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Effect of *Foeniculum vulgare* Mill. Fruits in Obesity and Associated Cardiovascular Disorders Demonstrated in High Fat Diet Fed Albino Rats

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Abstract: Present work is based on screening of *Foeniculum vulgare* Mill. Fruit extracts in high fat diet fed albino rats for their possible role in obesity and associated cardiovascular disorders. Three fractions prepared by successive solvent technique from methanol extract of *Foeniculum vulgare* Mill. fruits were administered at a dose of 300 mg/body weight by oral gavage and volatile oil obtained by hydrodistillation at a dose of 0.2 ml/body weight intraperitoneally once daily along with high fat diet to the female albino rats for six weeks (0-42 days). Normal control and high fat diet fed control groups were maintained simultaneously. Body weight of the experimental animals was estimated every week while lipid parameters and fat pad weights were estimated after 42 days after sacrificing the animals by euthanasia. Phytochemical studies were carried out of the above mentioned extracts. Results revealed that body weight and fat pad weights were reduced in drug fed animals in a variable pattern. Cholesterol and triglycerides levels, which were disturbed in high fat diet fed animals, improved in a significant manner. Maximum activity was observed with methanol fraction of the drug which contained maximum amount of phenolic (48.37 mg/g) and flavonoidal contents (21.44 mg/g). Based on the scientific reports, that antioxidant compounds play a vital role in the management and control of obesity via improvement in natural antioxidant defense and lipid metabolism, it is predicted that the observed activity may be due to this or more of these mechanisms. In conclusion, *Foeniculum vulgare*, a well known herb in Indian system of medicines has demonstrated to be effective in obesity and associated cardiovascular disorders.

Key words: *Foeniculum vulgare*, obesity, cardiovascular disorders, hyperlipidemia.

Introduction:

Obesity is a common nutritional disorder, presently considered as a major risk factor even more serious than diabetes because of its association with serious health disorders like coronary heart diseases, hypertension, diabetes, pulmonary dysfunction, osteoarthritis and certain types of cancer. [1-3] The prevalence of obesity is increasing world wide since last few years, and is shooting up to the alarming level not only in developed but developing countries as well. [4,5] Several efforts have been made by the scientific community in order to find out a possible solution for this health disorder from natural resources and as a result in the recent past, a few folklore medicinal plants have shown promising results acting through different mechanisms. Out of them, lipid and lipoprotein metabolism is the promising one in which, it has been observed that antioxidant drugs have a significant role in reducing the atherogenic lipoprotein profile in hyperlipidemia and atheroscelrosis. [6,7] Thus, antioxidant and cholesterol lowering activities of plants extracts or isolated components can be effectively utilized to reduce the development of obesity and associated cardiovascular disorders. [8]

Foeniculum vulgare (fennel) is a typical aromatic plant, biennial or perennial, therapeutically used as antibacterial, antifungal and in digestive problems like colic, indigestion and flatulence. [9, 10] Phytochemical evaluation conducted on fennel fruit describes the presence of major volatile components like estragole, fenchone, alpha-phellandrene and non volatile components like phenolic and flavonoid aglycons. [11,12] Fennel has strong antioxidant and free radical scavenging potential and also has positive effect against hypertension. [13,14] Scientific evidence is also found regarding the use of volatile components of this drug in weight management and food efficiency. [15] From this background, the present study was planned to screen the volatile and non volatile extracts of this drug in obesity and associated cardiovascular disorders in experimental high fat diet fed albino rats.

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Materials and Methods:

Plant Material:

Foeniculum vulgare Mill fruits were collected from local market Khari Baoli, New Delhi and identified from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen and identification certificate no. NISCAIR/ RHMD/Consult/2007-08/966/150 was kept in the department for future reference.

Extraction and Fractionation:

2 kg of the sample material was subjected for extraction by maceration using methanol as solvent for 3 days at 37^0C with occasional stirring in a closed vessel. The mixture was then filtered and the filtrate was concentrated using rota evaporator, then dried at room

temperature to get dried mass (275 g) which was stored at -20^{0}C prior to the use. This extract was then suspended in water and partitioned with n-hexane in a separating funnel. The mixture was shaken vigorously and n-Hexane layer was separated to get hexane fraction. Remaining mixture was again partitioned with chloroform in the same fashion to get the chloroform fraction. The remaining mixture left was lyophilized and re-dissolved in methanol and treated as methanol fraction. Each fraction was dried to get solid powder which was weighed and thereafter calculated the percentage yield. The three fractions were subjected for estimation of respective residual solvents by gas chromatography using standard protocols to maintain the WHO prescribed limits (data not shown). Volatile oil from the sample material was isolated by hydrodistillation (yield 1.4 % w/v), stored in a tightly closed glass container and used for further studies.

Phytochemical analysis:

Hexane, chloroform and methanol fractions were subjected for the presence/absence of various phytoconstituents through chemical tests using standard methods. Total phenolic contents were determined by Folin-Ciocalteu phenol reagent estimated as gallic acid equivalent and total flavonoidal contents were determined using rutin as reference standard. Volatile oil constituents of fennel were analyzed by a Thermo Finnegan having Trace GC Ultra as gas chromatograph equipped with Polaris Q mass spectrometer using electron impact. The gas-chromatographic (GC) temperature conditions were initially 70^{0}C for 3 minutes and then rose to 250^0 C at a rate of 3^{0}C min^{-1}, and finally held at that temperature for 5 min. The mass spectrometry (MS) was carried out with conditions described as: Ionization voltage, 70 eV; emission current, 250µA; mass range, 30-450 Da; ion source temperature, 200^{0}C microscans^{-3}. The MS fragmentation pattern was checked with those of other essential oils of known composition, with pure compounds and by matching the MS fragmentation patterns with NIST NBS75K and Wiley mass spectra libraries.

Preparation of Drug Samples:

Three fractions as obtained above were suspended in 0.3 % carboxy methyl cellulose (CMC) solution to get uniform suspension of concentration 60 mg/ml. The suspension was prepared daily to avoid any microbial contamination prior administration to the animals.

Test animals:

36 Female albino wistar rats weighing (100-150 g) were obtained from animal house, Jamia Hamdard, New Delhi. The animals were divided into six groups, kept in teflon cages and supplied with standard feed procured from Hindustan lever ltd *ad libitum.* The individual weight was recorded and dye marking was done on head, tail and back for proper identification. Neat, healthy and controlled conditions (22-28^{o}C temperature, 60-70% relative humidity) at 12-hr dark/light cycle, were maintained throughout the experiment. Experimental protocol complied with the rulings of the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) New Delhi, India (Registration no: 173/CPCSEA) and the experimental protocol was approved by the institutional ethical committee.

High Fat Diet:

High fat diet used for the experiment was procured from National centre for laboratory animal sciences at National institute of nutrition, Hyderabad. Each 5 kg feed comprised of casein (1.71 kg), L-cystine (0.015 kg), starch (0.86 kg), sucrose (0.86 kg), cellulose (0.25 kg), groundnut oil (0.125 kg), thallow (0.950 kg), mineral mixture (0.175 Kg) and vitamin mixture (0.05 Kg). The high fat diet was received in the form of pellets which was consumed by the experimental animals instead of regular food.

Experimental Protocol:

The animals were divided into six different groups having six animals in each group as : group A (Normal Control (NC)), group B (High Fat diet fed control (HFDC)), group C (High Fat diet + Volatile oil of *Foeniculum vulgare* (HFD+VO), group D (High Fat diet + Hexane fraction of *Foeniculum vulgare* (HFD+HF), group E (High Fat diet + Chloroform fraction of *Foeniculum vulgare* (HFD+CF), group F (High Fat diet + Methanol fraction of *Foeniculum vulgare* (HFD+MF). Drug fractions were administered through oral gavage at a dose of 300mg/kg body weight while volatile oil was administered at dose of 0.2 ml/kg body weight by intraperitoneal route. The drugs were administered once daily and continued up to 6 weeks (0-42 days). The dosage of all the drugs administered to the animals was selected on the basis of previously conducted studies as therapeutically active dosage and also on the basis of preliminary dose-response experiment conducted on animals (data not shown).

Parameters studied:

Body weight was monitored every week of the individual animal. On completion of the drug treatment for 42 days, before sacrificing the animals by euthanasia, the blood samples were collected by sino-orbital puncture thereafter, the animals were sacrificed and mesentric, perirenal and uterine fat pads were isolated and weighed. Organs like kidney, spleen, heart and livers were isolated and weighed. Blood samples were used for biochemical estimations like total cholesterol (enzymatic method), high density lipoprotein (phosphotungstate method), low density lipoprotein and triglycerides (enzymatic colorimetric method) according to the procedure mentioned in the respective kits purchased from standard suppliers.

Statistical Analysis:

The results were statistically analysed and expressed as mean ± S.D. Comparison between the treatment groups and control were performed by analysis of variance (one way ANOVA) followed by dunnets' 't' test. In all the tests, the criterion for statistical significance was considered to be $p < 0.05$.

Results:

Phytochemical Analysis:

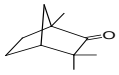
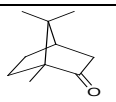
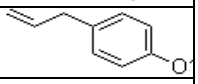
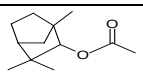
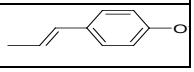
Preliminary phytochemical analysis revealed the presence of sterols, lipids and fats in hexane fraction, sterols, phenoilcs, flavonoids and glycosides in chloroform fraction and flavonoids and phenolic compounds in methanol fraction of fennel. Total phenolic and flavonoidal contents were found maximum in methanol fraction followed by chloroform and nil in hexane fractions of fennel ***(Table 1)***. Status of chief volatile constituents

obtained from GC-MS analysis of volatile oil is shown in ***(Table 2)***.

Table 1: **Constituents of Fennel fractions estimated by phytochemical screening**

Class	Hexane fraction	Chloroform fraction	Methanol fraction
Major phytoconstituents present	Sterols, lipids, fats	Sterols, phelolics, flavonoids	Phenolics, flavonoids
Total phenolic contents	Nil	10.51 mg/g	48.37 mg/g
Total flavonoidal contents	Nil	5.26 mg/g	21.44 mg/g

Table 2: **Chief constituents of Fennel essential oil analyzed by GC-MS**

S. No	Compound	Fennel		Structure of the Compound
		RT	Area%	
1	Fenchone	13.29	0.15	
2	Camphor	15.99	0.03	
3	p-allyl-anisole	18.21	4.41	
4	Fenchyl acetate	19.34	0.12	
5	Anethole	22.37	88.95	

Effect of Foeniculum vulgare on body weight:

Effect of drug on body weight of experimental animals was compared with normal control and high fat diet fed control groups. Data presented in ***(Table 3)*** reveals that the drug treatment with volatile oil restricted the elevation of body weight maximum on sixth week ($p<0.5$) but hexane fraction could not show any activity, however, significant improvement in the body weight was observed in the animal groups treated with chloroform and methanol fractions ($p<0.01$). Activity of methanol fraction was observed better than chloroform fraction.

Table 3: **Effect of Foeniculum vulgare on Body weight profile in albino rats during 42 days of treatment**

Group	Body weight (g)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
NC	104.6 ± 6.6	113.0 ± 8.8	123.6 ± 9.1	131.5 ± 8.3	136.8 ± 6.9	146.0 ± 10.5	152.1 ± 8.4
HFDC	108.6 ± 3.2	125.5 ± 6.7	146.2 ± 12.1	165.2 ± 8.4	176.2 ± 11.4	191.3 ± 15.6	213.6 ± 12.4
HFD + VO	106.3 ± 4.8	119.8 ± 6.2	137 ± 7.6	153.6 ± 5.4	175.5 ± 13.2	181.8 ± 9.6	190.0 ± 10.6
HFD + HF	108.2 ± 7.3	130.8 ± 6.6	150.2 ± 6	167.8 ± 7.2	174 ± 2.8	190.6 ± 8.4	220.5 ± 11.5
HFD + CF	111.8 ± 6.1	124.3 ± 12.6	145.6 ± 5.9	158.5 ± 8.4	173.0 ± 5.5	187.1 ± 10.2	185.2 ± 3.2[b]
HFD + MF	109.7 ± 5.4	120.7 ± 7.3	134.3 ± 6.8	149 ± 7.3	159.7 ± 5.2	172.0 ± 6.3[a]	181.2 ± 7.8[b]

Data expressed as Mean ± SD (n=6). Statistical significance in comparison to control,
[a] = P<0.5; [b]= P<0.01; [c]= P<0.001. One way ANOVA followed by Dunnet's t-test.

Effect of Foeniculum vulgare on organ weight and fat pad weights:

No significant changes in weights of heart, kidney, liver and spleen in *Foeniculum vulgare* drug treated albino rats as compared to normal control and high fat diet fed control groups were seen ***(Table 4)***, however, significant increase in mesentric, perirenal and uterine fat pad weights in high fat diet fed control groups as compared to normal control groups was seen. It was observed that volatile oil treatments have significantly reduced the mesenteric ($p<0.01$) and perirenal fat pad weights ($p<0.5$) but not uterine fat pad weights. Chloroform fraction resulted significant reduction in mesenteric fat pad weights only ($p< 0.5$). Treatment with methanol fraction was able to demonstrate highly significant effect in mesenteric and perirenal fat pad weights ($p<0.001$) and significant in uterine fat pad weights ($p< 0.01$).

Table 4: Effect of Foeniculum vulgare on organ weight profile in albino rats after 42 days of treatment

Group	Organ weight (g/100 g of animal weight)			
	Heart	Kidney	Liver	Spleen
NC	0.674 ± 0.087	0.861 ± 0.079	4.474 ± 0.124	0.657 ± 0.096
HFDC	0.681 ± 0.069	0.869 ± 0.068	4.843 ± 0.134	0.683 ± 0.084
HFD + VO	0.705 ± 0.045	0.879 ± 0.091	4.676 ± 0.236	0.668 ± 0.031
HFD + HF	0.693 ± 0.070	0.916 ± 0.023	4.938 ± 0.300	0.652 ± 0.053
HFD + CF	0.671 ± 0.032	0.849 ± 0.075	4.811 ± 0.416	0.675 ± 0.047
HFD + MF	0.660 ± 0.080	0.848 ± 0.094	4.843 ± 0.383	0.651 ± 0.041

Data are expressed as Mean ± SD (n=6). Statistical significance in comparison to control,
[a] = P<0.5; [b]= P<0.01; [c]= P<0.001. One way ANOVA followed by Dunnet's t-test.

Table 5: **Effect of Foeniculum vulgare on fat pad weight profile in albino rats after 42 days of treatment**

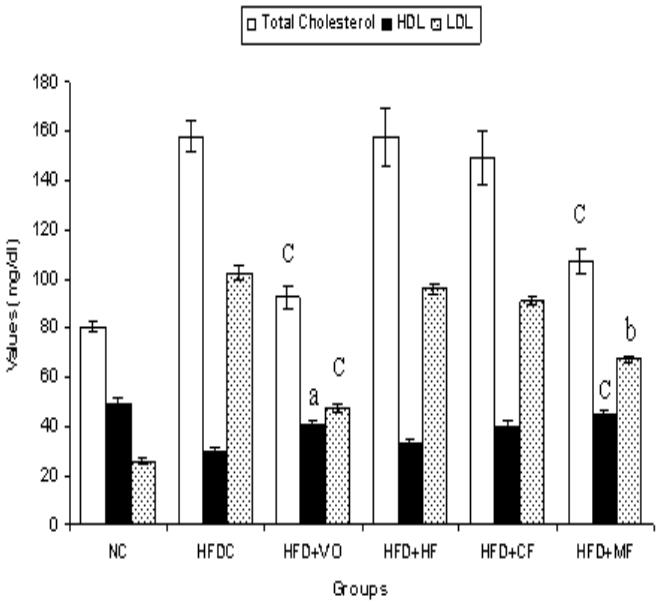
Group	Fat pad weights (g/100 g of animal weight)		
	Mesentric	Perirenal	Uterine
NC	0.264 ± 0.076	0.626 ± 0.056	0.221 ± 0.134
HFDC	1.760 ± 0.084	1.141 ± 0.068	0.671 ± 0.148
HFD + VO	0.867 ± 0.011 [b]	0.991 ± 0.174 [a]	0.762 ± 0.062
HFD + HF	1.470 ± 0.076	1.271 ± 0.210	0.627 ± 0.183
HFD + CF	0.958 ± 0.096 [a]	1.019 ± 0.242	0.619 ± 0.052
HFD + MF	0.502 ± 0.152 [c]	0.711 ± 0.036 [c]	0.438 ± 0.068 [b]

Data are expressed as Mean ± SD (n=6). Statistical significance in comparison to control,
[a] = P<0.5; [b]= P<0.01; [c]= P<0.001. One way ANOVA followed by Dunnet's t-test.

Effect of Foeniculum vulgare on lipid profile:

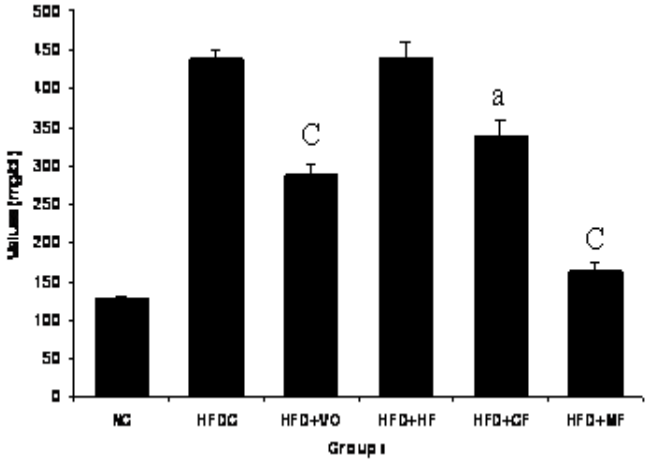
Data presented in ***(figure 1)*** show that total cholesterol and LDL levels were elevated while HDL levels were reduced in high fat diet fed control group animals. *Foeniculum vulgare* drug treatment has affected the lipid profile of animals in variable modes. Volatile oil treatment has significantly improved the lipid profile by decreasing TC (p<0.001) and LDL (P<0.001) levels and increasing HDL (p<0.5) levels. Methanol fraction treatment has resulted in significant variations at P<0.001, P<0.001 and P<0.01 levels respectively as compared to the control but this effect was not seen with other two drug schedules. Similar results were obtained with triglycerides levels in which the high fat diet has elevated the triglycerides levels immensely. Highly potential effect was observed with volatile oil and methanol fraction treatment (p<0.001) and significant effect with chloroform fraction (p<0.5) ***(figure 2)***.

Figure 1: **Effect of Foeniculum vulgare on cholesterol levels in albino rats after 42 days of treatment.**



Data are expressed as Mean ± SD (n=6). Statistical significance in comparison to control,
[a] = P<0.5; [b]= P<0.01; [c]= P<0.001. One way ANOVA followed by Dunnet's t-test.

***Figure 2*: Effect of Foeniculum vulgare on serum triglycerides in albino rats after 42 days of treatment.**



Data are expressed as Mean ± SD (n=6). Statistical significance in comparison to control,
[a] = P<0.5; [b]= P<0.01; [c]= P<0.001. One way ANOVA followed by Dunnet's t-test.

Discussion:

In the present study, the antiobesity effect of *Foeniculum vulgare* was studied using the dietary (high fat diet) animal models of obesity as they are reported to bear close resemblance to human obesity. [16] Studies have confirmed that overproduction or deficiency of lipoproteins leads to a disorder of lipoprotein metabolism called dyslipidemia, which is manifested by an elevation of serum total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels, and decrease of high-density lipoprotein cholesterol. [17] In support, present study shows that animals fed with high fat diet has significantly increased their fat pad weights, organ weights and resulted in disturbed lipid profile.

Present study demonstrates that simultaneous administration of *Foeniculum vulgare* volatile oil and high fat diet to the albino rats restricts the increase in body weight, fat pad weights and disturbance of TC, HDL, LDL and triglycerides. The effect of volatile components of the drug is in complete agreement with previously conducted studies in obesity and cardiovascular disorders. [14,15]

Various studies suggest that flavonoids and phenolic compounds are the class of phytoconstituents which are responsible for several therapeutic activities through various mechanisms including strong antioxidant potential.[18] Since, obesity also shows the decrease in antioxidant defense by lowering the levels of antioxidant enzymes i.e catalase, glutathione peroxidase and glutathione reductase [19], the epidemiological evidence suggests that dietary antioxidants play a vital role in the prevention and control of obesity by improving the natural antioxidant defense as well as by reducing the fat deposition in the adipose tissue and by exhibiting an inhibitory effect on lipase activity. [20-22] Additionally, they have antihyperlipidemic action by regulation of blood lipids. [23-25] Thus, it is hypothesized that the observed activity may be due to one or more of these mechanisms because chloroform and methanolic fractions of *Foeniculum vulgare* possessed significant amount of phenolic and flavonoidal contents. Moreover, the extent of activity was also observed better in methanol fraction which contains maximum amount of phenolic and flavonoidal contents.

Thus, the present study indicates the potential role of different phyto constituents for antiobesity and lipid lowering activity. Further studies are required to decipher the molecular mechanism of each constituent for its action.

Conclusion:

In conclusion, *Foeniculum vulgare* demonstrates promising action against obesity and disturbed cardiovascular disorders. Presentation of antiobesity potential of this drug through this study provides the scientific rationale for the folklore use of *Foeniculum vulgare* in Ayurveda for weight management and may provide greater contribution in the prevention and management therapies of obesity.

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Conflict of Interests:

Nil

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