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Haberlea rhodopensis (Friv.) reduces chromosomal aberrations in whole body irradiated rabbits

Svetlana Georgieva[1*], Borislav Popov[2], Svetlin Tanchev[1], Deyana Hristova[1]

*Corresponding author:

Svetlana Georgieva

[1]Dept. Genetics, Animal breeding and Reproduction, Trakia University, Stara Zagora 6000, Bulgaria.
[2]Department of Molecular Biology, Immunology and Medical Genetics, Faculty of Medicine, Trakia University, Stara Zagora 6000, Bulgaria

Abstract

Radioprotective effect of ethanolic extract *of Haberlea rhodopensis* have been studied by examining chromosome aberration in irradiated rabbits. Healthy adult New Zeland rabbits were injected intramuscularly (im) with 120 mg/kg body weight before and after irradiation or with double distilled water (DDW). They were exposed to whole body irradiation of 2.0 Gy gamma rays. After 24 h chromosomal aberrations were studied in the peripheral blood lymphocytes. Radiation (2.0 Gy) increased the number of aberrant cells from less than 3% in controls to almost 25%. Treatment with the total extract *of Haberlea rhodopensis* (HR) before and after irradiation resulted in a significant reduction in the percentage of aberrant metaphases as well as in the different types of aberration scored. Our results proved the ameliorating role of HR against radiation-induced chromosomal aberrations in rabbits.Further experimental studies using different cytogenetic and molecular biomarkers are needed to clarify the exact mechanisms of radioprotective action of the HR extract.

Keywords: chromosome aberrations, *Haberlea rhodopensis,* radioprotection

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Introduction

Ionizing radiation may cause cancer, death, and loss of neural function in humans and animals. It also induces mutation, chromosomal aberrations and apoptosis in cells [1, 2]. Therefore, to develop effective and nontoxic radioprotective and radiotherapeutic drugs, many sulphydryl compounds such as cysteine [3], cystamine [4] and WR-2721 [5] were synthesized and tested but none of them was found to be suitable for clinical applications due to their high toxicity. Hence, efforts have been made to develop effective, nontoxic, inexpensive and easily available radioprotective drugs of plants origin for human welfare and several plants extracts such as *Piper betel* [6], *Gingko biloba* [7], *Centella asiatica* [8], *Osimum sanctum* [9]. *Podophyllum hexandrum* [10], *Phyllanthus amarus* [11] have been tested and are also being tested.

Haberlea rhodopensis (Friv.) belongs to family *Gesneriaceae* and is a Balkan endemic relict that is widely distributed mainly in the Rhodope Mountains and some regions of the Sredna gora Mountains and the Balkans. *Haberlea rhodopensis* belongs to the group of extremely desiccation-tolerant (ressurection) plants which are capable of withstanding long periods of almost full desiccation and recover quickly on water availability [12]. Phenolic compounds, accumulated in high amounts in ressurection plants, are major contributors to the antioxidant activity of Haberlea rhodopensis extract (HR) [13]. The antimutagenic potential of HR was reported in a preliminary studies [14, 15, 16]. Therefore, the aim of present study is to investigate the protective role of the HR against induced chromosome aberrations in whole body irradiated rabbits.

Materials and methods

Animals

Twenty male New Zeland rabbits, 5 months old, weighing 3.5–4.0 kg body weight purchased from the Animal House of the Agricultural Faculty, Trakia University were used. Rabbits were given standard rabbits feeding pellets and water *ad libitum*. The experiment was approved by the Committee on Animal Experimentation at Trakia University, Stara Zagora, Bulgaria and was performed according to the recommendations of Directive 86/609/EC of November 24, 1986.

Irradiation

A cobalt teletherapy unit (Rocus M, ^{60}Co) at the Inter-District Cancer Dispensary, Stara Zagora, Bulgaria, was used for irradiation. Rabbits were placed in individual ventilated plexiglas cages and irradiated with 2.0 Gy γ-rays at a dose rate 89.18 cGy/min. While conducting the experiments and the data analysis, the guidelines and recommendations of the International Atomic Energy Agency [17] were followed strictly.

Preparation of extract

Fresh leaves of H. Rhodopensis were collected from their natural habitat (the vicinity of Asenovgrad, Bulgaria) during the flowering period in May-June. They were botanically identified in Department of Pharmacology and Pharmacognosy (Medical University, Sofia, Bulgaria) by botanist- phytotherapist. Voucher specimen was deposited in the Institute of Botany, Bulgarian Academic of Science, Sofia, Bulgaria. The leaves were cut into small pieces and dried at room temperature for 1 month. After grinding the leave pieces, the dry matter was macerated for 6 h in 70% ethyl alcohol and then was percolated for 48 h. The primary extract was concentrated by evaporating ethanol in a vacuum environment in order to reach a ratio of 5% ethanol and 95% water. The obtained extract was filtered through a 0.25 μm Millipore membrane (Millipore, USA) to remove emulsified substances, chlorophyll and other particles. The extract was standardized in accordance with the method for determining the relative density (Bulgarian Pharmacopoeia Roll 2, p.19). The amount of the extracted substance(s) ranged between 0.098 and 0.142 g/cm^3.

Acute toxicity studies

Having determined the LD50 of >1250 mg/kg body weight by Popov B [18] and showing that HRE was not acutely toxic at 1250 mg/kg body weight, in this study <1/10 of LD50 is used.

Study design

The animals were divided into following 4 groups of 5 animals each: Group 1 (control) untreated sham-irradiated rabbits; Group 2 irradiated rabbits; Group 3 HR (120 mg/kg body weight) pretreated rabbits; Group 4 HR (120 mg/kg body weight) treated rabbits. HR was administered intramuscularly (im) to rabbits 2 h before whole body exposure (group 3) and 30 minutes after whole body exposure (group 4). The rabbits from group 1 were injected (im) with double distilled water 2 h before irradiation.

Blood samples were obtained 24 h after irradiation from the marginal ear vein in sterile tubes with heparine for detection of chromosome aberrations.

Preparation of lymphocyte cultures

Micromethod of Evans HJ, [19] with modification for rabbits was used. Briefly, 0.5 ml of whole heparinised blood was incubated in 7 ml RPMI 1640 medium supplemented with 3 ml heat-inactivated normal calf serum (Sigma), 0.2 ml reconstituted phytohemagglutinin M (PHA-M, Gibco), 100E/ml penicillin and 50 μg/ml gentamicin. The cultivation flasks were thermostated in the dark at 39° C. All cultures were incubated for 48 h. Colcemid at a final concentration of 0.2 μg/ml was added at 46 h to block the cells at metaphase stage. At the end of the 48th h from the beginning of lymphocyte incubation, chromosomal preparations for detection of chromosomal aberrations were prepared. The slides were stained with 10% Giemsa and examined. 100 metaphases were scored for each rabbit. The data are presented as the number of chromosome aberrations /100 cells.

Statistical Analysis

The values were given as mean±SD. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the SPSS 13.0 software package for Windows. Post hoc testing was performed for inter-group comparison using the least significance difference (LSD) test. P-values < 0.05 was considered as significant.

Results and discussion

The results are presented in figure 1.

The sham-treated control group had 2.4±1.14 aberrant cells which consisted of only acentric fragments. Radiation significantly increased the frequency of aberrant cells 25.0±3.8, along with all types of aberration, as well as total aberrations 29.6±3.36. Pre-treatment with HR significantly reduced the percentage of aberrant cells 13.4±1.95, fragments, dicentrics and total aberrations 20.0±3.67 compared with irradiated group only. The treatment with HR after irradiation showed weaker reduction in frequency of chromosomal aberrations (22.0±6.32) and aberrant cells (20.8±3.8), compared to pre-treated group. The present data revealed that administration of HR ameliorate and improve the harmful effects of irradiation particularly when rabbits were pre-treated with the extract of HR.

Radiation is known to damage DNA and other molecules, causing gene mutation and chromosome aberrations. Scoring of chromosome aberrations gives direct assessment of genotoxicity of various physical and chemical agents. The use of certain chemicals may help to reduce/inhibit the genotoxicity, which in turn may inhibit mutagenesis and carcinogenesis.

group 1 (control) untreated sham-irradiated rabbits (n=5); group 2 irradiated rabbits only (n=5); group 3 HR (120 mg/kg body weight) pretreated and irradiated rabbits (n=5) ; group 4 HR (120 mg/kg body weight) treated rabbits after irradiation (n=5) .

statistical significance: * vs control group 1; ^ vs irradiated group 2.

Chromosome aberrations are highly quantifiable manifestations of radiation-induced damage to DNA that may be observed in the first post-irradiation mitosis, and studies conducted in animals employed scoring of chromosome aberrations as a method to quantify levels of radioprotection by various compounds [20,21].

The administration of HR prior and after irradiation resulted in significant decline in the frequency of chromosome aberrations,

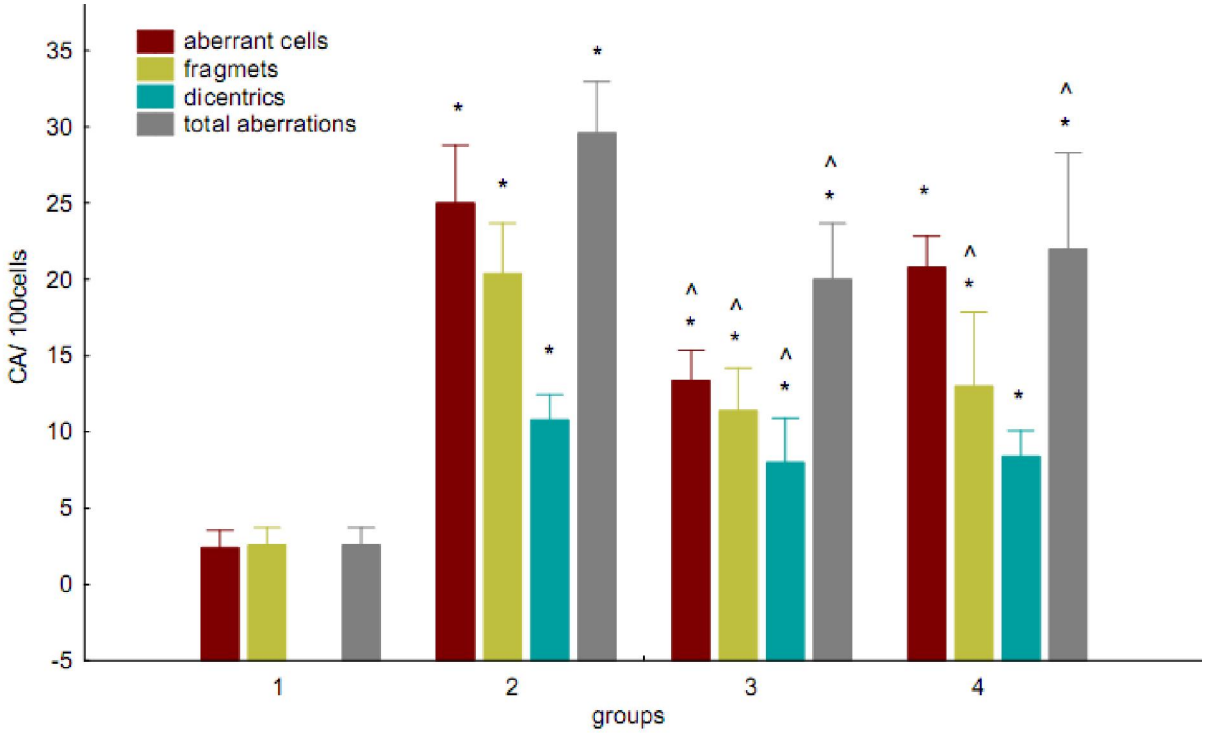

Figure 1: Frequencies of chromosome aberrations in rabbits irradiated to 2.0 Gy and treated with HR before and after irradiation.

when compared to γ-irradiated group. The extract injected before irradiation showed higher anticlastogenic activity than administered after irradiation.

The exact mechanism by which HR develops anticlastogenic potential is still unknown. But this may be due to the effective antioxidant potential and antiradical activity of HR [22, 23]. HR contains high level of flavanoid antioxidants, mainly phenolic acids, flavanoid-aglycones and glycosides [24]. Several studies also suggest that flavonoids may act as antioxidants, free radical scavengers, or radioprotectors [25, 26].

Conclusion

The data obtained in present study suggested that HR extract prevented DNA damage inflicted by gamma irradiation. Despite its limitation, this study has added new data to growing body evidence that some plant extract were effective radioprotectors. However, before their approval for clinical use, further experimental studies using at the same time different cytogenetic and molecular biomarkers and well designed clinical studies are needed to clarify the exact mechanisms of their radioprotective action and possible interactions.

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