Jundishapur Journal of Microbiology Research Article
Published online 2022 April Vol. 15, No.1 (2022)

Pre-clinical study of acute toxicity of biologically active additive "ascorbic-drop"

N.M. Rizaeva, R.A. Khusainova, K.S. Makhmudzhanova, B.S. Tulyaganov

Received 2022 **February** 2; **Revised** 2022 March 20; **Accepted** 2022 April 24

Annotation: The acute toxicity of the dietary supplement "Ascorbic-drop" was investigated in the scientific study that was provided, which was based on preclinical investigations. Pharmacological, biochemical, morphological, and statistical methodologies were all used to get the study's findings. We can discuss and provide pertinent information regarding a pre-clinical investigation on the acute toxicity of the biologically active additive "Ascorbic-drop" in this article.

Key words: biologically active additive, preclinical study, acute toxicity, dose, adsorbent, oral administration, microcirculatory changes, intoxication, trauma.

Biologically active food additives (BAA), along with specialized food products, are the most effective way to eliminate vitamin deficiencies, but provided that biological substances are contained in doses that correspond to the physiological needs of a person. Over the past few years, there has been a dynamic development of the market for medicines and dietary supplements. According to the Law of the Republic of Uzbekistan "On the quality and safety of food products" of August 30, 1997, biologically active additives (BAA) are given the following definition: these are concentrates of natural or identical to natural biologically active substances obtained during the processing of food raw materials or artificially and intended for direct ingestion or introduction into food products. Analysis of dietary supplements use, the impact of their action on the human body and animals, is relevant and timely [1,2,3].

As early as 2,500 years ago, the great physician of ancient Greece, Hippocrates, showed that by changing the diet and level of physical activity, you can heal or at least alleviate the course of many diseases. The experience of traditional and official medicine not only does not contradict the ideas of Hippocrates, but significantly expands and deepens them.

Dietary supplements to food and feed have entered our life relatively recently and a new branch of knowledge - pharmaconutriciology - began to develop rapidly.

The dietary supplement may include:

- vitamins (for example, vitamin A);
- minerals (for example, ferrum, magnesium);
- nutrients (for example, amino acids, fatty acids);
- substances of physiological action (for example, plant extracts, yeast spores, lactic acid bacteria).

Dietary supplements are not a substitute for a varied diet. For example, compared to a vitamin capsule, fresh fruits and vegetables provide a diverse range of nutrients that the body needs in the right ratios for absorption, and also make you feel full. In general, with a balanced and varied diet, a person

receives all the nutrients necessary for the normal functioning of the body. Dietary supplements should not be consumed without reason, since their excessive consumption can harm the body. It is also important that long-term use of unbalanced food and dietary supplements can impede the absorption of nutrients. For example, iron and calcium during assimilation compete with each other, therefore, it makes no sense to take calcium tablets with meat food and vice versa [4,5,6].

The objective of the tests: safety assessment of dietary supplements "Ascorbic-Drop" included the following volume of studies:

- determination of the oral average lethal dose;
- definition of cumulative (subchronic) action;
- study of the irritating effect on mucous membranes;
- study of sensitizing properties.

The object of the study is the toxic effect of **"Ascorbic-Drop".**

Toxicological assessment of dietary supplements "Ascorbic-Drop", the contents of which were intragastrically injected in the expected toxic dose to laboratory animals, followed by observation during the experiment to identify clinical signs of intoxication. This observation will provide information for the assessment and classification of risk.

Experimental studies were carried out on small laboratory animals (white rats and mice, guinea pigs) in accordance with the current regulatory and methodological framework. When extrapolating the obtained toxicological data from animals to humans, we took into account the interspecies differences in the toxic effect in experimental animals, the degree of toxicity and hazard of the additive, the peculiarities of a particular experiment (methods and methods of introducing a substance into the body, seasonal and circadian rhythms, etc.), factors of uncertainty. When assessing the irritating effect on the skin and mucous membranes of the eyes, the sensitizing effect was used direct transfer of experimental results to humans [7, 8, 9].

Experimental tests were carried out in compliance with the rules adopted by the European Convention for the Protection of Vertebrate Animals for Experiments or other Scientific Purposes (ETS № 123. Strastburg, 18.03.1986).

Research results and discussion

The dietary supplement "Ascorbic-Drop" was introduced in its native form. For intragastric administration of large doses, fractional administration was used. Experimental animals received the same dose in mg/kg per body weight of the object of research within observation hours (16-20 hours), the control received an adequate dose of distilled water. The animals were fed 3 hours after dosing.

On the basis of a comparative study of organs and tissues of animals, experimental and control groups, it can be concluded that the intragastric administration of the studied dietary supplements "Ascorbic-Drop" does not cause pathological changes and differences in the histological structure of internal organs in comparison with the control.

The results obtained were subjected to statistical processing using standard programs with an assessment of the significance of indicators (M±t) and differences according to the Student's t-test and methodological recommendations "Using the principles of evidence-based medicine in organizing and conducting hygienic research" based on Word 2010. Differences in the compared groups were considered reliable at a significance level of 95% ($p < 0.05$).

Mortality. During the period of the experiment, the death of the experimental animals was not observed.

Clinical features. There were no clinical signs of intoxication during the period of the experiment.

Body weight. Observations of the change in the body weight of the rats showed that throughout the experiment, the animals of the experimental groups gained weight and the degree of weight gain of the experimental animals did not significantly differ from the weight of the animals of the control group.

Acute toxicity. Under experimental conditions, the determination of acute toxicity of the studied dietary supplements "Ascorbic-Drop" was carried out on 2 types of laboratory animals (white outbred rats and mice) with a single intragastric intake of each drug in doses of 2000, 4000 and 6000 mg/kg weight of animals. During the period of the experiment, the death of the experimental animals was not observed. The maximum dose of the studied dietary supplements in the stomach of laboratory animals exceeded the maximum daily dose recommended for humans by 4-12 times. On the next day of observation, the animals added in body weight, maintained a normal reaction to external irritants, the general condition and behavior of the animals in both experimental groups was satisfactory. All animals were active and willingly ate food, the coat and visible mucous membranes did not change. The death of animals during the entire observation period was not noted. Thus, the average lethal dose of the studied dietary supplements "Ascorbic-Drop" for the animals taken into the experiment was not reached. No differences were found in the sensitivity of mice and rats to drugs depending on the species and sex.

Table 1

Lethal effects of dietary supplements when administered intravenously to laboratory animals

Name of dietary supplement	Dose mg/kg	number of animals in the group /number of dead animals	Clinical picture of intoxication	LD_{50}
"Ascorbic-Drop"	2000	6/0	No	Not reached
	4000	6/0	No	Not reached
	6000	6/0	No	Not reached

Thus, the results of toxicometry and observation data of experimental animals in the period after acute poisoning make it possible to classify dietary supplements as “Stop-Gas”, “Vitadetrim”, “Ascorbic-Drop”, “Levokhvartin”, “Stop-Bacteria” as low-hazard drugs (IV class of hazard according to SSt

12.1.007) and practically non-toxic according to the accepted hygienic classification (V class).

Effect on the mucous membranes of the eyes.

A single inoculation of 0.05 ml (2 drops) of dietary supplements "Ascorbic-Drop" in its native form into the conjunctival sac of the right eye of a guinea pig, the left one served as a control (in each group, 3 animals). There was no hyperemia, lacrimation, or blepharospasm under the influence of the supplements.

The average group total score of the severity of irritation of the mucous membrane (lir) after the termination of contact was 0 points in all samples.

Table 2

The results of assessment the effect on the mucous membranes of the eyes of the studied dietary supplements, (points)

Name of production	Conjunctivalhyperemia	Eyelids Swelling	Ptosis or blepharospasm	Discharge from the eye	lir, points
"Ascorbic-Drop"	0/3	0/3	0/3	0/3	0

Consequently, the research data obtained showed that the dietary supplement "Ascorbic-Drop" in the concentration of use does not irritate the mucous membrane of the eye (lir = 0 points).

Cumulative properties (subchronic experiment).

The cumulative ability of the studied dietary supplements "Ascorbic-Drop" was determined by the method of "subchronic toxicity" (according to Lim) on white rats weighing 110-120 g.

The dietary supplements under study were injected intragastrically for 28 days. The initial dose was the recommended single dose, followed by an increase every 4 days by 1.5 times, which was more than 9 times the recommended single dose. Control animals were injected with distilled water in an equivalent volume. The experimental animals were observed during the entire experiment according to the following indicators: survival during the experiment, general condition, activity of animals, food intake, water consumption, body weight dynamics, morphological composition of blood, biochemical blood parameters.

The animals taken into the experiment did not show any deviations in behavior during the entire observation period. Similarly to the control animals, they were active, tidy, ate food well and adequately responded to external stimuli. Signs of intoxication and lethal outcomes were not noted.

As can be seen from the data presented in the table below, the animals were divided into groups with the same initial weight. Observations of the change in the body weight of the rats showed the same weight gain, while the degree of weight gain did not differ in the experimental groups compared to the control.

Table 3

Dynamics of the body weight of rats (in % to the initial)

Observation periods	Group of animals	
	control, distilled water	"Ascorbic-Drop"
Before taking	100.0	100.0
After taking	128.0	126.5

In the study of hematological parameters of the peripheral blood of experimental animals, no significant changes were revealed in any of the studied parameters. Hematocrit (ratio of erythrocytes to plasma), hemoglobin content, thrombocrit (ratio of platelets in total blood volume), leukocyte and erythrocyte content in all experimental animals did not differ significantly from the control.

Table 4

Average indices of the morphological composition of rat blood with subchronic exposure of the studied dietary supplements

Groups	observation period	Hematological indicators				
		hematocrit,%	hemoglobin concentration, g/l	thrombocyte, %	leukocytes, $*10^9$/l	erythrocytes, $•10^{12}$/l
Control, distilled water	Before taking	33,8±1,2	131,8±4,2	0,459±0,04	14,65±0,53	6,67±0,13
	After taking	34,9±0,5	142,4±2,4	0,450±0,02	14,58±0,59	6,62±0,25
"Ascorbic-Drop"	Before taking	34,9±0,5	142,4±2,4	0,450±0,02	14,58±0,59	6,62±0,25
	After taking	36,6±1,1	136,8±2,3	0,438±0,05	14,61 ±0,39	6,80±0,18

As the results of studying the biochemical parameters of blood serum of experimental and control animals showed, the activity of transaminase enzymes (AsT, AlT) and alkaline phosphatase (ALP) of experimental animals did not significantly differ from those in the control group. Indicators of the total protein content (TP) of the control and experimental groups were also significantly the same.

Table 5

Biochemical parameters of the blood of white rats with subchronic exposure of the studied

dietary supplements

Groups	Statistical indicators	observation period, week	Biochemical indicators			
			Alt, U/l	AsT, U/l	ALP, U/l	TP, g/l
Control, distilled water	M ± t	1	54,2±2,5	116,0±5,26	36,2±7,5	66,2±0,7
		4	56,1±3,1,	114.8±5,4	33,4±4,9	66.1 ±0,3
"Ascorbic-Drop"	M ± t	1	50,2±3,7	112,4±5,17	32,6±5,6	62,0±0,5
		4	52,0±3,7	119,8±3,6	34,8±1,6	65,2±2,3

Pathomorphological studies were carried out the day after the last injection. According to the results of a macroscopic examination of the studied organs, no differences were ascertained between the experimental and control groups. On external examination, no excreta from natural openings were found. Rats are of the correct constitution, satisfactory nutrition. The coat is shiny, neat-looking, no foci of alopecia are found. The teeth are preserved. Visible mucous membranes are pale and shiny. The mammary glands of females were without seals to the touch, there was no secretion from the nipples. The male genitals are developed properly; there is no deformation or edema of the limbs.

At the end of the experiment, rats of control groups and animals receiving dietary supplements "Ascorbic-Drop" were euthanized by introducing into deep anesthesia with ether, and the state of internal organs was assessed visually during autopsy. Considering that no pronounced pathological changes were observed in rats of both the control and experimental groups, histological examination was performed of randomly selected tissue samples of rats, three from each group.

Tissue samples were fixed with neutral formalin, passed through alcohols of increasing strength, and embedded in paraffin. Paraffin sections were prepared, stained with hematoxylin-eosin and examined under a light microscope magnification.

Macroscopic pathoanatomical studies and determination of the relative mass coefficients of internal organs showed that dietary supplements "Ascorbic-Drop" did not cause toxic degenerative changes in lymphoid and most important internal organs.

According to the results of a macroscopic examination of the studied organs, no differences were ascertained between the experimental and control groups.

It was found that in all experimental groups, when the animals were dissected, the following was noted:

- the thoracic and abdominal cavities did not contain any effusion. The position of the internal organs of the thoracic and abdominal cavities was not disturbed. The parietal and visceral layers of the pleura and peritoneum are thin, shiny, smooth;

- the thyroid gland is reddish in color, of normal size and shape, of moderately dense consistency. The thymus is triangular, whitish, moderately dense, of normal size;

- the intima of the aorta is smooth, shiny, whitish in color. The diameter of the aorta is not changed. The leaves of the pericardium are thin, transparent, smooth. The size and shape of the heart do not represent changes. The left ventricle is contracted, the right one contains a small amount of dark liquid

blood. Heart valves are thin, shiny, smooth. The heart muscle in the section is of a uniform brown color, moderately dense;

- the lumen of the trachea and large bronchi is not changed, the mucous membrane is shiny, smooth, pale in color. Light airy, without seals to the touch, pale pink in color;

- the mucous membrane of the esophagus is shiny, smooth, pale in color. The stomach is of normal size and shape, filled with food contents. The mucous membrane of the glandular part of the stomach is folded, pinkish, shiny. The mucous membrane of the stomach is folded, pinkish, shiny. The mucous membrane of the small intestine is pale pink, shiny, smooth. The mucous membrane of the large intestine is grayish, shiny, smooth;

- the shape and size of the liver did not show any changes. The surface of the liver is smooth, uniform dark red color, the capsule is thin, transparent. Liver tissue on the cut is full-blooded, moderately dense;

- the pancreas is flat, pale pink, lobed, of moderately dense consistency;

-spleen is of normal shape, dark cherry color, moderately dense consistency. The surface of the organ is smooth, the capsule is thin. On a cut on a dark red background of the spleen, small grayish follicles are visible;

- the size and shape of the kidneys are not changed. The surface of the kidneys is brownish-brown, smooth, the capsule is thin, transparent, easily removable. On the section of the organ, the cortex and medulla are clearly distinguishable;

-adrenal glands are rounded, pale yellow in color, with a smooth surface, moderately dense. The section clearly shows the dark-colored medulla;

- the bladder is filled with clear urine. The mucous membrane of the bladder is smooth, shiny, pale in color;

- the body of the uterus of females is of normal density, size and shape. The horns of the uterus are thin, the mucous membrane is shiny, pale. The ovaries are dark red, with an uneven surface, moderately dense. The testicles of males are whitish in color, of normal size and density;

- the membranes of the brain are thin, transparent. The substance of the brain is of normal density, the surface of the brain is smooth. On the frontal sections of the brain, gray and white matter are clearly distinguished. The ventricles of the brain are of normal size, there is no extension.

During histological examination of preparations of the lungs, myocardium, liver, kidneys and gastric mucosa of experimental animals, dystrophic, inflammatory or necrobiotic changes of the above organs were not observed.

Table 6

Assessment of the cumulative effect of the studied dietary supplements in comparison with the control

Research	"Ascorbic-Drop"
General state	No
Hematological indicators	No
Biochemical indicators	No
Research of organs and tissues	No

Thus, the conducted studies of dietary supplements "Ascorbic-Drop" showed that daily intragastric administration in rats in an increasing dose for 28 days does not cause lethal effects, does not lead to significant changes in physiological parameters, does not cause dystrophic or destructive changes in the parenchymal organs and is not accompanied by irritation of the mucous membranes of the gastrointestinal tract. According to the integral indicators of subchronic toxicity, dietary supplements "Ascorbic-Drop" do not have the ability to cumulate and are non-toxic.

Study of the sensitizing effect.

The sensitizing effect of the studied dietary supplements "Ascorbic-Drop" was assessed by the scarification method.

In experimental animals, 10 days after the start of the synchronous experiment, sensitization is detected by setting a skin scarification test with a drop of each studied dietary supplement separately (test - antigen), the amount of which does not cause a visible reaction in animals, animals of the control group who received intragastrically distilled water, the resolving dose was applied similarly to experimental animals: a drop of the studied dietary supplement (-0.8 -1.0 g) was applied to the area of the lateral surface of the body, followed by an incision with a scarifier through a drop 1-1.5 cm long.

Sensitization is detected by the reaction of the skin at the site of scarification after 4-24-48 hours according to the appropriate scale.

Testing carried out after the scarification test of the studied dietary supplements "Ascorbic-Drop" showed the following: in all groups of animals the reaction was clearly negative (according to the rating scale: "-"), i.e. sensitization index (Is) was 0 points in each sample.

Table 7

The results of evaluating the sensitizing effect of the studied dietary supplements

Tested concentration	Hyperemia	Hyperemia and induration	Blister up to 5 mm, hyperemia вокруг	Blister up to 10 mm, lichenification	Is, points
Control, distilled water	0/6	0/6	0/6	0/6	0
"Ascorbic-Drop"	0/6	0/6	0/6	0/6	0

Consequently, dietary supplements "Ascorbic-Drop" do not have a sensitizing effect (Is = 0 points), i.e. do not provoke the development of allergies.

Conclusions

The tests have shown that dietary supplements "Ascorbic-Drop" - in the recommended doses of use, do not have a negative effect on the health of experimental animals, are not toxic (class 4 - low-toxic substances), do not have cumulative (hematological and biochemical blood parameters of experimental animals, as well as their weight fluctuated within physiological norms and did not differ from the control), irritating and sensitizing effects. No dystrophic, necrotic and inflammatory changes in animals observed in the experiment, as well as differences in the structure of their internal organs in comparison with the control, were found.

References

1. General toxicology / Ed. B.A. Kurlyandsky and V.A. Filova. - M.: Medicine, 2002. – p.606
2. Order of the Ministry of Health of the Russian Federation № 199n dated 01.04.2016 "On Approval of the Rules of Good Laboratory Practice". - Access mode: https://www.begalacts.ru/
3. Guidelines for experimental (preclinical) study of new pharmacological substances / ed. RU. Khabrieva. -2-ed., Rev. and additional - M.: JSC "Publishing House "Medicine", Moscow. - 832. - 2005
4. Menshikov, V.V. Laboratory research methods in the clinic. /V.V. Menshikov, L.N. Delectorskaya, R.P. Zolotnitskaya - M., 1987. – p.320
5. Tarkovskaya, I.A. Oxidized coal Text. study textbook for universities A. Tarkovskaya; Kiev: Naukova Dumka. 1981 . – p.200
6. Gomez-Serrano V., Piriz-Almeida F., Duran-Valle C. J., Pastor-Villegas J. Formation of oxygen structures by air activation. A study by FT-IR spectroscopy // Carbon. - 1999. - V.37. - pp. 1517-1528
7. Rational pharmacotherapy of diseases of the digestive system: a guide for practicing physicians / under the general. ed. V. T. Ivashkina.M.: Litterra, 2003 .- p.1046
8. Study of the effect of different sorbents on the state of the mucous membrane of the gastrointestinal tract P. B. Novikov, I. Ye. Trubitsyna, B. Z. Chikunova No. 12 (107) Endocrinology, Dermatology, Immunology, Allergology "Potential toxicity of aluminum-containing preparations".
9. Borodin Yu.I. On the functional interaction of sorbing substances with lymphatic structures. Problems of sorption detoxification of the internal environment of the body. / Materials of the international symposium. Novosibirsk. 1995. Pp.3-7