Antispasmodic Activity of Petroleum Ether Extract of Seeds of *Apium graveolens* (PEESAG) on Histamine Induced Contraction in Guinea Pig’s Ileum

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ABSTRACT:

Background: *Apium graveolens* has been used in Unani system of medicine as anti inflammatory, uricosuric, diuretic, carminative, anti dyslipidemic, abdominal discomfort etc. The aim of the present study was to determine the antispasmodic activity of *A. graveolens* in Guinea Pigs against histamine induced contractions.

Materials and Methods: Antispasmodic activity of PEESAG at the doses of 100, 200, 300, 400, 500 mcg/ml, 1 mg/ml, 2 mg/ml and 3 mg/ml against histamine at the dose of 10 mcg/ml in guinea pigs.

Result: The test drug produced antispasmodic effects 40%, 60%, 60%, 70%, 70% 80%, 90% and 100% at the different doses of 100, 200, 300, 400, 500 mcg/ml, 1 mg/ml, 2 mg/ml and 3 mg/ml respectively.

Conclusion: This result suggests that the PEESAG revealed antispasmodic activity as dose dependent i.e. maximum antispasmodic effect on 3 mg/ml of the dose.

Key Words: *Apium Agraveolens*, PEESAG, Antispasmodic activity, Histamine, Guinea Pig

INTRODUCTION:

*Tukhm Karafs* 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) and also known in Greek, as Fiturasaliyun, Akusaliyun and Samarniyun respectively [3]. There are many phyto chemical constituents have been documented viz; folic acids, isoquercitrin, Isoimperatorin, linoleic acid, furanocoumarins, alkaloids, flavonoids, β-carotene, 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]. There many Unani compound formulation available in the market for various disorders, which have active ingredient is *Tukhme Karafs* are Jawarish Zarooni Sada, Majoone-e, Majoone-e-Jograj Gugal, Sufoof-e- Mohazzil ,Dabeed-ul-Ward, Majoone-e-Nankhwah, Majoone-e-Reward, Majoone-e-Buqrat, Banadiq-ul- Buzzoor, Sikanjabeen Bazoori Moatadil [18,19].

MATERIAL AND METHODS:

Plant material and extraction procedure: The test drug was purchased 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 IlmulAdvia (Pharmacology) Govt. NizamiaTibbia college, Hyderabad. The solubility of the test drug was checked in propylene glycol dimethylsulphoxide (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 pig was 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 adreno receptor 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 3 consequent doses of histamine 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 histamine solution was added. After two minutes histamine 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, 400, 500, mcg/ml and 1,2,3,4 and 5 mg/ml bath concentrations of PEESAG solution against a fixed dose of histamine solution 10 mcg/ml bath concentrations 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 the spasmolytic activity also done in isolated Guinea pig ileum and Tyrode’s solution used. The test drug was used in dose of 100,200,300,400,500mcg/ml and 1 mg, 2mg and 3mg/ml against the histamine in dose of 10 mcg/ml antispasmodic activity recorded 40,60,60,70,70,80,90 and 100% respectively [Figure 1].

DISCUSSION:
Pain affects hundreds of millions of peoples throughout the world [5]. The mechanism of the results of this study is most probably explained by Ahmed et al., [21] in their study, and another study established that vasodilatory action of apigenin isolated from A. graveolans which diminished the contractions of ileum i.e. spasmolytic activity [22]. Histamine is known to act on the smooth muscle cells of the guinea pig ileum, interfering with specific receptors for these spasmodgens like acetylcholine, histamine [23].The possible mechanism of action of PEESAG is may be due to anticholinergic, antihistaminic and calcium-channel blocking activity of and presence phenylpropane constituents in tukhm karafs [24].The results of the present study suggest that tukhm karafs possesses persuasive spasmolytic activity on the smooth muscles of the gastrointestinal tract.

CONCLUSION:
Tukhm Karafs is revealed for its antispasmodic effect which may be due to its anticholinergic, antihistaminic, antiserotonergic or calcium channel blocking effect. It is concluded that uses of tukhm karafs helps in alleviate the spasmodic pain. Further explore is need to on human subjects on different doses.

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References:


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

Figure 1

Histamine = 10 mcg/ml

(Test drug PEESAG in mcg/ml)