

Evaluation of Antispasmodic Activity of Petroleum Ether Extract of Seeds of *Apium graveolens* Linn(PEESAG) on Acetylcholine Induced Contraction in Guinea Pigs Ileum- An experimental study

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ABSTRACT:

Background: *Apium graveolens* Linn. is one of the oldest medicinal plants which used since ancient time in Unani System of Medicine for the disease of gastrointestinal tract which acts as antispasmodic and carminative activity. The aim of this study was to determine the antispasmodic activity of *A. graveolens* in Guinea Pigs Ileum against acetylcholine induced contractions.

Materials and Methods: Antispasmodic activity of PEESAG at the doses of 100, 200, 500, 600, 800 and 900 mcg/ml against acetylcholine at the dose of 10 mcg/ml in guinea pigs.

Results: The test drug produced antispasmodic effect 80%, 90% and 100% at the dose of 600, 800 and 900 mcg/ml respectively.

Conclusion: This result suggests that the PEESAG revealed antispasmodic activity.

Key Words: *Apium Agraveolens*, Antispasmodic activity, Acetylcholine, Guinea Pig

INTRODUCTION:

Apium graveolens Linn. commonly known as Celery which belongs to the family of Apiaceae [1]. In Unani system of medicine the names of drugs are adopted from Persian or Arabic nomenclature. In Persian *Apium graveolens* Linn is known as Karafs. Hence the drug Karafs means the same as *Apium graveolens* Linn and the seeds are called as Tukhme Karafs [2]. Hussain writes that Karafs is the Celery of Europeans and the Udasaliyon of Greeks. He mentions five varieties of Karafs namely *Bustani*, *Jabli*, *Nabti*, *Sakhuri*, *Maiee* (Tari). *Bustani* is cultivated variety while *Jabli* grows in hills, *Sakhuri* on stony surface, *Nabti* in shady places and *Maiee* near water or ponds. According to Hussain, *Sakhuri*, *Nabti* and *Maiee* varieties of *Karafs* are called in Greek, as *Fiturasaliyun*, *Akusaliyun* and *Samarniyun* respectively [3]. There are many active chemical constituents have been reported such as; Beta-carotene, folic acids, Isoimperatorin, isoquercitrin, linoleic acid, furanocoumarins, flavonoids, alkanoids, phenolic compounds etc.[1, 4]. Different scientific activity are reported viz; anti-inflammatory, antispasmodic activity, Hepatoprotective activity, Hypolipidemic activity, Antioxidant activity, Anti-Depressant Activity, Spermatogenesis activity, Anti-hyperuricemic activity [5-17]. Unani compound formulation which have active ingredient is *Tukhme Karafs* are *Jawarish Zarooni Sada*, *Majoon-e-Dabeed-ul-Ward*, *Majoon-e-Jograj Gugal*, *Majoon-e-Nankhwah*, *Majoon-e-Buqrat*, *Majoon-e-Reward*, *Banadiq-ul- Buzoor*, *Sufoof-e- Mohazzil*, *Sikanjabeen Bazoori Moatadil* [18,19].

MATERIAL AND METHODS:

Plant material and extraction procedure: The test drug was procured from Begum Bazaar, Hyderabad and identity of the drugs was confirmed on the basis of description available in the Unani classical literature and botanical identification was done by botanist Central Research institute of Unani medicine, Hyderabad. The extract of the seed was obtained in petroleum ether by soxhlet apparatus at Deptt. Of Ilmu Advia (Pharmacology) Govt. NizamiaTibbia college, Hyderabad. The solubility of the test drug was checked in propylene glycol dimethyl sulphoxide (D.M.S.O.) and Tween 80 (Polysorbate), it is found that test drug was completely soluble in Tween 80. Hence Tween 80 is selected for vehicle.

Procurement of animals: Guinea pigs were procured from Deccan Medical College, Hyderabad (Tilangana). Animals were kept under standard laboratory condition i.e. 22-23 °C with 12 hour day and night cycle for acclimatization. The animals were supplied laboratory diet pellets and water at libitum for 5 days.

Methodology:

A healthy adult guinea pig weighing 400 gm was starved overnight and was killed by stunning (giving a smart blow behind the head) neck vessels cut and the animal bled out completely. Abdomen was opened through a midline incision so as to expose the ileo-caecal junction. The terminal ileum was cut after discarding 10 cm. Nearest to the ileo-caecal junction because of the presence of excitatory adrenoceptor near the ileo-caecal junction [20]. The mesenteric attachment was cut as close to the gut as possible without injury to it, for a distance about 20-25 cm. The intestine was cut across. An isolated luminal piece was cleaned and the lumen was thoroughly washed by running Tyrode's solution repeatedly with the help of a pipette. Frequent stretching, ballooning or handling of the intestine was avoided as much as possible.

A small segment of ileum about 4-6 cm length was cut and placed in Tyrode's solution in a petridish. A thread was passed through the lumen and the wall near the mesenteric attachment at each end with the help of a sewing needle in such a way so as not to obstruct the lumen of the intestine but kept intact and open for free passage of drug solution across the lumen, one end of the segment was tied securely to the tissue holder tube (oxygen bent tube) and transferred to the organ bath tub (already filled Tyrode's solution and bubbled with oxygen) which was to the reservoir bottle containing Tyrode's solution. The tissue holder tube was fixed in position with clamp the upper end of the tube was connected to the oxygen cylinder by means of a rubber for the supply of oxygen, kept a constant flow. The other end of the segment was fixed to a frontal writing lever which record on the smoked drum. The frontal writing lever was adjusted suitably for tension and magnification for maximum sensitivity the lever was nearly balanced and friction at the writing surface was reduced to a minimum by a smooth point.

The sensitivity of the tissue was tested by giving consequent doses of acetylcholine solution 10 mcg/ml bath concentrations and in between washing the tissue with Tyrode's solution, equal height of concentration were observed. Now the PEESAG 100 mcg/ml solution was added to make the bath concentration and was allowed two minutes to remain in contact before acetylcholine solution was added. After two minutes acetylcholine solution 10 mcg/ml bath concentrations was added and effect was recorded on the smoked drum. The procedure was repeated with the doses of 100, 200, 500, 600, 800, 900 and 1000 mcg/ml bath concentrations of PEESAG solution against a fixed dose of acetylcholine solution 10 mcg/ml bath concentration and effect was recorded. The tissue was washed two to three times with Tyrode's solution before administration of new dose of PEESAG and care was taken that the tissue had recovered from the effect of previous dose completely.

OBSERVATION & RESULTS:

Screening of antispasmodic activity also done in isolated Guinea pig's ileum and Tyrode's solution, first of all normal peristalsis recorded than 3 different consecutive contraction obtain by using acetylcholine in dose 10 mcg/ml good contraction observed. Tissue washed with Tyrode's solution, there after diluted PEESAG in dose of 100 mcg/ml tissue baths into inner bath. Against tissue bath washed with Tyrode's solution and test drug in dose of 100, 200, 500, 600, 800 and 900 mcg/ml against acetylcholine in the dose of 10 mcg/ml antispasmodic activity recorded 50%, 60%, 70%, 80% 90% and 100% respectively which showing in graph form [Fig.1 & 2]. .

DISCUSSION:

Concerning the effect of PEESAG on guinea pig's ileum, the present study demonstrated that the effect of PEESAG against tissue bath washed with Tyrode's solution and test drug at the doses of 100, 200, 500, 600, 800 and 900 mcg/ml against acetylcholine in the dose of 10 mcg/ml antispasmodic activity recorded 50%, 60%, 70%, 80% 90% and 100% respectively.

The mechanism of the results of this study is most probably explained by Ahmed et al., [21] in their study, and another study demonstrated that vasodilatory action mechanisms of apigenin isolated which reduce the contractions [22]. Acetylcholine is known to act on the smooth muscle cells of the guinea pig's ileum, interfering with specific receptors for these spasmogen[23].The possible mechanism of action of PEESAG is may be due to calcium-channel blocking activity of and presence of phenylpropane constituents in *tukhm karafs* [24].Thus the results of the present study suggest that *Tukhm Karafs* possesses potent antispasmodic activity on the smooth muscles of the gastrointestinal tract.

CONCLUSION:

Over centuries of cultivation, *Tukhm Karafs* has made its way into our daily food and more important it is used as medicinal herb. *Karafs* is grown in many areas around the world. It is also well established medicine in the AYUSH system of medicine. Various actions has been documented such as; carminative, anti-inflammatory, antimicrobial, antiparasitic, antiplatelet aggregation, antioxidant and analgesic effect as it is used to ameliorate pain in migraine, osteoarthritis and dysmenorrhoeal . It also has several cardiovascular and gastrointestinal

effects as its anti-ulcer, antispasmodic and anti-emetic effects. Its effects are due to its various active chemical constituents. *Karafs* is marketed for its antispasmodic effect which was evident in vitro studies on animal intestine and was revealed to be due to its anticholinergic effect. The antispasmodic effect of *karafs* was apparent in vitro experiment on Guinea pig ileum as it reduced the magnitude of acetylcholine induced contraction. From this study as well as what was mentioned in other studies, we can conclude that *Karafs* appears to be a herb that can be used for several purposes besides its use for its aroma in cooking, because it has nutrient and medical values. Further studies are required to find the biochemical and molecular mechanism of action of *Tukhm Karafs*.

ACKNOWLEDGEMENT:

The authors would like to acknowledge to all co-authors for the shaping this article.

FUNDING: Nil

CONFLICT OF INTEREST: None

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GRAPH SHOWING ANTI-SPASMODIC EFFECT OF PEESAG IN ISOLATED – GUINEA PIG ILEUM PREPARATION AGAINST ACETYLCHOLINE

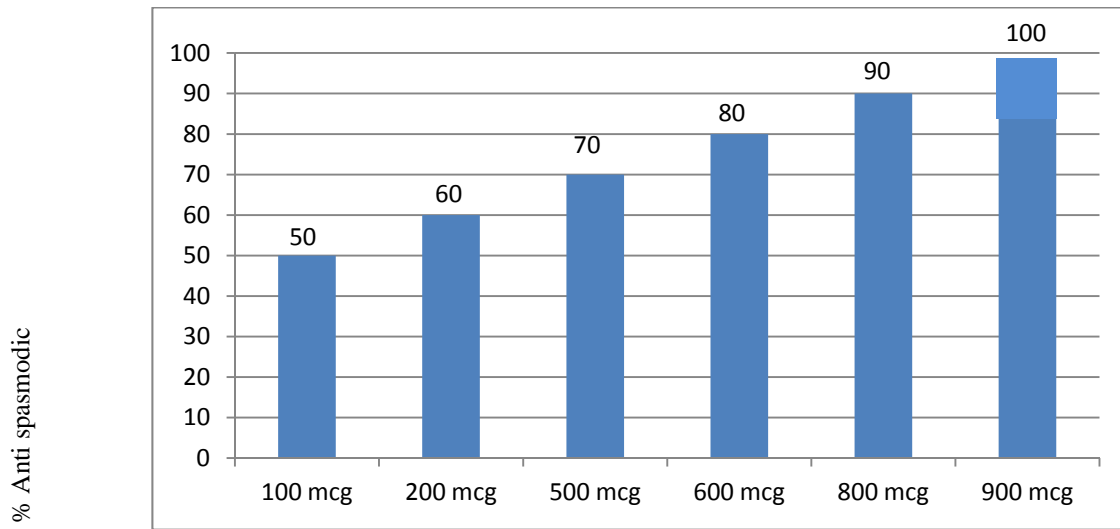


Fig. 1 (Test drug PEESAG in mcg/ml)

Ach = 10 mcg/ml

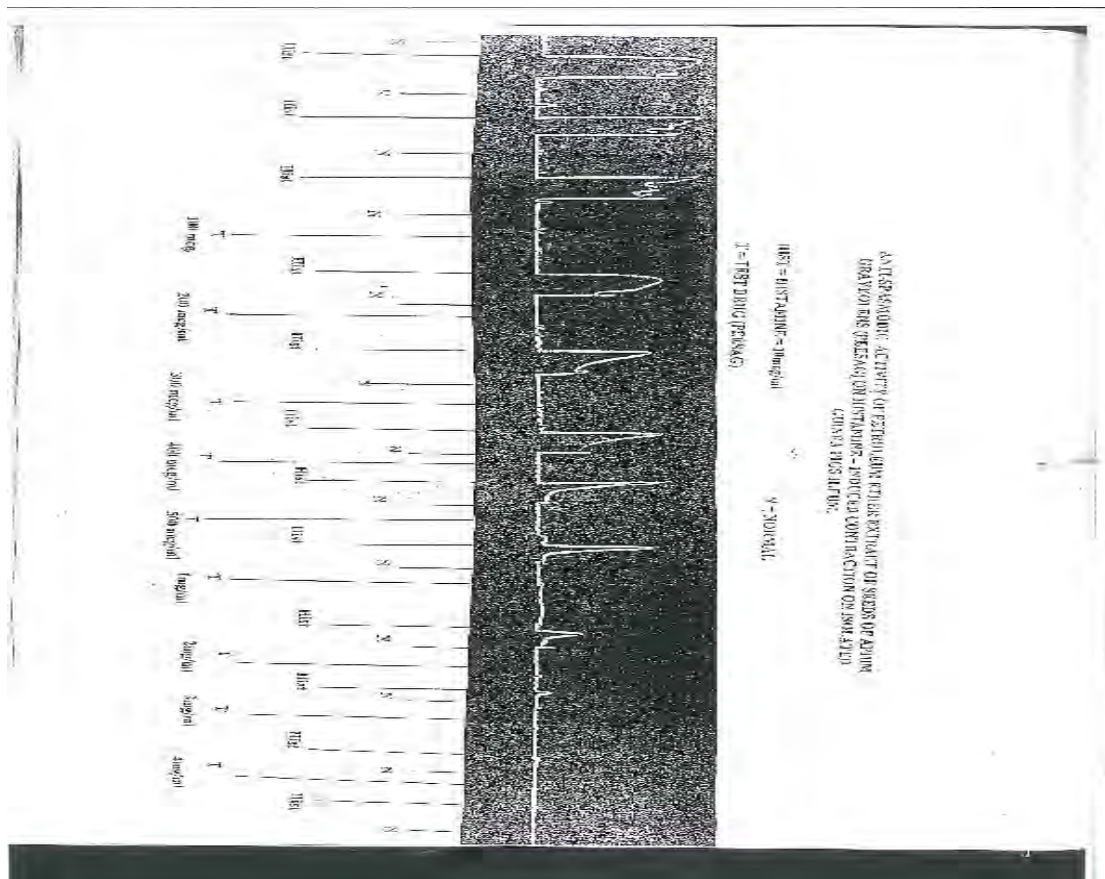


Fig. 2 (Antispasmodic Activity of Petroleum Ether Extract of Seeds of *Apium graveolens* (PEESAG) on Acetylcholine Induced Contraction in Guinea Pig's Ileum)

Note: N=Normal, Ach=Acetylcholine and T=Test Drug (PEESAG)