

Effect of Hexanic and Alcoholic Extracts of Fenugreek Seed in Male Diabetic Rats

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Article information	Abstract
<p>Article history: Received: 12 Mar 2012 Accepted: 16 May 2012 Available online: 29 Oct 2012 ZJRMS 2013; 15(6): 50-53</p> <p>Keywords: Diabetes Complications Trigonella Cholesterol Blood glucose</p> <p>*Corresponding author at: Department of Physiology, Zeinab Paramedicine -Nursing & Midwifery School, Guilan University of Medical Science, Langrood, Iran. E-mail: mahmood.abedinzade@gmail.com</p>	<p>Background: Diabetes mellitus is recognized with severe complications. Many herbal medicines have been recommended for treatment of diabetes problem. In this study, the effect of hexanic and alcoholic extracts of fenugreek (<i>Trigonella-foenum graecum</i>) on serum parameters was investigated in normal and streptozotocin-induced diabetic rats.</p> <p>Materials and Methods: This experimental study was carried out in 2011 at paramedical school of Guilan University of Medical Sciences, 48 male Sprague Dawley rats (230-300 gram) were divided into six groups: control, type 1 diabetic, and 2 diabetic groups that receive alcoholic extract and 2 groups receive hexanic extract of fenugreek (100, 200 mg/kg body weight) intraperitoneally for 28 days. For diabetes induction, streptozotocin (60 mg/kg/ intraperitoneally) was used. Blood glucose, total cholesterol, triglyceride (TG), urea, creatinine, uric acid, AST and ALT level was measured. Data were analyzed with spss software 16 and One Way ANOVAs and Tukey test. $p < 0.05$ was statistically significant.</p> <p>Results: fenugreek extract inhibit weight loss especially in diabetic groups that receive hexanic extract ($p=0.006$). blood glucose, total cholesterol, TG, urea, creatinin, uric acid, AST and ALT level was reduced significantly in diabetics groups that receive fenugreek extract ($p=0.001$). This effect was stronger in groups that receive Hexanic extract</p> <p>Conclusion: Fenugreek is a good candidate for reduction of diabetic complications.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Diabetes is a kind of disease that has been recognized since long time ago and there have been a lot of efforts in order to cure it. The term Diabetes is taken from Greek word Diabianin meaning increasing urine volume. Right now diabetes is increasing around the world and is considered as sixteenth factor of mortality in the world.

There are 150 million people suffering from diabetes now and this will raise up to 300 million people or more until 2025. Recent studies show that India has the most number of people affected by diabetes. Today, there are 25 million people suffering from diabetes in India, that is more than any other countries and this number will rise up to 57 million people until 2025 [1, 2].

Medical plants have always had relationship with humans since ancient times so that Iranians have had an advanced knowledge in identifying and applying medical plants since old times [3]. Fenugreek is one of the medical plants that has used in traditional medicine and in different nations and great medical effects have been mentioned for this plant. The name of this plant is taken from Greek word trigonou meaning triangle referring to the triangle- shape of follicles [4].

In ancient Egypt fenugreek was used in order to facilitate baby delivery and increase milk- feeder. In India fenugreek is methi and is used as spice to increase milk. The effects of fenugreek are as follows: expectorant, cardiac, astringent, increasing sexual desire, anti cramp,

decreasing blood cholesterol, blood glucose and blood pressure [5, 6]. In an animal research, done by vijaya kumar [7] in mice, comparing to Insulin, 15 mg/kg/ daily extract of fenugreek seed could decrease the amount of 115 unit insulin /kg in diabetic rats with low alloxane.

In another study aqueous- alcoholic extract of fenugreek could decrease plasma glucose either orally or via peritoneal injection in diabetic rats [8]. Medical plants are potential sources that can be used appropriately for medical, nutritional, economical applications, Also a lot of medical plants and their products are exported unrefined while the import of their products costs a lot [9]. Due to the different ingredients present in the aqueous and hexanic extract of fenugreek and because of no study has been done on the effects hexanic extract of *Trigonella foenum*. This study were designed to investigate the effect of hexanic and alcoholic extract of fenugreek seed on serum parameters in male diabetic rats.

Materials and Methods

In this study, first 48 male mature rats (230–300 gr) was purchased from pastor Institute in Amol and after transferring rats to paramedical faculty, they were kept in animal room for 7 to 15 days so that they yet close to their ideal weight in addition to having adaptation with environment. During this time rats kept in animal room under 12 hours light, 12 hours darkness, in $22 \pm 1^\circ\text{C}$ temperature.

Plant sample and its extraction method:

Fenugreek seed was supplied from grocery in Guilan province and then was identified and confirmed by herbarium part of agricultural research centre in Guilan province. In this study maceration method was used in order to extract efficient material. First fenugreek seed was powdered by quern then extraction procedures were done. In order to provide aqueous-alcoholic extract of fenugreek, first Ethylic Alcohol (96%) is diluted by distilled water, then for each gram of fenugreek powder, 5 ml of Ethylic alcohol is used.

In the beginning, a little alcohol is added to fenugreek powder for 3 days and the product is gathered in the previous container, in third procedure a little more of Ethylic alcohol is added to the powder for 2 days and the produced solution is added to the previous solutions, finally, Reni 2 powder is washed, then the produced material is transferred to the container of solutions.

The gathered solutions are strained by filter paper (watman) then is distilled to nearly dry phase, under 50°C temperature, with rotation-speed of 70 turns a minute, in vacancy the produced extract, act is Kept under 4°C temperature till the usage time. Extract solution with desirable concentrations is provided by solving it in physiologic normal saline solution [10].

To provide hexanic extract of Fenugreek: hexanic extract of the plant is extracted by maceration method. First 100 gram of fenugreek seeds powder was covered by hexan solvent and kept for 3 days, then, hexanic extract was taken out & gathered and again a little hexan was added to the powder for 2 days and the produced solution was gathered in the previous solution container, in third procedure, a little more hexane was added to fenugreek powder and after 2 days the produced solution was added to previous solutions, then final solution was cleared by filter paper (watman) and the produced extract was thickened nearly to dry phase under 40-47°C temperature, with rotation speed of about 60-70 turn a minute. And hexan was separated from the extract. Procedures: In this study 48 male Sprague dawley rats randomly were divided into 6 groups:

1-Control group, 2-Diabetic group that received aqueous-alcoholic extract of fenugreek (100 mg dosage) for 28 days, 3-diabetic group that received aqueous-alcoholic extract of fenugreek (200 mg dosage) for 28 days, 4-diabetic group that received hexanic extract of fenugreek (100 mg) for 28 days, 5-dialectic group that received hexanic extract of fenugreek (200 mg) for 28 days, 6-diabetic control group that received no treatment.

After dividing, blood glucose of all rats was measured by glucometer. Then the groups that should become diabetic received intraperitoneally streptozotocin (60 mg/kg/IP) and 48 hours later their blood glucose was measured by glucometer. Blood glucose >300 mg/dl considered as diabetic.

Then, according to divisions, they received aqueous-alcoholic extract 8 hexanic extract of fenugreek with different days. At the end of the experiment, in 28th day, rats were kept hungry about 12 hours and blood samples were gathered under slight anesthesia with Ether, by a little scratch in tale area blood glucose was measured by glometer. Total cholesterol and Triglyceride were measured by Rifai and coworkers [11] method, urea was measured by Thomas method [12], uric acid by Fossati method [13], creatinin Serum by Thomas method [14]. Amino acetat transfrase and serum alanin transfrase were studied by Mass & Henderson method [15]. Statistical Analysis: In this research, all data is presented in Mean±SD and for statistical analysis, SPSS 16 program & Tukey test were used. $p < 0.05$ was considered as meaningful level. This research has been conducted in compliance with all ethical issues.

Results

Weight changes during 28- day period in control and 4 treated groups are shown in table 1. Comparing to control group, there was a considerable and meaningful weight loss in diabetic rats ($p=0.05$). Using fenugreek extract (hexanic and aqueous-alcoholic) cause gain weight compared to diabetic animals ($p=0.001$).

Table1. Changes in weight and blood glucose in normal and diabetic rats and the effect of aqueous /hexanic extract of TF on the mentioned parameters

	Normal	Diabetic control	Aqueous 100	Aqueous 200	Hexanic 100	Hexanic 200	p-Value
Beginning weight	235±15	240±1	252±12	247±8	230±14	241±7	0.079
Final weight	252±17	*200±9	*224±9	*225±13	*230±10	*238±9	0.006
Beginning glucose	99±4	*560±15	*520±10	*510±7	*485±11	*460±7	0.001
Final glucose	97±3	*565±10	*503±9	*460±13	*435±9	*380±14	0.001

* $p < 0.05$, Significant difference from the control group.

$p < 0.05$, Significant difference from the diabetic control group.

Table2. Changes in serum parameters following intraperitoneal injection of hexanic/alcoholic extracts of fenugreek in all groups (all values are mean±SD)

	Control	Diabetic Control	Alcoholic 100	Alcoholic 200	Hexanic 100	Hexanic 200	p-Value
Cholesterol	70±7	*120±9	*80±9	*78±8	*76±8	*77±6	0.049
Triglyceride	74±6	*122±8	*89±4	*87±5	*83±7	*82±8	0.016
Urea	40±11	*119±9	*110±11	*97±7	*83±6	*71±9	0.021
Creatinin	0.05±0.07	*0.79±0.04	*0.71±0.06	*0.68 ±0.05	*0.66±0.03	*0.61±0.06	0.001
Uric acid	1±0.7	*1.5±0.09	*1.4±0.07	*1.31±0.05	*1.2±0.07	*1.13±0.08	0.001
ALT	81±11	*152±7	*143±9	*121±7	*98±6	*83±4	0.001
AST	125±8	*201±13	*181±8	*173±9	*150±6	*145±7	0.001

* $p < 0.05$, Significant difference from the control group. # $p < 0.05$ Significant difference from the diabetic control group

Recent research results showed that there is a considerable increase in blood glucose level after STZ injection ($p=0.007$), total cholesterol, triglyceride, urea, uric acid, creatinine, aminoacetate transferase, alanin transferase ($p=0.05$) (in all diabetic rats comparing to control group) (Table2). Receiving hexanic extract and aqueous-alcoholic extract of fenugreek Leaf have a great effect in glucose level, total cholesterol, triglyceride, uric acid, urea, creatinine, amino acetat transferase, and alanin teransferse in diabetic rats, this impact was more considerable in the group with hexaninc extract (200 mg/kg).

Discussion

The existing results showed that diabetes causes great increase in blood glucose, triglyceride, cholesterol, uric acid, urea, creatinin, AST and ALT in studied rats but both hexanic andaqueous-alcoholic extracts of fenugreek could decrease the mentioned parameters considerably. Significant weight loss was observed in all diabetic groups. But using aqueous-alcoholic and hexane TF improves weight gain in diabetic rat, while diabetic rats prevented of the extract have the progressive loss in weight [16].

Diabetes induction increase dramatically blood glucose in all diabetic groups, but fenugreek extract significantly reduced blood glucose levels in diabetic rats. It is likely that the beneficial effects of bioactive compounds in TF due to some of its 4-hydroxy-isoleucine content [17].

In agreement with current study, hypoglycemic effect of fenugreek seed has been proved in rats that became diabetic, dogs, mice and healthy volunteers and diabetic patients (type 1 and 2) [18-20].

Saponin, alkaloids and trigonelin available in fenugreek extract causes control of intestinal absorption of glucose under lab conditions [21].

Diabetes mellitus is the commonest metabolic complications in human beings. In this study, levels of cholesterol and triglycerides in diabetic controls rats has increased. Injection of aqueous- alcoholic and hexanic extract of fenugreek seed in diabetic rats caused considerable decrease of cholesterol and triglyceride. Previous studies showed that using fenugreek caused decrease in cholesterol and Triglycerides in diabetic rats [22] and hyper cholestrolemic patients [23, 24].

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Hypolipidemic action of extract can be caused by delaying lipid and carbohydrate absorption as a result of bioactive fibers existing in fenugreek seed [2]. In this study, serum urea level, creatinin and uric Acid in diabetic rats increased. It can be caused as a result of metabolic disorder effect in diabetes and the act of gezantinoxidase, lipid peroxidation [25]. On the other hand glycozilation of protein in diabetes causes high demolition of muscle and increase of porin level that is the main source of uric Acid [26].

In present study, hexaninc and aqueous extract of fenugreek could lower the mentioned parameters level. The mechanism of this action is not distinguished exactly but decrease in blood glucose level as a result of fenugreek extract (4-hydroxy isoleucine has insulino thropic effect and [17] argenin and tryptophan that have anti diabetic and hypoglycemic effect) can have positive effect on lipid metabolism and cause decrease in urea, cratinin and uric acid [27]. Increase the level of Liver Enzymes (ALT, AST) can show active liver injury. Inflammatory hepatocellular disorders can increase transe aminases level considerably [28]. Five different flavonoids naming vetixin, trisin, naringenin, cortsin and glicopiranosid are recognized from fenugreek seed, they can protect different types of cells against cell injury caused by oxidative stress [29].

Considering the current study, be able to conclude that aqueous-alcoholic & hexanic extract of Fenugreek seed has anti diabetic, hypoglycemic, hypolipidemic effects and decrease hepatic enzymes level. But more research is necessary to study plants (as a proper candidate) for curing diabetes mellitus.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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