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MANAGEMENT OF OXIDATIVE STRESS IN ANIMALS USING PREPARATION BASED ON *TRAMETES PUBESCENS*

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It has been determined that exposure to different stressors is associated with the development of oxidative stress, both in animals and in humans. The antioxidant defense system resists the development of oxidative stress. Basidiomycetes are the producers of a variety of biologically active compounds, including triterpene acids, exopolysaccharides, etc. Products derived from these species have immunostimulating, antitumor, antioxidant, antimetastatic action, and are effective for liver diseases treatment. The aim of the study, was to determine the antioxidant activity of Trametin to limit the stress-induced lipid peroxidation process. Antioxidant properties of Trametin (a product obtained by liquid-phase cultivation of the xylotroph fungus Trametes pubescens) were studied under experimental stress (decrease of the lighting intensity in piglets). It has been found that Trametin serves as an effective prevention of oxidative stress in stress conditions. It increases the level of unsaturation of lipids, reduces the concentration of primary and end products of lipid peroxidation, and also increases the total antioxidant activity of the blood, the activity of superoxide dismutase and reduces the glutathione level. Thus, the study of the effect of the preparation obtained by liquid-phase fermentation from fungus xylotroph of the genus Trametes pubescens showed a pronounced antioxidant effect on the model of dark stress in experimental animals, which can serve as a basis for its further application in veterinary and clinical medicine.

Key words: *oxidative stress, antioxidants, stress, animals*

КОРРЕКЦИЯ ОКИСЛИТЕЛЬНОГО СТРЕССА У ЖИВОТНЫХ С ИСПОЛЬЗОВАНИЕМ ПРЕПАРАТА НА ОСНОВЕ *TRAMETES PUBESCENS*

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Установлено, что воздействие различных стрессоров связано с развитием окислительного стресса, как у животных, так и у людей. Система антиоксидантной защиты противостоит развитию окислительного стресса. Basidiomycetes являются производителями различных биологически активных соединений, в том числе триполиэфирных кислот, экзополисахаридов и т.д. Продукты, полученные из этих видов, обладают иммуностимулирующим, противоопухолевым, антиоксидантным, антиметастатическим действием, эффективны при заболеваниях печени. Цель исследования заключалась в том, чтобы определить антиоксидантную активность траметина для ограничения вызванного стрессом перекисного окисления липидов. В условиях экспериментального стресса (снижение интенсивности света у поросят) изучались антиокислительные свойства траметина (продукт, полученный путём жидкофазного культивирования грибов-ксилотрофных Trametes pubescens). Было обнаружено, что траметин способствует эффективной профилактике окислительного стресса при стрессе. Он увеличивает уровень ненасыщенности липидов, уменьшает концентрацию первичных и конечных продуктов перекисного окисления липидов, а также увеличивает общую антиоксидантную активность крови, активность супероксиддисмутазы и снижает уровень глутатиона. Таким образом, изучение влияния препарата, полученного путём жидкофазной ферментации грибов-ксилотрофов рода Trametes pubescens, показало выраженный антиоксидантный эффект на модели темнового стресса у подопытных животных, что может служить основой для его дальнейшего применения в ветеринарной и клинической медицине.

Ключевые слова: *окислительный стресс, антиоксиданты, стресс, животные*

BACKGROUND

It is determined that exposure to different stressors is associated with oxidative stress development, both in animals and in humans [6, 7, 8, 11, 23]. The antioxidant defense system resists the development of oxidative stress [14, 15, 16]. Antioxidants include substances that neutralize aggressive radicals that cause the destruction or damage of cell membranes, nucleic acids and other vital structures and lead to cell death [17]. Decreased activity of antioxidant protective factors leads to weakening of antioxidant capacity in the body and lipid peroxidation activation and accumulation of toxic products [18, 19]. For determination of the antioxidant effect of any preparations, it is necessary to model the oxidative stress, since under normal redox status of a model system or all organism, this effect is very difficult to detect. This general methodological approach has long been used in experimental and clinical medicine [8, 11, 20, 21].

The search for universal, preventive preparation that increases resistance to a variety of adverse exogenous and endogenous factors of the environment is important [12, 16, 20, 21]. There are natural antioxidants (tocopherol, ascorbic acid, gallic acid esters, guaiac acid, etc.) and synthetic antioxidants (butyloxyanisole, butyloxytoluene, dodecylgallet, santohin, diludin, dibug, finozanic acid, etc.) [2, 6].

The wood-destroying fungi synthesize antioxidant substances as well. Basidiomycetes are producers of a variety of biologically active compounds, including triterpene acids, exopolysaccharides, etc. Products derived from these species have immunostimulating, antitumor, antioxidant, antimetastatic action, are effective for liver diseases treatment [1, 3, 4, 5]. We suggested that the product obtained by fermentation of a liquid phase-type xylotroph fungus *Trametes pubescens*, may be a promising redox regulator. **The aim** of the study was to determine the antioxidant activity of Trametin preparation to limit the stress-induced lipid peroxidation process.

MATERIALS AND METHODS

This experiment was carried out at the research farm of Irkutsk State Agricultural University in the village of Oek (Irkutsk region). The models were two-month piglets of Landrace breed hybrids with Yorkshire (n = 20) (hereinafter – animals). As a stress factor we used the disturbance of the light regime of the growing animals, reducing light intensity to 10 % of the normal level throughout the experiment. The animals were divided into 2 groups: intact animals (control group, n = 10) and animals treated with Trametin (experimental group, n = 10). All animals were kept on a standard diet. Trametin is a liquid-phase cultivation product of xylotroph fungus *T. Pubescens Pilat* strain 0663 (collection of the RAS Botanical Institute). It contains more than 100 types of different compounds (terpenes, aliphatic aldehydes, alcohols, organic acids and a number of others) [5]. This product was given with mixed feed at a dose of 18 grams per day (for 10 days). The blood was taken from the auricular vein in the morning before feeding. The material of this study was blood serum and erythrocytes (hemolysate).

The intensity of the lipid peroxidation processes (LPO) was determined by content products – diene conjugates (DC), ketodienes, conjugated trienes (KD-ST), and TBA-active products (TBARS) [9, 24]. The state of the antioxidant defense system (AOD) was estimated by total antioxidant activity (TAA) of blood [13], the content of α-tocopherol and retinol [2], superoxide dismutase (SOD) activity [22], reduced and oxidized glutathione (GSH and GSSG) [10]. We used spectrofluorometer Shimadzu RF-1501 (Japan) and a spectrophotometer Shimadzu RF-1650 (Japan). The study was performed in accordance with the ethical standards for experimental animals care and use – the European Convention for the Protection of Vertebrate Animals used for experimental and scientific purposes (1986), Guidelines on work with of experimental animals (Order of the Ministry of Health of the USSR N 775 as of August 12, 1977), Manual on experimental (preclinical) study of new pharmacological substances (2005), and the Rules of good laboratory practice (Order of the Ministry of Health of the Russian Federation as of August 23, 2010, N 708n). To analyze the results we used STATISTICA 6.1 Stat-Soft Inc, USA. We used visual and graphic approach and criteria for the consent of Kolmogorov – Smirnov with correction of Lilliefors and Shapiro – Wilk to determine the normal distribution. Testing of total equality variances was performed using Fisher's exact test (F-test). The data was presented as the mean (M) and the variance (σ). In the analysis of intergroup differences we used parametric (Student's t-test) and non-parametric (Mann – Whitney U-test). The significance was considered to be 5 % (0.05).

RESULTS AND DISCUSSION

Analysis of the effect of the test preparation on the intensity of LPO showed that Trametin has a positive effect on oxidation of fatty acids in serum lipids, which is seen from the higher level (2.62 times higher in comparison with the control) of double bonds in the experimental group (Fig. 1). The preparation produced marked effect on the content of the primary LPO products (CD): CD content decreased significantly by 2.08 times in comparison with the control (Fig. 1).

Trametin did not affect the concentration of the secondary LPO intermediates (KD and CT) (p > 0.05). Trametin intensively reduced the content of TBA-active LPO end products (by 1.81 times in comparison with the

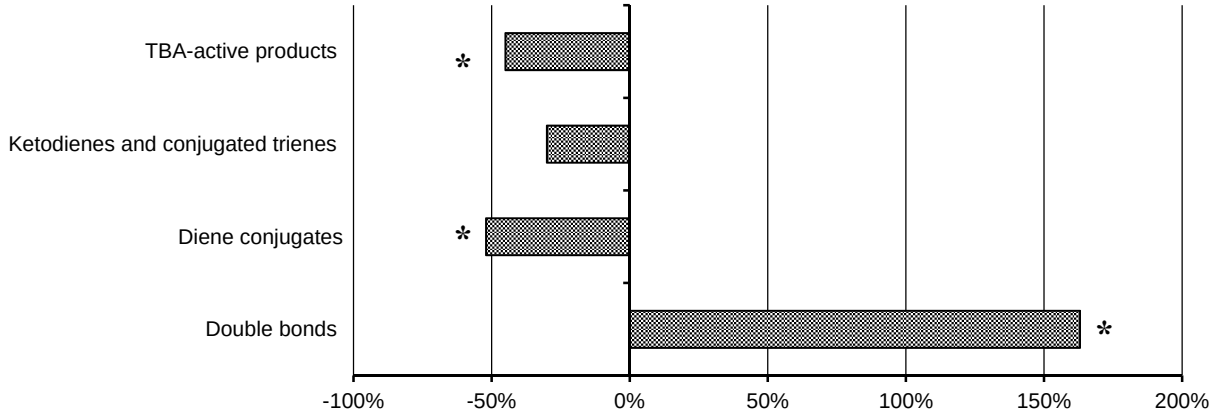

Fig. 1. The state of the components of the lipid peroxidation system with the addition of Trametin in stress-inducible LPO. * – statistically significant differences with the control (values taken as 0 %).

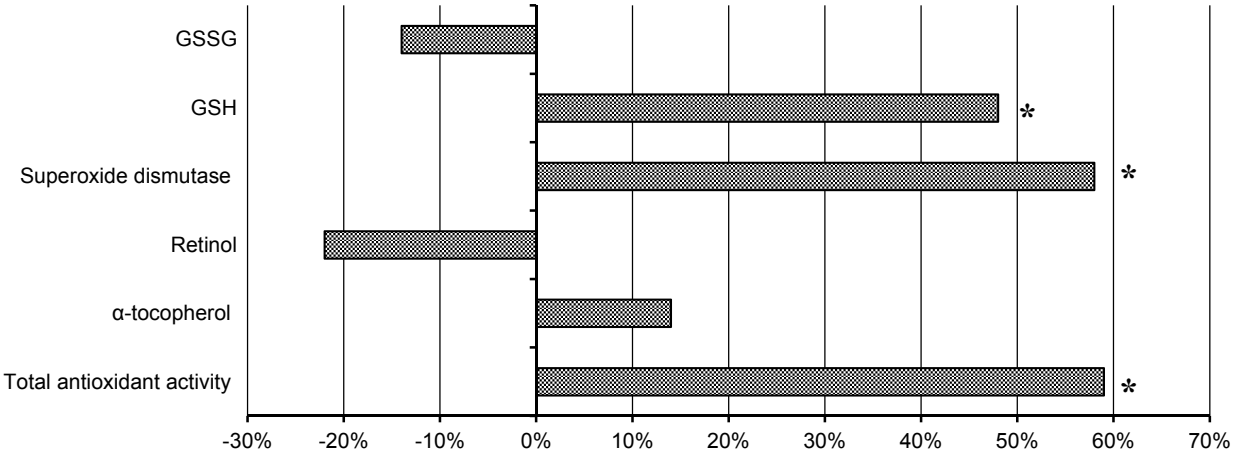

Fig. 2. The state of the AOD system with the addition of Trametin in stress-induced LPO. * – statistically significant differences with the control (values taken as 0%).

control; $p < 0.05$, Fig. 1). It should be noted that TBA-reactive products include a large group of highly reactive aldehydes and ketones (carbonyl compounds) inducing endogenous intoxication and mediating chemical modification of virtually all bioactive substances (nucleic acids, proteins, carbohydrates, vitamins) [1, 15, 19]. It can be concluded that Trametin protects unsaturated fatty acid from oxidation that determine its positive effect on LPO.

The status of LPO-AOD system can be fully evaluated only if the components that determine the power of antioxidant defense with its low-molecular and enzymatic links are simultaneously studied [17, 18].

When assessing changes in the AOP system we found that Trametin increases the control level of the total AOA by 1.59 times ($p < 0.05$) (Fig. 2). Determination of the activity of SOD, the key enzyme of redox metabolism, revealed an increase in this indicator by 1.58 times relative to the corresponding value in the control ($p < 0.05$). The content of non-enzymatic components of the AOD – α-tocopherol system and retinol did not differ significantly from the control ($p > 0.05$). The study of predominantly cellular antioxidants (GSH and GSSG) revealed that the content of the reduced tripeptide form in the experimental group was 1.48 times higher than the control values ($p < 0.05$) (Fig. 2), but Trametin had no significant effect on the concentration of the oxidized form of glutathione ($p > 0.05$).

We can assume that a positive shift in the AOD system (increased activity of SOD, as well as an increase in the content of the reduced glutathione), creates a sufficient buffer capacity for pro-oxidants and promotes effective prevention of oxidative stress [14, 21]. Among the important components of Trametin, the most interesting are organic acids (succinic, propionic, benzoic, fumaric, phenylacetic, oleic) and their ethers, in connection with their antioxidant activity. The methylated derivatives of phenolaldehydes and phenolic acids were found in free acids. It is well known that phenolic compounds are active metabolites of cellular metabolism and play a significant role in important physiological processes (respiration, growth, resistance to infectious diseases, growth and reproduction) [11, 19]. Probably, the noted effects of Trametin are due to the presence of phenolic compounds in the preparation, which directly react with free oxygen forms and organic peroxides [3, 4, 5]. At the same time, the most important acids in the composition of Trametin are amber and fumaric, which, by normalizing the mitochondrial oxidation of target cells, can also have a powerful antioxidant effect. We showed this fact in our earlier studies [20]. It should be noted that the antioxidant effect of Trametin, in our opinion, could be divided into two types: direct and indirect. The direct type is due to the presence in the preparation of phenolic compounds, which directly react with free oxygen forms and organic peroxides [5]. At the same time, Trametin contains such important substrates of the Krebs cycle as amber and fumaric acids, which, by normalizing the mitochondrial oxidation of target cells, can have a powerful mediated antioxidant effect [3].

Thus, the study of the effect of the preparation obtained by liquid-phase fermentation from xylotroph fungus *Trametes pubescens* showed a pronounced antioxidant effect on the model of dark stress in experimental animals, which can serve as a basis for its further application in veterinary and clinical medicine.

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