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Research Article

EFFECT OF *MISHREYA ARKA* (HYDRO DISTILLATE OF DILL) ON RAT ILEUM, AGAINST ACETYL CHOLINE INDUCED CONTRACTIONS

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KEYWORDS: *Arka Kalpana, Mishreya Arka*, *Shatpushpa*, Smooth muscles, Spasmolytic effect.

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ABSTRACT

Isolated tissue bath study is a classical and best way to evaluate the effect of any medication on smooth muscles. It allows the tissue to function as a system, it is close to how drug would act in body as whole. *Mishreya* (*Anethum sowa* Kurz) or dill is an important herb used as home remedy for common abdominal problems and described in Ayurveda as well as in other systems of medicines also like Unani and modern medicine. *Mishreya Arka* or hydro-distillate of dill is indicated in indigestion, stomach ache, uterine pain etc. To evaluate the effect of *Mishreya Arka* on smooth muscles of intestine, the present study has been planned. *Mishreya Arka* was prepared by following the Ayurvedic formulary of India. Isolated rat ileum was set on organ bath assembly with maintained temperature (37°C), nutrition and oxygenated. Contraction was induced by Acetylcholine. Then response of *Mishreya Arka* was observed at the dose of 0.5ml, 1ml, 1.5ml and 2ml/ 40ml Tyrode solution. Relaxations or spasmolytic effect was noticed on all the doses of *Mishreya Arka* against the contraction induced by Acetylcholine. The effective concentration for 50% effect (EC_{50}) of *Mishreya Arka* was 0.0315 ml/ml. It can be concluded that *Mishreya Arka* has spasmolytic effect on rat ileum.

INTRODUCTION

Anethumsowa Kurz is a herb belong to Apiaceae (Umbelliferae) family, which is very commonly used as home remedy in colic pain and other abdominal discomforts, especially in children. More or less it is very important and very widely used medicine in Ayurveda, Unani as well as modern medicine systems. In Ayurvedic terminology it is known with the synonyms of *Mishreya*, *Shatpushpa*, in *Unani Shibt*, *Sowa*, *Soa* etc. Common names are dill, *Sowa*, *Soa* etc.[1] It is indicated in indigestion, colic pain, stomach ache, uterine pain and other abdominal pain as per text of Ayurveda and Unani system of medicine.[2,3] In modern system of science also gripe water is a very commonly used remedy for relieve colic pain in babies and flatulence in young children and dill water is an important ingredient of it.[4]

Mishreya is used individually and in combinations with other drug in many formulations. Individually it is used in various dosage forms like *Churna* (Powder), *Taila* (Oil), *Phant* (Diffusion), *Kwatha* (Decoction) and *Arka* (Hydro-Distillate). Since main active constitute of *Mishreya* is its essential volatile oil, so for that *Arkakalpana* (Hydro-distillate) should be preferred, which is much palatable and pleasant than others. *ArkaKalpana* can be defined as a liquid preparation obtained by distillation of certain liquids or of drugs soaked in water using *Arkayantra* (Distillation assembly).[5] *Mishreya Arka* is also indicated in almost same indication like raw herb i.e. *Agnimandya* (Impairment of digestive fire), *Yoni Shoola* (Uterine pain), *Udara Shoola* (stomach ache), *Krimi Roga* (Helminthiasis/worm infection), *Su-e-Hazam/ Ajeerna* (Indigestion), *Qillat-ul-Baul* (Oliguria) and *Nafkh-e-Shikam* (flatulence) etc.[6,7]

These all conditions are related to smooth abdominal muscles. If any drug affects the movement of gastrointestinal muscles, it is important to understand in which manner it does

the same, so that its actions can be assumed much precisely. Considering these points, the present study *Mishreya Arka* has been selected to evaluate its effect on smooth gastrointestinal muscles.

To study the effect of any substance on smooth muscle, isolated organ bath assay is easy, convenient and reliable method. Isolated tissue bath assays are a classical pharmacological tools for evaluating dose concentration- response relationship in a myriad contractile tissue.[8] 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.[8] 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 *Mishreya Arka* on the contraction of smooth muscles, so it is planned to be carried out on the isolated rat ileum.

Materials and method

Mishreya Arka was prepared as per The Ayurvedic Formulary of India (AFI)[9] (Figure. 1&2) by using the simple distillation method. Total 50g of *Mishreya* fruits (Figure 1) were taken, soaked in 350ml of water and 250ml of *Mishreya Arka* was obtained (Figure 2). It was further analyzed as per The Ayurvedic Pharmacopeia of India (API).[10] The registration number of study was Aca/863/14-15.

Preparation of Physiological Salt Solution (Tyrode's Solution)

Tyrode solution was taken as physiological salt solution and used to keep the ileum. It was prepared by mixing of salt and glucose in distilled water as mentioned in Table 1. For composition of the same, previous study was followed.[11]

Table 1: Showing the composition of Tyrode's solution

Ingredient	Quantity
NaCl	16 g
KCl	0.4 g
MgCl	1.5 g
$NaHCO_3$	2 g
NaH_2PO_4	0.1 g
Glucose	4 g
$CaCl_2$	0.4 g
Distilled water	2 L

Preparation of Tissue

Ileum of Wistar rat was isolated and kept in the oxygenated Tyrode solution. Longitudinal strips of approximately 2-3cm long were prepared from the ileum. (Figure. 3)

Preparation of Organ Bath Assembly and Conduction of Study

In order to conduct the study first of all organ bath system was assembled (figure.4). 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^{0}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. 2cm, with silk thread was tied on the liver rod and suspended into inner chamber, filled with Tyrode solution, oxygenated and maintained temperature of 37^{0}C. The tissue was then given 3 successive washings with fresh Tyrode solution an allowed to relax for obtaining a stable base line.

After setting of above assembly, Acetylcholine (AcH) in dose of 10µml was poured on the suspended tissue to induce the contractions (Figure 5), these were auto recorded on kymograph paper (stuck on rolling drum) through the drawing pin attached to liver. The procedure was repeated till the ceiling effect was recorded (for 9 minutes maximum). Then 0.5ml of *Mishreya Arka* was poured on the tissue and readings were noticed for 2.5 minutes (Figure 6). In same manner the readings were taken with the doses of 1.0ml, 1.5ml and 2.0ml of *Mishreya Arka* against ACh induced contractions and the readings found on kymographs was collected (Figure. 7,8,9). Three times washings with Tyrode solutions were giving after every reading.

Data Analysis

To analyze the data the readings were 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 of drug and response from tissue (Dose-response curve; DRC). Finally effective concentration for 50% effect (EC_{50}) was calculated from the graph.

Results

Table 2: Composition of Tyrode's solution

Character	Observations
Color	Transparent
Odour	Typical *Mishreya* like
Taste	*Katu, Tikta*
Appearance	Clear watery
Clarity	Floating oil drops
pH	6.3
Specific Gravity	1.003
Refractive index	1.33

Relaxations against Acetylcholine induced contractions were noticed on all the dose of *Mishreya Arka*. As per Table no.3 relaxation of different intensity was noticed on different doses. The EC_{50} value of *Mishreya Arka* was found 0.0315ml/ml.

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

Dose of *Mishreya 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	0.5	18%
44.1.0 ml	0.025 ml	1.2	44.44%
1.5 ml	0.0375 ml	1.5	55.55%
2.0 ml	0.05 ml	2.7	100%

Figure 1: *Mishreya* Fruits

Figure 2 : *Mishreya Arka*

Figure 3: Isolated rat ileum

Figure 4: Organ bath assembly

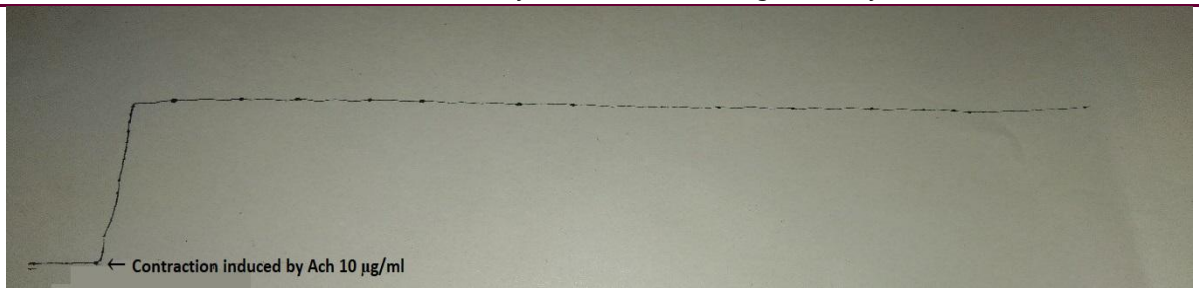


Figure 5: Acetyl choline induced contractions

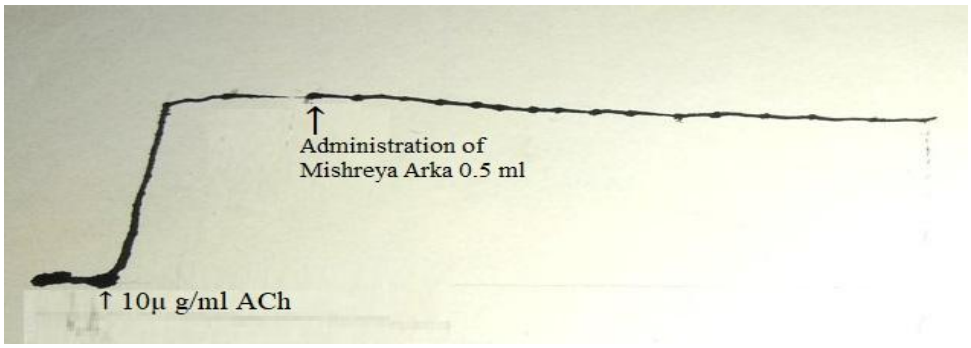


Figure 6: Effect of *Mishreya Arka* 0.5ml/40ml on ACh induced contractions

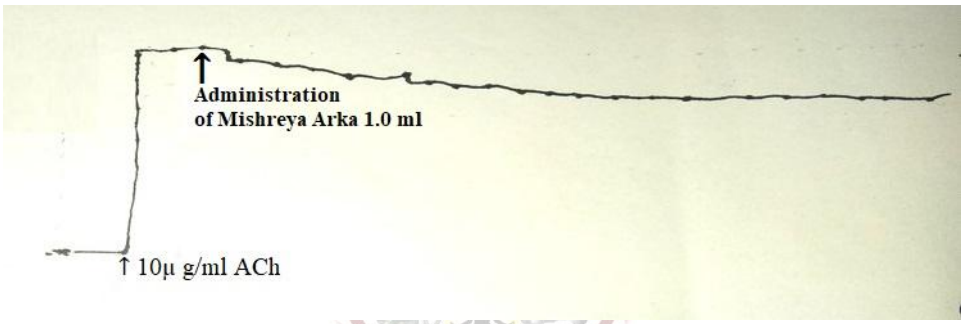


Figure 7: Effect of *Mishreya Arka* 1.0ml/40ml on ACh induced contractions

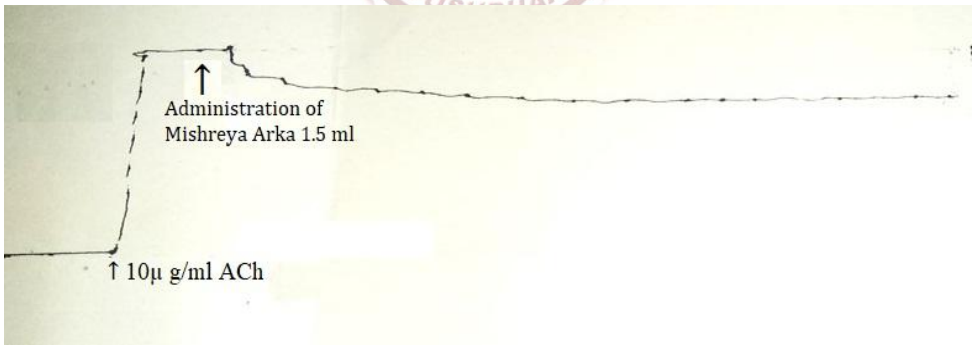


Figure 8: Effect of *Mishreya Arka* 1.5ml/40ml on ACh induced contractions

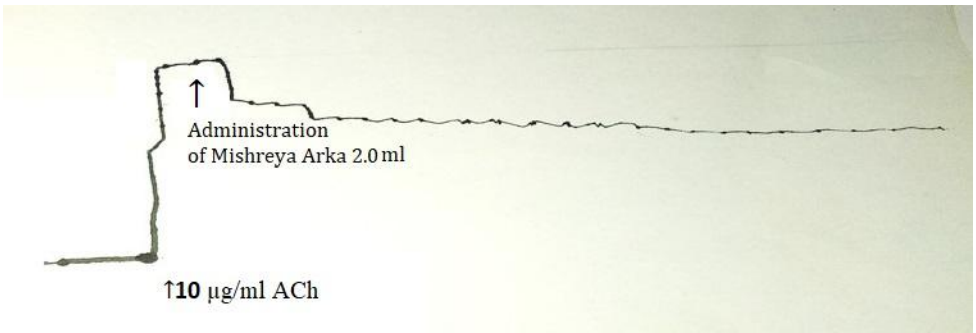
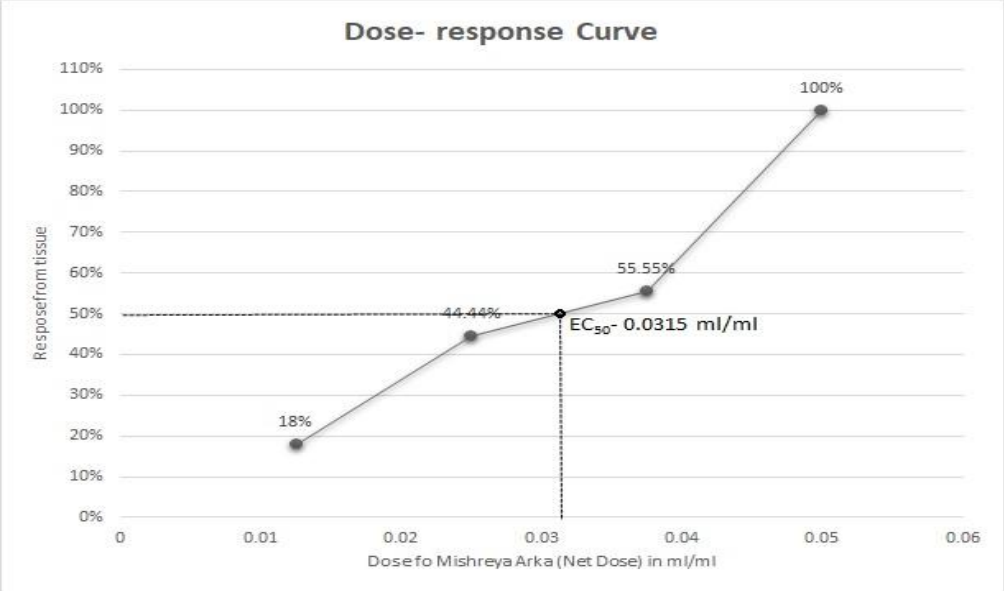


Figure 9: Effect of *Mishreya Arka* 2.0ml/40ml on ACh induced contractions



Graph 1: Showing Dose response curve

DISCUSSION

Anethumsowa and its hydro-distillate have been indicated in various disease conditions in text and had shown antimicrobial[12,13], anti-inflammatory[14], analgesic[15], gastro mucosal protective[16], anti-secretary effect[16], hyper-lipidemia[17] and increasing progesterone effects[18] in some earlier studies. It contains essential oils, fatty oil, moisture (8.39%), proteins (15.68%), carbohydrates (36%), fiber (14.80%), ash (9.8%) and mineral elements such as calcium, potassium, magnesium, phosphorous, sodium, vitamin A and niacin.[19,20] Fruits of *A. sowa* generally have 1-4% essential oil, which is an active constituent it. Essential oil comprising of major compounds as carvone (30-60%), limonene (33%), α-phellandrene (20.61%), including pinene, diterpene, dihydro-carvone, cineole, myrcene, paramyrcene, dillapiole, isomyristicin, myristicin, myristin, apiol and dillapiole.[21,22] Another study reveals that the major constituents of oil are limonene, 27.93%; carvone, 9.76%; dihydro-carvone, 26.74%; and dillapiole, 34.05%.[23]

Present study has been planned to test the efficacy of hydro-distillate on smooth muscles, regarding which it had shown potential in some earlier studies.[24,25] Dill seeds have been used as household remedy to relief digestive problems such as stomach ache, indigestion and flatulence. Dill water is believed to have a soothing effect and is given to babies to treat gripe, relieve hiccups and colic.[26] Isolated organ bath study technique is very suitable for testing any drug for these type of effects. 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.[27] 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.[8]

As the result of the study says that the *Mishreya Arka* relaxed the contractions induced by the ACh and showed antispasmodic or spasmolytic effect. The effect was found reversible in the experiment, which suggests that the effect was not due to any damage in intestine by *Mishreya Arka*. Finding of the study are similar to the earlier works as Ignjatović MJ et al. have reported the spasmolytic effect of methanolic extract of dill[28] and Nesari et al. found the same effect of hydro-alcoholic extract in their in vitro studies.[29] Findings of the present study suggested that the influence of *Mishreya Arka* on rat ileum is medicated by the effect on muscarinic receptors. The enteric nervous system regulated motor and secretory functions of the gastrointestinal system. Acetyl-choline is a very widely distributed neuro-transmitter that is released from excitatory cholinergic neurons and plays an important role in the stimulation of intestinal smooth muscle contractions. Activation of muscarinic receptor in the rat ileum by acetyl-

choline induces an increase in intracellular calcium concentration.[30-32] According to the literary sources, mono-terpenes have been isolated from dill.[33] The monoterpene carvone and limonene produced ileum relaxation[34] and have a protective effect against induced convulsion in experimental animals.[35,36] Carvone and limonene has shown the spasmolytic effective on Guinea pig trachea and rat aorta muscles, between them carvone was found more effective.[37] Spasmolytic effects of *Mishreya Arka* isolated rat ileum may be due to the presence of these physiologically active components.

CONCLUSION

It can be concluded by the current study that *Mishreya Arka* has relaxant or antispasmodic effect on the smooth muscles. It relaxes 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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