ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Use of Secondary Resources of Grape's Processing to Obtain Additives of Antioxidant Action.

Elena V Kuzminova[1], Marina P Semenenko[1]*, Nikolay N Kornen[2], Tatiana A Shakhray[2], and Elena P Viktorova[2].

[1]FSBSI "Krasnodar Research Center for Animal Husbandry and Veterinary Medicine", Krasnodar, Russia
[2]Krasnodar research Institute of storage and processing of agricultural products – branch of FSBSI "North-Caucasian Federal scientific center of horticulture, viticulture, winemaking", Krasnodar, Russia

ABSTRACT

The article presents the results of the study of additives obtained by various technologies from secondary resources of grapes processing on laboratory animals. The technology, which additionally includes the preliminary preparation of grape pomace for drying, by their short-term processing in an electromagnetic field of ultrahigh frequency leads to an increase in the content of micronutrients demonstrating antioxidant activity (with the maximum retention of carotenoid, tocopherol and phenol carboxylic acid concentrations). The use of "Grape powder" additives in laboratory rats in comparative aspect led to an intensification of protein and carbohydrate metabolism, stabilization of hepatocyte membrane structures and also contributed to the reduction of toxic lipoperoxidation products. The improvement in the biochemical profile of the blood had a positive effect on the growth rate of growing laboratory animals with the greatest efficiency in the group receiving the additive from the grape pomace using the special preparation of raw materials.
Keywords: veterinary pharmacology, laboratory rats, grape pomace, blood, biochemistry, antioxidants.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

One of the directions of the modern pharmaceutical industry is the application of resource-saving technologies, taking into account the use of waste from the processing of plant raw materials for the production of food, biologically active and feed additives. One of such raw materials is grape pomace formed as a result of processing of grape berries and consisting, basically, of skin (up to 75%) and seeds (to 25%).

Information on the biological activity of these natural compounds can serve as a basis for developing new formulations of nutraceuticals and pharmaceuticals that has an undoubted theoretical and practical interest for pharmacological science. At the same time, the most attention is paid to the development of technologies for processing grape pomace with the production of the target products of anthocyanins – natural dyes [1,2] and dietary fibers [3-5], including bacterial cellulose [3,4] and pectin [5].

However, grape pomace is not only the source of anthocyanins and dietary fiber, but also such micronutrients as P-active substances, phenol-bosonic acids, vitamins C, E, PP and provitamin A (β-carotene), which have an antioxidant activity. Some components of grape pomace are powerful antioxidants and realize their activity by means of different mechanisms: intercept active forms of oxygen; inhibit platelet aggregation and oxidative effects of myoglobin; bind free metal ions, limiting their catalytic prooxidant activity; restore the radicals of glutathione, etc. [6].

Taking this into account, it is advisable to use grape pomace as a raw material for the production of antioxidant preparations and additives [7].

The processes of free radical oxidation underlying the metabolism of all cells and determining the adaptive consistency of the organism to the action of damaging factors are not only a necessary link in the vital activity of a cell, but also act as a universal nonspecific link in the development of many pathological conditions. Excessive accumulation in the body of various products of peroxide oxidation of lipids, destructively affecting biological membranes, changing the activity of a large number of enzymes, affects the most important biochemical processes in the body that determine the main manifestations of its vital activity. Strict regulation of lipid peroxidation reactions is ensured by the coordinated functioning of the antioxidant defense system, which controls the level of active oxygen species, free radicals and molecular products of lipid peroxidation in the body [8].

The aim of the research is to study the effect of additives obtained by various technologies from secondary raw materials – grape pomace, on biochemical status and the accumulation of lipid peroxidation products in laboratory animals.

MATERIALS AND METHODS

As objects of research, two samples of the additive "Grape powder", obtained by various technologies from grape pomace, were taken.

The first sample of the additive "Grape powder No. 1" was obtained by the technology including infrared drying of pomace at a temperature of 45-50 º C to a moisture content of not more than 8%, cooling the dried pomace to a temperature of 20 º C and then grinding them at a temperature of 20 º C to a particle size of not more than 0,25 mm.

The second sample of the additive "Grape powder No. 2" was obtained using the above-mentioned technology, which additionally includes preliminary preparation of pomace for drying by short-term processing in an electromagnetic field of the super-high frequency, with the aim of transferring the bound moisture to the free one.

Table 1 shows the composition of micronutrient supplements having antioxidant activity contained in the test samples. From the data it can be seen that the content of components showing antioxidant activity in the sample No.2 is much higher than in the sample No.1. In the vitamin level with antioxidant action the most significant difference is found in the concentrations of beta-carotene on 41,5%, and tocopherols on 32,8%. The

mass fraction of phenol carboxylic acids in the second sample exceeds their content in the additive "Grape powder No. 1" in almost two times in all the studied parameters.

Table 1: Composition of antioxidants contained in the samples of additives "Grape powder"

Indicator name	Indicator value	
	Sample No. 1	Sample No. 2
Mass fraction, mg / 100 g: vitamin C	6,48	8,10
vitamin E (tocopherols)	11,45	15,20
beta-carotene	0,53	0,75
vitamin PP	2,65	3,40
Mass fraction, mg / 100 g: polyphenol compounds	3592,0	4943,5
catechins (vitamin P)	1020,0	1280,0
Leucoanthocyanins	715,0	820,6
Mass fraction of phenolcarboxylic acids, mg / kg: Gallic	213,4	433,9
Caffeic	240,6	497,1
4-hydroxybenzoic	70,8	153,5
3,4-dihydroxybenzoic	188,0	312,0

Inductor	impulses/min on 1×10^3 cells (24 hours)	impulses/min on 1×10^3 cells (72 hours)
Intact control	56,4±0,6	53,0±5,2
Control after TPA+ CH	706,0±16,6 <0,001	24,6±2,5 <0,02
IDU	598,0±10,0 <0,001	7,0±0,6 <0,001
TPA	445,8±9,8 <0,001	32,0±3,3 <0,05
Morpholinium	1,4±0,09 <0,001	0,45±0,002 <0,001
Lozeval	1,2±0,05 <0,001	0,5±0,004 <0,001

This can be explained by the optimization of the technology of obtaining additives from grape pomace due to the special preparation of the raw material by means of its short-time processing in an electromagnetic field of ultrahigh frequency, which reduces the duration of the drying process in 2 times and reduces the temperature effect on the dried material, which minimizes the loss of thermolabile micronutrients, demonstrating antioxidant properties.

The study of the effect of additives obtained from grape pomace on biochemical status and the accumulation of lipid peroxidation products was conducted on laboratory animals – white nonlinear rats of both sexes with an average body weight of 180,6 ± 2.1 grams, from which were formed three groups with 10 animals in each. The experiments were conducted in accordance with the requirements for the medical and biological experiment on the selection of analogues, the establishment of control, the observance of the same conditions of feeding and keeping animals during the work and the recording of the results.

Laboratory rats for adaptation were placed in special individual cells 4 days before the start of the experiment. Samples of additives were given to the animals during 30 days once daily, individually in the form of boluses, which were prepared immediately before each feeding at the amount of 2 grams per rat. Distilled water was used as the forming agent. Control animals were given boluses made from flour and water.

Scheme of the experiment: 1st group – additive "Grape powder No. 1", 2nd group – additive "Grape powder No. 2; 3rd group – control.

ISSN: 0975-8585

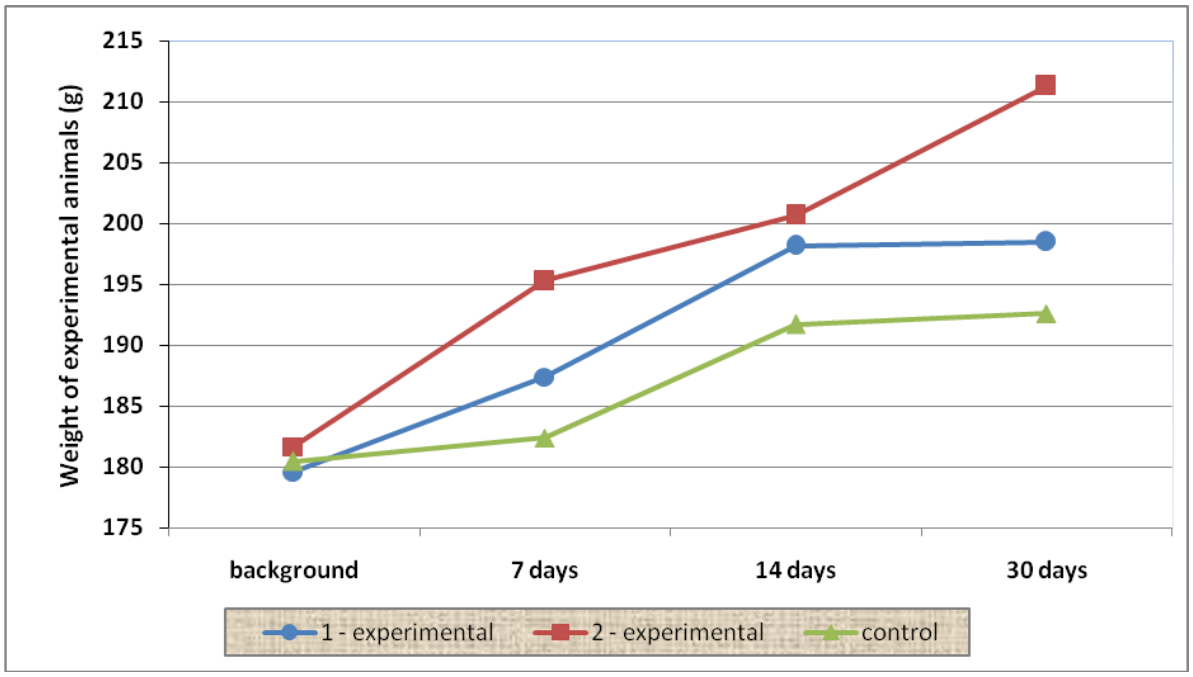
During the experiment the clinical status of rats, the intensity and nature of motor activity, the state of hair and skin and the color of the mucous membranes were regularly taken into account. The determination of body weight in all animals was carried out in dynamics: background and every 7 days. Blood for laboratory tests was taken 15 days later and at the end of the experiment to study the effect of additives on metabolic processes – the dynamics of biochemical indices, as well as the presence of an antioxidant effect – according to the level of diene conjugants, ketodienes and malonic dialdehyde.

Statistical processing of the results was carried out using special software packages. The study of quantitative characteristics was carried out by comparing the mean values of two sample populations with the Student's test and the significance level (p).

RESULTS

The biological effect of the additives "Grape powder" was studied by the dynamics of the body weight of laboratory animals. The results of the analysis of these data (Figure 1) showed that after the experiment the body weight in the experimental groups in average was: 198,5 ± 2,4 g (1st group) and 211,3 ± 1,8 g (2nd group), against 192,6 ± 2,4 g in the control group. When calculating the percentage of growth, the difference between the experimental rats and the control analogs was 3,8% and 9,6%, respectively, in groups, in favor of experimental animals.

Figure 1: Dynamics of the body weight of laboratory animals receiving the additives “Grape powder"



As a result of the evaluation of the effect of additives on the metabolic processes in the body of rats (Table 2), a dynamic increase in the level of total protein in blood serum of experimental animals was determined. However, when data were processed according to Student, the significant difference in the concentration of the total protein on the 30th day of the experimental period was 7,1% ($P \leq 0,001$) only in the second experimental group. The animals of the first group had a tendency to intensify protein metabolism with a difference of 4,5%.

The content of glucose in the blood of rats receiving "Grape powder" additives by the end of the experiment significantly exceeded the control indices, equal to 9,7 ± 0,33 mmol / l. The significant increase in the 1st group was 21,6%, in the 2nd group it was 36,6% ($P \leq 0,01$).

ISSN: 0975-8585

Table 2: Biochemical indicators of blood of laboratory rats with the use of additives "Grape powder" (M ± m; n = 5)

Indicators	1st group (sample № 1)		2nd group (sample № 2)		Control group	
	Days of experiment					
	15	30	15	30	15	30
Total protein, g/l	71,1±0,41	73,6±0,44	72,1±0,25	75,4±0,18*	70,3±0,56	70,4±0,31
Urea, mmol/l	7,5±0,15	7,3±0,31	7,4±0,39	7,5±0,26	7,2±0,17	7,3±0,46
Creatinine, mmol/l	47,1±1,25	46,8±1,46	46,5±2,35	49,2±2,37	44,1±0,81	47,7±1,19
Glucose, mmol/l	7,91±0,42	11,8±0,58**	7,60±0,23	12,3±0,14**	7,93±0,49	9,7±0,33
Cholesterol, mmol/l	1,40±0,05	1,43±0,08	1,41±0,04	1,44±0,02	1,39±0,06	1,42±0,14
Triglycerides, mmol/l	0,77±0,04	0,81±0,06	0,74±0,11	0,82±0,08	0,89±0,07	0,94±0,12
AST, IU/L	164,3±11,8	147,8±12,5	155,7±10,9	146,3±9,4	144,0±9,1	153,7±10,5
ALT, IU/L	86,7±3,11**	73,7±4,03*	85,3±4,21**	68,3±2,36*	93,6±3,35	89,5±2,0
Total bilirubin, umol/l	9,3±0,36	8,7±0,19	8,1±0,18	7,5±0,19	8,53±0,15	8,9±0,25
Calcium, mmol/l	2,51±0,06	2,63±0,08	2,58±0,02	2,61±0,15	2,53±0,05	2,57±0,09
Phosphorus, mmol/l	2,47±0,29	2,51±0,06	2,54±0,31	2,61±0,16	1,47±0,10	2,55±0,25

Note: * – probability value P ≤ 0,001, ** – probability value P ≤ 0,05 in relation to control

The presence of hepatoprotective properties in additives may be indicated by a dynamic decrease in the level of alanine aminotransferase in the blood of experimental animals of the 1st group on the 15th day of the experiment by 7,9% (P≤0.05), by 30th day – by 21,4% P≤0,001); of the 2nd group – by 9,7% (P≤0.05) and 31% (P≤0.001), respectively. Since this megabolite belongs to cytoplasmic enzymes, a decrease in its concentration indicates the stability of the membrane structures of hepatocytes.

For the rest of the biochemical indicators, a slight scatter in their content did not allow to reveal a reliable difference between control and experimental animals.

The antioxidant effect of the additives "Grape powder" in animals of experimental groups was manifested in a decrease in the concentration of the determined products of lipoperoxidation (table 3).

Table 3: Level of products of lipid peroxidation in blood of laboratory rats with the use of additives "Grape powder" (M ± m; n = 5)

Indicators	1st group (sample № 1)		2nd group (sample № 2)		Control group	
	Days of experiment					
	15	30	15	30	15	30
DC, AU/mg	0,106±0,03	0,110±0,05	0,104±0,02*	0,096±0,04*	0,118±0,05	0,121±0,09
KD, AU/mg	0,133±0,07	0,134±0,03	0,128±0,01	0,094±0,02*	0,141±0,08	0,145±0,10
MDA, umol/l	1,70±0,15	1,66±0,18*	1,52±0,23*	1,49±0,11*	1,71±0,14	1,82±0,16

Note: * – probability value P ≤ 0,05 in relation to control

Thus, in comparison with the control animals, the level of diene conjugates in the first experimental group decreased by 10% on average, in the second experimental group – decreased by 11,8% on the 15th day, by 20,4% on the 30th day; the level of ketodienes decreased in the first experimental group by 5,6% on the 15th day, by 7,5% on the 30th day, in the second experimental group the difference was 9,5% and 35,3%. The concentration of malonic dialdehyde in all rats receiving supplements decreased by 8,8% and 17,9%, respectively, in groups in comparison with the control group.

CONCLUSION

Thus, the use of "Grape powder" additives increases the antioxidant defense of the body that leads to a decrease in toxic lipoperoxidation products, and as a result, to the restoration of cell membranes,

normalization of biochemical processes and tissue structure functions, which manifests a positive effect on the metabolism in the body, optimization of protein and carbohydrate metabolism.

REFERENCES

[1] Lozovskaya T, Brenner-Weiss G, Franzreb M, Nusser M. Recovery of anthocyanins from grape pomace extract (Pinot Noir) using magnetic particles based on poly (vinyl alcohol) Celluslose Chemistry and Technology. 2012; 46: 427-433.

[2] Metivier RP, Francis FJ, Clydesdale FM. Solvent extraction of anthocyanins from wine pomace. J. Food Sci. 2015; 45: 1980-1999.

[3] Tataridis P, Apostolopoulos E, Moraitaki D, Spanou E, Nerantzis T, Papakonstantinou S. Utilization of grape pomace for the production of bacterial cellulose. e-Journal of Science & Technology. 2006; 3:114.

[4] Jozala FJ, Pertile RAN, dos Santos CA, Santos-Ebinuma V de C, Seckler MM, Gama FM, Pessoa A. Bacterial cellulose production by Gluconacetobacter xylinus by employing alternative culture media. Appl Microbiol Biotechnol. 2015; 99: 1181– 1190.

[5] Method of extraction of pectin from grape pomace: Pat. 2249600 of the Russian Federation, IPC C 08 B 37/06, 5/06 C 08 L / Donchenko LV, Vlasyk LG, Kvasenkov OI, Rodionova JL; applicant and patentee Kuban State Agrarian University. – No 2003113735/04; Appl. 13.05.2003; publ. 10.04.2005.

[6] Gribova NYu. Antioxidant activity of phenolic fractions of extracts of herbs. Bulletin of Donetsk University. 2008; 10 (1): 137-140.

[7] Tyapkina EV, Khakhov LA, Semenenko MP, Kuzminova EV, Antipov VA, Troshin AN, Fersunin AV. Rational use of medicines in veterinary medicine. Krasnodar, 2014, p. 57.

[8] Kuzminova EV, Semenenko MP, Ermakova TI. Application of antioxidants in poultry farming. International scientific and practical conference dedicated to the 60th anniversary of the State Scientific University of Krasnodar NIVI. Actual problems of veterinary medicine in modern conditions. 2006; 299-302.