# THE RADIOPROTECTIVE EFFECT OF PHASEOLUS VULGARIS PHYTOHEMAGGLUTININS EXERTED ON VITIA FABA GERMS

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The finding of effective radioprotectors is one of the most pressing and yet unsolved problems in radiobiology. In 1949, Barron reached the first practical achievement in the field of radioprotection, establishing the radioprotective effect of chemical compounds with a sulfhydryl group content (SH). Thousands of preparations have undergone detailed investigations ever since (3,5). The results attained however, are far from meeting the requirements for an ideal radioprotector. Most efficient for the time being prove to be the chemioprotectors of the thiol compounds (cystamine and aminoethylisothiuronium) (1). Some biopreparations also exert a radioprotective effect (8).

In 1963 Schrek and Stefani (12) reported the first results from investigations on the protective action of phytohemagglutinins (PHA) from Phaseolus vulgaris on lymphocytes of peripheric human blood, irradiated with X-rays or treated with nitrogen mustard ($HN_2$) in vitro (12). In their work they used PHA — p „Difko". More recent investigations by the same authors, carried out with the purpose of establishing the radioprotective action of PHA, confirmed their original findings and furthermore proved that the radioprotective effect of PHA is superior to that of cysteine (13). These results indicate that PHA has a strongly manifested radioprotective effect on leukocytes of peripheric blood of humans in tissue cultures.

Of particular interest is the question whether this PHA effect is specifically manifested merely in human lymphocytes, or it is a general biological phenomenon.

With the goal of making clear this issue, we undertook the present investigations on the radioprotective effect of PHA exerted on plants.

## Material and Method

In the experiment germs of the plant Vitia faba were used, as the latter exhibits a high degree radiosensitivity under natural conditions (2). Owing to these properties, it is recommended by Tank as a sample for primary selection of radioprotective compounds (4).

Experimentation was accomplished with Vitia faba germs, sort „Hyoska".

The seeds were kept submerged in water for 36 hours, subsequent to which their covering was stripped. Next, they were burried for 36 hours in moist

sand in order to provide for the correct orientation of their roots. The germs were distributed in groups of 20, depending on the daily growth of the roots, with a view to obtaining groups with equal daily growth. The further cultivation of the plants was carried out in acquarium, placing the germs over grid plates with suspended in the water roots. In two of the experimental groups (No. 9, 10; table 1) the seeds, following removal of the covering, were placed on filter paper, kept constantly moist by the PHA solution. With these groups the PHA action was begun before the time of outgrowing and was continued up to the moment of irradiation (84 hours).

PHA of Phaseolus vulgaris was used in the experiment (preparation Phaseolosaxin) diluted in physiological solution. The plants were exposed to the action of PHA one hour prior to irradiation, submerging their roots in PHA solution for one hour. The radioprotective effect of PHA was checked in three different concentrations: 1.5 mg %, 3 mg % and 10 mg %. The plants of the control group were treated with NaCl solution in concentration corresponding to that of the PHA. Groups of non-irradiated plants were secured for biological control purposes.

The experiment was carried out at room temperature (17—23 degrees C), and irradiation was carried out with Siemens apparatus in doses 200, 300 and 400 r (strength of current 10 mA, tension 180 kV, focus-to-object distance 30 cm, filter 0.5 mm Cu, air dose capacity 49.3 r/min).

The daily growth of the roots of the experimental groups contrasted to the daily growth of the controls was employed as an index of the protective effect, followed up to the 10th post-irradiation day.

Fourfold reproduction of the experiment was carried out with analogous results.

## Results

The results of the experiment are illustrated in table 1 and scheme 1.

The reliability of the difference between the growth of the roots in both experimental and control groups is determined with the aid of the t criterium. In this case P 0.05 corresponds to $t \geq 2086$.

It is evident from the data presented in the table that PHA exerts a substantial radioprotective effect in the experiment described.

In all the groups an arrest is marked of the growth or minimum growth of the roots during the initial 3—7 days following irradiation. About the 8—9th day after the radiation, the growth in the plants treated with PHA caught up with the normal daily growth of the non-iradiated plants.

The weakest protective effect of PHA is observed with 200 r radiation dose. In the latter case the reliability of difference is reduced on account of large fluctuations of arithmetical means, equally in experimental and control groups. In the plants irradiated with 300 r, the protective effect of PHA is optimal: inhibition of root growth is transitory while the daily growth is greater. With irradiation dose amounting to 400 r, the protective effect of PHA is insignificantly lowered as compared to that received with 300 r.

Radioprotective effect is more clearly manifested during one-hour treatment of the plants with PHA. The 84-hour-long treatment of the plants does not exert a significant effect on the ultimate result (table 1 , No. 9, 10).

The results are not equivalent in the three PHA concentrations tested. PHA in concentration 1.5 mg % exerts statistically reliable radioprotective effect merely with radiation dose amounting to 300 r, and not earlier than

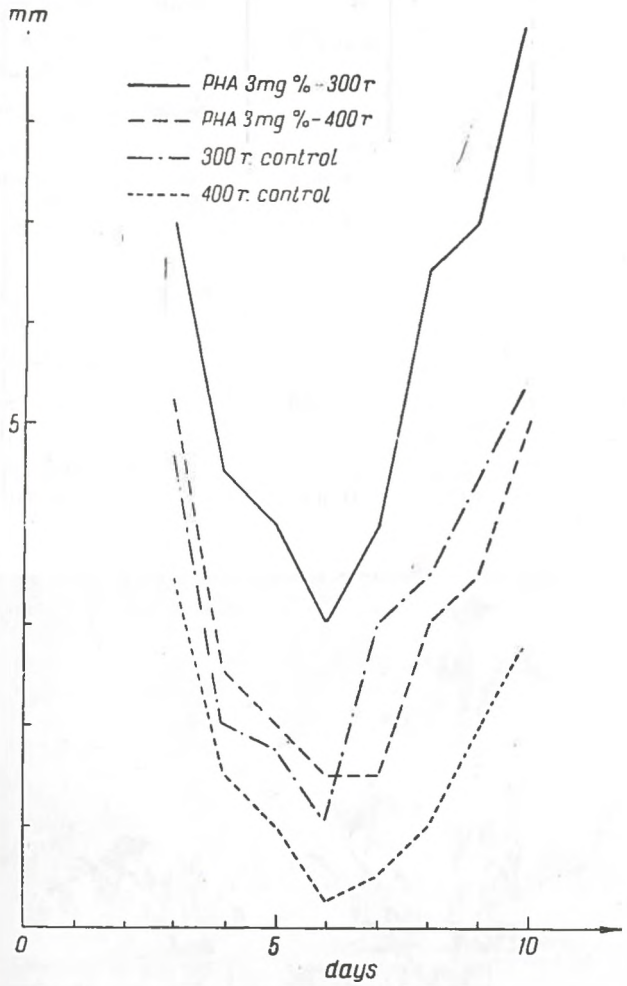


Diagram 1. The protective effect of PHA in concentration 3 mg %, duration of action 1 hour with radiation dose 300 r (1) and 400 r (2) (No. 2,3), compared to the corresponding controls (3 and 4).

Along the absciss — number days after irradiation; along the ordinate — mean daily growth of the roots in mm

the 7th day. Analogous results are noted in PHA concentration 10 mg %, with the only difference that in the latter case the protective effect is manifested as early as the fourth day (Fig. 1).

Table 1

| Experiment № | PHA concentration | Radiation dose | Duration of PHA effect | Number of days after irradiation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | | | | Reliability of the protective effect (t) | | | | | | | | | |
| 1 | 3 mg % | 200 r | 1 hour | — | — | — | — | — | — | — | 1.3 | 3.2 | 2.4 |
| 2 | | 300 r | 1 hour | 0.7 | 0.8 | 2.6 | 2.9 | 0.8 | 1.3 | — | 4.2 | 3.2 | 1.9 |
| 3 | | 400 r | 1 hour | — | — | — | 4.5 | 1.7 | 3.0 | — | 2.9 | 2.7 | 2.7 |
| 4 | 10 mg% | 200 r | 1 hour | — | — | — | — | — | — | — | — | 2.9 | 2.6 |
| 5 | | 300 r | 1 hour | — | — | — | 1.2 | 1.4 | 1.7 | 1.7 | 2.4 | 2.1 | 2.2 |
| 6 | | 400 r | 1 hour | — | — | — | — | — | — | — | — | — | 1.4 |
| 7 | 1.5 mg% | 300 r | 1 hour | — | — | — | — | — | — | 1.0 | 1.7 | 2.1 | 2.0 |
| 8 | | 400 r | 1 hour | — | — | — | — | — | — | — | — | — | — |
| 9 | 3 mg% | 300 r | 84 hours | — | 0.3 | 1.1 | 0.7 | 2.3 | 3.2 | 2.8 | 2.3 | 2.1 | 3.9 |
| 10 | | 400 r | 84 hours | — | — | 1.9 | 1.5 | 3.9 | 2.1 | 1.2 | 4.3 | 3.9 | 2.3 |

Fig. 1. Experimental group No. 3 (the plants are treated with 3 mg % PHA for a duration of one hour an, hour before irradiation with 400 r).
The supremacy in the height of the green part, number and size of the secondary roots in these plants is obvious as compared to the corresponding controls (the right-side group)

## Discussion

The results of the experiments described show that PHA in determined concentrations manifests a radioprotective effect upon the plant Vitia faba.

The radioprotective effect of PHA upon the Vitia faba germs is statistically reliable. The concentration 3 mg % proves to be optimal insofar effect exerted is concerned, whereas a rather weaker radioprotective effect was noted in lower and higher PHA doses (1.5 mg % and 10 mg % respectively).

The data reported indicate that PHA of Phaseolus vulgaris is a radioprotector equally for leukocytes of the peripheric human blood (12, 13) and plant organisms. The latter circumstance is a further corroboration of the radioprotective properties of PHA, assumed as a phenomenon of general biological sequence.

The mechanism of action of PHA is doubtlessly of particular interest. Our investigations on the amino acid content of the PHA utilized — Phaseolosaxin which represents a globulin, revealed the presence of 14 various amino acids, including cystine and glutamic acid (7). Presently, the radioprotective effect of cystine and glutamic acid has been likewise proved (1,5).

The stimulating effect of the PHA upon the synthesis of deoxy-ribonucleic acid (9, 10) and ribonucleic acid (15), blastemic transformation and division of lymphocytes in man, in vitro, has been established. Further studies, carried out by the authors, proved the stimulating effect of PHA upon the proliferation of granulation tissues in man and some animals (14), as well as upon phagocytosis of human leukocytes (6). All the data submitted characterize the PHA as a stimulator of cellular metabolism and reparative processes in the organism, properties readily explained with its radioprotective effect.

A full identification of the mechanism of the radioprotective effect of PHA exerted upon human leukocytes and the plant species employed is not possible, but very likely, phenomena are concerned of analogical character, effected at cellular level.

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## РАДИОПРОТЕКТИВНЫЙ ЭФФЕКТ ФИТОГЕМАГГЛЮТИНИНА ИЗ PHASEOLUS VULGARIS НА ЗАРОДЫШИ VITIA FABA

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РЕЗЮМЕ

Препарат Phaseolosaxin (фитогемагглютинин из Phaseolus vulgaris) в концентрации 3 мг % оказывает радиопротективный эффект на зародыши растения Vitia faba.

Авторы объясняют радиопротективное действие исследуемого препарата стимулирующим действием фитогемагглютинина на клеточный метаболизм и на репаративные процессы в организме.