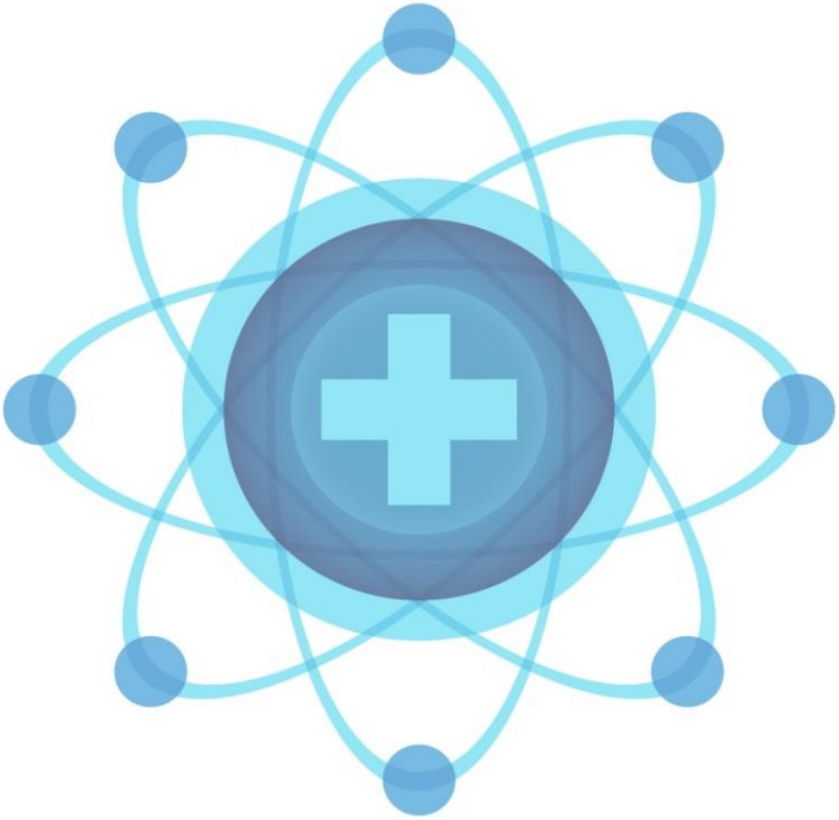

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Toxicological assessment of soy protein isolates "ALFA SOY 001"

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Abstract. The results of the toxicological assessment of soy protein isolate ALFA SOY 001 based on studies in the scope of the toxicological passport are presented. On the basis of studies, it has been established that, in terms of acute toxicity, it refers to low-toxic raw products, does not have a cumulative, irritating effect on the mucous membranes of the eyes (Iir = 0 points), and sensitizing effects (Is = 0 points). The results obtained allow us to conclude that with repeated oral intake of the soy protein isolate ALFA SOY 001 there is no systemic toxicological effect.

Keywords: soy protein isolates ALFA SOY 001, toxicology, acute toxicity parameters.

Conclusions: soy protein isolate ALFA SOY 001, produced by YUNUSJON AHLI LLC, Uzbekistan - in terms of acute toxicity, refers to low-toxic raw materials, does not possess cumulative (hematological and biochemical blood parameters of experimental animals, as well as their weight, fluctuated within physiological normal and did not differ from the control), irritating to the mucous membranes of the eyes (Iir = 0 points) and sensitizing effect (Is = 0 points). No dys-trophic, necrotic and inflammatory changes in animals observed in the experiment, as well as differences in the structure of their internal organs, were found.

A sharp acceleration in the growth rate of the world's population and an ever-increasing shortage of dietary protein has put before mankind with great urgency the search for its additional resources. In particular, the rationale for the use of soy products as food components, the toxicological assessment of the medical and biological safety of soy and soy protein isolate is being carried out. Soy is a unique plant with a high content of biologically active and highly nutritious protein. Whole soybeans are distinguished by a significant content of high-quality protein, fats, carbohydrates, fiber, polyunsaturated fatty acids, minerals and vitamins of groups B, D, E. In addition, the beans contain biologically active substances: phytosterols, flavanoids, saponins.

Soy products have important therapeutic and prophylactic properties, they have an antitumor, anti-sclerotic effect on the human body, stimulate the cardiovascular system, lower blood cholesterol levels, contain few calories, and are recommended for people with diabetes. Due to these properties, soybean products are widely used in the technology of special products for dietary and preventive nutrition [1, 2].

Soybeans are a unique source of high quality protein, with an average protein content of 38%. The biological value of soy protein is twice as high as that of other vegetable proteins, and approaches that of animal proteins. Soy protein has an

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optimal balance of amino acid composition and is as well absorbed as milk and meat protein. Soy lipids contain about 70% unsaturated fatty acids [5].

Soy protein isolates and concentrates are complete, high quality proteins that are highly digestible compared to animal proteins (casein). In fact, soy protein can serve as an important source of protein for both adults and children. While protein makes up 20 to 30% by weight of most legumes, it makes up roughly 35 to 38% by weight of soybeans. The amount of protein varies in different soy products: soy flour contains 50% protein, soy concentrate contains 70% protein, and soy isolates contain 90% protein. To date, many countries around the world have developed soy industrial production that produces textured protein, as well as other soy products (butter, milk, pasta, margarine, ice cream, chocolate, etc.) [1,2].

Soy isolate is obtained from a by-product of soybean oil production, which is a meal. However, given that soybean and products obtained from it are a source of various amino acids, including essential ones, meal is used to obtain protein, which in the form of a purified isolate is in great demand in the production of meat food products. In addition to the fact that soy isolate is a protein concentrate, it has valuable qualities for food production which are good gelation and the ability to create a stable protein-fat emulsion. All this very well characterizes soy isolate as a full-fledged raw product, which makes it possible to improve the composition of meat products in terms of balanced protein content.

Employees of YUNUSJON AHLI LLC in Uzbekistan have developed ALFA SOY 001 soy protein isolate, of their own production, which is made from non-genetically modified soybeans.

Soy protein isolate ALFA SOY 001 is used as a food additive in food production and is produced from non-genetically modified soybeans. TS 24179156-001:2019 was developed, produced by YUNUSJON AHLI LLC, approved by the Ministry of Health of the Republic of Uzbekistan and GOST standard.

According to the requirements of the Law of the Republic of Uzbekistan "On the sanitary and epidemiological well-being of the population" and the Law of the Republic of Uzbekistan "On the quality and safety of food products", all new food raw materials must be subjected to a special examination to assess their safety and harmlessness.

Purpose of the work: toxicological evaluation of ALFA SOY 001 soy protein isolate when administered intragastrically to laboratory animals in high doses, in contact with mucous membranes, as well as to assess possible general toxic effects in a sub acute experiment and determine sensitizing ability.

Materials and research methods.

The following materials were used: soy protein isolates ALFA SOY 001; 2 species of animals (outbred rats and mice) with a single intragastric administration at doses of 4000, 6000 and 8000 mg/kg of body weight; guinea pig;

The safety assessment of ALFA SOY 001 soy protein isolate included the following scope of studies:

- Determination of the oral average lethal dose [2];

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- Definition of cumulative (subchronic) action [1,6];
- Study of irritating action on mucous membranes [8];
- Study of sensitizing properties [5,7].

The studies were carried out in accordance with the legislative and regulatory and methodological documentation of the Republic of Uzbekistan [1, 2, 5, 8, 9].

Blood parameters were assessed on a semi-automatic biochemical analyzer "CYANS mart" with software (Cypress Diagnostics, Belgium) according to standard methods (AST, ALT, ALP, total protein - reagent kits Cypress Diagnostics, Belgium), hematocrit was determined on a hematocrit centrifuge (Cypress Diagnostics, Belgium). Belgium), a detailed analysis of peripheral blood was determined in the Goryaev chamber.

In the experiments, small laboratory animals (white rats and mice, guinea pigs) were used, convenient for direct extrapolation of the results obtained to the human body in accordance with the accepted methodology.

Animals received the same dose in mg/kg per body weight of the studied soy protein isolate in the form of an aqueous solution within hours of observation on an empty stomach, followed by feeding according to the method. During the entire period of the experiment, daily registration of behavioral reactions, clinical manifestations of intoxication, dynamics of weight and death of experimental animals was carried out.

Statistical processing was carried out with an assessment of the significance of indicators (M ± m) and differences according to Student's t-test based on Word 2010. Differences in the compared groups were considered significant at a significance level of 95% ($p<0.05$).

All surviving animals were euthanized at the end of the study by ether anesthesia and disposed of after macro- and microscopic morphological studies. No organ or tissue has been used for other purposes.

Research results

To determine the establishment of acute toxicity parameters of soy protein isolate ALFA SOY 001 - studies were carried out on 2 animal species (outbred rats and mice) with a single intragastric administration at doses of 4000, 6000 and 8000 mg/kg of body weight. The death of experimental animals was not observed not on the day of introduction of the isolate, nor in the next 14 days of observation. In view of the absence of death of experimental animals for the entire period of the acute experiment, it was not possible to calculate the average lethal dose, as well as to determine the differences in sensitivity to soy isolate of experimental animals by sex and species (Table 1).

Table 1

Lethal effects with intravenous administration

Name	Dose mg/kg	number of animals in the group / number of dead animals	LD_{50}

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Soy protein isolate ALFA SOY 001	4000	6/0	not reached
	6000	6/0	not reached
	8000	6/0	not reached

Thus, the results obtained for the assessment of the acute toxicity of soy protein isolate ALFA SOY 001 were classified as low-hazard substances (IV class) according to GOST 12.1.007.

At the next stage of the work, we studied the effect of soy protein isolate ALFA SOY 001 on the mucous membranes of the eyes of animals.

Evaluation of the possible effect on the mucous membranes, in the conjunctival sac of the right eye of a guinea pig (the left one served as a control: in the group of 3 individuals), soy isolate was inoculated once in the form of 2 drops of a 50% aqueous suspension.

The average group total score for the severity of mucosal irritation (Iir) after cessation of contact was 0 points in all samples (Table 2).

Table 2

The results of the evaluation of the action on the mucous membranes of the eyes of the product, (points)

Name of production	***Conjunctival hyperemia***	***Edema of the eyelids***	***Ptosis or blepharospasm***	***eye discharge***	***Iir, points***
Soy protein isolate ALFA SOY 001	0/3	0/3	0/3	0/3	0

Therefore, the research data obtained showed that soy protein isolate ALFA SOY 001 - does not irritate the mucous membrane of the eye (Iir = 0 points).

The next phase of research was devoted to the study of cumulative properties (subacute experience).

To assess the general toxic effect, a subacute experiment was carried out on rats. Animals were divided into 2 groups: experience and control. Animals in groups were selected approximately the same weight with a difference of no more than 20%.

The control group was on general vivarium food. The diet of the experimental group was based on the diet of the control group with additional intragastric administration of the studied Soy Protein Isolate ALFA SOY 001 - at the rate of 1/10 of the maximum tolerated dose when assessing acute toxicity, followed by an increase in the dose every 7 days by 1.5 times, the duration of the experiment was 28 days. A large volume of the test isolate was administered in a fractional manner.

During the experiment, the death of animals was not recorded, no signs of intoxication were observed. The animals responded adequately to external stimuli, were mobile, actively consumed food and water, kept neatness, the coat was smooth and shiny, visible mucous membranes had a normal color. Excrement was excreted according to feed intake.

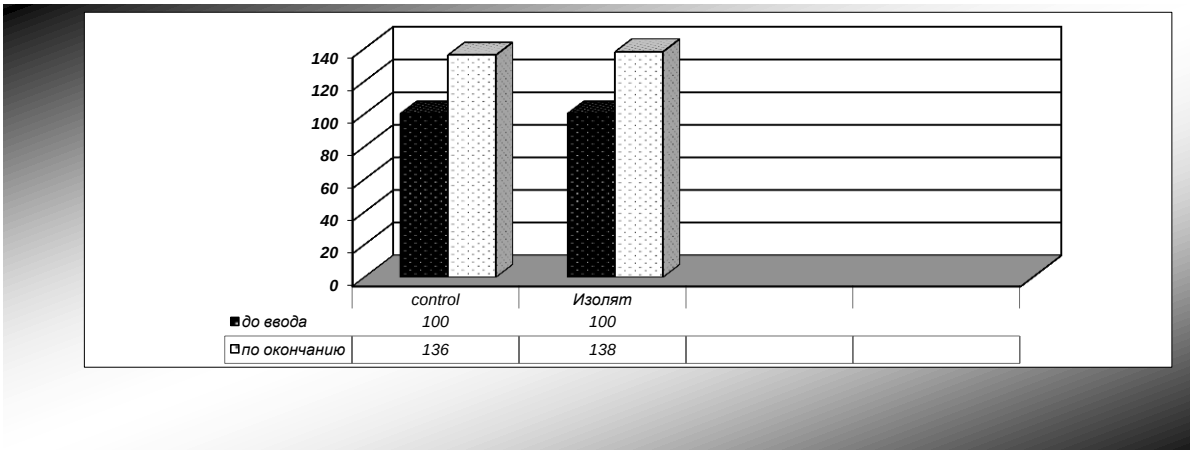

Figure 1. Change in the weight of rats, in % of the initial weight

A simple and rather sensitive indicator of the adverse effects of the test product on the body is the dynamics of animal body weight. Therefore, every 7 days the body weight of the animals was determined. At the same time, there were no significant differences between the control and experimental groups (Figure 1).

The morphological parameters of the blood of the experimental animals did not differ from those of the control group and were within the physiological limits for this species. (Table 3).

Hematocrit (ratio of erythrocytes and plasma), hemoglobin content, thrombocrit (ratio of platelets in total blood volume), leukocyte and erythrocyte counts in all experimental animals also corresponded to those of the control group.

Table 4

Average indicators of the morphological composition of the blood of rats under exposure to soy protein isolate

Groups	period observations	Hematological indicators				
		hematocrit, %	concentration hemoglobin, г/л	thrombocrit, %	leukocytes, $\cdot 10^9$/л	erythrocytes, $\cdot 10^{12}$/л
The control, distill. water	before the introduction	31,9±1,18	132,2±5,1	0,46±0,02	13,71±0,48	6,58±0,31
	at the end	32,3±0,20	134,4±3,2	0,44±0,04	14,28±0,69	6,45±0,35
Soy protein isolate ALFA SOY 001	before the introduction	33,8±1,21	133,6±4,3	0,45±0,01	14,38±0,51	6,35±0,21
	at the end	35,1±1,2	135,5±4,5	0,436±0,01	14,40±0,455	6,46±0,18
	at the end	31,9±1,18	132,2±5,1	0,46±0,02	13,71±0,48	6,58±0,31

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With repeated intake of the same raw materials, the body can accumulate the component itself, which comes in excess, or it affects the liver by interacting with the cells of the organ and thereby causing a response in the form of a violation of the biochemical status with a change in the parameters of amylase enzymes. Such a state can occur with functional cumulation, and a change in the biochemical parameters of the blood is an early sign of a violation of the body's activity.

Table 5

Biochemical parameters of the blood of white rats with infraspinatus exposure to isolate

Groups	Statistics	Observation period, week	Биохимические показатели			
			AlT, E/l	AcT, E/l	AlP, E/l	TP, g/l
The control, distill. water	M±m	Before the introduction	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
Soy protein isolate ALFA SOY 001	M±m	Before the introduction	50,2±3,7	112,4±5,17	32,6±5,6	62,0±0,5
		4	54,2±2,5	116,0±5,26	36,2±7,5	66,2±0,7

The results of biochemical analyzes of the blood of animals showed that the activity of transaminase enzymes (AST, ALT), alkaline phosphatase (ALP) and total protein (TP) of experimental animals were within the physiological limits and did not differ from the values of control animals.

At the end of the experiment, the experimental animals of both groups were euthanized by anesthesia with ether and the state of the internal organs was assessed visually during autopsy. Pathological changes in rats of both the control and experimental groups were not observed, the specific mass of internal organs in the experimental groups did not differ from the control.

When determining the relative mass of organs, convincing data on the presence of tissue edema, impaired blood supply or hemorrhages were not obtained. There were no significant differences between the groups in terms of gravimetric coefficients.

The conducted studies have shown that daily administration of ALFA SOY 001 soy protein isolate to rats at an increasing dose for 28 days does not have a general toxic effect in the sub-acute experiment.

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Table 6

General assessment of the action of the studied isolate in comparison with the control

Research	The control, distill. water	Soy protein isolate ALFA SOY 001
General state	missing	missing
Hematological indicators	missing	missing
Biochemical indicators	missing	missing
Morphological parameters of some organs and tissues (skin, mammary glands, lymph nodes, lungs, heart, esophagus, stomach, small intestine, large intestine, liver, spleen, kidneys, bladder)	missing	missing

During the experiment, the somatic status of the animals in both groups was the same, which leads to the conclusion that the Isolate of soy protein ALFA SOY 001 does not have any type of cumulation.

At the last stage of the work, we studied the sensitizing effect of the soy protein isolate ALFA SOY 001. The sensitizing effect of the studied soy protein isolate ALFA SOY 001 was assessed by the scarification method [4] with the detection of sensitization at the site of scarification after 4-24-48 hours according to the following scale:

Type of reaction	Reaction designation/points	Description of the reaction
negative	–/0	the size of the incision is the same as in the animals of the control group
dubious	±/1	hyperemia at the site of scarification
weakly positive	+/2	hyperemia, slight induration at the site of scarification
positive moderate	++/3	blister up to 5 mm, clearly visible and surrounded by hyperemia
sharply positive	+++/4	hyperemia, blister up to 10 mm, lichenification

Testing carried out after a provocative scarification test of the studied soy protein isolate ALFA SOY 001 showed the following: in all animals (6 animals), the reaction was clearly negative (according to the rating scale: "-"), i.e. sensitization index (Is) was 0 points.

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Table 7

Results of the evaluation of the sensitizing effect studied soy isolate (reaction/score)

Tested Concentration	*Hyperemia*	*Hyperemia and induration*	*induration up to 5 mm, hyperemia around*	Blister up to 10 mm, lichenification	Is, points
Control, distill. water	–/0	–/0	–/0	–/0	0
Soy protein isolate ALFA SOY 001	–/0	–/0	–/0	–/0	0

Therefore, soy protein isolate ALFA SOY 001 does not have a sensitizing effect (Is=0 points), i.e. do not provoke the development of allergies.

CONCLUSION

Based on the results of toxicological studies, it was established that the studied soy protein isolate ALFA SOY 001, produced by YUNUSJON AHLI LLC, Uzbekistan, in terms of acute toxicity, belongs to low-toxic raw materials, does 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 to the mucous membranes of the eyes (Iir = 0 points) and sensitizing effect (Is = 0 points).

Dystrophic, necrotic and inflammatory changes in the animals observed in the experiment, as well as differences in the structure of their internal organs, were not found.

Thus, the results obtained allow us to conclude that with repeated oral intake, soy protein isolate ALFA SOY 001, produced by YUNUSJON AHLI LLC, Uzbekistan, has no systemic toxicological effect.

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