ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF

DRYMARIA CORDATA WILDD CARYOPHYLLACEAE

C.C Baruah¹, S.K Pal¹, A.G Baruah², J.D Roy¹, B. Buragohain¹, R.S Bora³, L.C Lahon¹

¹Department of Pharmacology & Toxicology, College of Veterinary Science, AAU, Khanapara, Guwahati -781022. Assam, India.
²Department of Public Health, College of Veterinary Science, AAU, Khanapara, Guwahati 781022. Assam, India.
³Department of Statistics, College of Veterinary Science, AAU, Khanapara, Guwahati - 781022. Assam, India.

Summary

The analgesic activity of the leaves of methanolic extract of Drymaria cordata Willd (Caryophyllaceae) was investigated by non-narcotic models like acetic acid induced writhing syndrome test and narcotic models like hot plate, and tail flick models. Pretreatment with oral doses of 300, 600 and 900mg/kg body wt revealed significant (P<0.01) analgesic activity in acetic acid induced writhing syndrome as compared to the vehicle treated control group. However in narcotic models, the effect was not significant. The preliminary phytochemical investigation revealed the presence of glycosides, tannins, diterpenes, alkaloids and flavonoids, which might be responsible for its analgesic effect. From the above study, it can be concluded that analgesic activity of Drymaria cordata methanolic extract (DCME) might due to some of these constituents present therein and that the plant might have exerted its analgesic action by peripheral mechanism as evidenced by its significant analgesic effect on acetic acid induced writhing syndrome and little or no effect on hot plate or tail flick method. Further study on its mechanism of action and fractionation of active constituents may establish the preliminary finding.

Key Words: Drymaria cordata, methanolic, analgesic activity, phytochemical screening.
Introduction

Use of plant products is increasing in many segment of the population [1]. At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases. According to an estimate, 80% of the world’s population relied upon plants for their medication [2]. The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products [3].

Most of the drugs used at present for analgesic effect are synthetic in nature, prolonged use of which causes many side and toxic effects. Moreover, synthetic drugs are very expensive to develop. For the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested, whose cost of development ranges from 0.5 to 5 million dollars. On the contrary, many medicines of plant origin had been used since long time without any adverse effect. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [4].

Drymaria cordata L Willd (Caryophyllaceae) is a sprawling herb with procumbent and more or less ascending branched stems, often rooting at the lower nodes, quadrangular, glabrous or papillose especially in the upper internodes; internodes slender, generally 2-6 cm long. Leaves opposite, blades ovate to very broadly ovate, acute or subacute and shortly apiculate or even rounded at apex, cordate, truncate, or shortly cuneate at base, petals 3 mm long, white, ovules usually about 3, seeds 1.5 mm in greatest diameter, bluntly tubercled [5]. Its medicinal uses are antidote, appetizer, depurative, emollient, febrifuge, laxative and stimulant. The pounded leaf is applied to snake bites [6]. Studies on Drymaria cordata exhibited significant antitussive activity [7]. The antibacterial activity has also been evaluated. The anti-inflammatory activity of has also been studied [8,9].

Materials and Methods

Plant Material

The leaves of Drymaria cordata were collected in July-Sept, 2008 from the medicinal garden of the Department of Pharmacology & Toxicology, C.V.Sc. Khanapara, Guwahati and submitted to the Botanical Survey of India for identification. In addition, a voucher specimen (No AAU/CVSC/PHT/ 07-08/ 02) has also been deposited in the Herbarium of Botanical Survey of India, Meghalaya.

Preparation of Extract

The leaves were shade dried, cleaned from extraneous materials, mechanically grinded. They were powdered and soaked in 1000 ml methanol for 72 hours in separate beakers. Each mixture was stirred every 18 hr using a sterile glass rod. Filtrate was obtained 3 times with the help of Whatman’s filter paper No 1. The filtrate obtained was concentrated in Rotary Evaporator (Equitron) at 50°C-60°C under reduced pressure leaving a dark brown residue. The Drymaria cordata methanolic extract (DCME) thus obtained was transferred to a Petri dish and kept over hot water bath (50°C) until solvent was completely evaporated. It was stored at 4°C for further use. Recovery percent was recorded (8.7%).
Preliminary phytochemical screening [10]
The active fractions were subjected to phytochemical screening in order to find out the active ingredients.

Experimental animals
Albino male Swiss mice (18-22g) and Wistar rats weighing (120-122g) were used for the study. The animals were housed in colony cages and maintained under standard environmental conditions 25 ± 2°C temperature 12×12 h light : dark cycle ,and 45 –55% relative humidity, with free access to food and water ad libitum. The animals were fasted overnight and during the experiment. All experiment were carried out during the light period (08.00-16.00 h). The research was conducted in accordance with the ethical rules on animal experimentation, approved by ethical committee, Faculty of Veterinary Science, Khanapara, Assam Agricultural University.

Acute Toxicity studies [11]
Their safety value was also determined by studying acute toxicity and LD₅₀. An initial dose 460 mg/kg is administered and depending upon mortality two lower or higher geometrically spaced doses are given and LD₅₀ is read from the tables. The acute toxicity and gross effect of crude DCME was studied in albino mice by using ½ LD50. A total of four numbers of male albino mice were selected for the experiment. Animals were observed hourly for six hours and again after 24 hours. The parameters for motor activity and gross effect were determined after administration of DCME orally at a dose level of 2.5g /kg b. wt.

Treatment Regimes
The animals were divided into five groups, each containing six mice . The groups of mice were assigned to receive the following doses.

1. **Group I -Vehicle (distilled water 0.1mg/ml of body weight)**
2. **Group II -Morphine (1.5 mg/kg i.p)**
3. **Group III – DCME (300mg/kg body wt. p.o)**
4. **Group IV – DCME (600 mg/kg body wt. p.o)**
5. **Group V -DCME (900 mg/kg body wt. p.o)**

Drugs and Chemicals
Morphine (Drugs India Mfg. Dispur, Guwahati-5) was used as the standard analgesic agent for narcotic models and Indomethacin (Jagsonpal Pvt. Ltd., Faridabad) was used for non-narcotic model. Acetic acid was procured from Merck.

Evaluation of analgesic activity:

Acetic acid induced writhing test [12]
The analgesic activity of DCME was studied on chemically induced pain sensation in female albino mice (20-35 g). A total of thirty numbers of adult non pregnant female albino mice were selected which had shown stretching episodes in 20 minutes after intra peritoneal administration of acetic acid (0.7 % solution) at the dose of 70 mg/kg body weight. After 1 hour of pre-treatment with the test compound or the standard drug, mice were injected with acetic acid (0.7%) intra-peritoneally. Total numbers of stretching episodes for 20minutes in all the groups were recorded. Percent reduction in writhing syndrome was calculated and compared with the standard drug.

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Hot plate method [13]

Mice of either sex weighing between 20-30 g were kept on a hot plate (55± 0.5°C), lick their paws and try to escape within a few seconds. The reaction time is noted. Mice showing reaction time between 3-5 sec. were selected. Animals not responding in this period were discarded. The reaction time was recorded at 0, 30, 60, 90, 120 minutes following administration of the test compounds or the standard drug to determine the onset and duration of action and analgesic activity was determined by comparing with the control group.

Tail flick method [14]

The analgesic activity of DCME by was studied tail flick method using Nichrome wire analgesiometer in male albino rats (180-200 g). A total of thirty numbers of male albino rats were selected. Individually the tail of rats were placed over the hot wire and the time when the tail is withdrawn was recorded. The reaction time in each group of rat was determined at 0, 30 and 60 min following administration of the test compound or the standard drug and compared with the control group.

Statistical analysis

The statistical analysis of data was done using one-way analysis of variance by using the SPSS software (version 11.5). A probability less than 0.01 was considered to be statistically highly significant.

Results

Extractive value and preliminary phytochemical screening:

The extractive value of DCME is 8.7%. Phytochemical studies show the presence of glycosides by Benedict’s test, Molisch and NaOH test, tannins by ferric chloride and gelatin test, diterpenes, alkaloids by Wagner’s and Hager’s test and flavonoids by ferric chloride and lead acetate test for DCME.

Acute toxicity test

The DCME did not show any mortality up to 5000mg /kg body weight p.o. in rats. Hence the compound is safe. In acute toxicity study also there was no mortality at ½ LD₅₀ dose i.e. 2.5 g/kg body weight p.o. in rat after 24 hours. They did not show any change in the gross behavioral effect at the given dose.

Acetic acid induced writhing syndrome

At doses 300, 600 and 900mg/kg p.o of DCME showed 30%, 47% and 80% reduction in number of writhing, respectively. The effect of DCME showed dose dependent reduction in the number of writhing as compared to standard drug which was highly significant(P<0.01). The effect, at the dose 900mg/kg p.o( 80% reduction) is comparable to standard drug Indomethacin (82.7% reduction).
**Figure 1:** Effect of DCME in acetic acid induced writhing test

N=6; P<0.01, P< 0.01 vs vehicle (one way ANOVA)

**Hot plate method**

DCME pretreatment increased the response latency in the hot plate test which was not significant. The standard drug morphine increased the response latencies at various time intervals (P<0.01). The effect of DCME was dose as well as time dependent. Amongst all the doses used, DCME is most effective at 900mg/kg at 60 minutes, but not as comparable as the standard drug morphine.

**Figure 2:** Effect on DCME on the reaction time in the hot plate model.

N=6; P<0.01, P< 0.01 vs vehicle (one way ANOVA)
Tail flick method.
It was observed that the DCME showed dose dependent analgesic activity at the given dose. The effect was however not significant (p<0.05) between different dose of DCME.

NSS= normal saline, DC= Drymaria cordata, MP= morphine

![Graph showing effect on DCME on the reaction time in the tail flick model.](image)

Fig 2: Effect on DCME on the reaction time in the tail flick model.

N=6; P<0.05, P< 0.05 vs vehicle (one way ANOVA)

Discussion

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas, plants still hold their own unique place, by the way of having no side effects [15].

The DCME causes significant (P<0.01) reduction in the number of writhing in mice in dose dependent manner. Acetic acid-induced writhing is used to study the action of drug on peripheral nervous system. Acetic acid causes ‘algesia’ by liberating endogenous substances including serotonin, bradykinin, histamine and prostaglandin which may stimulate pain nerve ending [16]. Therefore, DCME might inhibit the synthesis and/or release of these endogenous substances. However in narcotic models like tail flick and hot plate method the effect of DCME was not as effective as the non-narcotic models suggesting it might not act through central mechanism, as these methods are normally used for evaluation of centrally acting analgesics. The methanolic extract of Capparis zeylanica Linn. Roots and Dendrophthoe falcate show analgesic activity which has been attributed to the presence of the flavonoids, alkaloids and other bioactive compounds [17,18]. Flavonoids are known to inhibit prostaglandin synthetase [18]. The phytochemical screening of DCME also shows the presence of steroids, triterpenes, tannins, diterpenes, alkaloids and flavonoids, which might contribute to its analgesic effect. Prostaglandins are involved in pain perception and are inhibited by flavanoids. The above results shows that DCME is more effective in decreasing pain due to peripheral mechanism, which mainly involves various endogenous substances like prostaglandins.
It might be possible that reduced availability of prostaglandins by flavonoids present in DCME might be responsible for its analgesic effect. From the above results it can be interpreted that analgesic activity of DCME can be attributed to the presence of flavonoids. Further, it is observed that the DCME acts peripherally as acetic acid induced writhing syndrome is significantly reduced ($P<0.01$) whereas, there is little or no effect on the narcotic parameters viz. hot plate and tail flick methods.

In conclusion, the present study demonstrated that DCME has intrinsic analgesic activity which needs to be further investigated with more information on the bioactive principles responsible for the action.

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