Essential oils are used for complementary treatment of migraine. Winsor type III microemulsion formulations containing peppermint oil and eucalyptus oil were prepared. In vitro and in vivo studies were carried out to compare the properties and efficacy of the developed formulations. The area of microemulsion existence obtained using Tween80/Span80/PEG400/Water system was studied for the two formulations. The developed formulations were characterized for pH, particle size and viscosity. The microemulsion was found to be stable on centrifugation at 6000 rpm for 10 min. The in vitro diffusion studies on developed formulations showed a release profile of 88% in 3 h. The results were supported by the findings of Ex vivo diffusion studies. Animal studies to quantify the sedative and calming effects of the intranasal formulations were carried. Peppermint oil formulations were found to be better for complementary treatment of migraine compared to marketed and eucalyptus oil containing formulations. However, all the developed formulations can be used for their antimigraine activity. The intranasal spray of peppermint oil is a cost effective formulation as the excipients used are easily available. Also, it is an efficient formulation which provides rapid onset of action and complementary treatment of migraine.

Keywords: Microemulsion, Antimigraine activity

INTRODUCTION

Migraine headaches are a common medical problem that physicians frequently encounter in their practice. A migraine headache is felt as a throbbing or pulsating headache that is often on one side (unilateral) and associated with nausea; vomiting; sensitivity to light, sound, and smell; sleep disruption; and depression. Attacks are often recurrent and tend to become less severe as the migraine sufferer ages. Migraine is the name given to severe headaches, normally lasting from 4 to 72 hours. During migraine, there is constriction or dilation of the blood vessels. Some of the factors that can trigger a migraine attack include anxiety, stress, lack of food or sleep, exposure to light and hormonal changes (in women). Migraine headaches are sometimes called vascular headaches. Vascular means having to do with the blood vessels. Studies suggest that a migraine is caused by swelling of the blood vessels in the scalp and tissues around the brain, causing more blood to pump through the brain. Most primary headaches slowly develop over minutes to hours. The pain experienced in headache is transmitted by the slowest of all unmyelinated nerves. Unmyelinated nerves lack a myelin sheath, or covering, and send impulses slowly.

Migraines can be disabling, leading to the individual’s suffering if not treated appropriately and quickly. There is a variety of medications and treatment approaches that can be used to relieve pain and any associated symptoms.

Since the sense of smell is altered and often heightened during a migraine, aromatherapy is definitely best used between attacks use at the use at the earliest stage of migraine. Aromatherapy is the practice of using essential oils extracted from plants for both physiological and psychological treatment. Lavender, Peppermint, Bay, Melissa, rosemary, Eucalyptus are some of the oils recommended for complementary treatment of migraine headaches. A central feature of the natural approach to migraine is to distinguish between a hot migraine, where the blood vessels are dilated and a cold migraine, where there is excessive constriction of the blood vessels. In first type a cold or perhaps just cool compress across the forehead will give relief and oils of Peppermint or Lavender can be used. The major active ingredient of peppermint oil is menthol that has soothing, stimulant and antidepressant effect. Aromatherapy using the intranasal route is the simplest and therapeutically effective route for migraine. Intranasal route is not only an alternative for systemic delivery of drug but preferentially also targets the drug to CNS. CNS penetration is favoured by low molecular weight and lipophilicity. Lipophilic entities can be smuggled across the blood brain barrier as lipophilic precursor's thus direct delivery of these molecules to the brain through cerebrospinal fluid. Drugs delivered intranasally are transported along olfactory sensory neurons to yield significant concentrations in the cerebrospinal fluid. Bioavailability of these drugs through nasal route is comparable to intravenous route. Neuronal connections between the nasal mucosa and the brain provides unique pathway for the non invasive delivery of therapeutic agents to the brain by bypassing the BBB (Blood Brain Barrier). BBB is a tight junction composed of single layer of cell which together separates the brain and the cerebrospinal fluid from the blood. Essential oils are lipophilic in nature and thus can pass the BBB through olfactory route and show their potential effects. Inhaled aromatherapy is a popular and gentle treatment to reduce mild anxiety. For developing formulations there are many critical factors which need to be taken into consideration. Also there are various types of formulations that are available for intranasal administration. However, microemulsions are preferred for intranasal delivery as they are thermodynamically stable, transparent system, dispersions of oil and water stabilized by an interfacial film of surfactant molecules. Microemulsions can cross the blood brain barrier due to their stable nature. Headaches may be alleviated because of microemulsion administration through nasal route by crossing the BBB. Hence attempts are made to develop microemulsion of peppermint oil for intranasal delivery.

In the present work we aim to develop intranasally administered microemulsion formulations of peppermint oil. Inhalation of these formulations will help in complementary treatment of migraine headaches. Efficacy of the developed formulations has been compared with the marketed formulation containing sumatriptan using motility models in rats as animal model.

MATERIALS AND METHODS

Peppermint oil, Eucalyptus oil (Vedic Life Sciences, Mumbai), Tween80, Span80 and PEG400 (S. D. Fine Chemicals Ltd. and Merck Ltd.), nasal spray pumps (Valois Pvt. Ltd.) All the other reagents and solvents were of analytical grade.

Experimental method

Microemulsion system was developed for peppermint as well as eucalyptus oil. In microemulsion, micelles that are colloidal aggregates formed above critical micelle concentration (CMC) are
formed. The core of micelle is an exclusive region where substances that are incompatible with the continuous phase can enter spontaneously in a process called solubilisation.

I) Formulation of Microemulsion using Pseudo ternary Phase Diagram:

In the present work an attempt was made to develop microemulsion formulations where, the oil phase and the water exist in equilibrium and surfactants are concentrated in surfactant rich Bicontinuous middle phase. In the present study combination of surfactant Tween 80: Span 80 (1:0.5) incase of peppermint oil, and Tween 80: Span 80 (1:0.5) was used as the surfactant medium in the diffusion cell. The receptor medium in the diffusion cell. Mucosa was sandwiched between the receptor compartment and donor compartment so that the dermal portion was continuously bathed with the receptor fluid maintained at 37 ± 1°C by circulating water bath and nasal mucosa side exposed to ambient temperature. The content of the receptor fluid was stirred continuously using a magnetic stirrer. Samples were withdrawn at different time intervals, replaced with same volume of fresh solution, filtered, and amount of drug was determined by HPTLC method detected at 540 nm for eucalyptus oil and 545nm for peppermint oil. A plot showing release pattern of the two formulations through sheep nasal mucosa as membrane was plotted as indicated in figure 4.

V) Histological studies:

Developed microemulsion formulations of the essential oils were applied for 24 hr on the excised sheep nasal mucosa mounted on the diffusion cell. Formulations were removed; mucosa was wiped off with tissue paper and fixed with neutral carbonate formalin solution in saline for atleast 72 hr before routine processing the tissue was sectioned vertically and each section was dehydrated and embedded in paraffin wax. Tissues were divided into small pieces and stained with hematoxylin and eosin. All sections were then examined under a microscope (10X). Nasal mucosa not treated with any formulation served as a control. Such studies have been carried out by Charoo et al, 2008 on excised skin to study the effect of transdermal formulations. Histopathological studies results are indicated in figure 5.

VI) In vivo efficacy studies of the developed microemulsion formulations:

Exhalation of essential oils provides calming and comforting effect to the patient which is necessary for migraine sufferers. To collect detailed information on the sedative effect of the oil, motility of the animals was ascertained after inhalation therapy compared to caffeine induced agitation 10. The effect was observed and compared for nasal formulations containing eucalyptus as well as peppermint oil.

Digital photoactometer was used to study the motility of the animals. Rats were divided in six each in three groups.

Group 1: Positive control – Caffeine

Group 2: Developed intranasal formulation of peppermint oil

Group 3: Developed intranasal formulation of eucalyptus oil

Group 4: Marketed formulation containing sumatriptan

Animals in the positive control group were agitated by injecting intraperitoneal 0.5mL of 0.1% solution of caffeine. Developed formulation and marketed formulation containing sumatriptan, 100 µL each were given intranasally and motility was noted for each of the animals using digital actophotometer. The effect on motility was recorded at predetermined time interval of 30, 60, 120 and 180 mins.

Percent reduction in the motility was calculated using the formula

\[
\% \text{ reduction} = \frac{\text{Caffeine treated group - Formulation treated group}}{\text{Caffeine treated group}} \\
\]

Percent reduction in the motility for all the three groups was compared by plotting graph of percent reduction in motility versus time.

RESULTS AND DISCUSSION

I) Formulation of Microemulsion using Pseudo ternary Phase Diagram:

With the help of tridraw software, pseudo ternary phase diagram were constructed for developed microemulsions. The amount of water incorporated for each of the oil: Smix ratio was noted. As depicted in ternary phase diagram, figure 1, area for microemulsion formation is the shaded region. Microemulsions obtained in this region are Winsor type III formulations, bicontinuous gels where water phase exist in equilibrium with oil phase. Approximately, 25% of water was incorporated when the ratio of Smix: oil was adjusted...
to 9:1 with peppermint oil. However, the amount of water in microemulsion formulation with eucalyptus oil was 20%. PEG 400 was present in the formulation in the concentration of 23%. This amount helped in decreasing the interfacial tension to very low values. Lowering of interfacial tension helped in greater incorporation of water. Hence, the amount of water incorporated in peppermint oil formulations increased from 25% to 28% and for eucalyptus oil formulation the water uptake capacity was found to be 26%. Figure 1 indicates the ternary phase diagram for formulations containing peppermint oil and eucalyptus oil with and without PEG 400. As seen in the figure when PEG 400 is added into the formulations the water uptake capacity is also increased.

![Fig. 1: Ternary phase diagram for microemulsion formulation Smix (Tween 80: Span 80 (1:0.5) and Cosurfactant PEG 400 (0.5gm))](image)

**II) Characterization of Developed formulations:**

Formulations containing Smix:oil in the ratio of 9:1 were selected for further characterization and evaluation.

Globule size: The size range of the developed formulation was found to be 300-400nm

Figure 2 indicates a comparative graph of the globule size for formulations containing essential oil. The globule size obtained for peppermint oil emulsion is smaller than eucalyptus oil, probably due to greater water uptake capacity by these formulations.

The viscosity obtained for formulations containing peppermint oil is greater compared to that of eucalyptus oil, hence it can be postulated that the formulations will be retained on the nasal mucosa for longer period of time and will not drain easily.

**III) In vitro in vivo drug diffusion study:**

Amount of the peppermint oil and eucalyptus oil as active diffused through cellulose acetate membrane was found to be approximately 80-90%. As indicated in the figure 3, the amount of active diffused through the membrane was almost similar. Thus the two formulations behave almost similar with respect to diffusion studies.

**IV) Ex vivo drug diffusion study:**

As indicated in the figure 4 amount of the active diffused through the sheep nasal mucosa was found to be approximately 70-80%. The amount of the drug diffused through the cellulose acetate membrane and nasal sheep mucosa differs marginally. The reason is the difference in the thickness between the two membranes. However the results indicate that a direct in vitro in vivo correlation can be developed for the intranasal delivery of the essential oils.

![Fig. 2: Globule size comparison for the formulations containing peppermint oil and eucalyptus oil.](image)
Other parameters for which the formulations were characterized are indicated in Table 1 with their results.

**Table 1: Physicochemical characterization of developed formulation**

<table>
<thead>
<tr>
<th>Characterisation of microemulsion formulations</th>
<th>Peppermint oil</th>
<th>Eucalyptus oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>Globule Size</td>
<td>350-400nm</td>
<td>350-400nm</td>
</tr>
<tr>
<td>pH</td>
<td>5.8-6.0</td>
<td>5.6-6.0</td>
</tr>
<tr>
<td>Stability</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>Oil content</td>
<td>97-107%</td>
<td>95-105%</td>
</tr>
<tr>
<td>Viscosity</td>
<td>200-400cps</td>
<td>120-250cps</td>
</tr>
</tbody>
</table>

![In vitro diffusion studies through cellulose acetate membrane](image1)

**Fig. 3: Diffusion studies of the essential oil containing formulations through cellulose acetate membrane**

![Ex vivo diffusion studies through sheep nasal mucosa](image2)

**Fig. 4: Diffusion studies of the essential oil containing formulations through sheep nasal mucosa**

**V) Histological studies**

A comparative diffusion study for Bicontinuous gels was performed on porcine nasal mucosa and further compared with untreated control group. The influence of active on the anatomical structure of the nasal mucosa was elucidated with the aid of light microscope findings. Section of the untreated control group showed normal uniform layered epithelium. No significant changes in the mucosal thickness were seen in biopsies from the skin section treated with formulations as shown in figure 5.
a) Control (Untreated nasal mucosa)  
b) Peppermint oil formulation treated nasal sheep mucosa  
c) Eucalyptus oil formulation treated nasal sheep mucosa

Figure 5: Histological studies:  

X In vivo efficacy studies of the developed microemulsion formulations:

A considerable reduction in % inhibition of activity was found with all the formulations as seen in figure 6. At the end of three hours 74% reduction in motility with formulations containing peppermint oil was seen. This activity was greater compared to marketed formulations containing sumatriptan and formulations prepared using eucalyptus oil. Thus the developed formulations have an efficacy to reduce the motility and produce calming and comfort in the excited animal.

Approximately 58% reduction in motility was seen with eucalyptus oil formulations and 68% reduction in marketed formulations containing Sumatriptan. Thus peppermint oil formulations are better for complementary treatment of migraine compared to marketed and eucalyptus oil containing formulations. However all the developed formulations can be used for their antimigraine activity.

CONCLUSION

Developed formulations showed potential in relieving migraine related headache. Essential oils are useful and effective approach in alleviating the pain of migraine sufferers. Developed intranasal formulation provides comfort and soothing effect. Amongst the developed formulations, peppermint oil containing preparations showed greater efficacy compared to the marketed and eucalyptus oil containing formulations. Thus peppermint oil formulations are better for complementary treatment of migraine compared to marketed and eucalyptus oil containing formulations. However, all the developed formulations can be used for their antimigraine activity. The effect needs to be further investigated by conducting detailed clinical trials.

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