COMPARATIVE STUDY OF ETHANOLIC AND AQUEOUS EXTRACTS OF *APIUM GRAVEOLENS* L. ROOT WITH FUROSEMIDE FOR ITS DIURETIC ACTIVITY & EXCRETION OF URINARY METABOLITES IN WISTAR RATS

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ABSTRACT: *Apium graveolens L. (root) is herb of Asian and European origin, used as renal protective and diuretic agents both as single remedy and in combination with other herbs. The aim of study is to scientifically validate its use. 40 Wistar rats of both sex were used, weighing between 180- 250 grams and divided into eight groups (n=5), half of the animals were subjected to acute renal injury by gentamycin (i.p.) for 14 days before the study according to international protocol. On day 15th, a group of 5 animals was given normal saline as control, another was kept as gentamycin control and three groups of normal animals were given oral calculated doses of ethanolic extract 50 mg/kg, agent aqueous extract 10g/kg and furosemide 40 mg/kg. Three groups of gentamycin induced acute renal insufficiency animals received same oral doses. All the animals were kept in diuretic cages for 24 hour under same conditions, their urine were collected and tested for volume, sugars, proteins, creatinine, sodium and potassium excretions. We found that the decoction and extract have significant diuretic activity without abnormal excretions of electrolytes, proteins or creatinine. Although, we found the herb in all conditions to cause less excretion of urinary sugars in all groups possibly due to some hypoglycaemic activity of herb.*

Keywords: *Apium graveolens* L. (root), Acute kidney injury, Diuretic activity, Urinary creatinine, Urinary sodium and potassium

INTRODUCTION:

The interest in health benefits and high effectiveness of medicinal herbal plants has been augmented because of their extensive use in therapeutic agents and preventions of multiple diseases. *Curcuma longa* L. has been proven to be antiseptic and anti-inflammatory [31] *Cannabis sativa* L. has aphrodisiac and stimulant characteristics [32], *Allium sativum* has been used for the treatment of hypertension and high cholesterol [33]. The uplift of these researches is being done to evaluate the exact efficacy of these medicinal plants. Furthermore, this growing era of research in medicinal plants has profoundly marked the front because of low comparable health outcomes and benefits from conventional therapeutic agents [1]. There are multiple therapeutic agents that have been found as diuretics among the medicinal plants, widely accepted for their imperative effects and benefits [2,3].

Many plants have different ingredients which promote, stimulate or depress the functions of various organs or systems of the human body [4]. For centuries, roots of many herbs have been traditionally assumed to have relatively higher concentrations of constituents and chemicals which can promote extraction of unwanted components and extra fluids from human body [4]. The belief has now been proved in this scientific era of evidence based research. The traditional physicians have used this phenomenon for the treatments of multiple diseases like high blood pressure, oedema, anasarca, kidney/urinary problems, renal stones etc. [4-6]. Many herbal roots had been consumed as single remedy or part of combination therapies to evacuate the human body of extra fluids. One of the best combinations used for this purpose is "Nuskha-e-Baikhain" [4] containing four roots of *Apium graveolins* L. (karfs), *Ferula communis* (saunf/badiyan), *Cichorium intybus* L. (kasni), *Andropogon laniger* (Azkhar). The combination is said to be antiphlegmatic, ecobolic, diuretic, lithontriptic, emmenagogue & deobstruent [5-8]. The combination specially is considered as tonic for kidneys. Each individual root has some therapeutic potential for the above functions but their individual capacity needs to be proven on scientific basis.

Seed of *Apium graveolens* L. (family Apiaceae) is commonly known as celery. This is locally also known as karfs, karnaulli or ajmod. Commercially celery is available as celery seed, celery flaks, vegetable, celery seed, and celery seed oleoresin. *Apium* is one of the lesser known herbs in western herbal medicine although it has been used for thousands of years in traditional herbal medicine [4-6]. The aerial parts of the *Apium* have been investigated in many aspects but the root is still a less scientifically studied part [9].

Apium graveolens L. has been used as an aphrodisiac, anthelmintic, antispasmodic, carminative, diuretic, emmenagogue, laxative, sedative, stimulant, and toxic[11]. It is known as mild diuretic and urinary antiseptic and has been helpful in the relief of flatulence and griping pains. In the medicinal –herbal market, its oil extract, ground seed or root are marketed as herbal and dietary supplement that "promote and regulate" healthy blood pressure, joint health and uric acid levels [10]. *Apium graveolens* L. seed extract works as hypolipidemic and antioxidant agent because of its free radical scavenging properties [12]. The chemical properties of plant comprises of flavonoids, carbohydrates, steroids, alkaloids, and glycosides in methanolic extract [12]. Root decoctions and tinctures have been used as a diuretic in hypertension and urinary disorder.

MATERIAL & METHODS:

The project was reviewed and approved by the Ethical Review Committees of the Faculty of Health & Medical Sciences, Hamdard University through letter number *HCMD/ ERC/15/2015*., and was conducted at the animal house of

HMI institute of Pharmacology, Hamdard University on Wistar rats**.**

The Plant Material

The fresh roots of *Apium graveolens* L. (5 kg weight) was procured through Hamdard Laboratories (Waqf) Pakistan, maintaining the best quality sampling. The herb was further identified & authenticated by Dr. Aftab Saeed of Department of Pharmacogonosy, College of Eastern Medicine, Hamdard University through letter number *HU/FEM/HRIUM/150915-A.*

Extraction

The fresh roots were cut in small pieces and shade dried at room temperature. The sample was grinded to a fine powder by electrical grinder. The fine powder after the formal permission from Dr. Mohammed Shakeel, Director of The Industrial Analytic Center, HEJ Research Institute of Chemistry, and International Center for Chemical & Biological Sciences, University of Karachi was submitted in the departmental laboratory. Ethanol (Merck) 100 % was used to prepare the extract through the Soxhlet extraction method. The aqueous extract of plant was prepared freshly at the time of administration by boiling 10 grams of dried powder in 50 ml of water for 5 minutes and then the aqueous extract was filtered through the filter paper and fed orally according to the weights of animals.

Animals & Study Protocol

Total 40 Wistar rats were purchased from the animal house of Dow Medical College, Ojha Campus (Karachi, Pakistan) & were acclimatized in the animal house of Dr. HMI Institute of Pharmacology and Herbal Sciences, Hamdard University for 15 days before the study. The average age of animals at the time of experiment was about six to seven weeks and with average weight between 150-225 gm. Animals of either sex were kept separately in groups of six animals in large boxes of Plexiglass material. The animals were kept in 12/12 hour dark & light cycles. The temperature of the animals' house was kept constant at 22 ± 2 degrees. The animals were freely fed standardized rat chow and tap water.

Twenty (20) animals of both sexes were randomly selected for diuretic study of kidneys with acute renal injury. Animals of gentamycin control group were given gentamycin i.p. 40 mg/kg for two weeks (14 days) before administering testing extracts [13,14]. At the day of experiment all the groups of animals were given single dose of respective drug and were placed in separate diuretic cages for next 24 hours. The floor of the cage, funnel and collecting beaker all were applied with thin layer of mineral oil (paraffin) to prevent loss through evaporation. Urine samples were collected and urinary volume, sugar, protein; creatinine, sodium and potassium were studied.

The animals were grouped (n = 5) as following:

1. Normal control rats on saline only
2. Control rats were injected with gentamycin i.p. 40mg/kg for 14 days for induction of AKI
3. **Study group on *Apium graveolens* L.**
   i. Ethanolic extract of *Apium* 50 mg/kg dose orally
   ii. Ethanolic extract of *Apium* 50 mg/kg dose orally after gentamycin i.p. 40 mg/kg 14 days for induction of AKI
   iii. Aqueous extract of *Apium* 10 gm/kg orally
   iv. Aqueous extract of *Apium*10 gm/kg orally after gentamycin i.p. 40 mg/kg 14 days for induction of AKI
4. Study group on Furosemide
   i. Furosemide 40 mg/kg orally
   ii. Furosemide 40 mg/kg orally after gentamycin i.p. 40 mg/kg 14 days for induction of AKI

Urine Analysis

The parameters analysed were total volume of urine in 24 hours, levels of creatinine, sugar, proteins, sodium and potassium in urine samples obtained from treated rats. The creatinine, proteins & sugars were analysed using the metro lab 1600 with diagnostic kits (Merck). While the electrolytes were analysed using the atomic resorption method under the guidance of Dr. Talat Mehmood (Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Abdul Haq Campus, Karachi).

Statistical Analysis

Statistical analysis was done by One way ANOVA followed by post hoc Sheffe/LSD test using Statistical package for Social Sciences; Version 19 (SPSS 19). The results are given as mean ± SEM. Significance of the results were considered when *p*-value was found less than or equal to 0.05.

RESULTS:

Ethanolic extract of *Apium graveolens* L. 50mg/kg body weight and decoction when compared with saline and gentamycin groups, one way ANOVA showed significant difference in the urinary volume between groups ($F=32.102$ (7, 32), $p<0.001$). On post-hoc analysis rise in the urinary volume was highly significant with *Apium* decoction (7.6 ml, $p<0.001$), ethanolic extract 50 mg/kg (15.8 ml, $p<0.001$) and furosemide (4.16, $p<0.001$). In animals previously treated with gentamycin and given decoction showed significant difference as compared to group treated only with gentamycin. Ethanolic extract and furosemide did not show significant difference when the tests were run between gentamycin control and drugs along with gentamycin.

Gentamycin was found to exert profound effect on all the parameters of urine namely rise in urinary sugar ($p<0.025$) and proteins ($p<0.001$), while fall in urinary creatinine ($p<0.025$), fall in sodium ($p<0.001$) and rise in potassium ($p<0.001$). Extract and decoction showed improvement in the excretion of urinary sodium even after gentamycin induced renal toxicity, potassium excretion was increased in all treatment groups as compared to control. Oral furosemide only showed significant rise in urinary potassium excretion ($p<0.001$).

Table 1.1: Diuretic Activity of Ethanolic and Aqueous extract of *Apium graveolens* L. root (Baikh Karafs)

S.No	Group	24 hr. Mean Urine Volume ml/day	Sodium excretion ppm (Mean ±SEM)	% change in Sodium excretion	Potassium excretion ppm (Mean ±SEM)	% change in Potassium excretion	Na/K Ratio	Mean Urinary Creatinine (mg/dl)
1	Control Saline Treated	7.2 ±1.2	417.75 ±131.87		146.77 ±42.35		3.2	151 ±31.16
2	Control Gentamycin 40 mg/kg/day	3.6 ±0.82	166.39 ±25.75	60% fall	263.73 ± 52.0	80% rise	0.2	67.7 ± 16.73
3	Ethanolic extract 50 mg/kg	9.4 ±0.65	471.67 ±116.21	13% rise	144.6 . ±26.39	1.5% fall	3.38	231.23 ± 69.79
4	Extract 50 mg/kg after Gentamycin	5.2 ± 0.78	190.5 ± 44.59	54% fall	156.8 ± 32.7	6.8% rise	1.25	143.64 ± 43.63
5	Aqueous extract 10 gm/kg	11.2 ± 1.64	365.9 ± 20.29	12% fall	239.6 ± 59.02	63% rise	1.6	180.55 ± 13.71
6	Aqueous extract 10gm/kg after Gentamycin	9.0 ±0.79	296.7 ± 31.84	29% fall	177.63 ± 52.06	21% rise	1.8	121.3 ±23.04
7	Oral Furosemide40 mg/kg	7.76 ±1.48	438.9 ±81.76	5% rise	253.53 ±24.33	73% rise	1.75	128.28 ±25.76
8	Oral Furosemide40 mg/kg after Gentamycin	4.2 ± 0.57	252.12 ± 50.3	40% fall	93.8 ±15.34	36% fall	2.68	88.19 ±15.93

DISCUSSION:

Acute kidney injury (formerly known as acute renal failure) is a syndrome characterised by the rapid loss of the kidney's excretory function and is typically diagnosed by the accumulation of end products of nitrogen metabolism (urea and creatinine) or decreased urine output, or both [16]. This term is used to include every type of injury from minor injury to major injury [17]. ARF is usually measured by serum creatinine levels, although it is not a very accurate measurement but it gives an idea about renal functions as it is an inversely proportional to GFR [18]. Acute renal injury is usually treated promptly after hospitalization but it is said that once ARF occurred, such a patient is more prone to chronic renal failure [16]. Gentamycin is a strong aminoglycoside agent used for severe gram positive and gram negative organisms. Its use is limited due to its nephrotoxicity [19], which can be the sole cause of mortality [20]. *In vitro* and *in vivo* studies show that gentamycin enhances the generation of reactive oxygen metabolites. Gentamycin is said to accumulate in the renal cortex, mostly in the proximal convoluted tubules, however, exact mechanism of toxicity is unknown [21]. Iron is important in models of tissue injury, presumably because it is capable of catalyzing free-radical formation [22] and gentamycin has been shown to cause release of iron from renal cortical mitochondria due to its structural alteration [23]. Scavengers of reactive oxygen metabolites as well as iron chelates provide protection in gentamycin-induced nephrotoxicity [22].

Extensive literature search shows that seeds of *Apium* is effective against several ailments namely inflammation, G.I disturbances, liver conditions and bones and joints [8-11]. Diuretic activity of *Apium* roots was not found till date. It has been found that *Apium graveolens* L. extract intake declines the oxidative stress [27]. Additional pharmacologically active response is found to be its antihypertensive activity [11,29].

Many plants have been proved to be nephro-protective against gentamycin induced nephro-toxicity [24-26]. *Apium* roots are used by ancient physicians as diuretic, renal and bladder lithotropic, anti-inflammatory, anti-infective and nephro-protective agent as single or combination agents [11]. Therefore, present study was designed to investigate the nephro-protective effect of ethanolic and aqueous extract of *Apium graveolens* L. in gentamycin induced nephro-toxicity.

As from the definition above, ARF is marked by serum creatinine levels and the urinary output. According to studies, urinary output is a non-specific marker of renal failure but it is said that urine output is said to occur long before biochemical changes [18]. According to some studies serum creatinine is not a specific marker of ARF, but still mild changes in the serum creatinine are associated with increased patient mortality [30]. Recent consensus shows that ARF has now two major diagnostic criteria viz. percent increase of serum creatinine $\geq$ 50% or/and reduction in urinary output of $<$ 0.5 ml/kg in 6 hours. It is found in some studies that use of furosemide in ARF usually deteriorate the condition further therefore it is wise to proceed cautiously [30].

Use of furosemide is usually in the need to rapidly evacuate the body of the fluid retention resulting due to ARF but in the case that use of furosemide is questionable use of safer

alternatives is necessary. Natural herbalists use *Apium* roots for the kidney ailments for years. To prove the use of its diuretic activity present study was designed and it provided fruitful scientifically proved data showed that fresh decoction of the roots exhibited powerful diuretic activity (p<0.001). Decoction showed 72% rise in the urinary output suggesting its strong diuretic activity. Gentamycin showed 45% fall in urinary output in 24 hours and comparatively pre-treatment of decoction prevented the urinary fall by 30% (p<0.001). Ethanolic extract of *Apium* root (50 mg/kg) showed 44% rise in the urinary output in normal animals. When compared with gentamycin induced animals 24% fall was prevented by the extract (p<0.001). The results were far better than furosemide as it prevented the fall by only 10% (NS). Again this is suggestive that furosemide if not contributing in further damage of the kidneys is not preventing the damage as well.

When urinary creatinine was calculated after 24 hours, it showed marked (59%) fall in the levels after gentamycin injection. Highest response to gentamycin induced renal damage as far as urinary creatinine levels are concerned was seen by 50 mg/kg dose of extract as it prevented the fall by 47% (-12% fall). Decoction prevented the fall in creatinine levels by 31% (-28% fall). The results were again very significantly better than furosemide i.e. it prevented only 9% fall(-46% fall) in gentamycin induced renal damage.

Urinary electrolyte excretion is a very important matter. In case of hypertension and other oedema related ailments sodium excretion along with water is required. But increased potassium excretion causes arrhythmias. Potassium sparing diuretics are not of good use as they have very mild diuretic activity. Our study showed enhanced potassium excretion when treatment given to normal animals. But in case of gentamycin induced renal injury, it was seen that gentamycin increased potassium excretion by 80% whereas extract of *Apium* prevented the potassium excretion significantly both when given to normal animals (1.5% fall, p = 0.005) and gentamycin induced animals (6.8% rise, p = 0.016) when compared to gentamycin control group. The levels remained in a range of saline control group. Furosemide also showed 36% fall in the potassium excretion; the results were significantly different from gentamycin control (p<0.001).

With the reversal of the injurious effects of gentamycin, it is evident that decoction and extract of *Apium graveolens* L. has a beneficial role in treatment and prevention of kidney damage. As it is known that *Apium* has documented radical scavenging activity [27] and gentamycin is thought to have a free radical mode of damage [22], the most probable mode of action of *Apium* extract may be prevention of radical formation or rapid neutralization of reactive species to prevent the chain reaction of radical induced damage. This also provides a positive clue that these extracts could be used in kidney and bladder conditions, as mentioned by many herbalists of Unani and Ayorvedic system of medicine. Present study also proves that root extract and decoction of *Apium graveolens* L.is a nephro-protective agent in rats.

CONCLUSION

On the basis of our study we can conclude that the ethanolic and aqueous extract of *Apium graveolens* L. (root) has comparatively significant diuretic activity against the standard drug furosemide. These functions should be further explored in pharmaceutical screening of new potential drug candidate for use in the long term treatment of essential hypertension, acute and chronic urinary tract infections and other cardiovascular diseases, where fluid depletion from the body without electrolyte disturbances especially serum potassium stabilization, is needed.

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