Development of a radioprotective drug based on substances of plant, microbial, zoogenic and inorganic origin

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Abstract. Environmental pollution with ecotoxicants makes humanity rethink its attitude to the world around it and accept the concept of the need to protect people and animals living on our planet. This paper presents the results of experimental studies of substances of natural origin for the selection of the most promising ones in order to create a multifunctional means of protecting farm animals in areas of anthropogenic pollution. In vitro experiments on lethally irradiated lymphocytes of the peripheral blood of animals, the radioprotective properties of phytogenic preparations were evaluated, and a three-component radioprotective composition was created. On lethally irradiated laboratory animals, the radioprotective activity of various compositions constructed from microbial biomasses and metabolic products of E. coli "PL-6", B. bifidum 1 bacterial strains, phyto- and zoo drugs preparations radiomodified at a dose of 4 kGy was tested. From the metabolites of the radiomodified bacterial strain B. bifidum 1 (R6), the phytopreparation "Turmeric" and the biologically active feed additive "Vita-Force M", the phytozoic microbial preparation FZMP was designed and tested on laboratory animals, which provided protection from radiation death to 80% of lethally irradiated laboratory animals, with 100% death of rats in the irradiation control group.

1. Introduction
The legacy of the 20th century for the modern world is global environmental pollution by artificial radionuclide’s, heavy metals, xenobiotics [1-14]. Currently, this is one of the main reasons for the emergence of acute and chronic diseases of various etiologies in farm animals with impaired hematopoietic, immune, cardiovascular, nervous, endocrine and other body systems [1; 7; 9]. For the correction of these violations, protective measures are often required in the form of the use of sorbents of toxic substances, haemostimulants, immunomodulators, immunostimulants, adaptogens, etc. [3; 4; 13]. An important role in protection is given to vitamins, which have no energy value, but are required to participate in biochemical processes [15-17].

Chemicals of a chemical nature do not possess such properties and, in addition, they have a number of negative qualities, namely, the effect of chemicals is often twofold and, in addition, chemical substances in large quantities are toxic [10; 16].

From this point of view, natural ingredients have significant benefits. The evolutionary development of animals has always proceeded in parallel with all other living organisms - plants, insects, microbes, and the impact of the components of the natural environment on them may be less aggressive, but more effective [6; 11; 15].

Biological preparations differ from chemical ones in softer and more prolonged action, harmlessness and effectiveness. The positive properties of drugs of natural origin include their low...
cost, harmlessness, the possibility of oral and unlimited long-term use, good compatibility with other means of prevention and treatment. Some of them have a corrective effect in relation to the organs and systems of the body in various diseases. One of the advantages of these drugs is that they can be used as general strengthening and immunity-enhancing substances, generally making the body healthier [8; 12].

In connection with the above, our research was aimed at searching for natural substances in order to create a means of polyfunctional protection of animals in case of anthropogenic pollution.

2. Materials and methods

Plant, microbial and zoogenic substances were used as objects of research.

In experiments in vitro, 14 samples of plant substances were tested: aloe, cloves, rhodiola rose, cabbage, currants, Kalanchoe, needles, carrots, beets, oregano, eleutherococcus, St. John's wort, wormwood, motherwort, willow weed, turmeric, plantain, lemongrass. A three-step ethanol-ether hydrolysis was used to dissolve turmeric. The rest of the herbal preparations were used in the form of aqueous extracts, which were subjected to dry gamma sterilization. The protective properties of plant substances were assessed by their ability to maintain the viability of lethally irradiated lymphocytes.

To assess the radioprotective properties of microbial preparations, cultures of E. coli "PL-6" (R10) and B. bifidum 1 (R6) radiomodified in doses of 4 kGy, as well as their consortium E. coli "PL-6" (R10) + B. bifidum 1 (R6) (2 options) with or without addition of phyto- and zoo-drugs (FP and ZP) to the formulation. The prophylactic and therapeutic qualities of microbial substances were assessed by their ability to maintain the viability of lethally irradiated (LD100/30) mice.

In the fourth series of experiments in vivo, the radioprotective properties of a phyto-zoomicrobial preparation (FZMP) based on metabolites of radiomodified bifid bacteria B. bifidum 1 (R6), turmeric and an aqueous extract of the biologically active feed additive "Vita Forze M" were evaluated.

For the research were used:

- Home and natural herbal preparations;
- Laboratory animals: white mice weighing 20 ± 2 g, white rats weighing 200 ± 30 g;
- Apizan (a zoo preparation of bee origin) produced by FGBNU "FCTRB-VNIVI";
- Biologically active feed additive "Vita-Force M" produced by FGBNU "FCTRB-VNIVI".

For gamma sterilization of preparations, their components and irradiation of cultures of microorganisms, we used a stationary gamma device "Researcher" with an exposure dose rate of 1.79e-2 A / kg; for irradiation of animals - a stationary gamma device "Puma" with an exposure power of 2.06e-5 A / kg.

The dose of gamma sterilization of the drugs was 10 kGy; lymphocytes - 5 kGy; cell cultures – 4 kGy. The lethal dose of radiation to laboratory animals was: mice - 7.7 Gy; rats 9.5 Gy.

The studies were carried out in the conditions of the "Radiobiology" department of the Federal State Budgetary Scientific Institution "FCTRB-VNIVI".

3. Results and discussion

Table 1 shows the results of a study of the radioprotective activity of phyto preparations in an in vitro experiment. It was found that biologically active substances contained in plant extracts contributed to the preservation of the viability of lymphocytes from 42.7% (aloe) to 79.7% (turmeric). In addition, such herbal preparations as decoction of beets (69.2%), carrots (67.5%), needles (64.8%) and some others had a high protective activity.

On the basis of three components - extracts of needles, plantain and Eleutherococcus, an experimental phyto-preparation (FP) was made, which was tested together with microbial substances (table 1).

In the second series of experiments, the laboratory strain of E. coli "PL-6", provided by the laboratory of diseases of young cattle FGBNU "FCTRB-VNIVI" and lyophilized drug
"Bifidobacterin" (B. bifidum 1) produced by the Moscow Research Institute of Epidemiology and microbiology them. G.I. Gabrichevsky, after rehydration, were subjected to increasing doses of gamma irradiation from 1 to 4 kGy with intermediate passages in mesopatamia broth under aerobic conditions at 37оС for 2 days (E. coli) and in Blaurock's liquid medium under anaerobic conditions - 4 days (B. bifidum).

Table 1. Survival of lethally irradiated (5 kGy) lymphocytes against the background of the use of tested herbal remedies.

| Number p/p | Test substances | Percent live lymphocytes | Number p/p | Test substances | Percent live lymphocytes |
|------------|----------------|--------------------------|------------|----------------|--------------------------|
| 1          | Aloe           | 42.7                     | 10         | Oregano        | 52.3                     |
| 9          | Carnation      | 47.5                     | 18         | Eleutherococcus| 49.2                     |
| 2          | Rhodiola rose  | 49.6                     | 11         | St. John's wort| 62.4                     |
| 7          | Cabbage        | 49.6                     | 16         | Sagebrush      | 48.4                     |
| 8          | Currant        | 51.7                     | 17         | Motherwort     | 56.7                     |
| 3          | Kalanchee      | 52.1                     | 12         | Fireweed       | 55.1                     |
| 4          | Needles        | 64.8                     | 13         | Turmeric       | 79.7                     |
| 6          | Carrot         | 67.5                     | 15         | Plantain       | 52.1                     |
| 5          | Beet           | 69.2                     | 14         | Schisandra     | 49.6                     |

Radiomodified strains were used to obtain corpuscular cells (centrifugates), metabolic products of bacteria (supernatants) and to produce a consortium of radioresistant bacteria - E. coli "PL-6" (R10) + B. bifidum 1 (R6).

To assess the radioprotective activity of the created compositions, experiments were carried out on 110 white mice, divided into 22 groups of 5 animals in each group. Mice of 20 experimental groups were injected with 0.1 cm$^3$ of test compositions subcutaneously in the thigh area 1 day before irradiation (prophylaxis) and 1 day after irradiation (treatment). Two groups of mice served as radiation control and biological control. Acute radiation sickness of severe degree was simulated by irradiation of mice on a gamma device "Puma" at a dose of 7.7 Gr. The research results are shown in table 2.

From table 2 it follows that during emergency prophylaxis (immunization) the most effective method of protecting animals was the use of compositions based on E. coli, B. bifidum and apizan (4th group), in emergency treatment - metabolic products, E. coli, B bifidum in combination with AF (group 20), which ensured 80% protection of animals against the background of lethal radiation (7.7 Gr) in prophylactic (“express immunization”) and express therapeutic application.

In the third in vivo experiment, the radioprotective activity of the consortium of radiomodified bacteria E. coli + B. bifidum of the preparation "Bifikol" was studied. The bacteria of the preparation, after their rehydration in physiological solution under aerobic conditions, were irradiated at a dose of 14, 140 and 300 Gr. The drug was injected parenterally into the inner thigh of mice at a dose of 0.1 cm$^3$ one day after lethal irradiation.

The research results are presented in table 3.

From table 3 it follows that microorganisms E. coli + B. bifidum of the drug "Bifikol" treated with gamma quanta at a dose of 300 Gr provided 80% protection of lethally irradiated animals with 100% death of animals in the radiation control group.

In the fourth series of experiments, the radioprotective properties of the constructed phytozoan microbial preparation (FZMP) were tested during prophylactic (one day) and therapeutic (one day later) use against the background of lethal irradiation of animals. White out bred rats were used as a model. The research results are presented in (table 4).
Table 2. Radioprotective properties of the tested drugs against the background of lethal irradiation of mice (7.7 Gr).

| Number p/p | Composition and method of application of the tested drugs | Number of animals (head) | Palo (goal) | SPJ (days) | Survived | Percent protection |
|------------|------------------------------------------------------------|--------------------------|-------------|-----------|-----------|-------------------|
| 1          | MB E. coli (PR)                                            | 5                        | 3           | 12.3      | 2         | 40                |
| 2          | MB B. Bifidum (PR)                                         | 5                        | 2           | 15.5      | 3         | 60                |
| 3          | MB E. coli + B. bifidum (PR)                               | 5                        | 3           | 16.7      | 2         | 40                |
| 4          | MB E. coli + B. bifidum + apizan (PR)                      | 5                        | 1           | 15.0      | 4         | 80                |
| 5          | MB E. coli + B. bifidum + AF (PR)                          | 5                        | 3           | 15.7      | 2         | 40                |
| 6          | E. coli PM (PR)                                            | 5                        | 2           | 13.0      | 3         | 60                |
| 7          | PM B. Bifidum (PR)                                         | 5                        | 2           | 12.0      | 3         | 60                |
| 8          | PM E. coli + B. bifidum (PR)                              | 5                        | 2           | 17.5      | 3         | 60                |
| 9          | PM E. coli + B. bifidum + apizan (PR)                      | 5                        | 2           | 17.5      | 3         | 60                |
| 10         | PM E. coli + B. bifidum + AF (PR)                          | 5                        | 2           | 17.5      | 3         | 60                |
| 11         | MB E. coli (L)                                             | 5                        | 3           | 15.3      | 2         | 40                |
| 12         | MB B. bifidum (L)                                          | 5                        | 3           | 9.0       | 2         | 40                |
| 13         | MB E. coli + B. bifidum (L)                               | 5                        | 3           | 15.6      | 2         | 40                |
| 14         | MB E. coli + B. bifidum + apizan (L)                       | 5                        | 2           | 18.5      | 3         | 60                |
| 15         | MP E. coli + B. bifidum + FP (L)                           | 5                        | 2           | 14.5      | 3         | 60                |
| 16         | PM E. coli (L)                                             | 5                        | 2           | 17.0      | 3         | 60                |
| 17         | PM B. Bifidum (L)                                          | 5                        | 2           | 14.5      | 3         | 60                |
| 18         | PM E. coli + B. bifidum (L)                               | 5                        | 2           | 19.5      | 3         | 60                |
| 19         | PME. coli + B. bifidum + apisan (L)                        | 5                        | 2           | 15.5      | 3         | 60                |
| 20         | PM E. coli + B. bifidum + FP (L)                           | 5                        | 1           | 20.0      | 4         | 80                |
| 21         | Exposure control                                          | 5                        | 5           | 12.5      | 0         | -                 |
| 22         | Biological control                                        | 5                        | 0           | -         | 5         | -                 |

Note: Legend: MB - microbial biomass; PM - metabolic products; PR - prophylactic use of drugs; L - therapeutic use of drugs; SPJ is the average life expectancy of dead animals.

Table 3. Radioprotective properties of E. coli + B. bifidum microorganisms radiomodified at a dose of 14, 140 and 300 Gy against the background of lethal (7.7 Gr) gamma irradiation of mice during the therapeutic use of the drugs.

| Number p/p | Composition and method of application of the tested drugs | Number of animals (head) | Palo (goal) | SPJ (days) | Survived | Percent protection |
|------------|------------------------------------------------------------|--------------------------|-------------|-----------|-----------|-------------------|
| 1          | Bifikol (E. coli + B. bifidum) without irradiation        | 5                        | 3           | 16.7      | 2         | 40                |
| 2          | Bifikol (E. coli + B. bifidum) 14 Gr                      | 5                        | 2           | 19.0      | 3         | 60                |
| 3          | Bifikol (E. coli + B. bifidum) 140 Gr                     | 5                        | 2           | 22.0      | 3         | 60                |
| 4          | Bifikol (E. coli + B. bifidum) 300 Gr                     | 5                        | 1           | 23.0      | 4         | 80                |
| 5          | Exposure control                                          | 5                        | 5           | 13.4      | 0         | -                 |
| 6          | Biological control                                        | 5                        | 0           | -         | 5         | -                 |

Note: SPJ is the average lifespan of dead animals.
Table 4. Results of testing the radioprotective effectiveness of the phytozoan microbial preparation FZMP on rats irradiated at a dose of 9.0 Gr (LD100 / 30).

| Number p/p | Animal groups          | Number of animals (head) | Palo (goal) | SPJ (days) | Survived | % protection |
|------------|------------------------|--------------------------|-------------|------------|----------|--------------|
| 1          | FZMP (prevention)      | 10                       | 3           | 17.4       | 7        | 70           |
| 2          | FZMP drug (treatment)  | 10                       | 2           | 19         | 8        | 80           |
| 3          | Exposure control       | 10                       | 9           | 4.9        | 1        | -            |
| 4          | Biological control     | 5                        | 0           | -          | 5        | -            |

Note: SPJ is the average lifespan of dead animals.

From table 4 it follows that the prophylactic use of the drug provided 70% protection of animals, therapeutic - 80% protection.

4. Conclusion

Thus, the following conclusions can be drawn:

- The tested phytogenic preparations in the in vitro experiment protected from death from 42.7 to 79.7% of lethally irradiated lymphocytes.
- Preventive use of the microbial biomass of a consortium of radiomodified microorganisms E. coli + B. Bifidum with the addition of apizan and therapeutic use of metabolites of radiomodified microorganisms E. coli + B. Bifidum with the addition of EP, a consortium of E. coli + B bacteria irradiated at a dose of 300 Gr Bifidum of Bifikol and FZMP based on phytopreparation, metabolites of B. Bifidum bacteria and biologically active feed additive Vita-Forze M provided 80% protection of lethally irradiated animals.
- The developed phytozoan microbial preparation FZMP can be used as a means of polyfunctional protection of animals in case of anthropogenic pollution.

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