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FATTY ACID PROFILE OF THE LIVER LIPIDS UNDER ACUTE FUNGICIDE ACTION AND INTAKE OF A BIOLOGICALLY ACTIVE PREPARATION IN RATS

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The widespread use of fungicides in agriculture leads to a negative consequences for both humans and the environment. The acute effect of fungicides containing tebuconazole alone or in combination with triadimefon on fatty acid (FAs) composition of the total lipids in the liver of rats was studied. FAs content was analyzed by gas chromatography with the use of flame ionization detector. The obtained results indicate that the acute effect of triazole fungicides one-time oral administration to rats is characterized by toxic reactions and is accompanied by the total liver lipids FAs profile redistribution, similar for the studied fungicides. In particular, the reduction in the total content of monounsaturated FAs (by 26% – 37%) and in the ω3/ω6 ratio was observed. It was demonstrated that the subsequent two-week introduction of the preparation based on Milk thistle seeds oil extract had a positive effect and improved the FAs profile of lipids in dynamics after fungicide intake.

K e y w o r d s: fatty acids, rat liver lipids, fungicides, triazoles, Milk thistle seeds extract.

Pesticides are the versatile group of chemicals which are widely used as plant protection agents. However, this increases the environmental and health risks associated with their use [1]. Pesticides, in particular, can accumulate in agricultural products with which they enter human and animal organisms. Similarly, pesticide-contaminated water and air have adverse effects on humans. The most numerous and important group of modern fungicides – are triazoles, systemic pesticides, used to protect plants from a wide range of fungal diseases. However, representatives of this group, in particular tebuconazole, are often detected in elevated concentrations in the environment.

Current data indicate diverse toxic effects of tebuconazole in vertebrates. In fact, the search for potential biochemical targets of pesticide toxicity is relevant today [2]. Thus, the study of the nature and mechanisms of biochemical action of tebuconazole fungicides is important as a typical example for assessing toxicity to vertebrates, including humans. Hematotoxic action is shown in acute intoxication of rats with tebuconazole [3]. Hepatotoxicity is also observed with signs of hepatocyte dystrophy [4]. In addition, studies of tebuconazole accumulation in HepG2 cells and associated lipid metabolic disorders led to suggestion that human exposure to tebuconazole may be a risk factor for the development of nonalcoholic fatty liver disease [5].

It is therefore important to study the composition of lipid fatty acids (FAs) in various tissues to understand the mechanism of tebuconazole toxicity, in particular, which is related to its effect on lipid metabolism. Lipid composition plays an important role in controlling the direction and functioning of various cellular processes. Moreover, modification of lipid FAs profile can accompany and characterize the effect of exogenous factors on organism [6], including pesticides [7]. On the other hand, the study of the possibility of regulating the FAs profile of rat liver lipids under the influence of pesticides in combination with a biologically active preparation contributes to the correction of metabolic abnormalities, which is important in the development of a set of measures to prevent the negative impact of pesticides on public health.

The aim of the current research was to study the toxic manifestations and fatty acid composition of the liver lipids in rats under acute exposure to triazole fungicides containing tebuconazole separately and in combination with triadimefon and the possibility of correction with biologically active preparation “OVA+”.

Material and Methods

Systemic fungicides of various pesticide formulations Azimuth and Azimuth Classic (manufactured by “Frandesa Ukraine”) in preparative form - emulsifiable concentrate (EC), which are registered in Ukraine and used to protect agricultural crops, were studied. Their stock solutions contained active ingredients in the following concentrations in Azimuth: tebuconazole, 125 kg/m^3 + triadimefon, 100 kg/m^3 and in single-component fungicide Azimuth Classic: tebuconazole 250 kg/m^3. The active ingredients of fungicides belong to the chemical class of triazoles. The patented preparation “OVA+” (production of NULES Ukraine) is obtained according to [8] by extracting substances from the seeds of Milk thistle (*Silybum marianum*) with corn oil, which are collected from an annual plant. Milk thistle seeds are a source of various biologically active substances: flavolignans, flavonoids, as well as vitamins, tannins, macro- and microelements, etc. It has a potential choleretic effect, stabilizes the functioning of the liver and digestive system, promotes the elimination of toxins from the body.

Female Wistar Han rats weighing 210-240 g, used in the studies, were kept under standard vivarium conditions of the State Institution “Institute of Occupational Medicine of the National Academy of Medical Sciences of Ukraine” under conventional temperature and light conditions. Rats were divided into four groups: control (Group I) and 3 experimental (Groups II, III, IV) of 7 animals each. Animals of Group II were given via gavage once orally 1200 mg/kg of an aqueous emulsion of the Azimuth fungicide (contained tebuconazole in combination with triadimefon). Animals of Groups III and IV were administered via gavage once orally 1200 mg/kg of an aqueous emulsion of the Azimuth Classic fungicide (contained tebuconazole). The control group (Group I) received an equivalent volume of distilled water. The rats of Group IV were orally given 4 drops (about 0.1 ml) of oily liquid of the “OVA+” preparation twice a day for each animal over the next 13 days. The used fungicide dose (1200 mg/kg), which is 1/4 LD_{50}, was calculated from the LD_{50} values for the active ingredients (reference data).

Toxicological studies were conducted in accordance with the principles and criteria set out in [9]. Toxic manifestations in animals were visually evaluated during the entire period of research, in particular, toxic reactions were determined and the beginning of the recovery period was noted. After 14 days the animals were decapitated under light ether anesthesia and the liver was used in research. Animal manipulations were carried out following the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), according to the general ethical principles of experiments on animals, adopted by the First National Congress of Ukraine on Bioethics (2001).

The liver tissue was homogenized and the lipids were subsequently extracted with a chloroform-methanol mixture to determine the fatty acid composition of total lipids. Hydrolysis and methylation of lipid FAs carried out according to [10]. Gas chromatographic analysis of FAs methyl esters was performed on a Trace GC Ultra gas chromatograph (USA) with a flame ionization detector. Experiment conditions: column temperature 140-240°C, detector temperature 260°C, analysis duration 65 min. Fatty acids were identified using a Supelco 37 Component FAME Mix standard. To quantify individual FAs the method of internal normalization was used and the relative content of FAs was expressed as a percentage of their total amount. Besides, the total quantities of saturated fatty acids (SFAs), unsaturated fatty acids (UFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), FAs of the ω3 and ω6 families were calculated.

The data were analyzed by computer software Origin 6.0 and Excel (Microsoft, USA) using Student’s *t*-test. Differences were considered significant when $P < 0.05$.

Results and Discussion

Studies on rats are carried out to assess the toxicity of chemicals used in industry and agriculture, as well as to establish the mechanisms of their toxic action [11]. With acute exposure to fungicides of the triazole group, toxic manifestations in rats were observed from the first hours after administration, which were most intense in the first four hours after administration, partially persisted until the second day and disappeared by the end of the study. Among the manifestations of toxic effects on animals reduced activity, depression, disheveled hair, ataxia, humpback posture, untidy appearance, constriction of the eye, salivation, respiratory failure, decreased appetite, etc were noted. The obtained results indicate a pronounced toxic effect of fungicides containing tebuconazole both individually and in combination with triadimefon at their acute action at a dose of 1200 mg/kg.

The fungicide toxic action is mediated by the processes of absorption and metabolism in the organism, which determines the involvement of the lipid component of cells. Important cellular structural and energy compounds - FAs play a significant role not only in metabolic processes, but also mediate protective reactions of the body under the action of exogenous factors [6, 12].

Chromatograms of FAs of rat liver total lipids were obtained in our researches. In the studied samples 21 short-, medium- and long-chain FAs with carbon chain length from C8 to C22 were identified. The main FAs and their relative content in total lipids of rat liver are shown in Table 1.

Minor components, the content of which did not exceed 0.1 %, are not presented in the Table. SFAs are mainly represented by palmitic (C16:0) and stearic (C18:0) acids, which are precursors of long-chain FAs. UFAs are heterogeneous, in particular, palmitoleic (C16:1) and oleic (C18:1ω9) have one saturated bond each, and such as arachidonic (C20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (C22:6ω3) are polyunsaturated.

The FAs composition of total lipids in rat liver tissue after fungicide administration did not differ qualitatively from the control. However, there was a similar redistribution of the content of both SFAs and UFAs for fungicides containing tebuconazole both individually and in combination with triadimefon (Table 1). Opposite changes in SFAs content, especially in the major palmitic (C16:0) and stearic (C18:0) acids, should be noted. Thus, the content of palmitic acid decreased by 28% and 27%, and the

Table 1. Fatty acid content of total lipids (% of total fatty acids) in rat liver tissues after acute peroral fungicide administration and preparation "OVA+" (M ± m, n = 7)

Fatty acid	Group I (control)	Group II	Group III	Group IV
C10:0 Capric acid	0.20 ± 0.02	0.14 ± 0.01*	0.09 ± 0.01*	0.20 ± 0.02
C14:0 Myristic acid	0.62 ± 0.05	0.28 ± 0.04*	0.33 ± 0.02*	0.62 ± 0.02
C15:0 Pentadecanoic acid	0.27 ± 0.02	0.23 ± 0.02	0.30 ± 0.03	0.25 ± 0.01
C16:0 Palmitic acid	21.79± 1.61	15.62 ± 1.10*	15.92 ± 1.21*	18.63 ± 1.32
C16:1 Palmitoleic acid	0.97 ± 0.07	0.42 ± 0.03*	0.30 ± 0.02*	0.59 ± 0.02
C17:0 Margaric acid	0.69 ± 0.04	0.62 ± 0.06	0.68 ± 0.05	0.57 ± 0.03
C17:1	0.12 ± 0.02	0.12 ± 0.02	0.14 ± 0.02	0.13 ± 0.02
C18:0 Stearic acid	16.74 ± 1.41	22.58 ± 1.76*	21.62 ± 1.88*	19.61 ± 1.12
C18:1ω9 Oleic acid	8.69 ± 0.41	6.72 ± 0.32*	5.80 ± 0.28*	7.35 ± 0.52
C18:2ω6 Linoleic acid	15.23 ± 1.21	15.20 ± 1.11	14.09 ± 1.04	14.93 ± 1.01
C20:0 Arachidic Acid	0.94 ± 0.06	0.27 ± 0.02*	0.32 ± 0.02*	1.03 ± 0.03
C18:3ω3 Linolenic acid	0.64 ± 0.04	0.52 ± 0.03*	0.56 ± 0.03	0.43 ± 0.02*
C21:0 Heneicosanoic acid	1.02 ± 0.04	0.76 ± 0.05*	0.52 ± 0.04*	0.90 ± 0.03
C22:0 Behenic acid	0.40 ± 0.02	0.54 ± 0.02*	0.64 ± 0.03*	0.43 ± 0.02
C20:3ω6 Eicosatrienic acid	1.08 ± 0.09	1.46 ± 0.06*	1.40 ± 0.03*	0.98 ± 0.05
C20:4ω6 Arachidonic acid	18.36 ± 1.11	23.57 ± 1.21*	25.94 ± 1.12*	21.00 ± 1.02
C20:5ω3 Eicosapentaenoic acid	0.90 ± 0.05	0.80 ± 0.03	0.63 ± 0.04*	1.00 ± 0.03
C22:5ω3 Docosapentaenoic acid	0.71 ± 0.03	0.67 ± 0.04	0.65 ± 0.02	0.67 ± 0.03
C22:6ω3 Docosahexaenoic acid	10.57±0.91	9.37±0.9	10.0±0.89	10.60±0.88

Note: in this and next table rats received fungicides: Group II – Azimuth (contained tebuconazole in combination with triadimefon), Groups III, IV – Azimuth Classic (contained tebuconazole) and preparation "OVA+" (Group IV). *$P < 0.05$ vs control content of stearic acid increased by 35 and 29% relative to the control in Groups II and III, respectively. At the same time, the total content of SFAs did not change significantly, and saturation index (SFAs/UFAs), which in control was 0.74, decreased to 0.68 and 0.70 (Table 2).

Moreover, analysis of FAs profile of total lipids indicates the decrease in the MUFAs content. In particular, the content of oleic acid (C18:1ω9), which can exhibit antioxidant properties [13], is reduced on average by 23% in Group II and 33% in Group III compared to control (Table 1). At the same time, MUFAs total content decreased significantly by 26% in Group II and 37% in Group III relative to the control (Table 2). It is known that ω9 FAs can partially replace those of the ω3 and ω6 families in lipids during the action of stress factors [12].

On the other hand, the PUFAs redistribution occurred in liver total lipids under acute fungicide action. In this case their total content increased insignificantly (Table 2). In ω3 FAs family the tendency to a decrease is observed in the content of C18:3ω3 and biologically active long-chain ω3 FAs (C20:5ω3 and C22:5ω3), which are characterized by the functions of an adaptive stabilizer of the cellular lipid bilayers [14].

As for the ω6 FA family, there was a clear upward trend in their total content, which increased by 16 and 20% compared to the control group in Groups II and III, respectively (Table 2). A significant differences were shown for a number of the ω6 family representatives. For the main constituent of the ω6 family, arachidonic acid (C20:4ω6), a significant increase by 28% and 41% was also found in groups II and III, respectively (Table 1). The accumulation of this ω6 FA, which, along with other reasons, is due to interaction abnormalities of the of FAs with membrane protein complexes, with proteins of exo- and endocinosis resulted from the modification in acute toxicity conditions, in turn can lead to an increase in the content of prostaglandins in cells [15].

Thus, fungicide delivery into the rat organism (Groups II and III) led to a redistribution of the relative content of FAs of the ω3, ω6 and ω9 families in total lipids of liver tissues. The revealed decrease in ω3/ω6, ω9/ω3 and ω9/ω6 ratios (Table 2) specifies the direction of FAs metabolism of liver lipids in view of the interconnection of these processes and the existence of competitive synthesis pathways in the metabolic process [16]. It is important to maintain the ratio ω3/ω6, since fatty acids of the ω6 and ω3 families are endogenous bioregulators, and therefore physiologically important for the animal organism [15]. Derivatives of ω6 FAs are highly active regulators of cellular functions that enhance membrane permeability and cause inflammatory processes. On the other hand, ω3 FAs metabolites which are antiag-

Table 2. The level of fatty acid saturation of total lipids (% of the total fatty acids) in the rat liver tissues after acute oral administration of fungicides and preparation "OVA+" (M ± m, n = 7)

ΣFAs	Group I (control)	Group II	Group III	Group IV
Σ SFAs	42.6 ± 3.2	41.0 ± 3.1	40.4 ± 3.3	42.2 ± 3.4
ΣUFAs	57.3 ± 4.1	58.9 ± 4.1	59.5 ± 4.2	57.7 ± 3.9
SFAs /UFAs	0.74	0.70	0.68	0.73
ΣMUFAs	9.8 ± 0.8	7.3 ± 0.5*	6.2 ± 0.4*	8.1 ± 1.1
ΣPUFAS	47.5 ± 3.2	51.6 ± 4.1	53.3 ± 4.2	49.6 ± 3.2
Σω6	34.7 ± 1.8	40.2 ± 2.1	41.8 ± 2.0*	36.9 ± 1.9
Σω3	12.8 ± 0.9	11.4 ± 1.0	11.8 ± 1.1	12.7 ± 1.0
Σω3/ Σω6	0.37	0.28	0.29	0.34
C20:4ω6/C18:2ω6	1.21	1.55	1.84	1.41
C22:6ω3/C18:3ω3	16.5	18.0	17.9	24.7
ω9/ω3	0.68	0.59	0.53	0.59
ω9/ω6	0.28	0.18	0.15	0.22

Note: FAs – fatty acids, SFAs – saturated fatty acids, UFAs – unsaturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids. *$P < 0.05$ vs control gregants, anti-inflammatory substances, contribute to the membrane stabilization [12].

It should be noted that modulation of the lipid PUFAs content can be associated with the processes of desaturation and elongation of fatty acids. It is known that adaptive changes in the degree of FAs unsaturation (ω3 and ω6) can occur through the participation of acyl-lipid Δ3- and Δ6-desaturases [17]. Changes in their activity can be seen by changes of the unsaturation indices (ratios C22:6ω3/C18:3ω3 and C20:4ω6/C18:2ω6). The increase in the C20:4ω6/C18:2ω6 ratio, which characterizes the activity of acyl-lipid Δ6-desaturase, is shown for Groups II and III (Table 2).

Thus, after administration of tebuconazole-containing fungicides, both individually and in combination with triadimefon, an imbalance in the PUFAs ratio is observed in total lipids of rat liver, which may reflect the direction of FAs metabolism.

Subsequently, the preparation "OVA+" was used to correct changes in the FAs content after tebuconazole action, which, along with other biologically active compounds, includes FAs [8]. Under experimental conditions (Group IV), the content of most individual FAs was stable or approaching the control level (Table 1). In particular, the content of the main SFAs: palmitic (C16:0) and stearic (C18:0) did not differ significantly from the control, as did the content of monounsaturated oleic (C18:1ω9) acid. The content of FAs of the ω3 and ω6 families stabilized at the control level. No differences were observed in the total content of SFAs, UFAs, FAs of the ω3 and ω6 families compared to the control (Table 2). Simultaneously, the decrease in the content of linolenic acid (C18:3ω3) and corresponding increase by 1.5 times of the ratio C22:6ω3/C18:3ω3 compared to the control reflect the activation of FAs metabolism of ω3 family in Group IV. Thus, the consumption of the preparation "OVA+" by animals that received the fungicide contributed to the normalization of the FAs composition of total liver lipids.

Analyzing the obtained results of the effect of triazole fungicides on rats, it should be noted that the greatest toxic manifestations occur in the first two days after their administration at a dose of 1200 mg/kg. Moreover, it is established that the administration of fungicides containing tebuconazole alone or together with triadimefon leads to similar modifications in the relative content of FAs in total lipids in the liver of rats. Taking into account the obtained results and scientific data [4], indicating the morphological manifestations of tebuconazole intoxication in female rats, characterized by signs of hepatocyte dystrophy, in particular, fatty degeneration and the appearance of signs of hepatic parenchyma necrosis, the own results may reflect the development of inflammatory or destructive processes. In this case, the impairment of FAs metabolism in the lipids of the liver tissue obviously acts as a causal mechanism for the formation of the syndrome of endogenous intoxication in the period after the fungicidal load of the animal organism. The established increase in the content of pro-inflammatory FAs of the ω6 family relative to anti-inflammatory fatty acids of the ω3 family in our researches can affect the manifestation of pathological changes in liver tissues under the fungicide load of animals, in particular due to changes in the functional activity of cell membranes [18].

The use of the preparation "OVA+", rich in biologically active substances, can cause an effect on FAs metabolism and have a positive effect in maintaining the FAs profile of lipids in dynamics after fungicide intake. Besides, the increase in ω3 FAs metabolism, occurred under these conditions, possibly prevents the involvement of ω9 acids in the synthesis of lipids (the content of C18:1ω9 is close to that in animals of the control group) due to inhibition of gene transcription of other enzymatic proteins in the liver [12].

Thus, studying the role of fatty acids in tissue lipids is important for understanding the biochemical mechanisms of adverse effects of pesticides on mammals and for finding ways to protect and repair tissues under pesticide load.

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ЖИРНОКИСЛОТНИЙ ПРОФІЛЬ ЛІПІДІВ ПЕЧІНКИ ЩУРІВ ЗА ГОСТРОГО ВПЛИВУ ФУНГІЦИДІВ ТА ВВЕДЕННЯ БІОЛОГІЧНО-АКТИВНОГО ПРЕПАРАТУ

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Широке використання фунгіцидів у різних сферах життєдіяльності людини призводить до негативних наслідків як для людини, так і довкілля. Метою роботи було дослідити жирнокислотний склад загальних ліпідів тканин печінки щурів за гострого впливу двох фунгіцидів: однокомпонентного, що містить тебуконазол та двокомпонентного – тебуконазол разом із триадимефоном. Аналіз метилових етерів жирних кислот (ЖК) проводили на газовому хроматографі з полум'яно-іонізаційним детектором. Одержані результати свідчать, що гостра дія триазольних фунгіцидів за одноразового перорального введення характеризується токсичними реакціями і супроводжується перерозподілом жирнокислотного профілю загальних ліпідів печінки, подібним для досліджуваних фунгіцидів. Встановлено зниження сумарного вмісту мононенасичених ЖК (на 26-37%) та співвідношення ω3/ω6. Показано, що подальше введення протягом двох тижнів препарату на основі олійного екстракту насіння розторопши плямистої покращувало жирнокислотний профіль ліпідів за дії фунгіцидів.

К л ю ч о в і   с л о в а: жирні кислоти, ліпіди печінки щурів, фунгіциди, триазоли, екстракт плодів розторопші плямистої.

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