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Original Article

Anti-spasmodic assessment of hydroalcoholic extract and essential oil of aerial part of *Pycnocycla caespitosa* Boiss. & Hausskn on rat ileum contractions

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Abstract

Pycnocycla caespitosa is an essential oil-containing plant naturally growing in southwest of Iran. The extract of this plant has been used as remedy in traditional medicine. Another species of *Pycnocyla* (*P. spinosa*) possessed antispasmodic activity. The pharmacological objective of this study was to look for relaxant effect of hydroalcoholic extract and essential oil of *P. caespitosa* on rat isolated ileum contractions for comparison with loperamide. The essential oil and the hydroalcoholic extract were prepared by hydrodistillation and percolation techniques, respectively. For antispasmodic studies a section of rat ileum was suspended in an organ bath containing Tyrode's solution. The tissue was stimulated with electrical field stimulation (EFS), KCl (80 mM) and acetylcholine (ACh 0.5 μM). The tissue was kept under 1 g tension at 37 °C and continuously gassed with O_2. The essential oil content in the aerial parts of *P. caespitosa* was found to be 0.16 % ml/g. The essential oil was analyzed by gas chromatography and gas chromatography–mass spectrometry. Seventy constituents, representing 97 % of the oil were identified. The major components of the oil were carvacrol (7.1%), β-eudesmol (6.4 %), ρ-cymene (5.7%), caryophyllene oxide (3.6%), α-pinine (1.4%) and α-phelandrene (1.1%). The hydroalcoholic extract of *P. caespitosa* inhibited the response to KCl (IC_{50} = 48 ± 3 μg/ml), ACh (IC_{50} = 61 ± 14.7 μg/ml) and EFS (IC_{50} = 77 ± 17 μg/ml) in a concentration-dependent manner. The essential oil of *P. caespitosa* also inhibited rat ileum contractions. The IC_{50} values for KCl, ACh and EFS were 9.2 ± 1.2 μg/ml, 7.6 ± 0.8 μg/ml and 6.4 ± 0.8 μg/ml, respectively. The inhibitory effect of both the essential oil and the extract were reversible. This research confirms the anti-spasmodic activity of both the essential oil and the extract of *P. caespitosa* on smooth muscle contraction of ileum.

Keywords: *Pycnocycla caespitosa*; Hydroalcoholic extract; Spasmolytic; Essential oil

INTRODUCTION

Genus Pycnocycla belongs to subfamily of Umbellales (Umbelliferae family) (1). Plant of this genus are stable and greenish bush type vegetation with soft cylindrical branched stem and thorns like leaves tapered to relatively long, straight large spines. At least eight different species of Pycnocycla exists in Iran and they have close morphological appearance (1,2). These include *P. spinosa, P. caespitosa, P. nodiflora, P. glauca, P. musiformis, P. flabellifolia, P. beshagardiana, P. aucherana,* and *P. acanthorhipsis* (3). Some of these vegetations have been used as folk medicine particularly in south of Iran. *P. spinosa* is an essential oil-containing plant with several pharmacological properties (4-10). *P. spinosa* extract is rich in chemical substance and contains alkaloids, flavonoids and saponins (6,7). It has shown antispasmodic activity both *in vitro* and *in vivo* (4-11). The hydroalcoholic extract of *P. spinosa* inhibits ileum contraction induced by various spasmogens including acetylcholine (Ach), serotonin, KCl and neuronal stimulation *in vitro* (4,5,11). In addition *P. spinosa* extract has anti-spasmodic activity on uterus and bladder smooth muscles(8,9).

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Furthermore, it has been shown that *P. spinosa* extract possesses antidiarrheal and anti-spasmodic activity *in vivo* (4,10-12). Other useful pharmacological activity of *P. spinosa* extract is its anti-colitis activity in animal model (13). Therefore, it is likely that other species may also have useful pharmacological activities.

Pycnocycla caespitosa Boiss. & Hausskn (umbelliferae) is another essential oil-containing species of Pycnocycla genus which grows in some parts of Khozestan and Kohgiloye and Boyerahmad provinces of Iran (3). The essential oil content in the aerial parts of *P. caespitosa* was reported to be 0.25 % based on the fresh weight. The major components of the oil were identified as β-eudesmol (20.3 %), 2,3,6-trimethyl benzaldehyde (13.2 %), Z-β-ocimene (6.1 %), α-pinene (5.9 %), spathulenol (4.6 %) and ρ-cymene (4.3 %) (14).

In Tange solak (a village in Kohkiloyah and Boyerahmed) this plant is known as Bonjeh Kharo and its boiled extract is traditionally used as aliment for alleviating dysmenorrhoea. It is also reported that the hydroalcoholic extract of *P. caespitosa* has both analgesic and anti-inflammatory activities in animal model of pain and inflammation (15,16).

Despite local use of *P. caespitose* extract as medicinal remedy, there are only a few scientific reports about pharmacological activity of this plant. Due to similarity between plant species, it is likely that the same as *P. spinosa, P. caespitosa* may also have anti-spasmodic activitiy. Therefore, the objective of this research was to investigate anti-spasmodic activities of *P. caespitosa* extract by using *in vitro* isolated tissue techniques.

MATERIALS AND METHODS

Plant materials

Aerial parts of *P. caespitosa* were collected in June 2013 from Choram in Kohgiloye and Boyerahmad a province of Iran. The plant was identified as *P. caespitose* Boiss. & Hausskn (umbelliferae) by the Botanist, Dr. Rahiminejad in the Biology Department at Isfahan University. A voucher specimen (3042) was authenticated and then deposited in the herbarium of Faculty of Pharmacy and Pharmaceutical Sciences at Isfahan University of Medical Sciences.

Extract preparation

The plant materials were dried in shade and grounded to powder using an electric mill. Hydroalcoholic extract of *P. caespitose* was obtained by percolation technique (17). In this method, 70% ethanol was used as solvent at 8 to 1 solvent to plant ratio. Initially plant powder was soaked for 2 h in 70% ethanol. Then the soaked material was packed inside a percolator apparatus and covered with a filter paper. A heavy glass plate was then placed on the filter paper to prevent material displacement. The percolator then was filled with 70% ethanol until the entire surface of the plant materials was covered. After 48 h, the percolator tap was opened and adjusted in such way that for the 400 g dried powder in the percolator (Isfahan, Iran), between 16 to 24 drops were eluted per minute. Simultaneously the percolator was taped up with 70% ethanol. This process was continued until the colour of the eluent faded. The percolated extract was then concentrated in a rotary (Hidolf, Germany) at 50 °C and dried in open air and the yield of the extract was calculated.

Analysis of the essential oil

The essential oil was prepared by hydrodistillation technique (18). For analysis, the essential oil was injected to gas chromatography–mass spectrometry (GC-Mass) apparatus (Agilent 6890, USA) equipped with a HP-5MS fused silica column (30 m × 0.25 mm: film thickness 0.25 µm) and interfaced with an Agilent 5975 mass selective detector. The oven temperature was programmed from 60 °C to 280 °C at a rate of 4 °C/min. Helium was used as the carrier gas at a flow rate of 2 ml/min. Other conditions of the instrument were as follows: ionization voltage, 70 eV; injector temperature, 280 °C; ion source temperature, 200 °C. Identification of oil components were based on GC retention indices relative to n-alkenes and computer matching with the WILEY275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (19).

Drugs and solutions

The extract of *P. caespitosa* was prepared as 20 mg/ml stock solution in dimethyl sulphoxide (DMSO). Further dilution was made in distilled water.

The essential oil of *P. caespitosa* was prepared as 5 mg/ml stock solution in DMSO and further diluted by distilled water. Loperamide (Sigma, Germany) was prepared as 1 mM stock solution in DMSO and further serial dilution was prepared in distilled water. ACh (Sigma, Germany) was prepared as 100 mM stock solution in distilled water (acidified with 0.1% acetic acid for stability) and further diluted with distilled water for the experimental use. KCl was prepared as 2 M stock solution. Tyrode's solution with the following composition NaCl, 136.9; KCl, 2.68; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaHCO_3$, 11.9; NaH_2PO_4, 0.42 and glucose 5.55 (mM) was made up in distilled water. Unless stated, all the chemicals were from Merck (Germany). Loperamide powder was a gift from Amin Pharmaceutical Company (Iran).

Spasmolitic studies

Male Wistar rats (170-250 g) bred in the Faculty of Pharmacy and Pharmaceutical Science animal centre (Isfahan) was used in this study.

The rats were killed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals, as recommended by the Ethics Committee of Isfahan University of Medical Science (20). Immediately after killing the rat, the abdomen was open and 10-15 cm of ileum was removed and placed in oxygenated Tyrode's solution at room temperature. The ileum then was cut into segments of 2-3 cm in length and set up in an organ bath and secured to an isotonic transducer for measuring contractile activity of the tissue. The tissue was subjected to 1 g tension and the transducer was connected to a Harvard physiograph apparatus (Harvard, England) for recording the contraction. Initially the physiograph was calibrated and then the tissue was adjusted and washed several times and allowed to relax to a stable baseline.

Contractions were induced in the ileum by addition of KCl (80 mM), ACh (0.5 μM) or electrical field stimulation (EFS; 6 V, 50 Hz, 1 s duration). Initially a series of pilot experiments were carried out to determine the effective ranges of the extract and the essential oil. After a stable and repeatable response was produced by the tissue, drugs were added into organ bath by 2 or 4 fold increments in concentration until a full concentration response curve was obtained. For each experiment 5-7 concentrations was tested. In the case of KCl, drugs were added in a cumulative manner while for ACh and EFS experiments, drugs were added in a non-cumulative manner. 30 s after addition of ACh the tissue was washed with fresh Tyrode's solution.

For the extract, a time interval of 15 min was chosen, while for the essential oil a time interval of 5 min was applied. Following achievement of the maximum response the tissues were washed with fresh Tyrode's solution for testing the reversibility of the drug action. All the experiments were conducted alongside a parallel vehicle treated time-matched control tissues.

Data analysis

Tissue response to spasmogens were assessed as amplitude of the contraction for each drug concentration and expressed as percentage of initial contraction in absence of drug for each tissue. The drug inhibitory concentration causing 50% of maximum response (IC_{50} value) was calculated by plotting a full concentration response curve for each tissue.

The data are expressed as mean ± standard error of mean (SEM). For statistical analysis, one way analysis of variance (ANOVA) was used for intra group variation and *Student's t-test* for inter group variation. $P<0.05$ was considered as statistically significant. SigmaPlot computer program (version 11) was used for plotting the graphs and statistical analysis.

RESULTS

Essential oil and the extract

Solid hydroalcoholic extract of *P. caespitosa* had dark brownish color. Based on the dried weight, the yield of extract was calculated to be 14.1% (W/W). The essential oil content in the aerial parts of *P. caespitosa* was found to be 0.16 % V/W (ml/g). The oil

was analyzed by GC and GC-MS. Seventy constituents, representing 97% of the oil were identified. The major components of the essential oil were carvacrol (7.1%), β-eudesmol (6.4 %), ρ-cymene (5.7%), caryophyllene oxide (3.6%), α-pinine (1.4%) and α-phellandrene (1.1%). 2H-3,9a-methano-1-benzoxepin was found as major constituents but it did not matched with the known components and might be a new compound. The rest of the compounds are summarised in Table 1.

Table 1. Constituents of essential oil of *Pycnocycla caespitose* Boiss. & Hausskn collected from southwest of Iran. The compounds are listed in order of their retention time (RT) on the HP-5MS. Kovates Indices (KI) were calculated using the Kovates equation.

NO	Compound	Quantity (%)	RT (min)	KI
1	α-thujene	0.17	3.66	930
2	α-pinene	1.39	3.80	939
3	sabinene	0.74	4.53	975
4	β-pinene	0.20	4.88	979
5	α-phellandrene	1.10	5.20	1003
6	α-terpinene	0.22	5.47	1017
7	ρ-cymene	5.74	5.72	1025
8	γ-terpinene	0.37	6.51	1060
9	linalool	0.53	7.64	1097
10	trans-verbenol	0.58	8.96	1145
11	terpineol-4	1.19	9.95	1177
12	cryptone	0.78	10.21	1186
13	cis- piperitol	0.50	10.49	1196
14	trans-piperitol	0.84	10.96	1208
15	trans-Carveol	0.31	11.25	1217
16	carvotanacetone	0.35	12.12	1247
17	piperitone	0.36	12.31	1253
18	thymol	0.53	13.59	1290
19	carvacrol	7.11	14.01	1299
20	α-copaene	0.20	16.03	1377
21	cis-jasmone	0.26	16.83	1393
22	β-caryophyllene	0.89	17.36	1419
23	α-humulene	0.18	18.38	1455
24	germacrene-D	0.40	19.22	1485
25	δ-cadinene	0.62	20.50	1523
26	2H-3,9a-methano-1-benzoxepin	20.56	21.76	*
27	hexenyl benzoate	1.53	21.99	1567
28	caryophyllene oxide	3.60	22.31	1583
29	salvia-4(14)-en-1-0ne	0.49	22.56	1595
30	hinesol	0.65	23.81	1642
31	β-eudesmol	6.39	24.19	1651
32	aristolone	0.77	25.10	1763

*KI is not known

Table 2. IC_{50} values of essential oil and extract of *Pycnocycla caespitosa* Boiss. & Hausskn on rat ileum contraction induced by KCl (80 mM), acetylcholine (ACh, 0.5 μM) and biphasic response to electrical field stimulation (EFS). Data are presented as mean ± SEM.

Contraction inducer	Essential oil (μg/ml)	Hydroalcoholic extract (μg/ml)	Loperamide (μg/ml)
KCl	9.2 ± 1.2	48 ± 3.0	0.5 ± 0.13
ACh	7.6 ± 0.8	61 ± 14	2.3 ± 0.40
EFS-1	6.4 ± 0.8	77 ± 17	0.4 ± 0.09
EFS-2	5.5 ± 0.5	33 ± 4.0	0.5 ± 0.08

Spasmolytic studies

Rat ileum suspended in the organ bath at 37 °C gradually relaxed to a stable baseline. Washing the tissue with fresh Tyrode's solution facilitated tissue relaxation. Addition of KCl (80 mM) caused a rapid contraction followed by a sustained contraction. Hydroalcoholic extract of *P. caespitosa* in a concentration-dependent manner inhibited the sustained contraction induced by KCl. Inhibitory effect of the extract started at bath concentration of 10 μg/ml and with 160 μg/ml of the extract in the bath the contractile response of KCl was completely abolished (Fig. 1). The essential oil of *P. caespitosa* also concentration-dependently reduced the contraction induced by KCl. The inhibitory effect of the essential oil started about 1 μg/ml bath concentration and total effect was achieved with 40 μg/ml bath concentration. Full concentration response for the essential oil and the extract are presented in Fig. 1 for comparison. The IC_{50} values are compared in Table 2. There was no statistically significant change in the contraction of vehicle-treated time-matched control groups (Fig. 1).

Addition of ACh (0.5 μM) into organ bath caused a single contractile response in the rat ileum within 30 s of contact time. Following washing the tissue with fresh Tyrode's solution the tissue quickly relaxed towards the baseline. Addition of ACh at 5 or 15 min intervals induced a reproducible contraction in the rat ileum. Both the hydroalcoholic extract and the essential oil of *P. caespitosa* concentration-dependently inhibited the phasic contraction induced by ACh. The inhibitory effect of the extract was seen at bath concentration of 20 μg/ml and at 320 μg/ml bath concentration the ACh response was abolished.

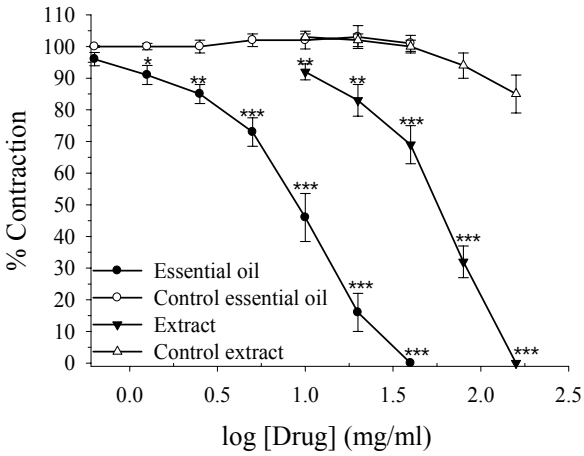

Fig. 1. Inhibitory effects of *Pycnocycla caespitosa* essential oil and its extract on the tension development to KCl (80 mM) in isolated ileum of rats. Ordinate scale: ileum contractile response expressed as percent of initial control responses. Abscissa scale: $\log_{10}$ concentration of test compounds. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). Asterisks show the statistically significant differences between the tested compound and the corresponding point in the vehicle treated time-matched control tissues (**P*<0.05, ***P*<0.01, ****P*<0.001; *Student's t-test*). The oscillation in the contractile response of the control tissues are not statistically significant (ANOVA). The maximum concentration of DMSO in the bath was 0.8 %.

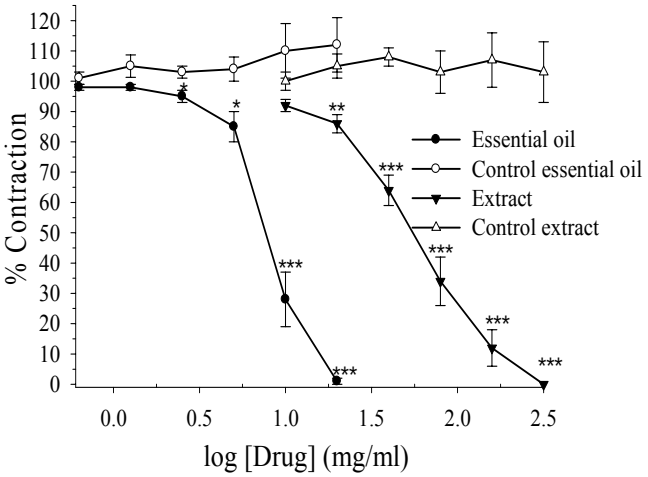

Fig. 2. Inhibitory effects of *Pycnocycla caespitosa* essential oil and its extract on the tension development to acetylcholine (ACh, 0.5 μM) in isolated ileum of rats. Ordinate scale: ileum contractile response expressed as percent of initial control responses. Abscissa scale: $\log_{10}$ concentration of test compounds. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n = 6). Asterisks show the statistically significant differences between the tested compound and the corresponding point in the vehicle treated time-matched control tissues (**P*<0.05, ***P*<0.01, ****P*<0.001; *Student's t-test*). The oscillations in the contraction of control groups are not statistically significant. The maximum concentration of DMSO in the bath was 1.6 %.

The inhibitory effect of the essential oil was observed with 2.5 μg/ml bath concentration and 100% inhibition was achieved with 20 μg/ml essential oil in the organ bath. Full concentration response curve for inhibitory effect of the extract and the essential oil of *P. caespitosa* are presented in Fig. 2 and their IC_{50} values are compared in Table 2. There was no statistically significant change in the repeated response of ACh in the vehicle-treated time-matched control tissues (Fig. 2).

Appliance of EFS produced a biphasic contraction in rat ileum as described before (21,22). The first contraction (EFS-1) was a rapid contraction which partially relaxed and then followed by the second slower contraction (EFS-2) which remained for longer duration before relaxing toward the baseline. After several repeated application of EFS stable responses were achieved and then drug effects were evaluated on the EFS responses.

The hydroalcoholic extract of *P. caespitosa* concentration-dependently attenuated the contractile responses to both EFS-1 and EFS-2 in a similar manner (Fig. 3). The inhibitory effects of the extract begin with 10 μg/ml bath concentration and the EFS responses were totally diminished with 320 μg/ml of *P. caespitosa* in the organ bath. The IC_{50} values are presented in Table 2 for comparison.

The essential oil of *P. caespitosa* also attenuated the EFS responses. However, the inhibitory effect of the essential oil was seen in a lower concentration than the inhibitory effect of the extract (Fig. 3). The inhibitory effect of the essential oil was observed at 2.5 μg/ml in the bath and complete inhibition was achieved with 20 μg/ml of the essential oil in the bath. The inhibitory effect of both the essential oil and the extract were reversible following washing the tissue with fresh Tyrode's solution. There were no changes in EFS-1 responses treated with the vehicle (DMSO) over the course of the study; however, the EFS-2 responses were slightly attenuated over the time (Fig. 3).

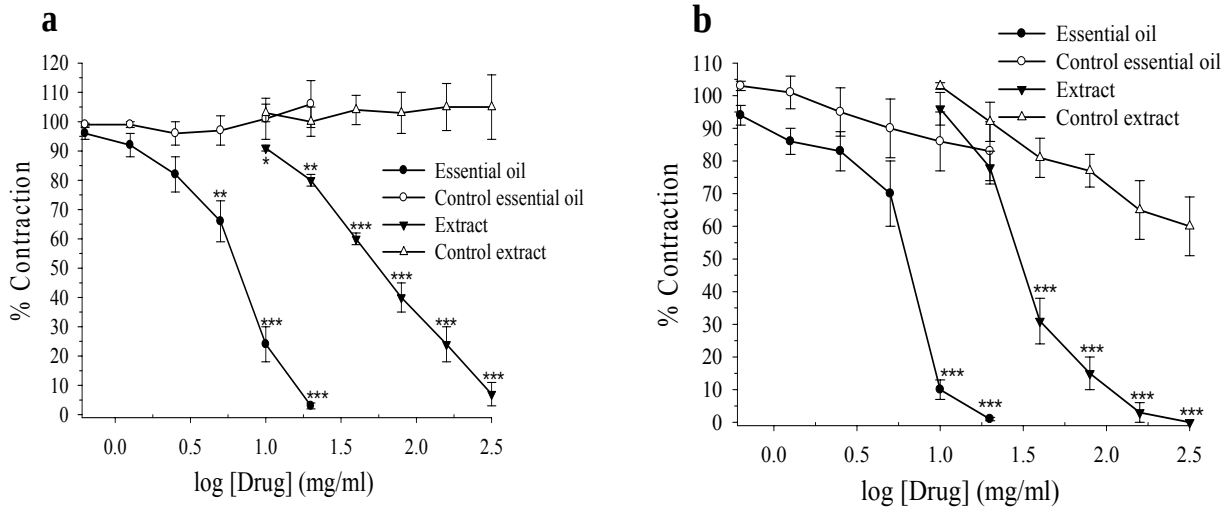

Fig. 3. Inhibitory effects of *Pycnocycla caespitosa* essential oil and its extract on the tension development to a; first and b; second contractile responses to electrical field stimulation (EFS, 6V, 50 Hz, 1 s duration) in isolated ileum of rats. Ordinate scale: ileum contractile response expressed as percent of initial control responses. Abscissa scale: $\log_{10}$ concentration of test compounds. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). Asterisks show the statistically significant differences between the tested compound and the corresponding point in the vehicle treated time-matched control tissues (*P<0.05, **P<0.01, ***P<0.001; *Student's t-test*). The oscillation in the EFS-1 contraction in the control groups are not statistically significant but the reduction in EFS-2 response is significant (ANOVA, P<0.01). The maximum concentration of DMSO in the bath was 1.6 %.

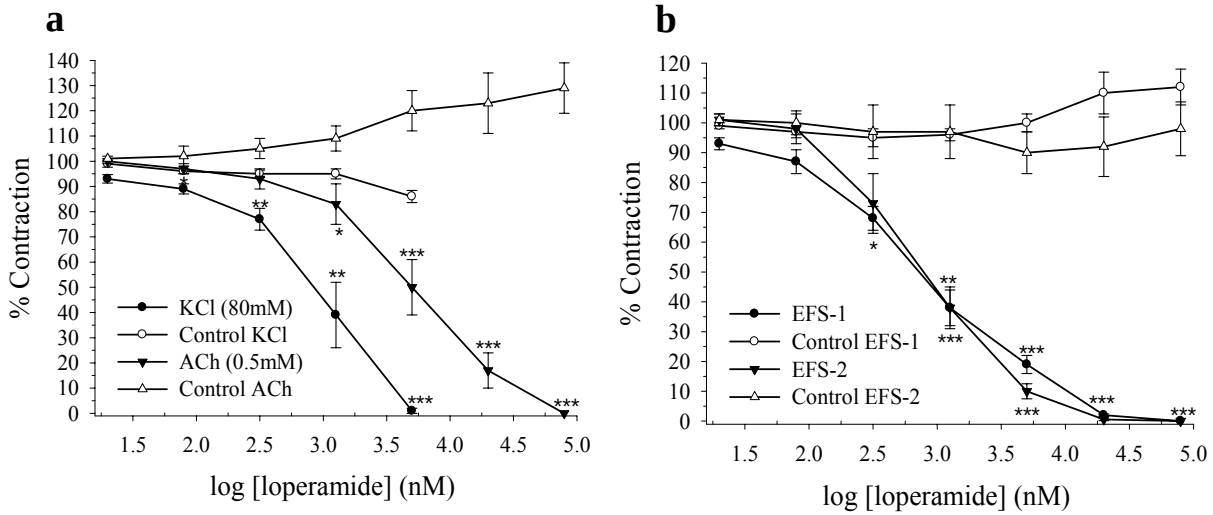

Fig. 4. Inhibitory effect of loperamide on tension development to a; KCl (80 mM), and acetylcholine (ACh, 0.5 μM) and b; to first and second contractile responses to electrical field stimulation (EFS, 6 V, 50 Hz, 1 s duration) in isolated ileum of rats. Ordinate scale: ileum contractile response expressed as percent of initial control responses. Abscissa scale: $\log_{10}$ concentration of test compounds. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). Asterisks show the statistically significant differences between loperamide and the corresponding point in the vehicle treated time-matched control tissues (**P*<0.05, ***P*<0.01, ****P*<0.001; *Student's t-test*). DMSO significantly potentiated the contractile responses to ACh (*P*<0.05, ANOVA). The reduction in KCl response in the control group was also significant (*P*<0.01, ANOVA). The oscillations in the contractions of other control groups are not statistically significant. The maximum concentration of DMSO in the bath was 2 %.

Loperamide was used as a standard antispasmodic drug for comparison. Loperamide inhibited the contractile responses to KCl, ACh and EFS in a concentration-dependent manner. The inhibitory effect of loperamide was started at concentration of 80 nM bath concentration. Loperamide with bath concentration of 5.12 μM, totally removed the response to KCl, while at this concentration still 50% of ACh response and 19% of EFS -1 response was remained. At higher concentration loperamide also totally removed the responses to ACh and EFS (Fig. 4).

DISCUSSION

The objective of the present research was to investigate the antispasmodic effect of both the essential oil and the hydroalcoholic extract of *P. caespitosa*. For this purpose contractions were induced in isolated ileum of rat in three different ways. High concentration of KCl (80 mM) was used as a method of activating voltage gated Ca^{2+} channels (23). External ACh stimulated the muscarinic receptors on the smooth muscle cells and the nicotinic receptors on the parasympathetic ganglions in the gut. This could result in further release of neurotransmitter from postganglionic cholinergic neurons (24). Neuronal stimulation by applying EFS causes release of various neurotransmitters in the enteric nervous system that is embedded within gastrointestinal smooth muscle layers (25,26). Neuronal stimulation has more resemblance with the natural activities of the gastrointestinal tract. Loperamide (used as the standard drug) inhibited the ileum contractions induced by KCl, ACh or neuronal stimulation. The inhibitory effect of loperamide is mediated via opioid receptors which exist on both the neurons and the smooth muscles of the gut (27). Stimulation of opioid receptors on the smooth muscle indirectly results in voltage gated Ca^{2+} channels inactivation (28) and that could explain the inhibitory effect of loperamide on KCl induced contraction. Activation of pre-synaptic opioid receptors on the gut neurons reduces neurotransmitters release (27,28). Therefore, the action of loperamide on both neuronal and smooth muscle sites can explain it inhibitory effect on

ileum contraction. Inhibitory effect of loperamide on ACh induced contraction was seen at higher concentrations than inhibition of KCl or EFS induced contractions. This is because activation of muscarinic receptors results in release of Ca^{2+} from intracellular stores (20).

The essential oil of *P. caespitosa* concentration-dependently inhibited rat ileum contractions induced by KCl, ACh or neural stimulation. Comparisons of antispasmodic activity with that of essential oil of *P. spinosa* indicated that their inhibitory effects were seen in a similar concentrations range (4,30). Analysis of essential oil of *P. caespitosa* revealed that it had many common constituents with the essential oil of *P. spinosa* (4). The common constituents include α pinene, α phellandrene, α-terpinene, ρ-cymene, γ-terpinene, linalool, α-copaene, β-caryophyllene, δ-cadinene and α-thujene. Existence of common substances could explain anti-spasmodic activity of both essential oils. Among constituents of essential oils, substance like α-pinene, β-pinene (31), geraniol, citronellol (21), β-caryophyllene (32), methyleugenol (33), eugenol (34), citral (geranial and neral) (35) limonene and α-terpineol (36) are reported to have antispasmodic activity on smooth muscles. Common constituents in the essential oils suggest that, they may have similar mechanism of action. Essential oil of peppermint also has some common constituents with the *P. caespitosa* essential oil. Mechanism of action of peppermint oil is investigated by electrophysiological methods including patch-clamp technique (37). Direct Ca^{2+} current recording has demonstrated that peppermint oil interacts with Ca^{2+} channels to bring about the relaxation of gastrointestinal smooth muscles and the effect of peppermint oil resembles that of the dihydropyridine calcium channel blockers (37).

The hydroalcoholic extract of *P. caespitosa* also has profound inhibitory effect on the contractions induced in rat ileum by KCl, ACh or neuronal stimulation. The inhibitory effect of the hydroalcoholic extract was seen at the same concentration ranges which were used for the extract of *P. spinosa* (4,11). Comparison of the IC_{50} values indicates that there are similarities between anti-spasmodic activities of these two species of Pycnocyla. For example at similar conditions, the IC_{50} values of hydroalcoholic extract of *P. spinosa* on KCl, ACh and EFS-induced contractions are 40 ± 7.3, 139 ± 17, and 73 ± 13 μg/ml, respectively (4,11). The inhibitory effect of hydroalcoholic extract of *P. spinosa* have been reported to be due to the active substances such as isovanillin, isoacetovanillon, 6-(4-hydroxy-3-methoxyphenyl)-hexanonic acid, and 3,7,10,14,15-pentaacetyl-5-butanoyl-13,17-epoxy-8-myrsinene, the latter being the most active compound (11,12,38). The constituents of the *P. caespitosa* extract are not determined yet but the similarities between plant species and the pharmacological activities may indicate that they may have similar constituents. Although, the essential oil of *P. caespitosa* was more potent than the extract which could be the results of the existence of more pure substances in the essential oil. As the solvent in the hydroalcoholic extract was evaporated it is likely that most of the essential oil is also lost. Thus, the inhibitory effect of the extract could not be due to the existence of the essential oil in the extract and other components could be responsible for the inhibitory effects.

CONCLUSION

In this study, we have demonstrated the relaxant effect of both the essential oil and the hydroalcoholic extract of *P. caespitosa* on ileum contractions. The antispasmodic effects were relatively similar to loperamide. Further investigations for identification of the active substances present in the extract are recommended.

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