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Evaluation of radicals scavenging, immunity-modulatory and antitumor activities of longan polysaccharides with ultrasonic extraction on in S180 tumor mice models

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ABSTRACT

Longan (*Dimocarpus longan* Lour.) is used as a traditional edible and medicinal material in China. In this paper, the effects of polysaccharides from longan pulp with ultrasonic extraction (UELP) on the radicals scavenging, immunity-modulatory and antitumor activities in S180 tumor mice models were investigated. UELP showed excellent scavenging activity on the hydroxyl radicals and a,a-diphenyl-1-picrylhydrazyl (DPPH) radicals, and obtained the almost complete scavenging effect. UELP with medium-dose (200 mg/kg) and low-dose (100 mg/kg) had potent immune-modulatory effects in S180 tumor mice model and exhibited significant effect on delayed-type hypersensitivity (DTH) response, macrophage phagocytosis and ConA-stimulated splenocyte proliferation as compared with model control treatment ($p < 0.01$). The results still showed that UELP had great antitumor effects, and maximum inhibition rate was obtained at medium-dose and low-dose (200 and 100 mg/kg).

1. Introduction

Polysaccharides from plant, epiphyte and animals extracts are an interesting source of additives for several industries, in particular food and drug industry [1]. They play important roles in the growth and development of living organisms, and have been widely studied in recent years due to their unique biological, chemical and physical properties [2]. Especially, the biological activities on the immunomodulatory and antitumor effects have attracted more attention recently in the biochemical and medical fields [3–5]. For example, lentinan, schizophyllan, and krestin have been accepted as immunoceuticals in several oriental countries, and it was proved that lentinan had excellent augmentations of lymphocytes proliferation, delayed-type hypersensitivity (DTH) responses and macrophage phagocytosis and antitumor activity on immunity-modulatory [6–8].

Longan (*Dimocarpus longan* Lour.) is an important tropical fruit in Southeast Asia, such as China, Vietnam and Thailand. It is taste, nutritional and favored by many consumers in the world [9]. Longan has also been used as a traditional Chinese medicine since ancient times, and great attentions have been paid for their great health effects [10], such as promoting blood metabolism, soothe nerves, relieve insomnia, etc. [11].

At present, there have been only a few reports on longan biochemical and physiological activities, and most of these reports focused on longan seeds and longan fruit pericarp. Previous reports depicted that longan seeds are administered to counteract heavy sweating and the pulverized kernel serves and possess potent radical scavenging, antioxidant and antityrosinase activity [12–14]. Other reports showed that longan peel extract with different extraction technology had excellent antioxidant and anticancer activity. Whereas, there are lacks in the research of the functional effects of longan fruit pulp [15,16].

Polysaccharides and lignin in the longan fruit have been considered the main functional compositions for these health effects [17]. Previous research showed that longan fruit pulp and pericarp contains a large quantity of polysaccharides [9,10]. The polysaccharides extracted from longan fruit pericarp were studied and which exhibited great functional effects on the radical scavenging, antioxidant, antiglycated and antityrosinase activity [18–20]. Whereas, the functional effects of polysaccharides in fruit pulp, especially the immunity-modulatory and antitumor effects *in vivo*, was not studied at present, and which was considered that the contents were higher than that in pericarp.

In this study, the main objective was to investigate the functional effects of polysaccharides from longan fruit pulp. Longan polysaccharides with ultrasonic extraction (UELP) were prepared and its effects on radicals scavenging activities, immunity-modulatory and antitumor effect in S180 tumor mice models were evaluated.

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2. Materials and methods

2.1. Materials and chemicals

Dried longan (*D. longan* Lour.) fruit was purchased from a commercial market of Guangzhou. The dried longan fruit was peeled, seeded and air-dried at 50 °C for balancing the water. Then, the dried longan pulp was grinded by a miller (A11 basic, ZKA®-WERKE, Germany), collected and stored in desiccator at room temperature (15–20 °C) until used.

2.2. Preparation of longan polysaccharides with ultrasonic extraction (UELP)

The crude longan polysaccharides with ultrasonic extraction were prepared according to the method described by Zhong and Wang [9]. In brief, dried longan pulp powder was extracted with distilled water (1:25 ratio, w/v) in an ultrasonic cell disintegrator (JY92-II, Xinzhi Bio-technology and Science Inc., Lingbo, ZheJiang Province, China) for 4.5 min at 680 W. Ice bathing was used to ensure the temperature of solution was below 50 °C in the whole extraction processing. After the extraction with ultrasonic treatment, the extracted slurry was centrifuged at 4200 rpm/min for 20 min to collect the supernatants, and the insoluble residue was treated again for 2–3 times as mentioned above. Supernatants were incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (Senco technology and Science Inc., Shanghai, China) at 55 °C under vacuum. Then, the pH value of concentrated solution was adjusted to 4.0 with 5% trichloroacetic acid (TCA) and holded at 4 °C for 2 h to remove the protein. Supernatants were collected by centrifugation at 4200 rpm/min for 20 min and mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) and kept overnight at 4 °C. The precipitate that formed was collected by centrifugation at 4200 rpm/min for 20 min and washed six times with dehydrated ethanol. The resulting extract was re-dissolved in less amounts of distilled water and centrifuged at 10,000 rpm/min for 15 min, supernatants was re-dissolved less amounts of distilled water again and freeze dried, giving the UELP.

2.3. Hydroxyl radical scavenging activity

0.5 mL of salicylic acid–ethanol solution (9.1 mM), 0.5 mL of UELP solution at different concentrations (5, 10, 15, 25, 30 and 35 mg/mL), 0.5 mL of Fe (II) solution (9.1 mM) and 3.5 mL of distilled water were piped in a 50 mL graduated tube, and the reaction was started by the addition of 5 mL H_2O_2 standard solution (8.8 mM/L) to the mixture solution. After the mixture solution was shaken vigorously and the color was in constant, the absorbance at 510 nm was read to express the radical scavenging effect with UV spectrophotometer (UV1201, bASCI Inc., Beijing, China). The hydroxyl radical scavenging activity percentage (P) was calculated as follows:

$$P\ (\%) = \left(1 - \frac{\text{absorbance of sample} - \text{absorbance of control}}{\text{absorbance of blank}}\right) \times 100 \quad (1)$$

where control and blank solution contains all reagents except that distilled water instead of H_2O_2 was used for the control while distilled water instead of sample was used for the blank. All tests were performed in triplicate and mean were centred.

2.4. DPPH radical scavenging activity

The radical scavenging effects of UELP on a,a-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, St. Louis, MO, USA) radical were estimated through the methods of Ebringerová et al with little modification [21].

UELP solution (1.5 mL) at different concentrations (5, 10, 15, 20, and 30 mg/mL) was added to equivalent aliquot DPPH (0.1 mM) in 95% ethanol. The reaction solution was shaken vigorously and incubated at room temperature (25 ± 3 °C) for 30 min, and then the absorbance of the resulting solution at 517 nm was measured. Black solution contains 95% ethanol instead of DPPH solution for the baseline correction. A lower absorbance represented a higher DPPH scavenging activity.

The DPPH radical scavenging percentage (P) was calculated as follows:

$$P\ (\%) = \left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right) \times 100 \quad (2)$$

where control solution contains equivalent distilled water instead of samples solution. All tests were performed in triplicate and mean were centred.

2.5. Animals treatment

Female BALB/c mice (6–8 weeks old, 20 ± 2 g) were obtained from the Experimental Animal Center of the Chinese Academy of Medical Sciences (Certificate No. SCXK-2004-0001, Beijing, China). S180 tumor cells, sheep red blood cells (SRBCs) and chicken red blood cells (CRBCs) used in experiment were purchased from the Experimental Animal Center of Peking University Health Science Center (Beijing, China). The animals were housed in polypropylene cages and maintained under controlled conditions of 12 h light/12 h dark photoperiod and 55 ± 5% relative humidity at room temperature (28 ± 2 °C). After fed in our facility for 1 week, 50 mice were induced S-180 tumor cells according to the reference Wu et al. with some modification [22]. All mice were subcutaneously implanted with 1×10^6 cells/mice on the right flank. 24 h after inoculation, all animals were randomly divided into the following 1 model control group, 1 positive control group and three experimental groups (each group contained 10 mice):

Group I (model control treatment) received orally the same volume of 0.9% normal saline once per day.
Group II (positive control treatment) injected intraperitoneally the lentinan (TAISHENG pharmaceutical Co. Ltd, Datong, Shanxi Province, China) at a dosage of 0.02 mg/kg body weight once in 2 days.
Group III (low dose of LP treatment) received UELP orally (dissolved in 2.0 mL distilled water) at a dosage of 100 mg/kg body weight once per day.
Group IV (medium-dose of LP treatment) received UELP orally (dissolved in 2.0 mL distilled water) at a dosage of 200 mg/kg body weight once per day.
Group V (high-dose of LP treatment) received UELP orally (dissolved in 2.0 mL distilled water) at a dosage of 400 mg/kg body weight once per day.

2.6. Evaluation of immunity-modulatory effects

2.6.1. Delayed type hypersensitivity (DTH) response by footpad measurement

All mice were sensitized intraperitoneally with 0.2 mL SRBCs suspension (2%, v/v, with normal saline) for injection at day 6, and then challenged with 20 μL of SRBCs suspension (20%, v/v, with normal saline) in the left rear-foodpad and PBS in the contralateral foodpad after 4 days.

The thickness of the left rear-footpad of mice before and 24 h after SRBCs challenge was measured by the vernier calliper, and the DTH response was expressed as the difference in thickness (0.001 cm) between both footpads.