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Effects of extracts and isolated compounds from safflower on some index of promoting blood circulation and regulating menstruation

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ABSTRACT

Ethnopharmacological relevance: Carthamus tinctorius is used as one of the Traditional Chinese Medicine (TCM) materials in prescriptions and composite to promote blood circulation to remove blood stasis, regulate menstruation and alleviate pain for over 2500 years. Modern pharmacological experiments have demonstrated that safflower has wide-reaching biological activities, including dilating coronary artery, modulating immune system, improving myocardial ischemia, anticoagulation and thromboprophylaxis, antioxidation, antihypoxic, antiaging, antifatigue, antiinflammation, anti-hepatic fibrosis, antitumor, analgesia, etc.

Materials and methods: Platelet aggregation of safflower extract and main constituents in safflower were determined by PAF-induced or ADP-induced platelet aggregation in vitro. Anticoagulation activity was measured by clotting assay of thrombin time (TT), prothrombin time (PT) and activated partial thromboplastin time (APTT) according to the methods provided by the biological reagents provider (Sun Biochemical). Antioxidant effects of safflower were assessed using DPPH radical-scavenging activity test, ABTS radical-scavenging activity test and ferric reducing antioxidant power test. In addition, rats ovary granulosa cell proliferation activity was used for the bio-activity index on regulate menstruation of safflower.

Results: Safflower extract at the concentration of 0.7 g/mL ($P<0.001$) and 0.5 g/mL ($P<0.01$) had significantly antagonistic effect on PAF-induced platelet aggregation, compared with negative control. And the anti-platelet aggregation of 0.7 g/mL safflower extract was significantly stronger than that of positive control ($P<0.001$). 0.7 g/mL of hydroxysafflor yellow A ($P<0.01$), anhydrosafflor yellow B ($P<0.05$), 6-hydroxykaempferol-3-O-rutinoside ($P<0.05$), keampferol-3-O-β-rutinoside ($P<0.01$) had significant effect on platelet aggregation compared with negative control. Safflower extract at the concentration of 0.5 g/mL ($P<0.001$) and 0.125 g/mL ($P<0.01$) could significantly inhibit ADP-induced platelet aggregation, compared with negative control. And antagonistic effect of safflower extract was significantly stronger than the effect of positive control ($P<0.001$). Adenosine ($P<0.001$), anhydrosafflor yellow B ($P<0.01$) and 6-hydroxykaempferol-3-O-rutinoside ($P<0.01$) at the concentration of 0.5 g/mL had significant effect on ADP-induced platelet aggregation compared with negative control. 0.125 g/mL of adenosine ($P<0.05$) had significant effect on ADP-induced platelet aggregation compared with negative control. The effect of 0.5 g/mL adenosine ($P<0.01$) and 6-hydroxykaempferol-3-O-rutinoside ($P<0.05$) was significantly stronger than that of positive control. Safflower extract at the concentration of 0.7 mg/mL ($P<0.001$) and 0.5 mg/mL ($P<0.001$) had significantly anticoagulation activity in PT, TT and APTT, compared with negative control. However, the respective compound didn't have significant effect on PT and TT at experiment concentration. At the concentration of 0.7 mg/mL, hydroxysafflor yellow A ($P<0.01$), 6-hydroxykaempferol-3,6,7-tri-O-β-D-glucoside ($P<0.05$), 6-hydroxyapigenin-6-O-glucoside-7-O-glucuronide ($P<0.01$), anhydrosafflor yellow B ($P<0.001$), 6-hydroxykaempferol-3-O-rutinoside ($P<0.05$) and keampferol-3-O-β-rutinoside ($P<0.05$) significantly prolonged APTT, compared with negative control. At the concentration of 0.5 mg/mL, hydroxysafflor yellow A ($P<0.05$), 6-hydroxyapigenin-6-O-glucoside-7-O-glucuronide ($P<0.05$), anhydrosafflor yellow B ($P<0.001$), 6-hydroxykaempferol-3-O-rutinoside ($P<0.05$) and keampferol-3-O-β-rutinoside ($P<0.05$) could significantly prolong APTT, compared with negative control. From the results of DPPH, ABTS radical scavenging activity test and Fe^{3+} reduction power test, 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL safflower extract had antioxidant effects. Every compound with each concentration (5 mg/mL, 2.5 mg/mL and 1.25 mg/mL) had significant effect on Fe^{3+} reduction power ($P<0.001$ vs. negative control). Safflower extract, cytidine, 6-hydroxy-

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kaempferol-3,6-di-O-β-D-glucoside-7-O-β-D-glucuronide, 6-hydroxykaemp-ferol-3,6,7-tri-O-β-D-glucoside and keampferol-3-O-β-rutinoside significantly promoted ovarian granulosa cell proliferation.
Conclusion: Based on previous researches, the activities of safflower extract and pure compounds isolated from safflower were studied in this paper. This study found some compounds with the effects of anti-platelet aggregation, anticoagulation, antioxidation and ovarian granulosa cell proliferation, and further revealed the possible pharmacological mechanism of safflower.

1. Introduction

Carthamus tinctorius (Safflower) is a plant species of asteraceae family (Siddiqi et al., 2009) which its flowers have applications in medical settings and food industry (Elias et al., 2002; Mass, 1986). In the theory of TCM, safflower is considered to promote blood circulation to remove blood stasis, promote menstruation and alleviate pain. It is mainly applied for blood-stasis syndrome with dysmenorrhea, amenorrhea, postpartum abdominal pain and mass, trauma and pain of joints, etc (Tang, 2003).

Many pharmacological experiments have demonstrated that safflower with its active compounds possesses wide-reaching biological activities. However, the present study was focused on investigating the pharmacological actions of safflower extraction and yellow pigment of safflower, monomer as hydroxysafflor yellow A and anhydrosafflor yellow B (Chen et al., 2000; Zang et al., 2002; Zhao et al., 2009; Wang et al., 2009; Lin et al., 2011; Pan et al., 2012; Nie et al., 2012; Zhou et al., 2014). More studies ought to be explored on the pharmacological effects and possible mechanisms of other constituents isolated from safflower.

In addition, the study of pharmacological activity about safflower is mainly concentrated on the anticoagulant activity, cardiovascular and cerebrovascular system (such as dilating coronary artery, antithrombosis, improving myocardial ischemia, protective effect on cerebral ischemia, protective effect on endothelial cells, etc), anti-fibrotic effect, antiinflammatory effect, antioxidant activity, immunomodulatory effects, neuroprotective effect, antiaging, antihypoxia, antifatigue, antitumor, analgesia, etc (Xiao, 2002; Zhou et al., 2014). More study was needed to investigate the impact of safflower on the uterus. Studies have indicated that gynecological blood stasis syndrome may be closely related to ovarian function and the oxidative damage of myometrium and endometria (Liu et al., 2015). The relationship between ovarian function and dysmenorrhea, amenorrhea is closely. Granulosa cells, primary cell type in the ovary, provide the structure support and microenvironment for the developing oocyte (Huang and Wells, 2010). Ovary granulosa cell proliferation test can be used to indirectly reflect the ovarian function.

This study of safflower extract and main constituents in safflower including nucleoside, flavonoids and chalcone flavonoids contains platelet aggregation, anticoagulation activity, antioxidant effects and ovary granulosa cell proliferation activity.

2. Materials and methods

2.1. Chemicals and materials

Carthami Flos were harvested from Xinjiang municipality in China, where were the genuine producing areas for this herbs. Their origins were authenticated by Professor Chungen wang as *Carthamus tinctorius* L.

Adenosine diphosphate (ADP), thrombin, Tris–HCl and kits for prothrombin time (PT), thrombin time (TT) and activated partial thromboplastin time (APTT) assay were commercial reagents from Sun Biochemical Co. Ltd. (Shanghai, China). PAF (Platelet-Activating Factor), DPPH (1,1-di-phenyl-2-picrylhydrazyl), ABTS (2,2′-azinobis-3-ethyl-benzothiazolin-6-sulfonic acid), TPTZ (2,4,6- tripyridyl-s-triazine), McCoy's 5A Medium (modified, with L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture), trypan blue and 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Shanghai, China). Pregnant Mare's Serum Gonadotropin (PMSG) was obtained from PROSPEC (Beijing, China).

Deionized water was purified by an EPED superpurification system (Eped, Nanjing, China). Other reagent solutions were of analytical grade. Cytidine, Adenosine, hydroxysafflor yellow A, -hydroxykaempferol-3,6-di-O-β-D-glucoside-7-O-β-D-glucuronide, 6-hydroxykaempferol-3,6,7- tri-O-β-D-glucoside, 6-hydroxyapigenin-6-O-glucoside-7-O-glucuronide, anhydrosafflor yellow B, 6-hydroxykaempferol-3-O-rutinoside and Keampferol-3-O-β-rutinoside were separated and purified by preparative HPLC in the Lab of National and Local Collaborative Engineering Center of Chinese Medicinal Resources Industrialization and Formulae Innovative Medicine, Nanjing University of Chinese Medicine. The purity of all compounds was above 90%, which was determined by HPLC-DAD. The structures (Fig. 1) were elucidated by their UV, MS, ^{1}H NMR (Zhou et al., 2009; Jiang et al., 2008; Fan et al., 2009; Kazuma et al., 2000; Fan et al., 2011).

2.2. Animals

Male New Zealand white rabbits (Qing Longshan Laboratory Animal, Nanjing, China) weighing 1.9–2.1 kg were used. They were housed in a conventional animal facility with free access to food and water where the environmental temperature (16 ± 3 °C) and relative humidity (70 ± 8%) were monitored and controlled. Female Sprague–Dawley rats (60–70 g) at the age of 21 days were obtained from experimental procedures strictly conformed to the Guide for the Care and Use of Laboratory Animals and the related ethics regulations of Nanjing University of Chinese Medicine.

2.3. Effect of PAF-induced or ADP-induced platelet aggregation

All the samples were weighed accurately and dissolved in deionized water (control) to obtain sample solutions of the concentration of 0.7 g/mL, 0.5 g/mL and 0.125 g/mL (crude drug dosage). Sample solutions of 0.7 g/mL and 0.5 g/mL were used for PAF-induced platelet aggregation. And sample solutions of 0.5 g/mL and 0.125 g/mL were for the other.

Blood was collected through a polyethylene cannula placed in the common carotid artery of male New Zealand white rabbits by a 10 mL plastic flask containing 3.8% sodium citrate (1:9, v/v). Platelet-rich plasma (PRP) was prepared by low-speed centrifugation of the blood at 800 rpm for 10 min and further centrifuged at 3000 rpm for 10 min to prepare platelet-poor plasma (PPP) (Ashida and Abiko, 1979).

Platelet aggregation test (PAgT) was performed by the turbidimetric method of Born and Cross using a four channel platelet aggregation and blood coagulation factors analyzer according to