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Original Article

Determination of Analgesic Potential of Ethanolic Extract of *Brassica campestris* Leaves in Rats

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ABSTRACT

Background: *Brassica campestris* plant, a prominent member of Brassicaceae family, has documented therapeutic potential in the common cold, as an antibacterial, antiprotozoal, and to some extent an analgesic in folk medicine. **Objectives:** The present study aimed to determine the antinociceptive activity and analgesic potential of the ethanolic extract of the leaves of the *Brassica campestris* plant in rats. **Materials and Methods:** The antinociceptive potential and analgesic effects of the *Brassica campestris* leaves extract were evaluated by using the hot plate test and formalin analgesia method. The data were presented as mean ± standard error of the mean (M ± SEM). One-way analysis of variance [ANOVA] followed by Tukey's multiple comparison tests were performed for statistical analysis. *P*-value was considered significant at < 0.05. **Results:** The withdrawal latency period was noticed to be gradually and significantly prolonged after 400mg/kg oral administration of *Brassica campestris* leaves extract reaching a maximum of 12.73 ± 0.61 sec ($p<0.05$) as compared to control rats (6.13 ± 0.09 sec) in hot plate test. The antinociceptive behavior of experimental rats was also found quite significant in both phases of formalin-induced pain (61 and 81% respectively; $p<0.05$) when administered to plant extract at the dose of 400mg/kg body weight as compared to control animals (25 and 40% respectively). **Conclusion:** *Brassica campestris* leaves may contain pharmacologically active constituents associated with antinociceptive potential and analgesic effects in rats. The further characterization of those active moieties may be valuable for the treatment of pain and for designing a new analgesic strategy.

Keywords: Analgesic potential, *Brassica campestris,* Formalin-induced pain, Folk medicine, Hot-plate test, Thermal hyperalgesia.

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INTRODUCTION

Human body pain is considered a major disabling accompaniment of many pathological and medical conditions and relief from pain is one of the most important therapeutic priorities for many treatment strategies.[1-2] Albeit; the clear definition of pain varies from one pathological state to the others;[1] however, usually defined as an unpleasant sensory and emotional experience associated with certain actual or potential tissue damage or the consequences of regarding such damages with the release of several inflammatory and pain mediators.[2] In contrast, the emotional sensation of pain is harder to determine in animals; hence, this concept is often excluded.[2] For the animals, pain is considered an aversive sensory experience caused by an actual or potential injury that may elicit protective motor and vegetative reflexes (reactions) resulting in learning avoidance and modulating species-specific behavior including social conduct.[3]

Various methods are used to evaluate the antinociceptive activity and analgesic potential of different pharmacological substances, plant extracts, and phytochemicals in laboratory animal models, particularly in mice.[2] The most used methods are the tail-flick method, the hot plate test, and the Formalin Analgesia assay (liking and biting method).[4] The tail-flick method is a nociceptive assay based on the measurement of the latency of the avoidance response to thermal stimulus in rodents.[2,5] The hot plate test is considered one of the most commonly used tests to evaluate the analgesic potential of pharmacologically active substances that act at the level of the spine and higher centers of pain perception in rats.[2,6] The test is also useful to determine basal thermal pain sensitivity or to evaluate putative genetic differences among animals without drugs.[2] The formalin chemical nociception is also a commonly used analgesic method that is included in a battery of analgesic tests.[2] Formalin-induced painful irritation can be measured as asymmetrically directed behaviors (e.g., licking and biting, lifting, flinching, and shaking) since they provide a reliable correlation of pain in the awake, freely moving rat.[1-2]

Analgesic drugs having a diverse classification including both opiates and non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat or reduce pain.[5,7] Many of the classical synthetic analgesics were originated from natural products;

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however, those acted by the mechanisms which are directly or indirectly associated with potential undesirable effects (e.g., peptic ulceration, gastrointestinal bleeding, addiction, respiratory distress, drowsiness, and nausea) in pain treated individuals.[8] Hence, medicinal plants of therapeutic potential are commonly used as alternative sources to treat a certain disease or disorder with lesser side effects in traditional, complementary, and homeopathic medicine.[9-11] Alternative medicine in the form of the whole plant or a part of the plant, extracts, and phytochemicals claim to possess safe and efficient drug fractions useful in various diseases and many disorders;[12-13] however, not all have been investigated for their reported therapeutic potential and observed efficacies.[14-16]

Brassica is a genus of plants in the mustard family (i.e., *Brassicaceae),* and the medicinal uses of the plants included in this family are quite evident in chronic cough, bronchial catarrh, anti-thyroid activity, and to some extent in tumors and cancer.[17-19] The traditional folk medicine practice reveals the treatment of the common cold while rubbing the oil of *Brassica campestris* plant on the throat and chest with mucus.[20-21] Analogously, the nutritional value of some plants is significant while containing soluble fibers and Vitamin C (a potent antioxidant);[20,22] although, much is lost during cooking but still available to a desirable extent of nutrition.[23] The plant genus is native to wild areas of Western Europe, the Mediterranean region, and temperate areas of Southeast Asia.[24] While many wild species are grown like weeds, especially in North America, South America, and Australia.[25] The *Brassicaceae* family comprises many plants, some of which have experimentally proven medicinal potential in animal models.[12] Some studies have been proven the analgesic potential of leaves of *Brassica oleracea* (L. var. capitate) in mice.[26] Similarly, another study demonstrates the antimalarial activity of 80% methanolic extract of the seeds of *Brassica nigra* (L.) against *Plasmodium berghei* infection in mice.[27] Sinigrin (allyl-glucosinolate or 2-propenyl-glucosinolate) is a natural aliphatic glucosinolate present in the plants of the *Brassicaceae* family.[28] The classical ayurvedic texts systematically described its potential therapeutic benefits for human health.[28] Several studies conducted to characterize the pharmacological activities of sinigrin have demonstrated its potential anti-cancer, antibacterial, antifungal, antioxidant, anti-inflammatory, wound healing, and bio fumigation properties.[29-31] *Brassica juncea*, (also known as Indian mustard), is enriched in redox-active polyphenols with antidiabetic activities.[32-33] A study was conducted to determine the antinociceptive and anti-hyperglycemic potential of methanol extract of *Brassica juncea* in Swiss albino mice.[34] Similarly, the methanolic extract of the plant was also exhibited dose-dependent glucose-lowering activity in oral glucose tolerance tests in mice.[33]

Other studies elucidate the antibacterial activity of root, stem, and leaves extract as determined by using the disk diffusion method against five pathogenic bacterial strains.[35-36] The ethanolic extracts of all plant parts were found to be involved in an antibacterial effect; however, the petroleum ether, methanolic and ethyl acetate extracts of root, stem, and leaves of *B. campestris* exhibited excellent antibacterial activity against all bacterial strains (i.e., *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli,* and *Staphylococcus epidermidis)* with the diameters of growth inhibition area in the range of 05 – 25 mm.[35-36] The benzene and chloroform extracts of the plant were assumed to be exhibited the least antibacterial activity against the tested bacterial strains.[35] This potential antibacterial activity of the *B. campestris* plant supported the use of plant seed oils to treat skin infections in Indian traditional medicine.[35-36]

The rationale for the evident therapeutic potential of the *Brassica campestris* plant in the folkloric literature and scientifically published work, the current study was aimed to investigate the analgesic potential and explore the anti-nociceptive effects of *Brassica campestris* leaf extract in adult albino rats. The preliminary findings of the study augment the potential anti-nociceptive effects of the plant extract in an animal model which could further be elucidated to transform the study outcomes for humans.

MATERIALS AND METHODS

Study Ethics and Approvals

This study was conducted at the Pharmacology and Toxicology Lab, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia. The ethical rules and regulations regarding animal handling and treatment protocol were followed as established by the Ethics Approval Committee of the Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia (REC/2491–21/CP/UQU-SA). The *Brassica campestris* leaves were purchased from the local market of Makkah, Saudi Arabia. It was identified and authenticated by Dr. Mohamed Ibrahim, Department of Pharmacognosy, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia.

Preparation of Plant Extract

The *Brassica campestris* dried leaves (500 g) were macerated and extracted with 1000 ml of 80% ethanol (v/v) by warming at 50°C for 1 hr 6 times. The collected solution was filtered through Whatman No-1 filter paper. The extract was evaporated to dryness under reduced pressure at 60°C by a Rotary vacuum evaporator followed by lyophilization to obtain the respective extract and about 41g residue was obtained. The extract was then stored in the refrigerator at 4°C until used for further analysis.

Animals

Male and female Albino rats weighing between 200 to 250g were utilized for the study under standard laboratory conditions. The animals were obtained from the animal house of King Abdulaziz University Medical Research Centre, Jeddah, Saudi Arabia. The rats were stocked randomly and distributed to treatment groups in polypropylene cages with husk as bedding. The rats were maintained at a temperature of 22 ± 2°C and 65% humidity. A 12:12 light: dark cycle was followed (Light on at 20:00). The rats were given free access to water and fed with a standard commercial pelleted diet (food and water *ad libitum*). The rats were adapted to the lab environment for at least one week and managed daily to familiarize them with the manipulation of the experimenter.[1] Experimental methods and treatment protocols used in this study were in accordance with the guidelines of the European Commission's directive (2010/63/EC) regulating animal research and reviewed by the Institutional Review Board Committee of the Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia, and agreed with the committee ethics guidelines.

Drugs

The following drugs were used and purchased from the international and local distributors of their manufacturers; Paracetamol (Bristol-Myers-Squibb®, Madrid, Spain), Normal Saline (NS; 0.9% NaCl; Pharmaceutical Solution Industries® (PSI), Jeddah, Saudi Arabia), and Formalin (Sigma Aldrich®; Darmstadt, Germany). Drugs were freshly prepared in an aqueous solution in appropriate concentration to administer in control as well as experimental animal groups, respectively.

Experimental Design and Treatment Protocol

The experimental rats were randomly divided into four groups of six rats each for the control (i.e., NS and paracetamol) and drug (*Brassica campestris* extract) treatment. The rats in Group 1 served as normal control and received a 0.9% NaCl injection intraperitoneally (i.p.). Group 2 received paracetamol (200 mg/kg) injected i.p. one hour after receiving

the last oral dose of NS. Group 3 and 4 rats were daily administered *Brassica campestris* extract by oral gavage in a dose of 200 mg/kg and 400mg/kg body weight for one week. We used the following equation to calculate the administered dose of NS, paracetamol, and *Brassica campestris* extract depending on the weight of the experimental animals.

$$\text{Amount} = \frac{\text{Weight of animals (g)} \times \text{dose (mg)}}{1000}$$

$$\text{Volume} = \frac{\text{Amount (mg)}}{\text{Concentration}}$$

At the end of the last drug treatment day (i.e., on day 7), all rats in the control and drug treatment groups were subjected to the hot plate test to determine the baseline withdrawal latency (see below) to evaluate the anti-nociceptive activity of the *Brassica campestris* extract. Furthermore, formalin-induced nociception was also evaluated by the licking and biting responses of the experimental rats in each group either treated with NS, paracetamol, or the *Brassica campestris* plant extract.

Hot-plate Test

The central anti-nociceptive potential of *Brassica campestris* leaf extract was determined by the hot plate test with slight modification by following the method of Sandrini *et al*., and Abdalla *et al*.[37-38] The hot plate test is useful to measure the intricate responses to an acute, noninflammatory, nociceptive input and is standard for studying central antinociceptive effects.[39] Briefly, each rat from the normal control group to the drug treatment group was placed onto the hot plate test apparatus (Harvard Apparatus® Ltd., Kent, United Kingdom). The temperature was fixed and maintained at 55 ± 0.5°C during the whole experiment and exposure to heat to the rat was continued till the animal showed withdrawal responses in the form of hind paw licking, shaking, lifting, or jumping off. The withdrawal latency or response period was the time gap between the moment when a rat was placed on the hot plate surface and when the animal licked, Shaked, lifted or jumped off any of its hind paws to avoid thermal pain. A cut-off time (the moments of removing the animal from the hot plate) of 30 sec was adjusted to nullify or minimize tissue damage and maintained throughout the experiment. Prior to measuring the withdrawal latency for control and *Brassica campestris* extract-treated animals, baseline withdrawal latency (i.e., pretreatment values) was determined just before NS, or paracetamol injection. For the NS, paracetamol, and drug-treated animals, the withdrawal latency period was again determined at 15, 30, 45, 60,75, and 90 min after. The prolongation in withdrawal latency responses was taken as an index for the antinociceptive potential of *Brassica campestris* leaves extract.

Rat Formalin Test

We performed the formalin-induced nociception test by following the same procedure as described by Liu *et al*., and Roca Vinardell *et al*.[1-2] with slight modifications. Prior to engaging the animal for the formalin test, each rat was placed in an individual standard cage for 15 min for three consecutive days. After that adaptation, the formalin-induced nociception was conducted by subcutaneously injecting 20 µL of 2.5% formalin (i.e., 0.92% formaldehyde) prepared in Phosphate buffer into the dorsal surface of the right hind paw (i.e., sub-planter surface) of each experimental rat. The test was conducted in three groups of mice (each group containing n=6) pretreated with either NS or *Brassica campestris* leaves extract (200mg/kg and 400mg/kg body weight of each rat in Group 3 and 4 respectively). The plant extract was orally administered 30 min before formalin injection, whereas the control group received intraperitoneally injected paracetamol (200mg/kg body weight of each animal). The formalin-induced nociception behavior of each animal was monitored for 45 min. The time (in seconds) spent by each rat in a group for licking and biting responses of the injected paw was considered as an index of nociception response (pain reflex). The time of licking/biting responses of the formalin injected hind paw was summed at 5-min intervals starting at time 0 min. Two phases of time spent for spontaneous licking and biting responses were recorded for each experimental rat; Phase 1 began immediately after formalin injection (0-10 min) indicative of neurogenic pain and recorded with a chronometer. Phase 2 started immediately thereafter at 10 min and last for 15-30 min. Maximum formalin elicited nociception responses were noticed around 20-35 min after 1% formaldehyde injection.

Statistical Analysis

The data values for both the hot-plate and formalin test were recorded as mean ± standard error of the mean (M ± SEM). The factors of variation for hot plate test results were NS and paracetamol treated rats while the data collected from the formalin test (phase 1 and 2) was comparable to the paracetamol treated animal group. Subsequently, a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was performed by using Minitab 17 for statistically significant variations. The p-value at the level of <0.05 was considered statistically significant where appropriate between control and experimental groups.

RESULTS

Analgesic Potential of *Brassica campestris* Leaves Extract against Thermal Hyperalgesia

The anti-nociceptive potential of *B. campestris* extract in rats as determined by the hot plate method is presented in Table 1. As depicted in the table, the normal withdrawal latency period (in sec) of control rats treated was 7.3±0.34 sec and it showed insignificant variation along with the whole experimental protocol after NS injection. However, the withdrawal latency period was noticed to be gradually and significantly prolonged after paracetamol injection (200mg/kg body weight i.p.) administration starting from 15 min after injection reaching a maximum of 26.62±0.84 sec after 90 min of the experiment. It started to gradually decline after that and reached 19.95±0.65 sec (data not shown) at the end of the withdrawal latency evaluation period (i.e., 120 min).

Withdrawal latency periods both for control and *B. campestris* leaves extract-treated rats were recorded for each animal group. Each animal group contained six rats which were individually evaluated for withdrawal latency period after NS (i.p.), paracetamol (200mg/kg i.p.) and *Brassica campestris* leaves extract (200mg/kg and 400 mg/kg p.o.) administration at different evaluation periods by hot plate test. Each experiment was performed in triplicate and data values were collected as mean (M)± standard error of the mean (SEM). Asterisk (*) at data values indicate significant differences in withdrawal latency period of drug administrated rats (*Brassica campestris* leaves extract) compared to its control animal (NS and paracetamol) groups at p<0.05.

The *B. campestris* leaves extract was not capable to prolong the withdrawal latency period of pain in experimental rats induced by the heating plate of hot plate test in experimental rats at certain dose levels. Figure 1 shows that no significant variations in the withdrawal latency period were noticed between control and experimental rats administered with 200mg/kg body weight plant extract. However, the withdrawal latency period was noticed significantly prolonged in the experimental rats administered to 400mg/kg plant extract at 90 min evaluation period (12.73±0.61; p<0.05) as compared to the control animal group (NS; 6.13±0.09). It indicates the anti-nociceptive potential of *B. campestris* leaves extract at a certain dose level in experimental rats to reduce thermal pain induced by the hot plate test. As expected,

Table: The analgesic potential of *Brassica campestris* leaves extract as determined by the hot plate test.

Withdrawal latency period evaluation (in min)							
	0 min	15 min	30 min	45 min	60 min	75 min	90 min
Drug treatment	Withdrawal latency period of rats against hot plate (in sec)						
Control (NS)	7.0 ± 0.81	8.7 ± 0.66	7.93 ± 0.45	7.1 ± 0.03	6.93 ± 0.26	7.5 ± 0.11	6.13 ± 0.09
Paracetamol (200mg/kg)	12.25 ± 0.57	20.5* ± 1.5	23.25* ± 1.14	24.37* ± 0.93	24.5* ± 1.27	26.37* ± 0.74	26.62* ± 0.84
Extract (200mg/kg)	7.43 ± 0.65	7.00 ± 0.74	7.01 ± 0.72	7.65 ± 0.91	6.68 ± 1.04	7.25 ± 0.90	8.48 ± 0.93
Extract (400mg/kg)	6.56 ± 0.31	7.53 ± 0.87	6.66 ± 0.95	9.23 ±1.23	9.62 ± 1.43	9.93 ± 1.25	12.73* ± 0.61

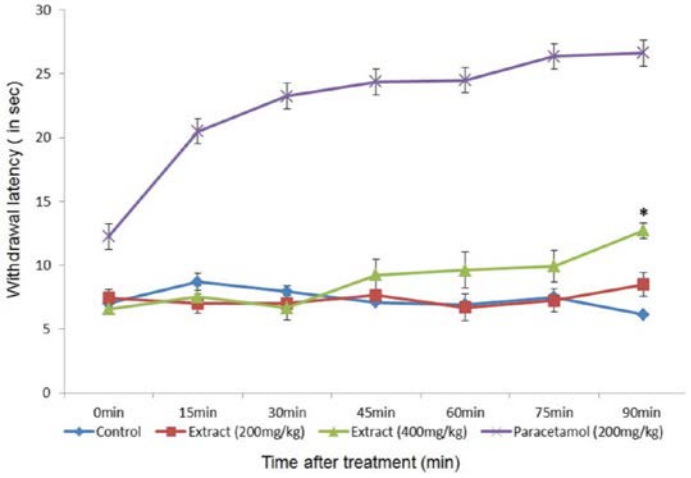

Figure 1: Anti-nociceptive effect of *Brassica campestris* leaves extract at 200 mg/kg and 400 mg/kg body weight (p.o.) in rats.

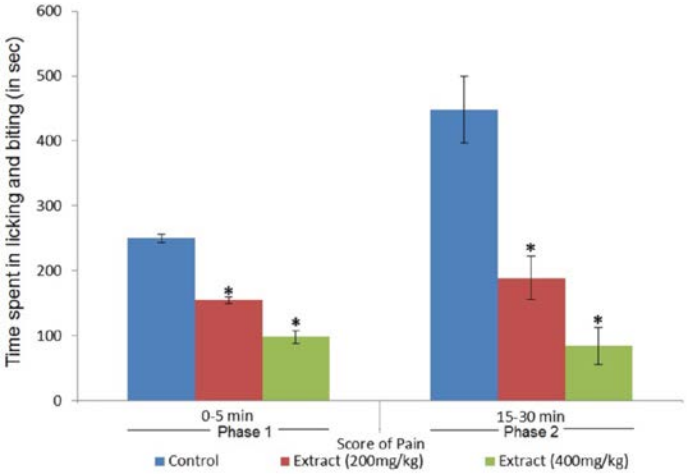

Figure 2: Antinociceptive effect of *Brassica campestris* leaves extract in rats in the formalin test.

Table 2: The analgesic potential of *Brassica campestris* leaves extract in formalin-induced nociception in rats.

	Phase 1 formalin-induced nociception		Phase 2 formalin-induced nociception	
	0-5 min		15-30 min	
Drug treatment	Licking and biting response (in sec)	% Inhibition	Licking and biting response (in sec)	% Inhibition
Control (Paracetamol 200mg/kg)	250 ± 6.06	25%	448 ± 51	40%
Extract dose (200mg/kg)	154.5* ± 49.4	38%	189* ± 32.9	57%
Extract dose (400mg/kg)	98* ± 9.81	60.8%	84* ± 28	81%

paracetamol administration (200mg/kg i.p.) significantly prolonged the withdrawal latency period (i.e., anti-nociceptive response) in treated rats at all evaluation periods (from 15 to 90 min).

Each trial was performed in triplicate with each rat in the control and plant extract treated animal group. Error bars indicate the SEM of 6 animals/group. Asterisk (*) at data values indicate a significant difference in the withdrawal latency period of *B. campestris* leaves extract administrated rats (400mg/kg p.o.) compared to control animals administered with NS (0.9%; i.p.) and paracetamol (200mg/kg; i.p.) groups at $p< 0.05$.

Antinociceptive potential of *Brassica campestris* leaves extract against formalin-induced pain

The *B. campestris* leaves extract was also evaluated for analgesic potential against formalin-induced pain in rats at 200, 400 mg/kg body weight administered dose while recording the anti-nociceptive effects in the early and late phases of the formalin test. Brassica extract was found to reduce the time spent in licking and biting responses in extract administered rats both in the early and late phases of the formalin test (Figure 2). A significant increase in pain inhibition stimulation was noticed in both phases of the formalin test where a pain inhibition stimulation of 38% and 61% of the early phase (0-5 min) and 57%, and 81% of the late phase (15-30 min) were recorded in experimental rats administered to 200 and 400mg/kg body weight dose of plant extract respectively. However, a higher percentage of scores in pain inhibition (i.e. 81%; $p<0.05$) was noticed in the late phase of the formalin test in the rat group administered with 400mg/kg body weight plant extract (Figure 2; Table 2). However, higher pain inhibition responses were observed in the late phase of the formalin test.

The analgesic potential of *B. campestris* leaves extract against formalin-induced nociception in rats was determined by the time spent in licking and biting responses of experimental rats. The antinociceptive responses were recorded for both phases of formalin-induced nociception. Each experiment was performed in triplicate and data values were collected as mean (M) ± standard error of the mean (SEM). The percentage inhibition of pain sensation in experimental rats after plant extract administration was also calculated. A higher percentage of pain sensation inhibition at 400 mg/kg bodyweight plant extract dose was demonstrated in the late phase of the formalin test. Asterisk (*) at data values indicate significant differences in time spent on licking and biting responses between extract administrated rats compared to control animals (paracetamol) at $p<0.05$.

Paracetamol (200 mg/kg i.p.) and different doses of *B. campestris* leaves extract (200 and 400 mg/kg p.o.) were administered 30 min before of formalin analgesia test. Each trial was performed in triplicate with each rat in control and plant extract treated animal groups. The time spent in

licking and biting responses was observed for two spontaneous phases of formalin-induced nociception over the 45 min test period. Error bars depict the SEM of 6 rats/group. Asterisk (*) at data values indicate significant differences in time spent in licking and biting responses of rats between extract administrated (200 and 400 mg/kg p.o.) and control group (paracetamol; 200mg/kg; i.p.) at $p< 0.05$.

DISCUSSION

Brassica campestris plant deserves particular attention from the agricultural, nutritional, and medicinal points of view.[25] It is common as a field crop and many varieties are grown and cultivated under several names in different geographical regions and a wide range of climates.[1-,20] The crop has wide acceptability in Central Europe, Asia, and the Mediterranean region.[24] It is a rich source of vitamin C, omega-3 fatty acids, and linolenic acid.[22-23] Irrespective of that, in some Arabian countries, it is used as an anti-scorbutic, antispasmodic, diuretic, anti-ulcers of the mouth, and against urinary disorders.[35,36,40] Folkloric literature and some published studies also demonstrate the antinociceptive and analgesic potential of the members of the *Brassicaceae* family.[26,29-31,41] Although *B. campestris* is widely utilized in folk medicine all over the world, the antinociceptive effects of this plant have not yet been fully elucidated. The studies of plant species that are conventionally used for the relief of pain are still elusive and used as logical research strategies to investigate novel analgesic compounds.[42]

Based on the above shreds of evidence of potential utilization of *Brassicaceae* family plants in traditional medicine, the current investigation was aimed to demonstrate the antinociceptive effects and analgesic potential of ethanolic extract of *Brassica campestris* leaves by using classical pharmacological models of pains in adult rats. In this study, the antinociceptive potential of Brassica leaves extract was investigated by using a hot plate test, and formalin-induced nociception assay. The study findings unveiled that the ethanolic extract of *B. campestris* leaves contains an antinociceptive effect at certain concentrations/doses when assessed by thermal models of nociception including hot plate test and neurogenic pain model (formalin-induced nociception). As depicted in Figure 1, the antinociceptive effect of plant extract was evident shortly after oral administration of extract manifested as prolongation in the withdrawal latency by 30 min after. However, this effect was noticed to reach a maximum after 70-90 min and after that started to fade gradually (data not shown). Our results showed that the Brassica extract significantly prolonged the time for thermal pain perception in experimental rats at the dose of 400mg/kg body weight ($p<0.05$) (Table 1). However, the anti-nociceptive effect showed insignificant variation at the dose of 200mg/kg body weight as compared to the control (Figure 1). The results of our study are in agreement with Uddin *et al*., and Hasan *et al*. who demonstrated that the root and leaf extract of different plants at certain bodyweight doses exhibited an antinociceptive effect in a classical pharmacological model of pain in adult mice when measured the animal responses by hot plate method.[43-44] The hot plate pain perception model has been used to study the centrally acting analgesic potential of chemical substances, phytochemicals, and plant extract.[38] In this model, sensory nerves sensitize the nociceptors, and the involvement of endogenous substances such as prostaglandins is minimized.[38] From the findings of the hot plate test, the extract showed an antinociceptive effect; however, not as pronounced as was noticed in the formalin-induced nociception model (Figure 2 and Table 2). Hence, the observations from the hot plate test might indicate that the analgesic activity of brassica extract might not be fully mediated through a central mechanism of antinociceptive perception in experimental rats. As the hot plate test is a specific central nociceptive test of pain perception, hence the *B. campestris* leaves extract might exert an analgesic effect at least in parts by interfering with central mechanisms of pain perception.

A dose-dependent significant antinociceptive effect of the brassica leaves extract was recorded in the formalin test as shown in Table 2. The formalin test is an appropriate pain model to distinguish the triggering mechanisms of the central and peripheral nociception in rats.[1,2] In this method, nociceptive behaviors of animals against nociception stimuli are converted to numerical values which vary as a function of time.[2] Furthermore, formalin-induced nociceptive behaviors determined by flinches or licking and biting responses provide a reliable correlation of pain perception in awake, freely moving rats. This biphasic model of formalin-induced pain behavior responses is represented by an initial acute phase (neurogenic;1-5 min) and followed by a late tonic phase, (inflammatory pain;15-30 min) respectively.[1] The acute phase pain perception is directly mediated by the effect of formalin on nociceptors, while the second tonic phase is elicited due to inflammatory responses. It has been demonstrated that this biphasic pain model displays the sites and mechanisms of action of tested analgesics by the modulation of neurogenic and inflammatory pain response mediators respectively.[1] As reported in this study, the nociceptive behavior of the experimental rats was significantly reduced in both phases of formalin-induced nociception when orally administered to *B. campestris* leaves extract in a dose-dependent manner. The reduction in time spent licking and biting of formalin injected hind paw in both phases of the formalin test indicated that the Brassica leaves extract possesses potential antinociceptive effect and strong analgesic activity. Our findings showed a weak antinociceptive effect at 200mg/kg body weight dose of plant extract in both phases of the formalin test (38 and 57% respectively; $p< 0.05$); however, a strong analgesic effect was noticed at the dose of 400mg/kg body weight in both phases (61 and 84% respectively; $p<0.05$). We also noticed that the analgesic effect at the dose of 400mg/kg body weight was more pronounced in the late phase of formalin-induced nociception (i.e., inflammatory pain response) as compared to the extract dose of 200mg/kg body weight (84% *vs.*57% respectively). We may also not rule out the release of endogenous opioids (e.g., enkephalins and endorphins function as powerful polypeptide analgesics) while inducing the thermal hyperalgesia by hot plate test and neurogenic nociception induced by formalin test in rats upon the stimulation of opiate receptors on nerve cells. However, determining either antinociception in experimental rats was induced by opiate analgesics or *B. campestris* leaves extract demands further investigation. However, the significant reduction of licking and biting activity of the experimental rats in both phases of the formalin test (Figure 2) after administering the plant extract at a dose of 400mg/kg extract might be attributed to its potent inhibition of the animal's visceral nociceptors which are highly sensitive to formalin-induced pain as well as the inhibition of the synthesis of the arachidonic acid metabolite involved in inflammatory pain induction. In this scenario, our results of the present investigation are concordant with previously published studies demonstrating the activation of opioid receptors in adult mice when administered to an extract of *Holoptelea Integrifolia, Argyreia Speciosa,* and *Microcos paniculata* barks and fruits extract.[45-46] Interestingly, the experimental control rats treated with paracetamol 200mg/kg i.p. showed a weak antinociceptive effect in both phases of the formalin test (25 and 40% respectively) However, it seems in line with previously published studies as Roca-Vinardell *et al*. demonstrated that paracetamol at the dose of 125 and 250 mg/kg exhibited week analgesic activity in the rat formalin test.[2] The suppression of neurogenic and inflammatory nociception in rats by the Brassica leaves extract in this study might imply that plant contains active analgesic moieties that may act both centrally and peripherally to suppress pain stimuli in rats.[45-46] These findings suggest that the leaves of this epiphytic plant can be used to manage acute as well as chronic pain;

however, extensive mechanistic and pharmacological studies are needed to evaluate this aspect of the *B. campestris* plant.

CONCLUSION

The *Brassica campestris* leaves extract possesses antinociceptive activity against the thermal hyperalgesia in rats induced by the hot plate model of nociception. The extract showed significant analgesic potential against formalin-induced pain in rats in both phases of the test. Further studies, of course, will be inevitable to find the pharmacological moieties responsible for this action and to elucidate precise underlying mechanisms responsible for these effects. Even though, the study findings potentiate the pharmacological basis to use the plant as an alternative source of an analgesic to reduce pain as well as to develop a novel analgesic strategy in folk and complementary medicine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

M: mean; **SEM:** standard error of the mean; **ANOVA:** analysis of variance; **NSAIDs:** non-steroidal anti-inflammatory drugs; **NS:** normal saline.

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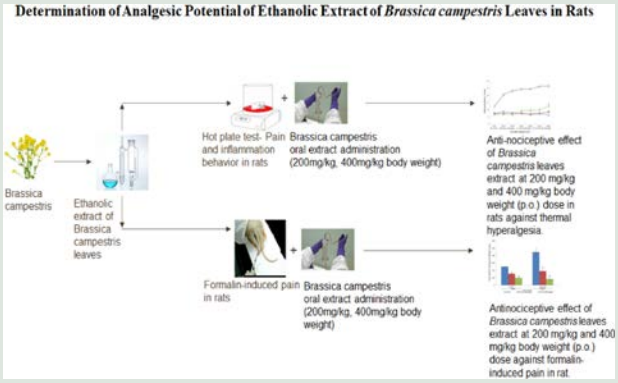
GRAPHICAL ABSTRACT



SUMMARY

Brassica campestris leaves extract shows antinociceptive potential against thermal hyperalgesia and formalin-induced nociception in rats. Further elucidation of functional moieties responsible for this therapeutic effect would be helpful to design and develop a novel analgesic strategy against pain in folk and complementary medicine.

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