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## COMPARATIVE STUDY OF THE RADIOPROTECTIVE PROPERTIES OF EXTRACTS OF CALENDULA, CHAMOMILE AND MILFOIL

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**Abstract:** There were studied radioprotective properties of extracts of calendula, chamomile and milfoil. It has shown that the injection of the extract of calendula in combination with extracts of chamomile and yarrow enhance the survival and the mean life span of irradiated animals. At the same time increase in average life span of animals is more than in the case of the use of calendula extract separately.

**Keywords:** *calendula, chamomile, milfoil, radioprotective properties, toxicity, lifetime*

### 1. Introduction

For many decades, promising direction of development of protection against the harmful effects of ionizing radiation extends search of natural radioprotective products. Medicinal plants have several advantages compared with synthetic means: low toxicity, broad spectrum of pharmacological activity, ability to influence on physiological processes in human body and respectively increase the body's natural defenses, and are characterized by a gradual increase in the pharmacological effect [1]. In addition, herbal remedies, obtained in the form of extracts, provide a complex effect on the patient because they contain biologically active substances of different groups in a concentrated form [2]. Expression of the pharmacological effects of extracts depends on their natural compounds.

In early studies [3-10] there were widely investigated the radioprotective effect of extracts, derived from medicinal plants of Azerbaijan: rutin separated from *Sophora japonica* and its complexes with metals on the life activity of plants, higher fungi and mammals.

Medicinal plant (*Calendula officinalis* L.) has a broad spectrum of pharmacological activity, which is due to the rich content of such biologically active compounds as carotenoids (carotene, lycopene, violaxanthine, citraxathine, rubixanthine, flavochromium), flavonoids (narcissin, izoquersitrin, ramnetin and izoramnetin-3-triglucoside), vitamins, essential oils, saponins, gummy substances, calenden, organic acids, unexplored alkaloids, phytosterols, enzymes and other substances in the flowers.

In the study of general and acute toxicity of calendula galenicals there were established that they are slightly toxic and have a marked inhibitory effect on motor activity and reflectory excitability of the animals. Effect of calendula preparations on the activity of cardiovascular system clearly manifested cardiogenic and hypotensive effects. With the introduction of calendula drugs to animals in high doses there have lowered arterial blood pressure by 30-40% from baseline, slowed heart rate and some increased the amplitude of heartbeats, there was marked deepening and slowing of respiration. Calendula preparations make slow the growth of tumors in cancer patients. Unpretentious flower of calendula is a whole well of useful properties that make it an indispensable drug.

Particularly good results are obtained by the use of calendula in combination with chamomile and yarrow. The integrated product has a choleric, antispasmodic and anti-inflammatory effect, eliminates cholestasis, improve biliary secretory function.

The aim of this work - to evaluate the radioprotective influence of extracts obtained from flowers of calendula (*Calendula officinalis* L.) and plant collection consisting of flowers of pot marigold (*Calendula officinalis*), chamomile (*Matricaria chamomilla*) and yarrow (*Achillea millefolium*).

## **2. Materials and methods**

Experiments were conducted on outbred mice weighing 20-25 g. The animals were kept in vivarium conditions on a standard diet. In experiments animals were used for experimental and other scientific purposes in accordance with the rules of the European Convention for the Protection of Vertebrate Animals.

Medicinal plants that are part of the collection, were collected in summer and autumn 2010 in the village Buynuz of Ismayilli region of Azerbaijan Republic.

To study the radiobiological activity there were obtained extracts of calendula and extract of plant collection containing calendula, chamomile and yarrow. Dried and powdered plant mass was extracted with chloroform or ethanol in a Soxhlet apparatus. Then the solvent was distilled off and the residue was passed through a column of silica gel. As eluent a mixture of chloroform: carbon tetrachloride 3:1 were used. Extracts was collected at the outlet of the column.

To determine the radioprotective activity of the obtained extracts there were performed test on a 30-day survival of irradiated animals. In the experiments, outbred mice weighing 20-25 g were used. Acute toxicity was determined with the introduction of the investigated extracts in an aqueous solution of a single dose intraperitoneally in full compliance with the standard technique in pharmacology. Observation of behavior and death of animals were registered during the first hour and then fixed death of the animals in subsequent five days after the injection. The animals were exposed to irradiation in  $\gamma$ -installation "RKHUND - 20000", radiation source  $\text{Co}^{60}$  at a dose of 7.5 Gy, dose rate 0,396 Gy/sec. The test extracts were administered to the animals in an aqueous solution for 30 minutes before exposure to irradiation at the dosage 300 mg/kg. Control animals at the same time and in equivolume quantities before irradiation were injected solvent - distilled water. To compare the radioprotective activity of the test extracts there were used standard radioprotector - cystamine (bis-( $\beta$ -aminoethyl) disulfide) in an optimal radioprotective dosage of 200 mg/kg, injected 30 min before irradiation.

Also a test was performed on a 30-day survival of irradiated mice at a dose of 9 Gy and determined the average dose at 50% survival of animals ( $\text{SD}_{50}$ ), with the injection of the extract and the average life expectancy (SPM) of irradiated animals.

In determining the acute toxicity after administration of extracts at doses 2000 and 1000 mg/kg there was registered a sharp decrease in respiratory function, the occurrence of clinical spasms and 100% death within 15 min. Reducing the dosage to 750 mg/kg resulted in 100% death of the animals with the same symptoms within an hour. Introduction of the extract at a concentration of 500 mg/kg caused an ambiguous effect: in some cases a 100% survival, in others - 50% death. The introduction of the dosage of 225 mg/kg did not result in a single case of death for all five days of observation. Thus, it could be concluded that the extracts are also refer to low toxic preparations;  $\text{SD}_{50}$  for it is in the range of 350-500 mg/kg.

## **3. Results and discussion**

Radiobiological experiment performed on mice injected with extract of calendula and complex extract (CPE) at a concentration of 300 mg/kg 30 min before irradiation at the dose 7,5 Gy and fixed lifetime of irradiated animals during the month. As a comparison of the radioprotective influence there were standard radioprotector - cystamine and a control group of animals, which instead of radioprotector were injected distilled water. The results of experiments are presented in the table.

Table. Results of radiobiological test

Variants	Number of mice	The number of surviving mice	Survival rate, %	ALE, days
Cystamine	20	16	80	8,32 ± 0,31
Calendula extract	20	11	55	13,4 ± 2,13
Complex extract (MCT)	20	12	58	15,52 ± 1,4
Irradiated control	20	0	0	8,4 ± 0,73
Biological control	20	20	20	30 or more

ALE - the average life expectancy

Study of radioprotective activity of extracts of calendula showed that the extract possesses radioprotective activity (55%) that is slightly inferior to the activity of the standard protector cystamine (80%), and extract of plant collection by reducing the lethal effect of ionizing radiation, possess radioprotective activity (58%) and increases the survival rate and average life lethally irradiated mice more than extract of calendula (58%) and less than cystamine (80%).

The obtained results show that, in injection of extract of calendula lifetime of animals significantly increase (nearly 2-times), although the survival rate after 30 days is slightly inferior to that of the introduction of standard radioprotector. In the case of a complex extract there is also occur an growth in life span of irradiated animals, but the survival rate after the end of the experiment - 30 days - more than extract of calendula.

Data obtained by us testify the prospects of using calendula extract individually and in the complex of extracts of chamomile and yarrow for the development of medicinal compositions for the prevention of local radiation injuries.

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