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Study of antinociceptive effects on acute pain treated by bioactive fractions of *Hyptis suaveolens*

Azmathunnisa Begum[1], Venkatesh Sama[1*], Jaya Prakash Dodle[2]

[1]*G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, 500028, Telangana, India*

[2]*Faculty of Pharmacy, University College of Chemical Technology, Osmania University, Hyderabad, Telangana, India*

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ABSTRACT

Objective: To investigate the ethanolic extract and its fractions of *Hyptis suaveolens* (*H. suaveolens*) for antinociceptive and central nervous system depressant effects.
Methods: Dried and coarsely powdered aerial parts of plant material were extracted in 80% aqueous ethanol. Further extract was fractionated using solvents of varying polarity. Analgesic properties was assessed using acetic acid-induced writhing and hot plate test and locomotor activity were performed in mice using hole board test.
Results: The petroleum ether and ethyl acetate extracts had produced significant analgesic properties and were found to be maximum when tested at 400 mg/kg. Both extracts significantly increased the latency time in hot plate test and the action was antagonised by naloxone. The naloxone was not able to alter *H. suaveolens* induced antinociceptive effect in writhing test.
Conclusions: From the point of central nervous system depressant and good protective effect on chemical and thermal pain stimuli, it indicates that *H. suaveolens* might have resulted from activation of opioid and/or peripheral receptors.

1. Introduction

Natural products derived from plants are important to cure various disease conditions. Side effects of various allopathic drugs and development of resistance to currently used drugs increased the emphasis on the use of plant materials as a source of medicine for a wide variety of human ailments. Incidentally, plants and herbs are persistently being studied for the discovery of novel therapeutic agents. India is one of the biodiversity centres with the presence of about 20000 plants having a good medicinal value and used by different traditional communities[1–3]. Traditionally, plants passed empirical testing against specific diseases and demonstrated that they are well tolerated in humans. However, many of these traditionally used medicinal plants were not investigated scientifically with regard to the modern medicine. Since the use of herbal drugs remains a good alternative to allopathic agents, it is important to conduct scientific evaluation of as many traditionally used plants as possible with reference to the modern system of medicine[4,5].

Hyptis suaveolens (L.) Poit. (*H. suaveolens*) (Lamiaceae) is a fast growing perennial herb found in dense clumps along road side and distributed in the tropical and sub-tropical region. It is used traditionally for the treatment of respiratory tract infections, cold, pain, inflammation, fever, skin diseases and diabetes[6,7]. The leaves of it are reported to be rich in essential oils and useful in antifungal, antibacterial and anticonvulsant activities[8–11]. The aerial parts are reported to contain antiplasmodial diterpenoids and triterpenoid heptadienic acid[12,13]. The leaves are also reported to possess antihyperglycemic activity in the management of diabetes mellitus[14]. Phytochemically, its leaves are reported to contain hentriacontane, hentriacontanone, lupeol, acetate and friedelin[15].

In view of the reported analgesic properties of *H. suaveolens*, the present study is conducted to evaluate antinociceptive effects of *H. suaveolens* and the action mechanism on several experimental models in mice. The activity of central nervous system (CNS) is also investigated in order to examine antinociceptive activity related to central depression action.

2. Materials and methods

2.1. Preparation of plant material and authentification

The aerial parts of *H. suaveolens* were collected from Rajendra Nagar, Hyderabad. The plant was identified and

*Corresponding author: Sama Venkatesh, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, 500028, Telangana, India.
Tel: +91 4023517222
E-mail: venkateshsama@hotmail.com

All experimental procedures involving animals were conducted in accordance to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals and all the procedures for investigating experimental pain in conscious animals in the study was approved by Institutional Animals Ethics Committee of G. Pulla Reddy College of Pharmacy, Hyderabad, India.

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authenticated by Dr. P.V. Prasanna, scientist and taxonomist of Botanical Survey of India, Hyderabad, India. A voucher specimen of *H. suaveolens* (AUB-HSL-2013) was maintained in the Department of Phytochemistry and Pharmacognosy of G. Pulla Reddy College of Pharmacy, Hyderabad, India. The aerial parts were cut, air dried and ground into powder.

2.2. Preparation of plant extract and its fractions

Dried powder material of *H. suaveolens* was extracted with 80% aqueous ethyl alcohol by maceration for 5 days. The percentage yield of crude ethanolic extract was 9.23. The concentrated aqueous ethanolic extract was suspended in 500 mL of distilled water and fractionated with petroleum ether (4 × 500 mL), chloroform (4 × 500 mL), ethyl acetate (4 × 500 mL) and *n*-butyl alcohol (4 × 500 mL). The percentage yields of petroleum ether, chloroform, ethyl acetate, *n*-butanol and left over aqueous fractions of *H. suaveolens* were 0.56, 0.59, 0.2, 0.55 and 0.574 respectively.

2.3. Chemicals

All the chemicals, reagents, solvents used were of analytical grade. Aspirin, morphine, diazepam and naloxone were purchased locally.

2.4. Animals

All experimental procedures involving animals were conducted in accordance to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals and all the procedures for investigating experimental pain in conscious animals in the study was approved by Institutional Animals Ethics Committee of G. Pulla Reddy College of Pharmacy, Hyderabad, India[16].

Male Swiss albino mice (25–30 g) were used in the present study. The animals were housed in standard laboratory conditions of temperature (21 ± 2) °C, relative humidity, 12 h dark and 12 h light cycles and had free access to feed and water *ad libitum* during the quarantine period. Besides, the animals were fasted for 6 h before experimentation, but had free access to water. All the extracts and drugs were administered orally as a fine suspension of 0.5% carboxy methyl cellulose (CMC).

2.5. Antinociceptive activity

2.5.1. Acetic acid-induced writhing test

The acetic acid-induced writhing test was carried out in prescreened mice[17,18]. The animals were divided into 15 groups of six animals each. Group 1 served as control and received vehicle CMC. Groups 2–11 received aqueous ethanolic extract, petroleum ether, chloroform, ethyl acetate and butanol fractions at an oral dose of 200 and 400 mg/kg of each extract. Group 12 served as positive control and received acetyl salicylic acid at an oral dose of 100 mg/kg. In an attempt to investigate the participation of opioid system in antinociceptive effects of *H. suaveolens*, a separate group of mice was pretreated with non-selective receptor antagonist, naloxone (5 mg/kg), which was injected 15 min before the administration of petroleum ether and ethyl acetate fractions (400 mg/kg, *p.o.*) for Groups 13 and 14. After 30 min of extract/drug administration, all the animals were given an *i.p.* injection of 0.6% acetic acid (volume of injection was 0.1 mL/10 g) and writhes produced were recorded for 30 min. Group 15 animals received naloxone (5 mg/kg) and aspirin (100 mg/kg).

2.5.2. Hot plate test

The temperature of Eddy's hot plate was maintained at (55.0 ± 0.2) °C. The basal reaction time of all the animals towards thermal heat was recorded. The animals which showed paw lickings or jumping responses within 5 s were selected for the study[19]. The pre-screened animals were divided into 15 groups of 10 animals each. Control animals were treated with CMC (Group 1). Groups 2–11 received aqueous ethanolic extract, petroleum ether, chloroform, ethyl acetate and butanol extracts at an oral test doses of 200 and 400 mg/kg. Group 11 served as positive control and treated with morphine (5 mg/kg, *p.o.*). The opioid receptor antagonist naloxone (5 mg/kg) was also tested along with petroleum ether and ethyl acetate extracts (400 mg/kg, *p.o.*, Groups 13 and 14) and morphine (5 mg/kg, *p.o.*, Group 15). All the substances were administered for 30 min before the beginning of the experiment. The latency time was measured before and at 30, 60, 120 and 180 min after the administration of the extract. The latency period of 20 s was defined as a cut-off mark and measurement was terminated if the latency exceeded the period to avoid injury.

2.5.3. CNS depressant activity

The activity of *H. suaveolens* on CNS was evaluated by performing assays of its effect on exploratory capacity (hole board test) and locomotory activity. In each experiment, the mice were divided into 13 groups consisting of six animals in each group. The animals of Group 1 were treated with vehicle and served as control. Aqueous ethanol, petroleum ether, chloroform, ethyl acetate and butanol extracts were administered to Groups 2–11 animals at an oral dose of 200 and 400 mg/kg as a fine CMC suspension. Groups 12 and 13 served as positive control and orally received diazepam (2 mg/kg) and morphine (10 mg/kg).

2.5.3.1. Hole board test

Hole board test described by Sonavane *et al.* was employed[20]. The animals were placed on a board (40 cm × 40 cm) with 16 holes symmetrically distributed in 4 rows. After finishing the oral administration of test and standard substance at 30 min, each animal was placed carefully in the centre of board and number of head pokes (head was dipped into the hole) for 5 min and recorded.

2.5.3.2. Locomotory activity

The locomotory activity was measured by actophotometer (INCO Photoactometer, Ambala, India). A count was recorded when the beam of light falling on the photocell of the actophotometer was cut off by animals. The basal activity score was obtained by placing each animal individually in actophotometer for 10 min. After administration for 30 min, each animal was placed carefully in the centre of actophotometer for recording the activity score[21,22].

2.6. Statistical analysis

All the values were expressed as mean ± SEM and analysed statistically by ANOVA followed by Dunnett's multiple comparison test. Values of $P < 0.05$ were considered to be significant[23].

3. Results

3.1. Effect of H. suaveolens on acetic acid-induced writhing test

The inhibitory effects of ethanolic extract of *H. suaveolens* and its fractions in writhing test were shown in Table 1.

Table 1
Effect of *H. suaveolens* on acetic acid-induced abdominal writhing in mice.

Groups	Treatment	Writhings	Inhibition (%)
1	Control	75.83 ± 0.47	–
2	Aqueous ethanol extract (400 mg/kg)	51.75 ± 0.89*	31.75
3	Aqueous ethanol extract (200 mg/kg)	55.50 ± 0.47*	26.80
4	Petroleum ether extract (400 mg/kg)	39.33 ± 0.66*	48.13
5	Petroleum ether extract (200 mg/kg)	61.00 ± 0.93*	19.55
6	Chloroform extract (400 mg/kg)	62.33 ± 0.61*	17.80
7	Chloroform extract (200 mg/kg)	71.00 ± 0.57*	6.36
8	Ethyl acetate extract (400 mg/kg)	44.66 ± 0.55*	41.10
9	Ethyl acetate extract (200 mg/kg)	62.66 ± 0.71*	13.17
10	Butanol extract (400 mg/kg)	70.33 ± 0.66*	7.25
11	Butanol extract (200 mg/kg)	71.66 ± 0.49*	5.49
12	Aspirin (100 mg/kg)	19.83 ± 0.60*	73.84
13	Petroleum ether + naloxone extract (400 mg/kg + 5 mg/kg)	40.83 ± 0.30*	46.15
14	Ethyl acetate + naloxone extract (400 mg/kg + 5 mg/kg)	46.10 ± 0.67*	39.20
15	Aspirin + naloxone (100 mg/kg + 5 mg/kg)	22.00 ± 0.57*	70.98

All values were expressed as mean ± SEM, $n = 6$; *: $P < 0.01$ compared with control.

Except butanolic fraction, all other extracts significantly ($P < 0.01$) inhibited the writhings in mice at both test dose levels. The maximum activity was observed in the animals receiving a test dose of 400 mg/kg. Among all extracts, petroleum ether extracts and ethyl acetate extracts had produced the maximum inhibitory effect with 48.13% and 41.0% protection when tested at 400 mg/kg. The administration of naloxone (5 mg/kg) along with petroleum ether, ethyl acetate extracts and aspirin demonstrated significant ($P < 0.01$) analgesic effects with percentage inhibition of 46.15, 39.20 and 70.98. None of the inhibitory effects of extracts were comparable with aspirin activity. These results indicated that naloxone had no effect on antinociceptive activity of *H. suaveolens* and aspirin.

3.2. Effect of H. suaveolens on the hot plate test

The results presented in Table 2 and showed the time course of antinociception produced by various extracts of *H. suaveolens*. Oral administration of all the extracts resulted in significant prolongation of latency time in hot plate test. Among all the extracts, petroleum ether and ethyl acetate extracts had produced higher latency time at 120 and 90 min respectively. The course of analgesic action was initiated from the 60 min of experiment. The animals received 400 mg/kg test dose had produced maximum antinociceptive properties. Chloroform and butanol extract were not able to produce any significant analgesic property. At 90 min, the mean latency time of petroleum ether and ethyl acetate extracts were (9.90 ± 0.50) s and (6.40 ± 0.40) s compared with

Table 2
Effect of *H. suaveolens* extracts in hot plate test(s).

Groups	Treatment	Latency time					
		0 min (pretreatment)	30 min (after treatment)	60 min (after treatment)	90 min (after treatment)	120 min (after treatment)	180 min (after treatment)
1	Control	2.10 ± 0.17	2.20 ± 0.13	2.00 ± 0.14	2.10 ± 0.17	2.20 ± 0.20	2.00 ± 0.14
2	AEHS (400 mg/kg)	2.10 ± 0.23	3.00 ± 0.33	4.60 ± 0.33**	5.30 ± 0.21**	5.30 ± 0.36**	3.20 ± 0.41
3	AEHS (200 mg/kg)	2.30 ± 0.26	2.50 ± 0.34*	2.80 ± 0.24*	3.20 ± 0.35*	2.80 ± 0.32*	2.30 ± 0.21*
4	PEHS (400 mg/kg)	2.30 ± 0.21	2.70 ± 0.26	6.20 ± 0.32**	9.90 ± 0.50**	8.90 ± 0.37**	4.10 ± 0.31**
5	PEHS (200 mg/kg)	2.20 ± 0.20	2.50 ± 0.16*	2.70 ± 0.21*	3.20 ± 0.20*	2.90 ± 0.17*	2.40 ± 0.16*
6	CHS (400 mg/kg)	2.00 ± 0.21	2.00 ± 0.25	2.50 ± 0.16	3.00 ± 0.25	3.20 ± 0.24	2.60 ± 0.26
7	CHS (200 mg/kg)	2.70 ± 0.15	2.70 ± 0.21	2.70 ± 0.26**	2.90 ± 0.23**	2.80 ± 0.24**	2.60 ± 0.22
8	EAHS (400 mg/kg)	2.00 ± 0.14	2.30 ± 0.15	3.70 ± 0.30**	6.40 ± 0.40**	5.90 ± 0.48**	3.50 ± 0.40*
9	EAHS (200 mg/kg)	2.50 ± 0.37	2.60 ± 0.23*	2.90 ± 0.31*	3.20 ± 0.20*	2.80 ± 0.20*	2.80 ± 0.46*
10	BHS (400 mg/kg)	2.20 ± 0.20	2.30 ± 0.33	2.30 ± 0.36	3.10 ± 0.31	2.80 ± 0.51	2.40 ± 0.47
11	BHS (200 mg/kg)	2.70 ± 0.33	2.70 ± 0.47*	2.80 ± 0.44*	2.90 ± 0.27*	2.90 ± 0.40*	2.70 ± 0.47*
12	Morphine (5 mg/kg)	2.70 ± 0.30	11.10 ± 0.43**	13.0 ± 0.49**	17.30 ± 0.55**	16.90 ± 0.64**	14.60 ± 0.40**
13	PEHS + naloxone (400 mg/kg + 5 mg/kg)	2.40 ± 0.26	2.40 ± 0.33*	2.50 ± 0.26*	3.20 ± 0.44*	3.10 ± 0.34*	2.30 ± 0.21*
14	LAHS + naloxone (400 mg/kg + 5 mg/kg)	2.30 ± 0.33	2.40 ± 0.33	2.40 ± 0.26	2.60 ± 0.30	2.50 ± 0.30	2.30 ± 0.39
15	Morphine + naloxone (5 mg/kg + 5 mg/kg)	2.10 ± 0.31	5.50 ± 0.50**	5.70 ± 0.51**	6.40 ± 0.33**	6.20 ± 0.46**	4.00 ± 0.39*

AEHS: Aqueous ethanolic extract of *H. suaveolens*; PEHS: Petroleum ether extract of *H. suaveolens*; CHS: Chloroform extract of *H. suaveolens*; EAHS: Ethyl acetate extract of *H. suaveolens*; BHS: Butanol extract of *H. suaveolens*; LAHS: Left over aqueous extract of *H. suaveolens*; *: $P < 0.05$; **: $P < 0.01$ compared with control. All values were expressed as mean ± SEM, $n = 10$.

(2.10 ± 0.17) s and (17.30 ± 0.55) s for control and morphine treated groups respectively. The analgesic effects of both extracts were not comparable with morphine activity during any course of time. Administration of naloxone (5 mg/kg) had reversed the analgesic properties induced by petroleum ether and ethyl acetate extracts. Similarly, the effect produced by morphine (5 mg/kg) was significantly blocked by administration of naloxone.

3.3. Hole board test

The results of hole board test was presented in Table 3. All the extracts had produced a significant dose dependent activity. The head dip responses were significantly reduced in animals treated with a dose of 400 mg/kg. Among all the extracts, aqueous ethanolic extract, ethyl acetate extract followed by petroleum ether extracts had produced maximum activity when compared to control. The action produced by aqueous ethanolic extract (400 mg/kg) was significantly high when compared to diazepam and morphine during the entire course of experiment.

3.4. Locomotory activity

Table 4 represented the results of *H. suaveolens* locomotor activity. Among all the extracts, petroleum ether and aqueous ethanolic (400 mg/kg) extracts had produced significant ($P < 0.01$) reduction in locomotor activity with percentage reduction of 64.17 and 59.89 respectively. Between the two test dose levels, animals received 400 mg/kg had produced maximum activity. Butanolic and chloroform extracts did not produced any action compared with control. The effect of diazepam and morphine was more marked with 69.48% and 73.41% reduction in activity and the action was comparable with animals treated with petroleum ether extract (400 mg/kg).

Table 3
Effect of *H. suaveolens* extracts on hole board test in mice.

Groups	Treatment	Head pokes/dips				
		1 min	2 min	3 min	4 min	5 min
1	Control	14.16 ± 0.47	15.33 ± 0.49	14.50 ± 0.42	14.50 ± 0.84	13.00 ± 0.36
2	AEHS (400 mg/kg, *p.o.*)	5.00 ± 0.36**	4.16 ± 0.47**	4.00 ± 0.36**	3.83 ± 0.54**	2.83 ± 0.47**
3	AEHS (200 mg/kg, *p.o.*)	11.83 ± 0.74	12.50 ± 0.61	12.66 ± 0.49	11.50 ± 0.61*	11.33 ± 0.33*
4	PEHS (400 mg/kg, *p.o.*)	9.33 ± 0.49**	8.66 ± 0.49**	7.83 ± 0.60**	4.16 ± 0.60**	3.50 ± 0.42**
5	PEHS (200 mg/kg, *p.o.*)	12.16 ± 0.74	11.50 ± 0.76*	10.16 ± 0.60**	11.00 ± 0.63**	10.66 ± 0.55**
6	CHS (400 mg/kg, *p.o.*)	5.83 ± 0.60**	6.00 ± 0.57**	5.66 ± 0.49**	5.33 ± 0.33**	5.00 ± 0.36**
7	CHS (200 mg/kg, *p.o.*)	15.00 ± 0.73	14.16 ± 0.70	14.00 ± 0.96	13.83 ± 0.87	14.66 ± 0.80
8	EAHS (400 mg/kg, *p.o.*)	6.00 ± 0.73**	5.66 ± 0.66**	5.16 ± 0.79**	4.83 ± 0.60**	3.83 ± 0.47**
9	EAHS (200 mg/kg, *p.o.*)	10.83 ± 0.74**	10.33 ± 0.49**	11.00 ± 0.77**	10.50 ± 0.42**	9.83 ± 0.60**
10	BHS (400 mg/kg, *p.o.*)	6.83 ± 0.87**	7.33 ± 0.80**	6.83 ± 0.60**	7.33 ± 0.42**	7.83 ± 0.70**
11	BHS (200 mg/kg, *p.o.*)	13.66 ± 0.55	13.50 ± 0.95	14.50 ± 0.56	14.16 ± 0.60	14.33 ± 0.76
12	Diazepam (2 mg/kg, *p.o.*)	5.66 ± 0.66**	5.33 ± 0.55**	5.33 ± 0.61**	4.66 ± 0.55**	4.50 ± 0.56**
13	Morphine (10 mg/kg, *p.o.*)	6.33 ± 0.66**	4.66 ± 0.42**	4.50 ± 0.47**	3.83 ± 0.47**	3.33 ± 0.49**

AEHS: Aqueous ethanolic extract of *H. suaveolens*; PEHS: Petroleum ether fraction of *H. suaveolens*; CHS: Chloroform fraction of *H. suaveolens*; EAHS: Ethyl acetate fraction of *H. suaveolens*; BHS: Butanol fraction of *H. suaveolens*; *: $P < 0.05$ compared with control; **: $P < 0.01$ compared with control. All values were expressed as mean ± SEM, $n = 6$.

Table 4
Effect of *H. suaveolens* on locomotor activity.

Groups	Treatment	Locomotor activity score in 10 min		Reduction in activity (%)
		Before treatment	After treatment	
1	Control	402.50 ± 0.76	396.00 ± 0.73	–
2	AEHS (400 mg/kg)	217.33 ± 0.66	87.16 ± 0.79**	59.89
3	AEHS (200 mg/kg)	242.50 ± 0.76	212.16 ± 0.60**	12.50
4	PEHS (400 mg/kg)	251.66 ± 0.94	90.16 ± 0.47**	64.17
5	PEHS (200 mg/kg)	192.33 ± 0.76	142.00 ± 0.57**	26.16
6	CHS (400 mg/kg)	200.50 ± 0.76	182.66 ± 0.84**	8.89
7	CHS (200 mg/kg)	262.16 ± 0.60	252.66 ± 0.49*	3.62
8	EAHS (400 mg/kg)	224.50 ± 0.76	136.33 ± 0.66**	39.27
9	EAHS (200 mg/kg)	317.50 ± 0.56	292.16 ± 0.60**	7.98
10	BHS (400 mg/kg)	307.50 ± 0.92	275.83 ± 0.60**	10.29
11	BHS (200 mg/kg)	331.66 ± 0.47	326.83 ± 0.60*	1.45
12	Diazepam (2 mg/kg)	217.50 ± 0.76	57.83 ± 0.60**	69.48
13	Morphine (10 mg/kg)	234.83 ± 0.47	71.66 ± 0.55**	73.41

AEHS: Aqueous ethanolic extract of *H. suaveolens*; PEHS: Petroleum ether extract of *H. suaveolens*; CHS: Chloroform extract of *H. suaveolens*; EAHS: Ethyl acetate extract of *H. suaveolens*; BHS: Butanol extract of *H. suaveolens*; **: $P < 0.01$ compared with control.

4. Discussion

The results of the present study indicate that oral administration of *H. suaveolens* extracts has produced significant analgesic properties (except butanolic extract) at 400 mg/kg. The petroleum ether and ethyl acetate extracts of *H. suaveolens* have produced high significant central analgesic (hot plate test) and protective effect on chemical (acetic acid injection) stimuli at 400 mg/kg.

Such an efficacy on these two stimuli is the characteristic of central analgesics like morphine, while peripheral analgesic (acetyl salicylic acid) is known to be inactive on thermal pain stimuli.

The petroleum ether and ethyl acetate extracts significantly inhibited the acetic acid-induced writhing in mice with percentage protection of 48.13 and 21.39 respectively, followed by aqueous ethanolic extract producing percentage protection of 31.97. This test was widely used for the evaluation of analgesics and involved the release of prostaglandins and phlogistic mediators like prostaglandin E_2 and prostaglandin $E_{2\alpha}$ and these levels were increased in peritoneal fluid of the acetic acid-induced mice. It was postulated that acetic acid acts indirectly by inducing the release of endogenous mediators which stimulates the nociceptive neurons that are sensitive to non-steroidal anti-inflammatory drugs and narcotics[24]. The hot plate test employed to verify the *H. suaveolens* extracts could show central antinociceptive drugs[25]. Among all the extracts, petroleum ether and ethyl acetate extracts had significantly increased in the latency time in hot plate test at dose of 400 mg/kg. Apparently, the effect largely depends on endogenous opioids. Although hot plate test is commonly used for the assay of narcotic analgesics, other drugs such as sedatives, muscle relaxants and psychometric drugs act centrally[19].

To determine the possible mechanism of action of petroleum ether and ethyl acetate extracts of *H. suaveolens* to produce analgesia, naloxone was used as a selective antagonist of opioid receptors. Naloxone apparently acts by antagonising the action of endogenous opioid involved in pain and stress[26]. The results indicated that naloxone markedly reversed the antinociceptive effect of petroleum ether and ethyl acetate extracts (400 mg/kg) and morphine (10 mg/kg) in heat induced pain (hot plate). The effect of petroleum ether and ethyl acetate extracts in acetic acid-induced pain model was unaffected by naloxone or the analgesic effects were not antagonised.

The results of antinociceptive study led us to carry out further investigation of *H. suaveolens* on CNS hole board test and locomotor activity in mice. The aqueous ethanol, petroleum ether and ethyl acetate extracts produced significant dose dependent activity on CNS. The mechanism underlined that this effect was unknown. However, from the point of CNS depressant and good protective effect on chemical and thermal stimuli, it indicated that *H. suaveolens* may have morphinomimetic properties. However, naloxone is not able to alter the *H. suaveolens* induced antinociceptive effect on writhing test. Thus, the observed antinociceptive activity of *H. suaveolens* might have resulted from the activation of peripheral and/or opioid receptors. The results of present investigation confirm the antinociceptive properties of *H. suaveolens* and the reports of traditional practices were stands to be correct. However, the exact mechanism of action is still not known at this stage and has to be established in various models.

Conflict of interest statement

The authors report no conflict of interest.

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