

Amelioratory effects of vitamin E against biochemical toxicity induced by deltamethrin in male rats

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Background/aim

Deltamethrin (DLM) is a synthetic pyrethroid insecticide known for its wide toxic manifestations. The present experiment pertains to the protective role of vitamin E (vit E) against biochemical toxicity following pesticide exposure during 30 days.

Materials and methods

Male albino rats were divided into four groups of six each: Group I served as control rats (0 mg (vit E) and 0 mg DLM/kg body weight), Group II received deltamethrin (7.5 mg/kg body weight). Group III received vit E (100 mg/kg body weight). Group IV received both deltamethrin (7.5 mg/kg body weight) plus vit E (100 mg/kg body weight).

Results

Exposure of rats to DLM induced significant increase in the levels of hepatic markers enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP); while acetylcholinesterase (AChE) was inhibited. Significant decrease in catalase (CAT) and glutathione S-transferase (GST) enzyme activities were observed in treated rats. Furthermore, renal markers such as urea and creatinine were increased in deltamethrin treated rats. Additionally, serum cholesterol, triglycerides, low-density lipoprotein (LDL) and the level of high-density lipoprotein (HDL) were significantly increased and decreased, respectively. Co-administration of vit E restored all the parameters cited above to near-normal values.

Conclusion

Our investigation showed that vit E acts as an effective antioxidant for DLM pesticide toxicity in reducing oxidative stress burden.

Keywords:

deltamethrin, enzyme activities, lipids, rats, vitamin E

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Introduction

The use of pesticides is an important procedure for enhancing the agricultural yield. However, the great consciousness, brought back upon their deleterious effects on human, animal and environmental health, lead to shortage their use by imposing various rules [1].

There are three types of pyrethroid insecticides: type I (T syndrome) pyrethroids produce abnormal sensitivity and coarse tremors leading to prostration. Type II (CS syndrome) pyrethroids produce ptialism and coarse tremors progressing to twisting movements of the neck and tail, whereas type I/II or TS pyrethroids produce signs of both whole-body tremors and salivation [2].

Previous studies have reported data indicating that these pyrethroids, which are widely used insecticides, induce oxidative stress through the generation of free oxygen radicals. Abnormal production of free radicals leads to damage of some macromolecules including proteins, lipids, and nucleic acids, and this is believed to be involved in the etiology of many chemicals and diseases [3–5].

Some experimental studies have shown that vitamins C and E (vit C and vit E) can be used to counteract pesticide toxicity [6,7]. Several biological defense mechanisms against intracellular oxidative stress are present in the organism such as antioxidant enzymes [superoxide dismutase, catalase (CAT), glutathione reductase, and glutathione transferase], and nonenzymatic antioxidants such as carotenoids, Vit E, vit C, and glutathione can also act to overcome the oxidative stress of the pesticides [8].

Antioxidant vitamins are the most important free radical scavengers in extracellular fluids, trapping radicals in the aqueous phase and protect biomembranes from peroxidative damage [6,9]. Some investigators have reported that the administration of vit E may be useful in controlling the toxic effect of insecticides and chemicals [10].

Therefore, the aim of the present study was

- (1) To evaluate whether deltamethrin (DLM) induced biochemical perturbations in rats.
- (2) To investigate the possible protective effects of vit E on deltamethrin induced toxicity and its role as inhibitor for free radicals generated following pesticide exposure during 30 days.

Materials and methods

Chemicals

DLM is a synthetic pyrethroid insecticide (Fig. 1) (purity over 98%) that was synthesized in the Laboratory of Applied Organic Chemistry, Chemical Industries Division, National Research Centre (Egypt) according to a known method [11]. DLM was administered orally at 1/20 LD₅₀ (7.5 mg/kg). Vit E (α -tocopheryl acetate) was supplied by Kahira Pharmacy and Chemistry (Cairo, Egypt).

Animals and drug administrations

Male albino Wister rats, 6 weeks old and weighing 120–160 g, were selected from an inbred colony maintained in the Animal House of the National Research Centre (Giza, Egypt) under controlled conditions of a temperature of $25 \pm 2^\circ\text{C}$, and a normal photoperiod (12 h dark: 12 h light). Animals were housed in cages throughout the experiment (with each cage housing six animals), fed on pellet diet and water *ad libitum*, and allowed to acclimatize to the laboratory environment for 7 days.

Experimental design

After 1 week of acclimation, rats were randomly divided into four groups, each containing six animals, and the route of administration selected for the study was oral (using oral feeding needles). The animals were grouped as follows: the rats in group I (control) received dimethylsulfoxide (1 ml); the rats in group II were administered DLM dissolved in dimethylsulfoxide (1 ml) at a dose of 7.5 mg/kg body weight corresponding to 1/20 LD₅₀; and the rats in group III received vit E only (100 mg/kg b.w.). Previous studies have shown that this dose was effective against the toxicity of DLM [7]; the rats in group IV received DLM at a dose 7.5 mg/kg b.w. plus vit E, respectively. Treatment duration was once a day daily for 1 month.

Biochemical analysis

At the end of the experimental period, animals were sacrificed by cervical decapitation, blood was collected from the arterial plexus, and divided into two parts: the first part was collected in a dry test tube, left at room temperature to clot, and then centrifuged at 3000 rpm for

10 min to separate the serum that was used for the assay of biochemical parameters. The other part was collected in heparinized tubes for the assay of cholinesterase activity in plasma. Plasma acetylcholinesterase enzyme activity was examined using acetylthiocholine iodide as a substrate according to Ellman *et al.* [12]. Blood parameters were determined using kits from Biodiagnostic Company, Egypt.

In serum, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to Reitman and Frankel [13]. Serum alkaline phosphatase (ALP) activity was measured by Kind and King [14]. CAT and GST enzyme activity was determined according to Aebi [15] and Habig [16], respectively. Serum samples were analyzed for the total protein (TP) concentration by the biuret method [17]. Serum albumin (ALB) concentration was determined using the Sigma diagnostics [18]. Blood urea nitrogen (BUN) concentration was determined according to urease-modified Berthelot reaction [19]. Serum creatinine determination was carried out according to Jaffe reaction [20]. Also, plasma was assayed for cholesterol, triglycerides by the method Carr *et al.* [21]. HDL and LDL were determined according to the methods of Warmick *et al.* [22] and Bergmeyer [23], respectively.

Statistical analysis

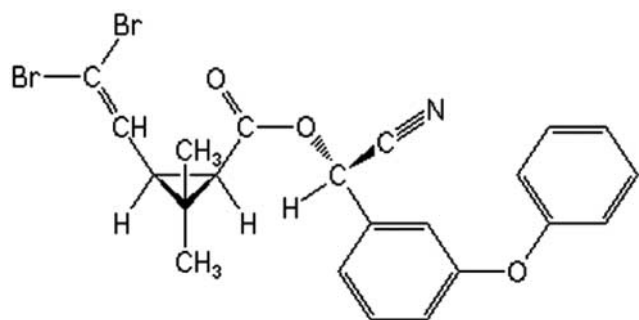
The obtained data from serum biochemical and enzymes analysis were statistically evaluated for the mean and standard error of the mean of each group. The significance of the changes between the tests and the control group was evaluated by the “*t*” test according to Sendecor and Cochran [24].

Results

The effects of DLM, vit E, and their combination on serum enzymes in the rats are shown in Table 1. The results indicated that the activities of ALT, AST, and ALP were significantly ($P < 0.01$) increased, whereas the activities of CAT, GST, and AchE were significantly ($P < 0.05$) decreased in the serum of rats treated with DLM for 30 days compared with the control group. However, treatment with vit E alone did not induce any significant change in the enzyme activities in the serum, whereas vit E in combination with DLM alleviated its negative effect on the activities of the above-measured enzymes (Table 1).

Renal profile biomarkers such as blood urea and creatinine showed a significant ($P < 0.01$) increase in the group of rats treated with DLM compared with the control rats at the end of the experiment. In addition, the presence of vitamin plus DLM led to a reduction in the elevation of urea and creatinine, and maintained normal values compared with the control group at the end of 30 days of treatment. In terms of the changes in total protein and albumin, a significant decrease was found in rats treated with DLM, whereas insignificant changes were found when these

Figure 1



Deltamethrin.

Table 1 Change in enzyme activities of rats after daily oral administration of deltamethrin, vitamin E, and deltamethrin plus vitamin E for 30 days

Enzymes	Experimental groups			
	Control	DLM	Vitamin E	DLM + vitamin E
ALT (U/l)	34.67 ± 2.58	52.50 ± 3.00**	36.50 ± 2.63	38.00 ± 2.98
AST (U/l)	91.30 ± 4.80	123.00 ± 5.60**	99.50 ± 4.85	106.30 ± 5.30
ALP (U/l)	62.67 ± 3.95	80.50 ± 3.48**	58.80 ± 3.40	63.50 ± 4.23
CAT (U/mg protein)	0.466 ± 0.03	0.27 ± 0.02*	0.50 ± 0.04	0.423 ± 0.02
GST (μmol/mg protein)	1.44 ± 0.08	0.95 ± 0.05*	1.55 ± 0.09	1.39 ± 0.06
AChE (μmol/min/ml)	2.20 ± 0.13	1.68 ± 0.28*	2.19 ± 0.25	1.96 ± 0.18

All data are expressed as means ± SD.

AChE, acetylcholinesterase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; DLM, deltamethrin; GST, glutathione S-transferase.

*Significant at $P < 0.05$ compared with the control.

**Significant at $P < 0.01$ compared with the control.

Table 2 Blood biochemistry of rats after daily oral administration with deltamethrin, vitamin E, and deltamethrin plus vitamin E for 30 days

Parameters	Experimental groups			
	Control	DLM	Vitamin E	DLM + vitamin E
Urea (mg/dl)	40.67 ± 2.58	74.25 ± 3.00**	41.00 ± 2.63	49.25 ± 2.98
Creatinine (mg/dl)	0.697 ± 0.08	1.11 ± 0.60**	0.72 ± 0.05	0.73 ± 0.30
TP (g/dl)	7.45 ± 0.95	5.65 ± 0.48**	7.78 ± 0.40	6.99 ± 0.23
ALB (g/dl)	3.79 ± 0.03	2.71 ± 0.02**	3.7 ± 0.04	3.59 ± 0.02

All data are expressed as means ± SD.

ALB, albumin; DLM, deltamethrin; TP, total protein.

**Significant at $P < 0.01$ compared with control.

Table 3 Blood lipid and lipoprotein profiles of rats after daily oral administration with deltamethrin, vitamin E, and deltamethrin with vitamin E for 30 days

Parameters	Experimental groups			
	Control	DLM	Vitamin E	DLM + vitamin E
Cholesterol (mg%)	103.33 ± 5.60	123.75 ± 6.74**	112.5 ± 5.32	98.75 ± 4.66
Triglycerides (mg%)	84.76 ± 4.98	135.50 ± 7.46**	93.75 ± 5.83	90.50 ± 5.00
HDL (mg%)	22.33 ± 1.67	14.50 ± 1.30**	25.75 ± 1.46	23.75 ± 1.80
LDL (mg%)	64.06 ± 2.38	80.90 ± 3.45**	68.00 ± 2.80	56.85 ± 2.34

All data are expressed as means ± SD.

DLM, deltamethrin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

**Significant at $P < 0.01$ compared with the control.

groups of rats were treated with vit E alone and in combination with DLM (Table 2).

The present data showed that plasma cholesterol, triglycerides, and low-density lipoprotein concentrations were significantly ($P < 0.01$) increased by DLM treatment, whereas the high-density lipoprotein level was decreased compared with the control animals (Table 3). Vit E alone caused an insignificant decrease in the lipid profile and minimized the toxic effects of DLM. Vit E in combination with DLM reduced the elevation in serum lipids and minimized the toxic effects of deltamethrin.

Discussion

Insecticides are chemicals used widely in agriculture, environmental, human and animal health fields. Exposure

to insecticides has been associated with many hazardous effects [25]. Determination of the common mechanism of toxicity in mammals is complicated by the number of potential biological target sites and effects exerted by various pyrethroid insecticides on these targets [26]. In the present study, we observed a significant increase in AST, ALT, and ALP activities in DLM-treated rats. ALT and AST are important indicators of liver damage in clinical finding. The increase in the activities of these enzymes may be because of the increase in the secretory activities of the hepatocyte cells, which were in agreement with the findings of El-Demerdash *et al.* [27] and Yousef *et al.* [7]. Biochemical analysis carried out by Tuzmen *et al.* [28] and El-Maghraby *et al.* [29] showed that administration of the chlorpyrifos and DLM caused damage in rat liver. The decrease in the activities of GST and SOD (Table 1) is in agreement with the results of Yousef *et al.* [7], who found oxidative stress and alteration in antioxidant enzymes in erythrocytes of

pyrethroid-intoxicated rats. (Pyrethroids [30] and other pesticides [31–33] have been reported to cause oxidative damage in studies conducted in various animals species by using at various doses for various periods. Dichlorvos causes subacute and subchronic hepatotoxicity, and vitamins C and E decreased dichlorvos toxicity, but did not confer complete protection [34]. Modulatory effects of DLM were recorded on antioxidant defense mechanisms and lipid peroxidation in fish liver and intestine [35]. In general, pesticide intoxication produces oxidative stress by the generation of free radicals and induced tissue lipid peroxidation in mammals and other organisms [36]. Reduced activities of antioxidant enzymes (CAT, SOD) after treatment of pesticides are important indicators for the toxicity of these chemicals [37]. Rahman *et al.*, [4] was determined to have reported induction in the level of lipid peroxidation and decrease in CAT activity following the administration of deltamethrin to albino mice for a period of 15 days at doses of 5.6 mg/kg and 18 mg/kg. Thus, significant increase in antioxidant enzyme levels in group 4 (DLM + vit E) when compared to group II (only deltamethrin) suggests protective potential of vitamin E. The decrease in AChE activity could be due to the decrease of the enzyme synthesis by the inhibitory nature of toxicant. Accumulation of pesticides in the liver is reported to disrupt lipid metabolism and increase serum cholesterol levels [38]. Significant increase in total cholesterol, triglycerides and LDL and a significant decrease in HDL in the toxic control indicate hepatopathy, cardiac damage as well as renal failure [39], which could be probably due to free radical-induced oxidative damage. In group 4, supplementation of vitamin E revealed a significant alternation in lipid profile to normal. This could be attributed to the antioxidant property of vitamin E [40]. In this study, the blood urea and serum creatinine levels were significantly increased in group 2 at the end of 4th wk, which could be attributed to the free radical induced oxidative damage by deltamethrin on kidney. Serum levels of creatinine and urea were used as indicator of renal function. Elevated blood urea is known to be linked with an increased protein catabolism to urea as a result of increased synthesis of arginase enzyme involved in urea production [41]. It can be concluded that vitamin E, as an antioxidant, has protective effect against deltamethrin adverse effects by scavenging free radicals generated following pesticides exposure and supplementation of vitamin E might be beneficial to deltamethrin exposed populations.

In our study, the blood urea and creatinine levels were significantly increased in the group treated with vit E plus DLM at the end of 30 days, which could be attributed to the free radical induced oxidative damage by DLM on the kidney. Elevated blood urea is known to be linked to an increased protein catabolism to urea as a result of increased synthesis of arginase enzyme involved in urea production [34]. It can be concluded that chronic exposure to DLM causes hepatic and renal toxicities which may be due to the release of free radicals and the lipid peroxidation that it induces. The use of vit E was found to reduce the

harmful effects of DLM in the mentioned parameters. Supplementation of vit E might be beneficial to diazinon-exposed populations [35]. Our findings show that DLM exerted adverse effects on some hematological and biochemical parameters. Vit E was observed to improve the hematological and biochemical changes induced by DLM. It can be concluded that vit E, as an antioxidant, may protect against the adverse effects of DLM by scavenging the free radicals generated following pesticide exposure.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

- Dikshit AK, Pachauri DC, Jindal T. Maximum residue limit and risk assessment of beta-cyfluthrin and imidacloprid on tomato (*Lycopersicon esculentum* mill). *Bull Environ Contam Toxicol* 2003; 70:1143–1150.
- Shafer TJ, Meyer DA, Crofton KM. Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. *Environ Health Perspect* 2005; 113:123–136.
- Parvez S, Raisuddin S. Copper modulates non-enzymatic antioxidants in the freshwater fish *Channa punctata* (Bloch) exposed to deltamethrin. *Chemosphere* 2006; 62:1324–1332.
- Rehman H, Ali M, Atif F, Kaur M, Bhatia K, Raisuddin S. The modulatory effect of deltamethrin on antioxidants in mice. *Clin Chim Acta* 2006; 369:61–65.
- Huang YL, Yu FZ, Si YW, Shi N. NF-E2 related factor 2 activation and heme oxygenase-1 induction by tert-butylhydroquinone protect against deltamethrin-mediated oxidative stress in PC12 cells. *Chem Res Toxicol* 2007; 20:1242–1251.
- Yavuz T, Delibas N, Yildirim B, Altuntas I, Candir O, Cora A, *et al.* Vascular wall damage in rats induced by methidathion and ameliorating effect of vitamins E and C. *Arch Toxicol* 2004; 78:655–659.
- Yousef MI, Awad TI, Mohamed EH. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. *Toxicology* 2006; 227:240–247.
- Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. *Br J Nutr* 2001; 85 (Suppl 2): S67–S74.
- Sulak O, Altuntas I, Karahan N, Yildirim B, Akturk O, Yilmaz HR, Delibas N. Nephrotoxicity in rats induced by organophosphate insecticide methidathion and ameliorating effects of vitamins E and C. *Pesticide Biochem Physiol* 2005; 83:21–28.
- Ateşşahin A, Yilmaz S, Karahan I, Piriñçi I, Taşdemir B. The effects of vitamin E and selenium on cypermethrin-induced oxidative stress in rats. *Turk J Vet Anim Sci* 2005; 29:385–391.
- Lee N, McAdam DP, Skerritt JH. Development of immunoassays for the II synthetic pyrethroids. 1. Hapten design and application to heterologous and homologous assays. *J Agric Food Chem* 1998; 46:520–534.
- Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; 7:88–95.
- Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; 28:56–63.
- Kind PR, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J Clin Pathol* 1954; 7:322–326.
- Aebi H. Catalase in vitro. *Methods Enzymol* 1984; 105:121–126.
- Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; 249: 7130–7139.
- Gornall AC, Bardawill CJ, David MM. Determination of serum proteins by means of Biuret reaction. *J Biol Chem* 1949; 177:751–766.
- Doumas B, Watson W, Biggs H. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chem Acta* 1971; 31:87–96.
- Charles J, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Analytical Chemistry* 1977; 49:464–470.
- Harry H, Abraham R. Estimation of creatinine by Jaffe reaction. A comparison of three methods. *Clinical Chemistry* 1968; 14:222–228.

- 21 Carr TP, Andresen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. *Clinical Biochemistry* 1993; 26:39–42.
- 22 Warmick GR, Benderson V, Albers N. Selected methods procedure for quantitation of high-density lipoprotein cholesterol. *Clin Chem* 1983; 10: 91–99.
- 23 Bergmeyer HU. *Methods of enzymatic analysis*. Vol. 8 3rd ed. Weinheim: Verlag Chemie; 1985. pp. 154–160.
- 24 Sendecor WG, Cochran WG. *Statistical methods*. 6th ed. Ames, Iowa, USA: Iowa State University Press; 1967.
- 25 Kanbur M, Liman BC, Eraslan G, Altinordulu S. Effects of cypermethrin, propetamphos, and combination involving cypermethrin and propetamphos on lipid peroxidation in mice. *Environ Toxicol* 2008; 23:473–479.
- 26 Wolansky MJ, Harrill JA. Neurobehavioral toxicology of pyrethroid insecticides in adult animals: a critical review. *Neurotoxicol Teratol* 2008; 30:55–78.
- 27 El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Role of α -tocopherol and β -carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters and semen quality of male rats. *J Environ Sci Health* 2004; B39:443–459.
- 28 Tuzmen N, Candan N, Kaya E, Demiryas N. Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver. *Cell Biochem Funct* 2008; 26:119–124.
- 29 El-Maghraby S, Taha H, Hassan N. Effect of Anthum Graveolens L. extract on biochemical and histopathological alteration of deltamethrin in rats. *Journal of Bioanalysis and Biomedicine* 2010; 2:8–12.
- 30 Prasanthi K, Muralidhara K, Rajini PS. Fenvalerate-induced oxidative damage in rat tissues and its attenuation by dietary sesame oil. *Food Chem Toxicol* 2005; 43:299–306.
- 31 Goel A, Dani V, Dhawan DK. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. *Chem Biol Interact* 2005; 156:131–140.
- 32 Sharma Y, Bashir S, Irshad M, Nag TC, Dogra TD. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *Toxicology* 2005; 215:173–181.
- 33 Sadowska WI, Wójcik N, Karowicz BA, Bieszczad BE. Effect of selected antioxidants in beta-cyfluthrin-induced oxidative stress in human erythrocytes in vitro. *Toxicol In Vitro* 2010; 24:879–884.
- 34 Ogutcu A, Suludere Z, Kalender Y. Dichlorvos-induced hepatotoxicity in rats and the protective effects of vitamins C and E. *Environmental Toxicology and Pharmacology* 2008; 26:355–361.
- 35 Dinu D, Marinescu D, Munteanu MC, Staicu AC, Costache M, Dinischiotu A. Modulatory. Effects of deltamethrin on antioxidant defense mechanisms and lipid peroxidation in *Carassius auratus gibelio* liver and intestine. *Arch Environ Contam Toxicol* 2010; 58:757–764.
- 36 Hazarika A, Sarkar SN, Hajare S, Kataria M, Malik JK. Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. *Toxicol* 2003; 185:1–8.
- 37 Jayakumar T, Ramesh E, Geraldine P. Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl₄-induced liver injury in rats. *Food and Chemical Toxicology* 2006; 44:1989–1996.
- 38 Kalender S, Ogutcu A, Uzunhisarcikli M, Acikgoz F, Durak D, Ulusoy Y, Kalender Y. Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. *Toxicol* 2005; 211:197–206.
- 39 Kaneko JJ, Harvey JW, Michael LB. *Clinical biochemistry of domestic animals*. 5th ed. New York: Academic Press; 1997. pp. 857–879.
- 40 Upasani CD, Balaraman R. Effect of vitamin E, vitamin C and spirulina on the levels of membrane bound enzymes and lipids in some organs of rats exposed to lead. *Indian J Pharmacol* 2001; 33:185–191.
- 41 Yanardag R, Sacan OO. Combined effects of vitamin C, vitamin E, and sodium selenate supplementation on absolute ethanol-induced injury in various organs of rats. *Int J Toxicol* 2007; 26:513–523.