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Liver and mucous secretion enzymatic biomarkers of *Eobania vermiculata* treated with some newly synthesized acrylamide derivatives

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Abstract

Background Acrylamide derivatives have a potential biological activity as well as acting as precursors in many organic syntheses. Moreover, acrylamides and their derivatives cause convulsions and diffused damage to different sections of the nervous system of infected animals. Novel copper and zinc chelates originated from (*E*)-3-(4-bromophenyl)-2-cyanoacrylamide **(L^1)**, and (*E*)-2-cyano-3-(4-nitrophenyl)acrylamide **(L^2)** were prepared, and their chemical skeletons were identified by infrared and mass spectra. The obtained compounds were screened in vitro against the brown garden snail, *Eobania vermiculata* using the contact method along 72 h. Stock solutions of tested compounds were prepared utilizing distilled water and DMSO mixture, and four concentrations of each compound were prepared (50, 150, 250 and 350 ppm). *Eobania vermiculata* snails were treated with LC_{50} concentrations of prepared compounds for 3 days, and live snails were used to estimate the level of some liver and mucous secretion enzymatic biomarkers: transaminases enzymes (Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)), Total Protein content (TP), Acid Phosphatases (ACP) and Alkaline Phosphatase (ALP).

Results The results demonstrated that the examined compounds have a relatively toxic effect toward the screened species. Zinc complexes displayed a higher toxicity than copper ones. The results authenticated considerable high effects of the synthesized compounds on investigated enzymes.

Conclusions The promising effects of Cu(II), Zn(II) complexes **(1, 2)** on stimulating the mucous secretion of tested snails are clear through the elevated levels of ALP and ACP enzymes of treated snails. The enhancement or reduction of AST, ALT level and TP content of treated snails demonstrated the effects of prepared compounds on liver functions of these species.

Keywords Acrylamides, Biomarkers, Land snails, Metal chelates

Background

Land snails are Gastropoda that belong to Mollusca class and Pulmonata subclass. These species have successfully invaded land and are considered one of the most diverse groups of animals in both habits and shape. Land snails are harmful pests that causing damage to many crops, vegetables and ornamental plants all over the world (Godan, 1983; Emara et al., 2022). Over the last few years, land snail species have increased in a rapid manner and a

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lot of species have been recorded in many Egyptian governorates (Emara et al., 2023a). Many snails are nocturnal that use the night cool and damp conditions. Under dry conditions, snails search for a suitable place for hiding. Some snails crawl up the stems of plants and fall into a dormancy state, then closing the shell entrance using a slime seal, by which snails also stick themselves to the chosen surfaces. Temperature degrees, photoperiod, relative humidity, habitat characteristics and food sources are the most important factors affecting the occurrence and survival of land snails (Martin et al., 2004; Metwally et al., 2002; Ramzy, 2009). The chemical control of land snails by using pesticides is still the most effective controlling technique, especially over large areas. Acrylamide derivatives have a potential biological activity as well as acting as precursors in many organic syntheses (Kariuki et al., 2022). Moreover, acrylamides and their derivatives cause convulsions and diffused damage to different sections of the nervous system of infected animals (Parod, 2005). This study aimed to prepare some copper and zinc chelates achieved from two acrylamide derivatives. The structure of target chelates was identified by mass and IR spectra, and their toxicity against *Eobania vermiculata* was assessed. The level of some important enzymatic biomarkers was assayed in order to study the efficacy of tested compounds on the liver and mucous gland of screened snails.

Materials and methods

Chemicals and instrumentations

(*E*)-3-(4-bromophenyl)-2-cyanoacrylamide (**L^1**) ($C_{10}H_7BrN_2O$) was purchased from Merck company. (*E*)-2-cyano-3-(4-nitrophenyl)acrylamide (**L^2**) ($C_{10}H_7N_3O_3$) was obtained from Sigma-Aldrich. $CuCl_2$ and $ZnCl_2$ salts and methanol were bought from commercial suppliers and used without any additional purification.

FT-IR spectra were specified within (4000–400 cm^{-1}) range using Nicolet FT-IR spectrophotometer. Mass spectra of synthesized metal constructions were performed at The Regional Center for Mycology and Biology, Al-Azhar University, on the direct inlet part of the mass analyzer in the Thermo Scientific GCMS model ISQ. Biochemical assays were conducted at Plant Protection Research Institute.

Synthesis of Cu(II), Zn(II) Constructions

The four metal chelates were prepared by the same procedures. A hot methanolic solution of metal chloride salt was added to a ligand methanolic solution, followed by continuous stirring at 65 °C for 4 h. The reaction temperature was then decreased to room temperature, and the mixture was allowed to settle, then filtered off and washed several times with methanol. The filtered off solid complexes were then dried under vacuum over anhydrous calcium chloride (Emara et al., 2022).

$Cu(L^1)Cl_2(H_2O)_2$, Mass spectrum (m/z) Found/calc.: 422.75/421.66 amu. IR (cm^{-1}): 3411 υ(NH)$_2$), 2209 υ(C≡N).
$Zn(L^1)Cl_2(H_2O)_2$, Mass spectrum (m/z) Found/calc.: 423.45/423.49 amu. IR (cm^{-1}): 3428 υ(NH)$_2$), 2212 υ(C≡N).
$Cu(L^2)Cl_2(H_2O)_2$, Mass spectrum (m/z) Found/calc.: 388.26/387.77 amu. IR (cm^{-1}): 3426 υ(NH)$_2$), 2224 υ(C≡N).
$Zn(L^2)Cl_2(H_2O)_2$, Mass spectrum (m/z) Found/calc.: 390.64/389.60 amu. IR (cm^{-1}): 3441 υ(NH)$_2$), 2221 υ(C≡N).

Tested herbivores

Eobania vermiculata individuals with nearly the same shell diameters were obtained from untreated infested nurseries located at Menouf city, Menoufia governorate, Egypt, during February 2022. The collected snails were then transferred to the laboratory at Sers Ellyan Agricultural Research Station at which in vitro trials were conducted. Snails were maintained at 27 °C and 65% R.H in small plastic boxes with a layer of moist optimal soil. Fresh green lettuce leaves were used to feed the reared snails along 14 days, allowing complete adaptation with laboratory conditions. Snails with approximately the same size were starved for 24 h before treatments.

Laboratory tests

Contact method was employed to run treatments according to (Mourad, 2014). Distilled water and DMSO mixture was used to prepare stock solutions, and four different concentrations; (50, 150, 250 and 350 ppm) of each tested compound were prepared. Petri dish containing 2 ml of each concentration was moved in circle for insuring equal spreading all over its surface. Used solvents were then evaporated under room temperature after leaving a layer of the tested compound on the Petri dish surface. Each treatment was run through three replicates, each with ten snails, along 3 days, and dead snails were taken away every day. A parallel control treatment was performed. The corrected mortality and sub-lethal concentrations were estimated using Abbott's formula (Abbott, 1925).

Biochemical assays

Preparation of homogenate samples

The reared *Eobania vermiculata* species were treated with LC_{50} concentrations of investigated chelates, and 3 replicates were conducted for each biochemical assay.

(BECKMAN GS-6R Centrifuge) was used to centrifuge homogenized live snails by distilled water at 6000 rpm at 5 °C for 10 min. After that, the supernatant fluid was divided into small partitions (0.5 ml) and stored at – 20 °C.

Total protein content determination

Total proteins were assessed (Bradford, 1976) via employing the standard of bovine serum albumin.

Determination of transaminase enzymes

Transaminase enzymes, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) activities were assayed using Reitman and Frankel (1957) method.

Determination of acid and alkaline phosphatase activities

Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) activities were determined according to the method described by Powell and Smith (1954).

Statistical analysis

Sub-lethal concentrations of investigated chemical compounds with slope and fiducial limits of each treatment were assessed by running Probit analysis program (Finney, 1971). The achieved data were subjected to analysis of variance method (ANOVA) and calculated as (Mean ± SD). Means were compared by LSD method for significance at the probability of 0.05 (Steel et al., 1981).

Results

Construction of metal chelates

IR spectral data of obtained metal constructions are shown in Fig. 1. Infrared spectra of $(\mathbf{L^1})$ and $(\mathbf{L^2})$ reveal bands at 3405, 3414 cm^{-1}, respectively, ascribed to the stretching vibration of amino group (Peng et al., 2021). $\upsilon(NH_2)$ of $(\mathbf{L^1})$ adopted shift to higher wavenumbers by (6, 23 cm^{-1}) after chelation with Cu(II) and Zn(II) ions, respectively. While, the stretching vibration of amino group of $(\mathbf{L^2})$ underwent shift to high wavenumbers by (12, 27 cm^{-1}) in Cu(II), Zn(II) complexes **(3, 4)**, respectively, authenticating the involvement of amino group in complexation. Moreover, the spectra of $(\mathbf{L^1})$ and $(\mathbf{L^2})$ show bands at 2225, 2232 cm^{-1}, respectively, due to $\upsilon(C\equiv N)$ (Kariuki et al., 2022). This spectral band suffered alternations in shape and position upon chelation, indicating its contribution in complexation process. These

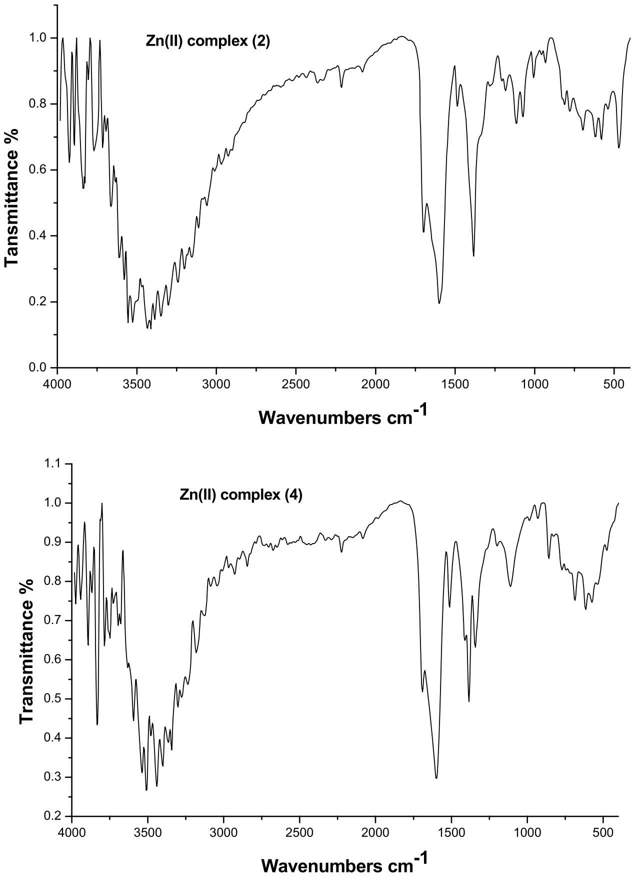
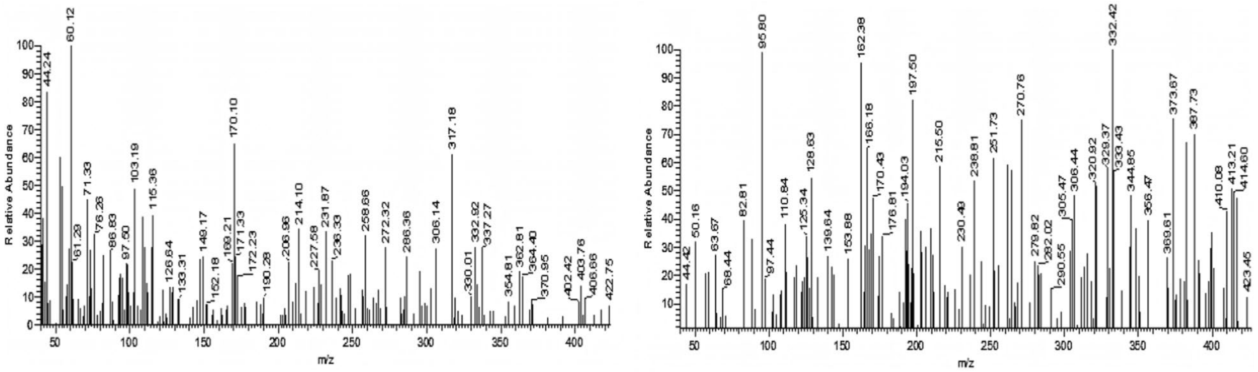


Fig. 1 IR spectra of synthesized metal constructions

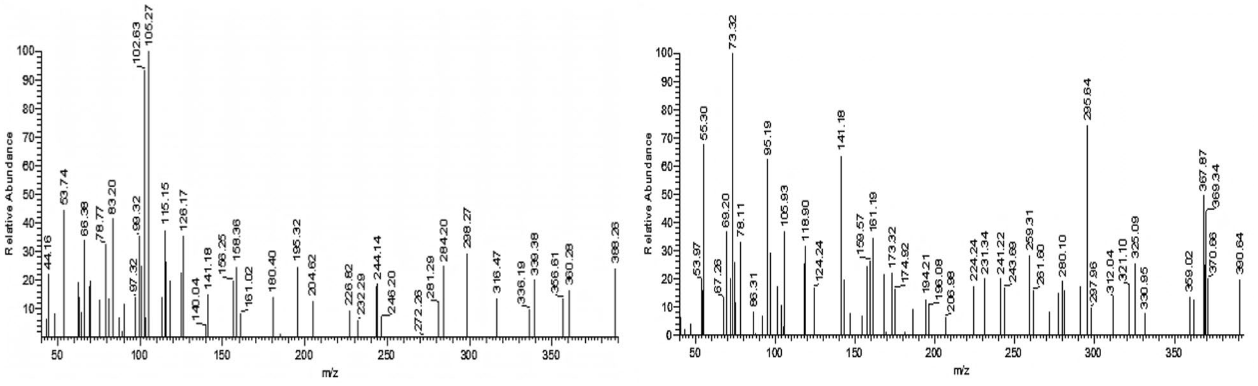
results conclude the neutral bidentate chelation mode of the two ligands toward copper and zinc ions via amino and nitrile nitrogen atoms.

Mass spectra of constructed metal complexes are shown in Fig. 2. Mass spectra of complexes **(1–4)** display characteristic molecular ion peaks at m/z = 422.75, 423.45, 388.26 and 390.64 amu, respectively, complying with their theoretical molecular weights. The aforesaid IR and mass spectral data



Cu(II) complex (1)

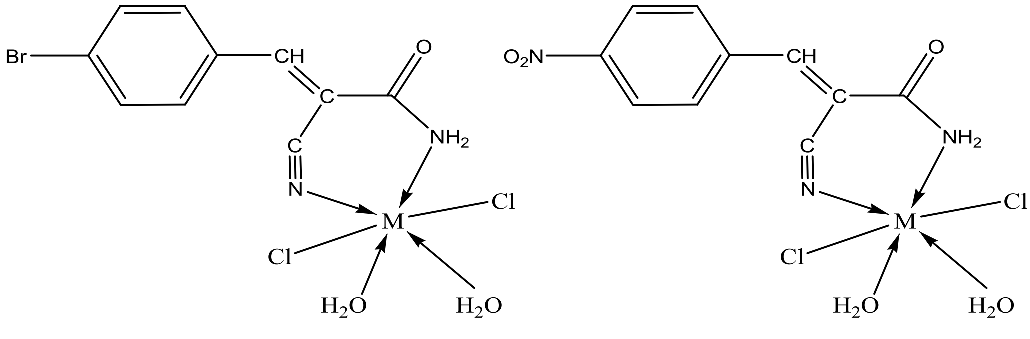
Zn(II) complex (2)



Cu(II) complex (3)

Zn(II) complex (4)

Fig. 2 Mass spectra of prepared metal complexes



Structure of complexes **(1, 2)**

Structure of complexes **(3, 4)**

(M = Cu, Zn)

Fig. 3 Proposed structures of prepared metal constructions authenticate the suggested structures for achieved metal chelates represented in Fig. 3.

Toxicity of metal chelates

Results of in vitro treatments are tabulated in Table 1 and displayed in Fig. 4. Laboratory trials data clarified the relatively toxic effects of the tested complexes toward the screened snails. Zinc complexes showed a higher toxicity than copper ones. The sub-lethal concentration of Cu(II) complexes **(1, 3)** is 365.16 and 332.80 ppm compared to 141.99 and 269.98 ppm for Zn(II) complexes **(2, 4)**, respectively. Susceptibility of land snails species toward examined compounds enhanced as the concentration and experiment period increased (Emara et al., 2023b).

Biochemical assay results

Total protein content

Total protein content of *Eobania vermiculata* is shown in Table 2 and illustrated in Fig. 5. The present results authenticated that the total protein level was inhibited in snails after exposure to LC_{50} concentrations of investigated complexes than the control one. The data clarified that TP content was reduced from 22.11 mg/g for control species upon treatment with complexes **(1–4)** to 15.61, 19.35, 21.76 and 15.19 mg/g, respectively.

Effect of tested complexes on liver transaminase enzymes (AST, ALT)

The gained data displayed in Table 3 and Fig. 5 reflect the promising effects of tested metal chelates on the activity of ALT enzyme. A considerable enhancement in the ALT activity was established after treatments with prepared chelates. The level of ALT enzyme was enhanced from 5.07 UL^{-1} in control snails to 18.00, 18.33, 21.57 and 24.80 UL^{-1} in complexes **(1–4)**, respectively. This indicates the high effects of tested complexes on this liver enzyme.

Table 4 and Fig. 5 illustrate the effect of investigated chelates on the activity of AST enzyme. The data reveal that AST activity of control snails was inhibited from 56.97 UL^{-1} to 44.63, 35.63 UL^{-1} in Zn(II), Cu(II) complexes **(2, 3)**, respectively, while Cu(II), Zn(II) complexes **(1, 4)** increased the activity of AST to 59.53, 61.83 UL^{-1}, respectively.

Effect of tested complexes on (ACP) and (ALP) enzymes

Eobania vermiculata snails were treated with LC_{50} concentrations of Cu(II), Zn(II) complexes **(1, 2)** in order to assess their efficacy on ALP and ACP enzymes. The achieved results are represented in Tables 5 and 6. It is obviously clear that the two tested chelates have a significant efficacy on ACP and ALP activity of treated snails. Cu(II) complex **(1)** increased the activity of ACP and ALP to 200.66 and 180.65 mg/g compared to 4.32 and 3.25 mg/g for control snails, respectively, whereas Zn(II) complex **(2)** enhanced ACP and ALP activity to 131.92 and 323.09 mg/g.

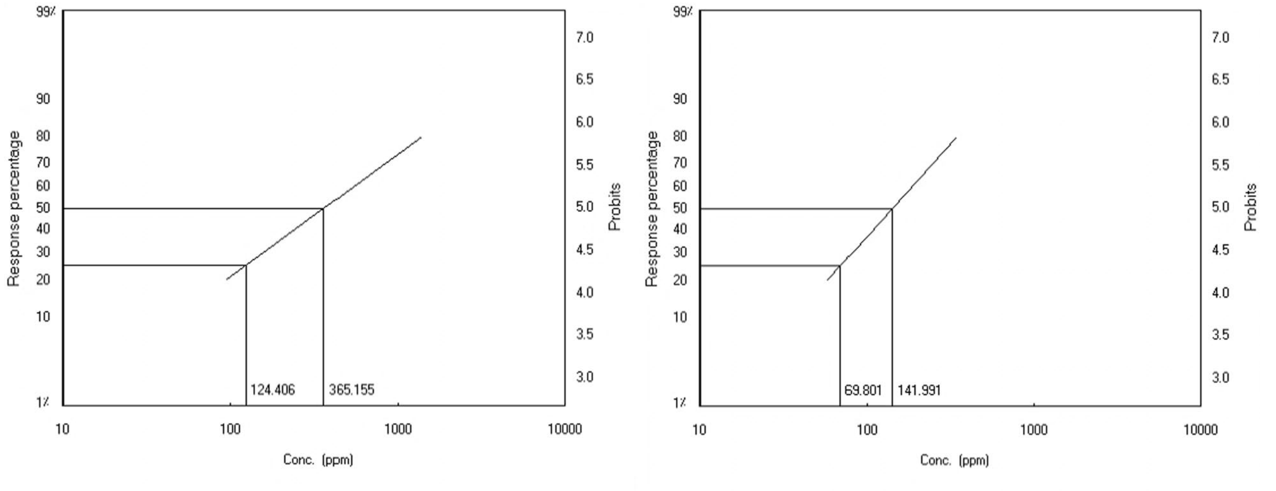
Discussion

Toxic influence

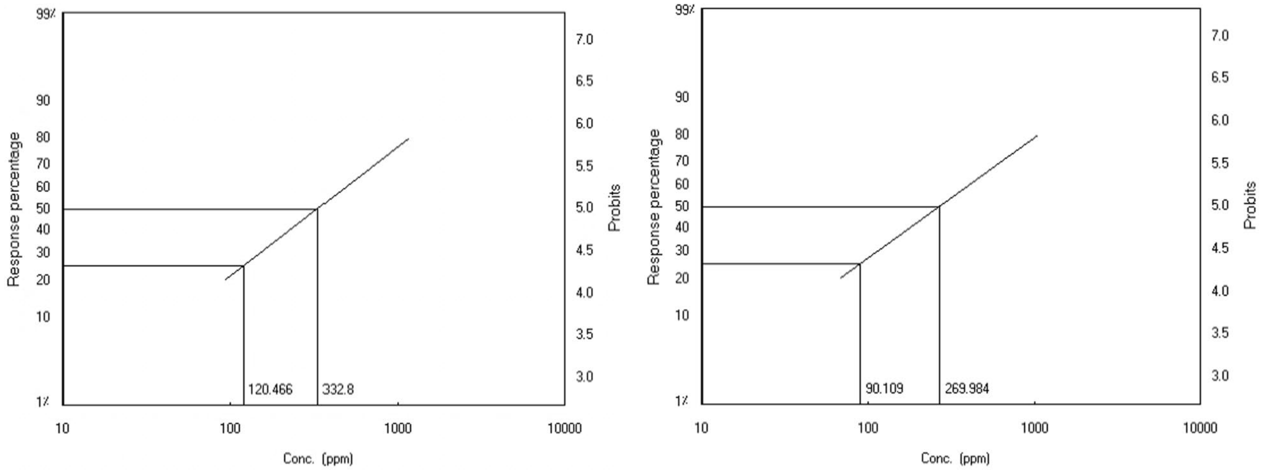
The presence of oxygen and nitrogen atoms in the structure of tested complexes is responsible for the toxicity of this set of compounds (El-Samanody et al., 2017). Additional reason for prepared chelates toxicity

Table 1 Toxicity data of tested metal complexes

No.	Complexes	Concentrations (ppm)	Corrected mortality (%)	LC_{50} (ppm)	Slope ± S.E
1.	$Cu(L^1)Cl_2(H_2O)_2$	50	16.6	365.16	1.44 ± 0.237
		150	16.6		
		250	30.84		
		350	64.28		
2.	$Zn(L^1)Cl_2(H_2O)_2$	50	16.6	141.99	2.19± 0.229
		150	52.24		
		250	66.67		
		350	83.33		
3.	$Cu(L^2)Cl_2(H_2O)_2$	50	16.6	332.8	1.53 ± 0.237
		150	16.6		
		250	33.33		
		350	66.67		
4.	$Zn(L^2)Cl_2(H_2O)_2$	50	16.6	269.98	1.42 ± 0.223
		150	33.33		
		250	43.79		
		350	61.36		



Cu(II) complex (1) **Zn(II) complex (2)**



Cu(II) complex (3) **Zn(II) complex (4)**

Fig. 4 Toxicity of tested metal chelates against *E. vermiculata*

Table 2 Efficacy of tested metal chelates on the TP content of *E. vermiculata*

No.	Complexes	TP (mg/g)			Mean ± SD (mg/g)
		R_1	R_2	R_3	
1.	$Cu(L^1)Cl_2(H_2O)_2$	16.96	15.36	14.52	15.61 ± 1.24
2.	$Zn(L^1)Cl_2(H_2O)_2$	20.11	17.96	19.97	19.35 ± 1.21
3.	$Cu(L^2)Cl_2(H_2O)_2$	20.58	22.68	22.03	21.76 ± 1.08
4.	$Zn(L^2)Cl_2(H_2O)_2$	15.33	16.53	13.71	15.19 ± 1.42
	Control	22.1	22.2	22.04	22.11 ± 0.22

is the presence of amino group in their skeleton, which has an ability to make many reactions inside cells and hence leads to the initiation of carcinogenic process (Garrigós et al., 2002). Also, bromide ions presented in complexes' structures elevate their toxicological activity. Toxicity of prepared copper and zinc constructions is related to the presence of metal ions that have ability to react with enzymes in cells (El-Samanody et al., 2017 and Emara et al., 2023c). Toxicological effects of Cu(II) ions are attributed to their high affinity for cysteine (protein free thiol groups), as copper ions are more likely binding to free thiol group of ALP enzyme, hence causing enzyme inactivation. Also, divalent copper ions are capable of binding and distorting the enzymes active site or even compete for Mg(II) and Zn(II) binding sites in these enzymes (Alnuaimi et al., 2012 and Emara et al., 2022). Moreover, zinc and cadmium have comparatively the same oxidation states, biological

Table 3 Efficacy of tested metal chelates on the ALT activity of *E. vermiculata*

No	Complexes	ALT (UL^{-1})			Mean ± SD (UL^{-1})
		R_1	R_2	R_3	
1	$Cu(L^1)Cl_2(H_2O)_2$	18.80	17.20	18.00	18.00 ± 0.80
2	$Zn(L^1)Cl_2(H_2O)_2$	18.20	16.60	20.20	18.33 ± 1.80
3	$Cu(L^2)Cl_2(H_2O)_2$	23.60	20.40	20.70	21.57 ± 1.77
4	$Zn(L^2)Cl_2(H_2O)_2$	240	25.80	24.60	24.80 ± 0.92
	Control	5.50	4.60	5.10	5.07 ± 0.45

Table 4 Efficacy of tested metal chelates on the AST activity of *E. vermiculata*

No	Complexes	AST (UL^{-1})			Mean ± SD
		R_1	R_2	R_3	(UL^{-1})
1	$Cu(L^1)Cl_2(H_2O)_2$	61.20	57.60	59.80	59.53 ± 1.81
2	$Zn(L^1)Cl_2(H_2O)_2$	45.30	44.00	44.60	44.63 ± 0.65
3	$Cu(L^2)Cl_2(H_2O)_2$	34.20	37.10	35.60	35.63 ± 1.45
4	$Zn(L^2)Cl_2(H_2O)_2$	65.20	58.70	61.60	61.83 ± 3.26
	Control	57.90	56.10	56.90	56.97 ± 0.90

Table 5 Efficacy of Cu(II), Zn(II) complexes **(1, 2)** on the ACP activity of *E. vermiculata*

No	Complexes	ACP (mg/g)			Mean ± SD (mg/g)
		R_1	R_2	R_3	
1	$Cu(L^1)Cl_2(H_2O)_2$	199.55	205.09	197.33	200.66 ± 3.99
2	$Zn(L^1)Cl_2(H_2O)_2$	109.75	150.77	135.25	131.92 ± 20.71
	Control	3.33	4.10	5.54	4.32 ± 1.12

Table 6 Efficacy of Cu(II), Zn(II) complexes **(1, 2)** on the ALP activity of *E. vermiculata*

No.	Complexes	ALP (mg/g)			Mean ± SD (mg/g)
		R_1	R_2	R_3	
1.	$Cu(L^1)Cl_2(H_2O)_2$	181.68	172.37	187.9	180.65 ± 7.82
2.	$Zn(L^1)Cl_2(H_2O)_2$	319.89	322.99	326.41	323.09 ± 3.26
	Control	3.39	3.25	3.11	3.25 ± 0.14

activity and cadmium can replace zinc present in metallothionein, thus preventing its free radical scavenger behavior in the cell (Jaishankar et al., 2014). Toxicological influence of zinc chelate is assigned from its binding nature with metal cations binding sites of enzymes (Alnuaimi et al., 2012). Its concentration increases 3000-fold when it binds to cysteine-rich protein such as metallothionein. The cysteine–metallothionein complex causes hepatotoxicity in the liver, and then it circulates to the kidney and gets accumulated in the renal tissue causing nephrotoxicity. Zinc ions have the ability to bind with glutamate, cysteine, aspartate and histidine ligands leading to the deficiency of iron.

Biochemical determinations

The reduction in TP level may be arisen from the imbalanced between the rates of degradation and synthesis of total protein in tissues of the body. This was the result established by (El-Shenawy et al., 2012) who stated that the TP level reduced in the digestive gland of *Eobania vermiculata*.

Quantitative estimation of enzymes reflects a reliable indicator for the imposed stress affected on the organism by environmental pollutants such as metals and their derivatives. A number of physiological roles

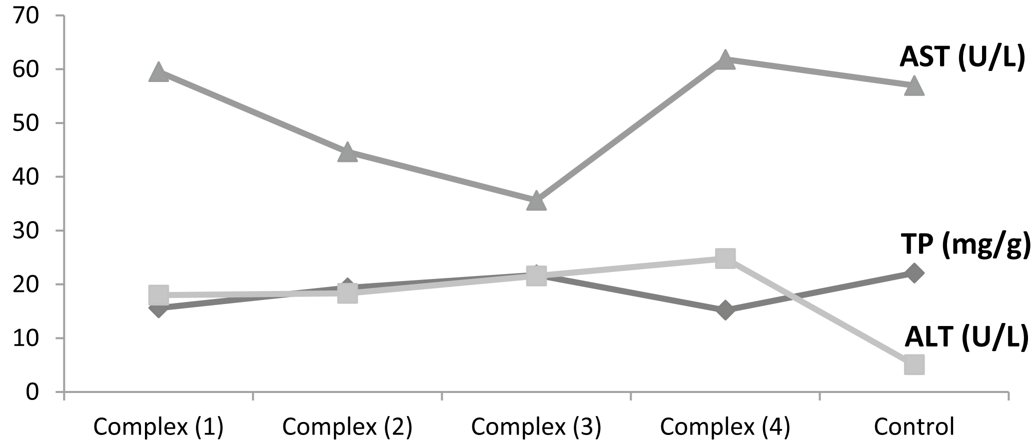


Fig. 5 AST, ALT levels and TP content of *E. vermiculata*

such as activity of many hydrolytic lysosomal enzymes are reduced by the action of heavy metals, even though these metals may also activate certain enzymes. Alterations in biomarkers represent an indicator for risk measurements of land snails and environmental conditions (Khalil, 2016). Aminotransferase enzymes are very active in the liver, and their activity can be detected in small amounts (Lehninger et al., 1992). AST, ALT and ALP enzymes are found in hepatic cells and in some tissues of the body like intestine and muscle, gill and heart. So, the reaction of treated snails toward toxic effects of chemicals needs adequate energy to get rid of the toxic stress which obtained via gluconeogenesis during breaking down the free amino acid to overcome the needed energy, leading to an increased transaminase enzymes activity (Neelima et al., 2013; Samanta et al., 2014). The toxic stress results in changes of cell permeability which causes enhancements in enzymes activity caused by leakage of the enzymes out of the damaged cells (Meenakshi et al., 2020). Banaee et al. (2016) emphasized that the increased activities of AST and ALT enzymes may happen because of an increase in permeability of cell membrane or damage of cell membrane of hepatocyte. ALT and AST activity alternations may also be due to the damage of digestive gland and cell necrosis resulted by the effect of insecticides and caused leakage out the enzymes out the cells (Kammon et al., 2010; Gaber et al., 2022). The elevated activity of enzymes is ascribed to degeneration and necrotic changes in the liver resulted by chemicals through which the enzymes leakage out the cells (Arfat et al., 2014). It is probable that tested metal constructions interact primarily with the liver and muscle tissue cell membranes. This leads to structure damage and changes in enzyme leakage and in metabolism of the constituents. So, metal chelates may pressurize those enzymes into plasma as a result of enzyme leakage breakdown. ALT and AST enzymes are principally located at the cytoplasm, and they will be secreted into the blood after hepatocellular injury, leading to an increase in their levels in the serum. Subsequently, this study states that all damages in land snails hepatopancreatic cells result in alteration in the serum AST and ALT levels (hemolymph). The obtained data are in agreement with Al-Attar (2005) who concluded that AST, ALT and ALP activity were enhanced significantly in the fish exposed to Cd ions. Also, the output results agree with El-Dafrawy et al. (2001) who established that when snails exposed to pesticides, a significant increase in the levels of AST and ALT enzymes was recorded in hemolymph and tissues of exposed snail than control ones; this may be ascribed to parasite growth inside the snail.

Acid phosphatase (ACP) and alkaline phosphatase (ALP) are important phosphatases which are different in their subcellular distribution. ALP activity is highly concentrated in plasma membrane enriched fraction, while ACP is associated with lysosomes. These enzymes have a very important role in bivalve immunity and are involved in many metabolic processes: protein synthesis, absorption, permeability, growth and cell differentiation and transport of nutrients, gonadal maturation and steroidogenesis (Ram et al., 1986). Alkaline and acid phosphatase enzymes have important roles in the defense mechanism of land snails (Saber et al., 1986). ALP is a hepatic microsomal enzyme that contributes in transport of membrane due to its effect on a number of phosphomonoesters of biochemical compounds such as glucose (Edqvist et al., 1992). Decline in the activity of ALP enzyme may be ascribed to the drop in glycogen biosynthesis resulted from lowered metabolic demands and electrolytic non-equilibrium caused by tissue over hydration (Shaffi, 1979). Alternations in endoplasmic mass that happens in the cell membrane are caused by the reduction in ALP activity (Edqvist et al., 1992). Thus, decline ALP activity could cause biosynthetic shifts and energy metabolism pathway of the exposed organism (Ovuru et al., 2000). ALP is an early marker of differentiation of cell in the osteogenic lineage in bivalve mollusc. The level of the activity of ALP enzyme is highly sensitive to pollution with heavy metals (Regoli et al., 1995). Phosphomonoesters which formed after hydrolysis of other major cell phosphates are hydrolyzed by the action of ACP enzyme.

In the present study, dead snails after treatments with complexes **(1, 2)** showed considerable body dryness due to the stimulation for mucous secretions caused by tested compounds. Snails secreted excess mucus in an attempt to face toxins. This fact is obviously clear from the elevated levels of ACP and ALP enzymes. Elevation in ALP activity affected on mucous production of the mucous gland, which in turn caused the stress to the gland, and hence leads to the mortality of the treated species. The mucous cell secretion displayed activity of ACP and ALP enzymes (Ning et al., 2005).

The present results are in agreement with those of Abdel-Hamid (2008) who stated that the activity of ALP was increased in the infected control group by 61.1% in *B. alexandrina* and 32.6% in *B. truncatus* more than the uninfected group. This may be a result of the liver damage caused by Schistosome parasite or its toxic metabolites. Our data are also complied with the obtained results of many other authors who concluded an enhancement in ALP activity in treated species (El-Dafrawy et al., 2001; Mahmoud et al., 2002).

Conclusions

Copper and zinc chelates originated from two acrylamide derivatives, namely (*E*)-3-(4-bromophenyl)-2-cyanoacrylamide (**L^1**) and (*E*)-2-cyano-3-(4-nitrophenyl)acrylamide (**L^2**), were prepared, and their chemical structures were elucidated. The toxicity of synthesized chelates was screened against *Eobania vermiculata* under laboratory conditions. The level of ALT, AST, ACP and ALP enzymes and TP content was assessed in order to investigate the effect of tested compounds on those vital biomarkers. The results demonstrated a considerable toxicity of investigated compounds, and their significant activity toward the assayed enzymes. It can be concluded that the tested metal chelates have a high efficacy on the liver enzymes as well as the mucous secretion of tested land snails.

Abbreviations

L^1	(*E*)-3-(4-Bromophenyl)-2-cyanoacrylamide
L^2	(*E*)-2-Cyano-3-(4-nitrophenyl)acrylamide
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
TP	Total Protein content
ACP	Acid Phosphatases
ALP	Alkaline Phosphatase
ANOVA	Analysis of Variance method

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Not applicable.

Author contributions

EE helped in conceptualization, data curation, chemical and in vitro analyses, investigation, methodology, resources, software, validation, writing the manuscript and final revision. ME was involved in investigation, resources, methodology, data curation, software and revision. RK helped in conceptualization, investigation, resources, methodology, data curation, software and revision.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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