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RESEARCH ARTICLE

Antioxidant capacity of omega-3-fatty acids and vitamin E against imidacloprid-induced hepatotoxicity in Japanese quails

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Abstract

Imidacloprid (IM) is a neonicotinoid insecticide, used in a wide range of agricultural activities worldwide. However, it results in ecosystem disturbances and signs of toxicity in human and animals. The current study was designed to elucidate the protective effects of omega-3-fatty acids (OFAs) and vitamin E (Vit E) against IM hepatotoxicity in Japanese quails. Seventy male quails (30 days old) were divided into seven groups ($n = 10$); G_1 −ve control; G_2 received IM (+ve control); G_3 received OFA; G_4 received Vit E; and G_5, G_6, and G_7 received OFA and/or Vit E with IM for 30 days, respectively. Blood and liver tissue samples were collected. Imidacloprid significantly ($p < 0.05$) increased serum levels of alanine transferase (ALT), aspartate transferase (AST), triglycerides (TGC), and low-density lipoprotein cholesterol (LDL-C), as well as liver tissue malondialdehyde (MDA) concentration. Moreover, IM caused a significant ($p < 0.05$) decrease in the levels of serum high-density lipoprotein cholesterol (HDL-C), as well as liver superoxide dismutase (SOD) enzyme activity and reduced-glutathione (GSH) concentration in comparison to the –ve control group. Histopathological changes in hepatocytes, including thick cell trabeculae with marked hydropic vacuolar degeneration of cytoplasm, were found in IM-treated group. Treatment with OFA and/or Vit E resulted in significant improvements in general body condition, serum HDL-C level, and liver tissue SOD enzyme activity and GSH concentration, as well as significant decreases in the levels of serum AST, ALT, TGC, LDL-C, and hepatic tissue MDA. In conclusion, OFA and Vit E have a protective effect against IM toxicity, especially in their combination.

Keywords Imidacloprid · Insecticide · Omega fatty acids · Oxidant/antioxidant makers · Vitamin E

Introduction

Imidacloprid (IM) is a new insecticide, belonging to neonicotinoids family (Kammon et al. 2012; Karunker et al. 2009), which has a worldwide distribution due to its use in both agricultural (Adejumo et al. 2015) and veterinary fields (Lyon et al. 1995). This is probably due to its selective toxicity to insects and apparent safety in humans and livestock (Stanneck et al. 2012). Besides the agricultural use of IM, it is used in the veterinary practice (Kammon et al. 2010) to control insects in poultry farms (Simon-Delso et al. 2015), where it is applied through spraying, painting of walls, or washing the walls and utensils (Lyon et al. 1995). However, many users are not aware of the therapeutic dose of IM and the possible ingestion of IM-sprayed seeds, plants, and water (Mason et al. 2013), which may precipitate IM overdose, toxicity, and even death in birds (Kammon et al. 2010).

Several outbreaks in migratory birds have been recorded in countries that used neonicotinoids for the first time, such as India, France, and the USA (Mason et al. 2013). Imidacloprid induces oxidative stress through increasing the production of free radicals and lipid peroxidation (Kobayashi et al. 2004; Sauer et al. 2014). The body tries to compensate these disturbances through increasing the activity of endogenous antioxidants, such as reduced glutathione (GSH) and superoxide dismutase (SOD) enzyme (Kapoor et al. 2010). However, the continuous production of free radicals leads to exhaustion of this antioxidant machinery (Kapoor et al. 2011). Moreover, it results in disturbances in the immune system functions and tissue integrity (Banerjee et al. 2001). Therefore, antioxidant supplementation may mitigate IM-induced toxicity (Kammon et al. 2012).

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Omega-3-fatty acids (OFAs) and vitamin E (Vit E) have been shown to alleviate the xenobiotic-induced oxidative stress (Abushouk et al. 2017; Grotto et al. 2011; Wefers and Sies 1988). Omega-3-fatty acids enhance the efficacy of antioxidant enzyme system (Ruiz-Gutierrez et al. 1999) and exert anti-inflammatory effects through decreasing leukotriene and prostaglandin production (Grotto et al. 2011; Lobo et al. 2016). Vit E is a fat-soluble vitamin, which contributes to numerous physiological processes related to health (DelCurto et al. 2013) through its antioxidant capacity, which prevents the production of free radicals in tissues (Wefers and Sies 1988).

Japanese quail (*Coturnix japonica*) is an interesting, domesticated bird for commercial meat and egg production (Ahmed et al. 2015a). Besides, Japanese quail birds are considered an environmental pollution indicator (US-EPA 1996) and an accepted model for assessing both acute and chronic effects of pesticides (OECD 1993; US-EPA 1996). Quails were used to examine the toxic effects of IM because cytochrome P450 enzymes' levels in humans, chicken, and quail livers are not high. Therefore, the compound stays for a longer period in the body resulting in higher tissue accessibility (Abu-Qare et al. 2001; Hansen et al. 2011) and more sensitivity to toxicity, compared to rats, which have a higher catalytic activity.

Therefore, the current study was designed to elucidate the toxic implications of IM, as well as the hepatoprotective and antioxidant effects of OFA and Vit E against IM-induced hepatotoxicity and oxidative stress damage in male Japanese quails.

Material and methods

Birds and experimental design

Adapted 70 male Japanese quails (30 days old) were used. They were housed in a battery system with adjusted environmental conditions (temperature 24 ± 2 °C and humidity 50 ± 10%). This study was carried out in strict accordance with the recommendations in the guide for the care and use of birds and the protocol was approved by the Ethical Committee of Animal Experiments at Suez Canal University. The birds were randomly divided into seven groups ($n = 10$); G_1 −ve control; G_2 +ve control receiving imidacloprid [IM, [1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine] (Quick Bayt®, Bayer pharmaceutical Co., Egypt) (Glover 2012) in water using gastric gavage at 3 mg/kg BW (1/10 LD_{50}; the acute oral toxicity of IM is 30 mg/kg) (Kammon et al. 2012); G_3 received OFA (a mixture of docosahexaenoic acid and eicosapentaenoic acid, El-Captain Co. Egypt) in the feed by 1% (Lopez-Ferrer et al. 2001) of the ration; G_4 received Vit E (α-tocopherol) (Pharco Co., Egypt) in the feed by 400 mg/kg diet (Chitra et al. 2014; Sahin et al. 2002); G_5, G_6, and G_7 received OFA and/or Vit E with IM, respectively. All treatments simultaneously continued for 30 consecutive days (Kadota and Miyamoto 1975).

Sampling

Blood sampling

After 30 days of the experiment, all birds were slaughtered and blood samples were collected into two different (EDTA and plain) tubes. The blood samples in the EDTA tubes were used for assessing the hematological parameters,while those in the plain tubes were centrifuged at 3000 rpm for 15 min for separation of sera. The collected sera were stored at − 20 °C until used for analysis of different biochemical parameters.

Tissue sampling

The liver was rapidly dissected out and washed free of blood with 0.9% NaCl solution. A part of the liver from the same lobe was fixed for 24 h in 4% paraformaldehyde in PBS pH 7.4 (Sigma-Aldrich Chemical). Then, it was kept in 70% ethanol for histopathological examination (Bancroft et al. 1996). The other part was frozen and stored at − 20 °C until analysis.

Biochemical analysis

Sera were used for kinetic estimation of liver tissue enzymes. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated according to Klauke et al. (1993), while that of alkaline phosphatase (ALP) was evaluated according to Bowers and McComb (1975). Serum low-density lipoprotein (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were determined according to Esteban-Salán et al. (2000) and Badimon et al. (1990), respectively. Moreover, we used kits from Randox Laboratories Ltd. (UK) to assess serum triglycerides (TGCs) according to Grundy et al. (2004).

Evaluation of liver lipid peroxidation and antioxidant enzymes markers

In frozen liver, hepatic lipid peroxidation was evaluated by measuring MDA concentration according to Tukozkan et al. (2006). Moreover, we assessed SOD enzyme activity according to Marklund (1979) and GSH level according to Akerboom and Sies (1981), using kinetic enzymatic assays (BioAssay Systems Co., USA).

Histopathological examination

The fixed liver samples were dehydrated through a series of ethanol-graded concentrations, clarified in xylene, embedded in paraffin, and sectioned at 4 μm. The liver sections were stained with hematoxylin and eosin for detection of histological alterations. The sections were examined by a calibrated standard digital Olympus® CX21 microscope camera (Tuscan ISH1000 digital microscope camera) with a resolution of 10 megapixels (3656 × 2740 pixel each image) and × 400 magnifications UIS optical system (Universal Infinity System, Olympus, Japan).

Statistical analysis

Data were tested for homogeneity distribution of variance, and statistical analyses were performed using the GraphPad Prism (version 5.01, San Diego, USA) and Minitab (version 17) software. The analysis was performed using one-way ANOVA followed by Dunn's tests. All data were expressed as means ± standard error of means (SEM) and a probability value of $p < 0.05$ was considered statistically significant.

Results

General health condition

Imidacloprid adminstration resulted in weight loss (Table 1), ruffled feathers, and signs of intoxication, including irregular locomotion, ataxia, spastic muscle contractions, dropped head, and sluggish movement.

Relative liver weight

Imidacloprid toxicity resulted in a significant ($p < 0.05$) decrease in relative liver weight in comparison to the −ve control group (Table 1). Treatment with OFA and/or Vit E led to a significant elevation in the relative liver weight in comparison to the +ve control group (IM).

Biochemical analysis

Liver function enzymes

Imidacloprid administration significantly ($p < 0.05$) increased serum levels of AST (59.14 U/L) and ALT (34.29 U/L), when compared with the −ve control group (34.14 and 24.14 U/L, Table 2), respectively. However, there was no significant increase in ALP level (Table 2). Treatment of IM-intoxicated quails with OFA resulted in a significant improvement in AST (36.57 U/L) level only, but no change in ALT level in comparison with the +ve control group. On the other hand, Vit E caused no significant improvements in both AST and ALT levels compared to the +ve control group. Interestingly, treatment of IM-intoxicated quails with a combination of OFA and Vit E resulted in a significant decrease in the levels of both AST (33.14 U/L) and ALT (24.42 U/L) compared with the +ve control group and restored their values within the normal range.

Lipid profile

Imidacloprid administration resulted in a significant ($p < 0.05$) increase in serum levels of TGC (169.57 mg/dl) and LDL-C (159.8 mg/dl) and a significant reduction in HDL-C (29.86 mg/dl) in comparison with the −ve control group (71.86, 47.7, and 46.29 mg/dl, respectively) (Table 2). Treatment with a combination of OFA and Vit E resulted in a significant ($p < 0.05$) decrease in TGC (77.14 mg/dl) and LDL (65.29 mg/dl) levels, as well as a significant ($p < 0.05$) increase in HDL (43.67 mg/dl) level in comparison with the +ve control group (Table 2).

Antioxidant marker enzymes and lipid peroxidation

Quails, intoxicated with IM, showed significantly lower hepatic tissue levels of GSH and SOD enzyme activity and increased tissue MDA content, compared to the −ve control group. Treatment of IM-intoxicated quails with either OFA or Vit E caused a significant reduction in MDA hepatic tissue content, as well as a significant increase in GSH concentration and SOD enzyme activity. The latter changes were more

Table 1 The protective effect of OFA and Vit E against the toxic effect of IM on the relative liver weight of male Japanese quails ($n = 10$)

	Control −ve	IM	OFA	Vit E	IM + OFA	IM + Vit E	IM + OFA + Vit E
Initial Bw	165.5 ± 7.20a	168 ± 3.20a	170.7 ± 3.27a	162.4 ± 3.71a	171.5 ± 2.92a	163.7 ± 1.50a	167.8 ± 3.6a
30 days Bw	200.5 ± 2.24a	134.9 ± 2.73b	205.7 ± 2.15a	197.9 ± 2.18a	178.9 ± 3.08ab	171.1 ± 1.34ab	190.4 ± 2.06ab
R. liver weight %	2.44 ± 0.03a	1.01 ± 0.06e	2.43 ± 0.08a	2.2 ± 0.02a	1.75 ± 0.04c	1.36 ± 0.07d	2.17 ± 0.01b

Values are expressed as mean ± SEM. Different letters (a, b, c, d, e) are significantly different ($p < 0.05$)

Bw body weight, *R* relative, *IM* imidacloprid, *OFA* omega-3 fatty acids, *Vit E* vitamin E

Table 2 Effect of OFA and Vit E against the toxic effect of IM on serum biochemical parameters of male Japanese quails ($n = 10$)

	Control −ve	IM	OFA	Vit E	IM + OFA	IM + Vit E	IM + OFA + Vit E
AST (U/L)	34.14 ± 4.2b	59.14 ± 4.63a	29.83 ± 3.05b	34.36 ± 1.44b	36.57 ± 3.13b	49 ± 4.6ab	33.14 ± 2.42b
ALT (U/L)	24.14 ± 2.38ab	34.29 ± 2.09a	25.86 ± 2.6ab	22.57 ± 2.16b	25.71 ± 2.39ab	26 ± 1.46ab	24.42 ± 2.52b
ALP (U/L)	427.4 ± 41.23ab	644.6 ± 45.7a	418.7 ± 36.28ab	387.7 ± 20.44b	541.1 ± 56.23ab	580.1 ± 89.61ab	342.7 ± 37.37b
TGC (mg/dl)	71.86 ± 5.95c	169.57 ± 7.89a	69.86 ± 9c	69.86 ± 6.46c	100.14 ± 11.46bc	116.43 ± 7.26b	77.14 ± 6.77c
HDL (mg/dl)	46.29 ± 2.87a	29.86 ± 3.49b	33.57 ± 3.48a	35.86 ± 3.44a	31.29 ± 4.49b	33.14 ± 4.35a	43.67 ± 3.7a
LDL (mg/dl)	47.7 ± 7.6b	159.8 ± 11.95a	67.86 ± 6.09b	53.5 ± 13.59b	130 ± 6.26a	150 ± 13.37a	65.29 ± 16.51b

Values are expressed as mean ± SEM. Different letters (a, b, c) are significantly different ($p < 0.05$)

ALP alkaline phosphatase, *ALT* alanine transferase, *AST* aspartate transferase, *HDL* high-density lipoprotein cholesterol, *IM* imidacloprid, *LDL* Low-density lipoprotein cholesterol, *OFA* omega-3 fatty acids, *TGC* Triglycerides, *Vit E* vitamin E

significant in IM-intoxicated quails receiving OFA and Vit E combination (Table 3).

Histopathological results

Compared to histological sections from the −ve control group (Fig. 1a), those from IM-intoxicated quails showed disturbed architecture, hepatocytes arranged in thick cell trabeculae with marked hydropic vacuolar degeneration of cytoplasm, congested vessels, and obliterated sinusoids (Fig. 1b). Treatment of IM-intoxicated quails with OFA (Fig. 1c) and Vit E (Fig. 1d) resulted in the liver hepatocytes showing abundant cytoplasm with residual mild to moderate hydropic degeneration and a slight distortion of architecture (the improvement was more marked in OFA group compared to Vit E group). The combination of OFA and Vit E restored the normal appearance of hepatocytes with abundant eosinophilic cytoplasm and small nuclei, arranged in thin cellular trabeculae in a lobular architecture, separated by thin-walled blood sinusoids (Fig. 1e).

Discussion

Due to the importance of quail meat for humans (Ahmed et al. 2015b), it is important to ensure the safety of these birds through controlling vector-borne diseases. Therefore, IM is used in a wide range of quail farms to control insects. However, farmers' unawareness of the safe IM use resulted in several cases of IM toxicity (Abdollahi et al. 2004). Besides, IM was classified as a toxic agent to birds with LD50 ranges between 25 and 50 mg/kg (Abd-El-Ghaney 2002; Berny et al. 1999). There is ample evidence to suggest that using insecticides in poultry/livestock houses causes residual contamination of animals' tissues which may later be consumed by humans (Eissa 2004; Shadnia and Moghaddam 2008; Wu et al. 2001). Therefore, in the current study, we evaluated the use of natural substances, such as OFA and Vit E, to alleviate IM hepatotoxicity.

The IM intoxication resulted in a retarded general health condition with sluggish movement. These results agreed with Bhaskar and Mohanty (2014) who reported a significant decrease in mice general performance for which IM-induced hypothyroidism was proposed as a possible underlying mechanism. Moreover, the irregular locomotion and spastic muscle contractions, observed in our study, may be due to IM mode of action as a nicotinic acetylcholine receptor (nAChRs) agonist (Kawahata and Yamakuni 2018; Stanneck et al. 2012). Treatment of IM-intoxicated quails with OFA and/or Vit E resulted in a significant improvement in health status in comparison with IM group. Further benefits, such as improving appetite, digestibility rate, absorption, metabolic rate, and immune response were reported for both compounds (OFA and Vit E) in former studies on chicken and broilers (Attia et al. 2017; Elzobier et al. 2016; Kammon et al. 2012; Saleh et al. 2009).

Oxidative stress is a biochemical disturbance in which production of free radicals, such as reactive oxygen species (ROS), exceeds the capacity of endogenous antioxidant

Table 3 The protective effect of OFA and Vit E against the toxic effect of IM on oxidative/anti-oxidative stress markers of male Japanese quails ($n = 10$)

	Control −ve	IM	OFA	Vit E	IM + OFA	IM + Vit E	IM + OFA + Vit E
GSH (mg/g tissue)	36.07 ± 0.98a	19.17 ± 0.88c	34.97 ± 0.97a	35.05 ± 0.90a	30.31 ± 0.70b	28.22 ± 1.26b	32.12 ± 0.96ab
SOD (U/g tissue)	35.07 ± 0.98a	19.17 ± 0.88c	34.97 ± 0.97a	35.05 ± 0.90a	30.31 ± 0.70ab	28.22 ± 1.26b	32.12 ± 0.96a
MDA (nMol/g tissue)	0.255 ± 0.019c	0.64 ± 0.04a	0.259 ± 0.021c	0.255 ± 0.020c	0.49 ± 0.038b	0.57 ± 0.038ab	0.27 ± 0.031c

Values are expressed as mean ± SEM. Different letters (a, b, c) are significant different ($p < 0.05$)

GSH reduced glutathione, *IM* imidacloprid, *MDA* malondialdehyde, *OFA* omega-3 fatty acids, *SOD* superoxide dismutase, *Vit E* vitamin E

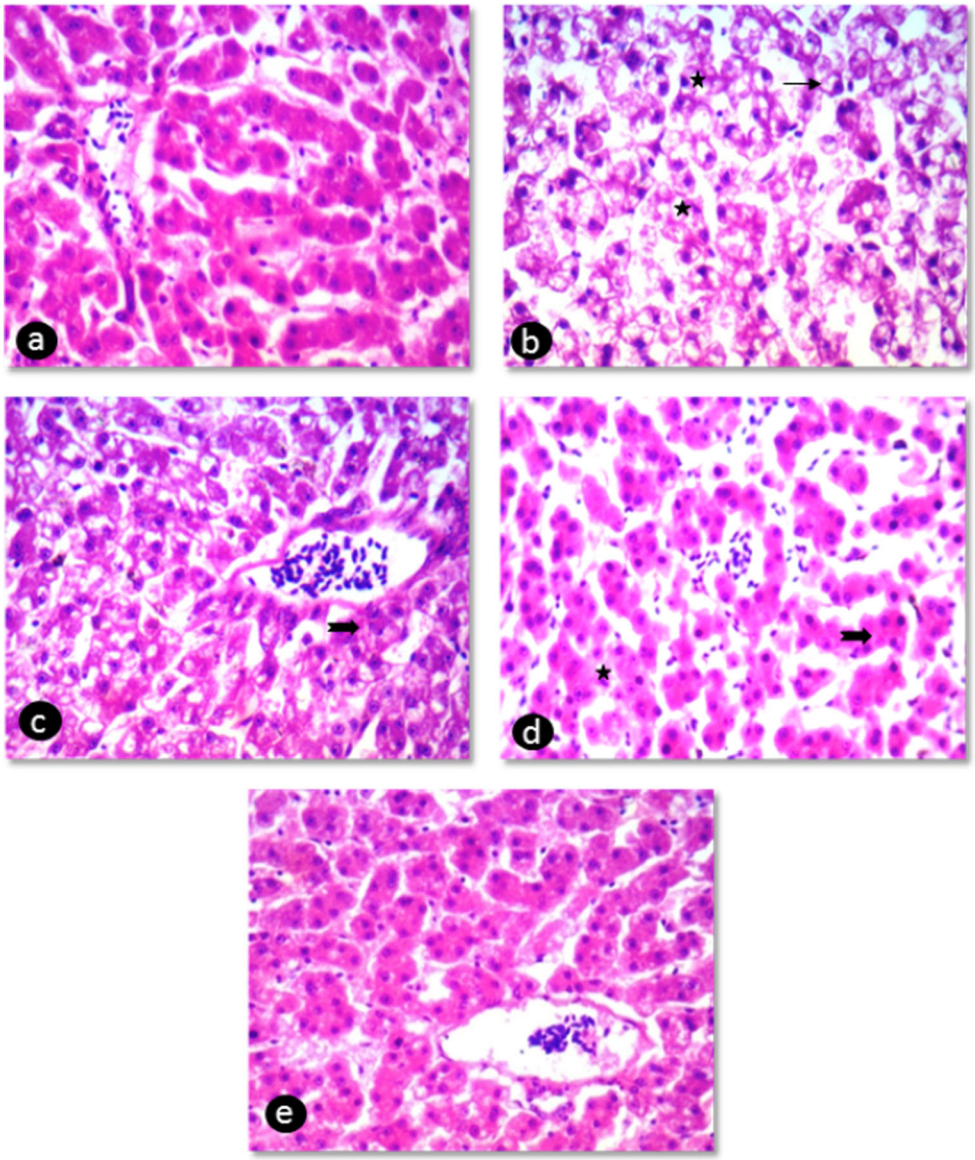

Fig. 1 Liver sections of Japanese quails. **a** Control –ve quails: The figure shows that hepatocytes had an abundant eosinophilic cytoplasm with small nuclei. These hepatocytes are arranged in thin cell trabeculae in lobules, separated by thin wall blood sinusoids. **b** Control +ve quails (IM toxicity): The figure shows that the liver had disturbed architecture, hepatocytes arranged in thin cell trabeculae (thin arrow) with marked hydropic vacuolar degeneration (black star) of cytoplasm. **c** OFA-treated quails against IM: The figure shows that hepatocytes had abundant cytoplasm (thick arrow) with residual mild hydropic degeneration and slight distortion of architecture indicating moderate improvement. **d** Vit E-treated quails against IM: The figure shows that hepatocytes have abundant cytoplasm (thick arrow) with residual moderate hydropic degeneration (black star) and mild distortion of architecture indicating mild improvement. **e** OFA + Vit E-treated quails against IM: The figure shows that hepatocytes had abundant eosinophilic cytoplasm with small nuclei. These hepatocytes are arranged in thin cell trabeculae in a lobular architecture, separated by thin wall blood sinusoids indicating marked improvement (H&E stain, × 400)

agents, causing membrane lipid peroxidation and damage to cellular organelles (Ahmed et al. 2017; Muthukumaran et al. 2008). Several pesticides have been shown to induce oxidative stress and lipid peroxidation through increasing the production of free radicals (Broznić et al. 2008; Etemadi-Aleagha et al. 2002). In the current research, IM administration resulted in a significant elevation in MDA hepatic tissue level and a significant decline in GSH and SOD enzyme levels in comparison with the −ve control group. According to earlier reports on IM toxicity, this may be attributed to the consumption of GSH and SOD to compensate for the increased free radical production (Duzguner and Erdogan 2010; Kapoor et al. 2011). Moreover, other authors reported that nicotine treatment induces the CYP2A1 and decreases GSH concentration and the activities of SOD and GPx in the liver and lungs of rats (Muthukumaran et al. 2008; Yamazaki et al.

1999). It is possible that agonists of nAChR, such as IM similarly induce oxidative processes.

Duzguner and Erdogan (2010) reported that exposure to IM for 2 h significantly altered the antioxidant capacity in the liver and the central nervous system. The authors also found that IM treatment significantly stimulated the xanthine oxidase and myeloperoxidase enzymes, which catalyze the synthesis of superoxide anion and hypochlorous acid in the liver and brain, respectively (Duzguner and Erdogan 2010). Moreover, oxidative stress (through stimulation of xanthine oxidase and myeloperoxidase enzymes) is a common product of ionic imbalance or elevated calcium, which have been reported in IM toxicity (Sharma and Rohrer 2007; Zayas et al. 2002).

In the present study, the antioxidant effectiveness against IM was more significant with the OFA-Vit E combination than the administration of either one. These results agreed with Sen et al. (1997) who recorded that OFA-Vit E combination is more effective in controlling oxidative stress than each one separately. Vit E has the ability to reduce lipid peroxidation (Erol et al. 2017) and decrease free radical production (Song et al. 2017), while OFA has the ability to halt inflammation (Bo et al. 2016). In turn, this leads to a marked decrease in MDA level and a significant improvement in the levels of GSH and SOD.

The liver is the major site of metabolism, including detoxification and activation of many substances (Hall 2015). Hepatic liver enzymes, such as ALT, AST, and ALP are useful indicators in the assessment of hepatotoxicity and liver damage (Koolman et al. 2005). As well known, oxidative stress, which is implicated in pesticide-induced toxicity, induces a cascade of biochemical changes (Abdollahi et al. 2004; Banerjee et al. 2001; Duzguner and Erdogan 2010). In agreement with the findings of previous studies (AL-Shinnawy and Abd-EL-Ghany 2009; Kammon et al. 2010), the present study showed that IM resulted in a significant decrease in relative liver weight and significant elevations in serum AST and ALT enzymes in comparison with the −ve control group. On the other side, El-Kashory (1999) noticed a reduction in ALT and ALP activities, when IM was used at a low concentration, either alone or combined with another insecticide as profenofos or carbosulfan in male albino rats (El-Kashory 1999). The difference may be attributed to the used IM concentration.

In our study, we noticed a significant reduction in serum levels of liver enzymes in IM-intoxicated quails after treatment with either Vit E or OFA; however, the drop was more significant with the latter one. These results agreed with Kammon et al. who clarified that OFA has a higher protective effect than Vit E because it protects the cells from the inlet and outlet, while Vit E has an outlet protection only (Kammon et al. 2012). Of note, the combination of OFA and Vit E against IM toxicity resulted in significant decreases in the serum levels of liver enzymes. Khanchandani (2015) explained that this combination can decrease the leak of liver enzymes to the serum through (1) the anti-inflammatory effect of OFA by regulating TNF-α and elevating the immune system reaction and (2) the ability of Vit E to bind the unsaturated fatty acids in the cell membrane, preventing lipid peroxidation (Khanchandani 2015).

Furthermore, treatment with OFA or Vit E, alone or in combination, significantly ameliorated the IM-induced increase in LDL-C and TGC serum levels and the decrease in HDL-C level. Popovic et al. showed that OFA improved liver phospholipids and improved plasma fatty acid profile and suggested that this effect is mediated by inhibiting the expression of hepatic genes encoding glycolytic and lipogenic regulatory enzymes (Popovic et al. 2012). On the other hand, Vit E exerts a role in preserving hepatocyte integrity, which are responsible for gluconeogenesis and this decreases lipid production (Bo et al. 2016). In agreement with the findings of Giudetti et al., the combination restored the serum levels of LDL-C, TGC, and HDL-C within the normal ranges (Giudetti et al. 2003).

On histopathological examination, the liver sections from IM-intoxicated quails showed disturbed hepatocytes' appearance with marked vacuolar degeneration of the cytoplasm. The vacuolation may be due to retention of fluid inside the hepatocytes resulting in what is termed hydropic degeneration or cloudy swelling which may be due to reduction of the energy necessary for regulation of membrane fluid transport (Mohan 2005), mild or short-term anoxia (Hruban et al. 1972), or metabolic stress (Eissa 2004). Similarly, previous studies found that IM administration resulted in marked dilatation, severe congestion of central veins, portal veins, and sinusoidal spaces in the liver and caused vacuolation and fatty changes in hepatocytes (Soujanya et al. 2013; Vohra and Khera 2015; Vohra et al. 2014). Ultra-thin sections of the liver revealed swollen nuclei, varied size and shape of mitochondria, disrupted chromatin, and rough endoplasmic reticulum, which could be due to IM-induced oxidative stress (Vohra and Khera 2015).

The current literature supports the use of natural antioxidants to reduce xenobiotics-oxidative stress (Abdel-daim et al. 2017; Eissa 2004). In the current research, OFA treatment resulted in a moderate improvement in IM-induced hepatic tissue histological damage, while Vit E treatment was less effective in this regard. The latter results are in consistence with those of Durak and colleagues who found that under an electron microscope, the cellular disturbances were still seen after Vit E treatment, but at a lower level than if the toxicant was administrated alone (Durak et al. 1996). A combination of OFA and Vit E against IM resulted in marked improvements in the liver tissue microscopic appearance. This confirms the findings of previous authors who recorded significant improvements in liver functions and appearance of vascular endothelial cells after treatment by OFA-Vit E combination (Bo et al. 2016; Ibrahim et al. 1997).

In conclusion, oxidative stress and lipid peroxidation play a major role in IM-induced hepatotoxicity and alterations in serum biochemical parameters. OFA and Vit E are potent antioxidants, which could ameliorate the hepatotoxic effects of IM and their combination provided near complete protection in terms of serum and tissue biochemical changes. Further research may focus on the nicotinic pathway of IM toxicity and its mechanism in the induction of oxidative stress.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Abbreviations ALP, alkaline phosphatase; ALT, alanine transferase; AST, aspartate transferase; GSH, reduced-glutathione; HDL, high-density lipoprotein cholesterol; IM, imidacloprid; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; OFA, omega-3 fatty acids; SOD, superoxide dismutase; TGC, triglycerides; Vit E, vitamin E

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