Research Journal of Agriculture and Biological Sciences, 4(5): 455-461, 2008

Hypoglycemic Effect of Dietary Fibre in Diabetic Rats

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Abstract: The present study concern the use of Egyptian grape (*Vitis vinifera*) leaves dietary fibre in treatment of Streptozotocin (STZ) diabetic rats. The chemical analyses of dried and milled grape leaves dietary fibre revealed the presence of higher amounts of total non–starch polysaccharides NSP (491.2±6.4 g/kg), contains cellulose (96.4±3.2 g/kg), insoluble and soluble NSP (337.4±3.4 and 153.8±2.8 g/kg, respectively). Chemical analysis of total NSP (Soluble and insoluble NSP) revealed the presence of higher amount of uronic acid (112.3±3.2 g/kg) as well as different amounts of monosaccharides, mainly consisiting of glucose, galactose, mannose, arabinose and xylose. The hypoglycemic and hypolipidemic effects of grape leaves dietary fibre (Calculated as total NSP) in Streptozotocin (STZ) diabetic rats as well as liver function test were studied over 8 weeks period. The diabetic rats were devided into 4 groups and each was administered with one of four different doses of total NSP (50,100,150 or 200 mg/kg, respectively). Higher significant decreases in the levels of glucose (160.1±9.4 and 210.1±10.8 mg/dl, respectively) and cholesterol (110.8±9.4 and 137.2±9.8 mg/dl, respectively) were observed in two groups (rats group 2 and 4) administered with dose of 100 and 200 mg dietary fibre/kg, particularly at 8 weeks. Significant decreases in the levels of glucose and cholesterol (340.4±8.9, 310±9 and 150.4±7.1, 145.2±9.6 respectively) were observed in other rat groups (group 1 and 3) administered with doses of 50, 150 mg dietary fibres/kg. Higher significant decrease of triglycerides levels (180.8±9.2 mg/dl) was observed in plasma of rats administered with dose of 100 mg/kg (group 2), while the other doses (50,150 and 200 mg/kg) exhibited gradually significant decreases (254.9±9.6, 366.8±8.6 and 366.7±9.4 mg/dl, respectively). The greatest reduction of glucose, cholesterol and triglycerides levels were observed in rats administered with doses of 100 and 200 mg/kg dietary fiber (61, 38% and 54, 49% and 23, 9% respectively), while less reduction was observed in rat groups administered with doses of 50 and 150 mg/kg dietary fiber (30, 16, 9 and 16, 19 and 6% respectively). Study on hypoglycemic effect of dietary fibre at different doses indicated higher significant reduced the plasma glucose level of the diabetic rats by 16-61%. Dietary fibre doses also had lowering effect on the activities of ALP, ALT and AST in serum of diabetic rats. Results showed significant decrease in the activity of ALP and ALT in plasma of diabetic rats administered with doses of 100 and 200 mg/kg dietary fibre (13.8±0.5, 36.3±5.1 and 17.2±1.1, 39.1±4.5 U/dl, respectively). Insignificant differences were observed in other 2 groups of diabetic rats administered with doses of 50 and 150 mg dietary fibre/kg (group1and 4). A marked reduction in ALP and ALT values (29 and 33%, respectively) were observed in group 2 more than the other three groups (9.3, 13, 12, 15% and 11, 28 % respectively). Result also showed gradually significant decrease in AST activities in plasma of all experimental rat groups throughout 8 weeks. According to these results, it can be concluded that the dietary fibre of grape leaves having hypoglycemic and hypolipidemic effects in STZ diabetic rats.

Key words: Hypoglycemic, dietary fibre, diabetic rats, liver function enzymes

INTRODUCTION

Dietary fibre has been defined previously as the plant polysaccharides and lignin, which are resistant to hydrolysis by digestive enzymes of man[33,53]. Dietary fiber has been one of the most enduring dietary interests of this decade world wide. It is a mixture of variety of polysaccharides, cellulose, hemicelluloses, pectins, gums, mucilages, algal polysaccharides and lignin has been found to have hypoglycemic effect[32,41]. Eastwood and Morris[14] and Galibois *et al*[20], reported that the main constituents of dietary fibre or un-available carbohydrate are non-starch polysaccharides (NSP), having physiological actions on gastrointestinal tract and inhibit absorption of glucose and its uptake[12,27,28,37,], studied the role of viscous and fermentable fibres in the treatment of diabetes mellitus. An increased dietary intake of plant fibre is currently being recommended for lowering of plasma lipids in persons with hyperlipidemia and improving the control

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of blood glucose in subjects with impaired tolerance[32]. Decreased glucose levels were observed in serum of animals receiving mixture of soluble and insoluble NSP[20,39]. Other workers reported lowering effects of different types of dietary fibres on plasma lipids and glycemic response in rats[13,35,38], they concluded that the plant polysaccharides shows both hypoglycemic and hypolipidemic activity. The greatest reduction in glycemic response to a test meal was observed with soluble fibre[39], that increases the viscosity of the gastrointestinal content[12] and therefore, retards the rate of carbohydrate digestion and absorption[7,40]. However, the solubility of dietary fibre (NSP) in water and diluted acids is physiologically important in animal and human studies[27].

Diabetes mellitus is one of the most common problems challenging the physicians in 21st century[4,8]. Diabetes mellitus has been defined as a chronic disease of carbohydrate metabolism, but lipid and Protein metabolism are also affected[2,34]. Other workers defined diabetes mellitus as a chronic disorder of glucose metabolism resulting from dysfunction of pancreatic beta calls and insulin resistance[42,47]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels[2,10,11,54]. Current use of dietary fibre in natural medicine which includes its uses for all types of liver and gall bladder disorders, diabetes and high cholesterol diseases[15,50,56]. Hence, the present study was designed to investigate the effect of dietary fibre on glucose and lipid levels in chemical induced (SZT) diabetic rats.

MATERIALS AND METHODS

Materials:

- Fresh leafy material of grape leaves (*Vitis vinifera*) was collected from an Egyptian local environment. They were cut into small pieces, and then dried in an oven at 60-80 °C until constant weight. Finally, the dried leaves were ground in a food grinder (mincer) to a very fine powder and stored at room temperature until used for chemical composition analyses.
- Streptozotocin (STZ) was obtained from Sigma chemical company and used as a diabetogenic agent.

Dietary Fibre Isolation and Analyses: Lipids were extracted with chloroform-methanol mixture (2:1 v/v), according to the method described by Folch *et al*[18]. Protein was extracted according to the method of Goel *et al*[22]. Defatted and deproteinized residue were collected and washed with distilled water and dried in freeze dryer at -60˚C, ground in grinder and used as a source of dietary fibres. (NSP) supplements Total non-starch polysaccharides (NSP), either soluble or insoluble and its uoronic acid components were estimated according to the method described by Englyst and cummings[16]. Qualitative and quantitative determinations of mono-saccharides in total NSP (Both soluble and insoluble NSP) were also measured[58].

Animals and Induction of Diabetes: One hundred and forty male albino rats (*Rattus norvgicus*), 9 weeks of age and each one weighing about 130 g, were purchased from the Egyptian Organization for Biological Products and Vaccines. The rats were then divided into 5 groups (28 rats /group) on the basis of their body weight and individually housed in wire screen cages. The rats had free access to food and tap water and maintained for a period of 8 weeks. The first group was injected intraperitoneally with 1ml citrate buffer (0.01 M), pH 4.5 and used as control group and the other 4 groups were made diabetic by intraperitoneally injection of the prepared solution of STZ (dissolved in 0.1 M citrate phosphate buffer solution, pH 4.5) in a dose of 65 mg/Kg body weight and used as experimental diabetic rat groups[5]. After 72 hours, blood samples were drawn using heparinized capillary tubes. The rats of diabetic groups (STZ intraperitoneally injection) having blood glucose more than 300 mg/dL were exclusive the study. The rats of 4 diabetic groups (second, third, fourth and fifth) were treated with a dose of 1ml solution of dietary fibre (containing 50, 100, 150 or 200 mg/ml, respectively) throughout experimental period (8 weeks). The dosage of a prepared dietary fibre solution can be given daily by oral route is 1 ml contained different concentrations of dietary fibre for a period of 8 weeks. All rats under the present study were fed on a commercial pellet diets obtained from animal laboratory NRC. Hypoglycemic and hypolipidemic effects were tested in plasma of diabetic rats group compared to control group.

Biochemical Assay: Blood samples of the experimental animals were collected every two week using heparinized tubes and plasma was separated by centrifugation at 1100 x *g* for 10 min. Chemical analyses of the separated plasma were done using Boehringer-Mannheim Kit for estimation of plasma glucose and cholesterol concentrations[51,52] and triglycerides[57]. Alkaline phosphatase (ALP) were estimated using Lab. kit[6]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also estimated using the kit of QCA[46].

Statistical Analysis: Data were expressed as mean values ± SE[17]. Statistical differences were considered significant at $p < 0.05$ and high significant at $p < 0.001$.

RESULTS AND DISCUSSION

Dietary Fibre Constituents: Chemical analyses of dried and milled dietary fibre (five samples) revealed the presence of higher amounts of total non–starch polysaccharides NSP (491.2±6.4 g/kg). The amount of insoluble NSP (337.4±3.4 g/kg), form gives the highest level (69 %) of total NSP, and nearly two times that of soluble NSP (153.8±2.8 g/kg). Chemical analysis of total NSP revealed the presence of cellulose (96.4±3.2 g/kg) and higher amount of monosaccharides (each soluble and insoluble NSP) mainly consisiting of glucose (84.7±5.2 g/kg), galactose (80.3±3.2 g/kg), mannose (62.2±1.2 g/kg), arabinose (41.7±1.4 g/kg), xylose (12.9±0.8 g/kg) and uronic acid (112.3±3.2 g/kg). These results are in the range with those reported by other investigators[43,45].

Generally, the major constituents of monosaccharides, usually arising from glucomannan, galacto-mannan, arabino-galactan[9] and side chains of pectic substances. The higher amounts of monosaccharides in total NSP under the present study comprising mostly galactose (80.3±3.2 g/kg) and mannose (62.2±1.2 g/kg) or galactose (80.3±3.2 g/kg) and arabinose (41.7±1.4 g/kg), that usually found in galactomannan (galactose and mannose units) arising from pectin and galactan and arabinogalactan side chain of pectic substances respectively. These dietary fibres (total NSP) resemble guar gum (gel fibre) in structure, are very viscous when dissolved in water[40,48], they demonstrate that the different effects of dietary fibres are type-dependent (dose, structure, soluble and insoluble NSP, level, particle size) and duration of experiment[44].

Dietary fibres of vegetable products may affect the bioavailability impairment of mineral absorption[59,60], which is traditionally ascribed to the uronic acid content of dietary fibre[27]. Similar results were obtained by other investigators using different fibre sources[23,44,45]. Dietary fiber of some plant contain large amounts of mannan is an unabsorbable poylsaccharide, composed of glucose and mannose, in 1:1.6 ratio, bound through beta-1,4-glycosidic linkages consumed in different countries. Recent interest in dietary fiber comes from the public's increased awareness of the effects of high fiber diets on health problems, such as diabetes, postprandial hypoglycemia, hyperlipidemias, various gastrointestinal disorders, some cancers and obesity. Studies in rats have shown dietary fibre gels around the food particles, interfering with the action of digestive enzymes and thus slowing the rate at which sugars and fats enter the blood stream. It has been suggested fiber modifies gut hormone response, as already demonstrated for glucomannan and suggested for glucagon. Fiber can also directly affect fat and protein metabolism[21,29,49,55].

Table 1: Glucose, total cholesterol, triglyceride, ALP, ALT and AST levels in plasma of normal and diabetic rats (Values are mean and standard error of 7 rats/group).

Component	Normal	Diabetic
Glucose	118.6 ±1.8	417.6±4.2
Total cholesterol	102.2± 1.4	178.5±6.3
Triglyceride	176.4±2.2	390.1±7.4
ALP(k & kU/dL)	10.4±0.4	19.4±0.4
ALT	24.5±0.6	54.5±2.8
AST	46.2±1.4	66.2±3.2

Serum Parameters: Results in Table (1) showed that the glucose, total cholesterol and triglycerides levels in rats group injected with citrate-phosphate buffer solution (0.1 M, pH, 4.50) were 118.6+1.6, 102.2+1.4 and 176.4+2.2 mg/dl respectively. ALP, ALT and AST levels were 10.4±0.4, 24.5±0.6, 46.2±1.4, respectively, used as control group. The diabetic 4 groups of rat injected interpritoneally with STZ have higher levels of glucose, cholesterol and triglycerides levels (417.6+4.2, 178.5+6.3 and 390.1+7.4 mg/dl, respectively), ALP, ALT and AST levels were 19.4±2.0, 54.5±2.8 and 66.2±3.2 respectively. These results showed more glucose concentration in plasma of diabetic rats than 300 mg/dl, and used as experimental diabetic rats (4 groups), maintained till experimental periods for 8 weeks. The high plasma level of triglyceride and total cholesterol in diabetic animals may be due to impaired liver function caused by the damage done by streptozotocin which acts either directly or indirectly by enhancing the plasma glucose level[8,55]. Result in Table (2) also showed that the diabetic rat groups (Group 2 and4) adminstered with different doses of dietary fibres (100, 200 mg/kg) have higher significant decreases in the levels of glucose (160.1±9.4 and 210.1±10.8 mg/dl, respectively) and cholesterol (110.8±8.4 and 137.2±9.8 mg/dl, respectively). Significant decreases in the levels of glucose and cholesterol (240.4±8.9, 310±9.1 and 150.4±7.1, 145.2±9.6, respectively) were observed in rats (Groups 1&2) administrated with doses of 50, 150 mg dietary fibres /kg.

Higher significant decrease of triglycerides levels (180.8±9.2 mg/dl) was observed in plasma of rats administered with 100 mg/kg (group 2), While, the other groups (1, 3 and 4) administrated with doses of dietary fibre (50,150 or 200 mg/kg, respectively), exhibited gradually significant decreases (354.9±9.6, 366.8±8.6 and 356.7±9.4 mg/dl respectively). However, the greatest reduction of glucose, cholesterol

Table 2: Glucose, total cholesterol, triglyceride, ALP, ALT and AST levels in plasma of diabetic rats treated with different doses of dietary fibre treatments. (values are mean and standard error of 7 rat / group).

Component	Time in weeks	Treated rat groups			
		Group 1 (50mg/kg)	Group 2 (100mg/kg)	Group 3 (150mg/kg)	Group 4 (200mg/kg)
Glucose (mg/dl)	2	402.2±6.2*	350.4±8.5**	388.2±9.5*	376.2±8.9**
	4	377.1±8.2*	290.6±7.9**	385.3±10.3*	330.4±8.8**
	6	350.6±9.2*	237.2±7.6**	381.6±11.8*	270.1±9.1**
	8	240.4±8.9**	160.1±9.4**	310.1±9.1**	210.6±10.8**
Total cholesterol (mg/dl)	2	164.2±2.6*	155.2±6.4**	164.2±5.2*	153.2±4.8**
	4	162.8±4.4*	142.6±6.4**	153.9±9.9*	146.6±7.9**
	6	152.9±6.2*	120.4±7.5**	148.1±8.4*	142.1±9.4**
	8	150.4±7.1*	110.8±8.4**	145.2±9.6*	137.2±9.8**
Triglygrides (mg/dl)	2	358.6±8.4*	336.1±7.4**	380.2±6.2	378.3±7.6
	4	357.4±8.4*	296.8±7.4**	368.9±9.9	368.0±8.9
	6	355.6±9.8*	240.4±7.8**	367.2±9.4336	358.5±8.6
	8	354.9±9.6*	180.8±9.2**	6.8±8.6	356.7±9.4
ALP(K&KU/dl)	2	18.6±0.4	17.9±0.6*	18.0±0.6	18.1±0.6*
	4	17.9±0.7	17.6±0.7*	17.7±0.8	17.8±0.8*
	6	17.6±0.9	16.8±0.8**	17.5±0.9	17.5±0.9*
	8	17.8±0.8	13.8±0.5**	17.1±1.2	17.2±1.1*
ALT(IU/L)	2	51.6±2.9	46.8±2.1*	52.9±3.2	50.4±4.4*
	4	49.4±3.1	43.6±4.3*	48.8±3.8	42.5±3.2*
	6	48.3±3.9	38.9±4.6*	47.4±4.2	41.8±5.4*
	8	47.2±3.6	36.3±5.1**	46.6±4.8	39.1±4.5*
AST(IU/L)	2	58.4±2.1*	52.7±3.6*	60.4±2.8*	59.4±4.8*
	4	53.7±3.1*	50.3±4.7*	58.4±3.2*	55.8±4.2*
	6	51.6±4.2*	49.8±5.3*	53.8±3.1*	53.2±3.8*
	8	49.0±5.2*	48.1±5.6**	50.6±5.8*	51.8±4.1*

* Significant (P<0.05)
** High significant (P < 0.01)

and triglycerides levels were observed in rats administered with 100 and 200 mg/kg dietary fiber (61%, 38% and 54%; and 49%, 23% and 9%, respectively), while less reduction was observed in rat groups administrated with doses of 50 and 150 mg/kg dietary fiber (30%,16%,9% and 16%, 19%, 6%, respectively). Study on hypoglycemic effect dietary fibre at different doses of 50,100,150,200 mg/kg body weight indicated significant reduced the plasma glucose level of the diabetic animals by 16- 61% as shown in Table 2. However, different changes were observed in plasma glucose, cholesterol and triglyceride levels throughout the 8 weeks compared to control group. Studies on normal subjects given glucose loads shown that 5-10grams of dietary fibres can be beneficial, depressing plasma glucose levels and the insulin response[26]. Its usefulness in the management of diabetes is thought to be due to dietary fibres ability to slow stomach emptying, modify responses of gastrointestinal hormones and delay glucose diffusion in the intestinal lumen. However, the results of the group 2 diabetic individuals showed a positive effect of

these dietary fibres in the reduction of blood glucose level, indicated that the dietary fibers are effective in the treatment and management of diabetes[25].

The plasma glucose lowering effect of dietary fibre can be explained by the fact that it might have increased glucose utilization in diabetic animals by promoting insulin secretion. This observation reported by other investigators[24]. Hypoglycemic and hypolipidemic activity of dietary fibre also reported by other investigators[20,31,38]. Plasma levels of triglyceride and total cholesterol have a significant correlation with the degree of diabetic control rats[21,30]. The substantial decrease in triglyceride and total cholesterol level in the diabetic animals by dietary fiber reinforces its hypoglycemic and hypolipidemic potential. Serum cholesterol and low-density lipoprotein cholesterol (LDL) levels were significantly reduced in the dietary fibre treated rat groups[3], glucomannan supplements are becoming important agents for weight control[11]. There is some concern the effect of dietary fibres on delay the transit time of carbohydrates may influence the bioavailability of minerals[7,59,60], reported that the effect of unavailable carbohydrates on the intestinal absorption of minerals (Calcium) in rats during a 7 to 8 week period and concluded that this compromise was partially due to the loss of calcium-binding protein caused by the gastrointestinal transit of large amounts of undigested food.

Liver Function: The present results (Table 1) showed higher significant increase in the levels of ALP, ALT and AST activities in diabetic rat group (19.4±0.4, 54.5±2.8 and 66.2±3.2 mg/dl respectively) compared to control group (10.4±0.4, 24.5±0.6 and 46.2±1.4 respectively). ALP, ALT and AST activities are increased when liver functions abnormally[10,45]. The biosynthesis of urea involves the action of several enzymes (transamination-oxidative determination–ammonia transport and reaction of urea), thus the study of liver function enzymes were done in the present study to find out the effect of these dietary fibre doses on liver functions enzymes activity. Results in Table 2 shows that all dietary fibre doses had lowering effect on the activities of ALP, ALT and AST in serum of diabetic rats compared to control group. These effects are mainly related to the presence of natural soluble and insoluble NSP of dietary fibre as well as its monosaccharides constituents[1,44], which had effects on intestinal digestion and absorption of nutrient[59,60]. Similar observation was found by other investigators[20,36,45].

Result in Table 2 showed significant decrease in the activity of ALP and ALT in plasma of rats (Groyup 2 & 4) administrated with doses of 100 and 200 mg/kg dietary fibre (13.8±0.5, 36.3±5.1 and 17.2±1.1, 39.1±4.5 U/dl, respectively). Insignificant differences were observed in other 2 groups of rats administrated with doses of 50 and 150 mg dietary fibre/kg (group 1and 4). A marked reduction (29% and 33% respectively) in ALP and ALT values were observed in group 2 (100 mg/kg), more than the other groups 1,2,3 (9.3,13 %, 12,15 % and 11,28 % respectively). Result also showed gradually significant decrease in AST activities in plasma of all experimental rat groups throughout 8 weeks. Similar results were reported by other investigators[19,45]. This signifies the fact that dietary fibre might play a corrective role in liver function either by reducing the blood glucose level or by some other mechanisms, which in turn reduced the level of triglyceride and total cholesterol in blood plasma of diabetic animals. Although the exact mechanism of dietary fibre is not properly understood, the above study suggests that a combination of mechanisms might be involved in exhibiting hypoglycemic and hypolipidemic effects of dietary fibre used in the present study.

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