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Technical Note

Korean Red Ginseng attenuates type 2 diabetic cardiovascular dysfunction in Otsuka Long-Evans Tokushima Fatty rats

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ABSTRACT

Extracts of ginseng species show antihyperglycemic activity. We evaluated the inhibitory effects of diabetic complications for Korean Red Ginseng (KRG), which is enriched in ginsenosides using Otsuka Long-Evans Tokushima Fatty (OLETF) rats. The animals were divided into one of four groups (n = 6∼9): Long–Evans–Tokushima–Otsuka rats (control rats), OLETF rats, rats given 200 mg/kg KRG, and rats given 400 mg/kg KRG. We examined the protective potential of KRG against type 2 diabetic illnesses. The results exhibited that KRG showed significant antihyperglycemic and antioxidative effects in KRG-treated OLETF rats. And, our results proposed the amelioration of cardiac function through normalized ejection fraction, fractional shortening, and vascular reactivity. Furthermore, histopathological abnormalities in the OLETF rats were prevented by KRG treatment.

The predominance of diabetes has been predicted to increase to 4.4% in 2030. This could lead to an increase of diabetic cardiovascular complications [1]. However, currently, there are no clearly established remedy for diabetic complication. Traditionally, ginseng (*Panax ginseng* Meyer) is used as an important herbal medicine in Far East Asia. In general, the major biological activities of Korean Red Ginseng (KRG) are known to include the alleviation of vascular dysfunction, antioxidant effects, and the cardiac protection [2–4]. Yet, there is no a sure proof presenting the potentials for cardiovascular effect of KRG against type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. In the present study, we use KRG extract manufactured by Korea Ginseng Corporation (Daejeon, Korea) from the roots of 6-year-old fresh ginseng cultivated in Korea. Six male Long–Evans–Tokushima–Otsuka (LETO) rats and 27 OLETF rats (Central Lab. Animal Inc., Korea), weighting about 200 ± 10 g, were used for one set of experiment agreeing to the "Guideline for Institutional Animal Care and Use Committees" of Chonbuk National University (Jeonju, Korea). Animals were randomly allocated into LETO rats, normal group, OLETF rats, OLETF+200 mg/kg KRG-treated group, and OLETF+400 mg/kg KRG-treated group. Administration of 200 mg/kg and 400 mg/kg KRG was conducted for 180 consecutive days (Fig. 1), and feed intake and plasma glucose were measured twice a month throughout 180 days. At the end of the experimentation, antioxidant analysis, echocardiography recording, and vascular reactivity were examined. Data are expressed as mean±standard error of mean. Comparison between the LETO, OLETF, and KRG experimental groups were analyzed by Student *t* tests and one-way analysis of variance. The probability values less than 0.05 were regarded as significant statistically.

As a result, compared with OLETF group, body weight gain was lesser in the 200 mg/kg and 400 mg/kg KRG group after 180 days of treatment (data not shown). In this respect, feed consumption of OLETF group was significantly increased than LETO after 75 days. However, administration of 200 mg/kg/day and 400 mg/kg KRG produced a significant decrease after 3 months in a dose-dependent manner ($^{*}p < 0.05$, $^{**}p < 0.01$ compared with OLETF, Fig. 2A). And, plasma glucose levels were significantly increased in OLETF control. But, the administration of KRG inhibited the elevation of fasting blood glucose with dose-dependent manner ($^{*}p < 0.05$, $^{**}p < 0.01$, Fig. 2B). The results indicated that 400 mg/kg KRG is more effective than 200 mg/kg in plasma glucose levels.

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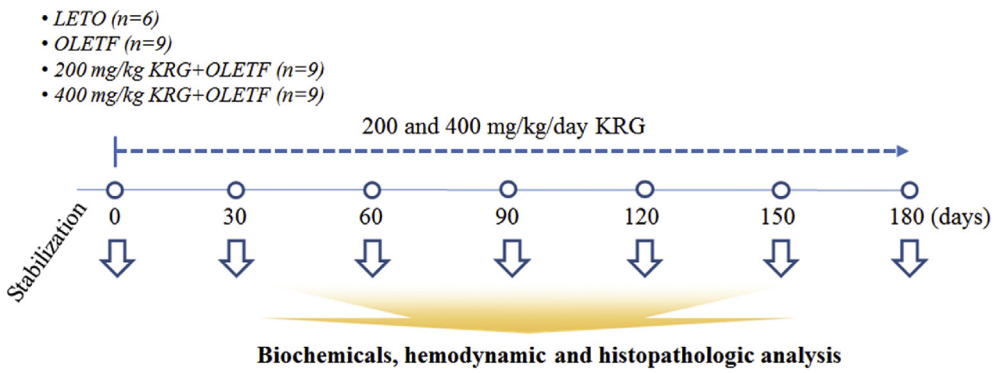

Fig. 1. Experimental protocol. All animal groups began with stabilization for 3 days. Then, animals were divided into LETO, OLETF, 200 mg/kg KRG + OLETF, which received 200 mg/kg of KRG for 180 days and 400 mg/kg KRG + OLETF, which received 400 mg/kg of KRG for 180 days. Antioxidant, biochemical, hemodynamic, vascular reactivity, and histopathologic studies were examined every 30 days for 180 days. LETO, Long–Evans–Tokushima–Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; KRG, Korean Red Ginseng.

And, using echocardiography, we have assessed the effect of KRG on cardiac function by evaluating an ejection fraction (EF) for the assessment of left ventricle systolic function and a fractional shortening (FS), another index for ventricle systolic function [5,6]. Currently, it was well known that EF is the most precious function index for clinical application [7]. As revealed in Fig. 3, OLETF group significantly reduced the average level of EF, since 2 months. Namely, the EF values were 92.53 ± 4.03%, 95.37 ± 3.71%, and 98.72 ± 3.24% in 3 months; 86.64 ± 4.37%, 89.32 ± 4.18%, and 92.53 ± 3.47% in 4 months; 81.71 ± 3.65%, 89.32 ± 3.69%, and 91.54 ± 3.73% in 5 months; 80.32 ± 4.03%, 87.56 ± 3.07%, and 89.54 ± 4.03% in 6 months, for OLETF control, 200 mg/kg KRG, and 400 mg/kg KRG groups, respectively, compared with LETO as 100% ($^{\#}p < 0.05$ compared LETO; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with OLETF, Fig. 3A). Until 2 months, there are no significant differences in EF for each group ($p > 0.05$). Also, there were significant decreases in the average level of FS of OLETF rats, since 2 months. In detail, the FS values were 91.65 ± 2.73%, 94.63 ± 1.67%, and 98.41 ± 2.14% in 3 months; 88.72 ± 2.26%, 92.47 ± 1.18%, and 94.35 ± 1.94% in 4 months; 87.35 ± 1.96%, 90.70 ± 2.24%, and 93.61 ± 2.17% in 5 months; 86.74 ± 2.27%, 89.65 ± 2.14%, and 90.47 ± 2.26% in 6 months, for OLETF control, 200 mg/kg KRG and 400 mg/kg KRG groups, respectively, compared with LETO as 100% ($^{\#}p < 0.05$ compared LETO; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with OLETF, Fig. 3B). Until 2 months, there are no significant differences in FS for each group ($p > 0.05$). To sum up, administration of KRG significantly inhibited the decreases of EF and FS levels, proposing that KRG could be an efficient to improve the cardiac ventricular dysfunction in OLETF rats. At the end of the experimental period, animals were anesthetized with ether and the thoracic aorta was isolated and placed with 4°C Krebs' buffer containing NaCl 118.5, KCl 4.74, $CaCl_2$ 2.5, KH_2PO_4 1.18, $MgSO_4$ 1.18, $NaHCO_3$ 24.9 (in mmol/L), and glucose 10.0. And, aortic tissue cross-sections (5 μm) in all groups were stained with normal hematoxylin and eosin stain. Compared with the LETO (Fig. 4A(a)), the dilated aorta in OLETF group showed an infiltration of inflammatory cells, thickening of intima, and extensive destruction (arrows, Fig. 4A(b)). In contrast, an infiltration of narrow thickening and a few extensive destructions of the medial elastic lamellae were observed in 200 mg/kg and 400 mg/kg KRG-treated group after 180 days (arrows, Fig. 4A(c) and (d), respectively). In succession, the contractile response of aorta was examined in all groups. The contractility for norepinephrine were represented as the percentage of the activity to the maximal contraction in 70 mM KCl. Cumulative dose of 10^{-10} to 10^{-5} M norepinephrine resulted in dose-dependent contractions in each group. In contraction response by 10^{-10} to 10^{-5} M norepinephrine, the maximum contraction or dilation value (V_{max}) of OLETF group were significantly increased than those of LETO control group, OLETF+200 mg/kg KRG, and OLETF+400 mg/kg KRG groups (Fig. 4B). Namely, the V_{max} were 69.37 ± 7.54%, 100.62 ± 9.75%, 97.84 ± 10.56%, and 89.72.490 ± 9.95% for LETO, OLETF control, OLETF+200 mg/kg KRG, and OLETF+400 mg/kg KRG groups, respectively ($^{*}p < 0.05$, $^{**}p < 0.01$ compared with OLETF, Fig. 4A). Moreover, we evaluated

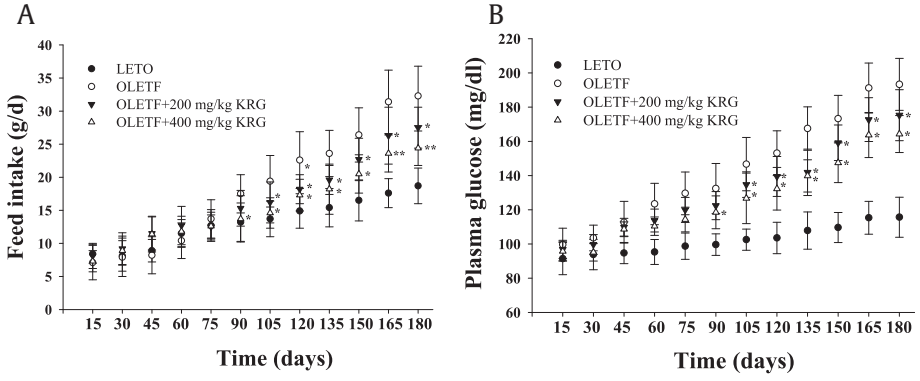

Fig. 2. Changes of feed intake and plasma fasting glucose in LETO and experimental groups. Feed intake (A) and plasma fasting glucose (B) were examined every 15 days for all periods of KRG treatment. Values indicate mean±standard error of nine rats per group except LETO (n = 6); $^{*}p < 0.05$, $^{**}p < 0.01$ compared with OLETF. LETO, Long–Evans–Tokushima–Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; KRG, Korean Red Ginseng.

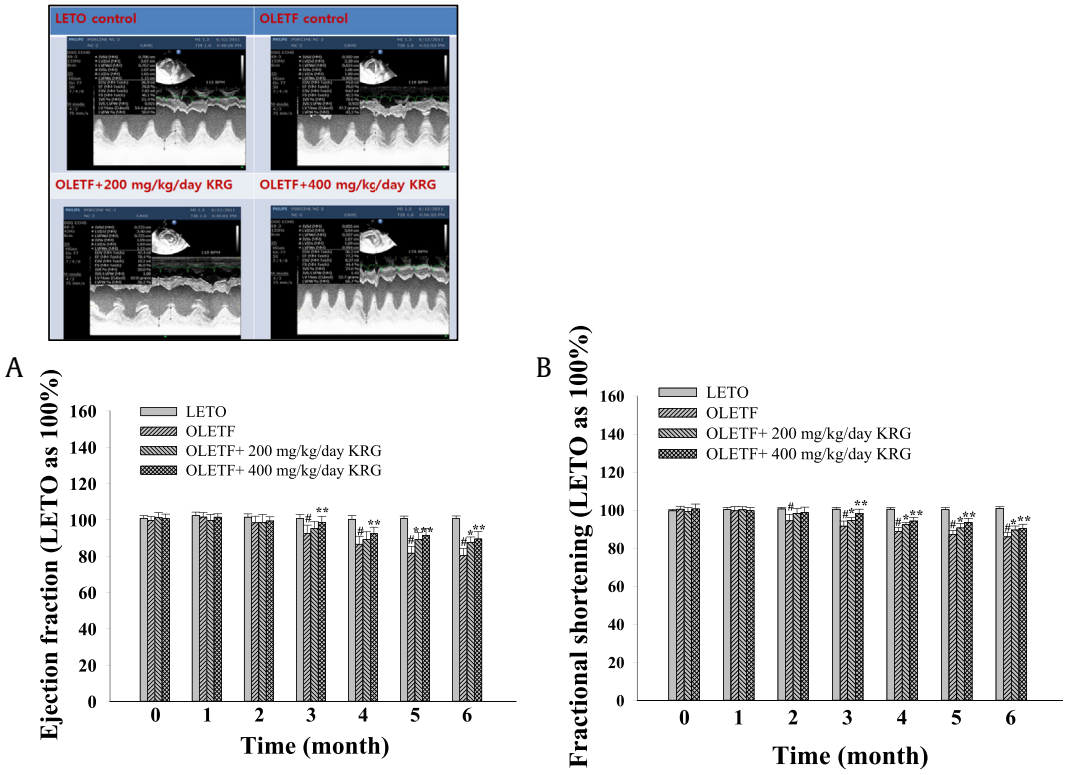

Fig. 3. The effect of KRG on ejection fraction and fractional shortening. After 180 days, echocardiography was performed on all groups, showing the images in upper square area. (A) Values of ejection fraction was expressed. (B) Fractional shortening was expressed, respectively. Values indicate mean±standard error of nine rats per group except LETO (n = 6); $^{\#}p < 0.01$ compared with LETO, and $^{*}p < 0.05$, $^{**}p < 0.01$ compared with OLETF. LETO, Long–Evans–Tokushima–Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; KRG, Korean Red Ginseng.

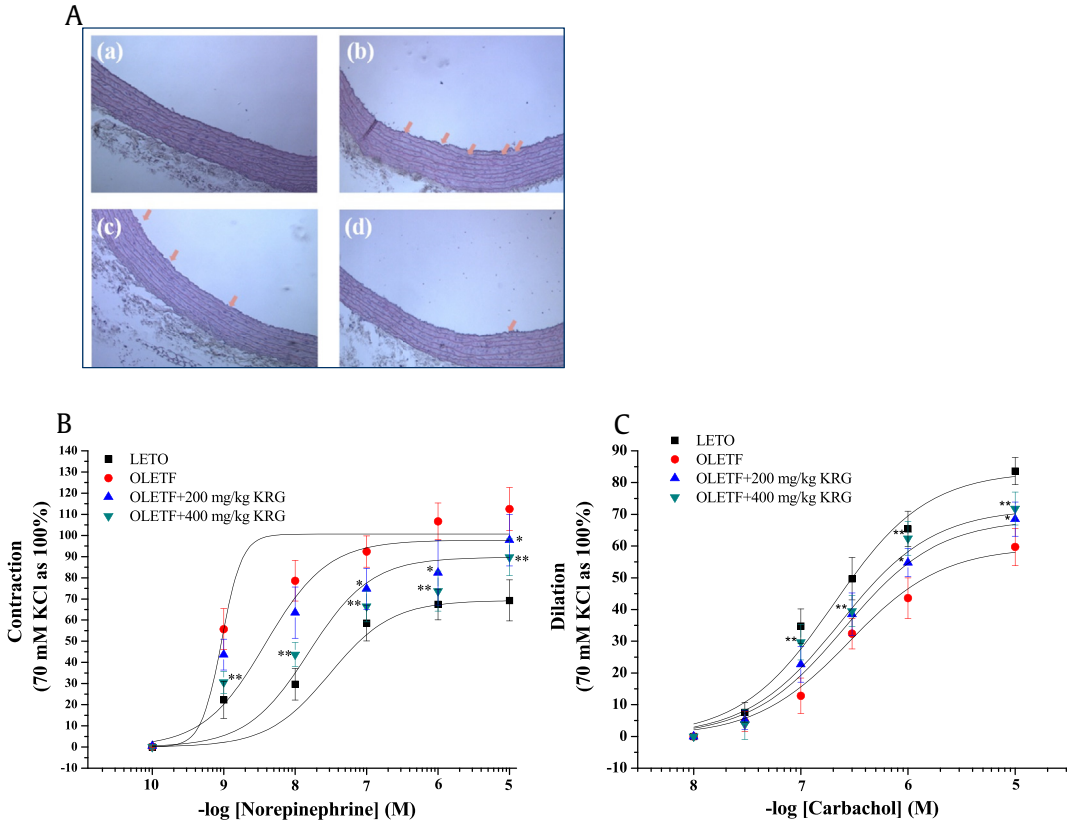

Fig. 4. The effect of KRG on vascular histopathological, contraction, and relaxation responses to norepinephrine and carbachol in LETO and experimental groups after 180 days of KRG treatment. (A) Transverse sections of aortic tissue were stained with hematoxylin and eosin in all groups. There is infiltration of inflammatory cells, extensive destruction of the elastic lamellae, and thickening of intima in OLETF group compared with LETO group. However, infiltration of only a few inflammation-like cells in adventitia in the 200 mg/kg and 400 mg/kg KRG-treated groups after 180 days. (B) Vascular contraction and (C) relaxation responses were shown in all groups. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with OLETF. LETO, Long–Evans–Tokushima–Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; KRG, Korean Red Ginseng.

Table 1
Effect of KRG on plasma malondialdehyde levels in type 2 diabetic OLETF rats.

	Mo	LETO control	OLETF control	OLETF +200 mg/kg KRG	OLETF +400 mg/kg KRG
Malondialdehyde (nmol/l)	0	4.75 ± 0.87	5.32 ± 0.75	6.02 ± 0.85	5.68 ± 1.03
	1	4.93 ± 1.04	5.79 ± 1.22	5.24 ± 1.15	5.96 ± 1.42
	2	5.01 ± 0.99	7.98 ± 1.24[a]	6.09 ± 1.05[b]	6.54 ± 1.38[b]
	3	5.21 ± 0.98	9.54 ± 1.32[a]	7.42 ± 1.04[b]	6.41 ± 1.54[c]
	4	5.62 ± 0.85	11.84 ± 2.56[a]	8.03 ± 1.19[c]	6.96 ± 1.32[c]
	5	5.37 ± 1.19	12.01 ± 1.93[a]	9.11 ± 1.63[c]	7.16 ± 1.02[c]
	6	5.78 ± 1.14	14.83 ± 2.24[a]	11.21 ± 1.63[c]	9.50 ± 1.83[c]

LETO, Long–Evans–Tokushima–Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; KRG, Korean Red Ginseng; SD, standard deviation.
Units are expressed as nmol/l for plasma malondialdehyde.
Results are expressed as the mean±SD in each group.
[a] Significantly different ($p < 0.01$) from LETO.
[b] Significantly no different ($p < 0.05$) from OLETF control.
[c] Significantly different ($p < 0.01$) from OLETF control.

Table 2
Effect of KRG on plasma GSH-Px levels in type 2 diabetic OLETF rats.

	Mo	LETO control	OLETF control	OLETF +200 mg/kg KRG	OLETF +400 mg/kg KRG
Glutathione peroxidase (U/ml)	0	1326.75 ± 93.78	1268.42 ± 99.53	1387.63 ± 93.54	1287.48 ± 99.47
	1	1423.54 ± 113.71	1335.74 ± 113.76	1299.43 ± 114.62	1295.75 ± 124.07
	2	1388.37 ± 105.6	1288.29 ± 138.4	1436.45 ± 97.48[b]	1536.73 ± 123.54[c]
	3	1407.64 ± 87.53	1163.74 ± 114.76[a]	1683.76 ± 143.07[c]	1599.76 ± 107.56[c]
	4	1454.62 ± 98.75	1275.37 ± 113.82[a]	1746.52 ± 127.53[c]	1607.54 ± 117.32[c]
	5	1493.13 ± 105.71	1299.73 ± 96.54[a]	1797.67 ± 107.5[c]	1828.51 ± 113.75[c]
	6	1477.29 ± 96.53	1197.35 ± 113.56[a]	1738.09 ± 97.52[c]	1940.17 ± 135.87[c]

LETO, Long–Evans–Tokushima–Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; KRG, Korean Red Ginseng; SD, standard deviation; GSH, glutathione.
Units are expressed as U/ml for plasma GSH-Px.
Results are expressed as the mean±SD in each group.
[a] Significantly different ($p < 0.01$) from LETO.
[b] Significantly no different ($p < 0.05$) from OLETF control.
[c] Significantly different ($p < 0.01$) from OLETF control.

the relaxation response by 10^{-8} to 10^{-5} M carbachol. As expected, endothelium-dependent relaxative activity by carbachol were lower in OLETF control compared with LETO rats. At that time, the V_{max} were 83.74 ± 5.17%, 59.75 ± 4.92%, 68.59 ± 6.04%, and 71.81 ± 5.13% for LETO, OLETF control, OLETF+200 mg/kg KRG, and OLETF+400 mg/kg KRG groups, respectively ($^{*}p < 0.05$, $^{**}p < 0.01$ compared with OLETF, Fig. 4B). Interestingly, the existing difference between OLETF rats with 200 mg/kg/day and 400 mg/kg KRG was not significant ($p > 0.05$) at the doses of 1×10^{-8} M to 1×10^{-5} M carbachol. Finally, these results are supportive of KRG being used as an agent normalizing aorta reactivity. Also, to antioxidant activity, lipid peroxidation was evaluated by quantifying malondialdehyde (MDA) levels, which was measured by the absorbance, spectrophotometrically [8]. As a result, in OLETF rats, there was a significantly increased MDA levels when compared with LETO ($^{a}p < 0.01$, Table 1). But, administration of 200 mg/kg and 400 mg/kg KRG decreased the MDA level compared with OLETF, dose-dependently ($^{b}p < 0.05$, $^{c}p < 0.01$, Table 1). Meanwhile, in the presence of H_2O_2, glutathione (GSH)-Px catalyzes reduced GSH. Activity of GSH-Px was analyzed by the analysis of GSH by an enzymatic response [9]. Namely, Activity of GSH-Px was significantly lowered in OLETF control compared to LETO ($^{a}p<0.01$, Table 1). However, in GSH-Px activity, there was a significant rise in groups administered 200 and 400 mg/kg KRG ($^{b}p < 0.05$, $^{c}p < 0.01$, Table 2) compared with OLETF group. In conclusion, our experiment proves primary evidence about the use of KRG against type 2 diabetic model. However, further study has been necessary to examine the biosafety and other additional effectiveness of KRG. Large-scale, potential, and more long-term studies are necessary to prove the right use of KRG against the risk of type 2 diabetic cardiovascular complications.

Conflicts of Interest

The authors report no conflicts of interest.

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References

[1] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27(5):1047–53.
[2] Sung J, Han KH, Zo JH, Park HJ, Kim CH, Oh BH. Effects of red ginseng upon vascular endothelial function in patients with essential hypertension. Am J Chin Med 2000;28(2):205–16.
[3] Jung CH, Seog HM, Choi IW, Choi HD, Cho HY. Effects of wild ginseng (panax ginseng C.A. Meyer) leaves on lipid peroxidation levels and antioxidant enzyme activities in streptozotocin diabetic rats. J Ethnopharmacol 2005;98:245–50.
[4] Li HX, Han SY, Ma X, Zhang K, Wang L, Ma ZZ, Tu PF. The saponin of red ginseng protects the cardiac myocytes against ischemic injury in vitro and in vivo. Phytomedicine 2012;19(6):477–83.
[5] Yang C, Wu K, Li SH, You Q. Protective effect of curcumin against cardiac dysfunction in sepsis rats. Pharm Biol 2013;51(4):482–7.
[6] Song J, Li H, WangY, Liu P, Li X, Tang C, Jin H, Du J. Left ventricular ejection fraction and fractional shortening are useful for the prediction of the therapeutic response to metoprolol in children with vasovagal syncope. Pediatric Cardiology 2018. May 16, https://doi.org/10.1007/s00246-018-1904-x.
[7] Razmaraii N, Babaei H, Mohajjel Nayebi A, Asadnasab G, Ashrafi Helan J, Azarmi Y. Cardioprotective effect of phenytoin on doxorubicin-induced cardiac toxicity in a rat model. J Cardiovasc Pharmacol 2016;67(3):237–45. Mar.
[8] Niehaus Jr WG, Samuelsson B. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968;6:126–30.
[9] Sedlak J, Lindsay RH. Estimation of total, protein bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192–205.