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PARAMETERS OF ACUTE AND CHRONIC TOXICITY OF FOOD ADDITIVE "BEEF FLAVORS №4"

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Relevance: the study of the toxicological characteristics of new food products, including food additives, currently remains relevant. The use of biologically active substances and various food additives in food products is possible only after toxicological studies have been carried out in accordance with modern scientific requirements, which allow us to assert the safety of the studied substances. In the toxicological assessment of food additives, the relationship between the dose and duration of exposure is revealed, and possible consequences are studied, a causal relationship is determined, which once again proves the relevance of the issue under consideration.

Aim of study of hematological, biochemical and histomorphological studies of internal organs.

Materials and methods of research. Toxicological studies were carried out on 150 white outbred adult rats of both sexes and 4 chinchilla rabbits kept in a vivarium with natural lighting, at a temperature of 22-24^{0}C, relative humidity of 40-50%, using a standard diet. For acute toxicity, 72 out of 150 rats (36 males and females each) were used. For the "chronic" experience, 72 animals of both sexes were also used. The control group consisted of 6 animals.

Results. Based on the examination of the scientific dossier, literature data and the results of toxicological studies of food additives "Beef Flavoring No. 4", it was established that, according to the parameters of acute toxicity in the intragastric route of intake, they belong to low-toxic substances (class IV).

Keywords: food additive, toxicological studies, acute and chronic toxicity.

Introduction. It is known that the latest achievements of modern medical science and the introduction of effective preventive measures are aimed at preventing a number of diseases associated with the conditions of modern human life. A special place is given to solving problems aimed at the environmental safety of food products. It has been established that various food additives in consumed food products, the toxicological properties of which have not been fully studied, can cause the development of poisoning or disease. Therefore, the study of the toxicological characteristics of new food products, including food additives, currently remains relevant [1, 6, 11, 12]. The use of biologically active substances and various food

additives in food products is possible only after toxicological studies have been carried out in accordance with modern scientific requirements, which allow us to assert the safety of the studied substances. Legislative documents on the use of food additives are based on the use of a certain "safe" amount both for single and long-term use [3, 4, 7, 9]. In the toxicological assessment of food additives, the relationship between the dose and duration of exposure is revealed, and possible consequences are studied, a causal relationship is determined, which once again proves the relevance of the issue under consideration [2, 5, 8, 10].

Materials and methods of research. Toxicological studies were carried out on 150 white outbred adult rats of both sexes and 4 chinchilla rabbits kept in a vivarium with natural lighting, at a temperature of 22-24^{0}C, relative humidity of 40-50%, using a standard diet. Before the start of the experiments, laboratory animals were examined, body weight, age, sex, motor activity and condition of the coat were taken into account. For acute toxicity, 72 out of 150 rats (36 males and females each) were used. For the "chronic" experience, 72 animals of both sexes were also used. The control group consisted of 6 animals.

Statistical studies were carried out based on standard clinical guidelines. Quantitative data are presented as arithmetic mean (M) ± standard deviation (SD) for a normal distribution and as median (Md) and quartiles (Q) or (SD) for other distributions. Significance level $P<0.05$ was taken as statistically significant changes. The results of the clinical examination were processed on a Pentium-IV personal computer using Statplus 9.0 office software with the calculation of the arithmetic mean of the studied indicator (M), its standard error (m), significance indicators (P) and Student's t-test. At the same time, methods and existing guidelines for statistical data processing in clinical and laboratory studies were taken into account (Zaitsev V.M. et al., 2003).

Results. Food additive "Beef Flavoring No. 4" produced by NESSE FASS GROUP LLC (Uzbekistan) is used in the production of all types of sausages and meat products to improve and ennoble the taste of the finished product. The composition includes ingredients and allergens (according to Regulation (EC) 1169/2011), table salt, maltodextrin (maltodextrin), cellulose (E460) as a stabilizer, emulsifier and thickener. This supplement is allowed in all countries, but the daily allowance has not been established. In the Republic of Uzbekistan, the allowable application rate is 15.0 g/kg of the product. 1-substituted sodium glutamate flavor enhancer (monosodium glutamate) - monosodium salt of glutamic acid, (E621) - flavor enhancer, is a white crystalline powder, highly soluble in water. The flavor enhancer is allowed in all countries. It has been established that the LD_{50} of monosodium glutamate for rats and mice is 15-19.9 g/kg of body weight; the allowable norm is 120 mg/kg of human weight. The optimal dose for the full disclosure of taste is 3.5–5 g. A person weighing 70 kg can eat about 8.5 g of monosodium glutamate without harm to health. Permissible application rate of 10.0 g/kg of the product is allowed in the republic.

Consistency of the food additive "Beef Flavoring No. 4" - free-flowing powder, light brown in color, smell - typical, fragrant, without foreign smell; the taste is typical, without aftertaste.

Dosage: 1-3 g per kg of raw material mass. Shelf life 12 months in original, undamaged packaging at room temperature, in a dark, dry place. Determination of the parameters of acute toxicity of substances was carried out under conditions of a single intragastric administration of food additives at doses of 1000, 2500 and 5000 mg/kg. For testing under conditions of acute toxicity, 3 groups of animals were selected:

1. For animals of the 1st group, when exposed to a dose of 1000 mg/kg, 40% solutions of two substances were prepared. Each animal was injected once with 0.5 ml/200 g of body weight.

2. For animals of the 2nd group, when exposed to a dose of 2500 mg/kg, 50% solutions of substances were prepared, each animal was injected once with 1.0 ml/200 g of body weight.

3. For animals of the 3rd group, when exposed to a dose of 5000 mg/kg, 33.33% solutions of substances were prepared, each animal was injected once with 3.0 ml/200 g of body weight. The experimental animals were observed for 14 days. Symptoms of intoxication in animals were not revealed, experimental animals reacted adequately to external stimuli. The hairline is shiny and smooth, no foci of alopecia or ulcers were found, visible mucous membranes were pale pink, unchanged. The death of animals was not detected when exposed to the maximum dose of 5000 mg/kg. Due to the absence of death of animals, the calculation of the average lethal dose (DL_{50}) was not possible, which made it possible to classify the studied additives according to the parameters of the degree of toxicity to class IV (low-toxic substances). When evaluating the skin-resorptive and local irritating effects, the results of studies on white rats showed that during the observation period of 3 weeks, no symptoms of intoxication and their death were detected. The animals remained active, willingly ate food, and responded adequately to external stimuli. Therefore, the studied food additives "Beef Flavoring No. 4" do not have a skin-resorptive effect. With repeated application of 20 skin applications of preparations to the clipped area of the back of white rats, it was found that food additives do not cause irritation of the skin, no symptoms of intoxication and death of animals were observed, and allowed us to conclude that food additives "Beef Flavoring No. 4" as well as in an acute experiment, they do not have skin-resorptive and locally irritating properties. Evaluation of the effect on the mucous membranes of the eyes of rabbits was carried out as follows. In the conjunctival sac of the left eye of rabbits, 2 drops of nutritional supplements were applied once, while the right eye served as a control. Observation for 7 days also did not reveal signs of an inflammatory reaction and is characterized by the absence of an irritating effect on the mucous membrane of the eyes of rabbits. We also studied the cumulative properties of food additives "Beef Flavoring No. 4" in terms of Lim on 12 white rats. For 3 weeks, food supplements were administered intragastrically daily at an initial dose of 450 mg, while every 5 days the dose was increased by 1.5 times. At the maximum dose of 3127.5 mg on the 21st day of

inoculation, the indicators of survival, general condition, and activity of animals, hematological parameters of peripheral blood and biochemical parameters of blood serum did not differ from those of the control group, which proves that the food additives "Beef Flavor No. 4" have no material properties and functional cumulation. The study of immunological activity in experimental animals in vivo is an important characteristic of the biological safety of food additives. The concentration of immunoglobulins of classes IgG, IgM in blood serum was determined by enzyme-linked immunosorbent assay. Serum samples were obtained from the peripheral blood of rats after exposure to food supplements at a dose of 200 mg/kg, 400 mg/kg and 1200 mg/kg.

The results obtained are presented taking into account the possible effect of the studied food additives on the content of immunoglobulins of the IgG and IgM classes in the blood serum of rats (Table 1). Thus, it was found that food additives "Beef Flavoring No. 4" at doses of 200 mg/kg, 400 mg/kg and 1200 mg/kg do not affect the content of immunoglobulins of the IgG and IgM classes in the blood serum of rats. The obtained results of the content of the studied immunoglobulins did not statistically significantly differ from those in the control group and, thus, do not have an immunostimulating or immuno-inhibitory effect on the body of experimental animals.

Table 1. The results of the influence of food additives on the content of immunoglobulins of the IgG and IgM classes in the blood serum of rats

Groups	Dose of food additives, mg/kg	concentration, IgG, mg/ml	concentration, IgM mg/ml
"Beef Flavoring No. 4"	200,0	3,12±0,10	0,10±0,02
	400,0	3,30±0,15	0,10±0,01
	1200,0	3,20±0,10	0,13±0,02
Control	-	3,30±0,15	0,10±0,01

The results of the study under conditions of chronic experience: the toxicity of food additives "Beef Flavoring No. 4" was studied under conditions of long-term intragastric administration at doses of 200, 400 and 1200 mg/kg. The results of the studies have shown that long-term administration of substances per os in the studied doses is well tolerated by experimental animals.

Indicators of general condition, behavior, weight gain, hematological and biochemical parameters of experimental animals did not differ from control values.

Table 2. Hematological parameters of blood in rats after long-term administration of food supplements per os "Beef Flavoring No. 4" at doses of 200, 400 and 1200 mg/kg

Groups, doses, mg/kg	Leukocytes, 10^9/l WBC	Absolute content of lymphocytes, 10^9/l	Absolute content of a mixture of monocytes, basophils and eosonophils, 10^9/l	Number of granulocytes, 10^9/l	Hemoglobin, g/l	Erythrocytes, g/l RBC	Hematocrit, % HCT	The average concentration of hemoglobin in the erythrocyte g/l MCHC	Platelets in absolute numbers, 10^9/l PLT	thrombocrit, % PCT
Control (intact)	14,95±1,11	6,5±0,43	2,67±0,28	5,2±0,40	139,3±5,45	6,57±0,27	38,37±1,59	365,8±5,66	619,7±45,65	0.560±0.06
200	14.55±1.08	6.08±0.48	2.47±0.28	5.53±0.52	134±5.58	6.10±0.31	36.13±1.31	367±5,55	612,3±45,60	0,570±0,06
400	14,4±1,17	6,40±0,61	2,50±0,35	5,47±0,67	140,5±5,63	6,86±0,31	37,65±1,67	358±5,96	586,2±42,2	0,570±0,05
1200	14.83±1.16	6.07±0.45	2.52±0.30	5.38±0.44	133.83±5.42	6.46±0.28	36.50±1.57	366,33±5,93	667,8±38,29	0,560±0,06

Table 3. Biochemical parameters of blood in rats after long-term administration of a food supplement per os "Beef Flavoring No. 4" at doses of 200, 400 and 1200 mg/kg

Groups, doses, mg/kg	Groups	Activity of alanine aminotransferase, ALT, U/l (at 37^0C)	Aspartate aminotransferase activity, AST, U/l (at 37^0C)	Alkaline phosphatase activity, ALP, U/l (at 37^0C)	Total protein, TR g/dl (at 37^0C)	Urea, mmol/l	Glucose, Glu, mmol/l
Control (intact)	Control (intact)	76,63±4,22	330,83±20,70	584,47±93,82	90,72±5,86	5,68±0,51	2,42±0,23
200	50 mg/kg	87,17±2,96	301,17±25,84	548,52±72,72	92,07±7,57	6,71±0,64	1,94±0,34
400	500 mg/kg	86,88±3,22	308,50±26,22	623,37±67,55	87,93±5,93	6,96±0,53	2,62±0,38
1200	1000 mg/kg	69,65±2,25	353,33±19,60	651,07±59,90	96,37±6,94	6,10±0,90	3,58±0,36

Thus, the observation of the dynamics of changes in the body weight of animals showed that with an initial body weight of 138.4 ± 1.4, after 30 days of intragastric inoculation, there was an increase in body weight up to 182 ± 1.2 (in percent, the increase averaged +16.2%).

The study of the dynamics of hematological parameters of peripheral blood after exposure to substances did not reveal statistically significant differences in the animals of the experimental groups compared with the control data.

The results of the study of biochemical parameters of blood serum of experimental and control animals after exposure to food additives.

Analysis of the obtained data showed that in experimental animals the indicators of total protein, total bilirubin, direct and indirect bilirubin, urea, cholesterol, ALT, AST, gamma-glutamyl transferase (γ GT) and glucose in blood serum did not differ significantly from control values. Thus, food additives "Beef Flavoring No. 4" with prolonged intragastric administration do not have a toxic effect on the hematological and biochemical parameters of experimental animals.

The research results given in the tables (Tables 2, 3) allow us to state the following.

So, in animals after prolonged intragastric exposure to food additives "Beef Flavoring No. 4" at doses of 200, 500 and 1200 mg/kg, there were no significant deviations from the norms and control intact values in hematological and biochemical parameters, renal and liver tests were normal.

Conclusion. Based on the examination of the scientific dossier, literature data and the results of toxicological studies of food additives "Beef Flavoring No. 4", it was established that, according to the parameters of acute toxicity in the intragastric route of intake, they belong to low-toxic substances (class IV). Food additives in the studied doses of 200, 400 and 1200 mg/kg do not have skin-resorptive and skin-irritating effects, do not irritate the mucous membranes of the eyes, do not show cumulative and allergenic properties.

The results of hematological, biochemical and histomorphological studies of internal organs confirm that the food additives "Beef Flavoring No. 4" with prolonged intragastric administration of 200, 400 and 1200 mg/kg do not have a toxic effect on the body of experimental animals. The results of toxicological studies allow us to make a conclusion about the safety of food additives "Beef Flavoring No. 4" for human health and can be approved for use in the prescribed manner in accordance with the appointments.

References:

1. Zaitsev V.M., Liflyandsky V.G., Marinkin V.I. Applied Medical Statistics: Textbook. - St. Petersburg, 2003. - 432 p. Zaytsev V.M., Liflyandskiy V.G., Marinkin V.I. Prikladnaya meditsinskaya statistika: Uchebnoye posobiye. - SPb., 2003. - 432 s.

2. Karkishchenko N.N. Classical and alternative models in drug toxicology // Biomedicine. - 2006. - No. 4. - S. 5-23.2. Karkishchenko N.N. Klassicheskiye i

al'ternativnyye modeli v lekarstvennoy toksikologii // Biomeditsina. - 2006. - № 4. - S. 5-23.

3. Makarov V.G. et al. Directory. Physiological, biochemical and biometric indicators of the norm of experimental animals. - St. Petersburg: Publishing house "LEMA", 2013. - 116 p. Makarov V.G. i soavt. Spravochnik. Fiziologicheskiye, biokhimicheskiye i biometricheskiye pokazateli normy eksperimental'nykh zhivotnykh. - SPB.: Izd-vo «LEMA», 2013. – 116 s.

4. Mironov A.N. Guidelines for conducting preclinical studies of drugs. Part 1. - M., 2012. - 944 p. Mironov A.N. Rukovodstvo po provedeniyu doklinicheskikh issledovaniy lekarstvennykh sredstv. Chast' 1. - M., 2012. - 944 s.

5. Sanotsky I.V. Methods for determining the toxicity and danger of chemicals. - M., 1970. - S. 161-163, 175-177. Sanotskiy I.V. Metody opredeleniya toksichnosti i opasnosti khimicheskikh veshchestv. – M., 1970. - S. 161-163, 175-177.

6. SanPiN No. 0283-10 "Hygienic requirements for food safety". - T., 2010. SanPiN № 0283-10 «Gigiyenicheskiye trebovaniya bezopasnosti pishchevoy produktsii». - T., 2010.

7. SanPiN RUz No. 0296-11 in the new edition "Hygienic requirements for the production, circulation and regulation of food additives" (2015, 2017). - T., 2017. SanPiN RUz № 0296-11 v novoy redaktsii «Gigiyenicheskiye trebovaniya k proizvodstvu, oborotu i normirovaniyu pishchevykh dobavok» (2015, 2017 gg.). - T., 2017.

8. SanPiN RUz No. 0338-16 "Hygienic requirements for the production and circulation of biologically active additives (BAA) to food." - T., 2016. SanPiN RUz № 0338-16 «Gigiyenicheskiye trebovaniya k proizvodstvu i oborotu biologicheski aktivnykh dobavok (BAD) k pishche». - T., 2016.

9. SanPiN No. 0366-19 "Hygienic standards for food safety". - T, 2019. SanPiN № 0366-19 «Gigiyenicheskiye normativy bezopasnosti pishchevoy produktsii». - T, 2019.

10. Khabriev R.U. Guidelines for the experimental (preclinical) study of new pharmacological substances. 2nd ed., revised. and additional - M., 2005. - S. 87-100. Khabriyev R.U. Rukovodstvo po eksperimental'nomu (doklinicheskomu) izucheniyu novykh farmakologicheskikh veshchestv. 2-izd., pererab. i dop. - M., 2005. - S. 87-100.

11. Khudaiberganov A.S., Kamilova R.T., Navruzov E.B., Khegai L.N., Basharova L.M. Guidelines for conducting toxicological studies of food and biologically active food supplements in animal experiments No. 012-3/0312 dated 18.06. - T., 2017. Khudayberganov A.S., Kamilova R.T., Navruzov E.B., Khegay L.N., Basharova L.M. Metodicheskoye rukovodstvo po provedeniyu toksikologicheskikh issledovaniy pishchevykh i biologicheski aktivnykh dobavok k pishche v eksperimente na zhivotnykh № 012-3/0312 ot 18.06. - T., 2017.

12. Parasuraman S. Toxicological screening // J. Pharmacol Pharmacother. - 2011 Apr-Jun; 2(2): 74–79.