PHYTOCHEMICAL INVESTIGATION OF ETHANOLIC SEED EXTRACT OF *ANETHUM GRAVEOLENS* AND ITS EFFECT ON GLUCOSE TOLERANCE IN RABBITS

HINA AKRAM MUDASSIR[1], MUHAMMAD BILAL AZMI[2] AND SHAMIM A.QURESHI[3]

[1]Department of Biochemistry, Federal Urdu University of Arts, Science and Technology, Karachi
[2]Quality Enhancement Cell, Dow University of Health Sciences, Karachi, Pakistan
[3]Department of Biochemistry, University of Karachi, Karachi, Pakistan

Abstract

The objective of the present study was to investigate the glucose tolerance and hypoglycemic activity along with the phytochemical constituents of ethanolic seed extract (ESEt) of *Anethum graveolens* in different groups of male rabbits (4/group). Orally administrated ESEt (10-500 mg/kg) was found non sedative and showed hypoglycemic activity by reducing the mean blood glucose level from (114 to 99 mg/dL) after 30 min. of administration while the doses (1000-3000 mg/kg) of the same extract showed itching along with hypoglycemia. In oral glucose tolerance test, ESEt at the doses of (50,100 and 150 mg/kg) showed significant hypoglycemic activity only at 30 min by decreasing blood glucose level from 175 to 49 mg/dL as compared to their control group. The phytochemical analysis showed the presence of alkaloids, carbohydrates, gallotannins, glycosides, saponins and steroids in the ESEt of *A.graveolens.* The results indicated that lower doses of ESEt of *A.graveolens* (10-500 mg/kg) were hypoglycemic but at higher doses (1000-3000 mg/kg), it produced itching along with hypoglycemia.

Introduction

Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia. In type-I diabetes, β-cells of pancreas lost insulin producing ability (insulin deficiency) where as insulin resistance or relatively reduced insulin secretion is the universal characteristic of type-II diabetes (Wadkar *et al*., 2008). The overall prevalence of diabetes worldwide was found 2.8% in the year 2000 and it is expected to increase to 4.4% by the year 2030 (Wild *et al.*, 2004). Pakistan is also at the high risk and facing the similar conditions (Hakeem and Fawwad, 2010).

Anethum graveolens (common name dill or soya) belongs to *family Umbelliferae.* This plant has been used for both its culinary and medicinal properties. Recent reports reveal that leaves of *A. graveolens* possesses anticancer, anti-diabetic, antioxidant, antisecretory, antispasmodic, cardioprotective, insecticidal and diuretic activities (Kaur and Arora, 2010; Sushruta *et al*., 2006) along with antihyperlipidemic and antihypercholesterolemic effects (Yazdanparast and Alavi, 2001). Where as its seed extract is aromatic, carminative, mildly diuretic, stimulant and stomachic (Hornok, 1992; Sharma, 2004) and have significant mucosal protective, anti-secretory and anti-ulcer activities (Hosseinzadeh *et al*., 2002).

The present study was designed for phytochemical screening of ethanolic seed extract of *A. graveolens* and to investigate its acute toxicity and effect on glucose tolerance in rabbits.

Materials and Methods

Experimental animal: Male rabbits (1-1.5kg) were purchased from the breading house of Dow University of Health Sciences (DUHS), Karachi and kept under usual management conditions in conventional animal house of Department of Biochemistry, University of Karachi. They were given standard laboratory diet with free excess to water *ad libitum*.

Plant material: Seeds of *Anethum graveolens* were purchased from local market and identified by expert of department of Botany, University of Karachi. The voucher specimen has been kept in our department (KU/BCH/SAQ/04).

Positive control: Glibenclamide (Daonil) purchased from Sanofi-Aventis Pakistan Ltd. and used as positive control in a dose of 5 mg/kg body weight.

Dimethyl sulphoxide (DMSO): DMSO of analytical reagent grade was purchased from Fisher (UK) and its 0.05% concentration in distilled water was used as vehicle for administering the dose of ethanolic seed extract of *A. graveolens* in experimental rabbits.

Preparation of Ethanolic Seed Extract (ESEt): 40 grams of seed powder of *A. graveolens* was soaked in ethanol (1L; 95%) for overnight at room temperature. Then it was filtered through Whatmann no 42 (125mm) filter paper and concentrated at 40°C till dryness in a rotary vacuum evaporator. Finally obtained brown residue termed as ethanolic seed extract (ESEt) (Qureshi *et al.,* 2009).

Qualitative Phytochemical screening: The ethanolic seed extract was screened for the qualitative determination of major constituents including alkaloids, carbohydrates, gallotannins, saponins, steroids and glycosides. For the determination of anthraquinones, ethanolic extract was shaken with 10 ml of benzene and filtered then 0.5 ml 10% ammonia solution was added to the filtrate and shaken. The appearance of violet color between layers indicated the presence of anthraquinones. Alkaloid detection was carried out by taking 2mg of ethanolic extract in a test tube then add few drops of Hager's reagent, formation of yellow precipitate (ppt) confirmed the presence of alkaloids. More confirmation of alkaloids was done by taking 2mg of ethanolic extract and acidified with 1.5% v/v HCl with the addition of few drops of Wagner's reagent, a yellow or brown ppt indicated the presence of alkaloids. For carbohydrates and glycosides, Molisch's test was done in which 2mg of ethanolic extract was shaken with 10 ml of water, filtered and filtrate was concentrated in which 2 drops of freshly prepared 20% alcoholic solution of α-naphthol and 2ml of sulphuric acid (concentrated) was added results in formation of red-violet ring or layer below the mixture indicates the presence of carbohydrates. Presence of gallotannins was confirmed by appearing green color in a test tube after adding few drops of 5% w/v $FeCl_3$ solution to 2ml of ethanolic extract and presence of phlobatannins was confirmed by the appearance of red ppt after boiling ethanolic extract with 1% aquous HCl. Saponins was determined by shaking 5ml ethanolic extract with a drop of sodium bicarbonate solution vigorously then left for 3 min, leads to the formation of honeycomb like froth. Steroids were identified by Salkowski reaction in which 2 mg of ethanolic extract was shaken with chloroform then sulphuric acid was added slowly along the sides of test tube results in the formation of red color. Libermann-Burchard's test was done in which 2mg of ethanolic extract was dissolved in acetic anhydrate, boiled and cooled then add 1ml of concentrated suphuric acid along the sides of the test tube, formation of pink color indicated the presence of triterpenoids (Harborne, 1973; Kodongala *et al.,* 2010).

Acute Toxicity: Overnight fasting rabbits were randomly divided into different groups (4/group). A single dose of each of 10, 50, 100, 500, 1000, 2000, and 3000 mg/kg of ESEt was individually administered orally to the rabbits of their respective test groups, where as rabbits in control group were treated with distilled water (1 ml/kg) orally. The rabbits in both control and test groups were than allowed free access to food and water, and their activity was observed over a period of 12 h for acute toxicity in terms of behavior (sedative or not), mortality rate and any other side effect such as itching. In addition, hypoglycemic activity was determined after 30 min of each dose of extract in its respective group with the help of glucometer (Optimum Xceed, Diabetes Monitoring System by Abbot). Percent blood glucose reduction in each test group with respect to control was calculated by using the following formula

$$\% \text{ Glucose reduction} = [(G_x - G_o) / G_o] \times 100$$

Where G_o = mean blood glucose level of control group at 30 min, G_x = mean blood glucose levels of each test group treated with different doses of ESEt of *A.graveolens* at same time interval.

Effect of ESEt of *A.graveolens* on Glucose Tolerance in Rabbits: Overnight fasting experimental rabbits were divided into different groups (4/group) according to the treatments; as follows,

1. Group I: Control group: treated with distilled water @1 ml/kg
2. Group II: Positive control: treated with glibenclamide @ 5 mg/kg
3. Group III: Test group: treated with ESEt @ 50 mg/kg in 1 ml 0.05% DMSO
4. Group IV: Test group: treated with ESEt @ 100 mg/kg in 1 ml 0.05% DMSO
5. Group V: Test group: treated with ESEt @ 150 mg/kg in 1 ml 0.05% DMSO

Each group after receiving its respective treatment orally was immediately administrated with glucose load @ 2 gm/kg body weight from same route. Blood glucose was monitored at 0, 30, 60, and 120 minutes with the help of glucometer in each of the group by pricking the ear vein of rabbits.

Statistical analysis: The results of present study are expressed as mean ± SD and analyzed by student's *t*-test (Graphpad Software, Quick Calcs Online calculator for Scientist). The differences were considered significant atleast at $p < 0.05$.

Results

I. Qualitative phytochemistry of ESEt of *A. Graveolens*: Qualitative phytochemical analysis showed the presence of alkaloids, carbohydrates, gallotannins, glycosides, saponins and steroids in the ESEt of *A. graveolens* (Table 1).

Table 1. Phytochemical analysis of ESEt of *A. graveolens*

Constitutents	*ESEt of A. graveolens*
Alkaloids	+
Anthraquinone	-
Carbohydrates	+
Gallotanin	+
Glycosides	+
Phalobotanin	-
Resins	+
Saponin	+
Steroids	+
Triterpinoids	-

II. Acute toxicity of ESEt of *A. graveolens*: The mean blood glucose level of control rabbits was found as 150 mg/dL after 30 min of oral administration of distilled water (1mL/ kg) and no sedation and mortality was found in the this group. The test groups treated with ESEt in doses of 10, 50, 100, 500, 1000, 2000 and 3000 mg/kg showed percent reduction in blood glucose with respect to control as 24, 21, 23, 34, 18, 43, 22, respectively with no sedation and mortality ($p<0.05$). However, at doses of 1000, 2000 and 3000 mg/kg of same extract itching was found in experimental rabbits of their respective groups (Table 2).

Table 2. Acute toxicity of ESEt of *A. graveolens* in Rabbits

Groups and treatments	Glucose level (mg/dL) at 30 min	% Reduction in glucose level with respect to control	Toxicity		
			Sedation	Itching	Mortality
Control (1ml distilled water)	150 ± 27.57	-	-	-	-
ESEt (10 mg/kg)	114 ± 13.11	24	-	-	-
ESEt (50 mg/kg)	118 ± 13.86	21	-	-	-
ESEt (100 mg/kg)	116 ± 14.14	23	-	-	-
ESEt (500 mg/kg)	99 ± 4.35*	34	-	-	-
ESEt (1000 mg/kg)	123 ± 22.36	18	-	+	-
ESEt (2000 mg/kg)	86 ± 28.61*	43	-	+	-
ESEt (3000 mg/kg)	117.5 ± 27.57	22	-	+	-

Values are expressed as mean ± S.D (n=4).
*$p<0.05$ represent significant differences when compared to control group.

III. Effect of ESEt of *A. graveolons* on oral glucose tolerance test in rabbits: The mean blood glucose level of control rabbits treated with distilled water (1 ml/kg) along with glucose load (2g/dL) were found as 150 ± 26.33 at 0 min, 252.3 ± 25.8 at 30 min, 185.6 ± 17.62 at 60 min and 92 ± 23.30 mg/dL at 120 min. Where as test rabbits treated with doses of ESEt of *A.graveolens* (50, 100 and 150 mg/kg) with same glucose load showed hypoglycemic activity at 30 min as compared to control. Positive control group treated with glibenclamide (5 mg/kg) showed blood glucose level from 81 to 111 mg/dL at 0, 30, 60 and 120 min (Table 3).

Table 3. Effect of ESEt of *A. graveolens* on Oral Glucose Tolerance of Rabbits

Groups	Treatments	Glucose levels (mg/dL) at different time intervals			
		0 min	30 min	60 min	120 min
Control	Distilled water (1mL/kg) + glucose load (2g/kg)	150 ± 26.3	252.3 ± 25.8	185.6 ± 17.62	92 ± 23.3
Positive control	Glibenclamide (5mg/kg) + glucose load (2g/kg)	96 ± 16.7	110.6 ± 6.66	85.3 ± 18.04	81 ± 10.44
Test	ESEt (50 mg/kg) + glucose load (2g/kg)	82 ± 34.7	149.3 ± 38.48*****	201.3 ± 79.51	152 ± 55.49
	ESEt (100 mg/kg) + glucose load (2g/kg)	81.3 ± 23.35	169 ± 9.17******	200 ± 23	126.67 ± 13.43
	ESEt (150 mg/kg) + glucose load (2g/kg)	127 ± 69	175.5 ± 13.44*****	220.5 ± 9.1	250.5 ± 41.7

Values are expressed as mean ± S.D (n=4). *$p<0.05$ and **$p<0.01$ represent significant differences when compared with control at their respective time.

Discussion

Herbal medicines have been the main source of primary health care in many nations and about 80% of the world's populations are still dependent on traditional medicines (Grover *et al*., 2002). Diabetes mellitus (DM), is the third killer of the mankind health along with cancer, cardiovascular and cerebrovascular diseases. It is one of the most challenging disease facing health care professionals today (Qi *et al*., 2010). Many natural products especially plants-derived medicines including *Zingiber officinale* (ginger), *Cyamospsis tetragonolobus* (Gowar plant) and *Grewia asiatica* (phalsa) are reported to produce hypoglycaemia by modifying glucose utilization and have been recommended for the treatment of this disease (Wadkar *et al*., 2008).

Previously *A. graveolens* also showed hypoglycemic activity, which support their traditional use for the treatment of various ailments including colic pain (Pulliah, 2002), intestinal spasms (Duke, 2001) along with hyperglycemia (Panda, 2008).

The acute toxicity revealed that doses of ESEt of *A. graveolens* at 10, 50, 100 and 500 mg/kg were safe and hypoglycaemic by reducing the mean blood glucose level from 24 to 34% after 30 min of administration as compared to control group while doses of same extract from 1000 to 3000 mg/kg induced hypoglycaemia along with severe itching (toxicity).

The prediabetic and hypergycemic condition are evaluated by oral glucose tolerance test (OGTT) (Islam *et al;* 2009). In the present study doses of ESEt of *A. graveolens* at 50, 100 and 150 mg/kg were found effective in reducing blood glucose level after 30 min of oral administration along with glucose load (2g/kg) as compared to control group. Where as at 60 and 120 min ESEt of *A. graveolens* produced hyperglycemia in experimental animals as compared to their respective controls. This hyperglycaemic finding of ethanolic seed extract was completely opposite to the hypoglycemic observation induced by ethanolic leaf extract of same plant (Panda, 2008). The decrease in blood glucose level at 30 min might be due to the extra-pancreatic action of extract through which it delays the glucose absorption in intestine (Qureshi *et al*., 2009). The phytoconstituents including alkaloids, gallotannins, saponins, steroids, resins and glycosides detected in ESEt of *A. graveolens* could also be responsible for their hypoglycemic activity as they have antioxidant activity and could counteract with free radicals (Sushruta and Dong, 2011). It is concluded that ethanolic extract (ESEt) of *A. graveolens* causes hyperglycemia at low doses where as at higher doses it produces toxic effects (itching) along with hypoglycemia. Whereas the oral glucose tolerance test in rabbits showed significant decrease in blood glucose level after 30 minutes as compared to control at the same time interval. Our results indicate that ethanolic seed extract of *A. greaveolens* is not a potent hypoglycemic agent.

References

Duke, J.A. (2001). Handbooke of Medicinal Herbs. London: CRC Press. p.42.

Grover, J.K., Yadav, S., and Vats, V. (2002). Medicinal Plants of India with anti-diabetic potential. *J. Ethnopharm* 81: 81-100.

Hakeem, R. and Fawwad, A. (2010). Diabetes in Pakistan: epidemiology, determinants and prevention. *J. Diabetology* 3:4.

Harborne, J.B. (1973). Phytochemical methods. A guide to modern techniques of plant analysis. Chapman and Hall, London. pp.49-188.

Hornok, L. (1992). Cultivation and processing of medicinal plants: Academic publications. p. 338.

Hosseinzadeh, H., Karimi, G.R. and Ameri, M. (2002). Effects of *Anethum graveolens* L. seed extracts on experimental gastric irritation models in mice. *BMC Pharmacol.* 2: 21-5.

Ishikawa, T., Kudo, M. and Kitajima, J. (2002). Water-soluble constituents of Dill. *Chem Pharm Bull.* 50: 501-507.

Islam, M.A., Akhtar, M.A., Khan, M.R., Hossain, M.S., Alam, A.H., Ibne-Wahed, M.I., Amran, M.S., Rahman, B.M. and Ahmed, M. (2009). Oral glucose tolerance test (OGTT) in normal control and glucose induced hyperglycemic rats with *Coccinia cordifolia* I. and *Catharanthus roseus* L. *Pharm. Sci.* 22(4): 402-404.

Kaur, G.J and Arora, D.S. (2010). Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family Umbelliferae - Current status. *J. Med. Plant Res.* 4: 87-94.

Kondongala, S.C., Hedge, V. and Prasanna, K.S. (2010). Phytochemical studies of stem bark of *Michelia champaca* Linn. *I.R.J.P.* 1(1): 243-46.

Panda, S. (2008). The effect of *Anethum graveolens* L. (dill) on corticosteroid induced diabetes mellitus: involvement of thyroid hormones. *Phytother Res.* 22(12): 1695-7.

Pulliah, T. (2002). Medicinal Plants in India. Vol. 1. New Delhi: Regency Publications New Delhi. p. 55-6.

Qi, L.W., Liu, E.H., Chu, C., Peng, Y.B., Cai, H.X. and Li, P. (2010). Anti-diabetic agents from natural products--an update from 2004 to 2009. Curr Top *Med. Chem*. 10(4): 434-57.

Qureshi, S.A., Nawaz, A., Udani, S.K. and Azmi, M.B. (2009). Hypoglycemic and hypolipidemic activities of *Rauwolfia serpentina* in alloxan-induced diabetic rats. *Int. J. Pharmacol.* 5(5): 323-326.

Sharma, R. (2004). Agrotechniques of medicinal plants. New Delhi: Daya Publishing House. p. 3-8.

Sushruta, K. and Dong, K. (2011). *Anethum Graveolens* Linn (Umbelliferae) Extract Attenuates Stress-induced Urinary Biochemical Changes and Improves Cognition in copolamine induced Amnesic Rats. *Trop. J. Pharm. Res.* 10:(1): 47.

Sushruta, K., Satyanarayana, S., Srinivas, N. and Raja, S.J. (2006). Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected Umbelliferous fruits used in culinary practices. *Trop. J. Pharm Res.* 5: 613- 617.

Wadkar, K.A., Magdum, C.S., Patil, S.S. and Naikwade, N.S. (2008). Antidibetic potential and Indian medicinal plants. *Journal of Herbal Medicine and Toxicology* 2(1): 45-50.

Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27: 1047-1053.

Yazdanparast, R. and Alavi, M. (2001). Antihyperlipidaemic and antihypercholesterolaemic effects of *Anethum graveolens* leaves after the removal of furocoumarins. *Cytobios* 105:185-91.