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Original Article

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF FORMULATION CONTAINING CAMPHOR, MENTHOL AND THYMOL

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ABSTRACT

Objective: To determine analgesic and anti-inflammatory activity of a oily formulation containing camphor, menthol and thymol as ingredients in wistar albino mice and rats.

Methods: Acute toxicity studies were done as per OECD 423 guidelines. No signs of toxicity were observed up to 2000 mg/kg. Based on the mortality rate dose was designed. Analgesic activity was performed in Swiss albino mice by Hot plate method. Anti inflammatory activity was performed in male Wistar rats by turpentine induced inflammation model.

Results: The formulation at a dose of (100 mg/kg & 200 mg/kg) produced a dose dependent significant analgesic effect in hot plate method ($p<0.001$). However the formulation at a dose of (250 mg/kg & 500 mg/kg) showed anti-inflammatory effect ($p<0.001$) when compared with the control group.

Conclusion: In the above investigations OFCMT (oily formulation of camphor, menthol and thymol) has shown tremendous protection from pain and Inflammation in experimental animals the above pharmacological effects may be due to the presence of monoterpenoids in the formulation.

Keywords: OFCMT, Anti-inflammatory, Analgesic.

INTRODUCTION

Herbal medicine has been used since ancient era for many centuries. In today's life there is need for efficacious therapy for pain and inflammation. In the search for new therapeutic options, novel bio-molecules of natural sources are to be investigated. Purpose of this study was to evaluate the analgesic and anti-inflammatory effects of OFCMT (Oily formulation of camphor, menthol and thymol).

One of the important and effective parts of herbal plants is essential oil and substances present in different parts of plants. Essential oils are components which are oil soluble that have effective smell and aroma and are separated by use of water and steam distillation and prepared by extraction with solvents and enzymatic hydrolysis [1]. Likewise OFCMT of oily nature is the combination of the three active ingredients (camphor, menthol and thymol), which was emulsified using excipients. The excipients used to solubilize drugs in oral and injectable dosage forms include pH modifiers, organic solvents, surfactants, water-insoluble organic solvents, triglycerides and phospholipids. The solvent system chosen was able to solubilize the drug at the desired concentration and an environment was provided where the drug has sufficient chemical stability. Each active individual ingredient of OFCMT has its own medicinal value. Camphor, a natural product derived from the wood of the tree *Cinnamomum camphora*, has a long history of use as antiseptic, analgesic, antipruritic, counter irritant and rubefacient [2]. Menthol is a natural compound of plant origin known to produce cool sensation. Menthol, the cooling natural product of peppermint, is widely used preparations for pain relief in sports injuries, arthritis, and other painful conditions. Thymol is a natural monoterpene phenol derivative cymene, isomeric with carvacrol, found in oil of thyme, and extracted from *Thymus vulgaris* and various other kinds of plants as a white crystalline substance of a pleasant aromatic odor and strong antiseptic properties [3].

However, limited information is available on the pharmacological properties of the above individual ingredients and mixture of the above in the form of formulation. There is no scientific report on the analgesic and anti-inflammatory effects of the above formulation to best of our knowledge. Based on the above claims, the present study is undertaken to evaluate analgesic and anti-inflammatory effect of the formulation.

MATERIALS AND METHODS

Oily formulation containing Camphor, Menthol and Thymol in 1:1:1 w/w ratio was provided by Limra Pharmaceuticals, Hyderabad as a gift sample.

Experimental animals

Healthy wistar albino rats weighing about (120-160 gm) and mice weighing about (50-60 gm) of either sex were obtained from animal house. The animals were maintained under standard conditions i.e., housed in polypropylene cages and maintained at a temperature 27±2 °C, relative humidity 65±10% under 12 hour light and dark cycle. The animals were acclimatized for 10 d in the animal house approved by the Animal ethical committee bearing registration No.1534/PO/A/11/CPCSEA, Anwar-ul-Uloom College of Pharmacy before carrying out the work.

Chemicals

Tween 80, cyclodextrin, Dimethyl Sulfoxide were obtained from Virchow Biotech Pvt Ltd. Turpentine oil, distilled water, and standard drugs diclofenac sodium and indomethacin (Ranchem Pharmaceuticals, Hyderabad)were obtained from the Drug store, Anwarul-Uloom College of Pharmacy, New Mallepally, Hyderabad.

Solubilization of OFCMT

Different solubilizing agents such as Dimethyl sulphoxide, Ethanol, Cyclodextrin, Hydroxypropyl methyl cellulose, Carboxymethyl Cellulose were used to solubilize the formulation. Finally fruitful results were obtained by adding tween 80 as surfactant. 12% of tween 80 was prepared in distilled water i.e. 12 gm of tween 80 was added to 88 gm of distilled water & stirred for 1 hour. OFCMT was added to the above solution in 1: 10 ratio with continuous stirring for 15 min.

Method of determination of acute toxicity

Acute oral toxicity study was performed as per OECD-423 guidelines category IV (acute toxic class method). Male wistar albino rats (n=3) selected by random sampling technique were employed in this study. The animals were kept on fasting for 4 h with free access to clean drinking water only.

The formulation was administered orally with maximum dose of 2000 mg/kg body weight by oral feeding needle. The mortality was observed for three days. If mortality was observed in 2 out of 3 animals or 3 out of 3 animals then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 3000 mg/kg of body weight [4].

Statistical analysis

The data was expressed as mean±SEM analyzed statistically using one way ANOVA procedures followed by Dunnett's test, P-value<0.005 was regarded as significant. P value<0.01 regarded as less significant and P-value<0.001 as highly significant.

Evaluation of analgesic activity

In the present investigation, analgesic activity of OFCMT was studied by using Eddy's hot plate method using diclofenac sodium as standard drug.

Hot plate induced analgesia [5]

The animals were placed individually in hot plate regulated at a temperature (55±2 °C) before the treatment and its reaction time was determined. After noting the initial reaction time, the treatment was given to each mouse. Then the mice were placed on the Eddy's hot plate under regulated temperature and the licking of the forepaws was recorded as the hot plate latency with the help of a stop-watch. The reaction time was re-determined after 0, 30, 60, 90 & 120 min after oral administration of standard and test drug.

The mice were divided into four groups with (n=6) and treated with the respective solutions as given below

Group-I: served as control & received 12% tween 80 in distilled water as vehicle at a dose of 10 ml/kg body weight orally.

Group-II: served as standard & received diclofenac sodium in distilled water at a dose of 50 mg/kg body weight orally.

Group-III: served as Test1 & received test drug 100 mg/kg body weight orally.

Group-IV: served as Test 2 & received test drug 200 mg/kg body weight orally.

Evaluation of anti-inflammatory activity

The anti inflammatory activity of FCMT was determined by turpentine induced inflammation [5, 6]. In this model, acute inflammation was induced by sub-plantar injection of 0.1 ml of turpentine oil one hour after oral administration of test drug at a dose of 250 & 500 mg/kg body weight, Indomethacin 10 mg/kg body weight, p. o and vehicle solution of 12% tween 80 in distilled water 10 ml/kg body weight, p. o. The volume of paw was determined at 0 h, pre and post treatment of turpentine oil, 1, 2, 3 and 4 h following the injection of turpentine oil. The changes in the volume of paw were measured by a plethismometer.

The rats were divided into four groups with (n=6) and treated with the respective solutions as given below.

Group-I: served as control & received 12% tween 80 in distilled water as vehicle at a dose of 10 ml/kg body weight orally.

Group-II: served as standard & received indomethacin in distilled water at a dose of 10 mg/kg body weight orally.

Group-III: served as Test1 & received test drug 250 mg/kg body weight orally.

Group-IV: served as Test2 & received test drug 500 mg/kg body weight orally.

RESULTS

Results of analgesic activity

Hot plate model

From the results of analgesic activity obtained it was observed that in the hot plate model the test group I i.e. OFCMT at a dose of 100 mg/kg showed an increase in the reaction time after 30 min of administration (2.98±0.41) when compared with the initial value (1.61±0.41) as shown in the table 1 and fig. no1. In 60 min the reaction time in the same group increased gradually (4.18±0.33) again when compared with the initial value. In 90 min the reaction time of group I increased gradually (4.80±0.22) when compared with initial value likewise after 120 min the reaction time of group I increased gradually (5.56±0.24) again when compared with the initial value. In 240 min the reaction time of OFMCT (Test 1) group increased (6.28±0.22) and was at the peak, slightly closer to the standard group as seen in the table.1. From the above results it was evident that OFCMT Test I group which received 100 mg/kg of OFCMT showed a highly significant analgesic effect (p value 0.001) in 240 min and the effect was slightly significant in 30, 60, and 90 and significant in 120 and 240 min.

In the Test II group i.e. OFCMT (200 mg/kg) there was a gradual increase in the reaction time after 30 min of administration (2.15±0.30) when compared with the initial (1.81±0.20). In 60 min the OFCMT in Test II showed an increase in reaction time again (3.58±0.31). In 90 min the reaction time of Test II group showed an gradual increase in the reaction time (4.45±0.29). The reaction time slightly increased in 120 min (5.26±0.29) when compared to initial. In 240 min the reaction time of (Test II) group increased (5.75±0.34) and was slightly closer to the standard group as seen in the fig no 1. From the above results it was evident that OFCMT Test II group which received 200 mg/kg of OFCMT showed a highly significant analgesic effect (p value 0.001) in 240 min and the effect was slightly significant in 30, 60, and 90 min and significant in 120 min and 240 min.

Results of anti-inflammatory activity

Turpentine induced inflammation

From the results of anti inflammatory activity it was observed that at 1 h after administration of OFCMT at a dose of 250 mg/kg in the test group–I, the paw volume of rats was (0.48±0.03) showed an significant decrease in inflammation when compared with initial paw volume at 0 h (0.55±0.02). In 4 h the paw volume of rats was (0.39±0.01) which showed a significant decrease in paw volume animals compared to standard group as seen in table 2.

In the test group II i. e OFCMT 500 mg/kg there was a gradual decrease in paw volume after 1h of administration of OFCMT when compared to initial paw volume at 0h (0.51±0.01) which showed a significant decrease in inflammation. In 4 h after administration paw volume was (0.41±0.02) which showed a significant decrease in paw volume of animals when compared to standard group.

Table1: Analgesic activity in mice using hot plate method at different intervals of time

Treatment group	Dose(mg/kg)	Reaction time in minutes (mean±SEM)					
		initial	30	60	90	120	240
Control	10 ml/kg	1.46±0.11	1.80±0.11	2.21±0.12	1.75±0.14	2.62±0.15	2.11±0.10
Diclofenac sodium	50 mg/kg	1.81±0.20	2.15±0.30	4.39±0.20	6.02±0.15**	8.10±0.12**	8.50±0.30***
Test-1	100 mg/kg	1.61±0.41	2.98±0.41	4.18±0.33	4.80±0.22**	5.56±0.24**	6.28±0.22***
Test-2	200 mg/kg	1.83±0.23	2.65±0.31	3.58±0.31	4.45±0.29**	5.26±0.29**	5.75±0.34***

**P<0.01: Significant, All the values are mean±SEM, n=6. One way ANOVA followed by Dunnets multiple comaparison test, ***p<0.001(INITIAL vs TEST I & TEST II) considered as extremely significant.

Table 2: Paw volumes of rats in different experimental groups

TreatmentGroup	Dose(mg/kg)	Decrease in paw volume of rats (in hs)				
		0h	1h	2h	3h	4h
Control	5 ml/kg	0.50±0.01	0.48±0.01	0.47±0.02	0.46±0.01	0.45±0.01
Indomethacin	10 mg/kg	0.76±0.02	0.62±0.01	0.57±0.01*	0.51±0.02**	0.49±0.01**
Test-1	250 mg/kg	0.55±0.02	0.48±0.03	0.44±0.02*	0.41±0.02**	0.39±0.01**
Test-2	500 mg/kg	0.51±0.01	0.48±0.01	0.46±0.02*	0.44±0.02**	0.41±0.02**

*P<0.01: Lesssignificant, All the values are mean±SEM, n=6. One way Analysis of Variance (ANOVA) followed by Dunnets multiple comaparision test, **p<0.01(Initial vs. test I & test II) considered as significant

DISCUSSION

Considering that the relief of painful symptoms typically involve pharmacotherapy and there are hundreds of essential oils that have not been assessed, which continue to be major source of bioactive compounds used in pain and inflammatory conditions the present investigation was carried out [7].

In order to solubilize OFCMT, several solvents were tested and it was found that in Tween 80 the solubility was good. Therefore it was used as solvent there are many useful solvents in laboratory which are toxic to biological organisms and consequently interfere with the activity being studied [8].

In order to demonstrate the effect of the tween 80, used for dispersion of OFCMT on the results of biological tests, a comparative study was carried out by administering 12% tween 80 to control group and test group which was treated with OFCMT dissolved in tween80.

Acute toxicity studies indicated that doses up to 2000 mg/kg were safe following oral administration in rats, where as in mice doses up to 1000 mg/kg were safe. Based on this study 250 and 500 mg/kg were used for the evaluation of anti inflammatory activity in rats and 100 mg/kg and 200 mg/kg were used for evaluation of analgesic activity in mice. Analgesic activity was performed by hot plate method. The significant reduction in reaction time of mice in hot plate model suggests its centrally mediated analgesic activity. Cholinergic system is also one of important mechanism which interferes in modulation of pain. Pain is considered as an unpleasant sensation which usually involves a protective mechanism for the body which occurs whenever any tissue is damaged [9]. The significant reduction in reaction time of mice in hotplate model suggests its centrally mediated analgesic activity [10]. The evaluation of central analgesic activity though hotplate test was selected due to several advantages such as sensitivity to strong antinociceptive and limited tissue damage [11].

Inflammation is a complex pathophysiological response to different stimuli. It can be treated and resolved by acting on the different mediators, enzymes, and pathways implicated in the process. [12] The inflammatory process occurs as a defensive response, which induces profound physiological adaptations triggered in an attempt to limited tissue damage and removes the pathogenic insult [13]. By activating the enzyme cyclooxygenase, the levels of prostaglandins, especially PGE2, increases markedly and its production provokes inflammation [14], pain and fever [15]. Turpentine induced edema was used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2h) of the turpentine induced inflammation model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the surrounding damaged tissue. The late phase is sustained by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages. Since OFCMT significantly inhibited paw oedema induced by turpentine in the second phase, these findings suggests a possible inhibition of cyclooxygenase synthesis by OFCMT and this effect is similar to that produced by non steroidal inflammatory drugs such as indomethacin.

Literature review evidenced the medicinal significance of monoterpenoids like menthol and thymol which were the constituents of OFCMT. Hence it can also be suggested that the analgesic and anti inflammatory activity of OFCMT might be due to the presence of monoterpenoids [16].

As OFCMT showed tremendous protection from pain and inflammation in experimental animal models. The study can be further extended for clinical trials and attempt can be made for the patency of the same.

ABBREVIATION

OFCMT-Oily formulation containing camphor, menthol and thymol, CPCSEA-Committee for the Purpose of Control and Supervision on Experimental Animals, OECD-Organisation For Economic Cooperation And Development, ANOVA-Analysis Of Variance, SEM-Standard Error Of Mean

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CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest

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