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Dietary fibres ameliorate decreased synthesis of heparan sulphate in streptozotocin induced diabetic rats

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Abstract

The role of dietary fibers in diabetes has been studied by several workers [1,2]. Long term dietary treatment with increased amounts of fiber-rich low-glycaemic index natural foods improves blood glucose and reduces the number of hypoglycemic events in type I diabetic patients [3,4]. On the other hand Rohrbach and Martin [5] and Cohen and Surma [6] described changes in the general and biochemical structure of renal tissues such as the glomerular basement membranes. One of these changes was the reduction and undersulfation of the glycoconjugate and glycosaminoglycan heparan sulfate, which plays an important role in renal structure and function [7,8]. The purpose of the present study was to determine specific effects of two types of dietary fiber on the composition of kidney glycoconjugates in an animal model of diabetes type I. Streptozotocin-treated diabetic rats were fed either a control diet or diets containing 10% wheat bran (insoluble dietary fiber) or 5% guar gum (soluble dietary fiber). Effects of these fibers on glycaemic control and nephropathy were assessed using previously described methodologies. The effect of dietary fiber in the glycoconjugate composition of kidneys of control and diabetic animals was studied by estimating their total hexose content, sulfated glycosaminoglycans, hexosamines and uronic acids. The activities of enzymes that participate in the synthesis of saccharides and glycoconjugates (L-glutamine-fructose-6-phosphate aminotransferase) and their degradation (N-acetyl-β-glucosaminidase and β-glucuronidase) were also evaluated. Results indicated that both soluble and insoluble dietary fibers ameliorated a significant increase in the activity of GFAT. Heparan sulfate was also isolated and quantified. Results indicated that the renal content of heparan sulfate decreased in diabetic animals and that this decrement was ameliorated by the ingestion of both soluble and insoluble fiber in the diet.

Keywords: Dietary fiber; Wheat bran; Guar gum; Diabetes; Glycosaminoglycans; Heparan sulfate

1. Introduction

Diabetes mellitus (DM) is one of the major metabolic diseases, which affects large number of people around the globe. DM, which is marked by sustained hyperglycaemia, has deleterious effects on various organs. Nephropathy is one such manifestations of diabetes, which affect the kidney. During diabetic nephropathy, the glomerular basement membrane is known to become thicker with a reduction in the contents of heparan sulfate and laminin [7,8,9] and an increase in type IV collagen [5] and reduced sulfation [6]. In recent years the role of mesangial cell expansion during diabetic nephropathy state is receiving much attention [10].

Apart from insulin and anti-diabetic drugs, fibers have become important nutritional components in the management of diabetes. Effect of fibers in the management of diabetes is well documented [2,11,12]. Dietary fibers (DF) play an important role in facilitating slow absorption of glucose. In recent years fermentation of DF to short chain fatty acids (SCFA) such as acetate, propionate and butyrate has gained a lot of interest. Butyrate in particular is shown to modulate activities of many cellular enzymes including enzymes involved in glycoconjugate metabolism such as sialyl transferase [13] and sulfotransferase [14].

Though food rich in DF is recommended to diabetics, its beneficial effect on diabetic nephropathy especially in relation to its effect on glycosaminoglycans such as heparan sulfate, which plays an important role in the process of filtration, is not known. In this investigation we have studied the effect of DF such as wheat bran (insoluble DF) and guar gum (soluble DF) on sulfated glycosaminoglycans.

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2. Materials and methods

2.1. Animals

Male wistar rats [OUTB-Wistar IND cft(2c)] were divided into three main groups. The rats were maintained on AIN-76 diet. I group of rats were fed with diet without fiber. II and III group of rats were fed with diet containing 10% wheat bran and 5% guar gum, respectively. Each group was subdivided into two sub-groups of which one of the subgroup served as control (6 rats) and the other served as diabetic group (14 rats). Diabetes was induced by a single i.p. injection of streptozotocin (55 mg/kg body weight) [15]. At the end of 35 days animals were sacrificed under ether anesthesia, kidneys were harvested, washed in cold saline, blotted dry, weighed and frozen until further analysis.

2.2. Estimation

Glomerular filtration rate (GFR) was estimated by measurement of creatinine in blood and urine (24 h collection). Creatinine was estimated by Folin's method [16]. GFR was estimated using the formula [17]:

$$\text{GFR} = \frac{\text{Urinary creatinine (mg/dL)} \times \text{urine volume (mL)}}{\text{Plasma creatinine (mg/dL)} \times 1440 \text{ (min)}}$$

Uronic acid was estimated in the urine by carbazole method [18] using appropriate controls.

2.3. Processing of kidney

Kidneys were freed of external fat, cut into small pieces and dried in acetone in cold at 4°C for about a month. Acetone was changed every week. Later, the tissue was defatted in Soxhlet extractor [19]. The tissue was powdered thoroughly using pestle and mortar. Powdered tissue samples were taken for estimation after hydrolyzing them in 2N trifluoroacetic acid (TFA) for total sugars, and uronic acids, 2N HCl for amino sugar and 60% formic acid for sulfates in sealed tubes at 100°C for 6-8 h in a oven. For protein estimation tissues were solubilised in 0.1N sodium hydroxide solution by repeated sonication and vortexing. Total sugars were estimated by phenol-sulfuric acid method using glucose as standard [20], uronic acid by carbazole method using glucuronic acid as standard [18], amino sugars by Ludoweig and Benmaman method using glucosamine as standard [21], sulfates by Dodgson's method [22] and proteins by Lowry's method [23].

Activities of L-glutamine-fructose-6-phosphate aminotransferase and β-N-acetyl glucosaminidase and β-glucuronidase were done as described earlier by Pogell and Gryder [24] and Kawai and Anno [25], respectively.

2.4. Isolation of glycosaminoglycans

GAGs were isolated as reported by Scott [26]. Briefly, powdered kidney tissues were subjected to papain digestion twice. The extract was then subjected to TCA precipitation and centrifuged. To the supernatant, 2 volumes of alcohol containing 2% potassium acetate was added to precipitate GAGs. This was done twice. The precipitate was solubilised in water and the aliquots used for various estimations, total sugars, uronic acid, amino sugars, sulfates as mentioned above.

Glycosaminoglycans were fractionated into heparan sulfate and chondroitin sulfate using chondroitinase ABC [27]. Sulfated GAGs were estimated using dimethylmethylene blue [28]. Agarose gel electrophoresis was carried out at 80V for 5 h [29]. GAGs corresponding to equal weights of the kidney tissue were loaded into the wells and run. The gel was then stained in toluidine blue and destained in acetic acid-water.

2.5. Statistical analysis

In the experiments wherein the samples were not pooled, statistical analysis was done using Students' 't' test [30].

Table 1
Effect of wheat bran and guar gum on fasting blood glucose, urine sugar and urine output in control and diabetic rats

Group	Fasting blood glucose (mg/dL)	Urine sugar (reducing) (g/24h)	Urine volume (mL/24h)
SFC	129.34 ± 6.62	0.01 ± 0.004	27.5 ± 10.60
SFD	426.80 ± 29.20[a]	11.63 ± 1.530[a]	122.8 ± 12.03[a]
BFC	121.58 ± 6.58	0.02 ± 0.010	10.0 ± 7.07
BFD	346.90 ± 18.84[b]	8.77 ± 1.450[b]	95.6 ± 13.14
GFC	121.12 ± 5.76	0.01 ± 0.002	15.0 ± 2.82
GFD	205.32 ± 19.36[b]	4.24 ± 0.490[b]	67.1 ± 6.54[b]

Values are mean ± SEM of 6 rats in control groups and 14 rats in diabetic groups.

[a] Statistically significant when compared to SFC at $p < 0.05$.

[b] Statistically significant when compared to SFD at $p < 0.05$.

SFC = Starch fed control; SFD = Starch fed diabetic; BFC = Bran fed control; BFD = Bran fed diabetic; GFC = Guar gum fed control; GFD = Guar gum fed diabetic.

Table 2
Effect of wheat bran and guar gum on the weight of the kidney, uronic acid excretion and glomerular filtration rate (GFR) in control and diabetic rats

Group	Weight of kidney (g/100g body wt)	Uronic acid excretion (mg/24 h)	GFR (mL/min)
SFC	0.64 ± 0.02	6.4 ± 1.56	0.70 ± 0.09
SFD	1.22 ± 0.04[a]	669.6 ± 127.45[a]	2.63 ± 0.17[a]
BFC	0.63 ± 0.02	6.3 ± 1.25	0.63 ± 0.09
BFD	1.07 ± 0.02[b]	385.0 ± 97.93[b]	3.33 ± 0.36
GFC	0.63 ± 0.02	4.6 ± 0.08	0.80 ± 0.05
GFD	1.10 ± 0.04[b]	332.2 ± 58.42[b]	1.53 ± 0.16[b]

Values are mean ± SEM of 6 rats in control groups and 14 rats in diabetic groups.
[a] Statistically significant when compared to SFC at $p < 0.05$.
[b] Statistically significant when compared to SFD at $p < 0.05$.
SFC = Starch fed control; SFD = Starch fed diabetic; BFC = Bran fed control; BFD = Bran fed diabetic; GFC = Guar gum fed control; GFD = Guar gum fed diabetic.

3. Results

Diabetes was induced in rats using streptozotocin. Control group had 6 rats and diabetic 14. Diabetic status was ascertained by measuring fasting blood glucose, urine sugar and urine output (Table 1). In diabetic animals there was a significant increase in urine sugar and fasting blood sugar and were ameliorated to considerable extents [15] in the experimental groups fed with either wheat bran (10%) or guar gum (5%). At the end of the experiment about 6-8 rats were used for further studies.

3.1. Effect on renal enlargement

Diabetes was found to affect the relative weight of kidney (Table 2). The relative weight of kidney increased in starch fed diabetic animals (SFD) when compared to starch fed controls (SFC). Both wheat bran and guar gum were effective in controlling renal enlargement during diabetes (BFD and GFD, respectively).

3.2. Effect on uronic acid excretion and glomerular filtration rate

Uronic acid excretion was found to increase during diabetes which was ameliorated by the presence of wheat bran and guar gum in the diet (Table 2). Increase in uronic acid excretion during diabetes is in tune with the reported literature [31].

Glomerular filtration rate (GFR) measured in terms of creatinine clearance, increased significantly in diabetic rats (Table 2).

3.3. Effect on glycoconjugates in kidney

Various constituents of glycoconjugates such as total sugars, uronic acids, amino sugars, sulfates and proteins were estimated in the kidney and the results revealed that there was a significant increase in total sugar and uronic acid contents during diabetes (SFD) when compared to SFC (Table 3). Increase in total sugar and uronic acid content was partially or totally prevented by the presence of wheat bran and guar gum in the diet which was statistically significant. There was not much of a change in amino sugar

Table 3
Effect of wheat bran and guar gum on the carbohydrate composition of kidney tissue in control and diabetic rats

Groups	Total sugars	Uronic acid	Amino sugars	Sulphates	Proteins
	(mg/g dry tissue)				
SFC	20.62 ± 0.51	1.50 ± 0.13	5.40 ± 0.27	6.41 ± 1.06	618.63 ± 23.14
SFD	25.29 ± 1.01[a]	2.53 ± 0.09[a]	6.01 ± 0.36	6.90 ± 0.20	540.31 ± 10.02[a]
BFC	20.22 ± 0.45	1.52 ± 0.19	4.86 ± 0.12	6.42 ± 0.90	560.31 ± 7.42
BFD	22.10 ± 0.46[b]	1.50 ± 0.09[b]	6.08 ± 0.50	6.51 ± 0.08	577.01 ± 6.43[b]
GFC	20.35 ± 0.79	1.62 ± 0.23	5.25 ± 0.54	6.53 ± 0.24	552.52 ± 10.91
GFD	20.87 ± 0.52[b]	2.08 ± 0.11[b]	6.10 ± 0.42	6.90 ± 0.18	558.43 ± 8.13

Values are mean ± SEM of 6 rats in control groups and 14 rats in diabetic groups
[a] Statistically significant when compared to SFC at $p< 0.05$.
[b] Statistically significant when compared to SFD at $p< 0.05$.

Table 4
Effect of wheat bran and guar gum on activities of renal enzymes in control and diabetic rats

Group	Glutamine fructose-6-phosphate aminotransferase	N-acetyl β-glucosaminidase (NAG)	β-Glucurnidase
	(μmoles of product formed/h/g protein)		
SFC	12.04 ± 1.12	2680 ± 108	1.89 ± 0.078
SFD	23.53 ± 1.12[a]	4408 ± 322[a]	2.13 ± 0.078
BFC	11.17 ± 1.34	2664 ± 461	1.62 ± 0.092
BFD	11.89 ± 0.65[b]	2613 ± 191[b]	1.76 ± 0.043[b]
GFC	11.60 ± 1.44	2385 ± 103	2.02 ± 0.032
GFD	9.58 ± 0.14[b]	4170 ± 143	1.68 ± 0.094[b]

Values are mean ± SEM of 6 rats in control groups and 14 rats in diabetic groups.

[a] Statistically significant when compared to SFC at $p < 0.05$.

[b] Statistically significant when compared to SFD at $p < 0.05$.

SFC = Starch fed control; SFD = Starch fed diabetic; BFC = Bran fed control; BFD = Bran fed diabetic; GFC = Guar gum fed control; GFD = Guar gum fed diabetic.

and sulfate content. There was a decrease in protein content during diabetes. In wheat bran and guar gum fed groups, not much change was observed between control and diabetic animals (BFC/BFD, GFC/GFD).

3.4. Effect on activities of renal enzymes involved in glycoconjugate metabolism

Activities of renal enzymes like L-glutamine-fructose-6-phosphate amino transferase (GFAT), involved in synthesis of amino sugars and N-acetyl β-glucosaminidase (NAG) and β-glucuronidase which are degradative enzymes of glycosaminoglycans were elevated during diabetes (Table 4). Activities of enzymes like GFAT and NAG nearly doubled in SFD when compared to SFC. Both wheat bran and guar gum in the diet were effective in completely preventing the increase of GFAT whereas only wheat bran in the diet was effective in preventing the increase in NAG activity. There was no change in the activity of β-glucuronidase during diabetes. In the fiber fed animals (BFD and GFD) the activity was less than SFD which was statistically significant.

3.5. Effect on glycosaminoglycans

For the isolation of glycosaminoglycans, kidneys were pooled. The composition of uronic acids, total sugars, amino sugars and sulfates were examined (Table 5) in isolated glycosaminoglycans. The uronic acid content of the isolated GAGs were found to decrease during diabetes which was ameliorated by the presence of wheat bran and guar gum in the diet. Same was the case with amino sugar content. Total sugar content of GAGs was found to decrease during diabetes when compared to SFD. Fiber in the diet further decreased the total sugar content in diabetic animals.

Table 5
Effect of wheat bran and guar gum on the composition of glycosaminoglycans of renal tissue in control and diabetic rats

Groups	Uronic acid	Total sugars	Amino sugars	Sulphate
		(μ/g dry tissue)		
SFC	1002	6028	672	1990
SFD	810	5606	512	1070
BFC	1003	7062	673	2000
BFD	955	4440	665	1800
GFC	1037	5789	692	2010
GFD	905	4003	711	1700

Values are the average of triplicates of 6-pooled samples in control rats and 8-pooled samples in diabetic rats.

SFC = Starch fed control; SFD = Starch fed diabetic; BFC = Bran fed control; BFD = Bran fed diabetic; GFC = Guar gum fed control; GFD = Guar gum fed diabetic.

Wheat bran and guar gum in the diet proved beneficial in preventing the decrease in sulfate content by 9.5 and 10.5%, respectively when compared to SFC.

Heparan sulfate was separated from other sulfated GAGs by treating with the enzyme chondroitinase ABC. The content of total sulfated GAGs was found to decrease by 41% during diabetes when compared to control animals (Fig. 1). This decrease was prevented to certain extent by the presence of wheat bran and guar gum in the diet. Both heparan sulfate and chondroitin sulfate were found to decrease during diabetes when compared to control animals. Both wheat bran and guar gum were beneficial in ameliorating the decreased synthesis of heparan sulfate, whereas only wheat bran was effective with respect to chondroitin sulfate.

Separation of GAGs was attempted by agarose gel electrophoresis. Equal amount of kidney samples were loaded. Heparan sulfate was the major component (Fig. 2). The other minor component corresponded to chondroitin sulfate B. The electrophorogram clearly shows decrease in the content of heparan sulfate during diabetes and its amelioration by wheat bran and guar gum.

4. Discussion

Renal enlargement is one of the common features which occurs during early stages of diabetes [32]. Increase in relative weight of kidney during diabetes has been reported by others. The degree of renal enlargement was correlated with the degree of glycaemic control [33]. Guar gum was more efficient in bringing about glycaemic control than wheat bran [15] but both were equally effective in controlling renal enlargement. This could be due to factors other than the viscous nature of guar gum, which may be presumably through short chain fatty acids (SCFA).

The increase in uronic acid excretion during diabetes may be due to degradation of proteoglycans. Increase in GAG excretion during diabetes has been reported [34]. Apart from uronic acid formed by degradation of proteo-

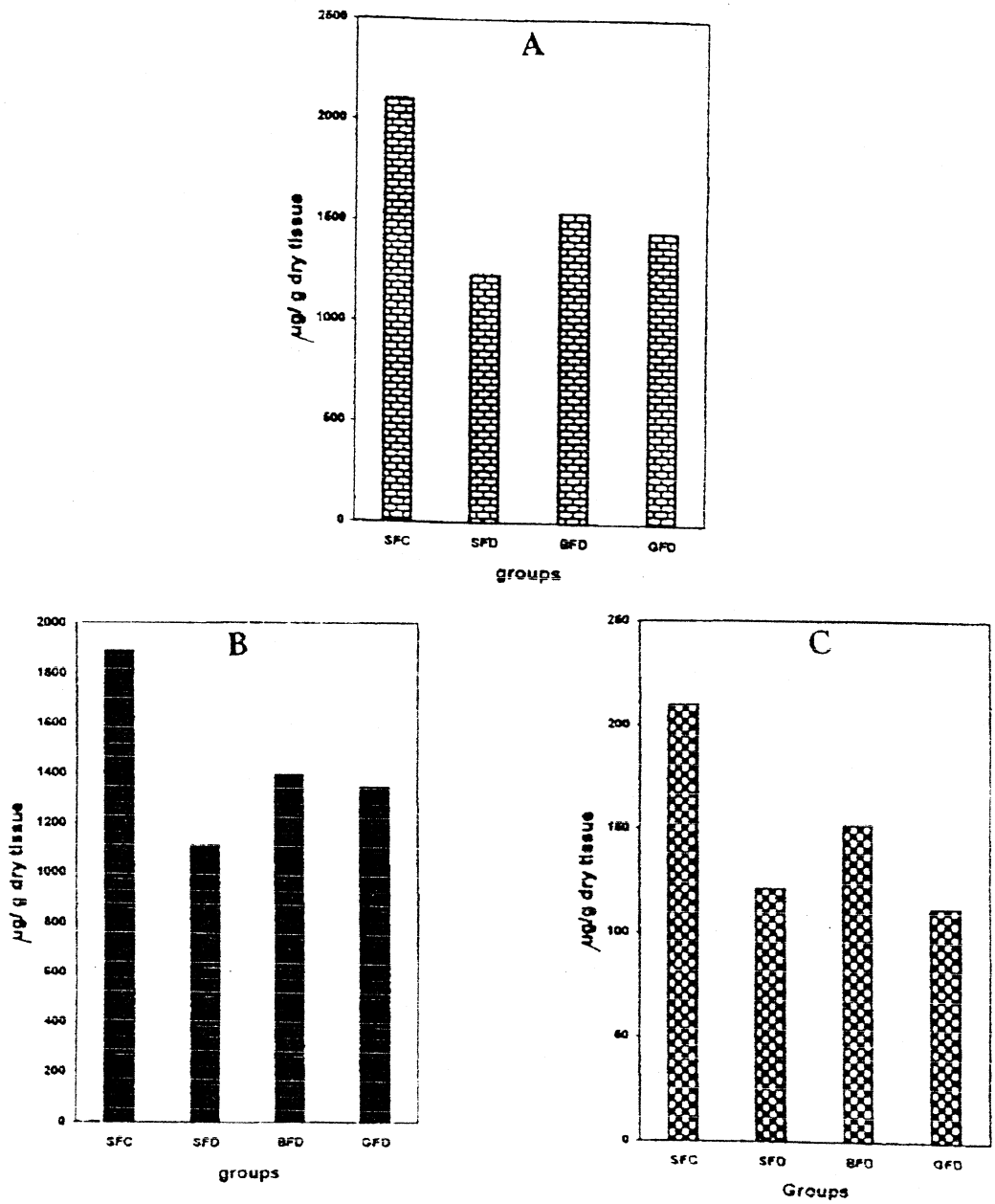

Fig. 1. Effect of wheat bran and gaur gum on total glycosaminoglycans (A), heparan sulfate (B) and chondroitin sulfate (C) of kidney tissue. Abbreviations as in Table 1.

glycans, this might also include conjugates of glucuronic acid.

In earlier stages of DM, a hypertrophy and hyperfunction of the kidney with typical 20-40% increases in kidney size and GFR was observed by Christiansen et al. [35]. Presence of wheat bran in the diet did not have any effect on GFR whereas guar gum was efficient in preventing the increase to certain extent. This may be due to more effective control of blood glucose by guar gum [15].

The increase in total sugar content during diabetes may be due to non-enzymatic glycation of tissue proteins [36]. The increase in uronic acid content during diabetes may be due to increment in the synthesis of small-sized glycosaminoglycans [37]. The decrease in protein content may be due to increase in gluconeogenesis during diabetes. A decrease in soluble proteins during diabetes in kidney has been reported [38].

Changes in the activities of enzymes involved in the glycoconjugate metabolism may be responsible for the quantitative changes in glycoconjugates in kidney. Renal sections from diabetic patients with diabetic nephropathy have shown significant increase in GFAT expression in glomerular epithelial cells [39]. Since UDP-sugars are important constituents of glycoproteins, increased synthesis of

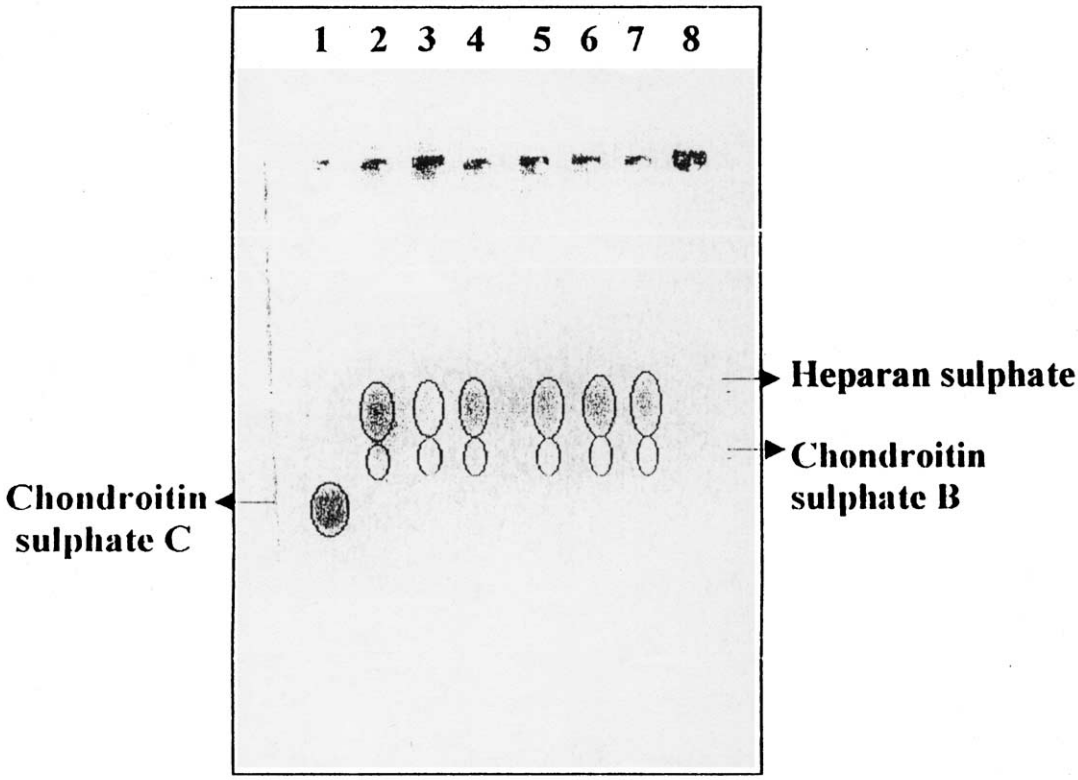

Fig. 2. Agarose electrophoresis of glycosaminoglycans isolated from kidney. Standard chondroitin sulfate C (Lane 1), SFC (Lane 2), SFD (Lane 3), BFC (Lane 4), BFD (Lane 5), GFC (Lane 6) and GFD (Lane 7). Abbreviations as in Table 1.

glucosamine points to the fact that there is a glycoprotein accumulation. Recent studies indicate that GFAT a key regulatory enzyme in the hexosamine biosynthetic pathway play an important role in regulation/development of diabetic vascular complications such as diabetic nephropathy [40]. Regulation of GFAT gene expression has been studied extensively and partially characterized by cloning the promoter region of mouse GFAT [41]. The existence of isoforms of GFAT has been recently elucidated from human cDNA showing 75.6% homology to the GFAT1 [42]. Thus changes in the GFAT expression may be one of the reasons for the thickening of glomerular basement membrane, which occurs during diabetic nephropathy. It was observed by Spiro [43] that there was an increase in UDP glucose, and UDP-galactose content in kidney during diabetes. The changes in NAG and β-glucuronidase activities reported by earlier workers are not very consistent. NAG activity has been shown to be elevated in diabetic mice [44] or decreased in diabetic rats [45]. β-Glucuronidase activity was shown to be unchanged or significantly elevated during diabetes depending on the diabetogenic agent used to induce diabetes [31]. The discrepancies in the reports may be due to various factors like diabetogenic agent used to induce diabetes, duration of diabetes and the strain of animals employed in the experiments.

GAGs are located as anionic sites in the lamina rarae of glomerular basement membrane and mesangial matrix where they serve as charge selective barriers in the filtration of macromolecules [46]. It is observed that heparan sulfate is the major GAG present in the kidney and amounted to 90% of the total GAGs present [47]. Our results are in agreement with the reported values. Previous workers have observed reduction in heparan sulfate during diabetes in both glomerular basement membrane [9] and renal cortex [48]. Our results agree with the earlier report and provide evidence to show that wheat bran (insoluble DF) and guar gum (soluble DF) ameliorate decreased synthesis of heparan sulfate.

The beneficial effect of fibers in ameliorating the symptoms of diabetic nephropathy may be either indirectly by production of short chain fatty acids (SCFA) like acetate, propionate and butyrate or by direct consequence of controlling blood glucose level which is a primary step in the control of diabetes. It has been suggested that some of the effects of high carbohydrate and high fiber diets on carbohydrate and lipid metabolism are mediated by the metabolism of SCFA in the liver [49]. Many authors have put forward the hypothesis of SCFA being involved in fiber induced metabolic changes [50,51]. Butyrate is shown to alter activities of many cellular enzymes including the enzymes involved in glycoconjugate metabolism like sialyl transferases and sulfotransferase, etc. Such alterations in the enzyme activities would lead to alteration in the synthesis/

degradation of many of the glycoconjugates such as heparan sulfate.

It can thus be concluded that dietary fibers both soluble and insoluble play an important role in maintaining the health, more so in ameliorating the severity of metabolic diseases like diabetes mellitus.

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