Bulgarian Journal of Veterinary Medicine (2010), **13**, No 3, 148–153

IN VITRO INCIDENCE OF CHROMOSOME ABERRATIONS IN GAMMA-IRRADIATED RABBIT LYMPHOCYTES, TREATED WITH *HABERLEA RHODOPENSIS* EXTRACT AND VITAMIN C

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Summary

Popov, B., R. Radev & S. Georgieva. 2010. *In vitro* incidence of chromosome aberrations in gamma-irradiated rabbit lymphocytes, treated with *Haberlea rhodopensis* extract and vitamin C. *Bulg. J. Vet. Med.*, **13**, No 3, 148–153.

Ionizing radiation produce deleterious effects in the living organisms and the rapid technological advancement has increased human exposure to ionizing radiation enormously. There is a need to protect humans against such effects of ionizing radiation. In this study, the effect of *Haberlea rhodopensis* and vitamin C on the frequency of chromosome aberrations in rabbits peripheral blood lymphocytes after *in vitro* gamma irradiation was compared. Results demonstrated that *H. rhodopensis* extract (at concentrations 1.0 μL/mL, 4.0 μL/mL and 8.0 μL/mL) reduced the frequency of chromosome aberrations, especially double chromosome fragments and dicentrics, as well as aberrant cells. *H. rhodopensis* extract applied at 1.0, 4.0 and 8.0 μL/mL was found to be more effective in reducing aberrant cells than vitamin C (1.0 μg/mL). The effect of *H. rhodopensis* and vitamin C on the frequency of dicentrics and double acentric fragments was similar. It can be concluded that the extract of *H. rhodopensis* at tested concentrations showed a radioprotective potential.

Key words: chromosome aberrations, *Haberlea rhodopensis*, rabbit lymphocytes, radioprotection, vitamin C

INTRODUCTION

The ample evidence on the negative effect of ionizing radiation on the living organisms, as well as the need of utilizing ionizing radiation in diagnostics, therapy, industry, energy production, inadvertent exposure during space flights, nuclear incidents etc. necessitate the availability of protection against the effects of such radiation.

Thiol-based synthetic compounds such as amifostine, are powerful radioprotective agents, but they have a limited use in clinical setting due to their side effects and toxicity (Kligerman *et al.*, 1984; Hosseinimehr *et al.*, 2002). It was established during the last two decades, that products derived from natural sources could be used as non-toxic radioprotectors (Jagetia *et al.*, 1986; Goel *et al.*, 2002; Pahadiya & Sharma, 2003; Kumar & Kuttan, 2004; Singh *et al.*, 2005). Plants and natural products have a number of advantages: they are not toxic at applied concentrations, are relatively cheap, can be applied orally and many of them are used in traditional medicine. If

these substances exhibit anti-inflammatory, antimicrobial, antioxidant, immunomodulating, anti-stress properties, they are likely to possess radioprotection qualities as well, and therefore they should be studied closely.

Haberlea rhodopensis is one of the so-called "resurrection plants" (Ushatinskaja, 1990) due to its ability to enter an anabiotic state in adverse environmental conditions (such as drying) and restore its initial state afterwards when conditions improve. The plant *H. rhodopensis* is not sufficiently studied, although data on its antioxidant potential (Yahubyan *et al.*, 2005) and antibacterial activity (Radev *et al.*, 2009) are reported. The examination of its phytochemical contents has revealed the presence of a significant amount of chlorophyll and some enzymes (superoxide dismutase, citrate dehydrogenase, etc.). The other species from the same family (Gesheriaceae) have also been found to contain flavonoids, flavonoid tannins, beta carotene, ascorbate, glutathione etc. (Klishev *et al.*, 1978).

The necessity of discovering natural substances with radioprotective potential underlined the goal of the current study – to examine the effects of *Haberlea rhodopensis* extract on the prevalence of chromosome aberrations in *in vitro* irradiated rabbit lymphocyte cultures.

MATERIALS AND METHODS

Preparation of the Haberlea rhodopensis extract

The total extract of the plant was prepared by maceration of the leaves for 48 h in 70% ethanol. Extract was obtained by evaporation of the ethanol until the drug-liquid phase ratio of 1:1.

The selection of optimal concentrations for treatment of lymphocyte cultures was done in a preliminary experiment, where rabbit lymphocyte cultures were treated with *H. rhodopensis* extract at 1, 2, 4, 8, 16, 38, 40, 50, 80, 100, and 500.0 μL/mL. The most optimal concentration was selected through the direct counting method in the Bürker counting chamber. In a cell culture treated with 8.0 μL/mL, the number of cells was 2×10^4 per mL – an amount which made the detection of chromosome aberrations more difficult, while also decreasing cellular vitality.

Lymphocyte cultures

The blood samples were obtained from the marginal ear vein of one donor – a male rabbit. The anticoagulant used was heparin, at a concentration of 30 IU/mL. The blood was transported to the laboratory for cytogenetic analysis immediately after collection and was processed using the micromethod of Hungerford (1965). Whole heparinized blood (0.5 mL) was incubated in 7 mL of cell culture medium RPMI 1640 with L-glutamine and HEPES buffer, 3 mL thermally inactivated normal calf serum, 0.2 mL resubstituted 2% PHA, 100 IU/mL penicillin, 50 μg/mL gentamicin.

Six cultivation flasks were prepared and placed into a thermostat in the absence of light at 39 ^{o}C. On the 1st hour after cultivation, *H. rhodopensis* extract was added to three of the flasks, at concentrations of 1 μL/mL, 4 μL/mL, and 8 μL/mL. To a fourth flask, 1 μg/mL of vitamin C was added and the fifth one remained untreated. After this treatment, all cultures (except the non-exposed control) were subjected to gamma irradiation at 2.0 Gy, after which they were incubated for another 71 hours.

Irradiation of the samples was

performed under water at 39 °C with Rokus gamma equipment. The dose rate was 2.0 Gy.

On the 70th hour, colchicine at a concentration of 0.5 g/mL was added to each flask. The classic lymphocyte culture processing protocol was used. By the end of the 72nd hour of lymphocyte culture cultivation, chromosome preparations were made for detection of chromosome aberrations. One hundred metaphase plates of each treatment were investigated – a total of 600. The aberrant cells number, the number of dicentric chromosomes, ring chromosomes, single and double fragments were counted.

The significance of differences was calculated by the two independent proportions test.

RESULTS

The results of the cytogenetic analysis are presented in Table 1. The irradiation of lymphocyte culture at 2 Gy induced the appearance of chromosome aberrations with highest frequency of double acentric fragments (Fig. 1) and dicentric chromosomes (Fig. 2).

The percentage of aberrant cells reached 12%, that of dicentrics – 9% and of double fragments – 21%. Chromosome aberrations per cell were 0.31.

The results of the cytogenetic analysis showed that the pre-irradiation treatment of lymphocyte cultures with *Haberlea rhodopensis* extract reduced the number of chromosome aberrations. After the treatment at 4 and 8 μL/mL medium, there were statistically significantly less aberrations (0.12 and 0.10, respectively) compared to irradiated control culture (0.31; P<0.001).

The frequency of double fragments was considerably decreased (P<0.05) when *H. rhodopensis* extract was added at 4 μl/ml. The culture treated at 8 μL/mL exhibited a greater extent of double fragments reduction (P<0.001).

The chromosome aberrations per cell in cultures treated at 4 μL/mL ressurection palnt extract were significatly less compared to irradiated

Table 1. Prevalence of chromosome aberrations (CA) in rabbit lymphocyte cultures after *in vitro* irradiation with 2.0 Gy, treated with different concentrations of *Haberlea rhodopensis* extract or vitamin C, and control cultures

Treatment	Aberrant cells	Dicentrics	Rings	Single fragments	Double fragments	CA/cell
HR extract 1.0 μL/mL culture	8/100	5/100	1/100	2/100	9/100**	0.17*
HR extract 4.0 μL/mL culture	8/100	4/100	1/100	1/100	6/100**	0.12***
HR extract 8.0 μL/mL culture	6/100	4/100	1/100	1/100	4/100***	0.10***
Vitamin C 1.0 μL/mL culture	9/100	4/100	2/100	0/100	5/100***	0.11***
Irradiated control	12/100	9/100	1/100	0/100	21/100	0.31
Non-irradiated control	2/100	0/100	0/100	2/100	0/100	0.02

*P<0.05; ** P<0.01; ***P<0.001 – statistically significant difference vs irradiated controls.

controls (0.12 vs 0.31; P<0.001). The same trend was observed when extract was added at 8 μL/mL (0.10 vs 0.31; P<0.001).

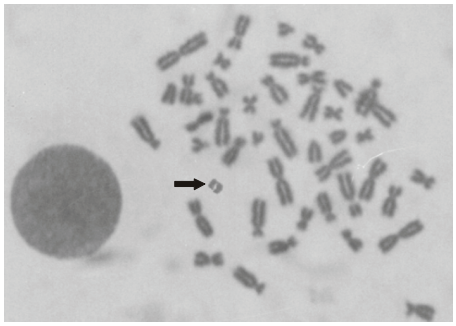

Fig. 1. Lymphocyte culture irradiated at 2 Gy. Double acentric fragment (arrow).

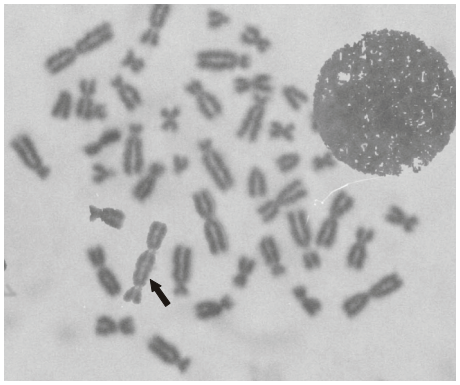

Fig. 2. Lymphocyte culture irradiated at 2 Gy. Dicentric chromosome (arrow).

Comparative analysis of the results from the culture treated with vitamin C and those treated with the *H. rhodopensis* extract confirmed the efficacy of this resurrection plant at doses of 4 μL/mL and 8 μL/mL with respect to the number of CA per cell. Regarding the reduction in the amount of dicentric chromosomes and double acentric fragments, the protective effect of the administration of vitamin C and *H. rhodopensis* extract was assessed to be nearly the same.

DISCUSSION

In general, the results of this experiment showed that *Haberlea rhodopensis* extract, applied *in vitro*, exhibited radioprotective properties at the tested concentrations.

A number of plants and herbs, applied *in vitro* (Jagetia *et al.*, 2003a; 2004) and *in vivo* (Emerit, 1995; Shobi & Goel, 2001) before or after irradiation, possess radioprotective properties, which prolong life (Mizina & Sitnikova, 1999), improve haematological parameters (Agrawala & Goel, 2002; Goel *et al.*, 2002; 2003), and reduce the negative effects of radiation on chromosome and DNA level (Jagetia *et al.*, 2003b; Jagetia & Venkatesha, 2006).

It is known that ionizing radiation induces reactive oxygen species (ROS) in the form of OH and H, as well as peroxide radicals (Jagetia, 2007), which trigger a cascade of events leading to DNA damage like single or double fragments, base changes, DNA-DNA or DNA-protein adducts. Double-chain fragments are the primary cause of cellular death following irradiation.

The probable mechanisms allowing certain plants and other natural substances to exhibit radioprotective properties are various (Jagetia, 2007). Most of the plants contain polyphenols, which neutralize free radicals, and the increase in cellular antioxidants in irradiated system is likely the primary mechanism of radioprotection (Decker, 1995). Polyphenols in plants can regulate the mRNA expression of antioxidant enzymes, such as catalase, glutathione transferase, glutathione peroxidase, and superoxide dismutase, thus countering oxidative stress caused by ionizing radiation. Plants and herbs can suppress the activation of the protein kinase C, mitogen-activated protein kinase, cytochrome P-450, as well as genes,

which are probably responsible for damage induced by ionizing radiation.

In conclusion, the evidence provided in the current study give reason to believe that the ressurection plant *Haberlea rhodopensis* extract possess a radioprotective potential, and therefore justify further *in vitro* and *in vivo* studies to confirm these data and to discover the mechanisms of the protective effect.

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Paper received 26.03.2010; accepted for publication 31.05.2010

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