



EVALUATION OF ANTI-CATARACT ACTIVITY OF FOENICULUM VULGARE SEEDS EXTRACT ON GOAT LENS

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ABSTRACT: Foeniculum vulgare fruits are routinely consumed for their carminative and mouth freshening effect. The plant was evaluated for aldose reductase inhibition and anti-diabetic action. Bioguided fractionation using silica gel column chromatography, HPLC, and GC-MS analysis revealed trans-anethole as the bioactive constituent possessing potent aldose reductase inhibitory action, with an IC₅₀ value of 3.8 µg/ml. Trans-anethole could effectively show anti-cataract activity through the increase in soluble lens protein, reduced glutathione, catalase and SOD activity on in vitro incubation of the eye lens with 55 mM glucose. Trans-anethole demonstrated noncompetitive to mixed type of inhibition of lens aldose reductase.

KEYWORDS: Trans-anethole; aldose reductase inhibitor; Foeniculum vulgare; anti cataract; anti-diabetic; Methanolic seed extract of Foeniculum vulgare (MSEFV).

INTRODUCTION:

Cataract dates to the 14th century. It comes from the Latin word '*cataracta*' which means portcullis. Cataract is generally identified by clouding of lens in eye and that's why it affects the vision of eye. It is very common in older people which having age around 80 or more. Sometimes cataract can occurs in both eyes but it never spreads from one eye to another eye. It is slowly developing disease commonly due to ageing and sometimes due to trauma or radiation. It involves mechanism in which accumulation of clumps of protein or yellow-brown pigment in the lens of eye which reduces transmission of light to the retina at the back of the eye.

CLASSIFICATION OF CATARACT:

The main types of cataract are age related cataracts which includes nuclear sclerosis, cortical and posterior subcapsular. In matured cataract the all lens protein is opaque but in immature cataract contain some transparent protein. In third type which is hyper mature cataract or Morgagnian cataract, the lens protein become liquid.

Cataract can also be classified by using lens opacities classification system (LOCS 3) and further it can be classified based on severity on a scale from 1 to 5. Some of the types of cataract including -

1. Posterior subcapsular cataract
2. Nuclear sclerosis cataract
3. Cortical cataract
4. Retro-illumination of cortical cataract
5. Intumescent cataract
6. Anterior subcapsular cataract

CAUSES AND RISK FACTORS OF CATARACT:

Some factors which are considered as risk-bearing factors for cataract such as ,

- i. Older age
- ii. Family history of cataracts
- iii. Greater exposure to sunlight
- iv. Living at a higher altitude
- v. Diabetes
- vi. High blood pressure
- vii. History of eye injury, inflammation, or surgery
- viii. Radiation treatments on your upper body
- ix. Smoking
- x. Drinking alcohol in excess
- xi. Long-term use of corticosteroid drugs

SYMPTOMS OF CATARACT:

- i. Blurry vision
- ii. Colors that seem faded
- iii. Glare – headlights, lamps or sunlight may seem too bright.
- iv. You may also see a halo around lights.
- v. Not being able to see well at night
- vi. Double vision
- vii. Frequent prescription changes in your eye wear

PLANT INTRODUCTION:

Fig 1- Fennel Plant



Fig 2- Fennel Seeds

Fennel (*Foeniculum vulgare*) is a traditional and popular herb with a long history of use as a medicine. A series of studies showed that *F. Vulgare* effectively controls numerous infectious disorders of bacterial, fungal, viral, mycobacterium, and protozoal origin. It has antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and oestrogenic activities. Some of the publications stated that *F. Vulgare* has a special kind of memory-enhancing effect and can reduce stress. Animal experiments and limited clinical trials suggest that chronic use of *F. Vulgare* is not harmful. Fennel may be consumed daily, in the raw form as salads and snacks, stewed, boiled, grilled, or baked in several dishes and even used in the preparation of herbal teas or spirits. A diet with desired quantity of fennel could bring potential health benefits due to its valuable nutritional composition with respect to presence of essential fatty acids. In recent years, increased interests in improvement of agricultural yield of fennel due to its medicinal properties and essential oil content has encouraged cultivation of the plant on large scale.

F. Vulgare is an upright, branching perennial herb with soft, feathery, almost hair-like foliage growing up to 6.6 ft. (2 m) tall. This plant looks similar to dill. It is typically grown in vegetable and herb gardens for its anise-flavored foliage and seeds, both of which are commonly harvested for use in cooking. It is erect and cylindrical, bright green, and smooth as to seem polished, with multiple branched leaves cut into the finest of segments. The leaves grow up to 40 cm long; they are finely dissected, with the ultimate segments fusiform (threadlike), about 0.5 mm wide. The bright golden flowers, produced in large, flat terminal umbels, with thirteen to twenty rays, bloom in July and August. Stem striate, leaves 3-4 pinnate, segments fusiform, up to 1.6 in. (4 cm) long; leaf bases sheathing. It has a green, sleek, and slippery stem with upright stiff branches and much divided leaves in linear segments. Rays are 5–30 numbers with 0.39–2.4 inches (1–6 cm) long. Flowers are small, yellow, and found in large flat-topped umbels. Fruits are oblong to ovoid with 0.12–0.2 inches (3–5 mm) long and 1.5–2.0 mm broad. The stylopodium persists on the fruit. The fruits are elongated and have strong ribs. The most esteemed fennel seeds vary from three to five lines in length and are elliptical, slightly curved, and somewhat obtuse at the ends. They are greenish-yellow, the colour of hay, from which the term fennel is derived. Wild fruits are short, dark coloured and blunt at their ends, and have a less agreeable flavour and odour than those of sweet fennel. Seeds ripen from September to October. This plant can reproduce from crown or root fragments but freely reproduces from seed.

Taxonomy-

- *Kingdom:* Plantae
- *Division:* Tracheophyta
- *Subdivision:* Spermatophytina
- *Class:* Magnoliopsida
- *Order:* Apiales
- *Family:* Apiaceae
- *Genus:* *Foeniculum*
- *Species:* *vulgare*

PHYTOCHEMICAL SCREENING TESTS:

- i. **Test For Alkaloid-** By adding 1ml of Dragendorff's reagent in the 2ml extract, an orange red ppt formed which indicates the presence of alkaloids.
- ii. **Test For Glycosides-** 0.5 mg of extract dissolved in 1ml of water and water and then aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides.
- iii. **Test For Triterpenoids-** A 5mg of dry extract dissolved in 2ml of chloroform and the 1ml of acetic anhydride added to it. Add conc. H₂SO₄ to it. Reddish violet colour of the solution indicates the presence of triterpenoids.
- iv. **Test For Steroids –** 1ml crude plant extract dissolved with 10ml of chloroform in a test tube and then by side of the test tube add equal amount of conc. H₂SO₄. The upper layer of solution turns red and sulphuric acid layer forms yellow with green fluorescence which indicates the presence of steroids.
- v. **Test For Saponins-** 15ml distilled water was added to extract, shake it vigorously until the formation of stable persistent froth which indicates the presence of saponins.
- vi. **Test For Flavonoids –** Few drops of dil. NaOH was added in the extract . A yellow solution that turns colourless shows the presence of flavonoids.
- vii. **Test For Tannins-** Add few drops of 1% lead acetate in the test tube containing little quantity of extract. Yellow precipitate appears which indicates the presence of Tannins.
- viii. **Test For Carbohydrates-** A small amount of extract mixed with Molisch's reagent and shake the mixture vigorously. After that add conc. H₂SO₄ by the sides of test tube. Absence of violet ring at the interface indicated presence of carbohydrates.

CHEMICALS AND REAGENTS:

Test drug: Methanolic seed extract of *Foeniculum vulgare* (MSEFV)

Chemicals: Sodium chloride (NaCl), Potassium chloride (KCl), Magnesium chloride (MgCl₂), Sodium bicarbonate (NaHCO₃), Calcium Chloride (CaCl₂), Glucose, Penicillin, Streptomycin , Ascorbic acid.

Instruments: Incubator, Wired mesh, Petri dish.

Dose Selection: *Foeniculum vulgare* seeds extract -15,30 and 60µg/ml

Standard Ascorbic Acid: 40µg/ml.

MATERIAL & METHODS:**Collection and Authentication of Plant –**

Ripe greenish brown seeds of cultivated funnel (*foeniculum vulgare*) were collected from Pune district, Maharashtra (India). The plant has been identified morphologically and authenticated by R.P.Ganorkar head of department of botany of Chandmal Tarachand College, Shirur,Pune, Maharashtra (India). The seeds were dried naturally during storage and their retained humidity was 11%. For the purpose of study they were milled and sieved in order to separate a fraction with particle size 0.1-1mm.

Collection of Goat Lens –

A Fresh goat eyeballs were obtained from the slaughter house and instantly transported to the laboratory at 0-4°C. The lens were detached by extra capsular extraction and incubated in unreal aqueous humor (NaCl 140 mM, KCl 5mM, MgCl₂ 2 mM,NaHCO₃ 0.5 mM, NaHPO₄ 0.5 mM, CaCl₂ 0.4 mM and glucose 5.5 mM) at room temperature and maintain pH 7.8 by addition of NaHCO₃). Penicillin G 32% and streptomycin 250 mg% added to the culture media to inhibit bacterial contamination.

Preparation of Extract (by Maceration)-

The seeds of *Foeniculum vulgare* were ground into a fine powder. Then, 500g powder was soaked into methanol with constant shaking for 7 days. It was assured that the methanol level must be 2–3 times more than the powdered material during the whole period of extraction. Afterward, the extract was filtered and the filtrate was rotary-dried at 40–50°C under reduced pressure to solidify the extract.

Introduce in-vitro cataract in goat lens-

Glucose at a concentration of 55 mM was used to induce cataracts. At high concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes over hydration and oxidative stress. This generates cataractogenesis. These lens were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM) served as normal control and 55 mM serve as toxic control) for 72hours.

PROCEDURE:

Study Design and Groups-

The Anti-cataract activity was carried out with the extracts of *Foeniculum vulgare* seed extract. The extract was taken in different doses. Goat lenses were divided into six groups of six lenses each and incubated as follows

- Group I:** Aqueous humor + Glucose 5.5 mM (Normal control).
- Group II:** Aqueous humor + Glucose 55 mM (Negative control)
- Group III:** Aqueous humor + Glucose 55mM + 40µg/ml Ascorbic acid. (Standard)
- Group IV:** Aqueous humor + Glucose 55mM + 15µg/ml MSEFV (Test I)
- Group V:** Aqueous humor + Glucose 55mM + 30µg/ml MSEFV (Test II)
- Group VI:** Aqueous humor + Glucose 55 mM + 60 µg/ml MSEFV (Test III)

Photographic Evaluation –

Lenses were placed on a wired mesh with the posterior surface touching the mesh, the pattern of mesh number of squares clearly visible through the lens was observed to measure lens opacity.

The degree of opacity was graded as follows:

- “0”: absence of opacity.
- “1”: slight degree of opacity.
- “2”: presence of diffuse opacity.
- “3”: presence of extensive thick opacity.

Preparation of Lens Homogenate -

After 72 hours of incubation, homogenate of lenses was prepared in tri buffer (0.23 M, pH- 7.8) containing 0.25×10^{-3} M EDTA and homogenate was adjusted to 10% w/v which was centrifuged at 10,000 G at 4°C for 1hour and the supernatant was used for the estimation of biochemical parameters.

BIOCHEMICAL PARAMETERS:**Chemicals Required:**

1. 10% TCA: 10 grams of Trichlor acetic acid is dissolved in 100ml of distilled water
2. 1N naoh: 4 grams of naoh is dissolved in 100ml of Distilled water.
3. Alkaline copper reagent (ACR):
4. SOLUTION A: 0.4 grams of naoh was dissolved in 100ml of distilled water. Then 2 grams of Na_2CO_3 was Added.
5. SOLUTION B: 0.5 grams of CuSO_4 was dissolved in 100 ml of distilled water. Then 1 gram of sodium
6. Potassium tartrate was added.
7. Solution A: 50 ml
8. Solution B: 1ml
9. Both the solutions were mixed properly.
10. Folin Phenol reagent: These is prepared by making 1:1 dilution i.e. 1ml of Folin phenol reagent: 1ml Of Distilled water

Estimation of total protein content:

To 0.1 ml of lens homogenate, 4.0ml of alkaline copper solution was added and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for color development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as $\mu\text{g}/\text{mg}$ lens tissue.

Total protein content was estimated by the Lowry method. The Method is having the following procedure. 1% of the lens Tissue was homogenized in 0.25M ice cold Sucrose solution. 0.5 ml of the crude Homogenate is thoroughly mixed with 1ml of Trichlor acetic acid. Then centrifuged at 1000 rpm for about 15 minutes. Discard the supernatant liquid. The pellet was dissolved in 1ml of 1N naoh. Then 4ml of Alkaline copper Reagent was added followed by 0.4 ml of Folin- phenol Reagent (Folin reagent) + Distilled Water { 1:1 }. Read the Colorimeter at 600nm.

STATISTICAL ANALYSIS:

All data were expressed as mean \pm SD. All data were analyzed with SPSS/10 student software. Hypothesis Testing methods include done way analysis of variance (ANOVA) followed by LSD. The values are Expressed as mean \pm S.D. and results were considered significantly different if $P < 0.05$. Statistical Variations are compared as-Normal Goat lens vs. Goat lens + Glucose 55mM, Goat lens + Glucose55mM Vs. Goat lens + Glucose55mM + MSEFV.

RESULTS AND DISCUSSION:**Phytochemical constituents of seed extract of *Foeniculum vulgare* -**

Components	Chloroform extract	Water Extract	Methanol extract	Aqueous: Methanol(20:8 V/V)
Alkaloid	-	+	+	+
Tannin	-	+	+	+
Saponin	-	+	+	+
Terpenoid	+	+	+	+
Flavonoids	-	+	+	+
Steroids	-	-	+	+

In-vitro Anti-cataract activity –

After 8 hr. Of incubation lenses with glucose 55mM shows opacification at periphery, on the posterior Surface of the lens. At the end of 72 hrs. Complete opacification is progressively increased to words the Center.

Degree of opacity on lens by glucose induced cataract -

Normal control - Zero degree opacity occurred, clear lens is obtained.

Negative control – presence of extensive thick opacity, because of high conc. of glucose induced cataractogenesis.

Positive control (Ascorbic acid 40µg/ml) – lenses show slight degree of opacity, clear lens was not found.

Test 1 (MSEFV 15µg/ml) – lenses show slight degree of opacity, clear lens was not found.

Test 2 (MSEFV 30µg/ml) – lenses show slight degree of opacity, clear lens was not found.

Test 3 (MSEFV 60µg/ml) – Zero degree opacity is occurred, clear lens is obtained. Test drug inhibits cataractogenesis.

Photographic Evaluation -

After 72 hours of incubation, transparency was maintained in the Group I (normal control group) but there was complete loss of transparency in the Group II (negative control group) indicating complete cataractogenesis. Group III (positive control group) containing lens treated with standard ascorbic acid were squares of the graph paper were visible through the lenses. Goat lenses of groups containing escalated doses of the methanolic seed extract of *Foenicullum vulgare* (Group IV, V) were less hazy and the squares of the graph paper were visible through the lenses indicating suppression of cataract formation. Group VI (containing 60µg/ml) was more effective in suppressing cataract formation than Group IV and Group V.

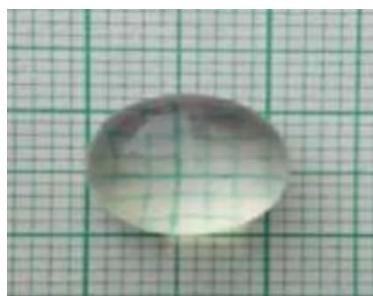


Fig a. Normal control(group 1)



Fig b. Negative control(Group 2)



Fig c. Positive Control

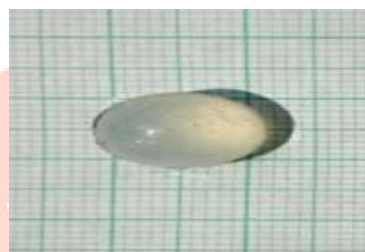


Fig d. Group 4



Fig e. Group 5



Fig f. Group 6

Fig 3: Effect of Methanolic extract of *Foenicullum vulgare* on goat lens in glucose induced cataract.

DISCUSSION:

Alteration of Na⁺/K⁺ ratio due to reduction in Na⁺/ATPase activity in the lens causes Radical scavenging activity. In Normal control, after 72 hr. of incubation of lens in aqueous humor and 55 mM of glucose. The lens was clear in because of low conc. of glucose that does not show any effect on lens and numbers of squares are clearly visible through lens. The lens showed zero degree of opacity. In Standard group, after 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose+ 40µg/ml Ascorbic acid std. drug, numbers of squares were not clearly visible through lens as compared to Test-3, the lens showed slight degree of opacity. In Test- 1 & Test -2, after 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose + 15 µg/ml and 30µg/ml MSEFV test drug, number of squares were not clearly visible through lens as compared to MSEFV 60 µg/ml test-3 drug, the lens show slight degree of opacity to cataractogenesis. This alteration in the Na⁺, K⁺ ratio changes the protein content of In Test- 3, after 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose + 60µg/ml MSEFV drug, number of squares were clearly visible through the lens .The lens showed absence of opacity, because the test drug inhibits cataractogenesis and oxidative stress.

CONCLUSION:

In the present study the MSEFV showed significant reduction of cataract at the dose of 100 µg/ml in goat lenses. The result support the traditional use of this plant in cataract conditions and suggests the presence of biologically active compounds which may be worth for further investigation.

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