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TOXICITY INDUCED BY IMIDACLOPRID AND INDOXACARB EXPOSURE IN MALE ALBINO RATS AND THE POSSIBLE PROTECTIVE EFFECTS OF VITAMIN E

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ABSTRACT

The present study aimed to evaluate the detrimental effects of exposure to imidacloprid at a dose of 22.5 mg/kg body weight (equivalent to 1/20 of its LD_{50}), indoxacarb (86.6 mg/kg bw, 1/20 of LD_{50}), and their combination on hematological and biochemical parameters in male Albino rats, administered through repetitive oral doses for 28 days. Additionally, the study focused on evaluating the protective role of Vitamin E in mitigating the hepatorenal toxicity induced by these insecticides. The exposure to imidacloprid and indoxacarb, along with their combination caused a notable decrease in body weight and liver weight, while kidney weight increased. Treatment with imidacloprid, indoxacarb, and their mixture significantly decreased red blood cell counts (RBCs), hemoglobin concentration (g/dL), and platelets (PLT). In addition, significant increases were observed in the levels of key liver and cellular enzymes, including serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). These enzymes are biomarkers of liver damage and cellular injury, and their elevated levels indicate hepatotoxicity and potential liver cell damage. Both insecticides and their combination caused increased levels of superoxide dismutase (SOD), which is a key enzyme that helps neutralize free radicals in response to

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oxidative stress. However, the levels of other crucial antioxidants like glutathione (GSH), glutathione peroxidase (GPx), and catalase (CAT) were significantly reduced, indicating an overall imbalance in the liver's oxidative defense system. Regarding the kidney functions, the exposure to imidacloprid, indoxacarb, and their combination resulted in significant changes in the serum levels of albumin, globulin (total protein), creatinine and uric acid in the treated rats. Also, the present results indicated that the administration with vitamin E has mild role in alleviating all these toxicological effects.

Keywords: Rats, Imidacloprid, Indoxacarb, Vitamin E, Liver function, Kidney function

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INTRODUCTION

Despite the numerous environmental and human health concerns linked to pesticide use, they remain the primary method for managing various types of pests. Currently, the most common strategy for controlling arthropods is the application of pesticides (**Lykogianni *et al.*, 2021; Zikankuba *et al.*, 2019**). Imidacloprid (neonicotinoid insecticides) has become a widely used insecticide for crop protection around the world over the past few decades, mainly due to its low persistence in soil and its effective insecticidal properties even at low application rates (**Correia *et al.*, 2001; Casida, 2001**). Globally, this insecticide is among the fastest growing in terms of sales, owing to its broad spectrum of insect control and its perceived safety for humans (**Chao and Casida, 1997; Matsuda *et al.*, 2001**). Its selective toxicity is due to its strong affinity for the insect nicotinic acetylcholine receptors, which is much higher compared to that in mammals (**Correia *et al.*, 2001; Nassar, 2016a; Tomizawa and Casida, 2005; Zhang *et al.*, 2000**). Indoxacarb (oxadiazine) is an insecticide primarily used to

control specific lepidopteran pests and also shows effectiveness against certain sucking insect pests (**Dinter and Wiles, 2000**). In Egypt, the Agriculture Pesticide Committee (**APC, 2020**) recommends its use on various fruits and vegetables targeting by these insect families. The insecticide operates by blocking sodium channels, which leads to paralysis and ultimately death of the pest, offering a lower risk profile (**Wing *et al*. 2000; McKinley *et al*. 2002**). The U.S. Environmental Protection Agency (EPA) classifies indoxacarb as a reduced-risk pesticide and considers it a replacement for organophosphates (**USEPA, 2000**).

Pesticides can lead to biochemical effects in organisms by either inducing or inhibiting enzyme activity. These effects can often be detected through monitoring biochemical changes, even before any noticeable adverse clinical health effects appear **(Sivapiriya *et al*., 2006**). Vitamin E is a naturally occurring non-enzymatic antioxidant found in biological systems. It plays a vital role in safeguarding cell membranes against lipid peroxidation caused by the overproduction of reactive oxygen species and reactive nitrogen species. Research has demonstrated that Vitamin E offers protective effects against various pesticides both *in vivo* and *in vitro* (**Saxena *et al*., 2011; Kammon, 2012; Niki, 2013; Zingg, 2015; Magdy *et al*., 2016; Sargazi *et al*., 2016**). The current study aimed to investigate the toxic effects of imidacloprid, indoxacarb and their mixture on male albino rats and explores the protective role of vitamin E in mitigating these effects through its antioxidant properties.

MATERIALS AND METHODS

Chemicals

Indoxacarb (Avaunt 15% EC) is manufactured by Du Pont De Nemours Co., and Imidacloprid 20% EC (Confidor) is manufactured by Bayer. The enzymatic assay kits, used for measuring various biomarkers such as AST, ALT, ALP, LDH, creatinine, albumin, uric acid, reduced

glutathione (GSH), GPx, SOD, and CAT, were sourced from Bio-Diagnostics Co., located in Dokki, Giza, Egypt. Vitamin E for this study was obtained from Pharco Pharmaceuticals Industries, Alexandria, Egypt.

Experimental Animals

Male Wistar albino rats, each weighing approximately 140 ± 10 grams and aged 90 days, were sourced from the animal breeding facility at the National Research Centre (NRC) in Dokki, Cairo, Egypt. Prior to the experiments, the rats underwent a two-week acclimatization period under laboratory conditions. They were housed in groups of five in plastic cages maintained at a controlled temperature of 25°C, a 12-hour light/dark cycle, and a relative humidity of 65–75%. The rats were fed a standard diet prepared by the NRC animal breeding house, comprising 60% maize, 20% soybean, 3% molasses, 1.5% brown dust, 0.5% salt, and 0.2% vitamins. This diet was obtained from the Animals Food Manufactory of the Agriculture Ministry in Embaba, Giza, Egypt. Water was provided ad libitum throughout the study. All procedures involving animal care and experimentation complied with the **OECD guidelines (2008)**.

Experiment design

A total of 40 adult male albino rats were used in this experiment, divided into eight groups with five rats each. The experimental setup was as follows: Control Group (Group 1): received only corn oil once daily. Group 2: treated daily with imidacloprid at a dose of 22.5 mg/kg bw ($1/20^{th}$ of the LD_{50}, with LD_{50} being 450 mg/kg body weight as per **WHO (2020)**. Group 3: administered indoxacarb at a dose of 86.5 mg/kg (1/20th of the LD_{50}, with LD_{50} being 1730 mg/kg body weight, based on **FAO (2005)** and **Tomlin (2005)**. Group 4: given vitamin E orally at a dose of 1000 mg/kg once daily, as suggested by **Selim *et al*. (2017)**. Group 5: exposed to a combination of $1/20^{th}$ of LD_{50} of both imidacloprid and indoxacarb. Group 6: treated with $1/20^{th}$ of LD_{50} of

imidacloprid along with Vitamin E at 1000 mg/kg. Group 7: administered 1/20th of LD_{50} of indoxacarb plus vitamin E at 1000 mg/kg. Group 8: received a combination of 1/20th of LD_{50} of both imidacloprid and indoxacarb, in addition to Vitamin E at 1000 mg/kg.

These treatments aimed to evaluate the effects of imidacloprid, indoxacarb, and vitamin E, both individually and in combination, on the health and biochemical parameters of the rats. The use of vitamin E was intended to assess its potential protective effect against any toxicity induced by the insecticides.

Before dosing, the rats were fasted for 18 hours to ensure the accuracy of the experiment. All animals were treated ethically, adhering to guidelines set by the animal rights and care committee, as required by the legislation at Damanhour University. The specified doses were administered through oral gavage once daily over a period of 28 days, allowing for consistent and controlled exposure to the substances being tested. This approach ensured that the study followed ethical standards while investigating the effects of the various treatments on the rats.

Blood and organs collection

At the end of the treatment period, the rats were weighed and fasted overnight with access to water. They were then sacrificed under ether anesthesia, and blood samples were collected from the aortic artery. For blood picture assays, blood was collected into EDTA-K_3 tubes. For serum separation, blood was collected in non-heparinized tubes, centrifuged at 1500 xg for 10 minutes using a Sigma K30 bench centrifuge, and the serum was stored at -20°C until further analysis.

Liver function biomarkers

Serum alkaline phosphatase (ALP) activity was measured using the method described by **Belfield and Goldberg (1971)**, a widely used technique for determining enzyme activity levels. The activities of

alanine aminotransferase (ALT) and aspartate aminotransferase (AST), key indicators of liver function, were determined using the method developed by **Reitman and Frankel (1957)**. The activity of lactate dehydrogenase (LDH), an enzyme involved in energy production and a marker of tissue damage, was measured according to **Oba and Uritani (1982)**.

Oxidative stress markers

Enzymatic and non-enzymatic antioxidants, including GSH, SOD, CAT, and GPx, were determined according to the methods described by **Vodovotz (1996)**.

Kidney function biomarkers

Creatinine concentration was determined using the method described by **Siest *et al.* (1985)**, while urea concentration was measured following the procedure outlined by **Fawcett and Scott (1960)**. Total protein levels were assessed using the method developed by **Weichselbaum (1946)**.

Statistical analysis

Data are presented as mean ± standard deviation (SD), with statistical significance defined at $p \leq 0.05$. One-way analysis of variance (ANOVA) was performed for data analysis using SAS software (2001), and the least significant differences (LSD) test was applied to compare treatment means.

RESULTS AND DISCUSSIONS

Effect of the tested insecticides the body weight and internal organs of male Albino rats

The current results revealed that after 28 days of repeated oral administration of imidacloprid, indoxacarb, and their combinations at 1/20 of the LD_{50}, there was no observed mortality in the treated rats. However, signs of toxicity such as mild tremors, diarrhea, glossy eyes, drooling, and emaciation were noted, especially towards the end of the exposure period. Additionally, most treatment groups exhibited signs of decreased appetite, which manifested as reduced body weight throughout the study.

The data presented in Table 1 showed significant differences in final body weight and weight gain between the control group (171 g) and the group treated with vitamin E (173.2 g) compared to the other treatments. The group treated with the combination of imidacloprid and indoxacarb had the lowest average final body weight (148.6 g) and body weight gain (3.36%), followed by those treated with imidacloprid alone (149.6 g), indoxacarb alone (155.2 g), indoxacarb plus vitamin E (155.8 g), and the combination of imidacloprid, indoxacarb, and vitamin E (156 g).

The observed decrease in weight gain among rats treated with these insecticides could be attributed to the chronic stress induced by the insecticides or reduced food consumption due to diarrhea and food avoidance, particularly towards the end of the exposure period. Similarly, **Mansour *et al.* (2008)** found significant decreases in body and kidney weights and increases in liver weights in male albino rats treated with various insecticides.

In this study, the liver and kidney weights of untreated rats were 5.25 g and 1.35 g for absolute weight, corresponding to 3.07% and 0.79% of body weight, respectively, as shown in Table 2. When compared to the control values, all treatments resulted in a significant increase in the relative liver weight, with the most notable increase observed in the group treated with a combination of imidacloprid and indoxacarb, which reached 6.03%. Conversely, all the tested insecticides led to a significant decrease in the relative kidney weight,

with the lowest values recorded in the group treated with imidacloprid and indoxacarb, showing 0.96 g and 0.65% relative to body weight. The inclusion of vitamin E in the treatment regimen led to a reduction in the overall weight loss of both the total body and the organs, which had been affected by the insecticides. In toxicological studies, as noted by **Akhtar *et al.* (2012)**, toxicants can be transported to various organs, where they may exert harmful effects. This necessitates the measurement of multiple biochemical parameters to assess the physiological functions and potential damage to organs and tissues. The findings from the current study are consistent with those reported by **Abouelghar *et al*. (2020)**, who observed an increase in the relative weights of the liver and kidneys in rabbits treated with Thiamethoxam. Similarly, **Nassar (2016b)** and **Reda (2018)** documented an increase in relative liver weight in mice following exposure to 2.6 mg/kg body weight of imidacloprid. Changes in kidney weight, as described by **Sellers *et al*. (2007)** may suggest renal toxicity, hypertrophy of renal tubules, or the presence of chronic progressive nephropathy, further emphasizing the potential for organ-specific toxicity from prolonged insecticide exposure.

Table (1): The effect of imidacloprid (Imida), indoxacarb (Indoxa), and /or Vit. E administration on body weight gain of male albino rats

Treatments	Initial body weight (g)	final body weight (g)	Body weight gain %
Control	140.4 ± 1.52^{abc}	171 ± 5.52^{a}	21.78 ± 3.25^{a}
Vitamin (E)	139.6 ± 1.94^{bc}	173.2 ± 2.68^{a}	24.08 ± 2.21^{a}
Imida	140.8 ± 3.42^{abc}	149.6 ± 1.52^{c}	6.29 ± 2.52^{cd}
Indoxa	139.4 ± 1.82^{bc}	155.2 ± 2.39^{b}	11.35 ± 2.06^{b}
Imida+Vit E	141 ± 3.39^{abc}	156.2 ± 1.92^{b}	10.82 ± 2.22^{b}
Indoxa+ Vit E	138.2 ± 5.4^{c}	155.8 ± 2.39^{b}	12.92 ± 5.87^{b}
Imida+Indoxa	143.8 ± 3.49^{a}	148.6 ± 2.7^{c}	3.36 ± 1.42^{d}
Imida+Indoxa+Vit E	143.2 ± 2.17^{ab}	156 ± 2.74^{b}	8.97 ± 2.94^{bc}
F values	1.8239	47.297	26.957
L.S.D	4.04063	3.80803	3.9747

The data are expressed as mean ± standard deviation (SD). Different superscript letters within the same column indicate statistically significant differences ($P \leq 0.05$).

Table (2): The effect of imidacloprid (Imida), indoxacarb (Indoxa), and /or Vit. E administration on liver and kidney weights of male albino rats

Treatments	Liver weight (g)	Liver weight Ratio	Kidney weight (g)	Kidney weight Ratio
Control	5.25±0.07^{d}	3.07±0.12^{d}	1.35±0.02^{a}	0.79±0.03^{a}
Vitamin (E)	5.2±0.02^{d}	3.01±0.05^{d}	1.34±0.01^{a}	0.77±0.01^{a}
Imidacloprid	5.93±0.05^{b}	3.97±0.06^{a}	0.99±0.01^{d}	0.66±0.01^{c}
Indoxa	5.55±0.03^{c}	3.58±0.08^{c}	1.03±0.03^{c}	0.66±0.03^{c}
Imida+Vit E	5.51±0.08^{c}	3.52±0.02^{c}	1.04±0.02^{c}	0.66±0.01^{c}
Indoxa+ Vit E	5.49±0.03^{c}	3.52±0.07^{c}	1.07±0.02^{b}	0.69±0.02^{b}
Imida+Indoxa	6.03±0.05^{a}	4.06±0.09^{a}	0.96±0.03^{e}	0.65±0.02^{c}
Imida+Indoxa+Vit E	5.88±0.06^{b}	3.77±0.09^{b}	1.02±0.11^{c}	0.66±0.02^{c}
F values	175.591	116.188	301.424	42.399
L.S.D	0.0675	0.10123	0.0256	0.0248

The data are expressed as mean ± standard deviation (SD). Different superscript letters within the same column indicate statistically significant differences ($P \leq 0.05$).

Hematological effects

The hematological data in Table 3 show that there was no significant difference in blood parameters between the Vitamin E-treated and the untreated rats. However, exposure to imidacloprid, indoxacarb, and their combination led to notable decreases in red blood cell (RBC) count, hemoglobin (Hg), and platelet (PLT) levels. In contrast, the white blood cell (WBC) count significantly increased in comparison to the control group. Administering Vitamin E to rats treated with these insecticides showed marked improvements in these hematological parameters, highlighting its protective role. The current

findings are consistent with those of **Nassar (2016b)** and **Abdelrasoul (2018)** who reported that abamectin, indoxacarb, and their combination led to significant decreases in RBC count and hemoglobin levels), packed cell volume (Pcv), and PLT in treated rats. Furthermore, an increase in WBC counts was observed in these rats compared to the control group.

Table 3. The effect of imidacloprid (Imida), indoxacarb (Indoxa), and /or Vit. E administration on red blood cells (RBCs), white blood cells (WBCs), hemoglobin concentration (Hg), and platelets (PLT) in male albino rats

Treatments	WBC ($\times 10^3$ /mm^3)	RBC ($\times 10^3$ /mm^3)	Hg (g/dL)	PLT ($\times 10$ /mm^3)
Control	9.56±0.48^{e}	7.76±0.29^{a}	13.02±0.53^{a}	533.57±33.72^{b}
Vitamin (E)	9.73±0.19^{e}	7.79±0.21^{a}	13.45±0.39^{a}	562.53±24.03^{a}
Imida	14.36±0.72^{b}	5.82±0.39^{c}	10.97±0.5^{d}	424.49±13.03^{d}
Indoxa	13.3±0.75^{c}	6.38±0.38^{b}	11.62±0.86^{c}	473.73±11.73^{c}
Imida+Vit E	13.01±0.53^{c}	5.85±0.15^{c}	11.8±0.34^{bc}	481.95±6.89^{c}
Indoxa+ Vit E	11.95±0.21^{d}	6.55±0.37^{b}	12.25±0.41^{b}	514.2±7.64^{b}
Imida+Indoxa	16.05±0.85^{a}	4.38±0.15^{e}	9.44±0.24^{e}	353.3±9.32^{f}
Imida+Indoxa+Vit E	14.99±0.24^{b}	5.13±.439^{d}	10.5±0.26^{d}	387.97±5.39^{e}
F values	112.5448	56.823	77.8233	217.427
L.S.D	0.9947	0.5147	0.5834	21.52238

The data are expressed as mean ± standard deviation (SD). Different superscript letters within the same column indicate statistically significant differences ($P \leq 0.05$).

Changes in hepatic function parameters (liver function)

The administration of vitamin E did not lead to any significant changes in the measured biochemical parameters compared to the untreated control rats, as shown in Table 4. However, exposure to imidacloprid, indoxacarb, and their combination resulted in elevated activities of liver enzymes such as ALP, ALT, AST, and LDH in the treated male albino rats. These enzymes serve as biomarkers of hepatotoxicity, and their increased activities suggest liver damage

caused by the insecticides. The results of elevated ALP, AST, ALT, and LDH levels typically indicate liver dysfunction or damage, as these enzymes are released into the bloodstream when hepatocytes (liver cells) are injured. The rise in these enzymes suggests that imidacloprid and indoxacarb cause hepatocellular damage, leading to the release of these enzymes into the blood serum. AST and ALT are particularly indicative of liver damage; their levels in the blood increase in proportion to the severity of liver cell injury. This response is consistent with findings by **Vinogradova *et al*. (1989)** and **Ncibi *et al*. (2008)** who noted that cellular destruction in the liver results in the release of these enzymes. Therefore, these enzymes are widely recognized as biomarkers for liver injury, as also supported by the findings of **Eissa and Zidan (2010)**. This information underscores the hepatotoxic effects of certain insecticides, as evidenced by the biochemical changes observed in this study. After 28 days of exposure to the insecticides, there were significant increases in the activities of these liver enzymes, indicating hepatic injury. However, when Vitamin E was administered alongside imidacloprid, indoxacarb, or their combination, it appeared to mitigate the adverse effects, improving most of the tested biochemical parameters. This suggests that Vitamin E may have a protective effect against the liver damage induced by these insecticides.

Table (4): The effect of imidacloprid (Imida), indoxacarb (Indoxa), and /or Vit. E administration on the activities of AST, ALT, ALP and LDH in the serum samples of male rats.

Treatments	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)
Control	36.97 ± 0.04^{f}	33.34 ± 0.91^{c}	98.13 ± 1.83^{c}	163.89 ± 2.69^{d}
Vitamin (E)	36.89 ± 0.29^{f}	35.57 ± 0.77^{c}	98.01 ± 0.33^{c}	164.85 ± 2.45^{cd}
Imida	63.69 ± 2.78^{e}	43.63 ± 0.69^{c}	156.26 ± 1.12^{ab}	205.95 ± 10.17^{abc}
Indoxa	174.47 ± 8.08^{b}	99.59 ± 8.22^{ab}	163.62 ± 8.44^{a}	212.51 ± 42.27^{ab}
Imida+Indoxa	195.63 ± 10.57^{a}	105.7 ± 17.98^{a}	172.72 ± 13.05^{a}	232.72 ± 17.19^{a}
Imida+Vit E	53.66 ± 2.62^{e}	39.61 ± 3.37^{c}	133.34 ± 10.52^{b}	181.47 ± 9.42^{bcd}
Indoxa+ Vit E	98.77 ± 6.94^{d}	79.42 ± 16.42^{b}	145.51 ± 18.86^{ab}	191.88 ± 12.39^{abcd}
Imida+Indoxa+Vit E	123.66 ± 11.1^{c}	86.56 ± 8.11^{ab}	151.56 ± 25.01^{ab}	205.58 ± 8.4^{abc}

F values	155.71	61.818	82.952	9.2752
L.S.D	16.264	19.925	33.482	42.5559

The data are expressed as mean ± standard deviation (SD). Different superscript letters within the same column indicate statistically significant differences ($P \leq 0.05$). AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase.

Hepatic oxidative damage parameters

The effects of imidacloprid and indoxacarb on lipid peroxidation and liver oxidative stress parameters in rats show significant oxidative damage (Table 5). In this study, both insecticides and their combination caused increased levels of superoxide dismutase (SOD), which is a key enzyme that helps neutralize free radicals in response to oxidative stress. However, the levels of other crucial antioxidants like GSH, GPx, and CAT were significantly reduced, indicating an overall imbalance in the liver's oxidative defense system. Neonicotinoids are known to worsen oxidative stress by reducing antioxidant enzymes like superoxide dismutase and catalase, which are linked to increased reactive oxygen species (ROS) (**El-Hak *et al*., 2022; Godbole *et al*., 2024; Kapoor *et al*., 2010**).

The administration of Vitamin E showed a protective role by reducing the toxic effects of these insecticides. Though it significantly improved the antioxidant parameters (SOD, GSH, GPx, and CAT), the levels still did not fully return to normal when compared to the control group, indicating partial protection against oxidative damage.

Vitamin E has been shown to offer neuroprotective effects, particularly against the neurotoxic impact of pesticides, as highlighted by **Abd El-Naeem and Ismael (2023)**. Due to its powerful antioxidant properties, Vitamin E can neutralize harmful reactive oxygen species (ROS), which are implicated in various health conditions such as cancer, arthritis, and cataracts **(Rizvi *et al*., 2014).** By scavenging free radicals and preventing oxidative damage to cells and tissues, vitamin

E plays a crucial role in reducing the risk of oxidative stress-related diseases and supporting overall health. Its antioxidant capabilities make it a valuable preventive measure against the harmful effects of environmental toxins and oxidative stress-induced disorders. This study suggests that while vitamin E can mitigate some of the oxidative damage caused by imidacloprid and indoxacarb, the liver's antioxidant system remains compromised after exposure to these pesticides.

Table (5): The effect of imidacloprid (Imida), indoxacarb (Indoxa), and /or Vit. E administration on the activities of SOD, CAT, GPx and GSH in the plasma samples of male rats.

Treatments	SOD (µmol/min/ml)	CAT (µmol/min/ml)	GPx (µmol/min/ml)	GSH (mg/g)
Control	106.18±1.18^{e}	0.54±0.01^{a}	0.85±0.02^{a}	63.31±4.24^{a}
Vitamin (E)	104.31±3.10^{e}	0.55±0.01^{a}	0.88±0.07^{a}	64.71±2.27^{a}
Imida	198.55±2.54^{bc}	0.35±0.02^{d}	0.45±0.02^{c}	42.49±2^{d}
Indoxa	219.73±16.24^{ab}	0.38±0.03^{cd}	0.42±0.02^{c}	45.67±1.48^{c}
Imida+Indoxa	243.92±32.71^{a}	0.29±0.03^{e}	0.39±0.05^{c}	47.14±1.99^{bc}
Imida+Vit E	150.42±17.20^{d}	0.44±0.01^{b}	0.65±0.01^{b}	49.87±1^{b}
Indoxa+ Vit E	172.49±10.80^{cd}	0.41±0.02^{bc}	0.61±0.02^{b}	38.46±2.24^{e}
Imida+Indoxa+Vit E	191.38±16.88^{bc}	0.35±0.02^{d}	0.58±0.09^{b}	41.66±1.02^{d}
F values	78.946	49.948	68.3429	95.54539
L.S.D.	29.3251	0.05162	0.1264	2.8918

The data are expressed as mean ± standard deviation (SD). Different superscript letters within the same column indicate statistically significant differences ($P \leq 0.05$). SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GSH: Glutathione

Kidney function biomarkers

The results presented in Table 6 of current study show that exposure to imidacloprid, indoxacarb, and their combination resulted in significant changes in the serum levels of creatinine, albumin, total protein, and uric acid in the treated rats. Specifically, creatinine levels increased significantly in the treated groups, with the highest levels observed in rats treated with the combination of imidacloprid and

indoxacarb, reaching 1.34 mg/dl. This increase in creatinine is a clear indicator of impaired kidney function, as creatinine is typically excreted through glomerular filtration. Elevated blood creatinine suggests that kidney function may have been compromised by the toxic effects of the insecticides. These findings are consistent with the work of **Lu (1996)**, which associates elevated creatinine with kidney dysfunction. Similarly, **Noaishi and Abd alhafez (2016)** also observed increased creatinine levels due to severe kidney damage following exposure to acetamiprid.

Total protein levels showed a marked decrease in the rats treated with the insecticides, particularly in those exposed to the combination of imidacloprid and indoxacarb. This decrease in total protein levels may reflect liver dysfunction, as the liver is the main organ responsible for protein synthesis. The results align with the findings of **Shallan *et al*. (2004)** who reported a significant reduction in total protein levels in rats treated with imidacloprid. Earlier studies, such as those by **Nachtomi and Alumat (1972)** and **Broda *et al*. (1976)** suggested that this reduction could be due to liver cell necrosis or an alteration in intracellular protein synthesis mechanisms. Changes in oxidative enzymes may further contribute to disrupting protein synthesis pathways. Additionally, treatment with imidacloprid, indoxacarb, and their combination resulted in a significant elevation of serum uric acid as compared to control in agreement with (**Salem *et al*., 2007**).

In summary, the toxic effects of imidacloprid and indoxacarb, both individually and in combination, have caused notable kidney and liver damage in the treated rats, as evidenced by elevated creatinine levels and decreased total protein levels, reflecting impaired kidney filtration and compromised liver function.

Table (6): The effect of imidacloprid (Imida), indoxacarb (Indoxa), and /or Vit. E administration on the levels of Albumin, Total protein, Creatinine and Uric Acid in the serum samples of male rats.

Treatments	Albumin (g/dl)	Globulin (g/dl)	Total protein (g/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
Control	4.72±0.09^{a}	4.05±0.07^{ab}	8.77±0.13^{a}	0.75±0.02^{e}	5.9±0.09^{e}
Vitamin E	4.68±0.32^{ab}	4.11±0.26^{a}	8.79±0.16^{a}	0.78±0.01^{de}	5.98±0.07^{e}
Imida	2.96±0.45^{d}	3.78±0.37^{ab}	6.74±0.36^{ab}	0.89±0.03^{bcd}	8.44±0.02^{b}
Indoxa	3.24±0.08^{cd}	3.54±0.02^{c}	6.78±0.09^{ab}	0.98±0.01^{b}	9.65±0.03^{a}
Imida+Indoxa	2.68±0.06^{d}	3.56±0.12^{c}	6.24±0.22^{b}	1.34±0.09^{a}	10.27±0.58^{a}
Imida+Vit E	3.63±0.04^{bcd}	3.66±0.05^{bc}	7.29±0.18^{ab}	0.81±0.04^{cde}	6.92±0.81^{de}
Indoxa+ Vit E	4.19±0.83^{abc}	2.93±0.78^{d}	7.12±0.82^{ab}	0.92±0.06^{bc}	7.23±0.81^{cd}
Imida+Indoxa+Vit E	3.36±0.83^{cd}	3.59±0.84^{c}	6.95±0.82^{ab}	1.22±0.07^{a}	8.18±0.03^{bc}
F values	39.1815	51.511	3.7726	49.83	94.6621
L.S.D.	0.8813	0.437	2.0484	0.16442	1.1782

The data are expressed as mean ± standard deviation (SD). Different superscript letters within the same column indicate statistically significant differences ($P \leq 0.05$).

CONCLUSION

In conclusion, imidacloprid, indoxacarb, and their combination caused significant hepatotoxic and nephrotoxic effects in rats, marked by changes in liver enzyme activity, kidney function, and oxidative stress markers. Vitamin E showed a mild protective effect, suggesting its potential in mitigating insecticide-induced toxicity.

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الملخص العربي

السمية الناجمة عن التعرض للإيميداكلوبريد والإندوكساكارب في ذكور الفئران البيضاء والآثار الوقائية المحتملة لفيتامين هـ

عواطف سعد منسي، مني عبدالنبي عبدالرسول، محمد مبروك رجب عطية

قسم وقاية النبات – كلية الزراعة – جامعة دمنهور – جمهورية مصر العربية

هدفت الدراسة الحالية إلى تقييم الآثار الضارة للتعرض للإيميداكلوبريد (22.5 مجم / كجم من وزن الجسم، 20/1 من LD_{50}) ، والإندوكساكارب (86.6 مجم / كجم من وزن الجسم، 20/1 من LD_{50}) ، ومزيجهما على المعايير الدموية والكيميائية الحيوية في ذكور الفئران البيضاء التي تم معاملتها من خلال جرعات فموية متكررة لمدة 28 يومًا. بالإضافة إلى ذلك، ركزت الدراسة على تقييم الدور الوقائي لفيتامين هـ في التخفيف من السمية الكبدية الكلوية الناجمة عن هذه المبيدات الحشرية. تسبب التعرض للإيميداكلوبريد والإندوكساكارب، جنبًا إلى جنب مع مزيجهما، في انخفاض ملحوظ في وزن الجسم ووزن الكبد، في حين زاد وزن الكلى. وأدت المعاملة بالإيميداكلوبريد والإندوكساكارب ومزيجهما إلى انخفاض كبير في عدد خلايا الدم الحمراء ، وتركيز الهيموجلوبين، والصفائح الدموية. بالإضافة إلى ذلك، لوحظت زيادات كبيرة في مستويات إنزيمات الكبد والخلايا الرئيسية، بما في ذلك الفوسفاتيز القلوي (ALP)، وألانين أمينوترانسفيراز(ALT) ، وأسبارتات أمينوترانسفيراز (AST) ، ولاكتات ديهيدروجينيز (LDH) . هذه الإنزيمات هي مؤشرات حيوية لتلف الكبد والإصابة الخلوية، وتشير مستوياتها المرتفعة إلى سمية الكبد وتلف خلايا الكبد المحتمل. تسبب كل من المبيدات الحشرية ومزيجها في زيادة مستويات إنزيم أكسيد الفائق ديسميوتاز(SOD) ، وهو إنزيم رئيسي يساعد في تحييد الجذور الحرة استجابة للإجهاد التأكسدي. ومع ذلك، انخفضت مستويات مضادات الأكسدة الحاسمة الأخرى مثل الجلوتاثيون (GSH) ، والجلوتاثيون بيروكسيديز(GPx) ، والكاتالاز (CAT) بشكل كبير، مما يشير إلى اختلال التوازن العام في نظام الدفاع التأكسدي للكبد. وفيما يتعلق بوظائف الكلى، أدى التعرض للإيميداكلوبريد والإندوكساكارب ومزيجهما إلى حدوث تغيرات كبيرة في مستويات الألبومين والجلوبيولين (البروتين الكلي) والكرياتينين وحمض البوليك في مصل الفئران المعالجة. كما أشارت النتائج الحالية إلى أن تناول فيتامين هـ له دور في تخفيف كل هذه التأثيرات السامة.