Anti-inflammatory of *Portulaca oleracea* L extract on LPS-stimulated RAW 264.7 macrophages

EP_330

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Introduction:

The inflammatory cell model established by inducing mouse macrophage RAW264.7 with lipopolysaccharide (LPS) was used to evaluate in vitro whether the chemical components of POL could inhibit the release of cytokines and inflammatory mediators, which could simply, sensitively and accurately verify whether it had anti-inflammatory activity.Furthermore, it provides scientific basis for further research on the anti-inflammatory, anti-allergic and eczema treatment of purslane chemical components.

Materials & Methods:

Portulaca oleracea L (POL) extract

POL Add 80% concentration of ethanol, 48℃~50℃, heat preservation stirring extraction for 2 hours . add white diatomite, stir it for 10 minutes,filter it with plate frame (press cloth + medium speed filter paper), filtrate is: wine red transparent and clear solution. The filtrate was filtered by 0.45μm membrane, and then filtered by 3000D molecular membrane. Then the concentrated solution was freeze-dried to obtain POL freeze-dried powdes(POP).

Cell viability

Cell viability was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.In brief, 5 × 10^3 cells/well of RAW264.7 cells were incubated with various concentrations of POP for 24H.Media containing 100 μg/ml of MTT solution were added to cells for 2 h at 37°C. The conditioned media were then removed and purple formazan crystals were solubilized with DMSO. The absorbance was determined at 540 nm using a spectrophotometer and all samples were assayed in triplicate.

Measurement of Pro-Inflammatory Cytokines and Nitric Oxide (NO)

To determine the levels of pro-inflammatory cytokines, RAW264.7 cells were pre-incubated with POP (1mg/ml,0.1mg/ml,0.01mg/ml） for 2H and then stimulated with LPS (2 μg/ml) and POP for 24h. Concentrations of TNF-α, IL-1β, IL-6 in the culture supernatants were analyzed using a commercially available mouse ELISA kit according to the manufacturer's instructions. All samples were assayed in triplicate. The concentration of nitric oxide in the culture supernatant was analyzed using the Griess reagent. In brief,150μl of the culture supernatant was mixed with 50 μl of Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylene diamine in 2.5% phosphoric acid solution) and incubated at room temperature for10 min. The absorbance at 540 nm was measured using a spectrophotometer. Nitrite production in each sample was determined from NaNO2 serial dilution standard curve.

Real-Time Reverse Transcription (RT)-PCR

To examine the effect of POP on anti-inflammation, we carried out real-time RT-PCR with known primers amplifying marker genes according to the manufacturer's instructions(TAKARA). RAW264.7 cells were pre-incubated with POP（1mg/ml,0.1mg/ml,0.01mg/ml） for 2H and then stimulated with LPS (2 g/ml) and POP for 24 h. After that, The total RNA was extracted by using TriZol (TAKARA) following the manufacturer's protocol. RNA con-centrations were determined using Qubit® RNA Assay kit (TAKARA). cDNA kit (TAKARA) was then used for cDNA synthesis following the manufacturer's protocol.The cDNA was stored at −20°C until use. Using Power SYBR Green PCR Mix (Invitrogen) following the manufacturer's pro-tocol, qRT-PCR reactions were performed in tripli- cates. Primers for the amplification of NF-κB p65,NF-κB p50,COX-2, and iNOS,GAPDH were purchased from Sangon Biotec. GAPDH was used as an endogenous control gene. qRT-PCR reactions were performed on a CFX -Connect Real Time PCR System(Bio-red). The mRNA expression levels were evaluated relative to the levels of GAPDH.

Results & Discussion:

To investigate the anti-inflammatory effect of POP, we performed the NO, IL-1 β, IL-6, TNF- α assay in the supernatant of RAW264.7 cells. As shown in Fig. A, LPS treatment increased NO, IL-1β, IL-6, TNF- α production more than eight-fold, whereas treatment with POP markedly reduced LPS- stimulated NO, IL-1 β, IL-6, TNF- α production in a concentration-related manner. These results suggest that POP peptide might have anti inflammatory activity in LPS-stimulated RAW264.7 cells.. We tested the anti- inflammatory effect of POP using a marker gene encoding iNOS , COX-2, NF-κb-p65 and NF-κb-p50 inflammatory response which promotes inflammatory response, by real-time RT-PCR (Fig. B). LPS can significantly increased the expression of iNOS, COX-2, NF-κb-p65 and NF-κb-p50 compared with the negative control without any LPS irradiation.

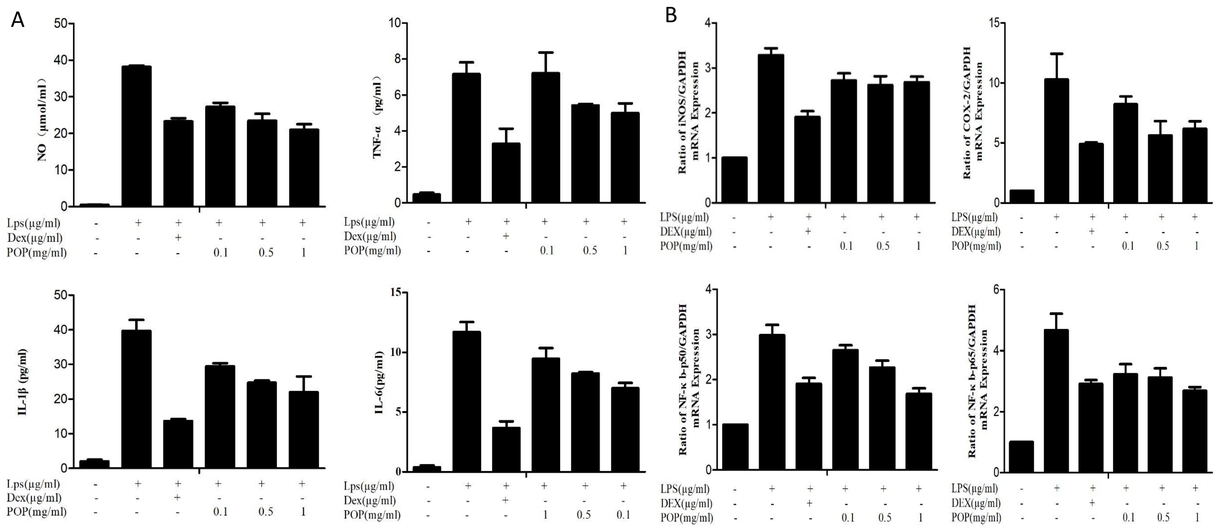

Fig A. Effects of POP on inflammatory factors and mediators(NO,TNF-α ,IL-1β,IL-6) in LPS-induced RAW264.7

Fig B . Effects of POP on the expression of iNOS, COX-2, NF-κb-p65 and NF-κb-p50 mRNA in RAW264.7 cells induced by LPS.

Discussion

POL is rich in nutrients, and also contains polysaccharides, polyphenols, flavonoids, alkaloids and other bioactive substances. However, it is not clear which class of substances or compounds have the biological activity. Most current purslane extracts are mixed, so that the sample color is extremely dark and unstable, which is very bad for end use. According to the literature, we found that almost all reports on POL activity focused on small molecule substances, so we used 3000D molecular membrane and retained substances under 3000 molecular weight. At the same time, freeze-drying method was adopted to dry the extract, so as to avoid destroying the activity of active substances in the drying process. Finally, we established an anti-inflammatory model of macrophages to evaluate the anti-inflammatory activity of POP, so we determine that the small-molecule POP has better anti-inflammatory activity.

Conclusions:

Low molecular Purslane extract has superior anti-inflammatory effects. Through experimental studies, it can be found that 10mg/ml of POP can almost completely scavenge free radicals, and different concentrations of POP have little effect on the survival rate of macrophages. ELISIA and RT-PCR were used to study the effects of POP on inflammatory factors and mediators, and it was found that at the dosage of 0.1, 0.5 and 1mg/ mL of POP, inflammatory factors and mediators were reduced to varying degrees, with a gradient effect. In conclusion, purslane freeze-dried powder has excellentanti-inflammatory ability.

Aknowledgments:

Thanks to the help of the researchers at huiwen R&D Center

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