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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF METAL COMPLEXES DERIVED FROM 2-(PHENYLAMINO)ACETOHYDRAZIDE LIGAND

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ABSTRACT

Metal complexes of the ligand, 2-(phenylamino)acetohydrazide (HL) with Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Cr(III) and Fe(III) ions were synthesized and characterized using elemental, spectral (IR, UV- VIS), and thermal analyses (TGA) as well as magnetic moment and molar conductance measurements. The ligand in the obtained solid complexes coordinates as a bi-dentate either as a neutral via the amino group nitrogen and carbonyl oxygen atoms or anilino NH or as a monobasic via the amino group nitrogen atom and enolic (C-O) oxygen atom in the enolimine-tautomeric form. The cytotoxic effect of these complexes was estimated against Hep-G2 cancer cell lines. Also, the in-vivo model of hepatic fibrosis was induced by the intraperitoneal (i.p) injection of carbon tetrachloride (CCl_4) in mice and the possible antifibrotic activities of these complexes were estimated. Moreover, liver function, histopathological examination of liver, and expression of caspase 3 in liver tissues were assayed. Results revealed that all the newly synthesized complexes exhibited cytotoxic effect on Hep-G2 cell lines. Also, administration of Cu(II) complexes to the CCl_4-intoxicated mice led to improvement the hepatic function and liver histology. Furthermore, the treatment of CCl_4-intoxicated with ligand and copper complexes led to a marked decrease in the caspase 3 expression in nucleus and cytoplasm of hepatic cells. In conclusion, Cu(II) complexes of hydrazide derivatives, specially Cu(II) perchlorate, showed anticancer and antifibrotic activities higher than the ligand. The potential activity of the tested complexes might be due to their cytotoxicity and ability to reduce the expression of apoptotic gene caspase-3 that helps in the repairing of liver cells.

KEYWORDS: Cytotoxic activity; Hep-G2; Fibrosis; Caspase-3; Hydrazide ligand; Metal complexes.

INTRODUCTION

The coordination chemistry of transition metals with ligands from the hydrazide family has been of interest due to different bonding modes shown by these ligands with both electron rich and electron poor metals. Schiff bases play an important role in inorganic chemistry as they easily form stable complexes with most transition metal ions. The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species[1-5].

The remarkable biological activity of acid hydrazides R–CO–NH–NH_2, a class of Schiff base, their corresponding aroylhydrazones, R–CO–NH–N=CH–R and the dependence of their mode of chelation with transition metal ions present in the living system have been of significant interest[6-12]. Schiff base metal complexes have been widely studied because they have industrial, antifungal, antibacterial, anticancer and herbicidal applications[13,14]. Hydrazides successfully provide various active potential donor sites, namely C=O, N-H and NH_2. Therefore, many metal complexes of hydrazides have been synthesized and characterized[15-20].

Liver fibrosis results from chronic damage to the liver in conjunction with the progressive accumulation of fibrillar extracellular matrix proteins. Hepatic fibrosis is characterized by increased deposition and altered composition of extracellular matrix (ECM). Its final stage is cirrhosis, with the liver architecture distorted by collagen bands and formation of islands of regenerating parenchymal cells[21].

Carbon tetrachloride (CCl_4) is a hepatotoxin, causing liver necrosis, fibrosis and cirrhosis when administered sequentially. CCl_4-induced fibrosis shares several characteristics with human fibrosis of different etiologies; thus, it is an adequate model of human fibrosis. CCl_4 induced liver damage had been thought to depend on the formation of reactive intermediates such

as trichloromethyl ($CCl_3^{\bullet}$) free radical produced by cytochrome P 450 mixed function oxidase system especially, P 450-2E1, the major human enzyme responsible for carbon tetrachloride bioactivation and further converted to a peroxy radical ($CCl_3O_2^{\bullet}$)[22].

Therefore, the present study was designed to prepare and characterize new metal complexes of ligand 2-(phenylamino)acetohydrazide and to investigate its antifibrotic activities against hepatic fibrosis induced in rats by CCl_4.

EXPERIMENTAL

Instrumentation and Measurements

Reagent grade chemicals were used without further purification. Elemental Micro analyses [C, H and N] were determined in the Micro analytical unit of Cairo University of Egypt. The IR spectra were measured using KBr discs on FT-IR Shimadzu spectrophotometer. Electronic spectra in solid states were recorded in Nujol mulls using Shimadzu spectrophotometer. The molar conductance of 10^{-3} M solution of the complexes in DMF was measured at 25ºC with a Bibby conductmeter type MCl at the Chemistry Department, Faculty of Science, Menoufia University. The resistance measured in ohms and the molar conductivities were calculated according to the equation:

$$\Lambda_M = VxKxg / Mw_* \Omega$$

Where: Λ_M = molar conductivity /$\Omega^{-1}cm^2mol^{-1}$, V = volume of the complex solution/ ml, K = cell constant (0.92/ cm^{-1}), Mw = molecular weight of the complex, g = weight of the complex/g, Ω = resistance/Ω. The thermal analyses (TGA) were carried out in air using Shimadzu DT-30 thermal analyzer. Magnetic susceptibilities were measured at 27°C using the modified Gouy method with a Johnson matthey balance. Magnetic moments were calculated using equation:

$$\mu_{eff} = 2.84\sqrt{\chi_M^{corr}.T}$$

Preparation of Ethyl 2-(phenylamino) acetate

Aniline (0.933g, 10 mmol in 30 mL ethanol) was added to a solution of ethylchloroacetate (1.22 g, 10 mmol in 50 mL of ethanol). Sodium acetate trihydrate (1.36 g, 10 mmol) dissolved in 15 mL distilled water was added to the mixture, then, it was refluxed with stirring for 6 hrs. The product was poured on crushed ice, and the solid precipitate which is formed was filtered off, washed several times with distilled water and dried over anhydrous $CaCl_2$.

Preparation of 2-(phenylamino)acetohydrazide (HL)

Hydrazine hydrate (2.5 g, 50 mmol) was added drop wise to a solution of ethyl 2-(phenylamino) acetate (1.79 g, 10 mmol) in 60 mL ethanol. The mixture was refluxed with stirring for 3 hrs, and then left to cool at room temperature. The formed precipitate was filtered off, washed several times with ethanol and dried over anhydrous $CaCl_2$.

Preparation of compounds' solutions

The stock solutions (1mM) of the newly synthesized complexes were prepared by dissolving each complex individually in 70% DMSO in water and kept at 4°C. Only, ligand and complexes of Cu(II) **2, 4 and 5** were solubilized and tested for their biological activities.

Cytotoxicity assay

The anti-hepatocellular carcinoma activity of complexes against Hep-G2 was estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-di phenyltetrazolium bromide (MTT) assay[23]. Briefly, cells were seeded in 96-well plates at 5×10^4 cells/well in the corresponding media supplemented with culture materials mentioned above. After 24 h of culture, phthalimide derivatives were individually added in triplicate in a range of 5–10000 µM, and the cells were further cultured for 24 h. The cells were then exposed to MTT (5 mg/mL in PBS) at a final concentration of 1 mg/mL in culture for 4 h. Formazan crystals formed during the incubation period were dissolved overnight at 37 °C by adding 10% SDS containing 0.02 N HCl. The absorbance was then measured at 570 nm. The half maximal growth inhibitory concentration (IC_{50} values) was calculated from the dose-dependent curve equation of each compound. The experiment was repeated three times.

Experimental animals and design

Adult male Swiss mice, weighing about 22-25 g, were housed at 23 ± 2°C in the animal house of Faculty of Science, Menoufia University, Egypt. They were maintained under standard condition and fed standard chow and water ad libitum. All experiments were carried out in accordance with protocols approved by the local experimental animal ethics committee. Mice were randomly divided into 7 groups of seven animals each. Normal group includes animals that were given only standard pellet diet and water ad libitium and served as normal controls. Positive control animals were i.p. injected with CCl_4 dissolved in corn oil (0.4 mg/kg B.W.) twice a week for 6 weeks. Animals in the third group were i.p. injected with DMSO (0.1 mg/kg B.W.) for 6 consecutive days. In order to evaluate the potential antifibrotic activities of the ligand and complexes **2**, **4** and **5**, animals were i.p. injected with CCl_4 (0.4 mg/kg, B.W) twice a week for six weeks, after that, they were i.p. injected with 10 µM of each complex for 6 consecutive days.

Blood and tissue samples collection

At the end of experiments, blood was collected in non-heparinized tubes and allowed to clot at room temperature. Serum samples were then obtained by centrifugation and were kept at –20°C until assayed. The liver was removed, washed with saline and divided into two parts. One part was stored in 10% neutral buffered formalin solution for histopathological examination and the second part was kept frozen at –80°C for further studies.

Assessment of liver function

Alanine transaminase (ALT) and aspartate transaminase (AST) activities in serum were estimated according to the method of Reitman and Frankel[24]. The level of serum albumin (Alb) was determined according to the method of Baure[25].

Histopathological examination of liver

Liver specimen was dehydrated in a graded alcohol series. After xylene treatment, the specimens were embedded in paraffin blocks. Four-micron thick sections were cut and stained with haematoxylin and eosin (H&E) staining and the changes were examined by light microscopy. Damaging effect of CCl_4-induced liver fibrosis (hepatic architecture, inflammatory infiltrates, portal tracts, hepatocytes arrangement, variations in hepatocytes size and nuclei) was investigated. Unintentional bias was prevented by coding mouse's tissue samples.

Immunohistochemical determination of α-smooth muscle actin and caspase-3

The serial sections were dewaxed, hydrated, and immersed in antigen retrieval (EDTA solution, pH 8). They were then treated with hydrogen peroxide 0.3% and protein block, followed by incubation with anti-caspase-3 (Santa cruz, 1:100 dilution) (Lab-vision; ready to use) at 4°C overnight. The slides were rinsed three times with PBS, incubated with anti-mouse IgG secondary antibodies (EnVision + System HRP; Dako) for 30 minutes at room temperature, visualized with diaminobenzidine commercial kits (Liquid DAB+Substrate Chromogen System; Dako), and finally counterstained with Mayer's haematoxylin. As a negative control procedure, the primary antibody was replaced by normal mouse serum. Immunolabeling reactions were scored for caspase-3, a distinct brown-coloured reaction either within the cytoplasm or the nucleus was observed. The positive cells were counted from seven high-power fields of 400x. Labeling index (%) was determined by the number of positive cells/total cells.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS) version 19 (SPSS, Inc., Chicago, IL). Data are presented as means with corresponding standard deviation (SD). Comparisons between different groups were performed by one-way analysis of variance (ANOVA). In all tests, the level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

All the prepared metal complexes are stable at room temperature, non-hygroscopic and partly soluble in most organic solvents and water, but completely soluble in dimethylformamide (DMF) or dimethylsulphoxide (DMSO) except complexes **6-10** are insoluble. Elemental analyses, physical data (table 1) and infrared spectral data (table 2), are consistent with the proposed structures as shown in figure (1). The elemental analyses confirmed that the complexes **2, 4, and 6 -11** are formed in 1:1 molar ratio between the metal ion and ligand whereas, complexes **5** and **12** are formed in 1:2 molar ratio between the metal ion and the ligand. Complex **3** is a binuclear complex.

The molar conductance

The molar conductance values of the metal complexes in DMF (10^{-3}M) are listed in table (1). The values of molar conductance show that all complexes are non-electrolytes[26]. This confirms that the anions in all these complexes are directly attached to the metal ion.

Infrared spectra

The most diagnostic infrared spectral bands of the ligand HL are depicted in table (2) and figure (2_{A-D}). The spectrum of ligand HL shows bands at 3460, 3342, 3309, 3200, 3106 cm^{-1}, assigned to ν(N-H) absorptions characteristic to NH and NH_2 groups. The spectrum also displays bands at 1651 and 1583 cm^{-1}, assigned to ν(C=O) and δ(NH_2), respectively.

The mode of bonding of the ligand, HL in the metal complexes has been deduced from the IR spectra. The most important diagnostic spectral bands of the ligand and its metal complexes are depicted in table (2). Results show that the infrared spectra of all complexes reveal that the band characteristic to δ(NH_2) is shifted to lower or to higher wavenumbers compared to that of the free ligand, indicating that the NH_2 group participates in coordination via the nitrogen atom in all metal complexes. IR spectra of metal complexes **4, 8, 10-12** lack absorption bands attributable to ν(C=O) and instead, a new intense band appears at ca. 1605-1629 cm^{-1}, assigned to ν(C=N), suggesting that the NH proton is likely lost via de-protonation induced by the metal and the resulting enolic oxygen participates in coordination.[27]

The above arguments indicate that the ligand in complexes **4, 8** and **10-12** behaved as monobasic bi-dentate, coordinating via the enolic oxygen atom and the nitrogen atom of NH_2 group. The ν(C=O) band appears in the spectra of complexes **2, 3, 5** and **6** at lower wavenumbers relative to that of the free ligand as a result of its involvement in bond formation with the central metal ion. This indicates that the ligand in This complex behaved as a neutral bidentate, coordinating through the carbonyl oxygen atom and the amine nitrogen atom. Moreover, ν(C=O) remains at the same position as that of the free ligand in the spectra of the two complexes **7** and **9** in addition the positions of the bands ascribed to ν(N-H) perturbed upon complex formation, indicating that the carbonyl oxygen does not participate in coordination and the aniline NH nitrogen atom involved in coordination beside the NH_2 nitrogen atom.

The spectra of the acetato complexes **5** and **10** show two absorption bands at 1620-1603 and 1400-1382 cm^{-1}, assigned to υ(C=O) and υ(C-O) , respectively. The

difference between these two bands about 220 cm^{-1} indicates that the acetate in these complexes coordinates to the metal ion as a monodentate ligand[28]. The acetate bands in infrared spectrum of complex 6 appear at 1507 and 1414 cm^{-1}. The difference between these two bands is 93 cm^{-1}, indicates that the acetate in these complexes coordinates to the metal ion as a bidentate ligand. Monodentate co-ordination of perchlorate ligand causes a lowering of symmetry,$Td \rightarrow C_{3v}$. The originally – degenrate modes υ_3 and υ_4 of the free perchlorate ion, are both split into two bands[29]. The infrared spectrum of the perchlorate $[CuClO_4LH_2O)].H_2O$ complex showed that the υ_3 band is split into two bands at 1114 and 1089 cm^{-1}, while υ_4 is also split into two bands at 630 and 605 cm^{-1}, indicating that the perchlorate coordinates to the metal ion as a monodentate ligand. The coordination of SO_4^{2-} to metal ions decreases the symmetry of the group and the ν_3 and ν_4 modes are split[30-32]. In the case, the SO_4^{2-} site symmetry is lowered from T_d to C_{3v} (monodentate coordination), both $\nu_3$and ν_4 each splits into two bands[31]. When the SO_4^{2-} site symmetry is lowered from T_d to C_{2v} (bidentate chelating or bridging co-ordination), again ν_3 and ν_4 each splits into three bands. The infrared spectrum of the chromium complex shows that the ν_3 splits into three bands at 1130, 1040 and 981 cm^{-1} and the ν_4 band also split into three bands at 754, 693 and 609 cm^{-1}, indicate that the sulphato ligand coordinates to the chromium ion as a bidentate ligand[33]. The infrared spectra of the hydrated complexes display a broad band at 3450-3405 cm^{-1}, assigned to ν(OH) of water molecule[34]. The IR spectra of all complexes display a new band at 574-426 cm^{-1}, assigned to ν(M-N)[35-37]. The spectra of all metal complexes except **7** and **9**, show a new band at 695- 659 cm^{-1}, assigned to υ(M-O)[35-37].

Magnetic and electronic spectra

The values of room temperature magnetic moments (μ_{eff} β.M. per metal atom) and electronic spectral bands for metal complexes in the solid state are listed in table (3) and showed in figure (3_{A-D}) .The data showed that, three bands were observed in the spectrum of the ligand at 270, 290 and 307 nm. The first two bands at 270 and 290 nm (omitted from table 3) are attributed to intraligand π→π* transition within the phenyl ring of the ligand which is almost unchanged upon complex formation. The third band at 307 nm ,ascribed to intraligand n→π* transition within the carbonyl group which showed a change upon complex formation indicating participation of this group in the complex formation[38,39]. In some complexes, new bands were observed in the 511-320 nm ranges, which are due to charge transfer electronic transitions from ligand to metal (LMCT) (O (σ,π →M)[40].

Copper (II) complexes **2, 4** and **5** show magnetic moment values 1.94, 1.97 and 1.87 β.M.; respectively. These values close to spin-only value for one unpaired spin (~1.73 B.M.). This indicates also that there is no any sort of molecular association of copper (II) ions in a square planar environment[29,39,40]. However, the room temperature magnetic moment value of the dimeric copper (II) complex **3** (0.42 β.M) is much lower than expected for spin only- value (1.73 β.M). This can be explained on the basis that a strong antiferromagnetic interaction between the two copper (II) centers in the dimeric as expected for all the dicopper (II) complexes [41]. The electronic absorption spectra of the Cu(II) complexes **2, 3, 4** and **5** showed a broad band at 710,550, 600 and 725 nm, respectively assignable to the transition $^2B_1g \rightarrow {}^2Eg$, suggesting a square planar geometry around the copper (II) ion.

The nickel(II) complexes were found to be paramagnetic, what ruled out a square-planar configuration. The values of magnetic moment for nickel (II) complexes **6** and **7** are 2.98 and 3.05B.M respectively, fall in the range reported for four-coordinate tetrahedral, five coordinate square pyramidal or trigonal bipyramidal, and six-coordinate octahedral configurations. The possible geometry for the complexes had been assigned from their electronic spectral studies. The electronic spectra of Ni(II) complexes **6** and **7** showed abroad weak band at 625-543 nm and a strong one at 580-480 nm ,assignable to $^3A_{2g}(F) \rightarrow {}^{3T}{}_{1g}(P)(\upsilon_3)$, $^3A_{2g}(F) \rightarrow {}^{3T}{}_{1g}(F)(\upsilon_2)$ transitions in octahedral environment[42-45].

Cobalt (II) complex **8** recorded a magnetic moment value was 4.08 β.M per metal ion ,suggesting high spin cobalt complex. The electronic spectrum of complex **8** displayed a broad band at 450 nm and a strong split band at 617 and 601 nm, assignable to $^4A_2 \rightarrow {}^4T_1(F)$ (v_2) and $^4A_2 \rightarrow {}^4T_1(P)$ (v_3) transitions respectively, indicates a tetrahedral arrangement around Co(II) ion[39,46].

Manganese(II) complex **9,** showed a value for magnetic moment equal to 5.95 β.M per metal ion, indicates a high spin manganese complex. The electronic spectrum of Mn(II) complex reveals very weak bands at 400, 550 nm, these bands are attributed to transitions $^6A_1 \rightarrow {}^4E_1(F)$ and $^6A_1 \rightarrow {}^4T_1(F)$ transitions respectively. These transitions characteristic to a tetrahedral environment for Mn(II) complex[39,46].

The magnetic moment value for chromium(III) complex **11** is 3.97 β.M per metal ion. This value is compatible with three unpaired electrons. The electronic spectrum of this complex showed four weak bands at 610, 580, 410 and 340 nm. , assigned to $^4B_{1g} \rightarrow {}^4E^ag$ (v_1), $^4B_{1g} \rightarrow {}^4B_{2g}$ (v_2), $^4B_{1g} \rightarrow {}^4E^bg$ (v_3) and $^4B_{1g} \rightarrow {}^4A1g$ (v_4) electronic transitions within a low symmetry octahedral Cr(III) complex[47].

Iron(III) complex **12** gave a magnetic moment value equal to.5.98 β.M per metal ion, corresponding to high spin d^5 configuration iron (III) complex. The electronic spectrum of this complex **12** gave bands at 295, 362 (br,s), 401, 440 (br,s), 530 and 580 nm. The first two bands are assigned to intraligand (π→π*/n→π*) transitions. The bands at 401 and 440 nm, assignable to

charge transfer from ligand to metal (LMCT). The last two bands at 580 and 530 nm are considered to arise from the $^6A_{1g} \rightarrow {}^4T_{1g}$ and $^4T_{2g}$ transitions, respectively, in the octahedral geometry around Fe (III)[48].

Thermal Analysis

Thermogravimetric analysis (TGA and DTG) of metal complexes are used to i. get information about the thermal stability of new complexes, ii. decide whether the water molecules are inside or outside the inner co-ordination sphere of the central metal ion and iii. suggest a general scheme for thermal decomposition of chelates.

In the present investigation, heating rates were suitably controlled at 10°C min^{-1} under nitrogen atmosphere and the weight loss was measured from the ambient temperature up to ~ 900°C. The TGA data are presented in table- 4. and shown in Figure 4 (A-C).

The TGA results for some of the solid complexes **6** and **8 -12** as shown in table (4) indicate that the results are in good agreement with the formulae suggested from the analytical, spectral and magnetic data (figure 1). A general decomposition pattern was concluded, whereby the complexes decomposed in three or four stages. The first stage is the loss of molecules of hydrated water at 23-162°C, the second decomposition stage is the loss of the coordinated water molecules at 100 -240°C, the third decomposition stage is an envelope stage as a result of overlaped processes include, the loss of anions, Cl^- or OAc^- anions, decomposition of complex to loss partially the ligands to form metal oxide mixed in some complexes with carbon residue.

Anticancer activity against Hep-G2 cells

The newly synthesized complexes were tested against HepG2 cells using MTT assay. IC_{50} values are reported in Figure 5. All the tested complexes showed activity towards HepG2 cell lines. The recorded IC_{50} values were 72, 49, 172 µM for complexes 2, 4, and 5, respectively compared to 1 (ligand) (565 µM).

Evaluation of liver function

As shown in figures 1 and 2, injection of CCl_4 to mice twice a week for 6 weeks resulted in a significant increase ($P < 0.05$) in the activities of ALT and AST as compared to those of normal control group. In addition, the capacity of liver to synthesize albumin is markedly deteriorated in mice after injection with CCl_4 as compared to that of normal control group. Results revealed that the ligand and Cu (II) complexes **2, 4** and **5** ameliorated the activities of ALT and AST as well as level of albumin. Complex **4** exhibited the highest ameliorative effect on the liver as compared to other tested complexes (Figure 6 and 7).

Histopathological examination of liver

The liver of control animals showed hepatic lobules which consisted from normal hepatocytes arranged in cords in radiating manner around the central vein. The hepatic cords was separated with endothelial lined the blood sinusoids. The portal area was located between each three hepatic lobules and consisted from hepatic artery, portal vein and bile duct (Figure 8-A).

The liver of animals treated with CCl_4 showed various hepatotoxic lesions starting from cell swelling till hepatic necrosis. The most prominent feature is the hepatic vacuolation consistent with fatty changes, as most of vacuoles were round in border and with clear lumen. The portal area showed marked degree of periportal fibrosis. The hepatic degeneration was mainly noticed in periportal areas associated with fibroblastic cell proliferation (Figure 8-B). The most interesting feature is the increase the fibroblastic proliferation which markedly extended into the hepatic lobules which forming small nodules consisted from hepatocytes (Figure 8-C). In addition, the hepatocytes within the centrolobular area showed marked vacuolation of hepatocytes (Figure 8-D).

The liver tissues of CCl_4-intoxicated animals treated with ligand showed remarkable heptopatoxicity features represented with marked vacuolar and fatty degeneration of hepatocytes (Figure 8-E). Animals treated with CCl_4 + complex **2** showed decreases in the hepatic degeneration with mild to moderate degree of portal fibrosis (Figure 8-F). Animals treated with CCl_4 + complex **4** revealed marked decrease CCl_4-induced lesions such as fatty changes and the periportal and intra-lobular hepatic fibrosis (Figure 8-G). Livers from mice treated with CCl_4 + complex **5** revealed marked decline in both hepatotoxicity and portal fibrosis (Figure 8-H).

Caspase-3 expression

The caspase-3 protein is a member of the cysteine-aspartic acid protease (caspase) family [49]. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes that undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. Caspase-3 is activated in the apoptotic cell both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways[50,51]. The zymogen feature of caspase-3 is necessary because if unregulated, caspase activity would kill cells indiscriminately[52].

Data of nuclear and cytoplasmic expression of caspase-3 is displayed in figure (9 A-F) and summarized in table 5. It was clearly noticed that caspase-3 immunostaining markedly increased within the CCl4-treated animal mostly within the centrolobular area. Treatment with ligand slightly reduces the expression of the caspase-3. Interestingly, the complex **2, 4,** and **5** markedly decrease the caspase-3 expression.

CONCLUSION

In the current study, novel metal complexes of the ligand, 2-(phenylamino)acetohydrazide (HL) with Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Cr(III) and Fe(III)

ions were synthesized. The possible antifibrotic activities and histopathological examination of Cu(II) complexes were estimated as well as their effect on liver function and caspase 3 and α-smooth muscle actin (α-SMA) expressions were assayed. Results revealed that administration of Cu(II) complexes to the CCl_4-intoxicated mice led to improvement in the hepatic function as well as liver histology. Moreover, treatment with these complexes led to a marked decline in the caspase-3 expressionin hepatic cells. Further study to investigate the molecular mechanism of these complexes was recommended.

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