The Protective Role of β -Carotene and Hesperidin on Some Hematological and Myocardial Measurements against Imidacloprid Toxicity in Albino Rats

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Abstract: Neonicotinoids including IM (Imidacloprid) are widely used as plant systemic insecticides. Several studies have indicated that pesticide toxicity may be associated with the enhanced production of ROS (reactive oxygen species). Both β -carotene (β C) and hesperidin (H) have an antioxidant property and quench free radicals. This study aimed to clarify the protective role of β -carotene and hesperidin as natural antioxidants on IM induced toxicity in hematological parameters and markers of cardiac muscle activity in male albino rats. The treatment of rats with IM showed a significant decrease in hemoglobin (Hb %), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), HCT (hematocrit) values and RBCs count comparing with control group. On the other hand, MCV (mean corpuscular volume), WBCs (white blood cells) and Plts (platelets) count pronounced a significant increase in IM group comparing to control. Also, α FP (plasma alpha fetoprotein), CEA (carcinoembryonic antigen), CK (creatin kinase), CK-MB (creatin phosphokinase myocardial band) and LDH (lactate dehydrogenase) clarify a significant increase in IM group comparing to control. Both β -carotene and hesperidin mitigate the deleterious effects of IM on previous parameters. β -Carotene and hesperidin may protect hematopoietic system and heart muscle against toxicity of IM. These improvements of the results clarify the protective effect of the used antioxidants. Conclusion: β -carotene and hesperidin, natural antioxidants, have a protective effect against IM evoked hematological and biochemical changes.

Key words: β -carotene, hesperidin, IM, blood cells, cardiac enzymes, cancer markers.

1. Introduction

Pesticide toxicity may be associated with the enhanced production of ROS (reactive oxygen species), which could explain the multiple types of toxic responses observed. The production of ROS is caused by a mechanism in which xenobiotics, toxicants and pathological conditions may produce oxidative stress and induce damage to the liver, kidney and brain tissue [1]. Immediate human health hazards from pesticides include mild headaches, flu, skin rashes, blurred vision and other neurological disorders, and rarely, paralysis, blindness, and even death long run health impacts include cancer,

infertility, miss-carriage, male sterility, birth defects, and effects on the nervous system [2]. Neonicotinoids are widely used in plant systemic insecticides developed in the last some decades, IM (Imidacloprid) was launched in 1991 for the first time by Bayer Crop-Science [3].

IM is an extensive pesticide used for crop protection worldwide from the last decade due to its low soil persistence and high insecticidal activity at very low application rate and binding affinity at the nAChR (nicotinic acetylcholine receptor) [4].

IM-treated rats showed a significant decrease in RBCs count, Hb content and MCHC (mean corpuscular hemoglobin concentration) values [5]. IM exhibited a significant increase in total leucocytes count [6]. Oral administration of IM to male albino rats led to a significant decrease in Hb, PCV, MCV

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(mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) [7].

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells, as oxidative stress might be an important part of many human diseases, in addition to these use of natural antioxidants in medicine. For many years chemists have shown that free radicals cause oxidation which can be controlled or prevented by a number of antioxidants substances [8].

Epidemiological studies have shown that populations that frequently consume vegetables containing vitamins A, C, and E and beta-carotene (β C) have a lower incidence and risk of developing cancer and heart disease. Naturally occurring carotenoids and flavonoids possess free radical scavenging properties and offer cytoprotection from oxidative injury. Beta-carotene (β C) and other carotenoids exert antioxidant functions such as the quenching of singlet oxygen and other electronically excited molecules that are produced by photoexcitation or chemiexcitation reactions. They further react with peroxy or alkoxy radicals [9].

Beta-carotene (β C) is found in many foods that are orange in color, including sweet potatoes, carrots, cantaloupe, squash, apricots, pumpkin and mangoes. Some green, leafy vegetables, including collard greens, spinach and kale are also rich in beta-carotene, in another study, β C was shown to decrease mitomycin C-induced sister chromatid exchange frequencies in lymphocyte cultures obtained from Down's syndrome patients, furthermore, β C and folic acid revealed positive effects on mitomycin C—induced DNA breaks and apoptosis of endometrial stromal cells, as

determined by ultra-structural examinations; accordingly, β C reduced caspase-3 activity [10]. The chemical abilities of β C to quench singlet oxygen and to inhibit peroxy free-radical reactions are well established, characteristics of β C as an efficient antioxidant play an important role in decreasing the incidence of cancer [11] and cardiovascular diseases [12].

Flavonoids are an important group of polyphenolic compounds which play a protective role by inhibition of enzymes of oxygen-reduction pathways and sequestration of transient metal cations and also quenching free radicals [13]. Hesperidin, a flavanone glycoside found abundantly in citrus fruits, possesses a wide range of pharmacological properties including potential anti-inflammatory and anticancer effects [14]. Hesperidin induces cell growth arrest apoptosis in a large variety of cells including colon and pancreatic cancer cells [15]. Hesperidin may be associated with potential benefits for the prevention of diseases, like decreased capillary permeability, inflammation, microbial infection and carcinogenic effects. A number of researchers have examined the antioxidant activity and radical scavenging properties of hesperidin using a variety of assay systems [16].

Hesperidin is a flavonoid found mainly in citrus fruits. Flavonoids are polyphenolic compounds, which directly quench free radicals and inhibit enzymes of oxygen-reduction pathways, hesperidin was also shown to have a wide spectrum of pharmacological effects including anti-inflammatory, anti-carcinogenic, antihypertensive and anti-atherogenic effects [17]. Hesperidin improves the vascular integrity, decreases capillary permeability and is given as a supplement to patients with blood vessel fragility, also shows anti-inflammatory and immunomodulatory effects [18].

Hesperidin has non-toxic activity in normal cells, while it suppresses cell proliferation in several cancer types. Hesperidin is known to have

anti-inflammatory, anti-viral, UV protecting, antioxidant, pro-apoptotic, anti-proliferative, anti-tumor properties, and protective effects against cerebrovascular disease and diabetes mellitus [19]. In addition, hesperidin as a radio-protective and chemo-protective therapeutic agent is expected to prevent invasion or metastasis of human cancers [20]. Anti-cancer effect of hesperidin was studied in tumor implanted animal models or culture cell lines of several cancer types, including colon cancer, bladder cancer, hepatocarcinoma cancer and breast cancer, but the mechanism of this compound was not understood [21].

2. Material and Methods

2.1 Material

2.1.1 Chemicals

(1). IM: was obtained from the Agricultural Research Center, Cairo, Egypt. It was dissolved in dist. water and administrated daily by oral at two different doses, high dose $1/5$ LD₅₀ (90 mg/kg b.wt.) and low dose $1/10$ LD₅₀ (45 mg/kg b.wt.) for 30 days.

(2). Beta-carotene (β C): was obtained from El-Goumhouria Co. for Trading Medicine, Cairo, Egypt. It was dissolved in corn oil and given daily orally in a dose of 20 mg/kg b.wt. before administration of IM for 30 days.

(3). Hesperidin (H): was obtained from Sigma Co. for Trading Medicine, Cairo, Egypt. It was dissolved in dist. water and given daily orally in a dose of 100 mg/kg b.wt. before administration of IM for 30 days.

2.1.2 Animals and Experimental Design

Fifty four adult male albino rats (*Rattus norvegicus*), weighting 140-160 g were used, housed in separated galvanized iron cages in a thermostatically controlled room under 12 h light/dark cycle. They were maintained on standard laboratory diet with free access to water.

Rats were randomly divided into the following groups:

Group I received the vehicle and was considered as

normal control (n = 6 rats).

Group II

A. Animal received a daily low dose of imidacloprid (45 mg/kg, $1/10$ LD₅₀) orally for 30 days (n = 6 rats).

B. Animal received a daily high dose of imidacloprid (90 mg/kg, $1/5$ LD₅₀) orally for 30 days (n = 6 rats).

Group III

A. The rats were treated daily with low dose of imidacloprid (45 mg/kg) and beta-carotene at dose level of 20 mg/kg [22] orally or 30 days (n = 6 rats).

B. The animals received a daily high dose of imidacloprid (90mg/kg) and beta carotene (20 mg/kg) orally for 30 days (n = 6 rats).

Group IV

A. The rats received a daily low dose of imidacloprid (45 mg/kg) and hesperidin at dose of 100 mg/kg [23] orally for 30 days (n = 6 rats).

B. The animals received a single daily high dose of imidacloprid and hesperidin (100 mg/kg) orally for 30 days (n = 6 rats).

Group V

A. The rats received a low dose of imidacloprid orally after administration of beta-carotene and hesperidin in combination for 30 days (n = 6 rats).

B. Each animal received a daily high dose of imidacloprid orally after administration of beta-carotene and hesperidin in combination for 30 days (n = 6 rats).

Both beta-carotene and hesperidin were pretreated orally daily for 2 weeks before imidacloprid treatment.

2.1.3 Samples Collection

At the end of the experiment, blood was withdrawn from retro-orbital plexus of eyes. Blood portion collected upon anticoagulant (EDTA) for the hematological measurements, and other portion was collected in other centrifuge tube for serum parameters, where blood sample was centrifuged at 4,000 rpm for 10 min by using centrifuge. Non-hemolized serum stored at -20 °C till for

biochemical analysis.

2.2 Methods

2.2.1 Determination of Complete Blood Count

Complete blood counts [hemoglobin (Hb %), HCT (hematocrit), RBCs count, MCV, MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), Plt (platelets) count and total WBCs (white blood cells) count were evaluated [24] using SYSMEX reagents and Cell blood counter (SYSMEX)—Model XT 2000 I, A double capillary, full automatic, Japan.

2.2.2 Determination of Cardiac Enzymes

Serum CK (creatine kinase), CK-MB (serum creatine phosphokinase myocardial band) and serum LDH (lactate dehydrogenase) were done [25] using BECKMAN COULTER reagents and BECKMAN COULTER analyzer—Model AU 480, Full automatic, Japan.

2.2.3 Determination of Tumor Markers

α FP (Alpha fetoprotein) and CEA (carcinoembryonic antigen) in serum were done [26] using Elecsys reagents and COBAS analyzer—Model E 411, Full automatic, Japan

2.2.4 Statistical Analysis

The data were presented as mean value $\pm$ SE (standard error). The significance of difference

between the mean values was evaluated using Student's t-test [27].

3. Results

3.1 Hematological Results

As shown in Table 1 or Fig. 1, the results indicated a significant decrease ($p \leq 0.05$) in Hb% and Hct of rats treated with IM comparing with control group, while both β -carotene or/and hesperidin groups showed significant increases comparing with IM group. On the other hand, WBCs and Plt count of rats treated with IM were significantly elevated comparing with control group, while both β -carotene or/and hesperidin groups showed significant decreases comparing with IM group.

The results presented in Table 2 and Fig. 2, pronounced a significant decrease ($p \leq 0.05$) in MCH, MCHC and RBCs count of rats treated with IM groups at the low and high doses, while MCV of rats treated with IM elevated significantly comparing with untreated group. Both beta-carotene or/and hesperidin reduced the deleterious effects of IM on blood measurements comparing with control group. On the other hand, MCH, MCHC and RBCs count of β C+H+IM group did not change significantly compared with control group.

Table 1 Protective effect of β -carotene and hesperidin against Imidacloprid toxicity on Hb%, Hct, WBCs and Plt in albino rats.

Group	C	Antioxidants and insecticide							
		IM		BC+IM		H+IM		BC+H+IM	
		1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀
Hb% (gm/dl)	16.90 $\pm$ 0.20	* 12.40 $\pm$ 0.46	* 13.63 $\pm$ 0.35	*a 14.00 $\pm$ 0.35	*a 14.70 $\pm$ 0.38	*a 13.80 $\pm$ 0.38	* 14.27 $\pm$ 0.28	*a 14.50 $\pm$ 0.36	*ac 15.47 $\pm$ 0.27
Hct (%)	51.67 $\pm$ 2.32	* 35.60 $\pm$ 1.08	* 38.72 $\pm$ 0.89	*a 39.47 $\pm$ 0.62	* 40.27 $\pm$ 1.27	*a 38.93 $\pm$ 1.03	* 40.03 $\pm$ 0.51	*a 41.70 $\pm$ 0.96	*a 42.02 $\pm$ 0.93
WBCs $\times 10^3/\text{mm}^3$	9.40 $\pm$ 0.56	* 18.70 $\pm$ 0.91	* 16.80 $\pm$ 0.69	*a 14.60 $\pm$ 0.49	*a 13.50 $\pm$ 0.66	*a 15.63 $\pm$ 0.52	*a 14.60 $\pm$ 0.44	*ac 13.97 $\pm$ 0.42	*ac 12.40 $\pm$ 0.59
Plt $\times 10^3/\text{uL}$	468.00 $\pm$ 41.80	* 1149.00 $\pm$ 102.15	* 991.00 $\pm$ 94.11	*a 715.00 $\pm$ 44.51	*a 684.00 $\pm$ 60.52	*a 761.67 $\pm$ 74.00	*a 742.00 $\pm$ 51.85	*a 701.00 $\pm$ 76.25	*a 693.00 $\pm$ 75.15

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Fig. 1 Protective effect of β -carotene and hesperidin against Imidacloprid toxicity on Hb%, Hct, WBCs and Plt in albino rats.

where:

C: Control, IM: Imidacloprid, BC: Beta-Carotene, H: Hesperidin.

Each value is the mean $\pm$ SE, $n = 6$.

*: Significant difference from Control group, $p \leq 0.05$.

a: Significant difference from IM group, $p \leq 0.05$.

b: Significant difference from BC+IM group, $p \leq 0.05$.

c: Significant difference from H+IM, $p \leq 0.05$.

Table 2 Protective effect of β -carotene and hesperidin against Imidacloprid toxicity on RBCs, MCV, MCH and MCHC in albino rats.

Group	C	Antioxidants and insecticide							
		IM		BC+IM		H+IM		BC+H+IM	
		1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀
RBCs $\times 10^6/\text{mm}^3$	8.35 ± 0.17	* 5.40 ± 0.54	* 5.73 ± 0.73	* 6.23 ± 0.63	* 6.43 ± 0.84	* 5.95 ± 0.50	* 6.42 ± 0.37	ac 7.43 ± 0.52	ac 7.87 ± 0.32
MCV (fl)	57.85 ± 3.57	* 93.00 ± 8.49	* 92.35 ± 7.25	* 75.20 ± 7.48	* 75.03 ± 9.75	* 77.38 ± 6.14	* 71.12 ± 4.16	a 68.97 ± 5.72	a 61.82 ± 4.83
MCH (pg)	27.10 ± 2.10	* 23.88 ± 2.27	* 24.80 ± 1.96	* 23.45 ± 2.52	* 24.33 ± 2.90	* 23.83 ± 2.16	* 24.13 ± 1.08	* 19.86 ± 1.43	* 25.13 ± 1.35
MCHC (g/dL)	37.55 ± 0.45	* 27.55 ± 1.02	* 30.30 ± 0.77	* 31.12 ± 0.76	* 32.67 ± 0.86	* 30.68 ± 0.84	* 31.72 ± 0.63	* 32.23 ± 0.81	* 34.38 ± 0.60

Fig. 2 Protective effect of β -carotene and hesperidin against Imidacloprid toxicity on RBCs, MCV, MCH and MCHC in albino rats.

where:

C: Control, IM: Imidacloprid, BC: Beta Carotene, H: Hesperidin.

Each value is the mean $\pm$ SE, n = 6.

: Significant difference from Control group, $p^ \leq 0.05$.

a: Significant difference from IM group, $p^a \leq 0.05$.

b: Significant difference from BC+IM group, $p^b \leq 0.05$.

c: Significant difference from H+IM, $p^c \leq 0.05$.

Table 3 Protective effect of β -arotene and hesperidin against Imidacloprid induced changes on cardiac muscle enzymes and cancer markers.

Group	C	Antioxidants and insecticide							
		IM		BC+IM		H+IM		BC+H+IM	
		1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀	1/5 LD ₅₀	1/10 LD ₅₀
CK (U/L)	117.00 $\pm$ 9.83	286.00 $\pm$ 9.06	239.00 $\pm$ 22.33	210.00 $\pm$ 10.17	181.00 $\pm$ 17.39	251.00 $\pm$ 13.33	213.83 $\pm$ 7.45	184.00 $\pm$ 7.77	175.17 $\pm$ 8.74
CK-MB (U/L)	22.00 $\pm$ 1.06	53.00 $\pm$ 4.94	48.00 $\pm$ 3.35	43.00 $\pm$ 3.69	35.00 $\pm$ 2.83	49.00 $\pm$ 2.61	39.17 $\pm$ 2.11	36.33 $\pm$ 2.96	30.00 $\pm$ 2.25
LDH (U/L)	350.17 $\pm$ 27.63	1001.17 $\pm$ 70.25	887.83 $\pm$ 63.71	707.33 $\pm$ 85.84	622.67 $\pm$ 54.54	921.67 $\pm$ 96.43	715.33 $\pm$ 48.61	630.83 $\pm$ 92.52	504.83 $\pm$ 43.40
α -FP (IU/mL)	3.60 $\pm$ 0.12	8.10 $\pm$ 0.60	7.70 $\pm$ 0.25	6.20 $\pm$ 0.51	5.40 $\pm$ 0.38	6.60 $\pm$ 0.27	6.08 $\pm$ 0.39	5.70 $\pm$ 0.35	4.98 $\pm$ 0.44
CEA (ng/mL)	2.80 $\pm$ 0.23	9.60 $\pm$ 0.56	7.12 $\pm$ 0.39	5.00 $\pm$ 0.73	4.20 $\pm$ 0.35	5.73 $\pm$ 0.30	4.80 $\pm$ 0.33	4.60 $\pm$ 0.34	3.70 $\pm$ 0.30

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Fig. 3 Protective effect of β -carotene and hesperidin against Imidacloprid toxicity on CK-Total, CK-Mb, LDH, α -FP and CEA in albino rats.

where:

C: Control, IM: Imidacloprid, BC: Beta Carotene, H: Hesperidin.

Each value is the mean $\pm$ SE, $n = 6$.

: Significant difference from Control group, $p^ \leq 0.05$.

a: Significant difference from IM group, $p^a \leq 0.05$.

b: Significant difference from BC+IM group, $p^b \leq 0.05$.

c: Significant difference from H+IM, $p^c \leq 0.05$.

3.2 Biochemical Results

From Table 3 or Fig. 3, the results showed a significant increase ($p \leq 0.05$) in CK, CK-Mb, LDH, α FP and CEA concentrations of rats treated with IM at the two used doses comparing with control. Both beta-carotene or/and hesperidin reduced the deleterious effects of IM on the cardiac enzymes and tumor markers.

4. Discussion

The obtained results showed a significant decrease in Hb, Hct, RBCs, MCH and MCHC and significant increase in MCV, WBCs and Plt of rats treated with IM comparing with control that reflects the deleterious and toxic effects of the IM upon these hematological data which may be due to marked increase of the ROS

that causes stress effects upon its production and haemopoiesis from the bone marrow or its toxic effects on the liver, bone marrow and other vital organs, this is in agreement with the finding of Refs. [5] and [6] who recorded that IM-treated rats showed a significant decrease in RBCs count, Hb content and MCHC values and significant increase in total leucocytes count, also this is in agreement with the finding of Ref. [7] who concluded that oral administration of IM to male albino rats induced a significant decrease in Hb, PCV, MCH and MCHC. On the other hand when β -carotene and hesperidin (separately or in combination) supplemented to rats given IM improved, the previous parameters and in addition to beside WBCs, Plt and MCV. The free radicals cause oxidation which can be controlled or prevented by both of β C (β -carotene) and hesperidin

(H) which improves the vascular integrity, decreases capillary permeability and cell death by several mechanisms, such as scavenging oxygen radicals [8, 28, 29]. Oral supplementation with antioxidants appears to be effective for radioprotection of hematopoietic cells and improvement of animal survival, and modulation of apoptosis [30]. The decrease of oxidative stress in blood by hesperidin was associated with amelioration of the hematology profile [31].

The present investigation showed that there is significant increase in both of CK-Total, CK-Mb, LDH, AFP and CEA comparing with control that reflects the deleterious and toxic effects of the IM upon these biochemical results which may be due to marked increase of the ROS or decrease of the natural humeral antioxidants that cause toxic stress effects on the heart and other vital organs [2, 9, 32].

On the other hand when β -carotene and hesperidin supplemented in separated or in combination reduced the changes in CK-Total, CK-Mb, LDH, AFP and CEA levels due to IM treatment that may reflect limiting of the toxicity of IM by the two antioxidants of the study. This is supported by the finding of Refs. [11] and [12] who found that β C (beta-carotene) has a lower incidence and risk of developing cancer and heart disease mediated by free radicals, via quenching singlet oxygen and to inhibit peroxy free-radical reactions. Our results are in agreement with the finding of Ref. [16]; Refs. [14] and [15] concluded that hesperidin possesses a wide range of pharmacological properties including potential anti-inflammatory and anticancer effects, hesperidin induces cell growth arrest apoptosis in a large variety of cells including colon and pancreatic cancer cells.

5. Conclusions

According to the results obtained in the present study, it is concluded that beta-carotene and hesperidin could be useful to attenuate biochemical and hematological changes induced by IM. Both

beta-carotene and hesperidin may protect the hematopoietic system and heart from toxic effects of IM via their antioxidant effects. Moreover, IM appears carcinogenic and the two natural antioxidants exhibited anticarcinogenic property. Beta-carotene and hesperidin may protect from environmental pollutants and insecticides effects.

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