes such as a decrease in blood pressure in short periods of treatment.

In 2009, Sivaprakasapillai [19] reported that the use of the GSE daily and for 4 weeks reduced arterial blood pressure in subjects with the metabolic syndrome. Another mechanism whereby various polymers of procyanidins in the GSE may be involved in lowering blood pressure is the inhibition of angiotensin-converting enzyme activity, which Ottaviani et al [20] observed in 2006 by examining the effects of various types of procyanidins in an in vitro setting on the endothelial cells of the human umbilical vein. Another express chemical pathway proposed by Alvarez et al [21] for the performance of the GSE function was the enhancement of endothelial NO bioavailability through superoxide production inhibition from endothelial NADPH oxidase.

In the present experiment, the discontinuation of lead, regardless of the use of grapes, may have modified the NO production process. We also observed that the response of aortic relaxation to sodium nitroprusside, as an exogenous NO, was similar in the different groups, indicating that the smooth muscles may not have changed during this period. Moreover, we observed similar relaxation responses in all the groups by adding exogenous NO.

One of the limitations of the current study was the limited time spent on lead and extract consumption as well as the lack of an examination of structural changes in the arteries. Further, in this study, due to limited funding, we were not able to measure the level of serum or tissue antioxidants, as well as the serum or tissue levels of lead.

CONCLUSIONS

The results of the present study showed that the use of the GSE, even after the occurrence of lead-induced hypertension, could be a useful treatment. We would, therefore, suggest that the extract and its polyphenols used in combination with other hypertension drugs.

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Conflict of Interest: None

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