



Antioxidant properties of the preparation Trametin on xylotrophe fungus *Trametes pubescens* (Shumach.:Fr.) Pilat.

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General Note



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ABSTRACT

The work represents the data from experimental research on the influence of the Trametin preparation, which is based on the products of the xylotrophe - fungi *Trametes* submerged cultivation, on the level of primary and intermediary products of lipid peroxidation in the animal blood serum during experimental colibacteriosis modelling. The data received indicates its significant

antioxidant activity in comparison with the activity of the Intestevit probiotic preparation and consequently there is a possibility of wider use of preparation based on xylophore fungus *Trametes* against several pathological processes.

Keywords: Basidiomycetes, Antioxidant properties, Gastrointestinal Diseases, Lipid Peroxidation

Abbreviations: RAS – Russian Academy of Sciences, NPK – Scientific and Production Company, ETC – European Treaty Community, FGBI – Federal Governmental Budget, Institution, CD - conjugated dienes, TBA - thiobarbituric acid, LPO - lipid peroxidation, AOA - antioxidant activity, AWM - average weight molecules, c. u. – conditional units

INTRODUCTION

Basidiomycetes are producers of a variety of biologically active substances: proteins, lipids, polysaccharides, organic acids, enzymes, vitamins, alkaloids (Belova, 1993; Gorshina et al., 2004; Chkhenkeli, 2006). Increasing attention of researchers is paid to the study of mechanisms of their influence on the human body and animals, including the study of their anti-oxidant activity (Li, 2005; Matsuzava, 2010; Nakamura et al., 2003; Wei et al., 2009; Yusoo, 2002). Fungi of the genus *Trametes*, as has been shown in earlier studies, synthesize substances with antioxidant effect: triterpene compounds, exopolysaccharides, etc., which are identified in the analytical studies, both in the mycelium of the fungus and in its culture fluid (Oleshko, Babitskaya, 1991; Fedorov, Chastov, 2005; Feofilova, 1998; Chkhenkeli et al., 2009; Chkhenkeli et al., 2010; Chkhenkeli et al., 2006). In this regard, the study the antioxidant activity of the preparation trametin obtained on the basis of the products of liquid-phase culture of the fungus *Trametes pubescens* (Shumach.: Fr.) Pilat. strain 0663 – causative of white wood rot from the Collection of the Botanical Institute of V.L. Komarov RAS (St. - Petersburg) and used in veterinary medicine (Habriev, 2005; Stepanova et al., 2004; Chkhenkeli, 2006; Chkhenkeli et al., 2009) for the prevention of massive gastrointestinal diseases.

Substantial interest as a promising antioxidant complex draws probiotic preparation Intestevit based on microorganism cultures *Bifidobacterium globosum*, *Enterococcus faecium*, *Bacillus subtilis* production NPK "Center for Medical - Veterinary Environmental Studies" (Blagoveshchensk, Russia), which can also be used for prevention of intestinal infections in calves of dairy period.

The aim of this work was to study the comparative antioxidant activity of drugs trametin obtained on the basis of the fungus *T. pubescens*, and Intestevit in modeling experimental colibacteriosis.

MATERIALS AND METHODS

Experiments were performed on mature white non-pedigree rats weighing 180.5 ± 10.6 g, kept in the vivarium of anatomy, physiology and microbiology chair on the standard diet. Investigations were carried out in compliance with the rules of laboratory practice in the conduct of preclinical studies in Russia (Oleshko, Babitskaya, 1991), as well as "the international recommendations of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (ETC № 126, 1986).

Modeling colibacillosis in rats was performed by intradermal administration to 1 ml of a suspension of biomass (in saline) of *Escherichia coli* pathogenic strain TI (1 billion microbial cells) isolated from pathological material in FGBI "Irkutsk Interregional Veterinary Laboratory" (Menshchikova, 2008).

6 groups were formed with 8 animals in each: 1) intact animals, 2) infected rat 3) rats which were given orally three times trametin 250 mg per 1 kg body weight for 3 days, then were infected and medication was given for 5 days in a same dose of study 4) infected animals being given trametin per os for 8 days 5) rats that before and after infection were treated orally by Intestevit (3 and 5 days) at a dose of 5 mg per kg of body weight; 6) infected animals, which were orally given Intestevit for 8 days.

After completion of the experiment the rats were killed by the dislocation of the head, the blood was collected, in the serum of which the primary content (conjugated dienes, CD) and secondary intermediates (TBK - active products) (Pershin, 1971) of lipid peroxidation (LPO) were determined. In addition, total value of antioxidant activity (AOA) and vitamin E in the serum were measured (Zhukova and Tanygin, 2008). To investigate the mechanism of disorder of LPO - AOA at colibacteriosis and its correction with known preparation and the one being examined concentration of lactic and citric acids in the serum was additionally measured (Krasnopolskaya et al., 2004), as well as the content of serum iron (Zhukova and Tanygin, 2008) and the average weight molecules (AWM) (Zenkov et al., 2001).

Statistical processing of the experimental results and evaluation of the reliability was conducted according to the Student t-test for a probability level of at least 95% with the use of the software package Microsoft Excel 97.

RESULTS

As it can be seen from the results shown in Table 1, bacterial infection causes marked changes in the intensity of free radical processes, which is reflected in a statistically significant increase in the concentration of conjugated diene units and TBA- active products (2.17 and 2.91 times respectively) as compared with those in the group of intact animals. Such a reaction of the whole organism on introduction of the infectious agent is in good agreement with the data of domestic and world literature indicating the increased generation of reactive oxygen species, particularly superoxide anion radical in the respiratory burst, which occurs as a response of macrophages and neutrophils to the introduction of the infectious agent (Shakhov, 2010).

According to this table, the treatment of bacterial infections of experimental preparation trametin almost completely normalized the value of CD and TBA-active products (in particular in the 4th test group) while when using intestevit in these experiments the observed reduction in the levels of these parameters is still higher than those in the group of intact animals ($P < 0.05$).

In the study in overall AOA and vitamin E as an essential component of this system, in conditions of infection (Table 1) their statistically significant reduction has been shown compared with the corresponding values in healthy animals. Comparing these results with data on indicators of LPO, one can make an unambiguous conclusion that animals infected with a pathogenic strain of *Escherichia coli*, are in a state of oxidative stress, which in this case is characterized not only by activation of LPO, but inhibition of the antioxidant defense system.

Analysis of factors of antioxidant protection in these experiments show (Table 1) that high efficiency in maintaining the redox state of the organism from infected animals has trametin; intestevit also has some protective properties that are identified in the 5th group of experiments, i.e. when prophylactic and therapeutic effects were combined.

To reveal the mechanisms of antioxidant action of preparations studied, we carried out additional studies that showed (Table 2) that lactic acid content in the blood serum of experimental animals infected with *Escherichia coli* bacterial infection ($P < 0.05$) increased 3.0 times and the concentration of citrate reduced 2.1 times ($P < 0.05$) compared with the corresponding values in the intact series.

According to modern concepts, such a combination of concentration of lactic acid and citric acid indicates a shift of exchange with hypoxic acidification of the internal environment by type of metabolic acidosis.

Table 1

Effect of preparations trametin and intestevit on parameters POL-AOP system during modeling of colibacteriosis in experimental animals, $M \pm m$

Experiment al groups*	LPO – AOD system parameters			
	CD, $\mu\text{mol/L}$	TBA- active products, $\mu\text{mol/L}$	AOA, c. u.	Vit E, $\mu\text{mol/L}$
1	1.32 \pm 0.08	1.08 \pm 0.06	15.6 \pm 1.2	8.35 \pm 0.66
2	2.87 \pm 0.21	3.14 \pm 0.26	7.3 \pm 0.4	5.21 \pm 0.39
3	1.47 \pm 0.10	1.36 \pm 0.12	16.8 \pm 1.9	9.06 \pm 0.74
4	1.54 \pm 0.12	1.01 \pm 0.09	17.9 \pm 1.9	8.01 \pm 0.62
5	1.76 \pm 0.10	1.90 \pm 0.13	13.5 \pm 0.9	8.06 \pm 0.70
6	2.09 \pm 0.18	2.45 \pm 0.11	11.5 \pm 0.8	6.05 \pm 0.52

* description of the experimental groups is given in the text

Table 2

Effect of trametin and intestevit on biochemical indicators of blood serum in experimental colibacteriosis infection, $M \pm m$

Experimental groups*	Indicators			
	Lactate, $\mu\text{mol/L}$	Citrate, $\mu\text{mol/L}$	Iron, $\mu\text{mol/L}$	AWM, E ₂₅₄
1	1.24 \pm 0.09	149 \pm 12	15.5 \pm 1.1	0.305 \pm 0.008
2	3.73 \pm 0.29	70 \pm 7	23.4 \pm 1.8	0.524 \pm 0.016
3	1.09 \pm 0.12	121 \pm 10	17.2 \pm 1.7	0.310 \pm 0.011
4	1.03 \pm 0.12	137 \pm 8	18.8 \pm 1.5	0.287 \pm 0.010

5	2.07 ± 0.17	107 ± 11	17.5 ± 0.9	0.375 ± 0.017
6	2.69 ± 0.16	120 ± 14	20.7 ± 1.2	0.409 ± 0.020

* description of the experimental groups is given in the text

DISCUSSION

The evidence of the metabolic acidosis with an infectious process is the increase of the concentration of iron in the blood serum of infected animals by 50.1 % ($P < 0.05$), which at an acidic pH value leaves the ferritin depot, activating free radical processes (Kondrakhin, 2004), including increase of the level of lipid peroxidation already mentioned by us.

When modeling a bacterial infection we observed the increase in average molecular weight (for E_{254}), which always accompanies the infection process, as one of its metabolic markers (Buinov and Vlasov, 1994). Furthermore, it has recently been found that between the AWM and the content of TBA-active products, in a state of oxidative stress, there is an almost linear dependence (Portyanaya, 1990).

Thus, the metabolism of active infection in experimental animals characterized by a marked exchange shift towards hypoxic conditions, which is accompanied by acidification of the internal environment, the output of iron from ferritin and subsequent activation of LPO processes, as well as increasing the concentration of AWM as a marker of endogenous intoxication and peroxidation of lipids.

Using the trametin preparation obtained from basidiomycetes prevents development of lactic acidosis caused by infection and reduction of the concentration of citric acid content in the serum which is formed aerobically in the cycle of three - carboxylic acids (Table 2). This naturally prevents accumulation of serum iron which is one of the trigger factors of free radical processes, which explains the stable level of lipid peroxidation products and the degree of endogenous intoxication when using this preparation.

The results obtained in the present series of studies confirm the data obtained in the study of the components of LPO and AOA system that indicate that (Table 2) intestevit is a less efficient antioxidant complex as compared with the preparation Levan-2. In this respect, our data are in good agreement with the already mentioned results of other studies that basidiomycetes secrete triterpenes, polyphenols and other compounds with antioxidant activity into the surrounding environment (Gorshina et al., 2004). It should also be noted that the content of lactic acid and citric acid, serum iron and AWM in serum of experimental animals using intestevit, although differing from the corresponding level of the parameters studied in infected animals, does not reach the values established for trametin. In addition, as shown by our special studies, the preparation trametin exceeds intestevit by their positive impact on hematological parameters, protein and carbohydrate metabolism.

CONCLUSION

Thus, while modelling experimental colibacteriosis in rats a significant decrease in the content of some primary and intermediates in the serum was observed when using the preparation trametin obtained on the basis of the liquid-phase fermentation product of the fungus - xylotrophe *T. pubescens*. This testifies to its antioxidant activity, and, consequently, to an wider use of the preparation in various pathological processes. Studied as a comparator preparation intestevit also shows the antioxidant effect in these conditions, but it is quantitatively less significant compared to trametin.

SUMMARY OF RESEARCH

1. When simulating experimental colibacteriosis in rats a significant reduction in the primary and intermediate products in serum has been observed while using the medicine trametin obtained by liquid-phase products of fermentation of the xylotrophe fungus *T. pubescens*.
2. This indicates its significant antioxidant activity, and, consequently, the possibility to enhance the range of the use of a drug for the treatment of various pathological processes.
3. Intestevit researched as a comparator drug also manifests the antioxidant effect in these conditions, but it is quantitatively less significant compared to Trametin.

FUTURE ISSUES

I believe that many researchers in the field of veterinary science and medicine have to pay attention to the unique properties of xylotrophe fungi of *Trametes* kind. The antioxidant properties explored in this paper, can significantly expand the range of applications of medicine based on wood-destroying fungi *T. pubescens*.

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Data and materials availability: All data associated with this study are present in the paper.

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