

Spasmolytic effect of traditional herbal formulation on guinea pig ileum

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ABSTRACT

Background: The herbal formulation consisting of *Andrographis paniculata* Nees., *Cassia fistula* L., *Foeniculum vulgare* Mill. and *Cuminum cyminum* L. is widely used by the local traditional practitioners in rural Northern Karnataka for spasmodic abdominal pain. **Objective:** The present study was undertaken to evaluate safety and spasmolytic effect of poly-herbal formulation. **Materials and Methods:** Acute toxicity studies were carried out in Swiss mice, as per the Organization for Economic Co-operation and Development (OECD) guidelines. The spasmolytic activity of the formulation was studied in isolated guinea pig ileum model using histamine and acetylcholine as agonists. The data were analyzed by one-way ANOVA, followed by Dunnetts *post-hoc* test and $P \leq 0.05$ was considered as significant. **Results:** The formulation did not show any adverse toxic effects and found to be safe. It also showed significant ($P < 0.05$) relaxation in different agonist like histamine and acetylcholine-induced contractions in guinea pig ileum. **Conclusion:** Antispasmodic activity of the herbal formulation can be attributed to its atropine-like activity. The present findings, therefore, support its utility in spasmodic abdominal pain.

Key words: Acetylcholine, histamine, spasmolytic activity, traditional formulation

INTRODUCTION

Traditional healers play an important role in health care delivery system, particularly for the primary health care, in rural parts of India. Many of these traditional herbal health practices are popular among the community and claimed to be effective. However, most of these formulations are not scientifically studied for their safety and efficacy. Hence, there is a need to evaluate the safety and therapeutic potentials of these formulations. One such multi-herbal traditional formulation has been evaluated in the present study. This formulation is being used for the treatment

of spasmodic abdominal pain in rural communities of Northern Karnataka, especially in Gokak and Hukkeri Talukas in Belgaum district of Karnataka in India. Local traditional practitioners claim that, the formulation is safe and effective in relieving abdominal pain. After detailed discussion with the practitioners, regarding the signs and symptoms of the pain, it was speculated that the formulation might be possessing anticholinergic/antihistaminic activity. Several reports are available on chemical components and biological activities of the ingredients in the formulation. The first ingredient of the formulation is *Andrographis paniculata* Nees. (*Acanthaceae*), locally known as *Nelaberru* and is mentioned in various Ayurvedic formulations for the treatments of fever, expectorant, diarrhea and inflammatory diseases.^[1] *A. paniculata* reported to contain flavones and glycosides. Its various extracts have been studied for antipyretic, anti-ulcerogenic, analgesic,^[2] antioxidant and anti-inflammatory activities.^[3] The plant is also reported for hepatoprotective,^[4] anti-hyperglycemic and renal protective activities.^[5] *Cassia fistula* L. (*Caesalpinaceae*) is the other plant in the formulation, which is known as *Kakeke*.^[6] In Ayurveda, the plant is reported for *Shoola*, *Gulma*, *Vibandha*, *Udavarta*, etc. Aragvadhadi Kvatha Churna is an important formulation mentioned in Ayurveda, in which *C. fistula* is a major ingredient.^[7,8] Flavones and glycosides are reported to be the major components in the plant.^[9] The hepatoprotective,^[10] hypoglycemic,^[11] antitussive^[12] and anti-inflammatory activities are reported for the plant.^[13]

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The third plant, *Foeniculum vulgare* Mill., (*Apiaceae*), commonly known as *Badesop*, contains trans-anethole as its major component. Indian pharmacopoeia of Ayurveda suggest its uses for various ailments such as *Shoola*, *Agnimandya*, *Kasa*, *Pravahika* and *Raktadosha*.^[7,14] The aqueous extract of *F. vulgare* has been reported for its anti-ulcerogenic activity.^[15] Analgesic,^[16] anti-inflammatory and antioxidant activities are also reported for various extracts of the plant.^[17] The fourth ingredient of the formulation is *Cuminum cyminum* L. (*Apiaceae*), known in local language as *Jeerige*.^[18] *C. cyminum* L. mentioned in Ayurveda for *Agnimandya*, *Atisara*, and *Krimiroga*. Hingvadi Curna, Jirakadyarista, Jirakadimodaka, Hinguvacadi Curna are the popular formulations comprising this plant.^[7,19] The reported major chemical components from the plant were α -pinene, 1, 8-cineole, cuminol, cuminic alcohol, γ -terpinene, safranal, *p*-cymene and β -pinene.^[20] The oil from *C. cyminum* has been reported for analgesic effect,^[21] while aqueous extract of the plant is reported for relaxant activity in guinea pig tracheal chain model.^[22] However, no reports are available on antispasmodic activity of the formulation as a whole, which is being used in the community for several generations. The present study was, therefore, undertaken to evaluate the safety and efficacy of the formulation.

MATERIALS AND METHODS

Documentation of the traditional practice

The selected traditional practitioners were frequently visited and interviewed to get the details on disease, diagnosis, treatment, formulation and proportions of the ingredients, method of preparation, mode of administration etc.

Plant material

The plants incorporated in the formulation, that is, *A. paniculata* Nees, *C. fistula* L., *F. vulgare* Mill. and *C. cyminum* L. were authenticated. Their voucher specimens were deposited in the Herbaria at Regional Medical Research Centre (ICMR), Belgaum. These specimens are numbered as RMRC-0007, 432, 501 and RMRC – 500 respectively. The parts of medicinal value from the plants were collected with the help of traditional practitioners.

Preparation of formulation

The formulation was prepared as per the processes employed in traditional practices. The ingredients were added in the same proportions, as in the traditional practice.

The method of preparation was standardized in consultation with the traditional practitioners. In the practice, 2 TSF of this mixture is given orally, early in the morning for 1-week. The coarse powders of *A. paniculata* stem (1.57 g), *C. fistula* stem bark (3.16 g), *F. vulgare* seeds (2.3 g) were mixed with *C. cyminum* seeds (2.4 g). As there are no models available to

evaluate the spasmolytic activity of the powdered material directly, the water extract of this formulation was prepared using cold maceration. The powder prepared is mixed thoroughly with 250 ml of distilled water for 7 days at room temperature with daily muddling up. The supernatant water was carefully decanted on 8th day and was used for the experiments.

Drugs and chemicals

Histamine and acetylcholine were procured from Sigma-Aldrich Chemical Co. calcium chloride (CaCl₂), magnesium sulfate, sodium chloride (NaCl), dextrose, sodium hydrogen carbonate (NaHCO₃), potassium chloride (KCl) and sodium dihydrogen phosphate (NaH₂PO₄) were procured from local supplier.

Animals

Guinea pigs of either sex weighing between 300 and 500 g were procured from Shri Venkateshwara Traders, Bengaluru, India. They were housed in galvanized cages under standard conditions (temperature 25°C ± 3°C, relative humidity 50–60% and natural light and dark cycle). The animals were fed with a balanced diet formulated in the department (cauliflower, cabbage, gram and wheat flour) and water was available *ad libitum*. The study was approved by Institutional Animal Ethics Committee, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Acute toxicity studies

Swiss mice weighing 20–25 g were used in the study. The animals were fasted overnight. A single dose (2000 mg/kg body weight) of herbal formulation was administered next day, between 10:00 and 11:00 am and the animals were observed as per Organization for Economic Co-operation and Development (OECD) guideline 423–2002.^[23]

Tissue preparation and spasmolytic studies

Overnight fasted (with free access of tap water) guinea-pigs of both sexes were sacrificed with halothane over anesthesia. The ileum was isolated. The terminal portions, of about 10–20 mm in length, were taken after discarding the portion nearest to the ileocecal-junction. The intestinal content was eliminated by washing with tyrode solution (composition NaCl 136.9; KCl 2.7; CaCl₂ 1.3; NaHCO₃ 11.9; MgCl₂ 1.1; NaH₂PO₄ 0.4 and glucose 5.6) as prescribed by Jabeen *et al.*^[24] The mesenteric residues were removed. 1.5 cm ileum tissue was mounted in 15 ml organ bath containing tyrode solution at 37°C, continuously bubbled with air. The tissue was allowed to relax for about 30 min with 1 g preload.^[25] Drug contact was allowed for 30 s, followed by washing for 3 min. Effect of acetylcholine and histamine, in a single dose (1 µg), was recorded individually in the

absence of herbal formulation. Similarly, effect of herbal formulation (0.5 ml) alone was tested in the absence of both acetylcholine and histamine. The test with a single dose of acetylcholine was repeated after wash period to confirm the tissue response. The tissue response for combination of herbal formulation (0.5 ml) and acetylcholine (1 µg) was recorded, followed by the test with a single dose of acetylcholine. Tissue response for a single dose of histamine was recorded, and test was conducted with a combination of herbal formulation (0.5 ml) and histamine (1 µg). Finally histamine, in a single dose, was tested again to confirm the tissue response. All the tests were carried out twice, and the washing was repeated before each test.

Statistical analysis

The percent of ileum relaxation induced by herbal formulation was calculated by assuming the contraction induced by spasmogens (acetylcholine and histamine) as 100%. The ileum relaxation or contraction was expressed as mean \pm standard error of the mean (SEM) results were analyzed using one-way ANOVA and Bonferroni *post-hoc* test by considering $P \leq 0.05$ as significant.

RESULTS

Acute toxicity

There was no mortality over a period of observation for 14 days in animals treated with a single dose of 2000 mg/kg. There were no other signs of toxicity and adverse effects. LD₅₀ was considered to be more than 2000 mg/kg.

Spasmolytic studies

The results of contraction and relaxation are expressed in mm (millimeter) and represented as mean \pm SEM [Table 1]. The herbal formulation reduced the ileum contraction induced by histamine and acetylcholine agonists [Figure 1]. The significant decrease ($P < 0.01$) was observed in histamine with herbal formulation (26.01 ± 3.53), compared to histamine alone (53.38 ± 8.29). Similar significant decrease ($P < 0.05$) was observed also in acetylcholine with herbal formulation (20.83 ± 4.24), compared to acetylcholine alone (44.38 ± 4.84). The herbal formulation alone did not showed any changes (contraction/relaxation) in the tissue.

DISCUSSION

The herbal formulation showed significant inhibition in the amplitude of spontaneous contraction of guinea pig ileum. Results showed that the herbal formulation is capable of inhibiting acetylcholine and histamine-induced contractions. The current study also demonstrated that the herbal formulation has neither relaxing nor

Table 1: Changes in treated and nontreated ileum contraction/relaxation

| Test | Mean \pm SEM (mm) | Percent change | P |
|----------------------------------------------|---------------------|----------------|-------------|
| 1 µg histamine | 53.38 \pm 8.29 | 48.79 | ≤ 0.01 |
| 1 µg histamine+0.5 ml herbal formulation | 26.01 \pm 3.53* | | |
| 1 µg acetylcholine | 44.38 \pm 4.84 | 47.02 | ≤ 0.05 |
| 1 µg acetylcholine+0.5 ml herbal formulation | 20.83 \pm 4.24** | | |

* $P < 0.05$, ** $P < 0.01$. SEM=Standard error of mean

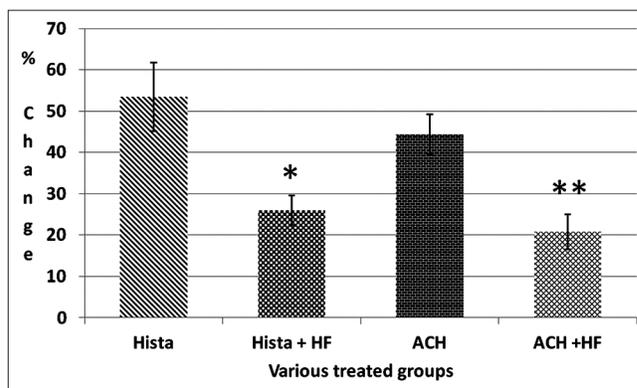


Figure 1: Effect of herbal formulation on ileum contraction. * $P < 0.05$, ** $P < 0.01$. Hista: Histamin, Hista + HF: Histamin + herbal formulation, ACH: Acetylcholine, ACH + HF: Acetylcholine + herbal formulation

contracting effect on ileum; it antagonizes the effect of spasmogens such as acetylcholine and histamine. Among the ingredients of the present formulation, only *C. cyminum* has been reported to possess relaxant like activity in guinea pig tracheal chain tissue.^[22] However, no such reports were available for the other ingredients. The observed antagonism against the spasmogens suggests that the spasmodic action of the formulation was produced by all ingredients of the used herbal formulation. The plants of herbal formulation are not yet reported for such relaxant activity except *C. cyminum*. The relaxant effect of formulation may be due to multiple plants.

CONCLUSION

Based on the data obtained, it can be concluded that, the traditional formulation is found to be safe and effective in the experimental model of the spasmodic pain. The study supports the claim of the traditional practitioners that this formulation can be used for the spasmodic pain treatment. Further work is being carried out to determine the responsible fraction of the plant by employing the suitable model.

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