Medical-biological evaluation of the safety of soy protein isolate

Abstract: It was found that soy isolate is non-toxic, does not cause local irritant effect on skin and conjunctiva, has no cumulation and sensitizing properties, can be attributed to non-toxic and low-hazard food additives.

Keywords: toxicology, food safety, soy isolate.

Soya is the most valuable universal culture. Its seeds contain 17–26% fat, 36–48% well-balanced amino acid composition of protein and more than 20% carbohydrates. Semi-drying soybean oil (iodine number 107–137) has a high content of physiologically active essential fatty acids (linoleic, oleic, linoleic, and others.). The quality of soy protein significantly exceeds many other plants, including cereals and oilseed. Soy protein is well absorbed by the body and its biological value approaches to proteins of animal origin, equivalent in composition to the animal protein. Isolates and soy protein concentrates are complete, high-quality proteins, which are well absorbed when compared to proteins of animal origin (i. e. casein). In fact, soy protein may serve as the only source of protein for adults and for children. While the protein compounds from 20 to 30% of the weight of most legumes, it is about from 35 to 38% of soybean weight. The amount of protein varies for different soy products: soy flour contains 50%of protein, soy concentrate contains 70% protein, and soy isolates contain 90% of protein. To date, in many countries around the world have developed the industrial soy production, producing textured protein, and other soy products (butter, milk, pasta, margarine, ice cream, chocolate, etc.). [1]. Simultaneously from soybeans have been isolated and studied in detail phytosterols, isoflavones, genistein, protease inhibitor, lecithin, allergens [2; 3].

Soy protein isolate (made in China) is used as a dietary supplement in sausage products. This food additive is permitted to be used as a natural moisture-retaining agent according to Directive 29 CFR 19112000FAO/WHO Joint Committee on Food Additives (JECFA).

The above was the basis for toxicological studies of soy protein isolate: to study general toxicity of soy protein isolate with assessment of the possible irritant action on mucous membranes, as well as its possible cumulative effects; to study possible allergenic activity [4–11].

To carry out the experiment from the samples of soy protein isolate they prepared aqueous solutions (50%) in the amounts of 0.3–5 ml per animal.

Animals received the same dose in mg/kg body weight of investigated object within the hours of observation (16–20 hours). Animal feeding was performed in 3 hours after dose introducing. The diet is balanced in content of proteins, fats and carbohydrates, a special diet for the test animals.

Determination of acute toxicity of investigated soy protein isolate under experiment was carried out on white mongrel rats with a single intragastric intake of each form of preparation in doses of 1500 to 9000 mg/kg. Introduction of soy protein isolate at higher doses was technically impossible due to poor solubility and physiologically limited quantity of introduction of preparation in rats' stomach.

The maximum dose of preparation under study on tested animals exceeded the recommended process dose≈ in 13 times; control animals received the equivalent quantity of distilled water.

To study the effect on conjunctiva there performed the single 0.05 ml inoculation of aqueous suspension of soy protein isolate into the conjunctival sac of the rabbit eye. The allergic effect of investigated soybean protein isolate was assessed by single intradermal injection 0.02 ml of solutions of each pharmacological form, diluted in saline 50% concentration solution with a tuberculin syringe into the outer surface of ear of guinea pigs (6 guinea pigs in experimental group and 6 — in control). For comparative purposes control animals were introduced 0.02 ml saline. Identification of sensitization was made on 12–14 days after the injection of soy protein isolate solution: the drop test of each type of preparation under study was applied on the lateral body surface in the dosage that exceeded sensitizing in 1.5–2 times (≈4.9 mg), 1–1.5 cm long incision through drop was made by scarification. Skin response on the spot of scarification was observed after 4–24–48 hours by the following scale:

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Notation of response</th>
<th>Description of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>–</td>
<td>incision sizes are the same as in control group of animals</td>
</tr>
<tr>
<td>ambiguous</td>
<td>±</td>
<td>redness on the spot of scarification</td>
</tr>
<tr>
<td>weakly positive</td>
<td>+</td>
<td>redness, small induration on the spot</td>
</tr>
<tr>
<td>moderate positive</td>
<td>++</td>
<td>blister up to 5 mm, clearly visible and is surrounded by hyperemia</td>
</tr>
<tr>
<td>strongly positive</td>
<td>+++</td>
<td>redness, blister up to 10 mm, lichenification</td>
</tr>
</tbody>
</table>

Section 7. Medical science
The animals of the control group were divided into 2 subgroups and there performed scratch test: the first subgroup — with saline, the second — with soy protein isolate in anaphylaxis-provoking dose.

Cumulative capacity of investigated soy protein isolate was determined in sub acute experiment by «sub chronic toxicity» on white rats weighing 150–180 g.

The solution to investigated soy protein isolate was introduced intragastrically within 28 days. Initial dose compounded 1/10 of the recommended process water with further increase every 5 days in 1.5 times that exceeding the process water more than in 7 times. Control animals received distilled water in equal volume. Experimental animals were monitoring throughout the experiment on the following parameters: survival during the experiment, general state, activity of animals, feed intake, water consumption, body eight dynamics, morphological blood composition, and biochemical blood value.

All surviving animals were killed at the end of the study by decapitation and liquidation after pathomorphological investigations. No organ or tissue was used for other purposes.

Statistical analysis was performed by Student and Fisher to define the criteria for reliability of laboratory research and methodological recommendations «Using the principles of evidence-based medicine in organizing and conducting health studies» based on Word 2003 [12–14].

Body weight, clinical signs of toxicity, death/paralysis were monitoring throughout the experiment.

Results

No death of the test animals was observed over the experimental period. No clinical signs of toxicity were during the time of the experiment.

Body weight of the experiment animals was not significantly different from the control animals' weight. During experimental period the death of test animals was not observed. However, after the introduction of high doses the animals showed the anxiety, became disheveled, there was observed cyanosis of ears and tail, short motor excitation which slowly passed into the decline in physical activity in 30–40 minutes. After [2] hours the symptoms of acute poisoning disappeared completely. The animals were at rest. After [3] hours, the test rats actively ate food, had a neat appearance. The following days of observation the rats added in weight, kept the normal reaction to external stimuli. No death of animals during the entire period of observation noted.

Thus, the median lethal dose of investigated soy protein isolate for the test animals was not achieved.

When studied the effect on conjunctiva under the influence of the dietary supplement the low–grade redness of eyes was noted, after 15–30 minutes it disappeared, that was explained with mechanical irritation of mucous membranes. After washing with water the irritation had passed.

Consequently, research findings showed that soy protein isolate under investigation in concentration close to the process water use does not influence irritant action on mucous membranes.

Study of allergenic action presented the following: testing that was conducted after scratch test in preparations under study showed that in the animals of experimental group which received the soy protein isolate the response was clearly negative (by scale: “–”). Consequently, the analyzed soy protein isolate in technological doses had not allergenic action.

Study results of cumulative capacity of investigated soy protein isolate showed that the introduction of tested doses of preparation did not affect the basic integral indicators: rats had a neat appearance, quite properly responded to external stimuli, daily consumption of dry food and water in all groups of animals corresponded to normal.

Dynamics of animal body weight who received soy protein isolate during the whole experiment had no differences with the control (Table 2).

Table 2. – Dynamics of rats' body weight (% of initial) which received soy protein isolate within 28 days of observation

<table>
<thead>
<tr>
<th>Observation periods, days</th>
<th>Animal Group</th>
<th>Control, water</th>
<th>Soy isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>127.5 ± 3.1</td>
<td>128.6 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>140.8 ± 5.1</td>
<td>136.8 ± 3.3</td>
<td></td>
</tr>
</tbody>
</table>

No signs of toxicity and deaths were observed during subacute trial.

The study of hematological parameters of peripheral blood of the test animals no significant changes in any of the studied parameters revealed. The total number of erythrocytes, platelets, hemoglobin content in all the test animals was not significantly different from the control (Tab. 3).

Table 3. – Parameters of peripheral blood of rats which received with preparation under study “SEDAREM” within 28 days

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Nº of animal</th>
<th>Erythrocytes, 10¹²/l</th>
<th>Hemoglobin, g/l</th>
<th>Platelets, 10⁹/l</th>
<th>Leukocytes, 10⁹/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, water</td>
<td>1</td>
<td>6.4</td>
<td>150</td>
<td>426</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.8</td>
<td>172</td>
<td>476</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.7</td>
<td>149</td>
<td>399</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.8</td>
<td>170</td>
<td>450</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.1</td>
<td>159</td>
<td>421</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>M ± m</td>
<td>7.15 ± 0.23</td>
<td>159 ± 3.8</td>
<td>439.5 ± 12.8</td>
<td>11.78 ± 1</td>
</tr>
<tr>
<td>Soy isolate</td>
<td>1</td>
<td>7.4</td>
<td>162</td>
<td>390</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.7</td>
<td>168</td>
<td>386</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.5</td>
<td>188</td>
<td>463</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.1</td>
<td>195</td>
<td>399</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.6</td>
<td>164</td>
<td>389</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>M ± m</td>
<td>7.75 ± 0.48</td>
<td>175.5 ± 5.5</td>
<td>397 ± 18</td>
<td>11.1 ± 0.6</td>
</tr>
</tbody>
</table>
The studied integral biochemistry blood parameters of experimental animals throughout the whole experiment were within the physiological oscillations.

When investigating the effect of soy protein isolate on the functional state of the central nervous system of rats in the «open» field conditions it was established that in the tested doses it did not reduce locomotor activity of rats in the number of squares crossed test and orientation response in the test of «mink reflex» compared to the control.

Macroscopic investigations of internal organs of rats received soy protein isolate within 28 days were without features: all organs were correctly positioned, the cavities without effusions and adhesions. Serous membranes were smooth, nitidous. Airway was available: the lungs were elastic, airy, on the incision spot of normal color. Heart, kidneys, liver, spleen, thymus, gastrointestinal organs, adrenals, testes were of normal shape, texture, color and sizes.

Thus, the conducted research of soy protein isolate showed that it did not affect the basic integral indicators.

Conclusions
1. Soy protein isolate is non-toxic, does not cause locally-irritating or system toxicity. It has no cumulating and sensitizing properties.
2. Macroscopic studies conducted at the end of the experiment showed that soy protein isolate with prolonged introducing in doses more than in 7 times higher than the recommended single process does not cause local irritant and systemic toxicity.
3. The test soy protein isolate can be attributed to non-toxic, (relatively harmless by S.D. Zaugolnikov) and low-hazard (IV class of danger according to State Standard Certifications 12.1.007) food additives.

References:
7. Standards for research on chemistry № 23 “Thorough toxicological evaluation — the classic method” of March 22, 1996.

Metabolic disorders in patients with adrenal incidentalomas

Abstract: In our study metabolic disorders was the basic component in manifestations of adrenal incidentalomas to be contributed by dislipidemia, high BMI, age, high levels of hormones and glucose, and to be confirmed by significant changes in the parameters as compared with those in the control group and those in group of patients without metabolic disorders. In addition, significant correlation was established between frequency of metabolic disorders and subclinical hormonal activity, high levels of cortisol and aldosterone, in particular.

Keywords: hormones, adrenal incidentalomas, metabolic disorders.

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