RESEARCH ARTICLE

Evaluation of antinociceptive and anti-inflammatory activity of stems of *Gynandropsis pentaphylla* Linn

Somnath N. Mule, Sandeep B. Patil, Nilophar S. Naikwade, Chandrakant S. Magdum
Department of Pharmacology, Appasaheb Birnale College of Pharmacy, Sangli, MS, India

The aim of the present study was to evaluate the analgesic and anti-inflammatory activity of the aqueous extract of stems of *Gynandropsis pentaphylla* (AEGP). The analgesic activity of the extract was evaluated for its central and peripheral pharmacological actions using Eddy's hotplate method and acetic acid-induced writhing respectively. The anti-inflammatory activity was evaluated by using Digital plethysmometer (UGO Basil, Italy 7140). The study was carried out using dose of 100 mg/kg i.p. The pharmacological screening of the extract showed significant dose-dependent analgesic activity with good anti-inflammatory profile.

Key words: *Gynandropsis pentaphylla*, analgesic, anti-inflammatory

INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation.[1] Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow.[2] Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation,[3] whereas prostaglandins are detectable in the late phase of inflammation.[4] A large number of Indian medicinal plants are attributed with various pharmacological activities because they contain a diversified class of phytochemicals. It is believed that current analgesia-inducing drugs such as opoids and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side-effects and potency.[5] As a result, a search for other alternatives seems necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel anti-inflammatory agents could be discovered. Scientific studies are required to judge their efficacy. Traditional and folklore medicines play an important role in health services around the globe. About three quarters of the world population relies on plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. Several plants have been used in folklore medicine.[7] The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. Ayurveda the traditional medicinal system in India, describes certain plants which strengthen the host immune system. *Gynandropsis pentaphylla* (family: *Cleomaceae*) is an annual, erect, branched, 0.6-1.2 m in height, stems and branches striate, white spreading hairs. Leaves 3-5 foliolate, petioles 5-7.6 cm long, and seeds muricate dark brown. The plant has been traditionally used as an anthelmentic and rubefacient Leaves are applied externally over the wounds to prevent the sepsis. The plant also used in the treatment of malaria, piles, rheumatism and in tumour. The decoction of the root is used to treat fevers.[8,9]

The juice of the root is used to relieve scorpion stings. The leaves, applied as a poultice, are used as a vesicant and rubefacient in the treatment of rheumatism. The juice of the leaves is a remedy for pain in the ear.The seeds are anthelmintic and rubefacient.[8,9] The whole plant is used in the treatment of scorpion stings and snake bites. In the present study, we investigated the analgesic and anti-inflammatory activity of the aqueous extract of *Gynandropsis pentaphylla* (AEGP) for the first time using popular preclinical screening models in laboratory animals.

MATERIALS AND METHODS

Plant Material

The stems of *Gynandropsis pentaphylla* were collected in the

For correspondence: Mule Somnath Navanath, Appasaheb Birnale College of Pharmacy, South Shivaji Nagar, Sangli-Miraj Road, Sangli - 416 416, (M.S.), India. E-mail: somu.mule@gmail.com
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month of July from local area of Sangli region, Maharashtra India. The plant material was taxonomically identified by Prof. V. B. Awale, Head of the Department of Botany, Dr. Patangrao Kadam Mahavidyalya, Sangli, India.

Preparation of Aqueous Extract of Stem

Stems were shade dried and extracted with chloroform water (water: choloroform; 80:20) for 72 h. The mixture was filtered and evaporated to dryness. The dark brownish semisolid mass obtained was stored in a well-closed, airtight and light-resistant container.

Animals

Adult male mice (20-35 g) were used for the antinociceptive experiments. Adult male wistar rats (150-200 g) were used to study the anti-inflammatory activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27° C ± 2° C), with access to food and water *ad libitum*. The experiment was carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the Institutional Animal Ethical Committee approved all the procedures. Experimental studies were undertaken according to their rules and regulations.[10]

Acetic Acid-induced Writhing

The antinociceptive activity of AEGP was assessed using writhing test (abdominal constriction test).[11] Acetic acid solution (10 ml/kg, 0.6% in normal saline) was injected intraperitoneally, and the contraction of abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 10 min beginning 5 min after acetic acid injection. The AEGP extract (100 mg/kg, i.p.) was administered 0.5 h before the acetic acid injection. Antinociceptive activity was expressed as the percentage inhibition of abdominal constrictions between control animals and mice pre-treated ($n = 5$) with the extract. In an attempt to investigate the participation of the opioid system in the antinociceptive effect of this plant extract, separate groups of mice ($n = 5$) were pretreated with non-specific opioid receptor antagonist, pentazocin (5 mg/kg, i.p.), injected 15 min before the administration of the acetic acid.

Hotplate Test

The hotplate test was performed to measure response latencies according to the method previously described.[12] The hotplate was maintained at 55.0 ± 0.2° C and the animals were placed into the perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, prior to and 30, and 60 min after administration of the extract (100 mg/kg, i.p.). A latency period of 20 sec was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

Anti-inflammatory Activity

AEGP was evaluated for anti-inflammatory activity by carageenan-induced rat paw oedema method.[13,14] Male wistar rats (150-200 g) were randomly distributed into three groups of five animals each. The first group served as a control, second group served as the standard (received aceclofenac sodium 10 mg/kg, i.p), while the third group received 100 mg/kg, body weight of AEGP respectively. After 1 h, 0.1 ml of 1% w/v suspension of carageenan was injected into the sub-plantar region of the right hind paw to all the three groups. The paw volumes were measured using plethysmometer (UGO Basile, 7140 Italy) every hour till 3 h after carageenan injection, and mean increase in paw volumes were noted. Thus oedema volumes in control (Vc) and in groups treated with test compounds (Vt) were calculated. The percentage inhibition was calculated by using the formula:[15]

$$\% \text{ Inhibition} = \frac{Vc - Vt \times 100}{Vc}$$

Where,

Vc = Oedema volume of Control

Vt = Oedema volume of Test

Statistical Analysis

The results are expressed as mean ± S.E.M. The statistical analysis was performed by analysis of variance (ANOVA) test.

RESULTS

Acetic Acid-induced Writhing

The results of AEGP on acetic acid-induced writhing test indicated a significant increase ($P < 0.01$) in reaction time, which is comparable to the reference drug pentazocine [Table 1].

Hot-plate Test

The results of the hotplate test indicated a significant increase ($P < 0.01$) in reaction time in 1 h comparable to the reference drug pentazocine [Table 2].

Anti-inflammatory Activity

The result of AEGP against carrageenin-induced paw oedema is shown in Table 3. AEGP (100 mg/kg, i.p.) gave significant ($P < 0.01$) reduction of rat paw oedema at all assessment times. The aqueous extract showed maximum inhibition of 46.93% at the dose of 100 mg/kg after 2 h of drug treatment in carrageenan-induced paw oedema whereas the standard drug showed 51.75% of inhibition.

DISCUSSION

The thermal stimuli in hotplate test and the writhing response of the animals to an intra-peritoneal injection

Table 1: Effect of aqueous extract of stems of *Gynandropsis pentaphylla* Linn on latency to acetic acid-induced writhing test

Time after administration (min)	Vehicle distilled water (10 ml/kg, i.p.)	Aqueous extract 100 mg/kg, i.p.	% Inhibition	Pentazocine 5 mg/kg i.p.	% Inhibition
30	67.8 ± 3.3	15.2 ± 3.114	77.58%	2.8* ± 0.58	95.82%
60	62.4 ± 1.12	25.4 ± 1.0	59.03%	6.5* ± 0.89	89.51%

Values are mean ± SEM. ($n = 5$); *$P < 0.01$

Table 2: Effect of aqueous extract of stems of *Gynandropsis pentaphylla* Linn on latency to hotplate test

Time after administration (min)	Vehicle distilled water (10 ml/kg, i.p.)	Aqueous extract	Pentazocine
		100 mg/kg, i.p.	5 mg/kg i.p.
30	9.2 ± 1.30	12.40* ± 0.13	15.2* ± 1.09
60	8.7 ± 0.37	11.89* ± 0.04	14.2* ± 0.3

Values are mean ± SEM. ($n = 5$); *$P < 0.01$

Table 3: Anti-inflammatory activity of aqueous extract of stems of *Gynandropsis pentaphylla* Linn on carrageenan-induced paw oedema in rats

		Mean paw volume (ml) ± S.E.M							
		0 h		1 h		2 h		3 h	
Treatment	Dose	EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
Control	–	1.846 ± 0.04	-	1.89 ± 0.01	-	1.99 ± 1.03	-	1.87 ± 0.04	-
Aceclofenac sodium	10 mg/kg	1.232 ± 0.03	33.26	1.034 ± 0.04	45.29	0.96 ± 0.04	51.75	0.91 ± 0.05	51.33
Aqueous extract	100 mg/kg	1.26 ± 0.07	31.74	1.088 ± 0.06	42.43	1.0562 ± 0.07	46.93	1.132 ± 0.10	39.46

Values are mean ± SEM. ($n = 5$); *$P < 0.01$

of noxious chemical are used to screen both peripherally and centrally acting analgesic activity. Acetic acid causes analgesia by liberating endogenous substances that excite the pain nerve endings.[16] From the results it is apparent that the AEGP showed a significant antinociceptive effect in the hotplate test and writhing response, which is comparable to that of the standard. Studies demonstrate that various flavonoids such as rutin, quercetin, luteolin, hesperidin and biflavonoids produced significant antinociceptive and anti-inflammatory activities.[17,18] There are also a few reports on the role of tannins in antinociceptive and anti-inflammatory activities.[19] NSAIDs can inhibit cyclo-oxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors.[20] The mechanisms of antinociceptive action of AEGP could be due to the presence of flavonoids and mediated through central and peripheral mechanisms.

Carrageenan-induced paw oedema was taken as a prototype of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin[21,22] and the delayed phase is sustained by the leucotrienes and prostaglandins.[23] Flavonoids and tannins are reported to inhibit PG synthesis.[24] Most of the non steroidal anti-inflammatory drugs (NSAIDs) have well balanced anti-inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity.

From the above discussion, the aqueous extract from the stems of *Gynandropsis pentaphylla* exhibited significant analgesic and anti-inflammatory activity. Further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities.

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