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The protective effect of Sider honey and Zinc on imidacloprid induced hepatorenal and hematological toxicity in rats

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Abstract: The present study aimed at evaluating the ability of antioxidants S.honey and Zn (2doses; high and low) for 30 days to ameliorate alterations caused by imidacloprid (IM) insecticide in damaged hepatorenal and hematological of rats. Rats oral administration with IM caused significant increase in hepatic markers enzymes (AST &ALT), kidney functions (urea, uric acid and creatinine) lipid profile (T.cholesterol, TG and LDL) concomitant with significant decreases of serum total protein, albumin and HDL level, as well as significant decreases in the number of erythrocytes, platelets and hemoglobin (Hb) levels together with significant increases of leukocytes number. Liver and kidney sections revealed extensive histological changes after IM treatment. On other hand, supplemented diet with S.honey and low dose of Zn (in separated and in combination) led to an obvious improvement of the injured times and ameliorating the damaging effects of IM, which might be due to their antioxidant properties and scavenging abilities against active free radicals. On the other hand, treatment with high dose of Zn, alone or in Combination with S.honey caused an adverse effects on the liver. Hence, further studies are needed to find out the appropriate doses of honey and Zn as antioxidants with fewer side effects.

Keywords: Sider honey, Zinc, lipid profile, liver enzymes, kidney functions, liver and kidney histological observation.

1. Introduction

Environmental pollution is a worldwide problem in a modern society. Pesticides are widely used to enhance the crop production and other benefits. They have raised concerns about potential adverse effects on the environment, human health and non-target animals. Unfortunately, the applications of these derivatives of pesticides are highly toxic to a number of non-target organisms [21]. Human health risks vary with the type of the pesticides and also with the extent of vulnerability. 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, birth defects and effects on the nervous system [53]. Pesticides are known to increase the

production of reactive oxygen species (ROS), which in turn prompt oxidative stress (OS) in different tissues [26,44].

Imidacloprid (IM) is a neonicotinoid insecticide first registered for agricultural usage in 1994 and classified under toxicity class II/III agents by the United States Environmental Protection Agency [52].

It acts on the nervous system by blocking postsynaptic acetylcholine receptors, which kills the insect [50]. Recently, the World Health Organization WHO (2009) has declared that neonicotinoid including IM induce toxicity in organisms [11,39,47]. The lipophilic nature of neonicotinoid facilitates their interaction with the cell membrane and leads to perturbations in the phospholipids bilayer structure, enhancing the production of ROS, which in turn generate OS in different tissues [33,39,48].

Antioxidants are any substance that delays, prevents or removes oxidative damage to a target molecule [24]. They are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats, and oil [25]. Honey consists of vitamin C, phenol compounds, catalase, peroxides and glucose oxydase enzymes so, it has antioxidant properties also, it contains flavonoids and carotinoids [13]. Antioxidant properties of honey act as an antidepressant during high emotional, physical and intellectual stress [29]. On the other hand, honey represents a natural product that does not carry side effect, which can be harmful to health. Many studies of the different indicate to protective effects of honey via its antioxidant activity against toxicity that induced hepatorenal and hematological toxicity by Abd El-Ghany et al. [2], Al-awar [3], Garba et al. [24], Mahaneem et al. [35] and Majid et al. [36].

Zn is known as an essential trace element necessary for protein metabolism. It is necessary for membrane integrity and also, involved in the structure and function of over 300 metalloenzymes. It has important functions in skin and connecting tissue metabolism as well as in wound healing [10]. It exerts its antioxidant effects indirectly by maintaining membrane structures, involving in the structure of SOD increasing the metallothionein concentrations and competing with redox reactive metals, iron and cuprous for critical binding sites [54]. It founds studies of indicate to role of Zn as antioxidant activity against toxicity that induced hepatorenal and hematological toxicity by Al-Sabaawy [6], Ambali et al. [7], Duran et al. [17], El-Ashmony [19], Kilic et al. [32], Mansour and Mossa [37] and Samir et al. [45].

Since, no data were available in the literature related to the protective effect of S.honey and Zn in combination (high dose and low dose) against the effects of insecticides. Hence the present study aims to evaluate the ability of antioxidants S.honey and Zn (in separated and in combination) to ameliorate the alteration caused by Imidacloprid insecticide on hepatorenal damage, hematological toxicity and the histological structure of liver and kidney tissues in rats.

2. Materials and Methods

2.1. Animals:

Forty five adult male albino rats weighing 300 - 350 g were obtained from the Zoo, Sana'a, Yemen. They were housed in stainless steel cages (five rats per cage) in a well-ventilated room, under standard conditions of humidity at room temperature and normal light dark cycle at Faculty of Education, Sana'a University for one week to adapt the laboratory environment before experimentation. The animals were fed on a diet composed of fresh vegetables, pea nuts, millet, dried bread, and water ad libitum during the study. The experimental animals were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH, 1978), and were approved by the Animal Experiments Local Ethics Committee at the Zoo, Sana'a, Yemen.

2.2 Chemicals

Chemicals used in this study were:

2.2.1 Imidacloprid (IM)

IM (70% W.P.) was obtained from general directorate of plant protection. Ministry of Agriculture and Irrigation, Sana'a, Yemen (Produced: KAMSUN Chemicals Co., Ltd. China).

2.2.2 Sider honey

National floral honey of Ziziphus spina- christi was bought from a beekeeper in Amran Province, Yemen.

2.2.3 Zinc (Zn)

Zn Sulfate (ZnSO4.7H2O), obtained from Sigma- Aldrich, to assess the antioxidant effect of the mineral.

2.3 Experimental Design

Animals were divided into seven groups of five animals each as follow:-

Group I: served as negative control group with a single daily dose of saline solution (5 ml) as oral administration for 30 days.

Group II: served as IM control group with a single daily dose of saline solution(5 ml) as oral administration for20 days, then a single daily dose of (1/5 of LD50 equal 90mg/kg bw/day) dissolved in distilled water) for 20 days.

Group III: was orally treated with a single protective dose of 5 ml/kg b.w/day of S.honey for 10 days, then treated with a single dose of IM (90 mg/kg bw), concomitant with S.honey (5 ml/kg b.w/day) for 20 days.

Group IV: was orally treated with high dose of Zn (50 mg /kg b.w/day) for 10 days, then treated with 90 mg/kg bw IM, concomitant with high dose of Zn (50 mg /kg b.w/day) for 20 days.

Group V: was orally treated with low dose of Zn (25 mg /kg b.w/day) for 10 days, then treated with 90 mg/kg bw IM, concomitant with low dose of Zn (25 mg /kg b.w/day) for 20 days

Group VI: It was orally treated with S.honey and Zn (5 ml/kg b.w/day &50 mg/kg b.w), respectively, for 10 days, then with 90 mg/kg bw IM, concomitant with S.honey and Zn (5 ml/kg b.w/day &50 mg /kg b.w) for 20 days .

Group VII: was orally treated with S.honey and Zn (5 ml /kg & 25 mg /kg b.w/day) respectively for 10 days, then with 90 mg/kg bw IM, concomitant with S.honey and Zn (5 ml/kg b.w/day & 25 mg/kg b.w/day) for 20 days .

2.4 Blood and Tissue Samples Collection:

At the end of the experiment, rats in all groups were fasted overnight for 12 h. Blood samples were taken from the eye and divided into two parts. The first was maintained in EDTA bulb and plain tubes for hematological assay whereas, the second was centrifuged at 3500 rpm for 20 min, and the serum was separated for biochemical tests. Animals were autopsied, Small pieces of the liver and kidney of each rat were removed, fixed in 10% formalin for 24-48 hours and kept in 70% alcohol for histological preparation.

2.5 Biochemical assay:

The serum of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein T.protein), albumin, urea, uric acid, creatinine, total cholesterol (T. cholesterol), high density lipoprotein - cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) levels were measured by spectrophotometry according to the standard protocol supplied by the Spin react commercial kits.

2.6 Hematological Assay

Estimation of RBCs, Hb, WBCs, lymphocytes and platelets were measured using automated hematology system (Sysmex America, Inc. Model:XE-2100).

2.7 Histological Preparation:

Tissues of liver and kidney were dehydrated in series of alcohol concentrations 80%, 90% and 100%, cleared in xylene, embedded in paraffin wax blocks at 58°C. Sections were cut at 4 microns thickness using rotary microtome and stained with hematoxylin and eosin [28] for histological examination under light microscope.

2.8 : Statistical Analysis

The statistical analysis was performed by Graph Pad Prism; continuous data were expressed as mean ±SE. Data was compared using one–way ANOVA. P value <0.01 and considered to be statistically significant

3. Results

3.1. Results of liver and kidney functions tests:

The administration of IM for 20 days caused significant rise in ALT and AST activity. Similarly serum level of urea, uric acid and creatinine were significantly higher in IM treated group compared to control group. In contrast, the level of T.Protein and albumin were significantly decreased, as compared to that of the control group. On the other hand, the G3 that was treated with IM concomitant with S.honey showed non-significant decrease in the level serum of AST, ALT, urea, uric acid and creatinine with non-significant decrease in serum levels of T.Protein and albumin compared to that of control group. The G4 that the treated male rats with IM and Zn (H.D), appeared a significant increase in the levels of AST, ALT, urea, uric acid and creatinine with significant decrease in the level of T.Protein and albumin compared to control group. The combined administration of IM and Zn (L.D) resulted in non-significant increase in serum levels of AST, urea and creatinine, when was significantly decreased in ALT level with significant decrease in uric acid when compared to controls. Meanwhile, results showed significant decrease in T.Protein and non-significant decrease in albumin compared to that of respective controls. The combined administration of IM, S.honey and Zn (H.D) to male rats resulted in a significant increase in serum levels of AST and ALT, whereas non-significant changes in urea, uric acid and creatinine when compared to that of the control group. Moreover, results showed significant decrease in T.Proteins and albumin, as compared to the control. The combined administration of IM, S.honey and Zn (L.D) significantly reduced the adverse effects of IM, and had non-significant decrease in the mean values of AST, ALT, urea, uric acid and creatinine than that of the control group. Meanwhile results showed a significant decrease in T.Protein and non-significant increase in albumin, as compared to control group (Table1& fig.1).

Table 1. The Effect of Antioxidants (S.honey and Zinc) on the Adverse Effects Induced of IM on L.F.T

Parameter / Experimental Groups	AST IU/L M±SE	%	ALT IU/L M±SE	%	T. Protein g/dl M±SE	%	Albumin g/dl M±SE	%
G1: Control	112.4±6.918	.....	42.40±2.315	.....	7.404±0.224	.....	4.36±0.161	.....
G2: IM	164.4±7.447*	46.3%	63.20±3.527*	49.1%	6.362±0.383*	14.1%	3.82±0.139*	12.3%
G3: IM+ honey	109.2±10.40$^{\#}$	2.9%	37.80±2.059$^{\#}$	10.9%	6.712±0.217^{NS}	9.4%	4.20±0.08367$^{\#}$	3.6%
G4: IM+ Zn(H.D)	183.5±8.332*	63.3%	55.00±2.739*	29.7%	6.575±0.214*	11.2%	3.80±0.1683*	12.8%
G5: IM+ Zn(L.D)	117.8±2.955$^{\#}$	4.8%	41.00±2.739$^{\#}$	3.3%	6.675±0.167*	9.9%	4.16±0.131^{NS}	4.2%
G6: IM+ honey+ Zn(H.D)	151.0±2.345*	34.3%	60.00±1.633*	41.5%	5.348±0.266*	27.8%	2.63±0.249$^{*\#}$	39.7%
G7: IM+ honey+ Zn(L.D)	100.8±5.313$^{\#}$	10.3%	41.00±2.160$^{\#}$	3.3%	6.688±0.163*	9.7%	4.475±0.1702$^{\#}$	2.7%

Values are expressed as mean ± SE; percentage of difference with control group. Comparisons are made between each group. *: significant different vs control group, $^{\#}$: significant different vs IM treated group, ^{NS}: not significant.

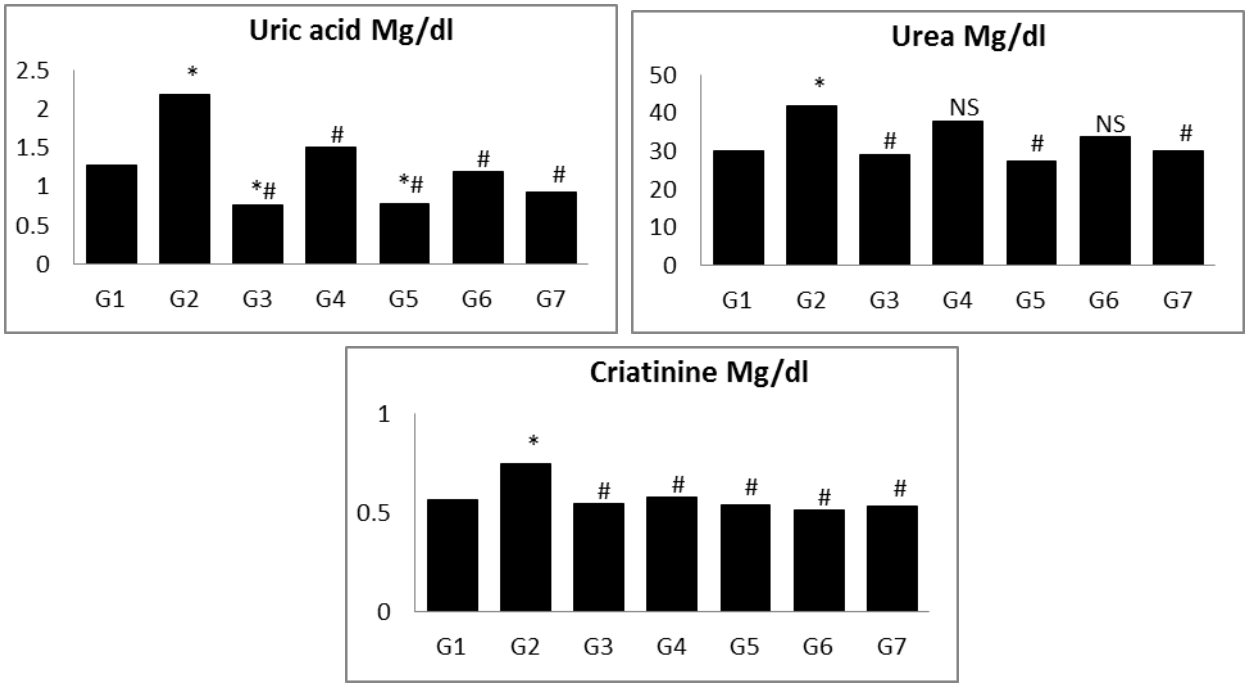

Figure 1. Vertical bars showing Mean± SE of serum of Urea, Uric acid and Creatinine in rats. *: significant different vs control group, $^{\#}$: significant different vs IM treated group, ^{NS}: not significant. G1: Control, G2 IM, G3: IM+honey, G4: IM+Zn(H.D), G5: IM+ Zn(L.D), G6: IM+honey+ Zn(H.D), G7: IM+ honey+ Zn(L.D).

3.2. Results of Lipid Profile:

As shown in table 2 in IM treated group, exhibited significant elevation in serum levels of cholesterol and TG with non-significantly decrease in HDL and significant increase in LDL, as compared to the control group. The combined administration of IM with S.honey in G3 and S.honey and Zn (L.D) in combination with IM in G7 revealed non-significant change in serum levels of cholesterol, TG, HDL and LDL, as compared to the control group. The administration of IM concomitant with Zn (H.D) in G4 and S.honey plus Zn (H.D) in combination to IM in G6 showed non-significant increase in serum levels of cholesterol and significant increase in TG, as compared to the control group. Moreover, results showed significant decrease in HDL with non-significant increase in LDL, as compared with to control group. The combined administration of IM and Zn (L.D) in G5 had non-significant change in serum levels of cholesterol, HDL and LDL with significant increase in TG, than that of the control group.

Table 2. The Effect of Antioxidants (S.honey and Zinc) on the Adverse Effects Induced of IM on Lipid Profile Levels.

Parameter / Experimental Groups	Cholesterol Mg/dl M±SE	%	Triglycerides Mg/dl M±SE	%	HDL Mg/dl M±SE	%	LDL Mg/dl M±SE	%
G1: Control	50.00±2.92	.....	83.20±5.17	.....	50.00±2.92	.....	83.20±5.17	.....
G2: IM	52.20±1.77 ^{NS}	4.4%	90.20±7.43 ^{NS}	8.4%	52.20±1.77 ^{NS}	4.4%	90.20±7.43 ^{NS}	8.4%
G3: IM+ honey	52.80±1.77 ^{NS}	5.6%	80.60±5.73 ^{NS}	3.1%	52.80±1.77 ^{NS}	5.6%	80.60±5.73 ^{NS}	3.1%
G4: IM+ Zn(H.D)	59.50±5.52 ^{NS}	19%	187.0±14.40 $^{*\#}$	124.8%	59.50±5.52 ^{NS}	19%	187.0±14.40 $^{*\#}$	124.8%
G5: IM+ Zn(L.D)	56.00±2.86 ^{NS}	12%	123.0±4.30 $^{*\#}$	47.8%	56.00±2.86 ^{NS}	12%	123.0±4.30 $^{*\#}$	47.8%
G6: IM+ honey+ Zn(H.D)	52.25±2.66 ^{NS}	4.5%	147.5±7.60 $^{*\#}$	77.3%	52.25±2.66 ^{NS}	4.5%	147.5±7.60 $^{*\#}$	77.3%
G7: IM+ honey+ Zn(L.D)	54.75±4.11 ^{NS}	9.5%	80.00±6.72 ^{NS}	3.9%	54.75±4.11 ^{NS}	9.5%	80.00±6.72 ^{NS}	3.9%

Values are expressed as mean ± SE; percentage of difference with control group. Comparisons are made between each group.*: significant different vs control group, #: significant different vs IM treated group, NS: not significant.

3.3. Results of Hematological Parameters:

As shown in fig. 2 in IM treated group, exhibited significant reduction in the number of RBC, platelets and Hb levels with significant elevation in in leukocytes number. The co-administration of S.honey with IM in G3 and Zn (H. D) with IM in G4 had a non-significant increase in the number of erythrocytes and Hb levels compared to the control group. Meanwhile, results showed significant increase in leukocytes number with significant decrease in platelets number compared to the controls. The combined administration of IM and Zn (L.D) in G5 had a non-significant change in numbers of erythrocytes, leukocytes, platelets and Hb levels, as compared to that of the control group. The co-administration of S.honey and Zn (H.D) in combination with IM in G6 revealed non-significant decrease in erythrocytes and platelets number, and significant decrease in Hb level with significant increase in leukocytes number of the treated male rats in comparison to that of the control group. The co-administration of S.honey and Zn (L. D) in combination with IM in G7 had a non-significant decrease in

erythrocytes, leukocytes number and in Hb level with significant decrease in platelets number compared to control group.

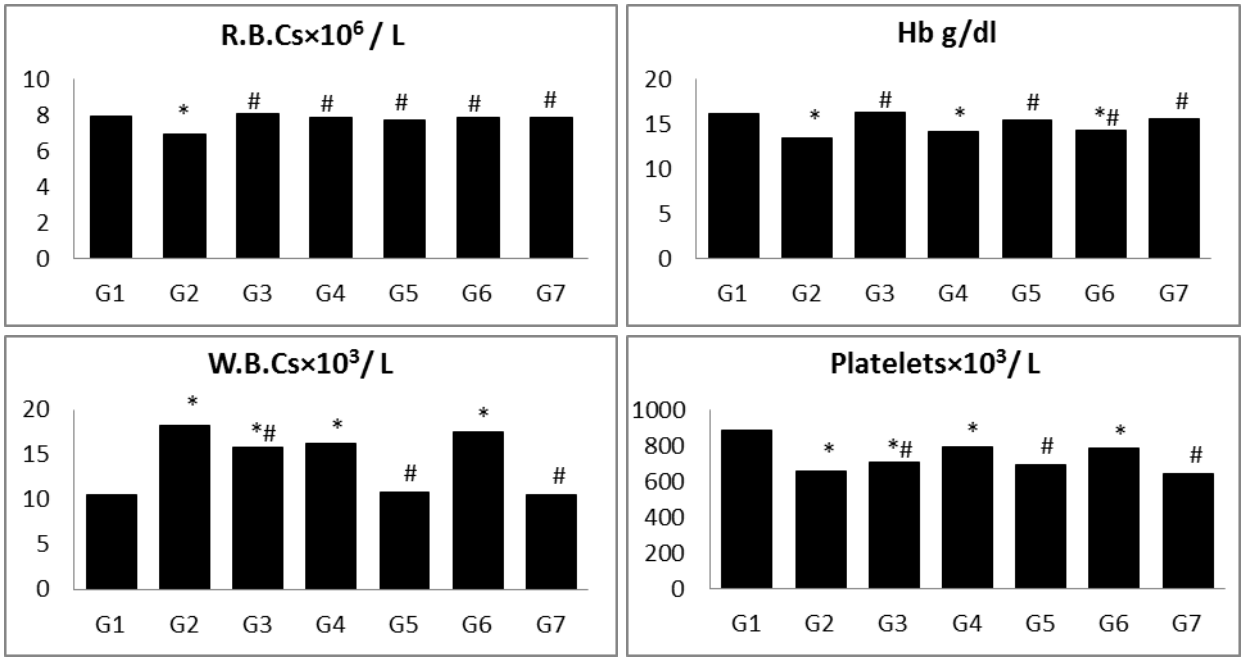

Figure 2. Vertical bars showing Mean± SE of RBCs, Hb, WBCs and Platelets in rats. *: significant different vs control group, #: significant different vs IM treated group. G1: Control, G2 IM, G3: IM+ honey, G4: IM+ Zn(H.D), G5: IM+ Zn(L.D), G6: IM+ honey+ Zn(H.D), G7: IM+ honey+ Zn(L.D).

3.4. Histological observation of liver:

Light microscopic examination of liver in the control rats showed healthy looked cells; normal lobular structure, hepatocyte plates were arranged in radiantly from central vein, blood sinusoids separate the plates from each other's, and Kupffer cells were clear (fig.3a). Examination of liver section of G2 (IM) showed histological changes; necrosis, amyloid, infiltration, and hydropic changes (fig. 3 b, c, d, e& f). Light microscopic examination of the liver section after administration of S.honey with IM in G3 (fig. 4b), Zn (L. D) with IM in G5 (fig. 4c) and S.honey and Zn (L. D) in combination with IM in G7 (Fig. 4d) revealed normal structure as in control group. Administration of IM with Zn (H. D) in G4 (fig. 5b) and S.honey and Zn (H. D) in combination with IM (fig. 5c) revealed severe necrosis, amyloid and hydropic changes.

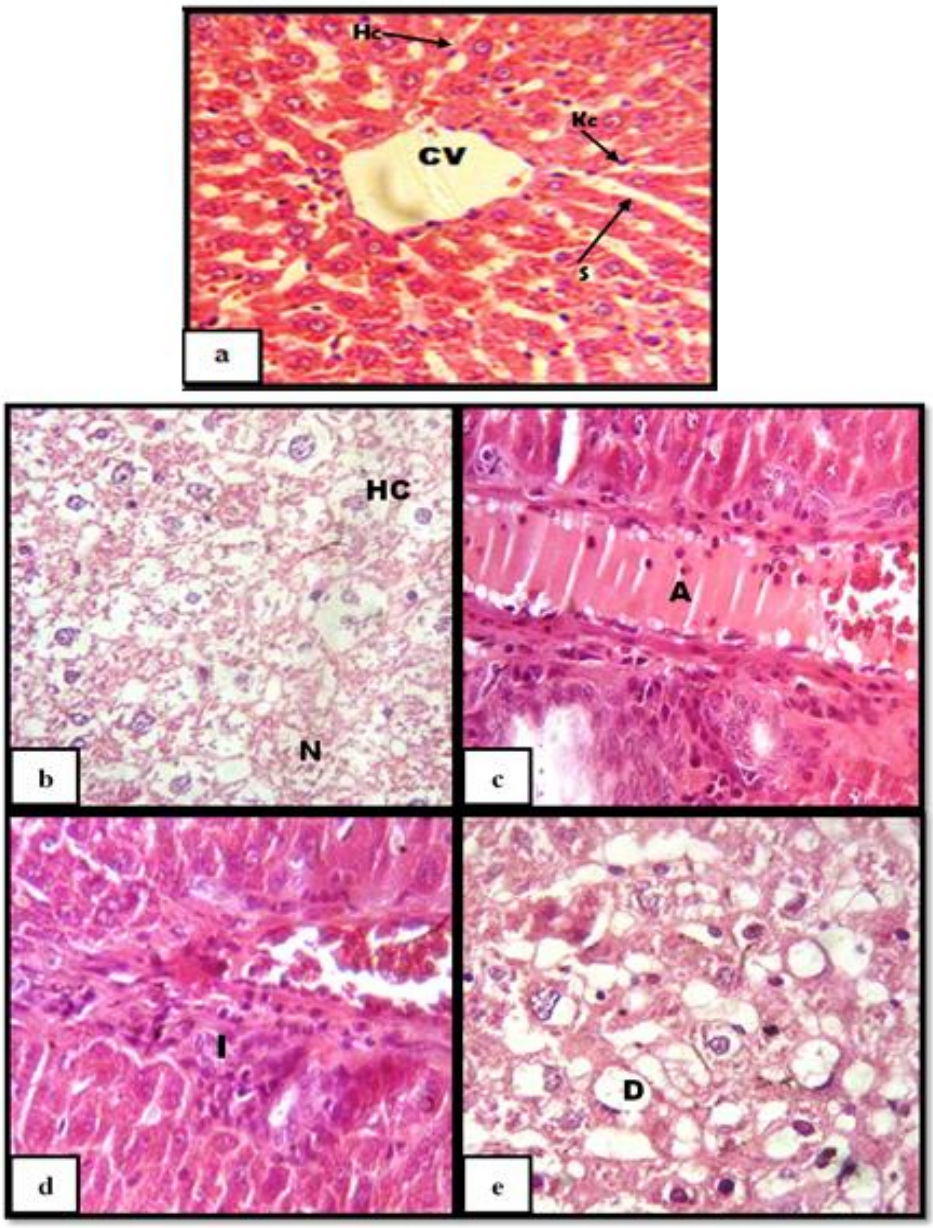

Fig.3:Photomicrographs of sections of liver. (a): control group showing a normal architecture without pathological alterations. Hepatocyte (Hc), Spherical nucleus (arrow), Sinusoids (S), Blood vessel (BV), and Kupffer cells (Kc). (b, c, d, e &f): IM group; showing obvious histopathological changes. Hydropic changes (HC), Necrosis (N), Amyloid (A), Inflammatory cells infiltration (I), Hepatocytes degeneration (D), Hemorrhage (H). (HE) stain (X400).

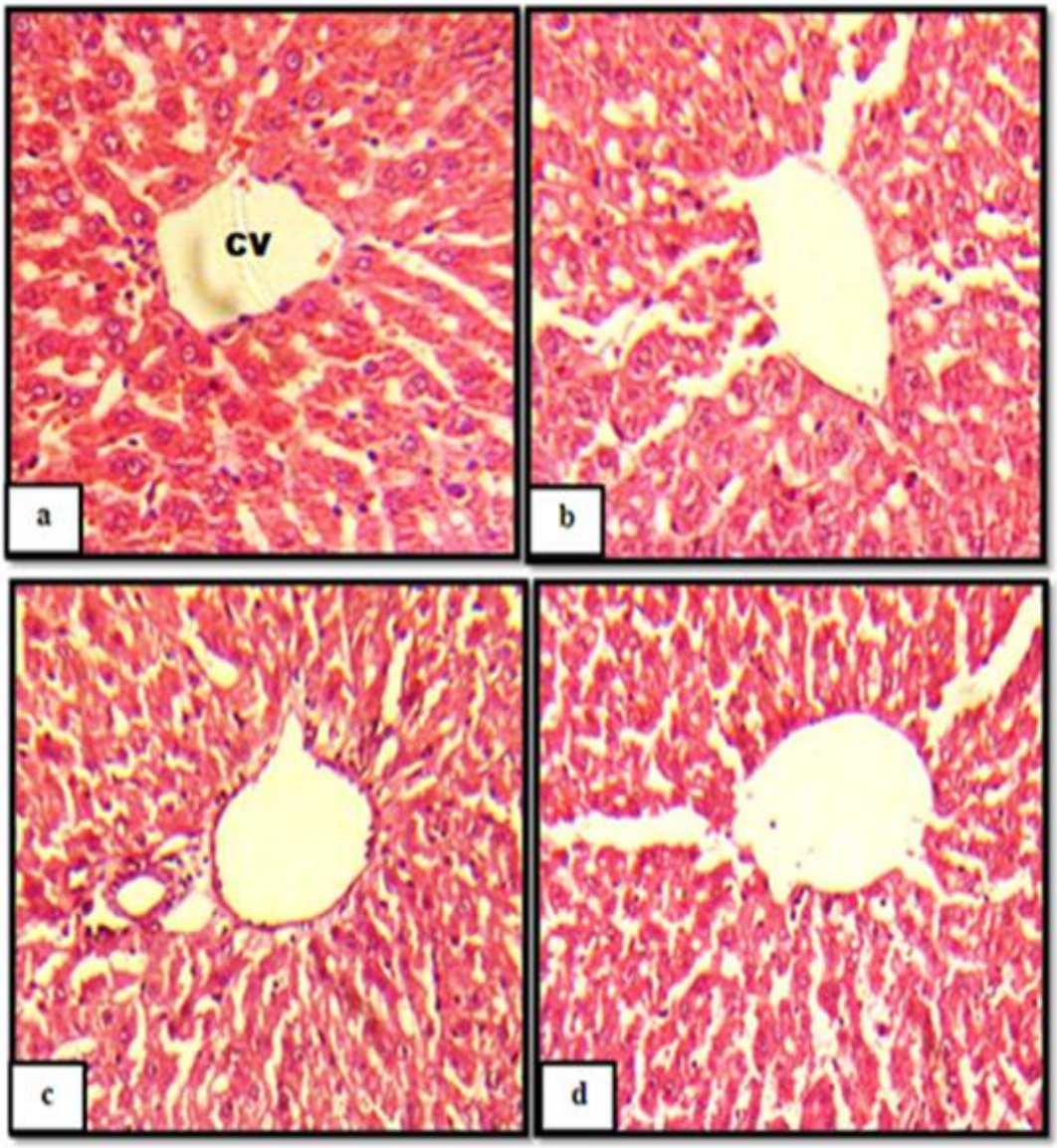

Fig. 4: Light Photomicrographs of sections of liver. (a): control group showing a normal architecture without pathological alterations. (b): IM + Sider honey; (c): IM + Zn (L.D); (d): IM + Sider honey + Zn (L.D), showing a normal liver structure as in control group. (HE) stain (X400).

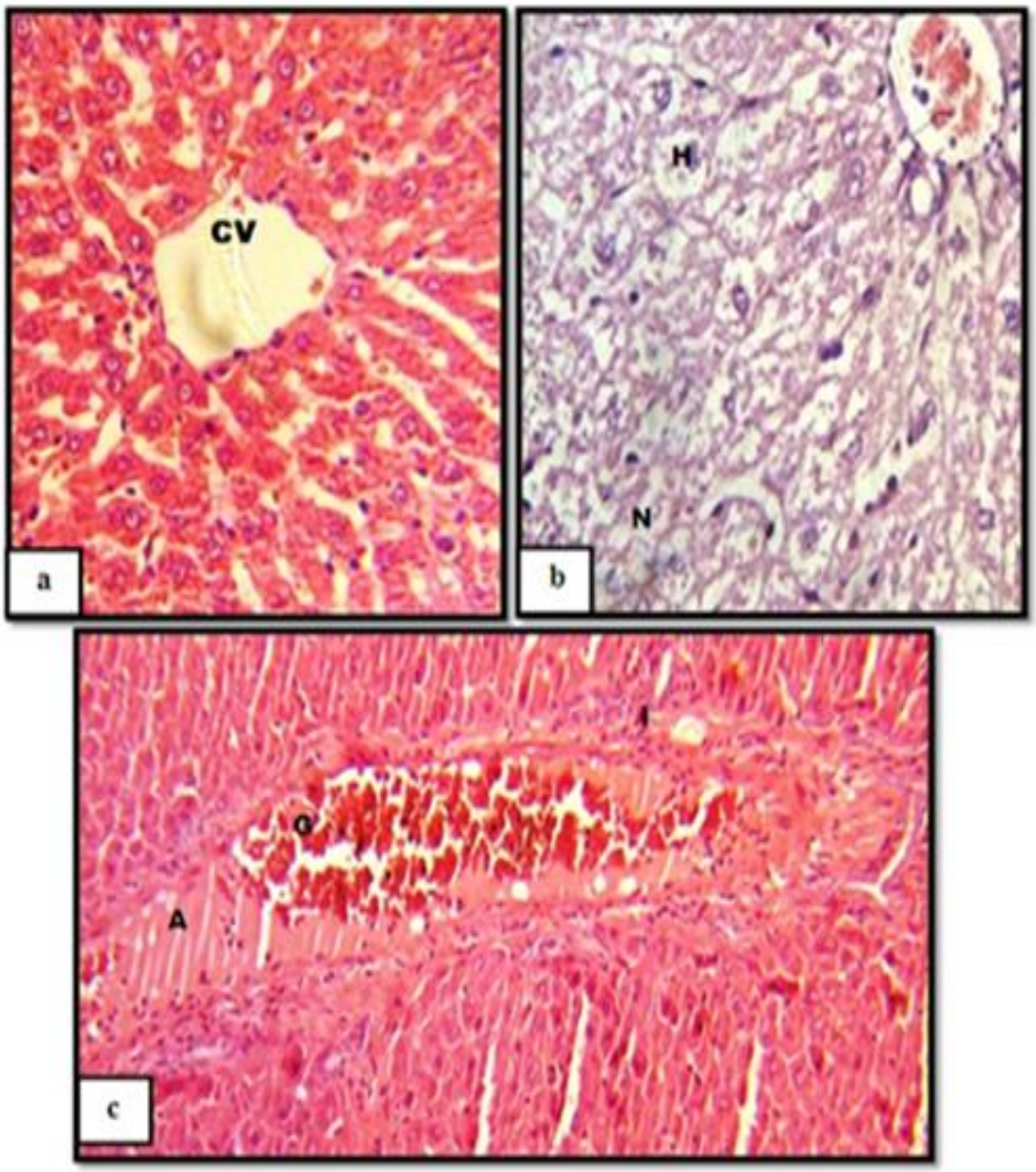

Fig. 5: Photomicrographs of sections of liver. (a): control group showing a normal architecture without pathological alterations. (b &c): IM + Zn (H.D) and IM + Sider honey& Zn (H.D), showing obvious histological changes as in IM group. Hydropic change (H), Necrosis (N), Amyloid (A), Inflammatory cells infiltration (I), Hemorrhage (G). (HE) stain (X400).

3.5. Histological observation of kidney:

kidneys in control rats appeared renal corpuscles each one is formed of glomerular tuft of blood capillaries surrounded by capsular space and Bowman's capsule, the proximal convoluted tubules was normal with intact distal convoluted tubules without pathological alterations. (Fig. 6a). Histological examination of the kidney after administration of IM revealed severe shrinkage of glomeruli and

glomerular degeneration, as well as hydropic changes, tubular degeneration and tubular necrosis were noticed, in addition to thickened blood vessels and little hemorrhage (Fig. b, c, d &e). Administration of S.honey with IM in G3 (fig.7b), Zn (H.D) with IM in G4 (Fig.7c) and Zn (L.D) with IM in G5 (Fig. 7d), in addition S.honey plus Zn (2doses; H.D in G6 or L.D in G7) combination with IM (fig. 7 e&f, respectively) showed nearly normal histological structures as in the control group.

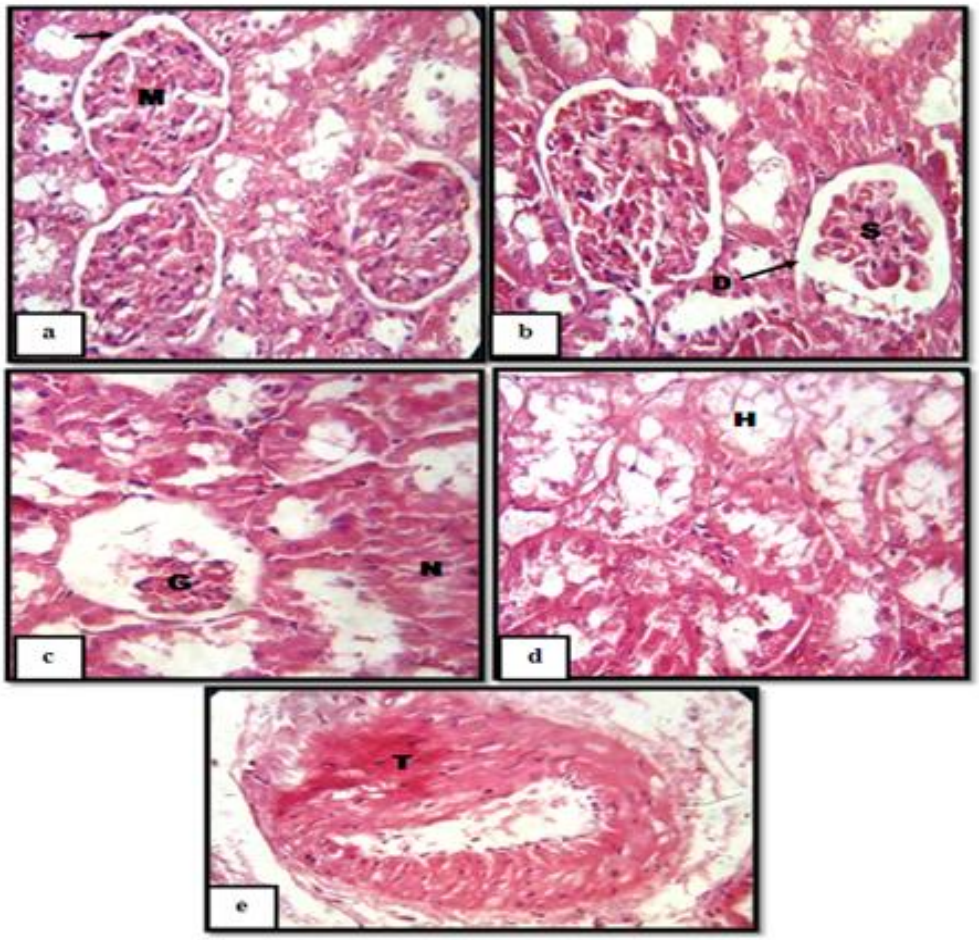

Fig. 6: Photomicrograph of sections of kidneys. (a) Control groups showing normal histological structures of Malpighian corpuscles with its glomerulus (M) Bowman's capsule (arrow). (b, c, d &e): IM showing dilatation of Bowman's capsule (S), glomerulus shrinkage (G), glomerular degeneration (D), Necrosis (N), Hydropic change (H) and Thickened blood vessels (T). (HE) stain (X400).

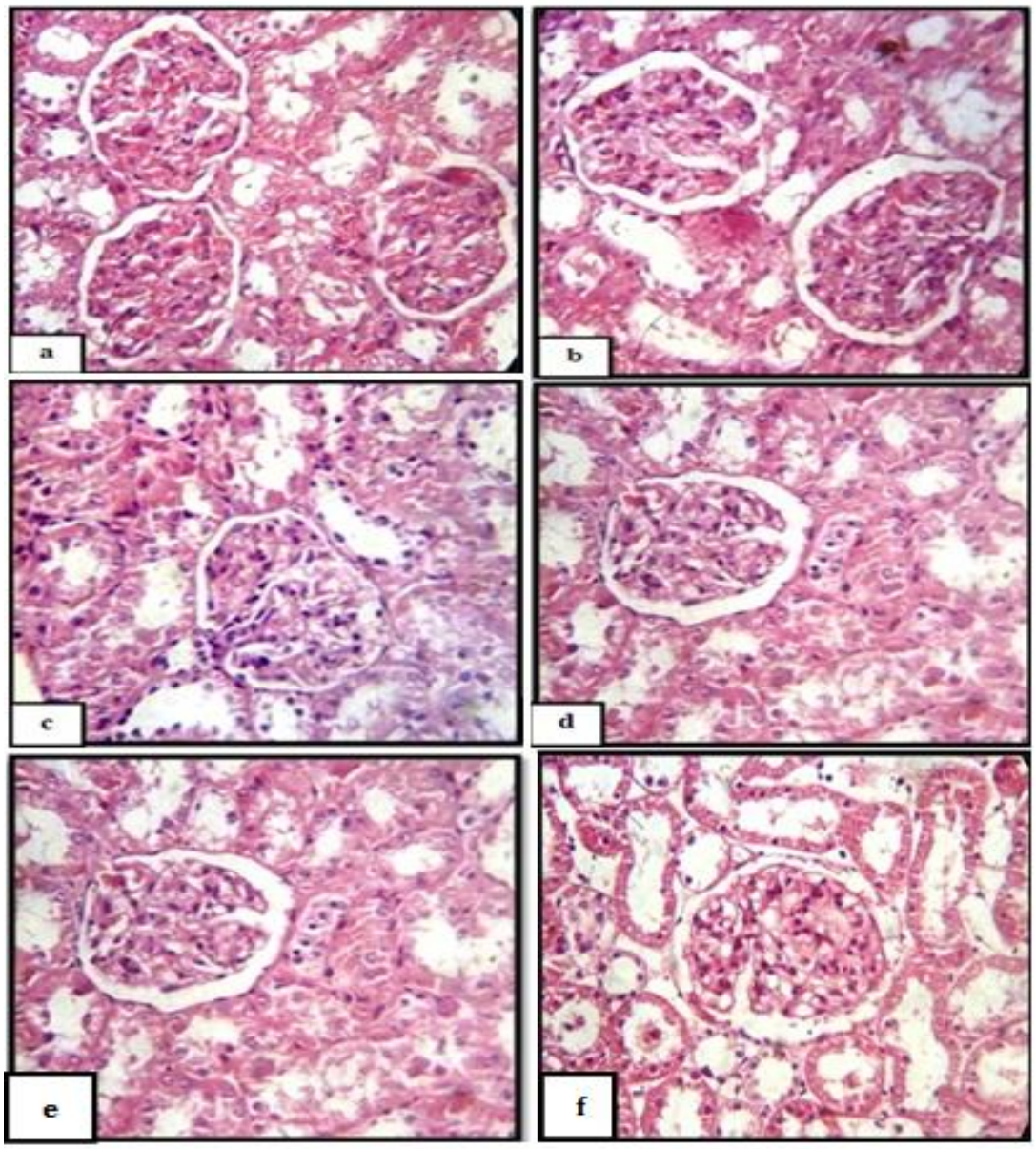

Fig. 7: (a) Photomicrograph of sections of kidneys. (a) Control groups showing a normal architecture without pathological alterations. (b): IM + S.honey; (c): IM + Zn (L.D); (d): IM + S.honey + Zn (L.D), (e): IM + Zn (H.D); (f): IM + S.honey + Zn (H.D); showing a normal kidney structure as in control group. (HE) stain (X400).

4. Discussion

Level of ALT, AST, T.Protin and albumin are the most sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity [49]. In our study administration of IM had significantly increased serum AST and ALT levels as compared to the control. This result might be attributed to increasing the permeability of cell membrane, that lead to release of transaminases into the blood stream because of degeneration and necrosis of hepatocytes, which might be attributed to ROS induced by IM according to Arfata et al. [8], Lonare et al. [34] and Soujanya et al. [47]. In the present study treatment with IM in rats resulted in a significant decrease in serum level of T.Protein and non-significant decrease in serum level of albumin compared to the control group. Our finding agreed with Priya et al. [42] who attributed the quantity of protein to the rate of protein synthesis or to rate of its degradation and the quality of protein may be due to impaired incorporation of amino acids in polypeptide chains. Halliwell and Gutteridge [24] found that IM had oxidative damage as the central mechanism of toxicity. Moreover, antioxidants can prevent cell damage caused by the action of ROS and free radicals [16].

In the present study, results revealed that administration of Sider honey ameliorate the changes that induce hepatotoxicity by IM. This indicated that Sider honey prevent liver damage, by its ability to eliminate free radicals generated by IM insecticide, as reported by Al-Awar [3] and Mahaneem et al. [35]. Zn is a trace element, was used to assess the antioxidant effect in this study by two doses (H.D) and (L.D). In general, results showed that low dose Zn reduced oxidative stress caused by IM more than high dose, which might be due to that Zn is a trace element. On the other hand, excessive Zn caused reverse effects, in addition, Zn of high dose caused toxicity on some liver function parameters more than IM. Our finding disagreed with some studies observed that Zn supplementation was found to offer protection against γ-irradiation induced hepatotoxicity [5], ethanol-induced liver damage [14], the different results might be referred to lower amount of doses, which used in those studies compared to that of the present study. The group treated by honey and Zn (H.D), respectively showed slightly reduction in the adverse effects induced by IM on serum AST and ALT levels, while it showed severe effects on TP and albumin. These results might give an explanation for the high percentage of deaths in this group, which might explain that both IM and increasing of Zn caused adverse effect. On other hand, the group treated with S.honey and Zn (L.D) respectively, improved serum AST, ALT and albumin levels than that of the control group, which might be due to the combination between artificial antioxidant and natural antioxidant in exact doses. our results have no supporting researches in previous literatures about the combination effects of honey and Zn against hepatotoxicity and nephrotoxicity, So this study could be considered as the first to achieve these results in this field.

In our results IM induced histological alterations in liver tissue. these result were in agreement with the results obtained by Duzguner and Erdogan [18] and Toor et al. [51]. Histological changes induced in liver by IM were ameliorated in rats which treated with S.honey, this refers to the antioxidant properties

of S.honey. This indicates that Sider honey prevented liver damage. These results were in conformity with Chandane et al. [15], Halawa et al. [23] and Al-Awar et al. [4]. Although, Zn is known as an essential element, produced severe structural changes in liver sections. Either when administrated alone or with honey, this result could be attributed to the amount of Zn dosage (50 mg/kg b.w). These findings indicated that the excessive of Zn supplement has adverse effects according to the suggestion of Yadrick et al. [54] who reported that excessive intake of Zn supplement has a potential risk to humans. Besides Caglar et al. [14], who suggested that Zn might increase the oxidative stress in healthy rats. Results in group 4 and 6 disagreed with some previous studies which insured that Zn prevented liver damage induced by cadmium [30], nickel [45] and alcohol [55]. The different results might be due to the difference amount of doses, methods of preparing doses and route of treatment. While healthy structure of liver section was observed either when Zn was administrated alone G5 or with S.honey G7, which might be attributed to the treatment with the low dose of Zn (25 mg/kg b.w/day).

In the present study effect of IM on lipid profile, showed non-significant increase in serum cholesterol and TG levels and significant increase in LDL level while there was a non-significant decrease in HDL when compared to the control group. These findings agreed with the study of Priya et al. [42] and Qadir et al. [43]. Our study, demonstrated that levels of lipid profiles such as cholesterol, TG, HDL and LDL were recovered after administration of S honey with IM when compared to the control group. These results indicated that the ameliorative effect of hone helped to reduce the damage induced by IM and produced significant improvement on liver functions, as reported by Al-awar [3] and Majid et al. [36]. The present study demonstrated that Zn has an improvement effect on serum HDL and LDL levels in both doses, but it has adverse effects on cholesterol and TG in the same doses. Our results are in agreement with many studies who reported that serum LDL and HDL levels restored normalcy following treatment with Zn of both doses, on the other hand the present study disagree with other studies which reported that Zn reduced serum TC, TG and LDL levels and increased HDL levels in patients with type 2 diabetes mellitus [19] and in hyperlipidemic patients [6]. S.honey with Zn in (H.D) caused adverse effect on lipid profile except LDL, these findings might be attributed to the high dose of Zn. On the other hand, the group treated with low dose of Zn with S.honey improved lipid profile restored to normalcy. This might explained the important role of combined S.honey and Zn as antioxidants and considering the appropriate dose of Zn with S honey.

The present investigation clearly demonstrated that administration of IM resulted in a significant increase in serum level of urea, creatinine and uric acid comparing to the control group. Our findings are in agreement with those reported by Arfata et al. [8], Priya et al. [42] and Qadir et al. [43]. On the other hand, Bhardwaj et al. [11], Helal et al. [27] and Kammon et al. [31] reported non-significant increase in uric acid and creatinine in animal treated with IM. The results of the present study might be attributed to the low renal functions which had been shown in the histological study as a result of severe shrinkage of

glomeruli and glomerular degeneration, as well as changes in water reabsorption ability of kidney, tubular degeneration and tubular necrosis. These actions might prevent the filtration of the waste products (urea, uric acid and creatinine) from the blood stream [12]. Rats' ingested honey had a significant improvement when compared to IM group. These findings were in agreement with other studies which suggested that honey exert protective effects against doxorubicin-induced nephrotoxicity [38], acetate [23], ochratoxin (OA)-induced OS in kidney [20] and paracetamol-induced nephrotoxicity [18]. These results might be attributed to the active role of S.honey which in turn reduced IM-induced toxicity.

Our results observed a toxic effect of IM on the structure of kidney, as nephrotoxicity. This study conformed those were recorded by several authors Arfata et al. [8], Bhardwaj et al. [11], Kammon et al. [31] and Soujanya et al. [47]. On the other hand, our results showed that in groups treated with (H.D) and (L.D) of Zn these was a significant decrease in nephrotoxicity, it has been noticed that treatment with IM. Histological changes on kidney which were different from those effects on liver, however Zn reduced histological changes either in (H.D) or (L.D). These results were in conformity with those reported by Duran et al. [17] who reported that Zn supplementation might have a protective effect from cigarette smoke-induced nephrotoxicity in rats and Mansour and Mossa [37] who observed that Zn had resulted in pronounced ameliorating effect against chlorpyrifos-induced kidneys damage in male and female rats. Also effects of S.honey with Zn in (H.D) or (L.D) together reduced IM effect that induced histological changes on kidney.

IM had adverse effects on hematological parameters that were studied in the present study. Our results agreed with results demonstrated by Qadir et al. [43] and Soujanya, et al. [48] who reported that administration of IM in fish and rats caused a significant decrease in RBC, Hb, platelets, PCV, MCV, MCH and MCHC. On contrast, the results of our study disagreed with the previous results obtained by Balani et al. [9], Bhardwaj et al. [11], Preeti et al. [41] and Shridhar [46] who observed non-significant change in haematological parameters after administration of IM. In the present study, supplementation of S.honey resulted in marked improvements in haematological parameters that might be attributed to the antioxidant properties that reduced toxicity induced by IM, which was in agreement with Abd El-Ghany et al. [2] who insured the ability of honey in ameliorating the side effects induced by gentamicin on hematological parameters, and Abd El-Baset and Abd El-Reheem [1] reported the benefit role of honeybee on the hematological parameters of rats exposed to cadmium chloride. Our findings revealed that alterations in values of RBCs, Hb, platelets and WBCs induced by IM were ameliorated by co-administration with Zn. The ameliorative effect of Zn may be have arisen from its antioxidant properties [7, 40]. These results were in agreement with Kilic et al. [32] and Ambali et al. [7] who found that, the combination between S.honey and Zn produced a significant improvement in some hematological parameters while others were insignificant. These two effects caused reduction in the adverse effects induced by IM. These results confirmed that combining two antioxidants had good results.

In conclusion:

Based on the present study, it can be concluded that, the first time S.honey and Zn (separated and combination) improve the hepatorenal and hematological alterations induced by IM intoxication. Moreover, the most protective effects were observed in male rats treated with S.honey alone, or in combination with Zn at low dose by Zn at low dose alone. Additionally, the antioxidant properties of S.honey and Zn support the bioactive roles of its protective effects on IM toxicity. To strengthen these findings, further experimental studies are needed to evaluate the effect of different doses of S. honey with low dose of Zn as therapeutic factors against the toxicity of IM and other toxicants.

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الأثر الوقائي لعسل السدر والزنك ضد سمية الإيميداكلوبريد على الكبد والكلى والمؤشرات الدموية في الجرذان

المخلص: هدفت الدراسة الحالية إلى تقييم قدرة مضادات الأكسدة; عسل السدر والزنك (منفصلة ومجتمعة) لمدة 30 يوماً في التخفيف من الأضرار الكبدية والكلوية والدموية المحدثة بالمبيد الحشري الإيميداكلوبريد في الجرذان. معاملة الجرذان بالإيميداكلوبراید عن طريق الفم أدت إلى زيادة معنوية في نشاط إنزيمات الكبد (AST & ALT) ومؤشرات وظائف الكلى (اليوريا، حمض اليوريك والكرياتينين)، ومستويات الدهون (الكوليسترول الكلي، الدهون الثلاثية و الدهون منخفضة الكثافة)، صاحب ذلك انخفاض معنوي في مستوى البروتين الكلي والألبيومين والدهون مرتفعة الكثافة، بالإضافة إلى ذلك انخفاض معنوي في عدد كريات الدم الحمراء والصفائح الدموية ومستوى الهيموجلوبين مع زيادة معنوية في عدد كريات الدم البيضاء، وكشفت المقاطع النسيجية للكبد والكلى أضراراً كبيرةً بعد معاملتها بالإيميداكلوبريد. من ناحية أخرى، أدى النظام الغذائي المكمل لكلٍ من عسل السدر والزنك بجرعة منخفضة (منفصلة ومجتمعة) عند استخدامهما كمرافق وقائي إلى تحسين الآثار الضارة للإيميداكلوبريد على كل المؤشرات الكيموحيوية والنسيجية المدروسة، والذي قد يكون عائداً لخصائصها المضادة للأكسدة وقدرتها على كسح الجذور الحرة، في حين أن المعاملة بالزنك بجرعة عالية لوحده أو في تركيبة مع عسل السدر سبب آثاراً سلبية على الكبد، وبالتالي هنالك حاجة إلى المزيد من الدراسات لتحديد الجرعات المناسبة من العسل والزنك كمضادات للأكسدة بدون آثار جانبية.

الكلمات المفتاحية: عسل السدر، الزنك، مستويات الدهون، وظائف وأنسجة الكبد والكلي، المؤشرات الدموية، الإيميداكلوبريد