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Genotypic Variability and Therapeutic Potential of Fennel (Foeniculum Vulgare Mill) Seed Extracts

Dolly Agarwal, Saxena SN*, Mishra BK, Krishna Kant, and John S

ICAR-National Research Centre on Seed Spices, Pacific University, Tabiji, Rajasthan, India

***Corresponding author:** Saxena SN, National Research Centre on Seed Spices, Immunology and Infectious Diseases, Arabian Gulf University, Manama, Kingdom of Bahrain, E-mail: bindayna@agu.edu.bh

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Abstract

Fennel (Foeniculum vulgare Mill.) is an important seed spice known for its culinary and medicinal potential. Genetic variation in therapeutic properties of fennel seeds may be helpful in developing specific genotypes for medicinal point of view. Present study was conducted by taking pure seeds of five fennel genotypes of different geographical origin. Methanol and hexane seed extracts of fennel genotypes Ajmer Fennel-1, Gujarat Fennel-2, Hisar Swarup, Rajendra Saurabh, and Rajasthan Fennel-101 were used on albino mice as source of drug for evaluating its therapeutic properties. Both seed extracts were found to possess anti-inflammatory, anti-diabetic, hypolipidemic and antihypertensive properties, which varied significantly among the five genotypes. Present study indicated that both methanol and hexane seed extract of fennel genotype Rajendra Saurabha and Hisar Swarup possessed more anti-inflammatory properties while methanol extract of genotype Rajendra Saurabha showed slightly more anti diabetic activity. Hexane extract of genotype RF-101 produced significant hypolipidemic properties. This study validated the use of fennel extracts for treating various ailments and fennel as an important spice provided suitable genotypes are identified for a particular therapy.

Keywords: Foeniculum vulgare; Genetic variability; Pharmacological properties; Seed extract; Seed spices.

Introduction

Foeniculum vulgare Mill (Apiaceae family) commonly known as fennel or Saunf grown in India as an annual herb crop. Fennel is native to the Southern Europe and Mediterranean region. It is cultivated in temperate and tropical regions of the world. It is considered an important medicinal plant in Asian countries. Each part of plant *viz.*, the bulb, young shoots, leaves and fully ripened and dried fruits are being used in culinary purposes and as traditional medicine for homemade remedies. Seeds of fennel are used for flavour in food products and an ingredient of cosmetics and pharmaceutical products [1].

Fennel herbal tea is a popular household remedy used for the treatment of gastrointestinal and respiratory tract [2]. The phytochemicals from the fennel include essential oil, fatty acid, phenylpropanoids, terpenoids, sesquiterpenes, coumarins, tannins, flavonoids, saponins, and other types of compounds. In a previous communication by author [3] a detailed analysis was carried out by taking five distinct fennel genotypes originating from different parts of India and was evaluated for intrinsic quality parameters and medicinally important compounds in crude seed extract.

Essential oil yield varied from 1-3% or even more. Various phytochemicals belonging to terpenic hydrocarbons, ether, alcohols, aldehydes, carboxylic acid and diverse functional groups were detected in essential oil. Among ethers, 4-allylanisole, anethol and estragol were the key compounds. Fennel seeds contain a fairly good amount of phenolic and flavonoid compounds which can be efficiently extracted using distilled water. Aqueous extract of fennel seeds contains rich phenolic compounds. Distilled water extract showed a good combination of phenolics, flavonoids and antioxidant activity (71.12 %) [3]. Many of them have antioxidant activities, like 3-caffeoylquinic acid, 4-caffeoylquinic acid, rosmarinic acid, eriodictyol-7-orutinoside, quercetin-3-o-galactoside, kaempferol-3-orutinoside and kaempferol-3-o-glucoside. Besides these compounds, fennel was reported to containing hydroxylcinnamic acid derivatives, flavonoid glycosides and flavonoid aglycones [4]. Some flavonoids like quercetin arabinoside were identified from F. vulgare [5].

Flavonoids such as quercetin, rutin and isoquercitrin were reported to have immunomodulatory activities. In addition, fennel seeds provided an excellent source of potassium, calcium, magnesium, iron, phosphorous and zinc. Seventeen fatty acids were found in flavourer oil including petroselinic acid (62.08%-66.71%), 10-Nonadecanone (4.70-22.80%) and polyunsaturated fatty acid (1.32-7.59%). The volatile oil of the foremost important fennel variety (var. dulce) contains anethol (50-80%), limonene (5%), fenchone (5%), estragol (methyl-chavicol), safrol, α-Pinene (0.5%), α-Phellandrine, camphene, β-Pinene, β-Myrcene and p-cymen. In contrast, the uncultivated form (var. vulgare) contains often more essential oil, but since it is characterized by the bitter fenchone (12% to 22%), it is of little value. Fennel has many biological activities because of its volatile and nonvolatile compounds. Fennel essential oil possessed carminative and stimulant activities as well as spasmolytic actions on the smooth muscles of experimental animals. Furthermore, it possessed analgesic, anti-inflammatory and antioxidant activities.

Oral administration of methanol extract of fennel exhibited inhibitory effect against acute and sub-acute inflammatory diseases and showed a central analgesic effect by inhibition of the allergic reactions. It significantly decreased the high density lipoprotein cholesterol level, thus decreasing the peroxidative damage. The essential oil of fennel exhibited antibacterial and antiviral activities. The aqueous and ethanol extracts of fennel exhibited potential antioxidant properties during *in-vitro* studies. Earlier publications from the author's laboratory established significant genotypic variations in antimicrobial activities and hepatoprotective properties of fennel seed extracts of different genotypes.

However, there are many reports indicating therapeutic potential of fennel plant parts but most of them are undertaken with seeds from the local market with no genetic purity of seeds. Present study was conducted for evaluating genotypic variation in therapeutic potential

of fennel seeds. Methanol and hexane seed extracts of five prominent fennel genotypes were used as source of phytochemical compounds for evaluating its therapeutic properties *viz*., anti-inflammatory, anti-diabetic, hypolipidemic and anti-hypertensive properties.

Materials and Methods

Plant materials

ICAR-National Research Centre on Seed Spices maintaining active germplasm centre for seed spices. Pure seeds of fennel genotypes (Ajmer Fennel-1, Gujarat Fennel-2, Hisar Swarup, Rajendra Saurabh, and Rajasthan Fennel-101) were obtained from seed store of ICAR-NRCSS, Ajmer and used for preparation of methanol and hexane seed extracts for this manifesto. All chemicals and reagents *viz.*, methanol, hexane, captopril were procured from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of seed extracts

Ground fennel seed powder (1 g) for each genotype was extracted with 5 ml of methanol and hexane separately. After filtration the residue powder was again soaked in respective solvent for 24 h. Obtained extracts were mixed together and volume was made to 10ml by adding the required amount of respective solvents. Dry crude seed extract was obtained by evaporating the solvent with rotary flash evaporator (JSGW, Ambala, India) and used as a crude drug for evaluation of medicinal activities.

Acute oral toxicity- acute toxic class method

The acute oral toxicity was carried out as per the guidelines set by Organization for Economic cooperation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The principle is predicated on a stepwise procedure with the utilization of a minimum number of animals per step to get sufficient information on the acute toxicity of the test substance to enable its classification.

Anti-inflammatory properties

Starved wistar (Rattus norvegicus) albino rats (150 to 200 gb.w.) were given 5 ml of water and seed extract uniformly. A subcutaneous injection of 1% carrageenan was given on the right hind paw and marked at the level of the lateral alveolus and immersed in mercury up to this mark. Before immersion paw volume was measured by using plethysmograph after 1-4 hr of carrageenan injection. Rats were divided into thirteen groups (six rats each) according to the treatment administered. Group I rats (only vehicle), group II (carrageenan 0.1ml of 1%mg/kg b.w., p.o.), group III (carrageenan 0.1 ml of 1%mg/kg b.w., p.o.) plus drug diclofenac (12.5 mg/kgb.w., p.o.), group IV-XIII (carrageenan0.1 ml of 1% mg/kgb.w., p.o.) with methanol and hexane extract of fennel seeds (400 mg/kg b.w., p.o.).

Hypolipidemic activity

Effect of fennel seed extracts on lipid profile parameters were evaluated using wistar albino rats divided in 19 groups. A group of rats were given a high cholesterol diet for 15 d while another group was given this diet along with standard medicine Simvastatin (100 mg/kg b.w., p.o.). Rats of group IV-XIX were given a high cholesterol diet along with seed extracts of different fennel genotypes (400 mg/kg b.w., p.o.). Control group rats (Group I) were given only vehicles. Lipid profile parameters viz., total cholesterol, triglycerides; LDL and HDL were observed after 16th d of treatment and compared with control.

Antidiabetic activity

Seed extracts (400 mg/kg,b.w., p.o.) and standard drug gilbenclamide were fed orally to experimental rats 30 minutes prior to loading glucose (2 g/kg). Control group of rats were given only water. Samples were collected from the tail vein after 30, 60, 120 and 240 min of glucose loading and level of glucose in treated as well as fasting was measured with standard Glucometer.

Anti-hypertensive properties

Anti-hypertensive properties of genotype GC-1 was evaluated by taking seed extracts in hexane and methanol on spontaneously hypertensive rats (SHR) and wistar rats (Rattus norvegicus), of 250 to 300g b.w. Prior to dividing the rats in groups, they were kept under controlled environmental conditions with ration and water as and when required for acclimatization. Wistar rats were used as normotensive control, given same volume of water (0.1ml/100mg) and divided into four groups with six rats each. Urethane 20% (0.8g/kg, i.p.) and pentobarbital sodium 1% (40mg/kg, i.p.) was used to anesthetize wistar rats. To facilitate drug and seed extract administration tracheostomy was created by cannulising right vena femoralis followed by bolus injection of heparin (30IU). Mean Artery Pressure (MAP) was observed with pressure transducer (Mod F-60, Narco Biosystems, Inc., Houston, Texas, USA) attached with Narcotrace 40 polygraph (Narco Biosystems, Inc., Texas, USA). The hemodynamic variables were allowed to stabilize for 15min before administration of test substances prepared in saline (0.9%). Acetyl Choline (ACh; 1.0µg/kg, i.v.) was chosen as standard drug. Group I-rats were given only vehicle while group II was given captopril 40 mg/kg, i.p. and group III-IV were given fennel seed extracts (200 mg/kg, i.p.).

Statistical analysis

All analysis were recorded observations was carried out for samples with three replications and results expressed in terms of means values. The data were analyzed in statistical software SAS 9.3. The comparisons between mean differences were done by using analysis of variance [14]. Statistical significance was calculated at p±0.05.

Anti-inflammatory properties

Paw edema volume of treated rats as a measure of inflammation and effect of seed extracts of fennel was presented in Table 1. Control group rats were measured at paw edema volume 3.4 ml during 0 to 4 hrs. Drug induced inflammation ranged from 3.3 to 6.8ml during observation period. Standard drug diclofenac at 12.5 mg/kg was able to reduce inflammation and bring this down at par with control (3.5ml after 4 h). Carrageenan treated rats of group IV to XIII were given either methanol or hexane seed extracts at 400 mg/kg. Results indicated that fennel seed extract irrespective of solvent and genotype showed excellent anti-inflammatory property at par with standard drug diclofenac. Effect of methanol seed extract of fennel genotype Hisar Swarup at 400 mg/kg was at par with diclofenac in reducing the edema volume up to 3.6ml after 4 hr whereas hexane seed extract of

genotype Hisar Swarup, Rajendra Saurbha and RF-101 reduced edema volume up to 3.7ml. Both the extract produced at par results with significant genotypic variation in anti-inflammatory property (Table 1).

Similar to present study [15] evaluated the analgesic and anti-inflammatory action of ethanolic seed extract of Foeniculum vulgare using Wistar rats and Swiss Albino mice by carrageenan- induced hind paw edema. They found significant ($p<0.001$) dose-dependent inhibition of pain response elicited by acetic acid and formalin tests and inhibition of edema development in the carrgeenan induced inflammation. The findings in this study suggest that the ethanolic extract of F. vulgare possess analgesic and anti-inflammatory activities possibly mediated through central and peripheral mechanisms. Choi and Hwang [9] gave oral administration (200 mg/kg) of Foeniculum vulgare fruit methanolic extract exhibited inhibitory effects against acute and sub-acute inflammatory diseases and type IV allergic reactions and showed a central analgesic effect. In a separated study Kooti et al., [16] showed that this plant has various pharmacological properties including antioxidant, anti-cancer activity, anti-inflammatory, antifungal, anti-bacterial and estrogenic effects which are probably due to the presence of aromatic compounds such as anethole, estragole and fenchone. Ozbac [17] reported anti-inflammatory activity of the Foeniculum vulgare Mill. essential oil and investigated its lethal dose in rats and mice with model of carrageenan induced rat paw edema and found an anti-inflammatory effect matching to that of etodolac at 0.050 and 0.200 mL kg⁻¹ doses.

In a published study with similar five fennel varieties by Agarwal et al. [4], seed essential and total oil content and its composition, total phenolic, flavonoid and antioxidant properties of crude seed extract were evaluated. The essential oil yield varied from 1.51 to 2.02%. Twenty one constituents belonging to terpenic hydrocarbons (7.64-12.96%), ether (9.05-88.73%), alcohols (0.0-0.06%), aldehydes (0.0-0.09%), carboxylic acid (3.22-6.32%) and diverse functional group (0.10-0.72%) were detected in essential oil. Among ethers, 4-allylanisole, anethol and estragol were the major compounds. Variation was observed for total phenolic and flavonoid content. Seventeen fatty acids were found in fennel seed oil including petroselinic acid (62.08 %-66.71%), 10-Nonadecanone (4.70-22.80%) and linoleic acid (1.32-7.59%). Oleic acid, stearic acid, eicosanoic acid, lenolenic acid and intermediate compounds cyclotetracosan, cyclohexadecan, 10-Nonadecanol were detected in one or other genotypes. However, results from present study revealed that anti-inflammatory effect is a result of combinatorial effect of various constituents rather than alone essential oil, phenolics and fatty oil.

Hypolipidemic activity

The study of the anti-cholesterol/ Hypolipidemic effect of methanol and hexane extract of fennel showed that treatment with the extract significantly reduced plasma lipid levels. It reduced triglycerides in fatty liver and facilitated blood flow in the coronary arteries by preventing the buildup of fatty deposit in arteries through reduction of plasma and liver fats.

All the four parameters affecting hypolipidemic properties were found to be influenced by both seed extracts of fennel genotypes as presented in Table 2. Total cholesterol was reduced up to 83.17 and 82.38 in methanol and hexane extract of genotype RF-101 respectively which is close to standard drug simvastatin (77.26). Level of triglycerides was also lowered in animals treated with either of seed extracts. Methanol and hexane seed extracts of RF-101 were able to reduce the triglycerides up to 127.16 and 126.18 respectively from 146.6 in animals fed with a high cholesterol diet. The level of triglycerides in animals fed with standard drug simvastatin was 109.1. Similarly, LDL level was also reduced by methanol seed extract of different fennel genotypes in the range of 67.13-77.04 as against 96.5 in high cholesterol fed animals and 48.2 in treated animals. Hexane seed extract was more effective in reducing LDL levels. Both the extract were able to increase the HDL of which methanol extract of AF-1 was most effective (34.16) followed by seed extract of GF-2 (33.62) and hexane extract of AF-1 (32.80).

In a previous study [18] it was found that high fat-diet supplemented with the three different levels of fennel seeds significantly improved serum levels of TL, TG, TC, LDL-C, VLDL-C, AST, ALT, ALP and MDA, and significant rise in serum HDL-C level, values of HDL-C/LDL-C ratio and improved activities of CAT, GPX and SOD enzymes compared to the positive control group. The aqueous extract of Foeniculum vulgare was used for the hypolipidemic and anti-atherogenic activity in mice by Mokkhasmit et al., [19]. In this study mice were divided into three groups, Control, hyperlipidemic and hyperlipidemic treated with fennel aqueous extract, administered by a force-feed. A significant decrease of plasma lipid levels occurred 24 h after treatment, plasma total , cholesterol, triglycerides, LDL- cholesterol and Apolipoprotein B decreased by 40%, 23%, 61% and 61%, respectively and increased in HDL-cholesterol and apolipoprotein A-I by 85% and 58%, respectively. Also, a histological study on heart alterations showed a marked decrease in lipid deposits. Fatty acids play an important role in lipid profile.

Agarwal et al. while analyzing FAME profile of these genotypes identified seventeen fatty acids and their isomers of which petroselinic acid was the major fatty acids found in the range of 62.08% in genotype Hisar Swarup to 66.71% in genotype AF-1. Significant quantity of 10-Nonadecanone was detected in all genotypes ranged from 4.70% in AF-1 to 22.80% in Hisar Swarup. Other fatty acids such as linoleic acid (9, 12 octadecadienoic acid) was detected only in Rajendra Sourabha and AF-1. Fennel seeds contain up to 20% fatty acids and petroselinic acid is a characteristic fatty acid of fennel oil. Petroselinic acid is a positional isomer of oleic acid, a monounsaturated omega-12 fatty acid occurs naturally in several animal and vegetable fats and oils. The level of petroselinic acid in fennel seed oil could be as high as 70 to 80% 48. The qualitative analysis of the acetone extract of fennel showed that polyunsaturated fatty acid (54.9%), hexadecanoic acid (5.4%) and monounsaturated fatty acid (5.4%) were major components in acetone extract. Occurrence of these fatty acids derivatives in fennel seed oil in significant quantities may be attributed to various pharmacological activities including antimicrobial and anti-carcinogenic activities 52. As compared to other common spices like ginger, chilli and pepper, fennel seeds contain high MUFA content, thus may be recommended for human diet.

Antidiabetic activity

Fennel seed extracts irrespective of genotypes were found effective in reducing blood glucose level in diabetic rats (Table 3). Methanol seed extract of fennel genotypes at 400mg/kg were able to reduce blood glucose level as Rajendra Saurbha (62.22%)>Hisar Swarupa (61.48%)> AF-1 (61.08%)> RF-101 (60.45%)> GF-2 (59.71%). In case of hexane seed extract genotype Hisar Swarupa showed

maximum reduction in glucose level (61.90%) from145.87 at 60 min to 120.44 at 240 min while genotype AF-1 showed minimum reduction in blood glucose 144.12 to 123.29 at 240 min (59.51%). In all the tested genotypes, maximum activity was recorded in methanol seed extract of Rajendra Saurbha and hexane seed extract of Hisar Swarupa. In the last two decades many studies have been conducted to find effective naturally occurring compounds which can substitute synthetic chemical drugs for diabetic patients. The traditional medicinally important natural sources include the spices which are commonly used folklore remedies to many ailments. Use of fennel seeds in whole or powdered form as well as its extracts has been found effective in reducing blood glucose levels.

Anti-diabetic property of fennel seed was also analyzed by Anitha et al., [21] in streptozotocin-induced diabetic rats. They studied metabolic enzymes in normal and streptozotocin-induced diabetic rats. They found that administration of the aqueous extract of *F. vulgare* to diabetic rats corrected the hyperglycaemia. Studies also reported that essential oil of *F. vulgare* corrected the hyperglycemia and pathological abnormalities in diabetic induced rats, which could be in part through its antioxidative effect and restoring of redox homeostasis [22-23]. In another study by Barros et al., [24] while evaluating the antioxidant potential of different parts of *F. vulgare* reported that fennel can improve rat glucose tolerance capacity. Another possible mechanism is due to its high source of nutrients like vitamin-C and potassium; it helps in lowering the blood sugar levels and also helps to increase insulin reactivity resulting in balancing the sugar. This makes the possibility of its inclusion in anti-diabetic drug industry.

Anti-hypertensive properties

Fennel seed extracts of genotype AF-1 were able to reduce mean arterial blood pressure (MABP). Methanol extract was more effective than hexane extract. MABP was recorded 87 and 100 mm of Hg after 1hr in treatment of methanol and hexane extract respectively as compared to 34 mm in captopril treated rats and 86mm in controlled animals. El Bardai et al., [25] studied the hypotensive effects of the water extract of Marrubium vulgare L. and Foeniculum vulgare L. Oral administration of Marrubium or Foeniculum extract lowered the systolic vital sign of SHR but not of WKY. In SHR, Foeniculum treatment increased water, sodium and potassium excretion as compare to Marrubium. Fennel lowered systolic vital sign in animals with laboratory-induced high vital sign. Chewing saunf seeds augments the nitrite content in saliva, which works as a natural remedy to maintain the blood pressure levels. In addition, notable amounts of potassium in fennel seeds, an important element of cells and body fluids assist in controlling acid-base balance, dilates the blood vessels, regulates heart rate and stabilizes blood pressure.

In a previous publication Agarwal et al., reported antimicrobial activity of methanol and hexane seed extract of similar fennel genotypes. The study showed significant anti-microbial activity and genotypic variation in anti-microbial effect of both the extracts. Methanolic extract of genotype AF-1 showed a good amount of antibacterial activity against both Gram positive and negative bacteria while methanolic extract of genotype RF-101 showed comparatively more antifungal activity.

Hepatoprotective activity of fennel seeds extract was also evaluated on male Wistar rats. Hepatotoxicity was induced with carbon tet 1 ml/kg s.c. subcutaneously. standard drug Silymarin@100 mg/kg was given along with CCl4. Increased levels of ALT, AST, ALP, Bilirubin total and bilirubin direct in blood serum of treated rats were found to reduce by administration of seed extract of different fennel genotypes. Hepatoprotective effect was more evident in total bilirubin and ALP levels where seed extracts of both AF-1 and GF-1 were found to reduce total bilirubin content. Hexane seed extract was found simpler than methanol seed extract. A significant genotypic variation was also observed, suggesting that specific genotypes may be identified for medicinal significance with respect to specific ailment.

Present study indicated that both methanol and hexane seed extract of fennel genotype Rajendra Saurabha and Hisar Swarup possessed more anti-inflammatory properties while methanol extract of genotype Rajendra Saurabha showed slightly more anti diabetic activity. Hexane extract of genotype RF-101 produced significant hypolipidemic properties. On the idea of earlier studies by author, genotype AF-1 and RF-101 showed significant antimicrobial activity regardless of solvent used while AF-1 and GF-1 were having more hepatoprotactive potential.

This genotypic variation could also be thanks to distinct morphology also as different chemical constitution of two genotypes also as agro-ecological and edaphic interventions. Genotype prefix with AF and RF are fennel varieties developed from state Rajasthan, India while, genotypes GF-2 is from state Gujarat, India showed significantly different phenotype. Hisar Swarupa and Rajendra Saurbha belong to the state Haryana and Uttar Pradesh respectively.

Relevant literature and work done on pharmacological potential fennel seeds and results of present study suggested that common fennel has potential to be used as an honest source of traditional medicine and it provides an interesting basis in pharmaceutical biology for the development/formulation of latest drugs and future clinical uses.

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