EFFECT OF YAVANI ARKA (HYDRO DISTILLATE OF TRACHYSPERMUM AMMI) ON RAT ILEUM, AGAINST ACETYL CHOLINE INDUCED CONTRACTIONS

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ABSTRACT

Yavani or Trachyspermum ammi is an important drug used in traditional systems of medicines to treat gastrointestinal disorders like indigestion, colic, and diarrhea. Yavani and its distillate (Arka) has been indicated in classics to treat Agnimandya (digestive impairment), Anidra (Insomnia), Atisara (Diarrhea), Klaibya (Impotency) and Grahani (Irritable Bowel Syndrome). To evaluate its effect on smooth muscle of gastro intestine, the present study has been planned. Isolated rat ileum was hanged and balanced in organ bath assembly; Tyrode solution (for nutrition), temperature of 37°C and Oxygen (through aerator device) were maintained in the assembly (inner tube, where tissue was mounted), to keep the tissue alive. Contraction was induced by Acetylcholine. Then the response of Yavani Arka was observed at the dose of 0.5 ml, 1 ml, 1.5 ml and 2 ml/40 ml Tyrode solution. Relaxation effect or antispasmodic activity of Yavani arka was observed on all the contraction. The Effective Concentration for 50% effect (EC50) was found to be 0.0165 ml/ml.


INTRODUCTION

Yavani (Trachyspermum ammi) is a well-known herb used in kitchen to as spices and also as home remedy to treat common abdominal problems1. The drug has also kept important place in the traditional medicinal systems i.e. Ayurveda and Unani, where it is used individually and in combinations in the form of powder, Arka (Hydro distillate), Asava- Arishtha (Fermented liquids), tablets, oils etc. Its constituents and oils are extracted and used in modern system of medicine also2,3. In Ayurveda text it is indicated in the treatment of Agnimandya (digestive impairment), Anidra (Insomnia), Atisara (Diarrhoea), Klaibya (Impotency) and Grahani (Irritable Bowel Syndrome) etc. Since it is an Aromatic drug and having volatile oil as one of important active constituent4, So Arka Kalpna, (the Hydro distillation) is very much compatible to use of it, it is very easy to intake (higher palatability). It is also indicated in Agnimandya (digestive impairment), Trikashoola (pain in sacral region) and abdominal discomforts5. Text of Ayurveda6 and Unani system of medicine7, both are having same type of indications, so for the present study Yavani Arka has been selected for the study.

Isolated tissue bath assays are classical pharmacological tool for evaluating dose concentration- response relationship in a myriad contractile tissue8. It is isolated organ bath system, which is used for over 150 years in the discipline of pharmacology. The versatility of this system has allowed scientists across the world to characterize receptors and receptor signal transduction, with this knowledge forming the basis of therapies that have treated millions of individuals with diseases or disorders such as hypertension, heart failure, diabetes, gastrointestinal disease, bladder dysfunction, asthma, and swallowing disorders, to name just a few9. To this day, the isolated tissue bath remains an important facet of drug development and basic research, as it allows the tissue to function as a system. Due to all this, it comes closer to examine that how drug would act in the body as whole. Since the present study is aimed to evaluate the effect of Yavani Arka on the smooth muscles, so it is planned to be carried out on the isolated rat ileum.

MATERIALS AND METHOD

Preparation and Analysis of Yavani Arka

Yavani Arka was prepared in the departmental lab, PG Department of Rasashastra & Bhaishajya Kalpna, National Institute of Ayurveda, Jaipur as per API10 (Fig. 1) and was analyzed as per API (The Ayurvedic Pharmacopoeia of India),(Table 1). Study was conducted after clearance form committee. The registration no. of study was Aca/863/14-15.

Table 1: Characters of Yavani Arka

<table>
<thead>
<tr>
<th>Character</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Transparent</td>
</tr>
<tr>
<td>Odour</td>
<td>Typical Yavani like</td>
</tr>
<tr>
<td>Taste</td>
<td>Tikta</td>
</tr>
<tr>
<td>Appearance</td>
<td>Clear watery</td>
</tr>
<tr>
<td>Clarity</td>
<td>Floating oil drops</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.004</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Preparation of physiological salt solution (Tyrode’s solution)

Tyrode solution was taken as physiological salt solution and used to keep the ileum alive. It was prepared by mixing of salt and glucose in distilled water as mentioned in Table 2. It was composed as per previous studies11.
Table 2: Composition of Tyrode’s solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>16 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.4 g</td>
</tr>
<tr>
<td>MgCl</td>
<td>1.5 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2 g</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>4 g</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2 L</td>
</tr>
</tbody>
</table>

Preparation of tissue

Ileum of wistar rat was isolated and kept in the oxygenated Tyrode solution at room temperature. Longitudinal strips of approx. 2-3 cm long were prepared from the ileum. (Fig. 2)

Preparation of organ bath assembly & conduction of study

In order to conduct the study first of all organ bath system was assembled. Outer chamber was cleared and filled with warm distilled water to maintain the temperature. Inner tissue bath is filled with Tyrode solution and continuously oxygenated by aerator; temperature of the chamber maintained at 37°C. The frontal pointing liver was fixed on mantle rod of organ bath. Kymograph paper was set on the rotating drum to record the readings. A piece of ileum approx. 2 cm, with silk thread was tied on the liver rod and suspended into inner chamber. The tissue was then given 3 successive washings with fresh Tyrode solution an allowed to be relax for obtaining a stable base line (Fig. 3).

After setting of above assembly, Acetylcholine (AcH) in dose of 10µlml was poured on the suspended tissue and contractions were induced, the graph of readings was found on Kymograph paper, place on rotating drum (Fig. 4). The procedure was repeated till the ceiling effect was recorded (for 9 minutes maximum). Then 0.5 ml of Yavani Arka was poured on the tissue and readings were noticed for 2.5 minutes (Fig. 5). In same manner the readings were taken with the doses of 1.0 ml, 1.5 ml and 2.0 ml of Yavani Arka against AcH induced contractions and the readings found on kymographs was collected (Fig. 6, 7, 8). Three times washings with Tyrode solutions were giving after every reading.

Data analysis

To analyze the data the reading was measured in centimeters. Maximum response was considered as 100% response and accordingly other readings were also calculated in percentage. Then a graph (Graph no. 1) was plotted in between dose & response (Dose-response curve; DRC). Finally, effective concentration for 50% effect (EC₅₀) was calculated from the graph.
RESULTS

Relaxations against Acetylcholine induced contractions were noticed on all the dose of Yavani Arka. As per table 3 relaxation of different intensity was noticed on different doses. The EC_{50} value of Yavani Arka was found as 0.0162 ml/ml.

<table>
<thead>
<tr>
<th>Dose of Yavani Arka</th>
<th>Net dose of Arka/Strength of Arka in 40 ml inner organ bath tube (v/v)</th>
<th>Response (Relaxation) in cm</th>
<th>% Response (Response/Max. response %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 ml</td>
<td>0.0125 ml</td>
<td>1.6</td>
<td>41%</td>
</tr>
<tr>
<td>1.0 ml</td>
<td>0.025 ml</td>
<td>2.8</td>
<td>71%</td>
</tr>
<tr>
<td>1.5 ml</td>
<td>0.0375 ml</td>
<td>3.2</td>
<td>82%</td>
</tr>
<tr>
<td>2.0 ml</td>
<td>0.05 ml</td>
<td>3.9</td>
<td>100%</td>
</tr>
</tbody>
</table>

DISCUSSION

Present study was focused on evaluation of relaxant effect of distillate of *Trachyspermum ammi* (Yavani Arka). Yavani is indicated in Agnimandya (digestive impairment), Anidra (Insomnia), Atisara (Diarrhea), Klaibya (Impotency) and Grahani (Irritable Bowel Syndrome). Its distillate (Arka) is also indicated in same. Its major component is essence which is mainly composed of thymol (49.0%), γ-terpinene (30.8%), p-cymene (15.7), β-pinene (2.1%), myrcene (0.8%), and limonene (0.7%). Along with the availability of modern medications, the propensity toward the traditional medications is progressively growing throughout the world. Isolated organ bath study technique was elected for the study design. The primary advantage of this technique is that the tissue is living and functions as a whole tissue, with a physiological outcome (contraction or relaxation) that is relevant to the body. It is a synthesis of steps i.e. drug-receptor interaction, signal transduction, second messenger generation, change in smooth muscle excitability, and change in tissue function. While other techniques allow study of each of these steps (e.g. radio ligand binding for drug affinity, measurement of second messengers), the isolated tissue bath technique allows for integration of all these steps. Another advantage is that retaining tissue function permits calculation of important pharmacological variables that are more meaningful in a tissue vs a cellular setting; it comes closer to how the drugs examined would work in the body as a whole.
As the result of the study says that the Yavani Arka relaxed the contractions induced by the Ach and showed antispasmodic effect. The main constituent of T. ammi is thymol²⁰ and in previous studies same effects were noticed by using different concentration of thymol²¹ and by aqueous & ethanolic extracts²²-²³. There are various mechanism involved for relaxation of gastrointestinal smooth muscles within the human body; these may be blocking action on excitatory pathways, such as cholinergic²⁴ and histaminergic²⁵ or may be through agonistic actions on inhibitory modulators such as adrenergic²⁶, purinergic²⁷, GABAergic²⁸, and nitric oxide²⁹. Thymol, which is reported to be present in the essential oil and Yavani Arka also contains essential oil. Anticholinergic activity of Thymol has been reported in earlier studies³⁰, where it showed significant relaxant effect on the smooth muscles. That may be most probable reason for the same effect, observed in the present study. Other probable reasons may be due to antihistaminic activity or calcium channel-clocking activity of some active part in Yavani Arka. Some reports are available in the favor of this, where thymol is reported for relaxant effect on isolated rat aorta³¹ and ventricular myocardium³², may be due to blocking of calcium channels.

CONCLUSION

It can be concluded by the current study that Yavani Arka has relaxant or antispasmodic effect on the smooth muscles. It relax the GIT muscles against contraction induced by Ach. Results supports that it can be used to treat abdominal discomfort, colic pain etc. Further clinical trials on human subjects are suggested on the basis of the current study, it can confirm the effects in more validated way.

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