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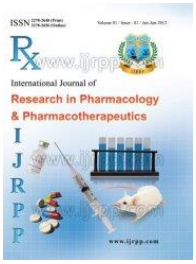

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Analgesic and anti-inflammatory activities of leaves of plectranthus vettiveroides

Mohammed Abdul Nazeer[1], Gayathri Nallathambi[2], Jeevanandham Somasundaram[3]

[1]Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam, Namakkal District, Tamil Nadu - 638 183, India
[2]Department of Pharmaceutical Analysis, Senghundhar College of Pharmacy, Tiruchengode, Namakkal District, Tamil Nadu - 637 205, India
[3]Department of Pharmacy, College of Medicine and Health sciences, Dilla University, Ethiopia
***Corresponding author: Mohammed Abdul Nazeer**
Email: abduln33@gmail.com

ABSTRACT

Plectranthus vettiveroides is an epiphytic orchid used extensively by the tribes of Karnataka in various diseases such as heart disease, leukoderma, skin allergy and rheumatism. It is used both internally and as an external application. This study aims to evaluate analgesic and anti-inflammatory activities of ethanolic extract of P.vettiveroides leaves powder in experimental animals. Shade-dried leaves were pulverized into fine powder. The analgesic activity of test drug was evaluated with tail-flick response and formalin-induced paw licking and anti-inflammatory activity with carrageenan-induced paw edema and formaldehyde-induced edema in Charles Foster albino rats. Statistically, the values were assessed with one-way analysis of variance followed by Dunnett's multiple t- tests and Student's t-test for paired and unpaired data. Administration of P.vettiveroides leaves powder showed significant increase in tail-flick response at 30 min (50.64%), at 60 min (106.13%), 120 min (80.01%), and 180 min (48.06%). Test drug produced no significant inhibition of carrageenan-induced paw edema at 1 h (36.14%) and 5 h (14.56%) compared to control group and did not produced any effect in formalin-induced edema. The present study concluded that the test drug has central analgesic activity against radiant heat-induced pain, moderate anti-inflammatory activity against carrageenan-induced acute inflammation.

Keywords: Analgesic, Anti-inflammatory, Carrageenan, P.vettiveroides, Formalin

INTRODUCTION

Traditional knowledge refers to innovations and practices of indigenous and local communities embodying traditional lifestyles as well as indigenous and local technologies. It includes Indian systems of medicine such as Ayurveda, Siddha, Unani and ethnic or folklore practices. India with 550 tribal communities belonging to 277 ethnic groups represents one of the richest heritages in the world.

The ethnic diversity in the country is represented by as many as 400 ethnic groups including the tribes and others. The tribal population is almost 7.5% of the total population of the country [1]. Due to inaccessibility to modern health care, the real knowledge of the usage of plants lies with the rural population of the country consisting of tribes, other forest dwellers and many villagers [2]. Ancient Ayurveda scholars always encouraged addition of new herbs into Ayurvedic material medical [3]. Pain associated with inflammation is most common manifestation found in arthritic or degenerative joint diseases. Many of the classical herbs have been screened for their analgesic and anti-inflammatory activities. At present, research interest in the area of ethno medicine or folklore have increased manifold, as many of them have led to novel drug discovery. According to the World Health Organization estimates, about 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health-care needs.

Plectranthus vettiveroides is also known as coleus vettiveroides, coleus zeylanicus, plectranthus zeynanicus (Lamiaceae). The main phytochemical constituents of the genus Plectranthus are diterpenoids, essential oils and phenolics. About 140 diterpenoids were identified from the coloured leaf – glands of plectranthus species. The main constituents of essential oils of plectranthus are mono and sesquiterpenes. Flavonoides seem to be rare in plectranthus, only two flavonoides were identified, 4', 7-dimethoxy -5, 6-identified, vone in plectra thus ambigns and chrysosplevetin from p.marruboides. Traditionally it has been used as an antibacterial, deodorant, cooling agent and also used against eye burning head ache and fever. The present research was designed to carry out analgesic and anti-inflammatory activity of ethanolic extract of leaves of Plectranthus vettiveroides (EEPV).

MATERIALS AND METHODS

Plant identification and collection

The plant was collected in July 2019 from Namakkal, Tamilnadu, India. The plant was identified by Joint Director of the Botanical Survey of India, Southern circle, TNAU Campus, Coimbatore, who authenticated the plant from information available in the literature. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy, J.K.K. Nattraja College of Pharmacy, Kumarapalayam, and Tamilnadu, India. The leaves were dried in the shade for 10–12 days. After complete drying, the dried leaves were pulverized to a coarse powder of 40 mesh size in a mechanical grinder. The powdered material was subjected to sohxlet extraction for 18 h at 50–55°C using ethanol and water. The extract was thereafter concentrated under vacuum and air-dried.

Animals

Charles Foster rats (Rattus norvegicus) of either sex weighing between 180 and 220 g were used for the experimentation. The rats were obtained from animal house attached to the institute. The experimental protocols were approved by Institutional Animal Ethics Committee in accordance with the guidelines formulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals, India. Six rats of either sex were housed in the cage made of polypropylene with stainless steel top grill. The animals were exposed to 12 h light and 12 h dark cycles with the relative humidity of 50%–70% and the ambient temperature was 22°C ± 03°C. All animals were kept in same environmental conditions. The rats were given food and water ad libitum.

EXPERIMENTAL DESIGN

There were four groups of animals for each model, namely

Group I – Normal control {Distilled water (5 ml/kg, PO)}, Group II – Vehicle control (Honey) – {450 mg/kg in distilled water (5 ml/kg, PO)}, Group III – Test drug (EEPV) (450 mg/kg body weight, 450 mg honey and 10 ml of distilled water was added, mixed well), Group IV – Reference standard.

ANALGESIC ACTIVITY

Analgesic activity was evaluated in two models, namely, tail-flick response and formalin-induced paw licking. The reference standard group received Pentazocine sodium (20.0 mg/kg PO) for tail-flick method and Diclofenac sodium (5 mg/kg PO) for formalin-induced paw licking.

TAIL-FLICK METHOD

The rats of either sex were placed on the analgesiometer so that constant heat intensity was applied to the lower third of the animal's tail. When the animal flicked its tail in response to the noxious stimulus both the heat source and timer were stopped and recorded as initial reading. Mean of three initial readings was taken and recorded. A cutoff time of 15 s was set to avoid tail damage [4]. The test drugs, vehicle and distilled water were administered to respective groups. One hour after test drug administration, tail-flick latency was again recorded at the intervals of 30, 60, 120, 180 and 240 min. The difference between actual values and initial values were registered for each time interval. The changes in tail-flick response were calculated and results were compared with control group.

Formaldehyde-induced paw licking response

Effect of test drug on the latency of paw licking, which represents pain threshold, was observed by formalin-induced pain in Charles Foster rats [5]. The test drug was administered for 5 consecutive days. On 5th day, 1 h after administration of test drugs, 0.1 ml of 2% v/v formalin was injected into the sub plantar surface of the left hand-paw of the rat. Immediately after the injection, animals were placed in a transparent plastic chamber (30 cm × 30 cm × 30 cm). Onset time of paw licking and number of licking of formalin-injected paw were considered as an index of pain or nociception. Animals were observed for onset time of licking and number of licking for total 30 min duration in periods of 0–10 min (early phase), 11–20 min and 21–30 min (late phase). The delay in onset and decrease in the frequency of paw licking after formaldehyde injection in test drug treated rats is considered to indicate analgesic effect of drug.

Anti-inflammatory activity

Anti-inflammatory activity was tested in two models, namely, carrageenan-induced paw edema and formaldehyde-induced paw edema. The reference standard group received phenylbutazone (100 mg/kg PO) for carrageenan-induced paw edema and diclofenac sodium (5 mg/kg PO) for formalin-induced paw edema.

ACUTE INFLAMMATION MODEL

Carrageenan-induced paw edema in rats

The test drug, vehicle and distilled water were administered to respective groups once daily for 5 consecutive days. On 5th day, before carrageenan injection, the initial paw volume of the left hind paw was measured using a digital plethysmometer. One hour after drug administration, paw edema was produced by injecting 0.1 ml freshly prepared 1% w/v carrageenan in sterile saline solution in to the sub plantar Apo neurosis of the left hind limb of rat. The intensity of edema formation was recorded after 1, 3 and 5 h of carrageenan injection.

SUBACUTE INFLAMMATION MODEL

Formaldehyde-induced paw edema in rats

The procedure of Brownlee [6] was employed to screen the anti-inflammatory activity of the test drugs against formaldehyde-induced hind paw edema in rats. The test drug, vehicle and distilled water were administered to respective groups once daily for 5 consecutive days. On 5th day, before formalin injection, the initial paw volume of left hind paw up to the tibiotarsal articulation was measured using a digital plethysmometer (model 520, IITC Life Science Inc.). One hour after the drug administration, 0.1 ml of 2% formaldehyde solution was injected beneath the plantar aponeurosis into the left hind paw. The intensity of edema formation was recorded after 24 h and 48 h of formalin injection.

Statistical analysis

The data are expressed as mean ± standard error of mean for six rats per experimental group. One-way analysis of variance was used to compare the mean values of quantitative variables among the groups followed by Dunnett's multiple “t”- test and Students “t”- tests for paired and unpaired data using Sigma stat software to determine significant difference between groups at $P < 0.05$.

RESULT

Percentile data showed that the administration of EEPV produced significant increase in tail-flick response up to 180 min, while it produced no significant increase in tail-flick response after 180

min in comparison to initial value and control group. Statistically, when compared to control group at 30 min (50.64%) and at 180 min (48.06%), EEPV produced significant activity. At 60 min (106.13%) and 120 min (80.01%), test drug showed highly significant activity. Standard drug produced highly significant activity at 60 min and 120 min (129.46% and 63.31%). Vehicle control group also showed mild analgesic response in comparison to initial values. Standard drug produced highly significant activity at all-time intervals in comparison to initial and control group [Table 1].

Table 1: Effect of EEPV on tail-flick-induced pain in Charles foster rats

Groups	Duration of latency of tail – flick responses recorded at different time intervals					
	Initial	30 min	60 min	120 min	180 min	240 min
Control	4.32±0.43	3.63±0.53	3.21±0.64	3.79±0.93	3.54±0.75	3.81±0.54
Vehicle control	4.70±0.23	5.37±0.63*	5.69±0.53*	4.56±0.32	4.00±0.64	3.73±0.54
EEPV	4.50±0.38	5.57±0.26*	6.81±0.64**	6.77±0.53	5.69±0.54**	4.72±0.23
Pentazocine Sodium	4.02±0.15	6.06±0.85**	7.55±0.87**	6.13±0.53	6.04±0.75**	4.12±0.87

Data: Mean±SEM *P<0.05, **P<0.01, ***P<0.001compared with initial (paired t test), *P<0.05, **P<0.01 when compared with the control group (ANOVA followed by Dunnett's multiple t test).

In formalin-induced paw licking, at early phase 0–10 min (4.61%) and at 11–20 min (60.42%), EEPV showed nonsignificant decrease in numbers of paw licking response. Whereas standard drug showed significant activity at 0–10 min (71.28%) and 21–30 min (63.70%) in comparison to control group [Table 2].

Table 2: Effect of EEPV on formalin-induced paw licking at different intervals in Charles foster rats

Groups	Dose (mg/kg)	Number of paw licking		
		0-10 min	11-20 min	21-30 min
Control	Q.S	11.78±1.45	5.19±1.54	10.14±0.43
Vehicle control	450	12.72±1.32	3.56±1.75*	13.32±2.32
EEPV	450	10.39±1.45	2.01±0.54**	12.01±1.43
Pentazocine Sodium	5	6.22±0.45@*	1.72±0.23**	2.72±0.54@@*

Data: Mean±SEM ***=<0.0001,**=<0.001, @P<0.02, @@P<0.01 when compared with the control group *P<0.05, when compared with the control group (ANOVA followed by Dunnett's multiple t test).

Test drug showed nonsignificant inhibition of carrageenan-induced paw edema at 1 h (35.22%) and 5 h (13.45%) compared to control group. Vehicle control treated group did not produce any significant change. Standard drug showed significant decrease at 3 h in comparison to control group [Table 3].

Table 3: Effect of EEPV on carrageenan-induced paw edema in Charles foster rats

Groups	Percentage increase in paw volume at different time interval after carrageenan injection		
	After 1 hr	After 3 hrs	After 5 hrs
Control	28.43±8.54	64.03±1.51	51.86±9.54
Vehicle control	29.19±6.12	61.44±9.81	47.40±7.43
EEPV	15.73±1.54	61.52±6.78	43.62±6.65
Phenylbutazone	15.60±2.09	30.01±1.64*	38.87±4.52

Data: Mean±SEM***=<0.0001, **=<0.001M, Increase *P<0.05, when compared with the control group (ANOVA followed by Dunnett's multiple t tests).

Administration of EEPV could not decrease the paw edema at 24 h while at 48 h (16.36%) showed nonsignificant decrease in comparison to control group. Standard treated group produced significant inhibition of formalin-induced paw edema in rats in comparison to control group [Table 4].

Table 4: Effect of EEPV on formaldehyde-induced paw edema

Groups	Percentage increase in paw volume at different time interval after formaldehyde injection		
	Dose	24 hrs	48 hrs
Control	Q.S	24.82±1.43	19.79±1.35
Vehicle control	450	29.44±6.43	21.12±6.77
EEPV	450	25.62±2.54	16.46±3.48
Diclofenac	5	16.47±2.43*	10.77±1.50**

Data: Mean±SEM ***=<0.0001,**=<0.001, *P<0.02, **P<0.01 when compared with the control group (ANOVA followed by Dunnett's multiple t tests).

The models investigating anti-nociception were selected based on their capacity to investigate both centrally and peripherally mediated effects. The tail-flick method investigates the central activity, while formalin-based study investigates both central as well as peripheral effect. Tail-flick responses are believed to be spinally mediated reflex. The effectiveness of analgesic agents in the tail-flick pain model is highly correlated with relief of human pain [7].

A tail-flick model is thermal-induced nociception, indicates narcotic involvement, which is sensitive to opioid μ receptors [8]. The drugs which prolong the reaction latency to thermally-induced pain in albino rats have central analgesic activity. In formalin-induced paw licking, animals present two distant nociceptive behavior phases, which probably involve different stimuli. The first phase initiates immediately after formalin injection and lasts for 3–10 min, representing neurogenic pain. The second phase initiates within 15–20 min after formalin injection, lasts 20–30 min and represents inflammatory pain. In the present study, drug-treated group showed significant increase in tail-flick response upto 180 min while produced nonsignificant increase in tail-flick response after 180 min in comparison to initial values and control group. It suggests that the drug has central analgesic activity in rats. Whereas, in formalin-induced paw licking, nonsignificant decrease was found at 11–20 min indicating that the drug has mild effect on inflammatory pain.

For scientific validation of arthritis claim, the anti-inflammatory study was also planned along with analgesic activity. Subcutaneous injection of carrageenan into the rat paw produces acute inflammation characterized by increased tissue water and plasma protein exudation with neutrophil extravasation and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways. Besides, carrageenan-induced acute inflammation involves the synthesis and release of mediators at the injured site. These mediators include prostaglandin, histamine, bradykinin, leukotriene, and serotonin which cause pain and fever. Inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effect will normally ameliorate the inflammation and other symptoms. In the present study, the standard drug phenylbutazone has suppressed the biphasic response of carrageenan-induced inflammation in rats. Administration of EEPV showed nonsignificant inhibition of carrageenan-induced paw edema at 1 h and at 5 h compared to control group. Vehicle control-treated group did not produce any significant change.

Inflammation induced by formaldehyde is biphasic, an early neurogenic component is mediated by substance P and bradykinin followed by a tissue-mediated response, where histamine, 5-HT, prostaglandin and bradykinin are known to be involved. The initial phase of the edema is due to the release of histamine and serotonin and the edema is maintained during the plateau phase by kinin-like substance and the second accelerating phase of swelling due to release of prostaglandin-like substances. Hence, it is speculated that apart from inhibition of chemical mediators, herbal drugs may also modulate pain response in the central nervous system (CNS). In the present study, the test drug

could produce only mild effect against formalin-induced edema.

The probable mode of action of P. vettiveroides leaves may be due to the presence of flavonoids (chrysin and quercetin), glycosides, tannin and phenolic compounds, and calcium which inhibit the causative factors of pain and inflammation such as histamine, 5-HT, seratonin, bradykinin, and prostaglandin. Flavonoids and phenolic compounds are reported to have good analgesic and anti-inflammatory activities. Free radicals also play an important role in the pathogenesis of inflammation and the compounds found in test drug have radical scavenging activity/anti-oxidant activity [9]. A number of flavonoids such as hesperidin, luteolin, and quercetin are reported to possess anti-inflammatory and analgesic effects [10]. Chrysin and quercetin are reported to have significant analgesic and anti-inflammatory activities [11]. The anti-inflammatory activities of flavonoids in vitro or in cellular models involve the inhibition of the synthesis and activities of different pro-inflammatory mediators such as eicosanoids, cytokines, adhesion molecules and C-reactive protein [12]. Besides, nonspecific CNS depression can also be attributed to tannin [13]. Further, the test drug is found to be a good source of calcium. Various experimental studies were carried out to validate role of calcium in anti-inflammatory activity [14]. Aspirin at sub-inflammatory dose when co-administered with these calcium salts showed significant anti-inflammatory response which was comparable with anti-inflammatory response of aspirin at therapeutic dose [15].

CONCLUSION

The result of the present study shows that P. vettiveroides leaves mixed with honey and water has central analgesic activity against radiant heat-induced pain, moderate anti-inflammatory activity against carrageenan-induced acute inflammation, and very mild or negligible activity against formalin-induced sub-acute inflammation and pain. Thus, it can be concluded that the drug may be more effective in acute pain.

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