ABSTRACT

**Object:** The present study was for screening of umbelliferae plants used to treat gastrointestinal spasm. Selection of volatile extracts of umbelliferae plants materials based on functional approach.

**Material and Method:** The umbelliferae family was found most effective against gastrointestinal spasm. Plants materials were procured from local market of Rajasthan. Volatile extracts isolated by hydro distillation. Functional ratio was calculated separately.

**Results:** Antispasmodic effect studied on guinea pig ileum *in vitro*; IC$_{50}$ was recorded. Volatile extracts inhibits spontaneous contraction of acetylcholine induced contraction of guinea pig ileum dose dependently. The volatile extracts of following plants *Trachyspermum ammi*, *Cuminum cyminum*, *Anethum graveolens* and *Foeniculum vulgare* induces dose dependent relaxation in guinea pig ileum has proved. A very effective value identified, when compared to antispasmodic drugs e.g. Atropine.

**Conclusion:** The current research discloses the reason behind selection of umbelliferae plants. This study validates volatile extracts of some plants as antispasmodic and functional ratio that helps in selection procedure.

**Key words:** Antispasmodic, functional ratio, atropine, guinea pig ileum.

INTRODUCTION

Gastrointestinal spasm is very common global problem in all age groups.[1] Various gastrointestinal disorders like abdominal pain, flatulence, colic, diarrhoea, constipation, bloating, cramping, Irritable bowel syndrome are due to abnormalities in motor function disturbance.[2-4] Majority of the above symptoms are due to spasm. Unfortunately no single drug has proven to be effective in treating functional gastrointestinal problems. In addition, the search for a truly effective and safe drug to control motility disturbances continues. [5]

Developing countries still dependent on rational medicinal plants infusions to treat various gastrointestinal problems. Based on this fact, we are focusing attention on medicinal plants. Because of great potential, these plants carry in combating various diseases. Therefore, antispasmodic activity of volatile extracts useful to treat gastrointestinal disturbance.

Ayurveda, an Indian system of medicine, cited several plants, which are useful against various gastrointestinal disorders without any side effects. On the basis of literature and various marketed formulation, the umbelliferae plants identified marginally different from other families. Various herbal formulation contains following umbelliferae plants *Criandrum sativum*, *Pimpinella anisum*, *Trachyspermum ammi*, *Apium graveolens*, *Cuminum cyminum*, *Anethum graveolens* and *Foeniculum vulgare*. All these plants have been used to treat various gastrointestinal disorders like abdominal pain, flatulence and colic. [7-9] In the present study, we have investigated the antispasmodic effect of umbelliferous plants volatile extracts on guinea pig ileum *in vitro*. 

*Corresponding Author: Saini N, Email: saininavdeep079@gmail.com*
Antispasmodic effect of these plants volatile extracts demonstrated by functional approach.

**MATERIAL AND METHODS**

**Collection and Authentication of Plants**

Plants of Umbelliferae (Apiaceae) family *Crianzum sativum, Pimpinella anisum, Trachyspermum ammi, Apium graveolens, Cuminum cyminum, Anethum graveolens and Foeniculum vulgare* were procured from the local market of Pratapgarh, Rajasthan. The plants materials were identified by Prof. S. K. Panday, Scientist, KNK College of Horticulture, Mandsaur, Madhya Pradesh, India. The voucher specimen is submitted in Department of Pharmacognosy for future reference.

**Chemicals and Reagents**

All chemicals, reagents and solvents were used for study procured from Puneet Enterprises, Ratlam, Madhya Pradesh, India. Atropine was purchased from Sigma-Aldrich Chemical, St. Louis, MO, USA and acetylcholine from Merck India Ltd., Mumbai, India. All other chemicals were purchased from Loba Chemie, Mumbai, India.

**Preparation of Volatile Extracts**

The dried fruits and seeds of umbelliferae plants were grinded to get fine powder using a grinder (Voltas-300). The grinded powder was then assembled for hydro-distillation to isolate volatile extract with the help of tap water. 500 g powdered plants material were hydro distilled with 1000 ml tap water at 100°C for 8 h. The extracts thus obtained were taken for the antispasmodic activity. The yield of the extracts was in range 2-4% of total dried material. The same procedure was repeated for other umbelliferous plants. Light yellowish coloured extract was obtained having characteristic odour and taste. Volatile extracts were dried over anhydrous sodium sulphate to remove moisture and stored in a dark glass bottle and kept at 4°C for further analysis.

**Determination of Functional Ratio**

The functional approach applied for screening umbelliferae plants as antispasmodic. It helps in determining the concentration of extracts for each plant giving half maximal inhibitory response (IC$_{50}$). After this determination, only the plants with the half maximal response lower than the mixture of plants were used in further studies. The new rational formulation can be prepared on the basis of functional ratio (Fr), which is considered as a unit of preparation of a new rational formulation. The Fr was obtained using the following equation:

$$Fr = \frac{\text{xIC}_{50}}{\text{yIC}_{50}}$$

xIC$_{50}$ represent the value of IC$_{50}$ of plant x, and yIC$_{50}$, the lowest IC$_{50}$ found for the most effective plant extract (y) among plants which composed traditional preparation.

**Preparation of Physiological Solutions**

Tyrode solution composition (mM): NaCl, 137; KCl, 2.7; CaCl$_2$, 1.8; NaH$_2$PO$_4$, 0.4; MgSO$_4$, 0.25; NaHCO$_3$, 11.9; glucose, 11.1 de Jalon solution composition (mM): NaCl, 154; KCl, 5.6; CaCl$_2$, 0.55; NaHCO$_3$, 6.0; glucose 2.78.

**Experimental animals**

Guinea pigs of average weight and either sex were used for in-vitro studies. The animals were maintained on synthetic pelleted feed (Lipton India Ltd., Mumbai, India) and water ad libitum, in a 12-h/12-h light/dark cycle at a temperature of 21-23°C. The study protocol was approved by Institutional Animal Ethical Committee (IAEC). CPCSEA guidelines were adhered to during maintenance and experiment.

**Animals for organ bath studies**

Male guinea pig (300-350 g b.wt.) after 24 h fasting were killed by stunning and bleeding. Ileum were removed and suspended under a constant tension of 1 g in 15 ml organ baths containing Tyrode at 37°C. Before the experimental procedures the organs were allowed for 30-40 min equilibration period with changes of medium at every 5 min. Volatile extracts were dissolved in 0.5% DMSO. 200 μl diluted volatile extracts were used as the maximum volume in the baths, at which volume there was no solvent effect. During the experimental phase volatile extracts were added to the bath and after 1 min incubation period the agonist was added. Contractions were recorded using isotonic transducer connected to a polyrite (Model 201, Recorders and Medicare, India).

**Isolated Guinea pig Ileum**

A portion of 2.5 cm guinea pig ileum was suspended in aerated Tyrode solution at 37°C.

**Antispasmodic activity of volatile extracts**

The amplitude of spontaneous contraction, frequency of spontaneous contraction, relaxation and contraction of tissue before and after addition of the volatile extract recorded cumulatively. Typical recording of the effect of volatile extract studied. In controlled condition, the frequency of
spontaneous contraction of ileum was close to 25 contractions per minute. The amplitude of spontaneous contraction of tissue without any addition of product was evaluated. This amplitude was stable before addition of 50 μL/mL volatile extract, the amplitude decreased with the augmentation of concentration. With concentrations of up to 1000 μL/mL, the frequency of contraction declined and became significantly blunted with 1550 μL/mL. Similarly activity of all volatile extracts of umbelliferae plants compared (IC50, Table1).

**Statistical Analysis**

All the data are expressed as mean ± standard error of mean (S.E.M., n = number of experiments), except for the IC50 (concentration of drugs causing half-maximal responses), which are presented as geometric means accompanied by their respective 95% confidence intervals. The statistical analyses were obtained by the one way analysis of variance (ANOVA), followed by the Dunnett's test where necessary. P<0.05 was considered significant. The concentration-response curves were analyzed by non-linear regression (Graphpad program for Windows version 5.01. Graphpad, San Diego, CA, USA).

**RESULTS**

The results of the present study clearly demonstrate that in vitro pre-treatment of guinea pig ileum with the volatile extract of plants induced dose dependent relaxation. In addition, this relaxation was accompanied by a reduction of frequency and amplitude of spontaneous contractions. Further addition of acetylcholine after maximal effect of volatile extract of plants showed that this effect was reversible. After confirmation of utility of volatile extracts of these plants, we focused our study on antispasmodic potency of volatile extract of umbelliferae plants. Among the various plants of umbelliferae, we observed that only four plants Trachyspermum ammi, Cuminum cyminum, Anethum graveolens and Foeniculum vulgare can provoke relaxation of guinea pig (Table 1). The effect of Anethum graveolens was more important (IC50=80.4±1.1 μL/mL) than Trachyspermum ammi, Cuminum cyminum and Foeniculum vulgare, whereas Coriandrum sativum, Pimpinella anisum and Apium graveolens exhibited weak effects (respectively IC50=747.1±1.2, 610.3±1.0 and 464.1±1.0μl/mL). The current research on the basis of functional ratio reveals that lowest IC50 with Anethum graveolens (IC50 of 80.4±1.1 μL/mL) and the value of 1 was given to this volatile extract whereas the highest functional ratio of 9.29 was attributed to Coriandrum sativum.

<table>
<thead>
<tr>
<th>S No</th>
<th>Name</th>
<th>IC50(μL/mL)</th>
<th>Functional ratio (Fr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anethum graveolens</td>
<td>80.4±1.1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Cuminum cyminum</td>
<td>96.7±1.2</td>
<td>1.20</td>
</tr>
<tr>
<td>3</td>
<td>Foeniculum vulgare</td>
<td>106.5±1.2</td>
<td>1.32</td>
</tr>
<tr>
<td>4</td>
<td>Trachyspermum ammi</td>
<td>111.5±1.2</td>
<td>1.38</td>
</tr>
<tr>
<td>5</td>
<td>Apium graveolens</td>
<td>464.1±1.0</td>
<td>5.77</td>
</tr>
<tr>
<td>6</td>
<td>Pimpinella anisum</td>
<td>610.3±1.0</td>
<td>7.59</td>
</tr>
<tr>
<td>7</td>
<td>Coriandrum sativum</td>
<td>747.1±1.2</td>
<td>9.29</td>
</tr>
</tbody>
</table>

Value of IC50 are expressed as geometric mean with 95% confidence interval

**DISCUSSION**

The present study shows that volatile extracts reduces spontaneous contractions on isolated guinea pig ileum. The absence of contractile activity of the drug itself on various smooth muscle preparations shows the lack of agonistic activity on muscarinic. The results obtained on isolated smooth muscle show the inhibition of acetylcholine induced contractions.

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Binding of Ach to muscarinic receptors in smooth muscles results in opening of receptor operated channels, thereby allowing sodium influx, which causes a depolarization of the cell membrane. This depolarization opens voltage dependent calcium channels and calcium ions enter the cell to induce the release of calcium from the sarcoplasmic reticulum. The cytosolic calcium thus binds to calmodulin, which results in contraction.[15,16]

Since these spasmogens have different modes of action, the antagonism elicited by volatile extract indicates that it might be acting at a common step in the contraction mechanism elicited by these agonists. The antagonism displayed was concentration dependent. Since acetylcholine effects were altered by the volatile extracts, it seems to be non-specific antagonism. Earlier research works on the extracts of individual ingredients of Trachyspermum ammi, Anethum graveolance, Cuminum cyminum and Foeniculum vulgare were credited for their antispasmodic activity. The essential oils of Foeniculum vulgare was reported to exhibit antispasmodic effect in rat uterus preparation[17-19] The combination of these
active constituents could be responsible for the observed antispasmodic effect of volatile extracts. Umbelliferae plants *Trachyspermum ammi*, *Anethum graveolance*, *Cuminum cyminum* and *Foeniculum vulgare* share common constituents i.e. α-pinene, limonene. The following compounds were obtained from the former plants. Anethole, and fenchone from, fennel (*Foeniculum vulgare*); Carvone in dill (*Anethum graveolance*), Thymol and carvacrol in ajwain (*Trachyspermum ammi*), limonene, linalool and 1,8-cineol in cumin (*Cuminum cyminum*). Several studies *in vitro* and *in vivo* showed that those compounds can regulate disturbances of gastrointestinal tract such as inhibition of guinea pig intestinal peristalsis.[20] Their effects depend on the mechanism involved and the tissues. The spasmogenic effect may involve the activation of muscarinic receptors, while the spasmolytic effect is predominantly due to blockade of the calcium channels.[21]

The present study reveals that different volatile extracts dose-dependently inhibited gastrointestinal spasm on guinea pig ileum *in vitro*. All these findings suggest that volatile extracts having non-specific antispasmodic activity, which can be used in the treatment of various non-specific spasm disorders.

**CONCLUSION**

All the above findings suggest that volatile extracts of *Trachyspermum ammi*, *Anethum graveolance*, *Cuminum cyminum* and *Foeniculum vulgare* comparatively more potent antispasmodic than others, which can be used in the treatment of various spasmodic disorders of gastrointestinal tract. The present study confirms the antispasmodic activity of the volatile extracts using modern pharmacodynamic experiments. Further studies are in progress for development of newer polyherbal formulation.

**ACKNOWLEDGEMENT**

We thank Professor G.K. Singh and Professor B. P. Nagori for advice. The study was made possible by cooperation between LMCP, Jodhpur and BRNCOP, Mandsaur, India for providing required facilities of this work. Authors are also grateful to Professor S. K. Panday, Scientist, KNK College of Horticulture, Mandsaur, Madhya Pradesh, India for authentication of plants materials.

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