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Chemical composition and mechanisms underlying the spasmolytic and bronchodilatory properties of the essential oil of *Nepeta cataria* L.

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ABSTRACT

Aim of the study: The study was aimed to investigate the chemical composition and pharmacological basis for traditional use of essential oil of *Nepeta cataria* L. (Limiaceae) (Nc.Oil) in gastrointestinal and respiratory disorders.

Materials and methods: Chemical analysis was carried out through GC-EIMS, ¹³C NMR and Kovats Retention Indices while pharmacological study was carried out in isolated tissues preparations.

Results: Four major components; 1,8-cineol (21.00%), α -humulene (14.44%), α -pinene (10.43%) and geranyl acetate (8.21%) were identified among the 27 compounds in Nc.Oil. In isolated rabbit jejunum, Nc.Oil, papaverine and verapamil inhibited spontaneous and high K⁺ (80 mM) precontractions, as well as shifted the Ca²⁺ concentration–response curves (CRCs) to right, indicating calcium channel blocking activity. In isolated guinea-pig trachea, Nc.Oil and papaverine inhibited carbachol (1 μ M) and K⁺ precontractions with similar potency, while verapamil was more potent against K⁺. Nc.Oil also potentiated isoprenaline inhibitory CRCs, similar to papaverine, indicating papaverine-like PDE inhibitor activity. In isolated guinea-pig atria, Nc.Oil caused cardiodepression at around 25–80 times higher concentrations, similar to papaverine.

Conclusions: These data indicate that *Nepeta cataria* possesses spasmolytic and myorelaxant activities mediated possibly through dual inhibition of calcium channels and PDE, which may explain its traditional use in colic, diarrhea, cough and asthma.

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1. Introduction

Nepeta cataria L. (family, Limiaceae; order, Lamiales), found in the Eastern Mediterranean, Southern Asia and China, is commonly known as “Catnip or Catmint” because of its irresistible action on cats. Due to lemony mint flavor it finds the ways in the herbal teas as well as in cooking. Medicinally, the plant is used in gastrointestinal and respiratory hyperactive disorders such as colic, diarrhea, cough, asthma and bronchosis (Duke, 2002; Baranauskiene et al., 2003).

Various compounds have been identified by different groups of workers in the essential oil of *Nepeta cataria*, the composition of which vary from region to region, variety, analytical and extrac-

tion methods, climatic conditions and vegetation period. The main constituents so far identified, include β -caryophyllene, caryophyllene oxide, 1,8-cineol, citronellol, geraniol, elemol, nerol, nerolidol, spathulenol, β -elemene, geranyl acetate, citronellyl acetate and geranial (Kalpoutzakis et al., 2001; Baranauskiene et al., 2003; Mortuza-Semmani and Saeedi, 2004; Schultz et al., 2004; Sajjadi, 2005). In addition, the plant also contained nepetalactones and alkaloids, such as, actinidine and iridomyrmecine (Kalpoutzakis et al., 2001). A limited number of studies exists on its biological activities; include antibacterial (Kalpoutzakis et al., 2001), antifungal (Nostro et al., 2001), analgesic (Aydin et al., 1998) and behavioral (Massoco et al., 1995).

Essential oil from *Nepeta cataria* L. grown in Pakistan has not been explored yet for its chemical composition or its biological effects related to medicinal use in hyperactivity of gut and airways disorders. This study was carried out to investigate the chemical composition of the essential oil and to explore the mechanism(s) involved in the medicinal use of the plant for hyperactive

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gut and respiratory disorders, such as colic, diarrhea, cough and asthma.

2. Materials and methods

2.1. Drugs and standards

The following reference chemicals were obtained from the sources specified: acetylcholine chloride, carbamylcholine chloride (carbachol), isoproterenol hydrochloride, papaverine hydrochloride and verapamil (Sigma Chemical Company, St. Louis, MO, USA). All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh in normal saline on the day of experiment.

The essential oil of *Nepeta cataria* was obtained from the HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan.

2.2. Determination of chemical composition of the essential oil

2.2.1. Instrumentation and identification

Gas chromatography using FID, was carried out on a Shimadzu gas chromatograph GC-17A hooked with Shimadzu Class GC-10 software and equipped with an SPB-5[®] (30 m × 0.53 mm ID × 0.50 μm filter thickness of 5% phenyl 95% methyl silicone) capillary column. The analyses were performed with an initial temperature 50 °C for 5 min, and then ramped with 3 °C/min to a final temperature 210 °C with final time 45 min. Injector with splitting ratio of 1:50 and FID were set at 300 °C. Carrier and make up gas was nitrogen with a flow of 28 and 40 mL/min at a pressure of 1 and 2.5 kg/cm², respectively. Kovats Retention Indices were also calculated (Kovats, 1965). Percentage composition was calculated using area normalization method. For GC-EIMS experiments a Hewlett-Packard 5890 gas chromatograph was combined with a Jeol, JMS-HX 110 mass spectrometer operating in EI mode with ion source at 270 °C and electron energy at 70 eV. Injector was set at 270 °C with splitting ratio 1:30. Analyses were performed on the aforementioned program on an equivalent column HP-5[®] (25 m × 0.22 mm and 0.25 μm film thickness). Mass spectral survey was performed using Mass Spectral Library (NIST, 1998). C NMR (BB and DEPT) spectra were recorded in CDCl₃ on a Bruker Aspect 3000 AM-300 spectrometer operating at 75 MHz (Kubeczka and Formacek, 2002).

2.3. Animals

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Research Council, 1996) and approved by the Ethical Committee of Aga Khan University Karachi. Local rabbits (1.5–2 kg) and guinea-pig (500–550 g) of either sex used in the study were bred and housed in the animal house of Aga Khan University under controlled environment (23–25 °C). Animals were given tap water *ad libitum* and fasted for 24 h before dissection.

2.4. Isolated tissue preparations

2.4.1. Rabbit jejunum

The isolated tissue experiments were carried out as previously described (Gilani et al., 2005a). The animals had free access to water but were fasted for 24 h before the experiment. The animals were sacrificed by cervical dislocation, the abdomen was cut open and the jejunal portion isolated out. Preparations 2-cm long were mounted in 10-mL tissue baths containing normal Tyrode's solution maintained at 37 °C and aerated with a mixture of 5%

carbon dioxide in oxygen (carbogen). The composition of Tyrode's solution, in mM, was: NaCl 136.9, KCl 2.7, MgCl₂ 1.1, NaHCO₃ 11.9, NaH₂PO₄ 0.4, glucose 5.6 and CaCl₂ 1.8 (pH 7.4). A preload of 1 g was applied and the tissues kept undisturbed for an equilibrium period of 30 min after which control responses to a sub-maximal concentration of acetylcholine (ACh; 0.3 M) were obtained and the tissue presumed stable only after the reproducibility of the said responses. Isotonic responses were recorded on Bioscience transducer and oscillograph. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing the relaxant (spasmolytic) activity directly without the use of an agonist (Gilani et al., 2005a).

2.4.1.1. Determination of calcium channel blocking activity. To assess whether the spasmolytic activity of the test substances was through calcium channel blockade (CCB), high K⁺ (80 mM), as KCl, was used to depolarize the preparations (Farre et al., 1991). K⁺ was added to the tissue bath, which produced a sustained contraction. Test substances were then added in a cumulative fashion to obtain inhibitory response curves, which is expressed as percent of the control response produced by high K⁺. Substances which produce relaxation against high K⁺-induced contractions may act through the antagonism of Ca⁺⁺ movements, possibly through voltage-dependent calcium channels (Bolton, 1979).

To confirm the calcium channel blocking (CCB) activity of the test substances, the tissues were allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca⁺⁺-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove calcium from the tissues. This solution was further replaced with K⁺-rich and Ca⁺⁺-free Tyrode's solution, having the following composition: NaCl 91.04, KCl 50, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42, glucose 5.6 and EDTA 0.1 mM. Following an incubation period of 30 min, control concentration response curves (CRCs) of Ca⁺⁺ were obtained. When the control CRCs of Ca⁺⁺ were found superimposable (usually after two cycles), the tissue was pretreated with the test substance for 1 h to test the possible CCB effect. The CRCs of Ca⁺⁺ were reconstructed in the presence of different concentrations of the test material.

2.4.2. Guinea-pig trachea

As described previously (Gilani et al., 2008), guinea-pigs were sacrificed by cervical dislocation. Tracheal ring strips were then mounted in a 20-mL tissue bath containing normal Krebs's solution, maintained at 37 °C and aerated with carbogen to study the tracheo-relaxant activity. The composition of Krebs's solution was (mM): NaCl 118.2, KCl 4.7, NaHCO₃ 25.0, CaCl₂ 2.5, KH₂PO₄ 1.3, MgSO₄ 1.2 and glucose 11.7 (pH 7.4). Under tension of 1 g the tissues were equilibrated for 1 h before the addition of any drug. Carbachol (CCh; 1 μM) and high K⁺ (80 mM) were used to induce sustained contractions and the relaxant activity of the test substances were assessed by adding in cumulative fashion. Responses were calculated as per cent of the agonists-induced contractions. Isometric responses were recorded via a force displacement transducer (model FT-03) on a Grass model 7 Polygraph.

2.4.2.1. Determination of phosphodiesterase inhibitory effect. As described previously (Gilani et al., 2005b), guinea-pig tracheal strips were suspended in normal Krebs's solution in 20-mL organ baths and control isoprenaline (0.003–1 μM) inhibitory CRCs were constructed against CCh-induced contractions. When the control CRCs of isoprenaline were found superimposable (usually after two cycles), the tissue was pretreated with the test substances for 30 min to test the possible potentiating effect. The CRCs of isoprenaline were reconstructed in the presence of different concentrations of the test material.

Table 1
Quantitative and qualitative analysis of essential oil of *Nepeta cataria* L.

Compound	% age	R.I.	Identification
α -Pinene	10.43	936	MS, R1 ^a , ¹³ C ^{b,c}
α -Fenchene	0.85	938	MS, RRT ^d
β -Pinene	1.80	977	MS, R1 ^a , ¹³ C ^{b,c}
α -Phellandrene	0.52	996	MS, R1 ^a , ¹³ C ^{b,c}
α -Terpinene	0.61	1021	MS, R1 ^a , ¹³ C ^{b,c}
1,8-Cineol	21.00	1042	MS, R1 ^f , ¹³ C ^{b,c}
β -cis-Ocimene	2.74	1054	MS, R1 ^f , ¹³ C ^b
β -trans-Ocimene	5.03	1061	MS, R1 ^f , ¹³ C ^b
Terpinene-4-acetate	1.86	1065	MS, RRT ^d
Linalool	1.28	1085	MS, R1 ^a , ¹³ C ^{b,c}
Terpinolene	0.90	1107	MS, R1 ^a , ¹³ C ^b
Terpinene-4-ol	2.29	1203	MS, R1 ^f , ¹³ C ^b
Caran-3 β -ol	2.14	1212	MS
E-Geraniol	6.80	1280	MS, R1 ^f , ¹³ C ^{b,c}
Bornyl acetate	0.37	1297	MS, R1 ^c , ¹³ C ^b
β -Bourbonene	0.21	1393	MS, R1 ^b , ¹³ C ^b
α -Copaene	1.69	1389	MS, R1 ^a , ¹³ C ^b
Geranyl acetate	8.21	1411	MS, R1 ^f , ¹³ C ^b
β -Caryophyllene	6.38	1436	MS, R1 ^a , ¹³ C ^{b,c}
α -trans-Bergamotene	0.21	1443	MS, R1 ^a , ¹³ C ^b
E- β -Farnesene	0.42	1453	MS, R1 ^b , ¹³ C ^b
α -Humulene	14.44	1472	MS, R1 ^f , ¹³ C ^b
Germacrene D	1.52	1497	MS, R1 ^f , ¹³ C ^{b,c}
α -cis-Bisabolene	0.23	1499	MS, R1 ^a , ¹³ C ^b
δ -Cadinene	1.09	1531	MS, R1 ^f , ¹³ C ^{b,c}
β -Caryophylleneoxide	2.37	1599	MS, R1 ^f , ¹³ C ^b
Santalol	2.22	1617	MS

^aSkaltsa et al., 2001; ^bKubeczka and Formacek, 2002, ^cViqar et al., 1992, ^dTopcu et al., 2005, ^eConstantin et al., 2001, ^fBaranauskienė et al., 2003, ^gMortuza-Semmani and Saeedi, 2004, ^hSajjadi, 2005, ⁱIoannou et al., 2007.

2.4.3. Guinea-pig atria

Spontaneously beating paired atria from guinea-pig were mounted in 20-mL tissue baths containing normal Krebs's solution maintained at 32 °C and aerated with carbogen, as described previously (Gilani et al., 2005a). After equilibrium period of 30 min, control responses of isoprenaline (1 μ M) and ACh (1 μ M) were obtained at least in duplicate. The test substances were then added cumulatively and the effect on force and rate of contractions was determined as percent of the pretreated control. Tension changes in the tissue were recorded via a force displacement transducer (model FT-03) using Grass model 7 Polygraph.

3. Statistics analysis

All the biological data expressed are mean \pm standard error of the mean (S.E.M.), and the median effective concentrations (EC₅₀ values) are given with 95% confidence intervals (CI). The statistical parameter applied is the student *t*-test with *P* < 0.05 noted as significantly different.

4. Results

4.1. Chemical composition

As a result of chemical investigation, the essential oil obtained from *Nepeta cataria* L., of North Western Pakistan, 27 compounds were identified (Table 1). The major components were 1,8-cineol (21.00%), α -humulene (14.44%), α -pinene (10.43%) and geranyl acetate (8.21%).

4.2. Effect on isolated rabbit jejunum

When tested in isolated rabbit jejunum preparations, the essential oil of *Nepeta cataria*, inhibited the spontaneous contractions (Fig. 1) with EC₅₀ value of 0.02 \pm 0.10 mg/mL; *n* = 4, thus showing

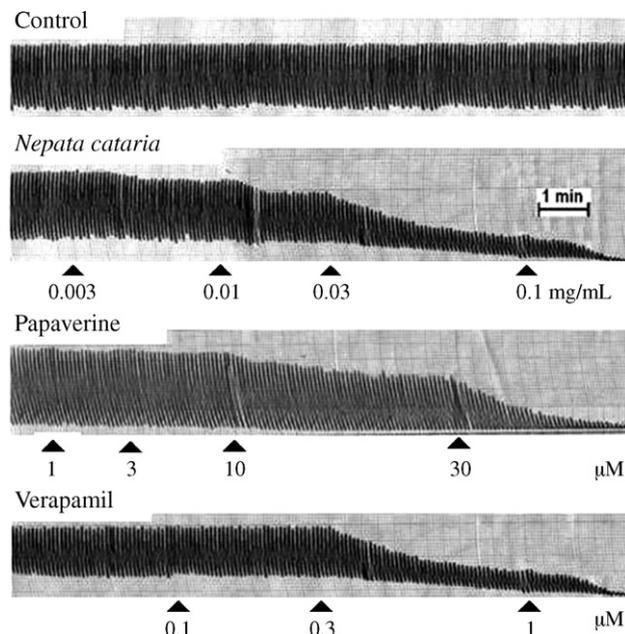


Fig. 1. A typical tracing showing inhibitory effect of the essential oil of *Nepeta cataria*, papaverine and verapamil on the spontaneous contractions in isolated rabbit jejunum preparations.

smooth muscle relaxant (spasmolytic) activity, similar to verapamil and papaverine (Fig. 2; Table 2). When tested against the high K⁺ (80 mM)-induced contractions, essential oil caused a relaxant effect with (*P* > 0.05) EC₅₀ value of 0.05 \pm 0.11 mg/mL; *n* = 5, similar to papaverine (Fig. 2C; Table 2), however, verapamil was relatively more potent (*P* < 0.05) against the high K⁺-induced contractions (Fig. 2E). Pretreatment of the tissues with Nc.Oil (0.01 or 0.03 mg/mL) caused a rightward shift in the Ca²⁺ CRCs (Fig. 2B), similar to papaverine (Fig. 2D) and verapamil (Fig. 2F).

4.3. Effect on isolated guinea-pig trachea

When tested in isolated guinea-pig tracheal strips against the carbachol (CCh; 1 μ M) and high K⁺-induced contractions, Nc.Oil caused inhibition of the induced contractions with EC₅₀ values of 0.05 \pm 0.14; *n* = 5 and 0.08 \pm 0.10 mg/mL; *n* = 6, respectively (Fig. 3A; Table 2). Papaverine also caused inhibition of the CCh and high K⁺-induced contractions showing similar potency (*P* > 0.05), with EC₅₀ values of 4.01 \pm 0.12; *n* = 5 and 6.11 \pm 0.20 μ M; *n* = 5, respectively (Fig. 3C; Table 2). Unlike the essential oil and papaverine, verapamil was found comparatively more potent against the high K⁺-induced

Table 2

Comparative inhibitory EC₅₀ values of the essential oil of *Nepeta cataria* (Nc. Oil), papaverine and verapamil in isolated rabbit jejunum, guinea-pig trachea and atrial preparations.

Preparations	Nc.Oil (mg/mL)	Papaverine (μ M)	Verapamil (μ M)
Rabbit jejunum			
Spontaneous	0.02 \pm 0.10; <i>n</i> = 4	7.63 \pm 0.04; <i>n</i> = 5	0.61 \pm 0.07; <i>n</i> = 3
High K ⁺ (80 mM)	0.05 \pm 0.11; <i>n</i> = 5	8.95 \pm 0.07; <i>n</i> = 3	0.07 \pm 0.12; <i>n</i> = 4
Guinea-pig trachea			
CCh (1 μ M)	0.05 \pm 0.14; <i>n</i> = 5	4.01 \pm 0.12; <i>n</i> = 5	0.28 \pm 0.08; <i>n</i> = 4
High K ⁺ (80 mM)	0.08 \pm 0.10; <i>n</i> = 6	6.11 \pm 0.20; <i>n</i> = 5	0.04 \pm 0.12; <i>n</i> = 4
Guinea-pig atria			
Force	1.70 \pm 0.11***; <i>n</i> = 3	21.04 \pm 0.06***; <i>n</i> = 3	0.35 \pm 0.08; <i>n</i> = 4
Rate	1.25 \pm 0.12***; <i>n</i> = 3	41.32 \pm 0.09***; <i>n</i> = 3	0.39 \pm 0.09; <i>n</i> = 4

Values represent \pm means S.E.M. of three to six determinations.

*** *P* < 0.0001.

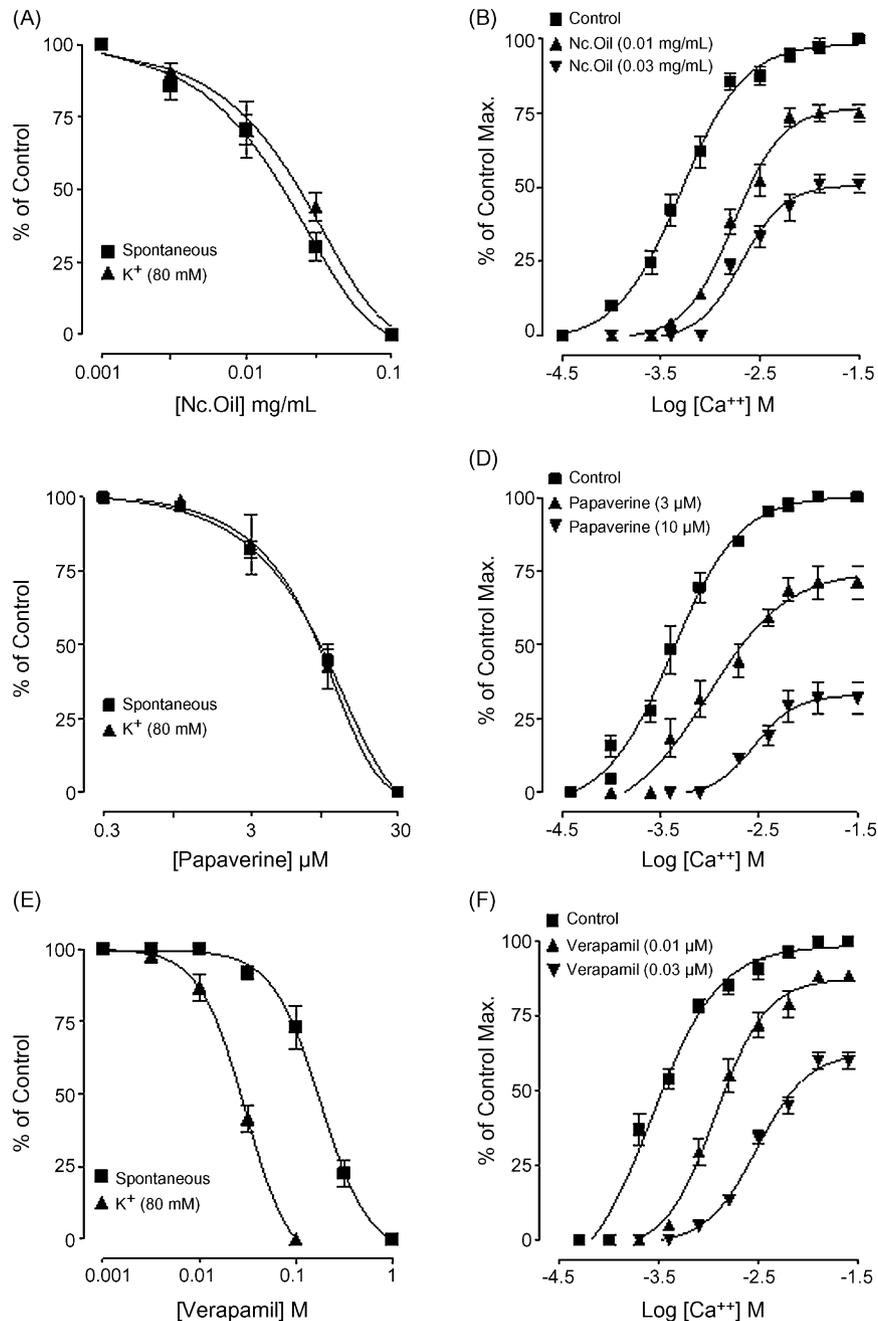


Fig. 2. Inhibitory effect of the (A) essential oil of *Nepeta cataria* (Nc.Oil) (C), papaverine and (E) verapamil on spontaneous and high K⁺-induced contractions in isolated rabbit jejunum preparations. B, D and F show the control concentration–response curves of Ca²⁺ in the absence and presence of the increasing concentrations of Nc.Oil, verapamil and papaverine, respectively, constructed in Ca²⁺-free medium. Symbols represent mean ± S.E.M. of four to five determinations.

contractions (Fig. 3E; Table 2). Pretreatment of the tracheal tissues with Nc.Oil (0.003 or 0.01 mg/mL) potentiated the isoprenaline-induced inhibitory CRCs, constructed on CCh-induced contractions (Fig. 3B), similar to papaverine (Fig. 3D), while verapamil was without effect (Fig. 3F).

4.4. Effect on isolated guinea-pig atria

In isolated spontaneously beating guinea-pig atrial preparations, the essential oil caused suppression of the force and rate of contractions at distinctly higher concentrations ($P < 0.001$), with respective EC₅₀ values of 1.70 ± 0.11 and 1.25 ± 0.12 mg/mL; $n = 3$ (Fig. 4A; Table 2). Papaverine also caused inhibition of both force

and rate of atrial contractions at higher concentrations ($P < 0.001$) than the smooth muscle preparations, with respective EC₅₀ values of 21.04 ± 0.06 and 41.32 ± 0.09 mg/mL; $n = 3$ (Fig. 4B; Table 2). However, verapamil caused relaxation of smooth muscle and cardiac preparations at similar concentrations ($P > 0.05$) with respective EC₅₀ values of 0.35 ± 0.08 and 0.39 ± 0.09 μM; $n = 4$ for atrial force and rate (Fig. 4C; Table 2).

5. Discussion

Nepeta cataria L. grown in Pakistan has not been explored yet for its chemical composition and here it is reported for the first time, the identification of 27 compounds along with four

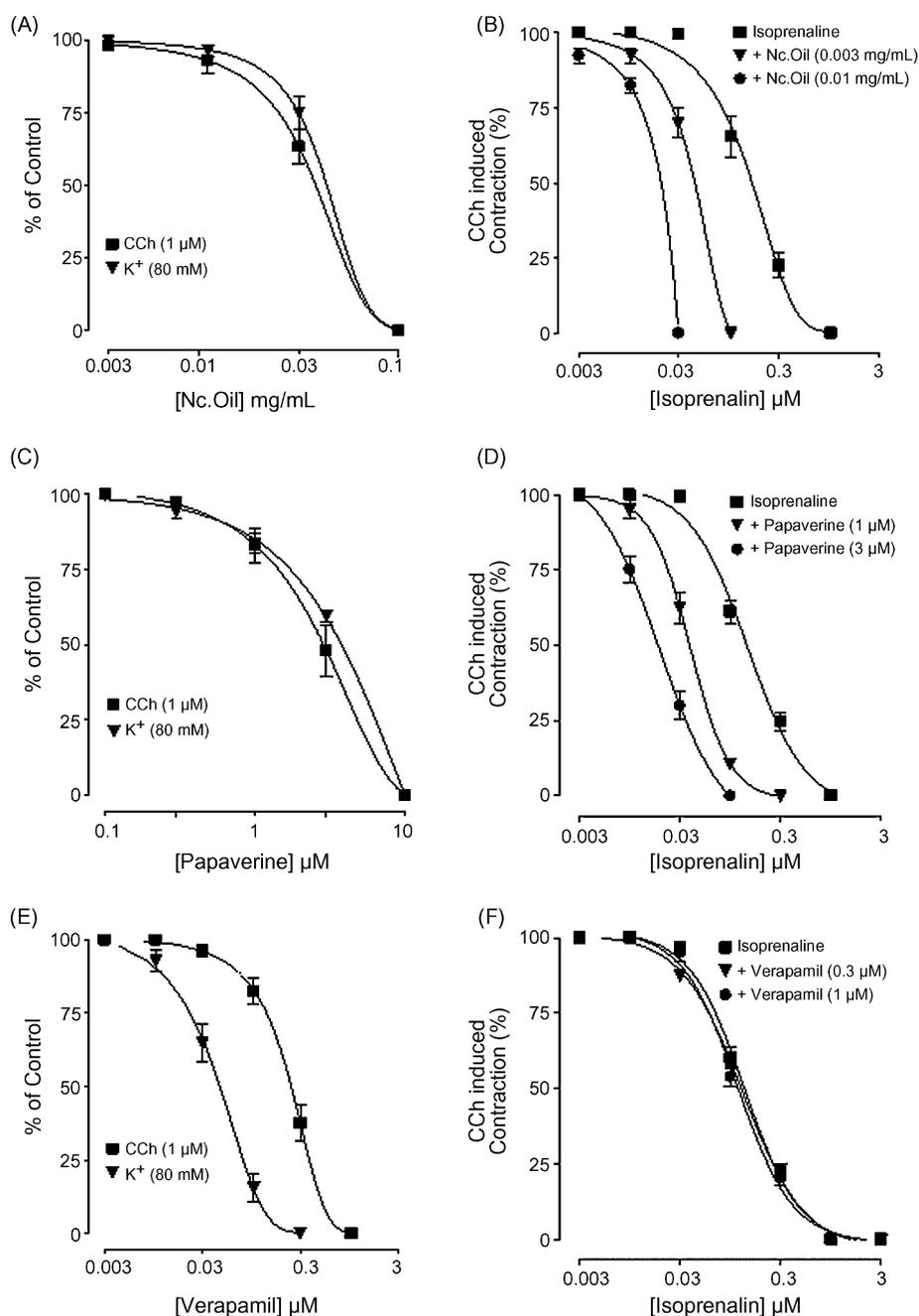


Fig. 3. Inhibitory effect of the (A) essential oil of *Nepeta cataria* (Nc.Oil), (C) papaverine and (E) verapamil on the carbachol (CCh) and high K⁺-induced contractions in isolated guinea-pig tracheal preparations. B, D and F show the inhibitory concentration–response curves of isoprenaline against carbachol (CCh)-induced contractions in the presence of different concentrations of Nc.Oil, papaverine and verapamil. Symbols represent mean ± S.E.M. of four to six determinations.

major components in its essential oil. The components mainly characterized by mass spectral survey (*vide infra*) resulted in the identification of constituents in the oil, which were further supported with Kovats retention indices (RI) cited in the literature and in a few cases with relative retention time (RRT) (Kubeczka and Formacek, 2002). Comparison of the ¹³C NMR spectra of the oil was found in good agreement with those recorded for the pure authentic compounds in literature. Several compounds were not reported from this species, such as linolenic acid, linoleic acid, oleic acid, palmitic acid, stearic acid and 7abtanepetalactone, although their presence has been demonstrated in other species, include *Nepeta viscida*, *Nepeta cilicica*, *Nepeta crinita*, *Nepeta aristata* (Kiliç et al., 2007) and *Nepeta crispa* Willd (Sonboli et al., 2004).

In line with the medicinal use of *Nepeta cataria* in hyperactive disorders of the gut, such as colic and diarrhea, its essential oil was tested in the isolated gut preparations. In rabbit jejunum, Nc.Oil inhibited spontaneous contractions thus showing spasmolytic effect. To see whether the spasmolytic effect of the essential oil is mediated through CCB, a high concentration of K⁺ (80 mM) was introduced to depolarize the tissue. The Nc.Oil caused relaxation of the K⁺-induced contractions, similar to papaverine, observed in spontaneous contractions ($P > 0.05$), suggesting that the spasmolytic effect is possibly mediated through calcium channel blockade. The presence of CCB constituent(s) was further evaluated by an indirect method when pretreatment of the tissues with Nc.Oil caused a rightward shift in the Ca²⁺ CRCs, similar

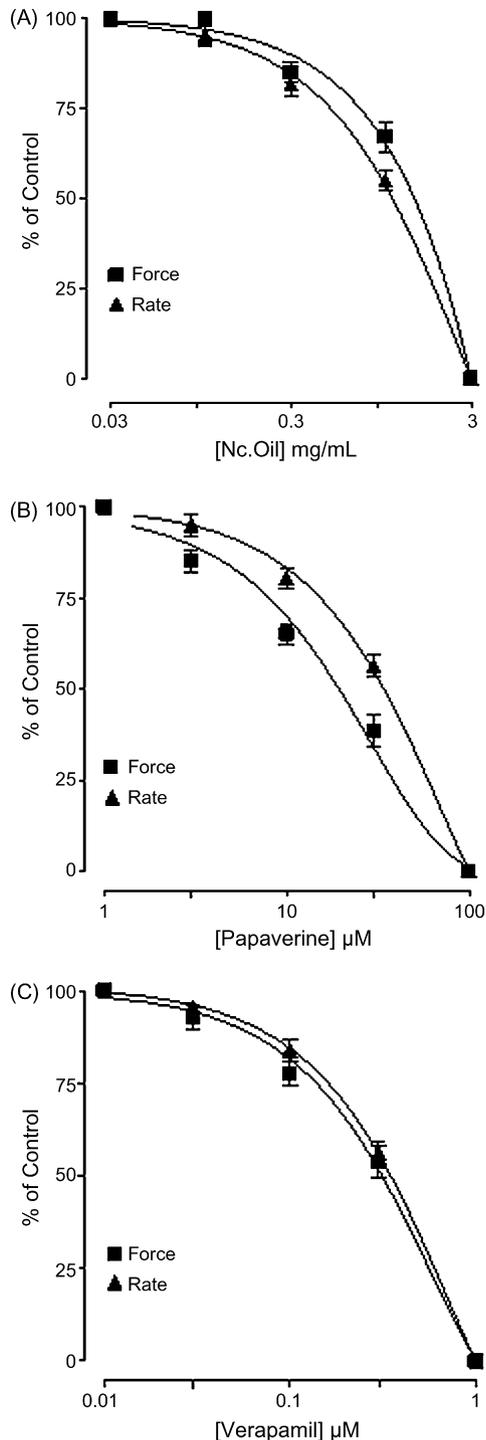


Fig. 4. Inhibitory effect of the (A) essential oil of *Nepeta cataria* (Nc.Oil), (B) papaverine and (C) verapamil on the force and rate of spontaneous contractions of isolated guinea-pig atria. Symbols represent mean \pm S.E.M. of three to four determinations.

to verapamil, a standard calcium channel blocker (Fleckenstein, 1977), which provides pharmacological basis for the medicinal use of the plant as antispasmodic and antidiarrheal as the CCBs are known to be useful in these conditions (Brunton, 1996). Interestingly, eucalyptol, an essential oil has also been reported to possess myorelaxant, vasodilator and cardiac suppressant activities partly through CCB mechanism (Soares et al., 2005).

A critical look at the potency of inhibitory effect in spontaneous and high K^+ -induced contractions revealed that the essential oil

was equipotent against both spasmogens ($P > 0.05$), similar to that of papaverine, a dual inhibitor of Ca^{++} influx (Boselli et al., 1998) and PDE (Boswell-Smith et al., 2006). This indicates the possible presence of PDE-like inhibitory constituent(s) in the essential oil, in addition to CCB, while, verapamil was more potent against the high K^+ -induced contractions, a typical characteristic of Ca^{++} antagonists (Godfraind et al., 1986).

Based on the therapeutic potential of CCBs and PDE inhibitors in the respiratory disorders, particularly asthma and cough (Mathewson, 1985; Triggle, 1992) and the possible presence of both constituents in the essential oil, it was further studied for its myorelaxant effect. In isolated guinea-pig tracheal preparations, Nc.Oil, papaverine and verapamil, caused inhibition of the CCh (1 μ M) and high K^+ -induced contractions, suggesting non-specific tracheal relaxation. When the inhibitory effect of essential oil was compared with that of papaverine, the pattern of potency was similar against both spasmogens as observed in the gut preparations, where as verapamil was distinctly more potent against the high K^+ , as expected from a Ca^{++} antagonist, which indicate that the essential oil possesses, papaverine-like, additional relaxant component(s) possibly, the PDE inhibitor. The PDE inhibitory effect was further confirmed when pretreatment of the tracheal tissues with Nc.Oil potentiated the isoprenaline-induced inhibitory CRCs, constructed on CCh-induced contractions, similar to papaverine, while verapamil was without such effect. These data indicate that the essential oil mediates the myorelaxant effect through dual inhibition of PDE and Ca^{++} channels.

There is evidence suggesting that agents, such as, isoproterenol, β -adrenergic receptor agonist (Chu, 1984; Kovarik et al., 2008) and PDE inhibitors, exert tracheal relaxant or myorelaxant activity by increasing the intracellular levels of cAMP or cGMP (Chu, 1984). The PDE inhibitors have been used for several decades in the treatment of asthma (Weinberger and Hendeles, 1996) and associated cough (Han-jing et al., 2004). Thus the co-existence of PDE inhibitory with CCB constituents, which are also considered useful in asthma and cough (Mathewson, 1985; Kamei and Kasuya, 1992) in the essential oil of *Nepeta cataria* offer therapeutic combination with synergistic potential.

The PDE inhibitors when used alone as myorelaxant are known to cause cardiac stimulation as a side-effect (Raeburn et al., 1993); CCBs on the other hand are known to cause relaxation of smooth muscle as well as cardiac depression. To see the outcome of the combined effect of CCB and PDE inhibition on the heart, the essential oil was studied in the cardiac preparations. In isolated guinea-pig spontaneously beating atria, the essential oil had no effect at the concentration which produced relaxation in the smooth muscle preparations but caused suppression of the force and rate of spontaneous contractions at distinctly higher concentrations (15–85 fold), similar to papaverine, while verapamil was equipotent ($P < 0.05$) in cardiac and smooth muscle preparations (Table 2). It is likely that the distinctly higher concentrations required for the cardiac depressant effect due to the presence of CCB in the essential oil, a pattern similar to that observed with papaverine, is due to the interference of PDE inhibitory constituents, which is known to be stimulant in the heart (Brain and Hoffman, 2001). If there had been any cardiac effect of the essential oil effect either stimulatory or inhibitory due to presence of PDE inhibitory or CCB, respectively at concentrations which produced spasmolytic, it would have been considered side-effect when used as spasmolytic or myorelaxant. The fact that the essential oil had no effect on the heart at doses required for its potential indications offer added advantage.

In summary, this investigation report for the first time the presence of 27 compounds, along with four major components in the essential oil of *Nepeta cataria* and the presence of antispasmodic

and myorelaxant activities mediated possibly through indirect dual blockade of calcium channels and PDE, provides pharmacological basis to its medicinal use in colic, diarrhea, cough and asthma.

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