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THE CENTRAL ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF THE METHANOLIC EXTRACT OF *CARTHAMUS OXYCANTHA*

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The plant extract and fractions of *Carthamus oxycantha* (Compositae) were assessed for analgesic and anti-inflammatory activities. Acetic acid and formalin-induced nociception, hot plate and carrageenan-induced rat paw oedema tests were employed to evaluate the analgesic and anti-inflammatory potential of the plant extract. The intraperitoneal (i.p.) administration of the methanolic extract (25–30 mg/kg), hexane (10–50 mg/kg, i.p.) and ethylacetate (50 and 100 mg/kg i.p.) fractions produced significant inhibition ($P < 0.01$) of the acetic acid-induced writhing in mice and suppressed formalin-induced licking response of animals in both phases of the test. In the hot plate assay the plant extract (100 mg/kg i.p.) increased pain threshold of mice. Pre-treatment of animals with naloxone (5 mg/kg i.p.) abolished the analgesic effect of the *C. oxycantha* in formalin and hot plate tests. *C. oxycantha* (50–200 mg/kg i.p.) produced marked anti-inflammatory effect in carrageenan-induced rat paw edema assay comparable to diclofenac. These findings suggest that *C. oxycantha* possesses central analgesic and peripheral anti-inflammatory properties, with analgesic effects associated with the opioid system.

Key words: *Carthamus oxycantha*, analgesic activity, anti-inflammatory activity, rodents, nociception, pain, edema, plant extract

INTRODUCTION

Carthamus oxycantha belongs to the family Compositae, locally known as kantiari. It is a medium size herb with orange yellow flowers and found in open places and mountains in Afghanistan, Iran, Tajikistan and Pakistan (1, 2). Flowers of the plant are used to treat cerebral thrombosis, male infertility, rheumatism and bronchitis (3, 4). *C. oxycantha* based medicines are beneficial in pain and swelling associated with trauma (2). It is mainly used in dressing ulcers and against itch (1). *C. oxycantha* herbage is valuable as green fodder in many countries. Other species of the genus *Carthamus* have several traditional applications in variety of health problems. *C. tinctorius* (Safflower) is used in abdominal colic, asthma, bronchitis, jaundice, rheumatism and as anti-pyretic and purgatives (5-7). *C. lanatus* is used as anti-tumor and sedative (8).

Some species of genus *Carthamus* have been reported for various pharmacological activities e.g. *C. oxycantha* for cholinimimetic (9), *C. tinctorius* for antihypertensive (10), antioxidant (11), calcium antagonistic (12) and anti-cancer activities (13). *C. lanatus* possesses analgesic, anti-inflammatory, antibacterial, antifungal and cytotoxic properties (14, 8).

The phytochemical investigation of various species of *Carthamus* have revealed the presence of several bioactive flavonoides, quinochalcone, tinctormine from leaves and flowers of *C. tinctorius* (12, 15, 16). Flavonoids, flavonoid glycosides and serotoninins from aerial parts of *C. lanatus* (8, 17). Flavanones, flavonoids and two novel glycosides have recently been reported from the aerial parts of *C. oxycantha* (18).

C. oxycantha is not thoroughly investigated for biological activities. Owing to the traditional uses and potential analgesic and anti-inflammatory properties of various members of the genus *Carthamus*, the present study was aimed at evaluating the analgesic and anti-inflammatory activity of *C. oxycantha* in mice and rats.

MATERIALS AND METHODS

Plant material

Fresh whole plants of *Carthamus oxycantha* were collected from the northern areas of Pakistan (Swat) in June 2003 and identified by Taxonomist, at the Department of Botany, University of Karachi. A voucher specimen (KUGH, No. 68189) was made from roots, leaves and flowers and has been submitted to the herbarium of the same department.

Extraction and fractionation

The plant material (leaves, flowers and roots) about 40 kg was cleaned, shade dried and coarsely ground. The powdered material was soaked in 70% aqueous-methanol (30% distilled water and 70% methanol) for 7 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on rotary evaporator under reduced pressure to a thick, semi-solid mass of dark brown color; i.e. the crude extract (Co.Cr), yielding approximately 800 g. Approximately 250 g of

the crude extract of *Carthamus oxycantha* was dissolved in about 300 ml of distilled water and portioned to get the hexane, chloroform and ethyl acetate and aqueous fractions.

Animals

All the experimental protocols were reviewed by institutional review board of council of medical research, College of Medicine, King Saud University Riyadh and complied with the National Institutes of Health guidelines for the care and use of laboratory animals. NMRI male mice (20–30 g) and Sprage-Dawley male rats (180–270 g) were obtained from the animal house facility of The Aga Khan University, Karachi and animal house facility of College of Medicine, King Saud University Riyadh, Saudi Arabia. The animals were housed in plastic cages under standard condition with 12/12 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 naloxone were purchased from the Sigma Chemical Co., (St. Louis Mo, USA). Morphine sulphate (MS Contin) from local pharmacy while thiopental sodium and formaline 37% were obtained from Abbot Laboratories, Karachi, Pakistan and Fluka Chemie, Switzerland respectively. All other chemicals used in experiments were of analytical grade.

Experimental methods

Acute toxicity tests

Male mice (20–25 g) were injected intraperitoneally (i.p.) different doses of the *C. oxycantha* extract (50, 100, 300, 500, 1000 and 2000 mg/kg; n=6). Animals were observed for 1–2 hours after administration of the extract for any acute toxicity behavioral symptoms. The number of deaths was counted at 48 hours after treatment. LD₅₀ value (dose of the extracts producing mortality in the 50% of the experimental animals) was determined by graphical method.

Writhing test

Male mice (20–25 g) were used in this experiment according to the method of acetic acid-induced writhing in mice (19). 30 min. after the administration of *C. oxycantha* extract (25–100 mg/kg i.p.), mice were given an i.p. injection of 0.7% v/v acetic acid solution (volume of injection 0.1 ml/10 gm). The mice were placed individually in transparent cages and five minutes were allowed to elapse. The number of acid-induced writhes were counted for 20 min. For the purpose of scoring, a writhe was indicated by stretching of the abdomen and/or simultaneous stretching of at least one hind limb. Control animals received normal saline (10 ml/kg, i.p.), diclofenac (10 mg/kg, i.p.) was used as a reference drug.

Formalin test

This test was performed by the method of assessing the formalin-induced paw licking response in mice (20), male mice (20–25 gm) were injected, 20 µl of 1% formalin prepared in 0.9% saline, subcutaneously into the dorsal hind paw and placed immediately in transparent box for observation. The duration of reaction time (paw licking or biting) was determined between 0–5 min (first phase) and 15–30 min (second phase) after formalin injection. Animals were treated (i.p.) with different doses of *C. oxycantha* plant extract (50 and 100 mg/kg) or diclofenac (10 mg/kg) or morphine (5 mg/kg), 30 min prior to

administration of formalin. Naloxone (5 mg/kg, i.p.) was administered 20 min prior to treatment of animals with plant extract or morphine. Control animals received the vehicle (0.1 ml/10 gm). The reaction time of the animals was compared to control group and expressed as percent inhibition.

Hot plate test

The hot plate test was performed by placing mice individually on hot plate and assessing their response to the thermal stimulus (21). The temperature of the hot plate was maintained at 52±0.8°C. Twenty four hours before the experiments each mouse was individually placed on the hot plate and the latency to a discomfort reaction (licking of the paw or jumping) was noted. The cut off time was 20 s to avoid thermal injury of the paw. Previously selected animals were given (i.p.), different doses of the plant extract of *C. oxycantha* (50 and 100 mg/kg) or morphine (5 mg/kg), 30 min. before their placement on the hot plate. Base line reaction time was taken before the treatment and 30, 60, 120 and 180 min after the administration of the treatment. Naloxone (5 mg/kg i.p.) was given 20 min before the administration of plant extract or morphine; control animals received same volume of vehicle. The latency time in second of the control animals was compared to that of treated animals.

Anti-inflammatory activity

The anti-inflammatory effect of plant extract was assessed against paw edema in rats induced by injecting 1% carrageenan (prepared in distilled water) into the sub-plantar tissue of the right hind paw (22). Male rats (180–270 g) divided randomly into 7 groups (n=6 per group) and injected subcutaneously into the plantar surface of the hind paw with 0.05 ml of freshly prepared 1% carrageenan (prepared in distilled water). Group 1–3 received different doses of plant extract (50–200 mg/kg), group 4 was administered the hexane fraction (100 mg/kg), group 5 received diclofenac (20 mg/kg). All treatments were given i.p., 30 min before the administration of carrageenan. The control animals (group 6 and 7) received same volume of the vehicle (saline or distilled water 1 ml/kg). Rat paw edema was assessed by volume displacement method (plethysmometer (Ugo Basile 7150) before and after carrageenan injection at 1, 2, 3 and 4 h. Difference in the paw volume, determined before and after injection of carrageenan indicated the severity of edema. The % inhibition of the inflammation was determined for each animal by comparison with controls and calculated by the following formula (23):

$$\% I = 1 - (dt/dc) \times 100$$

where “dt” is the difference in paw volume in the drug treated group and “dc” the difference in paw volume in control group and “I” = inhibition.

Statistical analysis

The results of the study are expressed as mean ±S.E.M. and statistical significance between control and treated groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. P<0.05 was considered statistically significant.

RESULTS

Acute toxicity test

The intra-peritoneal administration of various dose of the plant extracts did not cause any lethality up to 500 mg/kg. LD₅₀

value of *C. oxycantha* was 1830 mg/kg. Animals showed hypomotility, drowsiness at the dose range of 500–2000 mg/kg.

Writhing test

As summarized in Table 1, the intraperitoneal (i.p.) administration of various doses (25–100 mg/kg) of the methanolic extract of *C. oxycantha*, caused significant inhibition ($P<0.05$; $P<0.001$) of the nociception induced by acetic acid. The plant extract produced maximum protection of 89% at the dose of 100 mg/kg. The results were comparable to standard drug diclofenac sodium that produced 59% inhibition at 20 mg/kg i.p. (Table 1).

Among the various fractions of the plant extract the hexane fraction of *C. oxycantha* was the most potent with maximum protection of 56% obtained at 50 mg/kg, followed by ethyl acetate fraction producing 29% protection at 50 mg/kg (Table 1).

Formalin test

In the formalin test the plant extract of *C. oxycantha* (50 and 100 mg/kg i.p.) caused significant ($P<0.001$) inhibition of both phases of formalin induced pain. *C. oxycantha* (100 mg/kg i.p.) produced 62 and 69% inhibition of the formalin induced pain in the first and second phase respectively (Table 2). Pre-treatment of animals with naloxone (NLX) (5 mg/kg i.p.) abolished the inhibitory effect of *C. oxycantha* in the first phase with the licking time of the animals reverting almost equal to the control animals i.e. from 19 ± 4 (without NLX) to 45.8 ± 3 sec (with NLX), as shown in Fig. 1A. However the inhibitory effect of *C. oxycantha* in the second phase of formalin test, was partially reversed (Fig. 1B). Diclofenac sodium suppressed the reaction

time of the animals in the second phase of the test only. As shown in Fig. 1A and 1B, naloxone caused complete reversal of the analgesic effect of morphine in both phases of formalin test.

Hot plate test

In hot plate assay *C. oxycantha* (100 mg/kg i.p.) showed significant analgesic activity ($P<0.001$), similar to morphine (Table 3). *C. oxycantha* caused increased the latency time of animals from 8.2 ± 0.3 (control; saline treated) to 20 ± 0.5 s (extract treated). As shown in Table 3, the maximum analgesic effect of *C. oxycantha* and morphine in the hot plate test was

Table 1. Effect of the methanolic extract and fractions of *C. oxycantha* on acetic acid induced writhing in mice.

Treatment	Dose (mg/kg i.p.)	Number of writhing	% Protection
Control	-	73 ± 7	-
<i>C. oxycantha</i>	25	$46 \pm 5^*$	37
	50	$30 \pm 6^{**}$	59
	100	$8 \pm 2^{***}$	89
Hexane fraction	10	$51 \pm 3^{**}$	30
	25	$42 \pm 5^{**}$	42
	50	$32 \pm 7^{**}$	56
Ethylacetate fraction	50	$52 \pm 5^*$	29
	100	$24 \pm 4^{**}$	67
Diclofenac sodium	20	$30 \pm 6^{***}$	59

Values represent mean \pm S.E.M. of 5–20 observations. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$, compared to control.

Table 2. Effect of the methanolic extract and fractions of *C. oxycantha* on the formalin-induced licking response in mice.

Treatment	Dose (mg/kg i.p.)	Licking time (Sec)		% Inhibition	
		1 st Phase	2 nd Phase	1 st Phase	2 nd Phase
Control	-	50 ± 3	75 ± 5	-	-
<i>C. oxycantha</i>	50	$23 \pm 4^{***}$	$45 \pm 7^{***}$	54	40
	100	$19 \pm 4^{***}$	$23 \pm 5^{***}$	62	69
	50	$40 \pm 3^*$	$52 \pm 5^{**}$	20	30
Hexane fraction	100	$31 \pm 4^{**}$	$33 \pm 2^{***}$	38	56
	100	45 ± 4	$51 \pm 5^{**}$	10	32
Ethylacetate fraction					
Diclofenac sodium	20	47 ± 7	$27 \pm 5^{***}$	6	64

Values represent mean \pm S.E.M. of 5–30 observations. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$, compared to control.

Table 3. Effect of the methanolic extract of *Carthamus oxycantha* and morphine in the absence and presence of naloxone in hot plate test.

Treatment	Dose (mg/kg i.p.)	Latency time (seconds)				
		0h	0.5 h	1h	2 h	3 h
Control	10 ml/kg	6.3 ± 0.2	6.8 ± 0.2	7.2 ± 0.3	7.2 ± 0.2	6.8 ± 0.4
Morphine	5	6.5 ± 0.7	$8 \pm 0.3^{**}$	$11.2 \pm 0.7^{***}$	$12.6 \pm 0.5^{***}$	$11.7 \pm 0.3^{***}$
Morphine+ Naloxone	5 + 5	5.4 ± 0.2	5.5 ± 0.4	6.3 ± 0.2	6.5 ± 0.3	5.8 ± 0.6
<i>C. oxycantha</i>	100	8.2 ± 0.3	$11.3 \pm 0.2^{***}$	$17.2 \pm 0.7^{***}$	$20 \pm 0^{***}$	$18.3 \pm 0.4^{***}$
<i>C. oxycantha</i> + Naloxone	100 + 5	7.5 ± 0.6	6.5 ± 0.2	7.8 ± 0.4	8.2 ± 0.2	8.2 ± 0.3

Values represent mean \pm S.E.M. of 5–10 observations. ** $P<0.01$, *** $P<0.001$, compared to control.

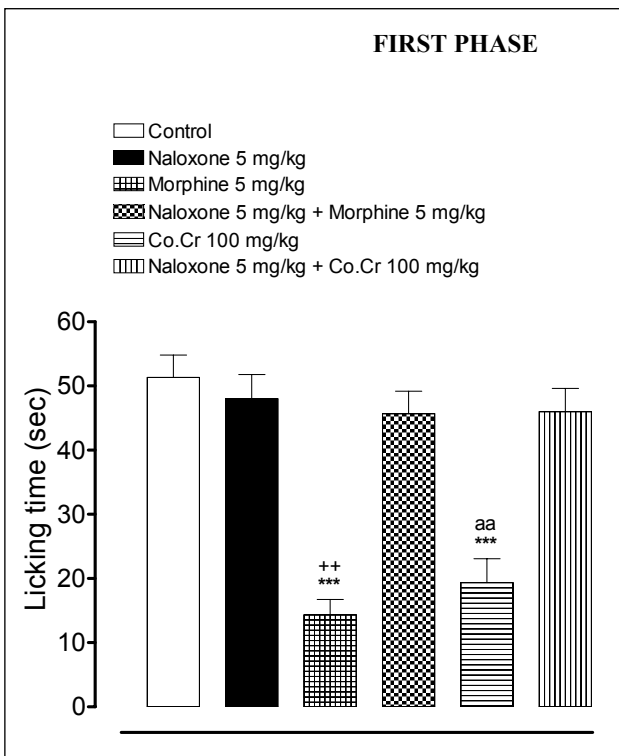


Fig. 1A. Effect of naloxone (5 mg/kg i.p.) on the analgesic effect of intraperitoneally administered crude extract of *C. oxycantha* (Co.Cr) and morphine on formalin induced licking response in first phase of the test.

Values are mean ± S.E.M. of 5–10 observation, **P<0.01, ***P<0.001, compared to control; ++P<0.01 compared to naloxone + morphine; aaP<0.01, compared to naloxone + Co.Cr.

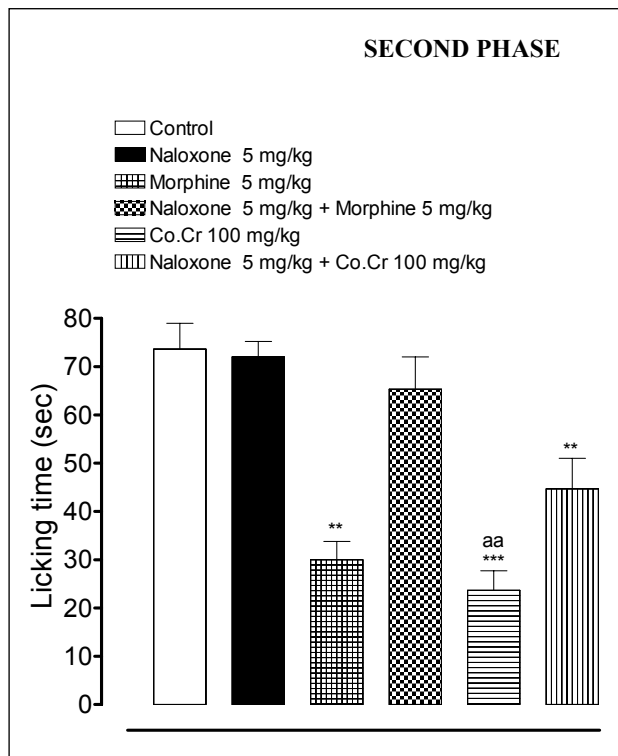


Fig. 1B. Effect of naloxone (5 mg/kg i.p.) on the analgesic effect of intraperitoneally administered crude extract of *C. oxycantha* (Co.Cr) and morphine on formalin induced licking response in second phase of the test.

Values are mean ± S.E.M. of 5–10 observations, **P<0.01; ***P<0.001, compared to control; aaP<0.01, compared to naloxone + Co.Cr.

Table 4. Effect of the methanolic extract and fractions of *C. oxycantha* on carrageenan induced paw edema in rats.

Treatment	Dose (mg/kg i.p.)	Initial paw volume (ml)	Paw volume at 3 h	Increase in paw volume	% Inhibition
Control		1.05 ± 0.02	1.21 ± 0.04	0.160	-
<i>C. oxycantha</i>	50	0.96 ± 0.03	1.06 ± 0.03	0.11*	31
	100	1.05 ± 0.02	1.12 ± 0.01	0.07**	56
	200	1.02 ± 0.03	1.07 ± 0.02	0.05***	67
Hexane fraction	100	0.96 ± 0.02	1.04 ± 0.01	0.08**	50
Control		1.04 ± 0.01	1.21 ± 0.02	0.170	-
Diclofenac sodium	20	1.10 ± 0.02	1.16 ± 0.02	0.06***	64

Values represent mean ± S.E.M. of 6-20 observations.

*P<0.05, **P<0.01 and ***P<0.001, compared to control.

observed at 2 h after treatment. Pre-treatment of the animals with naloxone (5 mg/kg i.p.) completely reversed the analgesic effect of *C. oxycantha*. Morphine (5 mg/kg i.p.) also increased the latency time of animals and its effect was abolished with naloxone (5 mg/kg) pretreatment.

Anti-inflammatory activity

Carrageenan (subplantar injection) produced a localized edema that reached to its maximum at the 3rd hour after injection. *C. oxycantha* (50–200 mg/kg i.p.) caused significant

inhibition of the carrageenan induced inflammation. The maximum anti-inflammatory effect of the plant extract was 67% at 200 mg/kg i.p. (Table 4). The difference between the paw volume of the control and extract treated animals was statistically significant (P<0.05, P<0.01) at the 3rd hour. Diclofenac sodium, a standard anti-inflammatory drug, at 20 mg/kg i.p. produced about 64% inhibition of the carrageenan induced edema (Table 4).

Based on the effect of various fractions of *C. oxycantha* in acetic acid and formalin induced pain models, only hexane fraction exhibited significant (P<0.01) analgesic activity, which

was further assessed for anti-inflammatory effect in the rat paw edema assay. As shown in Table 4, The anti-inflammatory effect of hexane fraction at 100 mg/kg was comparable to the parent methanolic extract of plant.

DISCUSSION

Various member of genus *Carthamus* including *C. oxycantha*, *C. tinctorious* and *C. lanatus* are traditionally used in the management of pain and inflammatory conditions (7). The current study was planned to validate folkloric uses of *C. oxycantha*, the least investigated plant of the genus *Carthamus*. The plant extract of *C. oxycantha* and its fractions (hexane and ethylacetate) caused significant inhibition ($P < 0.01$) of the acetic acid induced writhes in mice. Acetic acid is believed to increase the prostaglandins levels in the peritoneal fluid (24, 25) and widely employed tool for screening of novel analgesic agents. The observed analgesic effect of plant extract of *C. oxycantha* against acetic acid induced writhing suggest that it may have occurred through inhibition of prostaglandins action. Similarly, diclofenac, a standard non-steroidal anti-inflammatory drug, was effective in this test. Several medicinal plants used as analgesic such as *Quasia amara* (26), *Asparagus pubescens* (27) and *Melastoma malabathricum* (28) have been shown to decrease abdominal constriction induced by acetic acid.

The acetic acid-induced pain model though effective but lacks selectivity (29). Acetic acid indirectly releases endogenous mediators which stimulate neurons that are sensitive to other drugs such as narcotic and other centrally acting drugs (30). The analgesic effect of *C. oxycantha* extract was further investigated in the formalin and hot plate tests.

Formalin induced pain model is useful to elucidate the mechanism of pain and analgesia (31). Formalin induced pain involves two distinct phases, the first phase (neurogenic phase) in which pain is produced due to direct stimulation of the sensory nerve fiber by formalin and the second or late phase (inflammatory phase) in which the pain occurs due to release of inflammatory mediators such as histamine, serotonin, prostaglandin and bradykinin (20, 32). It is well established that centrally acting drugs such as narcotics inhibit both phases equally while peripherally acting drugs such as diclofenac inhibit the late phase (33, 34). In the present study *C. oxycantha* produced marked analgesia in both phases of the test similar to morphine, suggesting that central mechanism is involved in analgesic effect of the plant extract. Pre-treatment of animals with naloxone, an opioid receptor antagonist, abolished the analgesic effect of *C. oxycantha* in the first phase of formalin test, further strengthening the assumption of central mechanism of analgesia of the plant extract. The central pain inhibition of another species of genus *Carthamus*, *C. tinctorios* has already been reported (35, 36, 7) indicating that *C. oxycantha* may contain similar central analgesic constituents. However, naloxone pretreatment partially reversed the analgesic effect of *C. oxycantha* in the second phase of formalin test, suggesting that the plant extract may contain peripherally acting anti-inflammatory constituents (20, 33). The genus *Carthamus* is rich in flavonoids and flavonoid glycosides (4, 15, 18), which are common constituents in plants used in folk medicine and have been implicated for their analgesic and anti-inflammatory effects in several experimental studies (37, 38). Moreover, it is likely that the anti-inflammatory effect of plant extract has occurred *via* free radical scavenging properties of its flavonoids and glycosides contents (39).

The effect *C. oxycantha* in formalin test indicated central mechanism of analgesia, which was further confirmed in the hot

plate assay, a test considered suitable for such study (40, 41). *C. oxycantha* plant extract significantly prolonged the reaction time of animals provoked by heat stimuli. Similarly, morphine suppressed the thermal pain response of animals. Pretreatment of animals with naloxone, abolished the analgesic effect of both plant extract and morphine in the hot plate test suggesting the involvement of opioid pathway in the observed analgesic activity of the plant extract. However, additional mechanisms such as melatonin induced anti-nociception needs to be explored, which has been reported to release endogenous β -endorphins in the central nervous system (42).

Additionally, the marked inhibitory effect of plant extract of *C. oxycantha* in the second phase of formalin test, similar to diclofenac, indicates its peripheral anti-inflammatory effect (32). The anti-inflammatory effect of *C. oxycantha* was assessed in carrageenan-induced inflammatory edema in the hind paw of rats. Carrageenan, a mucopolysaccharide derived from Irish Sea moss *Chondrus*, produces experimental arthritis, is non-antigenic and does not produce any systemic effects (22). The intraperitoneal administration of the methanolic extract of *C. oxycantha* caused significant ($P < 0.001$) inhibition of the late phase edema induced by the sub-plantar injection of carrageenan with no inhibitory effect in the first phase of test. The carrageenan induced acute inflammation is believed to be biphasic; the early phase (1–2 h after carrageenan injection), in which the edema production is mediated by histamine and serotonin and the late phase (after second hour) the vascular permeability is maintained by bradykinin and prostaglandins (43, 44). These mediators participate in the inflammatory response, stimulate the nociceptors and induce pain (45). The second phase of the edema is sensitive to clinically effective anti-inflammatory drugs and has been frequently used to assess the anti-phlogistic effect of the natural products (46, 47, 48). In the present investigation the plant extract of *C. oxycantha* and its hexane fraction exhibited marked anti-inflammatory activity in the late phase of carrageenan induced edema test. Similarly the standard drugs, diclofenac, produced significant ($P < 0.001$) anti-edematous effect which is consistent with the previous reports (49, 50). Several studies have reported the inhibitory effects of plant extracts and standard drugs such as diclofenac sodium and aspirin, against inflammation induced by various phlogistic agents in experimental animal models (51-54). It is known that diclofenac, and aspirin reduces inflammation, swelling and inflammatory pain by inhibiting prostaglandin synthesis *via* inhibition of cyclooxygenase in arachidonic acid pathways (55-57). *C. oxycantha* produced similar analgesic and anti-inflammatory effects to that obtained with clinically used anti-inflammatory drugs, suggesting that the plant extract may contain constituents with similar properties to these drugs. Contents of flavonoids, sesquiterpene and phenolic compounds could be correlated with its observed anti-inflammatory effects (18, 58). This plant could be a potential new source for the discovery of analgesic and anti-inflammatory compounds (59).

In conclusion, *C. oxycantha* possesses analgesic and anti-inflammatory properties. It produced marked inhibition of the pain response of animals in the chemical (acetic acid, formalin) induced pain models and hot plate test. The analgesic constituents are concentrated in the hexane fraction. In the hot plate test, the analgesic effect of the plant extract was abolished by naloxone pre-treatment. *C. oxycantha* also produced profound anti-phlogistic effect similar to standard drugs diclofenac. These finding suggest that *C. oxycantha* plant extract contains constituents with central analgesic and peripheral anti-inflammatory properties. Moreover, our finding validate the folkloric uses of various member of the genus *Carthamus* including *C. oxycantha* in the management of pain and

inflammatory conditions. Further studies are required to identify the active principles responsible for analgesic and anti-inflammatory effects of the plant extract.

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