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Radioprotective effects of propolis and quercetin in γ-irradiated mice evaluated by the alkaline comet assay

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Abstract

The radioprotective effects of ethanolic extract of propolis (EEP) and quercetin on the white blood cells of the whole-body irradiated CBA mice were investigated. Irradiation was performed using a γ-ray source (^{60}Co), and absorbed dose was 9 Gy. The efficiency of test components was evaluated when given intraperitoneally (ip) at a dose of 100 mg kg^{-1} for 3 consecutive days before and/or after irradiation. Moreover, possible genotoxic effects of test components were also assessed on non-irradiated animals. For each experimental group leukocyte count was determined and the primary DNA damage in leukocytes was assessed using the alkaline comet assay.

The higher efficiency of EEP and quercetin was observed when given preventively. The results suggest that propolis and quercetin given to mice before irradiation protect their white blood cells from lethal effects of irradiation and diminish primary DNA damage as confirmed by the alkaline comet assay. Positive results obtained on gamma-irradiated mice given EEP and quercetin, complementary with our earlier observations on survival of irradiated mice, indicate that these compounds could be considered effective non-toxic radioprotectors. The exact mechanisms of radioprotection by these compounds and their effects on DNA repair processes are still to be elucidated.

Keywords: Propolis; Quercetin; Alkaline comet assay; White blood cells; Mice; Radioprotection

Introduction

Propolis is resinous substance manufactured by honeybees from leaves, buds and sap of trees and flower blossoms. Interest in propolis as harmless medicine has increased because of its broad spectrum of biological properties. Propolis shows antibacterial (Bankova, 2005), analgesic/anti-inflammatory (Ledon et al., 1997), antioxidant (Heim et al., 2002), prooxidant (Galati et al., 2002), immunoenhancement (Orsolic et al., 2002), antiproliferative activity in human tumor cells (Chen et al., 2004a, b), antitumor activity in mice (El-khawaga et al., 2003; Orsolic and Basic, 2003) and radioprotective effects in *in vitro* cultures (Liu and Zheng, 2002; Montoro et al., 2004; Orsolic et al., 2006). Major constituents of propolis are flavonoids, organic acids, phenols, various enzymes, vitamins and minerals (Bankova, 2005). Quercetin as flavonoid-aglycone class

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of polyphenols is found in fairly large amounts of fruits, vegetables, olive oil, red wine, tea and propolis as well, and has shown the highest antioxidant potential of all flavonoids. In a previous study greatest radioprotective effect was achieved with quercetin (Orsolic et al., 2007). Antioxidant activity of quercetin is based on direct free radicals scavenging ability, or stabilizing the reactive oxygen species (ROS) by reacting with the reactive compounds of the radical. Furthermore, antioxidant effects may be a result of a combination of radical scavenging and an interaction with enzyme functions. Radioprotective effects of quercetin, naringin, caffeic acid, chrysin, luteolin, orientin and vicenin, flavonoids also present in propolis are reported by authors (Shimoi et al., 1994; Devi et al., 1999; Arora et al., 2005; Orsolic et al., 2007; Benković et al., 2007). It has also been shown that a variety of sulfur containing natural products such as derivates of aminoacid cystein expressed radioprotective abilities (Patt et al., 1949).

Chemical composition of propolis mainly depends on plant sources around the hives from which honeybees collect it. After chemical analysis, propolis could be separated into chemo-types, and European, as well as Croatian propolis belong to ''poplar-type'' of propolis, which define poplars (*Populus* L. spp.) as the main sources of resinous propolis (Kosalec et al., 2004). Raw propolis is collected and extracted with ethanol to obtain ethanolic extracts of propolis (EEP). As compared with a water-soluble derivative of raw propolis (WSDP), EEP contains a higher proportion of lyphophilic compounds from the flavonoid-aglycones class, such as flavones and flavonols, and flavanones (Kosalec et al., 2004; Sobocanec et al., 2006). From the other side, WSDP contain mainly hydrophilic and polar compounds such as caffeic and ferrulic acids and their esters. Since EEP has been widely used in traditional phytomedicine, it was the main reason for its use in these studies.

Ionizing radiation in interaction with living cells causes a variety of changes depending on exposed and absorbed dose, duration of exposure and interval after exposure, and susceptibility of tissues (Sankaranarayanan, 2006). It is well documented that ionizing radiation through generation of toxic free radicals causes single strand breaks, double strand breaks, oxidative damage to sugar and base residues, chromosomal aberration and mutation lead to the cell death and are associated with an increased risk for numerous genetically determined diseases (Sankaranarayanan, 2006). Radiation effects are usefully exploited in radiotherapy for years. Since the radiation effects are not discriminate, transient and/or permanent injury to normal tissues and cells are unavoidable. Efforts to reduce toxicity to normal tissue cells and organs have led into searching for cytoprotective agents. Unfortunately, most of chemical radioprotectors have toxic side effects, which limit their use in medical practice. Investigations for effective and non-toxic compounds with radioprotection capability led to increasing interest in naturally occurring antioxidant such as propolis and its polyphenolic compounds.

The aim of this study was to evaluate radioprotective effects of propolis and quercetin administered preventively and therapeutically on the white blood cells of whole-body irradiated mice as well as on non-irradiated animals. The efficiency of test compounds was evaluated using the alkaline comet assay on white blood cells. Comet assay was selected since it was confirmed earlier as simple, rapid and sensitive technique for measuring DNA damage (McKelvey-Martin et al., 1993; Chen et al., 2004a, b).

Materials and methods

Animals

Male CBA mice, weighing from 20 to 22 g, from our conventional mouse colony were used. The animals were maintained on a pellet diet and water *ad libitum*. Animal studies were carried out according to the guidelines force in R. Croatia (Law on the Welfare of Animals, N.N. #19, 1999) and in compliance to the Guide for the Care and Use of Laboratory Animals, DHHS Publ. # (NIH) 86–123.

Treatment

Three independent experiments were performed. In the first mice were treated preventively with test components for 3 consecutive days. Test compounds were given intraperitoneally (*ip*) at dose of 100 mg kg^{-1} body weight and then mice were irradiated with γ-rays at dose of 9 Gy. The alkaline comet assay was performed on whole blood samples taken 30 min after irradiation. In the second experiment mice were irradiated with dose of 9 Gy and then treated *ip* for 3 consecutive days with test components at dose of 100 mg kg^{-1}. The comet assay was performed on whole blood samples on the third day after finishing the treatment. In the third experiment non-irradiated mice were treated with all tested compounds and the comet assay was performed on the third day after finishing the treatment. Appropriate negative and positive control groups (treated with chemical radioprotector AET) were also selected and handled in the same manner. Experimental groups were composed of five mice each.

Irradiation

Whole-body irradiation was performed using a ^{60}Co γ-ray source (situated at the Ruđer Bošković Institute,