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ORIGINAL CONTRIBUTION

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Increased binding of LDL and VLDL to apo B,E receptors of hepatic plasma membrane of rats treated with Fibernat

Summary *Background* Research has focussed on the hypocholesterolemic effects of certain types of dietary fiber such as enhancing conversion of hepatic cholesterol to bile acids or increase in catabolism of low density lipoprotein (LDL) via the apo B,E receptor. *Aim of the study* The effect of oral administration of a unique fibre cocktail of fenugreek seed powder, guar gum and wheat bran (Fibernat) and its varied effects on some aspects of lipid metabolism and cholesterol homeostasis in rats were examined. *Methods* Rats were administered Fibernat along with the atherogenic diet containing 1.5 % cholesterol and 0.1 % cholic acid. Amounts of hepatic lipids, hepatic and fecal bile acids and activity of hepatic triglyceride lipase (HTGL) were determined. Transmission electron microscopic examination of the liver tissue and extent of uptake of ^{125}I-LDL and ^{125}I-VLDL by the hepatic apo B,E receptor was carried out. *Results* Food intake and body weight gain were similar between the 3 different dietary groups. Fibernat intake significantly increased apo B,E receptor expression in rat liver as reflected by an increase in the maximum binding capacity (B_{max}) of the apo B,E receptor to ^{125}I-LDL and ^{125}I-VLDL. The activity of HTGL was increased by approximately 1.5-fold in Fibernat-fed rats as compared to those fed the atherogenic diet alone. A marked hypocholesterolemic effect was observed. Cholesterol homeostasis was achieved in Fibernat-fed rats. *Conclusion* Two possible mechanisms are postulated to be responsible for the observed hypocholesterolemic effect a) an increase in conversion of cholesterol to bile acids and b) possibly by intra-luminal binding which resulted in increased fecal excretion of bile acids and neutral sterols. The resulting reduction in cholesterol content of liver cells coupled with upregulation of hepatic apo B,E receptors and increased clearance of circulating atherogenic lipoproteins – LDL and very low density lipoprotein (LDL and VLDL) – is the main mechanism involved in the hypocholesterolemic effect of Fibernat. The results suggest that Fibernat's effect on plasma LDL concentration is also possibly mediated by increased receptor-mediated catabolism of VLDL. Thus, Fibernat therapy is an effective adjunct to diet therapy and might find potential use in the therapy of hyperlipidemic subjects.

Key words dietary fibre – apo B,E (LDL) receptor – ^{125}I-LDL – ^{125}I-VLDL – cholesterol homeostasis – bile acids

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Introduction

The atherosclerotic process is associated with the accumulation of LDL-derived cholesterol in the intima of susceptible arteries with the development of fatty streaks [1]. The B lipoproteins (apo B-100 and apo B-48) are particularly important because they play central roles in lipoprotein assembly, plasma lipid metabolism and atherogenesis [2]. The liver plays a central role in whole body cholesterol homeostasis as the site for cholesterol metabolism, excretion through bile, neutral sterol elimination and as the regulator of plasma cholesterol concentrations through the synthesis of VLDL

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and catabolism of LDL via the apo B,E (LDL) receptor [3]. Changes in hepatic receptor activity produce changes in plasma LDL levels [4].

Diet therapy to decrease plasma cholesterol levels reduces the mortality and morbidity from atherosclerotic disease. Previous studies exist on the efficacy of certain types of dietary fibre in combating or retarding the atherosclerotic process by a) enhancing hepatic cholesterol utilization by its biodegradation into bile acids [5] or b) by secondary mechanisms such as an increase in catabolism of LDL via the LDL receptor [6], as approximately 80 % of the LDL uptake by the liver is through the LDL receptor and is subject to feedback regulation [7]. Endocytosis of VLDL remnants is also mediated by the LDL receptor with apo E as the ligand [8]. A significant reduction in plasma cholesterol due to reduced VLDL and LDL cholesterol levels in LDL receptor deficient mice which were injected with recombinant adenovirus vectors encoding for human LDL receptor has been reported [9]. Under the influence of a hypocholesterolemic and anti-atherosclerotic drug, an upregulation of the LDL receptor activity in rats has been observed by our group [10].

The present study was carried out in rats to elucidate the hypocholesterolemic mechanisms of a dietary fibre cocktail – Fibernat which contains fenugreek seed powder, guar gum and wheat bran in the ratio of 70:15:15 respectively. Fenugreek seeds and guar gum are similar in chemical composition as they contain a large proportion of galactomannans which are said to be water-soluble fractions. It has been shown in rats that dietary galactomannans lowered plasma and liver cholesterol concentrations [11]. Water soluble fibres such as guar gum may exert cholesterol lowering effects; it is generally accepted that bile acid absorption in portal blood is reduced, thus limiting the capacity of bile acid to downregulate liver cholesterol 7 α-hydoxylase, the rate limiting enzyme of bile acid synthesis [12]. Wheat bran is an insoluble fibre and has no effect unless they displace foods supplying saturated fats and cholesterol [13]. With these points in view, it was of interest to investigate the varied effects of Fibernat (which has hitherto not been researched for its hypolipidemic effect) on some aspects of lipid metabolism in rats. Administration of Fibernat along with an atherogenic diet and its effect on the plasma lipoproteins, different lipid classes in hepatic tissue, as well as on LDL receptor-mediated uptake of β-lipoproteins (LDL and VLDL), hepatic bile acid concentrations and fecal elimination of bile acids and neutral sterols was studied. The activity of hepatic triglyceride lipase (HTGL), the enzyme responsible for conversion of triglyceride rich chylomicrons and VLDL particles into cholesterol rich remnant particles [14] and its uptake by the hepatic apo B,E receptor was determined.

Material and methods

Experimental animals and diets

Eighteen male albino rats (Wistar strain) obtained from the Frederick Institute of Plant Protection and Toxicology (FIPPAT) were fed on standard pellet feed (Hindustan Lever India Ltd., Bangalore, India) for an adaptation period of one week. Following this, they were divided randomly into 3 groups of six animals each with average weights of 120 g. Group I (FF) was fed the control diet, Group II was fed the atherogenic diet containing 1.5 % cholesterol and 0.1 % cholic acid and an excess of vitamin D_2 (1.25 million U. S. P. units/kg), i. e., 31.25 mg/kg of diet. Group III was fed the atherogenic diet + Fibernat (Table 1). The body weights of the rats were recorded every 48 hours during the experimental period of 6 weeks. Food intake was recorded over two 3-day periods.

The atherogenic diet was based on the recommendations of Bajwa et al. [15] with some modifications. Although the use of the rat as a model for atherosclerosis has been in decline because of the difference in response to diet (HDL being the major carrier of plasma cholesterol) and its resistance to atherosclerosis [16]), rats can be induced to develop atherosclerosis by administration of an excess of vitamin D_2 [15].

Estimation of hepatic lipids

At the end of the experimental period of 6 weeks, animals were sacrificed by cervical dislocation. Lipids were extracted from liver tissue [17] and total and free cholesterol and triglycerides were measured [18]. Ester cholesterol concentrations were calculated as the difference between total and free cholesterol.

Table 1 Composition of diets/kg

Diet Group	Group I (control) (FF)	Group II (Atherogenic diet)	Group III (Atherogenic diet + Fibernat)
Sucrose	550	534	434
Butter	250	250	250
Corn Oil	140	140	140
Cholesterol	0	15	15
Cholic acid	0	1	1
Fibernat	0	0	100
Mineral mix	50	50	50
Vitamin mix	10	10	10

Assay of hepatic triglyceride lipase

Activity of hepatic triglyceride lipase was determined by the method of Schmidt et al. [19] in a triethanolamine (TEA) buffer medium (1 M pH 8.5) using 10 mM stearic acid as the standard.

Estimation of bile acids in liver and fecal samples

Fecal samples of the last 5 days of the experimental period were collected from the animals from each group, dried, powdered and extracted for bile acids according to the method of Meyer [20]. Liver bile acids were extracted by the method of Okishio et al. [21]. Cholic and deoxycholic acid contents of the extracts were determined [22].

Preparation of rat liver tissue for electron microscopy

Specimens for electron microscopy were excised from the liver of the 3 groups of rats, washed in phosphate buffered saline, fixed in 2.5 % glutaraldehyde, washed in cacodylate buffer (vehicle osmolality 300 mosmol/kg) and post fixed in OsO_4 solution (10 g/l). Ultrathin (50–75 nm) sections stained with uranyl acetate and lead citrate were used for electron microscopy. From each animal, two different specimens were used. The micrographs were chosen randomly and examined at a final magnification of 4500 X.

Preparation of ^{125}I-LDL and ^{125}I-VLDL

Plasma separated from EDTA-anticoagulated fresh blood from normal humans was used for the separation of the lipoprotein classes by the method of Chung et al. [23], using a single discontinuous density gradient ultracentrifugation in a Beckman L8–60 model ultracentrifuge at 10 °C with a slow start and then at an increased speed of 100,000 g for 19 hours. The LDL fraction ($1.019 < d < 1.063$ g/ml) and VLDL (d~1.006 g/ml) were collected, subjected to exhaustive dialysis against 0.09 % NaCl and 0.01 % EDTA (24 h) and checked for purity on agarose gel. Lactoperoxidase labelling of LDL and VLDL with ^{125}I was done by the method of Thorell and Johnson [24].

LDL and VLDL binding assays

Pooled liver tissues from each dietary group were used for isolation of plasma membranes by the method of Emmelot [25]. The specific activity of 5' nucleotidase was assayed in the original homogenate and in the isolated membrane preparation. The apo B,E receptor activity was assayed by determining the extent of binding of radiolabelled human LDL (^{125}I-LDL) and radiolabelled human VLDL (^{125}I-VLDL) to hepatic plasma membranes of rats *in vitro*. Human LDL is considered to measure accurately the relative differences on LDL-receptor activity of the rat although it may not reflect the fate of rat LDL [25].

The binding assay was carried out in triplicate following the method of Kovanen [27]. Values for B_{max} (maximum binding capacity) and K_d (binding affinity) were calculated using Scatchard curves [28]. Simple linear regression analysis of the specific binding data was done [29]. The x-intercept represented B_{max}, and the inverse of the slope represented K_d.

Statistical analysis

Results were analysed by one-way Anova with Tukey's post hoc comparisons. A probability of 0.05 or less was considered as statistically significant.

Results

Food intake (measured as total food consumption per cage) was similar between the dietary groups though it should be noted that the number of observations was small. Body weight gain was comparable among the three groups which showed that Fibernet did not affect these two parameters.

Increases in plasma total cholesterol ($p < 0.01$), LDL cholesterol ($p < 0.01$), VLDL cholesterol ($p < 0.01$) and the atherogenic index ($p < 0.01$) were observed in Group II rats compared to control. Supplementation of Fibernat prevented an increase in these parameters significantly. Plasma cholesterol concentrations were lower ($p < 0.01$) by 24.5 % in Group III rats (Table 2). LDL-cholesterol (LDL-C) ($p < 0.01$) and VLDL-cholesterol (VLDL-C) ($p < 0.01$) were significantly lower in this group. HDL-cholesterol (HDL-C) concentrations were unaffected by dietary treatment. The atherogenic diet caused a large increase in hepatic cholesterol (Table 3) in Group II rats. However, hepatic cholesterol concentrations were significantly lower in Fibernat-treated rats ($p < 0.01$). There was a marked lowering of hepatic free ($p < 0.05$) and ester cholesterol ($p < 0.01$). Triglyceride concentrations were also significantly lower ($p < 0.01$) in this group.

The activity of HTGL lipase was significantly lower in Group II rats ($p < 0.01$) than rats fed the control diet. A marked increase in the activity of HTGL was observed in Group III rats ($p < 0.01$) compared to Group II rats.

Figs. 1–3 represent electron micrographs of Group I, Group II and Group III rats respectively. Numerous fatty

Table 2 Effect of Fibernat on plasma lipids and atherogenic index (LDL-C + VLDL-C/HDL-C) in control and experimental rats

Group	Plasma Cholesterol				Atherogenic index
	Total	HDL-C	LDL-C	VLDL-C	
I	80.7±5.3	38.2±2.1	30.3±2.0	9.8±1.0	1.04±0.3
II	130.0±2.04*	42.1±4.3	65.3±2.1*	22.3±2.8*	2.08±0.2*
III	98.2±3.1**	41.3±4.4	40.2±3.8**	18.3±1.1**	1.40±0.3**

Values are mean ± S.D. of 6 rats
Group II vs Group I – * $p < 0.01$
Group III vs Group II – ** $p < 0.01$

Table 3 Effect of Fibernat on hepatic lipids and hepatic triglyceride lipase activity in control and experimental rats

Group	Total cholesterol (mg/g)	Free cholesterol (mg/g)	Ester cholesterol (mg/g)	Triglycerides (mg/g)	Hepatic triglyceride lipase (µg FFA released/ min/mg/protein)
I	3.46±0.3	2.03±0.3	1.42±0.09	4.92±0.7	17.2±2.1
II	4.20±0.2*	3.10±0.3*	1.12±0.06*	6.93±0.4*	9.5±1.2*
III	3.7±0.1**	2.48±0.5***	1.06±0.03**	5.36±0.8**	15.7±2.2**

Values are mean ± S.D. of 6 rats
Group II vs Group I – * $p < 0.01$
Group III vs Group II – ** $p < 0.01$, *** $p < 0.05$

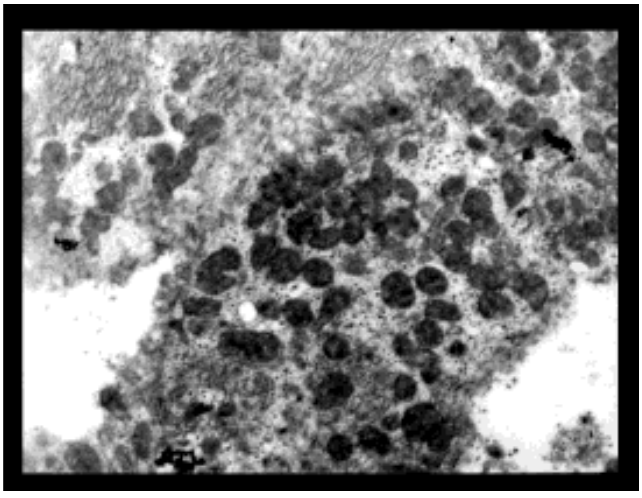


Fig. 1 Electron micrograph of control rat liver showing normal architecture (x 4500)

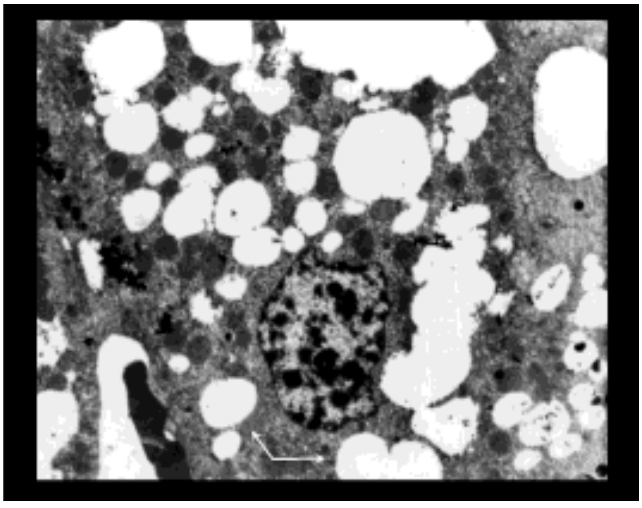


Fig. 2 Electron micrograph of the atherogenic diet-fed rat liver showing marked lipid vacuolation (x 4500)

changes such as fatty infiltration and accumulation of lipid in lipid vacuoles were observed in Group II rats and a marked alleviation of these changes in Group III rats.

Hepatic bile acids, fecal bile acids and elimination of neutral sterols in feces of rats increased significantly in both Group II and Group III rats (Table 4).

The specific activity of 5' nucleotidase which is a common marker enzyme for liver plasma membranes increased by about 3-fold above the original homogenate (data not shown). When LDL was electrophoresed on agarose gel, a single band of LDL was seen in contrast to three bands of whole plasma (data not shown). Hepatic plasma membrane proteins were electrophoresed along with molecular weight markers on a 7.5 % polyacrylamide gel. The apo B,E receptor corresponds to a protein of molecular weight of 160 kDa (data not shown). Autoradiography was performed in order to visualise binding of ^{125}I-LDL and ^{125}I-VLDL) to apo B,E receptor (data not shown). Figs. 4 and 5 represent the saturation curves for the specific binding of ^{125}I-LDL and ^{125}I-VLDL to hepatic plasma membranes from control and experimental animals. Fig. 6 represents the Scatchard plot of the specific binding data for apo B,E receptor with ^{125}I-LDL. The B_{max} (maximum binding capacity) of the apo B,E receptor was increased signifi-

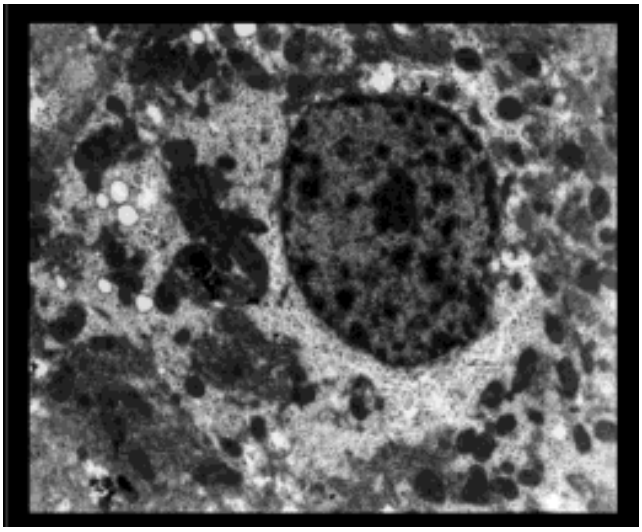


Fig. 3 Electron micrograph of Fibernat-fed rat liver. A marked reduction in lipid vacuoles was observed (x 4500)

cantly (by 37 %) ($p < 0.01$) in Fibernat-fed rats compared to Group II. The K_d (receptor-ligand binding affinity constant) was also modified by dietary treatment (Table 5). The atherogenic diet caused an increase in B_{max} of the receptor to ^{125}I-LDL by 24.4 %. In this case, K_d was not modified compared to that of control rats (Table 5).

Fig. 7 represents the Scatchard plot of the specific binding data for apo B,E receptor with ^{125}I-VLDL. In Group II rats, B_{max} of the receptors to ^{125}I-VLDL and K_d were not significantly different from that of control rats. The Fibernat-fed rats however displayed a dramatic increase in B_{max} of the receptor to ^{125}I-VLDL ($p < 0.01$) by 64 % as compared to Group II rats. In this case, the K_d was not modified (Table 6). Fig. 8 is a comparison of the B_{max} of apo B,E receptor to ^{125}I-LDL and ^{125}I-VLDL. B_{max} of ^{125}I-VLDL to the apo B,E receptors was comparable to the B_{max} of the same receptors to ^{125}I-LDL in Group I and Group III rats.

Discussion

In the present study, the effect of Fibernat on cholesterol homeostasis was studied with reference to regulation of plasma lipoprotein cholesterol concentrations, hepatic cholesterol pools, hepatic triglycerides, hepatic bile acids, fecal elimination of bile acids, neutral sterols and hepatic apo B,E receptor expression.

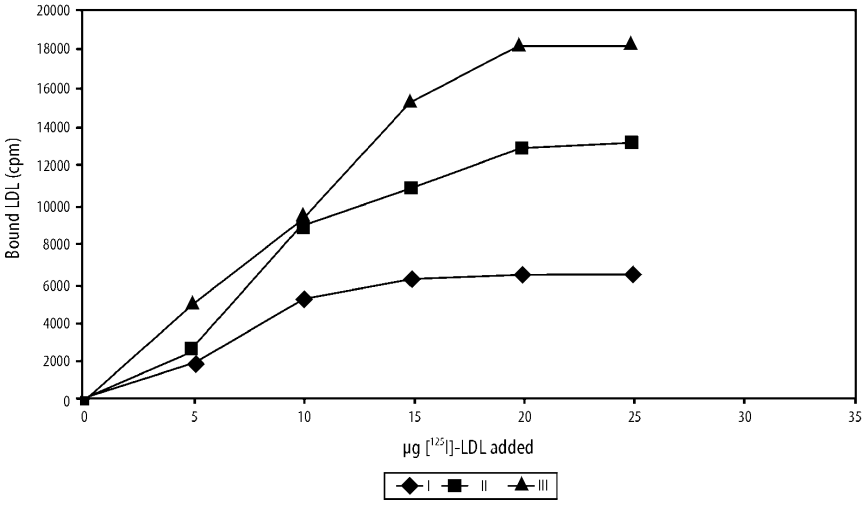
Rats were susceptible to the dietary cholesterol challenge. The hepatic cholesterol and triglyceride levels

Table 4 Effect of Fibernat on hepatic and fecal bile acids and neutral sterols in control and experimental rats

Group	Neutral Sterols mg/day/rat	Fecal bile acids (mg/g)		Liver bile acids (mg/g)	
		Cholic acid	Deoxycholic acid	Cholic acid	Deoxycholic acid
I	20.2±2.3	10.3±1.3	11.5±1.3	15.3±1.3	7.9±2.5
II	30.5±2.5*	23.3±2.5*	30.2±3.3*	21.2±2.6*	10.9±1.7
III	42.5±5.0**	49.9±2.3**	45.5±3.2**	29.5±3.2**	14.7±1.9***

Values are mean ± S.D. of 6 rats
Group II vs Group I – * $p < 0.01$
Group III vs Group II – ** $p < 0.01$, *** $p < 0.05$

Fig. 4 Specific binding of ^{125}I-LDL to rat liver plasma membranes. Saturation curves for the specific binding of ^{125}I-LDL to hepatic plasma membranes isolated from the control and experimental animals. Group I rats were fed the "normal" diet, Group II and Group III rats were fed the atherogenic diet. Group III rats received a 10 % fibernat supplementation for 6 weeks



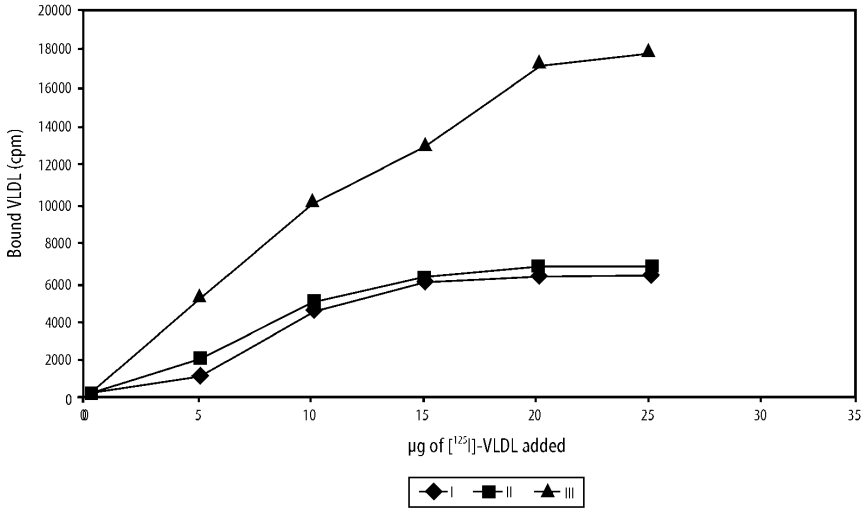


Fig. 5 Specific binding of ^{125}I-VLDL to rat liver plasma membranes. Saturation curves for the specific binding of ^{125}I-VLDL to hepatic plasma membranes isolated from the control and experimental animals. Group I rats were fed the "normal" diet, Group II and Group III rats were fed the atherogenic diet. Group III rats received a 10 % fibernat supplementation for 6 weeks

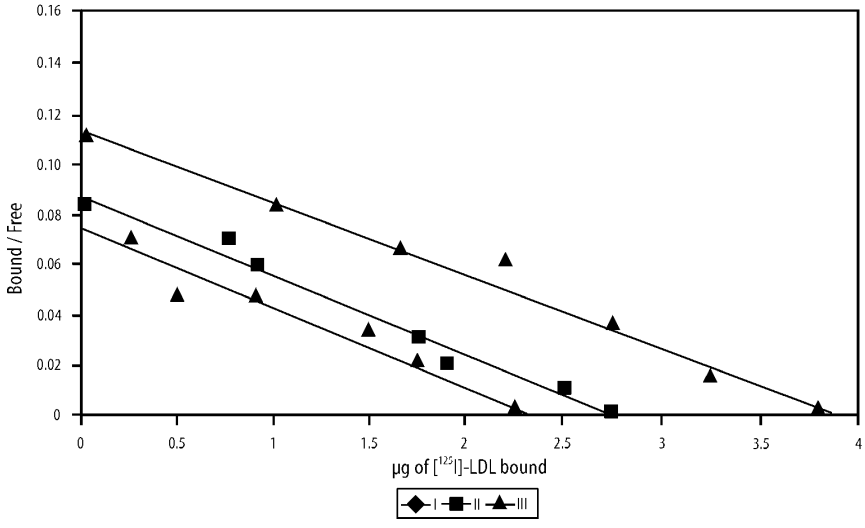


Fig. 6 Scatchard plots of the specific binding of ^{125}I-LDL to rat liver plasma membranes

Table 5 Scatchard analysis of the specific binding data of apo B,E receptor to ^{125}I-LDL from control and experimental rat liver plasma membrane

Group	K_d (µg)	B_{max} (µg LDL/mg protein)
I	29.41 ± 3.9	2.25 ± 0.31
II	21.77 ± 4.8	2.80 ± 0.17
III	37.03 ± 7.3*	3.85 ± 0.29**

Values are indicated as the mean ± S.D. of triplicate analyses
Group III vs Group II – ** $p < 0.01$, * $p < 0.05$

Table 6 Scatchard analysis of the specific binding data of apo B,E receptor to ^{125}I-VLDL from control and experimental rat liver plasma membrane

Group	K_d (µg)	B_{max} (µg VLDL/mg protein)
I	26.47 ± 9.3	2.10 ± 0.42
II	33.33 ± 10.8	2.25 ± 0.010
III	27.77 ± 11.1	3.70 ± 0.30*

Values are indicated as the mean ± S.D. of triplicate analyses
Group III vs Group II – * $p < 0.01$

were higher by 21.4 % and 68.2 % respectively in Group II rats compared to controls. This was evident in the electron micrographs of rat liver of Group II rats which showed a drastic increase in lipid vacuoles possibly due to an ingress and deposition of plasma lipids into the liver. The same phenomenon was observed in cholesterol and cholic acid fed animals [30]. In our study, plasma cholesterol levels increased by 61.1 %, LDL cholesterol increased by 115 % and VLDL cholesterol by 127.55 %. The increased hepatic cholesterol and triglyceride concentrations could be the source of increased VLDL and LDL cholesterol fractions in plasma.

There was an observed increase in hepatic bile acids, fecal elimination of bile acids and neutral sterols. Rats

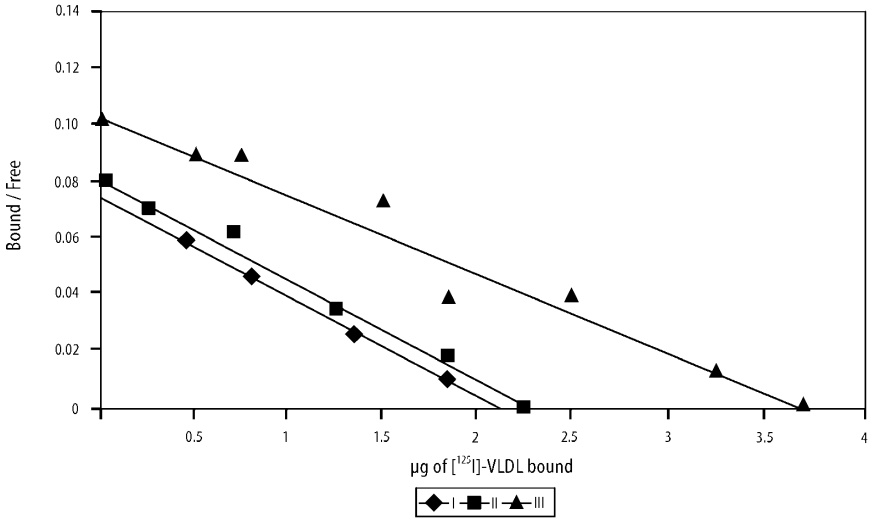


Fig. 7 Scatchard plots of the specific binding of ^{125}I-VLDL to rat liver plasma membranes

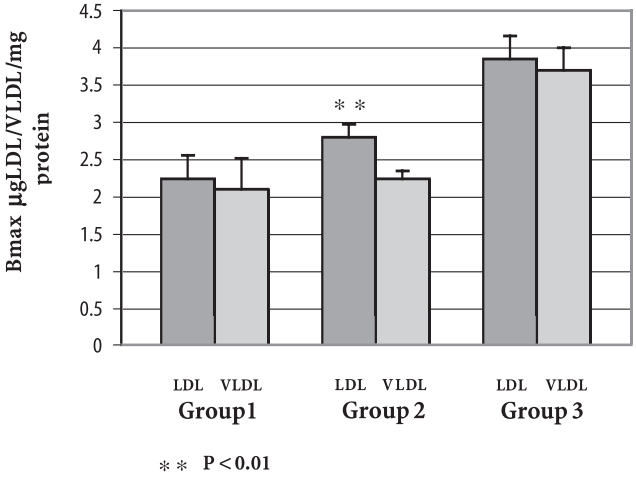


Fig. 8 Comparison of the B_{max} of apo B,E receptor to ^{125}I-LDL/^{125}I-VLDL

respond to an excess dietary cholesterol 'load' by increasing bile acid synthesis, thereby maintaining steady-state hepatic cholesterol concentrations. Control mechanisms in the rat prevent a disturbance in homeostasis by a reduction in the body's synthesis of cholesterol and by an increased conversion of cholesterol to bile acids which promote fecal sterol excretion [16].

Hepatic plasma membrane receptors (apo B,E) were electrophoresed along with molecular weight markers. The LDL receptor is a 160 kDa protein [31]. Binding of the receptor-ligand was in alignment with the 160 kDa molecular weight as visualized by autoradiography (data not shown).

Dietary cholesterol and fatty acids influence circulating concentrations of LDL-cholesterol in animals and humans by changing either LDL (apo B,E) receptor activity, the LDL cholesterol production rate, or both. The LDL (apo B,E) receptor activity is regulated by the sterol content of the hepatic cells. Normally, high amounts of cellular cholesterol suppress LDL-receptor activities and hence, LDL uptake by liver cells [32]. However, in this case, high levels of cholesterol in hepatic tissues cause a slight upregulation of the apo B,E receptor as seen by the increase (24.4%) in B_{max} of the receptors in Group II rats. Normal compensatory mechanisms, namely the negative feedback regulation that causes a suppression in LDL receptor (when the hepatic cholesterol concentration is high), have probably been overwhelmed due to cholesterol loading in the diet for 6 weeks. Because of this, accumulation of cholesterol in hepatic tissues has been observed as well as an increase in plasma total cholesterol.

A 38.4% lowering of LDL-C, 17.9% lowering of VLDL-C, a non-significant decrease in HDL-C, a 11.89% decrease in hepatic cholesterol and 29.3% decrease in hepatic triglyceride concentrations were observed in Group III rats compared to that of Group II rats. Rats (and hamsters) generally respond to dietary fibre by a modest reduction in LDL and a marked reduction in HDL [33]. Our observations however are in sharp contrast. An alleviation in fatty changes such as lipid-laden vacuoles in the liver of Group III rats was observed by electron microscopy. This is in contrast to the study by Mallki [30] wherein oat bran, a soluble fibre, accentuated liver fat accumulation. The observed dietary fibre-mediated reduction in hepatic cholesterol, whether attained by decreasing cholesterol absorption or interruption of the enterohepatic circulation of bile acids triggers a series of regulatory responses that culminate in the lowering of plasma LDL-cholesterol. Wheat bran (a component of Fibernat) an insoluble fibre, although not directly contributing to lower plasma cholesterol levels, could act indirectly by decreasing intestinal transit time and hence cholesterol and carbohydrate absorption. Rats fed Fibernat had lower hepatic triglyceride concen-

trations than rats fed the atherogenic-diet alone. The mechanism most likely associated with this hepatic triglyceride depletion may be related to the capacity of soluble fibre to delay sucrose absorption in the intestinal lumen [34]. In addition, it has been postulated that fructose skips the phospho-fructokinase regulatory step in glycolysis, increasing acetyl-CoA concentrations and therefore lipogenesis. Because fructose is one of the components of sucrose, it could be suggested that Fibernat, by inhibiting simple carbohydrate absorption, may decrease hepatic lipogenesis and therefore reduce hepatic triglyceride concentrations [35]. Also, in Group III rats, the fibre has been included in the diet at the expense of calories from sucrose following advocates of the necessity of complex carbohydrates replacing simple carbohydrates. The decrease in triglyceride concentrations could be partly attributed to this.

Fibernat intake caused a 37.5 % increase in the binding of ^{125}I-LDL to hepatic plasma membranes as compared to Group II rats. This was reflected by an increase in B_{max} which means an increase in the number of apo B,E receptors. K_d values were also significantly different in this group of rats. Synthetic pharmaceutical agents such as reserpine exert their anti-atherogenic effects partly by increasing LDL receptors in the liver [36]. The same phenomenon has been observed by administration of Tincture of *Crataegus* along with an atherogenic diet to rats [10]. Natural products such as guar gum, pectin and psylium increased the number of hepatic apo B,E receptors (B_{max}) in female guinea pigs when fed along with a diet containing a physiological dose of cholesterol. There was, however, no increase in K_d (binding affinity) [33]. Intake of pectin and psyllium resulted in a 45 % higher number of hepatic apo B,E receptors in male guinea pigs [37]. Some recent studies on mushroom and sugar beet fibre supplementation in rats [38, 39] demonstrate the enhancement of hepatic LDL receptor mRNA levels. In contrast soy-protein isolate inhibits atherosclerosis in mice by LDL receptor and plasma lipoprotein independent pathways [40]. The water soluble fractions of Fibernat namely fenugreek and guar gum could have mediated this increase in B_{max} of the receptors in Fibernat fed rats. An increase in B_{max} of apo B,E receptors to ^{125}I-LDL suggests that the hypolipidemic effect of the fibre is mediated through an increased LDL influx into the liver (which contributes to hepatic cholesterol pools) and subsequently, its catabolism to bile acids. This is substantiated by the finding that there is a significant increase in elimination of fecal bile acids as compared to the Group II rats. The fecal bile acids could be from two sources:

- An intra-luminal binding of bile acids by Fibernat and its subsequent excretion in feces.
- An increased conversion of cholesterol to bile acids in the hepatic cells and a subsequent elimination in the feces.

Water soluble fibres (both partially hydrolyzed and native guar gum) have the capacity to entrap bile acids in the small intestine and to increase their fecal excretion [41]. This could accelerate cholesterol oxidation to bile acids, resulting in a spill over of the body cholesterol pool due to fecal losses of sterols. Enhanced steroidal fecal excretion, greater intestinal bile acid absorption and portal flux to the liver were discussed as primary mechanisms of the lipid lowering effect of guar gum [12]. Studies in rats suggest that guar gum decreases plasma cholesterol concentrations by increasing bile acid production with little effect on cholesterol absorption [42].

In Group II rats, it was observed that the circulating plasma VLDL cholesterol concentrations were high and there was a slight (non-significant) increase in the VLDL binding as compared to control rats. The B_{max} of the receptors to ^{125}I-VLDL in atherosclerotic rats was less than B_{max} of receptors to ^{125}I-LDL in the same group although there is a hypothesis that LDL and VLDL bind with comparable affinity to the hepatic LDL receptor [43]. The metabolism of VLDL is by 2 mechanisms – a successive depletion of core triglycerides of VLDL by the enzyme lipoprotein lipase (LPL) converting it to Intermediate Density Lipoprotein (IDL) enriched in cholesteryl esters (CE) and retaining a portion of the original complement of apo E in addition to one copy of apo B-100. The fate of these particles is dichotomous at this point. Approximately half of these (those with a large complement of apo E) are endocytosed in the liver; the remainder are converted to cholesterol ester-rich daughter particles namely LDL [44]. Our finding leads us to two conclusions: i) in the atherogenic-diet fed rats, the delipidation cascade accounts for a greater proportion of the metabolism of VLDL to yield LDL particles. This probably is the explanation for the increased circulating plasma LDL cholesterol. Cholesterylester enriched plasma LDL was observed in Group II rats in our previous study (data not shown). ii) On account of VLDL being metabolised in this way, there is less VLDL (VLDL remnants) available for uptake by the apo B,E receptors (which is reflected by a decrease in B_{max} of hepatic receptor to VLDL as compared to B_{max} of hepatic receptor to ^{125}I-LDL). High cholesterol diets have been observed to upregulate LPL activity which has a major role in conversion of VLDL to LDL [45].

In Group III rats, the B_{max} of hepatic receptors to ^{125}I-VLDL increases and is comparable to the B_{max} of the receptor with the native ligand, i. e. LDL. In this group, VLDL metabolism is probably mediated through endocytosis via LDL receptor uptake, rather than the delipidation cascade. As a result, there is probably less conversion to LDL. It is also possible that due to less sucrose in the diet (being replaced partially by Fibernat), and hence fewer circulating triglycerides, there is proportionally less circulating VLDL.

This factor also probably contributes to lower circu-

lating plasma LDL cholesterol level in addition to other previously mentioned factors. Huff et al. [46] suggest that lower hepatic cholesterol concentrations are also associated with reductions in VLDL and LDL cholesterol. In a similar study, when Psyllium, a soluble fibre, was fed along with cholesterol-containing diets, it was observed that VLDL was not readily converted to LDL through the delipidation cascade [6].

In agreement with these results, in the Fibernat fed rats, HTGL was significantly higher than that of Group II rats. This could have an important metabolic meaning. It has been shown that HTGL enhances VLDL degradation in cultured cells by a LDL receptor-mediated mechanism leading to endocytosis and degradation [47]. This finding correlates well with the observed increase in VLDL uptake by the apo B,E receptor in Group III rats. In contrast, HTGL activity was significantly lower in Group II rats compared to that of control rats. In conditions with an impaired postprandial lipid clearing such as in coronary heart diseases, hepatic lipase deficiencies have been observed [48]. This probably explains the lower VLDL uptake by the apo B,E receptor in Group II rats.

Thus, these studies suggest that, in addition to plasma and hepatic cholesterol lowering, a possible interruption of enterohepatic circulation of bile acids, increased fecal elimination of bile acids and neutral sterols and increased uptake of circulating plasma LDL and VLDL by apo B,E receptors, there might be other primary mechanisms affecting lowering of hepatic cholesterol and that fibre effects on plasma LDL cholesterol concentrations are also possibly mediated though increased receptor-mediated catabolism of VLDL. The various constituents of Fibernat may act synergistically to bring about the observed effects.

The results obtained in this study suggest the unequivocal hypolipidemic effect of Fibernat in rats. Since an increased elimination of fecal bile acids and marked reductions in hepatic cholesterol pools of rats were observed upon intake of Fibernat, our research group is currently investigating the activity of cholesterol 7 α-hydroxylase which is the rate limiting enzyme for bile acids synthesis.

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