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Research Article

Assessment and Effectiveness of Fennel Powder Extract Based Cookies in Hyperglycemic Subjects

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Abstract

Background: In the current world, almost half of the total world's population is experiencing lifestyle complications that have deleterious effects on the human wellbeing. These life endangering endeavors are multiplying in magnitude of their adversity because of poor dietary habits. At present, there is a strong need to introduce the benefits of consuming functional foods for the treatment and prevention of disease related complications. The plants of *Umbelliferae Apiaceae* family are a rich treasure of bioactive polyphenols, imparting hypoglycemic, hypocholesterolemic, and anti-inflammatory, anti-cancer, anti-microbial, carminative and anti-allergic properties.

Objective: The present study was aimed to assess the effectiveness of fennel extract based cookies on hyperglycemic human subjects.

Keywords: Fennel; Phytochemicals; Polyphenols; Antioxidants; Proximate analysis; Sensory evaluation; Hyperglycemia

Introduction

Since the last few decades' people are increasingly centered on altering their nutritional approaches to struggle with the life style linked chronic health problems. Vegetables and fruits are highly rich sources of phytochemicals that are renowned for their health benefits [1]. These phytochemicals provide protection against various health issues such as different tumors [2], heart problems [3], elevated blood glucose levels [4], hypertension [5], inflammatory disorders [1], bacterial infections [6], viral infections [7] and other microbial infections [8], psychiatric illnesses [9], peptic ulcers [10], joints and bone related disorders such as osteoporosis [11]. Fennel plant, biologically known as *Foeniculum Vulgare*, is a familiar culinary spice that is also used as a therapeutic plant. It belongs to the family *Apiaceae* [12]. It is used in a wide range against gastrointestinal [13], endocrinal [14], reproduction [15], respiratory problems [16] and as a lactation improving agent [17]. Fennel is also employed in make-up [18] and medicinal products [13]. Fennel also imparts flavor to the edibles such as breads, pastries, pickles and cheese. The current study has proved that the nutritional syndromes can be corrected by healthful intakes. The functional and nutraceuticals foods are effective in the management of human health, on basis of their compositional profiling [19]. A review on detailed evaluation of *Foeniculum vulgare* concluded that it constitutes 9.5% protein, 6.3% of moisture, 13.4% minerals, 10% fats, 42.3% carbohydrates and 18.5% fiber. The vitamins and minerals composition of F. vulgare includes potassium, calcium, iron, sodium, riboflavin, phosphorus, thiamine, vitamin C and niacin. The phenolic contents include 4-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 1,3-O-di-caffeoylquinic acid, 5-O-caffeoylquinic acid, 1,5-O-di-caffeoylquinic acid and 1,4-O-di-caffeoylquinic acid [20]. Increased oxidative stress results in excessive production of Reactive Oxygen Species (ROS). It leads to the initiation of pathological conditions [21]. Elevated blood glucose levels, known as hyperglycemia, is a condition in which an excessive amount of glucose circulates in the blood plasma (American Diabetes Association). It occurs due to impaired insulin sensitivity of cells or insufficient insulin production. Persons with increased circulatory blood sugar concentrations are at risk of developing different complications endangering health and survival [22,23]. An experiment to assess the antimicrobial and antioxidant capacities of fennel vital oil using methanolic and ethanolic extracts indicated the presence of twenty three polyphenols with trans-anethole (69.87%) being the major one followed by fenchone (10.23%), estragole to be 5.45% and limonene (5.10%) [24]. A human trial to investigate the outcomes of Fennel in the management of hyperglycemic cases concluded that the mean values of blood sugar levels of individuals after two hours of fennel administration were lowered from 313.5 mg/dL ± 108.69 mg/dL to 262 mg/dL ± 88.69 mg/dL and from 279.33 mg/dL ± 96.24 mg/dL to 246.5 mg/dL ± 91.93 mg/dL for patients having 100 mg/kg body weight and 50 mg/kg body weight respectively [25].

Methodology Study Design

Quasi experimental study design

Settings: The study was conducted at following settings: Food Science and Technology Lab (Lab. No. 101) of University Institute of Diet and Nutritional Sciences (UIDNS), University of Lahore, Diabetic Center Services Hospital, Lahore.

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Sample size: 53 hyperglycemic subjects.

This was human based trial to study the effectiveness of fennel extract based cookies on the physiology (fasting blood glucose level at beginning (0 day) and 60 days respectively) of hyperglycemic subjects.

Sampling technique: Purposive sampling technique

Sampling selection: The samples were selected according to the inclusion and exclusion criteria.

Inclusion criteria: Male and female hyperglycemic patients attending the Services Hospital, Lahore were included in the study.

Exclusion criteria: Diabetic patients with other complications (diabetic neuropathy, diabetic nephropathy) and non- cooperative/ unwilling hyperglycemic subjects were not included in the study.

Equipments: Weighing Balance, Kjeldal Apparatus, Mixer, Oven, Rotary Evaporator, Orbital shaker, Baking Oven, Glucometer, 53 glucose meter strips for each interval, sterilized needles.

Collection and Preparation of Material

Ripe Fennel seeds sample were purchased from a local market of Lahore. The seeds of Fennel were subjected to shadow drying for 3 to 6 hours to remove adhering moisture followed by oven drying at 70°C and grinding to pass through 100 Mesh Screen Filter. Fennel seeds powder was packed in an air tight jar for further analysis.

Proximate analysis

The analysis for moisture content, ash content, crude protein, crude fat and crude fiber of fennel seeds powder was performed according to the methods of AOAC [26]. All the tests were executed in triplicates.

Preparation of fennel powder extract

The process of fennel seeds powder sample extraction was performed by using ethanol as solvent. Ethanol extracts of the fennel seeds (20% w/v) was prepared by shaking using orbital shaker for 24 hours at 300 rpm. The resultant supernatant was subjected to centrifuge at 9000 rpm for fifteen minutes. The coarse particles were filtered through proper filtration media for extract separation and then subjected to rotary evaporator for the recovery of solvents at 300 rpm [27,28].

Antioxidant indices

The powdered extract was evaluated for their antioxidant capacity through different tests including TPC (Total Phenolic Contents) and DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay as discussed below:

Determination of Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) in fennel powder extract was assessed using Folin-Ciocalteu method [29]. The mechanism is based on the phosphoric acid reduction to phosphorylated protein, and as the number of aromatic phenolic group upturns, the results of absorbance increases. For this purpose, fennel powder extracts (50 µl) was discretely added to nine tubes each having 250 µl Folin-Ciocalteu reagent and 750 µl (20%) sodium carbonate solution, and the final volume was filled with distilled water to 5 ml. After two hours, the absorbance was measured at 765 nm using a UV/ Visible Spectrophotometer (CECIL CE7200) against the control having all chemicals, except for the standard aqueous solution. Total polyphenols were calculated and values were stated as Gallic Acid Equivalent (GAE, mg Gallic acid/g) *via.* a following formula:

$$C = c \times V/m$$

m = Weight of fennel extract (ml)

V = Volume of extract (mL)

c = Concentration of Gallic Acid (mg/mL)

C = Total phenolic content (mg/ml fennel extract, GAE: Gallic Acid Equivalent")

Determination of DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging potential: DPPH (1, 1-diphenyl-1-picrylhydrazyl) is a potent and stable oxidizing agent that results in the yellow hydrazine (DPPH) formation that is related with the free hydrogen atoms uptake from part of phenolic antioxidants. The Atroozl protocol (2013) was used to find out the DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical removal (scavenging) activity from fennel powder extract. A sample solution was prepared by running 0.025 ml of sample fennel powder extract in 10 ml of the appropriate and suitable solvent. Freshly prepared DPPH solution (3 ml) mixed in an appropriate solvent (6×10-5M) with sample fennel powder extract (77 micro liter). Each sample was put in a dark place for 15 minutes at room temperature and an absorbance at 517 nm was obtained at the UV/ visible spectrophotometer. Similarly, pure absorbance of the sample was measured having the same amount of DPPH solution and solvent except for the sample, and the absorbance was assessed at the same wavelength on spectrophotometer. The activity of free radical scavenging can be illustrated as a calculated reduction of DPPH due to the identified amount of fennel powder extract.

$$\text{Reduction of absorbance (\%)} = [(AB - AA) / AB] \times 100$$

AB = Absorbance of blank sample at t = 0 minute

AA = Absorbance of tested sample extract solution at t = 15 minutes

Preparation of cookies

The cookies were prepared with 3.0% of fennel seeds powder extract added in the recipe. The recipe of cookies consisted of all-purpose flour, baking soda, brown sugar, butter and eggs along with 3% fennel ethanolic extract. After mixing the ingredients, the cookies were made and set on an oven tray. The cookies were subjected to baking in a pre-heat oven at 180°C for fifteen minutes.

Feeding trial

To explore the therapeutic potential of our developed functional fennel seeds powder extract based cookies against the lifestyle linked problem with special reference to hyperglycemia, 53 hyperglycemic patients were selected randomly from the Diabetic Center of Services Hospital, Lahore. Cookies were provided to the study volunteers for regular intake (4 cookies at mid meals in one dose daily). The patients' current medications were followed as prescribed. Anthropometry and blood analysis for circulatory glucose levels were determined at beginning (0 day) and at end (60^{th} day), respectively.

Data Collection Tool

Fasting Blood Sugar Level Test

Physical parameters feed intake: The selected hyperglycemic were advised to take 4 fennel seeds powder extract based cookies at mid-meal 1 dose daily for period of two months.

Determining circulatory glucose levels: Circulatory blood glucose concentration was measured using a Glucometer (Optimum,

Medisence). For taking the sample, the area of the skin to be used as a site of puncture was inspected wearing a pair of latex-free gloves and the puncture site was cleaned using alcohol swab. After calibration of Glucometer, the reagent strip was removed from the container and placed in the Glucometer with the test pad facing up. Then the skin puncture was performed using a sterilized needle. The blood drop was transferred to the reagent strips. Then, the timer on the Glucometer was immediately pressed to take reading. And final reading was noted down.

Data analysis: Data was entered and analyzed using SPSS version 25. Numerical data like age, blood glucose level etc. was presented in the form of Mean ± S.D. Categorical data like gender, diabetes status was presented in the form of F (%). After fulfilling the parametric assumptions, paired sample T-test and one sample T-test were used to compare BSL (Blood Sugar Level) value before and after intervention among hyperglycemic subjects.

Results

The present research was divided into four sections. Firstly, the proximate analysis of fennel seeds was performed; secondly, fennel extract was evaluated for its antioxidant potential. At third step, fennel extract based cookies were prepared for feeding trial of hyperglycemic patients. Finally, the effect of fennel extract based cookies on hyperglycemic subjects' blood sugar level was assessed with human trials.

Proximate analysis

The proximate analysis is performed to evaluate the compositional profiling of the product being selected for clinical experiments. The mean values of proximate analysis of fennel seeds powder; value of results of extraction yield of fennel powder using ethanol as solvent are tabulated in (Table 1 and 2). The proximate composition of fennel seeds powder in the graphical form is given in (Figure 1).

Blood glucose levels

The results tabulated have shown significant lowering of the circulatory fasting blood glucose concentrations of the hyperglycemic subjects in a period of two months with fennel extract based cookies (Figure 2) (Table 1-3).

Table 1: Proximate composition of Fennel Seeds Powder (%).

Proximate Composition	Percentage (%)
Moisture	8.44 (0.51)
Ash	9.93 (0.55)
Protein	8.51 (0.42)
Fat	9.87 (0.15)
Crude Fiber	13.09 (1.31)
Nitrogen Free Extract (NFE)	49.08 (3.04)

Table 2: Extraction yield of fennel seeds powder.

Solvents	Extraction Yield (%)
Ethanol	4.7 ± 0.18

Table 3: Results for antioxidant indices of fennel seeds powder ethanolic extract determined by TPC and DPPH assay.

Solvents used	TPC (mg GAE/g)	DPPH Assay (%)
Ethanol	104.1 ± 1.7	143.6 ± 4.3

Statistical interpretations on the blood glucose levels of hyperglycemic subjects

The mean ± Standard Deviation (S.D) values for initial fasting blood glucose levels (when fennel containing cookies as mid meal snack were not given) and final fasting blood glucose levels (after the treatment with fennel containing cookies as mid meal snack) of 53 hyperglycemic patients for a duration of two months *i.e.* 8 weeks feeding trial was subjected to evaluation using SPPSS version 25. The data was analyzed by using the statistical one sample and paired sampled T- test (Table 4-6).

Discussion

The use of Fennel as a therapeutic agent is quite evident. The investigation led by Syed et al. [13], concluded that the fennel seeds contain 8.8 g moisture, 15.8 g crude proteins, 9.91 g crude fats, 52.59

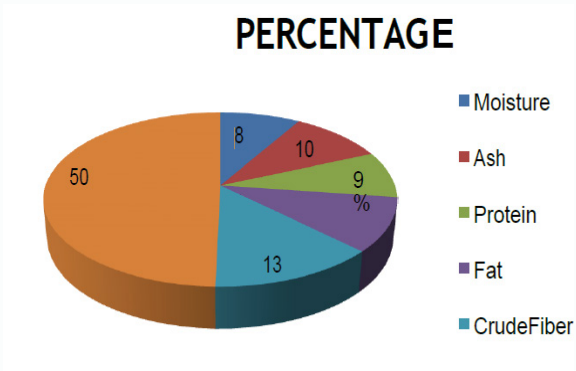

Figure 1: Graphical representation of the mean values of proximate analysis of fennel seeds powder.

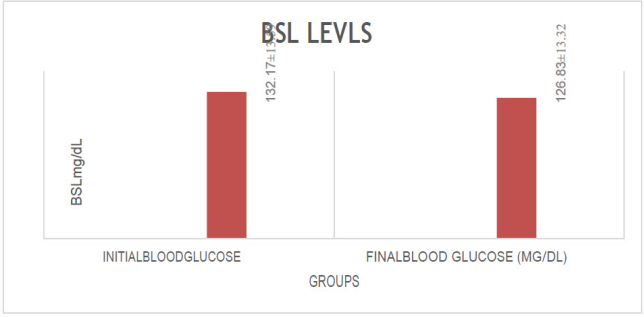

Figure 2: Graphical representation according levels of BSL.

Table 4: Descriptive statistics on mean blood glucose values of hyperglycemic subjects.

Pair	Mean	N	Std. Deviation	Std. Error Mean
Initial Blood Glucose	132.17	53	13.591	1.867
Final Blood Glucose	126.83	53	13.315	1.830

Table 5: Results of paired sample statistics on blood sugar levels of hyperglycemic subjects.

Paired Samples Test									
		Paired Differences						f	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
air 1	Final Blood Glucose (mg/dL) Initial Blood Glucose	-5.340	7.090	0.974	-7.294	-3.385	-5.483	2	.000*

*= Significant (P≤ 0.05 = Significant)

Table 6: Results of one sample statistics on blood sugar levels of hyperglycemic subjects.

One-Sample Test						
	Test Value = 0					
	t	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference Lower	Upper
Initial Blood Glucose	70.797	52	0	132.17	128.42	135.92
Final Blood Glucose (mg/dL)	69.344	52	0	126.83	123.16	130.5

*= Significant (P ≤ 0.05 = Significant)

g nitrogen free extracts per 100 g seeds sample respectively. Another study by Bukhari et al. [28], outlined the compositional profile of *Foeniculum vulgare* as containing moisture, protein, fat, fiber, ash and nitrogen free extract as 6.24% ± 0.24%, 9.38% ± 0.39%, 9.76% ± 0.34%, 18.21% ± 0.73%, 12.97% ± 0.51% and 43.44% ± 1.82%, respectively. The determination of total phenolic content of fennel by Faudale et al. [29], explored the values of total phenolic content using ethanol to be 104.0 mg ± 3.8 mg GAE/g. Also in the same study, the DPPH values of fennel using ethanolic and n- hexane solvents extracts were 116.8% ± 4.3% and 92.7% ± 2.1 % respectively. Hyperglycemia is a recognized risk factor for diabetes. Many herbs such as *Foeniculum vulgare* mill usually distinguished as fennel seeds due to its anti-diabetic activities have therapeutic properties to subordinate the elevated blood glucose level. In a study by Sania Zulfiqar in 2019 study, the hyperglycemic patients were distributed into three groups (G0, G1, and G2) that were prescribed to use a calculated quantity of fennel seeds at doses once daily on empty stomach in varying proportions. There was a significant decline in the blood glucose levels in diabetic patients after two hours of fennel administration. Before fennel administration, the mean values exhibited as 313.5 mg/dL ± 108.69 mg/dL and 279.33 mg/dL ± 96.24 mg/dL for patients having 100 mg per kg body weight and after 2 hours, the blood glucose levels for treated individuals showed mean values of 262 mg/dL ± 88.69 mg/dL for individuals having 100 mg per kg body. The mean values for control group showed as 272.16 mg/dL ± 89.84 mg/dL before and 330.5 mg/dL ± 91.87 mg/dL after two hours [25]. Another study conducted by Mhaidat et al. [30], in which anti hyperglycemic effects of *Foeniculum vulgare* extract were evaluated in streptozotocin-induced diabetes in rats. The controller and diabetic animals were cured by *F. vulgare* extracts (100 mg/kg). The results showed that action with *F. vulgare* abridged hyperglycemia in diabetic animals, and also condensed the hyperglycemia-associated elevation in levels of amylase, total cholesterol, ALT, AST, urea and creatinine signifying a defensive outcome on the liver.

Conclusion

The current research project confirmed the high antioxidant potential of fennel seeds owing to the presence of bioactive substances, known as 'phytochemicals', which are highly beneficial in imparting the health benefits and maintaining an integral physiological functioning of the human body. This fact was confirmed in our study by analyzing the blood glucose levels of hyperglycemic subjects for a period of two months by keeping them on feeding trial with fennel extract based cookies, which proved beneficial in lowering the circulatory blood glucose concentrations in the research subjects. Due to this certainty, the prepared fennel cookies, better to be termed as 'functional cookies', can be commercialized as ready- to-serve cookies to meet consumers' demands for a healthful snack.

Recommendations

It is recommended that Fennel is an extraordinary source of major and minor nutrients. It acts as an antioxidant, preventing the deteriorating health conditions such as hyperglycemia. Its bioactive composition makes it a potential candidate for inclusion in nutraceuticals industry. It is also strongly recommended to develop other functional foods containing fennel besides the cookies and to investigate their effects on various health states.

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