ANTI-INFLAMMATORY EFFECT OF ETHANOLIC EXTRACT OF CLEOME COLUTEOIDESBIOSS ON ACUTE AND CHRONIC INFLAMMATION IN ADULT ALBINO RATS

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ABSTRACT

The anti-inflammatory effect of the ethanolic extract of Cleome coluteoidesbioss was evaluated using the formalin-induced rat paw edema test in albino rats. Ethanolic extracts of the aerial part of this plant were prepared and intraperitoneally injected at dose rate of 50 mg/kg 30 min before formalin-induced inflammation on the first day, which was continued from the second to eighth day after measurement of paw thickness. The anti-inflammatory effects of the plants were monitored during 1h after formalin injection and then at 24h intervals for 8 days. Sodium Salicylate (SS), as a well-known anti-inflammatory agent (300 mg/kg), was used as a positive control. Statistical analyses using ANOVA and Newman–Keuls post-hoc tests revealed that similar to SS, the ethanolic extract of C. coluteoidesbioss at dose rate of 50 mg/kg significantly decreased the formalin-induced inflammation compared to control group (p<0.05). No significant difference was found between C. coluteoidesbioss and SS regarding the anti-inflammatory effect (paw thickness) from the second to eighth day of study. However, this difference was statistically significant on the first day of the study (1h after formalin injection). Furthermore, the median lethal dose (LD50) of C. coluteoidesbioss was greater than 5000 mg/kg body weight. The findings provided more evidence for the potential anti-inflammatory effect of C. coluteoidesbioss.

Keywords: Cleome coluteoidesbioss, Formalin test, Inflammation.

INTRODUCTION

About 2400 years BC, Hippocrates advised: “Let food be your medicine and medicine be your food” (Rishi 2006). Herbal medicines are used as a method of diseases treatment all over the world, especially in some countries like Iran. Advantages, such as less adverse effects, therapeutic efficacy and lower final prices make them a good alternative source for synthetic drugs. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are the most common drugs used to treat joints, bones, muscle inflammation and pain. Nevertheless, NSAIDs may cause gastrointestinal discomfort and even cause stomach ulcers (Sostres, Gargallo et al. 2010). In addition, widely used NSAIDs, such as naproxen, low-dose ibuprofen, and diclofenac in available doses without prescription, increases the risk of cardiovascular diseases (McGettigan and Henry 2011). Therefore, more studies are required to be performed on pharmacological and therapeutic effects of these routine medications.

Cleome is the genus from Cleomaceae family including 180-200 species of herbaceous annual or perennial plants and shrubs widely distributed in tropical and subtropical regions (Rassouli, Dadras et al. 2014). Cleomaceae is a small family of flowering plants including more than 300 species belonging to 9 genera among which, Cleome is the largest genus with about 180-200 species of medicinal, ethnobotanical, and ecological importance (Aparadh, Mahamuni et al. 2012). Some of these species are used as traditional drugs. Besides, several researches have determined that some species of this plant, such as C. chrysantha, C. viscosa, C. affinis, C. drosifolia, C. hirta, C. gynandropsis, C. chrysantha, and C. gynandra, had antioxidant, anti-inflammatory, antitumoral, antiviral, and antimicrobial activities (Hashem, Wahba 2000; Parimala Devi et al. 2004; Sodeifian, Ardestani et al. 2016).

The anti-inflammatory and anti-nociceptive activities of C. coluteoidesbioss have been pointed out in Iranian folk medicine to treat inflammatory and pain-related illnesses. Furthermore, C. coluteoidesbioss has noticeable separation in Iran and a limited number of studies have been done in this regard. Therefore, the present study aims to assess the anti-inflammatory effects of ethanolic extracts of C. coluteoidesbioss on acute and chronic inflammation by formalin test induced paw edema in rats.
MATERIALS AND METHODS

Plant material: The aerial parts of C. coluteoidesbioss were collected from Sabzevar located in Khorasan Razavi Province, Iran and recognized in herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Preparation of extracts: The aerial parts of this plant were washed with distilled water to remove the possible major surficial pollutions. They were dried in shade and powdered. The extract was prepared using 100 g of the powdered part of the plant, which added to ethanol 98% and distilled water (600 cc) and left for 48 h at room temperature. The supernatant was filtered through Whatman No. 1 filter and ethanol was evaporated at rotary evaporator under reduced pressure. The lyophilization of the final product was prepared on a Freeze dryer (EPSILON 2–6D; Martin Christ, Osterode am Harz, Germany).

Animals: Male albino Wistar rats weighing 250–300 g were used for formalin test induced edema. The animals were obtained from the Animal Unit, Faculty of Medicine, Aja University of Medical Sciences, Tehran, Iran and were housed at constant temperature (22 ± 2°C) in a light-controlled room (12-h light/dark cycle) and were fed with standard rodent diet.

Acute toxicity study: The lethal dose (LD₅₀) of the plant extract was determined by Lørke’s method (Lørke 1983). In the first phase of the LD₅₀ study, 13 rats were divided into 3 groups of 3 and were treated with the aqueous extract of the plant at doses of 10, 100, and 1000 mg/kg bodyweight intraperitoneally (IP) (Lørke 1983). They were observed for signs of toxicity for 24 h. The results of the first phase were used as a basis for selecting the subsequent doses. In the second phase, further specific doses (1600, 2900 and 5000 mg/kg bodyweight) of the extract were administered to three rats (one rat per dose) to further determine the correct LD₅₀ value. The median LD₅₀ was calculated using the second phase.

Effective dose 50: Non-fasted male albino Wistar rats were treated with different doses of plant extracts. To assess the anti-inflammatory activity of the plant extracts, 0.05 ml of a 2.5% formalin solution (Sigma-Aldrich, Germany) in distilled water were injected (intraplantar) followed by IP injection of the plant extracts. Furthermore, a fourth group of three rats was set up as control group and the equal amounts of distilled water-tween 80 (5%) was administered by intraplantar injection in animals in this group (a).

The rats were randomly distributed into six groups each containing three animals; (a) three dosages of plant extracts (10, 100, and 300 mg/kg) used in control rats and (b) three dosages of plant extracts (10, 100, and 300 mg/kg) used in rats with induced paw edema with formalin. This experiment was carried out for 96 hours and the edematogenic response was evaluated by the use of plethysmometer at 24 h intervals. As described by Trebien and Calixto (1989), edema was reported as the percentage of difference between the values obtained from the paws injected with formalin and distilled water-tween 80 (5%), as the controls, and plant extracts. Moreover, the anti-inflammatory activity of the plant extract was expressed as effective dose 50 (ED₅₀) defined as the amount of plant extract at which, the formalin-induced paw edema was reduced by 50%.

Formalin test induced rat paw edema: Anti-inflammatory activities of the extracts were determined by formalin-induced edema test in the rats’ hind paws according to the reported method. The animals were kept for 12 h of fasting before starting the experiment, but had free access to water. Approximately 0.05 ml of a 2.5% formalin solution (Sigma-Aldrich, Germany) in distilled water was prepared 1 h before the experiment and was injected into the plantar side of both hind paws of the rats. The lyophilized extracts were dissolved in distilled water-tween 80 (5%) and passed through a weighed filter paper and the filtrate was used for IP injection. Following filtration, the filtrate was dried and weighed again in order to obtain the real concentration of the extract.

The rats were randomly distributed into three groups each containing six animals; (a) control, (b) dosages of extracts (50 mg/kg), and (c) sodium salicylate (SS) (Sigma-Aldrich, Germany) as the reference drug (300 mg/kg). The control group only received distilled water-tween 80 (5%). It should be noted that SS powder was prepared in distilled water.

The paw thickness was measured from the ventral to the dorsal surfaces using a plethysmometer prior to formalin injection. It was also measured 1h after formalin injection and then at 24 h intervals for 8 days. The extracts solution or SS solution was injected IP 30 min before induction of inflammation. From the second to the eighth day, first the paw thickness of each rat was measured and recorded followed by immediate IP injection of special drugs for each group. The data were expressed as the percentage of increase in thickness.

Statistical analysis: All data were expressed as mean ± SEM. The study groups were compared using ANOVA and Newman–Keuls post-hoc test. All statistical analyses were performed using the SPSS statistical software (version 22, Chicago, IL, USA) and p<0.05 was considered to be statistically significant.

RESULTS

Acute toxicity (LD₅₀) of C. coluteoidesbioss: The acute oral toxicity of C. coluteoidesbioss in the male rats has been presented in Table 1. The results showed that no mortality had occurred in the first (with doses of 10, 100,
and 1000 mg/kg) and the second phase (with doses of 1600, 2900, and 5000 mg/kg) of the LD_{50} study. Thus, there is no toxicity just on the basis of mortality and the LD_{50} of C. coluteoidesbioss in the rats was greater than 5000 mg/kg body weight (Lorke, 1983).

**Effective dose 50:** The ED_{50} of C. coluteoidesbioss was 50 mg/kg.

**Effects of the ethanolic extract of C. coluteoidesbioss on formalin-induced paw edema:** The 50 mg/kg dose of C. coluteoidesbioss extract showed anti-inflammatory effects (paw thickness). In other words, this product significantly decreased the inflammation induced by formalin compared to the control rats all through the study (p<0.05) (Table 2). From the second to the eighth day of the study, no significant difference was found between C. Coluteoidesbioss and SS regarding their anti-inflammatory effects (paw thickness). However, this difference was statistically significant on the first day of the study (1 h after formalin injection) (Table 2).

### Table 1. The acute oral toxicity of Cleome coluteoidesbioss in the male rats.

<table>
<thead>
<tr>
<th></th>
<th>1st phase of the LD_{50} study</th>
<th>2nd phase of the LD_{50} study</th>
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<tbody>
<tr>
<td>Doses (mg/kg)</td>
<td>Mortality</td>
<td>Doses (mg/kg)</td>
</tr>
<tr>
<td>10</td>
<td>0/3</td>
<td>1600</td>
</tr>
<tr>
<td>100</td>
<td>0/3</td>
<td>2900</td>
</tr>
<tr>
<td>1000</td>
<td>0/3</td>
<td>5000</td>
</tr>
</tbody>
</table>

Figure 1. Increase in thickness (%) of the study groups on consecutive days of the study.

### Table 2. Percentage of increase in thickness of the groups on consecutive days of the study

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Sodium salicylate</th>
<th>Celome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>13^a</td>
<td>7^a,b</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>12^a</td>
<td>20^a</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>9^a</td>
<td>20^a</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>11^a</td>
<td>12^a</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>6^a</td>
<td>13^a</td>
</tr>
<tr>
<td>6</td>
<td>44</td>
<td>8^a</td>
<td>10^a</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>11^a</td>
<td>11^a</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>6^a</td>
<td>7^a</td>
</tr>
</tbody>
</table>

Data are expressed as the mean percentage of increase in thickness.

^a P<0.05 compared to the control value and ^b P<0.05 compared to the sodium salicylate value.
DISCUSSION

Different mediators such as 5-hydroxytryptamin, histamine, bradykinin and COX-2 products are involved in early phase of inflammation and neutrophil infiltration, as well as arachidonic acid (AA) production and free radicals activity involved in late phase of inflammation (Salvemini, Wang et al. 1996, Mantle, Edde et al. 2000). In formalin test, the late phase is thought to be secondary to inflammatory reactions (Hunskaar, Berge et al. 1986). Phospholipase A2 (PLA2) is involved in formation of potent inflammatory mediators, including the hydrolysis of ester bonds at the sn-2 position of membrane phospholipids, and release of fatty acids, such as AA and lysophospholipids (Ueno and Rosenberg 1990). Additionally, PLA2 was observed in synovial fluid of arthritic patients at the time of inflammation. PLA2 may also be involved in the process of inflammation. Hence, modulation of PLA2 activity is a very important target for design of anti-inflammatory drugs, especially chronic inflammatory conditions such as rheumatoid arthritis and asthma (Chandra, Prasanth et al. 2011).

The epoxyeicosatrienoic acids (EETs) formed by the epoxygenase pathway are released by endothelial cells in response to receptor agonists, such as acetylcholine or bradykinin, and act as local, paracrine hormones that relax vascular smooth muscles (Campbell, Gebremedhin et al. 1996, Fisslthaler, Popp et al. 1999). Node et al. (1999) described a new autocrine role for EETs in endothelial cells as anti-inflammatory mediators.

Researches have been carried out to recognize the way of inhibition of PLA2 by n-hexadecanoic acid. The enzyme kinetics study showed that n-hexadecanoic acid inhibited PLA2 in a competitive manner. It has been recommended that n-hexadecanoic acid might function as an anti-inflammatory agent by binding at the active site of PLA2. The asymmetric unit of the crystal contained one molecule of PLA2, one molecule of n-hexadecanoic acid, two calcium ions, and 58 water molecules (Muller, Lena et al. 2006).

Several possible mechanisms for sodium salicylate have been suggested its anti-inflammatory effects: sodium salicylate inhibits prostagland biosynthesis, which has been shown to interfere with inhibition by salicylate of cyclooxygenase-2-mediated prostanooid formation. Other possible action of sodium salicylate that are not directly related to cyclooxygenase inhibition. These effects target intracellular signaling mechanisms such as kinases, including the mitogen activated protein-kinases (MAPK) cascade (Aman and Peskar 2002).

In the recent research, extraction of an essential oil from C. coluteoidesboiss aerial parts was investigated using supercritical carbon dioxide (SC-CO2) technique and Clevenger. On the basis of their results, the main components extracted by Clevenger were Hexadecanoic acid (25.11%), 11,14,17-Eicosatrienoicacid (10.01%), alpha-Cadinol (8.24%), 2-Pentadecanone, 6,10,14-trimet (8.24%), and Hexadecanoic acid ethyl ester (8.12%). Furthermore, nineteen constituents identified using the SC-CO2 extraction method were Hexadecanoic acid (28.32%), 11,14,17-Eicosatrienoic acid(9.87%), alpha-Cadinol (7.98%), 2-Pentadecanone, 6,10,14-trimet(7.98%), and Hexadecanoic acid ethyl ester (8.94%) (Sodeifian, Ardestani et al. 2016). Thus, Hexadecanoic acid and Eicosatrienoic acid were presented as the main components extracted from C. coluteoidesboiss aerial parts (Sodeifian, Ardestani et al. 2016).

According to the results of mentioned studies (Sodeifian, Ardestani et al. 2016, Muller, Lena et al. 2006, Campbell, Gebremedhin et al. 1996, Fisslthaler, Popp et al. 1999, Node et al. 1999), essential oil of C. coluteoidesboiss aerial parts can be effectively used as an anti-inflammatory medication. Furthermore, the results of the present study indicated that while the ethanolic extract of C. coluteoidesboiss had significant inhibitory effects on formalin-induced inflammation, C. coluteoidesboiss at doses of 50 mg/kg reduced the inflammation of formalin significantly (Figure 1). This implies that the low dose (50 mg/kg) of C. coluteoidesboiss extract acted more efficiently compared to SS, as a NSAID, in the late phase (i.e., up to 60 min) of inflammation (Table 2, the first day of the study). This difference can be related to various anti-inflammatory mechanisms of C. coluteoidesboiss extract compared to SS in the late phase of inflammation. Nevertheless, further molecular surveys are required to conclude the exact mechanisms.

Yet, both C. coluteoidesboiss extract and SS showed similar anti-inflammatory effects in the chronic phase of formalin-induced inflammation (Table 2, the second to the eighth day of the study).

Furthermore, the LD50 of C. coluteoidesboiss in the rats was greater than 5000 mg/kg body weight. LD50 values greater than 5000 mg/kg are of no practical interest (Lorke 1983). Moreover, LD50 values greater than 5000 mg/kg may lead to use of the plant with anti-inflammatory properties and minimal side effects.

In recent years, there is an increasing attention in herbal therapy. It is supposed that the use of plant-derived active principles will offer people access to effective products for the prevention and treatment of diseases through self-medications (Bulus, et al. 2011). The findings provided more evidence for the potential anti-inflammatory effect of C. coluteoidesboiss.

One major criterion in the choice of herbal medicines for use in health services is safety (Bulus, et al. 2011). From the results of this study, it is assumed that extracts of C. coluteoidesboiss is safe for usage in
traditional medicine. It is now left for the therapeutic dosage to be determined for clinical applications.

In conclusion, the findings of the present study with complementary results of other studies (Sodeifian, Ardestani et al. 2016, Chandra, Prasanth et al. 2011, Muller, Lena et al. 2006, Campbell, Gebremedhin et al. 1996, Fisslthaler, Popp et al. 1999, Node et al. 1999) showed the anti-inflammatory activity of C. coluteoidesboiss. Nevertheless, further molecular surveys are required to conclude the exact mechanisms of the total extract and biologically active components of this plant by assessing the amount of oxidative stress parameters and inflammatory mediator in different phases of inflammation, especially chronic inflammation.

REFERENCES


