*Section A-Research paper*

# Experimental evaluation of activity of eugenol in reduction of pain in rats

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**Abstract:**

**Introduction:** According to the International Association for the Study of Pain "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage". Since aspirin can cause gastritis and opioids have abuse liability, there is need for a alternative analgesic drug or a treatment regime which will reduce need for the use of conventional NSAIDs in clinical practice and to reduce the devastating adverse effects on vital organs.

**Methodology:** In the current study, we carried out in vivo experimental analysis to see the analgesic potency of eugenol and its effect on writhing and the reaction time latency to painful stimulus by using Group Writing test and Tail clip method in Sprague Dawley rats.

**Results:** In our study 100mg/kg injection of eugenol in the peritoneum of rats has yielded the results in favor of eugenol pretreated group which showed statistically significant decrease than control group in writhing movements at all 0, 30,60 and 90 minute intervals. Eugenol showed statistically significant increase in mean reaction time compared to control in the Tail clip method.

**Discussion and Conclusion:** The present study supports the use of eugenol as an analgesic in dental and minor operative procedures in dentistry, particularly in dental cementing material so as to have local analgesic action. This can reduce use of analgesic drugs like aspirin and thus help to avoid side effects due to these drugs.

**Keywords: Pain; Analgesics; Eugenol; Analgesics; Pentazocine; Sprague Dawley Rats; Group Writing; Tail Clip Method.**

## INTRODUCTION

Pain is the most challenging symptom in Medical field and in the recent years, pain is considered as the fifth vital sign. Pain is a displeasing, agonizing and debilitating symptom that consists of sensory and emotional experience connected with factual or potential tissue damage or due to loss of an organ or body part. It can be mild, moderate or severe depending upon the intensity and can be of acute or chronic nature, which depends on the duration and etiology of pain. This is the most common symptom which drags a patient to the clinic or hospital and if not treated on time, this could result in loss of function, decreased quality of life and in extreme cases death due to shock. Hence diagnosing pain, identifying the cause of it and treating it remains the primary goal in medical practice.[1]

There are variety of drugs which are possible to get over the counter for the treatment of pain like NSAIDs such as Aspirin and Acetaminophen. Opioid Analgesics can also be prescribed by physicians for pain relief[2]. But considering adverse effect profile of long-term usage of NSAIDs and its harmful effects on vital organs like kidneys and heart, it is the need of the situation to opt for alternate pain-relieving regimes with minimal possible side effects.[2]

Since time immemorial clove oil is commonly used for its pain-relieving characteristics It contains eugenol which is the important component in the aromatic oil extract from cloves (Syzygium aromaticum).[3]

Eugenol is used in dental cementing material which is used for root canal treatment. It is particularly used in endodontic sealers which fill breach between pulp and dentine. Eugenol is supposed to decrease inflammatory pain which is present in this area.

When zinc oxide eugenol (ZOE) is put on to a dental cavity, small amount of eugenol diffuses through the dentine to the pulp. Thus application of ZOE temporary filling may lead to the relief

from pain. It may be concluded that eugenol may have potential for pain relief and having anti-inflammatory characteristics[5].

Eugenol in the current day practice is, thus used in the form of paste or mixture as a dental cement, filler, and restorative material.[6] Therefore we present this study which was undertaken to evaluate analgesic effect of eugenol on rat model through scientific experiments in support for its potential analgesic activity.

## MATERIAL AND METHODS

### ANIMALS AND THEIR MAINTENANCE

This study was conducted after due approval from the Institutional Animal Ethical Committee with necessary clearances to carry out the study

The rats of species Rattus norvegicus and strain Sprague-Dawely of either sex weighing 200-250 g are used in the present study. They were obtained from the animal house of Dr. D. Y. Patil Medical College, Pimpri, Pune. They were not used for experiment before.

Rats were fed with satisfactorily nutritive feed. Pellet and drinking water were given twice daily. In standard big polypropylene cages rats were sheltered in groups of three. Standard condition of temperature ($25^0C \pm 5^0$ C) and relative humidity (55 ± 10%) and 12/12-hour light / dark cycle maintained. Eugenol was obtained from Sigma Aldrich India which was given in a dose of 100mg/kg. Aspirin powder was obtained from Ajinkya Pharmaceuticals, Pune which was administered in a dose of 100mg/kg. Pentazocine obtained from Dr. D. Y. Patil Medical College Pharmacy, Pimpri and was given in a dose of 10mg/kg.

**Grouping for writhing test:**

6 rats were used per group

Group I- Normal saline or Control

Group II- Aspirin 100mg/kg

Group III- Eugenol 100mg/kg

**Grouping for tail clip method:**

6 rats were used in each group

Group I- Normal saline or Control

Group II- Pentazocine 10mg/kg

Group III- Eugenol 100mg/kg

**3) Procedure:**[5]

**Writhing test:**

Rats under the experiment were grouped as normal saline, eugenol and aspirin groups and were given the respective drugs orally. A volume of 4ml of 0.9% NaCl is injected in the peritoneum in all the rats under experiment, 30 minutes before the procedure. The rats responded with a peculiar stretching behavior, i.e. a succession of contractions occur that goes on along the abdominal wall sometimes along with extension of the hind limb. This reaction is called writhing. Number of writhes were recorded at 0, 30, 60 and 90 minutes of interval in each group. The values of eugenol and aspirin group were compared with normal saline. A comparison was also done between values of eugenol and aspirin.

**Haffner's tail clip method:**

In this method, mechanical stimulus is applied. This method is highly sensitive for centrally acting drugs. The rats were treated one hour before the procedure with drugs according to their respective groups. An artery clip is placed at the root of the tail of the mice to apply noxious stimulus. A quick response of animal is seen as biting the clip or tail, where clip has been placed. The time (reaction time) between application of the clip and response is noted by a stopwatch. Pentazocine is administered subcutaneously. After 0, 30, 60, 90 minutes, same procedure is repeated and reaction time is measured. Similar procedure is repeated and reaction time for eugenol is measured and compared with that of pentazocine and control. Reaction time of test animals greater than average reaction time for control group denotes positive response indicating analgesic activity.

**Statistical Analysis**

The data was compiled and analysis of variance (ANOVA) was applied as per statistical considerations for inter group comparison. Post hoc Tukey's test was applied for intra group comparison. p value <0.001 was considered statistically significant.

**Results**

**Table 1. Analgesic effect of Eugenol in comparison with aspirin and control by Writhing method**

| **Mean number of writhes** | **Control group (n=6)** | **Aspirin group (n=6)** | **Eugenol group (n=6)** | **p-value** |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | |
| **0 min** | 57.50 ± 7.15 | 38.00 ± 1.79 | 23.67 ± 1.51 | < 0.001* |

| | | | | |
|---|---|---|---|---|
| **30 min** | 52.17 ± 5.91 | 33.50 ± 4.28 | 15.67 ± 1.51 | < 0.001* |
| **60 min** | 48.67 ± 4.84 | 27.67 ± 3.45 | 11.33 ± 1.03 | < 0.001* |
| **90 min** | 44.00 ± 4.90 | 23.67 ± 3.88 | 7.33 ± 1.03 | < 0.001* |

**Table 2. Interpretation of Statistical Significance (P-Value) of Intra group comparison between Control Group, Aspirin Group and Eugenol group with respect to time in minutes**

**Note:** p value <0.001 is considered as statistically significant

| **Interpretation of Statistical Significance (P-Value) of comparison between Control Group, Aspirin Group and Eugenol group with respect to time in minutes** | | | | |
|---|---|---|---|---|
| **Mean Number of Writhes at (Time in Minutes)** | | Control group | Aspirin group | Eugenol group |
| **0 Minutes** | Control group | - | < 0.001* | < 0.001* |
| | Aspirin group | - | - | < 0.001* |
| | Eugenol group | - | - | - |
| | | | | |
| **30 Minutes** | Control group | - | < 0.001* | < 0.001* |
| | Aspirin group | - | - | < 0.001* |
| | Eugenol group | - | - | - |
| | | | | |
| **60 Minutes** | Control group | - | < 0.001* | < 0.001* |
| | Aspirin group | - | - | < 0.001* |

| | | | | |
|---|---|---|---|---|
| | Eugenol group | - | - | - |
| | | | | |
| **90 Minutes** | Control group | - | < 0.001* | < 0.001* |
| | Aspirin group | - | - | < 0.001* |
| | Eugenol group | - | - | - |

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**Figure 1. It was observed that the group of rats treated with eugenol showed considerable decrease in number of writhes as compared to group of rats to which aspirin was administered. This observation was also taken at the intervals of 0, 30, 60 and 90 minutes. All the observations were confirmed statistically.**

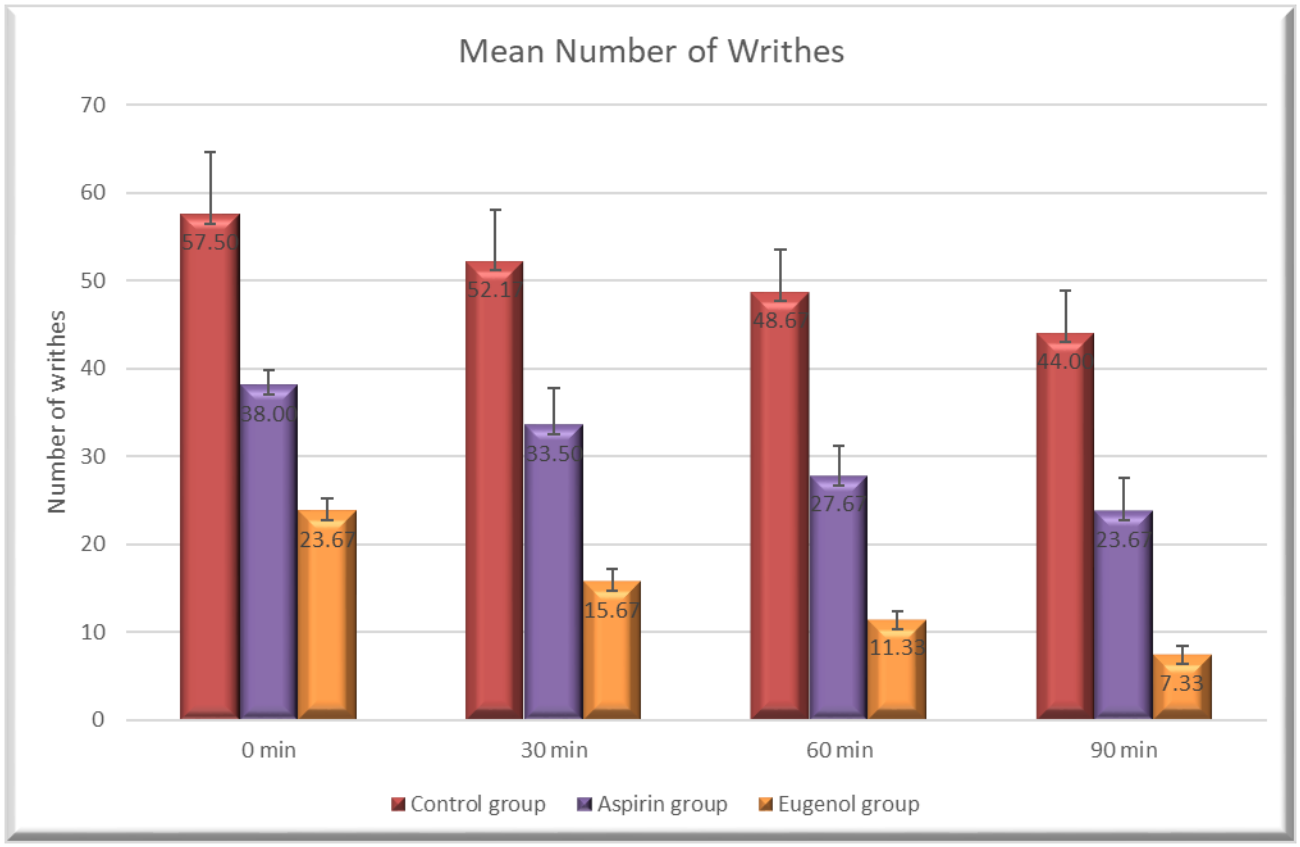

**Figure 2. The observation shows that percentage inhibition of writhing in group of rats with eugenol was greater as compared to group of rats with aspirin at all the intervals 0 min, 30 min, 60 min and 90 min.**

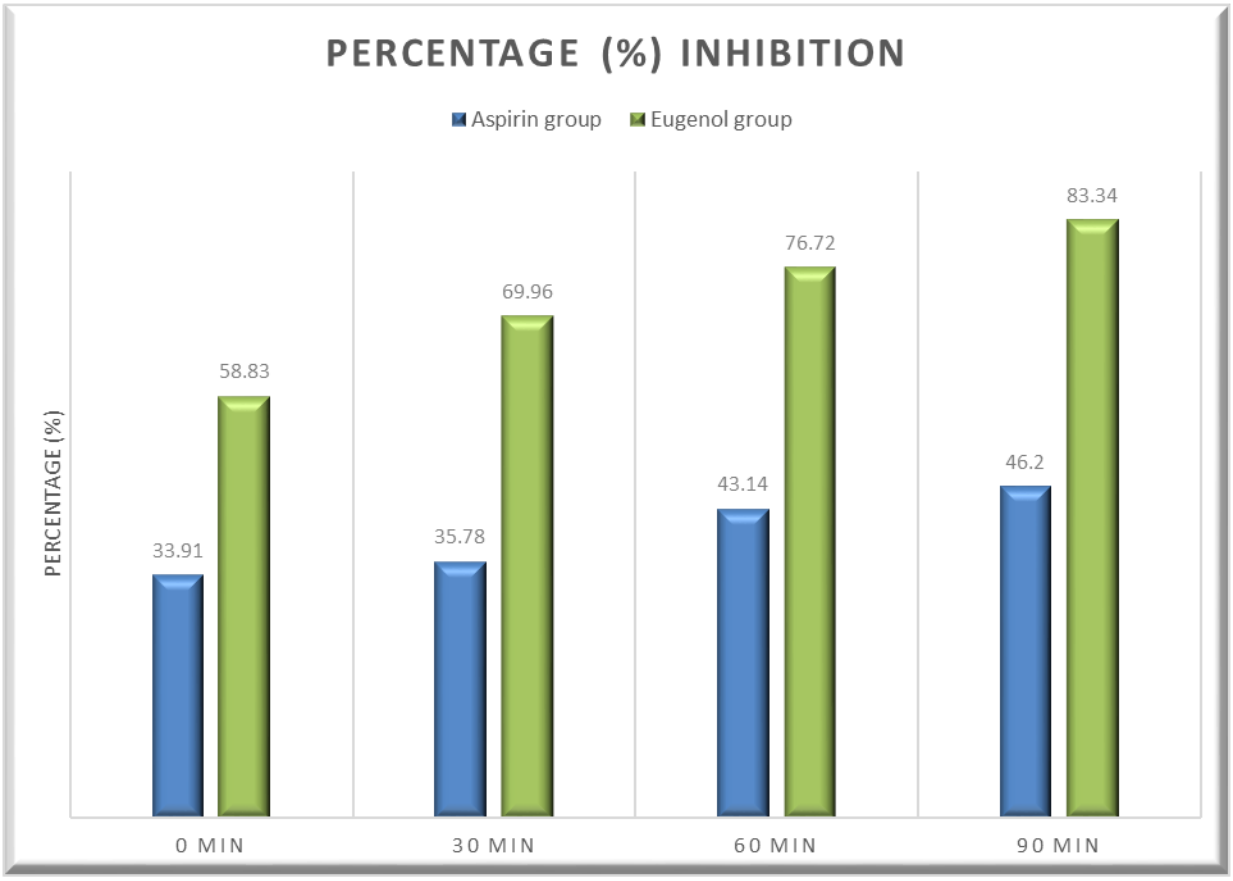


**Table 3. Analgesic effect of Eugenol in comparison with Pentazocine and control by Tail Clip Method**

| **Mean reaction time for** | **Control group (n=6)** | **Pentazocine group (n=6)** | **Eugenol group (n=6)** | **p-value** |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | |

| **Clip Method** | | | | |
|---|---|---|---|---|
| **0 min** | 20.70 ± 1.83 | 21.75 ± 2.32 | 21.37 ± 2.24 | 0.696 |
| **30 min** | 16.65 ± 2.01 | 25.23 ± 2.79 | 23.48 ± 3.19 | < 0.001* |
| **60 min** | 17.47 ± 1.89 | 28.13 ± 2.65 | 26.07 ± 1.49 | < 0.001* |
| **90 min** | 18.58 ± 2.07 | 30.48 ± 2.51 | 28.65 ± 1.73 | < 0.001* |

**Table 4. Interpretation of Statistical Significance (P-Value) of Intra group comparison between Control Group, Pentazocine Group and Eugenol group with respect to time in minutes** **Note:** p value <0.001 is considered as statistically significant

| **Interpretation of Statistical Significance (P-Value) of comparison between Control Group, Pentazocine Group and Eugenol group with respect to time in minutes** | | | | |
|---|---|---|---|---|
| **Mean Number of Writhes at (Time in Minutes)** | | Control group | Pentazocine group | Eugenol group |
| **0 Minutes** | Control group | - | 0.678 | 0.853 |
| | Pentazocine group | - | - | 0.948 |
| | Eugenol group | - | - | - |
| | | | - | - |
| **30 Minutes** | Control group | - | < 0.001* | < 0.001* |
| | Pentazocine group | - | - | 0.517 |
| | Eugenol group | - | - | - |
| | | | - | - |

| | | | | |
|---|---|---|---|---|
| **60 Minutes** | Control group | - | < 0.001* | < 0.001* |
| | Pentazocine group | - | - | 0.226 |
| | Eugenol group | - | - | - |
| | | | - | - |
| **90 Minutes** | Control group | - | < 0.001* | < 0.001* |
| | Pentazocine group | - | - | 0.322 |
| | Eugenol group | - | - | - |

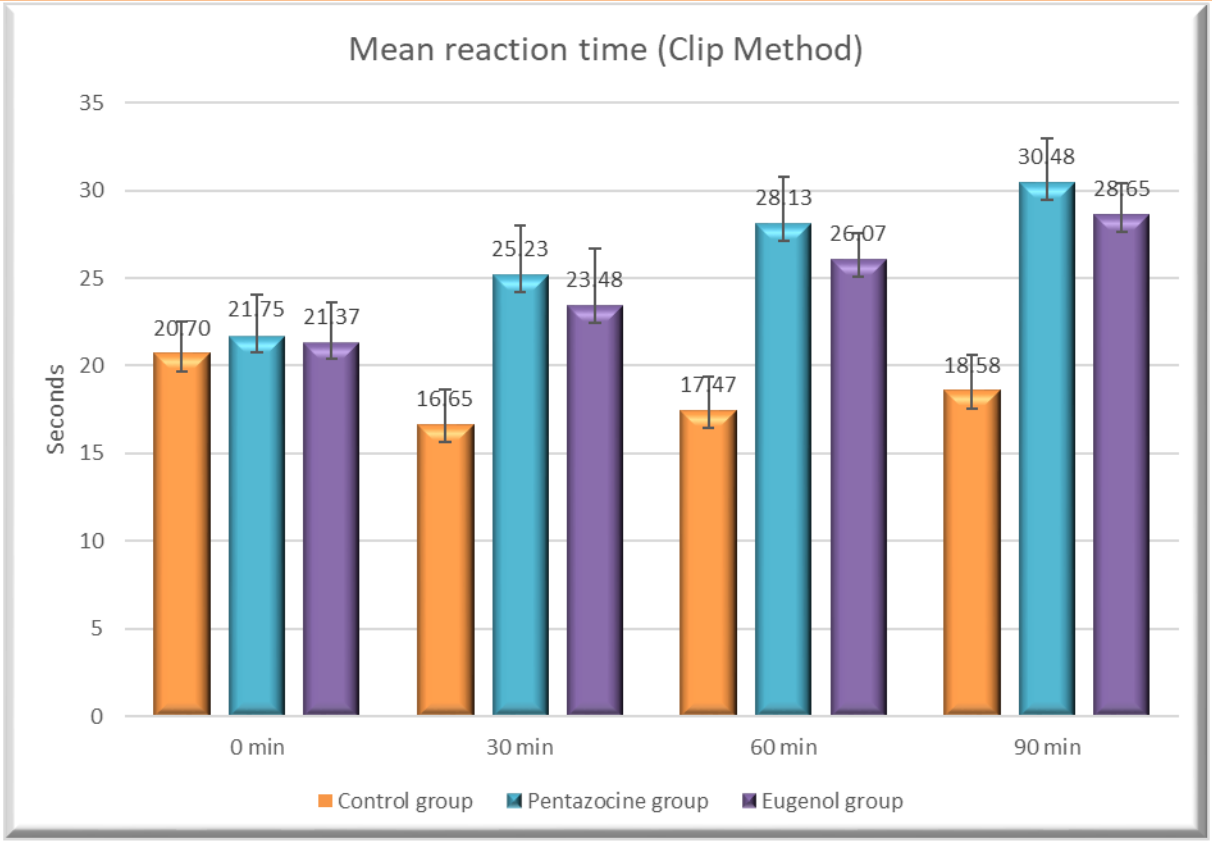


**Figure 3. Depicts Mean Reaction Time on Clip test, It is seen that there was an increase in mean reaction time or latency period in rats treated with pentazocine. This increase was found to be statistically significant p<0.001**

**Interpretation of Writhing test:**

Aspirin as well as eugenol pretreated group have shown statistically significant decrease than control group in writhing movements at all 0, 30,60 and 90 minute intervals. Aspirin pretreated group showed statistically significant decrease in number of writhing movements than Eugenol pretreated group.

Percentage inhibition of writhing in eugenol group was greater as compared to aspirin at all 0, 30, 60 and 90 minute intervals.

**Interpretation of Tail clip method:**

Pentazocine showed statistically significant increase in mean reaction time as compared to eugenol and control groups at 30, 60 and 90 minute intervals. Eugenol showed statistically significant increase in mean reaction time as compared to control group at 30, 60 and 90 minute intervals.

Writhing test signifies peripheral analgesic activity. Eugenol possesses peripheral analgesic activity according to present study. Tail clip method signifies central analgesic activity. According to present study the eugenol possesses negligible central analgesic activity.

**Discussion:**

Many plants are used as pain relieving medications traditionally, however scientific studies are lacking to prove their efficacy and effectiveness.

Eugenol, chemically 4-allyl-2-methoxyphenol is the most important chemical component of clove oil. It is derived from *Eugenia caryophyllus* and *Myristica fragrans* plants. [(9)]

It is found from this experiment that eugenol has an analgesic property when given in the dose of 100 mg/kg. When ortho eugenol was given 100mg/kg injection when given in peritoneum of rats it showed that mean number of writhes are reduced.[8] We have seen considerable reduction in number of writhes after intraperitoneal administration of 100mg/kg eugenol.

In our study 100mg/kg injection of eugenol in the peritoneum of rats resulted in significantly increased reaction time. It also showed increased latency period from thermal stimulus in hot plate test as compared to control. Another study on eugenol establishes peripheral analgesic activity but mild central analgesic activity .[9] First, there was ambiguity among different studies in defining the basic characterization of eugenol's analgesic properties. In this scenario, present study was carried out in rats. We have observed that eugenol possesses significantly higher peripheral analgesic activity and negligible central analgesic activity.

In another study, it is found that eugenol inhibits Ca(v)2.3 calcium channels present on dental primary afferent neurons. Endogenous expression of TRPV1 is absent. Thus inhibition of calcium channels is brought about by a mechanism that involves pathway other than capsaicin. This mechanism gives rise to pain relieving effect of eugenol.[10]

Also, there are voltage gated sodium channels in trigeminal ganglion which modulate dental pain. Eugenol is shown to have inhibitory effect on them. This may contribute to pain relieving action of eugenol.

There is an activation of transient receptor potential vanilloid 1 channels. This may be responsible for pain relieving action of eugenol.In an animal experiment, painful electrical stimulation was given to anterior tooth pulp and at the same time eugenol was given subcutaneously. Eugenol diminished the digastric electromyogram in this case. This proved that

there was blockade of conduction of action potential through inferior alveolar nerve. This proves that eugenol possesses pain relieving action.[9]

It has also been observed that eugenol inhibits COX -2 enzyme in cell line RAW264.7.[(12)] The present study gives opinion in favor of the use of eugenol as a pain relieving medication in dental pain. Thus it can be used for minor operative procedures in dentistry, particularly in dental cementing material so as to have local pain relieving action. This can reduce use of pain relieving drugs like aspirin and thus help to avoid adverse effects due to these drugs.

## CONCLUSION

Pain being the most challenging symptom in medical field, needs to be treated along with its root cause. Analgesics relieve the pain but also carry risk of renal adverse effects. Eugenol, an active component of clove has shown peripheral analgesic activity and negligible central analgesic activity in rats which were evident on models of Writhing test and Tail clip test. The present study gives opinion in favor of the use of eugenol as a pain relieving medication in dental pain. Thus it can be used for minor operative procedures in dentistry, particularly in dental cementing material so as to have local pain relieving action. This can reduce use of pain relieving drugs like aspirin and thus help to avoid adverse effects due to these drugs. However, pharmacokinetic studies for dosing is necessary.

**Conflict of Interest:** The authors don't declare any conflict of interest

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