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Indication of Toxic Radiolysis Products in Forage and Food Products Subjected to Radiation Sterilization.

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ABSTRACT

The question concerning food safety is of importance in connection with the expansion of radiobiological technologies (RBT) in the national economy. RBT are food and forage radiation preservation in order to increase their shelf life. The article is devoted to the methods of lifetime determination in radiotoxic substances formed in the body, plants and the environment after food radiation sterilization and assessment of their environmental harm. The problem is obvious, because it is known that irradiated forage has radiomimetic (similar to the ionizing radiation effects) properties with a short life. The existing methods of environmental safety assessment do not meet the modern requirements of radiation expertise, hygiene and radioecology. It fosters to search new approaches to solve this problem. Taking it into account, studies have been carried out to search and develop a new technology to manufacture highly sensitive and specific anti-beam diagnostics suitable not only for the diagnosis and prediction of the acute radiation sickness outcome, but also for radioecological monitoring of vet supervision facilities. The diagnostic allows to detect radiolysis (radiotoxic substances) toxic products in any samples transferred to the liquid phase immediately after irradiation at different doses and in the long term, in concentrations up to several nanograms in 1 ml. The obtained diagnosed experimental series of immune enzymometric conjugates (IEC) were used to indicate toxic antigenic substances in the serum of irradiated animals, in meat products obtained from irradiated animals at different doses of ionizing radiation, as well as in phytoextracts from irradiated plant products.

Keywords: cold method of sterilization, the plant radiolysis products, radiotoxic substances, radio protective, antigen, antigens, radiation, forage and food, safety, forecast.

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INTRODUCTION

An urgent question on indication of radiotoxic substances formed in the body, plants and the environment after the ionizing effect, as well as these technologies and products harm on human health and the environment arises along with the Federal program of radiation safety, as well as the expansion of radiobiological technologies (RBT) in the national economy.

One of such technologies is the radiation preservation to prolong the total volume of food by increasing agricultural products shelf life and their damage reduction. The question naturally arises about the safety of the products obtained through the use of these products in food.

It is known from the literature that toxic products of radiolysis with radiometric (antimitotic, cytotoxic, mutagenic, hematotoxic, etc.) properties are formed in irradiated forages and food products [2, 8, 10]. The existing methods of radiotoxic substances detection in irradiated objects to assess their environmental safety do not meet modern requirements of radiation hygiene. Therefore, the search for objective, express methods of radiotoxic substances detection is an urgent problem of radiation examination, hygiene and radioecology.

Taking into account the above mentioned, the objectives of our research were defined as following: to determine the optimal radiation and biotechnological treatment modes of various products, as well as to study the possible radiation and biological effects of gamma rays on food and forage using the immunochemical test system ELISA [4].

MATERIALS AND METHODS

Immunochemical test system (ELISA), that records in vivo the products of molecules oxidative modification in concentrations up to several ng in 1 ml of blood serum or other biological liquids samples, including cold processed method of forage and products sterilization transferred into the liquid phase with a high degree of specificity has been developed in previous studies.

Experiments to isolate immunodominant sensitine, which is also one of the antigens in the set of the developed test system (positive control) from irradiated potato tubers, have been conducted in the first series of experiments. Irradiation was carried out on a gamma-installation 'Puma' at the dose of $0.26 \cdot 10^{-4}$ C/kg and the absorbed dose of 800.0 Gy. The obtained extract protein was determined by Lowry. It was daily exposed at the room temperature by the method of ethanol extraction and partial purification of chloroform (A. M. Kousin' method) [2]. Quinoid radiotoxin, the obtained plant extract, was standardized with sterile saline solution (pH 7.2) to a concentration of 5 mg/ml, conjugated with a lipid carrier of Freund's incomplete adjuvant and used it for animals' hyperimmunization.

Animals' hyperimmunization was performed according to generally accepted immunization scheme. 7 days after the end of hyperimmunization samples of serum were taken. Having been determined antibody titers were used as raw material for diagnostic drug production.

Extraction, fractionation and purification of tissue preparations were carried out using the method of one-stage extraction of 1 n Na OH, as well as by fractional fractionation with ammonium sulfate of different saturation.

The content of quinines and lipids according to A. M. Kousin and others was determined in the radiotoxins obtained [2, 5]. Quinoid radiotoxins were identified by the method of paramagnetic resonance after H. D. Svistunenko [7, 9] in our modification (Ibragimova M. I. and others, 2001) [1, 6].

Globulins were isolated by precipitation with ammonium sulfate and subsequent chromatography on a column of DEAE-cellulose and on sephadex G-200 before antibodies conjugation from hyperimmune serum enzymes. Globulins were conjugated with horseradish peroxidase by means of one-stage glutaraldehyde method to obtain immunoenzymometric agents. Conjugates purification was carried out by dialysis on a column of sephadex. The fractions obtained were scanned photometrically on the spectrophotometer SF-46 at a wave length 280-403 nm. The specificity coefficient was calculated by the extinction ratio of 403/280 nm.

The obtained enzyme immunoassay conjugates were tested for activity and specificity in ELISA (direct variant and method of 'chess' titration). The results were taken into account visually and spectrophotometrically at a wavelength of 492 nm (scanning spectrophotometer 'Dinatech' manufactured in the USA) by the criterion of specificity coefficient equal to or exceeding 2.0. Homologous and heterologous antigens served as control.

Specific immunoassay peroxidase conjugates after their verification were packed in vials and lyophilized on the installation 'Lausanne' in the conditions developed by the staff of experimental biotechnology medicines laboratory.

Isopropyl-N-phenyl carbamate active experimental series of peroxidase were used to indicate radiation and toxic antigenic complexes in animal blood serum in meat and meat products of irradiated animals, as well as in extracts of irradiated plants.

RESULTS OF RESEARCH

During the test system (ELISA) arrangement in vitro it was established that it detects the presence of specific quinoid radiotoxin antigen (positive control – irradiated ethanol extract at a dose of 400 to 800 Gy of potatoes) up to the titer of 1:16384, which is equal to 280 ug/ml. RT is the amount of toxic substances in the first hole, and the negative results in heterologous (burn antigen) and negative (non-irradiated potatoes) controls. A titer of 1:8 or 0.070 mg/ml (or 70ug/ml) RT is considered to be diagnostic.

Potato tubers from sprouting were tested at radiation dose of 50, 100, and 150 Gy. All ethanol samples of irradiated potatoes were tested in ELISA with results 1:512, 1:1024 and 1:2048. This is the amount of toxic substances in the hole, with negative results in heterologous (burn antigen) and negative (irradiated potatoes) controls. Diagnostic titer 1: 8 or 0.070 mg/ml (or 70 ug/ml).

Further studies revealed that onion germination is delayed by γ-irradiation in the doses range of 70-100 Gy. It is established that ethanol extracts from onions give lower titers for 1 step of cultivation than other roots (1:256; 1:512) in ELISA. It can be explained by the presence of phytoncides and flavonoids in onion samples distorting the reaction results of the reaction.

Thus, the question arises in the course of experiments: if the dose of 100 Gy is the inhibitor of plant growth. We have previously determined that the titers of ELISA > 1:8 already catch mercury in toxic doses from the first hours of irradiation. Potato radiation dose to delay germination of 100 Gy recommended for the USSR by IAEA (1973) is harmless (the range of ELIZA titers in our studies 1:256-1: 2048). Sterilization method was tested at a late stage for potatoes storage (3, 4, 5 and 6 months). It is known that quinoid radiotoxin has mutagenic properties [3], but these properties are found in the substance isolated from raw potatoes within 1-4 days after irradiation. Storage of irradiated potatoes (when radiation is recommended to prolong shelf life) led to a decrease in titers of radio antigen in 4-6 months before the diagnostic titers. It was also interesting to determine the level of ELIZA titles after the heat treatment of irradiated products. Thermolabile RHW were completely destroyed when cooking even freshly released potatoes. The level of total RHW sharply reduced, but not to a safe level. Thermostable RHW are insensitive to high temperatures and continue to circulate in samples above the diagnostic titer in 6 months (research period). Thus it is necessary to check in vivo these samples mutagenic and toxic effect on laboratory animals both in the early and in the long term after irradiation.

It is necessary to use a dose of about 1500 Gy for grain gamma-processing (oats, barley) to prevent the development of the granary weevil, moth-mill moth and other pests. It stops a caterpillar transition into a chrysalis, when the effector (protein enzyme) has managed to penetrate into the caterpillar. Normally it happens 24-26 hours before pupation. Insect pests in food (grain, flour, etc.) happen to be infected with the postponed eggs and young larvae that are the most radiosensitive stages of insect development. It is necessary to use relatively low doses of radiation. Caterpillars irradiated at a dose of 300 Gy at any period of their life 48 hours before pupation (within 25 days) turned into 'eternal' caterpillars and could no longer perform derepression of genes for pupation [3].

Radiation doses to neutralize grain (barley, oats) from insects of 300 Gy and 700 Gy have been tested. ELISA titers immediately after irradiation were respectively 1:8192 and 1:16384 as in the positive control with quinoid radiotoxin. Practical radiation use for desinsection requires additional laboratory tests to find the optimal conditions for its further use.

As can be seen from the above, the diagnostic titer indicating products radiation treatment is considered to be the titer 1:8. RT is present in this sample. The toxic dose is captured in ELISA. Such a titer is already registered with a radiation dose of 1 Gy. Close to the maximum RT titers are an unfavorable sign and indicate a high mutagenic activity of irradiated products, i.e. they are an environmental hazard.

CONCLUSION

The possibilities of RHW indication in the irradiated products and their ecological safety assessment have been stated as a result of the carried researches. Antiradiation diagnosticum produced by the new technology is of higher activity and specificity. It is suitable for indicating radiation, toxic antigens and immunological toxic complexes for the diagnosis and prognosis of radiation sickness. It can also be applied in food and forage product sterilization processed by cold method.

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