PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 11 (11): 1004-1008, 2012
ISSN 1680-5194
© Asian Network for Scientific Information, 2012

Effect of Fenugreek (*Trigonella foenm greacum*) Seed Dietary Levels on Lipid Profile and Body Weight Gain of Rats

Atta Elmnan, A. Balgees and John L. Mangara
Faculty of Animal Production, University of Khartoum, Postal code 13314, Khartoum North, Sudan

Abstract: Thirty two healthy female Wister albino rats, average weight 144±5g and 3.5-4 months old were randomly divided into four groups T, TF1, TF2 and TF3 (with eight rats each). All groups were fed basal diet that contains sorghum floor, wheat bran, dry ground meat, maize oil and permix. However, TF1, TF2 and TF3 were supplemented with Fenugreek seed at 0.25, 0.50 and 0.75%, respectively. The experiment lasted for 12 weeks during which individual body weight was recorded weekly, while blood sample were collected 4 times during experimental period at weeks (6, 8, 10 and 12). Parameters measured were Total lipid, triglyceride, Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL). The results showed that fenugreek seed had a significant ($P<0.05$) decrease on plasma total, triglyceride and LDL. However, a numerical increase was observed in HDL with increasing levels of fenugreek seed. Long term administration fenugreek seed had a significant ($P<0.05$) decrease on plasma total lipid, triglyceride and LDL and a significant ($P<0.05$) increase was obtained on HDL in the last week. Progressive levels of fenugreek seed significantly ($P<0.05$) increase body weight gain.

Key words: Fenugreek seed, triglyceride, LDL, HDL, weight gain

INTRODUCTION

Fenugreek is an annual plant belonging to the family leguminasae, its Latin name is *Trigonella foenum graecum*. Fenugreek seeds are expected to have low toxicity to humans and animals since it is widely used for food and medicine (FAO, 1988). The seed is considered important both as food item and medicinal plant in many countries such as Egypt, India, Pakistan and Sudan (Mohiuddin *et al*., 1993).

Few years ago, the fenugreek *(Trigonella foenum graecum*) seeds were internationally believed to have restorative properties, the seed had high protein content (30%) and a good amount of lipid content (6.5%). Recently, fenugreek seed extracts have been described to contain steroid and saponins that increased food consumption, resulting in increased weight gain and reduced plasma lipids and cholesterol (Petit *et al*., 1995; Rao *et al*., 1996 and Sharma *et al*., 1996).

The current study is undertaken to investigate the effect of fenugreek seed (*Trigonella foenum graecum)* levels on lipid profile and body weight gain of rats.

MATERIALS AND METHODS

Experimental rats, site and duration: Thirty two adult female white albino rats average weight 144g and 3.5-4 months old were purchased from the Faculty of Pharmacy, University of Khartoum and transferred to the Faculty of Veterinary Medicine (Preventive Medicine Unit) University of Khartoum, Shambat. The experiment was conducted from 27th July to 27th October of the year 2009. After the animals were received, they were allowed 14 days of adaptation period, then initially weighed and allotted randomly 8 each to four treatment groups where increasing levels of fenugreek seed were administered at (T) 0% (TF1) 0.25% (TF2) 0.50% and (TF3) 0.75%. During the experimental period, body weight was individually recorded weekly.

Preparation of the experimental rations: Fenugreek seeds used in this study were obtained from the local market at Khartoum north (Bahri shabiya market). Five hundred grams of fenugreek seed were finely ground and added to the experimental diets as designed to each treatment with increasing levels 0, 0.25, 0.50 and 0.75%. Percent chemical composition of fenugreek seed is demonstrated in Table 1. The nutrient composition of the experimental and determined chemical composition of experimental diet (DM) are shown in Table 2 and 3 respectively. Four rations were formulated according to the nutrient requirements of the laboratory diet animals (rats) and randomly assigned to the experimental groups T, TF1, TF2 and TF3.

Chemical analyses: Fenugreek seeds and experimental diet were analyzed to their proximate components according the method of AOAC (1990). Plasma total lipid, triglyceride, low density lipoprotein and high density lipoprotein were determined using analytical methods described by (Frings and Dunn, 1970; Fossati and Prencipe, 1982; Assmann *et al*., 1984; Burstein *et al*., 1980), respectively.

Corresponding Author: Atta Elmnan, Faculty of Animal Production, University of Khartoum, Postal code 13314, Khartoum North, Sudan

Table 1: Percent chemical composition of fenugreek seeds (DM)

Component	Percentage (%)
Dry matter	94.11
Crude protein	28.60
Crude fibre	14.04
Ether extract	6.16
Ash	4.65
Nitrogen Free Extract	40.66
Metabolizable Energy (ME)	2368.17kcal/kg

ME: Calculated according to the equation of Lodhi *et al.* (1976)

Table 2: Percent composition of the experimental diet

Ingredient%	T	TF1	TF2	TF3
Sorghum	60.9	60.9	60.9	60.9
Wheat bran	8	7.78	7.55	7.30
Ground meat	13.07	12.98	12.89	12.83
Fenugreek seed	0	0.25	0.50	0.75
Maize oil	15.15	15.20	15.26	15.32
Salt	1	1	1	1
Premix	0.5	0.5	0.5	0.5
Di-Calcium phosphate	1.38	1.39	1.4	1.4
Total	100	100	100	100

T (0% F.S), TF1 (0.25% F.S), TF2 (0.50% F.S), TF3 (0.75% F.S), F.S (Fenugreek seed)

Table 3: Determined chemical composition of experimental diet (DM)

Component	T	TF1	TF2	TF3
Dry matter	96.53	95.30	95.27	95.09
Crude protein	13.35	13.05	12.77	12.96
Crude fibre	33.79	31.96	31.31	32.81
Ether extract	19.22	17.93	16.63	16.64
Ash	5.74	3.55	5.59	4.21
NFE	27.9	33.51	33.7	33.38
MEkcal/kg	3990.6	3990.5	3990.8	3991

T (0% F.S), TF1 (0.25% F.S), TF2 (0.50% F.S), TF3 (0.75% F.S), F.S (Fenugreek seed)

Blood chemistry

Blood sampling: The blood samples were collected four times during the experimental period from 6, 8, 10 and week 12. Blood was obtained from the medial canthus of the eye using capillary tube. The tip of the capillary tube was introduced into the medial canthus of the eye rotating the tube gently until the desired amount of blood was obtained. The blood was drown into a heparin tube 2 ml from each rat at every single collection and then centrifuged at 30°c for 5 minutes in order to obtained plasma for the determination of lipid profile. All samples of plasma were kept frozen at -20°c until analysis.

Statistical analysis: The data obtained from plasma samples and weight gain were subjected to statistical analysis of variance (ANOVA TABLE) for a completely randomized design, a Least Significant Difference (LSD) was carried out to test significant difference between the treatment means, using SAS (2000)

RESULTS

The effect of fenugreek seed levels on plasma lipid profile (mg/dl) in rats: As shown in Table 4 there was a significant difference ($p < 0.05$) among treatments on plasma total lipid, triglyceride and LDL concentration. Unsupplemented group (T) had the highest values of total lipids, triglyceride and LDL when compared to supplemented groups. Among the supplemented groups, the lowest value was recorded by (TF3). However, there was a numerical increase in HDL concentration among the treated groups, where group TF3 was higher than groups (TF2) and (TF1).

Table 4: Effect of fenugreek seed levels on plasma lipid profile of rats

Parameters	T	TF1	TF2	TF3	SEM
Total lipid	377.46^{a}	351.36^{b}	336.29^{c}	313.44^{d}	0.54
Triglyceride	118.34^{a}	109.02^{b}	85.16^{c}	76.72^{d}	0.75
LDL	82.33^{a}	73.45^{b}	66.46^{c}	62.33^{d}	0.45
HDL	35.17	35.51	36.18	36.56	0.47

T (0% F.S), TF1 (0.25% F.S), TF2 (0.50% F.S), TF3 (0.75% F.S), F.S (Fenugreek seed): (SEM) standard error of means.
$^{a-d}$Means with different superscripts in the same raw were significantly different ($P<0.05$).

Table 5: Long term effect of fenugreek seed levels on total lipid (mg/dl)

Week	T	TF1	TF2	TF3	SEM
W6	375.46^{a}	353.72^{b}	339.75^{c}	316.75^{d}	0.73
W8	377.48^{a}	351.90^{b}	336.96^{c}	314.52^{d}	0.60
W10	378.10^{a}	350.97^{b}	335.53^{c}	311.43^{d}	0.61
W12	378.81^{a}	348.85^{b}	332.92^{c}	311.08^{d}	0.91

T (0% F.S), TF1 (0.25% F.S), TF2 (0.50% F.S), TF3 (0.75% F.S), F.S (Fenugreek seed): (SEM) standard error of means.
$^{a-d}$Means with different superscripts in the same raw were significantly different ($P<0.05$).

Long term effect of fenugreek seed levels on plasma total lipid (mg/dl): The results of plasma total lipid of groups are shown in Table 5. The results indicated a significant ($p<0.05$) decrease in plasma total lipid concentration in (TF1) (TF2) and (TF3) when compared to (T). There was a periodic decrease in total lipid as (TF3) is lower than (TF2) (TF1) and (T).

Long term effect of fenugreek seed levels on plasma triglyceride (mg/dl) in rats: The results of plasma triglyceride of groups are shown in Table 6. The results indicated significant ($p<0.05$) decrease in plasma triglyceride concentration in groups (TF2) and (TF3) when compared to (T) and (TF1). However, week (10) demonstrated a significant decrease among the supplemented groups (TF1, TF2 and TF3). The last week displayed no significant difference in groups TF1, TF2 and TF3 but, a numerical decrease was obtained.

Long term effect of fenugreek seed levels on plasma Low Density Lipoprotein (LDL) (mg/dl) in rats: Fenugreek seed has a significant effect ($p < 0.05$) on Low Density Lipoprotein (LDL) on long term basis. This result is shown in Table 7, Group (T) reported the highest level of LDL and the last group (TF3) showed

Table 6: Long term effect of fenugreek seed levels on plasma Triglyceride (mg/dl)

Week	T	TF1	TF2	TF3	SEM
W6	119.88[a]	126.32[a]	88.51[b]	81.90[b]	2.1
W8	122.61[a]	118.77[a]	85.50[b]	77.88[b]	4.7
W10	117.60[a]	107.53[b]	84.21[c]	74.33[d]	1.4
W12	116.78[a]	79.63[b]	82.41[b]	72.76[b]	4.4

T (0% F.S), TF1 (0.25% F.S), TF2 (0.50% F.S), TF3 (0.75% F.S), F.S (Fenugreek seed): (SEM) standard error of means.
[a-d]means with different superscripts in the same raw were significantly different (P<0.05).

Table 7: Long term effect of fenugreek seed levels on low density lipoprotein

Week	T	TF1	TF2	TF3	SEM
W6	87.07[a]	74.73[b]	68.10[c]	63.43[d]	0.54
W8	83.20[a]	73.27[b]	67.53[c]	62.63[d]	0.57
W10	87.53[a]	72.90[b]	65.97[c]	62.33[d]	0.49
W12	81.50[a]	72.23[b]	64.23[c]	60.93[d]	0.84

T (0% F.S), TF1 (0.25% F.S), TF2 (0.50% F.S), TF3 (0.75% F.S), F.S (Fenugreek seed): (SEM) standard error of means.
[a-d]means with different superscripts in the same raw were significantly different (P<0.05).

Table 8: Long term effect of fenugreek seed on high density lipoprotein

Week	T	TF1	TF2	TF3	SEM
W6	34.10	33.77	32.96	34.93	0.61
W8	35.27	35.47	35.00	35.50	1.10
W10	35.97	36.13	37.17	37.43	0.76
W12	35.37[c]	36.67[bc]	38.37[ab]	39.60[a]	0.58

T (0% F.S), TF1 (0.25% F.S), TF2 (0.50% F.S), TF3 (0.75% F.S), F.S (Fenugreek seed): (SEM) standard error of means.
[a-d]Means with different superscripts in the same raw were significantly different (P<0.05).

Table 9: Effect of fenugreek seed levels on body weight gain (g/day)

Treatment	Body weight gain (g/day)+
T	0.17[a]
TF1	0.35[b]
TF2	0.39[c]
TF3	0.46[d]
SEM	0.01

T (0% F.S), TF1 (0.25% F.S), TF2 (0.50% F.S), TF3 (0.75% F.S), F.S (Fenugreek seed): (SEM) standard error of means.
[a-d]Means with different superscripts in the same colmn were significantly different (P<0.05).

lower level through out the period. Increased levels of fenugreek seed significantly (p<0.05) decreased low density lipoprotein.

Long term effect of fenugreek seed levels on high density lipoprotein (HDL) (Mg/dl): Table 8 showed the result of plasma High Density Lipoprotein (HDL). Weeks (6, 8 and 10) showed a numerical increase but, long term used of fenugreek seed reported significant (p<0.05) increase in HDL particularly at the end of 12 weeks.

The effect of fenugreek seed levels on body weight gain (g/day) of the rats: As shown in Table 9 There was a significant (p<0.05) difference on body weight gain. The higher increase was obtained in group (TF3) where the gain was (0.45g) while the lowest value is recorded by group (TF1) which had (0.34g) body weight gain.

DISCUSSION

The present study has been undertaken to manifest the effect of fenugreek seeds on lipid profile and body weight gain of rats. Parameters of lipid profile were done for all the experimental groups in which estimation was carried out after week 6, 8, 10 and 12 of the experiment. A Significant (p<0.05) decreased was obtained in plasma total lipid, triglyceride and LDL with numerical elevation in HDL in the supplemented groups. These results agree with those reported by Chaturred and Pant (1987); Awal *et al.* (1999); Al-Habori and Raman (1998); Prasanna (2000) and Wan *et al.* (2007).

There was a significant decreased on total lipid, triglyceride and LDL concentration on long term application of fenugreek seed in all the supplemented groups TF1, TF2 and TF3. However, long term administration of fenugreek seed had a significant increase on High Density Lipoprotein (HDL).

Low density lipoprotein and high density lipoprotein worked as antagonist, decreased in LDL, increased HDL which represents protection against atherosclerosis and coronary heart disease (Murray *et al.*, 2003; Oraby, 2005).

The reduction in total lipid, triglyceride, LDL and increase of HDL in this study may be due to crude fibre and saponins content in fenugreek seed. Madar *et al.* (1990) reported that fibre from fenugreek seed and brown rice were found to control lipid metabolic disorders. Al-Habori and Raman (1998) reported that the crude saponins extract was the most effective in reducing hypercholesterolemia.

Saponins and fibre content of fenugreek seed were found to reduce cholesterol absorption (Malinow *et al.*, 1977). The lipid profile is extracted in bile and then reabsorbed from the intestine. Therefore, it can be suggested that saponins may bind bile salts which are necessary for absorption of these substances or bind directly in the intestine and prevent reabsorption (Madar *et al.*, 1990). This may be due to the fact that saponins form insoluble complex with lipid profile (Rao *et al.*, 1996).

The presence of soluble fibre may block cholesterol absorption from the intestine (Lanksy, 1992). Saponins and crude fibre extracted from fenugreek seeds significantly resulted in reduction of cholesterol and triglyceride (El-Hussary, 1993). Fenugreek seed may reduce triglyceride by decreasing non Esterified Fatty Acids (NEFA) which are always the major component for this fraction of lipid profile (Reinila, 1981). The

hypolipidemic action could be the result of retardation of carbohydrate and fat absorption due to the presence of bioactive fibre in the fenugreek seed (Hannana *et al*., 2003).

In this study, fenugreek seed was found to increase body weight. This result agree with Rguibi and Belahsen (2006) and Wan *et al*. (2007). The effect of fenugreek seed on body weight gain may be due to the fact that, the seed is considered as an appetite stimulating agent (Blumenthal *et al*., 1998, Bruneton, 1999 and Borca *et al*., 2000), thus increased feed intake and a better utilization of nutrients in the diet lead to increase in body weight. Also Petit *et al*. (1995); Sharma *et al*. (1996) and Rao *et al*. (1996) reported that saponins found in fenugreek seed increased food consumption thus, resulted in increased weight gain.

In state of the high levels of antinutritive substances such as trypsin inhibitor, tannins, trigonella and saponins (Afifi *et al*., 1988), the rats never displayed any signs of toxicity which is in agreement with the findings of FAO (1988); Al-Habori and Raman (1998); Rao *et al*. (1996) and Sharma *et al*. (1996).

Summary: From the results, it can be suggested that fenugreek seeds could be considered as effective agent for hypolipidemic and weight gain purposes.

REFERENCES

Afifi, F.A., M.B. Mohammed and N.A. Elmahi, 1988. Fenugreek seed Extracts as a Protestants of wheat grains against stored products. Ann. Agric. Sci., 33: 1331.

Al-Habori, M. and A. Raman, 1998. Antidiabetic and Hypocholesterolaemic effects of fenugreek seed. J. Phytotherapy Res., 12: 233-242.

AOAC, 1990. Association of official analytical chemists. Official Methods of analysis (8th Ed) Washington D.C U.S.A.

Assmann, G., H.U. Jabs, U. Kohnert, W. Nolte and H. Schriewer, 1984. LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate. Clin. Chem. Acta, 140: 77-83.

Awal, M.A., M.U. Rashid, K.W. Ahamed, Z.S. Asadi and K. Islam, 1999. Effect of karela and fenugreek on lipid profile in hypocholesterolemic diabetic patients. Bangladesh J. Physiol. Pharmacol., 15: 6-8.

Blumenthal, M., W.R. Busse, A. Goldberg, J. Grunewald, T. Hall, C.W. Riggins and R. Rister, 1998. The complete German Commission E Monographs: therapeutic Guide to Herbal Medicines. America Botanical Council; Austin, Texas (Sigrid Klein, Primary Translator).

Borca, C., M. Manteghetti and R. Corss, 2000. 4 Hydroxyisoleucine: effects of synthetic and natured analogs on insulin secretion. Eur. J. Pharmacol., 390: 339-354.

Bruneton, J., 1999. Pharmacognosy: phytochemistry, medicinal plants (2nd Ed.). Intercept; London, Paris and New York.

Burstein, M., H.R. Scholnick and R. Morfin, 1980. Rapid method for the isolation of lipoprotein from human serum by precipitation with polyanious. Scand J. Clin. Lab. Invest., 40: 583-595.

Chaturred, V. and M.C. Pant, 1987. Effect of feeding *Trigonella foenum graecum* (methi) leaves on serum cholesterol, triglycerides and high density lipoprotein cholesterol in normal rabbits. Current Science, USA, 56: 600.

El-Hussary, N.A., 1993. Effect of fenugreek seed decoction on blood glucose, cholesterol and triglycerides level in normal and alloxan diabetic rabbits. Irags J. Vet. Sci., 6: 102.

FAO, 1988. Traditional food plants and nutrition. Paper no.42, Rome Italy, pp: 593.

Fossati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Chin. Chem., 28: 2077-2080.

Frings, C.S. and R.T. Duun, 1970. Determination of total lipids based on the color obtained by phosphovaniline after digestion in sulphuric acid conc. Anim J. Clin-Path, 53: 89-91.

Hannana, J.M.A., B. Rokeya and O. Faruque, 2003. Effect of soluble dietary fibre fraction *Trigonella foenum graecum* on glycemic, insuinemic lipidemic and platelet aggregation status of type 2 diabetic model rats. J. Ethnopharmacol., 88: 73-77.

Lanksy, P.S., H. Schulcher, J.D. Phillipson and W.D. Loe, 1992. Plant that lowers cholesterol. Acta-Horticulturae, 332: 131.

Lodhi, G.N., D. Singh and J.S. Ischhponani, 1976. Variation in nutrient content of feeding stuffs rich in protein and re-assessment of chemical method of metabolizable energy estimation for poultry. J. Agri. Sci., 86: 203.

Madar, Z., Y. Birk, A. Dovart, M. Waldman and H. Uzureau, 1990. Dietary fiber from legume seeds in control of blood lipids and glucose. Proceedings of the joint CEC-NCRD workshop held in Israel (G. nocar kibbutz) in January (1989) on lupine production and bio-processing for feed, food and other by-products. EUR. Publication No. 12641:115.

Malinow, M.R., R. Melaughlin, L. Papworth, C. Stafford, G.O. Kohler, A.I. Livingston and P.R. Cheeke, 1977. Effect of alfalfa saponins on intestinal cholesterol absorption in rats. Am. J. clin. Nut., 30: 2061.

Mohiuddin, S., A.O. Roshan, A. Zamir and A.Q. Salim, 1993. Laboratory evaluation of some vegetable oils as protectants of stored products. Pak. J. Sci. Indus. Res., 36: 377.

Murray, R.K., D.K. Granner, P.O. Mayes and V.W. Rodwell, 2003. Harper's Illustrated Bioch. 26th edition. Appleton and Lange Medical publication/MC Graw Hill.

Oraby, S., 2005. Oraby's Illustrated Reviews of Biochemistry for medical students and postgraduates part II eleventh Edn., pp: 139-153.

Petit, P.R., Y.D. Sauvaire, D.W. Hillarie, O.M. Lectone, Y.G. Baissae, G.R. Ponsin and G.R. Ribes, 1995. Steroid saponins from fenugreek seeds. Extraction, purification and pharmaceutical investigation on feeding behavior and plasma cholesterol. Steroids, 60: 666-724.

Prasanna, M., 2000. Hypolipidemic effect of fenugreek: a clinical study, Ind. J. Pharma, 32: 34-36.

Rao, P.U., B. Sesikeran, P.S. Rao, A.N. Naidu and V.V. Rao, 1996. Ramachandran E.P. short term nutritional and safety evaluation of fenugreek seed. Nutr. Res., 16: 1495-1505.

Reinila, A., 1981. Long term effects of untreated diabetes on the arterial wall in rat. Diabtologia, 20: 205-212.

Rguibi, M. and R. Belahsen, 2006. Fattening practices among Moroccan Saharawi women. East Mediterranean Health. J., 12: 619-624.

SAS, 2000. SAS/STAT Guide for Personal Computers (Version 8). Statistical Analysis Systems institute, Cary, NC, USA.

Sharma, R.O., A. Sarkar, D.K. Hazra, J.B. Misra and B.B. Maheshwari, 1996. Toxicological evaluation of fenugreek seeds a long term feeding experiment in diabetic patients. Phytotherapy Res., 10: 519.

Wan, L.X., S.L. Xuan, Z. Jian, H.L. Yong, L.W. Zhi and J.Z. Rui, 2007. Effect of *Trigonella foenum graecum* (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin induced diabetic rats. Asia. J. Clin Nutr., 16: 422-426.