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THE RADIOPROTECTIVE PROPERTIES OF POLYPHENOLS ON HUMAN LYMPHOCYTES

A. Leskovac¹, T. Momić¹, T. Janković², G. Joksić¹

¹Laboratory of Physical Chemistry, Vinča Institute of Nuclear Sciences, POB 522, 11001 Belgrade,

²Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade,
Serbia and Montenegro

Abstract

The aim of this study was to evaluate radioprotective properties of medical plants *Gentianella austriaca* and *Gentianella dinarica*. For this purpose human lymphocytes were irradiated using ⁶⁰Co γ rays and treated with different fractions of plant extracts, afterwards micronuclei (MN) and malondialdehyde (MDA) were measured. Polyphenols, fractions isolated from those plants have shown the protective effects, seen as significantly reduced incidence of micronuclei which was followed with reduced level of malondialdehyde. The results obtained in this study indicate that polyphenols isolated from *Gentianella austriaca* and *Gentianella dinarica* possess radioprotective properties possibly through reduction of the lipid peroxidation.

Introduction

Ionizing radiation induces chromosomal aberrations very efficiently. Main types of lesions that are induced in the DNA by ionizing radiation are single- and double strand breaks, base damages and DNA-protein cross-links. Injury to living cells is to large extent due to oxidative stress induced by ionizing radiation. Reactive oxygen species (ROS) and free radicals induced by partial reduction of oxygen (O₂) react with cellular macromolecules as lipids, proteins, and nucleic acids and damage them [1]. The most often assay used in genotoxicity testing is the induction of chromosomal aberrations. Instead, the in vitro micronucleus test is also suitable for evaluation of the genotoxicity [2]. Major biomarkers of oxidative damage to living cells are lipid peroxidation (LPO) products, such as malondialdehyde (MDA) as well as activity of myeloperoxidase and catalase.

The most important compounds present in plants, which are of considerable pharmacological significance, are the polyphenols. Polyphenols are very effective scavengers for free radicals and thus probably act to prevent oxidative damage [3].

The aim of this study was to evaluate the radioprotective effects of natural polyphenols, isolated from medical plants *Gentianella austriaca* (fractions 1, 2 and 3) and *Gentianella dinarica* (fractions 1, 2 and 3) on harvested human lymphocytes. Incidence of radiation-induced micronuclei and level of MDA was analyzed using cytochalasin block micronucleus (CBMN) test and thiobarbituric acid (TBA) test, respectively.

Experimental

Preparation of plant extracts: *Gentianella austriaca* and *Gentianella dinarica* were examined. The plant flowers were air dried and ground separately in a mixer. The finally powdered material was subjected to separate extractions with 70% ethanol. After removal of the ethanol under reduced pressure, the aqueous phase was evaporated to dryness, which was further separated onto different fractions.

Irradiation: Aliquots of heparinized whole blood were placed in sterile plastic test tubes in a 15 x 15cm Plexiglas container, and irradiated using ^{60}Co γ ray source, dose of 2 Gy. Irradiated samples were set up in cultures 1 hour after irradiation.

Micronucleus analysis: The assay is carried out on cultures of PHA-stimulated blood lymphocytes. Whole blood (0.5 ml) was added to 5 ml of Karyomax medium (Invitrogen-Gibco). One hour after stimulation 100 μl of water-soluble fractions of plant extracts were added to the cultures. Preparation of cultures for MN scoring was proceed according to method of Fenech [4].

MDA measurement: After 72 hours of incubation, parallel cultures were separated on Lymphoprep, lymphocytes were collected by centrifugation, washed in physiological saline, and were prepared for measurement MDA. The lipid peroxidation was indexed by measuring the MDA production, spectrophotometrically at 532 nm, using the 2- thiobarbituric acid (TBA) test [5]. Protein concentration was determined according to Lowry [6].

Results and Discussion

The purpose of this study was to assess the radioprotective properties of natural polyphenols, isolated from medical plants *Gentianella austriaca* (fractions 1, 2 and 3) and *Gentianella dinarica* (fractions 1, 2 and 3).

The results demonstrated that fractions of extracts isolated from *Gentianella* species significantly modulate the level of radiation-induced micronuclei and considerable change MDA production. As provided in Fig. 1A, the polyphenols from *G. austriaca*, particularly fractions 1 and 2 display the best radioprotective effects: the yield of radiation-induced micronuclei is reduced for 40,20% and 44,97%, respectively. The same fractions decrease MDA production for 15,97% and 25,62%, respectively. The similar decreases have shown fractions 1 and 2 from *G. dinarica*, (Fig. 1B). The yield of micronuclei is reduced for 27,78 % and 16,98%, respectively and the MDA production was reduced for 35,56% and 7,76%, respectively. On the opposite fraction 3 *G. austriaca* (Fig. 1A) and fraction 3 from *G. dinarica* (Fig. 1B), have shown insignificant increase of the yield of radiation-induced micronuclei which was followed with slight enhancement of MDA production. Statistical analysis demonstrated high positive, statistical significant correlation between incidence of micronuclei and MDA ($r = 0.93$, $p < 0.05$).

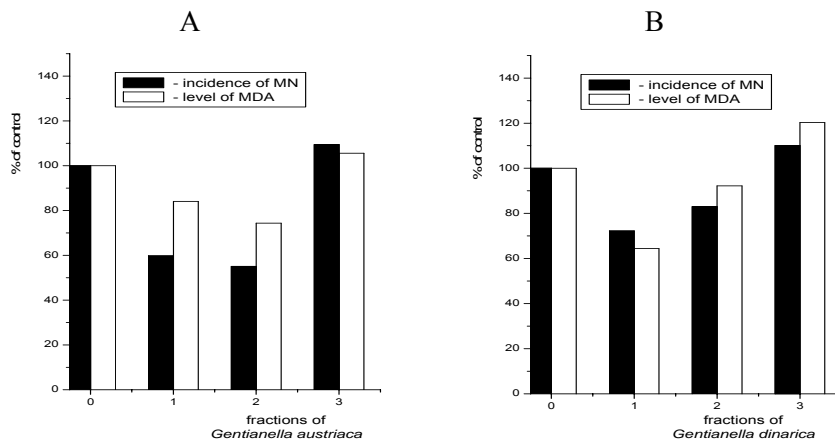


Figure 1. Incidence of micronuclei and level of MDA in human lymphocytes treated with extracts of A) *Gentianella austriaca* (fractions 1, 2 and 3) and B) *Gentianella dinarica* (fractions 1, 2 and 3)

Conclusion

This study indicates that polyphenols isolated from *Gentianella austriaca* and *Gentianella dinarica* possess radioprotective properties. The natural polyphenols obtained from *Gentianella* species are protectors against cellular damages and their benefit for human health should be examined further.

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