Journal of Medicinal Plants Research Vol. 5(19), pp. 4804-4813, 23 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Hypolipidemic effect of soluble dietary fiber (galactomannan) isolated from fenugreek seeds in WNIN (GR-Ob) obese rats

Punna Ramulu[1]*, Nappan Veettil Giridharan[2] and Paruchuri Udayasekhararao[1]

[1]Food Chemistry Division, National Institute of Nutrition, Indian Council of Medical Research, Jamai-Osmania (P. O.), Hyderabad-500604, India.
[2]National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Indian Council of Medical Research, Jamai-Osmania (P. O.), Hyderabad-500604, India.

Accepted 22 July, 2011

The hypolipidemic effect of a soluble dietary fiber isolated from fenugreek seeds rich in galactomannan was studied in WNIN/GR-Ob a mutant obese rat strain. The study consists of fifty five obese rats randomly divided into five groups of 6 males and 5 females in each group. Group I was fed with control diet, while Group II and IV were fed with diet containing 10 and 20% fenugreek seeds, whereas Group III and V received diet containing 2.5 and 5% galactomannan respectively for 9 weeks. The results showed significant ($p<0.05$) reduction in gain in body weight in experimental animals without any effect on their food intake. After 9 weeks, significant ($p<0.05$) reduction was observed in lipemic parameters (triglycerides and total cholesterol) in experimental groups from 3rd week onwards. The HMG-CoA reductase activity was significantly low in control group as compared with experimental groups. Concomitantly fecal neutral sterols excretion was significantly ($p<0.05$) higher in experimental groups, compared to control. The results which demonstrated the hypolipidemic effect of galactomannan extracted from fenugreek seeds for the first time in obese rats could be due to the increased activity of HMGA-CoA reductase, which is a key hepatic regulatory enzyme in experimental animals leading to excretion of bile acids and neutral sterols in feces.

Key words: Fenugreek seeds, galactomannan, soluble dietary fiber, hypolipidemic effect, obese rat, bile acids, neutral sterols, HMGA-CoA reductase.

INTRODUCTION

Cardiovascular disease (CVD) is one of the major killers, National Institute of Nutrition; short chain fatty acids (SCFA), prevailing in the developed as well as in developing coun-tries. This is attributed to modifiable (lifestyle-related) risk factors, and as well as to non-modifiable (constitutional) risk factors.

Major modifiable risk factors for CVD include hyper-lipidemia, hypertension, smoking, diabetes mellitus, obesity, physical inactivity, high intake of saturated fat and poor stress management. The non-modifiable risk factors are old age, family history of premature CVD and high blood pressure. Therapeutic implications of dietary fiber (DF) in CVD suggest that it can be used as an adjuvant. Hence increased intake of DF has beneficial effect in the treatment of specific cardiovascular related conditions (Jenkins et al., 2001). Hyperlipidemia is a dis-order, showing elevated levels of serum total cholesterol (TC) and triglycerides (TG) above the normal range.

To control hyperlipidemia, different strategies are used like anti-cholesterol drugs (Kritchevsky, 1999), restriction of calorie intake from saturated fat (Lairon, 1996),

*Corresponding author. E-mail: p_ramulu2001@yahoo.co.in. Tel: +91 40 27197243. Fax: 91 40 27019740.

Table 1. Chemical composition of isolated fraction from fenugreek seeds*.

Ingredient	g/100 g
Moisture	8.85
Protein	6.51
Fat	0.98
Ash	1.17
TDF	82.49
IDF	15.58
SDF	66.91

*Representative sample. TDF: Total dietary fiber, IDF: insoluble dietary fiber, SDF: soluble dietary fiber.

supplementation of polyunsaturated fatty acids (Jenkins et al., 1993) and also adding DF rich foods or supplements such as psyllium, guar gum, low cost bean gum, and fenugreek seed powder (Fernandez, 1995; Fernandez et al., 1994, 1995a, b).

Fenugreek seeds (FGS) are good sources of soluble dietary fiber (SDF) and their consumption have been shown earlier to bring about a significant reduction in serum and liver cholesterol levels (Sharma, 1986; Al-Habori et al., 1988; Al-Habori and Raman, 1998). The factors responsible for this effect have so far not yet been established, however, it has been suggested that total dietary fiber (TDF) might have played a key role in reducing the cholesterol levels through increased fecal excretion of bile acids and salts as well as inhibitors of hepatic cholesterol biosynthesis by short chain fatty acids produced by bacterial fermentation of SDF in the lower parts of large intestine (Kishimoto et al., 1995). In the present study FGS as well as galactomannan (GM) contained in the SDF were investigated for its hypolipidemic effects in a genetically obese rat model with impaired glucose tolerance (IGT) established at our center. For the first time attempts are made in this new obese mutant rat model (WNIN/GR-Ob), to screen the hypolipidemic effects of FGS. The WNIN/GR-Ob is isolated from the parental WNIN rat strain, and apart from physical symptoms of obesity, these animals also showed IGT, hypertriglyceridemia, hypercholesterolemia, hyperinsulinimia, and are leptin resistant as well (Giridharan et al., 1996, 1997).

MATERIALS AND METHODS

Chemicals

All the chemicals required for the analysis of serum and fecal parameters were obtained from indigenous sources, while HMG-CoA reductase (HMG-CoAR) activity in liver microsomes was performed using sigma chemicals.

Experimental animals

Obese rats (WNIN/GR-Ob) were obtained from National Centre for Laboratory Animal Sciences (NCLAS), at National Institute of Nutrition (NIN), Hyderabad, India. The experimental protocol of the study was cleared by the Institutional Animal Ethical Committee (IAEC).

Isolation of soluble dietary fiber from fenugreek seeds

Twenty five kilograms of FGS were purchased from the local super market, cleaned and powdered in a cyclone mill. The FGS powder was weighed in a plastic container and 10 to 12 volumes of dilute acetic acid solution was added and mixed thoroughly. Constant mixing of the sample with mechanical stirrer was performed for four hours at 4°C and transferred into one liter plastic bottles. Centrifuged at 4°C, using 3000 RPM for 30 min. Supernatant solution was quantitatively transferred and volume was measured, four volumes of 95% ethyl alcohol was added, mixed thoroughly and allowed to settle for one hour to get precipitation of SDF fraction.

It was filtered through muslin cloth and washed with 95% alcohol, ether and finally the residue was collected in a tray, after which it was dried overnight in hot air oven at 60°C and powdered in the cyclone mill. This isolated powder had 66.91% SDF (Table 1) enriched with galactomannan (Dea and Morrison, 1975; Evans et al., 1992) and used for the preparation of experimental diets for feeding obese rats.

Preparation of experimental diets

As described earlier (Sharma., 1986; Al-Habori et al., 1988; Al-Habori and Raman, 1998) 2.5 and 5% of isolated SDF fraction were incorporated into experimental diets (Table 2). Casein based laboratory diet was prepared according to Reeves et al. (1993). All the diets were made isonitrogenous by adjusting the protein content of all the diets to be uniform at 20% level and these were used for feeding animals for a period of 9 weeks.

Experimental design

The experiment was conducted in 50 days old WNIN/GR-Ob rats. Before the start of the experiment, animals were screened for their serum TG, TC levels and animals showing high fasting serum TG levels than the normal levels were taken for the experiment. The animals were divided into five groups; Group I - control, Group II - 10% FGS, Group III - 2.5% GM; Group IV - 20% FGS and Group V- 5% GM. Each group had 6 males and 5 females. The obese rats were housed individually in plastic cages in a room maintained with 20°C at 50% humidity. Diet and water were provided *ad libitum* for 9 weeks and the following parameters were studied.

Parameters

Weekly body weights and food intake of individual rats were monitored. Serum TG and TC levels of all the animals were measured at initial, 3, 6 and 9 weeks points. Fecal bile acids, neutral sterols and HMG-CoAR activity in liver microsomes after completion of feeding of diets were performed at 9 weeks. Feces

Table 2. Diet composition (g/100 g).

Ingredient	Group I	Group II	Group III	Group IV	Group V
Vitamin mixture	1	1	1	1	1
Salt mixture	4	4	4	4	4
Groundnut oil	5	5	5	5	5
Cellulose	5	5	5	5	5
Casein	29	25.5	29	22	29
Choline chloride	0.2	0.2	0.2	0.2	0.2
Galactomannan	0	0	2.5	0	5
Fenugreek seeds	0	10	0	20	0
Starch	55.8	49.3	53.3	42.8	50.8

All the diets were made isonitrogenous by adjusting the protein content of all the diets uniform at 20% level and these were used for feeding animals for a period of 9 weeks.

and food left by individual animals were collected during the last week of the experiment. The fasting blood was collected at initial, 3, 6 and 9 weeks and animals were sacrificed. Livers of the rats were collected, weighed and about 2 to 3 g of liver was taken in cold Tris-TEA buffer for isolation of liver microsomes as per Goldfarb and Pilot (1971), which were then stored at 75°C until the analysis.

Analytical and biochemical measurements

Determination of IDF, SDF and TDF contents of isolated fraction from FGS powder was by enzymatic and gravimetric method of Association of Official Analytical Chemists (Prosky et al., 1988). Isolation of SDF from fenugreek seeds was carried out as per Dea and Morrison (1975). Serum TG concentration was estimated by the procedure of Foster and Dunn (1973) and serum TC was estimated according to the procedure of Mayne (1994). While HMG-CoAR activity in liver microsomes was estimated as per Hulcher and Oleson (1973), bile acids in the fecal powder were estimated as per Dewael et al. (1977). Neutral sterols present in the feces were estimated by the procedure of Snell and Snell (1961).

Statistical analysis

The data were analyzed by one way and/or two way analysis of variance (ANOVA), paired `t' test and Duncan's multiple range test (Duncan, 1955).

RESULTS

Body weights

The average body weights of male and female obese rats during 9 weeks of experiment showed a significant ($p<0.001$) increase in the body weights from second week of feeding onwards in all the groups. At the end of 9th week, the average body weights of the male rats were 627 g in Group I, 553 g in Group II, 594 g in Group III, 568 g in Group IV and 586 g in Group V respectively. Whereas the average weights of the female rats were 483 g in Group I, 475 g in Group II, 440 g in Group III, 451 g in Group IV and 461 g in Group V. respectively.

The percent gain in body weight was significantly ($p<0.05$) low in experimental group males, that is, 74% in Group II, 75% in Group III, 86% in Group IV and 83% in Group V fed rats while It was high (97%) in control fed male rats respectively. Also the percent gain in body weight was significantly ($p<0.05$) low in experimental group females, that is, 69% in Group II, 63% in Group III, 58% in Group IV and 66% in Group V fed rats while It was high (75%) in control fed female rats respectively (Figure 1).

Food intake

There were no significant differences in food intake between control and experimental groups. The average daily food intake in the male rats was around 27 to 29 g while it was 23 g in females.

Lipid parameters

Serum triglycerides

Figure 2 gives the serum TG levels of the male and female obese rats in control and experimental groups. The initial TG levels in male obese rats were 3.69, 3.82, 3.80 3.32 and 3.99 mmol/L in control, Groups II, III, IV and V, respectively. While the initial TG levels in female obese rats were 3.34, 3.27, 3.52, 3.08 and 3.03 mmol/L in control, Groups II, III, IV and V respectively. After 3 weeks of feeding the diets, there was a significant ($p<0.05$) reduction in serum TG levels in all the experimental groups as compared to their respective

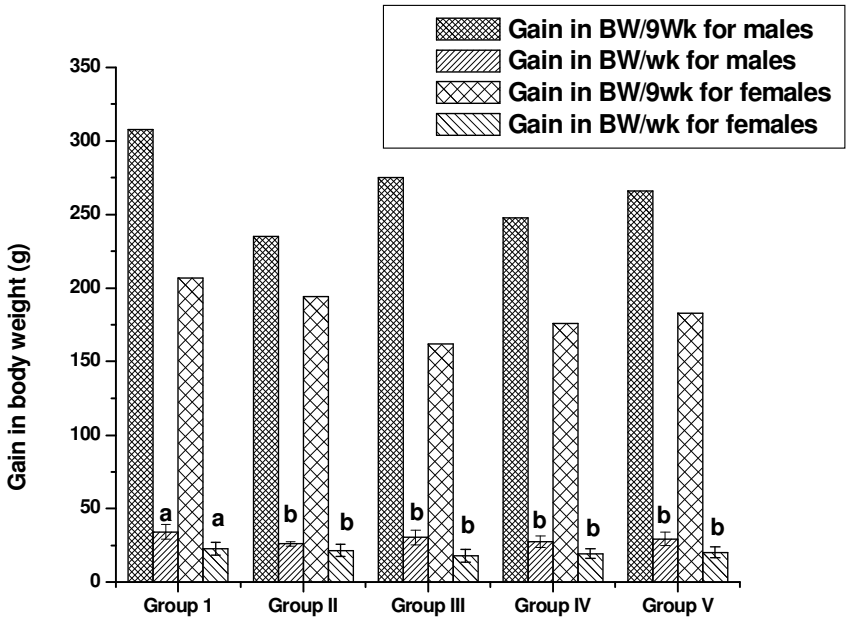

Figure 1. Effect of GM/FGS on gain in body weight (g) of obese rats. The average body weights of obese rats showed a significant ($p<0.05$) increase in all the groups. But percent gain in body weight was significantly ($p<0.05$) low in experimental rats compared to control rats. Values are Mean ± SE of 6 males and 5 females in each group. Values bearing different scripts are statistically different by Duncan's multiple range test at $p<0.05$. BW: Body weight Wk: Week.

initial values. However in contrast, TG values in control animals increased slowly and were significantly higher ($p<0.05$) by 9th week than their initial values.

After 9 weeks of feeding, there was a significant ($p<0.05$) reduction in serum TG levels as compared to their respective initial values observed in Group II (41%), in Group III (43%), in Group IV (48%) and in Group V (53%) fed males respectively, whereas a significant ($p<0.05$) increase was observed (7%) in Group I (control) males respectively. While after 9 weeks of feeding, there was a significant ($p<0.05$) reduction in serum TG levels as compared to their respective initial values observed in Group II (35%), in Group III (42%), in Group IV (45%) and in Group V (41%) fed females respectively, whereas a significant ($p<0.05$) increase was observed (16%) in Group I (control) females, respectively.

Serum total cholesterol

Figure 3 indicates the serum TC concentration of male and female obese rats of control and experimental groups. The initial serum TC values of male and female obese rats were not different between the different groups of obese rats. After 9 weeks of feeding, there was a significant ($p<0.05$) reduction in serum TC levels in all the experimental groups of rats, whereas significant ($p<0.01$) increase was observed in control rats from the 3rd week onwards till the end of 9th week.

At the end of 9th week, there was a significant ($p<0.05$) reduction in serum TC levels observed in Group II (12%), Group III (12%) Group IV 14%) and Group V (13%) males whereas significant ($p<0.01$) increase also observed in control (45%) males respectively. Also at the end of 9th week, there was a significant ($p<0.05$) reduction in serum TC levels observed in Group II (31%), Group III (25%) Group IV (25%) and Group V (25%) females whereas significant ($p<0.01$) increase also observed in control (16%) females respectively. Ninth week serum TC values between males and females were not different. However, initial serum TC values between groups of same gender as well as between genders showed high variation. These differences were much more than the final serum TC values of Groups II to V.

HMG-CoA reductase activity in liver microsomes

The HMG-CoAR activity in obese rat liver microsomes of control and experimental group animals are shown in

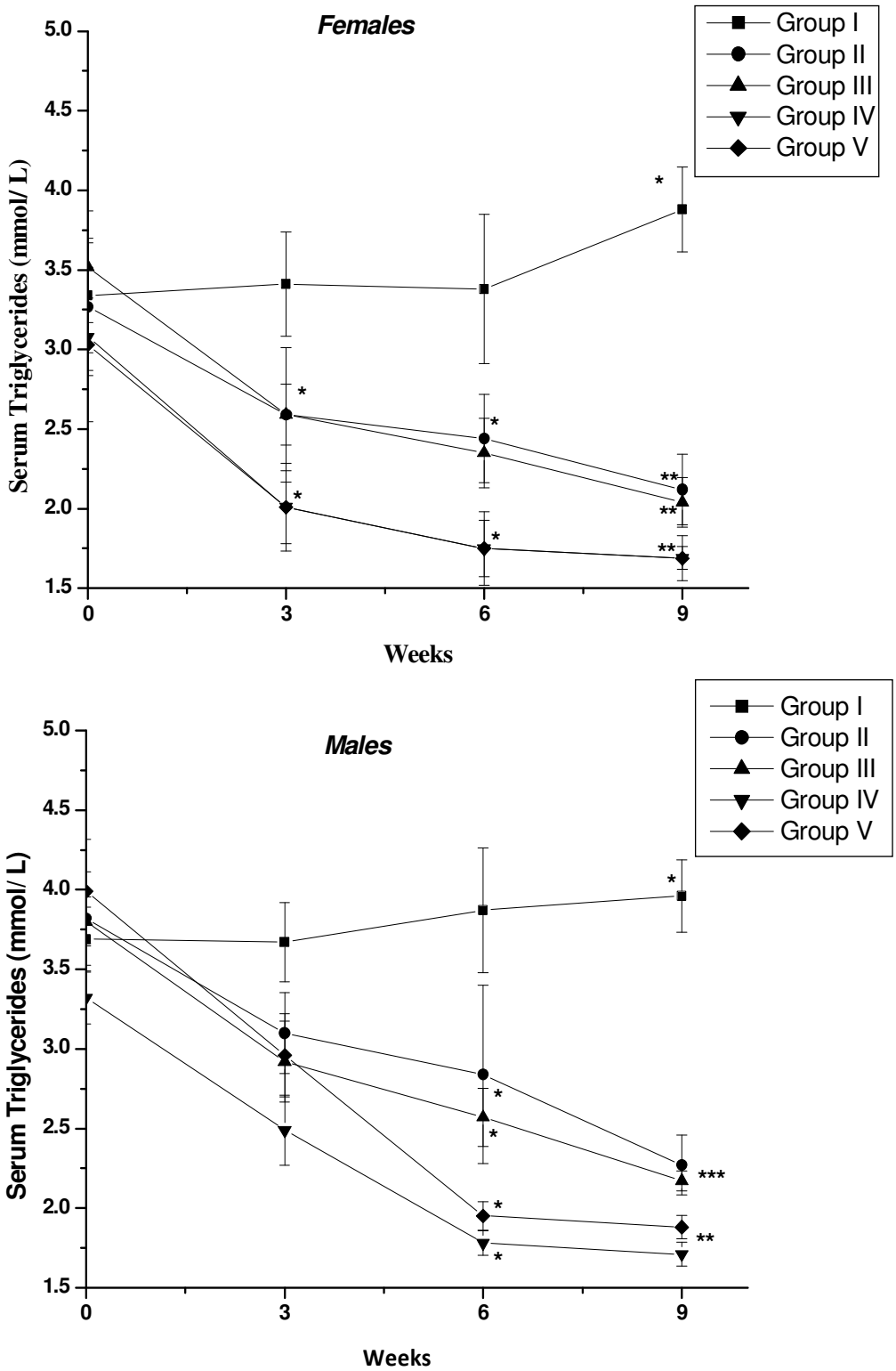

Figure 2. Effect of GM/FGS on serum triglycerides (mmol/L) in obese rats. Feeding of two levels of FGS or GM in the diet fed to rats has significantly ($p<0.01$) decreased the serum triglycerides levels in experimental animals compared to control animals. Values are Mean ± SE of 6 males and 5 females in each group. Paired 't' test *$p<0.05$ **$p<0.01$ ***$p<0.001$.

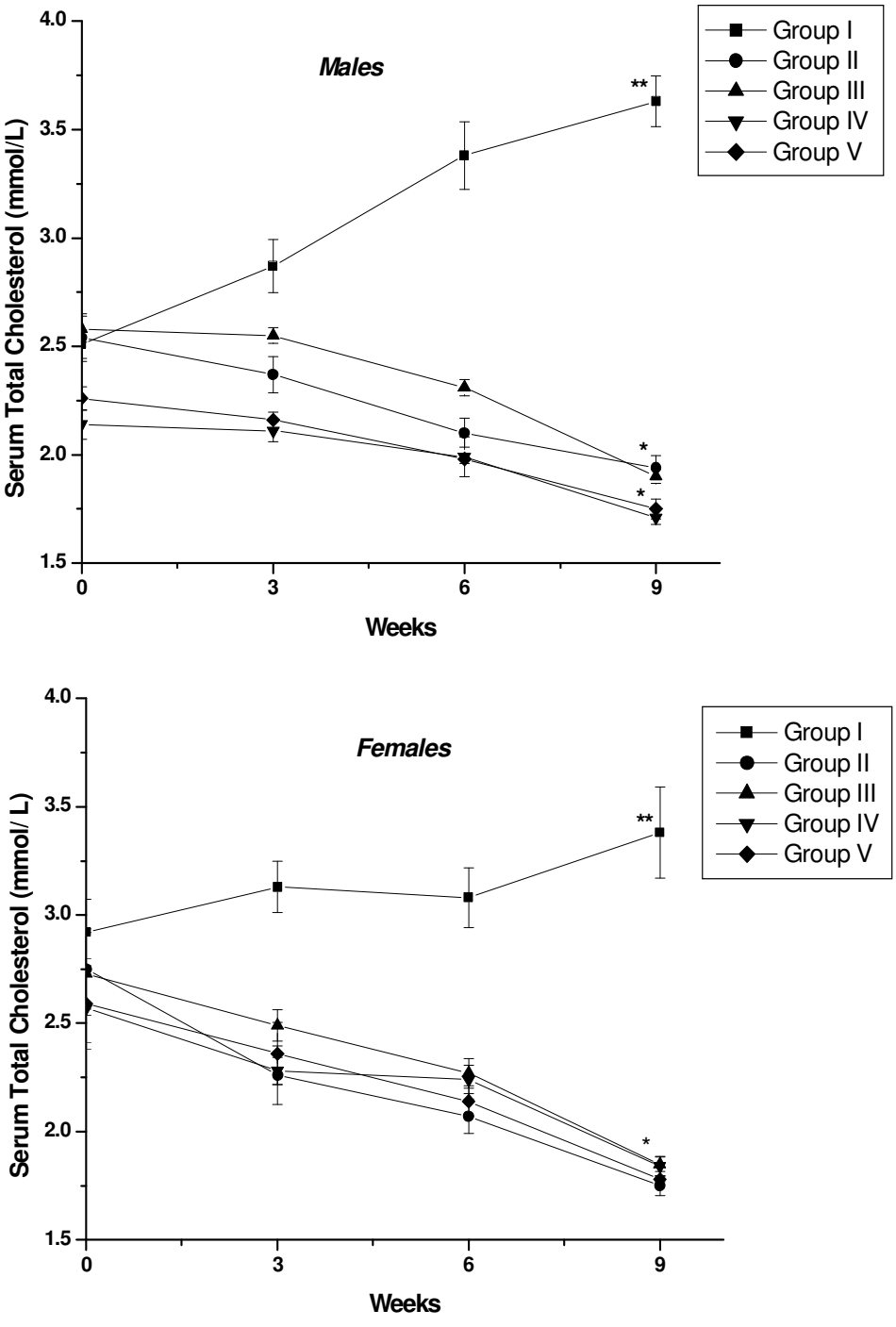

Figure 3. Effect of GM/FGS on serum total cholesterol (mmol/L) in obese rats. Addition of FGS or GM in the diet of experimental rats showed a significant ($p<0.01$) reduction in serum total cholesterol values compared to control rats. Values are Mean ± SE of 6 males and 5 females in each group. Paired't' test *$p<0.05$ **$p<0.01$.

Figure 4. The activity was low in control group as compared with experimental groups. Supplementation of the control diet with 2.5% (Group III) and 5%GM (Group V) brought a significant increase ($p<0.05$) in both male

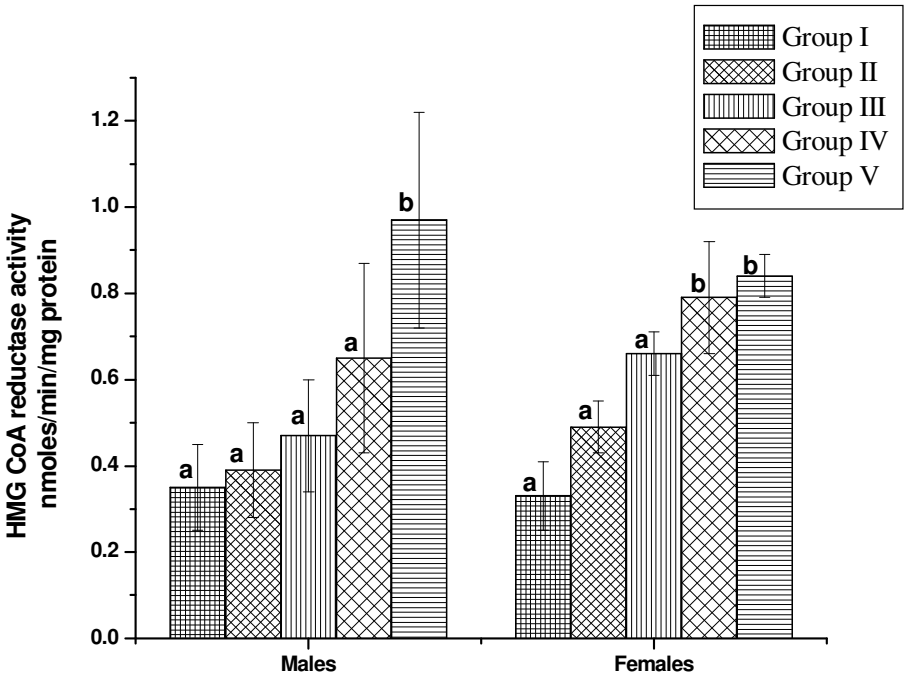

Figure 4. Effect of GM/FGS on HMG CoA reductase activity (n moles/min/mg protein) in liver microsomes of obese rats. FGS or GM mixed diets fed to experimental rats showed a significant ($p<0.05$) increase in HMG-CoA reductase activity compared to control rats. Values are Mean ± SE of 6 males and 5 females in each group. Values bearing different scripts are statistically different by Duncan's multiple range test at $p<0.05$.

Table 3. Effect of galactomannan/seeds of fenugreek on dry fecal weight (g) in obese rats.

Sex	Group I	Group II	Group III	Group IV	Group V
Males	2.69 ± 0.068^{a}	3.67 ± 0.165^{b}	3.27 ± 0.144^{bc}	4.05 ± 0.237^{bde}	3.28 ± 0.196^{bf}
Females	2.23 ± 0.093^{a}	3.04 ± 0.319^{b}	3.06 ± 0.047^{bc}	3.09 ± 0.192^{d}	2.97 ± 0.052^{e}

Values are mean ± SE of 6 males and 5 females in each group. Values bearing different scripts are statistically different by Duncan's multiple range test at $p<0.05$.The dry fecal weights for 24 h excretions were significantly higher ($p<0.05$) in all experimental group animals than control animals.

(34 to 100%) and female (140 to 194%) obese rats.

Fecal weights, bile acids and neutral sterol

The dry fecal weights for 24 h excretions were significantly higher ($p<0.05$) in all experimental group animals than control animals (Table 3). Fecal excretion of bile acids was significantly ($p<0.05$) higher in all the experimental groups than the controls. Fecal bile acid excretion was two-fold higher in Groups IV and V respectively (Figure 5). Fecal neutral sterols excretion was significantly ($p<0.05$) higher in 20% FGS and 5% GM fed groups than control groups respectively (Figure 5).

DISCUSSION

Earlier studies were done on the effect of FGS that was basically used by crude extract on experimentally induced (streptozotocin) diabetic rats (Xue et al., 2007). This is the first time the effect of SDF (galactomannan) from FGS, is tested using a genetically obese pre-diabetic rat model (WNIN/GR-Ob). As shown earlier, using crude extract, the SDF-galactomannan-per se reduced gain in body weight in experimental rats

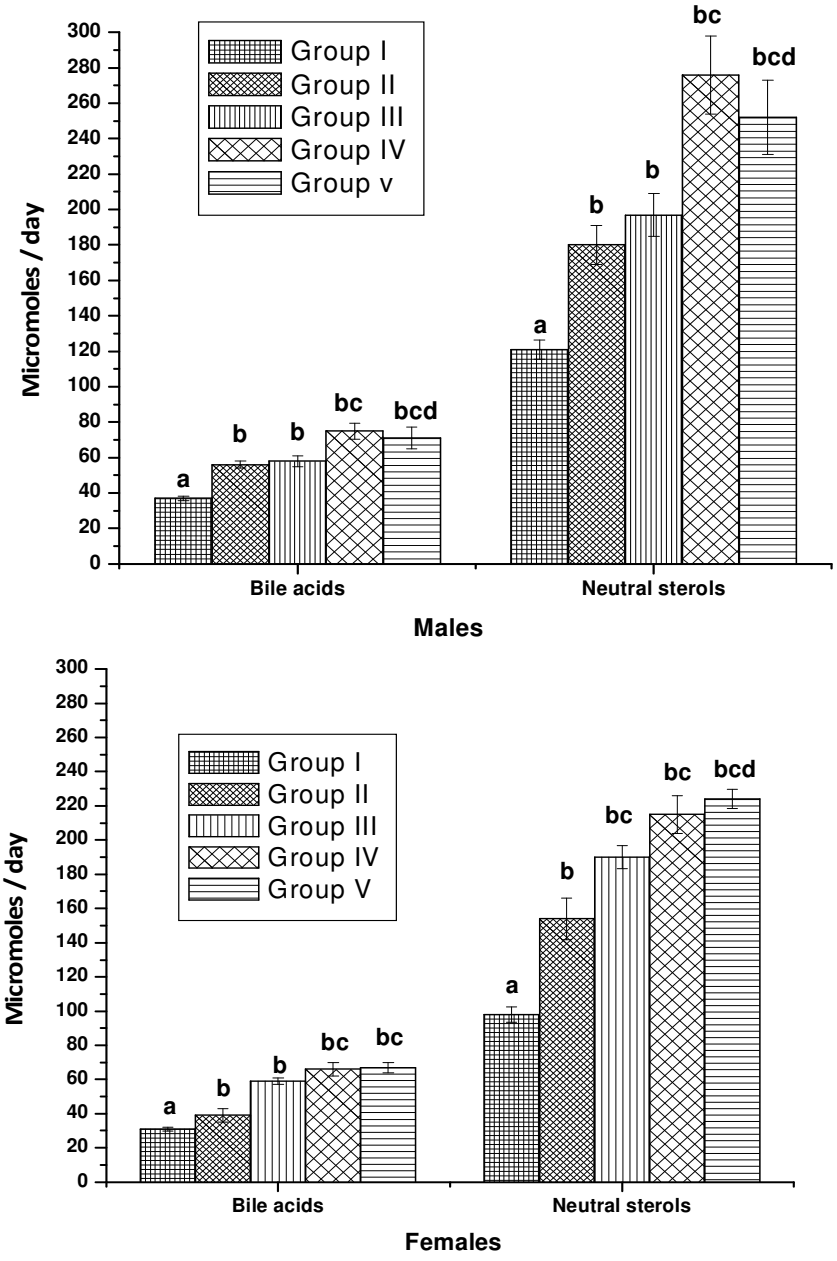

Figure 5. Effect of GM/FGS on bile acids and neutral sterols (µ moles /day) in feces excreted by obese rats. Supplementation FGS or GM in the diet fed to experimental animals showed a significantly ($p<0.05$) increased excretion of bile acids and neutral sterols in feces, compared to control animals. Values are Mean ± SE of 6 males and 5 females in each group. Values bearing different scripts are statistically different by Duncan's multiple range test at $p<0.05$.

compared to control rats. The reduction in body weight is reflected in terms of reduction in lipid parameters, like serum TG and TC. Compared to control, a significant ($p<0.05$) reduction in serum TG levels (35 to 53%) was observed in all experimental groups. This is the most positive effect reported so far, in FGS/SDF fraction and it is more than the effects observed earlier by Sharma et al. (1990, 1991). With respect to serum TC levels, the highest effect observed so far is in the range of 25 to 31% by Stark and Madar (1993), and Sharma et al.

(1986). Similar effects were seen in the present study also, with respect to cholesterol (25 to 31% reduction) in experimental groups as well. HMG-CoAR, a key regulatory enzyme is responsible for the cholesterol biosynthesis in liver. So far there is no study in literature on HMG-CoAR activity in animals fed with FGS/SDF (galactomannan) diet to rats. Although few studies were done using SDF from psyllium, guar gum and pectin (Fernandez et al., 1995c; Roy et al., 2000), they were in guinea pigs where four weeks feeding showed an increase in HMG-CoAR activity. In our studies too, similar increase in HMG-CoAR activity in liver microsomes was seen. We presume that the hypocholesterolemic effect of GM/FGS is not due to decreased cholesterol biosynthesis but by increased rate of catabolism of cholesterol. A direct proof for this should have been the measurement of hepatic cholesterol 7alpha-hydroxylase activity which unfortunately we did not do. However, there is indirect evidence in terms of increased fecal content in experimental rats, which is as a result of increased excretion of fecal bile acids and neutral sterols. In the present study, there was an increased excretion of feces in experimental groups, the highest being in Group IV obese rats. This could be due to high content of SDF and IDF present in Group IV supplemented diets than in other diets. Higher excretion of feces means higher excretion of fecal bile acids and neutral sterols, as shown earlier by Bhat et al. (1995) by feeding FGS and also by Sharma (1984) using different gums.

In summary, it could be conclusively said, that the earlier observation of hypolipidemic effect observed using FGS is mainly due to SDF (galactomannan) present in them. The possible mechanism of action of GM could be due to increased activity of HMG-CoAR and concomitant excretion of bile acids and neutral sterols in feces.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. B. Sesikeran, Director, NIN, Hyderabad for his help and encouragement. The authors wish to thank Mr. A Nadamuni Naidu, former Deputy Director, NIN for his help in statistical evaluation of the data.

Abbreviations: DF, Dietary fiber; **FGS,** fenugreek seeds; **GM,** galactomannan; **IGT,** impaired glucose tolerance; **IDF,** insoluble dietary fiber; **IAEC,** institutional animal ethical committee; **NCLAS,** National Centre for Laboratory Animal Sciences; **NIN, SDF,** soluble dietary fiber; **TDF,** total dietary fiber; **Wk,** week.

REFERENCES

Al-Habori M, Al-Aghabari A, Al-Mamary M (1988). Effect of fenugreek seeds and its extracts on plasma lipid profile: study on rabbits. Phytother. Res., 12: 572-575.

Al-Habori M, Raman A (1998). Antidiabetic and hypocholesterolemic effects of fenugreek. Phytother. Res., 12: 233-242.

Bhat BG, Sambaiah R, Chandrashehara N (1985). The effect of feeding fenugreek and ginger on bile composition in the albino rat. Nutr. Rep. Int., 32: 1145-1151.

Dea ICM, Morrison A (1975). Chemistry and interactions of seed galactomannans, In Advances in Carbohydr. Chem. Biochem., 31: 241-312.

Dewael J, Raaymakers CE, Endeman HJ (1977). Simplified quantitative determination of total fecal bile acids. Clin. Chim. Acta., 79: 465-670.

Duncan DB (1955). Multiple range and multiple F tests. Biometrics, 11: 1-42.

Evans AJ, Hood RL, Oakenfull DG, Sidhu GS (1992). Relationship between structure and function of a dietary fibre: a comparative study of the effects of three galactomannans on cholesterol metabolism in the rats. Br. J. Nutr., 68: 217-229.

Fernandez ML, Sun DM, Tosca MA, McNamara DJ (1994). Citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipoprotein metabolism: A dose-response study in guinea pigs. Am. J. Clin. Nutr., 59: 869-878.

Fernandez ML (1995). Distict mechanisms of plasma LDL lowering by dietary fiber in the guinea pigs specific effects of pectin, guar gum and psyllium. J. Lip. Res., 36: 2394-2404.

Fernandez ML, Riuz LR, Conde AK, Sun DM, Erickson S, McNamara DJ (1995a). Psyllium reduces plasma LDL in guinea pigs by altering hepatic cholesterol homeostasis. J. Lip. Res., 36: 1128-1138.

Fernandez ML, Jimenez MA, Romero AL, Erickson SK, McNamara DJ (1995b). Gender differences in response to dietary soluble fiber in guinea pigs: effects of pectin, guar gum and psyllium. J. Lip. Res., 36: 2191-2202.

Fernandez M, Sun DM, Tosca M, McNamara DJ (1995c). Guar gum effects on plasma low density lipoprotein and hepatic cholesterol metabolism in guinea pigs fed low and high cholesterol diets: a dose response study. Am. J. Clin. Nutr., 61: 127-134.

Foster LB, Dunn RT (1973). Stable reagents for determination of serum triglycerides by colorimetric hantzsch condensation method. Clin. Chem., 19: 338-340.

Giridharan NV, Harishankar N, Satyavani M (1996). A new rat model for the study of obesity. Scand. J. Lab. Animal Sci., 23: 131-137.

Girirdharan NV, Lakshmi CN, Raghuramulu N (1997). Identification of impaired- glucose tolerant animals from a Wister inbred rat colony. Lab. Animal Sci., 47: 428-431.

Goldfarb S, Pilot HC (1971). Improved assay of 3-hydroxy 3-methylglutaryl Coenzyme A reductase. J. Lip. Res., 12: 512-515.

Hulcher FH, Oleson WH (1973). Simplified spectrophotometric assay for microsomal 3-hydroxy-3- methylglutaryl Co A reductase by measurement of Co enzyme A. J. Lip. Res., 14: 625-630.

Jenkins DJA, Wolever TMS, Venkateshwer Rao A, Hegele RA, Mitchelli SJ, Ramsom TPP, Boctor DL, Spadafora PJ, Jenkins AL, Mehling C, Relle LK, Connelly PW, Story JA, Furumoto EJ, Corey P, Wursch P (1993). Effect of blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. N. Engl. J. Med., 329: 21-26.

Jenkins DJA, Kendall CWC, Vuksan V (2001). Viscous fibers, health claims, and strategies to reduce cardiovascular disease risk. Am. J. Clin. Nutr., 71: 401- 402.

Kishimoto Y, Wakabayashi S, Takeda H (1995). Hypocholesterolemic effect of dietary fiber: Relation to intestinal fermentation and bile acid excretion. J. Nutr. Sci. Vitaminol., 41: 151-161.

Kritchevsky (1999). Dietary fiber in health and disease: an overview. Asia. Pacific. J. Clin. Nutr., 8(Suppl): S1-S2.

Lairon D (1996). Dietary fibers: effects on lipid metabolism and mechanisms of action. Eur. J. Clin. Nutr., 50: 125-133.

Mayne PD (1994). "Plasma Lipids and Lipoproteins" in Clinical Chemistry in Diagnosis and Treatment. ELBS, 11: 223-243.

Prosky L, Asp NG, Schweizer TF, DeVries JW, Furda I (1988). Determination of insoluble, soluble and total dietary fiber in foods

and food products: Interlaboratory study. J. Assoc. Off. Anal. Chem., 71: 1017-1024.
Reeves PG, Nielsen FH, Fahey Jr GC (1993). AIN-93 purified diets for laboratory Rodents: Final report of the American Institute of Nutrition Adhoc committee on the reformulation of the AIN-76A Rodent diet. J. Nutr., 123: 1939-1951.
Roy S, Vega-Lopez S, Fernandez ML (2000). Gender and hormonal status affect the hypolipidemic mechanisms of dietary soluble fiber in guinea pigs. J. Nutr., 130: 600-607.
Sharma RD (1984). Hypocholesterolemic activity of fenugreek an experimental study in rat. Nutr. Rep. Int., 30: 221-231.
Sharma RD (1986). An evaluation of hypocholesterolemic factor of fenugreek seeds (*T. foenum graecum*) in rats. Nutr. Rep. Int., 33: 669-677.
Sharma RD, Raghuram TC, Sudhakar RN (1990). Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. Eur. J. Clin. Nutr., 44: 301-306.
Sharma RD, Raghuram TC, Dayasagar RV (1991). Hypolipidemic effect of fenugreek seeds: a clinical study. Phytother. Res., 5: 145-147.
Snell FD, Snell GT (1961). Colorimetric methodology analysis, Vol.3A, (Ed.) Van Norstrand, New York, pp. 350-356.
Stark A, Madar Z (1993). The effect of an ethanol extract derived from fenugreek (*Trigonella foenum graecum*) on bile acid absorption and cholesterol levels in rats. Br. J. Nutr., 69: 277-287.
Xue WL, Li XS, Zhang J, Liu YH, Wang ZL, Zhang RJ (2007). Effect of Trigonella foenum graecum (fenugreek) extract on blood glucose, blood lipid and hemorhelogical properties in streptozotocin induced diabetic rats. Asia. Pac. J. Clin. Nutr., 16(1): 422-426.