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Original Research Article

TOPICAL ANALGESIC ACTIVITY OF CLOVE GEL

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Abstract: Objective: To study the topical analgesic activity of gel containing clove. Methods: The essential oil was extracted from flower bud of *Eugenia cryphyllus*. Topical gel containing essential oil was investigated for analgesic activity in albino rat using hot-plate method. Diclofenac sodium gel was taken as standard drug. Results: The analgesic activity of topical gel preparation was observed in the early phase. The analgesic activity of topical preparation containing 5% *Eugenia cryphyllus* essential oil showed same analgesic activity compare to standard drug diclofenac sodium (7.833±0.31) at 120 minutes, whereas the test drug showed significant activity (8.166±0.65) than the standard drug (6.5±0.34) at 180 minutes of application of drug by hot-plate method ($p<0.001$). Conclusion: Hence it may suggest that the essential oil of *Eugenia cryphyllus* attribute potent analgesic activity.

Keywords: Clove gel, *in vivo*, hot plate method, tail immersion

Introduction: *Eugenia cryphyllus* generally called as clove is trees of height 8-12 meters have belonging to family Myrtaceae. Geographically it is found in east Indonesia and Brazil[1]. The main active bioconstituents are eugenol, gallic acid, phenolic acids like elagic, ferulic, caffeic and salicylic acids. Various forms of flavonoids also found in lower concentrations[2,3].

Clove possesses a broad range of pharmacological activity like Antioxidant activity[4], Antimicrobial activity[5], Antinociceptive[6] and Antiviral[7], for which it has given prime importance.

Syzygium aromaticum oil is applied for dental analgesic, dental caries and pyorrhoea. It was taken as popular remedy for sore throat, headache, dental and respiratory disorders, digestive system ailments and in traditional medicines of Australia and Asian countries[8]. Clove oil in the form of clove paste is employed for ulcer therapy[9]. In various respiratory disorders like colds, coughs, asthma, sinusitis,

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bronchitis and tuberculosis clove is recomended[10]. Clove oil boosts concentration and also increases the ability of thinking[11].

This volatile oil comprises more than twenty constituents, out of which eugenol (76.8%), β-caryophyllene (17.4%), α-humulene (2.1%), and eugenyl acetate (1.2%) considered as main constituents[12].

In the present investigation was carried out to observe the possible topical analgesic effect of clove oil in animal models *in vivo*.

Materials and Methods: Plant material and extraction of the essential oil: Clove bud was purchased from local market and the oil was extracted by using Clevenger apparatus by hydro-distillation method for 5 hours[13]. The oil was collected on glass bottle and kept for future study.

Preparation of herbal gel: Gel base was prepared by taking guar gum (600mg), xanthum gum (80mg) in water 100ml and was kept overnight to dissolve. Then it was stirred with the magnetic stirrer, maintain the temperature 40^{o} C and 80 rpm for 30 mins forming transparent gel. Two formulation was prepared by adding 2.5% and 5% clove oil to the above gel[14].

Animals: Healthy adult male albino rats weighing 170-200 g were used for the experiment study. Animals were maintained at 23-27°C with a 12 hrs light-dark cycle. They were allowed to standard laboratory feed and water. The animals were put into four groups contains six albino rats in each. The study protocol was approved by the Institutional Animal Ethics Committee (Registration No. 1376/ac/10/ CPCSEA). The experimental procedures were carried out in accordance of the ethical guidelines for investigations of experimental pain in conscious animals[15].

Analgesic activity: The analgesic activity of herbal formulations containing clove oil was carried out by modified hot plate method described by Padhan et al., 2017[16] and Abd Allah et al., 2011[17]. This is performed by using an electronically guarded hotplate (Eddy's hot-plate) heated to 55°C (± 0.1°C). Each rat of different groups was placed unrestrained on the hot-plate for basal reaction time measurement just before placebo or drug application considered as zero time. The test group animals were applied with gel (100 mg) topically on the hind paw of the albino rats at dose of 2.5% and 5% v/w, whereas standard group of animal treated with Diclofinac gel (100mg). After thirty minutes of drug administration, the applied surface of the skin was cleaned off with cotton. Before drug application to surface of each rat, they were placed on the hot plate to measure baseline and considered this as zero time. The pain threshold was measured after 30, 45, 60, 75, 90, 105 and 120 hours after drug application. All groups animal was observed for latency to lift and licking a hind paw or endeavour to jump from the apparatus. The cut-off time was set as 15 seconds to evade tissues damage of the surface exposure to hot plate.

Results:

Table 1: Analgesic activity of essential oil of *Eugenia cryophylus* flower bud by hot-plate method

	Time of treatment of the drug in minutes						
TREATMENT	0	30	60	90	120	150	180
CONTROL	3.5±0.22	3.5±0.34	3.667±0.21	3.667±0.21	3.33±0.33	3.667±0.17	3.833±0.22
TEST 2.5%	3.667±0.21	3.833±0.17	5.33±0.33	5.833±0.40*	6.33±0.33*	6.667±0.21	7.667±0.21*
TEST 5%	3.5±0.22	4±0.26	5±0.26	6.667±0.33*	7.833±0.17*	7.5±0.56*	8.166±0.65*
STANDARD	3.667±0.21	4.833±0.17	6.5±0.22*	7.166±0.17*	7.833±0.17**	8.166±0.60*	6.5±0.34*

n=6; *p<0.05, **p<0.001 values are expressed as mean±SEM. One-way ANOVA followed by Dunnett's t-test, all the groups are compared with control. SEM: Standard error mean

The clove essential oil containing herbal gel was showed analgesic activity in doses-dependent manner as well as latency time (Table 1). No significant antinociceptive activity was found at time 0 and 30 minutes after the treatment with both test groups compared with control. Whereas the basal reaction time increased notably after 90 minutes of the treated animals with topical ointment 2.5% (5.833±0.40) and 5% (6.667±0.33) doses. It was observed that both the test dose of 2.5% and 5% showed significant analgesic activity compared with control at 120 minutes i.e. 6.33±0.33 and 7.833±0.17 respectively. However, standard diclofenac ointment (1.16%) depicted a increased the basal reaction time significantly as compared to control group and have highest analgesic response after at 150 minutes (8.166±0.60) of stimuli. More over the test drug found significant activity at 180 minutes (8.166±0.65) as compared to standard drug diclofenac (6.5±0.34).

Discussion: The hot-plate test is generally inured to investigate nociception and analgesia in rodents. The standard method as described by Woolfe and MacDonald[18] and modified by Eddy et al., 1950[19], records latency for nociceptive responses in animals placed on a plate by keeping the temperature constant, usually about 55 _C[20].

Since 13th century clove was employment as analgesic for joint pain, toothache and antispasmodic[21]. In Ayurveda, Chinese medicine and Western traditional medicines clove has been taken as a preferable herbal remedy. This study validate the scientific prove of tradional and ayurvedic uses of Clove.

The results described topical administration of oil at both doses 2.5% and 5% significantly showed best activity at 180 minutes whereas the standard drug starts lowering of activity at the same time.

Essential oils obtained from plants are well concentrated mixtures of chemicals, both volatile and hydrophobic. The chief active constituents present in essential oils are chemically monoterpenes, sesquiterpenes, and phenylpropanoids[22].

Many aromatic plant containing essential oils possesses antinociceptive activity[23]. The mechanism of actions of antinociceptive drugs are based on the central nervous system (CNS) or the peripheral pathway. Eugenol also exhibits antinociceptive activity against chemical (acetic acid tests) and thermal stimuli revealed that eugenol mainly inhibits the peripheral pain mechanism[24, 25]. In certain studies eugenol possessed analgesic effects by suppress prostaglandins and other inflammatory intermediaries such as leukotrienes[26].

The other mechanism evolved has been attributed to the activation of calcium and chloride channels in ganglionar cells[27]. The voltage dependant effects of eugenol in sodium and calcium channels and in receptors expressed in the trigeminal ganglio also contributed to the analgesic effect of clove[28]. In some studies the analgesic effects of clove was due to eugenol which depresses compound action potentials in both A and C fibers[29]. So this may confirm the antinociceptive effect of eugenol probably acts by both opioid and alpha adrenergic receptors[30].

However, the variable response seen at different doses could also be due to the synergism of other bio-constituents available in the oil apart from eugenol[31]. The antinociceptive effect of essential oils is due to their major constituents and that synergism between such chemical constituents does occur[23].

It was also demonstrated that topical application of clove oil cream had a significant effect in patients suffering from chronic anal fissure[32]. Further study was recommended to develop a new formulation to alleviate the pain.

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