



# Antispasmodic Activity of Ether Extract of Seeds of *Apium Graveolens* linn in Rabbit Duodenum-A Research Study

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## INTRODUCTION:

Tukhm Karafs commonly known as Celery which belongs to the family of Apiacea. 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. 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. There are many phyto chemical constituents have been documented viz; folic acids, isoquercitrin, Isoimperatorin, linoleic acid, furanocoumarins, alkanoids, flavonoids,  $\beta$ -carotene, phenolic compounds etc. Different scientific activity are reported viz. anti-inflammatory, antispasmodic activity, Hepatoprotective activity, Hypolipidemic activity, Antioxidant activity, Anti-Depressant Activity, Spermatogenesis activity, Anti-hyperuricemic activity. There many Unani compound formulation available in the market for various disorders, which have active ingredient is Tukhme Karafs are Jawarish Zarooni Sada, Majoon-e-, Majoon-e-Jograj Gugal, Sufoof-e- Mohazzil, Dabeedul- Ward, Majoon-e-Nankhwah, Majoon-e-Reward, Majoon-e-Buqrat, Banadiq-ul- Buzoor, Sikanjabeen Bazoori Moatadil.




Celery (*Apium Graveolens*) is a marshland plant in the family Apiaceae that has been cultivated as a vegetable since antiquity. Celery has a long fibrous stalk tapering into leaves. Depending on location and cultivar, its stalks, leaves or hypocotyl are eaten and used in cooking. Celery seed powder is used as a spice.

Celery leaves are pinnate to bipinnate with rhombic leaflets 3–6 centimeters long and 2–4 cm broad. The

flowers are creamy-white, 2–3 mm in diameter, and are produced in dense compound umbels. The seeds are broad ovoid to globose, 1.5–2 mm long and wide.

Modern cultivars have been selected for either solid petioles, leaf stalks, or a large hypocotyl. A celery stalk readily separates into "strings" which are bundles of angular collenchyma cells exterior to the vascular bundles.

Wild celery, *Apium Graveolens* var. *Graveolens*, grows to 1 m tall. Celery is a biennial plant that occurs around the globe. It produces flowers and seeds only during its second year. The first cultivation is thought to have happened in the Mediterranean region, where the natural habitats were salty and wet, or marshy soils near the coast where celery grew in agropyro-rumicion-plant communities. North of the Alps, wild celery is found only in the foothill zone on soils with some salt content. It prefers moist or wet, nutrient rich, muddy soils. It cannot be found in Austria and is increasingly rare in Germany.

Cultivar	Image	Name	Part eaten
Celery		<i>Apium Graveolens</i> var. <i>Graveolens</i>	Primarily grown for its thick stalk.
Celeriac		<i>Apium Graveolens</i> var. <i>rapaceum</i>	The hypocotyl is eaten like a root vegetable.
Leaf celery		<i>Apium Graveolens</i> var. <i>secalinum</i>	Primarily eaten for its large leaves.

## Uses of Celery (*Apium Graveolens*)



Celery seed (*Apium Graveolens*) essential oil

Celery is eaten around the world as a vegetable. In North America and Europe the crisp petiole (leaf stalk) is used. In Europe the hypocotyl is also used as a root vegetable. The leaves are strongly flavored and are used less often, either as a flavoring in soups and stews or as a dried herb. Celery, onions, and bell peppers are the "holy trinity" of Louisiana Creole and Cajun cuisine. Celery, onions, and carrots make up the French mirepoix, often used as a base for sauces and soups. Celery is a staple in many soups. It is used in the Iranian stew khoresh.

### Leaves

Celery leaves are frequently used in cooking to add a mild spicy flavor to foods, similar to, but milder than black pepper. Celery leaves are suitable dried as a sprinkled on seasoning for use with baked, fried or roasted fish, meats and as part of a blend of fresh seasonings suitable for use in soups and stews. They may also be eaten raw, mixed into a salad or as a garnish.

### Seeds

In temperate countries, celery is also grown for its seeds. Actually very small fruit, these "seeds" yield a valuable essential oil that is used in the perfume industry. The oil contains the chemical compound a pole. Celery seeds can be used as flavoring or spice, either as whole seeds or ground.

### Celery salt

Celery seeds can be ground and mixed with salt to produce celery salt. Celery salt can be made from an extract of the roots or by using dried leaves. Celery salt is used as a seasoning, in cocktails (commonly to enhance the flavor of Bloody Mary cocktails), on the Chicago-style hot dog, and in Old Bay Seasoning. Similarly, combinations of celery powder and salt are used to flavor and preserve cured pork<sup>l</sup> and other processed meats as an alternative to industrial curing salt. The naturally occurring nitrites in celery work synergistically with the added salt to cure food.



Celery seeds

## Celery juice

In 2019, a trend of drinking celery juice was reported in the United States, based on "detoxification" claims posted on a blog. The claims have no scientific basis, but the trend caused a sizable spike in celery prices.

## Materials and Methods

The seeds of *Apium graveolens* linn (Tukhm-e-karafs) under present study. 3 kg seeds of *Apium graveolens* linn (Tukhm-e-karafs) purchased from a well-known and leading whole sales of Herbal medicine from Begum Bazar, Hyderabad, A.P. Fine powder of seeds obtained by grinding and proper identification was made on pharmacognostical basis. The macroscopically study of the herb was conducted by the naked eye. The size, shape, color and pharmacological and organoleptic character were observed. The seeds were identified with the help of literature.

The identity of the drug was confirmed on the basis of literacy description available in the Unani classical literature and moderate classical literature, the botanical identification was done by Botanist, Central Research Institute of Unani Medicine, Hyderabad.

Petroleum ether extract of seeds of *Apium gravelones* linn (PEESAG) was prepared by Soxhlet apparatus at Department of Ilmul-Advia (pharmacology) Govt. Nizamia Tibbi College, Charminar, Hyderabad. Oily fraction (extract) of Tukhm-e-Karafs was collected in a glass bottle and solubility checked in propylene glycol, dimethyl sulfo-oxide (D.M.S.O) and Tween-80 (polysorbate) and it is found that it was completely soluble in Tween-80.

Analgesic Activity is confirmed in albino mice by hotplate Tail immersion, Tail clip and writing method. Antispasmodic activity is confirmed in isolated preparation of Rabbit Duodenum and Guinea pigs ileum by Magnus Tissue bath. Animals were purchased from Deccan Medical College, Hyderabad,

A. P. India. Analgesic activity is confirmed in adult albino mice, by different methods i.e. Hot-plate, Tail-clip, Tail-immersion and writing reflex.

**Anti-spasmodic activity confirmed in isolated ileum piece of Guinea Pig and rabbit duodenum.****Pain and Analgesia**

(Latin-poena, a fine, a penalty) A sensation in which a person experiences discomfort, distress, or suffering due to irritation of or stimulation of sensory nerves, esp. pain sensors. Pain is one of the cardinal symptom of inflammation, it may vary in intensity from mild discomfort to intolerable agony. In most cases, pain stimuli are harmful to the body and tend to bring about reactions by which the body protects itself. Adaptation to pain stimuli does not readily occur. There are different types of pain, eg. Abdominal pain, pain in abdomen that increases usually with respiration. Experienced in a great variety of condition including appendicitis, broken ribs, intercostal neuralgia, wounds, herpes zoster, pleurisy, myalgia, acute peritonitis, colic, Hepatic, Gastric or Renal Ulcer, Gall-bladder disorder, Dental Pain, Cardiac pain. According to intensity & duration pain is divided into Acute, and Chronic

Pain, it has been said, is one of the "Nature's earliest signs of Morbidity". In general the concept of pain encompasses at least three components. Nociception, the body's detection and signaling of noxious events pain, the conscious perception or recognition of the nociceptive stimulus and suffering, the affective, behavioral, or emotional response to the pain. Acute pain is often the crucial response to the pain. Acute pain is often the crucial signals of the location and nature of the disorder In chronic pain, the quality of the discomfort and its neurologic basis seem to differ and its management becomes more complex

Pain in the gastrointestinal tract is produced because of distension or gm of smooth muscle, and traction on the mesenteric attachment Small that elicit pain are thought to do so by causing release of chemical substances into the tissues, Histamine. Brady kinin Prostaglandin's and potassium ions cause pain when Injected into the skin or when administered intra arterially The term "Painful as used to describe stimuli applied to animals is synonymous with the terms "Nociceptive" or "noxious" A Nociceptive must be carefully selected for screening analgesic compounds. The techniques employed for the various stimuli differ widely in applicability and Limitations

Human can distinguish and give verbal reports on a wide variety of painful sensations, animals can only display autonomic can only display autonomic or somato motor responses are most commonly employed in the experimental analysis of pain and they vary from one technique to another they include monosynaptic reflexes (such as tail flick) or poly synaptic reflexes (which include hick and jump, vocalization, and local contractor of the abdominal musculature). The polysynaptic reflexes involve a high degree of sensory motor condition.

A variety of nociceptive tests have been used in screening for analgesic activity objectively, depending upon the nature of the stimulus, they can be classified into thermal, mechanical, electrical and chemical methods

**Tween 80 (Polysorbate):**

In present study pet ether extract of seeds of Apium Graveolens Linn was dissolved in Tween 80 Tween 80 is a non-ionic surface active agent used as a solubilizing agent for water insoluble substances for injection Although it is claimed to be an inter-substance without any pharmacological actions The elimination of one molecule of water from the hexahydric alcohol sorbitol results in the formation of compounds termed

sorbitans, while elimination of another water molecule results in the formation of sorbides. By esterification of these with fatty acid, non-ionic surfactants are produced. These are termed polysorbate. These substances are active water in oil emulsifying agents, while their polyoxyethylene derivatives are non-ionic, oil in water emulsifying agents.

The polysorbate & their polyoxyethylene derivatives such as Tween 80 are widely used in pharmacy in the preparation of cream, ointment & suppository bases & for emulsification of fixed & mineral oils. They may be employed internally to improve the absorption of fat & fat soluble vitamins. They are also used as surface active agents in insecticides & fruit spraying solutions, as industrial detergents & in the manufacture of cosmetics, cream. They are devoid of any anti-septic activity (108).

## **Effect of Petroleum Ether Extract of Seeds of *Apium Graveolens* on Acetylcholine Induced Contractions in Rabble Duodenum**

### **Material**

1. Animal model Rabbit weighing 2.5kgs
2. Instruments Knife handle with blade, scissors, forceps beakers des cotton thread rubberconnecting tubes, turbierulin syringes, pipette pinch damp and petri dish
3. Apparatus Isolated organ bath Kymograph and oxygen cylinder etc.
  - A. Isolated organ bath consists the following Organ bath-Tube , Outer bath thermostat, Oxygen bent tube with tissue holder Frontal writing lever, Reservoir bottle, two upright stands and Thermometer
  - B. Kymograph: The kymograph consists of an electrically driven gear-box with a vertical spindle carrying a drum. The gear-box has clutch and controls which determine the speed of the drum. The kymograph smoked paper was wrapped smoothly round the drum with adhesive

### **Solutions**

1. Ringer locks solution
2. Acetylcholine solutions 1 mcg/ml
3. Petroleum ether extract of Seeds of *Apium Graveolens* (PEESAG)
4. Polysorbate
5. Distil water

## Composition of Ringer Lock solution in 1000 ml

Sr. No.	Drugs	Weight	Milligram
1	NaCl	0.9 gm	9000 m gm
2	KCl	0.42 gm	420 m gm
3	CaCl <sub>2</sub>	0.24 gm	240 m gm
4	NaHCO <sub>3</sub>	0.15 gm	150 m gm
5	Dextrose	1.0 gm	1000 m gm
6	Distil water	1 Liter	1000 ml

### Method

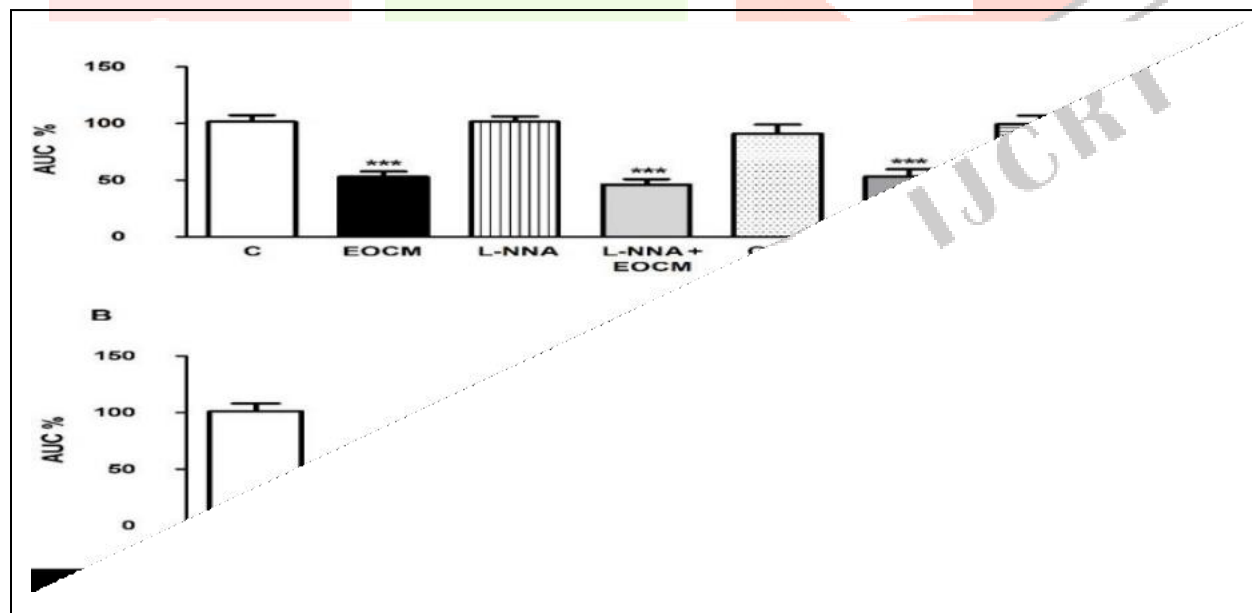
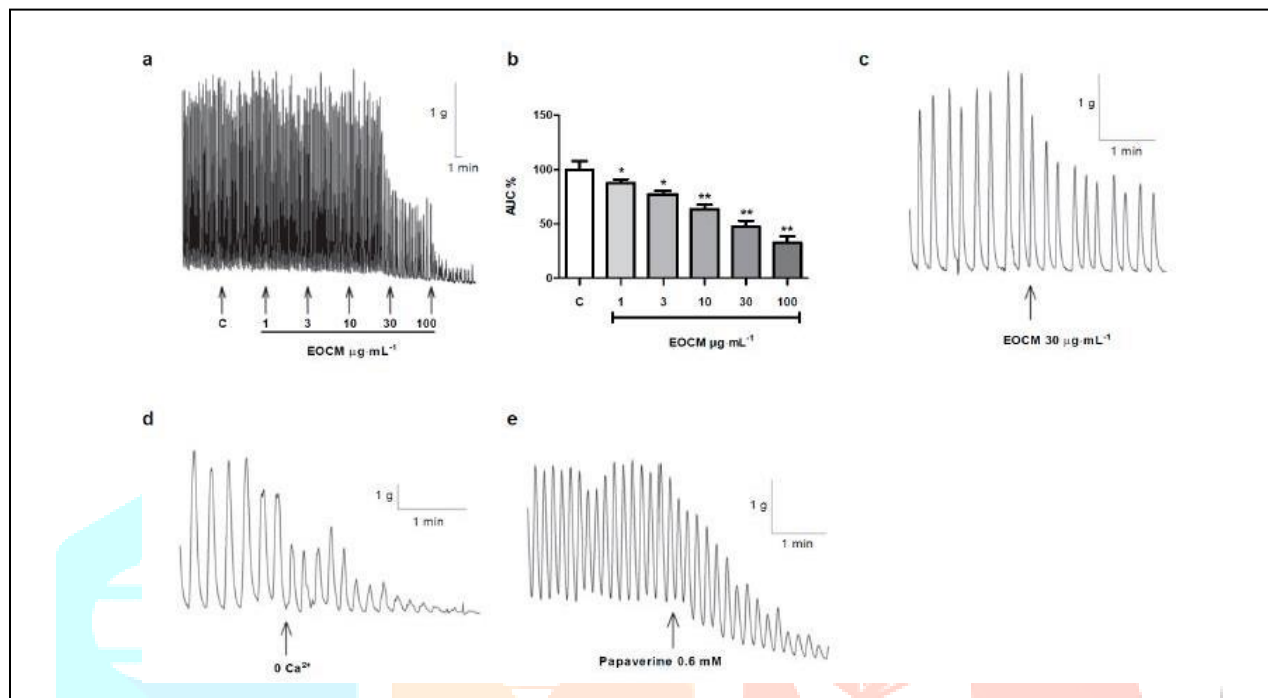
A healthy adult rabbit weighing 25 kg was starved overnight and was killed by stunning (giving a smart blow behind the head) Neck vessels were cut and the animal was bled out completely Abdomen was opened through mid-line incision so as to expose the duodenum The mesenteric attachment was cut, as close to the gut as possible without injury to it. The intestine was then cut across an isolated luminal piece was cleaned and the lumen was thoroughly washed by running Ringer Locke 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 duodenum about 4-6 cm length was cut and placed in Ringer Locke 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 duodenum which was kept open for the free passage of drug solutions across the lumen one end of the segment was tied securely to the tissue holder by (Oxygen bent tube) and transferred to the organ bath tube (already filled with Ringer Locke solution and bubbled with oxygen) which was connected to the Reservoir bottle containing Ringer Locke Solution. The tissue holder tube was fixed in position with clamps, the upper end of the tube for the supply of oxygen, kept at a constant flow. The other end of the segment was fixed to a frontal writing lever with recorded 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 contact surface was reduced to minimum by a smooth point

The capacity of organ bath tube was 15 ml. The tissue was adequately oxygenated. The temperature of the outer bath was maintained between 37°C throughout the experiment. Level of water in outer bath and Ringer Locke solution in the organ bath was the same. Tuberculin syringes were used to instill drug solution in the organ bath and not more than 1 ml Volume was put in at a time. The tissue was left over for 30 minutes before the drugs were put in the organ bath tube. The movements were recorded on a low moving drum.

The sensitivity of the tissue was seen by giving three consequent doses of Atropine solution 1 mcg/ml all concentration and equal height of action were recorded. Now the diluted PEEPAG solution 100mcg/ml was added to make the total concentration 1 mg/ml and was allowed 2 minutes to remain in contact before acetylcholine solution was added. After 2 minutes acetylcholine Solution 1 mcg/ml bath concentration was added and effect was recorded on the smoked drum. The procedure was repeated with doses of 100, 200 and 500 mcg/ml and

1,2,3,4 and 5 mg/ml bath concentration of PEESAG against a fixed dose of acetylcholine solution 1mcg/ml bath concentration and effects were recorded. The tissue was washed with ringer locke solution three to four times before administrative of new dose of PEESAG Tissue sensitivity was tested after washing with Ranger Locke solution.





## Discussion

The petroleum ether extract of celery seeds is evaluated as an analgesic drug in experimental animals by different methods i.e. Thermal, Mechanical and chemical, PEESAG used in 3 different doses in all the methods. The dose of test drug (PEESAG) kept constant 50, 75, and 100 mg/kg B. wt. of animals.

Tukhm-e-Karafs are used in Unani medicine since many years as analgesic drug and in present study it is confirmed that celery seeds are neither very potent nor very weak in efficacy but as an intermediate acting analgesic drug

Its efficacy also noted at different interval of time i.e. 15, 30, 45, 60 and 90 minutes after the test drug administration. Its peak analgesic effect observed at 45 and 60 minutes and came to near normal after 90 minutes.

All the animals tolerated well the above mentioned test drug. No any other abnormal activity noted. It is also observed that if the dose of test drug was reduced below 50 mg/Kb. B. wt. than the analgesic response of almost 0%.

In Hot plate method % MPE of standard drug (Pentazocine HCl) was 68 56% whereas it was 36 35, 44 25 and 15:71% in test drug in dose of 50, 75 and 100 mg/Kg B. wt. respectively

In Tail clip method % MPE of test drug was 27 44, 29 85 and 39 91 in different doses here weak efficacy of drug noticed

In Tail immersion method % MPE of test drug was 39 96, 44 17 and 53 58% respectively Standard drug has 61 16% MPE analgesic effect to chemical stimulus method Acetyladylic acid used in dose of 100 mg/kg BW and produced 73 34%, effect, what was 15 % 49% and 68 72% in test drug group. During experiment it is also observed that there was increase in urine output in almost all animals

### **Anti-spasmodic screening for PEESAG in isolated Gaines pig's leam and Rabbit duodenum**

Celery seeds also evaluated as a potent anti-spasmodic drug in isolated preparation of Guinea Pig and Rabbit duodenum.

In present study Acetyl choline (Ach) and Histamine first tested as a spasmodic agent and Acetylcholine and Histamine found more reliable and produced good contraction of isolated gut tissues, so omitted from study and the dose of Acetyl choline kept constant 10 mcg/ml in Guinea pig ileum and Histamine 10 mcg/ml in guinea pig ileum and Acetylcholine 1 mcg/ml in Rabbit duodenum because at this dose it produced maximum force of contraction

The test drug tested for Antispasmodic effect in isolated Guinea pig's ileum in dose of 100, 200, 300, 400, 500, 600, 800 and 900 mcg / ml against Ach 10 mcg/ml

The test drug produced antispasmodic effect 80%, 90%, 100%, in dose of 600 mg/ml 800 mcg/ml and 900 mcg / ml respectively

The test drug tested for antispasmodic effect in isolated Guinea pigs ileum in dose of 100, 200, 300, 400, 500 mcg/ml and 1, 2, 3, mg/ml against Histamine 10 mcg/ml The test drug produced spasmolytic effect 80, 90, 100% in dose of 1 mg 2 mg, and 3 mg/ml respectively.

The test drug (PEESAG) tested for Spasmodic Activity in Isolated Rabbit Duodenum in dose of 100, 200, 500 mcg/ml and 12, 3, 4, and 5 mg/ml against Acetylcholine 1 mcg/ml

The test drug produced Antispasmodic effect 100% in dose of 5 mg respectively It confirms the potent efficacy of Takhm-e-Karafts as Antispasmodic drug.

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