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HABERLEA RHODOPENSIS LEAF EXTRACT–INDUCED CHANGES IN THE DOSE-EFFECT CURVE FOR THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN PERIPHERAL BLOOD LYMPHOCYTES IRRADIATED IN VITRO WITH ^{60}Co.

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ABSTRACT
The aim of the present study is to investigate the dose-effect relationship for induced chromosomal aberrations in peripheral blood lymphocytes, irradiated *in vitro* after the application of a protector *in vivo* before the exposure. We used peripheral blood from male rabbits, the New Zealand breed. The animals were injected with *Haberlea Rhodopensis* (HR) extract (0.03, 0.06 and 0.12 g/kg b.w.) two hours before obtaining the blood samples. Then, the blood was irradiated with different doses of ^{60}Co gamma-rays: 1.0, 2.0 and 3.0 Gy. The established lymphocyte cultures were incubated for 48 hrs. at 39^{o}C in the presence of CO_2 to assess the chromosomal aberrations. We found a quantitative dose-dependent relationship for the recorded dicentric chromosomes. This relationship can be expressed with the following equations: $Y=(3.57\pm0.89D+(1.215\pm0.339)D^2$ for the irradiated samples; $Y=(2.97\pm0.9)D+(0.815\pm0.343)D^2$ for the samples with 0.03 g/kg HR; $Y=(3.31\pm0.14)D$ for the samples with 0.06 g/kg HR; and $Y=(2.52\pm0.14)D$ for the samples with 0.12 g/kg HR, where Y is the yield of dicentric chromosomes, and D is the ionizing radiation dose. These equations can enable us to make an assessment of the protective effect of the HR extract when the value of the applied radiation dose (D) is known.

Key words: radioprotection, chromosome aberrations, extract Haberlea Rhodopensis, dose-effect curve, γ-rays**.**

INTRODUCTION

The most sensitive biological method for the assessment of the ionizing radiation effect is the chromosomal aberration analysis in peripheral blood lymphocytes. The method provides a quantitative assessment of the dose as an equivalent dose in an evenly-distributed whole body irradiation with a threshold sensitivity of 0,1 Gy for gamma- or X-ray exposure (1, 2). The chromosomal aberrations - both the dicentric and ring-type – are considered to be "the gold standard" for the quantitative evaluation of radiation exposure in a biological way (1, 2). The elaboration and standardization of the cytogenetic techniques for the assessment of chromosomal aberrations is largely related to the requirements for an accurate and timely dosimetry in the event of a nuclear accident (3, 4). One of the method advantages is its ability to assess the individual radio-sensitivity of an organism, since the biological response to an ionizing radiation exposure is mainly dependent on the biological characteristics and genetic specificities of the irradiated individual, apart from the radiation source.

The comparison of the calibrating curves, published by various authors, most often based on the frequency of the found dicentric chromosomal aberrations, show a clear correlation between the number of aberrations

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and the absorbed radiation dose (2, 5, 6, 7). For the X-rays and gamma-rays, the correlation between the chromosomal aberration yield (Y) and the absorbed dose (D) is most frequently linear-quadratic. The international organizations for nuclear protection require that each laboratory performing biological dosimetry should work out and have its own calibrating curves (1, 2).

It has been found that the plant extract we used as a protector – a total extract of Haberlea Rhodopensis – possesses antibacterial (8), antioxidant (9) and anti-mutagenic activity (10, 11).

In the present study we investigated the number of chromosomal aberrations in peripheral blood lymphocyes from healthy donors, irradiated with various doses of ^{60}Co gamma-rays. The aim of the present investigation is an assessment of the dose-dependent correlation regarding the increase in the chromosomal aberration yield in relation to the gamma-irradiation dose and the application of different concentrations of the radioprotector, a total extract of HR, on peripheral blood lymphocytes from rabbits.

MATERIAL AND METHODS

We used whole peripheral blood from male rabbits, breed white New Zealand; the animals were the same age and body weight, and were taken care of under conditions complying with the requirements for human handling of animals. Blood was obtained from the marginal auricular vein of each donor. Two hours before obtaining the blood samples, the rabbits were injected with a total extract of HR in concentrations 0.03; 0.06 and 0.12 g/kg b.w. We subdivided the animals in 12 experimental groups (n=5). The blood was irradiated in glass vials made of neutral glass with a diameter of 25 mm. The irradiation was performed with a ROKUS-M gamma unit using ^{60}Co gamma rays; the dose intensity was 24 cGy/min., and the radiation doses 1.0, 2.0 and 3.0 Gy. When conducting the experiments and the data analysis, we strictly followed the guidelines and recommendations of the International Atomic Energy Agency (IAEA 1986, IAEA, 2001).

Following the irradiation, two lymphocyte cultures from each tube were prepared. The lymphocyte cultures were established in RPMI 1640 nutritional medium, supplemented with 20% bovine serum and incubated at 39^{o}C for 48 hours (12). The chromosomal analysis was performed after Giemsa staining. A minimum number of 500 cells for each dose was evaluated on a microscope for structural chromosomal aberrations. All types of chromosomal aberrations were recorded – deletions of the chromatid and chromosome type, dicentrics and exchanges. The slides from each donor were independently evaluated by different investigators.

The derived data were processed by using the appropriate statistical methods (variation and regression analysis, two-way ANOVA), applied with the statistical package STATISTICA 6. Stat. Soft.

RESULTS

Figure 1 illustrates the frequency of the aberrant cells in rabbit lymphocytes, irradiated in a dose range 1.0-3.0 Gy and pre-treated with an HR extract in concentrations of 0.03, 0.06 and 0.12 g/kg b.w.

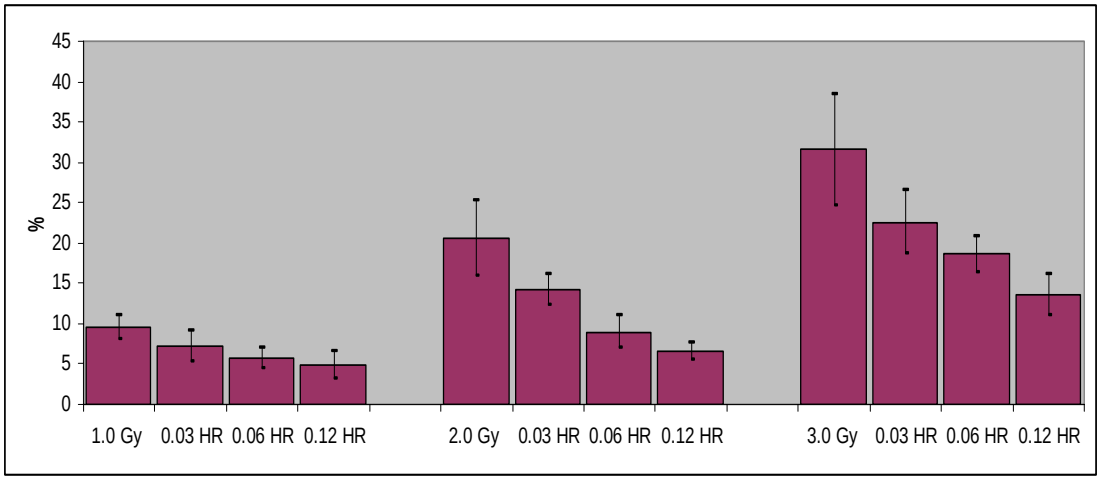

Figure 1. Frequency of the cells with chromosomal aberrations in rabbit lymphocytes, irradiated in a dose interval 1.0-3.0 Gy and pre-treated with an extract from HR.

The frequency of the aberrant cells induced by irradiation with doses of 1.0, 2.0 and 3.0 Gy is significantly lower in the rabbits treated with the HR extract in the three concentrations used ($p<0.01$) in comparison with the blood samples exposed to irradiation only. The results are presented with a mean value ± SD.

The results from the two-way ANOVA test regarding this parameter are presented in **table 1.**

Table 1. *Two way Anova (aberrant cells)*

Effect	Df effect	Ms effect	Df error	Ms error	F	p-level
Dose	2	1105.417	48	9.741	113.473	0.000*
HR	3	419.661	48	9.741	43.079	0.000*
Dose+HR	6	42.261	48	9.741	4.338	0.001*

In order to calculate the results from the conducted chromosomal analysis to determine the reducing effect of the extract, applied in different concentrations, upon the yield of chromosomal aberrations, we made an estimation of the number of dicentrics. The function of the increase in the yield of dicentrics as a result of irradiation in the dose range 1.0 – 3.0 Gy is shown in **figure 2.**

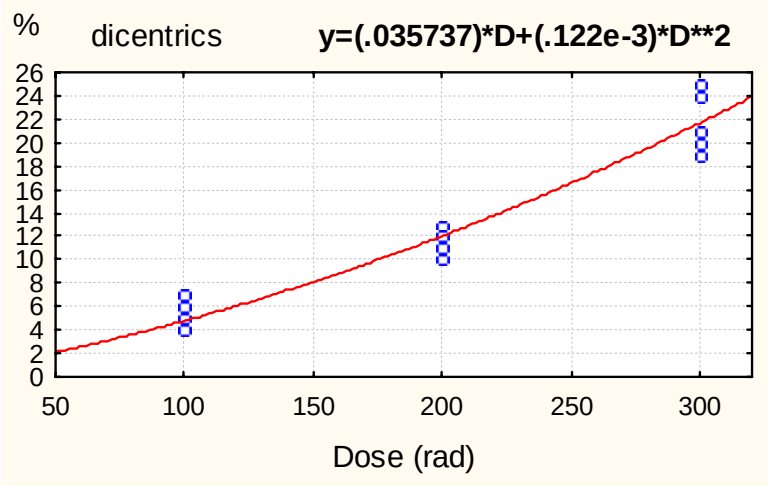

Figure 2. Dose-effect curve for the increase in the percentage of dicentrics as a function of the applied gamma irradiation dose.

The statistic processing of the data shows that the correlation dose-effect for dicentrics is best expressed with a linear-quadratic curve through the following equation: $Y=(0.0357\pm0.0089)D+(0.000122\pm0.000034)D^2$. To assess the quotients of this curve, we used the Nonlinear Regression procedure of the statistical package STATISTICA 6.

Table 2 shows the assessment of the quotients in the equations, as well as the lower and upper limits of the 95% confidence interval of the quotients to the dose.

Table 2. *Quotients of increase in the yield of dicentrics following irradiation in the dose range 1.0-3.0 Gy.*

Estimate	Standard error	t-value df=13	p-level	Lo. conf. limit	Up conf. limit
(α) 0.035736	0.00897	3.983054	0.001561	0.016353	0.055120
(ß) 0.000122	0.000034	3.585151	0.003325	0.000048	0.000195

The function of increase in the yield of dicentrics resulting from the protection of the rabbits with an HR extract before the irradiation of blood samples is shown on **figures 3 and 4**.

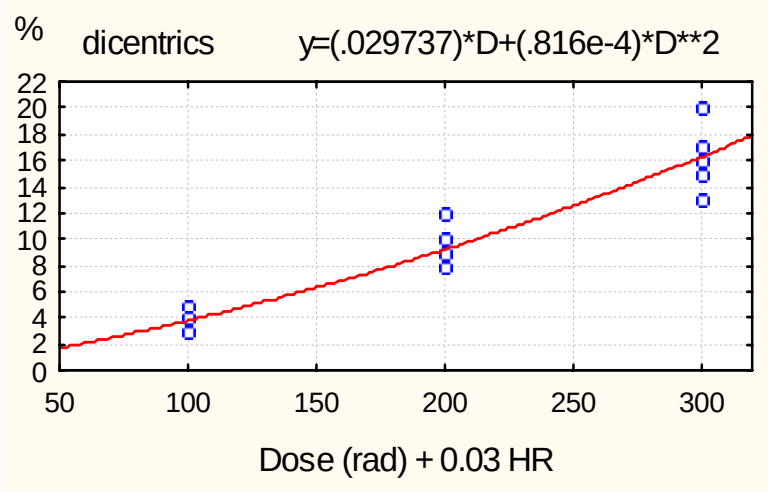

Figure 3. Dose-effect correlation in irradiated blood samples from rabbits pre-treated with an HR extract at a concentration 0.03 g/kg b.w.

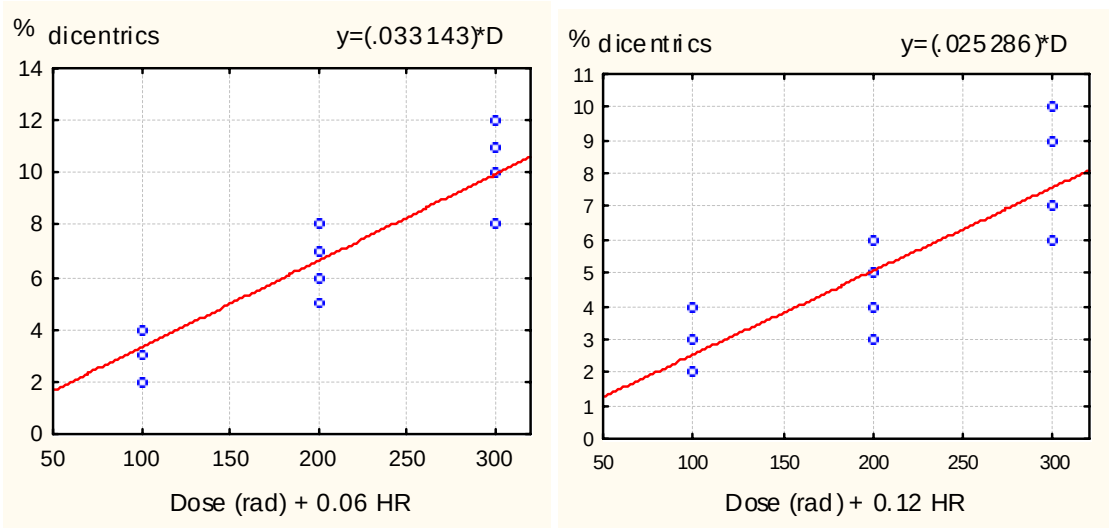

Figure 4. Dose-effect correlation in irradiated blood samples from rabbits pre-treated with an HR extract at a concentration 0.06 and 0.12 g/kg b.w.

The equation expressing the dose-dependent correlation in rabbits treated with an *Haberlea Rhodopensis* extract in concenyration 0.03 g/kg b.w. is linear-quadratic:
$Y=(0.0297\pm0.009)D+(0.000082\pm0.000034)D^2$.

The reliability of the quotients α ($p<0.006$) and ß ($p<0.03$) is statistically significant. The effect from the treatment with 0.03 g/kg b.w. is best revealed by a decrease in both quotients before the dose.

When applying higher concentrations of the extract, the correlation dose-effect acquires a linear manner. The equations describing the correlation after treatment of the rabbits with 0.06 and 0.12 HR g/kg b.w. are as follows: $Y=(0.0331\pm0.0014)D$ and $Y=(0.025\pm0.0014)D$, respectively.

Table 3 shows assessment of the quotients in the equations, as well as the lower and upper limits of the 95% confidence interval.

DISCUSSION

The assessment of dicentric chromosomes in lymphocytes from peripheral blood is the most sensitive biological dosimeter for the exposure to ionizing radiation. The threshold for sensitivity of this method is the equivalent whole-body dose of 0.1 Gy for irradiation with gamma- and X-rays (1,2).

According to the classical theory for the mechanism of occurrence of chromosomal aberrations following irradiation (13,14), these are regarded as a result of one or two ionizing

events. The aberrations that occur as a result of one ionizing event increase as a linear function of the dose, while these occurring as a result of two separate and independent ionizing events increase as a quadratic function of the dose. Dicentric chromosomes occur after an exposure to single events at low doses, while at higher doses they are mainly a result of two ionizing events (15,16). Therefore, at low doses the curve shows a linear course, whilst at higher doses it follows a steeper course (2). Our results for the dose correlation regarding the yield of dicentrics following irradiation with different doses gamma-rays show that it is linear-quadratic, and they are in full agreement with other published results regarding the dose correlations (6, 17, 18). Linear-quadratic is also the correlation for the yield of dicentrics in lymphocyes, irradiated in the same dose range, in rabbits that were pre-treated with 0.03 g/kg b.w. of an HR extract, albeit the quotients in the equations are decreased from 0.0357±0.0089 to 0.0297±0.009 for α and from 0.0357±0.0089 to 0.000082±0.000034 for β.

Table 3. *Quotients of increase in the yield of dicentrics following irradiation of peripheral blood from rabbits, pretreated with an HR extract, in the dose range 1.0-3.0 Gy.*

Treatment	Estimate	Standart error	t-value df=13	p-level	Lo. conf. limit	Up conf. limit
HR 0.03 g/kg b.w.	(α) 0.029737	0.009082	3.274225	0.006*	0.010116	0.049318
	(ß) 0.000082	0.000034	2.376516	0.033*	0.000007	0.000156
HR 0.06 g/kg b.w.	(α) 0.033142	0.001431	23.15044	0.00000*	0.030072	0.036213
HR 0.12 g/kg b.w.	(α) 0.025285	0.001444	17.50723	0.00000*	0.022188	0.028383

It must be pointed out that in the rabbits treated with 0.06 and 012 g/kg b.w. HR extract the correlation dose-effect is best expressed with a linear equation, something which is characteristic of irradiating with low doses (2).

The applied experimental model and the correlations we investigate provide us with further information about the effects of radioprotectors and can be used for the assessment of newfound and used in trials natural compounds with a potential radioprotective effect.

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