Adjuvant arthritis experiment

1. 1 Experimental Animals

Adult Imprinting Control Region (ICR) mice of both sexes (19-22g) and Sprague Dawley (SD) rats of female (180-220g) were used for the experiment. The animals were procured from the Hunan slack king of laboratory animal Co. Ltd. and maintained in the animal house of the College of Pharmacy, Hunan Chinese Medical University for 3 days for acclimation. They were housed in the standard cages under standard environmental conditions of room temperature at 25±1â„ƒ, relative humidity 55-65% , with 12h light-dark cycle and supplied with standard rodent food and water *ad libitum* . The animals were fasted (with free access to water) overnight before dosing for anti-inflammatory and anti-nocicepitive test. All experiments were conducted in accordance with the National Institutions of Health Guide for the Care and Use of Labortory animals. The experimental protocol was approved by the Committee on Animal Research, Zhejiang Academy of Traditional Chinese Medicine. All efforts were made to minimize the number of animals used and their suffering.

1. 2 Chemicals and Reagents

Dexamethaone acetate was purchased from Zhejiang Xianju Pharmaceutical Co., Ltd, (No. 140204). Aspirin was purchased from Bayer Healthcare Manufacturing S. r. l. (NO.). dimethylbenzene was purchased from Chengdu Kelong Chemical Co., Ltd, (No. 20130514). Carrageenin was purchased fromNanjing Oddfoni Biological Technology Co., Ltd., (BR Grade, No.). Sodium chloride was purchased from Sinopharm Chemical Reagent Co., Ltd, (No. 20130315). Freund`s Complete Adjuvant was purchased from MP

Biomedicals LLC, USA(No. 06858). Formalin was purchased from saline distilled waterã€,

2. Methods

Plant material Preparation and extraction

The rhizome of *Atractylodis Macrocephalae* were obtained in the Pan`an Country, Zhejiang Province, China. And it was authenticated by Professor Jinbao Pu, ExploitandResearchCenterofOfficinalSilkwormResources, Zhejiang Academy of Traditional Chinese Medicine. The plant materials were dried at 50â„ ƒ for 7 days and reduced to coarse powder with the help of a suitable grinder. About 500g of the dry rhizoma powder was extracted with 70% ethanol at 100â„ ƒ for 3h by Soxhlet extractor for three times. And the combined ethanolic extract was filtered by filter paper and concentrated by rotary evaporator (R-210, BUCHI, Swiss) at reduced pressure with 200bpm and at 65â„ ƒ temperature. Finally, a brownish-black colored residue was obtained and designated as crude ethanolic extract of *Atractylodis Macrocephalae* (EAM). The crude ethanolic extract was dissolved in freshly prepared 5‰CMC-N $_a$ solution at a concentration of 750, 1500 mg/ml respectively and refrigerated at 4â„ ƒ until used.

Phytochemical sceening

The freshly prepared extract of *A. Macrocephalae* (EAM) was qualitatively tested for the presence of chemical constituents such as Carbohydrate and Glycosides, Alkaloids, Phytosterols, gum and Mucilage, Saponins, reducing

sugar, Tannins and Phenolic Compounds, Flavonoids, Terpenoids, Triterpenes, ect. by the method described previously[ ].

Analgesic /antinociceptiveactivity

Hot plate test

The hot plate test was employed for measurement of analgesic activity as previously described by Chavan et al. with a slight modification. ICR mice of both sexes were put on the hot plate maintained at 55±0. 5â„ƒ, the time taken by the animals to lick a hind paw or jump out of the plate was taken as the hot-plate latency. Mice with baseline latencies of ï¼œ10s orï¼ž30s were eliminated from the study. If there is still no response after 60 seconds, remove the mice to avoid scald, and the pain threshold in 60 seconds. After the determination of baseline response latencies, mice were divided into five groups, and pretreated with EAM of 100, 200, 300mg/kg, aspirin(200mg/kg) and vehicle(0. 5% CMC-Na) twice a day for 3 days. Then they were placed on the hot plate to determination the latency time after 30, 60 and 90min of last administration.

Acetic acid-induced writhing test

The method used in the experiment was described by with a slight modification. ICR mice of both sexes were divided into 5 groups, and pretreated with EAM of 100, 200, 300mg/kg, aspirin (200mg/kg) and vehicle (0. 5% CMC-Na) twice a day for 3 days. The writhing were induced by injection intraperitoneally with 0. 1mL/10g body weight of 0. 6% acetic acid solution at 30min after the final administration. The typical symptom of the

writhing as following: abodoment indent, body and hind paw stretch, and haunch upwarping. The latency and the number of writhing of 15min were recorded, and the writhing inhibition ratio were permitted to express the percentage of protection. the writhing ratio= (control mean-treated mean)×100/control mean(Dongmo et al., 2005)

Anti-inflammatory activity

Carrageenan-induced rat paw edema

Carrageenan-induced paw edema was produced according to the method described by with a slight modification. Female SD rats were divided into 5 groups, and pretreated withEAM of 100, 200, 300mg/kg, dexamethaone (1. 2mg/kg) and vehicle (0. 5% CMC-Na) once a day for 6 days. At 30min after the final oral administration of EAM, each mouse was injected with 0. 1mL freshly prepared carrageenan (1% carrageenan suspened in saline) into sub-plantar tissue of right hind paw. As the control, 0. 1mL saline solutions were injected into that of right hind paw. And the measurement of paw size was done by means of thickness of paw using digital clearance gaugebefore injection and 30, 60, 120, 180, 240, 360min after injection. The percentage of inhibition were expressed as following:

Xylene-induced mouse ear edema

The procedure used was similar to that used by with slight modification. ICR mice were randomly divided into 5 groups. They were pretreated with EAM of 100, 200, 300mg/kg, dexamethaone(1. 8mg/kg) and vehicle(0. 5% CMC-Na) once a day for 6 days. 1h after final administration, 0. 02mL xylene was

applied to the anterior and posterior surfaces of the right ear. As a control, distilled water was applied to that of the right ear. 1h after xylene application, mice were used cervical dislocation to sacrifice and both ears were removed. Circular sections were taken by cork borer with a diameter of 9mm, and weighted. The degree of swelling was evaluated by the ratio *a/b* , which *a* was the weight of the right treated ear, and b was the weight of the left untreated ear. (treated ear weight- untreated ear weight)×100/untreated ear weight.

Freund’s complete adjuvant-induced inflammatory pain

The method of adjuvant arthritis rats was described by with a slight modification. SD rats were divided into 5 groups, and the rats in 3 doses of EAM groups andaspirin group were subcutaneously injected with 150μL CFA (MP Biomedicals LLC, USA) in the plantar surface of the right hind paw while under light ether anesthesia. As to control group, saline was applied to the subplantar of the right paw. All rats were orally administrated EAM (100, 200, 300mg/kg), aspirin (200mg/kg) and vehicle (0. 5% CMC-Na) once a day for 21 days. At 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 days after CFA or saline injection, the degree of swelling of right paw and the paw withdrawal latency (PWL) were measured as described previously. The animals were sacrificed at 21 st day and their blood and paw edematous tissue were obtained for next MDAã€ ¡ SODã€ ¡ NOã€ ¡ TNF-αã€ ¡ IL-1βã€ ¡ PGE2