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IN VITRO EFFECTS OF SOME PESTICIDES ON GLUTATHIONE-S TRANSFERASE ACTIVITY

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ABSTRACT

Human populations have been constantly exposed to pesticides due to their extensive use and presence in food and drinking water. Therefore, the aim of this study is to compare the inhibitory effect of three commonly used pesticides such as glyphosate (herbicide) and lambda-cyhalothrin and deltamethrin (insecticides) on glutathione S-transferase (GST) activity *in vitro* in human blood. GST enzyme activity was spectrophotometrically determined with observation of the formation of 1-chloro-2,4-dinitrobenzene-glutathione (CDNB-GSH) conjugate. GST activities were suppressed by all the pesticides tested; the deltamethrin was the most potent inhibitor, reducing GST activity *in vitro* in a dosage-dependent manner. The inhibition mechanism of pesticides on GST was different from each other. The inhibition types of glyphosate, lambda-cyhalothrin and deltamethrin pesticides were uncompetitive, mixed and competitive, respectively.

KEYWORDS:
Glutathione S-transferase, pesticides, environmental toxicity, inhibition.

INTRODUCTION

Today, conscious societies are aware of the importance of plant-derived foods in healthy nutrition. Besides, the increase of herbal nutritional needing due to the rapid increase of the world population, the development of greenhouse due to the intense demand for fresh fruits and vegetables in every season, climate change due to global warming and other ecological changes are provided suitable environments for the growth and diversification of some diseases and harmfulness in polyculture agriculture. This problem can be solved by taking measures that are not cost-effective, most importantly not causing environmental pollution, by producing high-quality products and yielding unit area [1]. In the world, pesticides are being used extensively in order to eliminate the harmful effects of agriculture areas and to obtain quality products. Pesticides used in agricultural struggle cause the product increase destructing of target organisms as well as damage to non-target organisms. However, these pesticides remain in the water, soil, fruits and vegetables for a long time without deterioration and cause environmental pollution and thus cause various damages that can reach human beings through the food chain [2-5]. Moreover, it is also stated that pesticides constitute a "risk factor" for breast cancer because of their lipophilic character, high bioaccumulation and estrogenic activity [2].

People meet pesticides in various forms. A large community, including producers, marketers, practitioners and finally consumers of pesticide agricultural products are exposed to pesticides acutely or chronically at different degrees. Pesticides entering the organism in various ways have negative effects on systems such as nervous system, endocrine system, immunity protection system, liver, heart and muscle [2]. An important system within the affected systems is the human defense system, i.e. the antioxidant system.

The most important feature of the antioxidant defense system is that all components of the system function in such a way as to create a synergy against reactive oxygen species [6]. For this reason, antioxidant enzymes have a vital important for regulating cell balance and their inductions are a consequence of the response to contaminants [7]. Antioxidant enzymes are the key components induced by oxidative stress and form endogenous enzymes (superoxide dismutase, glutathione peroxidase, glutathione-S transferases (GST), catalase, mitochondrial calcium chromoxidase system, hydroperoxidase) and exogenous enzymes (Vitamin E and C, some drugs) [8].

GST, an endogenous antioxidant enzyme, is an important group of enzymes involved in xenobiotic metabolism and detoxification of endogenous and exogenous substances. This enzyme protects cell membranes, DNA and proteins against reactive oxygen species that are triggered by environmental stress factors. GST is responsible for neutralizing mutagens, carcinogens and other toxic substances. It is known that GST is found in plants, insects, yeasts, bacteria and especially the liver, and plays a key role in detoxification [2].

Different pesticides have been reported to induce oxidative stress due to generation of free radicals and alteration in antioxidant defense mecha-

nism. For example, Souza et al. investigated biomarkers such as acetylcholinesterase, GST and catalase of pesticide exposure in placenta samples from pregnant women living in an area of agricultural exploitation with intensive pesticide application [9]; Ojha et al. the effect of commonly used organophosphate pesticides on lipid peroxidation and antioxidant enzymes in rat tissues [2]; Medina-Diaz et al. the effect of chlorpyrifos and methyl parathion on GST levels in HepG2 cells [10]; Gomez-Martin et al. the contribution of genetic polymorphisms of the pesticide-metabolizing enzymes paraoxonase-1 (PON1) and GST on N7-MedG levels [4]; Matic et al. the role of GST A1, M1, P1 and T1 gene polymorphisms and potential effect modification by occupational exposure to different chemicals in Serbian bladder cancer male patients [11]; Song et al. the effects of different pesticides on superoxide dismutase and GST activities [3]; Ezemonye and Tongo the effects of the organochlorine pesticide, endosulfan and the organophosphate pesticide, diazinon on the activity of GST of different tissues in the African common toad, Bufo regularis [12]; Kaya and Yiğit the changes in glutathione S-transferase, glutathione reductase and total glutathione in Vicia sativa L. "Selcuk-99" under flurochloridone stress [13]; Arslan et al. the suitability of using GST of *M. galloprovincialis* as potential biomarker of BPA in the environment [14]; and Kolarova et al. the primary cause of reproductive disturbances in salmonids from the Ticha Orlice river [15]. As can be seen from the above studies, no studies showing the effects of pesticides such as glyphosate (herbicide) and lambda-cyhalothrin and deltamethrin (insecticides) on GST activity have been found. Therefore, the aim of this study is to examine the effects of glyphosate (herbicide) and lambda-cyhalothrin and deltamethrin (insecticides) which cause environmental pollution and which can be passed into human body in different ways, *in vitro* on GST activity, which is an endogenous antioxidant enzyme. For this purpose, the inhibitory effects of pesticides such as glyphosate (herbicide) and lambda-cyhalothrin and deltamethrin (insecticides) on GST activity isolated from human blood were firstly investigated and then the inhibitory effects of pesticides were compared with previous studies in the literature.

MATERIALS AND METHODS

Materials. Blood samples used in this study were taken EDTA (ethylenediamine tetraacetic acid) tubes from healthy humans before each experiment. Chemicals, such as potassium dihydrogen phosphate, reducing glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Merck and Sigma. Enzyme activity was determined using a PerkinElmer Lambda 35 UV-Visible spectrophotometer.

Preparation of Hemolysate. Approximately 2 mL of venous blood was drawn by sterile vacuum injectors from healthy young humans for erythrocyte antioxidant enzyme activity measurements. Blood was transferred to eppendorf tubes and centrifuged at 2500 rpm for 15 min at +4 °C. The plasma remaining in the upper part was discarded, red blood cells were washed three times with 0.16 M KCl in +4 ^{o}C for 5 min at 2500 rpm, then diluted 1/5 with cold distilled water, centrifuged at 10000 rpm for 30 min at +4 °C and the erythrocytes were disintegrated [16].

Measurement of GST enzyme activity. GST catalyzes the reaction between 1-chloro-2,4-dinitrobenzene (CDNB) and the glutathione-SH group. GST activity was measured using the artificial CDNB and GSH. Kinetic analysis was made in assays containing various concentrations of GSH at fixed CDNB concentration. One unit of GST activity was defined as the amount of enzyme producing 1 µmol of GS-DNB conjugate/min at 340 nm and 37 °C [17].

Kinetic study of GST inhibition. GST inhibitions were determined kinetically in assays containing various concentrations of GSH at a fixed concentration of each pesticide. The results were then summarized in double-reciprocal Lineweaver–Burk plots and the inhibitory constant, Ki, was determined based on the reciprocal velocity versus reciprocal concentration [18].

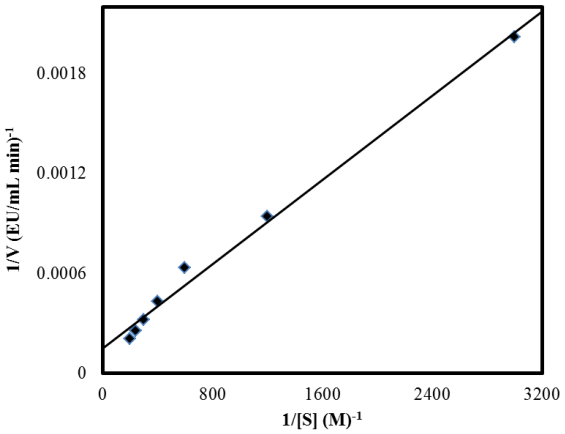

FIGURE 1
Lineweaver-Burk graph for human blood GST

RESULTS AND DISCUSSIONS

Kinetic analysis of GST activity. The kinetic parameters, V_{max} and K_m for various GSH concentrations at fixed CDNB concentration were determined using Lineweaver-Burk graphs (Figure 1). K_m and V_{max} values were calculated as $6x10^{-3}$M and 10.000 EU/mL min, respectively.

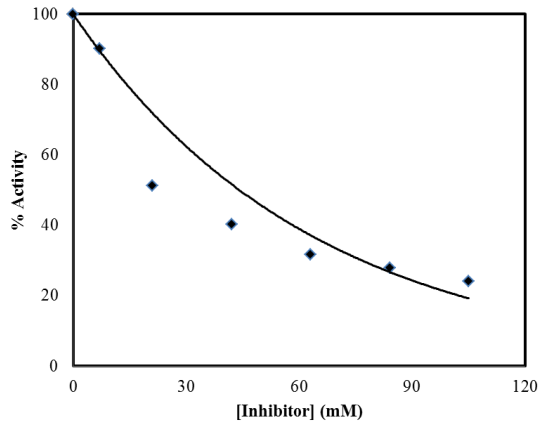

FIGURE 2
The graph of percent activity against glyphosate concentrations

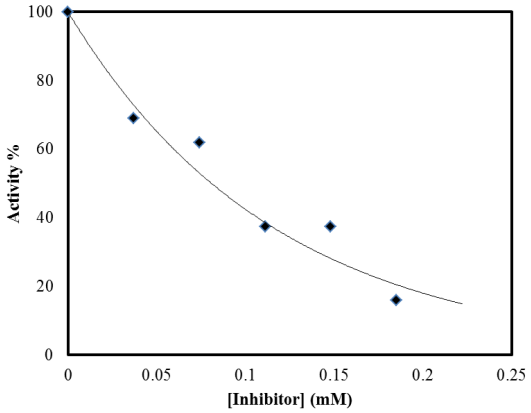

FIGURE 3
The graph of percent activity against lambda-cyhalothrin concentrations

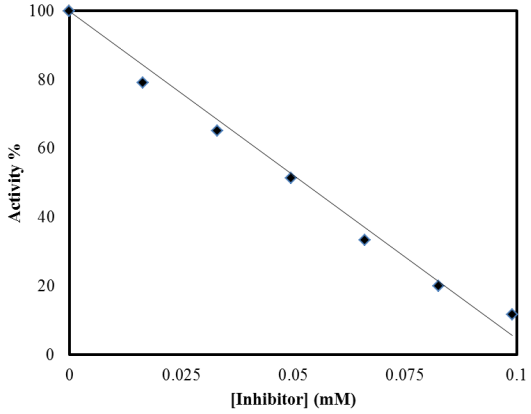

FIGURE 4
The graph of percent activity against deltamethrin concentrations

The effect of pesticides on GST activity. Many medicinal drugs and many pesticides have biologically important functions as a direct result of their effects on enzymes. Enzymes are large proteins (polymers of a-amino acids) which enable specific chemical reactions to occur at reasonable rates at moderate temperatures and at near neutral pH [19,20]. The reactions catalyzed by enzymes are responsible for such important biochemical processes. Consequently, the growth and replication of every living organism depends on the proper and coordinated functioning of a large number of enzymes [21]. It was found in literature that the effects of organic and/or inorganic compounds on different biochemical reactions were different. In some cases, a substance that is an activator for a reaction can act as an inhibitor for another reaction [22]. GST is a soluble protein with low molecular weight in various cells and tissues. GSTs are a family of detoxification enzymes that catalyze the conjugation of glutathione (GSH) with electrophilic compounds, thus preventing toxicity. Some GST isoenzymes have antioxidantase activity to defense against oxidative damage and peroxidative products of DNA and lipids [23, 24]. The toxicity of many exogenous compounds can be modulated by induction of GSTs. So they might be playing an important role in detoxifcation metabolism [3]. As can be seen from the results in Figures 2, 3 and 4, three pesticides have shown inhibitory effect on GST enzyme. As the concentration of the pesticides increased, the percentages of inhibition increased and the enzyme showed less activity. It was also found that the variation of GST activity was different from pesticide to pesticide. From the experimental results, the inhibition percentages at 7, 21, 42, 63, 84 and 105 mM concentrations for glyphosate were found as 10, 49, 60, 69, 73 and 76; those at 37, 74, 111, 148 and 185 μM concentrations for lambda-cyhalothrin as 31, 39, 63, 64 and 84; and those at 17, 33, 50, 66, 83 and 100 μM concentrations for deltamethrin as 21, 35, 49, 67, 80 and 88, respectively. When the experimental results were examined, it can be said that that deltamethrin inhibited enzyme activity more at low concentrations and the strongest inhibitor among the used pesticides was deltamethrin, followed by lambda-cyhalothrin and glyphosate pesticides, respectively. Again, the concentrations of inhibitor required to decrease the enzyme activity by 50% were calculated separately for each pesticide from the equations of the curves in Figures 2-4. These values may vary depending on the enzyme and its inhibitors. The IC_{50} values for the glyphosate, lambda-cyhalothrin and deltamethrin pesticides were determined as 43300, 58 and 51 μM, respectively. Among the tested pesticides, deltamethrin showed the highest inhibitory activity against human blood GST enzyme, whereas glyphosate exhibited the lowest activity. These results show that pesticides trigger oxidative stress in living cells and that GST enzyme activity decreases. Some researchers showed that the expression of GST was a crucial factor in determining the sensitivity of cells and organs in response to a variety of toxins in the aquatic organism, and dose-effect relationship [25,26]. It was also demonstrated that there was significant dose-effect relationship between the concentration of pesticides and GST activity [3]. The calculated IC_{50} values for the inhibitor-affecting substances on

TABLE 1
IC_{50} values of some chemicals and pesticides for glutathione S-transferase activity

Inhibitors	Substrates	IC_{50} (µM)	Enzyme sources	References
Tannic acid	CDNB	13,50	*M. troglodyte*	[27]
Quercetin	CDNB	13,55	*M. troglodyte*	[27]
Tannic acid	CDNB	8,19	*C. anachoreta*	[27]
Quercetin	CDNB	5,68	*C. anachoreta*	[27]
Glyphosate	GSH	43300	Human blood	In this study
Lambda-cyhalothrin	GSH	58	Human blood	In this study
Deltamethrin	GSH	51	Human blood	In this study

TABLE 2
Inhibition types and inhibition constants of some GST enzymes

Inhibitors	Substrates	[Inh] [M]	K_i [M]	K_i' [M]	Inhibition types	Enzyme sources	References
Quercetin	CDNB	-----	$5,93x10^{-6}$	-----	Competitive	*M. troglodyta*	[27]
Quercetin	CDNB	-----	$3,60x10^{-6}$	-----	Noncompetitive	*C. anachoreta*	[27]
Quercetin	GSH	-----	$6,70x10^{-7}$	-----	Competitive	*C. anachoreta*	[27]
Tannic acid	CDNB	-----	$7,93x10^{-6}$	-----	Competitive	*M. troglodyta*	[27]
Tannic acid	GSH	-----	$6,58x10^{-6}$	-----	Competitive	*M. troglodyta*	[27]
6,7-dihydroxy-3-(3',4'-dihydroxyphenyl)coumarin	CDNB	-----	$13,62x10^{-6}$	-----	Noncompetitive	Human placental	[31]
6,7-dihydroxy-3-(3',4'-dihydroxyphenyl)coumarin	GSH	-----	$7,54x10^{-6}$	-----	Mixed	Human placental	[31]
Ag^{+}	GSH	-----	$0,1x10^{-6}$	-----	Noncompetitive	turkey liver	[33]
Hg^{2+}	GSH	-----	$68x10^{-6}$	-----	Noncompetitive	turkey liver	[33]
Hypericin	GSH	-----	$248x10^{-6}$	-----	Uncompetitive	Rat	[30]
Hypericin	CDNB	-----	$150x10^{-6}$	-----	Noncompetitive	Rat	[30]
Deltamethrin	GSH	$1,7x10^{-5}$	$81x10^{-6}$	-----	Competitive	Human blood	In this study
Deltamethrin	GSH	$2,1x10^{-5}$	$5,1x10^{-6}$	-----	Competitive	Human blood	In this study
Lambda-cyhalothrin	GSH	$7,4x10^{-5}$	$1,3x10^{-5}$	$7,4x10^{-5}$	Mixed	Human blood	In this study
Lambda-cyhalothrin	GSH	$1,11x10^{-5}$	$1,1x10^{-5}$	$11,1x10^{-5}$	Mixed	Human blood	In this study
Glyphosate	GSH	$3,5x10^{-2}$	$3,8x10^{-3}$	-----	Uncompetitive	Human blood	In this study
Glyphosate	GSH	$5,3x10^{-2}$	$1,8x10^{-3}$	-----	Uncompetitive	Human blood	In this study

GST activity were given in Table 1 [27]. According to these results, the most effective inhibitor of GST was quercetin.

Inhibition Types. Oxidative stress is involved in pathophysiology of several toxins and diseases. The balance between the production of free radicals and antioxidant defenses in the body has important health implications. Reduction in the activities of antioxidant enzymes changes the redox status of the cells. *In vitro* inhibition is known to provide a useful tool for studying both the metabolism of xenobiotics catalyzed by GSTs and the involvement of GST in resistance. Reduction of the activity of the enzyme by a specific inhibitor may involve a single mechanism or may be a consequence of two or more inhibitor mechanisms. The enzyme binding of a specific inhibitor is very important in the interpretation of the obtained data [28]. In this study, kinetic inhibition parameters and types of inhibition of pesticides were determined with respect to GSH as a substrate.

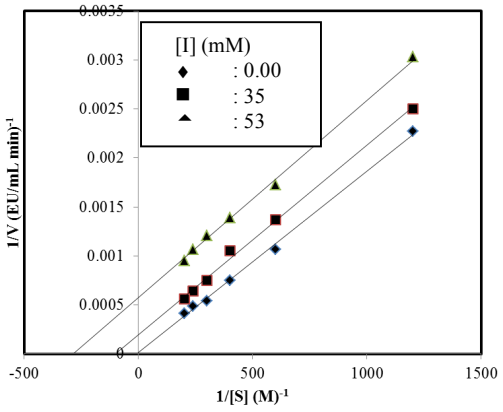

FIGURE 5
Lineweaver-Burk double reciprocal plots showing uncompetitive inhibition of human blood GST by glyphosate pesticide using CDNB as a substrate

Figure 5 showed that the inhibition type for glyphosate pesticide using GSH as a substrate was uncompetitive inhibition. Series of parallel lines in Lineweaver-Burk plot indicates uncompetitive inhibition. Uncompetitive inhibition requires that the inhibitor affects the catalytic function of the enzyme but not its substrate binding. In uncompetitive inhibition, inhibitor binds exclusively to the enzyme-substrate complex [29]. The dependencies obtained justified for this type inhibition. As seen from Table 2, Tuna et al. found that inhibition type for rat GST was uncompetitive for hypericin inhibitor when [CDNB] was used as the fixed and [GSH] was used as the varied substrate [30].

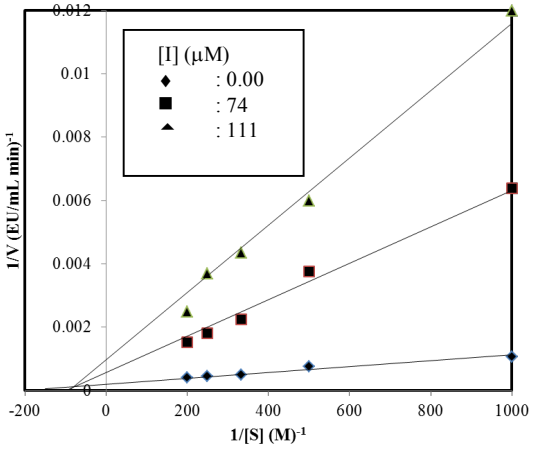

FIGURE 6
Lineweaver-Burk double reciprocal plots showing uncompetitive inhibition of human blood GST by lambda-cyhalothrin pesticide using CDNB as a substrate

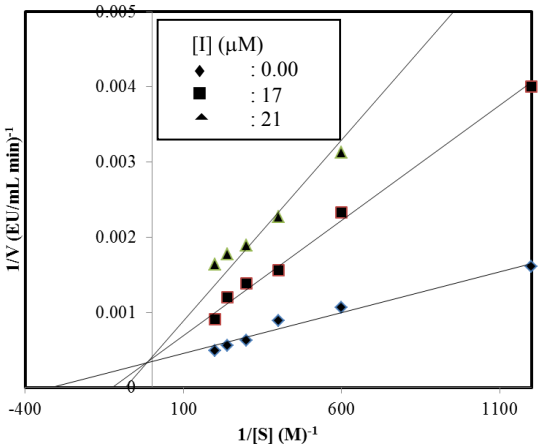

FIGURE 7
Lineweaver-Burk double reciprocal plots showing uncompetitive inhibition of human blood GST by deltamethrin pesticide using CDNB as a substrate

The type of inhibition may vary depending on the inhibitors and substrates used to decrease the activity of the enzyme. Lineweaver-Burk plot obtained for GST enzyme was shown in Figure 6 when GSH as substrate and lambda-cyhalothrin as inhibitor were used. When Figure 6 was examined, the curves intersect above the x-axis to the left of the y-axis. This suggested that the inhibition type was mixed-type inhibition. In this type of inhibition, inhibitor decreases the enzyme activity to bind both enzyme and the enzyme-substrate complex, and obtains two inhibition constants [22]. The inhibition constants obtained were given in Table 2. Similar result was found by Alparslan and Danış for human placental GST using GSH as substrate and 6,7-dihydroxy-3-(3', 4'-dihydroxyphenyl) coumarin as inhibitor [31].

Figure 7 shows the effect of deltamethrin pesticide on GST enzyme activity when GSH is used as the substrate. As seen from Figure 7, at certain inhibitor concentrations, the curves intersect on the y-axis. This indicates that the inhibition type is competitive type inhibition. In inhibition of this type, the enzyme competes with the substrate to bind to the active center of the enzyme. The substrate or the inhibitor binds to the active site of the enzyme. It is not possible to bind them together. The structure of inhibitor is similar to the substrate, so that binding of the substrate to the active site is prevented. In the competitive inhibition, inhibitor acts to reduce the concentration of free enzyme present for binding of the substrate. If the substrate concentration is increased, the effect of inhibitor can be eliminated [32]. Similar results were found by different researchers (Table 2) [33]. For *M. troglodyta* GST, Tang et al. found that inhibition type was competitive for quercetin and tannic acid pesticides when [GSH] was used as the fixed and [CDNB] was used as the varied substrate; and again, for *C. anachoreta* GST they found that inhibition type was competitive for quercetin acid pesticide when [CDNB] was used as the fixed and [GSH] was used as the varied substrate [27]. Results of the present study clearly showed a dose-dependent decrease in GST activities.

CONCLUSIONS

- Effects of three pesticides on GST activity were significant and different.
- GST activity were induced at low concentration and inhibited at high concentrations.
- Deltamethrin had the highest inhibition power on GST activity.
- The values obtained showed to be very strong inhibitors of pesticides when compared to the IC_{50} values obtained in the literature.
- The inhibition mechanism of pesticides on GST activity was different from each other.
- The inhibition types of glyphosate, lambda-cyhalothrin and deltamethrin pesticides on GST activity were uncompetitive, mixed and competitive, respectively.

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