EFFECT OF PROPOLIS AND ITS POLYPHENOLIC/FLAVONOIDS COMPOUNDS ON DNA DAMAGE INDUCED BY RADIATION TO MOUSE LYMPHOCYTES

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Summary

This study assessed the antioxidant potencies of several widespread flavonoids present in propolis and proplis alone. CBA mice were injected intraperitoneally (ip) with two preparation of propolis (water and ethanolic extract of propolis; WSDP or EEP) and propolis polyphenolic compounds (caffeic acid, naringin, chrysin, quercetin) at dose of 100 mg kg^{-1} body weight for 3 conecutive days before or after whole body γ-irradiation (WBI). Synthetic protector 2-aminoethylisothiouronium bromide hydrobromide (AET) was used as a positiv control. Mice were exposed to WBI with 9 Gy of ^{60}Co γ-radiation source. Therty min. after iradiation and/or treatment with test components we examined DNA damage of lymphocytes using the single-cell gel electophoresis assay (comet assay). The WBI of mice resulted in a significant elevation of DNA damage of lymphocytes as compared with unradiated mice.Pretreatment of mice with WSDP or EEP and flavonoids produced the reduction in oxidative DNA damage of lymphocytes as compared with control and they were ranked in decreasing order of potency as follows: naringin (2.98%); chrysin (16.84%); quercetin (33.67%); AET (48.52%); caffeic acid (49.51%); EEP (53.47%); and WSDP (54.46%), respectively. Also treatment with propolis and its polyphenolic/flavonoids compounds after irradiation resulted in a significant reduction of DNA damage as follows: caffeic acid (32.31%); AET (75.39%); naringin (78.46%); EEP (80%); chrysin (83.08%); quercetin (84.62%); and WSDP (89.24%). These data suggest that WSDP and EEP are more protective than flavonoids from propolis alone. Data are also consistent with the hypothesis that radioprotective activity of EEP and/or WSDP related to synergistic antioxidative effect of components present in EEP or WSDP.

Keywords: propolis, polyphenolic, lymphocytes

Introduction

Ionizing radiation is any electromagnetic wave or particle capable of producing ions in its passage through the matter, causing immediate chemical alterations in biological tissues. These alterations produce a metabolic disarrangement which after days or weeks can leed either to cell damage or ultimately to cell or organism death. Ionizing radiation damage is caused by either a direct interaction with target molecules or indirectly by formation of chemically and pharmacologically active elements produced mainly by water molecules. Water radiolysis generates molecules of hydrogen peroxide (H_2O_2), molecular hydrogen (H_2) and a number of higlly active free radicals such as hydrogen radical ($H^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), hydroperoxyl radical ($HO^{\bullet}$) and superoxide ($O_2^{\bullet}$) (1). The interaction of free radicals with sugar moieties leads to the cleavage of the sugar-phosphate backbone of DNA followed by single-strand breaks that undergo repair processes relativelly easily (2). On the other hand, double-strand breaks have more serious consequences. Double-strand breaks are well correlated with the cytotoxic effects of ionizing radiation and are considered the primary lesion involved in cellular death (2). If DNA repair mechanism, which are induced after exposure to ionizing radiation, are inefficient, the damaged DNA strand that are copied during replication lead to mutagenesis and carcinogenesis (3). The damaging effects of ionizing radiation lead to the cell death and are associated with an increased risk for numerous genetically determined diseases (4, 5).

Exposure of mammals to ionizing radiation causes the development of a complex, dose depedent series of potentially fatal physiologic and morphologic changes, known as hematopietic syndrome. Radiation induced destruction of lymphoid and hematopoietic systems is the primary cause of septicemia and death. Enhanced susceptibility to infections with opportunistic microorganisms occurs in parallel with progressive radiation induced atrophy of lymph nodes, spleen, and bone marrow (7).

It has been suggested that the therapeutic activities of propolis depend mainly on the presence of flavonoids (7-9). Several studies have confirmed the role of flavonoids in the deactivation of the free radicals (7-9), but very few data have been published on the effect of propolis and its polyphenolic componds used for protection from whole-body irradiation.

Immunomodulatory effects including increased hematopoietic activity of propolis have also been recorded (5). Since the increased hematopoietic activity could account for the improved hematopoietic tolerance to radiotherapy, data on the influence of propolis and and polyphenolic components from propolis on radioprotective ability could shed more light on this problem. Since 1949, a great deal of research has been conducted on the radioprotective action of chemical substance which reduced mortality if administered to mammals prior to exposure to a lethal dose of radiation.

The most remarkable radioprotectors are the sulfhydryl compouns, such as cysteine, and cysteamine (10). However, these compounds appear to produce serious side effects and are considered to be toxic at the doses required for radioprotection (10).

Here, we report the radioprotective ability of polyphenolic components from propolis and propolis alone in mice exposed to an acute WBI with 9 Gy using comet assay for observation of DNA damage in lymphocytes.

Materials and methods

Animal studies: Male and female CBA inbred mice from our conventional animal facility were used. In any experiment, mice were three months old, approximately 20 g body weight at the initiation of the experiment, and were maintained at 20^0C and at 12 L : 12 D photoperiod. All animals were maintained on a standard diet and water *ad libitum*.

Irradiation: WBI was performed with a cobalt-60 γ-radiation source (Teratron 780, Canada). Mice were placed in Plexiglass cages and irradiated in groups of five or six mice simultaneously. The source-to-skin distance was 291 cm with a dose rate of 0.0233 Gy/s at room temperature (23 ± 2°C). The mice were irradiated with a total dose of 9 Gy; time of irradiation was 390 sec.

Water-soluble derivative of propolis (WSDP) and ethanolic extract of propolis (EEP): WSDP and EEP was prepared by the methods described elsewhere (11, 12). According spectrophotometric analisis WSDP contains: flavones and flavonols 2.13%, flavanones and dihydroflavonols 9.06%, total flavonoids 11.19%, total **polyphenols** 70.48% and EEP contains: flavones and flavonols 1.6%, flavanones and dihydroflavonols 38.60%, total flavonoids 40.20, total **polyphenols** 84.40%.

Polyphenolic compounds: For experiments using polyphenolic compounds we used Caffeic acid (CA) – 3,4-dihydroxycinnamic acid (Aldrich–chemie, Milwaukee, WI, USA), Quercetin dihidydrate (QU) (Fluka, BioChemica, Switzerland), Chrysin and Naringin (Sigma, Germany).
All polyphenolic compounds were dissolved in water and quercetin was dissolved in ethanol.

Experimental procedure: Experimental design is shown in Table 1.

Table 1. Experimental design

I- Groups (Control groups) [ab]	II-Whole-body gamma radiation groups (Experimental groups)[a]
Control	Control + 9 Gy γ-ray
Test components[c]:	Test components[d] + 9 Gy γ-ray
AET[e]	AET + 9 Gy γ-ray
100 mg kg^{-1} of WSDP	100 mg kg^{-1} of WSDP + 9 Gy γ-ray
100 mg kg^{-1} of EEP	100 mg kg^{-1} of EEP + 9 Gy γ-ray
100 mg kg^{-1} of caffeic acid	100 mg kg^{-1} of caffeic acid + 9 Gy γ-ray
100 mg kg^{-1} of chrysin	100 mg kg^{-1} of chrysin + 9 Gy γ-ray
100 mg kg^{-1} of naringin	100 mg kg^{-1} of naringin + 9 Gy γ-ray
100 mg kg^{-1} of quercetin	100 mg kg^{-1} of quercetin + 9 Gy γ-ray

[a]Groups comprised of 5 mice each.
[b]Control mice were untreated and unirradiated.
[c]The test components were given to mice *ip* daily for 3 days, and the daily dose contained 100 mg kg^{-1} body weight.
[d]The test components were given to mice before or after irradiation *ip* daily for 3 days, and the daily dose contained 100 mg kg^{-1} body weight; 30 min. later of iradiation and/or treatment with test components we examined DNA damage of lymphocytes using the single-cell gel electophoresis assay (comet assay).
[e]AET; 2-aminoethylisothiouronium bromide hydrobromide (chemical protectors) was given to mice *ip* at dose of (1 mM kg^{-1}) one hour after injection mice were exposed to an acute whole–body gamma radiation dose of 9 Gy.

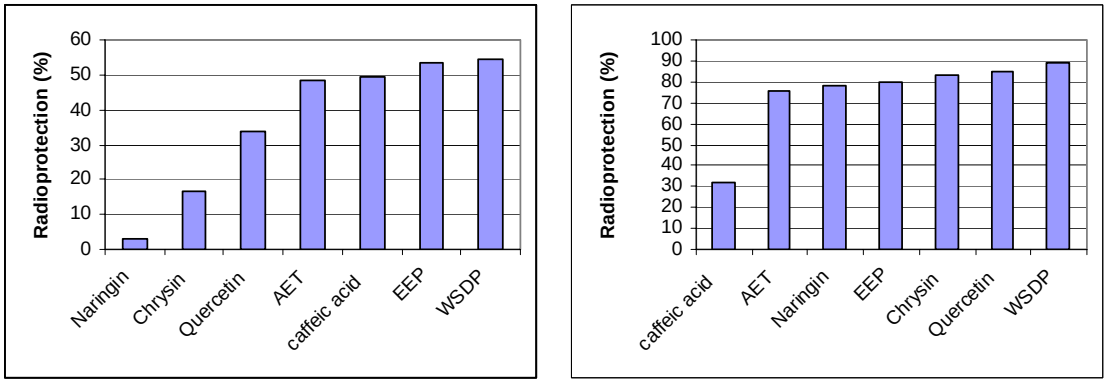
DNA damage determination by alkaline comet assay: The Comet assay was carried out under alkaline conditions, basically as described by Singh et al. (13). Slides received in assay procedure analysed.

Comet capture and analysis: A total of 100 randomly captured comets from each slide were examined at 400x magnification using an epifluorescence microscope (Zeiss) connected through a black and white camera to an image analysis system (Comet Assay II; Perceptive Instruments Ltd, UK). A computerized image analysis system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components and then evaluates the range of derived parameters. To quantify the DNA damage tail length (TL) and tail moment (TM) were evaluated. Tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometres. It was calculated from the centre of the cell. Tail moment was calculated as the product of the tail length and the fraction of DNA in the comet tail.

Statistical analysis: Both comet parameters measured in the exposed and control groups were evaluated using the non-parametric Mann–Whitney *U*-test and MANOVA on log transformed data. The level of significance was set at 5%.

Results

Result obtained using the lysed-cell comet assay are shown in Figures 1 and 2. Decreased damage is associated with decreased tail DNA content and indicates a protective effect of propolis and related polyphenolic compounds given to mice before and/or after irradiation with dose of 9 Gy of ^{60}Co γ–radiation sorce. Pretreatment of mice with WSDP or EEP and respective flavonoids produced the reduction in oxidative DNA damage of lymphocytes as compared with radiation control. The reduction of DNA damage was ranked in decreasing order of potency as follows: naringin (2.98%); chrysin (16.84%); quercetin (33.67%); caffeic acid (49.51%); EEP (53.47%); and WSDP (54.46%). Treatment with preparations of propolis and its polyphenolic/flavonoids compounds and EAT after irradiation also resulted in a significant reduction of DNA damage: caffeic acid (32.31%); AET (75.39%); naringin (78.46%); EEP (80%); chrysin (83.08%); quercetin (84.62%); and WSDP (89.24%). Propolis and related polyphenolic compounds given to mice preventively for three days before irradiation significantly ($p<0.05$) protected DNA from radiation-induced damages in the comet assay. Sygnificant decrease ($p<0.05$) in DNA damage of lymphocytes was observed with all test treatments after irradiation but caffeic acid.



A B

Figure 1. Radioprotective effects of propolis and its polyphenolic compounds on DNA damage induced with 9 Gy of ^{60}Co γ-radiation source. Mice were treated with test components (100 mg kg^{-1}) for 3 conecutive days before (A) or after (B)whole body γ-irradiation (WBI). Thirty minutes after iradiation and/or treatment with test components we examined DNA damage of lymphocytes using the single-cell gel electophoresis assay (comet assay).

Discusion

In this study, we have investigated the radioprotective effects of two preparation of propolis (WSDP or EEP) and its polyphenolic compounds (caffeic acid, naringin, chrysin, quercetin) in the whole body γ-irradiated (WBI) mice exposed to 9 Gy of ^{60}Co γ-radiation using comet assay. It is observed that proolis and its polyphenolic compounds causes a decrease in the tail moment showing radioprotective effects on DNA. Results showed that test components possess radioprotective effect similar or even better than AET. WSDP and EEP were the most effective against reduction of DNA damage of lymphocytes indicating synergistic antioxidative effect of different polyphenolic flavonoids components present in EEP or WSDP. Radioprotective effects of propolis and related compounds can be explained by their antioxidative activity. The antioxidant activities of propolis and its polyphenolic/flavonoid components are related to their ability to chelate metal ions and to scavage singlet oxigen, superoxide anions, peroxyl radicals, hydroxyl radicals and peroxynitrite (14).The exact mechanism of action in protecting mice from lethal effects of acute whole-body irradiation by propolis and its flavonoids is not known. However, scavenging of radiation free radicals may be one of the important mechanisms of radiation protection by test components. These observations confirm the results by (15), who showed that propolis and relted flavonoids scavenge hydroxyl and superoxide free radicals and lipid peroxides. Recently, flavonoids from propolis have been reported to elevate catalase, superoxide dismutase and glutathione peroxidase mRNA synthesis (16). The elevation of these enzimes by flavonoids may also be responsible for the observed protection against radiation-induced damage. By increasing the activities of antioxidant enzimes, flavonoids from propolis reduce the number of free radicals or ROS generated and increase the production of molecules protecting against oxidative stress. Experimental evidence has demonstrated that propolis and its polyphenolic/flavonoid components increased the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione (GSH) (17, 18).Several flavonoids have been reported to protect against DNA damage (19). The radical scavening abilities and effects of flavonoids from propolis on the activities of enzymes involved in antioxidative defense provide a reasonable explanation for the decreased of DNA damage in leukocytes of mice treated before or after irradiation with propolis and its flavonoids in this investigation. It is likely that more different co-operative and synergistic mechanisms of propolis and its polyphenlic compounds are included in protection of organism against radiation.

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