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RESEARCH

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In vitro molluscicidal activity and biochemical impacts of some thiophene derivatives against the glassy clover snail, *Monacha obstructa* (Pfeiffer)

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Abstract

Background The glassy clover snail, *Monacha obstructa* (Pfeiffer), is considered one of the major agricultural pests that ruin many field crops, vegetables, orchards of fruits, plants of ornament as well as many other plantations. Synthesis of the Schiff base ligand, namely bis-[4-benzylidene-thiophene-2′-yl] methane **(L)**, produced from the reaction between thiophene-2-carboxaldehyde and diaminodiphenylmethane (MDA), alongside its copper complex were conducted. The output chemical compounds were evaluated in vitro for their molluscicidal activity against the glassy clover snail, *M. obstructa* by performing the contact technique. Stock solutions were prepared via using (distilled water + DMF) mixture. Furthermore, the impact of these compounds on some critical biochemical indicators: cholesterol, total protein, and acetylcholinesterase (AChE), was evaluated.

Results The outcome results demonstrated the significantly higher molluscicidal activity of the Cu(II) chelate compared to its free ligand **(L)**, which in turn reveals the importance of metal chelation in enhancing toxicity against the target species. Particularly, the LC_{25} and LC_{50} values are (27.25, 34.65) and (17.88, 25.31) ppm for the ligand **(L)** and its copper construction, respectively. Additionally, the data confirmed the significant effectiveness of the tested compounds on the assessed biochemical indicators of treated snails. Total protein and cholesterol levels were elevated after treatment with both the ligand **(L)** and its copper complex while AChE activity increased after treatment with the ligand **(L)** and reduced upon the exposure to the Cu(II) chelate.

Conclusions The findings established that the copper complex exhibited a markedly higher molluscicidal activity compared to the free ligand **(L)**. Also, the results confirmed the significant effects of the investigated compounds on the assessed biochemical indicators of treated *M. obstructa* snails.

Keywords *Monacha obstructa*, Thiophene derivatives, Laboratory treatment, Biochemical parameters, Toxicity

Introduction

The notorious economic pests (land snails) are terrestrial gastropods that responsible for giving rise to damage for several horticultural and agricultural crops, especially in temperate and humid regions (Emara et al., 2023a; Rady, 2019). This damage in turn leads to the reduced quality of vegetables and fruits, resulting in significant economic losses. *Monacha obstructa* (Pfeiffer) is vastly located in natural habitats across the Middle East region,

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Mediterranean countries, along with many Egyptian Governorates (El-Halim et al., 2021; Emara, 2024a). This agricultural pest has a veterinary importance because it acts as an intermediate host for many parasites; the trematode parasite, *Brachylaima sp*., and the nematode parasite, *Angiostrongylus cantonensis* in Egypt, along with other parasitic diseases affecting humans and animals (El-Halim et al., 2021). Many methods are currently used for controlling such agricultural pests, but chemical treatment is still the most effective one (Emara et al., 2024b). Thiophene is a versatile and vital building block that contains sulfur atom in its chemical skeleton. It is important for manufacturing many pharmaceuticals that possessing potent anti-infective properties (Keri et al., 2017). Thiophene derivatives have been chosen because of their potential influence through the metabolism of cytochrome P450 (CYP450) (Dua et al., 2008). Enzymatic oxidation of thiophene is caused by various CYP450 enzymes, resulting in forming thiophene epoxides and sulfoxides, which in turn can interact with DNA nucleobases, forming DNA adducts. During replication of damaged DNA, mutations may occur in the newly synthesized DNA strands. This may contribute in the formation of tumor and lead to permanent mutations of gene (Machinist et al., 1995; Misra & Amin, 1990; Mizutani et al., 1994, and Sinsheimer et al., 1992). This study presents findings on the toxicological effects of some specific thiophene derivatives against *M. obstructa* species under laboratory conditions. Additionally, the impact of these compounds on some key biochemical indicators; acetylcholinesterase (AChE), cholesterol, and total protein levels, was assessed.

Methods

Tested compounds

Some reputable suppliers such as Sigma-Aldrich, Merck, and BDH were the main sources of the used chemicals. However, solvents employed in the experiments were obtained from commercial suppliers. The ligand, bis-[4-benzylidene-thiophene-2′-yl] methane **(L)**, and its copper chelate were synthesized and characterized as previously described (AbouEl-Enein, 2008). The structure of the synthesized ligand is depicted in Fig. 1.

Investigated land snails

Sers Ellyan Agricultural Research Station located in Menoufia Governorate, Egypt, was the infected natural habitat from which the healthy adult individuals of the glassy clover snail, *M. obstructa*, were manually collected during March, 2024. Upon collection, sacks of muslin were chosen to transport the collected snails to the laboratory at which the snails were housed in plastic aerated cages. These cages were filled with optimal, moist soil and the snails remained inside it along two weeks prior to the studies commencement to ensure acclimation. Along acclimation period, the fresh, green lettuce leaves were provided to the reared snails for nourishment.

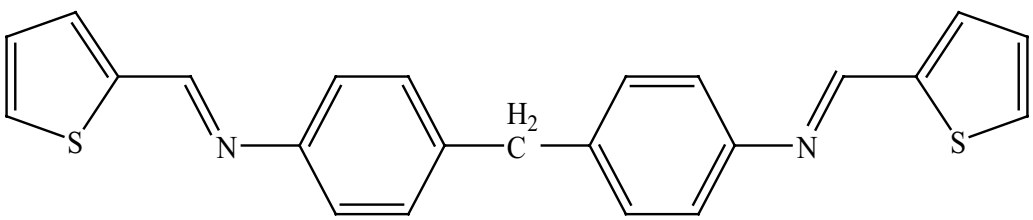
Toxicity tests

The median lethal concentrations (LC_{50}) of the ligand **(L)** and its Cu(II) construction were estimated employing the contact method following the protocol outlined by Mourad, (2014). The investigated compounds' stock solutions were prepared via using a mixture of distilled water and DMF (3:1 by volume). Screening test was performed, and then four different concentrations of each compound; (20, 30, 40, and 50 ppm) were prepared, alongside parallel control tests. Each treatment consisted of three replicates, and all experiments were conducted over a period of 72 h.

Biochemical assays

Preparation of homogenate samples

The exposure of the adult reared *M. obstructa* snails to the LC_{50} concentration of the tested compounds was performed for duration of 72 h. Additionally, parallel control tests were conducted. Each biochemical assay was performed via three replicates. After the treatment period, deceased snails were excluded from the analysis, and the shells of the surviving individuals were removed. The



Ligand (L)

Fig. 1 Chemical structure of the ligand **(L)**

remaining soft body of the live snails was then homogenized with distilled water at 6000 rpm and 5°C for 10 min using a BECKMAN GS-6R Centrifuge. Subsequently, the resulting supernatant fluid was divided into small aliquots (0.5 ml) and stored at − 20°C for further analysis.

Estimation of total protein levels

Total protein levels were estimated using the colorimetric method with Biuret reagent, following the technique outlined by Henry, (1964).

Determination of cholesterol concentration

Cholesterol concentration was assayed colorimetrically, utilizing the technique outlined by Ellefson & Caraway, (1976).

Assessment of acetylcholinesterase (AChE) activity

Acetylcholinesterase (AChE) activity in target snails was assessed kinetically, according to the method described by Burtis-Ashwood, (1994).

Statistical analysis

Sub-lethal concentrations of the investigated chemical compounds, along with their slopes and fiducial limits, were determined using Probit analysis program as described by Finney, (1971). The resulting data were then analyzed using the analysis of variance method (ANOVA). Mean values were compared for significance at a probability level of 0.05 using the LSD (least significant difference) method, following the approach outlined by Steel & Torrie, (1981).

Results

Toxicity evaluation

The yielded in vitro toxicity data of both the ligand **(L)** and its copper construction against adult individuals of *M. obstructa* snails are illustrated in Fig. 2 and displayed in Table 1. The ligand **(L)** reported LC_{25} and LC_{50} values as 27.25 and 34.65 ppm, respectively, with a slope of 6.46 ± 0.577, while the Cu(II) chelate revealed LC_{25} and LC_{50} values of 17.88 and 25.31 ppm, respectively, with a slope of 4.47 ± 0.523.

Biochemical parameters data

Content of total protein

The results of total protein content estimation of *M. obstructa* snails are presented in Table 2 and depicted in Fig. 3. The obtained data demonstrate a significant increase in the efficacy of the tested compounds on the total protein levels of treated snails. The total protein content of control snails was (1.85 ± 0.031 g/dL). Upon treatment with the ligand and its copper chelate, the total protein levels increased to (2.09 ± 0.062) and (2.15 ± 0.091) g/dL, respectively.

Cholesterol concentration

Data in Table 3 and Fig. 3 illustrate the response of cholesterol levels in treated snails to the ligand **(L)** and its

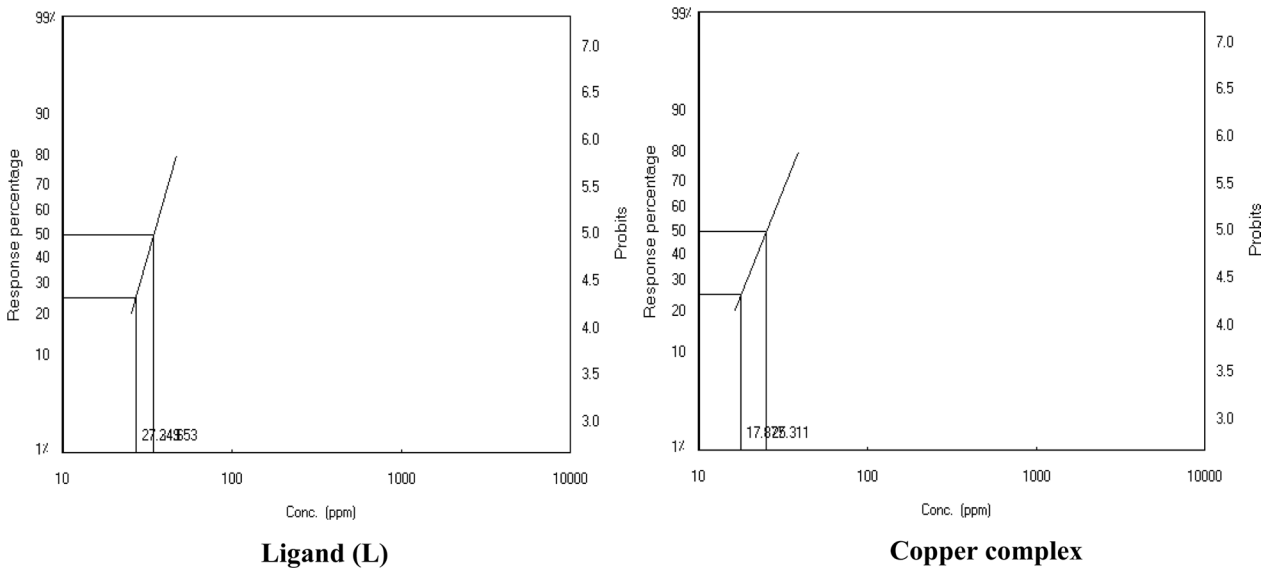


Fig. 2 Toxicity of tested compounds against *M. obstructa*

Table 1 In vitro toxicity data of the ligand **(L)** and its Cu(II) complex against *M. obstructa*

Compound	Concentrations (ppm)	Mortality (%)	LC_{25} (ppm)	LC_{50} (ppm)	Slope ± S.E
Ligand **(L)**	20	11.11	27.25	34.65	6.46 ± 0.577
	30	22.22			
	40	66.68			
	50	88.89			
Cu complex	20	22.22	17.88	25.31	4.47 ± 0.523
	30	77.78			
	40	88.89			
	50	88.89			

Table 2 Efficacy of the tested compounds on total protein content of *M. obstructa*

Compounds	Total protein (g/dL)			Mean ± SD (g/dL)
	R_1	R_2	R_3	
Ligand **(L)**	2.14	2.02	2.11	2.09 ± 0.062
Cu complex	2.23	2.05	2.16	2.15 ± 0.091
Control	1.86	1.82	1.88	1.85 ± 0.031

copper complex. The cholesterol concentration of treated snails displayed a significant susceptibility to the tested compounds. Treatment with the ligand **(L)** and its copper complex increased the cholesterol level to (47.49 ± 0.960) and (24.84 ± 0.933) mg/dL), respectively, compared to (4.76 ± 0.071 mg/dL) for control ones.

Acetylcholinesterase (AChE) activity

The results of AChE activity determination are displayed in Table 4 and depicted in Fig. 4. The outcome data indicate opposing effects of the ligand **(L)** and its copper construction toward the acetylcholinesterase (AChE) activity of treated snails. When *M. obstructa* snails exposed to the ligand **(L)** sub-lethal concentration, the AChE activity enhanced to 4974.54 ± 195.09 UL^{-1}, compared to 3310.56 ± 58.08 UL^{-1} for control snails. However, after exposure to LC_{50} of the copper complex, the AChE activity was inhibited, dropping to 1335.80 ± 58.15 UL^{-1}.

Discussion

Molluscicidal activity

The existence of sulfur and nitrogen atoms in the chemical skeleton of the ligand is responsible for its significant toxicity (El-Samanody et al., 2017a). Furthermore, the

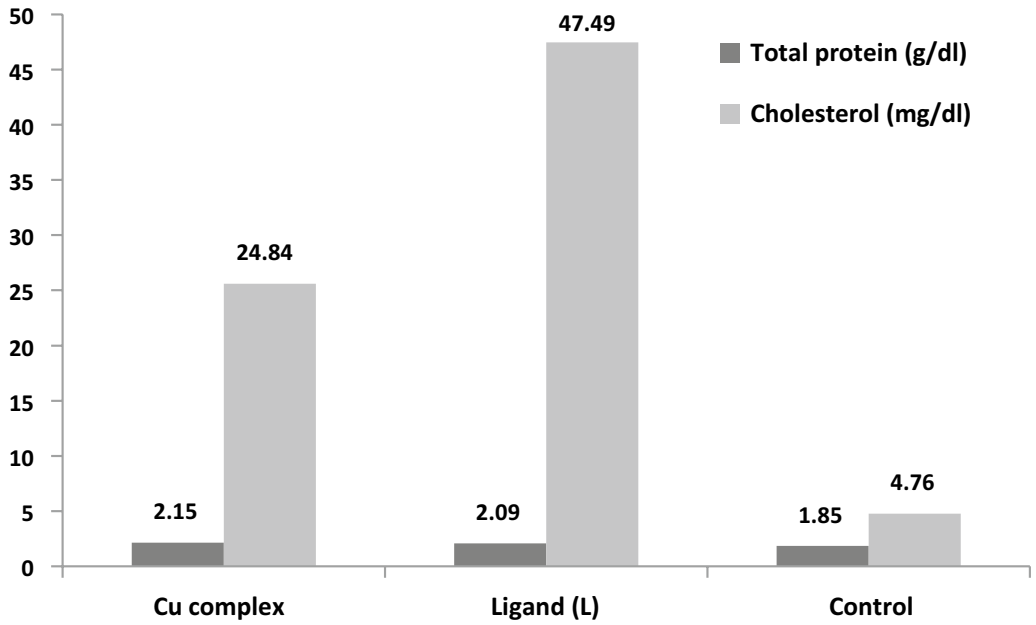


Fig. 3 Total protein level and cholesterol concentration of treated *M. obstructa* snails

Table 3 Impact of the tested compounds on cholesterol concentration of *M. obstructa*

Compounds	Cholesterol concentration (mg/dL)			Mean ± SD (mg/dL)
	R_1	R_2	R_3	
Ligand **(L)**	48.43	46.51	47.52	47.49 ± 0.960
Cu complex	26.52	24.68	25.33	24.84 ± 0.933
Control	4.84	4.70	4.75	4.76 ± 0.071

Table 4 Effect of the tested compounds on AChE activity of *M. obstructa*

Compounds	AChE activity (UL^{-1})			Mean ± SD (UL^{-1})
	R_1	R_2	R_3	
Ligand **(L)**	4783.92	5173.82	4965.87	4974.54 ± 195.09
Cu complex	1395.96	1279.90	1331.54	1335.80 ± 58.15
Control	3369.96	3253.90	3307.83	3310.56 ± 58.08

Cu(II) chelate demonstrated a higher toxicity than the free ligand because of enzyme inactivation caused by the interaction between copper cations and enzymes. Enzyme function may also be disrupted by the competition of Cu(II) ions with other metal cations such as magnesium and zinc (Alnuaimi et al., 2012; El-Samanody et al., 2017b). Additionally, oxidative stress may be triggered by metal ions accumulated in the snail hepatopancreas. This trigger occurs through several pathways; including activation of heme and xanthine oxidases, inhibition of electron transport chain, the respiratory chain, and enzymatic reactions. These effects pose significant risks to organisms, potentially leading to detrimental outcomes and even fatality (Bici et al., 2023). Overall, our findings suggest that the copper construction displayed a greater promising molluscicidal influence compared to its free ligand **(L)**, underscoring the significance of metal complexation in enhancing toxicity against target species (El-Samanody et al., 2017a; Emara et al., 2023b).

Thiophene-2-carboxaldehyde and diaminodiphenylmethane (MDA) bear resemblance to carbamates in their chemical structures: all sharing nitrogen and sulfur atoms within their chemical skeletons. This structural similarity is reflected in their comparable toxicity profiles against land snails. The current findings align with our earlier research (Emara et al., 2022), which highlighted the significant in vitro and in vivo toxicity of the ligand derived from the condensation reaction between methomyl and (MDA), as well as its metal chelates, against *Eobania vermiculata* snails. Moreover, Youssef, (2006) emphasized that methomyl produced the highest mortality rates for *Theba pisana*, *M. obstructa*, and *E. vermiculata* species after three days of treatment. This efficacy was attributed to the efficient fermentation of bait components. Furthermore, Mobarak, (2014) stated that methomyl and tannic acid influenced the activity of ACP and ALP enzymes along with cholesterol concentration that responsible for

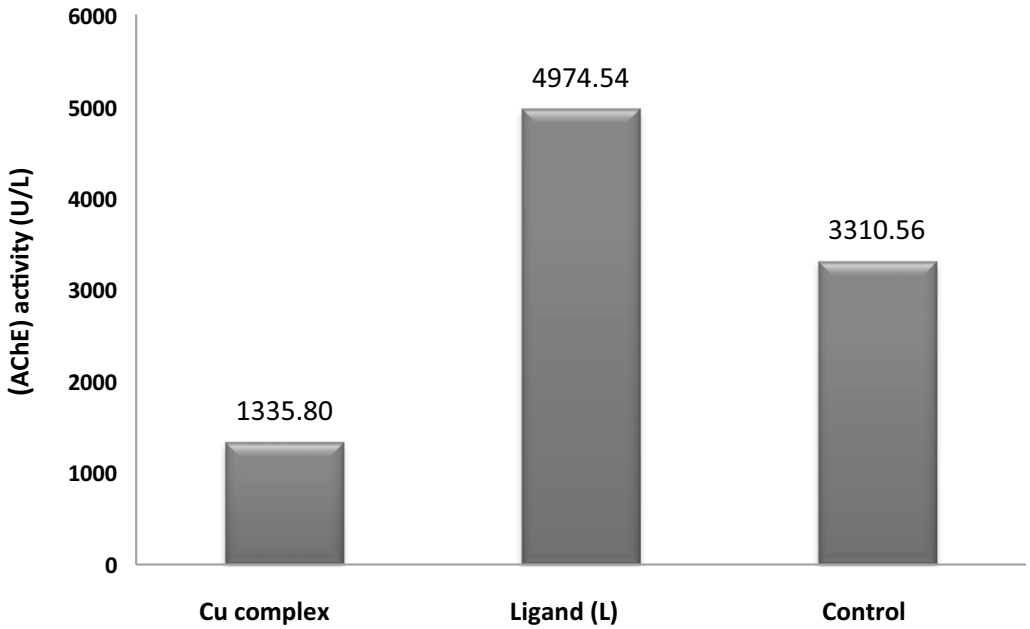


Fig. 4 Acetylcholinesterase (AChE) activity of treated *M. obstructa* snails

inhibiting the production of the shell of *E. vermiculata* and *M. obstructa* snails.

Biochemical assay

Total protein content

The observed fluctuations in total protein levels may stem from an imbalance between the synthesis and degradation rates (Emara et al., 2023a). Khater et al. (1990) suggested that the excess in total protein content could be attributed to heightened biosynthesis processes induced by elevated enzyme stress. The acquired findings align with those of Abd-El-All, (2004) who indicated that *E. vermiculata* snails treated with Niclosamide had elevated levels of total lipids and total proteins. Also, Sharaf et al. (2015) established that the levels of total protein and total lipid were increased after in vitro treatment of *Helicella vestalis* snail with Methiocarb and Chlorpyrifos.

Cholesterol concentration

Cholesterol, a lipid-waxy (fat-like) steroid, is a fundamental component of the cell membrane and is transported in the blood plasma of all animals (Mahan et al., 2012). It plays an important role in many body functions, serving as a precursor to certain hormones and aiding in the digestion of fats. In particular, a specialized form of cholesterol (7-dehydrocholesterol) that found in the skin can be converted into vitamin D upon exposure to sunlight. There are two primary sources of cholesterol; blood or serum cholesterol, primarily produced by the body, and dietary cholesterol, obtained from foods of animal origin (Mahan et al., 2012). Cholesterol is indispensable for maintaining proper membrane permeability and fluidity in mammalian cell membranes (Berry, 2004). While the body primarily synthesizes cholesterol, with a smaller contribution from diet, excessive levels of circulating cholesterol are strongly linked to the progression of atherosclerosis (Javiett, 1994). Atherosclerosis is characterized by the accumulation of cholesterol esters and other lipids in the connective tissues of arterial walls. Despite being the principal sterol synthesized by animals, small quantities of cholesterol are also produced by other eukaryotes such as plants and fungi. Cholesterol, being an amphipathic lipid, serves as an essential structural component of membranes. Cranton and Frackelton, (1984) highlighted additional roles of cholesterol, including its antioxidant properties.

The achieved results align with those reported by Beltagi et al., (2011) who observed that Thymol and Nicotine increased the levels of total protein, total lipid, and cholesterol in the hemolymph of *E. vermiculata* snails. In contrast, Kandil et al., (2014) reported findings that diverge from the observed increase in cholesterol levels. They found that the cholesterol level of *M. obstructa* responded differently to methomyl and a mixture of (abamectin + acetylsalicylic acid). Specifically, they noted a reduction in cholesterol levels to 201.5, 199.5, and 192.7 mg/dL for the first compound, and 207.2, 205.7, and 204.7 mg/dL for the second, after 1, 3, and 7 days of treatment, respectively, compared to 477.9 mg/dL for the control species. Notably, abamectin bioinsecticide exhibited a strong effect, reducing cholesterol levels from 477.9 mg/dL in the control to 0.476 mg/dl by the 7th day of treatment. Additionally, the combination of methomyl with acetylsalicylic acid resulted in even more severe reduction, lowering cholesterol to 0.0 after 3 days of treatment.

Acetylcholinesterase activity

Cholinesterase enzymes can provide crucial inhibition data, especially considering potential variations in substrates and inhibitor specificities among different species. Evidence suggests that acetylcholinesterase (AChE) possesses not only an anionic site in the catalytic center, but also peripheral anionic sites where ligands bind, exerting a regulatory role on enzyme activity (Eldefrawi, 1985). The ability of AChE to undergo ligand-induced conformational changes was initially proposed by Changeux, (1966) who observed that Gallamine inhibition of Torpedo marmorata AChE was not strictly competitive, a finding later confirmed by Moss and Henderson, (1998). This discovery led to the proposition that AChE harbors peripheral anionic sites, distinct from the catalytic site, where binding of cationic ligands could influence the enzyme's catalytic properties. The acetylcholinesterase (AChE) enzyme serves as a reliable biomarker across diverse aquatic and terrestrial organisms exposed to various pollutants, including insecticides. It remains a crucial component in biomonitoring programs aimed at assessing insecticide contamination. Acetylcholinesterase (AChE) plays a crucial role in degrading the neurotransmitter acetylcholine (ACh) within the synaptic cleft. AChE exhibits substrate preference by hydrolyzing ACh rather than other choline esters and shows substrate inhibition at high concentrations. There are two types of cholinesterase: AChE and butyrylcholinesterase (BuChE) (Yaqin, 2010). Sturm et al., (1999) highlighted the distinction between AChE and BuChE in terms of substrate and inhibitor binding. AChE has primarily been utilized as a biomarker in various organisms and is specifically classified as a biomarker of response to organophosphorus pesticide exposure (Walker et al., 2006). One overarching objective of this study was to investigate the potential impact of the tested chemical compounds on the AChE activity of *M. obstructa*, aiming to deepen our understanding of its neurotoxic effects. The importance of comprehending biomarker responses in land snails

had been emphasized (El-Shenawy et al., 2012). Putkome et al., (2008) stated that in the case of long exposure times and high concentrations, AChE is degraded, and tissue is altered. However, in the case of short exposure times and low concentrations, the organism is induced to synthesize more AChE than under normal conditions. Acetylcholinesterase (AChE) activity does not contribute to detoxification in vertebrates; instead, it is crucial in facilitating the transmission of nerve impulses throughout the body. The inhibition of this enzyme by various neurotoxic compounds results in an accumulation of the chemical messenger, acetylcholine, in the synaptic space. This accumulation sustains a continuous transmission of nerve impulses, ultimately leading to the demise of the individual (Smina et al., 2016).

Conclusion

The Schiff base ligand, bis-[4-benzylidene-thiophene-2′-yl] methane **(L)**, and its copper construction were prepared and evaluated for their in vitro molluscicidal activity against adult individuals of the glassy clover snail, *M. obstructa*. The findings indicated that the copper complex demonstrated a markedly higher molluscicidal activity compared to the free ligand **(L)**. Additionally, some critical biochemical indicators; including acetylcholinesterase (AChE), cholesterol, and total protein levels were measured. The acquired results confirmed the significant impact of the tested compounds on the assessed biochemical parameters of treated snails. In conclusion, the tested chemical compounds exhibited a notable molluscicidal activity against the land snails under investigation. However, further investigations are necessary to determine the most suitable application technique and mode of action for using these compounds to control *M. obstructa* species. Additionally, it is important to assess their impact on animals and humans' health.

Abbreviations

(L)	Bis-[4-benzylidene-thiophene-2′-yl] methane
AChE	Acetylcholinesterase
ANOVA	Analysis of variance method
LSD	Least significant difference
DMF	Dimethylformamide

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Author contributions

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

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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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References

Abd-El-All, S. M. (2004). Toxicity and biochemical response of *Eobania vermiculata* land snail to Niclosamide molluscicide under laboratory and field conditions. *Journal of Plant Protection and Pathology, 29*, 4751–4756.

AbouEl-Enein, S. A. (2008). Polymeric and sandwich Schiff bases complexes derived from 4,4′-methylenedianiline: Characterization and thermal investigation. *Journal of Thermal Analysis and Calorimetry, 91*(3), 929–936.

Alnuaimi, M. M., Saeed, I. A., & Ashraf, S. S. (2012). Effect of various heavy metals on the enzymatic activity of *E. coli* alkaline phosphatase. *International Journal of Biotechnology and Biochemistry, 8*, 47–59.

Beltagi, S. M., El-Shennawy, M. S., Elkatan, N. A., & Yousef, H. N. (2011). Physiological changes in the brown garden snail, *Eobania vermiculata* induced by sub-lethal doses of two botanical molluscicides. *Journal of Egyptian German Society of Zoology Comparative Physiology, 63A*, 375–397.

Berry, S. (2004). Lipid analysis: Isolation, separation, identification and structural analysis of lipids. *Nutrition Bulletin, 29*, 72–73.

Bici, M., Mazreku, I., Hyseni, B., Halili, J., Krasniqi, Y., & Bislimi, K. (2023). Effect of lead, nickel, and zinc pollution in some parameters of oxidative stress in hepatopancreas of snail *Helix pomatia L*. in power plant of Obiliq. *Journal of Ecological Engineering, 24*(9), 385–395.

Burtis-Ashwood. (1994). Tietz textbook of clinical chemistry. *European Journal of Clinical Chemistry and Clinical Biochemistry, 30*, 162–170.

Changeux, J. P. (1966). Responses of acetylcholinesterase from *Torpedo marmorata* to salts and curarizing drugs. *Molecular Pharmacology, 2*, 369–392.

Cranton, E. M., & Frackelton, J. P. (1984). Free radical pathology in age-associated diseases: Treatment with EDTA chelation, nutrition and antioxidants. *Journal of Holistic Medicine, 6*(1), 6–37.

Dua, H., Wang, J., Watzl, J., Zhang, X., & Hua, Z. (2008). Classification structure–activity relationship (CSAR) studies for prediction of genotoxicity of thiophene derivatives. *Toxicology Letters, 177*, 10–19.

Eldefrawi, A. T. (1985). Acetylcholinesterase and anticholinesterases. In G. A. Kekut & L. I. Gilbert (Eds.), *Comprehensive insect physiology, biochemistry and pharmacology: Insect control* (pp. 115–130). Pergamon.

El-Halim, S. M. A., Ali, I. H. H., El-Sayed, S. A. M., & Ali, R. F. (2021). Preliminary study on survey and population dynamic of the terrestrial snail *Monacha obstructa* (Pfeiffer) (Hygromiidae, Mollusca) at crop fields in Fayoum Governorate, Egypt. *Pakistan Journal of Biological Sciences, 24*, 928–938.

Ellefson, R. D., & Caraway, W. T. (1976). *Fundamentals of clinical chemistry*. Ed Tietz NW, p. 506.

El-Samanody, E. A., Emam, S. M., & Emara, E. M. (2017a). Synthesis, characterization, molecular modeling and biological activity of metal complexes derived from (E)-N′-(furan-2-ylmethylene) morpholine-4-carbothiohydrazide. *Journal of Molecular Structure, 1146*, 868–880.

El-Samanody, E. A., Polis, M. W., & Emara, E. M. (2017b). Spectral studies, thermal investigation and biological activity of some metal complexes derived from (E)-N′-(1-(4-aminophenyl) ethylidene) morpholine-4-carbothiohydrazide. *Journal of Molecular Structure, 1144*, 300–312.

El-Shenawy, N. S., Mohammadden, A., & Hessenan Al-Fahmie, Z. (2012). Using the enzymatic and non-enzymatic antioxidant defense system of the land snail *Eobania vermiculata* as biomarkers of terrestrial heavy metal pollution. *Ecotoxicology and Environmental Safety, 84*, 347–354.

Emara, E. M. (2024a). In vitro comparative study: Toxicity of *Calotropis procera*, *Nerium oleander* extracts, methomyl, and their mixtures on *Monacha obstructa*. *Menoufia Journal of Plant Protection, 9*(5), 199–209.

Emara, E. M., El-Sawaf, M. A., & Khalifa, R. F. (2022). The toxic effect of some newly constructed diaminodiphenylmethane derivatives applied in vitro and in vivo trials against *Theba pisana* in relation to mucus secretion. *Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control, 14*(1), 283–293.

Emara, E. M., El-Sawaf, M. A., & Khalifa, R. F. (2023a). Biochemical and toxicity effects of some 2-benzoylpyridine derivatives on *Eobania vermiculata* under laboratory conditions. *Egyptian Academic Journal of Biological Sciences (B Zoology), 15*(2), 35–42.

Emara, E. M., El-Sawaf, M. A., & Khalifa, R. F. (2023b). Laboratory and field toxicity evaluation of some methomyl derivatives against *Eobania vermiculata* infesting citrus trees. *Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control, 15*(2), 33–41.

Emara, E. M., El-Sawaf, M. A., & Khalifa, R. F. (2024b). Liver and mucous secretion enzymatic biomarkers of *Eobania vermiculata* treated with some newly synthesized acrylamide derivatives. *The Journal of Basic and Applied Zoology, 85*, 1–10.

Finney, D. J. (1971). *Probit analysis* (3rd ed., p. 318). Cambridge University Press.

Henry, R. J. (1964). *Clinical chemistry* (p. 181). Harper and Row Publishers.

Javiett, N. B. (1994). Bile acid synthesis from cholesterol: Regulatory and auxiliary pathways. *The FASEB Journal, 8*, 1308–1311.

Kandil, M. A., EL-Deeb, H. I., Eweis, E. A., Gabr, W. M., & Mobarak, S. A. (2014). Effect of acetylsalicylic acid on the physiological role of mucus gland of land snail species. *Egyptian Journal of Agricultural Research, 92*(1), 53–73.

Keri, R. S., Chand, K., Budagumpi, S., Somappa, S. B., Patil, S. A., & Nagaraja, B. M. (2017). An overview of benzo[b]thiophene-based medicinal chemistry. *European Journal of Medicinal Chemistry, 138*, 1002–1033.

Khater, A. A., El-Sheakh, A. A., El-Sheamy, M. K., & Hussein, M. Z. (1990). Biochemical effects of Lannate and Larvin on Tilapia nilotica fingerlings. *Egyptian Journal of Applied Sciences, 5*(8), 227–235.

Machinist, J. M., Mayer, M. D., Shet, M. S., Ferrero, J. L., & Rodriguez, A. D. (1995). Identification of the human liver cytochrome P-450 enzymes involved in the metabolism of zileuton (ABT-077) and its *N*-dehydroxylated metabolite, ABBOTT-66193. *Drug Metabolism and Disposition, 23*, 1163–1174.

Mahan, L. K., Escott-Stump, S., Raymond, J. L., & Krause, M. V. (2012). *Krause's food & the nutrition care process*. Elsevier/Saunders.

Misra, B., & Amin, S. (1990). Synthesis and mutagenicity of trans-dihydrodiol metabolites of benzo[b]naphtho[2,1-d]thiophene. *Chemical Research in Toxicology, 3*, 93–97.

Mizutani, T., Yoshida, K., & Kawazoe, S. (1994). Formation of toxic metabolites from thiabendazole and other thiazoles in mice: Identification of thioamides as ring-cleavage products. *Drug Metabolism and Disposition, 22*, 750–755.

Mobarak, S. A. (2014). Biochemical impacts of various compounds on the two land snails species, *Eobania vermiculata* and *Monacha obstructa*. *Egyptian Journal of Agricultural Sciences, 65*(1), 125–132.

Moss, D. W., & Henderson, A. R. (1998). Enzymes. In C. A. Burtis & E. R. Ashwood (Eds.), *Tietz Fundamentos de Química Clínica* (4th ed., pp. 275–325). Guanabara Koogan.

Mourad, A. A. (2014). Molluscicidal effect of some plant extracts against two land snail species, *Monacha obstructa* and *Eobania vermiculata*. *Egyptian Academic Journal of Biological Science, 6*, 11–16.

Putkome, S., Cheevaporn, V., & Helander, H. F. (2008). Inhibition of acetylcholinesterase activity in the golden apple snail (*Pomacea canaliculata Lamarck*) exposed to chlorpyrifos, dichlorvos or carbaryl insecticides. *Environment Asia, 2*, 15–20.

Rady, G. H. (2019). Survey and population density of land snails under different conditions at Qalubia and Sharkia governorates. *Annals of Agricultural Science, Moshtohor, 57*, 755–760.

Sharaf, H. M., Salama, M. A., & Abd El-Atti, M. S. (2015). Biochemical and histological alterations in the digestive gland of the land snail *Helicella vestalis* (Locard, 1882) exposed to Methiocarb and Chlorpyrifos in the laboratory. *Journal of Cytology and Histology, 6*, 327–332.

Sinsheimer, J. E., Hooberman, B. H., Das, S. K., Savla, P. M., & Ashe, A. J. I. (1992). Genotoxicity of chryseno[4,5-*bcd*]-thiophene and its sulfone derivative. *Environmental and Molecular Mutagenesis, 19*, 259–264.

Smina, A. H., Samira, B., Mohamed, D., & Houria, B. (2016). Evaluation of acetylcholinesterase, glutathione S-transferase and catalase activities in the land snail *Helix aspersa* exposed to thiamethoxam. *Journal of Entomology and Zoology Studies, 4*(5), 369–374.

Steel, R. G. D., & Torrie, J. H. (1981). *Principle and procedure of statistics. A biometrical approach* (2nd ed.). McGraw-Hill Book Company.

Sturm, A., da Silva de Assis, H., & Hansen, P. D. (1999). Cholinesterases of marine teleost fish: Enzymological characterization and potential use in the monitoring of neurotoxic contamination. *Marine Environmental Research, 47*, 389–398.

Walker, C. H., Hopkin, S. P., Sibley, R. M., & Peakall, D. B. (2006). *Principles of ecotoxicology* (p. 296). Taylor & Francis.

Yaqin, K. (2010). Potential use of cholinesterase activity from tropical green mussel, *Perna viridis* as a biomarker in effect-based marine monitoring in Indonesia. *Coastal Marine Science, 43*, 156–164.

Youssef, H. M. (2006). Molluscicidal and biochemical activity of some crude plant extracts against three types of land snails. *Journal of Plant Protection and Pathology, 31*(10), 6735–6742.

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