In Vivo Experimental Evaluation of Analgesic Efficacy of Eugenol in Experimental Rats

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ABSTRACT

Pain, considered as the fifth vital sign, is the most challenging symptom in medical practice, which often drags the patient to the doctor. Clinically, pain is categorized as acute and chronic depending on the duration, it is also categorized as somatic (Nociceptive) or neuropathic pain depending on the site and the organ structure involved. If not treated in time, it may result in debilitating sequelae and loss of function of the affected part. Analgesics are rampantly used in clinical practice and its harmful effects on vital organs like kidneys and heart on long term usage needs to be duly contemplated. It is the need of the situation to opt for alternate pain-relieving regimes with minimal possible side effects.

In the current study, in vivo experimental analysis was performed to see the analgesic potency of eugenol and its effect on writhing and the reaction time latency to painful stimulus by using Group Writhing test and Hot plate method in Sprague Dawley rats.

It was evident from this study that 100mg/kg injection of eugenol in the peritoneum of rats resulted in significantly decreased number of writhes compared to control and significantly increased latency period from thermal stimulus in hot plate test as compared to control. We have observed that eugenol possesses significantly higher peripheral analgesic activity and negligible central analgesic activity.

Thus, the present study favors the use of eugenol as a pain-relieving medication in dental pain. This can reduce use of pain-relieving drugs like aspirin and thus help to avoid adverse effects due to these drugs.

KEYWORDS: Pain; Analgesics; Eugenol; Analgesics; Sprague Dawley Rats; Group Writhing; Hot Plate method.

INTRODUCTION

Pain is appraised as 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 seek for medical attention and if not treated in time, this could result in loss of function, decreased quality of life and in extreme cases death due to shock. Hence diagnosing the pain, identifying its cause 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 the 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.[4]

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 pain relief. 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. Therefore the present study was undertaken to evaluate analgesic effect of eugenol on rat model through scientific experiments in support for its potential analgesic activity.

MATERIAL AND METHODS

This study was conducted after due approval from the Institutional Animal Ethical Committee with necessary clearances (*CPCSEA/DYPMC/IAEC/02/2019; dated 12/09/2019*) to carry out the study.

The rats of species *Rattus norvegicus* and strain Sprague-Dawley 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 (25^0 C ± 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 was given in a dose of 10mg/kg.

a) Grouping for writhing test:

Rats were divided in three groups each containing six rats:

Group I – Normal saline or Control

Group II – Aspirin 100mg/kg

Group III – Eugenol 100mg/kg

b) Grouping for hot plate:

Rats were divided in three groups each containing six rats:

Group I – Normal saline or Control

Group II – Pentazocine 10mg/kg

Group III – Eugenol 100mg/kg

Procedure: [6]

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 was 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 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.

Formula for computing percent inhibition is:

$$= \frac{Average\ writhes\ in\ control\ group - writhes\ in\ test\ group}{Writhes\ in\ control\ group} \times 100$$

Hot plate method:

This method has been used to a great extent to evaluate opioid analgesics which act centrally. The rats were previously treated with respective drugs one hour before the procedure according to their groups. Rats were kept on hot plate which was made up of electrically heated surface one at a time. Temperature of the hot plate was kept constant at 55-56 °C. Rats started jumping. Also withdrawal of paws observed. They started licking paws. The time period (latency period) between placing and responses were recorded by stopwatch. Normal saline, eugenol and pentazocine were administered subcutaneously and the latency period was recorded at 0, 30, 60 90 min subsequently. The values of pentazocine and eugenol groups were compared with values of normal saline group. Comparison was also done between values of eugenol and pentazocine groups.

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

Writhing test:

Table 1 shows analgesic effect of Eugenol in comparison with aspirin and control by Writhing method. Normal saline was administered to rats and number of writhes were counted at the intervals of 0, 30, 60 and 90 minutes. For the other group of rats, Aspirin was administered and writhes were counted at the intervals of 0, 30, 60, 90 minutes. It was found that the group of rats to which aspirin was administered showed considerable decrease in number of writhes.

Another group of rats were given eugenol and number of writhes were observed at the intervals of 0, 30, 60 and 90 minutes. It was noted that the number of writhes were considerably lower in the group which was treated with eugenol when compared to the group to which normal saline was given.

Table 2 interprets statistical significance (P-Value) of Intra group comparison between the Control, Aspirin and Eugenol groups with respect to time in minutes.

Figure 1 highlights the observation that percentage inhibition of writhing in group of rats with eugenol was greater as compared to group of rats with aspirin at the intervals of 0 min, 30 min, 60 min and 90 min.

Hot plate test:

Table 3 shows the analgesic effect of Eugenol in comparison with Pentazocine and normal saline by Hot Plate method. When mean reaction time or latency period was calculated in rats which were treated with pentazocine at 0, 30, 60 and 90 min intervals and compared with the mean

reaction time that was calculated in rats which were treated with normal saline, it was noted 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.

When mean reaction time or latency period was calculated in rats which were treated with eugenol, at 0, 30, 60 and 90 min intervals and compared with that in rats which were treated with normal saline, it was observed that there was an increase in mean reaction time or latency period in rats treated with eugenol. This increase was found to be statistically significant.

When mean reaction time or latency period was calculated in rats which were treated with pentazocine, at 0, 30, 60 and 90 min intervals and compared with that in rats which were treated with eugenol, it was evident 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.

Table 4 conveys the interpretation of Statistical Significance (P-Value) of Intra group comparison between Control Group, Pentazocine Group and Eugenol group with respect to time in minutes.

Writhing test signifies peripheral analgesic activity. Eugenol possesses peripheral analgesic activity according to the present study findings. Hot plate test signifies central analgesic activity. As per the observations of the present study, 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.

It is found from this experiment that eugenol has an analgesic property when given in the dose of 100 mg/kg.

A study by Fonsêca DV (2016)[7] concluded that, when ortho-eugenol was given as 100mg/kg injection in peritoneum of rats, it showed reduction in mean number of writhes. In the present study, we observed 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 by Kurian R (2006)[8] on eugenol establishes peripheral analgesic activity but mild central analgesic activity. Firstly, 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 and it was observed that eugenol possesses significantly higher peripheral analgesic activity and negligible central analgesic activity.

Furthermore, a study by Chung G (2008)[9], established 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.

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 conducted by the team of Park CK (2009)[10], 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.

It has also been observed by Kim SS (2003)[11] that eugenol inhibits COX – 2 enzymes in cell line RAW264.7. Thus it could be the possible mechanism of action of Eugenol in pain reduction.

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 Hot plate 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 are necessary.

TABLES & FIGURES

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 (p value <0.001 is considered as statistically significant)

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	-	-	-

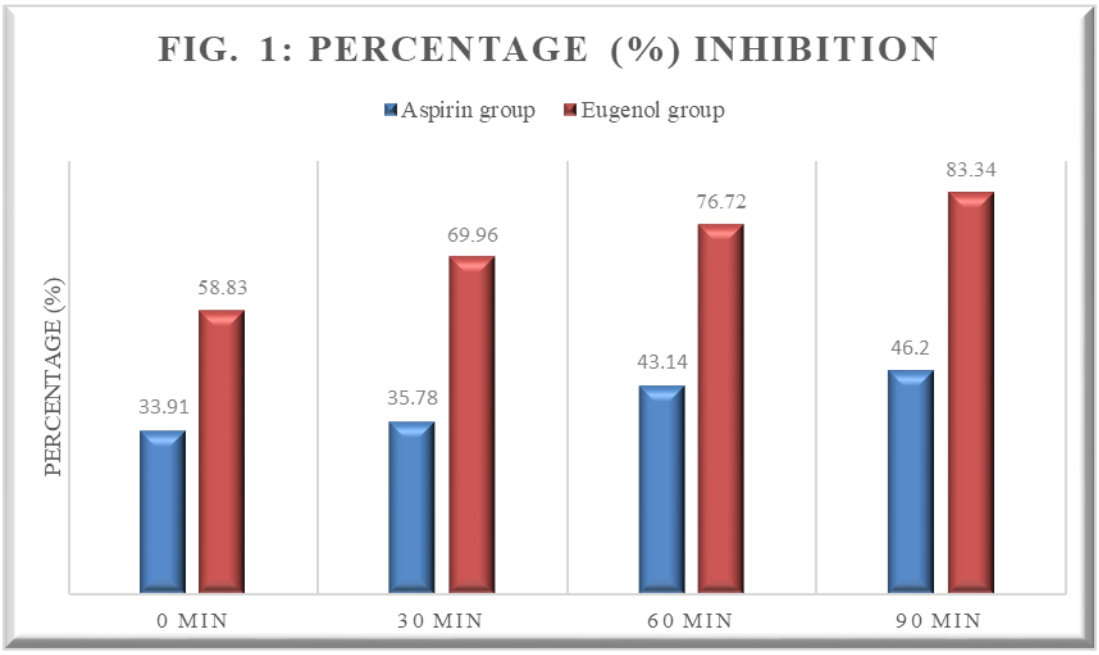
Table 3. Analgesic effect of Eugenol in comparison with Pentazocine and control by Hot Plate method

Mean reaction time for Hot Plate	**Control group (n=6)**	**Pentazocine group (n=6)**	**Eugenol group (n=6)**	**p-value**
	Mean ± SD	Mean ± SD	Mean ± SD	
0 min	7.00 ± 0.36	7.32 ± 0.65	6.67 ± 0.75	0.214
30 min	5.03 ± 0.61	7.68 ± 0.58	7.25 ± 0.96	< 0.001*
60 min	5.37 ± 0.99	8.45 ± 0.71	8.03 ± 0.56	< 0.001*
90 min	5.70 ± 0.28	8.72 ± 0.84	8.53 ± 0.42	< 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 (p value <0.001 is considered as statistically significant)

Mean Number of Writhes at (Time in Minutes)		**Control group**	**Pentazocine group**	**Eugenol group**
0 Minutes	Control group	-	0.648	0.619
	Pentazocine group	-	-	0.188
	Eugenol group	-	-	-
30 Minutes	Control group	-	< 0.001*	< 0.001*
	Pentazocine group	-	-	0.577
	Eugenol group	-	-	-
60 Minutes	Control group	-	< 0.001*	< 0.001*
	Pentazocine group	-	-	0.63
	Eugenol group	-	-	-
90 Minutes	Control group	-	< 0.001*	< 0.001*
	Pentazocine group	-	-	0.841
	Eugenol group	-	-	-

Figure 1. Percentage inhibition of writhing in group of rats



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