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# To Evaluate the Analgesic Efficacy of an Ethanolic extract of *Piper betle* Linn. (paan) and its Probable Mechanism of Action Using Animal Models.

**Datta A*, Bhalerao SV, Shidore PP, Tilak AV, Patil S, and Deshpande T.**

Department of Pharmacology Padmashree Dr. D. Y. Patil Medical College, Hospital & Research Centre, Sant Tukaram Nagar, Pimpri, Pune – 411018 Maharashtra, India.

## ABSTRACT

Use of *Piper betle* Linn. leaf was known for centuries for its curative properties, including analgesic, anticancer, antidiabetic, hepatoprotective effect. The analgesic efficacy of an ethanolic extract of *Piper betle* Linn. (Paan) was evaluated by Eddy's hot plate method and Haffner's tail clip method in Sprague-Dawley rats. In Eddy's hot plate method *Piper betle* Linn. extract in the doses of 100 & 200 mg/kg, significantly prolonged the reaction time (latency) in rats at 60 and 90 minutes as compared to control ($p<0.01$). In Haffner's tail clip method, *Piper betle Linn.* extract in the doses of 100 & 200 mg/kg, significantly prolonged the reaction time (latency) in rats at 60 and 90 minutes as compared to control ($p<0.01$). *Piper betle* Linn. extract in Eddy's hot plate & Haffner's tail clip method significantly prolongs the reaction time on heat stimulus which suggests that it elicits analgesic activity through its action on central pain receptors.

**Keywords:** Ethanolic extract of *Piper betle* Linn. (Paan), analgesic, Eddy's hot plate method, Haffner's tail clip method and Pentazocine

***Corresponding author***

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## INTRODUCTION

Pain is among the most personally compelling reasons for seeking medical attention. Psychological health and performance of social responsibilities in work and family life can be significantly impaired [1].

Pain was also one of the primary reasons for patients visiting the emergency department in more than 50% of cases [2] and about 30% of the patients, who are treated at a primary care practice, have some kind of medically defined pain problem, requiring the attention of a General Practitioner [3]. This complex clinical phenomenon which in most cases is a symptom when it occurs acutely, but a disease when it becomes chronic [4], can be diagnosed and categorised, based on the duration, intensity, type, source, or location in body. Various groups of drugs are used to alleviate pain, which can include Aspirin, Acetaminophen, many other Nonsteroidal anti-inflammatory drugs (NSAIDs) and Opioid Analgesics [5]. Some of these drugs are available over the counter and very commonly used in clinical practice. The overall, burden of unwanted side effects with these treatment regimens remains high. For example chronic use of NSAIDs is associated with gastric erosion and ulcers leading to upper GI bleed, while Opioid analgesics have dependence liability [6].

Herbal medicines are an integral part of Ayurveda and Ayurvedic medicine is a form of complementary and alternative medicine (CAM) which is practiced in India since thousands of years. World Health Organisation (WHO) too has recognised herbal medicine as an essential building block for primary health care of vast countries like India. Moreover natural plant products which are emerging as important alternative therapeutic options are cheap, abundantly available, and relatively less toxic as compared to the conventional allopathic medicines.

*Piper betle* Linn. (Piperaceae), a perennial climber, has its ethnomedicinal properties traced back to Sanskrit literature as early as 3000 B.C. It is mentioned as Vedanasthapana or analgesic herbal agent in ayurvedic texts. This twinning plant is cultivated extensively in the warm and moist parts of South India and Ceylon for its leaves. Regionally, also known as *Paan* in Hindi, Punjabi, Gujrati, Bengali *; Nagavelli* in Telugu ; *Vettilai* in Tamil . In Sanskrit, it is known as *Tambula, Nagavalli* [7].

Use of *Piper betle* Linn. leaf was known for centuries for its curative properties, including analgesic, anticancer, antidiabetic and hepatoprotective effects [8,9]. Its medicinal properties as a carminative, aromatic, digestive and stimulant are described in the Sushruta Samhita, a medico-scientific treatise on the indigenous Ayurvedic system of medicine [10]. The cytoprotective role of *Piper betle* Linn. as also its radioprotective, antimicrobial, antifungal and anti-inflammatory activity and anti-oxidant property are well documented [11-13].

Literature search showed no previous reference that has evaluated its analgesic potential. Thus, this study was undertaken to evaluate the analgesic efficacy of an ethanolic extract of *Piper betle* Linn and its probable mechanism of action using already established

animal models so as to find a safer and more effective alternative to the current medical treatment of pain.

## MATERIAL AND METHODS

### Animals and Their Maintenance

### Animals Used

The animals used in the present study were procured from the animal house of Pd. Dr. D. Y. Patil Medical College, Pimpri, Pune. They were experimentally naive. Species used is *Sprague Dawley* rats of both sex were used, weighing 150 to 200 g.

### Animal Feed

Animals were fed with commercially available Amrut rat pellet feed manufactured by Pranav Agro Food, Pune. The nutrition provided by the pellet feed was as follows:

Energy-3620 kcal/kg, crude protein-22.15%, crude fiber-62.48%, ash-5.11%, sand silica-1.15%. Drinking tap water supplied by Pimpri Chinchwad Muncipal Corporation was provided to the rats through the feeding bottles with stainless steel nozzle in each cage. Pellets were kept in the space provided for feed in the roofs of the cage. Food and water were replenished once daily.

### Animal Housing

Rats were housed in groups of three, in standard big polypropylene cages measuring 40×27.5×13.5 cm, having wire mesh top with provision for drinking water and space for pellets. Husk was used as bedding material in each cage. The water was maintained under standard condition of temperature ( 250C ± 50C). The relative humidity was maintained at ( 55 ± 10%) and 12/12 hour light / dark cycle was maintained. Apart from daily replenishment of food and water, the rats were left undisturbed.

The study was approved by the Institutional Animal Ethics Committee.

### Drugs and Chemicals Used

### Drugs Used

**Study drug:** An ethanolic extract of *Piper betle Linn.* (Paan) leaf of doses : 50, 100 & 200 mg/kg body weight. Authenticated leaves were obtained from Botanical Survey of India , Kolkata,West Bengal, India. The ethanolic extract was prepared in R.G.Kar Medical College & Hospital, Kolkata, India.

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**Standard Drug:** Pentazocine is given intraperitoneally in dose *of* 10mg/kg body weight. Source is Aventis Pharmaceuticals Ltd., India.

**PLAN OF THE STUDY**

The animals were divided into groups as per table 1.

| | | |
|---|---|---|
| Control | Group I | *0.5% Carboxymethylcellulose (CMC) solution* |
| Test | Group II | *Piper betle Linn. (Paan)* leaf extract, *50* mg/kg body weight |
| | Group III | *Piper betle Linn. (Paan)* leaf extract, *100* mg/kg body weight. |
| | Group IV | *Piper betle Linn (Paan)* leaf extract, *200* mg/kg body weight. |
| Standard | Group V | *Pentazocine* 10mg/kg body weight |

**Table 1: The animals were divided into the following groups**

**Routes of Administration**

*Piper betle* Linn. extract was given orally (p.o.) and was dissolved in 0.5% CMC (prepared by dissolving 100mg of carboxymethylcellulose powder in 20ml of distilled water) and freshly prepared prior to administration. Control group received an equivalent volume of 0.5% CMC, orally. Pentazocine was given in the dose of 10mg/kg body weight, intraperitoneal (i.p.). All animals were kept fasting overnight before conducting the test procedures.

**Evaluation of Analgesic Activity**

**Eddy's hot plate method**

Six screened SD rats were assigned to each of the 5 groups. After 30 minutes of test drug administration, each rat was individually placed on the hot plate with temperature adjusted to 52.50C±50C. The reaction time to heat stimulus in the form of paw licking or jumping was taken as an index of pain threshold; the cut off time was 30 sec in order to avoid damage to the paws. The procedure was repeated at 60, 90 & 120 minutes [14].

The values of prolongation of latency time of experimental groups were compared with control group for statistical analysis.

**Haffner's tail clip method**

Six screened SD rats were assigned to each of the 5 groups. The drug was administered 15, 30 or 60 min prior testing. An artery clip was applied to the root of the tail (approximately 1 cm from the body) to induce pain. The animal quickly responds to this noxious stimuli by biting the clip or the tail near the location of the clip. The time between stimulation onset and

response was measured by a stopwatch in 1/10 seconds increments. The length of time until response indicates the period of greatest activity after dosing [15].

The values of prolongation of latency time of experimental groups were compared with control group for statistical analysis.

## STATISTICAL ANALYSIS

The data was compiled and analyzed by using the statistical package, Graph Pad InStat. Results are expressed as Mean ± SD and statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by Holm's test. Value of $p<0.05$ was considered as statistically significant. The % Inhibition was calculated using the formula:

$$\frac{V1-V2}{V1} \times 100$$

[V1 = reading in the control group, V2 = reading in the standard or test group].

## RESULTS

### Eddy's hot plate method:

| Eddy's hot plate method (Mean±SD) | | | | |
|---|---|---|---|---|
| | Latency time (sec) | | | |
| **Group** | **0min** | **30min** | **60min** | **90min** |
| **Control** | 10.166±3.43 | 10.166±3.61 | 11.16±2.994 | 11.66±3.265 |
| **Pentazocine-(10mg/kg)** | 11±3.74 | **19.6±5.16*** | **20.16±5.56*** | **19.83±3.54*** |
| **PB-50mg/kg** | 9.166±3.65 | 10±3.22 | 11±3.34 | 10.16±3.37 |
| **PB-100mg/kg** | 13.5±3.78 | 14.5±3.674 | **17.5±3.5*** | **17.166±2.04*** |
| **PB-200mg/kg** | 12.5±3.78 | 14.33±4.5 | **16.6±5.71*** | 16.33±3.72 |
| *p<0.01, when compared with control group. | | | | |

**Table 2: Eddy's hot plate method latency intervals**

*Piper betle* Linn. extract in the doses of 100 & 200 mg/kg, significantly prolonged the reaction time (latency) in rats at 60 and 90 minutes as compared to control ($p<0.01$). While Pentazocine showed significant findings from 30 minutes onwards ($p<0.01$) when compared to control. (Table 2 & Graph 1)

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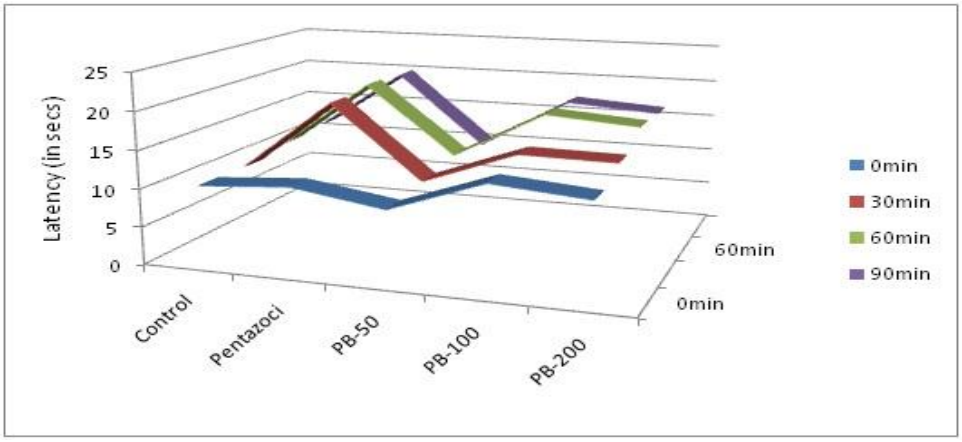
**Graph 1: (3D-Line) Eddy's hot plate method latency intervals**



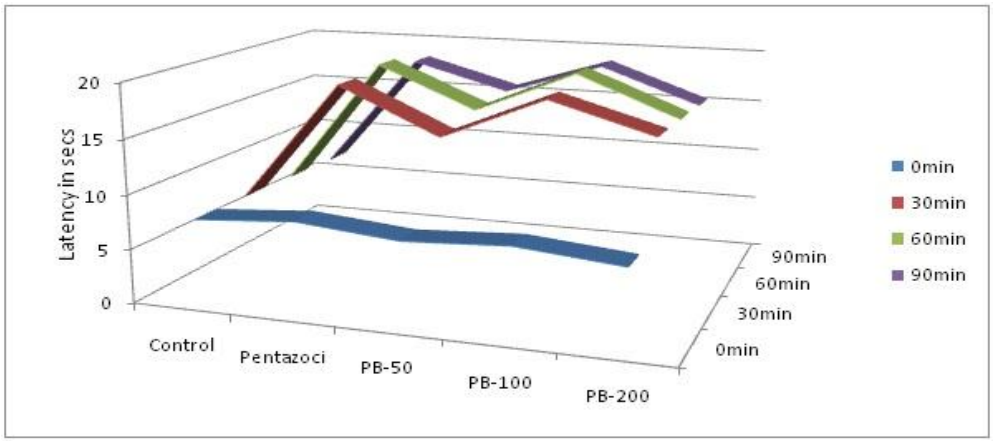

## Haffner's tail clip method

*Piper betle* Linn. extract in the doses of 100 & 200 mg/kg, significantly prolonged the reaction time (latency) in rats at 60 and 90 minutes as compared to control ($p<0.01$). While Pentazocine showed significant findings from 30 minutes onwards ($p<0.01$) when compared to control. (Table 3 & Graph 2)

**Table 3**: **Haffner's tail clip method latency intervals**

| Haffner's tail clip method (Mean±SD) | | | | |
|---|---|---|---|---|
| | Latency time (sec) | | | |
| **Group** | **0min** | **30min** | **60min** | **90min** |
| **Control** | 7.5±2.063 | 7.5±1.04 | 7.33±0.816 | 7.16±0.75 |
| **Pentazocine-(10mg/kg)** | 8.1666±1.60 | **18.66±4.718*** | **19.16±2.40*** | **18.166±2.92*** |
| **PB-50mg/kg** | 7.33±2.65 | 14.5±2.73 | 15.33±2.94 | 15.66±2.065 |
| **PB-100mg/kg** | 7.833±3.48 | 18.5±3.44 | **19.33±3.07*** | **18.66±1.966*** |
| **PB-200mg/kg** | 7±1.788 | 15.833±2.85 | **15.66±2.25*** | 15.33±1.366 |
| *p<0.01, when compared with control group. | | | | |

**Graph 2: (3D-Line) Haffner's tail clip latency intervals**



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## DISCUSSION

*Piper betle* Linn (Paan) is a semi-woody, climber and a common herb of tropical lands. Ayurveda praises its anodyne properties. *Piper betle* Linn. (Piperaceae) is widely recognized as a traditional medicinal plant not only in India but in many countries across Southeast Asia. Its leaves, and in a few cases leaf extracts, have been prescribed as an anti-stomatitic agent, antitussive, astringent, antiseptic, carminative, stimulant, tonic, expectorant, nerve stimulant, dental paste and inhibitor of nasal bleeding. Its ameliorative properties in elephantiasis and painful eye conditions have been documented. In more recent times, the pharmacological activities of *Piper betle* Linn. with regard to its anti-inflammatory, antimicrobial activity, antifertility properties, anti-ulcer properties, inhibition of platelet aggregation, radiation-induced stress and antidiabetic properties have been demonstrated. Some of the work done earlier did show the useful effects of *Piper betle*, including a sense of wellbeing [7].

The present study was undertaken to evaluate the analgesic efficacy of Paan to establish, it's yet another therapeutic potential. This study involved the use of well-established animal models of pain to confirm its analgesic efficacy. Eddy's hot plate and Haffner's tail clip method for evaluating the central action are believed to be reliable and one of the most widely used. The result from the present study shows that *Piper betle* Linn. has analgesic efficacy comparable to standard. It's probable mechanism of action is central.

Paan treatment significantly prolongs the reaction time on heat stimulus & mechanical stimulus; which suggests that Paan elicits analgesic activity through its action on central pain receptors. There was significant drowsiness observed in the test rats treated with Paan. It can provide a sense of well-being like central effect [7]. From this it can be reported that an ethanolic extract of *Piper betle* Linn. can produce analgesic activity through central mechanism of action.

This work scientifically confirms the traditional, folk and preliminary claims of *Piper betle* Linn. for its analgesic activities. This is also a step ahead in the direction of increasing the number of marketable drugs with traditional background with modern and scientific standard. Like most ayurvedic drugs, Paan has been used since ages by ayurvedic physicians and no serious adverse effect or drug reaction has been reported so far. Additional advantages of being cheap and easily available are an added benefit.

The burden of unwanted side effects with the various groups of drugs that are used to alleviate pain in allopathic medicine (as mentioned in literature survey) could be reduced if *Piper betle* Linn. (Paan) is used supplemental to the present analgesic regime. Paan could be especially advantageous in treating painful conditions with inflammation like arthritis along with traditional allopathic analgesic regimens. Moreover its supplemental use with analgesics would also result in reduction of dose and side effects of the primary analgesic agents leading to better patient compliance and tolerability.

Thus the results obtained in this study indicate that *Piper betle* Linn. (Paan) possesses potent analgesic properties, which are mediated via central inhibitory mechanisms. Moreover if the present finding could be confirmed to clinical situation then there may be possibility to develop a novel analgesic drug from the treasure of our traditional system of medicine. Future studies can be done by extracting the chemical components of *Piper betle* Linn. responsible for its analgesic efficacy. However further molecular pharmacological studies to find out the specific receptors involved and other biochemical investigations are needed to unwind its cellular mechanisms.

## CONCLUSION

From the present study we can conclude that, an ethanolic extract of *Piper betle* Linn. (Paan) leaf, exhibited analgesic effect at test doses of 100mg/kg and 200 mg/kg which may be mediated by central receptors. The onset of its analgesic effect after administration was seen at 30minutes, which peaked at 60 minutes and lasted beyond 90 minutes. It could be recommended as a supplemental add-on to drugs like NSAIDs used to manage chronic painful conditions like arthritis. It may help to decrease the dose and associated dose dependent adverse effects of NSAIDs. In addition it has anti-ulcer properties comparable to Omeprazole so it can reduce the possibility of ulcers on chronic use with any NSAID. Moreover additional advantage of being cheap and easily available is an added benefit with Paan.

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