Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *84* (5) 455–465 (2019)
JSCS–5198
UDC 632.95:577.15+611.018.5:615.279
Original scientific paper

Effects of hemazin SC 500 (terbuthylazine) on antioxidative enzymes in human erythrocytes *in vitro*

BILJANA DAVIDOVIĆ-PLAVŠIĆ[1]*, NATAŠA LUKIĆ[2], ALEKSANDRA NIKOLIĆ-KOKIĆ[3] and BILJANA KUKAVICA[1]

[1]*Faculty of Natural Sciences and Mathematics, University of Banja Luka, Mladena Stojanovića 2, 78000 Banja Luka, Bosnia and Herzegovina,* [2]*Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia and* [3]*Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia*

(Received 11 October, revised 1 December, accepted 21 December 2018)

Abstract: The aim of this work was to investigate the effect of the commercial formulation hemazin SC 500, an herbicide containing terbuthylazine as the active compound, on the isoenzyme patterns and activities of Cu-Zn superoxide dismutase (SOD1) and catalase (CAT), as well as on the glutathione *S*-transferase (GST) activity, in human erythrocytes *in vitro*. The human erythrocytes were treated with hemazin SC 500 over a broad range of terbuthylazine concentrations (37 nmol L^{-1}– –37 μmol L^{-1}) for 1 and 3 h at a temperature of 37 °C. Native electrophoresis of the control and treated samples revealed two SOD1 and one CAT isoform. Treatment did not affect the SOD1 and CAT isoenzyme profile, but induced a change in their activities. Terbuthylazine at lower concentration induced a significant increase of the total SOD1 activity and decreased the GST activity in samples incubated for 1 and 3 h. On the other hand, the highest increase in the CAT activity was observed for the sample treated for 1 h with a higher concentration of terbuthylazine. Hemazin SC 500 containing terbuthylazine induces changes in the erythrocyte antioxidative system whereby the response of individual enzymatic antioxidants depends on the concentration of the pesticide and the incubation time.

Keywords: herbicide; hemolysate; SOD1; catalase; glutathione *S*-transferase.

INTRODUCTION

Application of pesticides in agriculture is bidirectional. Pesticides lead to higher crop yields, enable sufficient quantities of food to be obtained and reduce the occurrence of life-threatening illnesses. On the other hand, pesticides could have toxic effects on animals and humans that are not the primary targets of pesticide application. Terbuthylazine (*N*-*tert*-butyl-6-chloro-*N*′-ethyl-1,3,5-triazine--2,4-diamine, $C_9H_{16}ClN_5$, Fig. 1) is the active component of hemazin SC 500, a

*Corresponding author. E-mail: biljana.davidovic-plavsic@pmf.unibl.org
https://doi.org/10.2298/JSC181011115D

selective herbicide from the class of chloro-triazine, and it inhibits photosynthesis in broad leaf weeds. It is applied in the protection of different crops, *e.g.*, maize, sugar cane, olives, pineapple, *etc*.

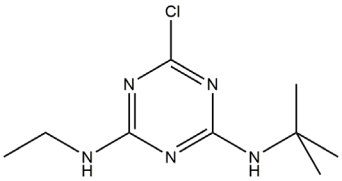

Fig. 1. Structural formula of terbuthylazine.

The prohibition of atrazine (European Union, 2006) resulted in a significant increase in terbuthylazine application. According to the U.S. EPA Office of Pesticide Programs (OPP), Carcinogen List, terbuthylazine exhibits slightly acute toxicity (category III), does not exhibit genotoxicity (classified in group D) and is not considered a carcinogen in humans.[1] As the data on terbuthylazine toxicity are incomplete or ambiguous, according to the International Agency for Research on Cancer (IARC), terbuthylazine is classified in group 3 of the Carcinogen List: unclassifiable. There are not many studies on the toxicity of terbuthylazine and its metabolism in human organism. However, in experiments on rats it was shown that the major metabolic pathway of terbuthylazine is hydrolytic removal of chlorine and mono- and di-dealkylation, as well as hydroxylation of one or both dialkylamino groups of amines. In addition, studies on rats showed that terbuthylazine is rapidly excreted from the body, completely metabolized and not accumulated in the tissue.[2]

It was shown in the literature that erythrocytes represent a good model for the study of the toxic effects of pesticides on the human organism.[3] Due to self-oxidation of hemoglobin and the presence of high content of polyunsaturated fatty acids, erythrocytes are susceptible to ROS (reactive oxygen species – superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$)) formation. There is evidence that pesticides result in increased production of ROS in erythrocytes.[4] Moreover, triazine pesticides (atrazine) show the same effect in erythrocytes.[5] On the other hand, to the best of our knowledge, there are no data on the impact of terbuthylazine on human erythrocytes. It was shown that terbuthylazine in algal cells leads to ROS formation.[6] Moreover, the major products of degradation of terbuthylazine, terbuthylazine-desethyl and hydroxyterbuthylazine,[7] caused the formation of ROS in carp[8] and red swamp crayfish.[9]

The main antioxidants in erythrocytes are antioxidative enzymes: copper–zinc superoxide dismutase (SOD1, Cu, Zn-SOD), catalase (CAT, EC 1.11.1.6) and glutathione *S*-transferase (GST, EC 2.5.1.18). The response of antioxidative enzymes in pesticide-treated erythrocytes has been demonstrated.[3,10] Hitherto,

there have been no reports published for the treatment of human erythrocytes with terbuthylazine *in vitro*.

Recent research showed that terbuthylazine lead to DNA damage[11] and DNA instability in a culture of leukocytes and inhibition of SOD1 activity in erythrocytes after treatment of whole blood.[12] Increases in the use of terbuthylazine and the lack of data on its toxicity point to the importance of examining its impact not only on humans, but also on other living organisms. In addition, a problem with the use of terbuthylazine could be its stability and the stability of its degradation products in soil and water,[7] which are extremely toxic to aquatic organisms.[8]

Therefore, the aim of the present study was to examine the changes in antioxidant activity enzymes in erythrocytes (SOD1 and CAT, as enzymes in the first line of defense against ROS, and GST, as an enzyme of biotransformation) after acute exposure to the commercial herbicide hemazin SC 500 with terbuthylazine as an active compound.

EXPERIMENTAL

Reagents

Commercial product hemazin SC 500 with 500 g L^{-1} terbuthylazine as the active substance was purchased from Agromarket (Serbia). Vacutainers for blood sampling with 3.2 % sodium citrate solution were purchased from Greiner Bio-One (Austria). Nitro blue tetrazolium (NBT) and tetramethylethylenediamine (TEMED) were purchased from Fisher Bioreagents (USA), riboflavin and glycerin from Semikem (B&H), toluene, ethylenediaminetetraacetic acid (EDTA), potassium hexacyanoferrate(III) and iron(III) chloride hexahydrate from Lach-Ner (Czech Republic). Hydrogen peroxide (30 %) was purchased from Carlo Erba (France). Glutathione (98 % purity) and 1-chloro-2,4-dinitrobenzene (CDNB, 99 % purity) were purchased from Acros Organics (USA). All the other reagents and chemicals used were of analytical grade.

Sample preparation

Human blood was obtained from healthy volunteers. All procedures were approved by the Ethical Committee of the Medical Faculty of the University of Banja Luka, B&H number 18/4.14/18. The average age of volunteers (6 men and 12 women) was 23 years (from 21 to 25). All volunteers were healthy, were not taking any therapies, were not smokers and had not come in contact with pesticides. On average, 6 mL of blood was taken from each person into a vacutainer containing citrate as an anticoagulant. The experiments were performed on the day of collection. The blood was centrifuged at 900 g for 10 min (Centric 200R, Tehtnica) and the plasma was separated. The precipitate of erythrocytes was washed three times with a cold physiological solution and used for treatment with hemazin SC 500.

Preparation of pesticides solution

In the experiments, a commercial herbicide hemazin SC 500 with a known concentration of terbuthylazine was used and therefore, the amount of herbicide used for treatment of erythrocytes was calculated based on the terbuthylazine concentration (37 nmol L^{-1}–37 μmol L^{-1}). The lowest selected concentration of terbuthylazine (37 nmol L^{-1}) in the experiments was based on data showing that the concentration of terbuthylazine in waterways on day on which terbuthylazine was applied on crops was 42 nmol L^{-1}.[13] On the other hand, the solubility of

terbuthylazine in water[14] is 39 μmol L^{-1} and therefore, 37 μmol L^{-1} was chosen as the highest concentration for the treated erythrocytes. Terbuthylazine was dissolved in a saline.

Treatment of erythrocytes with commercial herbicide hemazin SC 500 and lysis

The erythrocytes of 18 healthy volunteers were used in experiments. The experiments were repeated 3 times with 6 volunteers (samples) per experiment. Each of the 6 samples was divided into 4 aliquots (1 control and 3 treated). The results for one experiment with 6 volunteers are presented. The erythrocytes were treated with hemazin SC 500 calculated on terbuthylazine (control (0), 37 and 3.7 μmol L^{-1} and 37 nmol L^{-1}) for 1 and 3 h at a temperature of 37 °C under constant steering. Preliminary experiments showed that there was no lysis of erythrocytes for the selected concentrations of terbuthylazine under the chosen conditions. After incubation, the erythrocytes were centrifuged at 900 g for 10 min and the supernatant was discarded. Treated erythrocytes (washed 2 times with saline after treatment) were lysed with cold distilled water 1:3 (volume ratio) in order to prevent protein denaturation, and toluene 1:1 (volume ratio) to remove lipids. Then they were swirled in a vortex, and placed in the refrigerator for 1 h. The lysed erythrocytes were centrifuged at 900 g for 25 min and the lysed erythrocytes were used for further analysis, while membranes were removed. The hemoglobin (Hb) concentration was determined using the Drabkin and Austin method at 545 nm.[15]

Preparation of SOD1 fractions

From one part of the lysate, hemoglobin was removed using the Tsuchihashi method[16] and the remaining solution was used to determine the SOD1 isoenzyme pattern and activity. Prior to removal of the hemoglobin in the samples, its content was determined. The volume of each of the samples applied subjected to gel electrophoresis corresponded to an equal amount of hemoglobin.

Native polyacrylamide gel electrophoresis

For the separation of SOD1 isoforms, 10 % polyacrylamide gels, and for the separation of CAT isoforms, 8 % polyacrylamide gels were used. The buffer for electrophoresis (pH 8.3) contained 24.8 mmol L^{-1} tris and 192 mmol L^{-1} glycine. Before being loaded on the gel, the samples were mixed with a loading buffer (50 mmol L^{-1} tris pH 6.8, 10 % glycerol and 0.1 % bromophenol blue) in the ratio 1:3. After electrophoresis, the SOD1 isoforms were determined by specific staining. The gels were incubated in a staining solution consisting of 0.25 mmol L^{-1} NBT, 0.13 mmol L^{-1} riboflavin, 4 mmol L^{-1} tris buffer pH 7.8, 1 mmol L^{-1} EDTA and 2.72 mmol L^{-1} TEMED.[17] The CAT activity in the hemolysate was determined after native polyacrylamide gel electrophoresis and specific staining. Prior to staining, the gels were incubated in 0.003 % H_2O_2 for 5 min. The staining solution used for the CAT activity determination contained 1 % $FeCl_3$ and 1 % $K_3Fe(CN)_6$.[18] Quantification of SOD1 and CAT activities on the native gel were performed using the Image Master Total Lab TL 120 software (Nonlinear Dynamics Ltd., Durham, USA). The total SOD1 activity represents the sum of individual isoforms (SOD1 A and SOD1 B) activities for each sample.

Spectrophotometric determination of the GST activity

The GST activity in the hemolysate was determined according to the method of Habig *et al.*[19] This method is based on the binding of the substrate 1-chloro-2,4-dinitrobenzene (CDNB) to the sulfhydryl group of reduced glutathione (GSH) catalyzed by GST. The formation of the conjugate CDNB–GSH, with a maximum absorption at 340 nm, was measured during 150 s (every 30 s) at 37 °C. The GST activity is expressed in U g^{-1} Hb (μM GSH min g^{-1} Hb).

Statistical analysis

Data are given as mean ± *SE* for 6 healthy volunteers. For statistical analysis, the one-way ANOVA Tukey test was used for data comparison between the controls and the treated groups, and between the treated groups themselves. In each experiment, control blood samples and samples treated with terbuthylazine were taken from the same person. The experiments were repeated three times.

RESULTS AND DISCUSSION

The obtained results indicate that the SOD1, CAT and GST activities in human erythrocytes are changed on treatment with commercial herbicide hemazin SC 500 *in vitro*. Even though, active compound in hemazin is terbuthylazine at a concentration 500 g L^{-1}, hemazin also contains other components that are not listed in the product specification. In this study, focus was directed on the influence of the active component terbuthylazine on the antioxidative metabolism of erythrocytes, although the accompanying components in terbuthylazine could also contribute.

SOD1 activity

In all samples (control and treated erythrocytes) incubated for 1 and 3 h at 37 °C with terbuthylazine, two superoxide dismutase isoforms (labeled SOD1 A and SOD1 B) were detected with R_f values of 0.340±0.066 and 0.510±0.047 for SOD1 A and SOD1 B, respectively, where R_f is the ratio of the distance migrated by the SOD isoform to that migrated bromophenol blue (Fig. S-1A and B of the Supplementary material to this paper).

Available literature data showed that the change in the antioxidative metabolism of erythrocytes depends of the type (structure) and concentration of the pesticide and the treatment conditions.[3,20,21] Although terbuthylazine treatment did not lead to any changes in the isoenzyme profiles, it did induce changes in the SOD1 activity (Fig. 2). Terbuthylazine in all concentrations (except 37 μmol L^{-1}

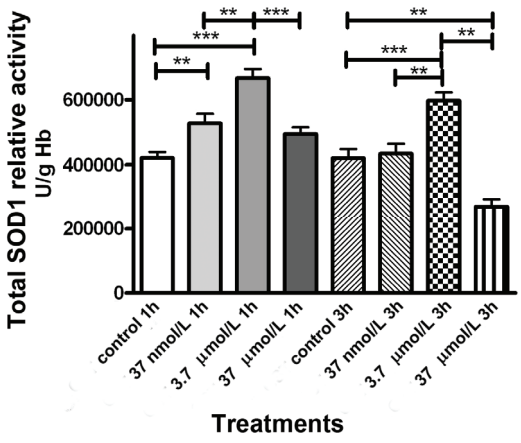

Fig. 2. Total SOD1 (sum of activities of SOD1 A and SOD1 B) relative activity in samples incubated for 1 and 3 h at 37°C. Quantification of SOD1 relative activity on the native gel was performed using Image Master Total Lab TL 120 software. Results are presented as mean ± *SE*. $^{**}p < 0.01$; $^{***}p < 0.005$.

incubated 1 h and 37 nmol L^{-1} incubated for 3 h) significantly changed the SOD1 activity in the samples incubated for 1 and 3 h compared to the controls (420694±18995 U g^{-1} Hb) for 1h and 3 h (420197±28530 U g^{-1} Hb). After 1 h of incubation, the SOD1 activity increased in the samples treated with concentrations 37 nmol L^{-1} ($p < 0.01$, 527926±29308 U g^{-1} Hb) and 3.7 μmol/L ($p < 0.005$, 667220±27949 U g^{-1} Hb), as well as after 3 h incubation with a concentration of 3.7 μmol L^{-1} ($p < 0.005$, 597906±25600 U g^{-1} Hb), Fig. 2. A significant decrease in SOD1 activity ($p < 0.01$, 270598±23388 U g^{-1} Hb) was measured only at the highest concentrations of terbuthylazine after incubation for 3 h. Statistically significant differences between treatments for both incubation periods were found between 3.7 μM and lower and higher terbuthylazine concentrations (Fig. 2).

Erythrocytes are exposed to oxygen radicals that are continuously generated primarily due to auto-oxidation of oxy-hemoglobin to methemoglobin.[22] An earlier examination of the activities of antioxidative defense enzymes *in vitro* showed that SOD1 possesses a constant specific activity and may be inhibited irreversibly by the cyanide ion (CN^-) and reversibly by H_2O_2 or by copper chelators such as DDC (diethyldithiocarbamate). Later it was shown that HS^- enhances the $O_2^{\bullet-}$ scavenging activity of bovine erythrocyte SOD1 by about twofold.[23] The present results show that at certain concentrations, terbuthylazine as chlorotriazine increases the SOD1 activity in erythrocytes (Fig. 2). In the work of Gultekin *et al*., it was shown that the decreases in SOD1 and CAT activities with increasing pesticide concentration were statistically significant and more pronounced for longer incubation periods.[24] However, the authors also showed that at lower pesticide concentrations, SOD1 activity increased (by 2 % after incubation for 1 h and 13 % after 4 h for a concentration of 0.01 g L^{-1} chlorpyrifos-ethyl). The treatment of commercial SOD1 with a low concentration of the same pesticides (0.01 g L^{-1} chlorpyrifos-ethyl) induced an increase in SOD1 activity by 1.34 %. As sulfur-containing compounds can activate SOD1 at certain concentrations and inhibit it at higher concentrations as a result of SOD1 damage,[25] according to the present results, it seems that it may also be true for certain chlorine-containing compounds (terbuthylazine). In other papers, it has also been shown that low concentrations of pyrethroid insecticide[20] and herbicide[10] lead to an increase in SOD1 activity in erythrocytes, whereas high concentrations decrease the SOD1 activity when compared to the control. The results of this paper related to the highest terbuthylazine concentration of 37 μmol L^{-1} showed that 3 h incubation leads to a decrease in SOD1 activity (36 %, $p < 0.01$). Statistically significant decreases in the SOD1 activity were demonstrated in the treatment of rat erythrocytes for 3 h with organochlorine insecticide endosulfan and the organophosphorus insecticide chlorpyrifos-ethyl.[4] Reduction of the activity may be due to oxidative protein changes that could result in increased susceptibility to proteolysis and protein denaturation.[26]

Catalase activity

A large amount of CAT, which participates in the defense against free radicals together with other enzymes for antioxidant protection in the cell, was present in the erythrocytes, and only the liver contained more of this enzyme. Catalase is effective at relatively high H_2O_2 contents, while low concentrations are removed by glutathione peroxidase. One CAT isoform with an R_f value R_{fCAT} = = 0.429±0.106 was detected in the control sample and the treated sample incubated for 1 and 3 h at 37 °C (Fig. S-2 of the Supplementary material).

A statistically significant increase in CAT activity (24 %) was observed in the samples treated for 1 h with 37 μmol L^{-1} of terbuthylazine (289699±7990 U g^{-1} Hb, $p < 0.01$, Fig. 3). In other treatments compared to control (233419±3719 U g^{-1} Hb) for 1 h and 3 h (229566±4950 U g^{-1} Hb), the CAT activity did not change significantly for either of the incubation periods. Between the treatments, statistical significance ($p < 0.005$) was detected among 37 nmol L^{-1} (223793±13887 U g^{-1} Hb) and 3.7 μmol L^{-1} concentration of terbuthylazine after 1 h incubation (Fig. 3).

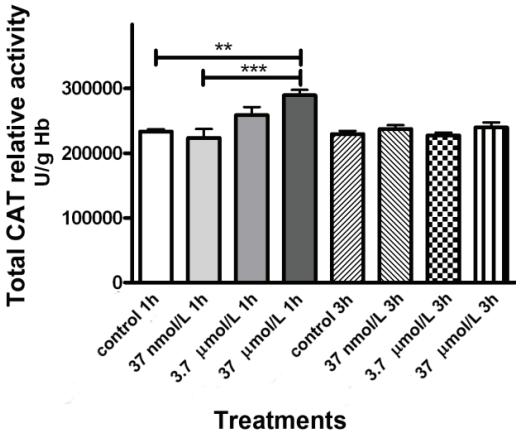

Fig. 3. Relative CAT activity in samples control (0) and groups treated with 37 and 3.7 μmol L^{-1} and 37 nmol L^{-1} terbuthylazine incubated for 1 and 3 h. Quantification of CAT relative activity on the native gel was performed using Image Master Total Lab TL 120 software. The results are presented as mean ± *SE*. $^{**}p < 0.01$; $^{***}p < 0.005$.

Santi *et al*. in the treatment of human erythrocyte for 1 h with the isoxazolidinone herbicide clomazone showed that all the tested concentrations of the herbicide led to a decrease in the CAT activity.[10] An Increase in the CAT activity was demonstrated in human erythrocyte treated for 1 h with the organophosphorus insecticide trichlorfon,[27] while the CAT activity remained unchanged for all tested concentrations of the organophosphate insecticide diazinon when treated for 1 and 3 h.[21] The obtained results showed that the CAT activity was the highest under exposure to the highest concentration of terbuthylazine (Fig. 3), which could be assumed to be the most pro-oxidative, as shown by other researchers.[28] Increased H_2O_2 concentration, resulting from the inhibition of CAT activity[29] with superoxide anion, can inhibit SOD1 activity.[30] It was shown

that the anti-oxidant system, at the level of coordinated expression, functions in the domain of positive correlation between SOD1 and CAT.

GST activity

In contrast to SOD1 and CAT, glutathione-dependent enzymes in the anti-oxidant system are separately regulated, probably *via* the concentration of (reduced) glutathione and the redox status of the cell. GST is an antioxidant enzyme, and, in addition, it belongs to the enzymes of Phase II biotransformation. GST possesses the ability to catalyze the conjugation of a reduced form of glutathione with xenobiotics for the purpose of detoxification.

A statistically significant reduction in GST activity for the lowest concentration of terbuthylazine after incubation with 37 nmol L^{-1} for 1 h (5.604±0.646 U g^{-1} Hb) and 3 h (5.024±0.815 U g^{-1} Hb) was observed compared to the control (7.910±0.796 U g^{-1} Hb) for 1 h and 3 h (9.374±0.648 U g^{-1} Hb), Fig. 4.

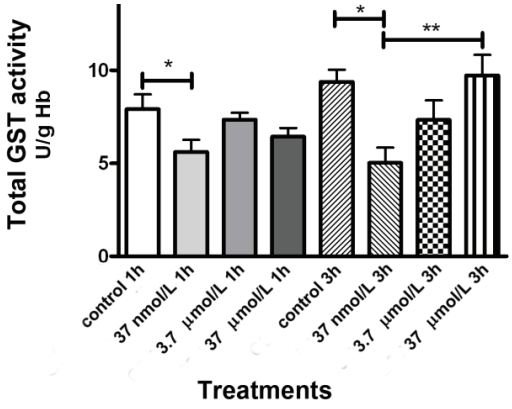

Fig. 4. GST (glutathione *S*-transferase) activity in samples control (0) and groups treated with 37 and 3.7 µmol L^{-1} and 37 nmol L^{-1} terbuthylazine incubated for 1 and 3 h. The results are presented as mean ± *SE*. *$p < 0.05$: **$p < 0.01$.

Treatment of erythrocytes with higher concentrations of terbuthylazine induced a statistically significant increase in GST activity ($p < 0.01$) compared to the lowest concentration, which was especially pronounced after 3 h of incubation (Fig. 4).

El-Demerdash measured decreased GST activity in rabbit erythrocytes for all concentrations of the synthetic pyrethroid insecticide lambda-cyhalothrin incubated for 4 h,[3] while treatment for 3 h with the organochlorine insecticide endosulfan and the organophosphorus insecticide chlorpyrifos-ethyl induced an increase in GST activity in rat erythrocytes.[4]

As already mentioned in the Introduction, there is no data on the metabolism of terbuthylazine in human cells, only in rat cells. Hitherto, the best studied mechanism of terbuthylazine detoxification is in plant cells, which, among others, involves conjugation with glutathione catalyzed with GST. In plants, chloro-triazines are metabolized at the chloro- or 2-position of the triazine ring

by hydrolytic dehalogenation *via* a non-enzymic constituent of plant sap to the corresponding hydroxytriazines.[31] Another important reaction of the chloro group involves an enzyme-mediated conjugation with glutathione to form a series of *S*-bound amino-acid conjugates. These compounds can rearrange to form *N*-bound amino-acid conjugates. A third metabolic reaction involves oxidation of the alkyl-amino side chains located at the 4- and 6-positions of the triazine ring, prior to sugar conjugation or *N*-dealkylation. In the case where the alkyl amino group contains a cyano group, hydrolysis leads to amide and carboxylic acid formation on the alkyl group. These three competing reactions can result in a complex mixture of Phase I metabolites (simple metabolites) and Phase II metabolites (conjugates of simple metabolites) that could occur either free or bound in various plant matrices.

Neefjes and coworkers showed that GST from erythrocytes is a marker of oxidative damage.[32] The herein obtained decrease in GST activity at the lowest concentration of terbuthylazine may be due to an induction of antioxidative metabolism, especially SOD1, which proved to be the most sensitive in response to different concentrations of terbuthylazine during acute exposure (Fig. 2). On the other hand, at high concentrations of terbuthylazine during 3 h incubation, the GST activity increased, which highlights the importance of GST in defense of ROS in pro-oxidative conditions when SOD is inhibited.

CONCLUSIONS

The obtained results showed the activation of SOD1 at lower concentrations of terbuthylazine and CAT at higher concentrations of terbuthylazine during acute incubation for 1 h. Meanwhile, GST, as an enzyme in the second stage of oxidative stress defense, is activated at higher terbuthylazine concentrations during 3 h of incubation. Such results may indicate the strategy of an organism in protection from oxidative stress. As a consequence of chronic exposure to low concentrations of pesticides, changes can occur in the antioxidant capacity at the systemic level. Due to the potential impact of accompanying compounds in the commercial preparation hemazin SC 500 on the antioxidative metabolism of erythrocytes, future research will be focused on the study of influence of pure terbuthylazine.

SUPPLEMENTARY MATERIAL

Representative native electrophoresis gel plates are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgement. This study was supported by the Ministry of Science and Technology of the Republic of Srpska, under Grant 19/6-020/961-24/15.

ИЗВОД

ЕФЕКАТ ХЕМАЗИНА SC 500 (ТЕРБУТИЛАЗИН) НА АНТИОКСИДАТИВНЕ ЕНЗИМЕ У ХУМАНИМ ЕРИТРОЦИТИМА *in vitro*

БИЉАНА ДАВИДОВИЋ-ПЛАВШИЋ[1], НАТАША ЛУКИЋ[2], АЛЕКСАНДРА НИКОЛИЋ-КОКИЋ[3] и БИЉАНА КУКАВИЦА[1]

[1]Универзитет у Бањој Луци, Природно–математички факултет, Младена Стојановића 2, 78000 Бања Лука, Босна и Херцеговина, [2]Универзитет у Новом Саду, Природно–математички факултет, Трг Доситеја Обрадовића 2, 21000 Нови Сад и [3]Универзитет у Београду, Институт за биолошка истраживања „Синиша Станковић“, Булевар деспота Стефана 142, 11060 Београд

Циљ нашег рада је био да се испита ефекат хербицида, комерцијалног назива хемазин SC 500, са тербутилазином као активном компонентом на изоензимски профил и активност Cu-Zn-супероксид-дисмутазе (SOD1) и каталазе (CAT) као и на активност глутатион-*S*-трансферазе (GST) у хуманим еритроцитима *in vitro*. Хумани еритроцити су третирани тербутилазином у широком опсегу концентрација (37 nmol/L–37 μmol/L) 1 и 3 h на 37 °C. Нативном електрофорезом су у контролним и узорцима третираним са тербутилазином детектоване двије SOD1 изоформе и једна CAT изоформа. Третман са пестицидом није довео до промјена у изоензимским профилима SOD1 и CAT, али је изазвао промјену њихове активности. Тербутилазин при ниским концентрацијама је индуковао значајно повећање укупне SOD1 активности и смањење GST активности у узорцима еритроцита инкубираним 1 и 3 h. С друге стране, највеће повећање CAT активности је измјерено у узорцима третираним 1 h са високим концентрацијама тербутилазина. Тербутилазин индукује промјене у антиоксидативном систему еритроцита при чему одговор појединачних ензимских антиоксиданата зависи од концентрације пестицида и времена инкубације.

(Примљено 11. октобра, ревидирано 1. децембра, прихваћено 21. децембра 2018)

REFERENCES

1. PAN Pesticides Database – Chemicals, http://www.pesticideinfo.org/Search_Chemicals.jsp (accessed 20 December 2017)
2. Pesticide Reregistration Status (https://archive.epa.gov/pesticides/reregistration/web/html/status.html)
3. F. M. El-Demerdash, *Toxicol. In Vitro* **21** (2007) 392 (https://doi.org/10.1016/j.tiv.2006.09.019)
4. R. Saxena, P. Garg, D. K. Jain, *Toxicol. Int.* **18** (2011) 73 (https://doi.org/10.4103/0971-6580.75871)
5. J. S. Bhatti, I. P. Sidhu, G. K. Bhatti, *Mol. Cell. Biochem.* **353** (2011) 139 (https://doi.org/10.1007/s11010-011-0780-y)
6. D. Spoljaric, A. Cipak, J. Horvatic, L. Andrisic, G. Waeg, N. Zarkovic, M. Jaganjac, *Aquat. Toxicol.* **105** (2011) 552 (https://doi.org/10.1016/j.aquatox.2011.08.007)
7. European Food Safety Authority (EFSA). *EFSA J.* **9** (2011) 1969 (https://doi.org/10.2903/j.efsa.2011.1969)
8. J. Velisek, A. Stara, D. Koutnik, J. Machova, *Biomed Res. Int.* 2014 (2014) Article ID 621304 (http://dx.doi.org/10.1155/2014/621304)
9. A. Stara, E. Zuskova, A. Kouba, J. Velisek, *Sci. Total Environ.* **566–567** (2016) 733 (https://doi.org/10.1016/j.scitotenv.2016.05.113)
10. A. Santi, C. Menezes, M. M. Duarte, J. Leitemperger, T. Lópes, V. L. Loro, *Interdiscip. Toxicol.* **4** (2011) 149 (https://doi.org/10.2478/v10102-011-0023-9)
11. M. Mladinic, D. Zeljezic, S. A. Shaposhnikov, A. R. Collins, *Toxicol. Lett.* **211** (2012) 62 (https://doi.org/10.1016/j.toxlet.2012.03.001)

12. D. Želježić, S. Žunec, M. Bjeliš, V. Benković, M. Mladinić, B. Lovaković Tariba, I. Pavičić, A. M. Marjanović Čermak, V. Kašuba, M. Milić, A. Pizent, A. Lucić Vrdoljak, N. Kopjar, *Environ. Sci. Pollut. Res.* **25** (2018) 19065 (https://doi.org/10.1007/s11356-018-2046-7)
13. B. R. Baillie, *N. Z. J. For. Sci.* **46** (2016) 16 (https://doi.org/10.1186/s40490-016-0072-0)
14. Terbuthylazine (compound). *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Terbuthylazine#section=Solubility (accessed 20 December 2017)
15. D. L. Drabkin, H. L. Austin, *J. Biol. Chem.* **112** (1935) 51 (http://www.jbc.org/content/112/1/51.full.pdf)
16. M. Tsuchihashi, *Biochem. Z.* **140** (1923) 65
17. C. Beauchamp, I. Fridowich, *Anal. Biochem.* **44** (1971) 276 (https://doi.org/10.1016/0003-2697(71)90370-8)
18. W. Woodbury, A. K. Spencer, M. A. Stahmann, *Anal. Biochem.* **44** (1971) 301 (https://doi.org/10.1016/0003-2697(71)90375-7)
19. W. H. Habig, M. J. Pabst, W. B. Jakoby, *J. Biol. Chem.* **249** (1974) 7130 (http://www.jbc.org/content/249/22/7130.full.pdf)
20. W. I. Sadowska, I. N. Wojcik, A. Karowicz-Bilinska, E. Bieszczad-Bedrejczuk, *Toxicol. in Vitro* **24** (2010) 879 (https://doi.org/10.1016/j.tiv.2009.11.022)
21. I. Altuntas, I. Kilinc, H. Orhan, R. Demirel, H. Koylu, N. Delibas, *Hum. Exp. Toxicol.* **23** (2004) 9 (https://doi.org/10.1191/0960327104ht408oa)
22. J. M. Rifkind, E. Nagababu, S. Ramasamy, L. B. Ravi, *Redox Rep.* **8** (2003) 234 (https://doi.org/10.1179/135100003225002817)
23. D. G. Searcy, J. P. Whitehead, M. J. Maroney, *Arch. Biochem. Biophys.* **318** (1995) 251 (https://doi.org/10.1006/abbi.1995.1228)
24. F. Gultekin, M. Ozturk, M. Akdogan. *Arch. Toxicol.* **74** (2000) 533 (https://doi.org/10.1007/s002040000167)
25. J. I. Toohey, A. J. Cooper, *Molecules* **19** (2014) 12789 (https://doi.org/10.3390/molecules190812789)
26. A. G. Kriebardis, M. H. Antonelou, K. E. Stamoulis, E. Economou-Petersen, L. H. Margaritis, I. S. Papassideri, *J. Cell. Mol. Med.* **11** (2007) 148 (https://doi.org/10.1111/j.1582-4934.2007.00008.x)
27. B. Karademir Catalgol, S. Ozden, B. Alpertunga, *Toxicol. In Vitro* **21** (2007) 1538 (https://doi.org/10.1016/j.tiv.2007.06.002)
28. S. Eroğlu, D. Pandir, F. G. Uzun, H. Bas, *Biol. Res.* **46** (2013) 33 (http://dx.doi.org/10.4067/S0716-97602013000100005)
29. Y. Kono, I. Fridovich, *J. Biol. Chem.* **257** (1982) 5751 (http://www.jbc.org/content/257/10/5751.full.pdf)
30. E. Pigeolet, P. Corbisier, A. Houbion, D. Lambert, C. Michiels, M. Raes, M. D. Zachary, J. Remacle, *Mech. Ageing Dev.* **51** (1990)283 (https://doi.org/10.1016/0047-6374(90)90078-T)
31. B. J. Simoneaux, T. J. Gould, *Plant Uptake and Metabolism of Triazine Herbicides*, in *The Triazine Herbicides 50 years Revolutionizing Agriculture*, H. M. LeBaron, J. E. McFarland, O. C. Burnside, Eds., Elsevier, Oxford, UK, 2008, p. 73 (http://base.dnsgb.com.ua/files/book/Agriculture/Pesticides/The-Triazine-Herbicides.pdf)
32. V. M. Neefjes, C. T. Evelo, L. G. Baars, C. E. Blanco, *Arch. Dis. Child. Fetal Neonatal Ed.* **81** (1999) F130 (http://dx.doi.org/10.1136/fn.81.2.F130).