

The Modifying Effects of Galactomannan from Canadian-Grown Fenugreek (*Trigonella foenum-graecum L.*) on the Glycemic and Lipidemic Status in Rats

Anchalee Srichamroen¹, Catherine J. Field¹, Alan B.R. Thomson², and Tapan K. Basu^{1,*}

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

²Division of Gastroenterology, Zeidler Leducor Center, University of Alberta, Edmonton, Alberta T6G 2X8, Canada

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Summary Using high sucrose-fed male Sprague-Dawley rats, a study was conducted to determine the effects of feeding Galactomannan (GAL), a soluble dietary fiber extracted from Canadian-grown fenugreek seeds, on blood lipid and glucose responses. Rats ($n = 8$, 175–200 g) were randomly assigned to one of three high sucrose diets containing 10% cellulose (control), 7.5% cellulose + 2.5% GAL, and 5% cellulose + 5% GAL, respectively for 4 weeks. After 3 weeks, an oral glucose tolerance test (OGTT) was performed on each rat. A week later blood samples were collected to determine the effect on blood lipids. A significant reduction in glycemic response was observed only in 5% GAL group at 120 min following OGTT, when compared with that of control and 2.5% GAL groups. The plasma level of insulin was also significantly reduced ($p < 0.001$) in 5% GAL-fed rats but at all times during OGTT. These animals also showed a reduction in body weight gain ($p < 0.05$) in parallel with less food intake ($p < 0.05$). All GAL-fed (2.5% and 5.0%) rats had significantly reduced plasma levels of triglycerides and total cholesterol in association with a reduction in epididymal adipose weight. Overall, this study demonstrated that feeding GAL from Canadian-grown fenugreek seeds has the potential to alter glycemic and lipidemic status and reduce abdominal fat in normal rats.

Key Words: Canadian fenugreek seed, galactomannan, glycemic status, lipidemic status, epididymal tissue

Introduction

Fenugreek (*Trigonella foenum-graecum L.*) is an annual dicotyledon plant belonging to the family Papilionaceae Leguminales and is thought to originate in India and Middle East. The seeds contain saponins, OH-isoleucine and galactomannans, which have been shown to exert beneficial health effects, specifically in diabetic and hypercholesterolemic animals and humans [1]. Among the potential active

components of fenugreek seeds is galactomannan (GAL), which is a guar gum representing approximately 50% of the seed weight [2].

GAL extracted from fenugreek seeds of South Asian origin has been shown to reduce postprandial blood glucose [3] and improve insulin sensitivity in both non-diabetic [4, 5] and diabetic subjects [6–8]. The decrease in postprandial blood glucose reduced the need for antidiabetic medication, including insulin, in diabetic subjects consuming diets containing guar gum. Feeding guar-GAL fibre has also been shown to reduce both total and LDL cholesterol levels in healthy and type 2 diabetic (T2D) subjects [9, 10]. However, these results are not consistent among all studies. A guar gum-containing diet, given for 4 weeks, failed to show an

*To whom correspondence should be addressed.
Tel: +1-780-492-5338 Fax: +1-780-492-4265
E-mail: tapan.basu@ualberta.ca

improvement in glucose control in T2D subjects [11], while a long-term ingestion of the fiber has been found to be associated with increased plasma insulin concentrations [12]. Further studies, involving healthy male subjects, also reported no appreciable effects of the fiber in modifying glycemic status [13]. It is possible that the discrepancies in glycemic responses to GAL intake are caused by the differences in the fiber concentrations, type of foods, and duration of the studies.

In recent years, fenugreek plant has been adapted to cultivation in a Canadian province, Alberta. Unlike the Asian variety of the plant, the potential health benefit of GAL of this Canadian fenugreek has never been studied. The present study was undertaken to examine the effects of feeding GAL, isolated and purified from Canadian-grown fenugreek seeds, in a high sucrose diet on blood lipids and glycemic responses in normal Sprague-Dawley rats. The high sucrose diet was chosen because of its established effects on increasing triglycerides (TG) in both plasma and adipose tissue [14].

Materials and Methods

Materials

GAL was extracted from fenugreek seeds (Amber variety) in our laboratory. Briefly, seed coat fraction (0.5 mm mesh) was dispersed in RO water, at a ratio of 1:40 (w/v) for 4 h. After centrifugation at $17,700 \times g$ for 30 min, the supernatant was mixed with absolute ethanol at a ratio of 1:1 (v/v) to precipitate the fenugreek gum, which contained 91.4% (w/w) GAL on a dry weight basis.

Animals and diets

Twenty-five male Sprague-Dawley rats (weighing 175–200 g) were obtained from Charles River Laboratories

(Ontario, Canada). The animals were housed two per cage at 20–22°C and subjected to a 12-h day/night cycle. After a week of acclimatization, animals were randomly divided into three groups, which were fed a diet containing 0% (control, $n = 9$), 2.5% (low, $n = 8$), and 5% (high, $n = 8$) GAL. Initial body weights of these animals were similar. All rats had *ad libitum* access to water and food during the experiment. The diets were high in sucrose (52% w/w), isocaloric and isonitrogenous, and they contained 10% w/w fibre, differed only in its composition as shown in Table 1. All diets contained some cellulose, a non-fermentable and non-soluble fiber, which was necessary to minimize the risk of soluble fiber-associated diarrhea. The body weight and the food intake were measured daily. The protocol of the study was approved by the Faculty of Agriculture, Forestry and Home Economics' Animal Policy and Welfare Committee (FAPWC), University of Alberta, Canada.

Oral glucose tolerance test

Animals were fed their respective diet for 3 weeks. Following 12 h fast, each rat received an oral gavage of 75% w/v glucose (3 g/kg BW). Blood samples were obtained from tail at 0, 15, 30, 60, and 120 min after the glucose challenge. Using the following calculation, the plasma and insulin responses were determined as the area under the curve (AUC) of plasma glucose and insulin:

$$\text{AUC} = 1/2(t_2 - t_1)(C_2 + C_1) + 1/2(t_3 - t_2)(C_3 + C_2) + 1/2(t_4 - t_3)(C_4 + C_3) + 1/2(t_5 - t_4)(C_5 + C_4),$$

Where C_1 , C_2 , C_3 , C_4 and C_5 are either plasma glucose or plasma insulin at times 0 (t_1), 15 (t_2), 30 (t_3), 60 (t_4) and 120 min (t_5), respectively, after glucose load.

Blood and tissue sampling

At the termination of the experiment (day 28), animals were fasted for 12 h and anaesthetized with halothane.

Table 1. Composition of diets

	Control	low GAL	high GAL
Ingredients (g/100 g of diet):			
Casein	20.0	20.0	20.0
Sucrose	52.0	52.0	52.0
Corn oil	5.0	5.0	5.0
Stearin	7.4	7.4	7.4
Linseed oil	0.5	0.5	0.5
DL-methionine	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2
Vitamin mix (AIN-93-VX)	1.1	1.1	1.1
Mineral mix (AIN-93-M)	3.5	3.5	3.5
Cellulose	10.0	7.5	5.0
Galactomannan	0.0	2.5	5.0

The composition of diets was modified from AIN 93G. Polyunsaturated fatty acid to saturated fatty acid (P/S) ratio was 0.4 based on approximate North American fat intake [31].

Cardiac puncture was performed to obtain sufficient blood for biochemical analyses. Tubes containing whole blood were centrifuged at 3000 rpm for 15 min at 4°C (Beckman J2-HC centrifuge, City, CA). The plasma samples were stored at -80°C until analyzed. At necropsy, liver, epididymal and peri-renal fat depots were excised, weighed, snap frozen in liquid nitrogen and then stored at -80°C until analysed.

Biochemical analyses in plasma and tissue

Plasma glucose and insulin concentrations were determined by a glucose oxidase method (Biopacific Diagnostic Inc., British Columbia, Canada) and a RIA kit [¹²⁵I] (Linco Research, City, MO), respectively. Plasma cholesterol and triglyceride were determined using commercially available enzymatic kits (Biopacific Diagnostic Inc.). Total free fatty acids (FFA) in plasma were measured by using NEFA-C Test Wako kit (Wako Pure Chemical Industries, City, VA).

Plasma lipoprotein fractions were separated by density gradient ultracentrifugation (Optima MAX Ultracentrifuge, Beckman coulter, City, CA) at 100,000 rpm for 3, 4, and 6 h to obtain the fraction of VLDL, LDL, and HDL, respectively, as described elsewhere (Wasan *et al.*, 2001). The different triglyceride and cholesterol molecules were fractionated as follows: VLDL had a density (d) of 0.095–1.006 kg/L, LDL had d = 1.006–1.063 kg/L, HDL had d >1.063 kg/L [15]. Triglyceride and cholesterol concentrations were determined for each of the fractions using enzymatic kits (Biopacific Diagnostic Inc.).

Lipid from liver and adipose tissues was extracted by chloroform/ methanol (2:1, v/v) solution as described by Folch *et al.* [16]. Triglyceride and cholesterol concentrations were measured using commercially available enzymatic kit (Biopacific Diagnostic Inc.).

Adipose tissue histology

Difference in epididymal histology was determined by analyzing two representative animals from each treatment group. Frozen epididymal adipose tissues were thawed and fixed in 10% (v/v) formalin. After 48 h of fixation, tissues

were dehydrated in graded alcohols, embedded in paraffin blocks, and cut into sections of 5 µm thick. Mounted sections were stained for light microscopy with haematoxylin and eosin using standard methods [17]. The samples were analyzed by an individual, who was blinded to the diet treatment that the animals received.

Statistical analyses

Results were reported as the mean ± SEM. Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls (SNK) test as a post-hoc analysis to identify significant differences among the groups. A *p* value <0.05 was considered statistical significance. Group means for body weight, plasma glucose and plasma insulin, AUC were calculated by repeated measurement analysis. The differences of AUC between time points were determined using repeated measurement analysis, and a SNK test was performed to identify differences.

Results

Food intake, body and tissue weights

Food intake was significantly reduced by 16%, and 12% for the high GAL group when compared with the control and low GAL groups, respectively (*p*<0.05, Table 2). Body weight of rats in all groups increased steadily with time, but the average body weight of the animals fed a diet containing high GAL diet was significantly lower on day 10 (by 6%) and on day 28 (by 11%) compared to the control and the low GAL groups (data not shown). Furthermore, the overall body weight gain in the high GAL group was significantly lower compared to the control and the low GAL groups (*p*<0.05, Table 2). There were no differences in overall body weight gain between the low GAL group and the controls. The ratio of weight gain to gram of food intake in all 3 groups did not differ significantly (Table 2).

The low and high GAL groups had significantly less epididymal adipose tissue than the control group by 23%

Table 2. Effect of galactomannan on food intake, weight gain, and adipose tissue weight

	Control	low GAL	high GAL
Food intake (g/d)	26.8 ± 0.8 ^a	25.6 ± 0.8 ^a	22.6 ± 0.8 ^b
Body weight gain (g)	165.8 ± 9.7 ^a	157.4 ± 10.3 ^a	124.2 ± 10.3 ^b
Body weight gain/ intake ratio	6.26 ± 0.1	6.10 ± 0.1	5.48 ± 0.1
Adipose tissue			
epididymal adipose tissue (g)	5.90 ± 0.3 ^a	4.57 ± 0.3 ^b	2.58 ± 0.3 ^c
% of body weight	1.5%	1.2%	0.7%
peri-renal adipose tissue (g)	1.38 ± 0.1	1.23 ± 0.1	0.91 ± 0.1
% of body weight	0.3%	0.3%	0.26%

Values represent means ± SEM of control (*n* = 9), low GAL (*n* = 8), high GAL (*n* = 8).

^{a-c} values in a row not sharing a common superscript differ significantly at *p*<0.05.

and 56%, respectively ($p < 0.05$, Table 2). However, there were no significant differences in the weight of peri-renal adipose tissue depot among these groups.

Oral glucose tolerance test

The average fasting plasma glucose concentration for the high GAL group was reduced compared to the low GAL and the control groups ($p < 0.05$, results not shown). After the OGTT, plasma glucose concentrations did not differ significantly at 15, 30, and 60 min, while the glucose concentrations in the high GAL group was significantly lower at 120 min as compared to the low GAL and the control groups ($p < 0.05$).

AUC between 0 and 120 min for glucose of high GAL group was significantly reduced compared to the control and low GAL groups (Table 3). Separate analysis of glucose AUC between 30 and 60 min confirmed that the AUC for the high GAL group was significantly lower than the control. Between 60 to 120 min, the AUC of both low and high GAL groups were significantly lower compared to the control group. There were no differences in glucose AUC at 15 min (AUC 0 to 15 min), 30 min (AUC 15 to 30 min) among all three groups.

Fasting plasma insulin in the high GAL group was lower than that of the low GAL and the control groups ($p < 0.001$, Fig. 1). After the OGTT, plasma insulin of the high GAL group was lower than the insulin levels of the low GAL and control groups at 15, 30, 60 and 120 min ($p < 0.001$). There were no differences in plasma insulin concentrations between the control and the low GAL groups.

Feeding both low and high GAL diets to rats resulted in significantly lower AUC for insulin between 0 and 120 min compared to the control (Table 4). The AUC for the high

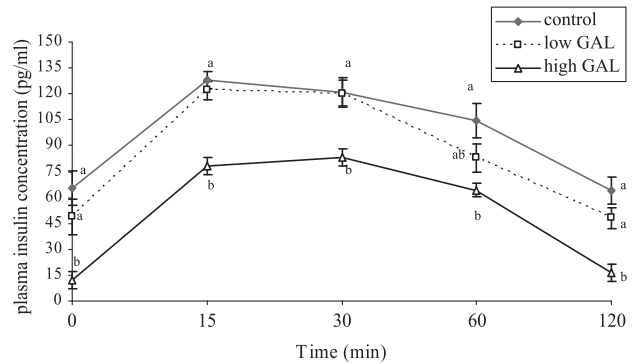


Fig. 1. Plasma insulin response to the OGTT in rats fed one of three diets for 3 weeks.

Values represent means \pm SEM of control ($n = 9$), low GAL ($n = 8$), high GAL ($n = 8$).

^{a-c} Values in the same time point not sharing the same superscript are significantly different ($p < 0.05$).

GAL group was significantly lower than the control at all measured time points. In low GAL group, compared to the control group, there was a significantly lower AUC for insulin at every measured time point, except at 30 min (AUC 15 to 30 min).

Triglyceride, free fatty acids and cholesterol concentrations

Both the low and high GAL groups had lower ($p < 0.05$) plasma triglyceride and cholesterol concentrations, compared to the control (Fig. 2), while plasma FFA in the low GAL group was significantly lower (Fig. 2). The plasma FFA was found to be reduced only in the low GAL group. VLDL triglyceride and cholesterol concentrations in plasma did not differ significantly among diet treatments (Fig. 3 and

Table 3. Area under the curve of plasma glucose after OGTT

Treatment	15 min	30 min	60 min	120 min	Total AUC (0 to 120 min)
Control	144.5 \pm 1.8	196.5 \pm 1.8	386.7 \pm 4.3 ^a	633.8 \pm 5.8 ^a	1361.5 \pm 12.5 ^a
Low GAL	149.7 \pm 1.8	199.3 \pm 1.8	364.4 \pm 4.3 ^b	597.4 \pm 5.8 ^b	1310.9 \pm 12.5 ^a
High GAL	144.5 \pm 1.8	191.3 \pm 1.8	348.1 \pm 4.3 ^b	559.8 \pm 5.8 ^c	1239.9 \pm 12.5 ^b

^{a-c} Different letters following means \pm SEM within a column indicate a significant difference at the 0.05 level.

Table 4. Area under the curve of plasma insulin after OGTT

Treatment	15 min	30 min	60 min	120 min	Total AUC (0 to 120 min)
Control	1.45 \pm 0.01 ^a	1.87 \pm 0.05 ^a	3.49 \pm 0.09 ^a	5.2 \pm 0.1 ^a	12.1 \pm 0.3 ^a
Low GAL	1.28 \pm 0.01 ^b	1.81 \pm 0.05 ^a	3.04 \pm 0.09 ^b	3.9 \pm 0.1 ^b	10.1 \pm 0.3 ^b
High GAL	0.73 \pm 0.01 ^c	1.20 \pm 0.05 ^b	2.37 \pm 0.09 ^b	2.8 \pm 0.1 ^c	7.1 \pm 0.3 ^c

^{a-c} Different letters following means \pm SEM within a column indicate a significant difference at the 0.05 level.

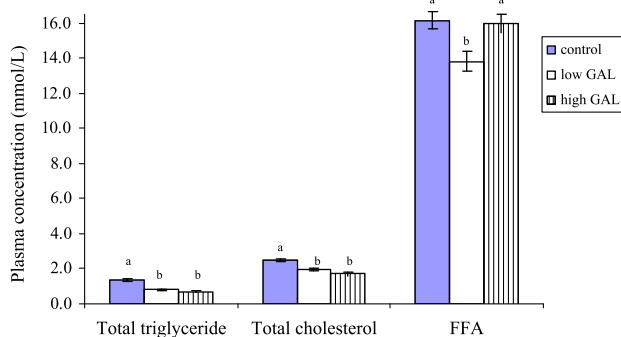


Fig. 2. Plasma levels of total-triglyceride, total cholesterol, and FFA in rats fed with different diets for 4 weeks. Values represent means \pm SEM of control ($n = 9$), low GAL ($n = 8$), high GAL ($n = 8$).
^{a,b} different superscripts indicate significant difference at $p < 0.05$ among control, low GAL and high GAL groups.

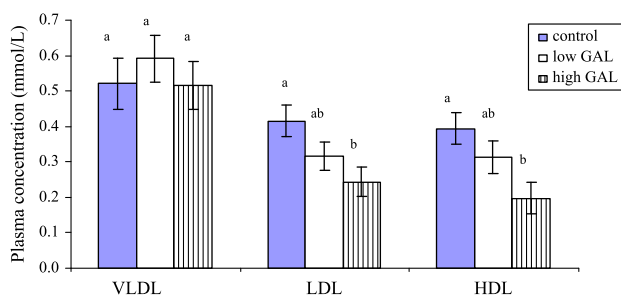


Fig. 3. Lipoprotein triglyceride levels in rats fed with different diets for 4 weeks. Values represent means \pm SEM of control ($n = 9$), low GAL ($n = 8$), high GAL ($n = 8$).
^{a,b} different superscripts indicate significant difference at $p < 0.05$ among control, low GAL and high GAL groups.

4). High GAL group, however, had significantly lower LDL- and HDL-TG concentrations than those of the control animals. The low but not the high GAL group had significantly lower concentrations of hepatic TG ($p < 0.05$) than the control (Fig. 5). Compared with the controls the hepatic cholesterol concentration was, however, lower in both low and high GAL groups.

Adipose fat

Epididymal triglyceride concentrations in the high GAL group were reduced by 20% when compared to the control group ($p < 0.05$, Fig. 6). Upon histological examination the epididymal adipose tissue of these animals was visibly different from the control and low GAL-fed animals (Fig. 7). The changes that were found to be present in the presence of GAL included clusters of large fat cells surrounded by small fat cells throughout adipose tissues, and capillaries that appeared to be filled with erythrocytes.

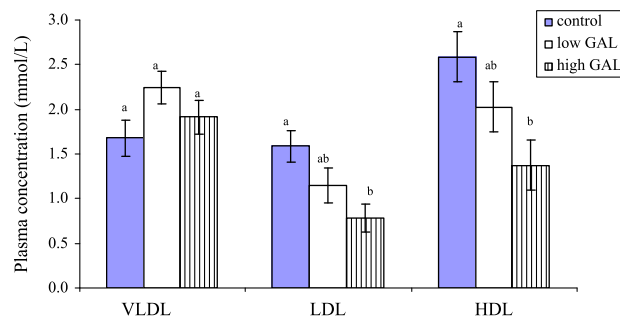


Fig. 4. Lipoprotein cholesterol levels in rats fed with different diets for 4 weeks. Values represent means \pm SEM of control ($n = 9$), low GAL ($n = 8$), high GAL ($n = 8$).
^{a,b} different superscripts indicate significant difference at $p < 0.05$ among control, low GAL and high GAL groups.

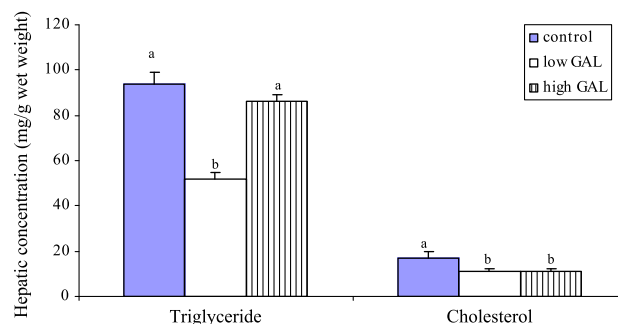


Fig. 5. Hepatic triglyceride and cholesterol levels in rats fed with different diets for 4 weeks. Values represent means \pm SEM of control ($n = 9$), low GAL ($n = 8$), high GAL ($n = 8$).
^{a,b} different superscripts indicate significant difference at $p < 0.05$ among control, low GAL and high GAL groups.

Discussion

Fenugreek is a native of the Indian sub-continent and the Eastern region. Its high adaptability to dry climate, high yield and their ability to fix atmospheric nitrogen (N) make it an ideal for short-term crop rotation in Northern countries, such as Canada. An examination in our laboratory has revealed that the Canadian-grown fenugreek seeds and the seeds of a fenugreek variety, grown in India, are of similar appearances, color, size, shape, odor, and anatomical features. The chemical composition (e.g., crude lipids, 4-hydroxyisoleucine, saponins) of Canadian fenugreek seeds has also been found to be very similar to that of the seeds of Indian origin. The Canadian variety, however, is significantly higher in crude protein and GAL contents (unpublished data).

In rats, a high sucrose diet has been reported to result

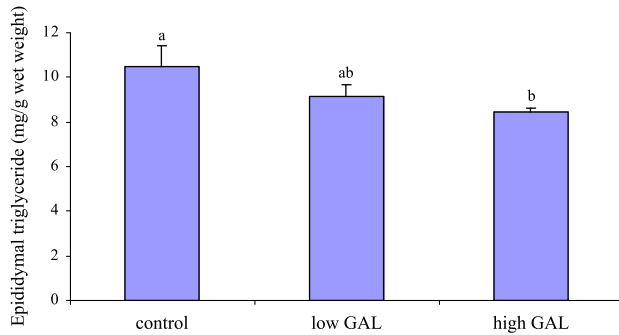


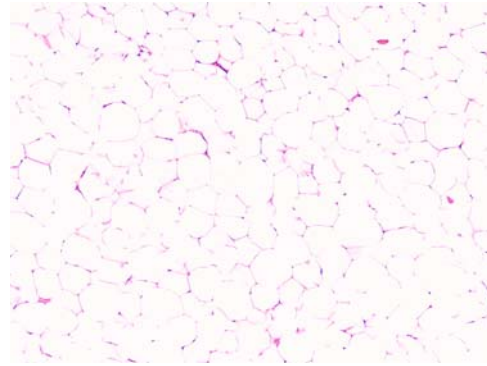
Fig. 6. Epididymal triglyceride level in rats fed with different diets for 4 weeks.

Values represent means \pm SEM of control ($n = 9$), low GAL ($n = 8$), high GAL ($n = 8$).

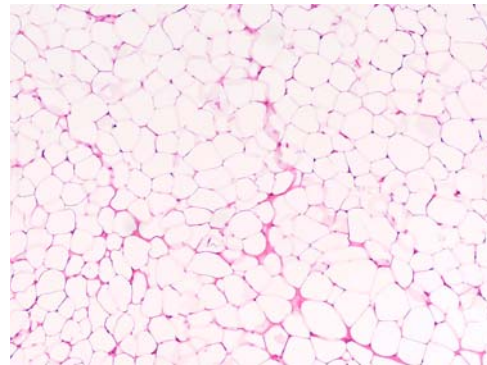
^{a,b} different superscripts indicate significant difference at $p < 0.05$ among control, low GAL and high GAL groups.

hypertriglyceridemia, hyperglycemia, and hyperinsulinemia [18, 19]. The diet is also known to impair insulin action, leading to hypertriglyceridemia [20, 21]. In the present study, an addition of 5% GAL to a high sucrose diet resulted in a marked reduction in food intake in parallel with body weight gain in rats, when fed for 4 weeks. GAL belongs to a family of seed gum; it represents polymers of galactose and mannose, and it is soluble in water [22]. Most soluble gum fibers, such as guar gum, xanthan gum, gum acacia and locust bean gum are viscous and that this property is thought to delay gastric emptying and consequently intestinal absorption [23]. However, the ratio of body weight gain to food intake in all 3 groups in this study was similar. These data indicated that the reduction in weight gain in the high GAL group was likely due to reduced food intake, rather than malabsorption. The fiber intake was also found to be accompanied by a decreased rise in plasma insulin, which is an important factor for appetite control [24]. It seems probable that the reduced food intake in the presence of GAL is caused by delaying in gastric empty (due to viscosity) and promoting satiety (due to insulin inhibition) of the soluble fiber. These results are in agreement with our previous *in vitro* study demonstrating reduced intestinal glucose uptake with increasing GAL concentration in lean and obese JCR rats [25]. This inhibition was found to be associated with an increase in the viscosity of the GAL-containing test solutions.

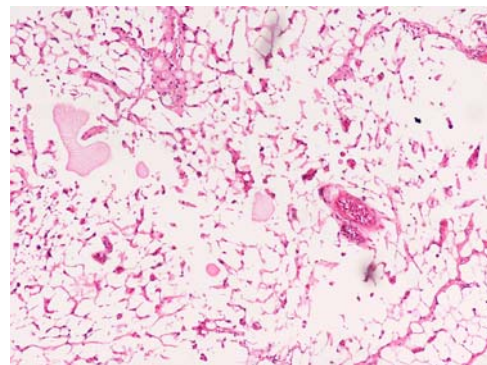
The present study was the first to demonstrate that feeding a GAL diet for 4 weeks to normal rats, markedly reduces the size of the abdominal adipose tissues compared with the control counterpart, fed a similar diet but containing an equivalent amount of cellulose as a fiber source. This result can be explained by a decrease in insulin level leading to reduced lipogenesis. The evidence of abdominal adipose tissue histology from rats fed with 5% GAL containing diet



a



b



c

Fig. 7. Histology of epididymal adipose tissues from rats fed a diet containing different levels of galactomannan for 4 weeks.

a) Rat fed with control diet b) Rat fed with low GAL diet c) Rat fed with high GAL diet

is suggestive of a high metabolic rate in the tissue, leading to a release of FFA into the circulation.

One of the plausible mechanisms linking visceral adiposity to insulin resistance is the liberation of non-esterified fatty acids (NEFAs) from visceral depots to the liver. These NEFAs in the liver, therefore, have a particularly important role in bringing about many features of insulin resistance. Stimulation of hepatic glucose synthesis may contribute to glucose

intolerance [26]. Stimulation of hepatic VLDL-TG secretion may contribute to impaired postprandial lipid metabolism (via competition with chylomicron-TG for peripheral clearance) and the development of hypertriglyceridemia. Given these facts, the high plasma and hepatic FFA in rats fed a diet containing 5% GAL for 4 weeks, observed in the present study, do not reflect insulin resistance. These rats had lower plasma TG, while VLDL-TG levels remained unchanged compared to the controls. The OGTT in these animals also remained unaffected. These results indicate that an underlying factor for the reduction of plasma insulin level in GAL-fed rats may increase lipolysis in adipose tissue, and consequently an increased plasma FFA levels and a reduced adipocyte size. The GAL-associated decrease is also accompanied by reductions in plasma and hepatic concentrations of triglycerides, total cholesterol, and LDL-cholesterol. These findings are in agreement with others, which reported similar effects using other viscous fibers [27, 28]. The hypocholesterolemic effects of soluble fibers may slow glucose absorption, because of their viscosity as well as by binding bile acids in the intestinal lumen leading to a decreased enterohepatic circulation [29]. Furthermore, viscous fibers have been shown to increase the number of LDL receptors in the liver of rats [30, 31]. An up-regulation of the LDL receptors, therefore, increases clearance of LDL cholesterol. The results of reduced hepatic cholesterol and plasma LDL cholesterol in the present study, thus, suggest the possibility of an increased clearance of cholesterol in the liver.

In summary, GAL at 2.5% and 5% (w/w) reduced plasma triglyceride, cholesterol and hepatic cholesterol concentrations. GAL at 5% (w/w) resulted in lower food intake, which may have enhanced the release of plasma FFA, and in turn, led to the least amount (i.e. weight) of epididymal adipose tissue. These results clearly demonstrate that GAL from Canadian-grown fenugreek, a novel source of dietary fiber, has the potential benefits in modifying both glycemic and lipidemic status as well as body weight.

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