



## Research Article

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### 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 (EC<sub>50</sub>) was found to be 0.0165 ml/ml.

**KEYWORDS:** Ajawain, Arq-e-ajavain. Ajavayana Arka. Arka Kalpana, Smooth muscle.**INTRODUCTION**

Yavani (*Trachyspermum ammi*) is a well-known herb used in kitchen to as spices and also as home remedy to treat common abdominal problems<sup>1</sup>. 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- Arishta (Fermented liquids), tablets, oils etc<sup>2</sup>. Its constituents and oils are extracted and used in modern system of medicine also<sup>3,4</sup>. In Ayurveda text it is indicated in the treatment of Agnimandya (digestive impairment), Anidra (Insomnia), Atisara (Diarrhea), Klaibya (Impotency) and Grahani (Irritable Bowel Syndrome) etc<sup>5</sup>. Since it is an Aromatic drug and having volatile oil as one of important active constituent<sup>6</sup>, So Arka Kalpana, (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 discomforts<sup>7</sup>. Text of Ayurveda<sup>8</sup> and Unani system of medicine<sup>9</sup> 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 tissue<sup>10</sup>. 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 few<sup>11</sup>. 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 Kalpana, National Institute of Ayurveda, Jaipur as per AFI<sup>12</sup> (Fig. 1) and was analyzed as per API (The Ayurvedic Pharmacopeia 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**

Character	Observations
Color	Transparent
Odour	Typical Yavani like
Taste	Tikta
Appearance	Clear watery
Clarity	Floating oil drops
pH	3.7
Specific Gravity	1.004
Refractive index	1.34

**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 studies<sup>13</sup>.

**Table 2: Composition of Tyrode's solution**

Ingredient	Quantity
NaCl	16 g
KCl	0.4 g
MgCl	1.5 g
NaHCO <sub>3</sub>	2 g
NaH <sub>2</sub> PO <sub>4</sub>	0.1 g
Glucose	4 g
CaCl <sub>2</sub>	0.4 g
Distilled water	2 L

**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 and allowed to be relax for obtaining a stable base line (Fig.3).

After setting of above assembly, Acetylcholine (ACh) in dose of 10µml 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 (EC<sub>50</sub>) was calculated from the graph.



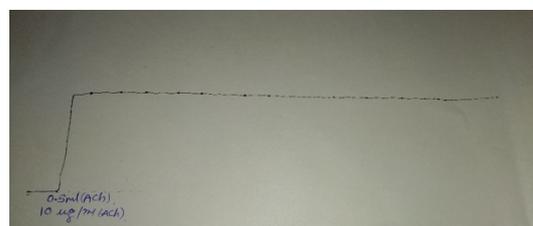
**Fig. 1- Yavani Arka**



**Fig. 2- Isolated Rat ileum**



**Fig. 3- Organ Bath assembly**



**Fig. 4 Contraction induced by ACh**

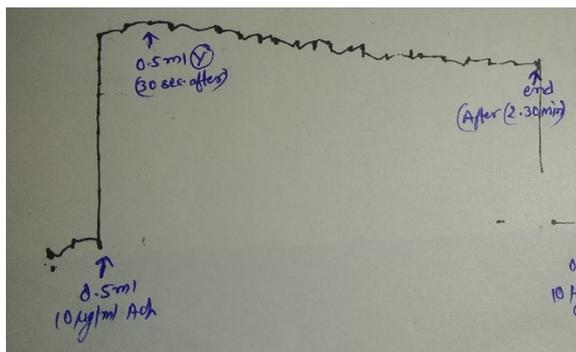


Fig. 5- Effect of Yavani Arka 0.5 ml/40 ml. against ACh induced contraction

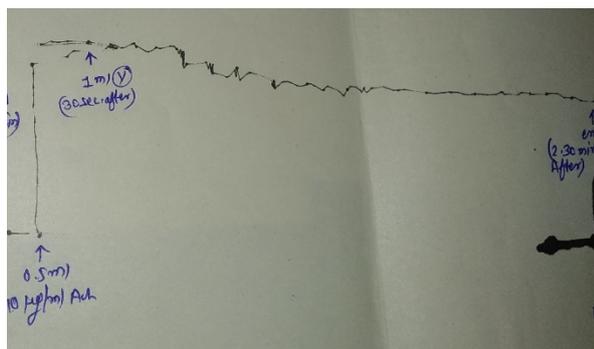


Fig. 6- Effect of Yavani Arka 1 ml/40 ml. against ACh induced contraction

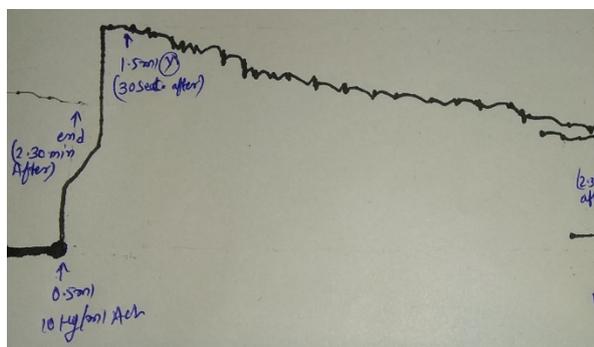


Fig. 7- Effect of Yavani Arka 1.5 ml/40 ml. against ACh induced contraction

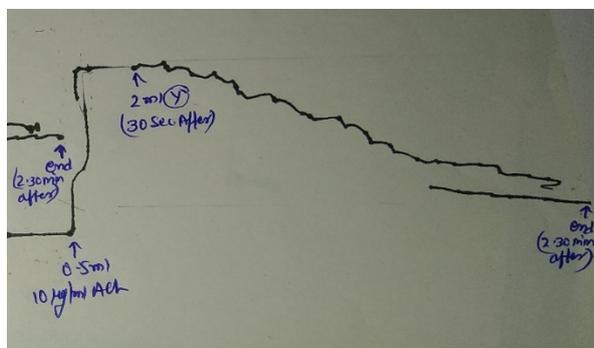


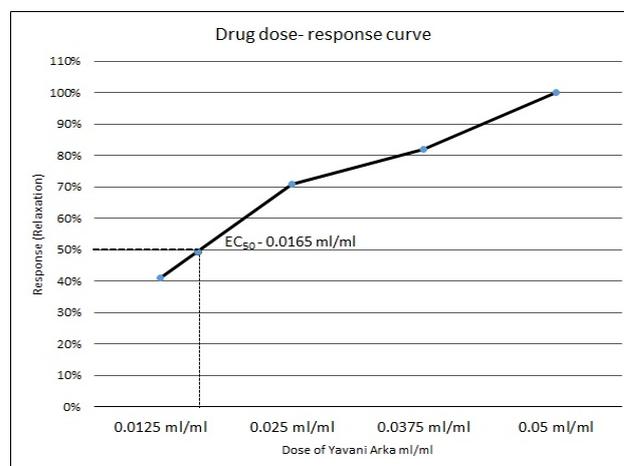
Fig.8- Effect of Yavani Arka 2 ml/40 ml. against ACh induced contraction

**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<sub>50</sub> value of Yavani Arka was found as 0.0162 ml/ml.

Table 3: Response of different doses of Arka on isolated tissue

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



Graph 1: Response on smooth muscles of different doses

**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)<sup>14</sup>. Its distillate (Arka) is also indicated in same<sup>15</sup>. Its major component is essence which is mainly composed of thymol (49.0%),  $\gamma$ -terpinene (30.8%), p-cymene (15.7),  $\beta$ -pinene (2.1%), myrcene (0.8%), and limonene (0.7%)<sup>16</sup>. Along with the availability of modern medications, the propensity toward the traditional medications is progressively growing throughout the world<sup>17</sup>.

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<sup>18</sup>. 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<sup>19</sup>.

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 thymol<sup>20</sup> and in previous studies same effects were noticed by using different concentration of thymol<sup>21</sup> and by aqueous & ethanolic extracts<sup>22,23</sup>. 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 cholinergic<sup>24</sup> and histaminergic<sup>25</sup> or may be through agonistic actions on inhibitory modulators such as adrenergic<sup>26</sup>, purinergic<sup>27</sup>, GABAergic<sup>28</sup>, and nitric oxide<sup>29</sup>. 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 studies<sup>30</sup>, where it showed significant relaxant effect on the smooth muscles. That may be most probable reason for the same effected, 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 aorta<sup>31</sup> and ventricular myocardium<sup>32</sup>, 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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