Subject Area:

Protective effects of antioxidant vitamins (C plus E) against oxidative damage induced by the insecticide imidacloprid in male rats

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Protective effects of antioxidant vitamins (C plus E) against oxidative damage induced by the insecticide imidacloprid in male rats

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Abstract

Introduction
Imidacloprid (IMC) is a synthetic neonicotinoid insecticide that is used in agriculture against harmful insects.

Aim
This study aimed to investigate the adverse effects of the IMC formulation on biochemical, hematological parameters, oxidative stress markers, pro-inflammatory cytokines, alpha-fetoprotein (AFP), and testosterone levels in male rats, and to determine the potential protective activity of vitamin C and E against IMC insecticides in rats.

Material and methods
The rats were divided into four groups. The first group was used as a control. The second, third, and fourth groups received vitamin C (200 mg/kg) plus vitamin E (200 mg/kg), IMC (45 mg/kg), and IMC plus vitamin C and E, respectively, for 28 days. At the end of treatment, blood and tissue samples were collected. Biochemical, hematological parameters, oxidative stress markers, pro-inflammatory cytokines, AFP, and testosterone were evaluated.

Results
The results elucidated that the IMC significantly caused elevation in the serum liver and kidney functions, lipid profile, AFP, and pro-inflammatory marker levels, as well as liver and kidney oxidative stress markers. While the serum testosterone level and complete blood count were disturbed, besides that, the liver and kidney antioxidant enzymes activities were decreased.

Conclusions
The protective effect of vitamins C plus E against IMC toxicity in the rat have a beneficial role in combating the adverse effects of IMC.

Keywords: Hematological, imidacloprid, inflammatory cytokines, oxidative stress, testosterone, vitamin E plus C

INTRODUCTION

Imidacloprid (IMC) [1-(6-chloro-3-pyridylmethyl)-N-nitro-2-imidazolidinimine] is a nicotine-chlorinated analog. In 1992, it was recorded for commercial use as a pesticide via the United States Environmental Protection Agency and became highly used worldwide. IMC is a soluble and nondegradable pesticide. So, it remains in the underground water for a long time leading to pollution of the environment and causing many harmful effects on nontarget organisms. IMC metabolites may leak into the food chain, which is an important prospective source of the probable adverse effects of IMC pollution [1]. Although IMC is not very toxic to mammals (median lethal dose (LC50)≈450 mg/kg body weight), it may have an effect on many of its organs, such as the heart and kidneys; besides

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that it may induce gastrointestinal irritation, neurological symptoms, or even death. Many studies were documenting other adverse effects like teratogenicity [1], mutagenicity [2], neurotoxicity [3], and immunotoxicity [4,5]. Also, there are reports recording that the accumulative exposure of the agrochemical environmental pollutants such as pesticides, herbicides, and heavy metals in mammals stimulate depletion in the antioxidant defense system and the generation of oxidative free radicals in mammalian' tissues. The extravagant generation of the oxidative free radicals in the tissues declines the antioxidant guard leading to functional integrity disturbance of mitochondrial membrane structures and other cytoplasmic organelles through peroxidation of phospholipids, proteins, and nucleotides [6]. Oxidative stress-induced tissue injury and activated the nuclear factor-kappa B (NF-KB) pathway, which upregulates the expression of the inflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and in turn, the inflammatory cytokines may further enhance oxidative stress [7]. Therefore, elevation of oxidative free radicals plays an important role in the pathogenesis of many vital organs [8]. Also, Bal *et al*. [9] documented that exposure to IMC increased germ cell apoptosis and fragmentation of seminal DNA and elevated the rate of sperm abnormalities. In addition, it decreased the serum testosterone level and impairs fertility.

Antioxidant supplementation may mitigate IMC-induced toxicity. So, the current study was designed to elucidate the role of vitamin C (i.e. ascorbic acid) plus vitamin E (i.e. α-tocopherol) supplementation in alleviating the toxic effect of IMC in male rats.

MATERIALS AND METHODS

Chemicals and reagents

IMC ($C_9H_{10}CIN_5O_2$) commercial formulation (Imidachem 35%SC) was purchased from Egyptchem Co., Shibin el Kom, Menofia, Egypt. Vitamin C ($C_6H_8O_6$) and vitamin E ($C_{29}H_{50}O_2$) were obtained from Pharco Co., Abdeen, Egypt. The biochemical assay kits including aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALK), albumin (ALB), urea, creatinine, uric acid, total protein (TP), cholesterol (CHOL), triglyceride (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were obtained from diagnostic systems GmbH (Holzheim, Germany) Company. Malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), and catalase (CAT) were purchased from Biodiagnostic Co. (Dokki, Cairo, Egypt). Erma Pce 210 n (Tokyo, Japan) was used for white blood cell count, red blood cell count, hemoglobin (Hb) concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count. In the differentiation of white blood cells, a smear of blood was taken, then fixed, and stained by a Wright-Giemsa stain from Sigma-Aldrich Company (St. Louis, Missouri, USA). Testosterone, TNF-α, IL-6, and alpha-fetoprotein (AFP) were measured by an ELISA kit, which was purchased from a bioassay technology laboratory (Shanghai, China).

Experimental animals

Male healthy Sprague-Dawley rats were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Giza, Egypt. All animals were preserved in polypropylene cages, with free access to a standard pellet diet and water. They were kept under laboratory conditions (48% relative humidity and 24 ± 3°C). The experimental rats were rested to acclimatize for 1 week before the start of the experiment. All studies were performed in accordance with the guidance for the care and use of laboratory animals, as adopted and promulgated by the Ethics Committee of the Scientific Research, GOTHI, Ministry of Health, Egypt (Regd. No. IMEOOO54, dated: 30/3/2021).

Experimental protocol

In all, 24 adult male Sprague-Dawley rats, weighing 150 ± 5 g, were randomly divided into four groups (six rats/each group); the first group of rats received corn oil (1 ml/rat) and served as the normal control group (group I). The second group of rats received vitamin C (200 mg/kg body weight) and vitamin E (200 mg/kg body weight) suggested by Uzun and Kalender (2011) [10], which were dissolved in distilled water and corn oil, respectively, by the oral route for 28 consecutive days. The rats of the third group were orally administered with IMC [45 mg/kg/body weight (1/10 of the lethal dose 50)] according to IPCS (2001) [11] for 28 days. Group IV rats received 1 ml of vitamin C plus vitamin E, then after 30 min received IMC for 28 days.

Collection of blood samples

At the end of the experimental period and a 12 h fasting period, blood samples were collected from the retro-orbital venous plexus of the animals with a fine sterilized glass capillary. The blood samples were collected from all groups into two types of vacutainer tubes; one of them contains EDTA for assaying complete blood pictures and the other without any additives. Samples without any additives were left to clot in clean and dry tubes and then centrifuged at 3000 rpm (600g) for 10 min at 4°C using HeraeusLabofuge 400 R (Kendro Laboratory Products GmbH, Germany) to get the serum for assaying biochemical parameters (AST, ALT, ALK, ALB, TP, urea, creatinine, uric acid, CHOL, TGs, HDL, and LDL), pro-inflammatory cytokines (TNF-α, IL-6), AFP, and testosterone levels according to kit instructions.

Evaluation of antioxidants in tissues

After blood collection, the laboratory rats were killed by cervical dislocation. The liver and kidneys were rapidly dissected out and cleaned. The tissues were cleaned with saline solution, weighed, cut into small parts, homogenized in 10% (w/v) ice-cold 100 mmol/l phosphate buffer (pH 7.4), and centrifuged at 4500 r/min for 15 min at 4°C, then the supernatant was collected and stored in -20°C until measurements of the antioxidant enzyme (CAT, SOD) as well

as the oxidative stress markers (MDA and NO) according to the methods described by Abdel-Wahhab *et al.* (2018) [12].

Statistical analysis

The study results were analyzed using SPSS, version 20 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, Illinois, USA). The results are expressed as the means ± SE; a probability level of less than 0.05 was accepted as statistically significant. The results from each experimental group were compared using a *t* test analysis.

RESULTS

In Table 1: serum liver biomarkers (ALT, AST, ALK, TP, and ALB) as well as kidney biomarkers (urea and creatinine) and uric acid were mainly measured for the evaluation of the liver and renal damage. ALT, AST, ALK, uric acid, urea, and creatinine concentrations were significantly increased in serum in IMC-exposed rats (group III) in comparison with the control group (group I), while TP and ALB concentrations were significantly decreased in group III in comparison with the control group (group I). The results indicated that administration of vitamin C plus E to rats received IMC (group IV) modulated significantly the level of liver and kidney biomarkers in comparison with the IMC-treated group (group III).

The level of CHOL, TG, and LDL were significantly increased in the IMC-treated group (group III) in comparison with the control group (group I) (Table 2). On the other hand, IMC decreased significantly the level of HDL compared with the control group (group I), while the lipid profile had modulated significantly in vitamin C and E plus the IMC-treated group (group IV) in comparison with the IMC-treated group (group III).

Table 3 shows a decreasing in the hematological indices values such as red blood cell, Hb, hematocrit, white blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, lymphocytes, monocytes, and eosinophils, but neutrophil values were increasing in the rats exposed to IMC (group III) in comparison

to the control group (group I). These hematological indices were statistically modulated in group IV in comparison to the IMC-treated group (group III).

The effects of IMC on antioxidant enzyme activities (CAT and SOD) in both the liver and kidney tissue are shown in Table 4. Activities of CAT (0.8 U/g tissue vs. 1.96 U/g tissues) and SOD (10.92 U/g tissue vs. 18.29 U/g tissues) in liver homogenate in group III were significantly declined compared with the control group (group I). In addition, the CAT activity (0.93 U/g tissue vs. 2.32 U/g tissues) and SOD (10.68 U/g tissue vs. 19.69 U/g tissues) in the kidney homogenate in group III were significantly diminished compared with the control group (group I). Administration of vitamin C plus vitamin E to rats received IMC (group IV) significantly recovered the activities of both of them in comparison to the IMC-treated group (group III) in both the tissues. The oxidative stress marker (MDA and NO) activities in liver and kidney homogenates were statistically elevated in the IMC-treated group (group III) compared with the control group (group I). However, these marker activities ameliorated with the treatment of vitamin C plus E (group IV) compared with the IMC-treated group (group III).

Effects of IMC on the level of AFP, TNF, and IL-6 in different male rat groups are shown in Figs. 1–3, respectively. Data showed an increment in AFP, TNF-α, and IL-6 levels after 28 days of IMC exposure (group III) in comparison to the control group (group I). Administration of vitamin C plus vitamin E to rats received IMC (group IV) decreased AFP, TNF-α, and IL-6 levels in comparison of the IMC-treated group (group III).

Serum testosterone hormone level was reduced in adult male rats exposed to IMC (group III) at a dose of 45 mg/kg/body weight for 28 days compared with the control group (group I), as shown in Fig. 4. Administration of vitamin C plus vitamin E to rats received IMC (group IV) corrected the serum testosterone level in comparison to the IMC-treated group (group III).

Table 1: Effects of vitamin C and E on the serum liver and kidney biomarkers, as well as the serum uric acid level in the imidacloprid-treated rats

Parameters	Groups			
	Group I	Group II	Group III	Group IV
ALT (U/l)	40.60±3.218	44.20±2.956	74.80±1.855[a, b]	62.00±2.302[a, b, c]
AST (U/l)	92.00±3.376	90.80±3.929	122.60±3.572[a, b]	103.60±2.976[a, b, c]
ALK (U/l)	82.400±3.749	89.400±4.864[a]	120.00±1.702[a, b]	107.80±2.354[a, b, c]
TP (g/dl)	6.180±0.107	6.460±0.234[a]	4.460±0.199[a, b]	5.480±0.1855[a, b, c]
ALB (g/dl)	3.400±0.130	3.480±0.107	1.780±0.275[a, b]	2.980±0.1562[a, b, c]
Urea (mg/dl)	41.00±1.817	42.600±1.600	66.800±2.939[a, b]	52.600±2.839[a, b, c]
Creatinine (mg/dl)	0.586±0.021	0.600±0.026	1.84±0.199[a, b]	0.844±0.052[a, b, c]
Uric acid (mg/dl)	4.00±0.330	4.14±0.250	6.080±0.215[a, b]	5.180±0.116[a, b, c]

Values are mean ± SE, *n*=6, group I: control group, group II: vitamin C-treated and E-treated group, group III: imidacloprid-treated group, group IV: vitamin C and E plus imidacloprid-treated group. ALB, albumin; ALK, alkaline phosphatase; ALT, alanine aminotransferases; AST, aspartate aminotransferases; TP, total protein. [a]*P* value less than 0.05 versus group I, [b]*P* value less than 0.05 versus group II, [c]*P* value less than 0.05 versus group III.

Table 2: Effects of vitamin C and E on the lipids profile in the imidacloprid-treated rats

Parameters	Groups			
	Group I	Group II	Group III	Group IV
CHOL (mg/dl)	141.00±2.43	141.40±2.713	200.00±1.703[a, b]	173.0±4.888[a, b, c]
TG (mg/dl)	205.80±1.685	209.40±1.860[a]	233.80±4.352[a, b]	219.20±2.498[a, b, c]
HDL (mg/dl)	37.40±2.581	48.60±2.542[a]	17.60±1.503[a, b]	27.80±1.497[a, b, c]
LDL (mg/dl)	52.80±3.993	55.600±3.919	87.400±2.249[a, b]	68.60±1.208[a]

Values are mean ± SE, *n*=6, group I: control group, group II: vitamin C- and E-treated group, group III: imidacloprid-treated group, group IV: vitamin C and E plus imidacloprid-treated group. CHOL, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride. [a]*P* value less than 0.05 versus group I, [b]*P* value less than 0.05 versus group II, [c]*P* value less than 0.05 versus group III.

Table 3: Effects of vitamin C and E on hematological parameters in the imidacloprid-treated rats

Parameters	Groups			
	Group I	Group II	Group III	Group IV
RBCs (10^6/cm)	4.670±0.097	4.834±0.056 [a]	4.054±0.059[a, b]	4.320±0.033[a, b, c]
WBCs (10^3/cm)	3.928±0.207	3.920±0.260	2.162±0.128[a, b]	3.008±0.173[a, b, c]
Hb (g/dl)	13.988±0.300	14.348±0.201	12.204±0.152[a, b]	12.912±0.106[a, b, c]
HCT (%)	41.952±0.905	43.044±0.603	36.424±0.310[a, b]	38.736±0.317[a, b, c]
MCV (fl)	89.720±0.107	89.22±0.781	89.374±0.333	90.200±0.192[a, b, c]
MCH (pg)	30.20±0.207	30.32±0.271	29.34±0.307[a, b]	29.80±0.192[a, b]
MCHC (%)	33.420±0.086	33.380±0.149	33.080±0.106[a, b]	33.20±0.071[a, b, c]
Platelet (10^3/cmm)	475.40±24.949	488.60 26.716	345.80±15.366[a, b]	381.60±10.906[a, b, c]
Neutrophil (%)	38.500±1.339	38.208±1.736	48.474±0.503[a, b]	44.904±1.703[a, b, c]
Lymphocyte (%)	45.220±1.750	45.648±1.439	34.612±0.912[a, b]	39.554±0.767[a, b, c]
Monocyte (%)	6.746±0.260	7.040±0.2801	5.178±0.177[a, b]	5.950±0.101[a, b, c]
Eosinophil (%)	1.350±0.069	1.368±0.062	1.072±0.022[a, b]	1.164±0.030[a, b, c]
Basophil (%)	0.608±0.037	0.648±0.032	0.454±0.014[a, b]	0.502±0.016[a, b, c]

Values are mean ± SE, *n*=6, group I: control group, group II: vitamin C- and E-treated group, group III: imidacloprid-treated group, group IV: vitamin C and E plus imidacloprid-treated group. Hb, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; WBC, white blood cell. [a]*P* value less than 0.05 versus group I, [b]*P* value less than 0.05 versus group II, [c]*P* value less than 0.05 versus group III.
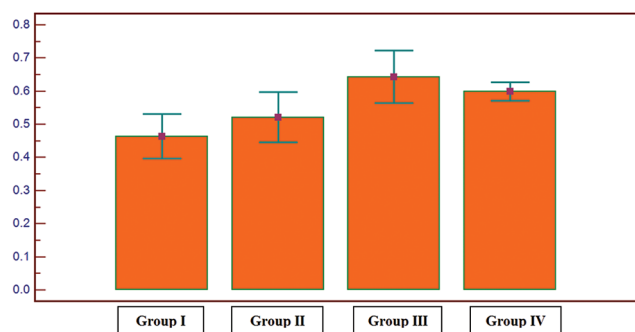

Figure 1: Effects of vitamin C and E on AFP in imidacloprid (IMC)-treated rats. Group I: control group, group II: vitamin C-treated and E-treated group, group III: IMC-treated group, group IV: vitamin C and E plus IMC-treated group. AFP, alpha-fetoprotein.
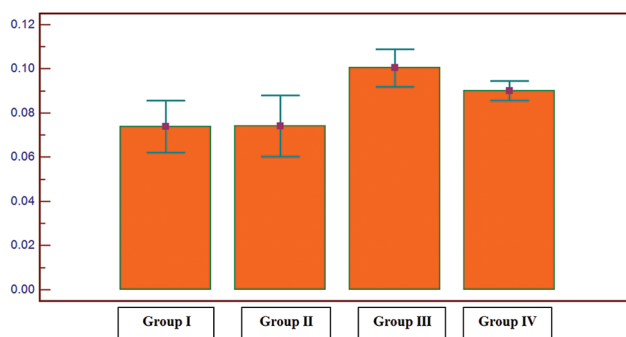

Figure 2: Effects of vitamin C and E on TNF-α in imidacloprid (IMC)-treated rats. Group I: control group, group II: vitamin C-treated and E-treated group, group III: IMC-treated group, group IV: vitamin C and E plus IMC-treated group. TNF-α, tumor necrosis factor α.

Discussion

IMC is one of the most commercially neonicotinoid insecticides, especially in developing countries, and it is one of the seven major insecticides marketing globally [13]. IMC accumulation in tissues has been associated with increased oxidative stress and overwhelms the antioxidant capacity, and leads to the production of oxidative free radicals (ROS/RNS) in mammalian tissues [14]. In the current study, IMC exposure produced oxidative stress in the liver and kidney tissues as evidenced by the significant elevation of MDA and NO with depletion CAT and SOD antioxidant enzyme activities. The elevation in the levels of MDA and NO in these vital organs is an indication of damage to all cell components, including proteins, lipid, and DNA as well as tissue injury. So, IMC

Table 4: Effects of vitamin C and E on hepatic and renal oxidative stress markers in the imidacloprid-treated rats

Groups	Liver				Kidney			
	CAT (U/g)	SOD (U/g)	NO (umol/g)	MDA (nmol/g)	CAT (U/g)	SOD (U/g)	NO (µmol/g)	MDA (nmol/g)
Group I	1.96±0.178	18.29±0.392	63.27±1.267	51.02±2.347	2.32±0.203	19.69±0.354	70.97±0.930	87.46±3.413
Group II	2.18±0.259	18.78±0.326	65.37±1.121[a]	51.90±2.496	2.38±0.227	20.21±0.297[a]	71.34±1.610	89.20±2.801
Group III	0.80±0.058[a, b],	10.92±0.337[a, b],	97.36±1.568[a, b],	90.30±1.770[a, b],	0.93±0.067[a, b],	10.68±0.345[a, b]	102.13±1.877[a, b]	115.12±2.459[a, b]
Group IV	1.05±0.045[a, b, c]	15.41±0.323[a, b, c]	78.26±2.912[a, b, c]	65.48±2.272a[b, c]	1.21±0.105[a, b, c]	15.02±0.405[a, b, c]	85.038±1.896[a, b, c]	101.80±1.834[a, b, c]

Values are mean ± SE, $n=6$, group I: control group, group II: vitamin C-treated and E-treated group, group III: imidacloprid-treated group, group IV: vitamin C and E plus imidacloprid-treated group. CAT, catalase; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase. [a]P value less than 0.05 versus group I, [b]P value less than 0.05 versus group II, [c]P value less than 0.05 versus group III.
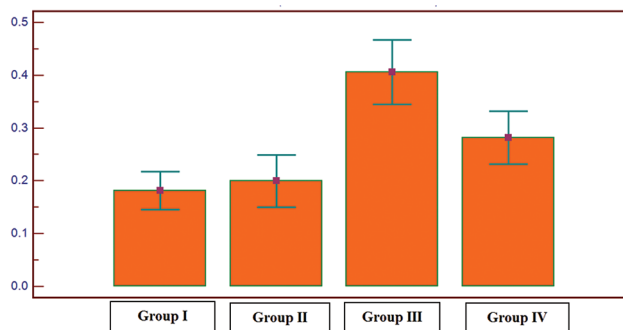

Figure 3: Effects of vitamin C and E on IL-6 in imidacloprid (IMC)-treated rats. Group I: control group, group II: vitamin C-treated and E-treated group, group III: IMC-treated group, group IV: vitamin C and E plus IMC-treated group. IL-6, interleukin 6.

exposure causes an elevation in serum liver and kidney functions as well as increasing the serum level of AFP due to hepatotoxicity and nephrotoxicity [15,16]. Uric acid is the end product of purine catabolism and its serum level has a relation with creatinine clearance decreasing and a correlation with the degree of renal tubulointerstitial damage [17]. In this study, the elevation of uric acid level may be related to either increasing protein degradation, which is involved in the uric acid formation, or the toxic effect of IMC on the kidneys.

Moreover, IMC induces toxicity throughout oxidant-mediated responses like apoptotic or necrotic cell death, membrane lipid peroxidation, metabolic perturbation, and deregulation of several signaling pathways as the NF-kB pathway [18]. High oxidative stress levels significantly upraised the mRNA expression of pro-inflammatory cytokines (IL-6, IL-8, TNF-α) [7]. In addition, IMC caused elevation oxidative stress in erythrocytes. Erythrocytes are targeted by the high oxidative stress due to the existence of Hb and polyunsaturated fatty acids. Oxidative stress is linked with raising the osmotic fragility of erythrocytes. Osmotic is caused by a lack of harmony in the manufacturing of reactive oxygen species and the capacity of the biological systems to quickly detoxify the reactive intermediates or readily remedies the resulting damage [19]. The explanation of the elevated serum lipid profile level in the case of exposure to IMC is that IMC enhances adipogenesis and the serum lipid profile parameters (CHOL, TG, and LDL) while decreases the serum HDL level through regulation of the lipid and glucose metabolisms through the AMP-activated protein kinase-α (AMPKα) pathway in the white adipose tissue and the liver [20]. In addition, the inflammation process is often accompanied by an increase in serum TG and CHOL levels [21].

IMC and its metabolites disrupted the steroid hormone biosynthesis through an imbalance of the cytochrome P450 (CYP) enzyme activities that play a key role in the synthesis and breakdown of several steroid hormones. So, exposure to IMC has a negative effect on the reproductive function in mammals. In males, it adversely affected their testicular function during early postnatal development and in
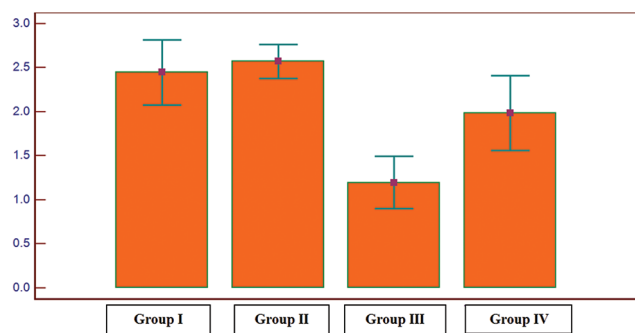
Figure 4: Effects of vitamin C and E on testosterone in imidacloprid (IMC)-treated rats. Group I: control group, group II: vitamin C-treated and E-treated group, group III: IMC-treated group, group IV: vitamin C and E plus IMC-treated group.

adulthood. Subchronic and chronic exposure to different doses of IMC can cause histological deterioration in the testicular tissue, apoptosis of germ cells, fragmentation of seminal DNA, deforms in sperm morphology, elevated sperm mortality and abnormality, diminished sperm count and motility, inhibited spermatogenesis, as well as decreased testosterone levels in male rats, all of which could drive to infertility [1].

Antioxidant vitamins such as vitamins C and E have various biological activities. Vitamin E is the most important lipophilic antioxidant and is naturally found in the cell membrane to maintain its stability; besides that, it is an important chain-breaking antioxidant [22]. Vitamin C is an important water-soluble antioxidant and plays an important role as a free-radical scavenger in extracellular fluids and in protecting both cytosolic and membrane components of cells from oxidant stress distraction. It directly reduces the pro-oxidative state through enzymatic and nonenzymatic reactions [23]. In the current study, serum liver and kidney function levels were at least partially normalized when vitamins C plus E were given together with IMC. Also, treatment with antioxidant vitamins neutralized ROS and serum lipid profile, thus preventing IMC-induced derangement in the antioxidant enzyme activities (Fig. 5). In addition, antioxidant vitamins act on downregulation of the pro-inflammatory cytokines (TNF-α and IL-6) expression through inhibition of NF-kB activation, which is activated by IMC [24]. Moreover, antioxidant vitamin supplementations elevate the testosterone level and increased the sperm quality [25,26]. In addition, the antioxidant vitamin supplementation modulated the serum lipid profile, erythrocytes, and platelets through reducing oxidative stress [27,28].

Conclusion

It can be concluded that vitamin C plus E supplementations have a protective role against toxicity induced by exposure to IMC. So, adequate dietary intake of vitamin C plus E by individuals who are exposed to pesticides and other environmental toxins is profitable in combating their negative effects.
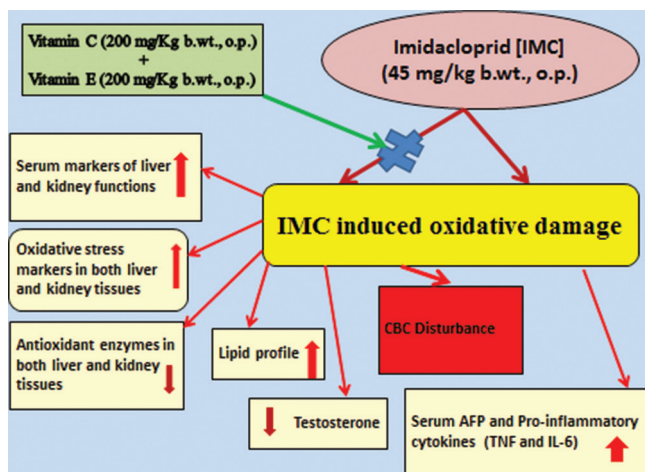

Figure 5: Effects of imidacloprid (IMC) on biochemical, hematological parameters, oxidative stress markers, pro-inflammatory cytokines, AFP, and testosterone levels in male rats, and potential protective activity of vitamin C and E against IMC insecticides in rats. AFP, alpha-fetoprotein.

Conflicts of interest
There are no conflicts of interest.

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