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ENZYMATIC AND NON-ENZYMATIC LINK COMPONENTS OF ANTIOXIDANT DEFENCE IN SUBCELLULAR FRACTIONS OF RAT LIVER UNDER THE INFLUENCE OF DIETHYL PHTHALATE

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Background. The antioxidant system is one of the protective cell systems. Changes in its functioning, after the introduction of xenobiotics into the body, will determine the further course of the intensity of free radical processes. Among xenobiotics, a prominent place belongs to phthalates, in particular diethyl phthalate (DEP) – the most common group of synthetic substances that are widely used as plasticizers in various industries.

Materials and Methods. For a series of experiments, white outbred rats were used, and cytosolic and microsomal fractions were isolated from the liver cells. The activity of such antioxidant enzymes as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9), glutathione *S*-transferase (GST, EC 2.5.1.18), and the concentration of reduced glutathione (GSH) were determined in the cytosolic fraction. GST activity was also studied in the microsomal fraction.

Results and Discussion. The administration of different doses of DEP for 14 days promoted the activation of antioxidant enzymes, regardless of the dose of xenobiotic administration. The use of DEP for 21 days led to a multidirectional effect of the xenobiotic on the enzymes of the antioxidant system in liver cells. The inactivation of the studied enzymes and depletion of the GSH pool were observed when DEP was administered at a dose of 5.4 mg/kg of body weight. The activity of antioxidant enzymes in liver subcellular fractions remained at a high level compared to the control when DEP was administered at a dose of 2.5 mg/kg of body weight. It was established that the

same trend of changes in GST enzyme activity was found in both the microsomal and cytosolic fractions of rat liver. The activity of the enzyme increased under the influence of both studied doses under the administration of DEP for 14 days. Administration of the xenobiotic for 21 days led to a decrease in GST activity when a high dose of DEP was administered.

Conclusion. The activation of antioxidant system enzymes occurs in response to a short-term intake of DEP. With an increase in the dose and duration of administration of the studied xenobiotic, inactivation of antioxidant enzymes was detected.

Keywords: antioxidant enzymes, cytosol, microsomal fraction, liver, diethyl phthalate

INTRODUCTION

Components of the enzymatic link of the antioxidant system of cells play an important role in reducing the intensity of free radical processes in the body under the influence of various exogenous and endogenous factors. Exogenous factors include xenobiotics, among which phthalates are the most common group of synthetic substances widely used as plasticisers in various industries (Smolinska-Kempisty *et al*., 2022; Fiocchetti *et al*., 2021). These substances have toxic properties and, therefore, are capable of disrupting the course of various metabolic pathways in cells with a probable increase in the development of free radical processes in many body systems, which can ultimately lead to various diseases (Goh *et al*., 2022; Diorditsa, 2019).

The most common phthalate used today in cosmetics, pharmaceutical and medical materials as well as the production of plastic tableware and children's toys is diethyl phthalate (DEP). This xenobiotic does not chemically bind to polymeric products in a stable manner, therefore it easily enters the body, having a negative impact on various organs (Cheng *et al*., 2012; Sharma *et al*., 2020).

One of the defence systems of cells is the antioxidant system, the key components of which are the following enzymes: superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9), glutathione *S*-transferase (GST, EC 2.5.1.18), glutathione reductase (GSH-Rd, EC 1.8.1.7) (Diorditsa, 2019). Changes in the functioning of these components will determine the further course of the intensity of free radical processes after the intake of DEP into the body. The questions of the manner of activation or inactivation of antioxidant enzymes in different subcellular structures after the intake of DEP remain open, which will allow for prediction and prevention of free radical oxidation of biomolecules in cells (lipids, proteins, nucleic acids).

The aim of the study was to evaluate the activity of antioxidant enzymes in subcellular fractions of rat liver under the influence of different doses and terms of DEP administration.

MATERIALS AND METHODS

The study was performed on white outbred rats weighing 120–160 g. The animals were kept and all manipulations were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Research and Scientific Purposes (Strasbourg, 1986) and the VII National Congress on Bioethics

"General Ethical Principles for Animal Experiments" (Kyiv, 2019) (the Minutes of the meeting of bioethics commission of ES Institute of Biology, Chemistry and Bioresources, Yuriy Fedkovich Chernivtsi National University No 2 dated September 29, 2023). At the beginning of the experiment, the animals were divided into three groups of 18 rats in each: group I – control, which included intact animals; group II – rats injected with DEP at a dose of 2.5 mg/kg of body weight; group III – rats injected with DEP at a dose of 5.4 mg/kg of body weight. DEP was administered orally for three weeks at doses that reflect the dose levels received by humans, namely 2.5 and 5.4 mg/kg b.w. (Wang *et al*., 2019). Animals were euthanized under light ether anaesthesia on days 14 and 21 after the start of DEP administration. Under chilled conditions, the liver was removed from the animals and the cytosolic and microsomal fractions were isolated (Borschovetska *et al*., 2016). The glucose-6-phosphatase activity was determined as a marker enzyme of the liver microsomal fraction.

In the cytosolic fraction, superoxide dismutase activity was determined by the method (Endröczi, 1990), which is based on the ability of SOD to inhibit adrenaline autooxidation. The absorbance value was measured at 347 nm every 60 seconds for three minutes from the moment when epinephrine was added. Superoxide dismutase activity is expressed in conventional units per 1 minute and mg of protein. Catalase activity was determined by the method described in (Góth, 1991), which is based on the ability of hydrogen peroxide (H_2O_2) to form a stable coloured complex with ammonium molybdate, the intensity of which was assessed spectrophotometrically at 410 nm. Catalase activity was expressed as μmol of peroxi-molybdenum/min per mg of protein. Glutathione peroxidase activity was determined by the increase in the amount of oxidised glutathione (GSSG) at 260 nm. The enzyme activity was expressed in μmol of GSSG/min per mg of protein (Borschovetska et al., 2016).

Glutathione S-transferase activity was determined in both cytosolic and microsomal fractions. The method is based on the ability of reduced glutathione (GSH) to interact with the substrate of 1-chloro-2,4-dinitrobenzenes (CDNB). The optical measurement of the formed GSH-CDNB conjugate was performed at 340 nm every 0.5, 1.5, 2.5 and 3.5 minutes. The enzyme activity was expressed as mmol of GSH-CDNB/min per mg of protein (Tymoshenko *et al*., 2012). The concentration of GSH, which interacts with 5,5′-dithiobis-2-nitrobenzoic acid (Elman's reagent) to form yellow products, was also determined in the cytosolic fraction. The GSH content was expressed as μmol/mg of protein (Salyha, 2013). Protein content was measured by Lowry's method.

Statistical analysis of the results was carried out using Excel software. All data are presented as mean (M) ± standard error (m). Statistical analyses were performed using two-way ANOVA test. A significance level of $P \leq 0.05$ was considered indicative of a statistically significant difference.

RESULTS AND DISCUSSION

The initiation of oxidative stress in liver cells is one of the mechanisms of xenobiotics' toxic effects, which is based on an imbalance in the antioxidant and prooxidant systems. It is the normal functioning of the cellular antioxidant defence system that maintains a safe intracellular concentration of oxidants, which prevents the damaging effects of reactive oxygen species (ROS) on cellular macromolecules (Borschovetska *et al.*, 2016).

One of such components of the antioxidant system is the antioxidant enzyme SOD, which is localised in the cytosol and regulates the first level of protection against oxidative stress by neutralising superoxide anion radical ($O_2^{\cdot-}$).

The results of the studies showed that in the cytosolic fraction of the rat liver under the action of DEP at a dose of 2.5 mg/kg of body weight administered for 14 days, the enzymatic activity of superoxide dismutase increased by 1.6 times compared to that of intact animals (P <0.01) (**Fig. 1**). At the same time, in animals that were administered DEP at a dose of 5.4 mg/kg of body weight for 14 days, SOD activity in the cytosolic fraction of the rat liver increased 2.4-fold compared with the control (P <0.001) (**Fig. 1**).

Presumably, SOD activation occurs in response to the generation of $O_2^{\cdot-}$ under the action of DEP, indicating a shift in the prooxidant-antioxidant balance in liver cells. At the same time, under the action of SOD, $O_2^{\cdot-}$ is neutralised with the formation of molecular oxygen (O_2) and H_2O_2, by oxidation and reduction (Trist *et al*., 2021).

On the other hand, under the influence of ROS, cells can express redox-sensitive genes, in particular SOD genes, which is necessary to protect cells from the toxic effects of DEP.

The administration of DEP for 21 days showed that in animals that were administered a dose of 2.5 mg/kg of body weight, superoxide dismutase activity in the cytosolic fraction continued to be maintained at a high level and was 1.4 times higher than in the control group (P <0.05) (**Fig. 1**).

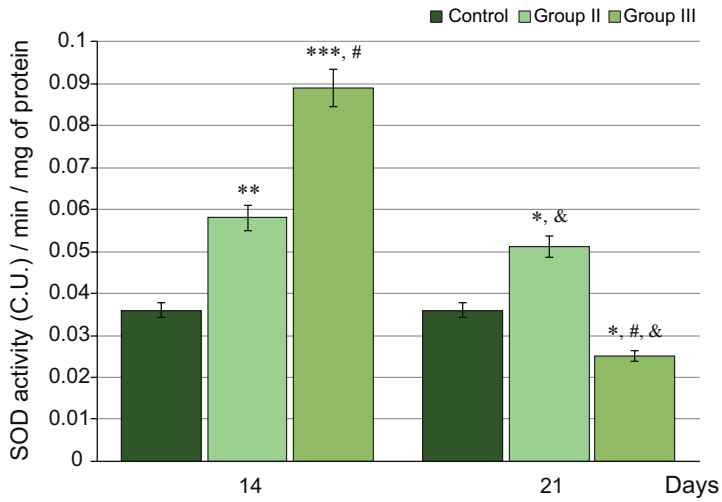

Fig. 1. Superoxide dismutase activity in the cytosolic fraction of rat liver under the influence of diethyl phthalate

Note (hereinafter): Control – intact animals (M±m, n=18); Group II – rats injected with diethyl phthalate at a dose of 2.5 mg per kg of body weight (M±m, n=18); Group III – rats injected with diethyl phthalate at a dose of 5.4 mg/kg of body weight (M±m, n=18); * – P <0.05; ** – P <0.01; *** P <0.001 – compared to the control group; # – statistically significant difference group III compared to group II; & – statistically significant difference compared to 14 days within the same group

When DEP was administered at a dose of 5.4 mg/kg of body weight, a 1.3-fold decrease in the enzymatic activity of SOD was observed compared to that of intact animals (P <0.05) (**Fig. 1**).

The decrease in the enzymatic activity of SOD is probably due to the accumulation of a large amount of H_2O_2, which is released during $O_2^{\cdot-}$ dismutation and is an inhibitor that leads to the inactivation of this enzyme (Zheng *et al*., 2013; Serbin *et al*., 2022).

In the next step, H_2O_2 is utilised by catalases, a family of enzymes that belong to the class of oxidoreductases and catalyse the reaction of H_2O_2 neutralisation to produce H_2O and molecular oxygen. The analysis of the study results showed that when DEP was administered for 14 days at a dose of 2.5 mg/kg and 5.4 mg/kg of body weight, a 1.3-fold ($P < 0.05$) and 1.7-fold ($P < 0.01$) increase in catalase activity in the cytosolic fraction of the rat liver was observed, compared with that of intact animals (**Fig. 2**).

From the obtained results it follows that in the initial stages DEP promotes the activation of CAT, an enzyme that effectively participates in one of the of the stages of antioxidant defence of the body.

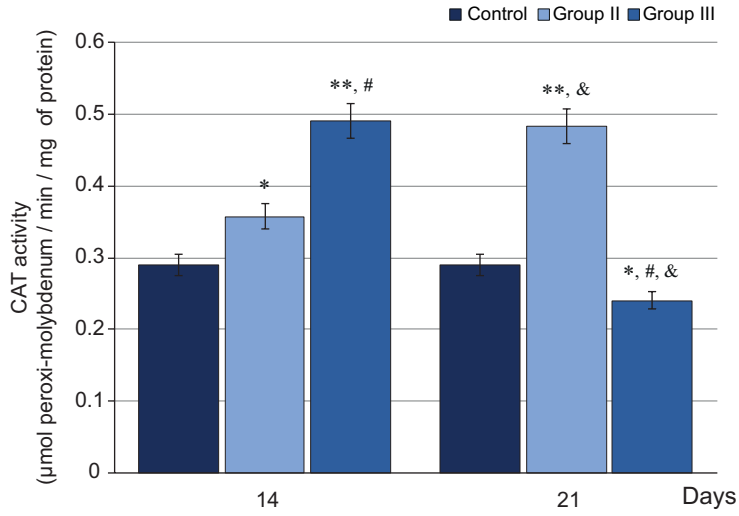

Fig. 2. Enzymatic activity of catalase in the cytosolic fraction of liver of rats under the influence of diethyl phthalate

The administration of DEP is likely to increase the generation of H_2O_2 in liver cells, which actively biotransforms the xenobiotic under study. An increase in the enzymatic activity of CAT may indicate the activation of antioxidant defence in liver cells in response to the initiation of oxidative stress under conditions of fourteen-day exposure to different doses of DEP (Diorditsa, 2019; Cecerska-Heryć *et al*., 2022).

The administration of DEP for 21 days at a dose of 2.5 mg/kg of body weight resulted in the fact that the enzymatic activity of CAT in the cytosolic fraction of the rat liver also remained at a high level and was 1.6 times higher than that of intact animals ($P < 0.01$) (**Fig. 2**). At the same time, in the cytosolic fraction of the liver of rats that were administered DEP at a dose of 5.4 mg/kg of body weight, the activity of CAT decreased by 1.2 times compared with the control ($P < 0.05$) (**Fig. 2**).

A reduced CAT enzymatic activity is likely to be accompanied by a reduced SOD activity, as these two enzymes function as synergists in the antioxidant defence of cells in response to xenobiotics. On the other hand, CAT can be inactivated by free radicals,

namely $O_2^{\cdot-}$. In addition, a decrease in the enzymatic activity of CAT may result from excessive amounts of H_2O_2 in liver cells, which also leads to inactivation of the enzyme (Samuni *et al*., 2016).

In addition to SOD and CAT, the enzymes of the glutathione system, among which GSH-Px and GST should be noted, demonstrate antioxidant properties. GSH-Px is a family of enzymes that belong to oxidoreductases, and their antioxidant functions are associated with the protection of the body against oxidative stress, namely, the neutralisation of H_2O_2, lipid hydroperoxides and organic hydroperoxides to H_2O or alcohol. During its functioning, GSH-Px uses reduced glutathione as a substrate in detoxification reactions (Zhang *et al*., 2020).

The analysis of the results showed that under the influence of DEP for 14 days at a dose of 2.5 mg/kg and 5.4 mg/kg of body weight, glutathione peroxidase activity in the cytosolic fraction of rat liver increased by 1.3 ($P < 0.05$) and 2.3 ($P < 0.001$) times, respectively, compared with that of intact animals (**Fig. 3**).

The increase in glutathione peroxidase activity is probably due to the increased production of H_2O_2 in liver cells under DEP administration, which is primarily metabolised in this organ. The formed H_2O_2, in turn, initiates peroxidation processes, which will lead to the formation of various hydroperoxides. This fact explains the increase in the enzymatic activity of GSH-Px with the subsequent utilisation of H_2O_2 and lipid hydroperoxides (Borschovetska *et al*., 2016).

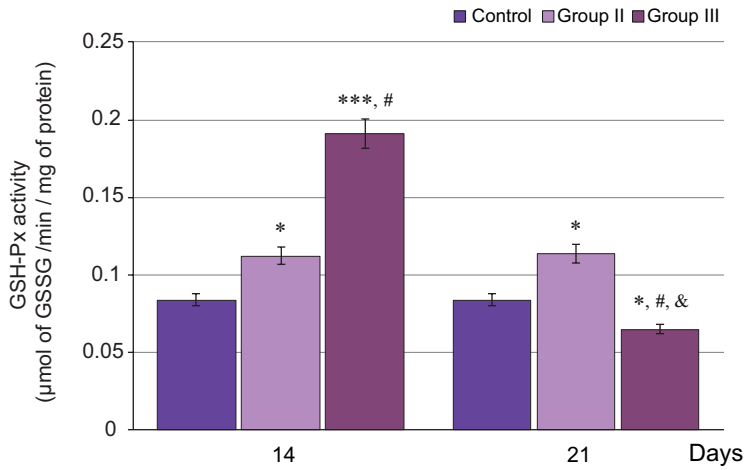

Fig. 3. Enzymatic activity of glutathione peroxidase in the cytosolic fraction of rat liver under the influence of diethyl phthalate

Longer-term administration of DEP at a dose of 2.5 mg/kg did not reduce the enzymatic activity of GSH-Px in the cytosolic fraction of the rat liver, since the studied index was 1.4 times higher than that of the control group on day 21 of the experiment ($P < 0.05$) (**Fig. 3**). At the same time, in the cytosolic fraction of the liver of rats that were administered DEP at a dose of 5.4 mg/kg of body weight, the activity of glutathione peroxidase decreased by 1.3 times compared to the control group ($P < 0.05$) (**Fig. 3**). This fact is likely to be a cellular response to the introduction of DEP, which can enhance

lipid peroxidation (LPO) in cells with subsequent imbalance of the redox state in cells. In addition, the activity of GSH-Px is directly proportional to the GSH content in cells, to which GSH-Px has a high affinity for GSH (Sekhar *et al*., 2022). To test this assumption under the conditions studied, we determined the level of GSH in the cytosolic fraction of rat liver.

The analysis of the results showed that a fourteen-day administration of DEP at a dose of 2.5 and 5.4 mg/kg led to an increase in the concentration of GSH in the cytosolic fraction of the rat liver by 1.5 ($P < 0.01$) and 1.8 ($P < 0.01$) times compared to of intact animals, respectively (**Fig. 4**).

This fact may indicate that GSH synthesis is carried out in the liver, and it acts as a substrate used for conjugation with the administered xenobiotic, as well as for the neutralisation of H_2O_2 and various ROS by-products (Salyha, 2013).

After a three-week administration of DEP, the content of GSH was 2.2 times higher than the control value in the cytosolic fraction of the liver of rats that were administered DEP at a dose of 2.5 mg/kg ($P < 0.001$) (**Fig. 4**). At the same time, in the cytosolic fraction of the liver of rats that were administered DEP at a dose of 5.4 mg/kg, the content of GSH decreased by 1.5 times compared with the control ($P < 0.01$) (**Fig. 4**). This fact indicates the depletion of the GSH pool in liver cells during a prolonged intake of DEP. Another reason for the decrease in intracellular GSH content may be the inactivating effect of DEP on enzymes involved in the reactions of maintaining GSH content in liver cells (Salyha, 2013). Thus, a decrease in the concentration of GSH in liver cells may be associated with its use in conjugation reactions in the second phase of xenobiotic metabolism under the influence of cytosolic and microsomal GST (Dong *et al.,* 2018).

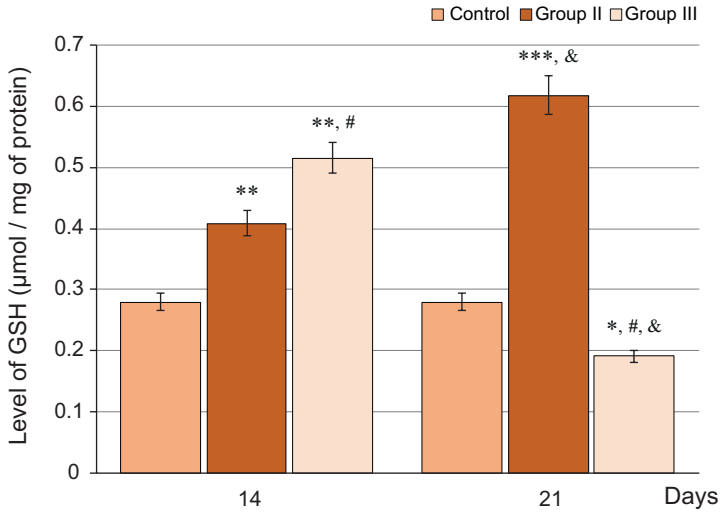

Fig. 4. The content of reduced glutathione in the cytosolic fraction of the liver of rats under the influence of diethyl phthalate

The results of our studies showed that in the microsomal fraction of the rat liver under the influence of DEP both at a dose of 2.5 mg/kg and 5.4 mg/kg, the enzymatic activity of GST increased compared to that of intact animals (**Fig. 5*A***). A similar

increase was detected in the cytosolic fraction (**Fig. 5*B***). Comparison of changes in indicators in groups II and III showed that a statistically significant difference between these groups was found only on the 21st day of DEP administration in both studied fractions (P <0.05) (**Fig. 5**).

The increase in glutathione transferase activity is likely to be a response to the introduction of a xenobiotic into the body. DEP serves as a substrate for conjugation with glutathione (Tymoshenko *et al*., 2012).

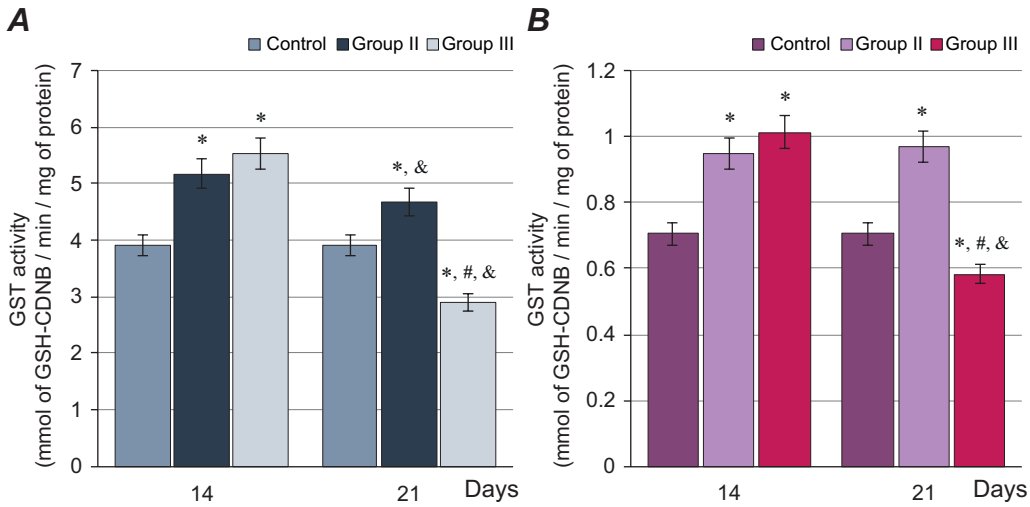

Fig. 5. Enzymatic activity of glutathione *S*-transferase in microsomal (***A***) and cytosolic (***B***) fractions of rat liver under the influence of diethyl phthalate

After three weeks of DEP administration, a decrease in glutathione transferase activity in the microsomal (**Fig. 5*A***) and cytosolic (**Fig. 5*B***) fractions was detected only in those animals that were administered DEP at a dose of 5.4 mg/kg.

Since GST and GSH-Px are among the key enzymes belonging to the glutathione system, their functioning is directly proportional to the content of GSH in cells which serves as a substrate for neutralisation of toxic compounds (Averill-Bates, 2023).

Thus, in both microsomal and cytosolic fractions of the rat liver, the same trend of changes in GST enzyme activity was found: under DEP administration for 14 days, the enzyme activity increased at both doses of DEP; administration of the xenobiotic for 21 days led to a decrease in GST activity at a high dose of DEP. Such a decrease, on the one hand, may be due to a decrease in the pool of endogenous conjugant GSH, and on the other hand, it may be due to free radical oxidation of GST (Tymoshenko *et al*., 2012; Averill-Bates, 2023).

CONCLUSION

Thus, the introduction of different doses of DEP for 14 days into the body of animals leads to the activation of the enzymes of the antioxidant system in liver cells, as indicated by the increase in the enzyme activities of SOD, CAT, GSH-Px, GST in subcellular fractions of the rat liver. The activation is more pronounced with an increase

in the xenobiotic dose. The increase in GSH-Px and GST activities occurs against the background of an increase in intracellular concentration of GSH.

The administration of different doses of xenobiotics for 21 days leads to a multidirectional effect of the latter on the enzymes of the antioxidant system in liver cells. High doses of DEP, namely 5.4 mg/kg of animal body weight, contribute to the inactivation of the studied enzymes and depletion of the GSH pool. However, the administration of DEP at a dose of 2.5 mg/kg of body weight for 21 days was not accompanied by a decrease in the activity of antioxidant enzymes in the cytosolic and microsomal fractions of the rat liver.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights. This article does not contain any studies with human subjects performed by any of the authors.

Animal Studies. All international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [O.V.; M.M.]; methodology, [O.V.; A.O.]; validation, [O.V.; M.M.]; formal analysis, [O.V.; A.O.; M.M.]; investigation, [O.V.; A.O.]; resources, [O.V.; A.O.; M.M.]; data curation, [O.V.; A.O.; M.M.]; writing – original draft preparation, [O.V.; A.O., M.M.]; writing – review and editing, [O.V.; M.M.]; visualization, [O.V.; A.O.; M.M.] supervision, [O.V.; A.O.; M.M.]; project administration, [M.M.]; funding acquisition, [-].

All authors have read and agreed to the published version of the manuscript.

REFERENCES

Averill-Bates, D. A. (2023) The antioxidant glutathione. *Vitamins and Hormones*, 121, 109–141. doi:10.1016/bs.vh.2022.09.002
Crossref ● PubMed ● Google Scholar

Borschovetska, V., & Shmarakov, I. (2016). Activity of hepatic antioxidant system under bisphenol administration and differential supplementation with retinoids. *Biolohichni Systemy*, 8(1), 28–34. doi:10.31861/biosystems2016.01.028 (In Ukrainian)
Crossref ● Google Scholar

Cecerska-Heryć, E., Krauze, K., Szczęśniak, A., Goryniak-Mikołajczyk, A., Serwin, N., Śleboda-Taront, D., Jacek, R., Heryć, R., Michalczyk, A., & Dołęgowska, B. (2022). Activity of erythrocyte antioxidant enzymes in healthy women depends on age, BMI, physical activity, and diet. *Journal of Health, Population and Nutrition*, 41(1), 35. doi:10.1186/s41043-022-00311-z
Crossref ● PubMed ● PMC ● Google Scholar

Cheng, L. J., & Cheng, T. S. (2012). Oxidative effects and metabolic changes following exposure of greater duckweed (*Spirodela polyrhiza*) to diethyl phthalate. *Aquatic Toxicology,* 109, 166–175. doi:10.1016/j.aquatox.2011.10.003
Crossref ● PubMed ● Google Scholar

Diorditsa, Y. (2019). Antioxidant system activity in liver or rats under conditions of acute hepatitis during correction with antioxidant complexes. *Visnyk of Lviv University. Biological Series*, 81, 12–20. doi:10.30970/vlubs.2019.81.02 (In Ukrainian)
Crossref ● Google Scholar

Dong, S.-C., Sha, H.-H., Xu, X.-Y., Hu, T.-M., Lou, R., Li, H., Wu, J.-Z., Dan, C., & Feng, J. (2018). Glutathione *S*-transferase π: a potential role in antitumor therapy. *Drug Design, Development and Therapy,* 12, 3535–3547. doi:10.2147/dddt.s169833
Crossref ● PubMed ● PMC ● Google Scholar

Endröczi, E., Hepp, J., Sasváry, M., Walentin, S., & Lévay, G. (1990). Dipeptidyl peptidase IV (DP IV) and superoxide dismutase activity in thymus-derived lymphocytes: effects of inhibitory peptides and Zn^{2+} *in vitro. Acta Physiologica Hungarica*, 75(1), 35–44.
Google Scholar

Fiocchetti, M., Bastari, G., Cipolletti, M., Leone, S., Acconcia, F., & Marino, M. (2021). The peculiar estrogenicity of diethyl phthalate: modulation of estrogen receptor α activities in the proliferation of breast cancer cells. *Toxics,* 9(10), 237. doi:10.3390/toxics9100237
Crossref ● PubMed ● PMC ● Google Scholar

Goh, C. J. H., Cui, L., Wong, J. H., Lewis, J., Goh, M., Kong, K. W., Yang, L. K., Alfatah, M., Kanagasundaram, Y., Hoon, S., & Arumugam, P. (2022). Diethyl phthalate (DEP) perturbs nitrogen metabolism in *Saccharomyces cerevisiae*. *Scientific Reports*, 12(1), 10237. doi:10.1038/s41598-022-14284-w
Crossref ● PubMed ● PMC ● Google Scholar

Góth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, 196(2-3), 143–151. doi:10.1016/0009-8981(91)90067-m
Crossref ● PubMed ● Google Scholar

Salyha, N. O. (2013). Activity of the glutathione system of antioxidant defense in rats under the action of L-glutamic acid. *The Ukrainian Biochemical Journal*, 85(4), 40–47. doi:10.15407/ubj85.04.040 (In Ukrainian)
Crossref ● PubMed ● Google Scholar

Samuni, U., Czapski, G., & Goldstein, S. (2016). Nitroxide radicals as research tools: elucidating the kinetics and mechanisms of catalase-like and “suicide inactivation” of metmyoglobin. *Biochimica et Biophysica Acta* (*BBA*) – *General Subjects*, 1860(7), 1409–1416. doi:10.1016/j.bbagen.2016.04.002
Crossref ● PubMed ● Google Scholar

Sekhar, K. R., Hanna, D. N., Cyr, S., Baechle, J. J., Kuravi, S., Balusu, R., Rathmell, K., & Baregamian, N. (2022). Glutathione peroxidase 4 inhibition induces ferroptosis and mTOR pathway suppression in thyroid cancer. *Scientific Reports*, 12(1), 19396. doi:10.1038/s41598-022-23906-2
Crossref ● PubMed ● PMC ● Google Scholar

Serbin, A., Strelkova, C., Koval, T., Kharchenko, O., & Andriichuk, T. (2022). Research of indicators of oxidative stress in the kidneys of immature rats with hyperhomocysteinemia. *Bulletin of Taras Shevchenko National University of Kyiv. Series: Biology*, 91(4), 5–9. doi:10.17721/1728.2748.2022.91.5-9 (In Ukrainian)
Crossref ● Google Scholar

Sharma, R., & Kaur, R. (2020). Elucidating physiological and biochemical alterations in giant duckweed (*Spirodela polyrhiza* L. Schleiden) under diethyl phthalate stress: insights into antioxidant defence system. *PeerJ*, 8, e8267. doi:10.7717/peerj.8267
Crossref ● PubMed ● PMC ● Google Scholar

Smolinska-Kempisty, K., Wolska, J., & Bryjak, M. (2022). Molecularly imprinting microfiltration membranes able to absorb diethyl phthalate from water. *Membranes,* 12(5), 503. doi:10.3390/membranes12050503
Crossref ● PubMed ● PMC ● Google Scholar

Trist, B. G., Hilton, J. B., Hare, D. J., Crouch, P. J., & Double, K. L. (2021). Superoxide dismutase 1 in health and disease: how a frontline antioxidant becomes neurotoxic. *Angewandte Chemie International Edition*, 60(17), 9215–9246. doi:10.1002/anie.202000451
Crossref ● PubMed ● PMC ● Google Scholar

Tymoshenko, M. O., Kravchenko, O. O., Gaida, L. M., Lynchak, O. V., Ruzhytska, N. I., & Ostapchenko, L. I. (2012). Glutathione transferase activity and reduce glutathione content in the cytosol of rat gastric mucosa cells under carcinogen N-methyl-N′-nitro-N-nitrosoguanidine treatment. *Biopolymers and Cell*, 28(5), 374–380. doi:10.7124/bc.000073
Crossref ● Google Scholar

Wang, Y., Zhu, H., & Kannan, K. (2019). A review of biomonitoring of phthalate exposures. *Toxics*, 7(2), 21. doi:10.3390/toxics7020021
Crossref ● PubMed ● PMC ● Google Scholar

Zhang, Y., Roh, Y. J., Han, S.-J., Park, I., Lee, H. M., Ok, Y. S., Lee, B. C., & Lee, S.-R. (2020). Role of selenoproteins in redox regulation of signaling and the antioxidant system: a review. *Antioxidants*, 9(5), 383. doi:10.3390/antiox9050383
Crossref ● PubMed ● PMC ● Google Scholar

Zheng, Q., Feng, M., & Dai, Y. (2013). Comparative antioxidant responses in liver of *Carassius auratus* exposed to phthalates: an integrated biomarker approach. *Environmental Toxicology and Pharmacology*, 36(3), 741–749. doi:10.1016/j.etap.2013.07.008
Crossref ● PubMed ● Google Scholar

СТАН КОМПОНЕНТІВ ФЕРМЕНТАТИВНОЇ ТА НЕФЕРМЕНТАТИВНОЇ ЛАНОК АНТИОКСИДАНТНОГО ЗАХИСТУ В СУБКЛІТИННИХ ФРАКЦІЯХ ПЕЧІНКИ ЩУРІВ ЗА ДІЇ ДІЕТИЛФТАЛАТУ

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Вступ. Антиоксидантна система – одна із захисних систем клітин, зміни функціонування якої будуть визначати подальший перебіг інтенсивності вільнорадикальних процесів після надходження в організм ксенобіотиків. Серед ксенобіотиків чільне місце належить фталатам, зокрема, діетилфталату (ДЕФ) – найпоширенішій групі синтетичних речовин, котрі широко застосовують у різних галузях як пластифікатори.

Матеріали та методи. Для проведення серії експериментів використовували білих безпородних щурів, із печінки яких виділяли цитозольну та мікросомну фракції. У цитозольній фракції визначали активності таких антиоксидантних ензимів як супероксиддисмутази (SOD, EC 1.15.1.1), каталази (CAT, EC 1.11.1.6), глутатіонпероксидази (GSH-Px, EC 1.11.1.9), глутатіон-*S*-трансферази (GST, EC 2.5.1.18), а також концентрацію відновленого глутатіону (GSH). У мікросомній фракції досліджували активність GST.

Результати. Введення різних доз ДЕФ протягом 14-ти діб сприяло активації антиоксидантних ензимів незалежно від дози введення ксенобіотика. Застосування ДЕФ протягом 21-ї доби призводило до різноспрямованої дії ксенобіотика на ензими антиоксидантної системи в клітинах печінки, оскільки після введення ДЕФ у дозі 5,4 мг/кг маси тіла тварин спостерігали інактивацію досліджуваних ензимів та виснаження пулу GSH, а після введення ДЕФ у дозі 2,5 мг/кг маси активність

антиоксидантних ензимів у субклітинних фракціях печінки залишалася на високому рівні, порівняно з контролем. Як у мікросомній, так і в цитозольній фракціях печінки щурів виявлено однакову тенденцію зміни ензимної активності GST: за умови введення ДЕФ протягом 14-ти діб активність ензиму підвищувалася за дії обох досліджуваних доз; введення ксенобіотика протягом 21-ї доби призводило до зниження активності GST за введення високої дози ДЕФ.

Висновки. Активація ензимів антиоксидантної системи відбувається у відповідь на нетривале надходження ДЕФ в організм. Зі збільшенням дози й терміну введення досліджуваного ксенобіотика виявлено інактивацію антиоксидантних ензимів.

Ключові слова: антиоксидантні ензими, цитозоль, мікросомна фракція, печінка, діетилфталат

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