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Investigation of the effects of some pesticides on carbonic anhydrase isoenzymes

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Abstract

The aim of this study was to investigate the inhibitory effects of some pesticides known to have harmful effects on human health on carbonic anhydrase isoenzymes. Therefore, carbonic anhydrase isoenzymes (hCA I and II) were purified from human erythrocytes. The isoenzymes were purified from human erythrocytes by using an affinity column that has the chemical structure of Sepharose-4B-4-(6-amino-hexyloxy)-benzenesulfonamide. The purity of the isoenzymes was checked by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDSPAGE). It was determined that the pesticides used in this study inhibit hCA I and hCA II isoenzymes at different levels in vitro. It was determined that the strongest inhibitor for the hCA I enzyme was Carbofuran (IC_{50}:6.52 μM; K_i: 3.58 μM) and the weakest one was 1-Naphtol (IC_{50}:16.55 μM; K_i: 14.4 μM) among these pesticides. It was also found that the strongest inhibitor for the hCA II enzyme was coumatetralil (IC_{50}:5.06 μM; K_i: 1.62 μM) and the weakest one was Dimethachlor (IC_{50} 14.6 μM; K_i: 8.44 μM).

KEYWORDS
carbonic anhydrase, enzyme inhibition, enzyme purification, pesticide

1 | INTRODUCTION

Pesticides are widely used in agriculture to satisfy a need for products due to the increase in the world population. They have both direct and indirect negative effects on environmental pollution and human health.[1–4] Agricultural workers are exposed to pesticides through direct skin contact, while other populations are exposed indirectly through the air, water, food, and dust, and this causes serious threats to human health such as diabetes, reproductive disorders, neurological dysfunction, cancer, and respiratory disorders.[5] Pesticides are classified according to their chemical structures (carbamates, organophosphates, organochlorines, etc.) and areas of use (herbicides, fungicides, insecticides, etc.).[6] Carbaryl, propoxur, carbofuran, and 1-naphthol are used as insecticides and belong to the carbamate class, which breaks down in the environment within weeks or months.[7] Pesticides belonging to the group of organophosphates contain phosphorus atoms in their active site. The phosphorus atom influences the hydrolysis and oxidation reactions during the degradation of pesticides. Chlorpyrifos, chlorpyrifos-methyl, and azinphos-ethyl belong to this group and are used as insecticides.[8–10] Organochlorines are carcinogenic and neurotoxic, have high persistence in the environment, and contain chlorine atoms. Dichlofluanid and dimethachlor are pesticides that belong to this group and are generally used as fungicides.[11] Tebucanazole is used as a fungicide in agriculture, it protects plants from diseases and prevents their spread.[12] Alachlor is used as a herbicide and causes cancer by activating tumor cells. Simazine keeps broadleaf grasses used as herbicides in agricultural fields under control by inhibiting them via the electron transport system.[13,14] Amitraz is an insecticide that causes the death of insects by reducing the activity of the central nervous system. At the same time, it is used in agricultural areas as a herbicide because it keeps the development of various plants under control. Dazomet is a pesticide from the group of fumigants, and it is used to prevent molds, fungi, and bacteria that damage soil structure. Coumatetralil is a pesticide that causes secondary poisoning of rodent and mouse species.[15,16]

Pesticides show their effects by inhibiting or activating the functions of many enzymes. Although pesticides used in agriculture to increase yields have positive effects on crops, their residues have

negative effects on the environment and human health. Therefore, the aim of this study was to determine the inhibition behavior of pesticides on CA isoenzymes purified from human blood erythrocytes.

Carbonic anhydrase (EC 4.2.1.1) is a metalloenzyme that occurs in many tissues of living organisms and has Zn^{+2} ion in its active site.[17,18] The most important task of this enzyme is to ensure that CO_2 formed in the cell is transported to other cells or excreted so that living cells can survive.[19–21] Since its other task is the transport of CO_2, it provides the accumulation of H^+ and HCO_3^- in tissues (kidney, eye lens, erythrocytes, brain). The resulting H_2CO_3 is rapidly converted to HCO_3^- and H^+ ions by the equilibrium reaction of the enzyme carbonic anhydrase. At the same time, H_2CO_3 forms a buffer between blood and cells.[22–24] There are 16 isozymes of the enzyme carbonic anhydrase in living organisms. Five are cytoplasmic (CA I, II, III, VII, and XIII), two are mitochondrial (CA, VA, VB), one is secretory (CA VI), four are membrane-bound (CA IV, IX, XII, and XIV), and three are noncatalytic (CA VIII, X, and XI).[25–27] hCA I is an isoenzyme found in human erythrocyte cells and is involved in the respiratory process. The hCA II isozyme is a very important enzyme for bone, brain, and kidney tissues. The isozyme hCA II ensures the reabsorption of Na^+ and water in the renal cortex; therefore, deficiency of this enzyme results in kidney stones and calcifications in the bones and brain. The enzyme hCA II is found in most cells, while hCA I is specific to erythrocytes.[28–30]

In this study, the CA (CA I, II) isoenzymes were purified from human erythrocytes in one step by affinity chromatography, and the inhibitory effects of some pesticides (Carbaryl, propoxur, carbofuran, 1-naphthol, chlorpyrifos, chlorpyrifos-methyl, azinphos-ethyl, dichlofluanid, dimethaclor, tebucanazole, alachlor, simazine, amitraz, dazomet, and coumatetralil) on these isoenzymes were investigated.

2 | EXPERIMENTAL

2.1 | Chemicals

The materials used in this study, including pesticides, Sepharose-4B, CNBr, protein assay reagents, and chemicals for electrophoresis, were supplied by Sigma Chemical Co. All of the other chemical materials used were of analytical grade. The study was approved by the local Institutional Ethics Committee, and informed consent was obtained from all participants.

2.2 | Purification of carbonic anhydrase isozymes from human erythrocytes by affinity chromatography and preparation of haemolysate

Blood samples (20 mL) were collected from healthy volunteers. First, blood samples were centrifuged at 1000× *g* for 20 min at +4°C, plasma and buffy coat were separated. Afterward, washed twice with 0.9% NaCl and hemolyzed three times with ice-cold water. The hemolysate was centrifuged again at 3000× *g* for 40 min at +4°C, and the pH was adjusted to 8.7 with a solid Tris base. The hemolysate was added to the affinity column containing Sepharose-4B-4-(6-amino-hexyloxy)-benzenesulfonamide, and the CA isoenzymes (hCA I and II) were eluted with 0.1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6), respectively.

2.3 | Protein determination

Quantitative protein determination was performed at 595 nm by the Bradford method.[31] Bovine serum albumin was used as a standard, and a purification table was prepared.

2.4 | SDS-PAGE

After purification by the enzyme affinity chromatography method, two different acrylamide concentrations were prepared: Stacking gel 3% and Separation gel 10%. SDS-PAGE was performed by the Laemmli method, and the purity of the enzyme was checked.[32]

2.5 | CA esterase activity assay

CA enzyme activity was measured by the method developed by Verpoorte et al.. According to this method, hydrolysis of 4-nitrophenyl acetate (NPA) to 4-nitrophenylate ion was performed by measuring for 3 min at 25°C using a spectrophotometer at 348 nm.[33] 4-nitrophenyl acetate solution was prepared fresh daily. The enzymatic reaction, with a total volume of 3000 µL, contained 800 µL of 0.05 M Tris-SO_4, 700 µL of 4-nitrophenylacetate, 400 µL of H_2O, and 100 µL of enzyme solution. A blank was obtained by preparing the same cuvette without enzyme solution. All pesticides were prepared at a concentration of 0.01 M and dissolved in dimethyl sulfoxide (DMSO). The impact of DMSO on the CA isoenzymes was assessed, and its effects were subtracted from all of the results.

2.6 | In vitro inhibition studies

For the inhibitory effects on the fifteen different pesticides, hCA I and hCA II, the activities of the enzymes were tested at least five different concentrations. The activity measured in the absence of pesticide was accepted as control and 100% activity. Activities were calculated and Activity%-[Inhibitor] plotted, and IC_{50} values were calculated from these exponential curve equations. For all pesticides, activity was calculated by measuring activity at three different inhibitor concentrations and five different substrate concentrations. K_i values and inhibition types of pesticides were determined by Lineweaver-Burk graphs.[34]

3 | RESULT AND DISCUSSION

In this study, the effects of some pesticides (Table 1, propoxur, alachlor, 1-naphthol, chlorpyrifos, simazine, dichlofluanid, chlorpyrifos-

TABLE 1 Structures of pesticides used in this study.

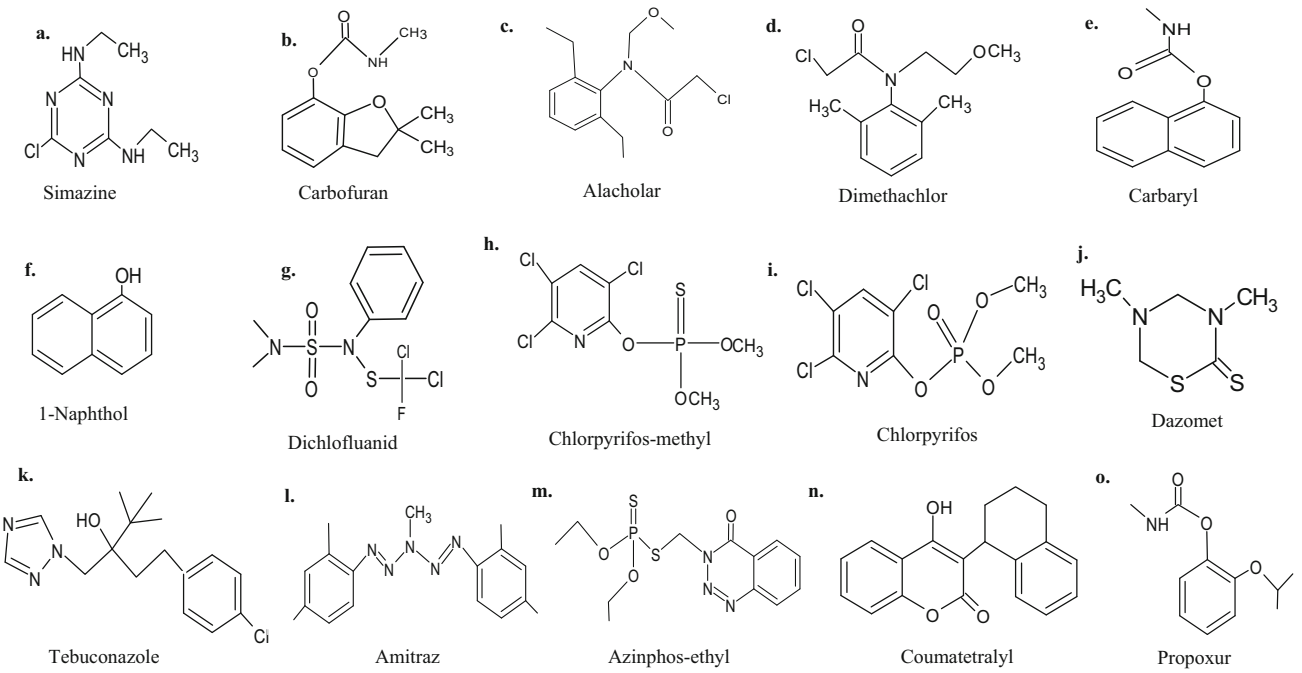

TABLE 2 Purification table of CA isozymes.

Purification step	Total volume (mL)	Activity (EU/mL)	Protein (mg/mL)	Total protein (mg)	Total activity (EU)	Specific activity (EU/mg)	Purificitaion fold	Yield %
Hemolysate	20	110	14.82	296.40	2200	7.42	–	–
hCA-I	10	156	0.22	2.20	1560	709.09	95.53	70.91
hCA-II	8	144	0.09	0.72	1152	1600	215.56	52.36

methyl, azinphos-ethyl, dimethachlor, tebuconazole, amitraz, dazomet, coumatetralil, and carbofuran, carbaryl) on CA isoenzymes were investigated. The reason for choosing these pesticides is that they reach humans through the food chain due to their uncontrolled nature. The enzyme CA was preferred in this study because it has an extremely important function in the construction of living organisms. The reaction of CA with CO_2 produces H^+ and HCO_3^- ions. This reaction occurs in many tissues, such as kidney, gastric mucosa, and lens of the eye.[35–37]

In this study, two CA isozymes were purified in a single step by Sepharose-4B-4-(6-amino-hexyloxy)-benzenesulfonamide affinity column chromatography. hCA I was purified 95.53-fold with a specific activity of 709.09 EU/mg and an overall yield of 70.91%, and hCA II was purified 215.56-fold with a specific activity of 1600 EU/mg and an overall yield of 52.36% (Table 2). CA isoenzymes were purified from human blood erythrocytes by affinity chromatography method. The purity of these isoenzymes was checked by applying SDS-PAGE, and a single band was observed. The molecular weights were found to be approximately 30 kDa, and this result was found to be in compliance with the literature.[38]

Esterase measurements were performed at five different suitable inhibitor concentrations for the hCA I and hCA II isoenzymes purified from human blood erythrocytes. IC_{50} values were determined by plotting % activity-[I] graphs of pesticides with inhibitory effects. K_i values and inhibition types of pesticides were determined by Lineweaver–Burk graphs. It was determined that the pesticides used in our study significantly inhibited the isoenzymes hCA I and II. When the effects of the pesticides on hCA I were examined, it was found that carbofuran showed the strongest inhibition (IC_{50}:6.52 μM, K_i: 3.58 μM), while 1-naphthol showed the weakest inhibition (IC_{50}:16.55 μM, K_i: 14.4 μM). There is no study in the literature regarding the effect of carbofuran pesticide on esterase activity of hCA I, but its effects on other enzymes have been reported. When the effects of the pesticides on hCA II were examined, it was found that Coumatetralyl showed the strongest inhibition (IC_{50}:5.06 μM, K_i: 1.620 μM), while dimethachlor showed the weakest inhibition (IC_{50}:14.6 μM, K_i: 8.44 μM).

Soydan et al. purified the enzyme carbonic anhydrase from honey bee (*Apis mellifera*) and found the inhibitory effect of Carbofuran pesticide on this enzyme (IC_{50}:0.0087 μM).[39] In another study, Assis et al. purified the Acetylcholinesterase enzyme from *Crassostrea rhizophora* gills and found the inhibitory effect of Carbofuran pesticide on this enzyme (IC_{50}: 0.17 mM), and in another study of the same group, carbonic anhydrase enzyme was purified from the gills of rainbow trout (*Oncorhynchus mykiss*) and the inhibitory effect of Carbofuran pesticide on this enzyme was found (IC_{50}:000.4 μM)[40,41] Table 3.

TABLE 3 Inhibition constants for pesticides.

Pesticides	IC_{50} (μM) (Esterase) hCA-I	IC_{50} (μM) (Esterase) hCA-II	K_i (μM) hCA-I	K_i (μM) hCA-II	Inhibition type
Propoxur	13.85	13.520	7.970	6.400	Competitive
Alacholar	16.320	9.060	12.470	4.050	Uncompetitive
1-Naphthol	16.550	10.200	14.400	5.190	Uncompetitive
Chlorpyrifos	15.400	12.750	10.750	5.900	Noncopetitive
Simazine	14.590	13.900	8.660	7.260	Competitive
Dichlofluanid	13.000	8.010	5.560	3.090	Noncopetitive
Chlorpyrifos-methyl	12.830	9.550	5.480	4.100	Uncompetitive
Azinphos-ethyl	13.980	10.950	8.320	5.680	Noncopetitive
Dimethachlor	13.700	14.600	7.530	8.440	Uncompetitive
Tebuconazole	13.300	9.850	7.350	4.500	Competitive
Amitraz	7.400	5.270	4.880	1.740	Uncompetitive
Dazomet	8.450	6.350	4.990	2.490	Noncopetitive
Coumatetralyl	6.550	5.060	3.660	1.620	Uncompetitive
Carbofuran	6.520	6.300	3.580	2.420	Uncompetitive
Carbaryl	6.820	5.350	3.800	1.920	Uncompetitive

In yet another study, Şentürk et al. purified the acetylcholinesterase enzyme and found the inhibitory effect of Carbofuran pesticide on this enzyme (IC_{50}:0.544 μM).[39] In another study, Shukor et al. purified acetylcholinesterase enzyme from walking catfish (*Clarias batrachus*), electric eel (*Electrophorus electricus*), and cattle (*Bos taurus*) and found the inhibitory effect of Carbofuran pesticide on these enzymes. (IC_{50}:6.66 μg/L, IC_{50}:6.20 μg/L, IC_{50}:20.94 μg/L, respectively).[42] Hiroshi et al. purified the Uridine Diphosphate Glucuronosyltransferase 1–6 enzyme from human liver and found the inhibition effect of 1-Naphthol pesticide on this enzyme (IC_{50}:28 μM).[43] When the effects of pesticides on hCA II were examined, it was found that Coumatetralil showed the strongest inhibition with an IC_{50} value of 5.06 μM, whereas dimethachlor had the weakest inhibition with an IC_{50} value of 14.6 μM. No study has been found in the literature regarding the effects of coumatetralil and dimethachlor on any enzyme. As the result of K_i constant; pesticides with competitive inhibitory properties are propoxur, tebuconazole, and dazomet. And the uncompetitive inhibitors are alachlor, 1-naphthol, dichlofluanid, amitraz, koumatetralil, carbofuran, and carbaryl. Pesticides with noncompetitive inhibitory properties are chlorpyrifos, simazine, chlorpyrifos-methyl, azinphos-ethyl, and dimetachlor.

The effect of the pesticides used in our study on the human enzyme carbonic anhydrase has not been previously investigated in the literature by esterase activity assays. However, some researchers have reported effects on different types of carbonic anhydrase enzymes or other enzymes. For example, Ceyhun et al. purified the enzyme carbonic anhydrase from the gills of *O. mykiss* and found the inhibitory effect of the pesticide propoxur on this enzyme with esterase activity (IC_{50}:0.420 μM).[44] Şentürk et al. purified glucose-6-phosphate enzyme from the gills of rainbow trout (*O. mykiss*) and found the inhibitory effect of propoxur pesticide on this enzyme (IC_{50}:12 μM).[39] In another study by the same group, the carbonic anhydrase was purified from honey bee (*A. mellifera*), and inhibitory effect of propoxur (IC_{50}:0.0321 μM), carbaryl (IC_{50}:0.0031 μM), and tebuconazole (IC_{50}:0.0030 μM) on this enzyme were found.[39] Holovska et al. purified the proliferative enzyme from Madin–Darby bovine kidney, rabbit kidney, pig kidney, and embryonic bovine lung cells and found the inhibitory effect of dichlofluanid pesticide on this enzyme (IC_{50}:0.134 mM; IC_{50}:2754 mM; IC_{50}:0.309 mM; IC_{50}:0.147 mM, respectively).[45] Çelik et al. purified the carbonic anhydrase from human blood and bovine blood and found the inhibitory effect of the pesticide chlorpyrifos-methyl on this enzyme with hydratase activity (IC_{50}:3.7 $\times$ 10^{-2} M, IC_{50}:63 mM, respectively).[46] Toni et al. purified the acetylcholinesterase from koi carp (*Cyprinus carpio*) and found the inhibitory effect of the pesticides chlorpyrifos-methyl, azinphos-ethyl, and amitraz on this enzyme (IC_{50}:4.45 μM; IC_{50}:4.46 μM; IC_{50}:25.77 mM, respectively).[47] Assis et al. purified acetylcholinesterase enzyme from *C. rhizophora* gills and internal organs and found the inhibitory effect of Carbaryl pesticide on this enzyme (IC_{50}:0.00013 mM; IC_{50}:0.0035 mM, respectively).[48] Shukor et al. purified acetylcholinesterase enzyme from Walking catfish (*C. batrachus*), electric eel (*E. electricus*), and cattle (*B. taurus*) and found the inhibitory effect of Carbaryl pesticide on this enzyme (IC_{50}:130.00 μg/L; IC_{50}:133.01 μg/L; IC_{50}:418.80 μg/L, respectively).[49] Anderson et al. purified acetylcholinesterase enzyme from housefly, American dog tick, American cockroach, and yellow fever mosquito and found the inhibitory effect of carbaryl pesticide on this enzyme (IC_{50}:1.2 μM; IC_{50}:1.8 μM; IC_{50}:0.4 μM; IC_{50}:1.2 μM, respectively).[50] Cochon et al. purified cholinesterase enzyme from black wolf (*Lumbriculus variegatus*) and freshwater snail (*Biomphalaria glabrata*) and found the inhibitory effect of Carbaryl pesticide on this enzyme (IC_{50}:0.10 mg/L; IC_{50}:1.77 mg/L, respectively).[51] Şentürk et al. purified the acetylcholinesterase enzyme and found the inhibitory effect of Carbaryl pesticide on this enzyme (IC_{50}:11.27 μM).[39]

In another study, some pesticides like nuarimol, fenarimol, and 2,4-D exhibited 50% inhibition of the enzymatic activity of goat

erythrocyte carbonic anhydrase (CA) even at the lowest concentrations.[52] In addition, no study has been found in the literature on the effects of Alachlor, dimethachlor, dazomet, or coumatetralil pesticides on any enzyme.

Carbamate derivatives are extensively used in the chemical industry for the production of agrochemicals, such as pesticides, fungicides, and herbicides. It was reported that carbamates can be substrates of Cas, which may hydrolyse their ester/thioester bonds with the formation of small molecules that can bind to the metal center. The carbamates and organophosphates investigated here possess ester bonds, which can be hydrolysed by the esterase activity of the a-CAs. In fact, it has been thoroughly documented that these enzymes are esterases/thioesterases/selenoestearses with carboxylic, phosphoric, thiocarboxylic, and even selenol esters.[53] It was reported that carbamates bind to the CA active site through their deprotonate carbamate nitrogen, which coordinates the catalytic metal ion. In this study, X-ray crystallography of the CA–carbamate interaction showed the direct binding of these compounds to a metal ion.[54]

4 | CONCLUSIONS

In conclusion, CA isoenzymes were purified from human blood erythrocytes, and we have investigated the inhibitory effects of some pesticides on CA isoenzymes. Within this group of pesticides, the strongest inhibitor for CA I was Carbofuran (IC_{50}:6.52 μM; K_i: 3.58 μM), while the strongest inhibitor for CA II was Coumatetralil (IC_{50}:5.06 μM; K_i: 1.62 μM). While pesticides offer an economical and efficient solution for agriculture, their use must be regulated due to their detrimental impact on environmental health.

AUTHOR CONTRIBUTIONS

Aybike Baltacı: Enzyme purification and inhibition studies **Kubra Cıkrıkcı:** Enzyme purification studies and article writing **Nahit Gençer:** Design the work, Evaluation of results, Writing-Reviewing and Editing.

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CONFLICT OF INTEREST STATEMENT

The data that supports the findings are reported in this manuscript and we are not planning to commercialization of research findings reported in this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings will be available in [repository name] at [DOI/URL] following an embargo from the date of publication to allow for commercialization of research findings.

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REFERENCES

1. Vioque-Fernandez A, de Almeida EA, Lopez-Barea J. Biochemical and proteomic effects in *Procambarus clarkii* after chlorpyrifos or carbaryl exposure under sublethal conditions. *Biomarkers*. 2009;14(5):299-310.
2. Koksal Z, Kalin R, Gulcin I, Ozdemir H. Inhibitory effects of selected pesticides on peroxidases purified by affinity chromatography. *Int J Food Prop*. 2018;21(1):385-394.
3. Caglayan C, Taslimi P, Türk C, Kandemir FM, Demir Y, Gulcin I. Purification and characterization of the carbonic anhydrase enzyme from horse mackerel (*Trachurus trachurus*) muscle and the impact of some metal ions and pesticides on enzyme activity. *Comp Biochem Physiol Part C*. 2019;226:108605.
4. Caglayan C, Taslimi P, Türk C, et al. Inhibition effects of some pesticides and heavy metals on carbonic anhydrase enzyme activity purified from horse mackerel (*Trachurus trachurus*) gill tissues. *Environ Sci Pollut Res*. 2020;27:10607-10616.
5. Rani L, Thapa K, Kanojia N, et al. An extensive review on the consequences of chemical pesticides on human health and environment. *J Clean Prod*. 2021;283:124657.
6. Jayaraj R, Megha P, Sreedev P. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdiscip Toxicol*. 2016;9(3–4):90-100.
7. Hacking I. Covariance structure analysis title on health-related indicators in home elderly people focusing on subjective health. 1991;43(10):8509-8515.
8. Tomlin C. *"The pesticides manual; A world compendium"; incorporating the Aquachemicals handbook*. British Crop Production Council; 1994:1341.
9. Bandara Gawarammana I, Roberts DM, Mohamed F, et al. Acute human self-poisoning with bispyribac-containing herbicide Nominee®: a prospective observational study. *Clin Toxicol*. 2010;48(3):198-202.
10. World Health Organization. *Promotion of Chemical Safety Unit & Food and Agriculture Organization of the United Nations*. WHO/FAO Data Sheet On Pesticides, No.72, Azinphos-Ethyl. World Health Organization; 1994.
11. Zhou X, Cao S, Li X, et al. Rapid determination of dichlofluanid residues in vegetables using dispersive-SPE sample preparation combined with gas chromatography–mass spectrometry. *J Chromatogr Sci*. 2016;54(5):858-863.
12. Muñoz-Leoz B, Ruiz-Romera E, Antigüedad I, Garbisu C. Tebuconazole application decreases soil microbial biomass and activity. *Soil Biol Biochem*. 2011;43(10):2176-2183.
13. Curren MS, King JW. Solubility of triazine pesticides in pure and modified subcritical water. *Anal Chem*. 2001;73(4):740-745.
14. Heydens WF, Wilson AG, Kier LD, Lau H, Thake DC, Martens MA. An evaluation of the carcinogenic potential of the herbicide Alachlor4 to man. *Hum Exp Toxicol*. 1999;18(6):363-391.
15. Picó Y, La Farre M, Tokman N, Barceló D. Rapid and sensitive ultra-high-pressure liquid chromatography–quadrupole time-of-flight mass spectrometry for the quantification of amitraz and identification of its degradation products in fruits. *J Chromatogr A*. 2008;1203(1):36-46.
16. Ahmed FE. Analyses of pesticides and their metabolites in foods and drinks. *TrAC Trends in Analytical Chemistry*. 2001;20(1):649-661.
17. Kumbhar D, Patil R, Patil D, et al. Target oriented selective synthesis of antibacterial active tyrosinase enzyme inhibitor coumarin Core derivatives. *Asian J Res Chem*. 2015;8(8):511-520.
18. Beydemir SÜ, Gülçin İ. Effects of melatonin on carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo. *J Enzyme Inhib Med Chem*. 2004;19(2):193-197.
19. Supuran CT, Scozzafava A, Casini A. Carbonic anhydrase inhibitors. *Med Res Rev*. 2003;23(2):146-189.
20. Boztas M, Cetinkaya Y, Topal M, et al. Synthesis and carbonic anhydrase isoenzymes I, II, IX, and XII inhibitory effects of

dimethoxybromophenol derivatives incorporating cyclopropane moieties. *J Med Chem*. 2015;58(2):640-650.
21. Göksu S, Naderi A, Akbaba Y, et al. Carbonic anhydrase inhibitory properties of novel benzylsulfamides using molecular modeling and experimental studies. *Bioorg Chem*. 2014;56:75-82.
22. Beydemir SÜ, Gülçin İ. Effects of melatonin on carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo. *J Enzyme Inhib Med Chem*. 2004;19(2):193-197.
23. Akbaba Y, Bastem E, Topal F, Gülçin İ, Maraş A, Göksu S. Synthesis and carbonic anhydrase inhibitory effects of novel sulfamides derived from 1-aminoindanes and anilines. *Arch Pharm*. 2014;347(12):950-957.
24. Akbaba Y, Akıncıoğlu A, Göçer H, Göksu S, Gülçin İ, Supuran CT. Carbonic anhydrase inhibitory properties of novel sulfonamide derivatives of aminoindanes and aminotetralins. *J Enzyme Inhib Med Chem*. 2014;29(1):35-42.
25. Akıncıoğlu A, Akıncıoğlu H, Gülçin İ, Durdagi S, Supuran CT, Göksu S. Discovery of potent carbonic anhydrase and acetylcholine esterase inhibitors: novel sulfamoylcarbamates and sulfamides derived from acetophenones. *Bioorg Med Chem*. 2015;23(13):3592-3602.
26. Hisar O, Beydemir Ş, Gülçin I, Küfrevioğlu ÖI, Supuran CT. Effects of low molecular weight plasma inhibitors of rainbow trout (*Oncorhynchus mykiss*) on human erythrocyte carbonic anhydrase-II isozyme activity in vitro and rat erythrocytes in vivo. *J Enzyme Inhib Med Chem*. 2005;20(1):35-39.
27. Kucukoglu K, Gul HI, Taslimi P, Gulcin I, Supuran CT. Investigation of inhibitory properties of some hydrazone compounds on hCA I, hCA II and AChE enzymes. *Bioorg Chem*. 2019;86:316-321.
28. Coban TA, Beydemir Ş, Gücin İ, et al. Sildenafil is a strong activator of mammalian carbonic anhydrase isoforms I–XIV, sildenafil is a strong activator of mammalian carbonic anhydrase isoforms I–XIV. *Bioorg Med Chem*. 2009;17(16):5791-5795.
29. Christie KN, Thomson C, Xue L, Lucocq JM, Hopwood D. Carbonic anhydrase isoenzymes I, II, III, and IV are present in human esophageal epithelium. *J Histochem Cytochem*. 1997;45(1):35-40.
30. Burish TG, Redd WH. Symptom control in psychosocial oncology. *Cancer*. 1994;74(S4):1438-1444.
31. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72(1–2):248-254. doi:10.1016/0003-2697(76)90527-3
32. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970;227(5259):680-685.
33. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. *J Biol Chem*. 1967;242:4221-4229.
34. Lineweaver H, Burk D. The determination of enzyme dissociation constants. *J Am Chem Soc*. 1934;56:658-666.
35. Karagecili H, İzol E, Kirecci E, Gulcin İ. Determination of antioxidant, anti-Alzheimer, antidiabetic, antiglaucoma and antimicrobial effects of Zivzik pomegranate (*Punica granatum*)—a chemical profiling by LC-MS/MS. *Life*. 2023;13(3):735.
36. Aytac S, Gundogdu O, Bingol Z, Gulcin İ. Synthesis of Schiff bases containing phenol rings and investigation of their antioxidant capacity, anticholinesterase, Butyrylcholinesterase, and carbonic anhydrase inhibition properties. *Pharmaceutics*. 2023;15(3):779.
37. Karagecili H, Yılmaz MA, Ertürk A, et al. Comprehensive metabolite profiling of Berdav propolis using LC-MS/MS: determination of antioxidant, anticholinergic, antiglaucoma, and antidiabetic effects. *Molecules*. 2023;28(4):1739.
38. Cuatrecasas P. Protein purification by affinity chromatography derivatizations of agarose and polyacrylamide beads. *J Biol Chem*. 1970;245(12):3059-3065.
39. Soydan E, Güler A, Bıyık S, Şentürk M, Supuran CT, Ekinci D. Carbonic anhydrase from *Apis mellifera*: purification and inhibition by pesticides. *J Enzyme Inhib Med Chem*. 2017;32(1):47-50.
40. Hisar O, Beydemir Ş, Bülbül M, Çiftçi M, Küfrevioğlu Öİ. *Gökkuşağı Alabalık (Oncorhynchus mykiss) Solungaçlarından Karbonik Anhidraz Enziminin Saflaştırılması Ve Bazı Kinetik Özelliklerinin Belirlenmesi*. Vol XVII. Ulusal Kimya Kongresi, Bk S17; 2003.
41. Akdeniz Ö. *Asetilkolinesteraz Ve Butirilkolinesteraz Enzimleri Üzerinde Bazı Pestisitlerin Etkilerinin incelenmesi*" Master's thesis. Ağrı Ibrahim Çeçen Üniversitesi, Fen Bilimleri Enstitüsü; 2019.
42. Tham LG, Perumal N, Syed MA, Shamaan NA, Shukor MY. Assessment of *Clarias batrachus* as a source of acetylcholinesterase (AChE) for the detection of insecticides. *J Environ Biol*. 2009;30(1):135-138.
43. Hiroshi HS, Ji HY, Lee H, Lim SR, Kim JH, Lee HS. Effect of efavirenz on UDP-glucuronosyltransferase 1A1, 1A4, 1A6, and 1A9 activities in human liver microsomes. *Molecules*. 2012;17(1):851-860.
44. Şentürk M, Ceyhun SB, Erdoğan O, Küfrevioğlu ÖI. In vitro and in vivo effects of some pesticides on Glucose-6-phosphate dehydrogenase enzyme activity from rainbow trout (*Oncorhynchus mykiss*) erythrocytes. *Pestic Biochem Physiol*. 2009;95(2):95-99.
45. Holovská V, Pistl J, Kovalkovičová N. In vitro effect of pesticides (dichlofluanid, endosulfan, simazine, tolylfluanid and triallate) on proliferative activity of animal derived cell cultures. *Acta Biol Hung*. 2007;58(1):61-74.
46. Çelik H. *Sığır böbrek karbonik anhidraz enziminin saflaştırılması, karakterizasyonu ve bazı doğal moleküllerle inhibisyonunun belirlenmesi*. Master's thesis. Ağrı Ibrahim Çeçen Üniversitesi, Fen Bilimleri Enstitüsü; 2017.
47. Toni C, Ferreira D, Kreutz LC, Loro VL, Barcellos LJG. Assessment of oxidative stress and metabolic changes in common carp (*Cyprinus carpio*) acutely exposed to different concentrations of the fungicide tebuconazole. *Chemosphere*. 2011;83(4):579-584.
48. De Souza PR, De Souza KS, De Assis CRD, et al. Acetylcholinesterase of mangrove oyster *Crassostrea rhizophorae*: a highly thermostable enzyme with promising features for estuarine biomonitoring. *Aquat Toxicol*. 2018;197:109-121.
49. Tham LG, Perumal N, Syed MA, Shamaan NA, Shukor MY. Assessment of *Clarias batrachus* as a source of acetylcholinesterase (AChE) for the detection of insecticides. *J Environ Biol*. 2009;30(1):135-138.
50. Anderson JA, Coats JR. Acetylcholinesterase inhibition by nootkatone and carvacrol in arthropods. *Pestic Biochem Physiol*. 2012;102(2):124-128.
51. Kristoff G, Cacciatore LC, Guerrero NRV, Cochón AC. Effects of the organophosphate insecticide azinphos-methyl on the reproduction and cholinesterase activity of *Biomphalaria glabrata*. *Chemosphere*. 2011;84(5):585-591.
52. Sinan S, Gencer N, Turan Y, Arslan O. In vitro inhibition of the carbonic anhydrase from saanen goat (*Capra hircus*) with pesticides. *Pestic Biochem Physiol*. 2007;88:307-311.
53. Soydan E, Olcay AC, Bilir G, et al. Investigation of pesticides on honey bee carbonic anhydrase inhibition. *J Enzyme Inhıb Med Chem*. 2020;35(1):1923-1927.
54. Simone GD, Angeli A, Bozdag M, et al. Inhibition of carbonic anhydrases by a substrate analog: benzyl carbamate directly coordinates the catalytic zinc ion mimicking bicarbonate binding. *Chem Commun*. 2018;54:10312-10315.

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