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PHYTOCHEMICAL SCREENING AND ANTINOCICEPTIVE EFFECTS OF *HYPTIS SUAVEOLENS* STEM

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ABSTRACT

The aim of the research work is to investigate the anti-nociceptive effects of petroleum ether and ethanolic extracts of *Hyptis suaveolens* (*H. suaveolens*) stems. Dried and coarsely powdered stem part of plant material was extracted in petroleum ether and ethanol and their analgesic activity was assessed using acetic acid-induced writhing and hot plate test methods.

The petroleum ether and ethanolic extracts had produced significant analgesic effects and was found to have shown the maximum at 400 mg/kg. Both the extracts have remarkably increased the latency time in hot plate test and acetic acid-induced writhing methods. The point of good protective effect on chemical and thermal pain stimuli indicates that the *H. suaveolens* might have resulted from activation of peripheral or opioid receptors.

Keywords: *Hyptis suaveolens;* antinociceptive effects; petroleum ether and ethanolic extracts.

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]. Traditionally, plants passed empirical testing against specific diseases and demonstrated that they are well toleratedin humans. However, many of these traditionally used medicinal plants were not investigated scientifically with regard to the modern medicine. Since the use ofherbal drugs remains a good alternative to allopathic agents, it is important to conductscientific evaluation of as many traditionally used plants as possible with reference to the modern system of medicine[2].

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[3]. The leaves of it are reported to be rich in essential oils and useful in antifungal, antibacterial and anticonvulsant activities[4]. The aerial parts are reported to contain antiplasmodial diterpenoids and triterpenoidheptadienicacid[5]. The leaves are also reported to possess antihyperglycemic activity in the management of diabetes mellitus[6]. Phytochemically, its leaves are reported to contain hentriacontane, hentriacontanone, lupeol, acetate and friedelin[7] 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 authentication

The stem parts of *H. suaveolens* were collected from Sattenapalli, Guntur District, Andhra Pradesh. The plant was identified and authenticated by Dr. P. Satyanarayana Raju, Department of Botany, University College of Sciences, Acharya Nagarjuna University, A.P, India. A voucher specimen of *H. suaveolens* (ANUCPS-HS-2019) was maintained in the Pharmacognosy lab, ANUCPS. The stem parts were cut, air dried and ground into powder.

2.2. Preparation of plant extract

Dried powder material (500 g) of *H. suaveolens* was extracted with petroleum ether and ethanol by soxhalet extraction6 hrs. The yield of the crude petroleum ether and ethanolic extracts were 5.86 g and 9.23 g respectively.

2.3. Chemicals

All the chemicals, reagents, solvents used were of analytical grade and they were purchased from Sigma Chemical Company, St. Louis, USA. Aspirin 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 Acharya Nagarjuna University College of Pharmaceutical Sciences, Guntur, A.P, India[8].

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. Phytochemical Screening

Standard screening tests[9] were employed in screening the extracts for different constituents. Conventional protocol for detecting the presence of alkaloids, tannins, flavonoids and steroids, etc. was utilized.

2.6. *Antinociceptive activity*

2.6.1. Acetic acid-induced writhing test

The acetic acid-induced writhing test was carried out in pre- screened mice[9]. The animals were divided into 8 groups of six animals each. Group 1 served as control and received vehicle CMC. Groups 2–7 received petroleum ether and ethanolic extracts at an oral dose of 100, 200 and 400 mg/kg of b.wt respectively. Group 8 served as positive control and received acetyl salicylic acid at an oral dose of 100 mg/kg. After 30 min of extract/drug administration, all the animals were given ani.p. injection of 0.6% acetic acid (volume of injection was 0.1 mL/ 10 g) and writhes produced were recorded for 30 min.

2.6.2. 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[10]. The prescreened animals were divided into 8 groups of 6 animals each. Control animals were treated with CMC (Group 1). Groups 2–7 received petroleum ether and ethanolic extracts at oral test doses of 100, 200 and 400 mg/kg. Group 8 served as positive control and treated with morphine (5 mg/kg, p.o.). All the substances were administered 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.

2.7. 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[11].

3. Results

3.1. Phytochemical Screening

Phytochemical screening of petroleum ether and ethanolic extracts of *H. suaveolens* revealed the presence of terpenoids, steroids and triterpenoids.

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

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

The petroleum ether and ethanolic extracts of *H. suaveolens* has significantly (P < 0.01) inhibited the writhings in mice at the dose of 400 mg/kg b.wttest dose levels. The maximum activity was observed in the animals receiving a test dose of 400 mg/kg. Among two extracts, petroleum ether extracts had produced the maximum inhibitory effect with 62.08%. the standard drug aspirin had produced the maximum inhibitory effect with 72.16%.

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

The results presented in Table 2 showed the time course of antinociception produced by various extracts of *H. suaveolens*. Oral administration of both the extracts resulted in significant prolongation of latency timein hot plate test. Both the petroleum ether and ethanolics extracts had produced higher latency time at 90 and 120 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. At 90 min, the mean latency time of petroleum ether and ethanolic extracts were (4.90 ± 0.87)s and (3.90 ± 0.86) s compared with(2.20±0.14) s and (11.41±0.47) 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.

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 ethanolic 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 ethanolic extracts significantly inhibited the acetic acid-induced writhing in mice with percentage protection of 62.08 and 41.24 respectively. This test was widely used for the evaluation of analgesics and involved the release of prostaglandins and phlogistic mediators like prostaglandin E_2 and these levels were increased inperitoneal fluid of the acetic acid-induced mice. It was postulatedthat 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[12]. The hot plate test employed to verify the *H. suaveolens* extracts could show central antinociceptive drugs[13]. Among the two extracts, petroleum ether extract 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[10].

The effect of petroleum ether and ethanolic extracts in acetic acid-induced pain model was unaffected by naloxone or the analgesic effects were not antagonised.

Thus, the observed anti- nociceptive 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.

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

Groups	Treatment	Writhings			Inhibition (%)
1	Control	74.27	±	0.68	–
2	PEEHS(100 mg/kg)	63.35	±	0.46*	14.70
3	PEEHS(200 mg/kg)	47.57	±	0.53*	35.95
4	PEEHS(400 mg/kg)	28.16	±	0.81*	62.08
5	EEHS(100 mg/kg)	69.75	±	0.72*	6.08
6	EEHS(200 mg/kg)	56.21	±	0.85*	24.31
7	EEHS(400 mg/kg)	43.64	±	0.91*	41.24
8	Aspirin (100 mg/kg)	20.67	±	0.67*	72.16

PEEHS: Petroleum ether extract of *H. suaveolens*; EEHS: Ethanolic extract of *H. suaveolens*;*: $P<0.05$; **: $P<0.01$ compared with control. All values were expressed as mean ± SEM, $n = 6$.

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	±	0.54	2.10	±	0.16	2.10	±	0.19	2.20	±	0.14	2.20	±	0.22	2.00	±	0.17
2	PEEHS (100 mg/kg)	2.20	±	0.78	2.40	±	0.52	2.70	±	0.71**	2.90	±	0.34**	3.00	±	0.27**	2.80	±	0.58
3	PEEHS (200 mg/kg)	2.30	±	0.58	2.50	±	0.34*	2.80	±	0.27*	3.20	±	0.41*	2.90	±	0.36*	2.80	±	0.14*
4	PEE HS(400 mg/kg)	2.20	±	0.27	2.90	±	0.45	3.70	±	0.65**	4.90	±	0.87**	6.50	±	0.67**	5.90	±	0.21**
5	EEHS(100 mg/kg)	2.10	±	0.31	2.40	±	0.47*	2.60	±	0.54*	2.80	±	0.12*	2.90	±	0.54*	2.60	±	0.87*
6	EEHS(200 mg/kg)	2.10	±	0.24	2.00	±	0.65	2.50	±	0.+65	3.00	±	0.98	3.20	±	0.32	2.70	±	0.32
7	EEHS(400 mg/kg)	2.20	±	0.17	2.70	±	0.32	3.20	±	0.45**	3.90	±	0.86**	4.60	±	0.68**	3.70	±	0.62
8	Morphine (5 mg/kg)	2.20	±	0.19	5.40	±	0.25	8.70	±	0.39**	11.41	±	0.47**	15.20	±	0.91**	17.50	±	0.87*

PEEHS: Petroleum ether extract of *H. suaveolens*; EEHS: Ethanolic extract of *H. suaveolens*;*:$P<0.05$;**: $P<0.01$ compared with control. All values were expressed as mean ± SEM, $n = 6$.

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