Anti-inflammatory activities of ethyl acetate extract of Polygonum jucundum and its phytochemical study

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Polygonum spp. (Polygonaceae) has long been used as folk medicines in China to treat bacterial infection, blood coagulation and cancer and inflammatory diseases. In this study, we aimed to demonstrate the ethnopharmacological activity of Polygonum jucundum. Its ethyl acetate extract (PJE, 200, 500 mg/kg, intraperitoneal (p.o)) produced a dose-related anti-inflammatory effects (P<0.05 to 0.001) of xylene-induced ear oedema in mice and acetic acid-induced vascular permeability models in mice and did not present acute toxicity at the dose of 3000 mg/kg. We also found that PJE dose-dependently diminished the production of nitric oxide (NO), release of tumour necrosis factor TNF-α and IL-6 in lipopolysaccharide (LPS) - activated RAW264.7 cells. These data suggested that the ethnopharmacological action of P. jucundum may be due to its negative modulation of macrophage-mediated inflammatory responses by suppressing NO, TNF-α and IL-6 production. Two new sesquiterpenoids, 2α, 3β-dihydroxycinnamolide (1) and 2α-hydroxyl-3β-angeloylcinnamolide (2), and five known flavonoids isolated from the active extract are speculated to account for the observed anti-inflammatory properties of this species. In vivo anti-inflammatory activities of compound 2 at doses of 50 to 200 mg/kg were also evaluated using xylene-induced ear oedema and acetic acid-induced vascular permeability models in mice. Thus, this plant may be developed as a new therapeutic remedy for various inflammatory diseases such as arthritis.

Key words: Polygonum jucundum, anti-inflammatory activities, sesquiterpenolides.

INTRODUCTION

Polygonum jucundum Lindx. (Polygonaceae) is an annual herb (50 to 90 cm), which is widely distributed in China and Japan (Li et al., 1998). The dry herb of P. jucundum is frequently used in traditional Chinese medicine with actions to remove heat, counteract toxicity and it can also be externally used for carbuncles (Li et al., 1998). The plants from genus Polygonum are well known for producing active compounds in oriental traditional medicine systems (Wang et al., 2006); for example, the ethanolic extract, sesquiterpenes and flavonoid glycosides from Polygonum viscosum are known to have anti-inflammatory properties (Datta et al., 2004), extracts and constituents from Polygonum spectabile Mart. showed antimicrobial, antiviral activity (Brand et al., 2010).

As part of our ongoing efforts in discovering active components in medicinal plants of genus Polygonum (Qi et al., 2005a, b; Lin et al., 2009), the present paper reports on the investigation of the anti-inflammatory activity of ethylacetate extract (PJE) from P. jucundum. In two acute animal models relevant to the inflammatory process.

Preliminary phytochemical process indicated that the active extract of PJE contain mainly two sesquiterpenolides: 2,3-dihydroxycinnamolide (1) and 2α-hydroxyl-3β-angeloylcinnamolide (2), along with five known flavonoids: isorhamnetin (3), luteolin - 3' - O - glucoside (4), apigenin - 7 - O - glucoside (5), quercetin - 3 - O - rhamoside (6) and kampferol (7). Furthermore, the in vivo anti-inflammatory effects of compound 2 were also investigated to analyze the possible anti-inflammatory...
properties of *P. jucundum*.

**MATERIALS AND METHODS**

**Preparation of the extract**

The aerial parts of *P. jucundum* Lind. was collected in Ju-rong country, Najing, Jiangsu province, China, in December 2007. The herb was authenticated by Prof. Mian Zhang (Department of Pharmacognosy, China Pharmaceutical University). A voucher specimen (No. PUL-0710) was stored at this Department.

**Phytochemical studies**

The dried minced plant (10 kg) was heated and refluxed with 85% ethanol for 2 h, 3 times, evaporating under reduced pressure to yield a dark brown tarry mass (PJM, 1.46 kg, yield 14.6%, w/w) at a temperature below 45°C. PJM was dissolved in water and was successively extracted with petroleum ether to remove chlorophyll, then extracted with acetyl acetate. The ethylacetate fraction (PJE, 285 g) was obtained for pharmacological and phytochemical studies. PJE (200 g) was subjected to column chromatography on silica gel and eluted by a mixture of petroleum ether and acetone (1:1) to afford compound 1 (2 mg), 2 (2 mg), 3 (5 mg) and 7 (15 mg). Their structures were confirmed by spectroscopic analysis and 1D and 2D NMR and HR-ESI-MS.

**Anti-inflammatory activity**

**In vivo studies**

Male Impaired cytokine response (ICR) mice, weighing 18 to 22 g, were obtained from the experimental animal center of China Pharmaceutical University. They were kept on standard laboratory chow with tap water ad libitum and the husbandry room was maintained at 22°C with a 12 h light and 12 h dark cycle.

**Xylene-induced ear oedema in mice**

The method was used according to previously described by Cao et al. (2010). After 15 min treatment, each animal received 50 µl of xylene on the anterior and posterior surfaces of the right ear lobe, the left ear was considered as control. One hour later, animals were sacrificed by cervical dislocation, and a diameter of 8 mm circular sections was taken from both ears with a cork borer and weighed. The degree of ear swelling was calculated based on the weight of left ear without applying xylene.

**Acetic acid-induced vascular permeability in mice**

This test was performed by the method described by Li et al. (2010). Male ICR mice were divided into four groups. The vehicle and drugs were administered orally to individual groups of mice, at a dose of 200, 500 mg/kg, once a day for 3 days. Group A received the same volume of normal saline orally as a vehicle control. 1h after the last treatment of drug, 0.2% Evan’s blue in normal saline was injected intraventrically into the tail vein at a dose of 0.1 ml/10 g body weight. And immediately each mouse was injected intraperitoneally with 0.2 ml of 0.6% acetic acid in normal saline. 30 min after intraperitoneal injection, the mice were killed by dislocating the neck and the abdominal wall was cut to expose the intestine. The abdominal cavity was washed using 5 ml of normal saline to collect pigments in a test tube. After centrifuging the contents of the tube to eliminate contaminants, the solution was subjected to colorimetry using a spectrophotometer at a wavelength of 590 nm. Control mice were treated similarly. The vascular permeability effects were expressed by the absorbance (A) of the total dye amount that leaked into the intraperitoneal cavity.

**Acute toxicity**

In this study, the acute o.p. toxicity of PJE was assessed using the limit test in the mice (Hayes, 1989). The limit dose (3000 mg/kg) for acute toxicity was used. In the test, male and female mice weighing 18 to 22 g each were used (n = 5 for each group).

**In vitro studies**

Raw 264.7 cell line was from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The mammalian cells were cultured in Dulbecco’s-modified Eagle’s medium with 10% heat-inactivated fetal bovine serum (FBS), 25 mM HEPES (pH7.5), 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were plated at a density of 1×10^4 and preincubated for 24 h at 37°C, and maintained in a humidified atmosphere containing 5% CO₂. The cells were incubated with culture medium (control), or stimulated with 5 µg/ml LPS in the presence of the different samples, for 24 h. Briefly, 170 µl of culture supernatants were collected and diluted with equal volumes of the Griess reagent [0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride and 1% (w/v) sulphanilamide containing 5% (w/v) H₃PO₄] during 10 min, in the dark. The absorbance at 550 nm was measured in an automated plate reader (SLT, Austria). Nitrite concentration was determined from a sodium nitrite standard curve.

**Nitric oxide measurement**

The production of NO was measured by the accumulation of nitrite in the culture supernatants, using a colorimetric reaction with the Griess reagent (Cruz et al., 2001; Zhou et al., 2006). The cells were plated at 0.3×10^4 cells/well in 96-well culture plates and then incubated with culture medium (control), or stimulated with 5 µg/ml LPS in the presence of the different samples, for 24 h. Briefly, 170 µl of culture supernatants were collected and diluted with equal volumes of the Griess reagent [0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride and 1% (w/v) sulphanilamide containing 5% (w/v) H₃PO₄]. The absorbance at 550 nm was measured in an automated plate reader (SLT, Austria). Nitrite concentration was determined from a sodium nitrite standard curve.
Table 1. Effect of PJE on xylene-induced ear edema and acetic acid-induced vascular permeability in mice (X±S, n = 10).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg/d)</th>
<th>Difference (mg)</th>
<th>Inhibition (%)</th>
<th>Absorbance (A)</th>
<th>Inhibition (%)</th>
</tr>
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<tr>
<td>Control</td>
<td>-</td>
<td>18.32±5.12</td>
<td>-</td>
<td>1.441±0.140</td>
<td>-</td>
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<tr>
<td>Aspirin</td>
<td>300</td>
<td>9.48±3.01**</td>
<td>48.25</td>
<td>1.135±0.015**</td>
<td>30.19</td>
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<tr>
<td>PJE</td>
<td>500</td>
<td>10.32±2.21**</td>
<td>43.65</td>
<td>1.006±0.003**</td>
<td>29.91</td>
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<tr>
<td></td>
<td>200</td>
<td>12.12±2.32**</td>
<td>33.84</td>
<td>1.010±0.025*</td>
<td>29.08</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± S.E.M., *P<0.05, **P<0.01 when compared with control group.

Table 2. The influence of PJE from *P. jucundum* on inflammatory factors NO, IL-6 and TNF-α.

<table>
<thead>
<tr>
<th>Section</th>
<th>Dose (µg/ml)</th>
<th>NO (µmol/L)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
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<tr>
<td>Blank</td>
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<td>7.28±0.01</td>
<td>75.91±9.72</td>
<td>2.58±0.80</td>
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<td>LPS</td>
<td>5</td>
<td>59.48±0.03***</td>
<td>277.51±23.19***</td>
<td>15.51±2.09***</td>
</tr>
<tr>
<td>PJE</td>
<td>30</td>
<td>17.44±0.03▲▲</td>
<td>206.74±10.07▲▲</td>
<td>10.01±2.43▲</td>
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<td></td>
<td>100</td>
<td>8.65±0.02▲▲</td>
<td>174.96±7.14▲▲▲</td>
<td>7.46±0.97▲▲</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>7.43±0.02▲▲</td>
<td>144.97±9.16▲▲▲</td>
<td>5.85±2.46▲▲</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001 compared with control group; ▲P<0.05, ▲▲P<0.01, ▲▲▲P<0.001 compared with model.

Inhibitory effects on LPS-induced TNF-α and IL-6 releases from RAW264.7 cells

The RAW 264.7 cells were seeded in 96-well plates with 1.0×10^5 cells/well and allowed to adhere for 1 h at 37°C in a humidified atmosphere containing 5% CO₂. After that the medium was replaced with a fresh medium containing 500 mg/ml of LPS together with the test samples at various concentrations and was then incubated for 48 h. The supernatant was transferred into 96-well plate and then TNF-α and IL-6 concentrations were determined using commercial enzyme immunoassay kits (BD Pharmingen) according to the manufacturer’s protocol. The test sample was dissolved in dimethyl sulfoxide (DMSO) and the solution was added to RPMI. The inhibition on TNF-α and IL-6 releases was calculated.

Statistical analysis

All the experiments were performed in duplicate. Results are presented as mean±standard error (SE) of the indicated number of experiments, and the means were statistically compared using the one-way ANOVA test, with a Dunnett’s post-test. The significance level was *p<0.05, **p<0.01, ***p<0.001.

RESULT AND DISCUSSION

In *vivo* and in *vitro* anti-inflammatory effects of PJE

As shown in Table 1, ethyl acetate-soluble portion (PJE) of 85% ethanol extract from *P. jucundum* at a dose of 200 to 500 mg/kg exhibited significant anti-inflammatory. Significantly inhibitory effect recorded with PJE at a dose of 500 mg/kg was comparable to that of aspirin. Xylenen cause instant irritation of the mouse ear, which led to fluid accumulation and edema characteristic of the acute inflammatory response. Suppression of this response was a likely indication of antiphlogistic effect. Similar result was obtained against another inflammatory model based on acetic acid-induced vascular permeability in mice (Table 1). Vascular permeability was induced by acetic acid, which can cause the increase of chemical mediators such as prostaglandin E₂ (PGE₂), histamine in peritoneal fluids, leading to the increase in vascular permeability. These results suggest that *P. jucundum* exerts an anti-acute inflammatory effect. The mechanism may be due to inhibiting the inflammatory mediators.

RAW264.7 cells, amurine macrophage cell line, are widely used to establish inflammatory model *in vitro* (Kang et al., 2006). In this study, we investigated *in vitro* the anti-inflammatory effects of PJE on the generation of several chemokines and cytokines, involved in the inflammatory process, such as NO, TNF-α and IL-6 in RAW264.7 macrophages. PJE significantly decreased the production of NO, tumor necrosis factor TNF-α and IL-6 production in a dose-dependent manner (Table 2).

Spectral data of two new sesquiterpenoids and its structural elucidation

2α,3β-dihydroxycinnamolide (3aS,4aS,6R,7R)-6,7-dihydroxy-8a-methyl-3α,4,4a,5,6,7,8,8a-octahydronaphtho [2, 3-c] furan-1(3H)-one, 1) White powder (CHO₂). Mp.172-174°C ultraviolet (UV)
(MeOH, \( \lambda_{\text{max}} \): 222 nm, [\( \alpha \rceil^2_{D} \) -9.6°(c = 0.1, MeOH). IR (KBr): 3279.6, 2967, 2923, 1775, 1697, 1257, 1178 cm\(^{-1}\).  

1H-NMR (\( \text{CD}_{3} \text{OD} \), 300 MHz): 1.90 (1H, dd, \( J = 9.2, 5.3 \text{Hz}, \ H - 1 \beta \)), 1.32 (1H, t, \( J = 8.9 \text{Hz}, \ H - 1 \alpha \)), 3.67 (1H, m, H - 2), 3.02 (1H, d, \( J = 10 \text{Hz}, \ H - 3 \)), 1.53 (1H, dd, \( J = 5.2, 12.1 \text{Hz}, \ H - 5 \)) 2.49 (1H, m, H - 6\( \alpha \)), 2.96 (1H, m, H - 9), 6.88 (1H, dd, \( J = 9.3, 3.3 \text{Hz}, \ H - 7 \)), 4.46 (1H, dd, \( J = 9.2, 9.2 \text{Hz}, \ H - 11 \beta \)), 1.07 (3H, s, H-13), 0.95 (3H, s, H-14), 0.90 (3H, s, H-15). 13C-nuclear magnetic resonance (NMR) ( \( \text{CD}_{3} \text{OD}, 500 \text{ MHz} \)): 46.9 (C - 1), 68.9 (C - 2), 84.4 (C - 3), 40.8 (C - 4), 50.7(C - 5), 26.5 (C - 6), 52.4 (C - 7), 128.8 (C - 8), 138.3 (C - 9), 36.5 (C - 10), 69.2 (C - 11), 172.8 (C - 12), 17.0 (C - 13), 29.3(C - 14), 15.0 (C - 15). HR - TOF - MS (m/z): 289.1410 [M+Na]+ (calcd. 289.1410 for \( \text{C}_{15} \text{H}_{22} \text{O}_{4} \)). Compound 1, white amorphous powder, showed positive reaction with a 10% vanillin-sulfuric acid solution in thin layer chromatography. The molecular formula \( \text{C}_{15} \text{H}_{22} \text{O}_{4} \) was determined by HR electrospray ionization mass spectrometry (ESI-MS) and 1D – NMR analysis, and with 5 degrees of unsaturation.

The UV spectrum showed absorption at 222 nm, meaning the presentation of a conjugated system. The Infrared (IR) spectrum revealed the absorptions of one olefinic group at 1775 cm\(^{-1}\) and double bonds at 1697 cm\(^{-1}\). The H-NMR spectrum showed one olefinic proton at \( \delta = 6.88 \) (1H, dd, \( J = 9.3, 3.3 \text{Hz}, \ H - 7 \)) and three methyl groups at \( \delta = 1.07 \) (3H, s), 0.95 (3H, s) and 0.90 (3H, s). The 13C-NMR, DEPT and HSQC spectra revealed the presence of 15 carbon atoms, including 3×CH\(_3\), 3×CH\(_2\), 5×CH and four quaternary carbons. The five methine carbon are including one double bond at \( \delta = 138.3 \), two oxygen - substituted methine carbon at \( \delta = 68.9 \) (C-2) and \( 84.4 \) (C-3), and two others carbon at \( \delta = 50.7 \) (C-5) and \( 52.4 \) (C-9); the four quaternary carbons including one carbonyl at \( \delta = 172.8 \) (C-12); one double bond at \( \delta = 128.8 \) (C-8); and other signals at \( \delta = 40.8 \) (C-4) and 36.5(C-10).

The HMBC spectrum displayed the cross-peaks from one olefinic proton at \( \delta = 6.88 \) (1H, dd, \( J = 9.3, 3.3 \text{Hz}, \ H - 7 \)) to carbonyl signal at 172.8 (C-12), a methylene carbon signal at \( \delta = 26.5 \) (C-6) and a methine carbon signal at \( \delta = 50.7 \) (C-5), from two singlets methyl signals at \( \delta = 1.07 \) (3H, s), \( \delta = 0.95 \) (3H, s, H-14) to carbon signals including one quaternary carbon signal at \( \delta = 40.8 \) (C-4), one oxygen-substituted methine carbon signal at \( \delta = 84.4 \) (C-3) and methine carbon signal at \( \delta = 50.7 \) (C-5). The long-range correlations from H-15 to C-10, C-1 and C-5, from H-5 to C-15, C-10, C-6, C-4 and C-1 were also be seen in HMBC spectrum, which were in accordance with the assignment of compound 1 (Figure 1). The relative configuration can be determined by NOESY spectral analysis, in which there were clear correlations between H-2/ H-3 and H-9/ H-5 (Figure 3).

Based on above analysis, compound 1 was indicated as being a tricyclic drimane sesquiterpenoid skeleton (Ayer et al., 1992).

**Figure 1.** Main correlations in 2D-NMR spectra of 1.
MeOH). IR(KBr): 3475, 3008, 2972, 2918, 1763, 1697, 1654, 1257, 1178, 955 cm$^{-1}$. $^1$H-NMR (CDCl$_3$, 300 MHz): 2.05 (1H, dd, $J = 12.3, 1.0$ Hz, H-1$\alpha$), 1.40 (1H, dd, $J = 12.3, 3.6$ Hz, H-1$\beta$), 3.87 (1H, m, H-2), 4.65 (1H, d, $J = 10.0$ Hz, H-3), 1.56 (1H, dd, $J = 12.0, 3.3$ Hz, H-5), 2.43 (1H, ddd, $J = 16.0, 3.9, 8.9$ Hz, H-3$\alpha$), 2.23 (1H, ddd, $J = 16.0, 12.0, 3.3$ Hz, H-6$\beta$), 2.58 (1H, d, $J = 12.0, 3.3$ Hz, H-6$\alpha$), 1.93 (3H, s, H-15), 6.12 (1H, ddd, $J = 9.2, 9.2$ Hz, H-11$\beta$), 1.03 (3H, s, H-11$\alpha$), 0.95 (3H, s, H-14), 0.88 (3H, s, H-15), 6.12 (1H, ddd, $J = 1.4, 1.9$, 14.5 Hz, H-1$\beta$), 2.02 (3H, dd, $J = 1.4, 7.2$ Hz, 3$´$-CH$_3$), 1.93 (3H, dd, $J = 1.9, 1.5$ Hz, 2$´$-CH$_3$). $^{13}$C-NMR (CDCl$_3$, 500 MHz): $\delta$ 46.2 (C-1´), 66.6 (C-2´), 83.6 (C-3´), 39.1 (C-4´), 48.9 (C-5´), 24.6 (C-6´), 135.6 (C-7´), 126.9 (C-8´), 50.6 (C-9´), 35.0 (C-10´), 66.7 (C-11´), 169 (C-12´), 17.1 (C-13´), 28.2 (C-14´), 14.3 (C-15´), 168.7 (C-1´), 127.5 (C-2´), 139.1 (C-3´), 15.8 (3$´$-CH$_3$), 20.6 (2$´$-CH$_3$). HR-TOF-MS m/z 371.1829 [M+Na]$^+$ (calcd 371.1829 for C$_{20}$H$_{29}$O$_5$Na). Compound 2 has molecular formula C$_{20}$H$_{29}$O$_5$ as established by HR-ESI/MS. IR spectrum showed strong absorptions of an unsaturated $\gamma$-lactone (1763 cm$^{-1}$) and OH group (3475 cm$^{-1}$) and O (3475 cm$^{-1}$). The $^1$H-NMR spectrum showed three methyl groups as singlets at $\delta$ 0.88 (3H, s, H-15), 0.95 (3H, s, H-14) and 1.03 (3H, s, H-13), which together with a high field signal at $\delta$ 1.56 (1H, dd, $J = 12.0, 3.3$ Hz, H-5) suggested the same skeleton as compound 1. The angeloyl fragment was indicated by its characteristic chemical shifts, two doublet methyl groups and one olefinic proton signal at $\delta$ 6.12 (1H, ddd, $J = 1.4, 1.9$, 14.5 Hz, H-3$\beta$) in the $^1$H-NMR spectrum. Location of the angeloyl group at C-3 is based on the long-range correlation in HMBC experiment from proton signal at $\delta$ 4.65 (1H, d, $J = 10.0$ Hz, H-3) to carbonyl signal at $\delta$ 168.7 (C-1$´$). The $^1$H-$^1$H COSY and especially the HMBC spectrum confirm the structure of 2 (Figure 2). It relative stereo-structure can also be confirmed by NOESY analysis, in which cross-peaks can be seen from H-2 to H-14-15, from H-3 to H-13, from H-6$\delta$ to H-15, from H-6$\alpha$ to H-9. Thus, the structure of compound 2 was confirmed and named as 2$\alpha$-hydroxyl-3$\beta$-angeloylcinnamolide.

To assess if the large amount of new compound 2 (3.5 g) is active component, in vivo anti-inflammatory experiments were conducted using xylene-induced ear oedema and acetic acid-induced vascular permeability in mice. Compound 2 exhibited significant anti-inflammatory activities at doses of 50 and 200 mg/kg after administration ($p<0.01$), respectively. Additionally, compound 2 showed significantly better activity at doses of 200 mg/kg than aspirin at the dose of 300 mg/kg ($p<0.01$) (Table 3). NO is produced by activated macrophages as a result of induction by several stimuli, including TNF-$\alpha$, IFN-$\gamma$ and LPS and may contribute to the pathological process in various acute and chronic inflammatory conditions (Kilbourn and Belloni, 1990). Therefore, the reduction of NO production may present a useful strategy for the treatment of a variety of inflammatory diseases, including some neurological disorders (Albina and Reichner, 1998; Boucher et al., 1999; Lee et al., 2006). In the present study, first we carried out the in vivo and in vitro anti-inflammatory effects of the ethyl acetate extract (PJE) from P. jucundum.

It is concluded that the present study may support the use in China traditional medicine of P. jucundum for treatment of inflammatory-related diseases through the inhibition of NO, TNF-$\alpha$ and IL-6 production or release. In this study, we demonstrate that PJE significantly inhibited NO production, TNF-$\alpha$, and IL-6 release from LPS-stimulated macrophages in dose-dependent manner.
Then we investigated phytochemical study of PJE. Two new sesquiterpenoids, 2α, 3β-dihydroxycinnamolide (1) and 2α-hydroxy-3β-angeloylcinnamolide (2), and five known flavonoids were obtained. Flavonoids are known to possess various biological activities, including anti-inflammatory properties (Hall et al., 1980) by suppressing LPS-induced NO production (Jin et al., 2000) or TNF-α inflammatory effects (Kim et al., 1999; Matsuda et al., 2003; Takada and Aggarwal, 2004; Yerra et al., 2005).

ACKNOWLEDGEMENTS

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REFERENCES


Table 3. Effect of compound 2 on xylene-induced ear edema and acetic acid-induced vascular permeability in mice (\(\bar{X}\pm S, n = 10\)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg/d)</th>
<th>Difference (mg)</th>
<th>Inhibition (%)</th>
<th>Absorbance (A)</th>
<th>Inhibition (%)</th>
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<tr>
<td>Control</td>
<td>-</td>
<td>18.1±2.31</td>
<td>0.52±0.190</td>
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<tr>
<td>Aspirin</td>
<td>300</td>
<td>9.2±2.32**</td>
<td>49.1</td>
<td>0.22±0.014**</td>
<td>57.65</td>
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<tr>
<td>PJE</td>
<td>500</td>
<td>11.7±2.50**</td>
<td>35.6</td>
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<tr>
<td>Compound 2</td>
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<td>44.8</td>
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<td>49.1</td>
<td>0.23±0.006**</td>
<td>55.75</td>
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Values are expressed in mean ± S.E.M., **P < 0.01 compared with control.