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Therapeutic Potential of Ni(II) Schiff Base Complex on CCl4 Toxicity

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Abstract

Carbon tetrachloride (CCl4) is a known ecological hazardous xenobiotic that could motivate hepatotoxicity. We aimed to examine, for the first time, the therapeutic potential of nickel(II) diacetyl monoxime-2-pyridyl hydrazone complex versus CCl4-induced hepatotoxicity in rats. We used six rat groups of ten animals each. The negative control, vehicle, normal rats injected i.p. with the complex (2.4 mg/kg/day), positive control rats injected i.p. with CCl4, and treated rats administered complex via injection at low and high concentrations of 1.2 and 2.4 mg/kg/day, respectively at the same time as CCl4 injection for short-term (3-weeks) and long-term (8-weeks) treatment. Intoxicated-rodents exhibited significant elevations in the liver index, hepatic serum markers, and oxidative stress with significant reductions in the hepatic, antioxidants, nucleic acids, and proteins. Complex co-treatment with CCl4 significantly suppressed the elevated liver enzyme activities, attenuated oxidative stress, reactivated antioxidant-system components, and amended the hepatic tissue injury. The complex high dose was more efficient than the low dose. Results of negative control were analogous to those of normal rats injected with the complex high dose. The histopathological analysis also supported the above findings. These results show that complex has good antioxidant and therapeutic properties, which can help in treating and preventing CCl4-induced hepatotoxicity.

Keywords: CCl4; Ni complex; Hepatotoxicity; Oxidative stress; GSH; SOD; Catalase.

1. Introduction

As a critical metabolic tissue, the liver encounters a pivotal role in the preservation, performance, and controlling of the body's homeostasis. Any lack of harmony in the activity of the liver results in its injury. Thus, for general body health keeping the liver healthy is a fundamental matter [1]. Hepatic injury is a common liver disease caused by continuous exposure to harmful metabolites such as drugs, viruses, bacteria, parasites, chemical toxins, alcohol, chronic diseases like diabetes, obesity, and tumor and may consequently lead to acute and chronic liver diseases as hepatitis, cirrhosis, hepatotoxicity [2-5]. Also, hepatic injury is coupled with systemic oxidative stress, which leads to cellular necrosis, fibrosis, tissue lipid peroxidation, along with depletion in glutathione levels [1].

Reactive oxygen species (ROS) are free radicals resulting from molecular oxygen and are able to extremely interact with vital cellular components e.g. fats and nucleic acids, causing their destruction. Cellular ROS are created either internally as side-products of aerobic respiration or owing to external-related sources such as xenobiotics. Extreme production of ROS crushes the cellular antioxidant defense system and generates oxidative stress (OS) which is known to play a critical role in the initiation and progression of liver injury [6].

Carbon tetrachloride (CCl4) is one of the xenobiotic materials that gives rise to hepatotoxicity in both animals and man [7]. CCl4 releases to the ecosystem as one of the components in the water of industrial wastes, water-free liquid solvents used in dry-cleaning laundries and fire extinguishers liquids [8]. CCl4, in its metabolic pathway, trichloromethyl (CCl3·) and trichloromethylperoxyl (CCl3O2·) radicals have formed that start peroxidation of lipids and contribute to liver damage. Then, hepatic cells activate their defense mechanism to avoid damaging effects of ROS by means of internal antioxidants superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), which are highly induced in OS [9,10]. Accordingly, natural/synthetic antioxidants

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have drawn a lot of attention for their potential hepatoprotective roles.

Recently, hepatic injury is still a global health problem. Currently, many drugs for preventing and treating hepatic injury have been developed; unfortunately, the current drugs often have limited efficacy and sometimes with serious side effects [11]. Consequently, great interest from researchers to seek powerful and less/non-toxic liver protective drugs has been observed globally. Besides, metals/metal complexes application in therapeutics is a field of increasing importance [12].

Actually, transition metal complexes have been used as therapeutic agents against numerous diseases [13]. In this regard, our research team is greatly interested. We, for instance, used cobalt-quercetin complex [14] against diabetes, iron(III) 3-oxo-N-(pyridin-2-yl)butanamide complex [15], cobalt(II) diacetyl monoxime-2-hydrazinopyridine complex [16], a set of metal complexes derived from 2-hydrazinobenzothiazole [17] and iron-rifampicin complex [18] against cancer. They provide the purpose as free radical scavengers and thereby gain importance as antioxidant agents. Nickel has been notable as an essential trace element for humans. In-depth, the inhibitory actions of nickel complexes have inspired authors to discover its role as a potential antioxidant of the highly reactive oxygen resulting free radicals [19]. Moreover, such antioxidant property acquired by some nickel complexes may also support treating a variety of pathophysiological abnormalities that mostly result from ROS.

Schiff base ligands, such as diacetyl monoxime-2-pyridyl hydrazone, have great respect due to their prevalent biological applications attributed to their variable bioactivities such as antitumor and anti-carcinogenic properties [20]. In this regard, nickel(II) diacetyl monoxime-2-pyridyl hydrazone complex, synthesized and characterized by Basu et al. [21], was investigated in solution by our team [22]. We elucidated that it can hinder Ehrlich solid tumor progression in mice without serious adverse effects towards the normal tissues and it exhibited both good SOD-mimic activity and high anti-hemolytic effect that indicate its redox capacity through scavenging ROS including superoxide radicals. According to the literature, the impact of this Ni complex on CCl_4 toxicity was not investigated until this moment. Thus, in continuation of our interest, the present study aimed to examine, for the first time, the possible protective effect of nickel(II) diacetyl monoxime-2-pyridyl hydrazone complex against CCl_4-induced liver toxicity in rats and to prospect the underlying mode of action.

2. Experimental

2.1 Chemicals

All solvents and chemicals procured for the experiments were used as received. CCl_4 and nickel chloride were obtained from Sigma (St. louis, MO, USA). Diacetyl monoxime-2-pyridyl hydrazone ligand was generously provided by Prof. Mohamed M. Hassanien (Chemistry Department, Faculty of Technology and Education, Beni-Suef University, Beni-Suef, Egypt). All the further chemicals and reagents used in the experiments were of the highest pure analytical grade available.

2.2 Complex preparation

The complex was prepared in solution according to Saad et al. [22] by mixing two volumes of 1 x 10^{-4} M diacetyl monoxime-2-pyridyl hydrazine ligand dissolved in absolute EtOH with one volume of 1 x 10^{-4} M nickel chloride dissolved in bi-dist. water. The prepared $[Ni^{2+}(L)_2]^{2+}$ complex exhibits stability constant of 6.5 x 10^8 observed spectrophotometrically at 390 nm.

The complexation ratio (1:2) was confirmed by the continuous variation technique. The addition of nickel chloride solution decreased the absorbance band of the ligand at 285 nm and a novel distinctive band of the complex emerged at 390 nm. The plot at 390 nm versus the ligand mole fraction displayed a peak of absorbance at X = 0.66 proving the complexation occurs in a 1:2 ratio.

2.3 Rats and treatment

The study conducted on 60 healthy adult male Wistar rats weighing 161–220 g (about one month age) supplied from the Theodor Bilharz Research Institute, Giza, Egypt. The animals were housed for about one month prior to experimental use and they were nourished with the standard pellet diet and water. Rodents were housed at standard laboratory conditions agreeing with the criteria defined in the Guide for the Care and Use of Laboratory Animals intended by the National Academy of Sciences and published by the National Institute of Health, and approved by the Chemistry Department, Faculty of Science, Damietta University, Egypt.

The effects of CCl_4 and the complex on male rats were studied by random grouping of the sixty animals into six-separated groups (ten animals/group). The first group (control) did not receive any treatment. The second group (vehicle group) was injected daily i.p. with the equivalent solvent amount as that of the normal-complex treated rats group and with olive oil 0.5 ml/kg/week. The third group (normal+Dose 2.4) was injected i.p. with the freshly prepared complex at a dose of 2.4 mg/kg/day. Rats in groups four, five, and six were treated with 50% CCl_4 in olive oil (0.5 ml/kg/week via i.p. injection) [23]. Rats in group four did not get any additional treatment and used as positive controls (CCl_4), whereas rats in group five and

group six were i.p. injected with the complex at low (one-twentieth of the LD_{50}) and high (one-tenth of the LD_{50}) concentrations of 1.2 and 2.4 mg/kg/day, respectively [22] at the same time as CCl_4 injection. The treatment was done for 21 days and 56 days where each group was subdivided into two equal subgroups, one of them was sacrificed after 3 weeks and the other was sacrificed after 8 weeks. All animals were being fasted for 8 hours then sacrificed under diethyl ether anesthesia.

Quickly, cardiac blood specimens were collected, left to settle, and sera were harvested after centrifugation at 4500 rpm for 15 min then stored at -20 $^{\circ}$C until used for the assays of liver markers. The liver was detached and cleaned using ice-cold saline. A small piece from the liver was cut and fixed in 10% formaldehyde for histopathology assay. The residual liver pieces were homogenized in ice-cold PBS (pH 7.2), centrifuged at 4500 rpm for 15 min at 4 °C to get (10%, W/V) homogenate for the estimation of hepatic oxidative stress markers, and liver content of nucleic acids and total protein content.

Body weight was recorded each week during the experiment. Rats' initial body weights (BW) on day 0, final BW on day 21 and day 56 respectively, and the net final BW were recorded. BW gain = (final BW - initial BW) and % BW change = [BW gain / initial BW] $\times$ 100 were determined.

2.4 Liver index

The body mass of each rat was measured earlier to being sacrificed. Similarly, the liver weight was measured directly after sacrificed. The liver index = [liver weight / body mass] $\times$100.

2.5 Serum markers of hepatotoxicity

Serum hepatic markers including activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), and levels of protein (albumin (ALB) and total (TP)) were assayed by using available assay kits (Biodiagnostic, Giza, Egypt).

2.6 Oxidative damage assays in liver homogenate

To evaluate the degree of cellular damage in hepatocytes, the malondialdehyde (MDA) content, a degrading product of lipid peroxidation, was determined in tissue according to the thiobarbituric acid method as described by Ohkawa et al. [24]. Liver glutathione (GSH) level was estimated by the method depending on the breakdown of 5,5′-dithiobis(2-nitrobenzoic acid) resulting in yellow color. Total antioxidant capacity (TAC) was estimated as stated in the kit guidelines (Biodiagnostic, Giza, Egypt). The activity of SOD was assessed as stated by Dechatelet [25]. Hydrogen peroxide degradation was the basis of measuring the activity of catalase (CAT) [26].

2.7 Nucleic acids in liver tissue

Hepatic RNA concentration was assayed by the way of Mejbaum using orcinol reagent to develop a greenish color [27]. Hepatic DNA content was estimated by the method of Dische & Schwarz [28]. The DNA in the supernatant interacts with diphenylamine to yield blue colored complex.

2.8 Hepatic total protein

Hepatic TP content was estimated according to the procedure of Lowry et al. [29].

2.9 Histopathological assay

A sample of liver obtained after decapitation was cleaned in saline and fixed at once in 10% formalin, dehydrated by passing in ascending series of alcohol, cleared with xylene, and embedded in paraffin wax. Sections of the tissues of 5–6 μm thickness were prepared and stained with hematoxylin and eosin (H&E) dye. The pathologist assessing liver sections was uninformed of the rats' treatment.

2.10 Statistics

All the data were expressed by means ± standard error (SE) and the statistical analysis was achieved by SPSS version 22. One-way analysis (ANOVA) was applied for testing the significance of the treatment. Differences between groups' means were determined by Tukey's test and correlation analysis was done using RStudio version 1.3. P values < 0.05 are significant.

3. Results

3.1 Effects on body weights

During the 3 weeks or 8 weeks experiment, normal, vehicle, and normal treated with the complex groups revealed insignificant alterations in the weight gain. While CCl_4 group exhibited a serious decline in weight gain when compared with the normal (p=0.0001). At the end of treatment, CCl_4 rats treated with the complex showed a dose-dependent increase in body weight change percentage compared with the CCl_4 group [p=0.002 and p=0.0001, respectively for the 3-week experiment (Fig. 1A), and p=0.06, and p=0.0001, respectively for the 8-week experiment (Fig. 1B)].

3.2 Effects on liver weight and liver index

At the end of 3 weeks or 8 weeks of treatment, the liver weight increased (p=0.001) in CCl_4 rats as compared to the control ones. Administration of complex with CCl_4 at all doses reduced liver weight in comparison with the CCl_4 group in a dose-dependent mode. Also, vehicle, normal administrated with 2.4 dose, and control groups displayed insignificant variances in the weight of the liver (Fig. 2A,B).

Liver index here is a measure of the degree of liver injury prompted by CCl_4. At the end of either 3 weeks or 8 weeks of treatment, the liver index of the CCl_4 intoxicated group was greater than that of the normal group (p=0.0001; Fig. 2A,B). It was declined by complex administration with CCl_4 at the doses of 1.2 and 2.4 mg/kg (p=0.0001) for 3 weeks (Fig. 2A), and significantly further improved towards normal at

the end of 8 weeks of treatment. Treatment of intoxicated rats with 2.4 mg/kg dose showed an insignificant change in the liver index compared to the normal group. Also, the liver index of normal rats administered 2.4 mg complex/kg was not different from those of the normal and vehicle groups indicating that the complex was safe for the longtime treatment (Fig. 2B).

3.3 Effects of on serum parameters

Table 1 showed that administration of CCl_4 for 3 weeks led to rising (p=0.0001 versus negative control) in activities of hepatic enzymes (ALT, AST, and ALP). A further significant rise in their activities is shown in Table 2 regarding the administration of CCl_4 for 8 weeks. By administration of 1.2 or 2.4 complex dose with CCl_4, these changes in liver enzymes were significantly reduced compared to the CCl_4 group. In-depth, the higher dose showed a better protective effect than the lower dose on liver injury in rats according to ALT, AST, and ALP levels. Moreover, no significant differences in these enzymatic levels were observed between the CCl_4+2.4 dose and the control group, indicating normalization of liver functions (Table 1 and Table 2).

On contrary, the current data showed that CCl_4 in the 3 weeks experiment (Table 1) and in the 8 weeks experiment (Table 2) significantly reduced serum levels of TP and ALB as measured with respect to the control group. However, administration of complex at all doses with CCl_4 caused insignificant changes in these levels as compared to the control group or normal treated with the high dose of the complex.

Our results showed significant positive correlations between the liver enzymes. But both TP and ALB were negatively correlated with ALT, AST, and ALP (Table 3 and Table 4).

3.4 Effects on hepatic oxidative stress

To inspect the protection mechanisms behind the Ni complex on the liver intoxication by CCl_4 in vivo, the levels of MDA and antioxidants in the rats' liver tissues were determined. The present data shown in Table 1 and Table 2 indicate that giving CCl_4 for 3 weeks or 8 weeks initiated significant accretion in the hepatic MDA level as lipid peroxidation index, and significant down-regulation of TAC, SOD, CAT, and GSH levels with respect to normal, vehicle, or normal+dose 2.4 groups. Whereas, co-treatment of CCl_4 with 1.2 mg/kg and 2.4 mg/kg doses of the complex for 3 weeks (Table 1) strengthened the hepatic antioxidant protection mechanism by increasing the antioxidants levels (p<0.05), at the same time, inducing significant fall in the MDA level as regard to those of the CCl_4 group. The higher dose of complex co-administered with CCl_4 showed a nonsignificant change in hepatic antioxidants compared to normal rats.

After 8 weeks of treatment, when the CCl_4+dose 1.2 and CCl_4+ dose 2.4 groups were compared to the only CCl_4-given group, it was found that the GSH levels increased in both (p<0.05). There was a significant decrease in TAC, CAT, and SOD between the CCl_4+1.2 dose group and the healthy control group. But, CCl_4+complex treatment (2.4 mg/kg) insignificantly decreased the activities of TAC, CAT, and SOD as compared to the healthy control group. There was no significant difference in GSH, CAT, SOD, and TAC between the control negative group and normal+2.4 mg/kg dose group (Table 2).

MDA was significantly positively correlated with liver enzymes and antioxidants but was negatively correlated with nucleic acids, TP, and ALB. While the antioxidant enzymes were correlated negatively with liver enzymes and MDA, and positively with each other and with nucleic acids, serum TP and ALB (Table 3 and Table 4).

3.5 Effects on nucleic acids and protein

In Table 5, the results obtained after 3 weeks or 8 weeks of treatment indicated that comparing to the normal, vehicle, and normal+2.4 dose, CCl_4 intoxication triggered significant reductions in DNA, RNA, and protein contents in the liver. However, administration of the complex (1.2 and 2.4 mg/kg) along with CCl_4 significantly increased liver DNA, RNA, and protein content in respect to untreated CCl_4–intoxicated rats depending on the dose. No significant changes were observed in DNA, RNA, and protein contents in the liver between 2.4 mg/kg of complex combined with the CCl_4, normal+dose 2.4, and normal control groups.

In addition, a significant positive correlation was detected between the liver DNA, RNA, protein content, and serum TP and ALB (Table 3 and Table 4).

3.6 Effects on histological analysis of liver

After 21 days of treatment (Fig. 3A), the livers in the normal control, normal rats treated with 2.4 mg/kg complex, and vehicle groups were further found to have normal lobular morphology and hepatocytes with well-defined sinusoids. The hepatic injury in the rats treated with CCl_4 manifested as congestion of portal area blood vessels associated with hepatic steatosis and sinusoidal cell activation. But, focal aggregation of mononuclear cell inflammatory in the livers of 1.2 mg/Kg treated rats with CCl_4. The livers of the rats treated with 2.4 mg/kg complex with CCl_4 showing mild focal aggregation of mononuclear cell inflammatory cell infiltration.

Table 1: Biochemical parameters of rats groups at the end of 3 weeks treatment

|	Measurements	Normal groups			Intoxicated groups		
		Control	Vehicle	Normal+Dose 2.4	CCl$_4$	CCl$_4$+Dose 1.2	CCl$_4$+Dose 2.4
Serum liver markers	ALT (U/ml)	37.2 ± 1.40	38.2 ± 1.35	36.8 ± 1.90	55.6 ± 2.04[a,b,c]	46.2 ± 0.9[a,b,c,d]	34.8 ± 1.40[d,e]
	AST (U/ml)	71.4 ± 1.40	72.8 ± 1.35	72.4 ±1.53	82.6 ±1.47[a,b,c]	79.0 ± 1.40[a,c]	74.0 ± 1.50[d]
	ALP (IU/l)	68.4 ± 1.20	70.4 ± 1.32	69.4 ± 1.30	81.0 ± 1.40[a,b,c]	72.4 ± 1.43[d]	70.0 ± 1.90[d]
	Albumin (g/dl)	3.25 ± 0.02	3.26 ± 0.01	3.21± 0.03	3.0 ± 0.03[a,b,c]	3.17 ± 0.01[d]	3.22 ± 0.02[d]
	TP (g/dl)	7.27 ± 0.11	7.02 ± 0.20	7.35 ± 0.20	6.11 ± 0.13[a,b,c]	7.05 ± 0.11[d]	7.36 ± 0.15[d]
Oxidative stress markers	TAC (mM/g)	1.73 ± 0.02	1.68 ± 0.07	1.71 ± 0.02	1.24 ± 0.05[a,b,c]	1.5 ± 0.03[a,c,d]	1.80 ± 0.03[d,e]
	CAT (µ/g)	69.14 ± 1.4	68.17 ± 1.36	68.19 ± 1.5	48.48 ± 1.50[a,b,c]	59.01 ± 1.9[a,b,c,d]	67.76 ± 2.6[d,e]
	GSH (µM/g)	0.3 ± 0.012	0.29 ± 0.015	0.28 ± 0.009	0.16 ± 0.014[a,b,c]	0.24 ± 0.008[d]	0.30 ± 0.01[d,e]
	SOD (%)	60.6 ± 2.5	62.4 ± 2.80	57.4 ± 1.60	38.2 ± 2.80[a,b,c]	57.6 ± 2.30[d]	62.2 ± 1.80[d]
	MDA (mM/g)	1.56 ± 0.11	1.69 ± 0.13	2.15 ± 0.15	3.07 ± 0.26[a,b,c]	2.15 ± 0.12[d]	1.93 ± 0.13[d]

Data represented as mean ± SEM
[a]Significant when compared with the control healthy rat group.
[b]Significant when compared with the vehicle treated rat group.
[c]Significant when compared with the normal rat treated with 2.4 mg/kg dose of Ni complex group.
[d]Significant when compared with the CCl$_4$ injected rat group.
[e]Significant when compared with the CCl$_4$ injected rat treated with 1.2 mg/kg dose of Ni complex group.
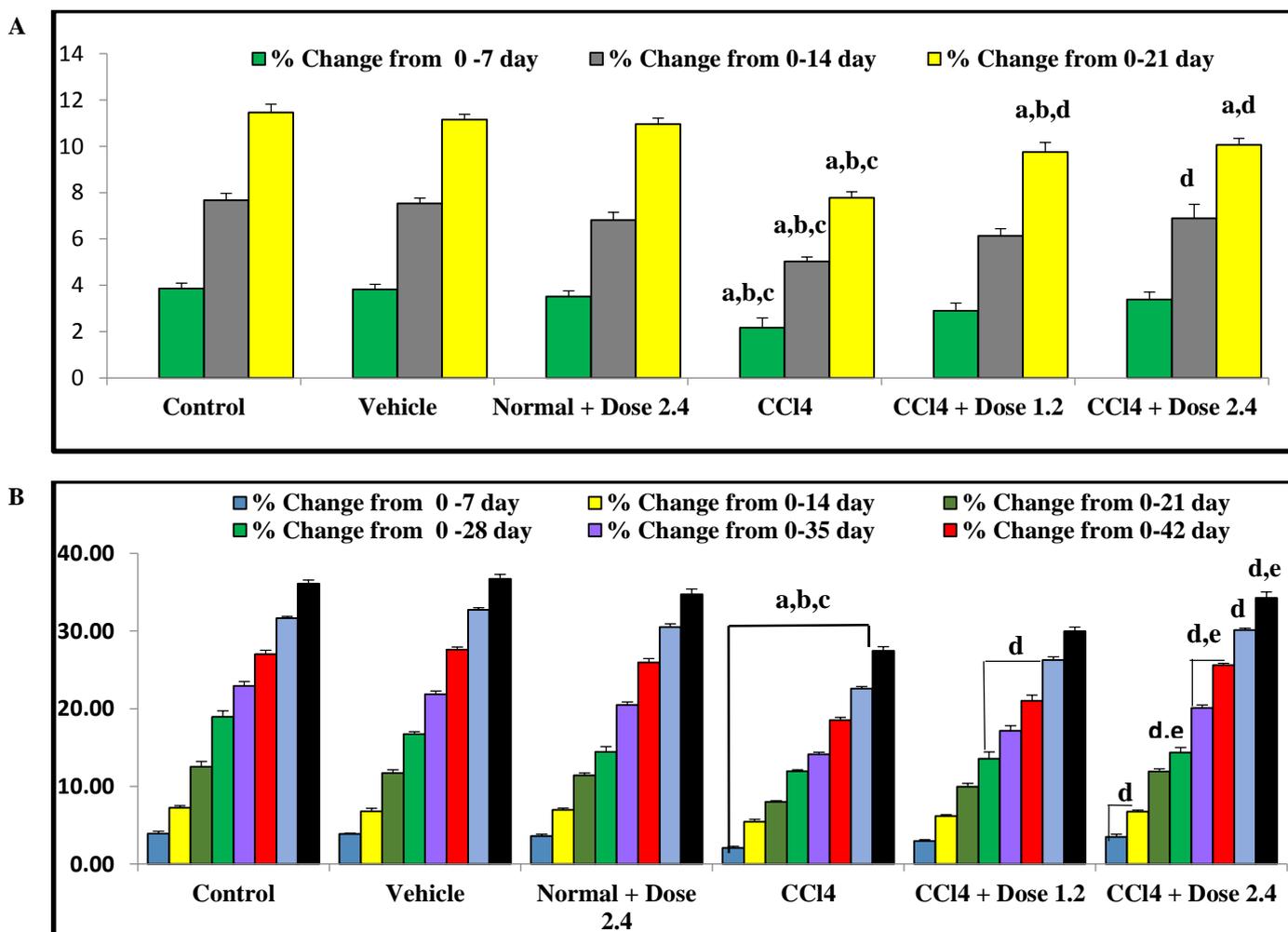
Fig. 1. (A) Bar graphs showing the percentage of body weight change in all studied groups throughout the 3 weeks of treatment. (B) Bar graphs showing the percentage of body weight change in all studied groups throughout the 8 weeks of treatment. Significant alterations are expressed relative to untreated rats (marked with asterisks a), to Vehicle (marked with crosses b), to Normal+Dose 2.4 (marked with crosses c), to CCl$_4$ (marked with ampersand d), and to CCl$_4$+Dose 1.2 (marked with ampersand e). Statistical difference was considered significant p<0.05.
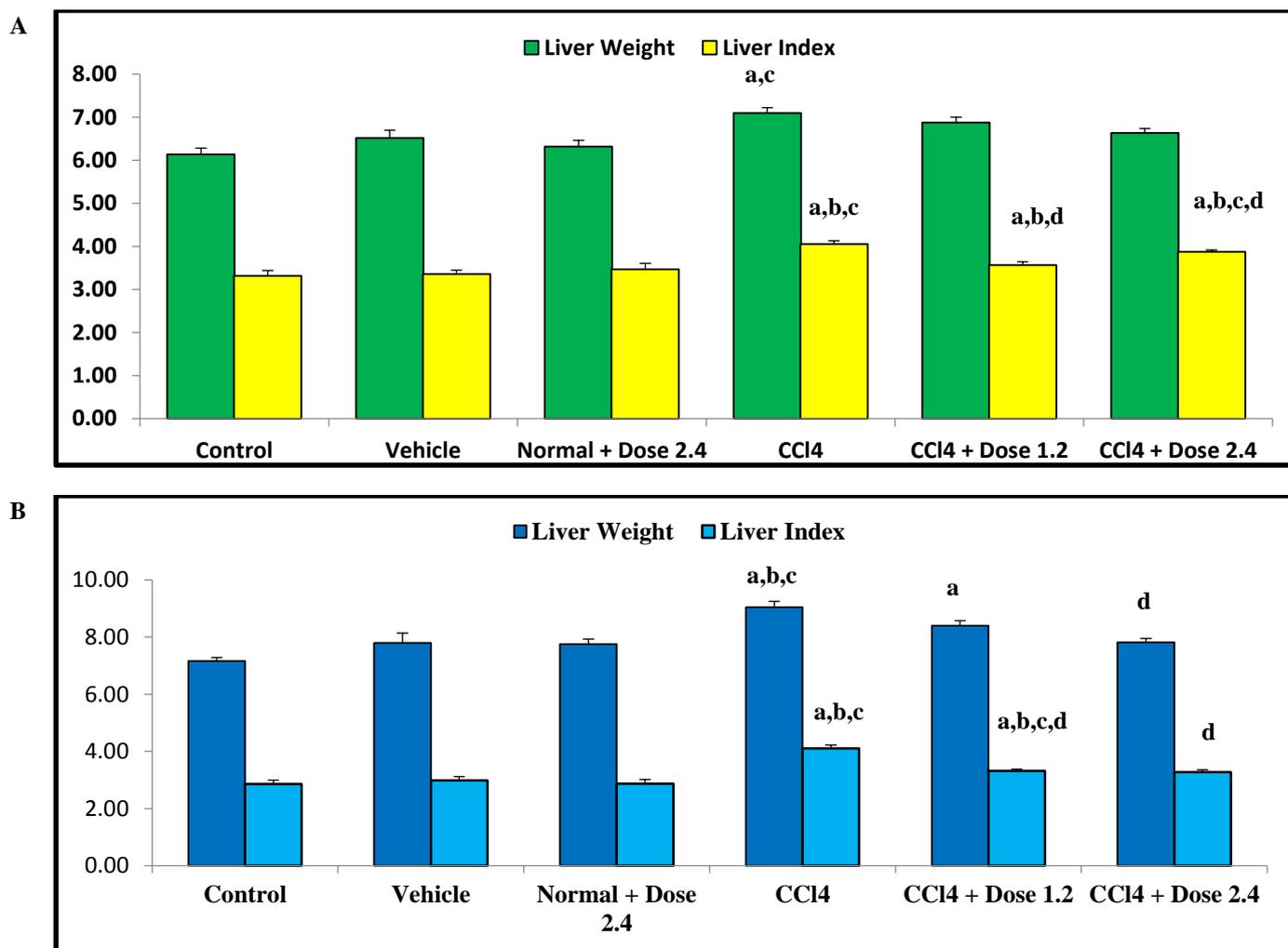
A

B

Fig. 2. (A): Bar graphs showing the change in liver weight and liver index in all studied groups throughout the 3 weeks of treatment. (B): Bar graphs showing the change in liver weight and liver index in all studied groups throughout the 8 weeks of treatment. Significant alterations are expressed relative to untreated rats (marked with asterisks a), to Vehicle (marked with crosses b), to Normal+Dose 2.4 (marked with crosses c), and to CCl₄ (marked with ampersand d). Statistical difference was considered significant p<0.05.

Table 2: Biochemical parameters of rats groups at the end of 8 weeks treatment

	Measurements	Normal groups			Intoxicated groups		
		Control	Vehicle	Normal+Dose 2.4	CCl₄	CCl₄+Dose 1.2	CCl₄+Dose 2.4
Serum liver markers	ALT (U/ml)	38.2 ± 1.10	40.2 ± 1.15	42.8 ± 1.70	67.6 ± 1.50[a,b,c]	48.8 ±1.80[a,b,d]	41.0 ± 2.2[d,e]
	AST (U/ml)	72.2 ± 1.70	70.6 ± 1.70	70.0 ± 1.30	89.4 ± 1.90[a,b,c]	79.6 ± 1.4[a,b,c,d]	77.4 ± 0.9[c,d,e]
	ALP (IU/l)	68.8 ± 1.56	69.0 ± 1.70	69.8 ± 1.30	86.0 ± 1.76[a,b,c]	75.2 ± 1.20[d]	70.2 ± 1.70[d]
	Albumin (g/dl)	3.22 ± 0.02	3.18 ± 0.03	3.21± 0.030	2.93 ± 0.02[a,b,c]	3.14 ± 0.01[d]	3.19 ± 0.03[d]
	TP (g/dl)	7.16 ± 0.11	6.95 ± 0.10	7.5 ± 0.220	6.09 ± 0.15[a,b,c]	6.96 ± 0.07[d]	7.18 ± 0.11[d]
Oxidative stress markers	TAC (mM/g)	1.94 ± 0.04	1.93 ± 0.01	1.97 ± 0.10	0.87 ± 0.04[a,b,c]	1.60 ± 0.03[a,b,c,d]	1.80 ± 0.07[d]
	CAT (μ/g)	71.88 ± 2.1	70.03 ± 2.7	63.31 ± 2.5	41.9 ± 1.90[a,b,c]	60.4 ± 1.12[a,b,d]	69.24 ± 1.50[d]
	GSH (μM/g)	0.28 ± 0.02	0.28 ± 0.02	0.3 ± 0.008	0.13 ± 0.008[a,b,c]	0.22 ± 0.007[c,d]	0.27 ± 0.02[d]
	SOD (%)	61.6 ± 2.08	59.6 ± 2.70	63.8 ± 1.50	34.2 ± 1.800[a,b,c]	50.2 ± 2.10[a,b,c,d]	58.6 ± 1.70[d]
	MDA (mM/g)	1.56 ± 0.08	1.46 ± 0.14	1.99 ± 0.06	3.23 ± 0.130[a,b,c]	2.16 ± 0.20[b,d]	1.95 ± 0.12[d]

Data represented as mean ± SEM
[a]Significant when compared with the control healthy rat group.   [b]Significant when compared with the vehicle treated rat group.
[c]Significant when compared with the normal rat treated with 2.4 mg/kg dose of Ni complex group.
[d]Significant when compared with the CCl₄ injected rat group.
[e]Significant when compared with the CCl₄ injected rat treated with 1.2 mg/kg dose of Ni complex group.

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Table 3: Pearson correlation analysis of serum marker as well as hepatic nucleic acids and antioxidants in all studied groups throughout the 3 weeks of treatment

		ALT	AST	ALP	ALB	TP	TAC	CAT	GSH	SOD	MDA	DNA	RNA	Tissue TP
ALT	R	1	0.76	0.78	-0.83	-0.64	-0.86	-0.87	-0.76	-0.76	0.7	-0.57	-0.8	-0.69
AST	R		1	0.62	-0.76	-0.5	-0.69	-0.81	-0.7	-0.53	0.63	-0.55	-0.6	-0.54
ALP	R			1	-0.76	-0.7	-0.76	-0.72	-0.69	-0.79	0.58	-0.59	-0.74	-0.63
ALB	R				1	0.62	0.73	0.78	0.77	0.71	-0.75	0.62	0.78	0.67
TP	R					1	0.63	0.63	0.65	0.61	-0.65	0.55	0.75	0.62
TAC	R						1	0.8	0.79	0.83	-0.62	0.72	0.83	0.74
CAT	R							1	0.84	0.77	-0.66	0.65	0.77	0.73
GSH	R								1	0.78	-0.72	0.67	0.7	0.74
SOD	R									1	-0.62	0.7	0.76	0.73
MDA	R										1	-0.56	-0.68	-0.45
DNA	R											1	0.7	0.68
RNA	R												1	0.81

Where R= Pearson correlation. Red numbers indicate significant negative correlations, and black numbers indicate significant positive correlations ($p \leq 0.0001$).

Table 4: Pearson correlation analysis of serum marker as well as hepatic nucleic acids and antioxidants in all studied groups throughout the 8 weeks of treatment

		ALT	AST	ALP	ALB	TP	TAC	CAT	GSH	SOD	MDA	DNA	RNA	Tissue TP
ALT	R	1	0.81	0.88	-0.79	-0.71	-0.85	-0.89	-0.8	-0.85	0.88	-0.78	-0.82	-0.81
AST	R		1	0.71	-0.73	-0.63	-0.79	-0.71	-0.67	-0.89	0.7	-0.75	-0.82	-0.84
ALP	R			1	-0.76	-0.69	-0.86	-0.84	-0.72	-0.82	0.81	-0.72	-0.78	-0.72
ALB	R				1	0.73	0.79	0.81	0.78	0.81	-0.74	0.7	0.75	0.77
TP	R					1	0.73	0.61	0.73	0.78	-0.54	0.65	0.74	0.56
TAC	R						1	0.81	0.83	0.88	-0.78	0.75	0.92	0.73
CAT	R							1	0.78	0.74	-0.87	0.76	0.79	0.78
GSH	R								1	0.75	-0.76	0.67	0.77	0.64
SOD	R									1	-0.67	0.81	0.87	0.8
MDA	R										1	-0.58	-0.7	-0.72
DNA	R											1	0.78	0.71
RNA	R												1	0.74

Where R= Pearson correlation. Red numbers indicate significant negative correlations, and black numbers indicate significant positive correlations ($p \leq 0.0001$).

After 56 days, a mild degree of hepatic vacuolation was observed in the liver of the vehicle-treated group. Notably, the livers of the normal rats treated with 2.4 mg/kg complex revealed hepatic vacuolation mostly consistent with fatty degeneration. Nevertheless, the CCl4 group showing marked periportal hepatic necrosis and a severe degree of hepatic vacuolation associated with focal aggregation of mononuclear inflammatory cells. However, treated rats with 1.2 mg/kg complex with CCl4 showed mild liver damage. Interestingly, the 2.4 mg/kg complex with the CCl4 group showed a mild degree of hepatic steatosis congestion of blood sinusoids (Fig. 3B).

Table 5: Dose-dependent effects of nickel(II) diacetyl monoxime-2-pyridyl hydrazone complex on DNA, RNA and total protein levels in rat liver tissues

	Groups	3 Weeks treatments			8 Weeks treatments		
		DNA (µg/ml)	RNA (µg/ml)	TP (g%)	DNA (µg/ml)	RNA (µg/ml)	TP (g%)
Normal groups	Control	64.38 ± 2.6	111.8 ± 1.60	8.03 ± 0.2	59.9 ± 1.20	107.3 ± 2.9	8.12 ± 0.30
	Vehicle	61.93 ± 2.1	107.3 ± 1.90	7.8 ± 0.10	56.46 ± 2.5	112.6 ± 2.4	7.90 ± 0.28
	Normal+Dose 2.4	60.15 ± 2.6	108.09 ± 2.7	8.2 ± 0.24	59.07 ± 2.7	112.9 ± 2.4	7.85 ± 0.22
Intoxicated groups	CCl4	45.7 ± 1.07[a,b,c]	87.90 ± 1.80[a,b,c]	6.1 ± 0.18[a,b,c]	40.22 ± 2.3[a,b,c]	76.41 ± 1.5[a,b,c]	5.9 ± 0.16[a,b,c]
	CCl4 +Dose 1.2	61.23 ± 2.20[d]	100.56 ± 2.04[a,d]	7.32 ± 0.19[d]	55.40 ± 2.30[d]	101.2 ± 1.8[b,c,d]	7.21 ± 0.27[d]
	CCl4 +Dose 2.4	61.84 ± 2.80[d]	110.13 ± 1.90[d,e]	7.92 ± 0.20[d]	59.23 ± 2.08[d]	108.9 ± 3.06[d]	7.93 ± 0.10[d]

Data represented as mean ± SEM
[a]Significant when compared with the control healthy rat group.   [b]Significant when compared with the vehicle treated rat group.
[c]Significant when compared with the normal rat treated with 2.4 mg/kg dose of Ni complex group.
[d]Significant when compared with the CCl4 injected rat group.
[e]Significant when compared with the CCl4 injected rat treated with 1.2 mg/kg dose of Ni complex group.
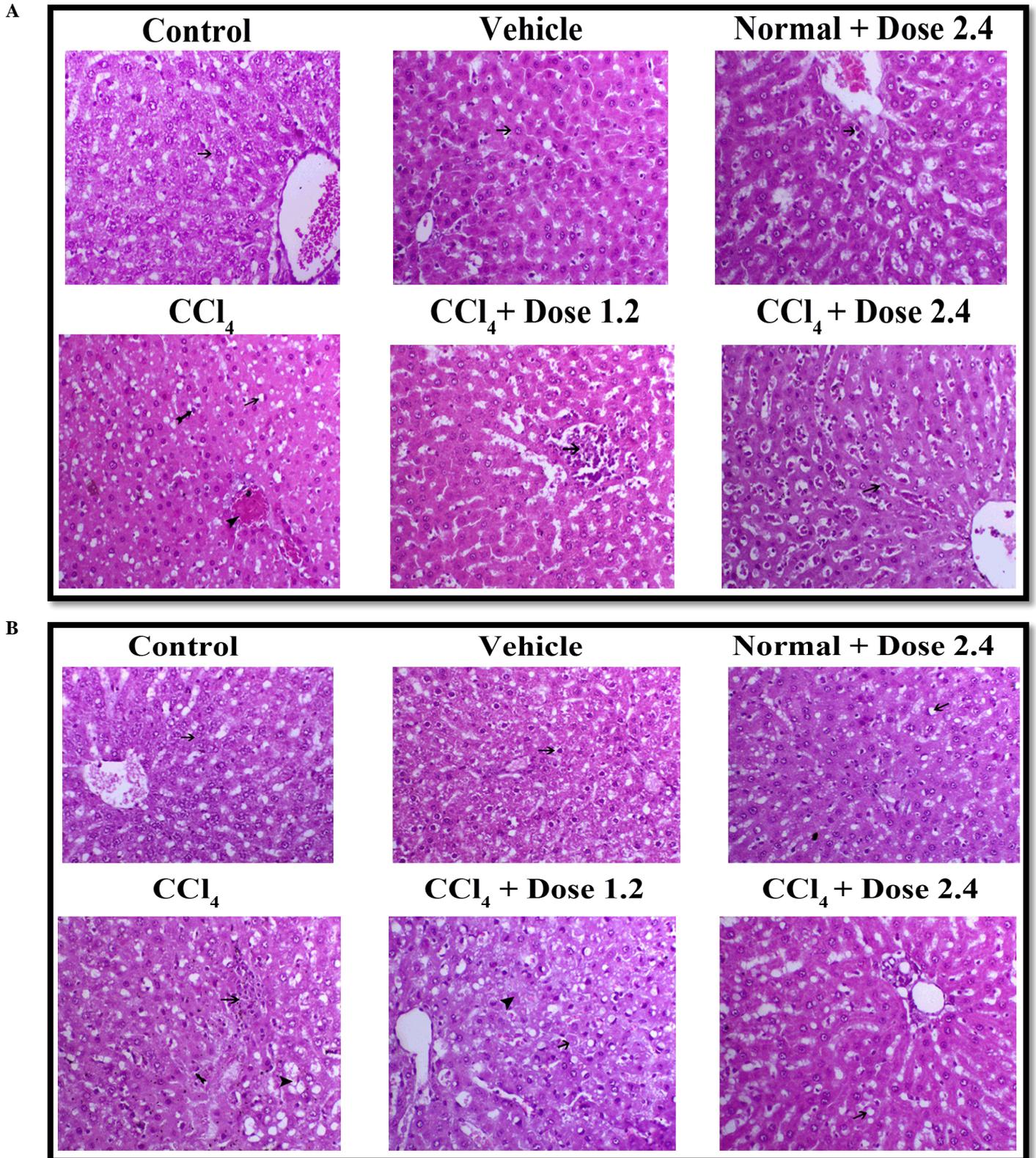
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Fig. 3: Liver histopathology. (A) Liver structure (H&E, X100) of all studied groups after 3 weeks treatment. (B) Liver structure (H&E, X100) of all studied groups after 8 weeks treatment.

4. Discussion

CCl_4 is a frequently used model for the investigation of hepatoprotective activity on various experimental animals. The liver damage caused by CCl_4 is parallel to that made by viral hepatitis [30]. In the current study, CCl_4 injection was connected with marked declined body weight gain, enlarged liver, and high liver index as outcomes from CCl_4 toxicity linked with hepatic deterioration. These findings greatly support that, alteration in liver and body weights post-CCl_4 administration is a considerable factor in the potency check of CCl_4-related liver deterioration. This is in consistent with Lee et al. [30] and Yang et al. [31]. Here, all CCl_4–intoxicated rats who co-treated with the complex gradually gained body weight and restored their near-normal/normal liver weight, body weight, and liver index too at the end of the experiment based on the used dose and treatment duration. The higher concentration of complex exerted the most favorable outcome, followed by the lower concentration with the longer treatment duration. These favorable improvements put Ni complex between the potential potent inhibitors of CCl_4-prompted weight alterations.

In the current work and in line with Ouassou et al. [32] and Sharma and Shukla [33], the more time of rats exposure to CCl_4 exerted the more AST, ALT, and ALP rise in sera definitely at the expense of their presence within hepatic tissue signifying their infiltration through hepatic cell membranes or even their release into the bloodstream following cell death. Interestingly, the increase in the complex dose co-administered with CCl_4 to 2.4 mg/kg produces a pronounced significant decrease in these activities. This drop reflects the conservative power on the cell membrane and the damage-healing ability of the Ni complex that helps liver enzyme activities back within normal. This explanation is also in harmony with that reported in Al-Mehdar et al. [34]. ALB is one of serum proteins; it constitutes one-half or more of serum TP in healthy body, is responsible for adjusting oncotic pressure, and attaches to many substances circulating in the blood. In this study, the CCl_4 group displayed decreased ALB and TP as another symptom of the CCl_4-persuaded deterioration to hepatic cell organelles involved in protein synthesis confirming limited liver synthetic function. A similar result was reported by Shehu et al. [35]. Returning to the present study, rats subjected to CCl_4 suffer from marked reductions in hepatic nucleic acid (DNA&RNA) content and hepatic protein level. Reactive species originated from CCl_4-metabolism can attack nucleic acids and proteins resulting in their destruction. It is widely accepted that ''administration of CCl_4 results in dislocation of ribonucleoproteins from rough endoplasmic membranes causing an inhibition in protein synthesis'' [36]. These events agree with the drop in liver ability to make ALB and other proteins. Ni complex administration with CCl_4 restored nucleic acid levels, kept liver functionality, and reversed protein levels around their normal ranges. Saad et al. [22] demonstrated the liver-protecting feature of the Ni complex against tumor-mediated liver damage in mice.

Once CCl_4 is injected, trichloro-radicals are produced in the hepatocytes by cytochrome P450 enzymes, which by their turn initiate the chain reaction of lipid peroxidation that is frequently measured via the catabolic product MDA. In our study, the doubled hepatic MDA content of the CCl_4-treated group is a clear indication of lipid oxidative degradation up-lifting that ultimately led to declined overall antioxidant capacity along with the concomitant destruction of liver tissue depending on the exposure time to CCl_4 exactly as we detected. The latter is supported by the seen congestion of portal area blood vessels associated with hepatic steatosis and sinusoidal cell activation post-21-days of CCl_4 exposure and the marked periportal hepatic necrosis with severe degrees of hepatic vacuolation and focal aggregation of mononuclear inflammatory cells seen post-56-days of CCl_4 exposure. These risky changes refer to the failure of protective mechanisms to cope with or even to delay the free radical formation. This confirms previous reports such as Peng et al. [37] and Zhang et al. [38]. Our tested Ni complex, as an antioxidant compound, offered a concentration-dependent fall in the liver's MDA content accompanied by a rise in the overall liver's antioxidant capacity too. This can be interpreted by trapping reactive species and inhibition of lipid peroxidation and its propagation in the liver that translated into the histological improvements seen in the Ni complex-co-treated liver sections.

Particularly, due to their precious roles in the systematic cellular defense in health and disease, more concern from research teams including us have been given currently to investigate the status of antioxidant enzymes, SOD and CAT and/or their variations. As well as the GSH molecule which has a unique compound role as antioxidant as it acts as a hunter of a wide variable group of reactive species. Examples are Abd El Azeem et al. [39], Chambliss et al. [40], EL-Shahat et al. [41], Habib et al. [42], Saad et al. [43], etc. In the current investigation, it is worth mentioning that CCl_4-intoxication depleted CAT, GSH, and SOD from hepatic cells confirming similar results of Yang et al. [44] and Chao et al. [45]. In this way, the protection of the liver was toppled and liver damage became faster, easier, and persistent. Fortunately, concomitant injection of Ni complex (at the high-dose) with CCl_4 recovered and maintained these fundamental antioxidants at their normal ranges. This ascertained the interesting antioxidant characteristics of the complex as studied earlier by us, which

currently have been proposed as an effective therapeutic mechanism towards hepatic damages including the induced hepatotoxicity by CCl_4 in the current rat model. Moreover, the obtained negative correlations between the three liver enzymes with these three antioxidants are additional evidence and suggest healing and regeneration of hepatocytes. By the way, the good antioxidant property was also reported as one of the mechanisms contributing to hepatoprotective effect by researchers Arun and Asha [46].

Regarding time-factor, as introduced above in our study, long-term exposure to CCl_4 brings more hepatic damage as expected; this was obvious biochemically on the level of all the studied parameters; more worse values of the studied parameters were observed for the 8-weeks experiment with respect to those observed for the short-term (3-weeks) experiment. In spite of this worsening, Ni complex remained able to protect the liver, neutralize CCl_4 toxicity, and improve liver analysis results, which in turn indicates the recovery of the liver tissue structure and the restoration of its function with no serious adverse effects linked to the complex.

The complex safety was assured in both the 3-weeks and the 8-weeks experiment since all biochemical results of normal control rats and normal rats treated with the high dose of the complex were comparable with no significant difference between them. For more confirmation, liver sections of the healthy rats treated with the complex at the high dose were examined. They did not disclose any significant microscopic alterations relative to normal control tissue.

About the dose-effect, both the low-dose (1.2 mg/kg) and the high-dose (2.4 mg/kg) of Ni complex showed appreciated reversal of CCl_4 toxicity in either short-term or long-term experiment however, the high-dose was much better as it was able to re-normalize liver performance. These findings were confirmed too by the lesser degrees of liver cell damage exerted by the Ni complex in liver tissue sections. These positive actions may be attributed to the complex antioxidative power since it mitigated and reduced oxidative stress burden resulting in decreased cellular deterioration and ameliorated hepatic functional performance. Finally, both our histopathology and biochemical results were well matched and ascertained our interpretations and suggestions.

5. Conclusion

Finally, our study concluded that long-term exposure to CCl_4 stimulates greater damage to the liver while the complex co-treatment with CCl_4 protected the liver from the CCl_4 toxicity without any serious adverse effect. It seems that the antioxidative criteria of the complex are behind its exerted positive actions.

6. Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

7. Formatting of funding sources

8. Acknowledgments

9. Ethical approval

The study protocol was approved by Chemistry Department, Faculty of Science, Damietta University, Egypt.

10. References

[1]   Shirani K., Yousefsani B.S., Shirani M. and Karimi G., Protective effects of naringin against drugs and chemical toxins induced hepatotoxicity: A review. *Phytother Res,* **34**(8), 1734-1744 (2020).

[2]   Attallah A.M., El-Far M., Abdel Malak C.A., Farid K., Omran M.M., Yahya R.S., Saad E.A., Albannan M.S., Attallah A.A., El Basuni M.A., Ali I.S., Abed S.B. and El Naggar M.A., A simple diagnostic index comprising epithelial membrane antigen and fibronectin for hepatocellular carcinoma. *Ann Hepatol*, **14**(6), 869–880 (2015).

[3]   El-Emshaty H.M., Saad E.A., Gouida M.S. and Elshahawy Z.R., Associations between CD133, CK19 and G2/M in cirrhotic HCV (genotype-4) patients with or without accompanying tumor. *Biocell,* **42**(2), 55–60 (2018).

[4]   Saad E.., Habib S.A. and Eltabeey M.S., Diagnostic performance of AFP, autotoxin and collagen IV and their combinations for non-invasive assessment of hepatic fibrosis staging in liver fibrosis patients associated with chronic HCV. *IJPQA*, **8**, 165–173 (2017).

[5]   Ren X., Xin L.T., Zhang M.Q., Zhao Q., Yue S.Y., Chen K.X., Guo Y.W., Shao C.L. and Wang C.Y., Hepatoprotective effects of a traditional Chinese medicine formula against carbon tetrachloride-induced hepatotoxicity in vivo and in vitro. *Biomed & Pharmacother*, **117**, 109190 (2019).

[6]   Aayadi H., Mittal S.P., Deshpande A., Gore M. and Ghaskadbi S.S., Protective effect of geraniin against carbon tetrachloride induced acute hepatotoxicity in

Swiss albino rat. *Biochem Biophys Res Comm*, **487**(1), 62-67 (2017).

[7] Acharya K., Chatterjee S., Biswas G., Chatterjee A. and Saha G.K., Hepatoprotective effect of a wild edible mushroom on carbon tetrachloride-induced hepatotoxicity in rat. *Int J Pharm Pharm Sci*, **4**(3), 285-288 (2012).

[8] Dassarma B., Samanta S. and Nandi D., Toxicokinetics of carbontetrachloride (CCl4) exposure on hepatorenal system. *Euro J Biomed Pharmace Sci*, **4**(1), 520-525 (2017).

[9] Aniya Y., Koyama T., Miyagi C., Miyahira M., Inomata C., Kinoshita S. and Ichiba T., Free radical scavenging and hepatoprotective actions of the medicinal herb, Crassocephalum crepidioides from the Okinawa Islands. *Biological and Pharmaceutical Bulletin*, **28**(1), 19-23 (2005).

[10] Saad E.A., Curative and protective effects of L-arginine on carbon tetrachloride-induced hepatotoxicity in mice. *Biochem Biophys Res Commun*, **423**, 147-151 (2012).

[11] Sun J., Wen X., Liu J., Kan J., Qian C., Wu C. and Jin C., Protective effect of an arabinogalactan from black soybean against carbon tetrachloride-induced acute liver injury in rat. *Int J Biol Macromolecules*, **117**, 659-664 (2018).

[12] Bruijnincx P.C. and Sadler P.J., New trends for metal complexes with anticancer activity. *Current opinion in chemical biology*, **12**(2), 197-206 (2008).

[13] Sakurai H., Overview and frontier for the development of metallopharmaceutics. *J Health Sci*, **56**(2), 129-143 (2010).

[14] Hassanien M.M., Saad E.A. and Radwan K.H., Antidiabetic Activity of cobalt–quercetin complex: A new potential candidate for diabetes treatment. *J Appl Pharm Sci*, **10**(12), 044–052 (2020).

[15] Saad E.A., Elsayed S.A., Hassanien M.M. and AL-Adl M.S., The new iron(III) 3-oxo-N-(pyridin-2-yl)butanamide complex promotes Ehrlich solid tumor regression in mice via induction of apoptosis. *Appl Organometal Chem*, **34**, e5282 (2020).

[16] Saad E.A., Hassanien M.M., El-mezayen H.A. and ELmenawy N.M., Regression of murine Ehrlich ascites carcinoma using synthesized cobalt complex. *Med Chem Commun*, **8**, 1103–1111 (2017).

[17] Elsayed S.A., Saad E.A. and Mostafa S.I., Development of new potential anticancer metal complexes derived from 2-hydrazinobenzothiazole. *Mini-Rev Med Chem*, **19**(11), 913-922 (2019).

[18] Saad E.A., Kiwan H.A., Hassanien M.M. and Al-Adl H.E., Synthesis, characterization, and antitumor activity of a new iron-rifampicin complex: a novel prospective antitumor drug . *J Drug Deliv Sci Technol*, **57**(C), 101671 (2020).

[19] Jia L., Xu J., Xu X.M., Chen L.H., Jiang P., Cheng F.X., Lu G.N., Wang Q., Wu J.C. and Tang N., Synthesis, Crystal Structure, Antioxidant Activity, and DNA-Binding Studies of a Novel Ni (II) Grid Complex with a Rigid Bistridentate Schiff Base Ligand. *Chemical and Pharmaceutical Bulletin*, **58**(8), 1077-1080 (2010).

[20] Selvaganapathy M. and Raman N., Pharmacological activity of a few transition metal complexes: a short review. *J Chem Biol Ther*, **1**(02), 2572-0406 (2016).

[21] Basu C., Chowdhury S., Stoeckli-Evans H. and Mukherjee S., A new chromogenic agent for iron(III): synthesis, structure and spectroscopic studies. *J Chem Sci*, **122**(2), 217e223 (2010).

[22] Saad E.A., Hassanien M.M. and El-lban F.W., Nickel(II) diacetyl monoxime-2-pyridyl hydrazone complex can inhibit Ehrlich solid tumor growth in rat: A potential new antitumor drug. *Biochem Biophys Res Commun*, **484**(3), 579-585 (2017).

[23] Sahreen S., Khan M.R. and Khan R.A., Hepatoprotective effects of methanol extract of Carissa opaca leaves on CCl4-induced damage in rat. *BMC Complement Altern Med*, **11**, Article number: 48 (2011). https://doi.org/10.1186/1472-6882-11-48

[24] Ohkawa H., Ohishi N.and Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, **95**(2), 351-358 (1979).

[25] Dechatelet L.R., McCall C.E., McPhail L.C. and Johnston R.B., Superoxide dismutase activity in leukocytes. *The Journal of clinical investigation*, **53**(4), 1197-1201 (1974).

[26] Sinha A.K., Colorimetric assay of catalase. *Anal Biochem*, **47**(2), 389-394 (1972).

[27] Mejbaum W., Estimation of small amounts of pentose especially in derivates of adenylic acid. *Hoppe-Seyler's Z. Physiol Chem*, **258**, 117-122 (1939).

[28] Dische Z. and Schwarz K., Mikromethode zur Bestimmung verschiedener Pentosen nebeneinander bei Gegenwart von Hexosen. *Microchimica Acta*, **2**(1), 13-19 (1937).

[29] Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J., Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275 (1951).

[30] Lee Y.S., Cho I.J., Kim J.W., Lee M.K., Ku S.K., Choi J.S. and Lee H.J., Hepatoprotective effects of blue honeysuckle on CCl4-induced acute liver damaged rat. *Food Science & Nutrition*, **7**(1), 322-338 (2018).

[31] Yang J., Li Y., Wang F. and Wu C., Hepatoprotective effects of apple polyphenols on CCl4-induced acute liver damage in rat. *Journal of Agricultural and Food Chemistry*, **58**(10), 6525-6531 (2010).

[32] Ouassou H., Bouhrim M., Daoudi N.E., Mekhfi H., Ziyyat A., Legssyer A., Aziz M. and Bnouham M., Evaluation of Hepatoprotective Activity of Caralluma europaea Stem Extract against CCl4-Induced Hepatic

Damage in Wistar Rats. *Adv Pharmacol Pharm Sci,* **2021**, Article ID 8883040, 8 pages (2021).

[33] Sharma N. and Shukla S., Hepatoprotective potential of aqueous extract of Butea monosperma against CCl4 induced damage in rats. *Exp Toxicol Pathol*, **63**(7-8), 671-676 (2011).

[34] Al-Mehdar A.A., Dammag M.A. and Hussien T.A., Assessment of hepatoprotective activity of Caralluma cicatricose against CCl4-induced liver damage in rabbits. *J Drug Discov Therap*, **3**, 1-10 (2015).

[35] Shehu Z., Uba G., Alhassan A. and Bala M., Hepato-protective Effects of Crude Phenol and Petroleum Spirit Root Extracts of Calotropis procera (Sodom of Apple) on CCL4 Induced Hepatotoxicity in Albino Rats. *Int J Curr Microbiol App Sci*, **8**(2), 2733-2743 (2019).

[36] Krithika R. and Verma R.J., Solanum nigrum confers protection against CCl4-induced experimental hepatotoxicity by increasing hepatic protein synthesis and regulation of energy metabolism. *Clinical Phytoscience*, **5**(1), 1-8 (2019).

[37] Peng Y., Gan R., Li H., Yang M., McClements D.J., Gao R. and Sun Q., Absorption, metabolism, and bioactivity of vitexin: recent advances in understanding the efficacy of an important nutraceutical. *Crit Rev Food Sci Nutr*, **61**(27), 1-16 (2020).

[38] Zhang X., Kuang G., Wan J., Jiang R., Ma L., Gong X. and Liu X., Salidroside protects rat against CCl4-induced acute liver injury via down-regulating CYP2E1 expression and inhibiting NLRP3 inflammasome activation. *International Immunopharmacology*, **85**, 106662 (2020).

[39] Abd El Azeem R.A., Zedan M.M., Saad E.A., Mutawi T.M. and Attia Z.R., Single-nucleotide polymorphisms (SNPs) of antioxidant enzymes SOD2 and GSTP1 genes and SLE risk and severity in an

Egyptian pediatric population. *Clin Biochem*, **88**, 37–42 (2021).

[40] Chambliss J.M., Ansar M., Kelley J.P., Spratt H., Garofalo R.P. and Casola A., A Polymorphism in the Catalase Gene Promoter Confers Protection against Severe RSV Bronchiolitis. *Viruses*, **12**, 57 (2020).

[41] El-Shahat R.A., El-Demerdash R.S., El Sherbini E.S. and Saad E.A., HCl-induced acute lung injury: a study of the curative role of mesenchymal stem/stromal cells and cobalt protoporphyrin. *J Genet Eng Biotechnol*, **19**(1), 41 (2021).

[42] Habib S.A., Saad E.A., Al-Mutairi F.M., Alalawy A.I., Sayed M.H. and El-Sadda R.R., Up-regulation of antioxidant status in chronic renal failure rats treated with mesenchymal stem cells and hematopoietic stem cells. *Pak J Biol Sci*, **23**(6), 820–828 (2020).

[43] Saad E.A., El-Gayar H.A., El-Demerdash R.S. and Radwan K.H., Hepato-toxic risk of gum Arabic during adenine-induced renal toxicity prevention. *J Appl Pharm Sci*, **8**(12), 104–111 (2018).

[44] Yang L., Wang C.-z., Ye J.-z. and Li H.-t., Hepatoprotective effects of polyprenols from Ginkgo biloba L. leaves on CCl4-induced hepatotoxicity in rats. *Fitoterapia*, **82**(6), 834-840 (2011).

[45] Chao W.-W., Chen S.-J., Peng H.-C., Liao J.-W. and Chou S.-T., Antioxidant activity of graptopetalum paraguayense E. Walther leaf extract counteracts oxidative stress induced by ethanol and carbon tetrachloride Co-induced hepatotoxicity in rats. *Antioxidants*, **8**(8), 251 (2019).

[46] Arun M. and Asha V., Preliminary studies on antihepatotoxic effect of Physalis peruviana Linn.(Solanaceae) against carbon tetrachloride induced acute liver injury in rats. *Journal of Ethnopharmacology*, **111**(1), 110-114 (2007).