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EXPERIMENTAL EVALUATION OF ANALGESIC EFFECT OF CLOVE OIL WITH COMPARISON OF TRAMADOL IN MICE

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ABSTRACT:

Background: Pain, the most common reason for patients, is an unpleasant sensation. It is generally caused by inflammatory reaction in the tissue or tissue damage and produced emotional reaction. Clove oil is an important aromatic spice, which belongs to the Myrtaceae family, contains high number of phenolic compounds with several biological activities, including analgesic, anticonvulsant, antibacterial, antifungal, insecticidal and antioxidant properties. Eugenol is the major compound in Clove essential oil. It is used as a local antiseptic and analgesic and anesthetic. **Aim:** To study the analgesic activity of clove oil in comparison to tramadol in mice. **Methods:** This experimental study was done in central animal house, Department of Pharmacology, Gandhi medical college, study duration from 18th august 2022 to 1st october . Three months old Male albino mice(n=15) were divided into three groups: 1) control group treated with normal saline, 2) standard group treated with inj.Tramadol 50mg/kg(Zydus corza zydus health are Ltd)., 3) Test group treated with Inj.clove essential oil 0.1 ml/kg (Dwibhashi bapamma Ayurvedic Nilayam Pvt.Ltd.) 15 min before testing in hot plate. Analgesic effect of clove oil and Tramadol had been seen with Hot plate method as a basic method to induce their respective doses. Repeated measures of ANOVA with Post-hoc Tukey's HSD test were used for statistical analysis. **Results:** Repeated measures of ANOVA with Post hoc Tukey's HSD test showed that maximum analgesic effect in group treated by 10% Clove essential oil was significantly higher (P value<0.05) than Normal saline group and similar to Tramadol group. **Conclusion:** Present study results showed that Clove oil may possibly act as analgesic agent from heat with stimulates the nociceptive mechanism. Clove essential oil has analgesic action similar to Tramadol in mice using hot plate test.

Key words: Normal saline, Hot plate, Nociceptive, Phenolic compounds, Eugenol.

INTRODUCTION

Pain, the most common reason for patients, is an unpleasant sensation. It is generally caused by inflammatory reaction in the tissue or tissue damage and produces emotional reaction. At present most of the people determine to select medicinal plants to relief pain instead of drugs because it has fewer toxic effects, and can treat a variety of diseases[1]. Various herbal extracts can be used as analgesic remedies. Clove oil is an important aromatic spice, which belongs to the family Myrtaceae. Clove is cultivated in India, Madagascar, Sri Lanka and Malaysia[2,3]. Clove oil contains high number of phenolic compounds with several biological activities including

analgesic, anticonvulsant, antibacterial, antifungal, insecticidal and antioxidant properties, Eugenol is the major compound in CEO, with at least 50% of CEO. At least thirty compounds have been identified in the Clove Essential Oil (CEO)[4,5,6,7]. The remaining 10 to 40% of CEO are eugenyl acetate, β-caryophyllene, and α-humulene. Less than 10% corresponds to minor or trace components such as diethyl phthalate, caryophyllene oxide, cadinene, α-copaene, 4- (2-propenyl) -phenol, α-cubebene, γ-muurolen[8,9]. Eugenol ($C_{10}H_{12}O_2$; 2-methoxy-4-(2-propenyl) phenol), is an allyl chain-substituted guaiacol. It is a clear, pale yellow, oily liquid which is slightly soluble in water and soluble in organic solvents. In dental plasters, fillings, and cements either clove oil or eugenol have been used for their topical analgesic properties[10]. In addition to these indications, it is used as a local antiseptic and anaesthetic. Current available literature states that 10%clove oil has potent analgesic activity in comparison to morphine, indomethacin, tramadol in combination, but not in comparison to tramadol alone as it doesn't produce dependency in comparison to above mentioned analgesic drugs. Therefore, the Present study had been planned to evaluate the Analgesic activity of 10% clove oil in mice in comparison with Tramadol.

MATERIALS AND METHODS

The present study deign is an Experimental and comparative, and this was conducted in Central animal house, Department of Pharmacology, Gandhi medical college, study duration from 18th august 2022 to 1st October 2022. [After the approval of IAEC and IAEC Reg. no. 28/GMC/IAEC/428/GO/Re/S/2001/CPCSEA]

ANIMALS AND DRUGS: Male albino mice (n=15), three months old, weighing 30 grams that were used. All mice were housed in 4-6 mice per standard cage room temperature (23±1°C) on a 12 h light/dark cycle. Food was withheld overnight before the experiments while water was still provided. All experiments were done during the day time. The experimental protocol dated 18th February 2022, was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under Ref.No.28/GMC/IAEC/428/GO/Re/S/2001/CPCSEA, as per CPCSEA guidelines animal handling and further procedures to evaluate analgesic effect of clove oil in mice have done in accordance with Gandhi Medical College, Animal Ethical Committee Acts, Reg, No:428/GO/Re/S/2001/CPCSEA. The clove oil was bought from Dwibhashi Bapamma Ayurvedic Nilayam Pvt. Ltd. The composition of the clove oil was 100% natural, and Tramadol was from (Zydus corza zydus health care Ltd). No other chemicals or anaesthetic agents used to evaluate analgesic activity of test drugs. Study medication had been tested in albino mice those already in facility with 3 days of acclimatization period for physiologic, psychologic and nutritional stabilization prior to experimental evaluation.

EXPERIMENTAL DESIGN:[11] Pilot study was performed in total number of animals (n=6) mice in each group n=2 mice was used to analyze analgesic action of clove oil compared to tramadol for basic Evaluation of analgesic effect of clove oil had been seen in n=15 mice which was divided into three groups: Control group(n=5), Standard group(n=5), test group(n=5). Group 1 treated with Normal saline 5 ml/kg, Group2 Standard treated with Inj. Tramadol 50 mg/kg, Group3-Test Treated with Inj. Clove oil 0.1ml/kg 15 mins before testing in hot plate. The volume of Injection induced 10 ml/kg (i.p.).

NOCICEPTIVE TEST: [11] To assess nociceptive response hot plate method was used as it is a feasible and standardized method to test effectiveness I of analgesic in basic pain research. In hot plate method, animals were placed on the hot plate with temperature setting controlled at 55±0.2 ° C and the Cut-off time was 60 seconds. Nociceptive response was defined as licking forepaws. Time duration between placing the animals on hot plate and licking forepaws were considered as reaction time. The hot plate was performed in seven(n=7) readings with 15 min interval from base line to 90 mins. All three groups were subjected to above mentioned time duration with the respective dosage of drugs.

STATISTICAL ANALYSIS: Analgesic effect of Normal saline, Tramadol and Clove oil were observed. The obtained data presented as Mean ±SD. Statistical comparison of Basal reaction time between three groups was done with One way analysis of Variance ANOVA with Post-hoc Tukey's HSD test in Microsoft excel. Difference was considered statistically significant when p=<0.05.

RESULTS

In the present day, it was demonstrated Analgesic effect of clove oil with comparison to tramadol in Mice using Hot plate method. The study animals were grouped into three and they were subjected to respective doses of study medication and then placed on the hot plate with temperature setting Controlled at 55 ±0.2° C. Cut-off

time was 60 seconds. The basal reaction time in mean±SD between three study groups from base line time 0 to 90 mins had been shown in Table no 1. As it is mentioned below table no1/ figure no1- Comparison between groups shows maximum analgesic effect with 10% Concentration of clove oil (test group) was higher than tramadol (standard group) and normal Saline (Control group).

In Present study observed basal reaction time between the different study groups from Base line to 90 mins duration with Mean±SD. Control group showed minimum response at Base line time with 13±0.63 and maximum response at 90 mins with 15.6±0.49. Standard group showed minimum response at Base line time with 13.2±0.74 and Maximum response at 90 mins with 18.2±0.74. Test group showed minimum response at Base line time with 13.6±1.02 and Maximum response observed at 90 mins with 19.4±1.02. The maximum effect of study medication in three different study groups had been compared applying ANOVA with Post-hoc Tukey's HSD test i.e., shown in Table no.2,3,4 and figure no.1

Repeated measures of ANOVA with Post-hoc Tukey's HSD test showed that maximum analgesic effect in three groups' animals treated by Normal saline, Inj. Tramadol 50 ml/kg, Inj. Clove oil (10%) 0.1 ml/kg, shown in figure no.1

Among these animals treated by Clove oil (Test group) was significantly higher than Normal saline (Control group) and similar to Tramadol (Standard group) when P value < 0.05, shown in table no.4

DISCUSSION

In the present study Analgesic effect of clove oil was evaluated using Hot plate method in comparison to Tramadol by measuring heat threshold in mice. In hot plate method, animals were placed on the hot plate with temperature setting controlled at 55 ±0.2°C. The unrestrained animals were placed on the plate until nocifensive behaviour is observed and the cut-off time was 60 seconds. The nocifensive response was observed i.e., Licking forepaws or withdrawal and moving hind paws, during study procedure if the animals were not reactive to test, they had been removed after above mentioned time point to avoid tissue damage in animal.

The present study results states that 10% concentration of clove oil had been showed maximum effectiveness which was similar to study reported by Hossieni et al. conducted a study on analgesic effect of clove oil in Mice using hot plate Method, showed that 10% concentration of clove oil was the most effective in Mice comparison with other two doses of clove oil (2%,5%) concentrations [11].

Kamakar Asl et al. done a study on Analgesic effect of the aqueous and ethanolic extract of clove oil in mice, observed maximum nociceptive effect in animal groups treated with 50mg/kg of Clove oil was significantly higher in mice than the Naloxone dose 100mg/kg, 200 mg/kg treated animals[12], which is similar to present study results i.e.,10% clove oil is more analgesic effect than tramadol 50mg/kg.

A study done by Pouya Tayebi et al. Tramadol effect on morphine dependency and analgesia in mice, concluded analgesic effect of Tramadol doses of 25 mg/kg; 50 mg/kg was higher in mice than Morphine 10 mg/kg[13]. In the present study Comparison between groups shows maximum analgesic effect with 10% Concentration of clove oil (test group) was little more during the experiment with tramadol (standard group) and normal Saline (Control group) but statistically it shows insignificant to prove statistical significance may require more sample size.

Kanyarat sueksakit et al. conducted a study of syzygium aromaticum on analgesic activity in rats, observed analgesic effect of Indomethacin 5% w/w was higher with tail flick method in rats than clove oil of different doses (2%,5%,10%)[14]. A study conducted by Yousef A. Taher et al. experimental evaluation of anti-inflammatory, antinociceptive and antipyretic activities of clove oil in mice, concluded nociceptive effect of morphine 5mg/kg was significantly higher in mice than clove oil 0.03% [15]. The analgesic effect of eugenol has been attributed to its capability for inhibiting the prostaglandins and other inflammatory mediators such as leukotrienes [16].

The analgesic studies revealed that clove oil exhibited potent analgesic effect against tramadol[12], clove oil may possibly act as analgesic agent from heat with stimulates the nociceptive mechanism. Hence, the analgesic effect of clove essential oil which was shown in the present study should be considered for further evaluation.

CLINICAL IMPLICATIONS:

Clove oil may have benefits for dental and topical application for treating headache, ear pain, tooth ache, infections and even fighting cancer pain.

LIMITATIONS

- Sample size is the one of the limitation, Hence the present study had been planned for basic evaluation of analgesic effect of 10% clove oil.
- Analgesic effect to be evaluated with multiple doses of clove oil for further clinical implication

CONCLUSION

Present study results showed that analgesic effect of 10% concentration of clove essential oil is effective like Tramadol in mice. The role of clove oil and its analgesic effect which was shown in the present study should not be ignored. However, clove is an aromatic spice and high number of phenolic compounds with several biological activities needed to be investigated further to know its exact mechanism for clinical implications.

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TABLES

Study groups	Baseline	15 mins	30 mins	45 mins	60 mins	75 mins	90 mins
Control group	13±0.632	13.4±0.49	13.6±0.49	14.6±0.49	14.2±1.16	15.4±0.8	15.6±0.49
Standard group	13.2±0.74	14.2±0.74	14.6±0.74	15.8±0.74	16.6±0.8	17.8±0.74	18.2±0.74
Test group	13.6±1.02	15.4±1.02	16.2±1.32	17.2±1.16	18.4±1.02	19±0.632	19.4±1.02

Table 1; Basal reaction time between the different study groups from Base line to 90 mins duration with Mean±SD.

Groups	Mean±SD	Standard error	Normality
Control group	14.2±0.99	0.37	0.7065
Standard group	15.8±1.85	0.70	0.9171
Test group	17±2.08	0.78	0.8896

Table 2; Comparison of analgesic effect of study medication applying ANOVA with Post-hoc Tukey's HSD test.

Table 3;

Source	DF	Sum of Square	Mean Square	F Statistic	P-Value
Groups (Between groups)	2	26.4686	13.2343	4.5205	0.02566
Error (Within groups)	18	52.6971	2.9276		
Total	20	79.1657	3.9583		

Repeated measures of ANOVA with Post-hoc Tukey's HSD test showed that maximum analgesic effect showed with significant P-value (0.02566).

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
Control Vs Standard group	2.3857	0.2372168	Insignificant
Control Vs Test group	4.2413	0.0199883	P<0.05 Significant
Standard Vs Test group	1.8556	0.4082317	Insignificant

Table 4; Post hoc Tukey's HSD test applied within the study groups (P<0.05)

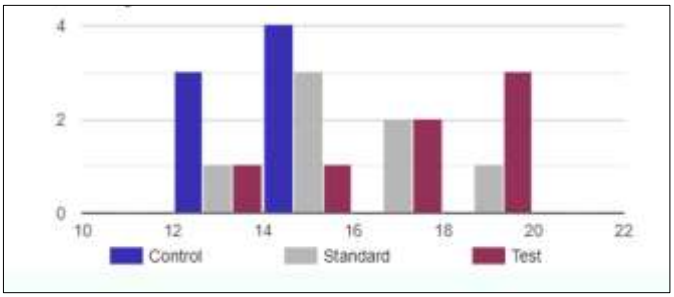

Figure 1; Comparison of analgesic effect of study medication in three study groups