

## Original Article

# EFFECT OF ETHANOLIC EXTRACT OF CLOVE (*Eugenia Caryophyllata*) ON PAIN IN MICE

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### ABSTRACT:

**Background:** Pain is a natural phenomenon. There are several pharmacological medicines available in the market for pain relief. Ethanolic extract of clove is a natural ingredient and can be as beneficial as a pharmacological drug for pain relief.

**Objective:** To determine the effect of *Eugenia caryophyllata* flower buds ethanolic extract on experimentally induced pain in albino mice.

**Material and Methods:** This randomized trial was done on 90 male albino mice. An intraperitoneal injection of 0.6% acetic acid was induced for the writhing test as a chemical model of nociception. Mice were divided randomly into three equal groups. Group A was considered as a control group (n=30) and normal saline was infused; group B was given *Eugenia caryophyllata* flower bud ethanolic extract (n=30) and group C was given an intraperitoneal injection of indomethacin (n=30). In these mice, abdominal contractions (writhings) were counted. SPSS version. 22 was used to analyze the data.

**Result:** The mean number of writhing in each of the three groups of mice was 16.80 writhings in group A, 4.90 writhings in group B, and 4.60 writhings in group C. Ethanolic extract of clove and indomethacin significantly reduced ( $p<0.05$ ) the number of writhing.

**Conclusion:** The *Eugenia caryophyllata* ethanolic extract significantly reduces the pain in mice. This analgesic effect is almost similar to that produced by indomethacin.

**Key Words:** Clove, Pain, Mice

## INTRODUCTION:

Pain is a natural emotional and sensory experience, related to the actual or potential tissue injury. It is an essential feature of defense mechanisms of the body to lessen the physical harm.<sup>1</sup>

Analgesics or opioids are the most common therapeutics which can help to relieve the pain. Numerous researches have already been done on these to relieve pain.<sup>2</sup>

The bioactive composites present in edible as well as medicinal herbs are valuable molecules for the synthesis of many medicines containing activity against many syndromes, especially involved in inflammation that is associated with oxidative stress.

Numerous such herbs have a considerable inhibitory effect on inflammatory response and oxidative stress and can help protectively to increase the quality of life after initiation of taking diets, which is rich in such components.<sup>3</sup>

There are several natural herbs, which have analgesic properties and are traditionally used without any sort of adverse effects. Clove is the most valuable spices which have been used for several centuries, especially in tropical countries for food preservation and medicinal purposes.<sup>4</sup>

*Eugenia caryophyllata* (Clove), is a medicinal plant, which is traditionally used to avert the pain. It is commonly found in

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tropical countries. Conventionally, flower buds of the clove are used in medicine for the management of rheumatic pains, sciatica, headache, neuralgia, toothache, indigestion, nausea, loss of appetite, hiccup, vomiting, paralysis, and skin disorders.<sup>5</sup>

Clove is used as a medicinal herb, from several years in Chinese traditions. Cloves have antiseptic, antifungal, antibacterial and anti-viral action.<sup>6,7</sup> Chemical composition of clove bud is diverse. It is composed of carbohydrates, fat, proteins, and water. It also has minerals including sodium, calcium, potassium, iron and phosphorous it also contains some vitamins like riboflavin, thiamine, ascorbic acid, niacin, and vitamin A.<sup>8</sup>

Several properties of clove bud including antiseptic, antifungal, antiviral, antibacterial, antipyretic, anti-oxidant, anti-allergic, anticonvulsant, anti-mutagenic, insecticidal and natural anti-helminthic have been studied extensively in the world,<sup>9,10</sup> but to our knowledge, in Pakistan, no research has been done on the above-stated properties of clove. The effects of *Eugenia caryophyllata* extracted oil and Ethanolic extract on pain and inflammation are encouraging.<sup>11</sup>

In our country, the abuse of analgesic drugs has increased in previous years. This may be because of the over-the-counter sale and purchase of these medicines. These drugs are causing an increase in gastro-intestinal tract problems including gastritis, gastric ulcers, bleeding, and renal damage, and failure of some other organs. Medicinal herbs can be a good replacement for pharmacologically based analgesics and are also safe and cost-effective.

## MATERIAL AND METHODS:

This Randomized controlled trial was done at the Department of Physiology, Services Institute of Medical Sciences, Lahore. The trial was done on 90 adult male albino mice, Mice were distributed in three equal groups of 30 mice in each group mice were kept in a cage for a week beforehand initiation of the trial. Atmospheric conditions were maintained at the  $24\pm 2^{\circ}\text{C}$  and dark: 10-12

hours darkness and 12-14 hours light. All mice were given standard pellet diet ad libitum which was commercially available and tap water in clean bottles was given.

*Eugenia caryophyllata* flower buds (3000g in dried condition) were obtained from the local market. The extract of *Eugenia caryophyllata* flower buds was made using ethanol and standardized by using the facilities available at Applied Chemistry Research Centre, PCSIR Labs, Lahore.

The mice were divided randomly into three groups. Each contained 30 mice:

Group A (Control): given intra-peritoneal normal saline, 10ml/Kg

Group B (Experimental): given intra-peritoneal ethanolic extract of *Eugenia caryophyllata* flower buds, 50mg/Kg

Group C (Reference): given intra-peritoneal indomethacin, 3mg/Kg

After the administration of the trial drug, each mouse was transferred to a separate, transparent glass case and trained for 30 minutes. Then, acetic acid (0.6%) in a dose of 10ml/Kg was injected intraperitoneally and writhings (abdominal contractions) were counted for 15 minutes. Percentage inhibition of writhing was calculated to observe the analgesic effect by using the following formula:

$$\text{Inhibition (\%)} = (1 - \text{Wt/Wc}) \times 100$$

Where Wt and Wc represent the number of writhings in experimental (*Eugenia* extract or indomethacin) and control group, respectively. SPSS v. 22 was used to analyze the data and the mean number of writhing was compared by using one-way ANOVA tests.

## RESULTS:

The mean number of writhing per 15 minutes was  $16.80\pm 0.21$  in the control group,  $4.90\pm 0.14$  in the *Eugenia* extract group, and  $4.60\pm 0.18$  in the indomethacin group. The percent inhibition in the *Eugenia* group was 70.83% while 72.62% in the indomethacin group ( $p=0.000$ ), indicating a highly significant analgesic effect in these groups. (Table 1)

**Table 1.** Comparison of three groups for body weight, number of writhing, and percentage inhibition of writhing among mice.

Parameter	A (Control)	B (Eugenia (Caryophyllata))	C (Indomethacin)
	n=30	n=30	n=30
Number of writhing (in 15 min)	16.80±0.21	4.90±0.14*	4.60±0.18*
Percent Inhibition (%)	0.00	70.83	72.62

\*p=0.000, highly significant in comparison to control

## DISCUSSION:

Pain is usually defined as ache prolonged for  $\geq 3$  months.<sup>12</sup> Opioids are used for effective control of pain, but the evidence available in literature does not recommend the prolonged use of such opioids for the treatment of chronic non-cancerous pain. The patients taking opioids for a prolonged period have a high risk of opioid use disorders also some other adverse outcomes.<sup>13-15</sup> Side effect such as addiction should be avoided.<sup>16</sup>

For medical use, several herbs can be taken orally, sublingually, or even topically and can also be smoked; inhaled; combined, or cooked with food or drinks. These can be used in herbal form, extracted naturally from the plant, or prepared synthetically.<sup>2</sup> Eugenol (4-allyl-2-methoxyphenol) is the phenolic compound from phenylpropanoids class and the main component of the clove.<sup>17</sup> Eugenol is used in the food industry as a preservative, primarily because of antioxidant properties, for flavoring of foods, and also in cosmetics.<sup>18</sup> Furthermore, clove is also known for its anti-inflammatory activities, which might

be due to anti-inflammatory actions of eugenol.<sup>19</sup>

In the present study, intraperitoneal administration of the ethanolic extract of clove flower buds significantly decreased the number of writhing (abdominal contractions) as compared to the control with the percentage inhibition being 70.83%. This inhibition (i.e. 70.83%) is more than that reported by Daniel (61.6%) who used essential oil of *Eugenia caryophyllata* but this inhibition is less than that reported by Tanko et al. (i.e. 75%) who used the ethanolic extract of clove flower buds and observed maximum effect by the same dose which has been used in this study (i.e. 50 mg/Kg).<sup>20</sup> The percentage inhibition of writhing caused by the reference drug indomethacin was 72.62%.

Taher et al. found that in the mice given *Eugenia caryophyllata* extracted oil, acetic acid-induced writhing was reduced significantly by 87.7% ( $p < 0.01$ ) than 77.7% produced ( $p < 0.01$ ) by 100 mg/kg, intraperitoneal aspirin injection. Additionally, *Eugenia caryophyllata* oil, as well as indomethacin, have anti-inflammatory effects, i.e. 50.6% ( $p < 0.05$ ) and 70.4% ( $p < 0.01$ ), respectively, to avert the edema of mouse foot, which was induced by using carrageenan.<sup>21</sup>

Clove extract is enriched with polyphenol, because of its antioxidant property, the clove extract is capable to inhibit the secretion of advanced glycation end products and protein glycation. Such findings recommend the use of clove extract for some diabetic complications.<sup>5</sup>

Daniel et al in 2009, used essential oil of *Eugenia caryophyllata* flower buds. In an acetic acid-induced writhing test to determine the analgesic effects, 61.6% inhibition was achieved.<sup>22</sup>

Eugenol is extensively used in dentistry as a local analgesic agent, owing to its ability to lessen the toothache. Interestingly, eugenol shares many pharmacological activities with local anesthetics. It inhibits voltage-gated sodium channels and sensory receptors

which are involved in the perception of pain, the transmission of action potential.<sup>23</sup>

Ethanol extract of the *Eugenia caryophyllata* flower buds possesses a potent analgesic effect and it can be used as a traditional remedy for different pain disorders.

### CONCLUSION:

The ethanol extract of *Eugenia caryophyllata* significantly reduces pain in mice. This analgesic effect is almost similar to the effect of indomethacin.

### ACKNOWLEDGMENT:

We pay special thanks to Dr. Zaheer-ud-Din Khan, Professor & HOD, Department of Botany, Government College University, Lahore for identifying the type of flower buds used in the experiment. We are also thankful to Applied Chemistry Research Centre, PCSIR Labs, Lahore for extracting the ethanol extract of clove.

### AUTHOR'S CONTRIBUTION:

ST: Conception of idea and study design

HJQ: Supervisor of the research work

AA: Data collection

SM: Data analysis

HHP: Data collection

WT: Drafting the article

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