

# The acute X-ray irradiation effects in *Salvia officinalis L. in vitro* plants



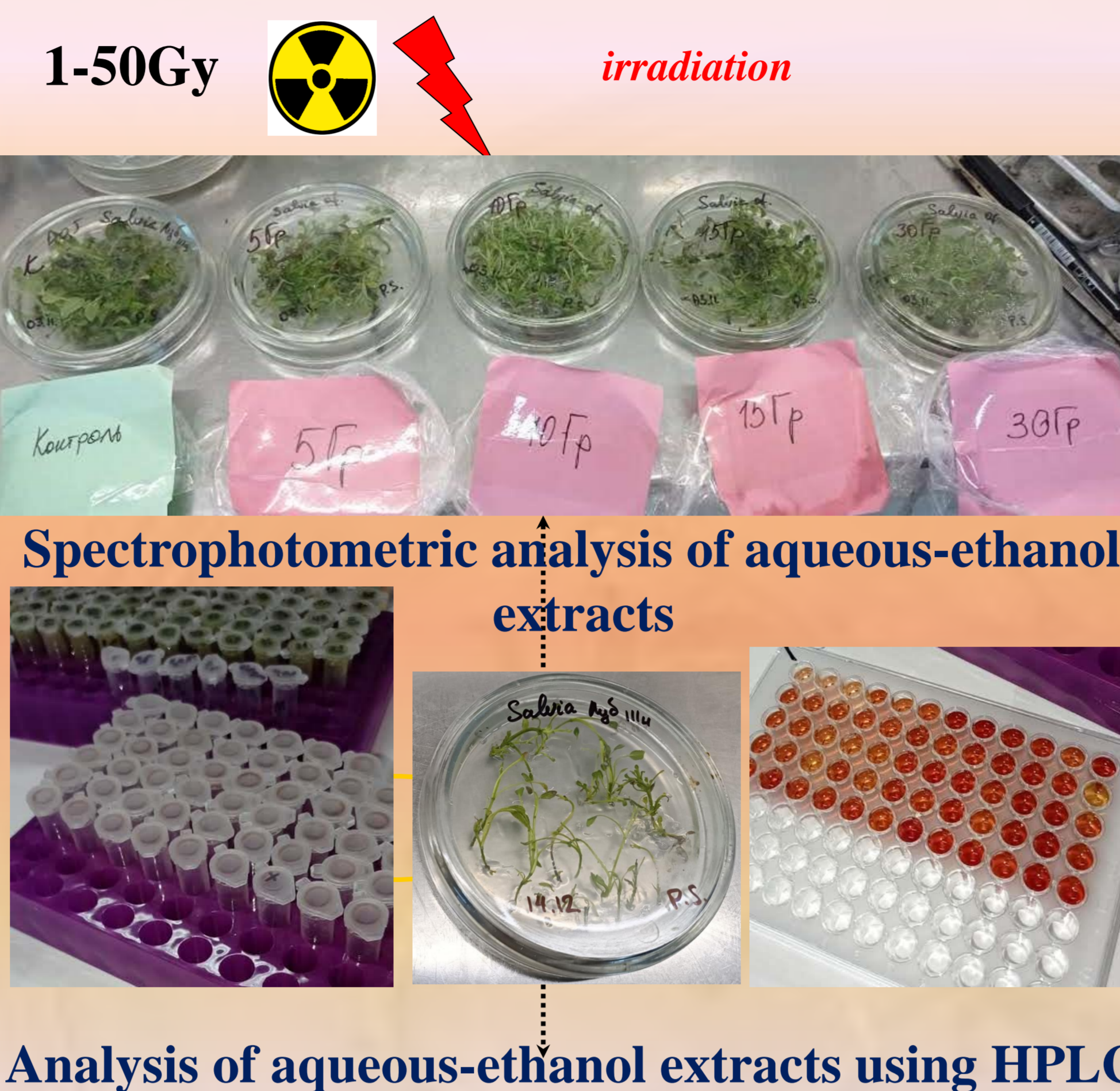
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In continuation of studies of the effect of ionizing radiation (IR) on the processes of secondary metabolism in medicinal plants, to investigate the effect of IR on the accumulation of secondary metabolites in medicinal *in vitro* plants culture was proposed. So the aim of the work was to identify radiation doses capable of stimulating the biosynthesis of pharmaceutically valuable substances.

The results of research conducted since 1932 (Breslavskaya L.P., Berezina N.M., Kuzin A.M., Batygin M.F., etc.) indicate the possibility and prospects of using ionizing radiation as a stimulating factor that accelerates the emergence of seedlings (increases germination energy), the beginning of flowering, and increases the yield and green mass of a number of agricultural crops. [1]. There are also many works in the world on the study of the stimulating effect of radiation on yield and content of useful substances in plants. In particular, it has been shown that ionizing radiation of both seeds and individual parts (shoots, etc.) of plants, plants in *in vitro* culture, leads to an increase in the content of phenolic compounds, flavonoids, vitamins in a number of cultural and medicinal plants.[2-5]. It has been experimentally confirmed that under the influence of stress factors, in particular ionizing radiation, the intensity of secondary metabolism in medicinal plants should increase, and, accordingly, their pharmacological value will increase.[6].

**Pic. 1. Experimental study of the effect of irradiation on the accumulation of secondary metabolites by *Salvia officinalis L.***



## Materials and methods

The work used *Salvia officinalis L.* of the medicinal variety Shans, presented by the Research Station of Medicinal Plants of the NAAS of Ukraine (village of Berezotocha). Plants were grown *in vitro* on hormone-free Murashige-Skooga medium supplemented with 30 g/l sucrose. Irradiation was carried out on a RUM-17 X-ray unit at doses of 5 Gy, 10 Gy, 15 Gy and 30 Gy for *Salvia officinalis L.* at a dose rate of 1.42 cGy/s. Samples for determination of the content of secondary metabolites – phenols and flavonoids were taken one, three and six weeks after irradiation. Plants were freeze-dried and extracted with 70% ethanol.

Extraction of the total flavonoids was carried out by maceration of a portion of the crushed material with 70% ethanol in a quantitative ratio of 1:100 at 24 °C for 72 h. Quantitative assessment of the content of secondary metabolites in extracts from medicinal raw materials was determined by measuring the optical density of the flavonoid complex and aluminum chloride on a CF-46 spectrophotometer (RF) at a wavelength of 410 nm.

The concentration of the total flavonoids was determined according to a calibration graph and expressed in mg of rutin per 1 g of dry weight. Qualitative analysis of the extracts was performed on a Shimadzu HPLC10Avp high-performance liquid chromatograph (Japan) in the Department of Genetic Engineering of the Institute of Biotechnology of the National Academy of Sciences of Ukraine. The results of three replicate experiments were averaged and calculated using standard tools of the Microsoft Excel 2003 program (USA).

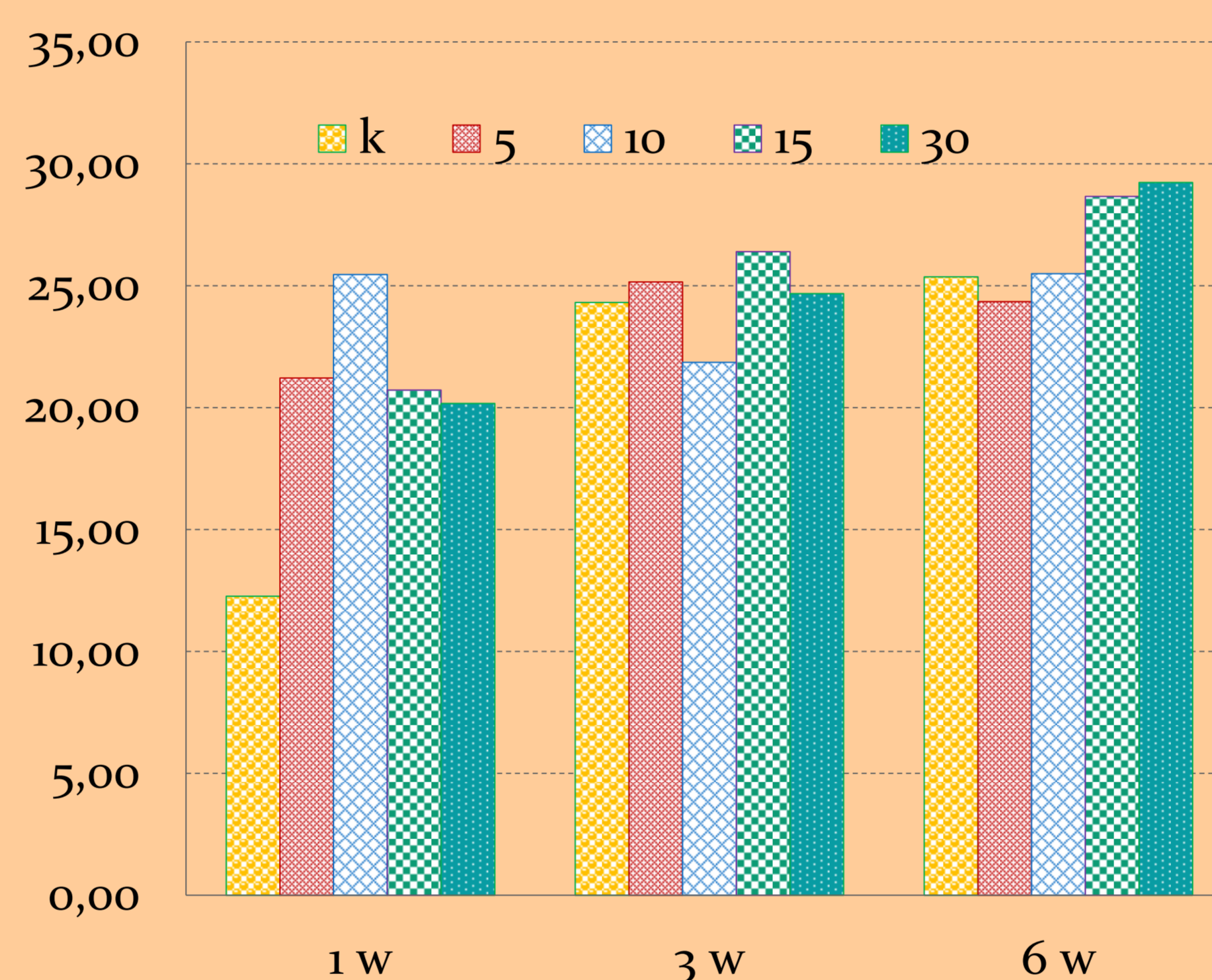
## Results and discussion:

The changes in the content of pharmaceutically important secondary metabolism substances in plant extracts of *Salvia officinalis L.*, grown *in vitro* and exposed to IR was investigated. For 1-week-old *Salvia officinalis L. in vitro* plants an increase in the yield of phenolic compounds, flavonoids and rosmarinic acid in ethanol extracts was observed for all irradiation doses compared to the control.

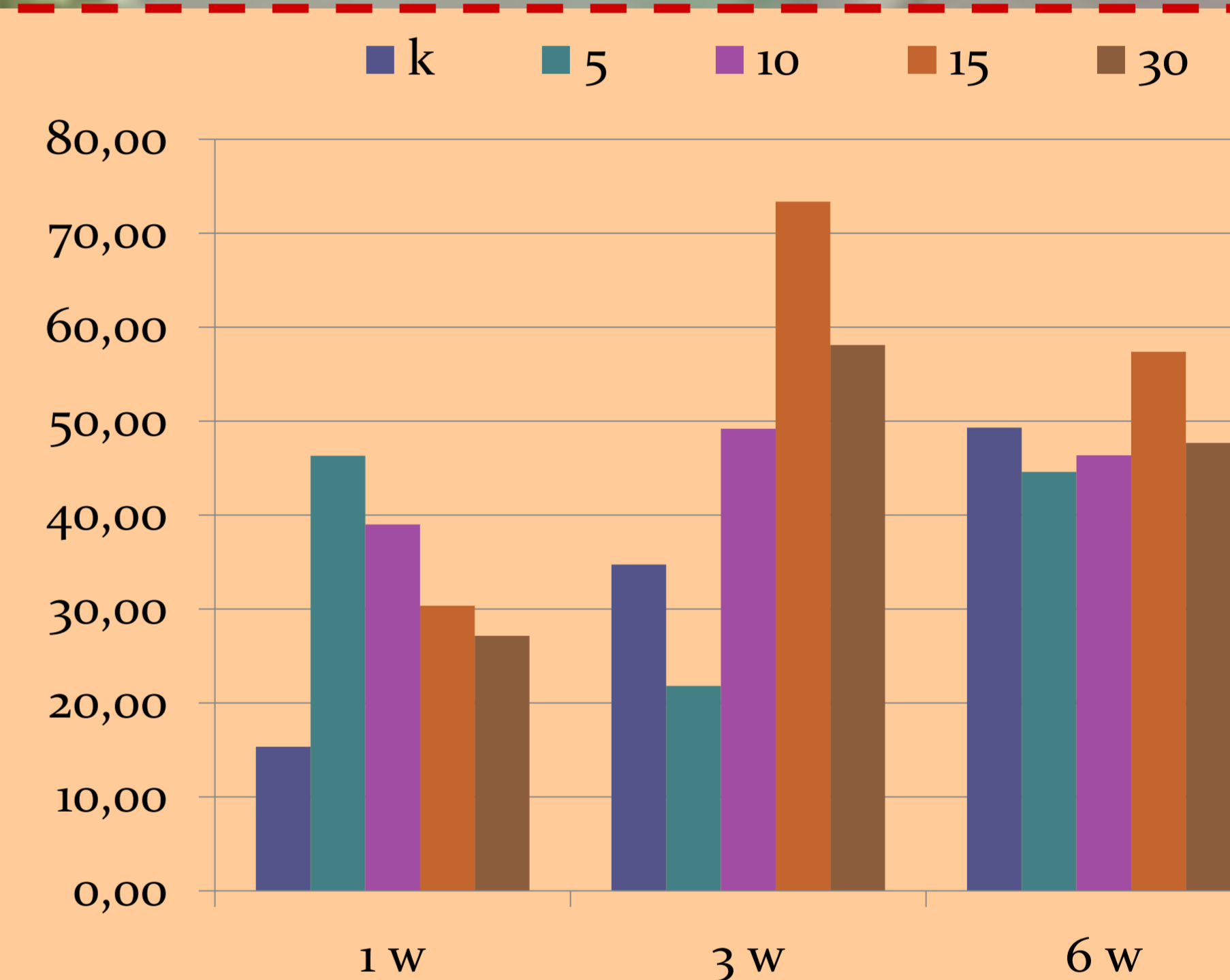
The greatest stimulation of the release of phenolic compounds into ethanol fr *Salvia officinalis L. in vitro* plants extracts was observed irradiated at a dose of 10 Gy.

A significant increase in the content of flavonoids in extracts from irradiated *Salvia officinalis L. in vitro* plants was observed one and three weeks after irradiation. After 6 weeks, no significant difference was observed. The greatest increase in the yield of flavonoids in extracts from irradiated plants compared to controls was observed for 5 Gy both after a week and after three weeks. In addition, after 3 weeks, a significant increase was also observed for the irradiation dose of 15 Gy. After 6 weeks after irradiation, a slight increase was observed for the variants irradiated at doses of 15 Gy and 30 Gy.

**mg of ferulic acid per 1 g of dry weight**



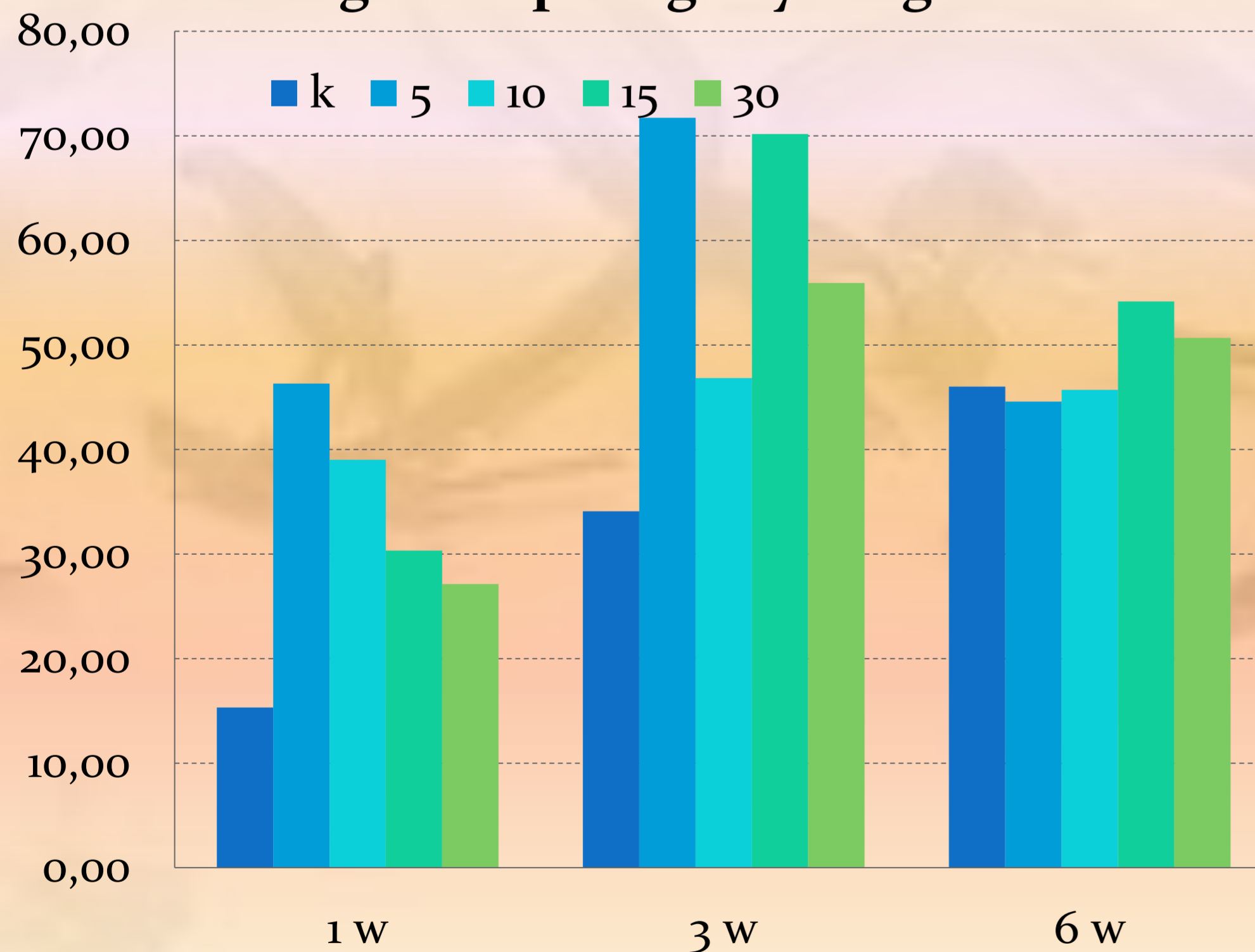
**Pic. 2. Dose dependence of the phenolic compounds by ferulic acid in aqueous-ethanolic extracts from *Salvia officinalis L.***



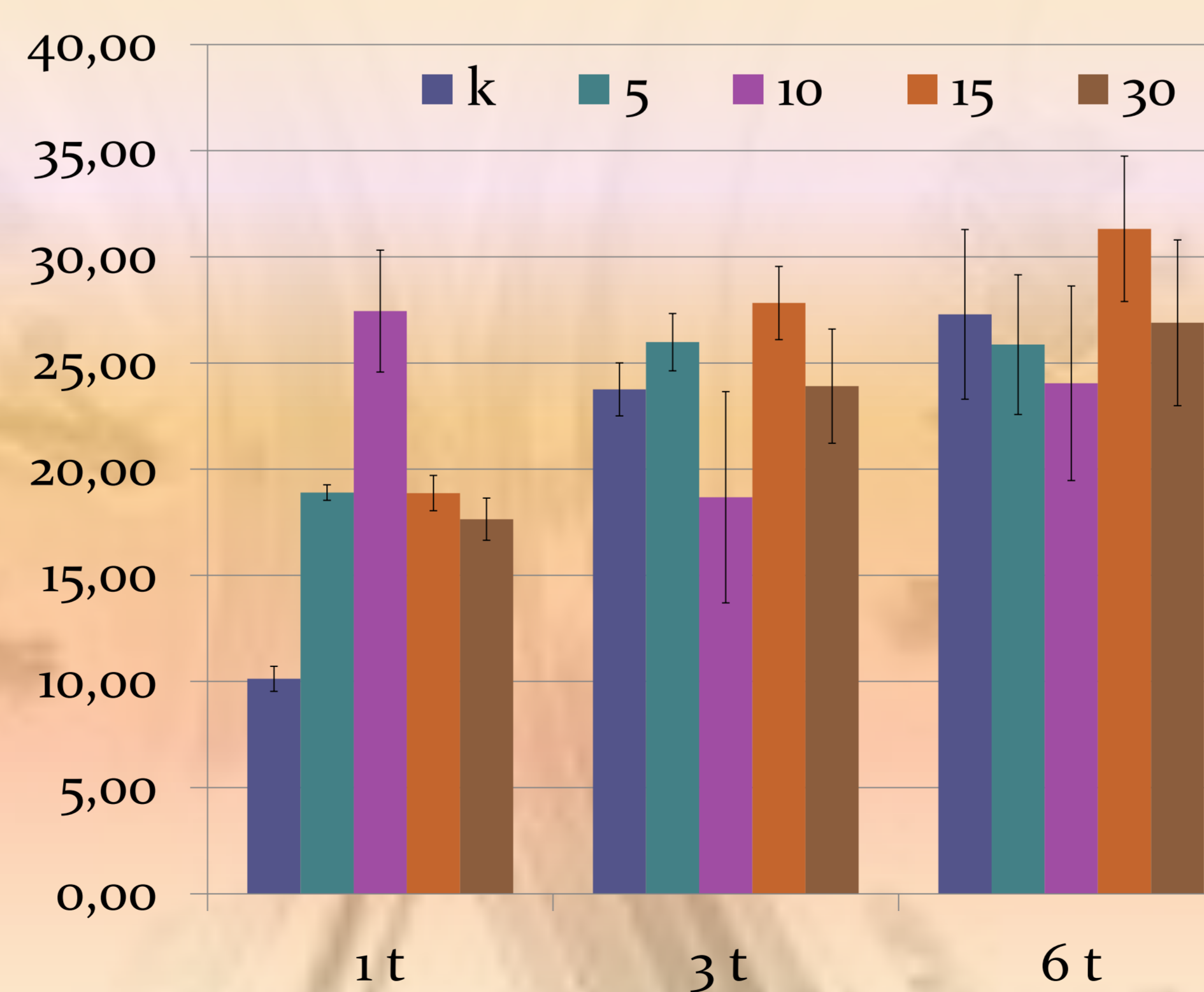
**Pic. 3. Dose dependence of the total flavonoid content in plant extracts of *Salvia officinalis L.***

A significantly higher release of rosmarinic acid into ethanol extracts for the irradiated variants is observed a week after irradiation. Moreover, the use of irradiation at a dose of 10 Gy exceeds the control level by 4 times. For three and six weeks after irradiation, the effect was not preserved.

**mg rutin per 1 g dry weight**



**Pic.4. Dose dependence of the flavonoid by rutin content in plant extracts of *Salvia officinalis L.***



**Pic.5. Dose dependence of the rosmarinic acid content in plant extracts of *Salvia officinalis L.***

## CONCLUSIONS

An increase in flavonoid content in extracts from irradiated sage plants grown *in vitro* was observed one and three weeks after irradiation. An increase in phenolic content in extracts from plants irradiated at doses of 5, 15, and 30 Gy was observed one week after irradiation.

Thus, confirmation of the possibility of using ionizing radiation to modify the accumulation of pharmaceutically valuable substances in *Salvia officinalis L. in vitro* plants culture was obtained.

## References

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