8.1 INTRODUCTION

Coughing is a normal physiological response to irritation of the laryngeo-tracheobronchial system caused by mechanical or chemical stimulation. It may be painful, fatiguing, and requires suppression by antitussive drugs. Cough is the most common symptom for which individuals seek medical advice (Schappert, 1997; Burt and Schappert, 2004). An acute cough is often the most prominent symptom of common cold, which in itself is the most frequent illness to afflict mankind. Nocturnal bouts of cough, especially in children with upper respiratory tract infections adversely affect sleep (Paul et al., 2004; Lee and Birring, 2010). In USA, the direct and indirect costs of the common cold afflictions have been estimated to the tune of US$40 billion per annum (Fendrick et al., 2003).

The most effective antitussives available so far are centrally acting opioid derived such as codeine (1), dihydrocodeine, and pholcodeine (2) which have side effects like nausea, vomiting, drowsiness, respiratory depression, constipation, and often physical dependence at their effective doses, Fig. 47. The other antitussives with a diverse chemical architecture such as a phenanthrene derived dextromethorphan (3), nociceptin, an endogenous peptide ligand SR48968 (4), a dual NK2 and NK3 receptor antagonist NS1619 (5), a K⁺ channel opener having hypotension as the major adverse effect and many more have been developed.

![Chemical structures](image-url)
Antiussive drugs basically act by increasing the threshold of cough by acting on CNS or reduce the tussal impulse by acting peripherally in the respiratory tract.

Menthol have been used either directly as pharmaceutical agents or as flavoring agent for pharmaceutical products for example it has been used in tooth paste mouthwash, shaving cream, cigarettes, and oral pharmaceutical preparations, and over-the-counter drugs for common cold (Gelal et al., 1999). Peppermint oil, in which menthol is the major constituent, is a naturally occurring carminative, which relaxes gastrointestinal muscle and is used for the treatment of irritable bowel syndrome (IBS) (Samerville et al., 1984; Rees et al., 1979). Mint tea, which contains a high content of menthol among other ingredients, is a herbal remedy for the treatment of digestive disorders (Gelal et al., 1999). Menthol is a specific stimulant of cold receptors; increases neural discharge by inhibiting the efflux of calcium from the cold receptor, and thus increase the afferent activity of cold sensors. Thus, inhalation of menthol may act to inhibit respiration through stimulation of upper airway cold receptors, and menthol may act to inhibit cough through this mechanism (Gelal et al., 1999). Camphor and menthol are common ingredients found in topically applied analgesics and rubifacients for the treatment of minor muscle aches and pains (Valdez et al., 1999)
8.2 AIM OF WORK

Menthol (8) and camphor (9) are members of a class of monoterpenoids also known collectively as volatile or aromatic oils, which have been widely used in the symptomatic treatment of upper respiratory tract infections (Laude et al., 1994). Both experimental animal and clinical studies convincingly evidenced the antitussive activity of menthol and camphor against citric acid induced cough models (Packman and London, 1980; Laude et al., 1994; Morice et al., 1994). Recently reported croomine-type alkaloids (6 and 7; Lin et al., 2008) have been found to exhibit strong antitussive activity. These compounds also possess azepino moiety fig. 48. Thus the aim of the present study was nitrogen insertion in the structures of menthol and camphor, as azepine nucleus has already been proved to be an important structural feature for antitussive activity.

![Dehydrocroomine (6)](image1)

![10-hydroxycroomine (7)](image2)

**Figure 48**

![menthol (8)](image3)

![menthol lactam (10)](image4)

**Figure 49**

![camphor (9)](image5)

![camphor lactam (11)](image6)

**Figure 50**
8.3 RESULTS AND DISCUSSION

8.3.1 Synthesis of menthol lactam

\[
\begin{align*}
\text{8} & \xrightarrow{\text{a}} \text{12} & \text{OH} & \text{10} \\
\text{b) } & \text{a} & \text{b} & \text{c}
\end{align*}
\]

- a) CrO\(_3\)/H\(_2\)SO\(_4\), acetone
- b) NH\(_2\)OH·HCl/NaOH, methanol
- c) C\(_6\)H\(_5\)SO\(_2\)Cl/NaOH, THF

Figure 51

8.3.2 Synthesis of camphor lactam

(9) \quad (11)

a= hydroxylamine-O-sulfonic acid/glacial acetic acid

Figure 52

For the synthesis of compound 10, 8 was oxidized to menthone (12) by chromic acid, which underwent oxime formation with NH\(_2\)OH followed by Beckmann rearrangement by tosylation in alkaline medium. The synthesis of camphidone (11) was carried out, by treatment of camphor with hydroxylamine-O-sulfonic acid involving a Beckmann like rearrangement of camphor (Krow and Szczepanski, 1980).
8.3.3 Pharmacology

The antitussive activity of synthesized menthol and camphor lactams was compared with that of menthol and camphor against coughing induced by citric acid in conscious guinea pigs. (Laude et al., 1994) demonstrated that both guinea pigs and humans respond to similar concentrations of citric acid and well correlated with a concentration-response relationship, although it has been associated with the difficulties in calculating the actual inhaled dose. The present author conducted the following set of experiments on guinea pigs for the evaluation of 10 and 11 as antitussive activity. There were no sign of irritation or adverse behavioral responses observed in the guinea pigs on exposure to aerosolized test solutions employed during the study.

8.3.3.1 Pharmacological evaluation

Effect of aerosolized menthol (8) and menthol lactam (10) on cough frequency and latency induced by citric acid in guinea pigs

The pre-treatment of aerosolised menthol for 5 min at doses (15 μg/l, 30 μg/l and 60 μg/l) significantly reduces cough response induced by citric acid as compared with vehicle exposed group. Similarly, prior exposure of aerosolised menthol lactam for 5 min at doses (15 μg/l, 30 μg/l and 60 μg/l) has dose dependently reduced cough response induced by citric acid (fig. 53)
**Figure 53.** Effect of aerosolized menthol and menthol lactam on citric acid induced cough frequency. Values are expressed as mean ± SEM. a= p< 0.05 Vs VC, b= p< 0.05 Vs menthol 15μg/l, c= p<0.05 menthol lactam 15μg/l; d= p<0.05 menthol lactam 30μg/l; e: p<0.05 menthol 60μg/l. Abbreviations; VC, vehicle control; Ment, menthol; Ment.Lact, menthol lactam.

Further, latency to initial cough response was significantly increased only with doses (30μg/l and 30μg/l), but not with a dose (15μg/l) of menthol pre-treatment. In addition, prior exposure to menthol lactam at all the doses employed in the present study significantly increased latency to initial cough response induced by citric acid (Fig 54).

**Figure 54.** Effect of aerosolized menthol and menthol lactam on latency time of cough induced by citric acid. Values are expressed as mean ± SEM. a= p< 0.05 Vs VC; b= p< 0.05 Vs menthol lactam 15μg/l; c= p<0.05 Vs menthol lactam 15μg/l; d= p<0.05 Vs menthol lactam 30 μg/l; e: p<0.05 menthol 60μg/l. Abbreviations; VC, vehicle control; Ment, menthol; Ment. Lact, menthol lactam.
Effect of aerosolized camphor and camphor lactam on number of coughs reduced and latency time increased by citric acid in guinea pigs

The pre-treatment of aerosolised camphor for 5 min at doses (125 μg/l, 250 μg/l and 500 μg/l) significantly reduces cough response induced by citric acid as compared with vehicle exposed group. Similarly, prior exposure of aerosolised camphor lactam for 5 min at doses (125μg/l, 250μg/l and 500μg/l) has dose dependently reduced cough response induced by citric acid (Fig. 55).

![Cough frequency graph](image)

**Figure 55.** Effect of aerosolized camphor and camphor lactam on citric acid induced cough frequency. Values are expressed as mean ± SEM. a: p< 0.05 Vs VC, b: p< 0.05 Vs camphor 250 μg/l, c: p<0.05 camphor lactam 125μg/l, d: p<0.05 camphor lactam 250μg/l, e: p<0.05 camphor 500μg/l. Abbreviations; VC, vehicle control; Camp, camphor; Camp. Lact, camphor lactam.

In addition, camphor lactam (500μg/l) was noted to produce a greater inhibitory cough response as compared to camphor at the same dose. Further, latency to initial cough response was only significantly increased with prior exposure of camphor at a dose (500μg/l), but not at doses (125μg/l and 250μg/l). In addition, prior exposure to menthol
lactam at doses (250μg/l and 500μg/l) has significantly increased latency to initial cough response induced by citric acid (Fig. 56).

**Figure 56.** Effect of aerosolized camphor and camphor lactam on latency time of cough induced by citric acid. Values are expressed as mean ± SEM. a: p< 0.05 Vs VC; b: p< 0.05 Vs menthol lactam 15μg/l; c: p<0.05 Vs menthol lactam 30 μg/l. Abbreviations; VC, vehicle control; Camp, camphor; Camp. Lact, camphor lactam.

8.4 EXPERIMENTAL

8.4.1 Oxidation of menthol: synthesis of (2S, 5R)-(−)-2-isopropyl-5-methylcyclohexanone (12)

To a stirred solution of menthol (6 g, 3 mmol) in acetone (10 ml), Jones reagent (20 ml) was added dropwise. The progress of reaction was monitored by TLC. The reaction was quenched with water, extracted with chloroform (30x3ml) and concentrated under reduced pressure to afford the crude product. The pure product (12) (yield 5.1 g, 86%) was obtained by fractional distillation of the crude product.

Bp 207–209°C; IR (KBr, ν): 2956, 1710 (C=O) cm⁻¹, ¹H NMR (CDCl₃, 200 MHz, TMS at 0): δ 0.84 (3H, d, J = 6.81 Hz), 0.91 (3H, d, J = 6.81 Hz), 0.95 (3H, d, J = 6.43 Hz), 2.34 (1H, m), 1.71 (2H, m), 1.71(6H, m); MS (EI): m/z (rel. intensity) 154 (M⁺).
8.4.2 Oximation of 12: synthesis of (2S, 5R)-(-)-2-isopropyl-5-methyl cyclohexanone oxime (13)

A mixture of 12 (3.00 g, 0.02 mol), sodium hydrogen carbonate (2.00 g, 0.02 mol) and hydroxylamine hydrochloride (1.50 g, 0.02 mol) in methanol (15 ml) and distilled water (2 ml) was heated to 65°C for 3 h. After completion of the reaction (TLC) the mixture was diluted with distilled water (15 ml) and then extracted with hexane (30x3ml). The extract was washed successively with 5% NaHCO₃ and saturated NaCl solutions and dried over anhydrous sodium sulfate. After evaporation of solvent the crude product was distilled under reduced pressure to afford the pure product 13 (2.24 g, 68%).

Mp 54–56°C, IR (KBr, ν): 3288 (–OH), 1711 (C=NOH), 1456 (s) cm⁻¹; ¹H NMR (CDCl₃, 200 MHz, TMS at 0): δ 0.95 (9H, m), 1.86 (1H, m), 1.45 (8H, m), 2.21 (N–OH); MS (EI): m/z (rel intensity) 169 (M⁺).

8.4.3 Synthesis of (2S, 5R)-(-)-2-isopropyl-5-methyl-1-azacycloheptane-7-one (10)

13 (3.38 g, 0.02 mol) was added to 2.40 g (0.06 mol) NaOH in distilled water (15 ml) and THF (15 ml) solution. The mixture was stirred for 8 h under cooling at 10°C. Tosyl chloride (6.50 g, 0.04 mol) in THF (5 ml) was added drop wise. Reaction mixture was additionally stirred under cooling for 4 h and then for 2 h at 50°C. After evaporation of solvent the mixture was diluted with distilled water and extracted with diethyl ether (15 x 3 ml). Extract was washed with 5% NaHCO₃ solution and then with saturated NaCl. Removal of the solvent under reduced pressure afforded crude product which was then purified by column chromatography (hexane:ethyl acetate:70:30) on silica (60–120 mesh) to yield the pure product 10 (1.5 g, 44%).
Mp 121–122ºC, IR (KBr, v): 3415, 3079, 2965, 2922, 1665, 1637, 1456, 1373, 776 cm⁻¹;
¹H NMR (CDCl₃, 200 MHz, TMS at 0): δ 0.94 (6H, dd, J = 1.18 and 1.14 Hz), 1.01 (3H, d, J = 6.65 Hz), 1.30 (2H, m), 1.86 (4H, m), 2.38 (2H, m), 3.17 (1H,m), 5.5 (1H, bs, D₂O exchangeable proton); ¹³C NMR (CDCl₃, 50 MHz, TMS at 0): δ 18.99 (C-1), 18.93 (C-9), 24.85 (C-10), 30.48 (C-8), 32.63 (C-4), 33.06 (C-50), 39.34 (C-6), 45.01 (C-3), 59.16 (C-7), 177.12 (C-2); MS(EI): m/z (rel. intensity) 169 (M⁺).

8.4.4 Synthesis of (1S)-1,8,8-trimethyl-3-azabicyclo[3.2.1]octan-2-one (11)
Camphor (2.77 g, 0.03 mol) and hydroxylamine-O-sulfonic acid (3.08, 0.03 mol) were dissolved in 60 ml glacial acetic acid and refluxed for 24 h. The reaction mixture was concentrated under reduced pressure and adsorbed on silica (60–120 mesh) for column chromatography. The desired product (1.75 g, 57%) was eluted from the column (hexane:ethyl acetate:65:35).

IR (KBr, v): 3310, 3220, 1667 cm⁻¹; ¹H NMR δ (CDCl₃, 200 MHz, TMS at 0): 5.90 (1H, D₂O exchangeable proton), 3.44 (m, 1H, J = 11.1, 3.5, 1.2, 1.6 Hz), 2.99 (m, 1H, J = 11.1, 2, 2.0 Hz), 2.22-1.42 (m, 5H), 1.10 (s, 3H), 1.04 (s, 3H), 0.96(s, 3H); ¹³C NMR (CDCl₃, 50 MHz, TMS at 0): δ 13.39, 19.32, 23.05, 27.76, 37.96, 42.32, 43.71, 47.20, 52.27, 178.98; MS (EI): m/z (rel intensity) 167 (M⁺).

8.4.5 Pharmacological Evaluation

Menthol lactam and Camphor lactam were evaluated for antitussive activity using citric acid induced cough model on guinea pigs.

The activity was done in collaboration with Pharmacology department ISF College of Pharmacy, Moga, with the help of Mr. B.V.K. Reddy
8.4.5.1 Selection criteria of animals

Animals were maintained in animal house and were exposed to normal cycles of day and night under standard conditions, temperature 25 ± 2°C and relative humidity 55–65%. Animals underwent a screening procedure before pre-treatment with drugs. On the first day after a 3 min acclimatisation period was animal are firstly exposed to normal saline and subsequently 5 min later they were exposed to aerosolised 7.5 % citric acid for a period of 10 min. Animal selection criteria was done based on the following, either number of coughs are < 7 and > 15 after 7.5% citric acid exposure or if any animal coughed on exposure of aerosolised saline were excluded as this was taken as an indication of infection or hyper reactivity. Cough challenge was given at the same time of day for each animal and minimum of 24 h was allowed between challenges to eliminate any short term prophylaxis (Laude et al., 1994).

8.4.5.2 Antitussive assay

Animals were allowed free access to food and water up to the time of testing. Each animal was placed in a Perspex chamber, dimensions 30 cm X 20 cm X 20 cm and exposed to a nebulised aqueous solution of 7.5% w/v citric acid for a period of 10 min. The output of the nebuliser (Inco Laboratories, Ambala, India) was 0.25 ± 0.02 ml solution per minute and same nebuliser was used throughout the experiment. The animals were watched continuously by the trained observer, who blinded to the treatment given and number of coughs and latency time to initial cough response was noted. Coughs could easily be distinguished from sneeze since there is a clear difference in sound as well as in the behaviour of the animals (Fox, 1996).
Figure 57. Perspex chamber, with nebuliser, was used for the whole Antitussive evaluation of synthetics

8.4.5.3 Experimental protocol

The protocol of the present study was approved by the Institutional Animal Ethical Committee. Albino guinea pigs weighing 400-600 g of either sex were selected and thirteen groups (n=6) groups were employed in the present study. All the drugs were initially dissolved in dimethyl sulfoxide (DMSO) and then diluted to final concentration in a normal saline and treatment was given by exposure in nebulised form for 5 min. 1 min later the pre-treated animals in all groups were exposed to nebulised citric acid (7.5 w/v) in normal saline for 10 min. The experimental protocol was employed as following:

Group I: Vehicle Control (0.01 % DMSO in normal saline) pre-treated;

Group II: Menthol (15μg/l) pre-treated;

Group III: Menthol (30μg/l) pre-treated;

Group IV: Menthol (60μg/l) pre-treated

Group V: Menthol lactam (15μg/l) pre-treated;
**Group VI:** Menthol lactam (30μg/l) pre-treated;

**Group VII:** Menthol lactam (60μg/l) pre-treated;

**Group VIII:** Camphor (125 μg/l) pre-treated;

**Group IX:** Camphor (250 μg/l) pre-treated;

**Group X:** Camphor (500 μg/l) pre-treated;

**Group XI:** Camphor lactam (125μg/l) pre-treated;

**Group XII:** Camphor lactam (250μg/l) pre-treated;

**Group XIII:** Camphor lactam (500μg/l) pre-treated.

There were no sign of irritation or adverse behavioural responses observed in the guinea pigs on exposure to aerosolized test solutions employed the present study.