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ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF *R.A.P. (RADIX ANGELICAE PUBESCENTIS)* ETHANOL EXTRACTS

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Abstract

The objective of this paper was to study the anti-inflammatory and analgesic effects of *Radix Angelicae Pubescentis* (R.A.P) ethanol extracts. Three classic anti-inflammatory models and two analgesic models were used in this research. In anti-inflammatory tests, all the extracts have a certain inhibition on the acute inflammation induced by xylene, however, 60% ethanol extract significantly inhibited the inflammation in the three models. In analgesic experiment, compared with the blank control group, the comparisons between R.A.P. groups and control group had significant difference ($p < 0.01$). The incubation period in mouse writhing test or the tail-curl immersion tests could be extended greatly.

Keywords: Heracleum, Soxhlet extraction, anti-inflammatory, analgesic

Introduction

Radix Angelicae Pubescentis (R.A.P.) is mainly produced in Sichuan, Hubei, Anhui and other provinces in China. Usually its excavation takes place early spring or late fall. It is common to remove the fibrous roots and sediment, half dried above the heated mud. They were piled for 2 to 3 days, then heated to dry absolutely when they become soft. Modern research wrote that R.A.P. have anti-inflammatory, analgesic and sedative effects; inhibition of platelet aggregation; antihypertensive effect, but not lasting for a longer time (Zhu et al., 2007; Liu et al., 1994; Zhang et al., 2008).

Columbianetin, Columbianetin acetate, Columbiadin, osthol, isoimperatorin, bergapten, xanthotoxin, Columbianetin- β -D-glucopyranosid, etc are the main compounds from the ethanol extracts of the R.A.P. (Lin et al., 2011). Bergapten and xanthotoxin has photosensitive and anti-tumor effects. At the same time, it also can cure headache induced by cold weather, rheumatism, lassitudache etc (Deng et al., 2004; Zhang et al., 2002; Li et al., 2011). This paper evaluates the mechanism of the anti-inflammatory and analgesic activities of the alcohol extract of R.A.P..

Materials and Methods

Reagents

Aspirin enteric-coated tablets (Beijing Shuguang Pharmaceutical Limited Company), Glacial acetic acid (AR, Beijing yili fine chemicals limited company), xylol (Beijing chemical plant), Diclofenac diethylamine Cream (Beijing Novartis Pharmaceutical Limited Company), Absolute alcohol (Sinopharm Chemical Reagent Co., Ltd).

Instruments

Precision electronic balance (BSA124S, Sartorius), Soxhlet extractor (Hang zhou huier instrument equipment co., LTD), Gas bath thermostats oscillator (ZD-85, Changzhou guohua electric appliance co., LTD), 6 mm puncher (deli, China, No 0104)

Drug Preparation

The sources of the three different concentrations of ethanol (40%, 60%, 80%) of the test extract solution were got by diluting the absolute alcohol with distilled water, alcohol extracts were prepared by soxhlet extraction, extract ointment was stored in the refrigerator (4 °C), adjusted to 20 °C when used. R.A.P. was purchased from Liaoning Benxi Senxiu Medicinal Processing Limited Liability Company (harvested in October 2010) in May 2011. The specimen was identified by Dr. Yunpeng Diao of Dalian Medical University. It is affiliated to the umbelliferous plants, dried marshmallow root of *Angelica pubescens*. The voucher specimens were kept in crude drugs specimen room (1050112). The research period was from May 2011 to October 2011.

Animals

Healthy male, Kunming mice were purchased from The Animal Centre of Capital Medical University, weight ranged

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from 18g–22g. All animals had been used only for this research, not for any other purpose. Animal ethical consideration was considered in using the animals.

Anti-inflammation tests (Xu et al., 2012; Deng et al., 2004)

The R.A.P. extract effects on xylene-induced mouse ear edema

50 mice were randomly divided into blank control group, the aspirin group, the extracts group. R.A.P. groups (40%, 60%, 80%, three different concentrations of ethanol extracts group, and 10 mice in each group. Administration of ethanol group was 1.5g/kg, the positive control was 0.03g/kg aspirin suspension, the blank control was the same volume of distilled water, once a day, continued for seven days, and 30 minutes after the last administration. 20 μ L xylene was coated on both sides of each mouse right ear to induce inflammation. Left ear being a normal ear control, inflammation was assessed after one hour, ears were cut off along the pinna baseline, 6mm diameter hole puncher to punch down auricle at the same position of the double ear, and they were immediately weighed on an analytical balance, in order to calculate swelling (mg) and inhibition rate.

Swelling degree (mg) = weight of right auricle - weight of left auricle

Swelling inhibition rate (%) = $\frac{\text{Average swelling degree of swelling in blank group} - \text{Average degree of swelling in extracts group}}{\text{Average swelling degree of swelling in blank group}}$

The R.A.P. extract effects on the mice pettitoes swelling by egg white

50 male mice were randomly divided into three groups and we adopted oral administration as the route of drug administrations. Distilled water without drugs as a blank control group, 1.5 g/kg of the 40%, 60%, and 80% R.A.P. ethanol extract as dose group (containing three groups) and 0.005g/kg dexamethasone as a positive control group were administered to mice. All of the drugs were administered once a day, and continued for three days. We marked at the back of the right ankle joint inflammatory. And test the volume of the marked ankle joint for two times, and the average value was used as the normal joint volume. 30min after the last administration, 0.1ml 10% the fresh egg white was injected around the marked zone for each group mouse, and test the volume after inflammatory response. Thus the joint volume change values were worked out.

Joint swelling volume(ml) = Volume before inflammatory - Volume after inflammatory.

Mouse tampon granulation swelling test

The left groin skin of 50 healthy male mice was disinfected with 75% ethanol, and a small hole was cut out with ophthalmic scissors. Autoclaved cotton balls (10 \pm 0.5) mg were soaked with gentamicin, implanted in left subcutaneous armpit. Animal groups and the methods of administration are same with the xylene-induced mouse ear swelling test. Drugs were administered once a day, for seven days. 24h after the last administration, cotton balls and the connective tissue around cotton balls were taken out, and the fat removed. The processed tissues were dried and weighed, so the granulation weight can be worked out by the tissue weight minus the weight of cotton balls, Granulation Index (mg/10g) = granulation weight / body weight \times 10.

The analgesic experiments (Song et al., 2006; Fan et al., 2009)

Methods of acetic acid-induced writhing and tail-immersion test

Acetic acid-induced writhing and tail-immersion tests were used to study the analgesic effects. Fifty mice (20 \pm 2) were used, female and male in equal proportion. The whole mice were randomly divided into five groups, ten mice in each group. Consisting of blank group, R.A.P. groups (40%, 60%, 80%), Aspirin groups. The blank group received 0.2ml normal saline /one mouse; aspirin group, 20 mg/ml, in 0.2ml per mouse of the test substance. The dose of extracts was 1.5g/kg (1.5g extracts / mouse weight), one hour later, 0.2ml 0.6% glacial acetic acid was injected, while the writhing times in 15min was recorded immediately. Analgesic rate was worked out by the following formula.

Analgesic rate = $\frac{\text{blank control group average writhing times} - \text{average extract group writhing times}}{\text{blank control group average writhing times}} \times 100\%$.

The method of tail-immersion test was the same with acetic acid-induced writhing, the only difference was the recording time, which was 0.5h after the administration to record the analgesic effect of the drug in mice.

Results

Anti-inflammatory experiments

Xylene-induced mice ears edema

Table 1 shows that all of the extracts have a certain inhibition on the acute inflammation induced by the xylene, however, 60% ethanol extract significantly inhibited the inflammation, thus the mouse ear was significantly reduced. From the Table we also found that the dose-effect relationship with the blank control group, was statistically significant different (P <0.05).

Table 1: R.A.P. ethanol extract of mouse ear edema induced by xylene

Group	Dose (g·kg ⁻¹)	The number of animals	The degree of swelling (mg)	Inhibition rate *100
Blank		10	12.14±2.56	
40% ethanol extract	1.5	10	8.21±1.74	32.37
60% ethanol extract	1.5	10	6.52±1.09*	46.29
80% ethanol extract	1.5	10	8.05±3.64	33.69
Voltaren	0.02	10	5.23±2.05**	56.92

Compared with blank control, **P* < 0.05, ***P* < 0.01, n=10

Mice pettitoes swelling by egg white

The anti-inflammatory effect of extracts in this test are shown in **Table 2**. The restraining effects of the three extracts were all good, and the longer the period after administration, the better the inhibitory effect. 60% ethanol extracts didn't exhibit obvious inhibitory effect until the second hour, and continued until the fourth hour. And the other two extracts also showed some low depressor level.

Table 2: Heracleum alcohol extract of egg white induced mice paw edema

Group	Dose (g·kg ⁻¹)	Foot volume before inflammation (ml)	Foot volume after inflammation (ml)			
			30min	60min	120min	240min
Blank	—	1.21±0.07	0.92±0.13	0.85±0.11	0.74±0.12	0.73±0.09
40%	1.5	1.18±0.15	0.83±0.15	0.75±0.08	0.72±0.09	0.65±0.02
60%	1.5	1.21±0.12	0.80±0.14	0.70±0.04	0.53±0.07**	0.42±0.03
80%	1.5	1.34±0.07	0.84±0.16	0.73±0.12	0.65±0.06	0.51±0.07
Dexameth- asone	0.005	1.22±0.10	0.52±0.19	0.62±0.21*	0.39±0.18**	0.24±0.11*

Compared with blank control, **P* < 0.05, ***P* < 0.01, n=10

Mouse tampon granulation swelling test

The data of granulation index in this test are shown in Table 3. The results showed that 40% and 80% ethanol extracts almost had the same depressor effects on the granulation growth, however the 60% ethanol extract had a more obvious restraining action. As a whole, the ethanol extracts of the R.A.P. had a satisfying inhibition effect.

Table 3: Ethanol extracts in mouse tampon granulation swelling test ($\bar{X} \pm s$)

Group	Dose g·kg ⁻¹	Granulation weight mg	Granulation index mg·(10g) ⁻¹
Blank group	—	19.15±5.82	9.58±2.32
40% ethanol extract	1.5	14.30±5.33	7.15±2.56
60% ethanol extract	1.5	12.95±4.21*	6.47±2.08*
80% ethanol extract	1.5	13.80±4.08*	6.90±1.95*
Dexamethasone	0.005	11.72±4.52**	5.86±2.14**

Compared with blank control, **P* < 0.05, ***P* < 0.01, n=10

Analgesic experiments

Acetic acid-induced writhing tests

The data of the analgesic effect of the R.A.P. on the mice are shown in Table 4. R.A.P. groups and aspirin group writhing reaction times were all significantly lower than the control group. Observation at 60 minutes after administration

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showed that all of the three extracts could extend the incubation period of the mouse writhing induced by acetic acid. Compared with the blank control group, the comparisons had significant difference ($p < 0.01$). 60% ethanol extracts had the biggest inhibition rate, and the other extracts group had lower inhibition compared with the Aspirin group. In brief, the ethanol extracts of the R.A.P. had a excellent inhibitory effect on the pain induced by acetic acid in mice.

Table 4: Effect of Heracleum extract on the writhing action induced by acetic acid

Group	Dose (g·kg ⁻¹)	The number of animals	The number of Writhing	Inhibition rate %
Blank group		10	24.20±5.36	-
40% ethanol extract	1.5	10	14.26±2.41	41.07
60% ethanol extract	1.5	10	10.31±3.26*	57.40*
80% ethanol extract	1.5	10	12.63±2.58	47.81
Aspirin group	0.2	10	8.72±4.53**	63.97**

Compared with blank control, * $P < 0.05$, ** $P < 0.01$, n=10

Analgesic effects of the alcohol extract in tail-curl in mice tail immersion tests

Table 5 shows that the three kinds of ethanol extracts had analgesic effects after 60 minutes of administration, in which the tail-curl latency in mice tail immersion tests was prolonged. Each extract group compared with the control group exhibited significant difference. 60% and 40% extracts at 120min still existed analgesic effects ($P < 0.05$).

Table 5: Effect of ethanol extracts on mice in tail-immersion test

Group	The incubation periods (s)	The incubation period after Administmiceion (s)			
		30min	60min	90min	120min
Control	7.5±0.3	7.6±0.2	7.4±0.6	7.7±0.5	8.1±0.4
40% ethanol extract	7.4±0.5	7.6±0.3	7.9±0.7	10.2±0.1**	10.3±0.5**
60% ethanol extract	7.8±0.4	8.2±0.2	8.8±0.6	9.5±0.4**	9.9±0.9**
80% ethanol extract	7.6±0.7	7.4±0.3	7.5±0.5	8.4±0.6	8.5±0.4

Compared with blank control, * $P < 0.05$, ** $P < 0.01$, n=10

Discussion

Three classic anti-inflammatory models and two analgesic models were used in this research found that *Radix Angelicae Pubescentis* (R.A.P) ethanol extracts had satisfying anti-inflammatory and analgesic effects. Just like paclitaxel extracted from the yew roots had broad-spectrum anti-cancer effects (Wang et al., 2006), specific compounds may need to be extracted by methods of Natural Medicinal Chemistry. The major component of Angelica was coumarin., most of other components were volatile oils. Dominant parts of edible *Aralia elata* were diterpene, triterpenes, phytosterol, organic acid, saccharide. Inflammation was the defense reaction mainly to the danger caused by various injurious factors. The pathological process of the R.A.P. extracts was generally divided into three periods: capillary permeability in early phase, exudation and oedema; leucocyte migration in middle phase; the connective tissue were proliferated in late stage (Zhou et al., 2012).

Some research tested the anti-inflammation of R.A.P.'s volatile oil in mice macrophage inflammatory reaction model induced by lipopolysaccharide (LPS), and found that it could inhibit the hydrolysis activity of the N-acylethanolamine-hydrolyzing acid amidase (NAAA), increase the level of PEA, decrease the expression of the inflammation factor (Sun et al., 2011). At the same time, the research demonstrated that the effect of *Angelicae pubescentis radix* (APR) on inflammation was mediated by the inhibition of NAAA activity, which increases the cellular endobioactor PEA levels and decrease pro-inflammatory factor. The results suggested that APR can serve as a nature NAAA inhibitor.

The foot - plate swelling either in primary and secondary stage could be obviously inhibited by DHJST (Wang et al., 2008). Ostarthritis in knee can also be relieved (Yu et al., 2010). The therapeutic effects of DHJST (a traditional Chinese herbal medicine used to treat osteoarthritis), containing the R.A.P., on cartilage degradation in a rabbit model of osteoarthritis was also investigated. DU HUO JI SHENG TANG (DHJST) exerted significant effect on osteoarthritis in rabbits, and mechanisms are associated with inhibition of VEGF and HIF-1 α expression (Chen et al., 2011).

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Though researchers have done many effective and reliable results from many meaningful trials, the scientific and systematic methods of getting the satisfying extracts especially for some active compounds still need much work, and the clear action mechanism on anti-inflammation or analgesic of the compounds isolated from R.A.P. are also waiting for the scientific answers.

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