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Analgesic, antipyretic, and anti-spasmodic activities of solvent fractions derived from *Chenopodium ficifolium* on albino mice

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ABSTRACT: Albino mice, hot plat-induced spasm, charcoal-induced spasm, and brewer yeast-induced pyrexia were used to assess the analgesic, anti-spasmodic, and antipyretic activities. The experimental groups received doses of 100 and 200 mg/kg of chloroform, n-hexane, ethyl acetate, and aqueous fractions. The n-hexane fraction at a dosage of 200 mg/kg notably prolonged the reaction time ($P<0.01$) and mitigated paw edema only at the 200 mg/kg level across all models. Significant suppression of rectal temperature was noted with a higher dosage (200 mg/kg) of chloroform friction. Analgesic activity was prominently exhibited by the ethyl acetate fraction, yielding results on par with the established standards. These findings underscore the promise of these fractions in mitigating pain-associated reactions, Brewer's yeast-induced hyperthermia, and charcoal-induced spasms. Enhanced efficacy was evident at dosages of 100 mg/kg and 200 mg/kg of the organic fraction obtained from *Chenopodium ficifolium*, particularly in the management of fever, pain, and spasmodic conditions.

Keywords: *Analgesia, pyrexia, spasmodic, C. ficifolium, albino mice*

INTRODUCTION

Chenopodium ficifolium, an annual herbaceous plant, belongs to the Chenopodiaceae family. It is frequently referred to as Fig-leaved Goosefoot and shares synonymous names, such as *Chenopodium serotinum*. This plant typically reaches heights ranging from 20 to 70 cm, with exceptional specimens reaching 150 cm [1]. The stem of the plant is typically upright, displaying green stripes and occasional yellow or red shades. In some cases, red markings rarely appeared in the leaf axils. Plants tended to branch out, particularly in lower sections. The inflorescence is terminal, extensively branched, and lacks leaves in the uppermost portion. The seeds were roundish in outline, black in color, and measure approximately 0.8-1.0 mm [2]. Although it was previously considered an archaeophyte weed in Europe, *C. ficifolium* is now found in most temperate crop-growing countries [3]. Globally, a significant proportion of the population relies on traditional medicine, including the use of herbs to treat various illnesses. These herbal traditions have laid the groundwork for modern medicine [4]. Pharmacology is a broad field within the realm of natural science that is concerned with the study of drug actions. Medicines encompass internally derived molecules, whether natural or synthetic, that exert biochemical or physiological effects on cells, tissues, organs, or entire organisms [5]. Pharmacology explores the positive and negative effects of natural and synthetic substances on living organisms [6]. Medicinal plants contain naturally occurring biologically active compounds or phytochemical constituents that are used in diverse pharmacological applications [7]. Analgesics, also known as painkillers, are drugs used to alleviate pain. Pain is an unpleasant physiological sensation caused by an injury or tissue damage [8]. Chemicals, such as prostaglandins and leukotrienes, can trigger pain sensations. Formalin and acetic acid are responsible for transmitting pain signals to the peripheral and central nervous systems [9]. Drugs that provide pain relief are referred to as analgesics. Despite their analgesic properties, these drugs have several side effects, including constipation, dry mouth, vomiting, and respiratory depression [10]. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as paracetamol (salicylates) and opioids such as morphine and oxycodone are categorized as analgesics [11]. According to the International Association for the Study of Pain (IASP), pain is defined as an unpleasant sensory and emotional experience associated with real or potential tissue damage, or described in terms of such damage [12]. Alternatively, pain can be construed as the conscious recognition of nerve impulses elicited by noxious sensations within the brain [13]. Pain exhibits variability in intensity, character, and duration, ranging from mild to severe, intermittent to continuous, and sharp to dull, respectively. It can also be localized, widespread,

superficial, or deep [14]. Anti-spasmodic agents inhibit muscle contraction. In the gastrointestinal tract, these agents prevent spasms of the stomach and intestine by blocking the receptors to which acetylcholine binds, thus inhibiting cholinergic nerve impulses [15]. Anti-spasmodics are muscle relaxants used to relieve spasms in the stomach, intestine, and bladder. Although anti-spasmodic drugs such as dicyclomine and hyoscyamine alleviate stomach spasms associated with Irritable Bowel Syndrome (IBS), they can cause constipation and are not prescribed for this reason. These substances are frequently used to treat a range of gastrointestinal ailments such as spasms, diarrhea, and IBS, which affect a substantial portion of the population [16]. Fever refers to an increase in the body's typical temperature, and signifies an active mechanism that counteracts external environmental fluctuations. Infections, tissue damage, inflammation, graft rejection, and other diseases can lead to an increase in body temperature [17]. Fever is a complex, physiological disorder. Conditions such as bacterial infections, brain tumors, and dehydration can influence temperature regulation. An increase in body temperature is influenced by an increase in Prostaglandin E2 (PGE2) concentration. Antipyretic medications suppress the function of cyclooxygenase (COX), consequently diminishing PGE2 levels and decreasing fever [18].

METHOD AND MATERIALS

Collection of plant

Chenopodium ficifolium plant specimens were collected in the vicinity of Shandong, China, in April 2023. Its identification was achieved by consulting the Flora in China. The validation was conducted by comparing it with the exemplar archived in the Department of Pediatrics at Shandong Provincial Third Hospital, affiliated with Shandong University, China.

Dry and powder

The upper sections of the plants underwent a shade-drying procedure lasting 25-30 days at ambient temperature. Subsequently, the dehydrated plant matter was fragmented into small sections and pulverized into a powder with an electric grinder [15].

Mice collection

Albino male mice weighing between 2 and 30 g were sourced from Shandong Provincial Third Hospital. Mice, aged 5 to 7 weeks, were obtained from the Experimental

Animal Laboratory of Shandong Provincial Third Hospital, which is affiliated with the Department of Pediatrics at Shandong Provincial Third Hospital, Shandong University. The study followed the ARRIVE guidelines (https://arriveguidelines.org) and was conducted ethically in compliance with UK standards. Animal (Scientific Procedures) Act of 1986, EU Directive 2010/63/EU, or the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The research protocol involving animal use was approved by the Animal Experimental Biosafety Committee of Shandong University. Prior to the experiment, the mice underwent a seven-day acclimatization period to adjust to the laboratory environment.

Plant materials

In April 2023, *C. ficifolium* leaf material was collected from the local Jinan area of the Wuyingshan Middle Road, Tianqiao District, Jinan, Shandong, China. The plant was identified and verified at the Department of Pediatrics of Shandong Provincial Third Hospital.

Preparation of extract and fraction

The extraction and fractionation methods described [19]. The powdered material (1 kg) was extracted by maceration with methanol (1.5 L) at room temperature. After seven days of agitation in an electrical shaker, the mixture was filtered through filter paper. The filtrate obtained was evaporated to produce a methanolic extract. Subsequently, 100 g of this extract was dissolved in a solution comprising 400 mL of distilled water and 400 mL of n-hexane in a separating funnel.The mixture was shaken for 20 min, after which the n-hexane portion was separated using a separating funnel and then dried. The aqueous, chloroform, and ethyl acetate fractions were obtained using the same process and subjected to the same drying process. The obtained fractions were used for biological investigations.

Analgesic activity by hot plate method

The method employed to evaluate analgesic activity [20] involves assessing the response of mice and rats to heat, which induces notable sensitivity in their paws, even at temperatures not damaging to the skin. Observable reactions included leaping, paw withdrawal, and paw licking. The hot-plate temperature was maintained at 56 ± 1°C [21]. after a 12-hour

fasting period, albino mice of both sexes were divided into five groups for the experiment. Groups 1 and 2 were designated as the negative and positive controls, respectively, in relation to the extract. Groups 3, 4, and 5 were administered doses of 100, 200, and 400 mg/kg of filtered fractions derived from n-hexane, ethyl acetate, chloroform, and aqueous solutions, respectively. Both the experimental substances and prescribed medications were administered orally. Subsequently, each animal was placed individually on a hot plate and the time until licking or jumping was recorded using a stopwatch.

$$\textbf{Percentage (\%)} = \frac{\textit{Treated No} \times 100}{\textit{Controle No}} - 100$$

Antipyretic effect

Brewer's yeast induced pyrexia

Brewer's yeast-induced fever was induced using a method outlined in a prior study [22]. A suspension of 15% Brewer's yeast in distilled water was formulated and given to ten cohorts of male and female mice. The control group was treated with saline water, whereas the second group received 10 mg/kg standard paracetamol. The other groups were administered n-hexane, chloroform, ethyl acetate, or aqueous fractions at dosages of 100 and 200 mg/kg. Rectal temperatures were measured using a thermometer at 1, 2, 3, and 4-hour intervals following the administration of the doses. The pre-drug temperature readings were then compared with post-Brewer's yeast rectal temperatures.

$$\textbf{Percent reduction (\%)} = \frac{B - Cn}{B - A} \times 100$$

B denotes the temperature after the onset of fever; Cn denotes the temperature recorded at intervals of 1, 2, 3, and 4 h; and A represents the typical body temperature.

Activities of anti-spasmodic

The anti-spasmodic activity of the extracts was evaluated using the Charcoal Meal method following the procedure outlined by Kerner and Prudic [16]. The animals were randomly divided into 10 groups: the first group served as a control and received only distilled water, whereas the second group was administered a standard drug, atropine, at a dose of 10 mg/kg.

The remaining four groups were administered fractions of n-hexane, chloroform, ethyl acetate, and aqueous solutions at doses of 100 mg/kg, while the other four groups received the same fractions at doses of 200 mg/kg. Subsequently, all animals were orally administered with 10% deactivated charcoal, followed by the respective drug doses. After one hour, all animals in each group were humanely euthanized and their intestines were removed. The distance traveled by charcoal from the stomach to the rectum was measured and expressed as a percentage of the total length, calculated using the following formula:

$$\textbf{Intestinal Transit (\%)} = \frac{D}{L}\mathbf{100}$$

D denotes the length of the charcoal meal (cm) and L denotes the total intestinal length (cm).

Statistical analysis

In each experiment, three separate trials were conducted, and the results were presented as the mean ± standard error of the mean (SEM) of three independent studies (n = 3). Bar graphs were generated using GraphPad Prism, version 8 [23].

RESULTS

In the current study, an investigation encompassing the biological activities and comprehensive quantitative as well as qualitative phytochemical analysis of *C. ficifolium* was undertaken.

Pharmacological activity

Analgesic activity

Significant increases in the reaction time ($P<0.01$) were noted across all fractions of C. ficifolium when exposed to various concentrations of n-hexane, ethyl acetate, chloroform, and aqueous fractions. Notably, the ethyl acetate fraction of C. ficifolium at a dose of 200 mg/kg exhibited a higher percentage of mean reaction time than the other fractions (Figure 1). Moreover, when comparing the reaction time of the tested dose with that of the standard drug morphine, the observed analgesic effect was less pronounced at the test drug dosage of 200 mg/kg. Compared to the standard drug morphine, which resulted in 193.2% inhibition, the inhibition of the test drug was still comparable.

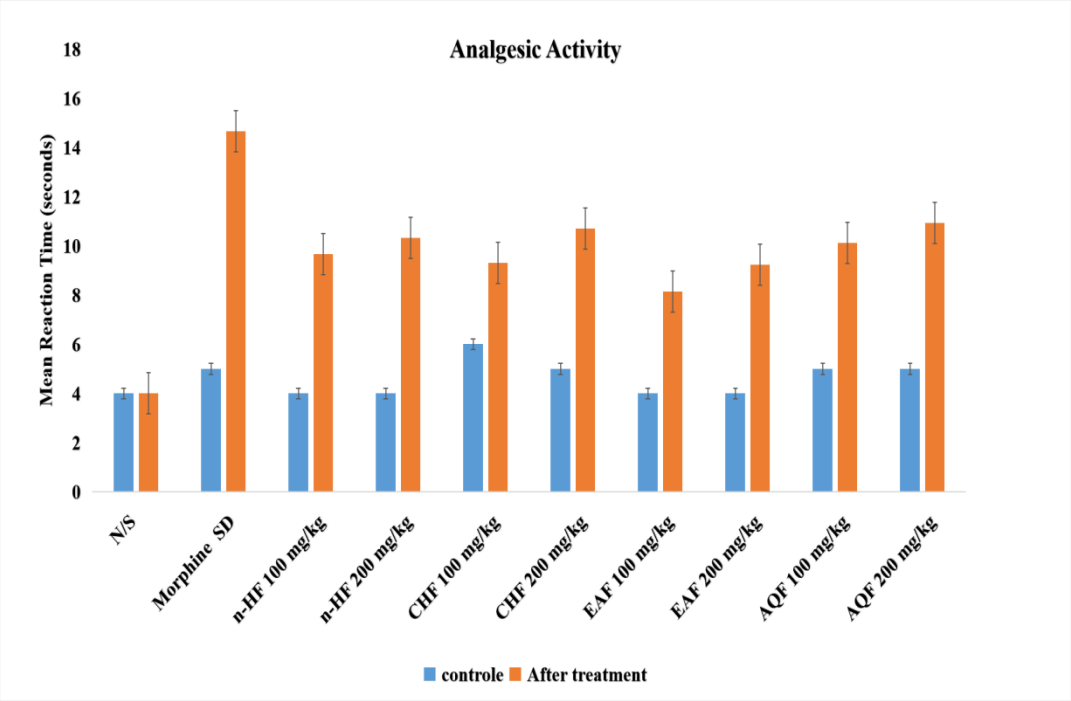

Figure 1. For analgesic activity, control, and post-treatment, the X-axis shows different treatments; for example, normal strain (N/S), morphine standard drugs (Morphine SD), n-hexane fraction (n-HF), chloroform fraction (CHF), aqueous fractions (AQF), ethyl acetate fraction (EAF), and Y-axis shows different mean rectal times (seconds). Error bars in the graph indicate standard error of the mean.

Antipyretic activity

This study explored the effect of various fractions of *C. ficifolium* (n-hexane, ethyl acetate, chloroform, and aqueous) on brewer's yeast-induced fever. Administration of these fractions to mice showed notable antipyretic effects against brewer's yeast-induced fever, as indicated by a decrease in rectal temperature. Significant inhibition ($P<0.05$) of rectal temperature was observed with both lower and higher doses of all fractions, 4 h after brewer's yeast injection. However, only the higher dose (200 mg/kg) of the chloroform fraction exhibited highly significant ($P<0.05$) inhibitory activity against rectal temperature caused by the subcutaneous injection of brewer's yeast. The inhibitory effects of CHF, EAF, NHF, and AQF were not identical to the standard but were comparable (Figure 2).

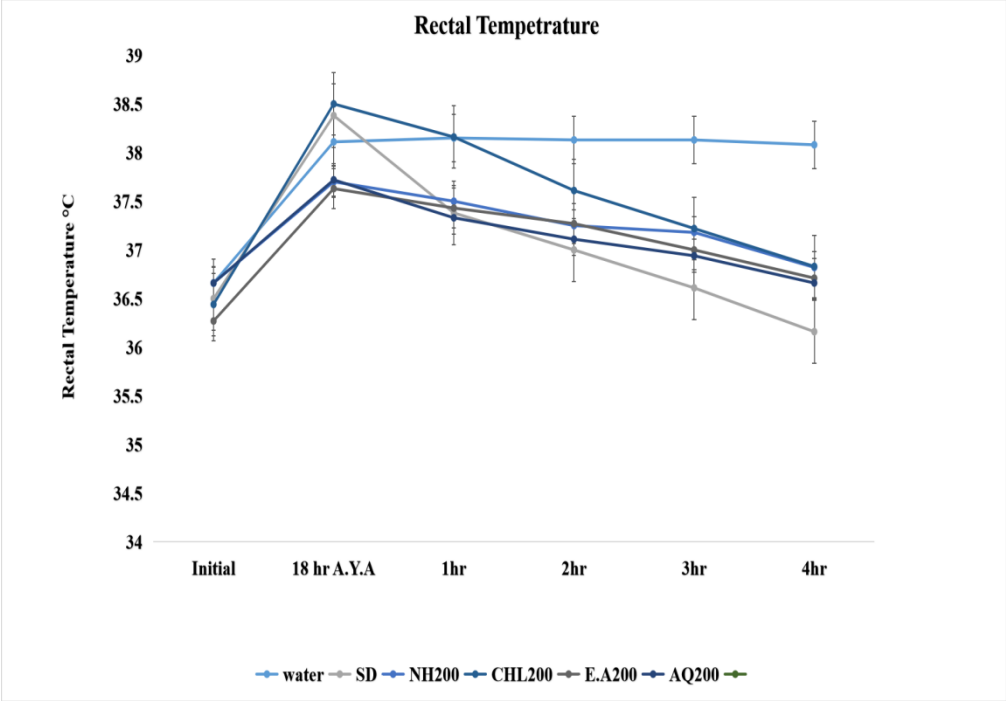

Figure 2. The antipyretic activity of rectal temperature at 200 mg/kg D/F fraction, X-axis shows different times (hours) after yeast administration (AYA), standard drugs (SD), n-hexan fraction (NH), and Y-axis shows different rectal temperatures (°C). Error bars in the graph indicate standard error of the mean.

Anti-spasmodic activity

All fractions of *C. ficifolium* exhibited a significant ($P<0.05$) ability to control intestinal motility at various concentrations of n-hexane, ethyl acetate, chloroform, and aqueous fractions. The inhibitory effect of the Ethyl Acetate fraction of *C. ficifolium* was noteworthy, with an inhibition of 60%. This inhibition was significantly higher at doses of (100 mg/kg and 200 mg/kg) than at the other fractions (Figure 3 and 4). Notably, when compared to the standard drug aspirin, which resulted in 72.97% inhibition, the inhibition observed with the Ethyl Acetate fraction was not equal, but still comparable.

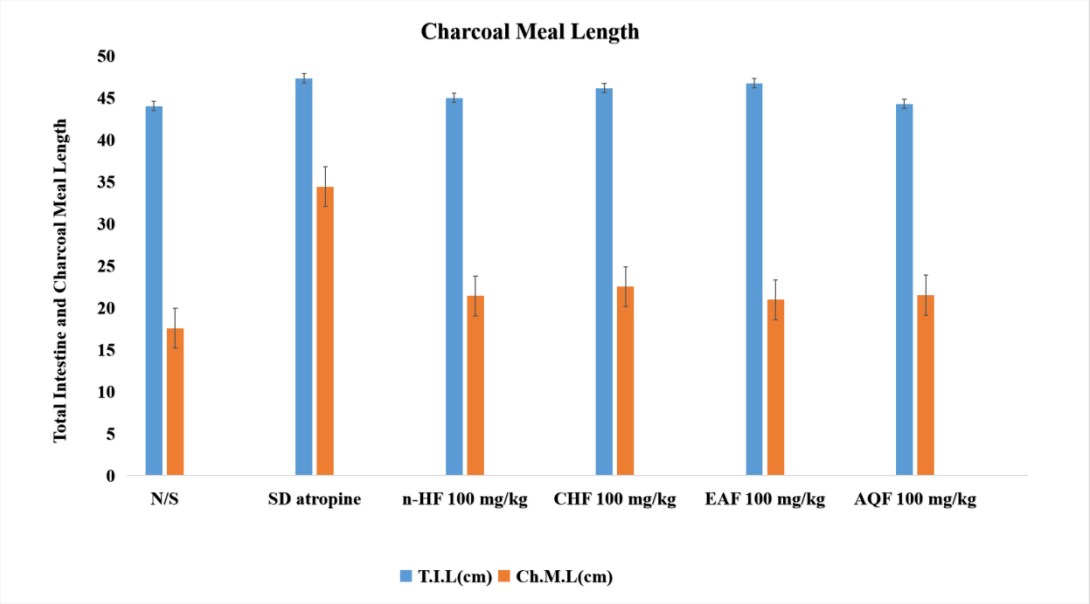

Figure 3. The anti-spasmodic activity of charcoal meal length by 100 mg/kg D/F fraction, X-axis shows different treatments such as normal strain (N/S), morphine standard drugs (Morphine SD), n-hexane fraction (n-HF), chloroform fraction (CHF) and aqueous fractions (AQF), ethyl acetate fraction (EAF), and Y-axis shows different total intestine length (T.I.L) cm, and charcoal meal length (T.I.L). Error bars in the graph indicate standard error of the mean.

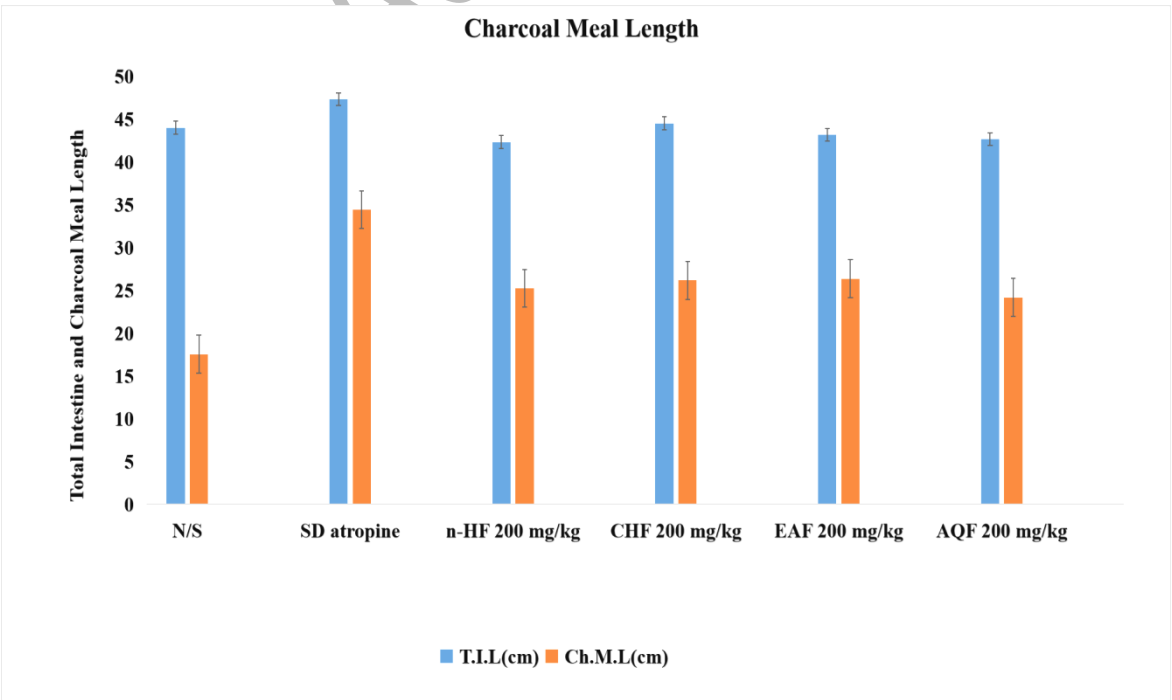

Figure 4. The anti-spasmodic activity of charcoal meal length by 200 mg/kg D/F fraction, X-axis shows different treatments; for example, normal strain (N/S), morphine standard drugs (Morphine SD), n-hexane fraction (n-HF), chloroform fraction (CHF) and aqueous fractions (AQF), ethyl acetate fraction (EAF), and Y-axis shows different total intestine length (T.I.L) cm, and charcoal meal length (T.I.L). Error bars in the graph indicate standard error of the mean.

DISCUSSION

In this study, we explored the pharmacological effects of the n-hexane, ethyl acetate, chloroform, and aqueous fractions derived from C. ficifolium, specifically focusing on their analgesic, antipyretic, and antispasmodic properties. The experimental data suggest that *C. ficifolium* possesses analgesic, antipyretic, and antispasmodic activities. The extract induced a reduction in the number of writhes, indicating potential inhibition of prostaglandin synthesis and release, along with other endogenous chemicals. This peripheral mechanism may mediate the analgesic action.

The hot plate method [20] is a widely used thermal nociception model for assessing the efficacy of drugs or compounds as central analgesics. The effect of any drug or molecule in this pain model suggests the possibility of a centrally acting antinociceptive activity. This approach is considered selective for centrally-acting analgesics [24]. Nociceptors in this paradigm are sensitized by sensory nerves with minimal involvement of endogenous compounds such as prostaglandins [25]. The present study revealed that the methanolic fraction of *C. ficifolium* exhibited significant ($P<0.05$) antipyretic potential at Brewer's yeast-activated body temperature. Similar results were observed across all fractions, indicating a reduction in body temperature comparable to that of standard drugs. This underscores traditional claims regarding the antipyretic efficacy of the methanolic fraction of *C. ficifolium*. To assess the anti-spasmodic activity of the methanolic extract of *C. ficifolium*, a comparison was made with the standard drug atropine sulfate, using the charcoal meal method. The effects of these substances on intestinal motility were evaluated using the charcoal meal test in mice [26]. This fraction exhibited substantial inhibitory effects on the intestinal motility. Remarkably, the efficacy of 10 mg/kg atropine was similar to that of 200 mg/kg. The methanolic fraction of *C. ficifolium* has demonstrated its potential to reduce and inhibit contractions induced by various spasmogens, using diverse pharmacological approaches. Previous studies have attributed the anti-spasmodic activity to the extracts of *Z. officinale, Apium graveolens, and Foeniculum*

vulgare. For instance, *Z. officinale* was found to hinder colonic motility in rats [7]. This spasmolytic effect is linked to inhibition of prostaglandin biosynthesis and serotonergic activity. Similarly, the active constituents of *Apium graveolens* counteracted the norepinephrine-induced contractions in rat aortic preparations. Essential oils from *Foeniculum vulgare* have been reported to exhibit anti-spasmodic effects in rat uterine preparations [27].

The current study showed that *C. ficifolium* inhibited gastrointestinal tract motility, resulting in reduced intestinal transit. These results highlight the promising prospects of *C. ficifolium* as a significant resource for creating innovative spasmolytic medications and warrant further investigation into its anti-spasmodic attributes.

LIMITATIONS

Albino mice were used as test subjects to evaluate the effects of solvent fractions. Animal models offer valuable insights, and although their findings may not always correlate directly with human responses, they remain crucial for scientific understanding. Further research is required to validate our findings in humans. This study focused solely on the medicinal plant, *C. ficifolium*. It is important to note that different plant species exhibit different pharmacological activities. Therefore, the general applicability of these results to other plant species or herbal remedies is limited. In this study, we evaluated the effects of different solvent fractions (chloroform, n-hexane, ethyl acetate, and aqueous) on *C. ficifolium* growth. Other fractions or components within the plant may have different or additional therapeutic activities, which were not investigated in this study. Two different doses (100 mg/kg and 200 mg/kg) of the fractions were administered to the test groups. These doses were selected on the basis of known standards, and the optimal dosage for therapeutic efficacy in humans may differ. Further studies are required to determine the appropriate dose and the potential side effects. This study evaluated the analgesic, antipyretic, anti-spasmodic, and antioxidant activities of these fractions. However, other pharmacological activities and potential interactions with existing drugs have not been explored. Therefore, the broader therapeutic potential and safety profile of *C. ficifolium* fractions remains to be fully elucidated. Although this study provides promising preclinical evidence, the efficacy and safety of *C. ficifolium* fractions in human patients have not yet been established through clinical trials. Further research is necessary to bridge the gap between preclinical findings and clinical applications.

FUTURE PERSPECTIVES

This study investigated the analgesic, antipyretic, anti-spasmodic, and antioxidant properties of the various fractions. These findings indicate that the n-hexane fraction administered at 200 mg/kg notably prolonged the reaction time and offered protection against paw edema. Additionally, the chloroform fraction at a higher dose (200 mg/kg) significantly suppressed rectal temperature, whereas the ethyl acetate fraction displayed considerable analgesic effects. *C. ficifolium* displayed resilience against analgesic, pyretic, and spasmodic effects induced by diverse agents. This study suggests a need for further exploration to assess the potential anticancer and antidiabetic properties of *C. ficifolium*. Moreover, future studies should investigate the medicinal properties of various solvent fractions of *C. ficifolium* for potential therapeutic applications.

CONCLUSION

This study highlights the pharmacological activities of *C. ficifolium*, including its analgesic, antipyretic, and antispasmodic properties. The results indicated that various fractions of *C. ficifolium* exhibited significant effects in experimental models, revealing potential therapeutic benefits in pain management, fever reduction, and alleviation of intestinal spasms. The analgesic activity of *C. ficifolium* was demonstrated through an increase in the reaction time, indicating its potential to alleviate pain. Similarly, its antipyretic activity was evidenced by a reduction in body temperature, suggesting its ability to lower fever. The anti-spasmodic activity of *C. ficifolium* is demonstrated through its inhibition of intestinal motility, indicating its potential in treating conditions related to gastrointestinal spasms.

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Authors' contributions

Y.S. designed the experiment and drafted the manuscript. M.Z. supervised the study. All authors reviewed the manuscript. S.A. provided guidance in data interpretation.

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Data availability

All processed data used in this study can be obtained from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests.

Ethical approval

The research trials were conducted within the Department of Pediatrics and were approved by the Experimental Ethics Committee of Shandong Provincial Third Hospital, affiliated with Shandong University, located at No.11 Wuyingshan Middle Road, Tianqiao District, Jinan, Shandong 250031, China. All procedures strictly adhered to applicable guidelines and regulations. All methods were reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

Informed consent

This article does not contain any studies with human participants performed by any of the authors.

Consent to publish

All the authors have consent to publish.

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