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Whole body radioprotective activity of an acetone–water extract from the seedpod of *Nelumbo nucifera Gaertn.* seedpod

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ABSTRACT

Procyanidins extracted with acetone–water from lotus (*Nelumbo nucifera Gaertn.*) seedpod (LSPCs) were evaluated for *in vivo* radioprotective activity against whole body gamma irradiation in Swiss albino mice. Pretreated with LSPCs 200 mg/kg by intragastric (*i.g.*) for 15 days was found to be the most effective dose in preventing radiation sickness, reducing radiation-induced mortality, increasing mean survival time and elevating radiation median lethal dose (LD_{50}) from 8.9 to 10.5 Gy, indicating a dose modifying factor (DMF) of 1.18. Further, administered LSPCs at a dose of 200 mg/kg could effectively maintain spleen index close to normal, stimulate endogenous spleen colony forming units, promote the levels of red blood cells (RBC), white blood cells (WBC), platelets and hemoglobin in peripheral blood, and prevent spleen and skin damage in irradiated mice, reduce the level of radiation-induced micronucleated polychromatic erythrocytes in bone marrow, maintain the polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) ratio (*P*/*N* ratio) and significantly decrease bone marrow chromosomal damage. Alternatively, pretreated with LSPCs (200 mg/kg) significantly decreased the lipid peroxidation (LPO) level, and elevated the activities of endogenous antioxidant enzymes in liver after irradiation. Thus LSPCs possess a strong whole body radioprotective activity, and it may be used as a radioprotector.

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1. Introduction

Ionizing radiation is an electromagnetic wave or particle capable of producing ions in its passage through matter, causing immediate chemical alterations in biological tissues. These alterations disrupt metabolic pathways, which can lead, after days or weeks, to cell damage and, potentially, cell dysfunction and death (Hosseinimehr, 2007). Radiation attenuates the endogenous antioxidant enzymes, which are considered as the first line defense mechanism in the maintenance of redox balance and normal biochemical processes (Sun et al., 1998). The exposure of mammals to ionizing radiation, such as gamma-radiation, can cause the development of a complex, dose-dependent series of potentially fatal physiologic and morphologic changes, known as hematopoietic syndrome (Lahouel et al., 1987). Radiation-induced destruction of lymphoid and hematopoietic systems are the primary cause of septicemia and death. Enhanced susceptibility to infections with opportunistic microbes occurs in parallel with progressive radiation-induced atrophy of lymph nodes and the spleen (Orsolić et al., 2007). Recently, the synthetic agents WR2721 (amifostine), OK-432 (picibanil) and ethiofos have been investigated for their efficacy in protecting against radiation-induced tissue damage (Hosseinimehr, 2007). However, these agents have the potential to cause serious side effects including decreased cellular function, nausea, hypotension and death (Bogo et al., 1985; Satoh et al., 1982). Alternatively, natural plant extracts that can protect cells and tissues against ionizing radiation, without obvious side effects, would be a considerable adjunct to successful radiotherapy (Hosseinimehr et al., 2003, 2009; Jagetia and Baliga, 2004; Park et al., 2008). Such as procyanidins extracted from grape seed exhibit a radio-protective effects against chromosomal damage induced *in vivo* by X-rays (Castillo et al., 2000).

Nelumbo nucifera Gaertn., commonly known as lotus, is a perennial aquatic plant grown and consumed throughout Asia. Almost

Abbreviations: CAT, catalase; CFU, colony forming units; DMF, dose modifying factor; GSH-Px, glutathione peroxidase; HE, hematoxylin and eosin; *i.g.*, intragastric; LD_{50}, median lethal dose; LPO, lipid peroxidation; LSPCs, procyanidins from lotus seedpod; MPCE, micronucleated polychromatic erythrocytes; NCE, normochromatic erythrocytes; NS, normal saline; *P*/*N*, polychromatic erythrocytes/normochromatic erythrocytes; PCE, polychromatic erythrocytes; RBC, red blood cells; ROS, reactive oxygen species; SOD, superoxide dismutase; WBC, white blood cell.

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all parts of the plant are eaten as vegetable and are used for various medicinal purposes in oriental medicine. Recently several bioactive compounds such as alkaloid (Kashiwada et al., 2005; Lu et al., 2008; Sugimoto et al., 2010; Zheng et al., 2010), triterpenoid (Chaudhuri and Singh, 2009), flavonoids (Jung et al., 2003; Lin et al., 2009a,b; Deng et al., 2009; Guo et al., 2010), and polyphenols (Park et al., 2009) have been extracted from rhizomes, seeds, flowers and leaves of this plant, which can account for the pharmacological effects of different parts of *N. nucifera Gaertn.* (Mukherjee et al., 2009), including antidepressant (Sugimoto et al., 2010), antifertility activity of seed of *N. nucifera* (Mazumder et al., 1992), anti-HIV (Kashiwada et al., 2005), anti-obesity (Ono et al., 2006; Ohkoshi et al., 2007; Wu et al., 2010), antioxidant activity (Wu et al., 2003; Rai et al., 2006; Jung et al., 2003; Lin et al., 2009a,b; Sohn et al., 2003), hypoglycemic activity (Mukherjee et al., 1997b; Mani et al., 2010), anti-inflammatory (Liu et al., 2004; Mukherjee et al., 1997a), anti-diarrheal (Talukder and Nessa, 1998), sedative effects (Sugimoto et al., 2008), antipyretic potential (Sinha et al., 2000), immunomodulatory activity (Mukherjee et al., 2010), anti-atherosclerosis (Ho et al., 2010), hepatoprotective effects (Sohn et al., 2003; Lin et al., 2009b), and enhancing learning and memory (Yang et al., 2008).

Lotus seedpod is usually discarded, except when occasionally used as a traditional medicine with hemostatic function and eliminating bruise. It has been reported that lotus seedpod is an another important natural source of oligomers and polymers of catechin and epicatechin, which are also denominated procyanidins (Ling and Xie, 2002). Procyanidins from lotus seedpod (LSPCs) were first isolated and characterized by our laboratory, which were constituted by a variable number of flavan-3-ols units linked together through C_4–C_8 (or C_6) interflavanoid bonds, and the oligomeric procyanidins are considered to be the main active constituents of LSPCs (Ling et al., 2005). Previous research indicated that LSPCs contain monomers, dimers, and tetramers of proanthocyanidins, in which the amounts of dimmers are greatest, and catechin and epicatechin are the base units (Ling et al., 2005). As a part of our research, we have found LSPCs possess a wide range of biological effects including scavenging free radicals (such as hydroxyl radical, hydrogen peroxide, hydroperoxyl radical, and superoxide anion radical), inhibiting the formation of lipid peroxidation (LPO) in soybean oil system, or from erythrocyte, liver mitochondria, and liver homogenates of rat *in vitro*, increasing the activities of SOD, GST and decreasing the level of LPO in liver and plasma of CCl_4 toxic mice (Ling and Xie, 2002; Duan and Xie, 2003; Ling et al., 2005), keeping red blood cell membrane from lipid peroxidation and promoting the regeneration of natural vitamin E (Duan et al., 2005), protecting against experimental myocardial injury (Zhang et al., 2004) and ethanol-induced liver damage (Li et al., 2005), suppressing the growth and inducing apoptosis in the cancer cells *in vitro* (Duan et al., 2010), and improving learning and memory abilities (Gong et al., 2008; Xu et al., 2009).

It has been confirmed that radiation-induced normal tissue damage is manifested as a result of the increased production of reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, hydroperoxyl radical and superoxide anion radical, due to the radiolysis of water (Pandey et al., 2006). Considering the excellent antioxidant capacity and radical scavenging activities, LSPCs may be a promising radioprotective compound. However, the effect of LSPCs on irradiated animal survival and hemopoietic system has not been established. Therefore, we investigated the effect of LSPCs administration to Swiss albino mice before whole body gamma irradiation on the radioprotective activities of LSPCs through histopathology, micronuclei assay, chromosomal analysis, and the measurement of lipid peroxidation and antioxidant enzymes levels in this study.

2. Materials and methods

2.1. Preparation of LSPCs

Lotus seedpod was collected from Honghu Lantian Lake (Hubei, China). This variety of *N. nucifera Gaertn.* was named Number 2 Wuhan plant and authenticated by the Department of Botany, Wuhan Plant Institute of the Chinese Academy of Science.

LSPCs is a kind of procyanidins compounds extracted from lotus seedpod. Commonly, these phenolic compounds are extracted with aqueous-ethanol. However, our previous research work indicated that aqueous-ethanol was defective in extracting oligomeric procyanidins in lotus seedpod, although the total phenolic compounds content was higher than the other solvents, such as aqueous-methanol, ethyl acetate and acetone–water. Conversely, acetone–water possessed obvious advantage in extracting oligomeric procyanidins. Oligomeric procyanidins are the main activated substance with special physiological functions, and it has been reported acetone–water can be used to extract oligomeric procyanidins (Palazzo de Mello et al., 1999). Therefore, we select acetone–water as the extraction solvent to extract LSPCs from lotus seedpod.

LSPCs was extracted, purified, and characterized by the method described previously (Ling et al., 2005). Briefly, the lotus seedpod was extracted three times with acetone/water (V/V, 7:3), then the acetone–water extract was purified by Sephadex LH-20 column chromatography, with a purity of >98%, and the main molecular weight distribution of LSPCs was confirmed to be in the range 291.1–1155.3, the LSPCs polymerization was $\leqslant 4$ and contained monomers, dimers and tetramers of procyanidins in which the amounts of dimers were greatest and catechin and epicatechin were the base units, which were consistent with Ling et al. (2005). For all experiments, final concentrations (50, 100 and 200 mg/kg) of the tested compound were prepared by diluting the stock with normal saline.

The residue of acetone in the extract (LSPCs) was determined by headspace capillary gas chromatography (HP5890 type, Hewlett-Packard, US). The chromatographic column was HP-Wax, bonding polyethylene glycol 30 m × 0.32 mm × 0.50 μm. A temperature program starting at 50 °C for 3 min followed by a 20 °C/min ramp to 120 °C for 4 min, and the temperature of detector and injector was 200 °C. The carrier gas was nitrogen, and the detector was flame ionization detector (FID). Under these conditions, the minimum detection limit of acetone was 0.002%, and the acetone residue in LSPCs was not detected (tested three times). According to the regulations of International Conference on Harmonization (ICH), the residue of acetone in the pharmaceuticals for human use is restricted of $\leqslant$0.5%. Consequently, the security maybe posed from acetone has been excluded.

2.2. Animals

All experiments were carried out on random bred male Swiss albino mice, aged 6–8 weeks and weighing 25 ± 2 g, from the Laboratory Animal Research Center of Hubei Province. The mouse colony was maintained under conditions of controlled temperature (23 ± 2 °C) and humidity (50 ± 5%), and a 12 h light/dark cycle. The animals were housed in sanitized polypropylene cages containing autoclaved paddy husk as bedding. They had free access to standard mouse food and water. Animals were treated humanely in compliance with the guidelines of the National Institutes of Health, and the protocol conformed to the Institutional Animal Ethical Committee.

2.3. Irradiations

Mice were placed in well ventilated Perspex boxes of dimensions 23.5 cm × 23.5 cm × 3.5 cm, partitioned into 3 cm × 3 cm × 11 cm cells for individual animals. They were exposed to whole body irradiation from a ^{60}Co Gammatron teletherapy unit (Theratron-780, Hubei Academy of Agricultural Sciences) at a dose rate of 1.14 Gy/min and a source to surface distance of 100 cm. The irradiation facility was provided by Hubei Academy of Agricultural Sciences Atomic Energy Graduate School.

2.4. Treatment of mice with LSPCs and survival studies

To determine whether LSPCs conferred an advantage after lethal whole body irradiation, the effect of LSPCs on the survival of mice was investigated. LSPCs dissolved in NS were used for administration (*i.g.*) daily at doses of 50, 100 and 200 mg/kg to animals for 15 consecutive days before irradiation. The number of surviving mice was recorded daily up to 30 days post-irradiation, and the data were expressed as percentage survival. Radiation doses between 8 and 10 Gy were used to study the effect of LSPCs on survival, and a dose of 4 Gy was used to evaluate the effect of LSPCs on bone marrow, lipid peroxidation and antioxidant enzymes (Coleman et al., 2003). All of the mice in the experiments were randomly divided into six groups as follow: Group I (control) and II (radiation alone) orally received normal saline but no LSPCs; Group III was only given with LSPCs 200 mg/kg (LSPCs200); Group IV was radiation plus LSPCs 50 mg/kg (radiation + LSPCs50); Group V was radiation plus LSPCs 100 mg/kg (radiation + LSPCs100); Group VI was radiation plus LSPCs 200 mg/kg (radiation + LSPCs200). Each group consisted of 12 mice.