

Effect of *Nigella sativa* Oil on the Imidacloprid Induced Toxicity in Male Albino Mice

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ABSTRACT

Imidacloprid (IMC) is a systemic insecticide being the most frequently detected neonicotinoid in fruits and vegetables. So, this study was carried out to evaluate the biochemical and the histological alterations associated with IMC toxicity in male albino mice (*Mus musculus*). Also, the role of *Nigella Sativa* oil (NS oil), (4 ml/kg bw) on the adverse effect of IMC was investigated. The animals were orally administered with 1/50 LD₅₀ IMC (2.6 mg/kg bw) for 28 days (5 doses/week). Hepatic and pancreatic toxicity was monitored by quantitative measurements of the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), α -amylase activities, glucose, total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels. Creatinine and urea levels were used as the biomarkers of kidney damages. The results showed that imidacloprid administration caused a significant increase in the body and the organs weights. Also, the ALT Activity, glucose, urea, creatinine, total cholesterol, triglyceride, and HDL cholesterol levels were increased. Whereas the AST and α -amylase activity showed significant reduction when compared with the control group. The effect of IMC on the previous parameters was confirmed by the histological changes of liver and kidney. In addition, NS oil supplementation showed a significant reduction of IMC adverse effect. This result indicated that imidacloprid has the ability to damage liver and kidney functions and cause histological lesions in mice at the tested sublethal dose. In addition, NS oil supplementation attenuated the IMC adverse effect which confirms its protective influence.

Key words: Imidacloprid; Biochemical Studies; Histopathological Examination; Hepatotoxicity; Nephrotoxicity; *Nigella sativa*; Lipid profile.

INTRODUCTION

Imidacloprid is a potent neurotoxic insecticide that acts as agonists on the nicotinic acetylcholine receptors (Tomizawa and Yamamoto, 1993). It was extensively used in agriculture and veterinary medicine (Ware and Whitacre, 2004). Lee Chao and Casida (1997) and Tomizawa et al (2005) mentioned that the IMC characterized by high selective insecticidal activity at low application rate, low soil persistence and apparent safety in humans. Also, it was absorbed rapidly from the gastrointestinal tract and eliminated via urine and feces (Kidd and James, 1991). IMC classified as a moderately hazardous pesticide (Class II) Meister (1995) and WHO (2005).

The liver is an important target organ for the toxicity of drugs and xenobiotics due to its connection with the gastrointestinal tract as well as for the singularity and complexity of its anatomical structure and metabolic functions. It can be considered a target organ of numerous chemicals either in the workplace or at home Arafa et al., (2003), and Piñeiro-Carrero and Piñeiro (2004). The activities of serum AST and ALT have been commonly used in the diagnosis of liver diseases as well as in the detection of tissue damage caused by environmental pollution Palanivelu et al., (2006).

Both aminotransferases are highly concentrated in the liver. AST is also diffusely present in the heart, skeletal muscle, kidneys, brain and red blood cells, while ALT has low concentrations in skeletal

muscle and kidneys Wroblewski (1958); an increase in serum ALT levels is, therefore, more specific for liver damage. In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity) Rej R (1989). Pesticides may damage liver cells and liver transaminases may be used to monitor liver damage.

Hyperglycemia is a metabolic disorder associated with high blood sugar occurs due to the pancreas does not produce enough insulin or when the body cannot use the insulin effectively Alpha-amylase is an enzyme that plays an important role in the digestion of starch and glycogen. Alpha-amylase catalyses the breakdown of polysaccharide into monosaccharide and only monosaccharide form of food can absorb in the stomach. so the determination of glucose level and α -amylase activity serve as useful biochemical tools to understand etiology of pesticide-induced hyperglycemia (Powers, 2011, Arumugam, Manjula, Paari, 2013, and Pallavi et al., 2015)

Urea, commonly referred to as blood urea nitrogen (BUN) when measured in the blood, is a product of protein metabolism. The BUN is considered a non-protein nitrogenous (NPN) waste product. Urea accounts for the majority (up to 80%–90%) of the NPNs excreted by the body. The body's dependency on the renal system to excrete urea makes it a useful analyte to evaluate renal function. Creatinine also, an NPN waste product synthesized in the liver, pancreas, and kidneys from the

transamination of the amino acids arginine, glycine, and methionine. Creatinine produced from the breakdown of creatine and phosphocreatine, it is freely filtered by the glomerulus, these characteristics make creatinine a useful endogenous marker for creatinine clearance and can also serve as an indicator of renal function Price and Finney (2000).

Lipophilic nature of the majority of pesticides may render them with the ability to interact with the serum and tissue lipids. So, that triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol levels have been reported to be useful tools for determination of pesticides-induced hyperlipidemia Gupta et al., 1994 and Slotkin et al., (2005).

Blood, being the medium of intercellular transport, it rapidly comes in direct contact with various tissues in the body; therefore, the physiological state of an organism is always reflected in the contents of the blood (Narra, 2017). So, the biochemical parameters can indicate toxicity with great potential for environmental monitoring of health (Narra, 2016). Likewise, histopathological changes in animal tissues is a sensitive and rapid method commonly used to detect the toxic potential and risk assessment of chemicals in the environment (Adams, 2002; Crissman et al., 2004; Mela et al., 2007).

Nigella sativa is an amazing herb with a religious background and commonly grows in Mediterranean coasts and Asia. It contains many active components including nigellidine, nigellimine, nigellidone, alphahederin, thymoquinone, thymohydroquinone, dithymoquinone, thymol, and carvacrol (Randhawa and Alghamdi, 2002 and 2011). Most of the potent activity of *Nigella sativa* herb associated with its volatile oil and a protein component. Salem, (2005) and Randhawa, (2008) reported that *N. sativa* has a wide spectrum of pharmacological actions which include liver tonics, antidiabetic, antihypertensive, diuretics, digestive, anti-diarrheal, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic and bronchodilator

Due to the increasing application of IMC in agriculture to control insect pests and its likely hazard for consumers by intake of fruits and vegetables with pesticide residues, therefore, this study was planned to investigate the effects of IMC sublethal dose on male albino mice by means of quantitative analysis of some biochemical parameters and histopathological alterations to highlight the potential adverse effects of such environmental contaminants. Also, the protective role of *Nigella Sativa* oil against imidacloprid-induced toxicity was investigated.

MATERIALS AND METHODS

1. Chemicals

Imidacloprid technical grade (97%) was obtained from Zhejiang Rayfull Chemicals Co., China. *Nigella Sativa* oil was obtained from Egyptian Natural Oils Co., Cairo. All other chemicals were purchased from Sigma – Aldrich Co.

2. Animals

Male albino mice weighing, 30 ± 3 g were obtained from the animal house, Faculty of Agriculture, Alexandria University. Animals were housed in groups each of five mice in a stainless steel cages and kept under laboratory conditions; $25 \pm 2^\circ\text{C}$, 65–75% humidity and light 12 h light/12 dark. Animals allowed balanced standard rodent diet and water *ad libitum* for two weeks before being experimented. All procedures involving animals were performed in accordance with the guidelines of the standard procedures established by OECD guideline (2008).

3. Experimental design

The animals were divided into four groups, each five animals and orally treated by gavage with 5 doses /week for 4 weeks as follows:

Group, C: Corn oil (1 ml/kg bw) used as control. Group, NS oil: *Nigella Sativa* oil (4 ml/kg bw). Group, IMC: Imidacloprid in a dose equivalent to 2.6 mg/kg bw ($1/50$ LD₅₀; 130 mg/kg bw obtained from WHO, 2004). Group, IMC+NS oil: Imidacloprid ($1/50$ LD₅₀) plus *Nigella Sativa* oil (4 ml/kg bw). The animals were weighed weekly. At the end of the experiment (4 weeks), blood was collected in plain tube for serum preparation. Then liver, pancreas, and kidney were quickly removed and weighed individually. The % of relative organ weight was calculated (organ weight/body weight) X 100. Liver and kidney were fixed immediately in 10% buffered formalin for histopathological examination.

4. Biochemical studies

The collected, blood allowed to clot for 30 min at room temperature and centrifuged at 4,000 g using Sigma 3K30 bench centrifuges for 15 min to separate the serum which stored at (-20°C) until used for the determination of biochemical parameters by using BioMed diagnostic kits (Germany).

4.1. Measurement of AST and ALT activity

The transaminases AST and ALT were measured by the Reitman and Frankel, (1957) method. The generation of hydrazone formed with 2, 4-dinitrophenylhydrazine in both transaminase reactions was measured at 546 nm.

4.2. Measurement of serum glucose level and α -amylase activity

Serum glucose was measured by employing Trinder, (1969) enzymatic method, uses glucose

oxidase to catalyze the oxidation of glucose to hydrogen peroxide and gluconic acid. Hydrogen peroxide, when combine with 4-aminoantipyrine and phenol forms a red quinoneimine dye, having an absorbance maximum at 510 nm. The intensity of the red color is proportional to the amount glucose in the sample

A-amylase activity was measured calorimetrically according to Kurrle-Weitenhiller *et al.*, (1996). A-amylase catalyzes the hydrolysis of the EPS substrate (Ethylidene Protected Substrate) p-nitrophenyl-maltoheptaoside 4, 6-ethylidene blocked (ethylidene-G7PNP) forming 2 ethylidene-G5 + 2 G2PNP + 2 ethylidene-G4 + 2 G3PNP + ethylidene-G3 + G4PNP. The α -glucosidase hydrolyses all fragments of G2PNP, G3PNP and G4PNP into p-nitrophenol (PNP) and glucose (G). The increase of absorbance, due to PNP formation, is proportional to the activity α -amylase in the examined sample measured at 410 nm.

4.3. Measurement of serum urea and creatinine concentrations

urea concentrations were measured by the Fawcett and Scott, (1960) method. Serum urea hydrolyzed by urease to form ammonium and carbonate. In alkaline medium, the ammonium ions react with the salicylate and hydrochloride to form a green colored indophenol (2,2 dicarboxylindophenol). The intensity of the color produced is directly proportional to urea/BUN concentration measured at 578 nm.

Serum creatinine was estimated by Jaffes method (1886). In alkaline medium serum creatinine reacts with picrate to produce an orange color that absorbs light at 492 nm. The increase in the absorbance rate is directly proportional to the concentration of creatinine in the specimen.

4.4. Measurement of serum triglycerides, total and HDL cholesterol levels

Serum total and HDL cholesterol levels were measured by Richmond, (1973) and Grove, (1979) methods respectively. Under the action of cholesterol esterase cholesterol esters resolve in free cholesterol and fatty acids, and cholesterol oxidase then converts the cholesterol to H_2O_2 and cholest-4-en-3 one. In the presence of peroxidase H_2O_2 oxidatively couples with 4 aminoantipyrine and phenol to produce a red quinoneimine dye, having an absorbance maximum at 510 nm. The intensity of the red colour is proportional to the total cholesterol in the specimen

Triglycerides levels in serum were measured spectrophotometrically by Fassati and Prencipe, (1982) method. TG was hydrolysed by enzyme lipase into glycerol and free fatty acids and then liberated glycerol convert to glycerol-3- phosphate by glycerol kinase. Glycerol-3-phosphate oxidized by glycerol-3- phosphate oxidase into dihydroxyacetone phosphate and hydrogen

peroxide. Finally, the hydrogen peroxide is monitored in the presence of horseradish peroxidase with 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone as the chromogenic system. The high absorbance of this chromogen was at 500 nm. Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) cholesterol were calculated according to Friedewald's equation 1972.

5. Histopathological examination

Samples of liver and kidney were fixed in 10 % buffered formalin. Then, dehydrated by standard procedures and embedded in paraffin, sections approximately 5 μ m thick were cut, stained with hematoxylin and eosin (H&E) stains and examined by light microscope (Drury and Wallington 1980).

6. Statistical analysis

All results were expressed as mean \pm standard error (SE). The data was analyzed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls Test. The criterion for statistical significance was set at $p < 0.01$. These tests were performed using the computer software CoStat program, version 6.2, 2002.

RESULTS AND DISCUSSION

1. Body and organs weight

It was noticed that there was no mortality occurred in any group of animals during the experimental period (4 weeks). Table (1) IMC treated group showed a significant increase in mice's weight during the experimental period compared to control. The relative liver, pancreas, and kidney weights were markedly increased by 10.2, 36.1 and 10.7 % respectively as a percentage of control (Table 2). The obtained results are in line with those of William *et al.*, (2007), who suggested that excessive weight gain is associated with the environmental pollutants, including pesticides. Simons *et al.*, (1995) cited that the changes in either absolute or relative organ weight after administration of a chemical or drug is an indication of the toxic effect of that chemical. The present study revealed an increase in relative liver weight in IMC treated mice which could be related to IMC toxic effect, and those are consistent with Eissa and Zidan, (2009).

Liver enlargement could be induced as a result of; 1) the maintenance of the liver normal functional capacity (Robinson and Yarbrough, 1978), 2) the accumulation of abnormal amounts of fat, predominately triglyceride, a result of an imbalance between the rate of synthesis and the rate of release of triglyceride by the parenchymal cells into the systemic circulation (Plaa, 1980), or 3) the increased demands for the detoxification of toxic compounds, (Okuna *et al.*, 1976). In contrast; Bhatnagar and Jain, 1986 stated that the increase in liver weight is not necessarily considered toxic lesions since this effect is observed in a large number of compounds.

Table 1: Body weight pattern of male albino mice orally administrated with IMC (2.6 mg/kg bw), NS oil (4 ml/kg bw) and their combination.

Animal Group	Body weight (gm) / Weeks				
	Zero	First	Second	Third	Fourth
C	29.4 ^a ± 0.87	31.00 ^b ± 0.47	32.4 ^b ± 0.96	33.0 ^b ± 0.61	34.8 ^b ± 0.30
NS oil	29.2 ^a ± 0.63	29.75 ^b ± 0.56	31.4 ^b ± 0.96	31.6 ^c ± 0.60	33.8 ^c ± 0.42
IMC	29.4 ^a ± 0.77	33.50 ^a ± 0.34	34.2 ^a ± 1.32	34.6 ^a ± 0.51	36.2 ^a ± 0.63
IMC+NS oil	28.2 ^a ± 0.49	30.75 ^b ± 0.30	32.0 ^b ± 1.0	33.0 ^b ± 0.57	33.4 ^c ± 0.66

Values are expressed as means (5 mice ± Standard Error (SE))

Values in the column with different letters are significantly different at (p ≤ 0.01).

Zero: the day of application.

Table 2: Relative organs weight of male albino mice orally administrated with IMC (2.6 mg/kg bw), NS oil (4 ml/kg bw) and their combination.

Animal Group	Liver			Pancreas			Kidney		
	Weight (g)	Relative Weight (%)	% Change ^a	Weight (g)	Relative Weight (%)	% Change ^a	Weight (g)	Relative Weight (%)	% Change ^a
C	1.7 ^{ab} ±0.06	4.9 ^a ±0.06	0.0	0.126 ^a ±0.003	0.36 ^a ±0.008	0.0	0.49 ^a ±0.003	1.40 ^a ±0.012	0.0
NS oil	1.73 ^{ab} ±0.03	4.8 ^a ±0.17	-4.1	0.128 ^a ±0.004	0.38 ^a ±0.006	-5.5	0.47 ^a ±0.008	1.39 ^a ±0.012	0.71
IMC	1.95 ^b ±0.03	5.4 ^c ±0.12	-10.2	0.178 ^c ±0.001	0.49 ^c ±0.017	-36.1	0.56 ^b ±0.006	1.55 ^c ±0.001	-10.7
IMC+NS oil	1.6 ^a ±0.06	5.0 ^b ±0.23	-2.0	0.143 ^b ±0.002	0.43 ^b ±0.012	-19.4	0.48 ^a ±0.006	1.44 ^b ±0.004	-2.9

Values are expressed as means (5 mice) ± Standard Error (SE)

Values in the column with different letters are significantly different at (p ≤ 0.01).

^a Percentage of increase (-) in treatment weights compared to control

Otherwise, Sellers *et al.*, 2007 reported that changes in kidney weight may reflect renal toxicity, tubular hypertrophy or chronic progressive nephropathy.

2. Biochemical studies

The toxic effects of the repeated sublethal dose of IMC on the tested biochemical parameters in mice serum were illustrated in table 3. The results in Table (3) showed significant (p < 0.01) decrease in AST activity of IMC treated mice by about 70% from control, whereas a significant elevation in ALT activity by 1.76 fold was observed. Moreover alterations in AST and ALT activities were positively modulated by NS oil supplementation

compared to control. Serum AST and ALT activities were believed to be a sensitive index for hepatocellular damage Peng *et al.*, 2007. The liver dysfunction may be due to the changes in the liver enzyme activities, biosynthesis and altered membrane permeability permitting enzyme leakages into the blood Mansour and Mossa, 2010. Helling *et al.*, 1995 ascertain that hepatic damage is considered pathologically irreversible, the ALT elevation may render the liver to be more susceptible to other pathogen/toxicants (Nayak *et al.*, 1996 and Choudhary *et al.*, 2003).

Table 3: Serum parameters of male albino mice orally administrated with IMC (2.6 mg/kg bw), NS oil (4 ml/kg bw) and their combination.

Animal Group	Parameters (mean ± SE; n=5)					
	AST (U/ml)	ALT (U/ml)	Glucose (mg/dL)	α-Amylase (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
C	230 ^c ±0.52	43 ^a ±0.50	94 ^a ±1.45	246 ^c ±0.67	26 ^a ±0.87	0.47 ^a ±0.006
NS oil	228 ^c ±1.5	44 ^a ±0.45	96 ^a ±0.88	245 ^c ±2.3	27 ^a ±0.33	0.47 ^a ±0.003
IMC	69 ^a ±0.57	76 ^c ±0.2	215 ^c ±1.44	183 ^a ±1.44	35 ^b ±0.58	2.5 ^c ±0.057
IMC+NS oil	105 ^b ±1.7	52 ^b ±0.87	102 ^b ±1.50	211 ^b ±0.57	28 ^a ±0.50	0.95 ^b ±0.006

Values in column with different letters are significantly different at (p ≤ 0.01).

α -amylases, starch hydrolyzing enzymes, are synthesized by animals, plants, and microorganisms Tamura *et al.*, 2007. Data in Table (3) show that α -amylase activity was significant ($p < 0.01$) reduced under IMC toxication condition by 26.6% from control, whereas glucose level was significantly augmented by 2.28 fold compared to control. Co-administration of NS oil with IMC significantly modulated ($p < 0.01$) the α -amylase activity and glucose level by 1.16 and 1.06 folds, respectively which close to control value. The significant decrease in amylase activity, which thought to be a consequence of IMC toxic effect, was in agreement with those reported by Shetti and Kaliwa, (2017). In contrast; some cells show increased catabolism to meet the energy demand under stress induced by the xenobiotics like pesticides (Ivanova-Chemishanska, 1982). The present data are in agreement with those of Kulkarni and Kaliwal, (2011) who reported an increase in amylase activity of immobilized and free *Escherichia coli* following monocrotophos and cypermethrin exposure.

Serum urea and creatinine levels usually used as clinical indicators of nephrotoxicity. Table (3) showed that IMC treatment caused a significant increase in urea and creatinine levels by 1.3 and 5.3 fold respectively, whereas NS oil co-administration greatly mitigate urea and creatinine levels near the control value. The deamination of amino acids in the liver produces urea which transported through the blood to the kidneys where it is excreted in urine (Harvey *et al.*, 2011). In the present study, IMC exposed mice showing an elevation of serum urea

which could be explained by 1) disturbance in protein metabolism, 2) impairment in its synthesis as a result of the impaired hepatic function and 3) decrease in the filtration rate of the kidney. In this context, Khan *et al.*, 2008; Eissa and Zidan, 2009 documented that, pesticide exposure caused cytotoxic changes in the hepatic and renal biochemical markers which were positively correlated with the present data. The normal breakdown of muscle tissue produced creatinine, a waste product, which is normally filtered from the blood and excreted with urine. Also, Gowda *et al.*, (2010) and Khan *et al.*, (2012) revealed that the increase in creatinine level in response to IMC exposure indicates renal disease and may be a result of the impaired glomerular function.

The present data exhibited a significant elevation of total cholesterol, triglyceride and HDL levels in IMC treated mice by 2, 1.3 and 1.4 fold, respectively from control Table (4), and consequently increment in LDL and very low-density lipoprotein VLDL values, estimated according to Friedewald *et al.*, (1972). Co-administration of NS oil with IMC significantly declined the IMC impact near to control value. The increase in cholesterol level may be a sign of liver damage which could be referred to; increased synthesis of cholesterol in the liver (Abdou and El Mazoudy 2010 and Padma *et al.*, 2012), the effect of pesticides on the permeability of liver cell membrane (Kalender *et al.*, 2005) and or the blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum (Aldana *et al.*, 1998).

Table 4: Lipid profile of male albino mice orally administrated with IMC (2.6 mg/kg bw), NS oil (4 ml/kg bw) and their combination.

Animal Group	Parameters (mean \pm SE; $n=5$)				
	TG Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)
C	151 ^b ± 0.57	97 ^a ± 0.58	70.6 ^a ± 0.28	-3.8 ^c ± 0.03	69.4 ^a ± 0.15
NS oil	147 ^a ± 0.88	101 ^a ± 0.7	71 ^a ± 1.15	0.6 ^d ± 0.1	67.6 ^a ± 0.4
IMC	204 ^d ± 0.57	197 ^c ± 0.58	99 ^c ± 0.86	57.2 ^b ± 0.5	93.8 ^c ± 0.53
IMC+NS oil	190 ^c ± 0.87	133 ^b ± 1.4	77 ^b ± 1.16	18 ^a ± 0.4	87.4 ^b ± 0.35

Values in the column with different letters are significantly different at ($p \leq 0.01$).

LDL and VLDL were calculated according to Friedewald's equation 1972.

LDL = TC - HDL - TG / 5. VLDL = TG / 2.175.

TC: Total cholesterol, TG: Triglyceride

HDL: High-density lipoprotein cholesterol

LDL: Low-density lipoprotein cholesterol

VLDL: Very Low-density lipoprotein cholesterol.

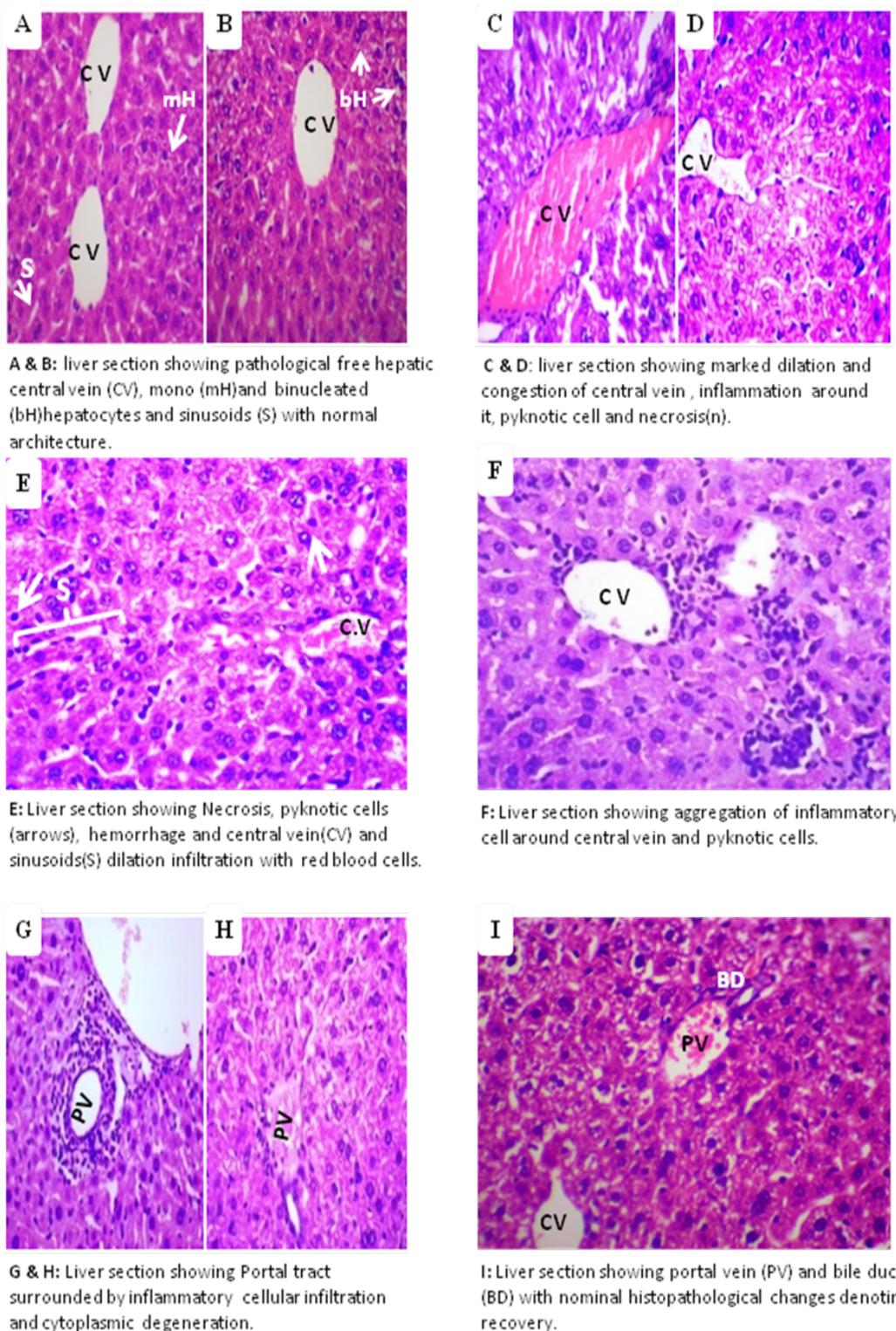


Fig. 1: Liver section showing many histological abnormalities of male albino mice orally administrated to IMC (2.6 mg/kg bw) (C- H), NS oil (4 ml/kg bw)(B) and their combination (I) comparing with control (A). (H & E 400X).

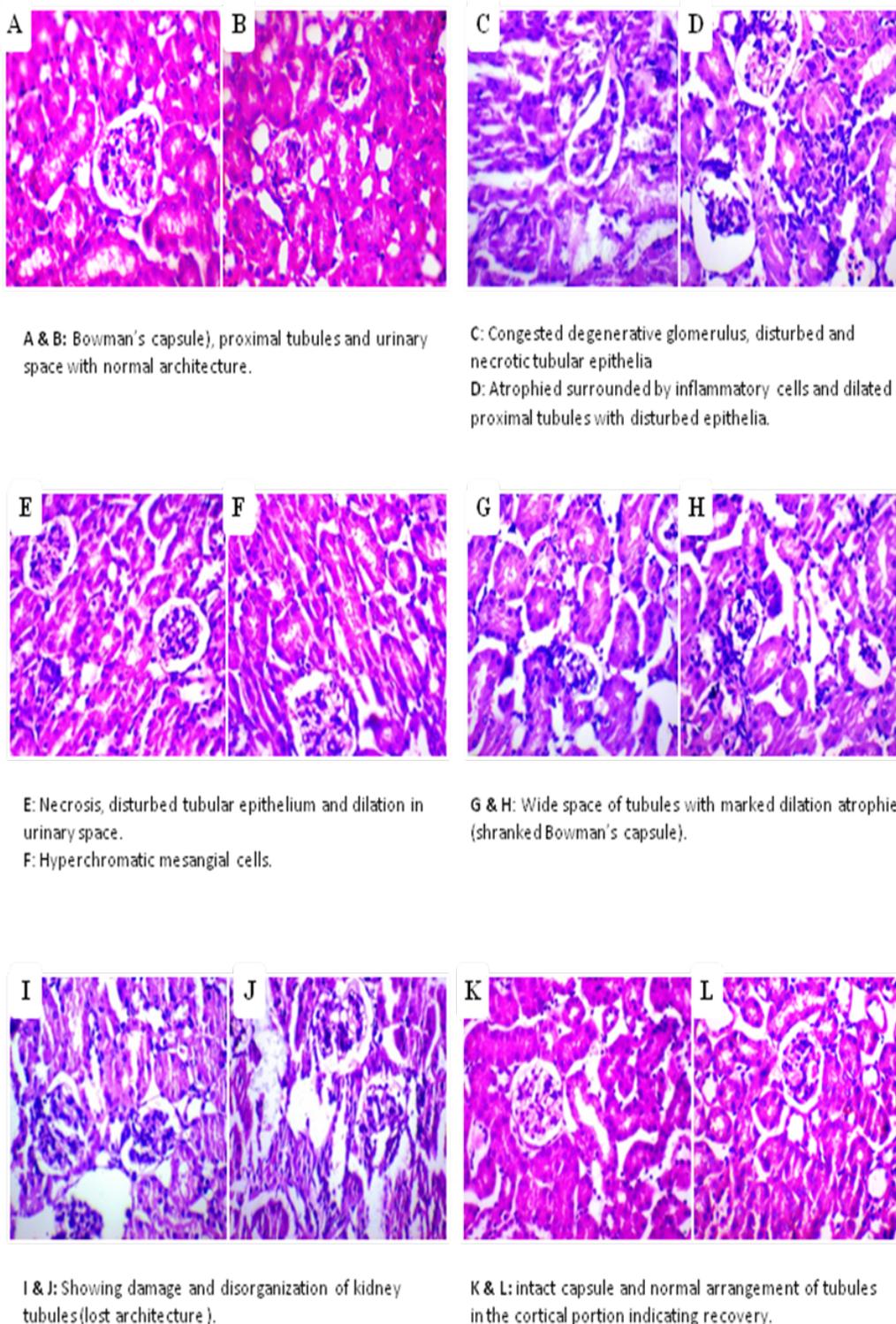


Fig. 2: Kidney section showing many histological abnormalities of male albino mice orally administrated to IMC (2.6 mg/kg bw)(C- J), NS oil (4 ml/kg bw) (B) and their combination (K, L) comparing with control (A). (H & E 400X).

Ashgar *et al.*, (1994) observed that methyl parathion elevate total cholesterol level in male rabbits, which is consistent with our findings. In contrary, Ananya *et al.*, 2003 stated that some pesticides cause a decrease in total cholesterol level. Shakoori *et al.*, (1988), documented that HDL-C is mainly synthesized in the liver and intestinal cells, it plays an important role in cholesterol efflux from tissues and return it to the liver for removal as bile acids. It has been established that the elevated serum or plasma HDL-C levels are antiatherogenic (McGill *et al.*, 1981). Ryhanen *et al.*, 1984 observed that serum HDL-C levels were increased in the dichlorvos treated rats which in consent with our results, whereas the HDL-C level reduction are associated with an increased risk for coronary artery disease (Stein 1987). Salem, (2005) and Saleem *et al.*, (2012) mentioned that NS oil has hypoglycemic and nephroprotective effects, due to its ability to decrease hepatic gluconeogenesis as well as lowered serum creatinine and blood urea nitrogen values which consistent with the present findings.

3. Histopathological examination

histopathological assessments are regularly used for identifying organ abnormalities related to chemical exposure (Crissman *et al.*, 2004). Fig. (1 A, B) illustrated the normal architecture and a pathological free mice liver section showing mono and binucleated hepatocytes with granular cytoplasm. Sinusoids (S) are evident between the cords of hepatocytes, Kupffer cells (K) are associated with sinusoidal lining cells of liver control (A) and NS oil (B) groups. The IMC treated mice showed degenerative changes in the hepatocytes in the form of marked dilation, congestion, hemorrhage (Fig.1 C, D). Also, the results clarify infiltration with red blood cells, pyknotic cell, necrosis (E) and hepatic aggregation of the inflammatory cell around central vein (F) while portal inflammation and cytoplasmic degeneration were represented in (Fig.1 G, H).

The observation in this study are in agreement with those of Choudhary *et al.*, (2003) who revealed that endosulfan treated rats exhibited liver degenerative changes include binucleated cells, hypertrophy of hepatocytes, lymphocytic infiltration in the central vein and dilation of sinusoidal spaces with the irregular nuclear shape. Also, Kostka *et al.*, (2000) detected that in rats, treated with permethrin and DDT separately; liver section of permethrin - treated rats presented, pyknotic nuclei, acidophilic cytoplasm, and cell with nuclear fragmentation, whereas DDT causes cytoplasmic vacuolization and hepatocyte necrosis and all the previous mentioned symptoms are in line with our findings. At the present work co-administration of NS oil implies that hepatocytes, central vein, portal vein (PV) and bile duct (BD) showing nominal histological changes attending recovery (Fig.1 I), meaning that NS oil

lightened the lesions caused by IMC treatment in mice, this data is in concomitant with, Kapoor, (2009).

Fig. (2). represent kidney sections from the control group (A) and NS oil-treated mice (B) showed intact histological structure of glomeruli and renal tubules. However, abnormalities in kidney of treated mice were detected in glomeruli and in convoluted tubules (Figure 2C-J). The main characteristic findings were the appearance of; congested degenerative glomerular tuft, disturbed and necrotic tubular epithelia, dilated atrophied, infiltration in between the degenerated tubules with fibrosis and hyalinosis between the tubules in a focal manner, wide space dilated proximal tubules, hyperchromatic mesangial cells, shrunken Bowman's capsule, damage and disorganization of kidney tubules (lost architecture) were also observed. The present findings of mice kidney tissues treated with IMC revealed histological changes, similar signs of toxicity were also reported earlier by Kammon *et al.*, 2010 and Marrs 2012. Co-administration of the NS oil with IMC showed marked improvement in kidney histological structure compares to the IMC group alone (Fig. 2 K, L). Khajavi rad *et al.*, 2008 and Mohammadian *et al.*, (2008) indicated that NS oil has significant preventive and disruptive effects against renal calculi and CaOx deposits and that in line with our present data.

CONCLUSION

The present study concluded that IMC at the sublethal dose, 2.6 mg/kg bw, induced cytotoxic changes in the liver, pancreas, and kidney as manifested by biochemical results and histopathological findings, NS oil attenuates the IMC adverse effects, due to antioxidant and cytoprotective property, so we highlight its use as a food supplement.

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الملخص العربي

تأثير زيت حبة البركة *Nigella sativa* على السمية المستحثة بمبيد الإيميداكلوبريد

في ذكور الفئران البيضاء

رضا خميس عبد الرازق على

قسم بحوث سمية المبيدات للتديبات - المعمل المركزى للمبيدات - مركز البحوث الزراعية - الاسكندرية

الإيميداكلوبريد (IMC) هو مبيد حشرات جهازى يستخدم على نطاق واسع في كل من الزراعة والطب البيطري، ويعتبر الإيميداكلوبريد من أكثر المبيدات النيونيكوتينويدية التي تم رصد متبقياتها فى الفواكه والخضروات، لذا وضعت هذه الدراسة لتقييم التغيرات البيوكيميائية والنسجية المرتبطة بسمية IMC في ذكور الفئران البيضاء *Muss musculus*. أعطيت جرعات فمية من IMC تمثل خمس الجرعة المميته لنصف الحيوانات المعاملة (١/٥٠ من قيمة LD₅₀) وتعادل ٢,٦ مجم / كجم من وزن الجسم ولمدة ٢٨ يوماً (٥ جرعات / أسبوع). تم رصد السمية على الكبد والبنكرياس والكلى عن طريق تقدير كل من الأسبارتات امينوترانسفيراز (AST)، والأنين امينوترانسفيراز (ALT) وكذا الألفا أميليز ومستوى كل من الجلوكوز، والكوليسترول الكلي، والدهون الثلاثية، ومستويات الكوليسترول عالي الكثافة (HDL) والكرياتينين واليوريا بالإضافة الى دراسة تأثير زيت حبة البركة (*Nigella Sativa oil*)، (٤ مل / كجم من وزن الجسم) للتخفيف من الآثار السلبية للـ IMC. وأظهرت النتائج أن الإيميداكلوبريد سبب زيادة معنوية في كل من وزن الجسم للحيوانات المعاملة ووزن الأعضاء النسبي، أيضا حدث زيادة فى نشاط انزيم ALT وارتفاع مستويات كل من الجلوكوز، اليوريا، الكرياتينين، والكوليسترول الكلي، الدهون الثلاثية والكوليسترول عالي الكثافة (HDL) بينما حدث انخفاضا ملحوظا فى نشاطى كل من AST والألفا أميليز بالمقارنة بالمجموعة الضابطة. تم تأكيد التأثير السلبي للـ IMC على القياسات البيوكيميائية السابقة بالتغيرات التشريحية في كل من الكبد والكلى، كما أظهرت المعاملة بزيت حبة البركة اختزالاً ملحوظاً لتأثير المبيد السلبي. وخلصت الدراسة الحالية إلى أن مبيد الإيميداكلوبريد المستخدم بجرعة تمثل خمس قيمة الـ LD₅₀ لديه القدرة على إحداث تغييرات سامة لخلايا كل من الكبد والكلى كما تجلى من النتائج البيوكيميائية والنسجية، وان زيت حبة البركة حد من التأثيرات الضارة للـ IMC لماله من خصائص مضادة للاكسدة وحامية للانسجة.