Zahedan J Res Med Sci. 2015 December; 17(12):e5200. doi: 10.17795/zjrms-5200

Published online 2015 December 26. **Research Article**

Effects of Selenium on Antioxidant Activity and Recovery From Sciatic Nerve Ischemia–Reperfusion in Adult Rats

Abolfazl Zendedel,[1] Sana Delavari,[2] Hassan Ahmadvand,[3] Kourosh Ghanadi,[1] and Mohammadreza Gholami[2,*]

[1]Department of Internal Medicine, Lorestan University of Medical Sciences, Khorramabad, IR Iran
[2]Razi Herbal Medicines Research Center, Department of Anatomy, Lorestan University of Medical Sciences, Khorramabad, IR Iran
[3]Department of Biochemistry, Lorestan University of Medical Sciences, Khorramabad, IR Iran

*Corresponding author: Mohammadreza Gholami, Razi Herbal Medicines Research Center, Department of Anatomy, Lorestan University of Medical Sciences, Khorramabad, IR Iran. Tel: +98-2166165327, E-mail: gholami.mr@lums.ac.ir

Received 2014 August 31; **Accepted** 2014 November 30.

Abstract

Background: Selenium is an antioxidant trace element that is capable protects various tissues against damage induced by ischemia-reperfusion injury.
Objectives: This study examines the selenium effect on antioxidant activity and recovery from sciatic nerve ischemia-reperfusion in adult rats.
Materials and Methods: In this experimental study, 80 adult male Wistar rats weighing 250 - 300 g were used. They were divided into ten groups (n = 8 per group). The first group received sham surgery only. The second group received 0.2 mg/kg selenium without ischemia-reperfusion surgery and eight remaining groups were divided to four control groups and four experimental groups which the latter groups received 0.2 mg/kg selenium. All ischemia groups were rendered in ischemic for 3 hours and reperfused for various times of 3, 7, 14 and 28 days. Half of the groups had experimental selenium (0.2 mg/kg) intraperitoneal injection treatment immediately after ischemia. According to a schedule for each group of rats bled and then centrifuged, and serum was prepared for assessment of activity levels of glutathione peroxidase (GPX), catalase (CAT), nitric oxide (NO), paraoxonase (POX), and malondialdehyde (MDA).
Results: Comparison of the serum GPX, CAT, NO, POX, and MDA levels in the groups that received selenium with corresponding control group showed that selenium can increase plasma activity levels of GPX, POX and decrease activity levels of NO.
Conclusions: Selenium with decreased activity levels of NO and increased activity GPX and POX can decrease ischemia-reperfusion injury in lower limb especially sciatic nerve.

Keywords: Selenium, Ischemia-Reperfusion, Peripheral Nerve, Antioxidant

1. Background

The hip joint dislocation, trauma and drug injection may lead to ischemia-reperfusion injury which will have adverse effects on the lower limb especially sciatic nerve and branches. Ischemia plays an important role in the production and development of pathological changes in various neuropathies including peripheral neuropathy particularly sciatic nerve [1-3]. Pathophysiology ischemia-reperfusion is platelet aggregation, oxygen free radicals and the interaction between endothelial cells and leukocytes [1-3]. This phenomenon leads to endothelial damage, capillary occlusion and deficiency of oxygen reaching to neural tissue [1-3]. Many efforts have occurred to reduce the effects of ischemia-reperfusion injury. effects of antioxidants such as lipoic acid [4] and melatonin [5] and some medications such as statins [6-8] and cooling [9] has been studied to reduce reperfusion injury of nerve damage which this material did not provide objectives of the study. Today, the emphasis is on the proper use of antioxidants to reduce ischemia-reperfusion injury. Antioxidant is a molecule that prevents the oxidation of other molecules. Antioxidants protect cells from the dangers of free radicals by destroy free radicals and inhibit other oxidation reactions [5, 10-13]. The key enzymes defense system include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), nitric oxide (NO) and paraoxonase (POX) [5, 10-13]. These enzymes are necessary for cell viability even in normal circumstances. These enzymes can be induced, an important feature, under conditions of oxidative stress. Each enzyme has a unique function. Superoxide anions, first derivative of free radicals, convert by SOD into water and oxygen [5, 10-13]. Glutathione, the major nonprotein thiol in aerobic organisms, is abundant intracellular non-enzymatic antioxidant. Malondialdehyde (MDA) is one of frequently used markers of lipid peroxidation [5, 10-13]. Superoxide dismutase-catalase enzyme complex are the first line of cellular defense against free radical toxicity [14]. Exposure of cells to oxidative stress can in-

duce this complex. These enzymes act synergistically with each other. Recently, selenium has consideration to reduce damage caused by ischemia-reperfusion. The effect of antioxidants on antioxidant enzymes to reduce cell damage caused by ischemia-reperfusion is one of the most important debates. Selenium, a trace element, has antioxidant and neuroprotective effects [14]. Selenium protects DNA, lipids and proteins against free radicals [14]. Selenium reduces lipoproxidase and hydroxides through a similar activity of glutathione peroxidase [15]. The protective effect of selenium against ischemia-reperfusion has been shown in several studies [15]. Researcher show beneficial effects of selenium on ischemia-reperfusion of brain [12, 16].

2. Objectives

The main goal of the present study was to examine if selenium induced serum antioxidant enzymes such as GPX, CAT, NO, POX and MDA in ischemia-reperfusion to the sciatic nerve at reperfusion phase.

3. Materials and Methods

This experimental study was performed at Razi herbal medicine center of Lorestan University of Medical Sciences. All experiments were performed in accordance with principles of laboratory animal care. All animal were obtained from Razi herbal medicine center of Lorestan University of Medical Sciences. Every effort was made to minimize the number of animals used and their suffering. Eighty adult male Wistar rats weighing 250 - 300 g were used. The animals were acclimatized for one week to the condition of our laboratory before the commencement of the experiment. The animals were exposed to 12 hours light and 12 hours dark cycle at a room temperature of 22°C. The animals had free access to standard laboratory chow and water ad libitum. The rats were divided into ten groups (n = 8 per group) (Table 1). The first group received sham surgery only (Table 1). The second group received 0.2 mg/kg selenium without ischemia-reperfusion surgery and eight remaining groups were divided to four placebo groups and four experimental groups which the latter groups received 0.2 mg/kg selenium (Table 1). The rats were anesthetized once intraperitoneally with ketamine HCl (50 mg/kg) and xylazine (5 mg/kg) [17] in accordance with the protocol approved by the animal care and use committee.

The animals were placed in supine position on a heated mat during the operation and recovery. Right femoral vessels were exposed through an inguinal incision and were dissected free the femoral nerve under operating microscope. The trifurcation of the sciatic nerve into peroneal, tibial and sural branch were rendered near-completely ischemic by occluding the femoral artery and vein with a silk suture 6 - 0 using slipknot technique [13, 18] for rapid release and reperfusion was achieved by release of these ligature. In all groups the vascular ligature was removed after 3 hours of ischemia [13, 18] and the sciatic nerve was allowed to reperfusion for 3, 7, 14 and 28 days in both the control and experimental selenium treatment groups. After ischemia and release of ligature, inguinal incision was sutured with silk 0 - 2. Selenium was administered in all experimental selenium treatment groups with a single dose of 0.2 mg/kg prior to vascular ligature via intraperitoneal. Selenium (0.2 mg/kg) or the same volume of distilled water was injected via intraperitoneal for selenium and vehicle-injected groups, respectively. The animals were placed under heating lamps until they recovered from anesthesia. According to a schedule for each group of mice blood samples were obtained from hearts and allowed to clot for 20 minutes in laboratory temperature and then centrifuged at 3000 rpm for 10 minutes for serum separation.

3.1. Biochemical Study

3.1.1. Levels of Nitrite

Serum levels of nitrite were measured by measuring accumulation of nitrite in serum using the Griess reaction with sodium nitrate as the standard. Briefly, 50 μL samples of serum were mixed with equal volumes of 1% sulphanilamide and 0.1% N-1-naphthylethylene diamine-dihydrochloride in 0.5% H_3PO_4. After 10 minutes at room temperature, the absorbance at 540 nm was measured in a microplate reader. Nitrite concentrations were calculated by comparison with a standard calibration curve with sodium nitrite ($NaNO_2$: 0 - 110 μmol/L).

3.1.2. Levels of Malondialdehyde (MDA)

The serum levels of MDA as a product of lipid peroxidation which reacts with thiobarbituric acid (TBA) analyzed using Ohkawa et al. method [19]. Absorbance was measured spectrophotometrically at 532 μm and MDA concentration was expressed as nmol/mg pr [19].

3.1.3. Activity of Catalase (CAT)

CAT activity was estimated following the method of Sinha et al. [20]. The reaction was started by the addition of 20 μL of sample in 2 mL of 30 mmol/L hydrogen peroxide (H_2O_2) in 50 mmol/L potassium phosphate buffer (pH = 7.0). Enzyme units are expressed as mmol/L of consumed H_2O_2 per minute g or mL.

3.1.4. Activity of Glutathione Peroxidase (GPx)

The GPX activity in the serum was determined using a GPX assay kit (Randox Lab., Ltd., UK) according to the manufacturer's protocol.

3.1.5. Determination of Paraoxonase (PON) Activity

PON activity was determined using paraoxon as a substrate and measured by increases in the absorbance at 412 nm due to the formation of 4-nitrophenol as already described [21]. The activity was measured at 25°C by add-

ing 50 mL of serum to 1 mL tris-HCl buffer (100 mM at pH = 8.0) containing 2 mM $CaCl_2$ and 5 mM of paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated using molar extinction coefficient 17100/Mcm.

Kurskal-Wallis test, Mann-Whitney U test and Dunn's multiple comparison tests were chosen for analysis variables (SPSS-22). Results are presented as mean ± SD. Results considered significant at $P \leq 0.05$.

4. Results

4.1. Determination of GPX Activity

Comparison of serum GPX levels in the groups received reperfusion showed that in the group 5, selenium received with 7 days reperfusion, significantly higher than the other groups (P = 0.041) (Table 2). Comparison of serum GPX levels in the groups received reperfusion showed that in the group 4, 3 days reperfusion without selenium injection, significantly lower than the other groups (P = 0.041) (Table 2). Comparison of the serum GPX levels in the groups that received selenium showed that levels of GPX increased until days 14 and then decreased. Comparison of serum GPX levels between groups selenium treated with the control corresponding groups suggests that the only difference between group 5, selenium received with 7 days reperfusion, and group 6, 7 days reperfusion without selenium injection, are statistically significant (P = 0.029) (Table 3).

4.2. Results of Serum Nitrite Levels

Comparison of serum NO levels in the groups received reperfusion showed serum NO levels is lower in the group 7, 14 days reperfusion with selenium treated, than the other groups (P = 0.047) (Table 4). Comparison of serum NO levels between groups selenium treated with the untreated corresponding groups suggests that difference between group 7, selenium received with 14 days reperfusion, and group 8, 14 days reperfusion without selenium injection, (P = 0.006) and also between group 9, 28 days reperfusion with selenium treated, and group 10, 28 days reperfusion with selenium untreated, are statistically significant (P = 0.002) (Table 5).

4.3. Results of Serum POX Levels

Comparison of serum POX levels in the groups received reperfusion showed serum POX levels is lower in the group 3, 3 days reperfusion with selenium treated, than the other groups (P = 0.001) (Table 6). Comparison of serum POX levels in the groups received reperfusion showed that in the group 9, selenium received with 28 days reperfusion, significantly higher than the other groups (P = 0.001) (Table 6).

Comparison of serum POX levels between groups selenium treated with the untreated corresponding groups suggests that difference between group 3, selenium received with 3 days reperfusion, and group 4, 3 days reperfusion without selenium injection, (P = 0.001) and also between group 5, 7 days reperfusion with selenium treated, and group 6, 7 days reperfusion with selenium untreated, are statistically significant (P = 0.029) (Table 7).

4.4. Results of Serum MDA and CAT Levels

Comparison of serum MDA levels in the treated groups with selenium and corresponding untreated groups do not statistically significant. Comparison of serum CAT levels in the treated groups with selenium and corresponding untreated groups do not statistically significant.

Table 1. Features of Study Groups

Groups (N = 8)	Selenium Administration[a]	Ischemia	Reperfusion Times, d
1	-	-	-
2	+	-	-
3	+	+	3
4	-	+	3
5	+	+	7
6	-	+	7
7	+	+	14
8	-	+	14
9	+	+	28
10	-	+	28

[a]Administration of selenium before ischemia.

Table 2. Plasma Concentrations of GPX Compared at Different Times of Reperfusion With Injection of Selenium Which Groups Compared With Each Other's Using Kurskal-Wallis Test[a]

Study Groups	Concentration of GPX	
	Mean of Ranks	Mean ± SD
3 days ischemia-reperfusion with selenium injection	2.75	912.7 ± 123.9
7 days ischemia-reperfusion with selenium injection	11.75	1439.5 ± 206.1
14 days ischemia-reperfusion with selenium injection	9.5	1342.6 ± 209.4
28 days ischemia-reperfusion with selenium injection	10	1311.7 ± 196.4

[a]P Value < 0.041.

Table 3. Plasma Concentrations of GPX Compared at Different Times of Ischemia-Reperfusion With and Without Injection of Selenium Which Each Group Compared With Placebo Using Mann-Whitney U Test

Study Groups	Concentration of GPX		P Value
	Mean of Ranks	Mean ± SD	
3 days ischemia-reperfusion			0.34
With selenium injection	3.5	979.1 ± 93.4	
Without selenium injection	5.5	912.7 ± 123.9	
7 days ischemia-reperfusion			0.029
With selenium injection	2.5	1017.5 ± 124.3	
Without selenium injection	6.5	1439.5 ± 206.1	
14 days ischemia-reperfusion			0.68
With selenium injection	4	1303.5 ± 271.2	
Without selenium injection	5	1342.5 ± 209.4	
28 days ischemia-reperfusion			0.88
With selenium injection	4.75	1368.2 ± 260.1	
Without selenium injection	4.25	1311.7 ± 196.4	

Table 4. Plasma Concentrations of NO Compared at Different Times of Reperfusion With Injection of Selenium Which Groups Compared With Each Other's Using Kurskal-Wallis Test[a]

Study Groups	Concentration of NO, nmol/min/mL	
	Mean of Ranks	Mean ± SD
3 days ischemia-reperfusion with selenium injection	12.5	1.26 ± 0.37
7 days ischemia-reperfusion with selenium injection	18.6	1.47 ± 0.79
14 days ischemia-reperfusion with selenium injection	8	1.86 ± 0.34
28 days ischemia-reperfusion with selenium injection	9.8	1.88 ± 0.22

[a]P Value < 0.041.

Table 5. Plasma Concentrations of NO Compared at Different Times of Ischemia-Reperfusion With and Without Injection of Selenium Which Each Group Compared With Placebo Using Mann-Whitney U Test

Study Groups	Concentration of NO		P Value
	Mean of Ranks	Mean ± SD	
3 days ischemia-reperfusion with selenium injection			0.81
	6.83	1.36 ± 0.42	
	6.18	1.26 ± 0.37	
3 days ischemia-reperfusion without selenium injection			0.95
	7.38	1.63 ± 0.34	
	7.67	1.47 ± 0.79	
7 days ischemia-reperfusion with selenium injection			0.006
	3.67	1.12 ± 0.33	
	9.33	1.86 ± 0.34	
7days ischemia-reperfusion without selenium injection			0.002
	8	1.18 ± 0.25	
	15	1.88 ± 0.22	

Table 6. Plasma Concentrations of POX Compared at Different Times of Reperfusion With Injection of Selenium Which Groups Compared With Each Other's Using Kurskal-Wallis Test[a]

Study Groups	Concentration of POX, nmol/min/mL	
	Mean of Ranks	Mean ± SD
3 days ischemia-reperfusion with selenium injection	5.5	75.7 ± 8.4
7 days ischemia-reperfusion with selenium injection	17.6	106.1 ± 14.6
14 days ischemia-reperfusion with selenium injection	26.6	120.5 ± 20.8
28 days ischemia-reperfusion with selenium injection	32.2	125.2 ± 16.5

[a]P Value < 0.001.

Table 7. Plasma Concentrations of POX Compared at Different Times of Ischemia-Reperfusion With and Without Injection of Selenium Which Each Group Compared With Placebo Using Mann-Whitney U Test

Study Groups	Concentration of POX, nmol/min/mL		P Value
	Mean of Ranks	Mean ± SD	
3 days ischemia-reperfusion			0.001
With selenium injection	5.6	16.9 ± 7.1	
Without selenium injection	15.4	75.7 ± 8.4	
7 days ischemia-reperfusion			0.029
With selenium injection	7.6	92.3 ± 19.3	
Without selenium injection	13.4	106.1 ± 14.6	
14 days ischemia-reperfusion			0.79
With selenium injection	10.1	120.5 ± 20.1	
Without selenium injection	10.9	118.03 ± 20.9	
28 days ischemia-reperfusion			0.14
With selenium injection	12.5	137.6 ± 7	
Without selenium injection	8.5	125.2 ± 16.6	

5. Discussion

Sciatic nerve ischemia led to nerve damage but the injury caused by reperfusion is more severe and induced nerve fibers degeneration. Previous studies have shown that nerve fibers degeneration reaches its maximum at 14 days reperfusion. Studies showed that nerve fibers regenerated until 28 days of reperfusion. Use of various antioxidants such as simvastatin reduces the damage caused by reperfusion. Use of antioxidants against oxidative stress induced by ischemia-reperfusion injury changes in the antioxidant activity. Selenium is an antioxidant that can help prevent damage induced by ischemia-reperfusion. In the present study observed that selenium with reduce serum level of NO and increase serum level of GPX and POX can reduce damage induced by oxidative stress. Ansari et al. demonstrated that selenium can prevent the degeneration of neurons in the brain caused by ischemia [22]. They also showed that selenium can influence the activity levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and monoamine oxidase (MAO-A and MAO-B) have a significant impact on the brain [22].

Results of present study showed that selenium cannot influence the activity levels CAT but can influence the activity levels of GPX significantly. Venardos et al. demonstrated that selenium can increase the activity of thioredoxin reductase and glutathione peroxidase, which leads to reduced myocardial injury induced by ischemia-reperfusion [23]. Results of their study were consistent with the results of present study because both studies showed that selenium can influence the activity levels of GPX. Heyland et al. demonstrated that the use of selenium, because of antioxidants properties, in chronic patients who are susceptible to damage by free radicals, reduce the mortality and morbidity while others oral antioxidants and vitamins have no such effects [24]. Tinggi et al. demonstrated that selenium can prevent the damage induced by ROS and NOS [25].

Turan et al. showed that selenium can reduce ischemia-reperfusion injury in the heart by increase GPX activity levels and reduce MDA [15, 26]. The above-mentioned outcomes are consistent with results obtained in this study. We suggest that cellular and molecular studies be done about the effect of selenium on the damage induced by sciatic nerve ischemia-reperfusion.

Taken together, it can be said that selenium can in-

creased plasma activity levels of GPX, POX and decreased activity levels of NO. It is concluded that selenium, which is an essential part of our diet, might be helpful in protection against damage in lower limb ischemia.

Acknowledgments

This study approved with grant number 1837 in the Lorestan University of Medical Sciences. Special thanks to Lorestan University of Medical Sciences for the financial support, khorramabad, Iran. The authors thank the head and staff of Razi herbal medicines research center of Lorestan Medical University.

Footnotes

Authors' Contribution: All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest: The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

Funding/Support: This paper had been done by personal expenses.

References

1. Gray C, Nukada H, Jackson DM, McMorran PD, Wu A, Ma F. Neuroprotective effects of nitrone radical scavenger S-PBN on reperfusion nerve injury in rats. *Brain Res.* 2003;**982**(2):179–85. [PubMed: 12915253]
2. Kihara M, Schmelzer JD, Kihara Y, Smithson IL, Low PA. Efficacy of limb cooling on the salvage of peripheral nerve from ischemic fiber degeneration. *Muscle & Nerve.* 1996;**19**(2):203–9. doi: 10.1002/(SICI)1097-4598(199602)19:2<203::AID-MUS12>3.0.CO;2-V. [PubMed: 8559170]
3. Kwitko AO, Pieterse AS, Hecker R, Rowland R, Wigg DR. Chronic radiation injury to the intestine: a clinico-pathological study. *Aust N Z J Med.* 1982;**12**(3):272–7. [PubMed: 6956295]
4. Mitsui Y, Schmelzer JD, Zollman PJ, Mitsui M, Tritschler HJ, Low PA. Alpha-lipoic acid provides neuroprotection from ischemia-reperfusion injury of peripheral nerve. *J Neurol Sci.* 1999;**163**(1):11–6. [PubMed: 10223404]
5. Sayan H, Ozacmak VH, Ozen OA, Coskun O, Arslan SO, Sezen SC, et al. Beneficial effects of melatonin on reperfusion injury in rat sciatic nerve. *J Pineal Res.* 2004;**37**(3):143–8. doi: 10.1111/j.1600-079X.2004.00145.x. [PubMed: 15357657]
6. Gholami MR, Abolhassani F, Pasbakhsh P, Akbari M, Sobhani A, Eshraghian MR, et al. The effects of simvastatin on ischemia-reperfusion injury of sciatic nerve in adult rats. *Eur J Pharmacol.* 2008;**590**(1-3):111–4. doi: 10.1016/j.ejphar.2008.05.050. [PubMed: 18586025]
7. Gholami MR, Abolhassani F, Pasbakhsh P, Akbari M, Sobhani A, Sohrabi D, et al. The effects of simvastatin on functional recovery of rat reperfused sciatic nerve. *Pak J Biol Sci.* 2007;**10**(23):4256–60. [PubMed: 19086581]
8. Vaughan CJ, Delanty N. Neuroprotective properties of statins in cerebral ischemia and stroke. *Stroke.* 1999;**30**(9):1969–73. [PubMed: 10471452]
9. Mitsui Y, Schmelzer JD, Zollman PJ, Mitsui M, Kihara M, Low PA. Hypothermic neuroprotection of peripheral nerve of rats from ischemia-reperfusion injury: intraischemic vs. reperfusion hypothermia. *Brain Res.* 1999;**827**(1-2):63–9. [PubMed: 10320694]
10. Ostadalova I, Vobecky M, Chvojkova Z, Mikova D, Hampl V, Wilhelm J, et al. Selenium protects the immature rat heart against ischemia/reperfusion injury. *Mol Cell Biochem.* 2007;**300**(1-2):259–67. doi: 10.1007/s11010-006-9391-4. [PubMed: 17187170]
11. Ozacmak VH, Sayan H, Arslan SO, Altaner S, Aktas RG. Protective effect of melatonin on contractile activity and oxidative injury induced by ischemia and reperfusion of rat ileum. *Life Sci.* 2005;**76**(14):1575–88. doi: 10.1016/j.lfs.2004.08.031. [PubMed: 15680167]
12. Ozbal S, Erbil G, Kocdor H, Tugyan K, Pekcetin C, Ozogul C. The effects of selenium against cerebral ischemia-reperfusion injury in rats. *Neurosci Lett.* 2008;**438**(3):265–9. doi: 10.1016/j.neulet.2008.03.091. [PubMed: 18490106]
13. Saray A, Can B, Akbiyik F, Askar I. Ischaemia-reperfusion injury of the peripheral nerve: An experimental study. *Microsurgery.* 1999;**19**(8):374–80. [PubMed: 10594912]
14. Beck J, Ferrucci L, Sun K, Walston J, Fried LP, Varadhan R, et al. Low serum selenium concentrations are associated with poor grip strength among older women living in the community. *Biofactors.* 2007;**29**(1):37–44. [PubMed: 17611292]
15. Turan B, Saini HK, Zhang M, Prajapati D, Elimban V, Dhalla NS. Selenium improves cardiac function by attenuating the activation of NF-kappaB due to ischemia-reperfusion injury. *Antioxid Redox Signal.* 2005;**7**(9-10):1388–97. doi: 10.1089/ars.2005.7.1388. [PubMed: 16115044]
16. Erbil G, Ozbal S, Sonmez U, Pekcetin C, Tugyan K, Bagriyanik A, et al. Neuroprotective effects of selenium and ginkgo biloba extract (EGb761) against ischemia and reperfusion injury in rat brain. *Neurosciences (Riyadh).* 2008;**13**(3):233–8. [PubMed: 21063330]
17. Milcan A, Arslan E, Bagdatoglu OT, Bagdatoglu C, Polat G, Kanik A, et al. The effect of alprostadil on ischemia-reperfusion injury of peripheral nerve in rats. *Pharmacol Res.* 2004;**49**(1):67–72. [PubMed: 14597154]
18. Saray A, Apan A, Kisa U. Free radical-induced damage in experimental peripheral nerve injection injury. *J Reconstr Microsurg.* 2003;**19**(6):401–6. doi: 10.1055/s-2003-42637. [PubMed: 14515234]
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;**95**(2):351–8. [PubMed: 36810]
20. Sinha BK, Antholine WM, Kalyanaraman B, Eliot HM. Copper ion-dependent oxy-radical mediated DNA damage from dihydroxy derivative of etoposide. *Biochim Biophys Acta.* 1990;**1096**(1):81–3. [PubMed: 2176549]
21. Azizi F, Rahmani M, Raiszadeh F, Solati M, Navab M. Association of lipids, lipoproteins, apolipoproteins and paraoxonase enzyme activity with premature coronary artery disease. *Coron Artery Dis.* 2002;**13**(1):9–16. [PubMed: 11917194]
22. Ansari MA, Ahmad AS, Ahmad M, Salim S, Yousuf S, Ishrat T, et al. Selenium protects cerebral ischemia in rat brain mitochondria. *Biol Trace Elem Res.* 2004;**101**(1):73–86. doi: 10.1385/bter:101:1:73. [PubMed: 15516704]
23. Venardos K, Harrison G, Headrick J, Perkins A. Effects of dietary selenium on glutathione peroxidase and thioredoxin reductase activity and recovery from cardiac ischemia-reperfusion. *J Trace Elem Med Biol.* 2004;**18**(1):81–8. doi: 10.1016/j.jtemb.2004.01.001. [PubMed: 15487768]
24. Heyland DK, Dhaliwal R, Suchner U, Berger MM. Antioxidant nutrients: a systematic review of trace elements and vitamins in the critically ill patient. *Intensive Care Med.* 2005;**31**(3):327–37. doi: 10.1007/s00134-004-2522-z. [PubMed: 15605227]
25. Tinggi U. Selenium: its role as antioxidant in human health. *Environ Health Prev Med.* 2008;**13**(2):102–8. doi: 10.1007/s12199-007-0019-4. [PubMed: 19568888]
26. Turan B, Hotomaroglu O, Kilic M, Demirel-Yilmaz E. Cardiac dysfunction induced by low and high diet antioxidant levels comparing selenium and vitamin E in rats. *Regul Toxicol Pharmacol.* 1999;**29**(2 Pt 1):142–50. doi: 10.1006/rtph.1998.1227. [PubMed: 10341144]