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Evaluation of antiobesity activity of *Apium graveolens* stems in rats

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ABSTRACT

It is a serious public health problem throughout the world, affecting both developed societies and developing countries Moreover, obese and overweight patients are at higher risk from coronary artery disease, hypertension, hyperlipidemia, diabetes mellitus, cancers, gall bladder disorders, cerebrovascular accidents, osteoarthritis, restrictive pulmonary disease and sleep apnoea. aim of the present study was to evaluate antiobesity activity of ethanolic and aqueous extracts of *"Apium graveolens". Apium graveolens* (Family: umbelliferae) is a well known plant of Indian medicinal system. The traditional seeds of *Apium graveolens* used in India to treat bronchitis, asthma, liver and spleen diseases. Wistar albino rats of either sex, 4- 5 weeks old and weighing around (200-250g) were used in this study. The preliminary phytochemical analysis of the aqueous extract of *Apium graveolens* revealed the presence of Alkaloids, Carbohydrates, Glycosides, Flavonoids, Coumarins, Saponins and Tannins. Acute oral toxicity studies of the aqueous extract of *Apium graveolens* did not exhibit any sign of toxicity up to 2000 mg/kg body weight. Based on the body weight, organs weight and biochemical parameters revealed that aqueous extract of *Apium graveolens* having anti-obesity activity.

Keywords: Hypertension, Hyperlipidemia, Osteoarthritis, Patients, Pulmonary disease.

1. INTRODUCTION

Obesity is defined medically as a state of increase in fat mass and it occurs when unilocular adipocytes show hyperplasia or hypertrophy following macrophage infiltration of fat tissue [1]. It is a serious public health problem throughout the world, affecting both developed societies and developing countries [2]. More than two third of the American population is now considered obese or overweight, and the prevalence of obesity in children is escalating dramatically, signifying even greater medical harm in the decades to come [3,4]. Moreover, obese and overweight patients are at higher risk from coronary artery disease, hypertension, hyperlipidemia, diabetes mellitus, cancers, gall bladder disorders, cerebrovascular accidents, osteoarthritis, restrictive pulmonary disease and sleep apnoea [5,6]. Although a number of pharmacological approaches for treatment of obesity have been investigated, but only few are safe and all of these have adverse effects [7]. So alternative is to discover antiobesity drugs from plants and hence, aim of the present study was to evaluate antiobesity activity of ethanolic and aqueous extracts of *"Apium graveolens".*

Apium graveolens (Family: umbelliferae) is a well known plant of Indian medicinal system. The traditional seeds of apium graveolens used in India to treat bronchitis, asthma, liver and spleen diseases. The results also showed that, celery leaf extracts of suspension produce anti pyretic effect[8]. The plant has been reported to produce Hepatoprotective [9], Hypocholesterolaemic [10], Anti-inflammatory [11] activities. Aqueous celery extract for 8 weeks treatment causes a significant reduction in serum total cholesterol (TC) level in growing genetically hypercholesterolaemic (RICO) rats[9]. However, there are no reports regarding the effectiveness of *Apium graveolens* in obesity and its mechanism of action of anti-obesity activity is poorly understood. Therefore, this study has been designed to investigate the anti-obesity activity of *Apium graveolens* along with its mechanism of action in wistar rats.

2. MATERIALS AND METHODS

2.1. Experimental animals

Wistar albino rats of either sex, 4- 5 weeks old and weighing around (200-250g) were used. The animals were obtained from Drug testing laboratory (DTL) Bangalore, Karnataka.

Protocol of the experiments and animal usage was discussed in the Institutional Animal Ethical Committee meeting and permission has been obtained to carry out the parameters selected for the study. Animals were maintained in suitable nutritional and environmental condition throughout the experiment. Housed six animals per cage made up of polypropylene with paddy husk as bedding. The animals were acclimatized for 10 days under standard husbandry condition i.e. room temperature 25 ± 2ºC, relative humidity: 65 ± 10 %, 12 hr. light / dark cycle. They were provided feed, with rodent pellet diet (Venkateshwara enterprises Bangalore) and water *ad libitum* under strict hygienic condition. The bedding material of the cages was changed on alternative days.

2.2. Drugs and chemicals

Anaesthetic ether(Sigma solvents and Pharmaceuticals,Mumbai.), Ethanol(Sd fine chem, Mumbai), Sibutramine(Micro Labs, Bangalore), Kits for estimation of Glucose, Cholesterol, Triglyceride, BUN(Swemed Diagnostics Yelachenahalli,Bangalore) were used in the present study.

2.3. Collection of plant material:

The fresh leaves of *Apium graveolens* were collected from Amruth Kesari Depot D.PG. Complex Bangalore-53, and authenticated by Regional Research Institute Ashoka Pillar, Bangalore, India.

2.4. Preparation of extract of *Apium graveolens*

The fresh leaves are thoroughly washed and shade dried. The dried leaves were crushed to coarse powder using a hand mill and sieved using the sieve number 10, extracted by cold maceration using distilled water and few ml of chloroform to avoid fungal growth for aqueous extract. The extract was concentrated by evaporating at room temperature and air dried. Dark brownish black solid weight of yield obtained was 3g/100ml for aqueous extract.

2.5. METHODS

2.5.1. Preparation of extract solution

The above obtained aqueous extract was dissolved in distilled water (vehicle). All preparations (doses) were freshly prepared on the day of experiment and administered to the animals.

2.5.2. Preliminary phytochemical analysis

Preliminary phyto-chemical studies were carried out for the aqueous extract of *Apium graveolens* to find out the presence of different phyto-chemical constituents like carbohydrates, proteins, fats and oil, alkaloids, glycosides, terpenoids, flavonoids, tannins and polyphenols.

2.5.3. Acute toxicity studies

Acute oral toxicity was carried out for aqueous extract of *Apium graveolens* using acute toxic class method, according to OECD guidelines No. 423. Healthy adult Swiss albino mice (female) weighing between 25 to 35 g were used for the study. Animals were divided in to four groups of three animals each and kept fasted for overnight. The different doses like 5, 50, 300, 2000 mg/kg b.w were administered to animals of Group I, II, III, and IV respectively. After administering the extract to different groups the behavioral changes in the animals like body temp, CNS activities, micturition and defecation etc were observed for 24 hrs.[12]

2.5.4. Laboratory models employed for the evaluation of antiobesity activity

2.5.4.1. Cafeteria diet -induced obesity

Albino rats were weighed and selected for the experiment depending on weight. The rats were be divided into four groups of 6 rats each. The group I served as normal control and were maintained only on normal pellet chow and water ad libitium. The II, III & IV groups were provided with cafeteria diet (Table 1) for 40days. The Group II served as cafeteria diet control. The III group animals were administrated with received Standard (Sibutramine 5mg/kg)i.p. and Group IV animals were administered aqueous extract *Apium graveolens* P.O. The treatment was continued for 40 days. Body weight and body temperature were measured on every 5days till 40 days. On 40th day animals were sacrificed by cervical dislocation and the weight of organs such as liver, spleen and adrenal gland were recorded per 100 g body weight of animal after washing them with alcohol and measured serum lipid levels, LDL, HDL, and VLDL, cholesterol and triglyceride. [13]

Table - 1: Compositions of different ingredients for cafeteria diet preparation for g/100 g

Cafeteria	Quantity
Condensed milk	40g
Bread	40g
Chocolate	15g
Biscuits	30g
dried coconut	30g
Cheese	40g
Boiled potatoes	50g

2.5.4.2. Data and statistical analysis

Data obtained were expressed as mean ± standard error of mean (SEM). The significance was determined by applying one-way ANOVA using prism graph pad software. The statistical differences in the sample means were considered significant at p<0.05.

3. RESULTS

3.1. Preliminary phytochemical analysis

The preliminary phytochemical analysis of the aqueous extract of *Apium graveolens* revealed the presence of Alkaloids, Carbohydrates, Glycosides, Flavonoids, Coumarins, Saponins and Tannins.

3.2. Acute toxicity study

Acute oral toxicity studies of the aqueous extract of *Apium graveolens* did not exhibit any sign of toxicity up to 2000 mg/kg body weight. Since there was no mortality of the animals found at highest dose. Hence dose for the present study was selected randomly that is 200 and 400 mg/kg.

3.3. Effect of *Apium graveolens* on body weight in Cafeteria diet -induced obese rats

Consumption of high fat diet for 40 days significantly increased the body weight of rats compared to control group. Aqueous extract of *Apium graveolens* at dose 400mg/kg significantly(*P*<0.05) decreased the body weight in Cafeteria diet -induced obese rats on 15th, 30th,35th and 40th day compared to obesity induce group. Treatment with sibutramine to obesity induce group(C) shown significantly(*P*<0.001) decreased the body weight in cafeteria diet induced obese rats on 30th, 35th and 40th day compared to obesity induce group. (Table 2)

Table - 2: Effect of treatment of *apium graveolens* on body weight of animals of Cafeteria and atherogenis diet model .n=6

Treament dose mg/kg Body weight	1st day	5th day	10th day	15th day	20th day	25th day	30th day	35th day	40th day
Control group	180	185.17 ± 0.478	189 ± 0.25	192.62 ± 0.25	195.62 ± 0.55	204.33 ± 0.55	215.51 ± 1.61	223.83 ± 1.20	232.33 ± 1.11
Cafeteria diet(A)	180.83 ± 0.40	185.05 ± 0.50	196.83 ± 0.47	218.83 ± 1.49	225.52 ± 1.95	235.17 ± 0.70	257.67 ± 2.64	281.67 ± 3.22	311.83 ± 3.78
Aqueous extract 400mg(B)	181.51 ± 0.42	185.33 ± 0.66	195.33 ± 1.02	211.33 ± 1.411	227.17 ± 339.71	233.01 ± 1.00	244.33 ± 0.981	251.05 ± 0.988	259.17 ± 0.913
Sibutramine 5mg/kg(C)	181.66 ± 0.76	185.33 ± 0.88	194.33 ± 0.49	211.51 ± 1.71	237.16 ± 1.70	251.66 ± 0.49	250.33 ± 1.31	261.16 ± 1.08	265.66 ± 0.84

Table - 3: Effect of aqueous extract of *Apium graveolens* 400mg/kg on cafeteria n=6

No of animals	glucose	Cholesterol	HDL	LDL	VLDL	Triglyceride
Control group(a)	58.68 ± 2.52	73.23±1.29	12.12± 0.30	44.76±1.76	16.39± 0.15	81.97±1.97
Cafeteria diet (b)	94.20±20**	99.00±2.92***	16.75± 0.65**	47.16±2.97**	33.41± 1.61*	167.11±8.08***
Aqueous extract 400mg(c)	66.27±1.58**	73.71±2.99***	16.51± 0.33ns	38.43±3.05**	18.34±0.32***	91.71±1.62***
Sibutramine 5mg/kg(d)	63.13±2.13***	76.39±1.79***	17.56± 1.25***	41.34±1.92***	17.80±0.20***	88.71±1.29***

Significance at **P<0.01***P<0.001, as compared to control group

3.4. Effect of *Apium graveolens* on the ratios of weight of liver, heart and kidney to the weight of animal

Consumption of Cafeteria diet for 40days has shown significant increase in the weight of heart and liver organ and no significant increase in kidney weight as compared to control group. Treatment with dose of aqueous extract of *Apium gravelones* 400mg/kg to cafeteria fed rats produced significant reduction in liver weight ratio(P<0.01), heart weight ratio(P<0.01) and weight on kidney ratio(P<0.001) compared to normal fed control. Treatment with sibutramine (5mg/kg) produced significant reduction in liver weight ratio, heart weight ratio and kidney weight ratio(P<0.001) compared to normal fed control. The values were summarized in table 3.

3.5. Effect of *Apium graveolens* on Biochemical parameter in Cafeteria diet -induced obese rats

Cafeteria diet significantly increased the levels of glucose, triglycerides, cholesterol and vldl levels. Treatment with dose of aqueous extract of *Apium gravelones* of 400mg/kg to cafeteria fed rats for 40 successive days significantly decreased the glucose, cholesterol, ldl, vldl and triglycerides and increased the hdl cholesterol levels. Treatment with Sibutramine 5mg/kg to cafeteria fed rats for 40 successive days significantly decreased the glucose, cholesterol, hdl, vldl and triglycerides.

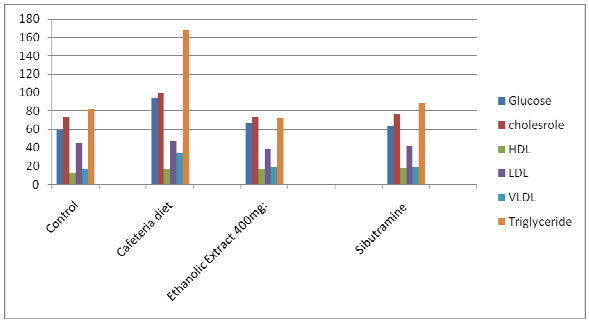

Figure - 1: Effect of aqueous extract of *Apium graveolens* 400mg/kg on cafeteria biochemical parameters n=6.

4. DISCUSSION

We examined the anti-obesity effect of *Apium gravelones* aqueous extrct in high-fat diet-fed rats, since this metabolic model of obesity reproduces human obesity better than the genetic obese models. In the present study Wistar rats fed a high-fat diet had significant increased body weight, adipose tissue weight and levels of serum glucose, triglycerides and total cholesterol compared to rats fed a normal chow diet.

Cafeteria diet- induced obesity model is the simplest obesity-induction model and possibly the one that most closely resembles the reality of obesity in humans [14]. The results of the present study showed that rats fed with a variety of highly palatable, energy rich, high carbohydrate cafeteria foods elicited significant increase in body weights and serum cholesterol, triglycerides, glucose. Cafeteria diets have been previously reported to increase energy intake and cause obesity in humans as well as in animal [15]. The cafeteria diet has been reported to induce hyperplagia in rats which further results in higher fat stores [16].

In this study, antiobesity-like effect of aqueous extract of *Apium gravelones* might be due to –(i) weight reducing effect of the extract (ii) enhanced thermogenesis since obesity is associated with defective thermogenesis [17]. It has been reported that tannins and flavonoids may be responsible for prevention of obesity [18]. Phytochemical screening indicated the presence of alkaloids, glycosides, carbohydrates, sterols, polyphenolic compounds, tannins and flavonoids in aqueous extract of *Apium gravelones*. However further studies are required to find out the component(s) responsible for antiobesity activity. Thus aqueous extract of *Apium gravelones* can be explored further for its potential treatment in obesity.

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