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Original article

Elm bark extract improves immunomodulation and ameliorates oxidative stress in irradiated mice

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ABSTRACT

Background: Radiation therapy can result in side effects such as cellular and tissue inflammation and organ damage. Previously, elm bark (EB) extract has been reported to exert antioxidant and anti-inflammatory effects. In this study, we investigated whether EB administration could improve immunocompetence and ameliorate radiation-induced intestinal damage in irradiated mice.

Methods: After exposure to radiation of 15 Gy, mice were orally administered with EB extract (0 mg/kg, 50 mg/kg, and 500 mg/kg body weight) every other day for 2 weeks, and there was a 2-week washout period. The proliferation of splenocytes and cytokine production of macrophage were measured as indices of immune activity, and histological grade and antioxidant levels in radiation-induced intestinal injury were measured.

Results: Radiation exposure reduced the T-cell proliferation in splenocytes and the levels of interleukin-1β and interleukin-6 from macrophage at Week 2. The supplement of EB extract at low concentration (50 mg/kg body weight; EB-50) tended to enhance T-and B-cell proliferation in irradiated mice. The histological grades of the small intestine were induced by radiation exposure, whereas histological grade of the EB-50 group was lower than that of the irradiated control at Week 4. The EB-50 treatment reduced the level of glutathione at Day 5 and Week 2 and reduced myeloperoxidase activity at Week 4, suggesting that EB-50 may counteract the intestinal inflammation caused by radiation exposure.

Conclusion: Our results indicate that EB extract (50 mg/kg body weight) protects against radiation damage, at least in part, by improving immunomodulation and ameliorating oxidative stress in irradiated mice.

1. Introduction

Radiation therapy is a cancer treatment that uses high doses of radiation to destroy tumors and kill cancer cells [1]. However, it can harm normal cells by damaging their DNA and generating reactive oxygen species, which can result in side effects such as organ damage, cellular and tissue inflammation, and potential for secondary cancer [2]. Increased reactive oxygen species production by radiation exposure upregulates inflammation via induction of nuclear factor kappa B activity and production of cytokines, including tumor necrosis-alpha (TNF-α), interleukin (IL)-1β, and IL-6 [3,4]. The small intestine is one of the organs damaged by radiation therapy, with symptoms such as pain, nausea, and diarrhea [5]. Small intestine damage is a good indicator of overall damage caused by radiation treatment. As such, reduction of small intestine damage indicates the effectiveness of radiation damage treatment [6]. Multiple drugs on the market reduce damage caused by radiation therapy by reducing radiation-induced oxidative stress and inflammation in normal cells [2]. Some natural compounds have displayed promising results in a reduction of damage to normal cells affected by radiation therapy, potentially with fewer deleterious side effects than their synthetic counterparts [2,7].

Elm (*Ulmus davidiana* var. *japonica*) trees (Fig. 1) are widely distributed in South Korea, and their root barks and stems have long been used as a traditional medicine for inflammation, edema, and cancer [8]. The first record of elm trees is found in a Korean document called *Samguksagi* (三國史記)—The Story of Ondal at the end of the 12th century. It was written that elm bark (EB) was used for famine relief [9]. A document called Dongui bogam (東醫寶鑑)

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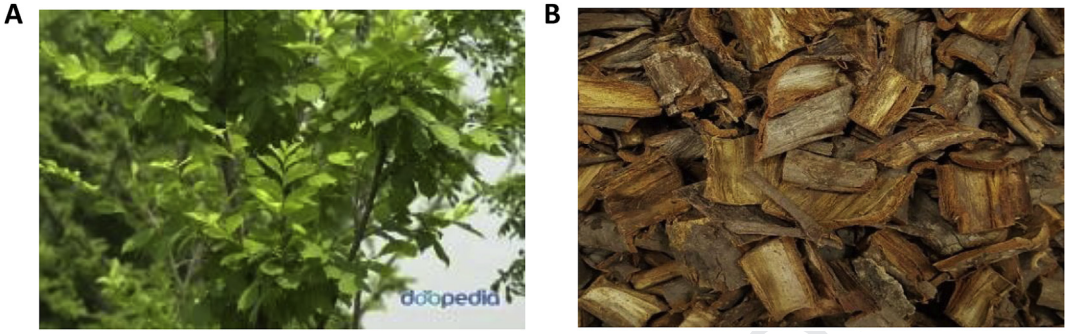

Fig. 1. Elm tree and elm bark. (A) Elm tree. The elm tree, which resembles a zelkova tree, is 20 m high and 60 cm in diameter, and has small hairs. Leaves are broad obovate with sharp ends, leaf edges are double sawteeth, the front side is rough, and the back side has strong hairs (http://terms.naver.com/entry.nhn?docId=1078384&cid=40942&categoryId=32343). (B) Elm bark. The elm bark is called "*yupi*" or "*yugunpi*" in Oriental medicine and contains a large amount of mucus and tannin, which is used as anti-inflammatory medicine.

from the 16th century stated that EB had diuretic properties and that it was used as a remedy for edema, constipation, and insomnia [10]. This statement demonstrated the importance of EB in traditional Korean medicines.

EB extract is rich in glycoproteins, terpenoids [11], lignan, and neolignan glycoside [12], as well as bioactive compounds such as (+)-catechin, (+)-catechin-7-*O*-β-D-apiofuranoside, and (+)-catechin-(4α→8)-(+)-catechin [13]. EB has been reported to exert antioxidant and anticancer effects *in vitro* [13,14]. In particular, the anti-inflammatory properties of EB extract have been reported in some studies in animal models. Inflammatory cytokine production, intracellular immunoglobulin-A, and Th1 and Th17 responses were decreased in small intestinal lamina propria cells isolated from mice fed with EB extract [15,16]. Glycoprotein isolated from EB showed anti-inflammatory activities via the inhibition of inducible nitric oxide and cyclooxygenase 2 in dextran sulfate sodium-induced colitis model [17]. Also, our previous study showed that EB enhanced the immunomodulation properties by inducing splenocyte proliferation and cytokine production from activated macrophages in mice [18].

In this study, we hypothesized that EB administration would improve immunocompetence and ameliorate radiation-induced intestinal damage in irradiated mice. This is the first study regarding the effects of EB extract on immune-enhancing and radiation injury in irradiated mice. The results may help in developing natural products for improving radiation therapy.

2. Methods

2.1. Study design

This study was approved by the Institutional Animal Care and Use Committee at Ajou University. A total of 108 male Imprinting Control Region mice (6 weeks old, 33–37 g) were supplied by Daehan BioLink (Eumseong, Korea). The animals were kept under standardized conditions (20–24°C and a 12-hour light/dark cycle) and given free access to chow diet and water. This study was a $2 \times 3 \times 3$ factorial design with two kinds of radiation treatments [nonirradiation (NC) or irradiation (IR)], three different concentrations of EB extract [0 mg/kg, 50 mg/kg, and 500 mg/kg body weight (bw)], and three time points (Day 5 and Weeks 2 and 4). After a week of acclimation, mice were allocated to six groups ($n = 18$ per group; Fig. 2). Half of the mice received an external 15-Gy dose of 6-MV proton irradiation on the abdominal region under anesthesia (xylazine and ketamine, 5:8 volume/volume, intraperitoneal injection) at the Department of Radiation Oncology, Ajou University of Medicine (Suwon, Korea) [19]. The dose of 15 Gy was chosen based on our previous study to induce inflammation [20]. EB extract (0 mg/kg, 50 mg/kg, and 500 mg/kg bw) was administered by oral gavage in a volume 200 μL every 2 days for 2 weeks, and there was a 2-week washout period to determine the long-lasting effects of EB extract. Body weight was recorded daily. Mice were intraperitoneally injected with 2 mL of 4% thioglycollate 3 days prior to termination for collecting macrophages, and killed by cervical dislocation at one of the three time points ($n = 6$ per group, Day 5 and Weeks 2 and 4 after irradiation). The spleen, peritoneal lavages, and small intestine were collected and weighed on these time points. Splenocytes and macrophages were isolated from spleen and peritoneal lavages, and small intestine samples were excised for histological and biochemical analysis.

2.2. Preparations of EB extract

EB was purchased from Kyungdong market (Seoul, Korea). The EB extract was prepared as described in our previous study [18]. Briefly, the bioactive compounds from EB were extracted with methanol three times. The solvent was evaporated and dissolved in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) and 0.01% dimethyl sulfoxide (Sigma, St. Louis, MO, USA) as a final concentration (0 mg/kg, 50 mg/kg, 500 mg/kg bw).

2.3. Splenocyte proliferation assay

Mitogen-induced splenocyte proliferation is a marker of B- and T-cell immune response [21]. Splenocytes were isolated from the spleen as previously described [18,22] and resuspended in RPMI 1640 medium containing 10% FBS and 1% penicillin. Cells were seeded onto a 96-well plate (5×10^5 cells/well) and incubated for 48 hours at 37°C under 5% CO_2 with concanavalin A (T cell mitogen; 5 μg/mL) or lipopolysaccharide (B cell mitogen; 1 μg/mL). The level of splenocyte proliferation was determined by performing a 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide colorimetric assay, which is used to assess cell cytotoxicity [23]. After 48 hours of incubation, 10 μL/well 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide solution (5 mg/mL phosphate-buffered saline) was added and incubated again for 4 hours. After aspirating the medium, 150 μL dimethyl sulfoxide was added, and the absorbance was measured at a wavelength of 540 nm.

2.4. Cytokine production in mice peritoneal macrophages

Cytokines play an important role in the immune response, and macrophages are the main modulators of cytokines [24]. Mice macrophages were isolated from peritoneal lavages [18].

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