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Influence of the Herbal Preparation "Licorice Oil" on the State of Hematopoiesis in Rats Under Ionizing Irradiation

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The radioprotective effect of the herbal preparation "Licorice oil" on the hematopoietic system and oxidative stress was studied. The experiment was carried out on 30 male Wistar rats, divided into 3 groups. The first group is control group, the second is irradiation with 6Gy, third group - a week before irradiation and two weeks after, received "Licorice oil" intragastrically at a dose of 2.5 ml/kg of body weight. Gamma irradiation significantly reduced the number of erythrocytes, hemoglobin, hematocrit, leukocytes, thrombocytes in peripheral blood and bone marrow cellularity. The frequency of micronuclei in polychromatophilic erythrocytes of the bone marrow has significantly increased. The level of lipid peroxidation in the blood increased against the background of a significant decrease in the activity of antioxidant enzymes. The introduction of "Licorice oil" for 21 days provided a protective effect. In application of "Licorice oil", there was an increase in the number of cellular elements in the peripheral blood and against the background of a decrease in the frequency of micronuclei in the bone marrow. The activity of antioxidant enzymes in blood plasma increases against the background of a decrease in the amount of peroxidation products. The herbal preparation "Licorice oil" exhibits antioxidant activity, reduces genotoxicity and cytotoxicity under gamma irradiation. "Licorice oil" can be used to prevent radiation damage.

Keywords: Blood; Bone Marrow; Gamma Radiation; Micronuclei; Oxidative Stress; Phytopreparation "Licorice Oil",

Radiotherapy is one of the most effective methods of treating malignant neoplasms. However, the side effects associated with radiotherapy limit its use. The most common side effect is suppression of the hematopoietic system, which is composed of rapidly proliferating precursor cells in the bone marrow. Hematopoietic injury causes myelosuppression and dose-dependent depletion of circulating blood cells, which lead to anemia and increased susceptibility to infection[43,45,13]. Radiation exposure leads to dose-dependent defects in the lymphoid and hematopoietic systems through a complex cascade known as hematopoietic syndrome, which can lead to septicemia and death [20]. It is well known that ionizing radiation also has an unprecedented long-term effect on cellular pathways, that leads to genomic instability, which can subsequently manifest itself in hereditary diseases or various forms of malignant neoplasms[50,6].

Ionizing radiation causes radiolysis of water in cells, resulting in the formation of highly reactive free radicals. Known as reactive oxygen species (ROS), they lead to a disproportion between prooxidants and antioxidants[6], causing oxidative damage to vital cellular structures[8,23,36,84], which leads to many pathological conditions[37,73]. Consequently, free radicals and changes in vital structures require the development of countermeasures to minimize radiation damage. Protection of biological systems from ionizing radiation has paramount importance in case of planned as well as unplanned accidental exposure to radiation[3].

Many synthetic drugs have been tested in both in vitro and in vivo models to mitigate damage caused by ionizing radiation[42]. However, at a clinically effective concentration (dose), they are toxic and cause side effects. In most cases, toxicity appears in promising radioprotective agents, which limits their usefulness and applicability[52].

Therefore, it is necessary to study alternative sources, especially natural ones, that will be used as effective and safe radioprotectors. Because of their low toxicity in an effective dose with minimal side effects, herbal products with antioxidant properties bind free radicals[34], leading to a minimum of radiation damage to normal tissues, are considered to be radioprotective[83].

The ability of bioactive phytocompounds to simultaneously have a multifactorial effect is unique. Among them, phytolipophilic drugs play a special role. They more easily penetrate cell membranes, have a higher activity and specificity of action, are stable and retain their pharmacological activity for a long time[53]. One of them is a phytopreparation oil extract from licorice roots - "Licorice oil" (RK-M-#011042).

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Naked licorice (Glycyzrhiza glabza L.), due to its chemical composition, occupies one of the leading places among medicinal plants in terms of the scale of industrial collection, the breadth and value of the therapeutic effect. Licorice root preparations have a wide spectrum of pharmacological action - antioxidant, wound healing, antiallergic, antimutagenic, antiviral, antitumor, anti-inflammatory, antimicrobial, immunotropic, hepatoprotective, antidote, etc. [56,46,32,1,72,76,80]. And the main medicinal properties are due to the composition of its root: glycyrrhizic and glyceretic acids (6-23%); flavonoids (about 30%) - liquiditin, licurazide, quercetin and others (up to 5.3%); mono- and disaccharides (up to 20%); pectins (4-6%); phenol carboxylic acids; coumarins (up to 2.5%); alkaloids; essential oil (up to 0.03%); steroids (extriol); organic acids (up to 4.3%); macro- and microelements[15].

The main active ingredient of licorice root is the triterpene saponin glycyrrhine, which is, in active form, mixed potassium-calcium-magnesium salts of tribasic glycyrrhizic acid. It has been established that glycyrrhizic acid has anti-inflammatory, antiallergic, antiviral, hepatoprotective, immunomodulatory, antiulcer effects, and has antioxidant properties[74,75,54,79,68,49,51]. In addition, the presence of hydrophilic and hydrophobic fragments in the glycyrrhizic acid molecule gives it unique surface-active and gel-forming properties that contribute to the expansion of the spectrum of biological activity[54,70].

An oil extract of licorice has been developed and patented in Kazakhstan. The antioxidant properties of "Licorice oil" have been studied, as a result of which the drug is recommended for the prevention and complex treatment of diseases characterized by an increase in the intensity of lipid peroxidation[32,34].

The study of the influence of the herbal preparation "Licorice oil" on oxidative stress and hematopoiesis in chromium intoxication showed that the drug had a hemo- and immunostimulating effect[47,71], showed hemorheological and antioxidant activity[24].

An aqueous extract of licorice root under inhalation exposure to industrial uranium ore optimizes the balance ratio of lipid peroxidation products and antioxidant protection in the lung tissue[48,14] and has a corrective effect on the Krebs cycle in the lungs[5]. However, there is no detailed study of the effect of licorice root extract, especially the herbal preparation "Licorice Oil"

on oxidative stress and hematopoietic systems, while hematopoietic syndrome is considered the main cause of death in animals after general body irradiation. Thus, finding them above, the present study was conducted to study the role of the herbal remedy "Licorice oil" in mitigating the adverse radiation effects on the blood system and oxidative stress.

MATERIAL AND METHODS

The work was performed on 30 male Wistar rats weighing 170-190 g. The animals were kept in standard conditions in the vivarium of the Scientific and Practical Center of the Non-Commercial Joint Stock Company "West Kazakhstan Marat Ospanov Medical University" (Aktobe, Republic of Kazakhstan) on a standard diet with free access to food and water. The experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Purposes (Strasbourg, 1986). The experiment program was discussed and approved by the regional ethical commission of the university.

10 days after acclimatization, the rats were randomly divided into 3 groups: group 1 - control, group 2 - irradiation, group 3 - before and after irradiation, which received the drug "Licorice oil" intragastrically at a dose of 2.5 mg / kg of body weight.

The dosage of the herbal preparation "Licorice oil", the method of application and the duration of use is justified by the literature data[31,32]. The process of irradiation of animals of groups 2 and 3 was carried out on a Teragam radiotherapy device (Czech Republic) with total gamma rays of ^{60}Co at a dose of 6 Gy, with a power of 0.54 Gy/ min. The distance from the source to the skin of animals is 70 cm.

Animals in all groups are euthanized at the end of the experimental period by instant decapitation under light ether anesthesia to prevent stress. Part of the blood was collected in tubes with EDTA, an anticoagulant, and centrifuged at 2200g for 10 min. Collected plasma samples were stored at - 20°C. Erythrocytes obtained from blood samples were washed three times with 5 volumes of phosphate-buffered saline (pH -7.4) by centrifugation at 1500 rpm. Storage at – 80^{0}Ñ. Another part of the blood was collected for biochemical analysis.

Hematological analysis. The cellular composition of the blood was studied using a BF-580 hematological analyzer (China). The bone marrow cellularity was determined by flushing the bone marrow from the femur and calculating the number of cells (myelokaryocytes) per femur in the Goryaev chamber according to the standard method.

Micronuclear analysis. Micronucleus in the bone marrow: the preparation of the test preparations was carried out by the standard method[66]. A smear was made from a suspension prepared for cytogenetic preparations and stained by the Papenheim method using the May-Grunwald fixative and Giemsa paint[22]. The resulting preparations were encrypted and subjected to microscopic cytogenetic analysis. The number of micronuclei (MN) in polychromatophilic erythrocytes (PCE) of the bone marrow was counted. Analyzed - 3000 PCEs from each animal.

Biochemical analysis. Lipid peroxidation. Determination of diene conjugates (DC) was carried out by a generally accepted method[55] modified [19] by the ultraviolet spectrum of the first oxidation products of polyunsaturated lipids with an absorption maximum at 233 nm; the molar extinction coefficient is $2{,}210^{-5}$Ì$^{-1}$cm^{-1}. The content of DC was expressed in units of optical density (ODU) per ml (ODU/ml). The content of malonic dialdehyde (MDA) was determined using thiobarbituric acid (TBA) according to a modified method[2]. The principle of the method: at high temperatures in an acidic medium, MDA reacts with 2-TBA to form a colored trimethine complex with an absorption maximum at 532 nm. The MDA level was expressed in ìmol/L.

Antioxidant blood defense system. The content of sulfhydryl (SH) groups in blood plasma was determined by the method[17]. The amount of thionitrophenyl anion formed in the sample is directly proportional to the amount of SH-groups that reacted with 5,5– dithiobis. After 40 minutes, the optical density of the sample was measured spectrophotometrically at 412 nm. The number of SH groups was expressed in ìmol/L. The serum glutathione (GSH) level was determined according to the method[17] modified in[29].

Catalase activity (CAT) was measured

by the method[30]. The reaction was started by the addition of 2.0 ml of hydrogen peroxide to 10 ìl of the hemolysate and, after 10 minutes, was stopped by the addition of 1.0 ml of 4% ammonium molybdate. The absorbance of the sample was measured at 410 nm. The enzyme activity was expressed in units of activity per mg protein (U min/mg protein). One unit of catalase activity was defined as the activity to decompose 1 ìmol of hydrogen peroxide per minute (60s).

Superoxide dismutase (SOD) activity in erythrocytes was determined by the method[4]. As a unit of SOD activity, we took the amount of enzyme necessary to inhibit the decrease in nitroblue tetrazolium (NBT) by 50%, and the activity was expressed as U/mgPt.

The activity of glutathione peroxidase (GPx) was determined by the method[39] by the oxidation of NADPH•H2 in the conjugated glutathione reductase reduction reaction on a spectrophotometer at 340 nm. Results are expressed as nmol oxidized NADPH min/mg protein or U min/mg protein. Protein content was determined by the method of Lowry et.al.[40].

Statistical analysis. The results are expressed as mean values. The significance of the differences in mean values was assessed using the Student and Wilconson – Mann – Whitney tests. Differences were considered statistically significant at $r<0.05$.

RESULTS AND DISCUSSIONS

Gamma irradiation (6 Gy) led to a pronounced and persistent change in the peripheral blood picture, caused mainly by the emptying of the bone marrow (table 1), which is characterized by a decrease in the level of cellular elements in the blood and bone marrow cellularity by 41%, 50%, 25%. 42%, 35% and 46% compared to the control group. Prophylactic and therapeutic use of the drug "Licorice oil" caused a significant increase in the peripheral blood of the number of cellular elements and bone marrow cellularity by 25%, 34%, 19%,

Table 1. Influence of "Licorice oil", gamma-irradiation on the parameters of peripheral blood and bone marrow cellularity in male rats. (mean ± standard deviation - M ± σ, n = 10)

	RBS	ÍB	HTC (%)	WBC	PLT	Bone marrow cellularity
Control	5.3±0.945	124±25.303	40.3±4.444	8.1± 1.265	449±91.644	262±41
γ- irradiated rats (IRR)	3.12±0.729^{x}	62±11.015$^{\delta}$	30.3±6.312^{x}	4.7± 1.899^{x}	290±82.172^{x}	142±25.2^{x}
"Licorice oil"	3.99±0.79^{o}	83±15.779^{o}	36.16±7.585	6.53±0.949^{o}	386±69.674^{o}	195±34.09^{o}

Note: x - $p<0.05$ in comparison with control data; 0 - $p<0.05$ compared with data from irradiated rats

Table 2. Influence of "Licorice oil" on the formation of micronuclei in erythrocytes of the bone marrow in rats exposed to 6 Gy gamma radiation. (Ìì± σ in a group of 10 rats)

Group	Indicators Number of analyzed cells	The number of cells with micronuclei, ‰	anti-mutagenic effect, %
Control	3000	4.32±1.044	
γ- irradiated rats (IRR)	3000	21.6±4.734^{x}	
"Licorice oil"	3000	11.26±2.853^{o}	48%

Note: Each value represents the mean ± standard deviation (M ± σ) of 10 animals; x - $p<0.05$ in comparison with control data; 0 - $p<0.05$ compared with the data of irradiated rats; the antimutagenic effect was calculated using the formula - AME= $\frac{M_1-M_2}{M_1}\times 100$, M1 - the number of cells with micronuclei under ã-ray irradiation; M2 - the number of cells with micronuclei with the introduction of "Licorice oil" (a week before and two weeks after irradiation).

Table 3. Influence of "Licorice oil" on oxidative stress in rats with gamma irradiation

	DC	MDA	SH-gr	GSH	SOD	Cat	GP_x
Control	1.1±0.25	1.31±0.173	360±47.434	2.23±0.33	3.0±0.508	71±8.225	13±3.157
γ- irradiated rats (IRR)	1.76±0.41^{x}	1.69±0.189^{x}	270±37.997^{x}	1.62±0.1^{x}	2.4±0.411^{x}	50±6.326^{x}	7.0±1.9^{x}
"Licorice oil"	1.3±0.33^{0}	1.42±0.129^{0}	340±44.355^{0}	2.1±0.09^{0}	2.9±0.475^{0}	63±6.667^{0}	12±2.851^{0}

Note: x - $p < 0.05$ in comparison with the data of the control group; 0 - $p<0.05$ - compared with the data of irradiated animals. Each value represents the mean ± standard deviation (M ± σ) of 10 animals.

39%, 33% and 37%, respectively, compared with the data of the irradiated group.

Micronucleus test. The effect of gamma irradiation and the results of the protective action of the herbal preparation "Licorice oil" on PCE with micronuclei in the bone marrow are presented in Table 2. Single total g-irradiation at a dose of 6 Gy is accompanied by the induction of cytogenetic disorders in the cells of the bone marrow, which is manifested by an increase in micronuclei in the erythrocytes of the bone marrow 5.0 times (21.6±4.734, ‰) in comparison with the control data.

The increase in the rate of formation of micronuclei was 500%. With the therapeutic and prophylactic use of Licorice Oil, the frequency of micronuclei in polychomatophilic erythrocytes of the bone marrow decreased in comparison with the data of the irradiated (11.26±2.85 ‰). This indicator statistically differed from the clastogenic effect of gamma irradiation (6 Gy) ($p < 0.001$), and the reduction was 48%. The rate of micronucleus formation decreased 1.9 times as compared to the data of irradiated animals. Therefore, we can conclude that the prophylactic and therapeutic use of the herbal remedy "Licorice oil" has shown the ability to modulate the mutagenesis induced by gamma radiation in the direction of reducing the damaging effect. The antimutagenic effect was 48%.

The data presented in table 3 showed that whole body gamma irradiation in rats resulted in a significant decrease in the activity of antioxidant enzymes in erythrocytes. The activity of superoxide dismutase, catalase and glutathione peroxidase decreases by 20%, 30% and 46%, respectively, in comparison with the data of the animals of the control group. Also, the concentration of GSH and SH-groups in the blood decreases significantly, respectively, by 27% and 25% against the background of an increase in the level of diene conjugates and malonic dialdehyde in plasma by 60% and 29%, respectively, in comparison with the control. Therapeutic and prophylactic administration of the herbal preparation "Licorice oil" caused an increase in the activity of erythrocyte enzymes SOD, CAT, GPO, the content of GSH and SH blood groups by 21%, 26%, 71%, 30% and 26%, respectively, and a decrease in the level of DC and MDA in blood plasma by 26% and

16%, respectively, compared with the data of the irradiated.

Ionizing radiation has negative health effects, including dysfunctions of the hematopoietic system[38,9], immune dysfunction[81], genetic mutations[64,58] and oxidative stress[78,82,11]. Radiation damage resulting from the formation of reactive oxygen species is the result of oxidative damage to vital cellular molecules and structures, including proteins, lipid, DNA, and membranes[62] by free radicals (ROS). In order to achieve the best therapeutic effect during the treatment of tumors, normal tissues must be protected from radiation damage. Therefore, the development of antioxidant-based biologics is necessary to prevent and / or treat radiation hazards[65].

In the present study, the radioprotective effect of the herbal preparation "Licorice oil" was studied. Ionizing radiation has well-documented effects on blood cells, and suggests that these effects contribute to the hematopoietic syndrome observed in animals and humans after exposure to general body radiation[7]. Hematopoietic syndrome is considered the leading cause of death in animals after general body irradiation and mainly occurs within 30 days after exposure[9,59]. In the present study, the levels of erythrocytes, hemoglobin, hematocrit, leukocytes and platelets were reduced in rats irradiated with ã-radiation compared with control, which indicates erythropenia, leukopenia and thrombocytopenia in these animals.

Depletion of the bone marrow is observed (bone marrow cellularity decreased by 46% in comparison with the control data). Bone marrow cellularity most adequately characterizes the degree of radiation damage to the body. Bone marrow depletion, caused by direct destruction of hematopoietic stem cells, and a decrease in the incorporation of iron and hemoglobin binding to the erythrocyte membrane, resulting in anemia[44]. Our results showed that the use of "Licorice oil" before and after irradiation led to an increase in the number of erythrocytes, hemoglobin, hematocrit, leukocyte and platelet content compared with the levels in the irradiated animals. The bone marrow cellularity increased by 37% in comparison with the data of the irradiated animals. All this indicates a hemo-stimulating effect. The hemostimulating effect of the phytopreparation can be associated with both increased proliferation, the activity of surviving stem cells, and migration to the bone marrow from the peripheral blood and thymus[71]. Research by T.G. Razina[57] showed that glyceram, which is a monoammonium salt of glycyrrhizic acid, stimulates granulo- and erythrocytopoiesis under conditions of hemodepression. Guryantseva L.A. et al[21] in their experiments found that glyceram normalizes the structural and functional organization of the bone marrow, providing intensive maturation of colony-forming units under conditions of myelosuppression caused by the administration of cyclophosphamide.

To date, enough information has been collected on the role of free radicals in the mechanism of induced mutations. Our results showed that total gamma irradiation of the whole body of animals leads to the induction of cytogenetic abnormalities in bone marrow cells, which is manifested by an increase in the frequency of micronuclei in polychromatophilic bone marrow erythrocytes. Phytopreparation "Licorice oil", when administered before and after irradiation, significantly reduces the amount of micronuclei. The anti-mutagenic effect was 48%.

Our results show that whole body gamma irradiation led to a significant increase in the level of DC in the blood plasma and a marker of LPO intensity - MDA in the blood plasma, as well as a significant decrease in the activity of SOD, CAT, GPO in erythrocytes, and a decrease in the GSH and SH groups in the blood. This decrease is caused by damage to cell membranes and changes in dynamic membrane permeability due to increased peroxidation after irradiation, release of intracellular enzymes into the bloodstream[61], and increased use of antioxidant enzymes in the body to detoxify radiation free radicals[33]. These results, characterizing the intensity of oxidative stress, are consistent with previous results[27,18,16,58]. Reduced glutathione, blood SH-groups, thiol constituents of the second line of cellular defense of the antioxidant system (AOS) act as a direct reactive scavenger of free radicals. A decrease in the levels of GSH, SH-groups reflects their increased need for cells to combat reactive oxygen species formed after irradiation[69]. However, a decrease in GPx activity may be associated with a decrease in the availability of GSH, which is a GPx substrate and is required for catalysis[67].

The introduction of "Licorice oil" before

and after irradiation reduced the content of DC and MDA in the blood. The mechanisms by which a phytopreparation inhibits lipid peroxidation most likely include direct scavenging of initiating radicals. In our previous studies, it was shown that the drug "Licorice oil" plays an important role in the prevention of chromium-induced oxidative stress, and enhancement of the cellular antioxidant system of the blood[24] and exhibits hemorheological and antioxidant activity. Also, among workers of chromium production, the use of "Licorice oil" activates the compensatory reactions of the body aimed at inhibiting lipid peroxidation, stimulates antiradical activity[25].

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The positive effect of the herbal remedy "Licorice oil" is a systemic manifestation of a number of mechanisms. Apparently, we can talk about an antistress effect[26], including a stabilizing effect on certain links of hematopoiesis, it is possible that biochemically active compounds of a phytopreparation can act as a "trap" of free radicals, thirdly, about an immunomodulatory effect[12, 77, 60, 71]. The formation of a certain relationship in the hypothalamo-pituitary-adrenal system under the influence of the nootropic properties of "Licorice oil" contributes to the inclusion of protective mechanisms that determine, together with immunomodulatory, antioxidant, antimutagenic, the body's resistance to the effects of ã-irradiation.

CONCLUSION

Thus, our results show that the herbal preparation "Licorice oil" exhibits antioxidant activity, reducing the production of free radicals and restoring the imbalance of prooxidant-antioxidant homeostasis during therapeutic and prophylactic use. Apparently, the explanation of the observed changes in antioxidant homeostasis after the introduction of "Licorice oil" is that the phytopreparation increases the power of the antioxidant system in neutralizing and removing the formed radicals of oxidative stress. The drug reduces the level and intensity of lipid peroxidation, has an anti-mutagenic effect. Phytopreparation "Licorice oil" has a hemo-stimulating effect on the blood system, manifested in an increase in the cellular components of blood and bone marrow cellularity, due to the stimulation of migration, proliferation and maturation of hematopoietic cells.

It can be assumed that the protective effect of the phytopreparation is mainly due to the presence of glycyrrhizic acid in its composition, which has a corticoid effect[5,62], maintaining at a higher level the process of energy supply to the structures responsible for the implementation of the adaptive reactions of the organism.

The results of this study show that under conditions of therapeutic and prophylactic use (before and after irradiation), Licorice Oil controls the production of free radicals and protects against oxidative damage by inhibiting blood lipid peroxidation and enhancing the antioxidant system.

We assume that Licorice Oil, due to its multifaceted influence, may represent a promising radioprotector that deserves an integral assessment of its antiradiation effect.

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Conflict of interest

No conflicts of interest.

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