Biomedical Research 2016; 27 (2): 373-376 *ISSN 0970-938X*
www.biomedres.info

Anti-allergic effect of *Fructus cnidii* ethanol extracts.

Jinshu Yin[1], Haiyun Shi[2], Xiaorong Li[3], Xueyan Wang[2*]

[1]Department of Otolaryngology, Beijing Shijitan Hospital, Capital Medical University, PR. China

[2]Department of Allergy, Beijing Shijitan Hospital, Capital Medical University, PR. China

[3]Department of Pharmaceutical preparation, Beijing Shijitan Hospital, Capital Medical University, PR. China

Abstract

This study was mainly to observe the anti-allergic effect of *Fructus cnidii* ethanol extract. Three methods were chose to observe the anti-allergic effect of *Fructus cnidii* including rat Passive Cutaneous Anaphylaxis (PCA) tests, rat skull periosteum mast cell degranulation tests, and histamine released factors in rat peritoneal mast cells sensitized mast cells with vitro techniques. The PCA inhibition ratio, the mast cell degranulation ratio and relevant inhibition ratio of drugs, histamine release ratio and relevant inhibition ratio of drugs were all the main indicators needed to dectet. Results showed that *Fructus cnidii* ethanol extracts could significantly inhibit the PCA reaction in rats, mast cell degranulation in rat skull periosteum as well as the release ratio of histamine. At last, we could come to the conclusion that *Fructus cnidii* ethanol extract has an obvious anti-type I hypersensitivity effect.

Keywords: Passive cutaneous anaphylaxis, Periosteum, Mast cells, Antiallergics, *Fructus cnidii*.

Accepted January 20, 2016

Introduction

Type I allergy reaction was a kind of disease having higher incidence as well as vast influence among present hypersensitivities [1]. However, mast cell degranulation reaction was a key step in the process of allergy, also was closely associated with the inflammatory response. Its mechanism was to inhibit or block a particular part in the reaction, including: protect and stabilize the target cell membrane, reduce degranulation ratio and release of allergy mediators, suppress IgE formation, neutralize allergens and so on. *Fructus cnidii* was a traditional Chinese medicine, mature dry fruit of *Cnidium monnieri* (L.) Cuss belonging to the umbelliferous *Cnidium cusson* plants. It was mainly for the treatment on various skin diseases especially for pruritus disease. In many topical treatment of pruritus compound, Cnidium also was used as medicine [2], commonly used in anti-fungal, anti-viral, deworming, ezema [3] and so on. In view of few studies on the ethanol extract of Cnidium, we analyzed its anti-type I hypersensitivity effect, these work may lay a strong foundation for the subsequent extraction and separation work.

Materials and Methods

Fructus cnidii was purchased from Beijing Tong Ren Tang, identified by Medical Laboratory of Capital Medical University, it was belonging to the umbelliferous *Cnidium Cusson* plants. Jidesheng pills was chose as positive control pill, produced by Nantong Jinghua Pharmaceutical Co., Ltd., batch number Z32130099, Size: 0.4 g × 20 × 3 packets. SPF grade Wistar rats, were purchased from the PLA Military Academy of Medical Sciences Laboratory Animal Center, Certificate of Conformity: SCXK-2012-008. SPF grade SD rats were purchased from Capital Medical University Experimental Animal Department, quality certificate number (SCSK Beijing 2012-0032). Evans blue reagents, lot was 20130105, produced by Sinopharm Chemical Reagent Co., Ltd., Acetone, Tianjin Kermel Reagent Co., Ltd., its Batch number was 20130207. UV2000 spectrophotometer produced by Unico company. Statistical data were all expressed as mean ± standard deviation. Intergroup comparison by t-test.

Effects of Fructus cnidii extract on rat PCA

Preparation: 30 Wistar male rats, weitht 180 ~ 220 g, 12 rats took the tail vein injection of saline, the remaining 18 rats took 1% fresh egg white liquid by tail vein injection, 50 mg/kg, at the same time, took intraperitoneal injection 5% Al $(OH)_3$ gel, 2 ml/per rat. The serum was separated 11 d after sensitization, centrifugation, -20°C in preservation. Another 60 Wistar male rats, weight 180 ~ 220 g, were randomly divided into six groups, the blank control (distilled water), model group (distilled water), *Fructus cnidii* extract low, medium and high dose groups (respectively containing crude drug 5.53, 11.06, 22.12 g/kg), traditional Chinese medicine Jidesheng *Fructus cnidii* positive control pills group, took by oral, Once/day for 7 days [4]. On the 6th day of drugs given, animal mid-line hair was removed respectively at the back, the prepared antiserum was diluted with 1:8 dilution saline, then injected intradermally

antiserum 0.1 ml/depilation point. Sensitization after 48 h (30 min after the last administration), the rats were injected with 0.5% evans blue protein injection (containing 1 ml% fresh egg white liquid) 1 ml/per rat, rats were sacrificed after 30 min, the dorsa locus coeruleus skin was cut out into pieces, 5 ml acetone-saline (7:3) mixed solution, centrifuged at the next day, and the supernatant was measured with spectrophotometer at a wavelength of 610 nm, the intergroup difference was compared [5], the percentage inhibition calculated and the results are shown in Table 1.

Rat skull periosteum mast cell degranulation experiments

Antiserum preparation: 4 SD rats weighing 180 ~ 220 g composed of male and female in equal. 5% fresh egg white liquid allergens were given by tail vein injection every other day, a total of five times. Blood was taken at the last 11 d after sensitization, antiserum containing IgE was prepared and stored at -4°C [6]. Antiserum was diluted to 1:10 with saline in use. Another 60 SD rats were composed of male and female in half weighing 180 ~ 220 g, were randomly divided into six groups, group and dose were same with the above part. Lavage 1 time/day, used for seven days. At 5d, control group injected with normal saline and the other groups were injected subcutaneously at head with anti-ovalbumin serum, the serum was 1:10 dilution, 0.2 ml/per rat. Sensitization after 48 h, each rat were intravenously 0.5% evans blue protein injection (containing 5% fresh egg white liquid), 1 ml/per rat. 30 mins later, were killed by cervical dislocation [7], the skull cap was cut, immediately immersed in ethanol, one hour later, fixed anhydrous methanol solution overnight, dyed with 0.18% neutral red ethanol solution for 1 h, skull periosteum stripped, put on the slide, calculate degranulation of mastocyte ratio and inhibition ratio. The results were shown in Table 2.

Extraction of rat peritoneal mast cells and culture

Normal SD rats were sacrificed and soaked in 80% ethanol, limbs were fixed on the operating table, calcium-free Tyrode's solution 20 ml (0.8 g NaCl, 0.02 g KCl, 0.005 g $NaH_2PO_4.H_2O$, 0.11g D-Glucose 100 ml), open the abdominal cavity, draw peritoneal fluid, centrifuged at 1500 rpm for 10 min, the supernatant was removed, take the cell suspension was slowly added 3:8 percoll gradient separation medium, 2500 rpm centrifuge 15 min, 30% and 80% junction cells were collected, washed for 3 times with PBS 4°C, centrifuged at 1500 rpm for 8 min, cell density were adjusted to 5 × 106 ~ 6 × 106/ml. Trypan blue showed viability greater than 95%, toluidine blue staining showed more than 90% purity [8].

Grouping

Blank control group, model group, *Fructus cnidii* extract (150, 75, 32.5,16.25 mg/L) and sodium cromolyn (0.3 mg/L). Drugs are formulated with serum-free RPMI 1640 medium, each tested in triplicate.

Drug intervention and stimulation of mast cell degranulation by stimulant

Cells were inoculated in 96-well culture plate with 5 × 106/ml cell solutions, 100 μl each well, pretreated for 12h (10 μl/well), 10 μl stimulant compound 48/80 (C48/80) was added (final concentration of 20 mg/L), incubated 2h at 37°C, centrifuged at 1500 rpm for 5min, and the supernatant was centrifuged, 120 μl PBS added, set in -80°C refrigerator repeated freezing and thawing for three times, got the supernatant after centrifugation(cell lysate). Histamine content was measured and calculated its release ratio [9]. Release ratio = content in supernatant/(content in supernatant+content in cell lysate) × 100% Data was analyzed with SPSS 17.0 statistical software, expressed by $\bar{x} \pm s$, intergroup differences were compared using SNK test, if $P<0.05$, the data was considered statistically significant.

Table 1. *Effects of Fructus cnidii extract on rat PCA.*

Group	Dose (g/kg)	OD(/ × 100)	Inhibition ratio (%)
Blank control	-	1.15 ± 0.58	-
Model control	-	11.9 ± 4.35Δ	-
Low dose extracts	5.53	9.32 ± 2.05	21.7
Middle dose extracts	11.06	7.55 ± 1.21*	36.6
High dose extracts	22.12	6.37 ± 1.48*	46.5
Jideshengshe pills	0.72	8.52 ± 1.83*	28.4

Notes: compared with blank control, ΔP<0.01; compared with model control, *P<0.05.

Table 2. *Rat skull periosteum mast cell degranulation test.*

Group	Dose (g/kg)	Degranulation (%)	Inhibition (%)
Blank control	-	6.51 ± 2.30	-
Model control	-	28.35 ± 11.58	-
Low dose extracts	5.53	25.64 ± 12.47	9.6
Middle dose extracts	11.06	17.52 ± 10.15	38.2
High dose extracts	22.12	13.66 ± 4.37	51.8
JiDeShengShe tablets	0.72	16.18 ± 5.63	42.9

Notes: compared with blank control, ΔP<0.01; compared with model control, *P<0.05.

Result

Effects of Fructus cnidii extract on rat PCA

The results showed that Fructus Cnidi iethanol extract could inhibit rat Passive Cutaneous Anaphylaxis (PCA) significantly, and the activity of middle dose group and high doses group were more active than the positive control (table 1).

Rat skull periosteum mast cell degranulation tests

Seen from Table 2, Degranulation in the model control group was significantly higher than the control group, the difference was significant (P<0.01), shows that successful model. *Fructus cnidii* extract high-dose group degranulation of mast cells was significantly lower than the model group, the difference was significant (P<0.05), showing that *Fructus cnidii* extracts could inhibit degranulation mast cell in skull periosteum greatly.

Effects on the histamine release in rat peritoneal mast cells

Rat peritoneal mast cells were stimulated by C48/80, histamine release ratio was significantly decreased (P<0.01), different doses of *Fructus cnidii* extract could significantly reduce the ratio of histamine release level which may originally rise (P<0.01), the action exhibited a dose-dependent manner (table 3).

Table 3. *Effects on the histamine release in rat peritoneal mast cells ($\bar{x} \pm s$, n=6).*

Group	Dose (mg/L)	Release ratio (%)	Release quantity (ng/L)	Total content (ng/L)
Blank control	-	29.48 ± 2.45	1.65 ± 0.07	5.6
Model control	-	58.52 ± 15. 24[2]	2.57 ± 0.41[1]	4.39
Fructus cnidii extracts	150	18.36 ± 4.50	0.67 ± 0.23	3.65
	75	25.59 ± 3.89	1.45 ± 0.18	5.67
	32.5	29.15 ± 2.56[4]	1.60 ± 0.17[3]	5.49
	16.25	40.62 ± 5.11[4]	1.73 ± 0.15[3]	4.26
Sodium cromoglycate	200	35.75 ± 5.18[4]	2.35 ± 0.18[4,3]	6.57

Note: Compared with blank control, [1]P<0.05, [2]P<0.01; Compared with model control [3]P<0.05, [4]P<0.01; concentration of Sodium cromoglycate was 0.3 mg/L.

Discussion

In this paper, traditional chinese medicine *Fructus cnidii* was extracted by 75% ethanol solvent, studied the effect of different extracts on mast cell histamine release in mast cell and the degranulation, the impact on rat Passive cutaneous anaphylaxis (PCA), inquired into the effects of extracts on type I allergic action. Type I hypersensitivity [10] is a common clinical disease which could also trouble the life quality of patients seriously. There [11] are various problems in the present anti-allergy medications, thus research and development of such drugs is currently a hot topic There are a lot of possible targets for anti-allergy drugs, at the same time, the associated treatments mainly focused on several aspects, including hormonal drugs (phospholipase A2 inhibitory proteins (lipid cortex protein) inducted, such as budesonide, prednisone, beclomethasone dipropionate, etc.; antihistamines (H1 receptor blockers), such as diphenhydramine, promethazine, mizolastine etc.; mast cells and basophils targeted drugs (lower mast cells and basophils number and inhibit its activity), such as bepotastine, Olopatadine Hydrochloride, Bepreve etc [12].

The experiment proved that *Fructus cnidii* ethanol extracts could strongly inhibit mice PCA reaction, suggesting that it could obviously inhibit the formation of IgE antibodies; *Fructus cnidii* ethanol extracts were effective on specific parts of the Type I hypersensitivity including mast cells degranulation, allergy mediators, sensitive organs etc., showing that it also could protect the target cell membrane, inhibit the release of biologically active substances, and to antagonize the effects of biologically active substances on the function of the effector organs. [13] According to modern pharmacological studies, *Fructus cnidii* have anti-mutagenic, anti-tumor, anti-arrhythmia effects. And its monomer Osthole shows obvious heart inhibition and blood vessels dilation effects. The content of Osthol and other monomers of *Fructus cnidii* could be well analyzed by HPLC [14], HPLC-MS, like Imperatorin, bergapten etc [15]. Relationship between the concentrations of these monomers and anti-allergic inhibition may be statistical. Perhaps that will provide a better foundation for discovery of anti- allergy drug, which is also the next step of our research.

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***Correspondence to:**

Xueyan Wang

Department of Allergy

Capital Medical University

PR. China