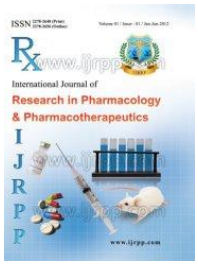
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278 -2648 ***IJRPP |Vol.4 | Issue 1 | Jan-Mar-2015***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Research article ***Open Access***

Anti-obesity and anticoagulant activity of ethanol extract of *Trigonella foenum-graecum* L

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ABSTRACT

Aim

To study the antiobesity and anticoagulant activity of ethanol extract of *Trigonella foenum-graecum* L. (Fenugreek) in male wistar rats.

Methods

Trigonella foenum-graecum L. was first extracted with ethanol and subjected to phytochemical analysis and its anti-obesity activity was studied in male wistar rats by feeding with cafeteria diet for 42 days in diet induced model and by administering a single intra peritoneal injection of Triton X-100 in chemical induced model. In diet induced model, weight of the animals was measured once in a week and parameters like total cholesterol (TC), triglycerides (TG), LDL, VLDL, HDL levels, clotting time were measured on 43rd day. In chemical induced model, the above parameters were measured on 8th day.

Results and Discussion

Preliminary phytochemical analysis revealed the presence of saponins, flavonoids, phyosterols, alkaloids, cardiac glycosides, coumarin glycosides, proteins and tannins & phenolic compounds. Significant increase in body weight, TC, TG, LDL, VLDL levels and reduction in HDL levels and clotting time was seen in animals fed with cafeteria diet. Treatment with standard drug orlistat and fenugreek extract significantly suppressed the increase in body weight and normalized the lipid levels and prolonged the clotting time.

Conclusion

From the results, it could be concluded that the ethanol extract of *Trigonella foenum-graecum L*. had significant anti-obesity and anticoagulant activity by maintaining the normal levels of physical and biochemical parameters and by prolonging the clotting time.

Keywords: *Trigonella foenum-graecum L*., cafeteria diet, obesity, clotting time, orlistat**,** body weight, total cholesterol**.**

INTRODUCTION

Obesity is termed as an excess body weight with an abnormally increased body fat and it is said as an imbalance between energy intake (feeding) and energy expenditure (physical activity) leads to obesity [1]. WHO has declared obesity as global epidemic [2, 3]. Now it is recognised as a major threat to the population worldwide. According to a survey conducted by WHO, there are over 1.6 billion overweight adults in which about 400 million are obese clinically [4, 5]. The popular method used clinically for the fat assessment and classification of obesity is by measuring the body mass index (BMI). BMI is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2). The plant *Trigonella foenum-graceum L.* (Fenugreek) is an annual, leguminous plant belongs to the family Fabaceae commonly grown in India, Nepal Afghanistan, and in the Mediterranean regions of the world. It is used as spice, condiment, seeds are used as antipyretic and leaves are useful to treat external and internal swellings and burns and to prevent hair falling off (yunani). The earlier studies conducted using this plant reported that this plant shown anticancer [6], antiplasmodial [7], analgesic & neuro pharmacological activities [8]. The major chemical constitutents found in this plant are Flavonoids, saponins (Diosgenin), alkaloids, Scopoletin, γ-Schisandrin, steroids, mucilage & gum galactomannan, p-Coumaric acid and Hymecromone. Due to the increased availability of fast food everywhere, which is high in caloric content and the increased sedentary life style had led to rise in obesity which is also associated with an underlying risk factor for various diseases like diabetes along with insulin resistance, hypertension, arthritis, cardiovascular diseases and various endocrine disorders. Obesity is a pro-inflammatory and prothrombotic state. It is stated that obesity may cause coagulation via leptin, by increasing the coagulation cascade activity and inhibiting the fibrinolysis [9]. In the Framingham offspring study conducted in 3230 subjects, cardiovascular risk factors and association with selected prothrombotic factors were assessed and the results was that in subjects with increased BMI and waist-hip ratio there was increased levels of fibrinogen and PAI-1 [10].

Although obesity is one of the major health problems there is no an effective drug to treat obesity because they all have undesirable side effects. Sibutramine and orlistat are the two drugs which are approved by FDA for obesity and weight management. Bariatric surgery also used for weight loss but only for the people who have BMI of $40kg/m^2$ or more and when the diet, exercise and drug therapy are not working. However, it is believed that botanicals provide a safer and natural way to human body in both pharmaceutical and nutraceutical aspect. Under the guidelines of the US Food and Drug Administration, drugs from plants can be developed much faster and less costly than conventional single-entity pharmaceuticals [11]. Therefore the present research was undertaken to investigate the antiobesity and anticoagulant effect of ethanol extract of *Trigonella foenum-graecum L.*

MATERIALS AND METHODS

Collection of plant material

Whole plant (with roots) of *Trigonella foenum-graecum L.* was collected from nearby market in Warangal, Telangana state and authenticated by Dr. Md. Mustafa, Department of Botany, Kakatiya University, Warangal.

Experimental animals

The present study was conducted using male wistar albino rats (120-150g) procured from Sanzyme Ltd, Hyderabad. All rats were allowed to free access to water ad libitum and a standard pellet diet (Pranav Agro Industries Ltd. India). The animal room was air conditioned at 23±1°c and maintained according to a 12h light-dark cycle. All the rats were acclimatized to the laboratory conditions for about one week prior to initiation of the study. Experiment was conducted according to the principles and guidelines provided by committee for the purpose of control and supervision of experiments on animals (CPCSEA). The research protocol was approved by Institutional Animal Ethics Committee (Approval No.06/SPIPS/IAEC/14).

Chemicals

Triton X-100 (Sigma Aldrich), Orlistat drug (Biocon Limited, Dehradun), Biochemical kits for Total cholesterol, Triglycerides, HDL-Cholesterol

(Transasia Bio-medicals LTD, Solan, HP-ERBA Diagnostics).

Preparation of ethanol extract of *Trigonella foenum-graecum L.*

Fenugreek whole plant (with roots) was collected from nearby market, washed and shade dried. 230g of powder is taken and extracted with ethanol using soxhlet apparatus at 50°c for 48hrs.The filtrate extract were then evaporated to dryness at 30°C under reduced pressure. Percentage yield was found to be 16.32% (37.55g). This extract was dissolved in 10% Tween 20 and used for further studies.

Phytochemical analysis

The plant extract was evaluated for the presence of carbohydrates, saponins, alkaloids, flavonoids, coumarin glycosides, cardiac glycosides, proteins, phytosterols, tannins and phenolic compounds by using standard procedures [12].

Experimental procedure

Obesity was induced using two methods [13] as follows.

1. Cafeteria diet induced obesity

This experimental study was conducted for 42 days in which cafeteria group received cafeteria diet, other groups (group 3, 4 & 5) received test and standard drugs along with cafeteria diet after half an hour of feeding. Cafeteria diet consists of combination of three diets-(a) Condensed milk (8 g) + bread (8 g); (b) Chocolate (3 g) + biscuits (6 g) + dried coconut (6 g) and (c) Cheese (8 g) + boiled potato (10 g) {this composition is for single rat}.

Animals were divided into five groups in which each group consists of six animals.

Group 1: Normal control (fed with normal pellet diet & vehicle).
Group 2: Disease control (fed with cafeteria diet).
Group 3: Cafeteria diet + TFG extract low dose (250mg/kg).
Group 4: Cafeteria diet + TFG extract high dose (500mg/kg).
Group 5: Cafeteria diet + standard drug (orlistat 30mg/kg).

2. Chemical induced obesity

In this model obesity was induced in rats using Triton X-100. After the acclimatization period rats were fasted overnight for 18 hours and they are administered with single intraperitoneal (IP) injection of Triton X‑100 (100 mg/kg) solution, freshly prepared in physiological saline solution. 72 hours after the injection of Triton X-100, two of the induced groups were treated with the test drug.

Rats were divided into five groups in which each group contains six rats.

Group 1: Normal control (Vehicle).
Group 2: Disease control (Triton X-100, 100mg/kg).
Group 3: Triton X-100 + TFG extract low dose (250mg/kg).
Group 4: Triton X-100 + TFG extract high dose (500mg/kg).
Group 5: Triton X-100+ standard drug (orlistat 30mg/kg).

Anticoagulant Activity of ethanol extract of *Trigonella foenum-graecum L.*

For screening of anticoagulant activity, blood samples were collected from diet induced groups on 43rd day and from chemical induced groups on 8th day through retro orbital plexuses under light ether anaesthesia. In order to determine the Clotting time Lee and White method [14] (Capillary tube method) was used.

Through one end of capillary tube blood was collected from retro orbital plexus and at every 30 seconds, a small piece of capillary tube was broken. To measure the time stop watch was used and this process was repeated until fibrin thread appeared, at the broken end of the capillary tube. The time interval between pricking the retro orbital plexuses and first appearance of the fibrin thread was recorded. This time was noted as the clotting time of blood.

Estimation of Physical parameters

Once in a week the body weight of rats in different groups was taken regularly for a period of 42 days.

Estimation of biochemical parameters

On 43rd day in diet induced model and on 8th day in chemical induced obesity model blood was collected using retro orbital puncture method and the serum was separated by centrifugation at 3000rpm for 15 minutes. Estimation of various biochemical parameters like Total cholesterol (TC), HDL-Cholesterol, Triglycerides (TG) was done using the serum samples. The Low Density Lipoprotein was calculated by using Friedwald equation [15]: Total Cholesterol – HDL - (Triglycerides/5). The Very Low Density Lipoprotein was calculated using formula: Triglycerides/5. Clotting time was measured using capillary tube method.

Statistical Analysis

Statistical analysis was performed by using One-way ANOVA followed by Bonferroni's multiple comparison tests in Graph pad prism version 5.3 software. All the results were expressed as Mean ± SEM. For all the tests $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

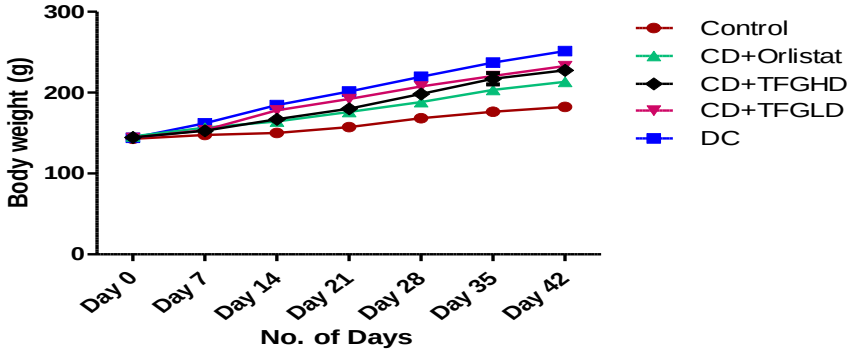
Preliminary Phytochemical screening.

The preliminary phytochemical screening of ethanol extract of *Trigonella foenum-graecum L.* revealed the presence of saponins, flavonoids, carbohydrates, alkaloids, cardiac glycosides, coumarin glycosides, phytosterols, tannins and phenolic compounds and proteins.

Effect of ethanol extract of *Trigonella foenum-graecum L.* on body weight in rats fed with Cafeteria diet

There was a significant increase in body weight in the group of animals fed with cafeteria diet (CD) compared with control group. In Orlistat treated group and in fenugreek extract treated group the body weight was significantly reduced when compared with disease control group (Figure 1).

Figure 1: Effect of ethanol extract of Trigonella foenum-graecum on body weight in rats fed with cafeteria diet for 42 days



In this graph we can observe that the group received cafeteria diet showed more increase in body weight than control. Orlistat and Fenugreek extract treatment along with cafeteria diet showed a marked suppression of increase in body weight than cafeteria diet fed group. From the two doses given, fenugreek extract in high concentration showed marked activity than low dose.

Effect of ethanol extract of *Trigonella foenum-graecum L.* on biochemical parameters in rats fed with Cafeteria diet

In the group of animals fed with cafeteria diet there was a significant increase in total cholesterol(TC), triglycerides(TG), LDL and VLDL cholesterol levels and decreased levels of HDL cholesterol when compared to control group animals. Treatment with standard drug Orlistat and test drugs significantly normalised the levels of TC, TG, LDL, and VLDL and increased levels of HDL levels when compared to disease control group (Table 1).

Table 1: Effect of ethanol extract of Trigonella foenum-graecum on biochemical parameters in rats fed with cafeteria diet for 42 days

GROUPS	TC (mg/dl)	TG (mg/dl)	LDL(mg/dl)	VLDL(mg/dl)	HDL(mg/dl)
Control	163.6±1.514	50.89±1.748	8.147±0.8727	10.18±0.3491	144.6±1.992
Disease control	213.7± 2.401*** [a]	119.7± 2.145***[a]	101.4± 1.791***[a]	24.00± 0.4367***[a]	88.32± 2.540***[a]
CD+ Orlistat	175.3± 2.436***[b]	68.28± 1.977***[b]	32.16± 3.516***[b]	13.65± 0.3961***[b]	131.2± 1.818***[b]
CD+ TFGLD	198.9± 1.738***[b,c]	89.24± 3.936***[b,c]	63.76± 3.586*** [b,c]	17.84± 0.7874***[b,c]	117.2± 2.433***[b, *c]
CD+ TFGHD	187.9±2.2 84***[b,**c]	104.5± 3.033**[b,***c]	63.82± 3.327***[b,c]	20.89± 0.6065**[b,***c]	103.2± 4.403**[b,***c]

All values are expressed as Mean±SEM; n= 6, *p<0.05 , ** p<0.01, *** p<0.001, a- compared to control group, b- compared to disease control group, c-compared to standard group, CD- Cafeteria diet, TFGLD= *Trigonella foenum-graecum L*ow dose, TFGHD=Trigonella foenum-graecum high dose.

Effect of ethanol extract of *Trigonella foenum-graecum L.* on biochemical parameters in rats treated with Triton X-100

Chemically obesity was induced by injecting Triton X-100 (100mg/kg i.p). In rats treated with only Triton X-100 showed significant increase in lipid levels like Total cholesterol, triglycerides, LDL, and VLDL levels where as decrease in the levels of HDL cholesterol when compared to control group. Treatment with standard drug Orlistat and test drugs significantly normalised the levels of TC, TG, LDL, and VLDL and increased levels of HDL levels when compared to disease control group (Table 2).

Table 2: Effect of ethanol extract of Trigonella foenum-graecum on biochemical parameters in rats treated with Triton X-100

Groups	TC(mg/dl)	TG (mg/dl)	LDL(mg/dl)	VLDL(mg/dl)	HDL(mg/dl)
Control	162.6±4.262	53.11±2.750	4.722±0.5451	10.62±0.5487	147.5±4.832
Disease control	222.3± 4.105***[a]	171.4± 2.898***[a]	101.2± 2.103***[a]	34.28± 0.5796***[a]	86.76± 4.683***[a]
CD+ Orlistat	185.2± 2.796***[b]	61.00± 5.033***[b]	34.35± 4.833***[b]	12.20± 1.006***[b]	138.5± 2.104***[b]
CD+ TFGLD	203.6± 2.519*[b, c]	107.0± 6.733***[b,c]	53.48± 3.118***[b,*c]	21.40± 1.347***[b,c]	128.7± 1.120[ns]
CD+ TFGHD	211.7± 4.463[ns]	86.93±7.534***[b,*c]	74.63± 6.062***[b,c]	17.38± 1.506***[b,*c]	119.7± 2.959***[b,**c]

All values are expressed as Mean±SEM; n= 6, *p<0.05,p<0.01, ***p<0.001.**

Effect of ethanol extract of *Trigonella foenum-graecum L.* on clotting time

Anticoagulant activity was performed using Lee & White Method (capillary tube method) by collecting blood after treatment period on 8th day from triton X treated groups and on 43rd day from cafeteria diet induced groups and the clotting time was recorded. In both models ethanol extract of fenugreek showed significant prolongation in the clotting time compared to control groups. Among the two doses given, Fenugreek ethanol extract in high concentration showed marked activity than low dose (Table 3).

Table 3: Effect of ethanol extract of *Trigonella foenum-graecum* on clotting time.

		Clotting time (sec)	
S.No:	**Groups**	**Cafeteria diet fed rats**	**Triton X-100 treated rats**
1	Control	137.2±6.069	136.7±8.913
2	Disease control	90.83±7.973**[a]	101.7±7.149*[a]
3	CD+TFGLD(250mg/kg)	281.8±7.525***[b]	232.0±4.235***[b]
4	CD+TFGHD(500mg/kg)	313.2±7.227***[b]	339.2±8.863 ***[b]

Values are expressed as mean±SEM, n=6, *p< 0.05,p<0.01,***p<0.001, a-compared to normal control, b- compared to disease control.**

DISCUSSION

Obesity is defined as an excess of adipose tissue that imparts major health risk [16]. It is the serious risk factor for development of cardiovascular diseases, diabetes mellitus along with insulin resistance, endocrine disorders and mainly increasing the risk of overall mortality[17]. The preliminary phytochemical screening of ethanol extract of *Trigonella foenum-graecum L.* showed the presence of carbohydrates, saponins, flavonoids, phyosterols, alkaloids, cardiac glycosides, coumarin glycosides, proteins and tannins & phenolic compounds. In the present study, the groups which received only cafeteria diet significantly increased the body weight compared to control group which received standard pellet chow diet and the animals which received standard and test drugs treatment showed suppression against increase in body weight compared to cafeteria diet fed animals was shown in Figure 1. Among the two doses of plant extract the high concentration (500mg/kg) showed marked suppression of increase in body weight compared to low dose. As shown in Table 1 & 2, there was observed a Significant elevation in levels of total cholesterol, triglycerides, LDL, VLDL and reduction in HDL cholesterol levels in rats fed with cafeteria diet which was a sugar/fat content diet and in Triton X-100 treated rats compared to control group. Treatment with standard drug orlistat and ethanol extract of fenugreek significantly normalised the levels of triglycerides, total cholesterol, LDL, VLDL and increased the levels of HDL-cholesterol levels when compared to cafeteria diet fed group and Triton X-100 treated groups. In this study the antiobesity potential of ethanol extract of *Trigonella foenum graecum* L. was compared with a FDA approved antiobesity drug Orlistat.

In case of obese people there are larger numbers of circulating micro vesicles (fragments of damaged cells) that bear the tissue factor. It is stated that obese people also have higher levels of coagulation proteins like prothrombin, factor VII, factor VIII, and Von Willebrand factor. The Plasminogen activator inhibitor-1, which is a fibrinolysis inhibitor, is also present in higher levels in people with obesity which leads coagulation of blood. Anticoagulant activity of plant extract was studied using Lee and White method. The group of rats fed with cafeteria diet and rats treated with Triton X-100 significantly reduced the clotting time compared to control group. The rats treated with test drug significantly prolonged the clotting time compared to cafeteria diet fed and Triton X-100 treated groups and control group as shown in Table 3. In both the models the high concentration of plant extract (500mg/kg) showed significant effect on clotting time.

As obesity is becoming globally a serious health problem and due to complications of currently available anti-obesity drugs there is a need for the development of drugs by identifying the potential phytochemicals from the traditional medicinal plants.

CONCLUSION

In the present study, the antiobesity activity of *Trigonella foenum-graecum* L. was observed through decrease in body weight and normalised levels of total cholesterol, triglycerides, LDL, VLDL and increase in HDL-cholesterol compared to disease control groups. Anticoagulant activity of plant extract was found through prolonged clotting time. From the

results, we can conclude that ethanol extract of *Trigonella foenum-graecum L*. have significant anti-obesity and anticoagulant activity. These activities may be due to the presence of various phytochemical constituents.

Further validation is needed for anti-obesity and anticoagulant potential and also to ascertain the exact molecular mechanism involved and to find out the particular components responsible for these activities.

ACKNOWLEDGEMENT

We are grateful to the management of St.Peter's Institute of Pharmaceutical Sciences, Warangal for supporting and providing facilities for research.

CONFLICTS OF INTEREST: There are no conflicts of interest.

REFERENCES

[1] Pilch PF and Bergenhem N. Pharmacological targeting of adipocytes/fat metabolism for treatment of obesity and diabetes. Mol Pharmacol. 2006; 70(3):779-85.

[2] Caballero B. The global epidemic of obesity: An overview, Epidemiol Rev, 2007; 29:1-5.

[3] Data and statistics [http://who.int/research/en/],(2012)

[4] Drew B, Dixon A and Dixon J. Obesity management: update on orlistat, Vasc Health Risk Manag.2007; 3: 817-821.

[5] The Global Epidemic [http://iaso.org/iotf/obesity/obesity the globalepidemic/] (2012)]

[6] Alshatwi A A, Shafi G, Noorul Hasan T, Naveed Ahmed S, K Khalid Khoja K**.** Fenugreek Induced Apoptosis in Breast Cancer MCF-7 Cells Mediated Independently by Fas Receptor Change. Asian Pac J Cancer Prev. 2013;14 (10**)**:5783-5788.

[7] Palaniswamy M, Pradeep B.V, Sathya .R, Angayarkanni.J. In Vitro Anti-plasmodial Activity of Trigonella foenum–graecum L. eCAM 2010;7(4)441–445.

[8] Akter M, Afroze M, Khatun A. Evaluation of analgesic, neuropharmacological and cytotoxic activity of *Trigonella foenum-graecum Linn*. Int. cur.r Pharm.j. 2011, 1(1): 6-11.

[9] Margaret A. Allman-Farinelli, Obesity and Venous Thrombosis: A Review. Seminars in thrombosis and hemostasis. 2011;37(8):903-907.

[10] Rosito GA, D'Agostino RB, Massaro J, et al. Association between obesity and a prothrombotic state: the Framingham Offspring Study. Thromb Haemost 2004;91(4):683–689.

[11] Raskin I, Ribnicky D, M Komarnytsky S, Ilic N, Poulev A, Borisjuk N, et al.,Plants and human health in the twenty-first century. Trends Biotechnol. 2002, 20, 522-531.

[12] Khandelwal K.R. Preliminary phytochemical screening. Practical Pharmacognosy Techniques and Experiments, 9th edition..Pune: Nirali Publications 2000. P.149-156.

[13] Vishnu Priya K, Himabindu P, A.B.Raju, Evaluation of antiobesity activity Of Fucus vesiculosus. Indian Journal of Research in Homoeopathy. 2013; 7(3):126-32.

[14] Bhavesh L,Synthesis and pharmacological screening o some analogue havingaryl/ heteroaryl nucleus having alkylaminohydroxypropoxy side chain", thesisPhD.2012.Avaliablefrom: http://etheses. saurashtrauniversity.edu /id/eprint/787

[15] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Am Assoc Clin Chem.1972;18:499‑502.

[16] Harsh Mohan, Environmental and Nutritional diseases. In: Text book of pathology,6th edition. New Delhi: Jaypee Brother's Medical Publishers (P) Ltd; 2010:243-245.

[17] Gurevich P T, Soumya P, Emilia W , Marek Los. Obesity: Pathophysiology and Clinical Management. Current Medicinal Chemistry. 2009, 16, 506-521.