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Research Article
Peripheral Analgesic and Anti-inflammatory Activities of the Methanolic Extracts of *Conyza bonariensis* and its Fractions in Rodents Models

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Abstract

Background and Objective: Several species of *Conyza bonariensis* (*C. bonariensis*) and related species are used traditionally in the management of pain and inflammatory conditions. The current study was aimed to evaluate the analgesic and anti-inflammatory activities of *C. bonariensis* in animal models of pain and inflammation. **Methodology:** For the analgesic effect, acetic acid and formalin-induced models of nociception were employed while the anti-inflammatory effect was assessed in the carrageenan-induced rat paw edema test. The data were analyzed by student's t-test and one-way ANOVA followed by Tukey-Kramer multiple comparisons. **Results:** The intraperitoneal (i.p.) administration of the methanolic extract (50-100 mg kg^{-1}), butanol, chloroform and hexane fractions (25-50 mg kg^{-1} i.p) produced significant inhibition ($p<0.01$) of the acetic acid-induced writhing in mice and suppressed formalin-induced licking response of animals in the second phase of the test. *C. bonariensis* (50-200 mg kg^{-1} i.p.) produced marked anti-inflammatory effect in the carrageenan-induced rat paw edema assay comparable to indomethacin. Among various fractions of the plant, hexane and butonalic acid exhibited significant ($p<0.05$) anti-inflammatory effects. **Conclusion:** It is concluded that *C. bonariensis* possesses analgesic and anti-inflammatory properties and that the bioactive bhytochemicals appeared to be concentrated in the hexane and butonalic fractions.

Key words: *Conyza bonariensis*, writhing test, formalin test, carrageenan induced edema, peripheral analgesia

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cony*za bonariensis* belongs to family *Asteraceae* and found in the local range lands of subcontinent and Middle East[1]. Various parts of C*. bonariensis* are used in a variety of ailments in the South Asian traditional system of medicine. Leaves are used as laxative, roots used in diarrhea[2], cough and flowers are considered as aphrodisiac. Phytochemical studies of C*. bonariensis* revealed several bioactive constituents including flavonoids, flavonoid glycosides, cyanin glycoside, taraxeryl acetate, β-sitosterol, campesterol, stigmasterol and ergosterol. *Conyza filaginoides*, a realted specie known as simonillo in Mexico is used in the relief of painful digestive disorders such as dyspepsia, hepatic and biliary colic and has been shown to have analgesic and anti-inflammatory properties[3].

The plant has also been investigated pharmacologically for anticonvulsant[2], antitumor, hypoglycaemic[4] and estrogenic[5] activities. The essential oil from *C. bonariensis*[6] and extracts of some related species of the plant such as *C. Canadensis*[7] and *C. floribunda*[8] have shown marked anti-inflammatory effects. The genus *Conyza* is popular for its medicinal uses as an anti-inflammatory and is rich in bioactive phytochemical constituents[9].

The current study was aimed to validate the traditional uses of *C. bonariensis* in the management of pain and inflammatory conditions.

MATERIALS AND METHODS

Animals: NMRI/Balb-C mice (20-25 g) and wistar rats (200-250 g) of either sex were obtained from the animal house facility Department of Pharmacology, College of Medicine, King Saud University, Riyadh. The animals were housed in plastic cages under standard condition with 12 h light: dark cycle with free access to food and water.

Chemicals: The following chemicals were used in the experiments: Acetic acid, arachidonic acid, carrageenan, diclofenac sodium, indomethacin and were purchased from the Sigma Chemical Co., (St. Louis Mo, USA). Formalin 37% purchased from Fluka Chemie, Switzerland, respectively.

Plant materials: The whole plant of *Conyza bonariensis* (L.) Cronq. (*Asteraceae*) was collected from Oghi, Mansehra, Pakistan, in November, 2013 and identified by Mr. Jan Alam (Taxanomist) at the Department of Botany, University of Karachi, Pakistan. A voucher specimen (KUH G. H. No. 68220) has been deposited at the herbarium of the above department.

Extraction and fractionation: The air dried whole plant of *Conyza bonariensis* (35 kg) was extensively extracted with MeOH (50 L×3) at room temperature. The extract was evaporated to yield the residue (815 g), which was partitioned between n-hexane (110 g), $CHCl_3$ (90 g), EtOAc (95 g), n-BuOH (495 g) and water (25 g). The extracts were stored in freezer at -80°C.

Experimental methods

Acute toxicity tests: Different doses of the plant extract of *C. bonariensis* were injected intraperitoneally (i.p) to separate groups of six animals. The animals were observed for 1-2 h after administration of the extracts for any acute toxicity symptoms e.g., behavioral symptoms. After 48 h, the highest dose that did not induce any mortality was considered as the maximum non-fatal dose. The number of deaths was counted 48 h after treatment. LD_{50} value (dose of the extracts producing mortality in the 50% of the experimental animals) was determined wherever applicable by graphical method.

Writhing test: Male adult mice were used in this experiment according to the method of Erol and Demirdamar[10], 30 min. After the administration of the extracts, mice were given an i.p. injection of 0.7% v/v acetic acid solution (volume of injection 0.1 mL/10 g). The mice were placed individually into glass beakers and 5 min were allowed to elapse. The number of writhes produced in these animals was counted for 20 min. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Control animals received normal saline (10 mL kg^{-1}, i.p.) and diclofenac (10 mg kg^{-1}, i.p.) was used as a reference drug.

Formalin test: The formalin induced pain test was perform according to method described by Hunskaar and Hole[11], mice were injected, 20 µL of 1% formalin in 0.9% saline, subcutaneously into the dorsal hind paw and placed immediately in transparent box for observation. The duration of paw licking was determined between 0-5 min (first phase) and 15-30 min (second phase) after formalin injection. The time in seconds spent in licking and biting responses of the injected paw was noted. Animals were treated (i.p) with different doses of the plant extracts, 30 min prior to administration of formalin. Diclofenac (10 mg kg^{-1}, i.p.) and

morphine (5 mg kg^{-1}, i.p.) were used as reference drugs. Naloxone (5 mg kg^{-1}, i.p.) was administered 20 min prior to the extracts or morphine injections. Control animals received the vehicle (0.1 mL/10 g). The paw licking time of the animals was compared to control group and represented as percent inhibition.

Rat paw edema assay (anti-inflammatory activity): The carrageenan induced hind paw edema test was conducted according to the Winter *et al.*[12]. Rats divided randomly into different groups of 5-8 animals were injected subcutaneously into the plantar surface of the hind paw with 0.05 mL of freshly prepared 1% carrageenan (prepared in distilled water). Different doses of plant extracts were injected i.p., 30 min before the administration of carrageenan. Indomethacin was used as positive control. The control animals received same volume of the vehicle. Rat paw edema was assessed by volume displacement method (plethysmometer (UgoBasile 7150)) before and after carrageenan injection at 1, 2, 3 and 4 h. Difference in paw volume, determined before and after injection of the phlogistic agent indicated the severity of edema.

The percentage inhibition of the inflammation was determined for each animal by comparison with controls and calculated by the following formula[13].

$$I\ (\%) = \frac{dt}{dc} \times 100$$

Where "dt" is the difference in paw volume in the drug treated group and "dc" the difference in paw volume in control group. "I" stands for inhibition.

Statistical analysis: The results of the study were expressed as Mean±SEM and statistical significance between control and treated groups evaluated by student's t-test and one-way ANOVA followed by Tukey-Kramer multiple comparison test. $p<0.05$ was considered significant.

RESULTS

Acute toxicity test: The intraperitoneal administration of various dose of the plant extracts did not cause any lethality in mice. The maximum non-fatal dose of *C. bonariensis* was 2 mg kg^{-1}.

Writhing test: The intraperitoneal (i.p) administration of the crude extract *C. bonariensis* (50 and 100 mg kg^{-1}) caused significant inhibition ($p<0.001$) of the nociception induced by acetic acid with maximum inhibition 72%. The results were comparable to standard drug diclofenac sodium that produced 62% inhibition at 10 mg kg^{-1} i.p (Table 1).

Among various fractions of the plant extract, the butanolic fraction was the most potent producing 80% protection at 50 mg kg^{-1}. The chloroform and hexane fractions (50 mg kg^{-1}) caused 75 and 44% inhibition of the acetic acid induced writhing respectively (Table 1).

Formalin test: In the formalin test, the plant extracts of *C. bonariensis* had no appreciable effect in the first phase but caused dose dependent inhibition of the licking responses in the late phase of formalin test. As shown in Table 2, *C. bonariensis* extracts protected the animal from painful stimulation of formalin with maximum effect of 61% at 200 mg kg^{-1}. Unlike the effect of chloroform fraction that demonstrated activity in the first phase, the butanolic fraction of *C. bonariensis* produced significant suppression of the nociceptive responses in the first and second phases of the formalin test with 40 and 63% inhibitions, respectively. Diclofenac (10 mg kg^{-1} i.p) produced 67% inhibition of the formalin induced nociception in the second phase of the test.

Anti-inflammatory activity: The crude extracts and fractions of *C. bonariensis* were assessed in the carrageenan induced edema model in rats. The injection carrageenan when injected into subplantar region of the rats paw produced a localized edema that reached to its maximum at the 3rd h after injection. The localized inflammatory response to carrageenan was sustained for 4 h and gradually declined after this time.

As shown in Table 3, *C. bonariensis* produced marked reduction in carrageenan induced edema (79% at 200 mg kg^{-1}) at the 3rd h of the carrageenan administration. The difference between the paw volume of the control and extracts treated animals was statistically significant ($p<0.05$, $p<0.01$) at the 3rd h of observation. The early phase of the edema (1-2 h) was not affected by the plant extracts (data not shown). The standard drugs indomethacin, at 20 mg kg^{-1} i.p. produced about 66% inhibition of the carrageenan induced edema (Table 3).

The effect of various fractions of the plant extracts was quite variable. Hexane fraction was the most potent producing 47% inhibition of carrageenan edema at 100 mg kg^{-1} . At the same dose butanolic and chorofrom fraction exhibited 37 and 11% of the anti-inflammatory effect (Table 3).

Table 1: Effect of the methanol extract of *C. bonariensis* and its fractions on acetic acid-induced writhing in mice

Treatments	Dose (mg kg^{-1})	Number of writhing	Protection (%)
Control	-	73±7	-
C. bonariensis	50	38±5**	48
	100	20±4***	72
Butanol fraction	25	37±5**	49
	50	14±4***	80
Chloroform fraction	25	43±3**	41
	50	18±2***	75
Hexane fraction	50	41±6**	44
Diclofenac sodium	10	28±4***	62

Values represent Mean±SEM of 10 observations, **p<0.01 and ***p<0.001, compared to control

Table 2: Effect of the methanol extract of *C. bonariensis* and its fractions on the formalin-induced licking response in mice

		Licking time (sec)		Inhibition (%)	
Treatments	Dose (mg kg^{-1}) i.p.	1st Phase	2nd Phase	1st Phase	2nd Phase
Control	-	50±3	75±5	-	-
C. bonariensis	100	50±2	47±3***	-	37
	200	48±4	29±5***	4	61
Chloroform fraction	50	51±6	60±5*	-	20
	100	47±3	31±2***	4	59
Butanol fraction	25	53±5	35±5***	-	53
	50	30±3***	28±4***	40	63
Diclofenac sodium	10	44±6	25±4***	12	67

Values represent Mean±S.E.M of 12 observations, *p<0.05and ***p<0.001, compared to control

Table 3: Effect of the methanol extract of *C. bonariensis* and its various fractions on carrageenan induced paw edema in rats

Treatments	Dose (mg kg^{-1}) i.p	Initial paw volume (mL)	Paw volume at 3 h	Increase in paw volume	Inhibition (%)
Control		0.98±0.01	1.16±0.02	0.180	-
C. bonareinsis	50	1.0±0.02	1.14±0.03	0.140*	26
	100	0.98±0.01	1.04±0.03	0.06***	68
	200	0.98±0.01	1.02±0.02	0.04***	79
Hexane fraction	50	0.97±0.02	1.10±0.02	0.130*	32
	100	0.98±0.02	1.08±0.01	0.100**	47
Butanolic fraction	100	1.0±0.02	1.12±0.01	0.12*	37
Chloroform fraction	100	0.98±0.04	1.15±0.02	0.17	11
Control		1.04±0.01	1.31±0.02	0.270	-
Indomethacin	20	0.96±0.01	1.05±0.02	0.09**	66

Values represent Mean±SEM of edema volume of 15 observations, *p<0.05, **p<0.01 and ***p<0.001, compared to control

DISCUSSION

This study was aimed to assess the analgesic and anti-inflammatory activities of *C. bonariensis*. For the anti-nociceptive effects, the two pain models such as acetic acid induced writhing and formalin induced licking test were used[14]. Clinically used analgesic drugs such as diclofenac and ibuprofen have been reported to inhibit the acetic acid induced writhing[15,16]. In the current study, the intraperitoneal administration of the plant extracts of *C. bonariensis* caused significant inhibition (p<0.01) of the acetic acid induced writhes. Acetic acid increases the prostaglandins levels in the peritoneal fluid[17]. The inhibitory effects of the plant extracts similar to the effect of diclofenac sodium suggest that it may have occurred via inhibition of prostaglandins action. The analgesic effect was predominantly seen with butanolic and chloroform fractions of the plant extracts indicating that analgesic components are concentrated in these fractions. Similar to the effects of plant extracts, diclofenac sodium, a standard non-steroidal anti-inflammatory drug showed significant activity in this test. Medicinal plants that are used as analgesic in traditional medicine such as, *Asparagus pubescens*[18] and *Melastoma malabathricum*[19], have been shown to decrease abdominal stretching/constriction induced by acetic acid[20].

The acetic acid induced pain test is non-selective[21].The mechanism of analgesia of the plant extract and its fractions was further investigated in the formalin induced pain assay. Formalin induced pain models useful to understand the mechanism of pain and analgesia[22]. Formalin induced pain involves two distinct phases, the first phase called the neurogenic phase, where pain is due to direct stimulation of

the sensory nerve fiber by formalin while in the second or late the pain is due to release of inflammatory mediators such as prostaglandin and bradykinin[11,23,24]. The crude extracts and fractions of *C. bonariensis* inhibited formalin induced pain response in the second phase of formalin test indicating their peripheral action of analgesia. However butanolic fraction additionally exhibited significant analgesic effect in the first phase of the formalin test, which suggests central mechanism of its analgesic effect. This may explain the greater potency of the butonolic fractions in acetic acid and formalin tests. The peripherally acting drugs such as aspirin and diclofenac[25], known to attenuate pain by inhibition of cyclooxygenase in arachidonic acid pathways[26]. The analgesic effect of the plant extract in chemical induced pain models suggests NSAIDs like activity of *C. bonariensis* plant extracts.

The speculation was further confirmed when *C. bonariensis* plant extract and it fractions caused significant inhibition of the carrageenan induced paw edema in rats. C*. bonariensis* and some of its fractions (hexane and butanol) caused significant inhibition of the late phase edema induced by the sub-plantar injection of carrageenan. The carrageenan induced acute inflammation is biphasic, in the early phase (1-2 h after carrageenan injection), edema production is mediated by histamine and serotonin while in the late phase (after 2nd h) inflammatory response is maintained by bradykinin and prostaglandins[27]. These mediators are well established for their role in inflammation, stimulate the nociceptors and induce pain[28]. Most of the clinically effective anti-inflammatory drugs are effective in the late phase and has been frequently used to assess the anti-phlogistic effect of the natural products[29,30]. In the present investigation, *C. bonariensis* and its fractions exhibited marked anti-inflammatory activity in the late phase of carrageenan induced edema test similar to indomethacin, a standard non-steroidal anti-inflammatory drug (NSAID)[31,32]. Several natural products and NSAIDS have been reported to inhibit inflammation induced by various phlogistic agents in experimental animal models[33-35]. NSAIDS such as indomethacin reduces inflammation and arthritic pain by inhibiting prostaglandin synthesis and/or production[36]. Therefore it is likely that *C. bonariensis* may contain NSAIDs-like constituents responsible for the observed analgesic and anti-inflammatory properties. *Conyza dioscaridis*, a related specie has been reported for analgesic and anti-inflammatory activities[37.] These data substantiate the therapeutic potential of the genus *conyza*, in pain and inflammatory conditions. These findings suggest that *C. bonariensis* could prove to be a potential new source of natural drugs with analgesic and anti-inflammatory activities. Further studies are required to investigate the active constituents and possible biochemical mechanisms of the observed analgesic effect of the plant extracts.

CONCLUSION

The results from our current study determine that *Conyza bonariensis* possesses analgesic and anti-inflammatory properties. The plant extract and its fractions produced marked inhibition of the pain response in animals in the chemical (acetic acid, formalin) induced pain models and carrageenan induced paw edema test. The analgesic and anti-inflammatory effects were similar to that observed with standard NSAIDs suggesting the presence of NSAIDs like constituents in *C. bonariensis*, while anti-inflammatory activity was concentrated in the hexane and butanol fractions.

SIGNIFICANCE STATEMENTS

This study substantiates the traditional use of *C. bonariensis* as a remedy of pain and inflammatory conditions. The findings of this study will help natural product researcher to identify the active constituents of this plant as possible novel analgesic and anti-inflammatory drug candidate with good safety and tolerability profile.

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