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ORIGINAL RESEARCH

Haematological and hypoglycemic potential *Anethum graveolens* seeds extract in normal and diabetic Swiss albino mice

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Abstract

Aim: The present study investigates the hypoglycemic and hematopoetic potential of the seed extracts of *Anethum graveolens*.

Context: *A. graveolens* (Apiaceae) is an Indian traditional herb. Leaves and seeds of this plant are known for their medicinal properties. Leaves are best used in fresh form while seeds are used in dried form.

Materials and Methods: Control and alloxan-induced diabetic mice were orally treated once in a day with aqueous and ethanol extracts for the period of 15 days and the effect of both extracts on body weight, organ weight and blood glucose level were determined. For the hematological study, aqueous (3.04 g/kg), ethanol extracts (6.98 g/kg) and carvone (100 mg/kg body weight) were orally administered once in a day to male Swiss albino mice. While control mice were supplemented with normal saline.

Results: A significant decrease ($P<0.05$) in blood glucose level after administration of aqueous extract was observed along with a significant increase in body and organ weight in alloxan induced diabetic mice after the treatment with plant extracts. Moreover carvone and the aqueous extract significantly increased ($P<0.05$) red blood cell count (11.94 and 10.42%), hemoglobin (15.55 and 15.06%), Mean Corpuscular Haemogolobin (MCH) (2.80 and 4.80%), Mean Corpuscular Haemoglobin Content (MCHC) (8.16 and 9.57%) when compared with control. While in ethanol extract no such changes were observed except in WBC, which was increased by 9.43%.

Conclusion: The result suggests an anti-diabetic property of the aqueous and ethanol extracts. Both extract and carvone also have beneficial effect on hematological parameters.

Keywords: aqueous extract, blood glucose level, ethanol extract

Introduction

Anethum graveolens L. (Apiaceae), commonly known as "dill seed", is an annual herb found in Mediterranean region, Europe, and Central and South Asia. *A. graveolens* is used both as a medicine and an aromatic herb and is shown to have therapeutic properties [1]. It is used in Iranian folk medicine as an anti-hypercholesterolaemic plant [2]. It has traditionally been used for gastrointestinal ailments, such as flatulence, indigestion, stomachache colic, and to tract intestinal gas [3].Various different compounds have been isolated from the seeds, leaves, and the inflorescence of this traditional herb; For example; seventeen different volatile compounds have been isolated from this herb [4]. Carvone and limonene are monoterpenes, which are present as the main constituent of dill oil from fruits [5]. á-Phellandrene, dill ether and myristicin are the compounds, which form the important odor of dill herb [6,7]. The presence of flavonoids, phenolic compounds and essential oil constituents in seeds of *A. graveolens* make it an important component for the preparation of gripe water [8] *A. graveolens* has some pharmacological effects, such as anti-microbial [9-11], anti-spasmodic, anti-secretary, and mucosal protective effects [3], *A. graveolens* has significant lipid lowering effects and is a promising cardioprotective agent [12] and antioxidant properties have also been reported [13].

Ingestion of some plant material (either in the raw form or their extracts) has been reported to cause anaemia, which may result from confiscation of RBC in the spleen, impaired red blood cell production or primary bone marrow dysfunction [14,15]. Diabetes mellitus is a chronic disorder of carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorders that have hyperglycemia as a common feature [16]. It affects over 100 million people worldwide [17]. Its conventional treatment is oral hypoglycemic agent/insulin therapy [18]. However, several herbs are now being used in the management of diabetes mellitus, although the active principles have been isolated [19-21]. In this study, we sought to verify the effect of the administration of aqueous and ethanol extracts of *Anethum graveolens* seeds on blood glucose level and few haemoglobin parameters, which could serve as indices of anaemia and bone marrow function.

Materials and Methods

Plant material and preparation of extracts: Author collected seeds from Jawaharlal Nehru Krishi Vigyan

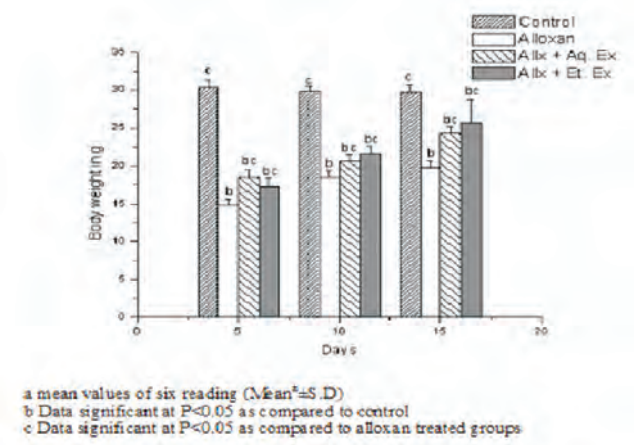

Figure-1. Effect of Aq.Ex and Et.Ex of A graveolens L. on body weight expressed as mean ± SD.

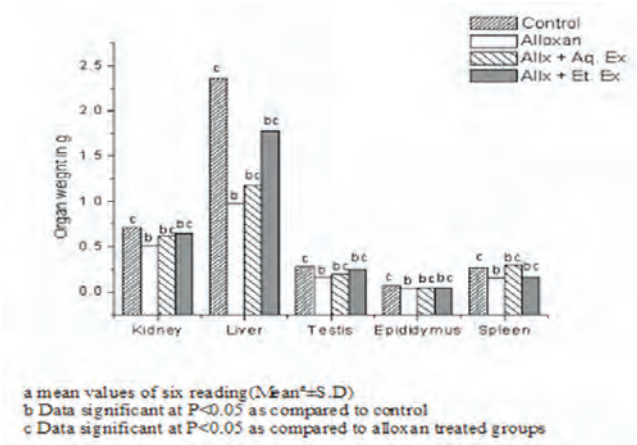

Figure-2. Effect of Aq.Ex and Et.Ex of *A graveolens* L. on organ weight expressed as mean ± SD.

Kendra Jabalpur, M.P., India. Rajasthan University Herbarium incharge Roop Singh properly identified the plant with voucher specimen in the herbarium of Department of Botany, Rajasthan University, Jaipur. All the samples were air dried in shade and then powdered. The seed powder was extracted using maceration with ethanol (80 v/v) and water for 3 days and, subsequently, the mixture was filtered (Wattman filter paper 1) and concentrated under reduced pressure by rotaevaporator at 40 °C. Aquous extract (Aq. Ex.) was autoclaved and then both the extracts ethanol (Et. Ex) as well as aqueous extract were stored at 4 °C and further used for experimentation.

Experimental animals : A total 20 adult male Swiss albino mice (age 12-14 weeks) weighing 30 g were used in this study. The mice were housed in university animal house facility with controlled temperature (22 ± 2°C) under a 12/12 h light/dark schedule. Mice were maintained under hygienic conditions in well-ventilated room of animal house. All the animals were fed twice a day with animal pellet feed (Hindustan Lever Limited, Mumbai). Tap water was provided *ad libitum*. Animals in each group were housed in polypropylene cages. General body weight of the animals was monitored regularly during entire tenure of the experiment.

Ethical consideration: Animals were maintained according to the Guidelines of Institutional Animal Ethics Committee.

Induction of diabetes: Diabetes was induced by a single intravenously injection of alloxan monohydrate prepared freshly in normal saline (70 mg/kg body wt.) [22]. Mice were monitored for blood glucose level after 48 h. The mice with blood glucose value of <250 mg/dl were considered diabetic. Blood sample were collected from tail vein and estimation was done by using glucometer (Dr. Morepen Gluco Monitor).

Experimental design and treatment schedule: Twenty mice selected for study were divided into 5 groups of 4 animals each. Groups were as Group 1: Untreated animals (control), Group 2: Alloxan Treated (70 mg/kg of B.W), Group 3: Diabetic Mice given with Aq.Ex at 3.04 g/kg for 15 days, Group 4: Diabetic Mice given with Et. Ex at 6.98 g/kg for 15 days, and Group 5: Diabetic mice given with Carvone 100 mg/kg for 15 days.

Fasting and PP blood glucose level, body weight were recorded periodically. The blood samples were collected from tail vein for measuring blood glucose level and for hematological parameters. Blood sample was collected from retro-orbital plexus using micro-capillary technique in EDTA, which is used as anti-coagulant [23].

Determination of hematological parameters: RBC and WBC counting was done with the help of neubaurs chamber. Packed cell volume (PCV) with Wintrobe hematocrit tubes, Haemoglobin by Sahli's method [24] and certain hematological indices (MCV, MCH and MCHC) were calculated with formula;

MCV (Mean Corpuscular Volume)

MCV in cubic microns=PCV X 10/RBC (in million per cubic mm)

MCH (Mean Corpuscular Haemogolobin)

MCH in Picograms=Haemoglobin in g/100 ml x 10/RBC (in million per cubic mm)

MCHC (Mean Corpuscular Haemoglobin Content)

MCHC in (g/dl)=Haemoglobin in g/100 x 100/ PCV

Characterization of phytoconstituents: For characterization of compounds HPLC, IR and NMR were performed. HPLC of the isolated compound was carried out to confirm its nature. Infrared (IR) spectroscopy is mainly used in biomedical research for intermediate sized molecules such as drugs, metabolic intermediates and substrates. NMR spectra are of great value in elucidating chemical structures

Statistical analysis: Results are expressed as mean ± S.D. Statistical significance between the different groups was determined by one way Analysis of Variance (ANOVA) using the SPSS (Ver. 16). Post hoc testing was performed for inter-group comparisons using the Tukey multiple comparison test at $P<0.05$. Whenever sphericity was significant, degree of freedom and F-value are corrected by Huynh Feldt epsilon.

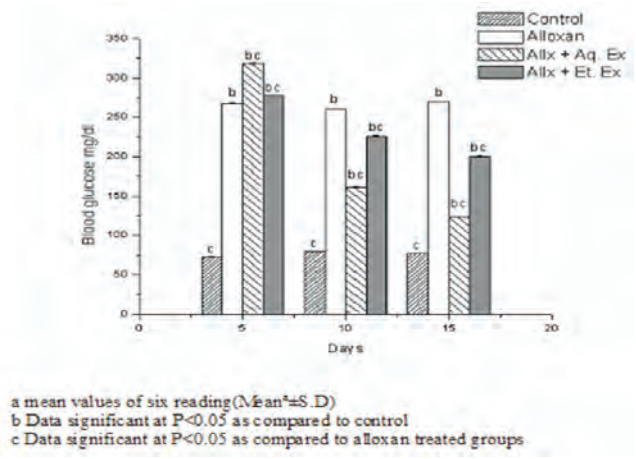

Figure-3. Effect of Aq.Ex and Et.Ex of *A graveolens* L. on blood glucose level (fasting) expressed as mean ±SD.

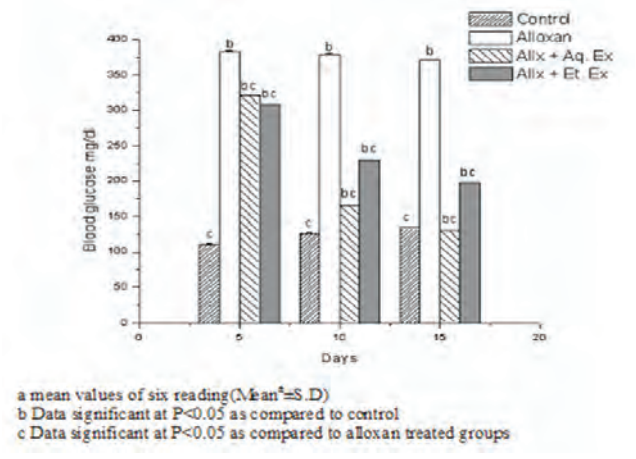

Figure-4. Effect of Aq.Ex and Et.Ex of *A graveolens* L. on Blood glucose level (PP) expressed as mean ±SD.

Table-1. Effect of Aq.Ex and Et.Ex of *Anethum graveolens* L. on hematological parameter expressed as mean ±SD.

Hematological Parameters	Control	Allx + Aq.Ex	Allx + Et.Ex	Carvone
WBC (x 10^3/ ì l)	20.13 ± 0.12^{cd}	23. 2 ± 0.14 ^{bd}	22.03 ± 0.12^{bc}	23.48 ± 0.41^{bd}
RBC (x 10^6/ ì l)	9.21 ± 0.02^{cd}	10.17 ± 0.48^{bd}	8.96 ± 0.06^{bc}	10.31 ± 0.36^{bd}
HGB (g/dl)	12.41 ± 0.14^{cd}	14.28 ± 0.19 ^{bd}	13.16 ± 0.10^{bc}	14.34 ± 0.36^{bd}
HCT (%)	42.73 ± 0.67^{cd}	46.67 ± 0.25^{bd}	41.02 ± 0.24^{bc}	46.67 ± 0.33^{bd}
MCV (FL)	46.32 ± 0.14^{cd}	45.58 ± 0.19^{b}	45.48 ± 0.35^{b}	45.29 ± 1.72^{b}
MCH (pg)	13.53 ± 0.12^{cd}	14.18 ± 0.14^{b}	14.52 ± 0.33^{b}	13.91 ± 0.55^{b}
MCHC (g/dl)	28.41 ± 1.92^{cd}	31.13 ± 0.08^{b}	32.15 ± 0.30^{b}	30.73 ± 0.80^{b}
PLT (ì l)	1281.33 ± 31.42^{cd}	857.33 ± 10.20^{bd}	808.33 ± 14.43^{bc}	1267.5 ± 9.35^{bd}

a - mean value of six readings (Mean ± SD)
b - Data significant at P<0.05 as compared to control
c - Data siginificant at P<0.05 as compared to aqueous extract treated groups
d - Data significant at P<0.05 as compared to ethanolic extract treated groups

Results

Effect of extract on body and organ weight: Administration of vehicle (distilled water) in alloxan induced diabetic mice resulted in gradual decrease in body weight during the period of 15 days (Figures 1A and 1B). Aq.Ex and Et. Ex of Anethum did not cause any decrease in body weight and doses were helpful for alloxan induced diabetic mice for regaining their body weight. Organ weight was also significantly recovered (*P<0.05*) due to treatment (Figure-2).

Effect of extract on blood glucose level: Figures 3 and 4 are showing level of blood glucose. There was a significant increase in the blood glucose level was observed in alloxan induced diabetic mice after 48 h of alloxan monohydrate intraperitoneal injection. Experimental studies revealed that the aqueous extract of *Anethum* seeds shows a significant decrease in blood glucose level in alloxan induced diabetic mice from 5th day onwards.

Effect of extract on hematological parameters: The effect of aqueous, ethanol extract of *A. graveolens* and of carvone on hematological parameters of control and experimental mice is shown in Table-1. Results indicates, that the administration of plant aqueous extract and carvone produced significant increase (*P<0.05*) in the RBC and factors relating to it (HGB, HCT, MCH and MCHC). Though it significantly decreased (*P<0.05*) platelets and MCV number. Whereas ethanol extract showed no significant change in RBCs and its relating indices. Only HGB, WBC was slightly increased although number of platelets decreased.

Phytochemical analysis: HPLC chromatogram showed prominent peaks at retention time 17.188 and 46.71% area (Figure-5). NMR results shows 6.9 ppm due to aromatic protons formed as multiplet, 4.8 ppm doublet due to olefinic proton, 2.7 ppm due to the saturated ring protons A- broad spectrum, 1.76 ppm two singlet due to two methyl protons. An IR spectrum exhibits a peak at 2924.5 cm^{-1} for CH group. 1673 cm^{-1} exhibits a peak for C=O group, 1440-1371 cm^{-1} for CH bond, (1250-1230 cm^{-1}) 1247.8 cm^{-1} due to C-O-C stretching, 1110-1057 cm^{-1} for C-O bond, 897 cm^{-1} for C=C group.

Discussion

Herbal medicine basic tenets are the interactions between different constituents occur in such a fashion that their activity is enhanced and the likelihood of adverse effects is reduced. Such interaction is generally additive or truly synergistic because the compounds present in the plant interact and produce an effect greater than the contribution to each of them singly. For inducing diabetes alloxan was used which is â - cytotoxic to pancreatic beta cells. Induction of diabetes decreases the weight of mice.

Dose of alloxan varies with the species for induction of diabetes. From the results summarized in Figure 1 and 2, it is clear that the extracts of the plant

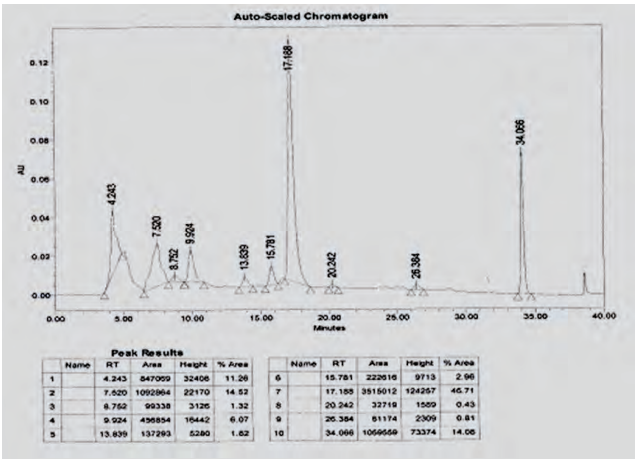

Figure-5. HPLC OF Aq.Ex of *A. graveolens*.

taken for the study have no affect on the body weight of the control mice but it has significant effect on the alloxan induced diabetic mice, however the weight of testis and epididymis inclined significantly in treated animals. Moreover, after the treatment, the weight of kidney, liver and spleen, increased in the case of *A. graveolens*.

According to Adebayo et al [25], an increase in the body weight and organs is an indication of inflammation and decrease is an indication of cell constriction. According to this statement, the increase in the weight of testis, epididymis and other organ observed in present study might be due to the phenomena of inflammation.

Alloxan causes diabetes through its ability to destroy the insulin producing â cells of the pancreas [26, 27]. It leads to cell necrosis and also cause time and concentration dependent degenerative lesions of the pancreatic â cells which results in the increased blood glucose level [28, 29].

Administration of aqueous extract of *A. graveolens* orally for the period of 15 days produced significant ($P<0.05$) decrease in blood glucose level of alloxan induced diabetic male mice, as it was earlier reported. While the effect of ethanolic extract exhibits slight lesser antidiabetic activity [30-32].

For Haematological parameters, it was also found that aqueous extract has positive effect on the haemopoeitic system of mus musculus. It was showing significant increase in RBC, HCT. The rise in production of RBCs (erythropoiesis) shows that aqueous extract of anethum and carvone have the probablility of provoking erythropoietic release from kidney, which act as a humoral regulator for RBC production [33, 34], as earlier reported in case of *Mangifera indica L*. [35]. The percentage increase in HCT was from 42.73% for control to 46.57% for group 3 and 46.67% in 5th group mice. The raised haematocrit is an indication of increased haemoconcentration. Administration of aqueous extract and carvone also significantly increased the haemoglobin level which shows that it will help in increasing the oxygen carrying capacity of the blood. RBC and HGB play a major role in transport of respiratory gases [36]. Aqueous extract and carvone also significantly increased WBC count as compared to control, which indicates that plant aqueous extract and carvone, is working as a booster dose for immune system. It was found that MCV was not showing any significant change while MCH and MCHC were increased from 13.53 to 14.18 pg and 28.41 to 31.13 g/dL, respectively. But there was a decrease in platelets count, which might be results from the inhibitory effect on thrombopoietin [37,38]. MCV, MCH and MCHC values have meticulous significance in anaemia diagnosis in most animals [39]. Increased count of RBC and its associated parameters suggest that stage as polycythemia [40]. Therefore it can be concluded that aqueous extract of Anethum may not have any unfavorable effect on the bone marrow, kidney and haemoglobin metabolism, since the value for RBC are not greatly affected [41]. While in the case of ethanol extract no change was observed on RBCs and its related indices except HGB, which 12.14 g/dL was for control and 13.51 g/dL was seen in case of ethanol extract treated mice. Ethanol extract showed slight increase in WBC count whereas no significant effect was found on RBC and its indices. Similarly platelets count was also significantly decreased. This implies that the ethanol extract does not posses any potential of inducing anaemia throughout the 15 days period of administration. HPLC, NMR and IR peaks depict the presence of carvone in aqueous extract of seeds. The phytochemical screening of the seed showed the presence of, tannins terpinoids saponins flavonoids, anthraquinones and alkaloids [42, 43].

Conclusion

From the above results, it may be concluded that *Anethum graveolens* aqueous extract is constitute of carvone which is responsible for its potency for curing diabetes and can be used for the treatment of anaemia. While ethanol extract is having lesser anti-diabetic activity and no significant affect on Haematological parameters. Thus, aqueous extract is more potent than ethanol extract.

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Competing interests

Authors declare that they have no competing interest.

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