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Gastrointestinal, selective airways and urinary bladder relaxant effects of Hyoscyamus niger are mediated through dual blockade of muscarinic receptors and Ca(2+) channels

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Gastrointestinal, selective airways and urinary bladder relaxant effects of *Hyoscyamus niger* are mediated through dual blockade of muscarinic receptors and Ca$^{2+}$ channels

Anwarul Hassan Gilani* a, Arif-ullah Khan a,b, Mustafa Raoof a, Muhammad Nabeel Ghayur a,c, Bina S. Siddiqui d, Waseem Vohra d, Sabira Begum d

Abstract

This study describes the spasmolytic, antidiarrhoeal, antisecretory, bronchodilatory and urinary bladder relaxant properties of *Hyoscyamus niger* to rationalize some of its medicinal uses. The crude extract of *H. niger* seeds (Hn.Cr) caused a complete concentration-dependent relaxation of spontaneous contractions of rabbit jejunum, similar to that caused by verapamil, whereas atropine produced partial inhibition. Hn.Cr inhibited contractions induced by carbachol (1 μM) and K$^{+}$ (80 mM) in a pattern similar to that of dicyclomine, but different from verapamil and atropine. Hn.Cr shifted the Ca$^{2+}$ concentration–response curves to the right, similar to that caused by verapamil and dicyclomine, suggesting a Ca$^{2+}$ channel-blocking mechanism in addition to an anticholinergic effect. In the guinea-pig ileum, Hn.Cr produced a rightward parallel shift of the acetylcholine curves, followed by a non-parallel shift with suppression of the maximum response at a higher concentration, similar to that caused by dicyclomine, but different from that of verapamil and atropine. Hn.Cr exhibited antidiarrhoeal and antisecretory effects against castor oil-induced diarrhoea and intestinal fluid accumulation in mice. In guinea-pig trachea and rabbit urinary bladder tissues, Hn.Cr caused relaxation of carbachol (1 μM) and K$^{+}$ (80 mM) induced contractions at around 10 and 25 times lower concentrations than in gut, respectively, and shifted carbachol curves to the right. Only the organic fractions of the extract had a Ca$^{2+}$ antagonist effect, whereas both organic and aqueous fractions had anticholinergic effect. A constituent, β-sitosterol exhibited Ca$^{2+}$ channel-blocking action. These results suggest that the antispasmodic effect of *H. niger* is mediated through a combination of anticholinergic and Ca$^{2+}$ antagonist mechanisms. The relaxant effects of Hn.Cr occur at much lower concentrations in the trachea and bladder. This study offers explanations for the medicinal use of *H. niger* in treating gastrointestinal and respiratory disorders and bladder hyperactivity.
INTRODUCTION

*Hyoscyamus niger* Linn. (Solanaceae), commonly known as ‘Henbane’ and ‘Ajwain-i-khurasani’ locally in Pakistan, grows wildly throughout the Himalayan range at altitudes of 8000–11,000 feet and in many other parts of the world [1]. Its seeds are used in traditional medicine for treating a variety of ailments including asthma, cough, colic, diarrhoea and genito-urinary complaints such as irritable bladder [2,3].


Hyoscine and hyoscyamine, previously identified as the anticholinergic constituents in *H. niger*, are considered responsible for its antispasmodic effect [13]. However, it is generally believed that the plant contains multiple compounds acting on different sites. The aim of this study was to see if *H. niger* has an antispasmodic effect on the gut, airways and urinary bladder through a combination of mechanisms. Plants with medicinal use in hyperactive gut and airway disorders usually possess a Ca$^{2+}$ channel-blocking action in combination with other spasmylytic mechanisms [14–16]. In this investigation, we report the presence of a combination of anticholinergic and Ca$^{2+}$ antagonist effects in *H. niger* seed extract, studied in tissues relevant to gastrointestinal, airway and urinary systems. Activity-directed fractionation revealed that the anticholinergic component(s) was widely distributed both in the organic and aqueous fractions, while Ca$^{2+}$ antagonist was concentrated only in the organic fraction. Apart from the crude extract and its resultant fractions, the pure compounds of *H. niger* namely β-sitosterol, scopoline, hyoscymine, coumarin, chlorogenic acid and vanillic acid were also studied. β-sitosterol was found to exhibit spasmylytic effect via Ca$^{2+}$ antagonist mechanism.

MATERIALS AND METHODS

Plant material, preparation of crude extract and fractions

The seeds of *H. niger* (5 kg) were purchased from the local market, cleaned and coarsely ground. The powdered material was extracted four times with 98% methanol at room temperature. The soaked material was filtered through a muslin cloth and then through Whatman qualitative grade 1 filter paper [17]. This procedure was repeated thrice and the combined filtrate was evaporated with a rotary evaporator under reduced pressure to a thick syrupy mass, the crude extract (Hn.Cr), at a yield of approximately 19.6%. Activity-guided fractionation of the parent extract was carried out by using solvents of increasing polarity. Hn.Cr was partitioned between ethyl acetate and water. The ethyl acetate phase was dried over Na$_2$SO$_4$ (anhydrous), then concentrated at reduced pressure to yield an ethyl acetate residue. The water layer was dried to obtain the aqueous fraction (Hn.Aq). The ethyl acetate residue was treated with petroleum ether to obtain the petroleum ether fraction (Hn.PE) and insoluble fraction. The latter was treated with ethyl acetate to give the ethyl acetate fraction (Hn.EtAc). The fraction left after extraction with ethyl acetate was dissolved in methanol to obtain the methanolic fraction (Hn.MeOH). Stock solutions of the plant materials were prepared in 10% ethanol, and subsequent dilutions in distilled H$_2$O/saline.

Chemicals

Acetylcholine chloride, atropine sulphate, β-sitosterol, carbachol, chlorogenic acid, coumarin, dicyclomine, loperamide hydrochloride, scopolamine, vanillic acid and verapamil hydrochloride were purchased from Sigma Chemicals Co (St Louis, MO, USA). Hyoscyamine sulphate and castor oil were obtained from Chromadex (Santa Ana, CA, USA) and KCl Pharma (Karachi, Pakistan) respectively. Chemicals used for making physiological salt solutions were potassium chloride (Sigma Chemicals Co), calcium chloride, glucose, magnesium chloride, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate (Merck, Darmstadt, Germany) and sodium chloride from BDH Laboratory Supplies (Poole, UK). All chemicals used were of analytical grade and solubilized in distilled H$_2$O except, β-sitosterol which was dissolved in 10% ethanol; subsequent dilutions were

prepared in distilled H₂O. Vehicles used had no effect on tissue contractility in control experiments.

**Experimental animals**

Animals used in this study. adult rabbits (1.0–1.2 kg), guinea-pigs (500–550 g) and BALB/c mice (20–25 g) of either sex and local breed, were housed at the Animal House of the Aga Khan University and maintained at 23–25 °C. Animals were given tap water ad libitum and a standard diet consisting of (g/kg): flour 380, chokar 380, molasses 12, sodium chloride 5.8, nutrivet L 2.5, potassium meta-bisulphate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150. Rabbits and guinea-pigs had free access to water, but food was withdrawn 24 h prior to experiments. They were killed by a blow on the back of the head and cervical dislocation respectively. Experiments performed complied with the recommendations of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council [18] and approved by the Ethics Review Committee of the Aga Khan University.

**Isolated tissue preparations**

*Rabbit jejunum*

The jejunum was dissected, kept in Tyrode’s solution and cleaned of mesentery tissues [19]. Segments about 2 cm long were suspended individually in a 10-mL tissue bath filled with Tyrode’s solution, at 37 °C and aerated with CO₂ (carbogen). An initial load of 0.7 g was applied to the tissue and isotonic contractions were recorded with a Bioscience transducer coupled to a Harvard oscillograph (Harvard Apparatus, Holliston, MA, USA). Tissues were allowed to equilibrate for at least 30 min before the addition of any drug, then stabilized with a sub-maximal concentration of acetylcholine (0.3 μM) at 3-min intervals until constant responses were recorded. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing the testing of relaxant (spasmolytic) activity directly without the use of an agonist.

For the determination of Ca²⁺ channel-blocking activity, high K⁺ concentration (80 mM) was used to depolarize the preparations as described by Farre et al. [20]. K⁺ was added to the tissue bath, which produced a sustained contraction. Test materials were then added in cumulative fashion to obtain concentration-dependent inhibitory responses [21].

To confirm the Ca²⁺ antagonist action of the test substance, the tissue was allowed to stabilize in normal Tyrode’s solution, which was then replaced with Ca²⁺-free Tyrode’s solution containing ethylenediaminetetra-acetic acid (EDTA: 0.1 mM) for 30 min in order to remove Ca²⁺ from the tissues. This solution was replaced with K⁺-rich and Ca²⁺-free Tyrode’s solution, having the composition (mM): KCl 50, NaCl 91.04, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42, glucose 5.55 and EDTA 0.1. Following an incubation period of 30 min, control concentration–response curves of Ca²⁺ were constructed. When the control Ca²⁺ concentration–response curves were super-imposable (usually after two cycles), the tissue was pretreated with the plant extract for 60 min to test for a possible Ca²⁺ antagonist effect. The concentration–response curves of Ca²⁺ were reconstructed in the presence of different concentrations of the test material.

**Guinea-pig ileum**

The ileum was dissected and kept in Tyrode’s solution. The segments, about 2 cm long, were mounted individually in a 10-mL tissue bath, filled with Tyrode’s solution, at 37 °C and aerated with carbogen. An initial load of 0.7 g was applied to the tissue and isotonic contractions were recorded with a Bioscience transducer coupled to a Harvard oscillograph (Harvard Apparatus, Holliston, MA, USA). An equilibrium period of 30 min was required before administration of drugs. After equilibration, each tissue preparation was repeatedly treated with sub-maximal concentrations (0.3 μM) of acetylcholine at 3-min intervals until constant responses were recorded. Control acetylcholine concentration–response curves were then constructed by addition of increasing concentration of agonist and washing between doses. Acetylcholine curves were then re-determined in the presence of increasing concentrations of test and control drugs as described previously [22].

**Guinea-pig trachea**

Trachea was dissected and kept in Kreb’s solution. The tracheal tube was cut into rings, 2–3 mm wide, each containing about two cartilages. Each ring was opened by a longitudinal cut on the ventral side opposite the smooth muscle, forming a tracheal chain with smooth muscle in the centre and cartilaginous portions on the edges. The preparation was mounted in a 20-mL tissue bath containing Kreb’s solution at 37 °C, aerated with carbogen. The composition of Kreb’s solution was (mM): NaCl 118.2, NaHCO₃ 25.0, CaCl₂ 2.5, KCl 4.7, KH₂PO₄ 4.9.
1.3, MgSO₄ 1.2 and glucose 11.7 (pH 7.4). A tension of 1 g was applied to the tracheal strips continuously throughout the experiment. The tissue was equilibrated for 1 h before the addition of any drug. In some preparations, carbachol (1 μM) or K⁺ (80 mM) were used to stabilize the respective preparations until constant responses of each agonist were achieved (usually three to four concentrations). When sustained contractions were obtained, the relaxant effect of the test substance was assessed by adding it in a cumulative fashion. The carbachol cumulative concentration–response curves were plotted using increasing concentrations of agonist. When a threefold increase in concentration produced no further increment in response, the tissue was washed to re-establish the baseline tension (usually 30–35 min). The carbachol curves were then replotted in the presence of increasing concentrations of an extract [23]. Isometric responses were recorded on a Grass model 7 Polygraph (Grass Instrument Company, Quincy, MA, USA).

Rabbit urinary bladder
The whole urinary bladder was divided into four vertical strips [24]. Each preparation was mounted in a 20-mL tissue bath containing Krebs–Henseleit solution (mM): NaCl 119, NaHCO₃ 25.0, CaCl₂ 2.5, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ 1.17, EDTA 0.027, glucose 5.5 and HEPES 10 aerated with carbogen to yield a pH of 7.4 at 37 °C. A tension of 1 g was applied continuously throughout the experiment. Tissues were equilibrated for 1 h before the addition of any drug. As was the case with trachea, in some preparations carbachol (1 μM) and K⁺ (80 mM) were used to stabilize the respective preparations until constant responses of agonists were achieved. Once sustained contractions were obtained, inhibitory effects of the plant material were assessed by adding it in a cumulative fashion. The carbachol concentration–response curves were plotted in the absence and presence of increasing concentrations of an extract. Isometric responses were recorded on a Grass model 7 Polygraph.

In vivo experiments
Castor oil-induced diarrhoea
Mice were fasted for 24 h before the experiment. Animals were housed in individual cages and divided in four groups, each containing five mice. The first group received saline (10 mL/kg p.o.) and served as a negative control. Extract doses were selected on trial basis and then two increasing doses of the extract were given orally. A group of mice was treated with loperamide (10 mg/kg p.o.), as a positive control. One hour after treatment, each animal received 10 mL/kg of castor oil orally through a feeding needle [25]. Afterwards, the cages were inspected for the presence of diarrhoea droppings; their absence was noted as a positive result, indicating protection from diarrhoea at that time.

Intestinal fluid accumulation
Different groups of overnight fasted mice were treated with increasing doses of extract intraperitoneally through detachable U-100 insulin syringe with 25G × 1” (0.50 × 25 mm) needle, 60 min before oral administration of castor oil (10 mL/kg). The mice were killed 30 min later by cervical dislocation and the entire intestine was removed and weighed with care, not allowing any intestinal fluid to leak out [25]. The results were expressed as (Pᵢ/Pᵣ) × 1000 where Pᵢ is the weight (g) of the intestine and Pᵣ is the weight of the animal.

Statistical analysis
Data are mean ± standard errors of mean (SEM, n = number of experiments) for the median effective concentrations (EC₅₀ values) with 95% confidence intervals. The statistical parameters applied were Student’s t-test for intestinal fluid accumulation, and chi-squared test for antidiarrhoeal activity. Difference of P < 0.05 was considered statistically significant. Concentration–response curves were analysed by nonlinear regression using GraphPad program (GraphPAD, San Diego, CA, USA).

RESULTS
Effect on jejunum
Figure 1a compares inhibitory effects of Hn.Cr with those of dicyclomine, verapamil and atropine on the spontaneous contractions of isolated rabbit jejunum. The respective EC₅₀ values were 5.6 μg/mL (4.1–7.6, 95% confidence interval, n = 5), 0.53 μM (95% CI 0.40–0.70, n = 4), 0.23 μM (95% CI 0.17–0.32, n = 5) and 0.001 μM (95% CI 0.0008–0.01, n = 4). The spasmolytic effect was concentration-dependent with the exception of atropine, where further increments in the concentration beyond 0.3 μM did not show additional inhibition. When tested against carbachol (1 μM) and K⁺ (80 mM)-induced contractions, Hn.Cr inhibited (Figure 1b) carbachol-induced contractions at a much lower concentration with EC₅₀ value of 7.2 μg/mL (95% CI 4.4–12, n = 4) as compared with 300 μg/mL (95% CI 209.8–428.4, n = 4) for K⁺. Dicyclomine also showed a similar pattern of inhibition (Figure 1c) with respective
Figure 1 (a) Tracing showing spasmolytic effect of the crude extract of *Hyoscyamus niger* (Hn.Cr), dicyclomine, verapamil and atropine on spontaneous contractions and concentration–response curves representing comparison of (b) Hn.Cr; (c) dicyclomine; (d) verapamil and (e) atropine for the inhibitory effect against carbachol (CCh) and K⁺-induced contractions in isolated rabbit jejunum preparations. Symbols represent mean ± SEM, n = 4–8.
EC$_{50}$ values of 0.13 (95% CI 0.11–0.19, $n = 6$) and 3.2 $\mu M$ (95% CI 1.9–5.3, $n = 5$). Verapamil on the other hand, was more potent against K$^+$-induced contractions with EC$_{50}$ value of 0.04 $\mu M$ (95% CI 0.03–0.05, $n = 5$), compared with carbachol [0.16 $\mu M$ (95% CI 0.13–0.19, $n = 5$)] as shown in Figure 1d. Atropine relaxed the carbachol-induced contractions potently with EC$_{50}$ value of 0.003 $\mu M$ (95% CI 0.002–0.004, $n = 8$), without any effect on K$^+$ (Figure 1e), as was expected. When tested for the possible interaction with Ca$^{2+}$ channels, Hn.Cr caused a rightward shift of the Ca$^{2+}$ concentration–response curves (Figure 2a), similar to that caused by verapamil (Figure 2b) and dicyclomine (Figure 2c).

**Effect on ileum**

Hn.Cr at 1.0 $\mu g/mL$ caused a rightward parallel shift in the acetylcholine concentration–response curves without suppressing maximum contractile response, followed by a non-parallel shift with suppression of the maximum response at 3.0 $\mu g/mL$ (Figure 3a). Dicyclomine (0.03–0.1 $\mu M$) also showed a similar pattern of shift (Figure 3b), while verapamil (0.01–0.03 $\mu M$) produced a non-parallel rightward shift with suppression of the maximum response (Figure 3c) and atropine (0.01–0.03 $\mu M$) caused a rightward parallel shift without suppression of the maximum contractile effect (Figure 3d).

**Effect on castor oil-induced diarrhoea**

Hn.Cr exhibited a dose-dependent (300–1000 mg/kg) inhibitory effect against castor oil-induced diarrhoea in mice. The negative control treatment (saline) did not protect the animals from diarrhoea. Pretreatment with the plant extract led to 40% protection from diarrhoea at 300 mg/kg and 80% protection at 1000 mg/kg ($P < 0.05$ vs. saline group). Loperamide (10 mg/kg) led to complete protection from diarrhoea in the positive control group (Table I).

**Effect on intestinal fluid accumulation**

When tested against castor oil-induced intestinal fluid accumulation in mice, Hn.Cr had a dose-dependent (300–1000 mg/kg) antisecretory effect (Figure 4). Intestinal fluid accumulation in the saline-treated group was 114.4 ± 0.77 g, while in castor oil-treated group it was...
122.6 ± 0.70 g ($P < 0.001$ vs. saline group). Hn.Cr at the doses of 300 and 1000 mg/kg reduced the castor oil-induced fluid accumulation to 118.6 ± 1.10 g ($P < 0.05$ vs. castor oil group) and 114.4 ± 0.70 g ($P < 0.001$ vs. castor oil group) respectively.

**Effect on trachea**

In tracheal preparations, Hn.Cr inhibited carbachol (1 μM) contractions at low concentrations with $EC_{50}$ value of 0.6 (95% CI 0.3–1.0, $n = 5$) and 6.2 μg/mL (95% CI 4.2–9.1, $n = 5$) as shown in Figure 5a. Similar to that in ileum, Hn.Cr produced a rightward parallel displacement of the carbachol curves without suppression of the maximum contractile response at 1.0 μg/mL, followed by a non-parallel shift with suppression of the maximum effect at 3.0 μg/mL (Figure 5b).

**Effect on urinary bladder**

Hn.Cr inhibited carbachol (1 μM) and K⁺ (80 mM) contractions with respective $EC_{50}$ values of 0.6 (95% CI 0.3–1.0, $n = 5$) and 6.2 μg/mL (95% CI 4.2–9.1, $n = 5$) as shown in Figure 5c. Hn.Cr caused a rightward parallel shift of the carbachol curves without suppression of the maximum contractile response at 1.0 μg/mL, followed by a non-parallel shift with the suppression of maximum effect at 3.0 μg/mL (Figure 5d).

**Effect of fractions**

Hn.PE, Hn.EtAc and Hn.MeOH inhibited carbachol (1 μM) and K⁺ (80 mM) contractions of rabbit jejunum

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**Figure 3** Concentration–response curves of acetylcholine (ACh) in the absence and presence of (a) crude extract of *Hyoscyamus niger* (Hn.Cr); (b) dicyclomine; (c) verapamil and (d) atropine in isolated guinea-pig ileum preparations. Symbols represent mean ± SEM, $n = 3–6$.

**Table 1** Effect of the crude extract of *Hyoscyamus niger* (Hn.Cr) on castor oil (c.oil, 10 mL/kg)-induced diarrhoea.

<table>
<thead>
<tr>
<th>Treatment (p.o.)</th>
<th>No. mice/five with diarrhoea</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (10 mL/kg) + (c.oil)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Hn.Cr (300 mg/kg) + (c.oil)</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>Hn.Cr (1000 mg/kg) + (c.oil)</td>
<td>1*</td>
<td>80</td>
</tr>
<tr>
<td>Loperamide (10 mg/kg) + (c.oil)</td>
<td>0**</td>
<td>100</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 compared to saline group, chi-squared test.

122.6 ± 0.70 g ($P < 0.001$ vs. saline group). Hn.Cr at the doses of 300 and 1000 mg/kg reduced the castor oil-induced fluid accumulation to 118.6 ± 1.10 g ($P < 0.05$ vs. castor oil group) and 114.4 ± 0.70 g ($P < 0.001$ vs. castor oil group) respectively.
DISCUSSION

*Hyoscyamus niger* is known to contain tropane alkaloids, i.e. hyosine and hyoscymine, which are well known for their anticholinergic effect [13]. In view of the wide distribution of Ca$^{2+}$ antagonists in medicinal plants, which usually exist in combination with other spasmyotics [14–16], the present study was undertaken to see whether the antispasmodic action of *H. niger* is the result of a combination of anticholinergic and Ca$^{2+}$ antagonist mechanisms. When tested in isolated rabbit jejunum preparations, the plant extract completely inhibited spontaneous contractions, like that caused by verapamil, a standard Ca$^{2+}$ channel blocker [26]. Atropine, a muscarinic receptor antagonist [27], caused partial relaxation of spontaneous contractions. Gastrointestinal motility is regulated by multiple physiological mediators, mainly acetylcholine, histamine, 5-hydroxytryptamine, bradykinins, prostaglandins, substance P and cholecystokinin which achieve their contractile effects through an ultimate increase in cytosolic Ca$^{2+}$ [28]. Hence, substances that evoke non-specific inhibition, like Ca$^{2+}$ antagonists, are considered to more effectively suppress gut motility, whereas muscarinic receptor antagonists produce partial inhibition, as cholinergic innervation is one of the several mechanisms responsible for regulating intestinal motility [29,30]. This suggests the presence of a non-specific spasmyotic component(s), most likely Ca$^{2+}$ antagonist in *H. niger* in addition to the anticholinergic constituents (hyosine and hyoscymine). This speculation was strengthened when the plant extract reversed both carbachol and K$^{+}$-induced contractions (being more potent against carbachol), indicating the presence of at least two different spasmyotic mechanisms, similar to dicyclomine, a dual blocker of muscarinic receptors and Ca$^{2+}$ influx [31]. Verapamil, however, was found to be more potent against the contractions of K$^{+}$ than carbachol, and atropine relaxed carbachol-induced contractions only.

High K$^{+}$ (>30 mM) is known to cause smooth muscle contractions through opening of voltage-dependent L-type Ca$^{2+}$ channels, thus allowing influx of extracellular Ca$^{2+}$ [32] and a substance that inhibits high K$^{+}$ contractions is considered as a blocker of Ca$^{2+}$ influx [33]. The Ca$^{2+}$ antagonist effect was further confirmed when *H. niger* extract caused a rightward shift in the Ca$^{2+}$ concentration–response curves similar to that caused by verapamil and dicyclomine.

The concept of a dual mode of inhibition involving anticholinergic and Ca$^{2+}$ channel blockade received
additional support from acetylcholine concentration–response data in guinea-pig ileum, as this preparation is considered more useful to quantify contractile responses of an agonist in the presence of an inhibitor, thus allowing exploration of the nature of antispasmodic effect [22]. The plant extract at a low concentration caused a rightward parallel shift in the acetylcholine curves without suppressing the maximum response, a characteristic of a competitive or specific antagonist, like atropine [27], then at higher concentration caused a non-parallel shift with suppression of the maximum effect, pointing towards an additional non-competitive inhibition [34], as expected for Ca$^{2+}$ antagonists [35,36]. Dicyclomine also shifted the acetylcholine curves similar to that of extract, while verapamil resulted in a rightward but non-parallel shift with suppression of the maximum contractile effect at both concentrations used. Atropine caused a rightward parallel shift of the acetylcholine curves without suppression of the maximum response. This confirms the presence of a combination of muscarinic receptors and Ca$^{2+}$ channel-blocking activities in H. niger, similar to dicyclomine.

In view of the medicinal use of H. niger in diarrhoea, its extract was tested for possible protective effect against castor oil-induced diarrhoea in mice. Diarrhoea induced by castor oil is a result of the action of ricinoleic acid formed during hydrolysis [37], which affects transport of electrolytes and water and generates giant contractions of the transverse and distal colon [38]. The observed antidiarrhoeal effect of H. niger extract, following oral administration, could have resulted from a local or a combination of local and systemic actions. H. niger also protected the mice against castor oil-induced intestinal fluid secretions. This is expected, as both anticholinergic drugs and Ca$^{2+}$ antagonists possess an antidiarrhoeal and antisecretory actions [39–41].

As H. niger has uses in hyperactive respiratory and urogenital ailments, the plant extract was further studied for possible bronchodilatory and urinary bladder relaxant activity. In trachea and bladder preparations, as with the gut, the extract inhibited both carbachol and K$^+$-induced contractions and displaced carbachol curves to the right in a parallel fashion without suppression of the maximum response at low and higher concentrations it

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**Figure 5** (a, c) represents concentration-dependent inhibitory effect of the crude extract of Hyoscyamus niger (Hn.Cr) on carbachol (CCh) and K$^+$-induced contractions, while (b) and (d) show CCh concentration–response curves in the absence and presence of Hn.Cr in isolated guinea-pig tracheal and rabbit urinary bladder preparations. Symbols represent mean ± SEM. $n = 4–6$. 

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caused a non-parallel shift with suppression of the maximum effect. Interestingly, the plant extract was found to be a more potent spasmolytic in trachea and bladder than in the gut (Table II). Tissue-selective behaviour of *H. niger* could be due to a Ca\(^{2+}\) antagonist component, as Ca\(^{2+}\) channels are known to be heterogeneous and different Ca\(^{2+}\) antagonists have selectivity for different organ systems [20,42]. A parallel shift of the cholinergic agonists’ concentration–response curves constructed in different tissues by similar concentrations of the plant extract may rule out any possibility of the tissue-selective nature of the anticholinergic component, as expected from the non-selective profile of anticholinergic constituents of *H. niger*, i.e. hyoscine and hyoscyamine [43]. Alternatively, a possibility exists that a difference in physiological modulators among various tissues and/or the extent of their regulatory influences [44] may cause plant constituents to exert a better synergistic interaction in trachea and bladder compared with the gut.

The presence of a Ca\(^{2+}\) channel-blocking component in *H. niger* reported here for the first time may enhance the medicinal benefits of the plant, as Ca\(^{2+}\) antagonists have the potential to depress cardiac arrhythmias, which usually occur when anticholinergics are used alone [45]. In smooth muscles, a combination of anticholinergic and Ca\(^{2+}\) antagonist components could synergize, making the plant more effective for therapy against spasmodic conditions [16]. For instance dicyclomine, which possesses dual muscarinic receptors and Ca\(^{2+}\) channel-blocking action, is considered to be a more efficacious spasmolytic, compared with pure anticholinergics [46].

The activity-directed fractionation study revealed that the Ca\(^{2+}\) antagonist component, as opposed to the anticholinergic was concentrated in the petroleum ether fraction (around 10 times more potent than the parent extract), while the aqueous fraction was devoid of it. This observation supports our previous findings that the Ca\(^{2+}\) channel-blocking component is concentrated in the organic fractions [47,48]. The relative potency difference

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**Figure 6** Concentration-response curves showing effect of the *Hyoscyamus niger* fractions: (a) petroleum ether (Hn.PE); (b) ethyl acetate (Hn.EtAc); (c) methanolic (Hn.MeOH) and (d) aqueous (Hn.Aq) on carbachol (CCh) and K\(^{+}\)-induced contractions in isolated rabbit jejunum preparations. Symbols represent mean ± SEM, n = 3–5.
against carbachol and K⁺-induced contractions in the organic fractions was less than that in the parent extract, probably because the Ca²⁺ antagonist component is entirely concentrated in these fractions, while anticholinergic component is distributed in both organic and aqueous fractions. Among the tested plant constituents, β-sitosterol was found to possess Ca²⁺ antagonist effect, though the presence of additional compounds with Ca²⁺ channel-blocking mechanisms cannot be ruled out.

In conclusion, H. niger offers an interesting combination of spasmolytic mechanisms (anticholinergic and Ca²⁺ antagonist) which might be responsible for its medicinal use in treating disorders of the gut and airways, and urinary bladder hyperactivity. The selectivity for the latter two preparations presents an interesting scenario, which warrants further studies to find out whether this tissue-selective behaviour of the plant is due to the selectivity of the Ca²⁺ antagonist component or the better synergistic interaction of the plant-active constituents in trachea and urinary bladder.

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