STUDY OF THE IMMUNOMODULATORY EFFECT OF POLYSACCHARIDES FROM THE TURNIP *BRASSICA RAPA* SEED

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https://doi.org/10.5281/zenodo.11390458

Abstract. *When organizing the conditions for conducting research on the immunocorrecting properties of pharmacological agents, the most discussed issues are the choice of methods, doses and administration schemes of the drug under study. The choice of a targeted assessment of the immunotropicity of pharmacological substances is developed on the basis of information about the physico-chemical and biological properties of the drug under study.*

The Laboratory of Pharmacology and Screening of biologically active substances of the Institute of Bioorganic Chemistry has accumulated experience in studying the immunomodulatory and immunocorregulating effects of pharmacological agents using an integrated approach. This approach includes the study of the effect of the studied compound both with a single administration in a wide range of doses, and with a course administration in doses selected with a single administration and recommended for clinical study of a potential drug. A single administration of the test drug simultaneously with the antigen allows you to identify a direct effect on the cells of immune organs.

Keywords: *immunotropicity, Brassica rapa, water-soluble polysaccharides, hypolipidemic, cardioprotective.*

Based on these judgments, when testing for immunotropicity of a substance from *Brassica rapa* of plant origin, we proposed to use a step-by-step approach. In assessing the immunomodulatory effect of the substance, priority was given to functional methods of immunological research.

Vegetables of the *Brassicaceae* family are considered part of the human diet, consumed by people all over the world. Many studies have also shown that there is an inverse relationship between the consumption of *Brassicaceae* vegetables and the risk of chronic diseases, especially cardiovascular diseases, also in cancer, Alzheimer's disease, cataracts and age-related functional decline [1, 2].

Brassicaceae plants grow in Europe, Russia, Central Asia, the Middle East and are now widely cultivated as sources of vegetables and oils all over the world. The *Brassicaceae* family has about 3,500 species and includes 350 genera [3]. *Brassica rapa* – turnip is one of the common representatives of the *Brassicaceae* family. There are several varieties of turnips growing in Uzbekistan, such as Namangan, Samarkand, Muyassar, etc. Brassica gara contains biologically active compounds such as vitamins, polyphenols, polysaccharides, carotenoids, alkaloids, isothiocyanates, indoles, terpenoids, tocopherols and antioxidant enzymes [4]. *Brassica rapa* has shown a variety of biological activity, including antioxidant [5], antitumor [6], antidiabetic [7],

anti-inflammatory [8], antimicrobial [9], hypolipidemic, cardioprotective [10], hepatoprotective [11], nephroprotective [12] and analgesic effect [13].

Materials and methods. *Plant material*. Turnip seeds *Brassica rapa* L. were collected in July 2020 in the territory of Republic of Uzbekistan (Namangan region, Mingbulak district). *Degreasing and removal of low molecular weight impurities*. The crushed seeds were degreased with petroleum ether in the Soxlet apparatus for 72 hours. To remove low molecular weight impurities and coloring substances, the raw materials were extracted in a Soxlet apparatus with a mixture of chloroform – ethanol 96% (1:2). The raw material was dried in air to remove the smell of solvents.

Extraction of water-soluble polysaccharides. Water-soluble polysaccharides were extracted from defatted seeds three times with water in a water bath at 95ºC (the ratio of raw materials and extractant 1:20, 1:15, 1:15). The duration of each extraction was 2 hours. The aqueous extracts were combined and evaporated on a rotary evaporator at a temperature of 50°C of 1/5 of the volume. From the resulting concentrate, water-soluble polysaccharides were precipitated by adding a fourfold volume of 96% ethanol and left at 4 ° C overnight. The precipitate was separated by centrifugation, washed with ethanol and freeze dried.

Deproteinization of polysaccharides. Deproteinization of the sum of polysaccharides was performed according to the Sevag method [14]. A 3-fold volume of $CHCl_3$–n-BuOH (4:1 ratio) was added to an aqueous solution of the sum of polysaccharides and transferred to a separating funnel. The funnel was shaken vigorously for 5 min and the mixture was kept for 3 hours to achieve equilibrium of the two phases. The organic phase with residual proteins (lower layer) was removed. This procedure was repeated 6 times. Turnip seed polysaccharides were precipitated with three volumes of ethanol from the aqueous phase. After filtration, the precipitate was washed with absolute ethanol and dried in air.

Determination of the content of polysaccharides. The quantitative content of polysaccharides was determined by the Phenol-sulfuric acid method [15].according to the calibration graph for glucose. The optical density was measured using a METASH UV-5100 spectrophotometer (Shanghai, China).

Immunological tests. The study of the effect of the substance on animal immunity was carried out on model reactions of evaluating antibody formation in mice during immunization with antigens (humoral immune response) and induction of delayed-type hypersensitivity reaction (HR) to antigen (cellular immune response):

To study the effect of the drug on humoral immunity, the number of antibody-forming cells (AFC) in the spleens immunized sheep erythrocytes (ShE) of mice by the method of Jerne, Nordin. As a result of seeding the spleen cells of mice immunized with antigens (ShE) using complement - guinea pig serum, plaques of local hemolysis were clearly marked on an opaque background of agarose – transparent zones with a diameter of about 1 mm, representing antibody-forming cells with sheep erythrocytes lysed around. The number of IgM–antibody producing cells was determined by the number of plaques on the cup. 0.05 ml of a suspension of spleen cells was sown per cup, that is, 1/200 part of the splenic suspension contained in 10 ml of Hanks' solution. To determine the absolute number of AFC accumulating in the spleen of mice, the number of AOCS calculated over the entire cup was multiplied by 200. To recalculate the number of AFC per 1 million nucleated cells of the spleen, the number of millions of cells for the entire spleen was calculated in the Goryaev chamber. Dividing the absolute number of AFC by the number of

millions of cells, the number of AFC was determined by 1 million. nucleated cells of the spleen (NC spleen). Mice of the experimental groups on the day of immunization were intragastrically injected with an aqueous solution of the drug at a dose of 5, 0.5 and 0.05 mg/kg in a volume of 0.2 ml. The control group of mice received water in the same volume.

Evaluation of the immune action of the substance from Brassica rapa

Evaluated function	Model reactions	Immunological tests
Humoral immune response	Evaluation of antibody formation in mice during immunization with test antigens	Determination of antibody-forming cells to ShE in the course of local hemolysis in agarose gel (Erne method)
Cellular immune response	Induction of delayed-type hypersensitivity reaction (HR) to an antigen	The reaction of HR to ShE

2) To study the effect of the drug on cellular immunity, a delayed-type hypersensitivity reaction (HR) was used. When setting up the HR reaction, the antigen is injected twice for sensitization for resolution. Sensitization by protein antigens causes the formation of antigen-specific T-lymphocytes, which, upon repeated administration of the antigen, specifically interact with it and secrete a number of pro-inflammatory cytokines.

White mongrel mice weighing 18-24 grams were used in the experiments. To develop the HR reaction , mice were subcutaneously immunized with 1x10 sheep erythrocytes in 0.2 ml of saline solution. On the 5th day, a permissive dose of 1x108 ShE in 0.05 ml of saline solution was injected into the pad of the hind paws of mice, and saline solution in the same volume was injected into the contralateral paw as a control. After 24 hours, the local inflammatory reaction was evaluated by the difference in the weight of the experimental and control paws and the reaction index was calculated according to the formula:

$$\frac{P_{experience} - P_{control}}{P_{control}} \times 100\%.$$

On the day of immunization, an aqueous solution of drugs – 5 mg/kg - was administered intravenously to mice in a volume of 0.1 ml / mouse. The control group of mice received intragastrically distilled water in the same volume [15]. In the studies, the comparison drug was a pharmaceutical preparation of plant origin – Immunal at a dose of 60 mg/kg.

Results and their discussion. *Isolation of polysaccharides from Brassica rapa.* Extraction with water s were used to isolate polysaccharides. Polysaccharides (conventionally named BSP) were precipitated from aqueous solutions with the addition of ethanol in a ratio of 1:4 (by volume). The yield of polysaccharides was 1.6%. Further, the quantitative determination of the carbohydrate composition of the isolated polysaccharides by the phenol-sulfuric acid method was carried out. The total amount of carbohydrates was 30.3%, which indicates the presence of impurities in the composition of the isolated polysaccharide. Further, the isolated polysaccharides were deproteinized by the Savage method. After deproteinization, the amount of protein in the samples

was determined by the Lowry method. The results showed that the isolated polysaccharide contains trace amounts of proteins and peptides.

Results of immunological tests. Assessment of the effect of *Brassica rapa* substance on humoral immunity. Based on the results of the physico-chemical properties of the investigated substance from *Brassica rapa* and the developed targeted immunological tests, we obtained results evaluating the humoral and cellular immune response of the action of this substance.

The results of the conducted immunological experiments (Table.1) showed that 5900±1700.25 AFC accumulated in the spleens of mice of the control group, when calculated for 1 million nucleated cells of the spleen, the number of AFC was 55.5±20.5. In experimental groups of mice receiving the drug at a dose of 0.05; 0.5 and 5 mg/kg, the number of AFC per spleen increased to 9160±1477.06; 12000±3480.33 and 19080±12391.6, respectively (RI=1.5; 2 and 3.2 times). In terms of 1 million spleen cells, the number of AFC was - 70.8±8.05; 73.17±20.5 and 120.3±83.7, respectively.

The results of studying the effect of the substance from *Brassica rapa* in doses of 0.05, 0.5 and 5 mg/kg on antibody formation in the spleen showed that the substance in all the studied doses did not significantly affect the number of NC in the spleen, but significantly increased the number of antibody-forming cells (AFC) both in terms of the spleen and on 10^6 NC spleen.

The results of the experiments showed that the substance *Brassica rapa* in the studied doses, compared with the pharmaceutical herbal preparation Immunal, has a pronounced stimulating effect on the process of antibody formation in mice. A significant increase in antibody formation was revealed after intragastric administration of *Brassica rapa* substance at doses of 0.05, 0.5 and 5 mg/kg, which proves the stimulating effect of the drug at doses of 0.05 and 0.5 mg/kg. Thus, the experimental results showed that the drug in the studied doses has a pronounced stimulating effect on the process of antibody formation in mice, that is, on the humoral immunity of animals.

Evaluation of the effect of *Brassica rapa* substance on cellular immunity. Studies have shown that the inflammatory response index (RI) in the control group of mice was 23.11% (Table 2). A dose of 0.05 mg/kg caused a decrease of 15.45±2.99 mg/kg of the difference in the weights of the experimental and control paws in mice, at a dose of 0.5 mg/kg - a significant decrease of 9.95±1.07 (p=0.047), respectively. The substance *Brassica rapa* at a dose of 5 mg/kg caused a significant decrease of 8.20± 2.97 (p=0.045) in the mass difference of the experimental and control paws in mice, as in the case of the above doses.

Under the influence of all studied doses of *Brassica rapa* substance, there was a significant tendency to decrease the difference in the weights of the experimental and control paws and a tendency to decrease the index of the inflammatory reaction with the development of delayed hypersensitivity.

The results obtained indicate that the substance *Brassica rapa* in doses of 0.05, 0.5 and 5 mg/kg showed reliable results in reducing the difference in the weights of the experimental and control paws, as well as in the index of the inflammatory reaction. Under the action of the substance *Brassica rapa* in all studied doses (0.05; 0.5 and 5 mg/kg) in relation to the comparison drug Immunal at a dose of 60 mg/kg, a dose-dependent decrease (decrease in the weight difference of the experimental and control paws) of the index of the reaction to inflammation was observed and there was a significant tendency to decrease the development of delayed hypersensitivity reactions, especially at doses of 0.5 and 5 mg/kg.

Table 1

Effect of Brassica rapa substance on antibody formation

Groups	Doses in mg/kg	Number of mice	Number of AFC per spleen M±m	AFC for 1 million NC spleen M±m	RI
Control		5	5900±1700,25	55,5±20,5	
Immunal	60 mg/kg	5	7360±1275,02	62±14,5	1,2
Substance *Brassica rapa*	0,05 mg/kg	5	9160±1477,06	70,3±10,9	1,5
Substance *Brassica rapa*	0,5 mg/kg	5	12000±3480,33	73,17±20,5	2
Substance *Brassica rapa*	5 mg/kg	5	19080±12391,6	120,3±83,7	3,2

Table 2

The effect of the substance from Brassica rapa on the inflammatory response

Substance, doses, n=5	**The difference in the weight of the paws in mg/kg**	**Reaction Index (RI), %**
Control - saline solution	25,43±5,81	23,11
Immunal 60 мг/кг	15,22±10,34	12,75
Substance *Brassica rapa* 0,05 мг/кг	15,45±2,99	13,9
Substance *Brassica rapa* 0,5 мг/кг	9,95±1,07 **p=0.047**	8,7
Substance *Brassica rapa* 5 мг/кг	8,20±2,97 **p=0.045**	6,8

**P<0.05 relative to the original data*

Conclusions. Thus, as a result of the experiments conducted on the effect of the drug on humoral and cellular immunity, it was revealed that after a single administration of the studied drug simultaneously with the antigen (ShE) at doses of 0.05 and 0.5 mg/kg, a significant increase in antibody formation was detected in mice and there was no significant increase in delayed-type hypersensitivity reaction in mice in all studied doses according to in relation to control. When studying the immunomodulatory properties of the substance *Brassica rapa*, it was shown that the substance does not cause changes in immunity, detected by its effect on lymphoid organs. Thus, the results of a comprehensive study allow us to conclude that the substance of the potential drug *Brassica rapa* has a stimulating effect on the humoral and cellular immune response.

Based on the studies conducted to identify the immunomodulatory activity of the substance from turnip seeds *Brassica rapa*, it can be concluded that polysaccharides are the main effective immunocorrectors. Thus, the revealed immunomodulatory activity of the substance *Brassica rapa*

allows for a targeted search for new effective immunomodulatory drugs among substances of plant origin.

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