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EVALUATION OF ANTI-NOCICEPTIVE EFFECT OF ETHANOLIC EXTRACT OF *VANILLA PLANIFOLIA* SEEDS IN ANIMAL MODELS OF PAIN

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Keywords:

Vanillin, Paw licking, Hot plate, Acetic acid, Writhing

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ABSTRACT: Background: Pain is warning signal of the underlying pathology and is also essential for the diagnosis. *Vanilla planifolia* (VP) as per literature has analgesic activity. Analgesics like NSAIDs and Opioids are used for treating pain. Adverse effects limits their long-term use. Therefore, there is always a need of more effective and safe drugs. **Objective:** To evaluate the analgesic activity of ethanolic extract of *Vanilla planifolia* seeds. **Material and Methods:** Wistar rats weighing 150-200gms and mice 30-40 gms of either sex were included in the study. Hot plate model and acetic acid-induced writhing test was used. Animals were divided into four groups -Vehicle control, Standard control, VP Low Dose and VP High Dose. Drug treatment was given 1hr prior to the test & response to the thermal stimuli was noted on eddy's hot plate. Drug treatment was given 1 h before and number of writhes was measured, at different time intervals 30 min up to 240 min after the injection of 1% acetic acid in writhing test. Data analyzed with Graph pad prism 6. **Results:** Reaction time in control rats was reduced, showing hyperalgesia. In the standard control group reaction time was significantly high ($p<0.001$) at 30,60 & 90 min. VPLD & VPHD showed significant increase in reaction time at 60 ($p<0.001$) and 90($p<0.001$). Percentage inhibition of writhes after drug administration was seen in both the VP-treated groups. **Conclusion:** Ethanolic extract of VP seeds showed an analgesic effect in both animal models. The analgesic effect was comparable to pentazocine in hot plate method and to Diclofenac in writhing test.

INTRODUCTION: Pain is a universal concept and according to the International Association for the Study of Pain (IASP), pain is defined as "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [1]. Pain is the foremost cause of disability and disease burden universally.

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Particularly when chronic, it significantly reduces health, increases suffering, and compromises the quality of life of individuals [2, 3]. Pain is always associated with inflammation, an unpleasant sensation but essential for diagnosis. Pain is categorized into mild, moderate and severe and can range from annoying to debilitating.

Mild to moderate pain is usually treated with Non-steroidal anti-inflammatory drugs. Still, they are associated with many adverse effects like epigastric pain, nausea, vomiting, gastric ulcers, bleeding, melena, etc. In contrast, severe visceral pain treated with opioids shows increased tolerance and dependence liability, which limits their long-term use. Therefore, there is always a need for more

effective and safe drugs [4]. Many natural substances and herbal products are claimed to be effective in reducing pain and inflammation [5]. *Vanilla planifolia* (VP) is an aromatic plant; as per the literature, it has analgesic activity. Vanillin has been shown to have diverse bioactivities benefits for human. *Vanila planifolia* belongs to the family Orchidaceae and is available for commercial and medicinal purposes [6]. The active substance in *Vanilla planifolia* is vanillin, a methyl-protocatechuic aldehyde (4-hydroxy-3-metoxy benzaldehyde) maximum part is present in *Vanilla beans*. This herbal agent is claimed to be effective as an anti-inflammatory and have an analgesic activity [7]. At the same time, no toxic effects have been observed in rats. The present study aimed to evaluate the analgesic activity of the ethanolic extract of *Vanilla planifolia* seeds.

Objectives: To evaluate the analgesic activity of ethanolic extract of *Vanilla planifolia* seeds. To compare it with standard drug pentazocine &diclofenac sodium.

MATERIALS AND METHODS: The study was started after obtaining approval from the Institutional Animal Ethics Committee of Bharati Vidyapeeth Medical College (Approval Letter No. IAEC / BVDUMC / 1607 /2020/002/ 008) Pune.

Chemicals: Organic vanilla seeds powder was obtained from authentic Ayurvedic oushadhalaya., Pentazocine, Diclofenac, Acetic acid, and Normal saline- obtained from the Chemist.

Standard Drug: Market preparation of Pentazocine, Diclofenac ampoule was used as a positive control.

Preparation of Extract [8]: The Soxhlet apparatus was filled with 300ml of ethanol into the round bottom flask. The thimble containing vanilla sample (100mg) was kept in an extraction tube & attached to the flask containing solvent. A condenser unit was attached to the extraction tube, water passed through it, and the Soxhlet apparatus was fixed on the heating mantle. The heating mantle was switched on & the temperature was set as per requirement & the flask containing solvent was heated. The solvent start evaporating & falls in the extraction tube after condensing. Remove extraction from the heating mantle and cool the solution transferring it into the porcelain dish. The rectangular water bath is filled with water & heated at the temperature as per requirement [generally set 100°C]. The extract was placed in the porcelain dish on the rectangular water bath. After some time, the water temperature gradually evaporates ethanol, and the agellylike substance remains in the porcelain dish. After complete drying, it was used for the study.

Animals Used: Albino Wistar Rats of either sex weighing - 150-200 gms were used for the study for hot plate method and mice of either sex weighing - 50-60 g were used for writhing test. Animals were obtained from B.V.D.U. Medical College, Central animal house, recognized by CPCSEA(Regd.No.258/PO/ReBi/S/2000/CPCSE), Pune 43. Housed at 25°C+ 2°C in clean polypropylene cages in batches of three animals per cage. 12 h day and night cycle maintained. Rodent food from Pranav agro-industries and aqua guard water given *ad libitum*.

Methods:
For Anti-nociceptive Activity:

1. Centrally acting - Hot plate method

2. Peripheral acting-Writhing test

Centrally Acting - Hot Plate Method [9, 10]: 24 Adult wistar albino rats of either sex weighing (150-200 g) were used in the study.

Animals were divided into four groups (*n*=6).

Baseline reading of paw licking was taken using Hot plate model.

Group	Treatment
I	Vehicle control (Saline)
II	Standard control- Pantazocine (1.1mg/kg body weight)
III	Vanilla seed extract X(100mg/kg body weight)
IV	Vanilla seed extract 2 X(200mg/kg body weight)

Vehicle/drug treatment was given orally, according to the groups to all the animals 1 h prior to the test. The temperature was stabilized at around 55 degrees C. Each rat was placed on hot plate **Fig. 1** & reaction time in the form of form of paw licking or jumping at 30, 60 & 90 min was noted. Normally animals show such a response in 6-8 sec. A cut off period of 10 sec was observed to avoid damage to

the paws. Pain threshold data of vehicle control and test was analysed.

FIG. 1: EDDY HOT PLATE ANALGESIOMETER (PAW LICKING RESPONSE)

Peripheral Acting- Writhing test [11, 12]: 24 albino mice of either sex weighing between (50-60 g) were used in the study. Animals were divided into four groups of 6 animals each as follows,

Group	Treatment
I	Vehicle control- Normal saline (1ml/100g)
II	Standard Control- Diclofenac sodium (10 mg/kg body weight)
III	Vanilla seed extract X (100mg/kg body weight)
IV	Vanilla seed extract 2 X (200mg/kg body weight)

The animals were treated orally with diclofenac sodium (10mg/kg), normal saline (1ml/100g), and *Vanilla planifolia* (100 and 200mg/kg). Writhing was induced by intraperitoneal injection of 1% (1ml/100g) acidic acid solution 1 h after the drug treatment.

The writhes **Fig. 3** abdominal constrictions and hind limbs stretching were counted for each 30 min. up to the 240 min. for each test group after the injection of acetic acid solution.

The anti-nociceptive activity was calculated as the percent maximum possible effect (% MPE)

% MPE = Mean writhes control – Mean writhes test / Mean writhes Control × 100

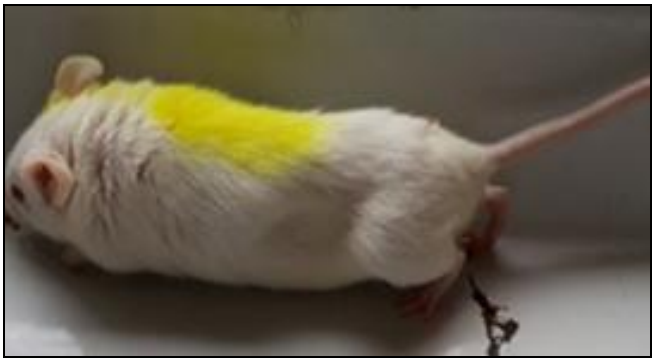

FIG. 2: WRITHES IN MOUSE FROM CONTROL GROUP

Statistical Analysis: Statistical analysis was carried out with GraphPad Prism 6.0 software. Analysis of Variance (ANOVA) followed by Tukey's test was used for multiple comparisons. P value <0.05 was considered as statistically significant.

RESULTS:

Centrally Acting - Hot Plate Method: Reaction time in the normal control rats was reduced, showing hyperalgesia. In standard control, pentazocine-treated rats' reaction time was significantly high at 30, 60 & 90 min. Improvement was seen in both drug-treated groups. Low-dose *Vanilla planifolia* did not show an increase in reaction time at 30 min, but at 60 ($p<0.001$) and 90min, a significant increase was observed. A high dose of Vanilla Planifolia showed an increase in reaction time at all intervals. The activity of *Vanilla planifolia* was comparable to that of pentazocine.

TABLE 1: EFFECT OF *VANILLA PLANIFOLIA* ON THERMAL STIMULUS INDUCED PAIN

Group Treatment	Dose (mg/kg)	Reaction time in seconds at different Time		
		30 Minute	60 Minute	90 Minute
Normal control (VC)	Saline-1ml	4.00±0.632	4.16±0.408	4.66±1.032
Standard Control Pentazocine	1.1mg/kg	6.50±0.547***	8.66±0.516***	9.66±0.516***
Vanilla planifolia Low Dose (VPLD)	100mg/kg	5.33±0.816	6.66±0.816***	7.33±0.516***
Vanilla planifolia High Dose (VPHD)	200mg/kg	6.33±0.816***	7.33±0.816***	9.16±0.752***

Values expressed as Mean ± SD, Data analyzed by one way ANOVA followed Tukey's test**,** *p<0.05, **p<0.01, ***p<0.001 comparison with control.

Peripheral Acting- Writhing test: Percentage inhibition of writhes after drug administration was measured. The highest inhibition was seen with the Diclofenac sodium. VPLD & VPHD showed marked inhibition compared to control, but inhibition efficacy was less than Diclofenac sodium. Duration of inhibition remained up to 4 h, and a gradual increase in the activity was seen.

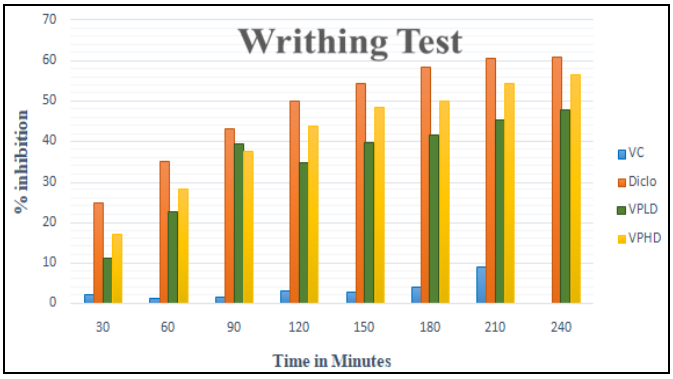

FIG. 4: EFFECT OF *VANILLA PLANIFOLIA* ON WRITHING IN MICE. Values expressed as Mean ± SD, Data analyzed by one way ANOVA followed Tukey's test, *p<0.05, **p<0.01, ***p<0.001 comparisons with control

DISCUSSION: International association for the study of pain has divided pain into three broad categories – acute pain, chronic pain, and cancer pain. Uncontrolled pain gives a constant feeling of discomfort to patients affecting their psychological and functional well-being. The mechanism of pain has two components Central and peripheral. Severe visceral pain has spinal and supraspinal components, whereas the peripheral mechanism mainly involves prostaglandins. Prostaglandin synthesis inhibitors like Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat the mild to moderate pain induced by the PGs, bradykinins, and other pain mediators.

Centrally acting agents, opioids are used to treat severe pain like visceral pain, fracture, and burn pain. Additionally, there is a list of drugs that are given as adjuvants or prescribed in special pain conditions, like Antidepressants, Anticonvulsants, Local anesthetics, Steroids, Alpha -2 agonist etc. In the present study pentazocine, κ receptor agonist, a commonly used opioid analgesic for postoperative and chronic pain and with less abuse liability, was used as standard drug for assessing the central analgesic activity of *Vanilla planifolia* seeds extract and for the peripheral activity a non-steroidalanti-inflammatory drug, Diclofenac sodium was used.

The hot plate is the well-known method used for testing the central analgesic activity of the test compound **Fig. 1**. In the present study, Low dose *Vanilla planifolia* showed an increase in reaction time at 60 and 90 min **Table 1**. The onset of action of VPLD was after 60 min but in high dose, analgesic activity was seen from half an hour of drug treatment. Rathnakar UP *et al.* evaluated the analgesic activity of VP at a dose of 10 mg/kg and 100 mg/kg demonstrating a significant increase in reaction time and Naloxone pretreatment was used to check the central analgesic activity probably mediated through opioid receptors [13]. The writhing test is used to test peripheral analgesic activity. Acetic acid is an inducer for writhing movements, characterized by the contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body due to algesia (pain) caused by the liberation of endogenous substances like prostaglandins and other mediators, which stimulates pain nerve endings.

Fig. 2 Acetic acid activates visceral and somatic nociceptors in the peritoneum and causes inflammation [14]. Acetic acid-induced writhing is a highly sensitive and useful test for analgesic drug development. Treatment with VP decreased the number of writhes at 30 min after the treatment. VPLD & VPHD showed marked inhibition of number of writhes in comparison with control but the efficacy of inhibition was less than Diclofenac sodium. Duration of inhibition remained up to 4 hrs and gradual increase in the activity was seen. Park SH *et al.* study results suggest that vanillin exerts a selective antinociceptive property in the acetic acid induced visceral inflammatory pain model and concluded that antinociceptive effect of vanillin may be mediated by α2-adrenergic and opioid receptors [15].

Beaudry F *et. al* evaluated the effect of vanillin using thermal sensitivity and mechanical allodynia using the sciatic nerve constriction model Neuropathic pain [16] vanillin was observed effective in reducing the effect on mechanical allodynia and not in neuropathic pain, Yrbas M reported antinociceptive activity of vanillic acid may be due to the involvement of serotonergic and adrenergic system [17]. So, multiple mechanisms are involved in pain relief, and further studies are required to evaluate the exact mechanism of anti-nociceptive action.

CONCLUSION: *Vanilla planifolia* ethanolic extract showed anti-nociceptive effects in two different animal models of pain. In both models, VP showed a strong analgesic effect, so it can be concluded that VP has a central and peripheral analgesic effect. The analgesic effect was

comparable to pentazocine in the hot plate method and to Diclofenac in the writhing test.

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CONFLICTS OF INTEREST: Nil

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