



## Effects of *Pithecellobium clypearia* Benth extract and its main components on inflammation and allergy

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### ABSTRACT

The ethanol extract of *Pithecellobium clypearia* Benth (PCE) was characterized to be rich in polyphenols by HPLC analysis, and investigated for its anti-inflammatory and anti-allergic activities. In our assay, PCE showed anti-inflammatory activity in both acute and auto-immune inflammation animal models. Administration of PCE can effectively inhibit the croton oil-induced ear edema and capillary permeability, the carrageenin-induced paw edema, and the liver injury caused by *propionibacterium acnes* plus lipopolysaccharide. PCE was also found to possess anti-allergic activity in inhibiting the DNFB-induced delayed hypersensitivity reaction. Meanwhile, seven main components (**1–7**) from PCE were studied for their effect on histamine release stimulated by compound 48/80 from rat peritoneal mast cells in vitro. Compound **2** ((–)-epigallocatechin-7-gallate), **3** ((–)-5, 7, 3', 4', 5'-pentahydroxyflavan), and **5** ((–)-tetrahydroxyflavan-7-gallate) showed significant inhibition effect on histamine release.

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### 1. Introduction

*Pithecellobium clypearia* Benth is a member of the Mimosaceae family growing in the south of China [1]. *P. clypearia* is recorded in the Pharmacopeia of China [2], and used for the treatment of upper respiratory tract infections, pharyngitis, laryngitis, acute tonsillitis, acute gastroenteritis, and bacterial dysentery. One product manufactured from the aqueous extract of *P. clypearia* has been clinically used as an anti-inflammation drug in China for many years. Phytochemical investigation of *P. clypearia* resulted in the isolation of tannins and flavonoids [3]. Previous pharmacological research on *P. clypearia* only revealed its antiviral activity [4,5]. Although the extract of *P. clypearia* has been identified to be effective for anti-inflammation in clinics, there is no experimental evidence to support its clinical usage. In this study, we first demonstrate the anti-inflammatory and anti-allergic effects of the extract of

*P. clypearia* in vivo, and test the effect of compounds isolated from *P. clypearia* on histamine release stimulated by compound 48/80 from rat peritoneal mast cells.

### 2. Materials and methods

#### 2.1. Plant material

The twigs and leaves of *P. clypearia* were collected at Conghua in Guangdong province of China, in October 2004, and identified by Bai-ying Liu (Associate chief pharmacist, Guangdong Institute of Drug Control). A voucher specimen (YGXYPC-2004) is deposited in Traditional Chinese Medicines and Natural Products Research Centre, Shenzhen, China.

#### 2.2. Extract and isolation of *P. clypearia*

The ethanol extract of *P. clypearia* (PCE) were partitioned with CHCl<sub>3</sub>, EtOAc, and *n*-BuOH, successively. The EtOAc extract was subjected to Diaion HP-20, Sephadex LH-20, MCI gel CHP-20P, and ODS column chromatography to yield 7 compounds [3]:

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gallic acid (**1**); (–)-epigallocatechin-7-gallate (**2**); (–)-5, 7, 3', 4', 5'-pentahydroxyflavan (**3**), ethyl gallate (**4**), (–)-tetrahydroxyflavan-7-gallate (**5**), myricitin-3-O- $\alpha$ -L-rhamnopyranoside (**6**), quercetin-3-O- $\alpha$ -L-rhamnopyranoside (**7**) (Fig. 1).

### 2.3. HPLC analysis of *P. clypearia*

Chemical components containing in PCE was analyzed by a HPLC system coupled with UV detector at 254 nm. Sample solution was prepared by dissolving 10 mg PCE in 1 ml MeOH. HPLC analysis was performed on Shimadzu SPC-10AT VP and a Shim-pack VP-ODS column (4.6  $\times$  250 mm, 5  $\mu$ m) at flow rate of 1.0 ml/min. Oven temperature is 30  $^{\circ}$ C. Two mobile phase solvents were used: H<sub>2</sub>O (A) and MeOH (B). A elution profile was: 0–5 min, 10%–20% B in A (linear gradient); 5–10 min, 20%–35% B in A (linear gradient); 10–20 min, 35% B in A (isocratic manner), 20–22 min, 35%–40% B in A (linear gradient); 22–35 min, 40% B in A (isocratic manner); 35–50 min, 40%–65% B in A (linear gradient). The retention times were observed for compounds **1**–**7** at 8.1, 17.0, 17.4, 21.0, 29.7, 30.2, 42.3 min, respectively (Fig. 2).

### 2.4. Animals

Seven-week-old male KM mice were purchased from the Center of Medical Laboratory Animal, Guangdong Province (Certificate of approval: 2003A023), China. All mice were kept in a specific pathogen-free animal room under the controlled condition of temperature (23  $\pm$  1  $^{\circ}$ C) and lighting (12 h dark-light cycle), and provided with standard laboratory diet and tap water. The animals were allowed to acclimatize to the environment for 1 week before the experiment.

### 2.5. Chemicals and materials

Croton oil, carrageenin, evans blue, compound 48/80, and DNFB were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). *O*-phthalaldehyde (OPA) and thiofluor were the products of Pickering Laboratories (Mountain View, CA). Cyclosporin A (CsA) and dexamethasone acetate (DEX) were obtained from Sigma chemical (St. Louis, MO, USA).

### 2.6. Croton oil-induced ear edema in mice

The croton oil ear test was performed according to references [6,7]. A total of 25  $\mu$ l of an acetonic solution containing 75  $\mu$ g of croton oil was applied to the inner surface of the right ear for each mouse (about 1 cm<sup>2</sup>). The left ear remained untreated. The animals were sacrificed by cervical dislocation 4 h later and a plug (8 mm in diameter) was removed from both the treated and the untreated ear. The difference in weight between the two plugs was taken as a measure of edematous response. DEX (1 mg/kg, served as the reference) and PCE (125 mg/kg, 500 mg/kg) were orally administered to mice twice at 10 min and 1 h prior to the application of croton oil, respectively.

### 2.7. Croton oil-induced capillary permeability in mice

Mice were treated with croton oil as described in the Croton oil-induced ear edema in mice section. 3 h after blazing, 0.2 ml of 1% evans blue was injected via the tail vein. The animals were sacrificed by cervical dislocation 1 h later and a plug (8 mm in diameter) was removed from ear. The plugs were soaked in 1 mL of 1 N KOH at 37  $^{\circ}$ C for 24 h, added 9 ml 0.6 N H<sub>3</sub>PO<sub>4</sub>-acetone (5:13), and centrifuged at 3000 rpm for 15 min. The supernatants were detected at 620 nm [8].

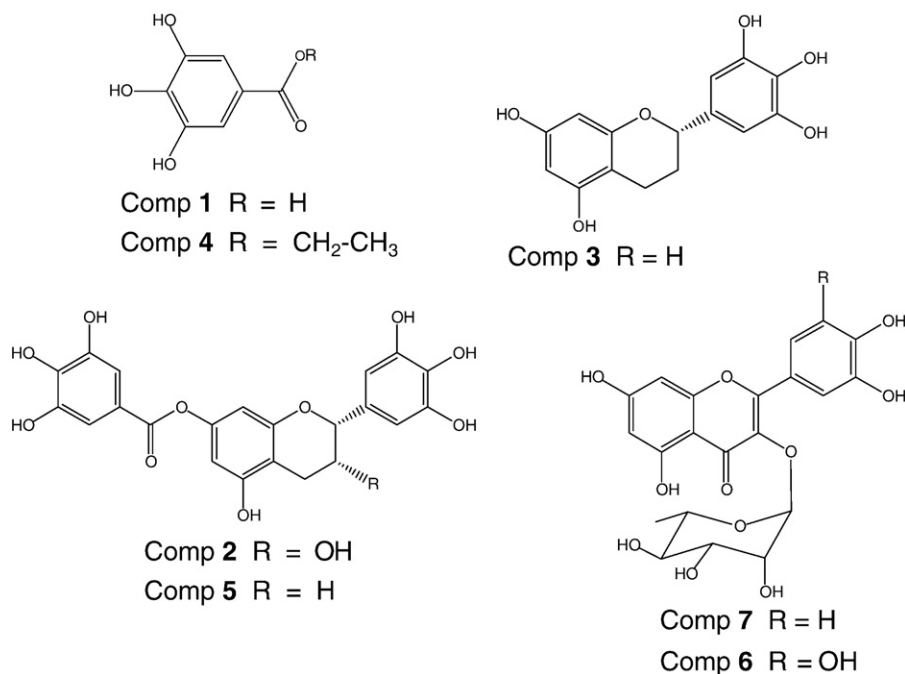


Fig. 1. The structures of compounds 1–7 from *Pithecellobium clypearia* Benth.

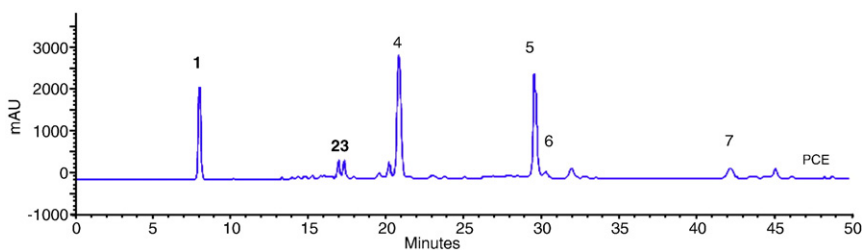


Fig. 2. The HPLC fingerprint analysis of the ethanol extract from the leaves and stems of *P. clypearia*.

## 2.8. Carrageenin-induced paw edema in mice

The carrageenin-induced paw edema model in mice was used to study the effect of PCE on acute inflammation [9]. DEX (1 mg/kg, served as the reference) and PCE (125 mg/kg, 500 mg/kg) was orally administrated to mice twice at 10 min and 1 h before injecting 10  $\mu$ l of carrageenin (2.0%, w/v) into subplantar area of the right hind paw respectively. Measurement of paw size was carried out with caliper rule (Digimatic, Mitutoyo 500, Japan) 4 h following carrageenan injection.

## 2.9. *P. acnes* plus LPS-induced liver injury

PCE (125 mg/kg, 500 mg/kg) and Cs A (1 mg/kg, served as the reference) were orally administrated to mice for 7 d, while the normal control group and *P. acnes*-LPS control group received water only. Heat-killed *P. acnes* was injected through the tail vein at 0.3 mg/mouse, and after 5 d, LPS (0.1  $\mu$ g/mouse) was injected intravenously. The animals were sacrificed for analysis of liver injury 5 h after the LPS injection [10]. Blood samples were taken from the hearts of the mice under anesthesia with diethyl ether into a tube containing 2% sodium heparin. The tubes were centrifuged at 5000 rpm for 5 min and the supernatants were used as samples. All samples were stored at  $-20^{\circ}\text{C}$  until the assay. Plasma ALT activity, which is a marker of hepatocyte injury, was determined with a MK<sub>3</sub> microplate reader (LabSystems Co., Finland) at 492 nm.

## 2.10. Inhibitory effect on DNFB-induced delayed hypersensitivity reaction (DHR)

The DNFB-induced DHR was performed as Dai et al. described [11]. Animals were randomly divided into four groups. The control group was treated with normal saline (0.2 ml/10 g i.g.). PCE (125 and 500 mg/kg) and Dex (1 mg/kg, served as the reference) were respectively administrated to the three test groups for 7 d. 50  $\mu$ l of 1% DNFB in acetone-olive oil (4:1) was injected on the back of mice for sensitization after 6 h of administering PCE on the first day. 25  $\mu$ l of 1% DNFB was applied to the right ear of the mice after 1 h of the last administration. 24 h later, ear discs were removed from both ears of the mice using a cork borer with the diameters of 8 mm. The weight difference between the left and the right ear disc was used as an indication for the degree of hypersensitivity.

## 2.11. Histamine analysis in vitro

Isolation of rat peritoneal mast cells was performed as described by Sullivan et al. [12]. Briefly, cells from a peritoneal lavage were washed with mast cell medium (MCM: 150 mM NaCl, 3.7 mM KCl, 3 mM dibasic sodium phosphate, 3.5 mM monobasic potassium phosphate, 0.9 mM CaCl<sub>2</sub>, 0.1% glucose, 0.1% bovine serum albumin, pH 6.8) and 10 units/ml heparin and separated by centrifugation (600 rpm) on a single step gradient of MCM.

Purified mast cells were preincubated with 250 mM CaCl<sub>2</sub> and compounds isolated from PCE before activation by 5  $\mu$ l 0.05 mg/ml compound 48/80. Reaction was stopped 10 min later by chilling the tubes in ice bath, then centrifuged at 6000 rpm for 2.5 min. The supernatants were then treated with 20  $\mu$ l of 60% perchloric acid (PCA), and centrifuged at 13,000 rpm for 2.5 min. The resulting supernatants were collected for histamine determination.

Histamine levels were analyzed by HPLC combined with a 4-channel CoulArray electrochemical detector. After derivatizing with *o*-phthalaldehyde, histamine levels in samples were detected on a Capcell Pak C18 column (3  $\mu$ m, 3.0  $\times$  50 mm) eluted with a mobile phase consisting of 22% methanol and 13% acetonitrile in 100 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 6.8). The cell was set at +550 mV.

## 2.12. Statistics

The data were presented as mean  $\pm$  SEM. Statistical analysis of data was performed using SPSS 13.0 statistical package program for windows. One-way analysis of variance (ANOVA) was applied to analysis for difference in data of biochemical parameters among the different groups followed by Dunnett's significant Post-Hoc test for pair-wise multiple

Table 1

Effects of PCE on ear-swelling and capillary permeability of mice induced by croton oil.

Group	Dose (mg/kg)	n	Ear-swelling (mg)	Inhibitory rate (%)	Absorbance
Control	–	7	16.71 $\pm$ 3.59	(–)	0.65 $\pm$ 0.20
PCE	125	7	15.07 $\pm$ 5.15	(9.81)	0.54 $\pm$ 0.24*
	500	7	12.71 $\pm$ 4.20*	(23.94)	0.52 $\pm$ 0.25*
DEX	1	7	0.08 $\pm$ 0.26**	(99.52)	0.20 $\pm$ 0.15**

Data are mean  $\pm$  S.E.M. \* $p$  < 0.05, \*\* $p$  < 0.01 as compared to control group.

**Table 2**  
Effects of PCE on edema hind paw of mice induced by carrageenan.

Group	Dose (mg/kg)	n	Edema hind paw (mm)	Inhibitory rate (%)
Control	–	7	1.11 ± 0.32	–
PCE	125	7	0.97 ± 0.38	12.61
	500	7	0.83 ± 0.30*	25.23
DEX	1	7	0.25 ± 0.26**	77.48

Data are mean ± S.E.M. \* $p < 0.01$ , \*\* $p < 0.001$  as compared to control group.

comparisons. Differences were considered to be statistically significant at  $p < 0.05$  level.

### 3. Result

#### 3.1. Anti-inflammatory effects of PCE

As shown in Table 1, PCE produced a dose-related inhibition of ear oedema with topical application of croton oil. Topical application of croton oil-induced cutaneous inflammation at the ears of mice, which caused significant increase in ear plug weight of the right ear compared with the untreated left ear. As a positive control, DEX (1 mg/kg) gave rise to a significant inhibition of 99.52% in ear plug weight. PCE at 500 mg/kg produced inhibition of ear oedema by 23.94%.

PCE at doses of 125 and 500 mg/kg also showed inhibitory effects on croton oil-induced capillary permeability in mice (Table 1).

In the carrageenan-induced oedema test, the paw volumes and percentages of inhibition by PCE and DEX are shown in Table 2. A maximum increase of paw circumference,  $1.11 \pm 0.32$  mm was obtained after 4 h following carrageenan injection in control mice. PCE significantly decreased the carrageenan-induced oedema at doses of 500 mg/kg and achieved its maximal inhibitory effects of 25.23% ( $p < 0.05$ ) at 4 h after carrageenan injection. As a reference control, dexamethasone (DEX) significantly inhibited the hind paw swelling at a dose of 1 mg/kg and attained its maximal effect at 4 h after carrageenan injection.

#### 3.2. Effect of PCE on *P. acnes* plus LPS-induced liver injury

ALT measurement was performed using plasma obtained from mice 5 h after LPS administration. As shown in Table 3, the ALT activity in the *P. acnes*-LPS-induced liver injury mice was significantly higher than that in the normal mice ( $p < 0.001$ ). Treatment with PCE can significantly reverse the

**Table 3**  
Effect of PCE on plasma ALT activity in mice treated with *P. acnes* + LPS.

Group	Dose (mg/kg)	n	ALT activity (IU/l)	Inhibitory rate (%)
Normal control	–	7	26.80 ± 3.10	–
<i>P. acnes</i> -LPS alone	–	7	209.36 ± 13.57 <sup>#</sup>	–
<i>P. acnes</i> -LPS + PCE	125	7	67.40 ± 23.25**	67.81%
<i>P. acnes</i> -LPS + PCE	500	7	92.73 ± 29.70*	55.71%
<i>P. acnes</i> -LPS + Cs A	1	7	54.76 ± 16.52*	73.84%

Data are mean ± S.E.M. <sup>#</sup> $p < 0.001$  as compared to control group. \* $p < 0.05$ , \*\* $p < 0.01$  as compared to *P. acnes*-LPS group.

**Table 4**  
Effects of PCE on ear-swelling of mice induced by DNFB.

Group	Dose (mg/kg)	n	Ear-swelling (mg)	Inhibitory rate (%)
Control	–	7	27.48 ± 5.63	–
PCE	125	7	26.62 ± 3.76	3.13
	500	7	22.43 ± 1.45*	18.38
DEX	1	7	18.25 ± 0.26**	33.59

Data are mean ± S.E.M. \* $p < 0.05$ , \*\* $p < 0.01$  as compared to control group.

ALT activity with the inhibition rate of 67.81% ( $p < 0.01$ ) at doses of 125 mg/kg.

#### 3.3. Anti-allergic effects of PCE on ear-swelling induced by DNFB in mice

As shown in Table 4, the treatment with DNFB in sensitized mice resulted in a biphasic skin reaction. As a positive control, DEX (1 mg/kg) gave rise to a significant inhibition of 33.59% in ear plug weight ( $p < 0.01$ ). PCE at 500 mg/kg produced inhibition of ear oedema by 18.38% ( $p < 0.05$ ).

#### 3.4. Effect of compounds 1–7 isolated from PCE on histamine release from rat peritoneal mast cells induced by compound 48/80

Histamine level was 4.66% in spontaneous release group and 42.99% in compound 48/80 stimulated group. Compounds 2, 3, 5 and PCE clearly reduced the histamine release induced by compound 48/80 from rat peritoneal mast cells (Table 5).

### 4. Discussion

Based on the early chemical study, the HPLC analysis was conducted on the ethanol extract of *P. clypearia*. Seven phenolic compounds accounting for 77.2% total peak area were detected, including gallic acid, (–)-epigallocatechin-7-gallate, (–)-5, 7, 3', 4', 5'-pentahydroxyflavan, ethyl gallate, (–)-tetra hydroxyflavan-7-gallate, myricitin-3-O- $\alpha$ -L-rhamnopyranoside, quercitin-3-O- $\alpha$ -L-rhamnopyranoside.

In the present study, the anti-inflammatory and anti-allergic effects of PCE were evaluated by different animal models. The results demonstrate that PCE can play a significant effect in the inhibition of inflammatory and allergic processes.

**Table 5**  
Effect of compounds isolated from PCE on histamine release from rat peritoneal mast cells induced by compound 48/80.

Compound	Dose (500 $\mu$ g/ml)		Dose (50 $\mu$ g/ml)	
	Histamine release %	Inhibition (%)	Histamine release(%)	Inhibition (%)
Compound 48/80	42.99 ± 2.1	–	–	–
Compound 48/80 + 1	44.93 ± 3.4	– 5.06	44.43 ± 3.5	– 3.76
Compound 48/80 + 2	21.29 ± 1.8	56.61	28.94 ± 3.2	36.66
Compound 48/80 + 3	33.53 ± 2.5	24.68	34.33 ± 4.3	22.59
Compound 48/80 + 4	41.92 ± 3.2	2.79	40.34 ± 2.9	6.91
Compound 48/80 + 5	31.03 ± 2.7	31.20	35.63 ± 3.3	19.20
Compound 48/80 + 6	39.74 ± 3.2	8.48	41.32 ± 4.1	4.36
Compound 48/80 + 7	37.30 ± 2.3	14.84	40.83 ± 3.7	5.64
Compound 48/80 + PCE	32.34 ± 3.0	27.79	36.92 ± 3.2	15.84

Data are means of six experiments.

The effects of PCE on acute inflammation were studied on croton oil-induced ear edema and carrageenan-induced paw oedema. It was seen that high dose of PCE reduced inflammation caused by croton oil and carrageenan. Application of croton oil and carrageenan can induce significant inflammatory responses characterized by oedema, neutrophil infiltration, prostaglandins production and increases in vascular permeability [13,14]. The action of croton oil and carrageenan was believed to be involved in or to be dependent on arachidonic acid release and metabolism by both cyclo-oxygenase and lipoxygenase enzyme pathways [15]. Thus, results obtained in our assay suggest that PCE have similar pharmacological properties with those of lipoxygenase and cyclo-oxygenase inhibitors, which might be related to the polyphenols containing. The polyphenols have been recognized as potent inhibitors of lipoxygenase and cyclo-oxygenase [16–18].

Taking into account that PCE's inhibition on the paw oedema and ear edema induced by several mediators might be involved in allergic response, we also evaluated the anti-allergic actions for PCE. The data indicated that PCE can significantly reduce ear-swelling induced by DNFB.

The effect of PCE on *P. acnes* plus LPS-induced auto-immune active hepatitis was investigated as well. When mice was pretreated with PCE once a day for 5 d before intravenous LPS injection, plasma ALT activity was significantly decreased compared with normal controls. LPS can increase histidine decarboxylase (HDC) activity, leading to the production of histamine [19–21]. Histamine is known as one major chemical mediator in the regulation of inflammatory responses, immunity and allergic reactions [22,23]. In our early report, histamine has been found to play an important role in auto-immune active hepatitis [10]. Therefore, we tested the effect of PCE and compounds 1–7 isolated from PCE on histamine release induced by compound 48/80 from rat peritoneal mast cells in vitro. As a result, (–)-epigallocatechin-7-gallate (**2**), (–)-5, 7, 3', 4', 5'-pentahydroxyflavan (**3**), and (–)-tetra hydroxyflavan-7-gallate (**5**) showed good inhibitory effect, which is consistent with previous report that polyphenols showed potent inhibitory effect on the histamine release [24]. Base on above analysis, we proposed that inhibition of PCE on auto-immune active hepatitis, acute inflammation and allergic response maybe due to its inhibitory effect on the histamine release.

In conclusion, PCE inhibited auto-immune inflammation, acute inflammation and allergic response *in vivo*, and significantly inhibited histamine release induced by compound 48/80 from rat peritoneal mast cells. Although the anti-inflammatory and anti-allergic activities of PCE are relatively weaker than those of reference control, PCE is still considered to be useful as an anti-inflammatory herbal medicine due to its few side effects. The current finding supports the clinical application of PCE in China as an inflammation drug.

## References

- [1] The Editorial Board of Flora of China, Chinese Academy of Science. Flora of China [M], vol. 39. Beijing: Science Press; 1988. p. 50.
- [2] Pharmacopeia Committee of the Ministry of Public Health, China. Pharmacopeia of the People's Republic of China 1992. Beijing, China: Chemical Industry Press; 1992. p. 158.
- [3] Guo X-Y, Wang N-L, Bao L, Li Y-H, Xu Q, Yao X-S. J Chin Pharmaceu Sci 2007;16:208.
- [4] Li Y-L, Leung K-T, Yao F-H, Ooi LSM, Ooi VEC. J Nat Prod 2006;69:833.
- [5] Li Y-L, Li K-M, Su M-X, Liang J-T, Chen Y-W, Zhang Y-W. China J Chin Mater Med 2006;31:397.
- [6] Tubaro A, Dri P, Delbello G, Zilli C, Della Loggia R. Agents Actions 1985;17:347.
- [7] Zitterl-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavec M, Della Loggia R, Tubaro A, et al. J Ethnopharmacol 1997;57:139.
- [8] Katayama S, Shionoya H, Ohtake S. Microbiol Immunol 1978;22:89.
- [9] Winter CA, Risle EA, Nuss GW. Proc Soc Exp Biol Med 1962;111:544.
- [10] Kurihara H, Fukami H, Asami S, Shibata H, Kiso Y, Tanaka T, et al. Biol Pharm Bull 2003;26:1393.
- [11] Dai Y, Hang B-Q, Meng Q-Y, Ma S-P, Tan L-W. Acta Pharmacologica Sinica 1988;9:562.
- [12] Sullivan TJ, Parker KL, Stenson W, Parker CW. J Immunol 1975;114:1473–9.
- [13] Rao TS, Currie JL, Shaffer AF, Isakson PC. Inflammation 1993;17:723.
- [14] DiRosa M, Giroud JP, Willoughby DA. J Pathol 1971;104:15.
- [15] Gamache DA, Povlishock JT, Ellis EF. J Neurosurg 1986;65:679.
- [16] Hong J, Smith TJ, Ho CT, August DA, Yang CS. Biochem Pharmacol 2001;62:1175.
- [17] Laughton MJ, Evans PJ, Moroney MA, Michele A, Hoult JRS, Halliwell B. Biochem Pharmacol 1991;42:1673.
- [18] Gryglewski RJ, Korbut R, Robak J, Swies J. Biochem Pharm 1987;36:317.
- [19] Funayama H, Mayanagi H, Takada H, Endo Y. Infect Dis 2001;184:1566.
- [20] Katoh Y, Niimi M, Yamamoto Y, Kawamura T, Morimoto-Ishizka T, Sawada M, et al. Neurosci Lett 2001;305:181.
- [21] Funayama H, Mayanagi H, Takada H, Endo Y. Arch Oral Biol 2000;45:787.
- [22] Schneider E, Rolli-DerKinderen M. Trends Immunol 2002;23:255.
- [23] Bachert C. Allergy 2002;57:287.
- [24] Osawa K, Miyazaki K, Imai H, Arakawa T, Yasuda H, Takeya K. Nat Med (Tokyo) 1999;53:188.