Dragon's blood and its extracts attenuate radiation-induced oxidative stress in mice

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Dragon's blood (DB) possesses great medicinal values due to the presence of several phenolic compounds. This study was designed to investigate the effects of DB and its extracts (DBEs) on oxidative stress in mice exposed to whole body ^{60}Co-γ irradiation (4 Gy). DB and DBEs were intragastrically administered to mice for 5 d prior to radiation. The antioxidant activities, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels in liver and spleen were measured using kits. Furthermore, DB and DBE effects were determined by organ indices and histology of liver and spleen. Our results indicated that the DB and DBE-treated groups showed a significant decrease ($P < 0.05$) in levels of MDA in liver and spleen compared with the irradiation-only group. Moreover, the activity of SOD, CAT and the level of GSH in liver and spleen tissue were enhanced significantly ($P < 0.05$) in the DB and DBE groups. DB and DBE also had a significant effect on the recovery of thymus indices. The histological observations of groups having treatment with DB and DBE indicated significant reduction in the radiation-induced damage to the liver and spleen, together with improvement in the morphology of the liver and spleen. These results suggest that DB and DBE treatment prevents radiation-induced oxidative stress injury and restores antioxidant status and histopathological changes in the liver and spleen, but there is need for further study to explore the precise molecular mechanism and strategy for optimal practical application of DB and DBE.

Keywords: dragon's blood; radioprotective effects; irradiation; oxidative stress

INTRODUCTION

Accidental exposure to radiation and the therapeutic application of ionizing irradiation are the main triggers for the production of reactive oxygen species (ROS) in cells [1]. Superoxide anions, hydrogen peroxide, and hydroxyl radicals are the principal types of ROS that react with macromolecules, resulting in cell dysfunction, mortality, and ultimately tissue damage [2]. Proteins, nucleic acids and lipids are the major targets for ROS, generating DNA strand breakage, DNA–protein cross linking, and lipid peroxide production [3, 4]. These toxic products initiate a cascade of events on the molecule level, which alter the cytokine content of the microenvironment and affect the balance of antioxidant systems such as glutathione and enzymatic antioxidant (superoxide dismutase and catalase) defense systems [5–7]. Although there are endogenous antioxidant defenses in the cells to scavenge ROS, reducing the radiation-induced free radicals and preventing

lipid peroxidation, they are insufficient to scavenge all of the free radicals induced by radiation [8, 9]. Traditional herbs appear to have value in reducing free radical toxicity and offering protection against radiation-induced damage [10–13]. Herbal medicines are considered to be less toxic and cheaper than synthetic compounds at optimum doses [14–16]. Therefore, there is an urgent need to screen promising natural products for new radioprotective agents. Dragon's blood (DB), a bright red resin obtained from *Dracaena cochinchinensis* (Lour.) S.C. Chen (China), was originally used for hemostasis [17–19]. More recently, DB has been found to be rich in phenolic compounds and to offer benefits for the treatment of blood stasis, inflammation, oxidative stress, immune suppression and tumors [20–26]. In our study, we aimed to investigate whether DB and dragon's blood extracts (DBEs) have radioprotective activity in mice irradiated by ^{60}Co γ-rays. We focused on determining DB and DBE effects on immune organ indices, antioxidant status and histological observations in liver and spleen.

MATERIALS AND METHODS

Animals

We obtained 6–7-week-old male BALB/c mice weighing 22–25g from the Institute of Laboratory Animal Science at the Chinese Academy of Medical Sciences (Beijing, China). All animal experiments followed the guidelines of the Institutional Animal Care and Use Committee. Mice were kept in well-ventilated cages and maintained under a 12-h light/dark cycle at room temperature (22–24°C). They were provided with normal laboratory chow and water *ad libitum*.

Drug preparation and administration

DB (No. 20061120) was obtained from Beijing BIT&GY Pharmaceutical R&D Co. Ltd. DB powder was weighed into the reagent bottle and dissolved in 83% ethanol until completely dissolved at room temperature. The solution was diluted to attain 50% ethanol and kept for 24 h at room temperature to separate into a clear supernatant and a precipitate. The precipitate was dissolved in 83% ethanol and the above process was followed once again. Both the primary and secondary clear supernatants were combined, evaporated and lyophilized. Finally, the 50% ethanol extract (DBE) was prepared for the experiment. The main biological activity of DB and DBEs appears to be due to the presence of a number of phenolic compounds (Fig. 1).

BALB/C mice were randomly divided into four groups of ten mice each: control group, irradiation group, DB treatment + radiation group (DB group), DBE treatment + radiation group (DBE group). Both DB (375 mg/kg b.w.) and DBE (300 mg/kg b.w.) were dissolved in 0.5% sodium carboxymethycellulose (CMC-Na). Mice in the DB or DBE groups were given a daily intragastric administration of doses of 375 or 300 mg/kg body weight from 5 d before irradiation till sacrificed. For the control and irradiation groups, the same 0.5% CMC-Na solvent was similarly administered. After 5 d, all mice, except the control group, were exposed to the whole body 4-Gy ^{60}Co-γ irradiation. Mice were sacrificed by cervical dislocation on Days 1, 3, 7 and 28 post-irradiation. Tissues were removed immediately and stored at −80°C for determination of immune organ indices and antioxidant status, and observation of histology in the liver and spleen.

Whole-body irradiation

Mice were placed in well-ventilated Plexiglas boxes and treated with whole body 4-Gy ^{60}Co-γ irradiation. The source of radiation was ^{60}Co-γ and was provided by the Academy of Military Medicine (Beijing, China). The animals were exposed to whole-body radiation at a rate of 1.42 Gy/min at a distance of 400 cm from the source.

Organ indices

On Days 1, 3 and 7 after whole-body irradiation, mice were weighed, sacrificed by cervical dislocation and the liver, thymus and spleen were removed. The liver, thymus and spleen indices were calculated as follows:

Liver, thymus or spleen index = liver, thymus and spleen weight/body weight × 1000.

Preparation of homogenates and determination of antioxidant status

To test the radioprotective effects of DB and DBEs against oxidative stress injury in liver and spleen exposed to whole-body irradiation, the activities of SOD, CAT, GSH and MDA in liver and spleen tissue were measured using commercial kits. On Days 1, 3 and 7 post-irradiation, livers and spleens were removed and homogenized in a 10-fold volume of phosphate buffer solution (PBS, pH7.4) using a homogenizer. The homogenates were centrifuged at 4000 r/min for 20 min and the supernatants were measured according to kit instructions (Nanjing Jiancheng Bioengineering Institute, China).

Histopathology

To evaluate the effect of DB and DBEs on radiation-induced organ damage, livers and spleens were collected from mice on Days 7 and 28 after irradiation. Both liver and spleen tissues were removed, washed in PBS, fixed in 4% paraformaldehyde solution and then embedded in paraffin. Samples were cut into 5-μm sections and stained with hematoxylin and eosin (H&E) [27].

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA), using an SPSS Statistical program (version 17.0). Results were expressed as mean ± standard deviation (SD) of 10 mice in each group. A P-value < 0.05 was considered significant. The differences between the means were considered to be statistically significant if $P < 0.05$.
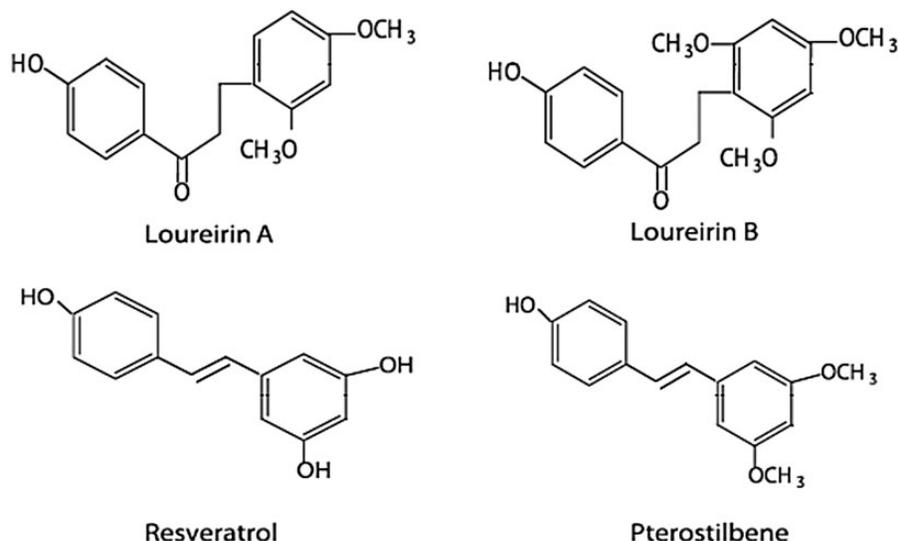
Fig. 1. Main phenolic compounds of Dragon's Blood (patent of BIT&GY: A Dragon's Blood extract and its preparation method and uses, Patent Application No. 200810182971.7).

RESULTS

Effects of DB and DBEs on body weight and organ indices

We investigated the body weight changes of each group after whole-body ^{60}Co-γ irradiation. Compared with the control, the weights of the irradiation group showed a significant decrease on Day 3 ($P < 0.01$) and a moderate increase on Day 7. However, the DB and DBE-treated groups showed a marked increase on Day 3($P < 0.01$) following irradiation (Fig. 2).

As the liver, thymus and spleen are vital organs, it was very important to test the effect of DB and DBEs on these organs after irradiation. Compared with the control, the irradiation group showed a significant increase in the liver indices and decreases in the spleen and thymus indices on different days after irradiation. The liver indices of the DBE-treated mice were significantly lower than those of the irradiation-only group on Day 7 ($P < 0.001$) following irradiation. The DB group demonstrated a clear tendency for decreasing liver indices, but this was not statistically significant. The thymus indices for the DB and DBE groups were significantly higher than that for the irradiated group on Day 7 ($P < 0.05$). However, DB and DBE had no significant effect on the recovery of spleen indices (Fig. 3).

Effects of DB and DBEs on the antioxidant status of irradiated mice

In this mouse model, it was observed that the level of MDA decreased markedly, whereas there was a significant increase in the levels of SOD, CAT and GSH in DB and DBE-administered groups compared with the irradiation-
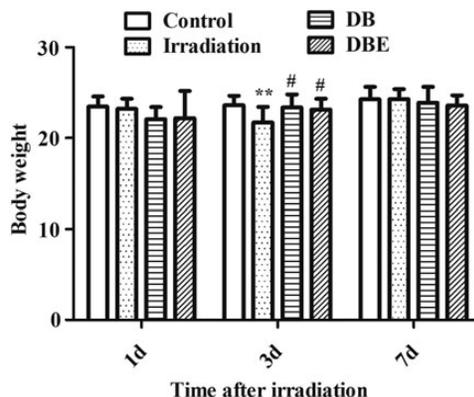

Fig. 2. Effects of DB and DBE on body weight of irradiated mice. The bars represent standard deviation. Significant differences were observed in the DB and DBE-treated groups compared with those in the irradiation group on Day 3. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with the control group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, compared with the irradiation group.

only group. The levels of lipid peroxides (MDA) showed a marked elevation in the liver and spleen after irradiation, but the mice treated with DB and DBEs revealed significantly lower levels of lipid peroxides (MDA) in the liver on Days 1 ($P < 0.05$), 3 ($P < 0.001$) and 7 ($P < 0.01$), and in the spleen on Days 1 (DB $P < 0.01$; DBE $P < 0.05$), 3 ($P < 0.05$) and 7 (DB $P < 0.01$; DBE $P < 0.05$) in comparison with the irradiation-only group (Figs 4A and 5A).

Whole-body irradiation produced a significant decrease in the levels of SOD activity in the liver and spleen. DB and
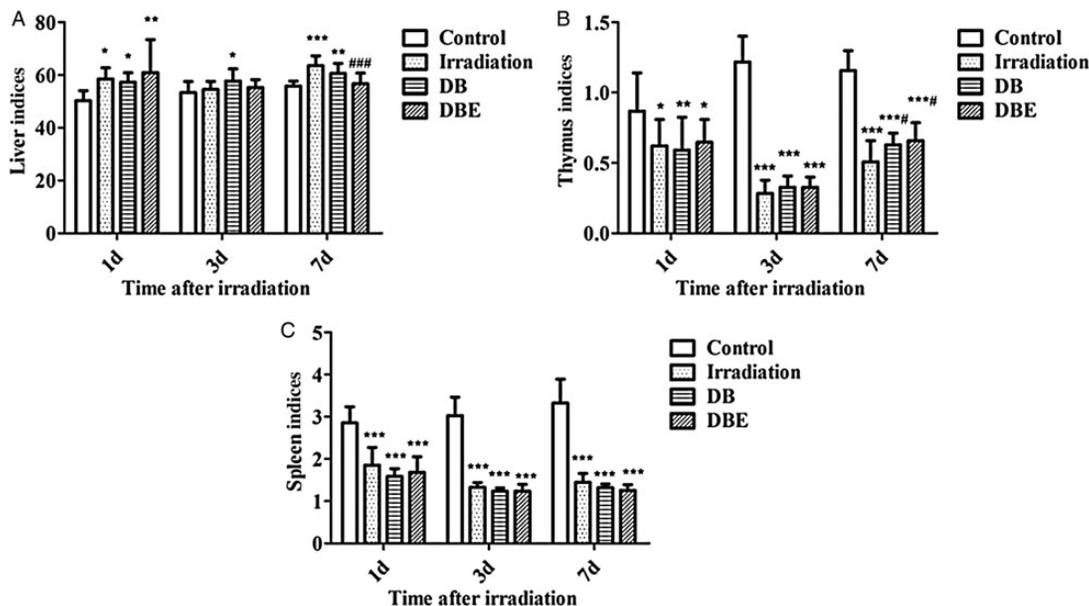
Fig. 3. Effects of DB and DBE treatment on liver, thymus and spleen indices. The bars represent standard deviation. (**A**) DBE-treated mice had significantly lower liver indices compared with those in the irradiation group on Day 7. (**B**) The thymus indices in the DB and DBE-treated mice showed a significant increase on Day 7 after irradiation compared with those in the irradiation group. (**C**) DB and DBE had no significant effect on the recovery of spleen indices. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with the control group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, compared with the irradiation group.
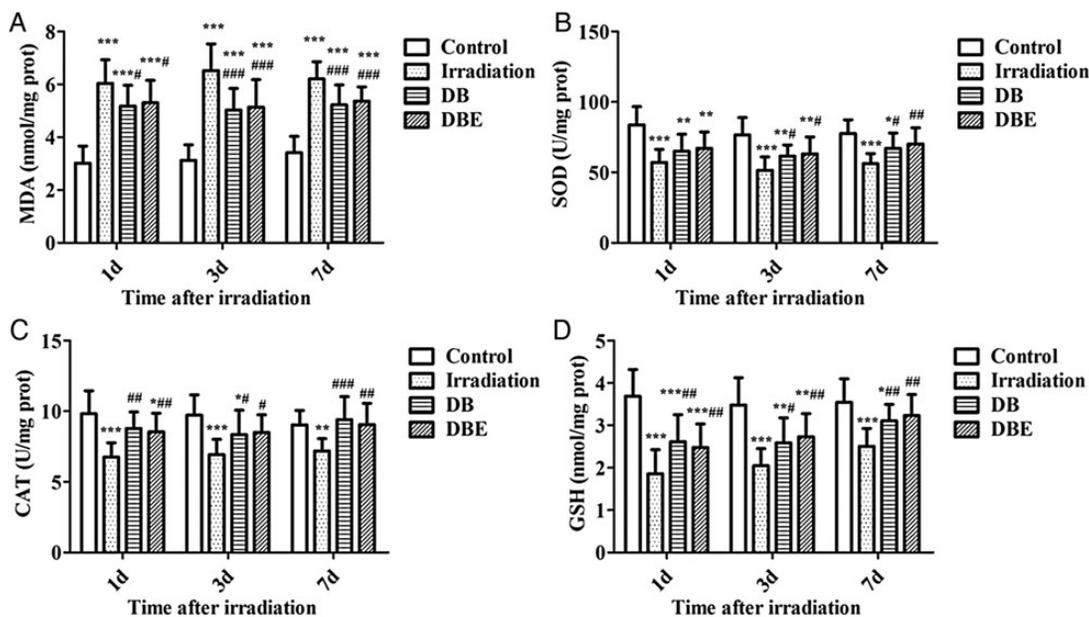

Fig. 4. Effects of DB and DBE on the liver antioxidant status of irradiated mice. The bars represent standard deviation. DB and DBE-treated groups showed significantly lower levels of MDA (**A**), higher activity of CAT (**C**), and even higher levels of GSH (**D**) in the liver on Days 1, 3, 7 after irradiation. They also revealed markedly higher activity of SOD (B) on Days 3, 7. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with the control group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, compared with the irradiation group.

DBE-treated groups showed significantly higher levels of SOD activity in the liver on Days 3 ($P < 0.05$) and 7 (DB $P < 0.05$; DBE $P < 0.01$), and in the spleen on Days 1 (DB $P < 0.01$; DBE $P < 0.05$), 3 (DB $P < 0.01$; DBE $P < 0.001$) and 7 (DB $P < 0.01$; DBE $P < 0.05$) in comparison with the irradiation-only group (Figs 4B and 5B).
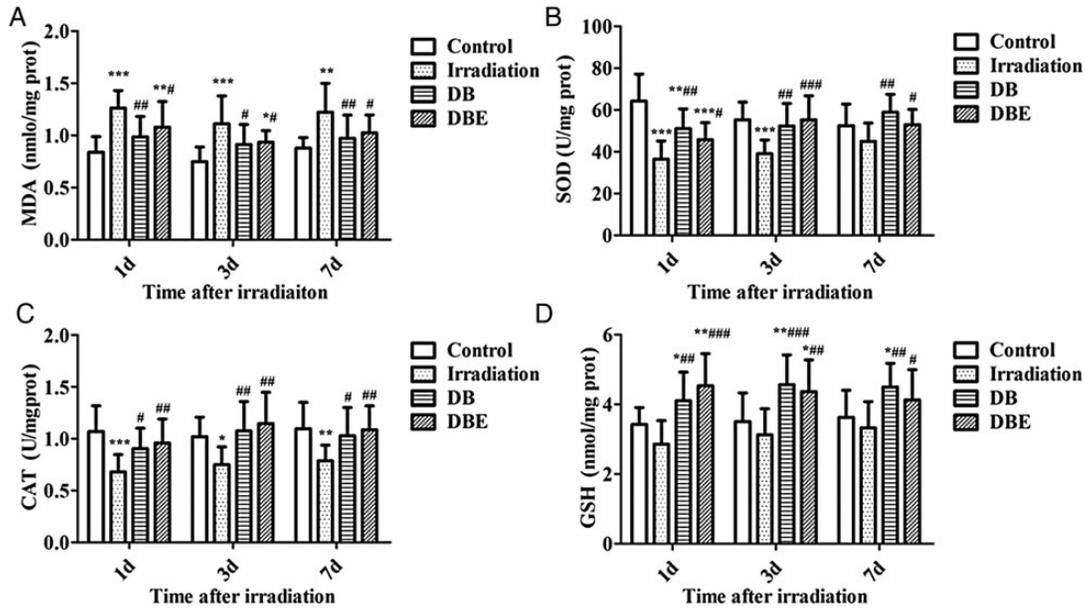
Fig. 5. Effects of DB and DBE on the spleen antioxidant status of irradiated mice. The bars represent standard deviation. The administration of DB and DBE significantly reduced the MDA level (**A**) and markedly increased levels of SOD (**B**), CAT (**C**) activity and GSH (**D**) in the spleen on Days 1, 3, 7 after irradiation. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with the control group; $^{#}P < 0.05$, $^{##}P < 0.01$, $^{###}P < 0.001$, compared with the irradiation group.

The activity of CAT was found to be markedly decreased in the liver and spleen after irradiation. Compared with the irradiation-only group, the administration of DB and DBEs significantly elevated the level of CAT activity in the liver on Days 1 ($P < 0.01$), 3 ($P < 0.05$) and 7 (DB $P < 0.001$; DBE $P < 0.01$), and in the spleen on Days 1 (DB $P < 0.05$; DBE $P < 0.01$), 3 ($P < 0.01$) and 7 (DB $P < 0.01$; DBE $P < 0.05$) (Figs 4C and 5C).

Radiation induced a significant decrease in the levels of non-enzymatic antioxidant GSH in the liver, but the GSH level showed a moderate decrease in the spleen. GSH levels revealed a marked enhancement in the liver on Days 1 ($P < 0.01$), 3 (DB $P < 0.05$; DBE $P < 0.01$) and 7 ($P < 0.001$), and in the spleen on Days 1 (DB $P < 0.01$; DBE $P < 0.001$), 3 (DB $P < 0.001$; DBE $P < 0.01$) and 7 (DB $P < 0.01$; DBE $P < 0.05$) after DB and DBE administration (Figs 4D and 5D).

Effects of DB and DBEs on the histology of liver
The control group revealed normal cellular characteristics of liver, including normal parenchyma, unremarkable hepatocytes, canaliculi and canals of Hering *et al*. (Fig. 6A). The liver histology of the irradiation-only group, however, showed hepatocyte edema, inflammatory cell infiltration and focal areas of necrosis (Fig. 6B and C). The liver histology of mice treated with DB and DBEs together with gamma irradiation exposure showed better cellular architecture than that of the irradiation-only group (Fig. 6D and E).

Effects of DB and DBEs on the histology of the spleen
Spleen sections were stained with H&E for the histopathological assessment. The spleen histological sections of the control group revealed normal cellular characteristics with red and white pulp, as shown in Fig. 7A. Whereas the spleen histology of the irradiation-only group indicated an increase in extramedullary hematopoiesis and a decrease in lymphocytes in the white pulp (Fig. 7B), it was clear that in the DB and DBE treatment groups there was less radiation-induced damage in the spleens and improved spleen morphology (Fig. 7C and D).

DISCUSSION

Radiation is known to produce reactive oxygen species (ROS), which are implicated in the process of DNA damage, cell killing and tissue damage of organs [28, 29]. Our previous studies revealed that DB has radioprotective properties, including reduction of oxidative stress, inflammatory cytokines and neuronal apoptosis after whole-brain irradiation of rats with either heavy ions or γ-rays [30]. In the present study, the radioprotective effect of DB and DBEs in preventing γ-radiation-induced MDA, and in attenuating the inhibition of SOD, CAT and GSH was assessed in the liver and spleen of male BALB/c mice.

Our results demonstrated that 4 Gy of whole-body irradiation caused a significant increase in the MDA level,
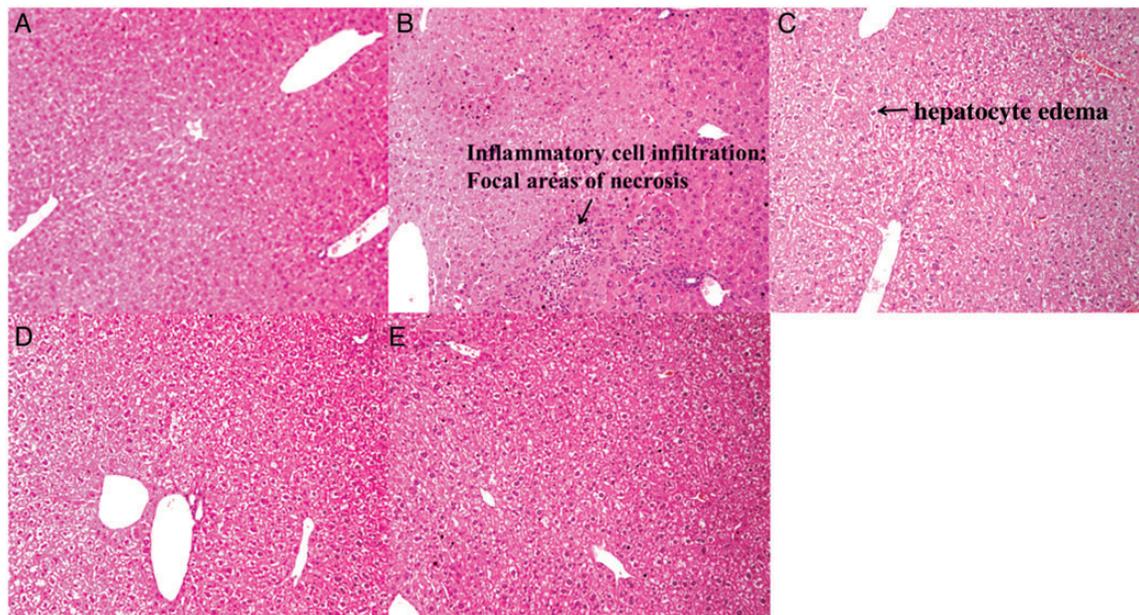
Fig. 6. Effect of DB and DBE treatment on liver morphology. Photomicrographs (×100) of liver sections stained with H&E demonstrating the protective effect of DB and DBE on liver exposed to irradiation. (**A**) Control group; (**B**) and (**C**) irradiation-only group; the liver histology of the irradiation group showed inflammatory cell infiltration, focal areas of necrosis (B, black arrow) and hepatocyte edema (C, black arrow); (D) DB group; (**E**) DBE group.

whereas the activities of antioxidant enzymes (SOD and CAT) and antioxidant molecular levels (GSH) were markedly decreased in the liver and spleen. Radiation-induced ROS and free radicals react with the molecules of cell membranes and induce lipid peroxidation (LPO) products (MDA), which play an important role in direct biological damage such as mutagenic and carcinogenic damage [31, 32]. Administration of DB and DBEs effectively decreased MDA levels in the liver and spleen of irradiated mice. This clearly indicates that DB and DBEs effectively reduced the radiation-induced oxidative stress. It has been suggested that ROS are responsible for radiation-induced toxicity, therefore destruction of ROS by SOD and CAT would ameliorate such toxicity, which means that the enzymes would be able to scavenge the ROS generated [33, 34]. Treatment with DB and DBEs increased the activity of these enzymes and thus may help to control the production of ROS due to radiation exposure. GSH, as an antioxidant, has been considered as the most accurate single indicator of cell health, as GSH depletion represents vulnerability to oxidant attack [35].

In this study, markedly elevated levels of GSH were observed in the liver and spleen of mice treated with DB and DBE, which may be a factor responsible for the inhibition of MDA generated from LPO. In addition, the significant increase in GSH protects cellular proteins against oxidative damage through the glutathione redox cycle and also directly detoxifies ROS induced by irradiation [36].

These observations confirmed our previous report, in which we observed that DB and DBE decreased the MDA level and increased the activity of antioxidant enzymes (SOD and CAT) and the level of antioxidant molecules (GSH) in the serum of mice exposed to radiation. Simultaneously, DB and DBEs reduced the irradiation-induced chromosomal aberrations in bone marrow cells [37]. Abundant phenolic compounds contained in DB were reported to execute free radical scavenging and antioxidant capacities [23], which may be responsible for the increased antioxidant status in irradiated mice treated with DB.

The liver and the spleen have been reported to be highly radiosensitive hematopoietic organs. The former is the primary organ responsible for drug metabolism, detoxifying damaging electrophiles generated during oxidative stress, and the latter is the main organ involved in the development of the immune response [38, 39]. Morphological study of the tissues revealed better liver and spleen architecture in irradiated mice treated with DB and DBEs as compared with irradiation-only treatment. The decrease in pathological changes might be correlated with a lower level of oxidative stress induced by DB and DBE treatment, which suggests that DB and DBE might promote the hematopoietic functions of the liver and spleen.

Ion radiation also induces a variety of immune changes, which are associated with bone marrow suppression, reduction in the number of immune cells, and microcirculatory disturbances [40]. The decreased thymus and spleen indices,
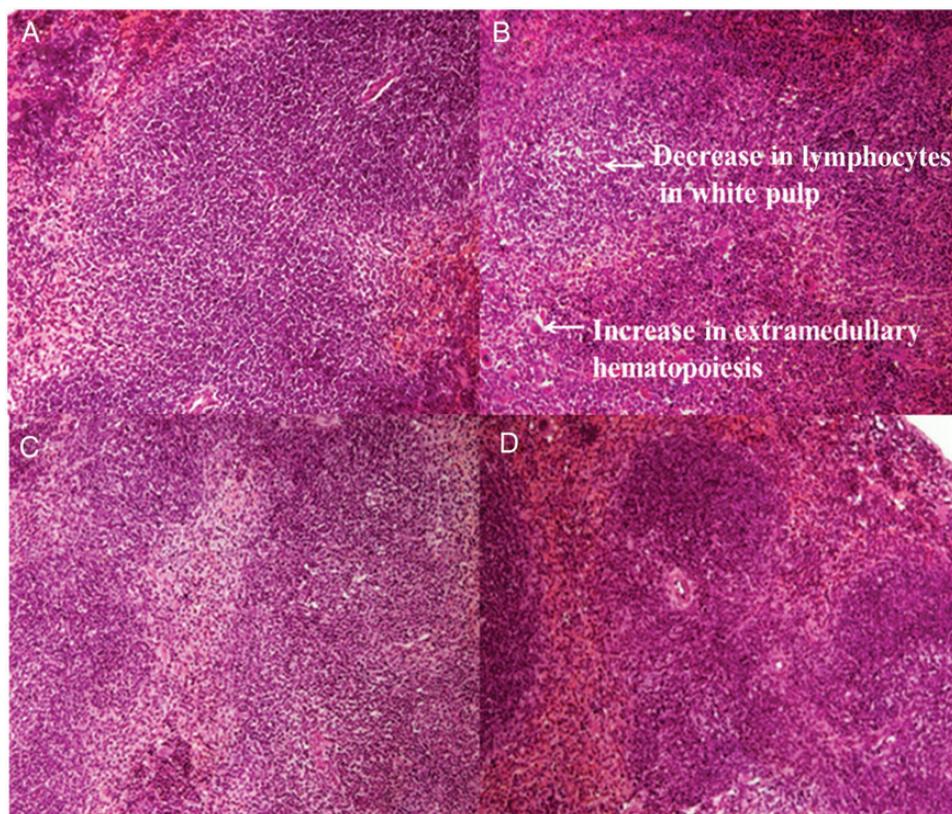
Fig. 7. Effect of DB and DBE treatment on spleen morphology. Photomicrographs (×100) of spleen sections stained with H&E demonstrating the protective effect of DB and DBE on the spleen of irradiated mice. (**A**) Control group; (**B**) irradiation-only group; the spleen histology of the irradiation group showed an increase in extramedullary hematopoiesis and a decrease in the lymphocytes in the white pulp (B, white arrow); (**C**) DB group; (**D**) DBE group.

and increased liver indices in the irradiation-only group, were good indicators of radiation-induced change in immune function. The administration of the DBEs markedly reduced the liver index, suggesting that DBE could reverse the radiation-induced liver swell. DB and DBE-treatment could antagonize the decrease of the thymus index. These data demonstrated that DB and DBEs might reduce the trend to atrophy of the thymus, which would help to improve the immune function of the thymus. However, DB and DBEs had no influence on the spleen index.

CONCLUSION

In conclusion, DB and DBEs administration prior to radiation can effectively mitigate oxidative stress in the liver and spleen, potently enhance immunity via the thymus, and improve the morphology of liver and spleen histology. The protective mechanism of DB may be attributed to its ROS scavenging activity and to regulating the activity of antioxidant enzymes. Despite the lack of clinical studies, the result of the present study and our previous data suggest that DB has the potential to protect tissues from radiation injury and

is a candidate for further development as a radiation countermeasure. Further experiments and clinical trials are necessary to validate this.

FUNDING

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