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ORIGINAL ARTICLE

Mechanism of Antidiabetic Action of Compound GII Purified from Fenugreek (*Trigonella foenum graecum*) Seeds

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Abstract To study the mechanism of action of water soluble compound GII purified from fenugreek (*Trigonella foenum graecum*) seeds which was shown earlier to have antidiabetic effect in the subdiabetic, moderately and severely diabetic rabbits. In rabbits (1–1.5 kg bw) diabetes was induced by intravenous injection of 80 mg/kg bw of alloxan. They were fed with GII at a dose of 50 mg/kg bw daily once in the morning for 15 days in the subdiabetic and moderately diabetic and 30 days in the severely diabetic rabbits. Serum total cholesterol (TC), triglycerides (TG), LDL + VLDL cholesterol [(LDL + VLDL)C], HDL cholesterol [(HDL)C], total tissue lipids, glycogen and enzymes of carbohydrate metabolism (glycolysis, gluconeogenesis, polyol pathway) hexokinase, glucokinase, pyruvate kinase, malic enzyme, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, aldose reductase and sorbitol dehydrogenase and antioxidant enzymes glutathione peroxidase, glutathione reductase and superoxide dismutase were estimated. Liver and kidney function parameters were also estimated. Treatment with GII for 15 days in the subdiabetic and moderately diabetic rabbits and for 30 days in the severely diabetic rabbits (i) decreased the elevated lipids TC, TG, (LDL + VLDL)C and increased the decreased (HDL)C, (ii) decreased the elevated liver and heart total lipids, TC and TG, (iii) increased the decreased liver and muscle glycogen, (iv) increased the decreased hexokinase, glucokinase, pyruvate kinase, malic enzyme, glucose-6-phosphate dehydrogenase, superoxide dismutase, glutathione peroxidase, (v) decreased the increased glucose-6-phosphatase, sorbitol dehydrogenase, aldose reductase. Results thus show that treatment with GII compound purified from fenugreek seeds for 15 days in the subdiabetic and moderately diabetic and 30 days in the severely diabetic rabbits corrects the altered serum lipids, tissue lipids, glycogen, enzymes of glycolysis, gluconeogenesis, glycogen metabolism, polyol pathway and antioxidant enzymes. Histopathological abnormalities (fatty infiltration and other cellular changes) seen in the pancreas, liver, heart and kidneys were repaired after treatment with GII. In fact partially damaged pancreas was repaired. Liver and kidney function test results were normal in the GII treated animals indicating that GII treatment is safe and free from any side effects.

Keywords GII from fenugreek seeds · Mechanism of action · Serum and tissue lipids · Glycogen · Glycolysis · Gluconeogenesis · Antioxidant enzymes

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Introduction

In our previous communications [1–3] we reported the purification of compound GII from fenugreek (*Trigonella foenum graecum* Linn) seeds and demonstrated it to be useful in the subdiabetic, moderately diabetic and severely

diabetic rabbits. We also showed that another advantage with this compound is that it is useful in intermittent therapy instead of daily therapy and its antidiabetic activity persists for at least 2 weeks after stopping treatment with the compound. In this communication we report its mechanism of action in the subdiabetic, moderately diabetic and severely diabetic rabbits by studying its effect on serum lipids and enzymes of carbohydrate metabolism.

Methods and Materials

Chemicals

The biochemicals were purchased from Sigma chemical Co., St Louis, USA. Kit for the estimation of serum insulin was from Boehringer Mannheim Immunodiagnostics, New Delhi and other chemicals from British Drug House (India), New Delhi.

Animals

Rabbits (1–1.5 kg) were purchased from M/S All India Chemical and Scientific Company approved by our college. They were fed on pellet diet of Hindustan Lever Ltd., Mumbai, India. Food and water were given ad libitum. They were acclimatized to the laboratory conditions for at least 15 days. Permission to carry out the studies was taken from the Committee in-charge of the animal house.

Induction of Diabetes and Treatment

It was induced in rabbits by alloxan 80 mg/kg intravenously as reported earlier [1]. They were treated with GII purified from fenugreek seeds at a dose of 50 mg/kg bw daily once in the morning for 15 or 30 days.

Estimations

Serum cholesterol, triglycerides, LDL + VLDL cholesterol [(LDL + VLDL)C], HDL cholesterol [(HDL)C] were estimated using the methods of the kits from Ranbaxy Diagnostics, New Delhi, India. Total tissue lipids were extracted with chloroform: methanol (1:2 V/V) and estimated by the method of Folch et al. [4]. Glycogen was isolated from the liver and the muscle and estimated by the method of Carrol et al. [5]. Blood glucose and serum insulin were estimated as described earlier [2, 3].

Enzymes of carbohydrate metabolism: The methods used for the estimation of various enzymes were, hexokinase and glucokinase of Gumma and McLean [6], pyruvate kinase of Gutman and Bernt [7], malic enzyme by reduction of NADP+ at 340 nm, glucose-6-phosphatase (G6Pase) of Harper [8], glucose-6- phosphate dehydrogenase (G6PD) by the method of Zink and Lenland as described in WHO technical report series [9], aldose reductase by the method of Pottinger [10], sorbitol dehydrogenase by the reduction of NAD+ and the antioxidant enzymes glutathione peroxidase (GPx) and glutathione reductase and superoxide dismutase by the method of Marklund and Marklund [11].

The liver function was assessed by serum bilirubin, serum proteins, serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase and the renal function by estimating serum urea and serum creatinine by methods described in our earlier communication [2].

Results

Subdiabetic, moderately diabetic and severely diabetic rabbits were treated with GII at a daily dose of 50 mg/kg bw for 15, 15 and 30 days respectively. The serum lipids, tissue lipids and tissue enzymes of carbohydrate metabolism were analysed and the changes are described below.

In the untreated subdiabetic rabbits there was increase (Table 1) in the values on day 1 of serum TC to 86.8 ± 6 mg/dl, TG to 120.4 ± 8 mg/dl and (LDL + VLDL)C to 62.8 ± 4 mg/dl when compared to healthy control (normal) values of 54.5 ± 5 mg/dl, 72.5 ± 7 mg/dl and 22.1 ± 2 mg/dl respectively for TC, TG and (LDL + VLDL)cholesterol. After treatment for 15 days with GII (50 mg/kg bw) the values came down to normal or slightly below normal in the subdiabetic rabbits on day 15. In the untreated moderately diabetic rabbits, there was increase on day 1 in TC (76.4 ± 5 mg/dl), TG (245.3 ± 2 mg/dl) and (LDL + VLDL) cholesterol (53.2 ± 5 mg/dl), which came down to 39.2 ± 2 mg/dl, TC 81.1 ± 5 mg/dl, (TG), 13.3 ± 1 mg/dl (LDL + VLDL) cholesterol on day 15, which are in the normal range. But in the untreated severely diabetic rabbits, the changes were more severe. TC, TG and (LDL + VLDL)C increased on day 1 to 180.4 ± 16 mg/dl, 360.5 ± 41 mg/dl and 168.4 ± 15 mg/dl respectively. After treatment with GII (50 mg/kg bw) for 15 days the values came down significantly but still above the normal values. So the treatment with GII same dose was continued for 30 days when all the values of TC, TG, (LDL + VLDL)C came down to the normal values. (HDL)C which decreased to 11.6 ± 2 mg/dl in the untreated severely diabetic rabbits increased considerably after 15 days treatment with GII to 23.1 ± 2 mg/dl but increased to the near normal value of 28.7 ± 2 mg/dl after 30 days treatment with GII at the same dose.

Tissue lipids were estimated in the liver and heart of all the three types of diabetic animals (Tables 2 and 3). In the liver of the untreated subdiabetic rabbits there was

Table 1 Effect of GII (50 mg/kg bw) treatment for 15 days on the serum lipids of the subdiabetic, moderately diabetic and severely diabetic rabbits

Lipid	TC (mg%)	TG (mg%)	(LDL + VLDL)C (mg%)	(HDL)C (mg%)	(HDL)C/TC
Normal	54.5 ± 5	72.5 ± 7	22.1 ± 2	32.4 ± 3	0.59
Subdiabetic					
Untreated day 1	86.8 ± 6	120.4 ± 8	62.8 ± 4	24.0 ± 2	0.27
Treated day 15	48.3 ± 4*	87.4 ± 10*	18.4 ± 2*	29.9 ± 3*	0.62
Moderately diabetic					
Untreated day 1	76.4 ± 5	245.3 ± 12	53.2 ± 5	23.2 ± 2	0.30
Treated day 15	39.2 ± 2*	81.1 ± 5*	13.3 ± 1	25.9 ± 2*	0.66
Severely diabetic					
Untreated day 1	80.4 ± 16	360.5 ± 41	168.4 ± 15	11.6 ± 2	0.06
Treated day 15	79.2 ± 8*	110.1 ± 9	56.1 ± 7*	23.1 ± 2*	0.31
Treated day 30	48.6 ± 3*	78.4 ± 4*	19.9 ± 2*	28.7 ± 2*	0.59

* $P < 0.01$ day 1 values are untreated

negligible change in the total tissue lipids, TC and TG (Table 2) and treatment with GII 50 mg/kg bw brought down slightly and kept these values within the normal range. In the untreated moderately diabetic rabbits total lipids increased to 56.7 ± 5, TC to 4.7 ± 0.4 and TG to 5.8 ± 0.4 mg/g liver tissue. But after treatment with GII 50 mg/kg bw for 15 days the values came down to normal level with a change of 49, 45 and 59% of total lipids, TC and TG respectively. But in the liver of the severely diabetic untreated rabbits the lipid parameters were higher than in the moderately diabetic rabbits. The values were total tissue lipids 58.4 ± 5, TC 4.9 ± 0.4 and TG 5.8 ± 0.5 mg/g tissue. Treatment with GII brought the TC and TG to normal range. Total lipids were still higher than normal (41.0 ± 0.4 mg/g). Since the liver lipids of the untreated subdiabetic rabbits were in the normal range, lipids of the heart were not studied. But in the heart of the moderately and severely diabetic rabbits there was slight increase in the total lipids, TC and TG (Table 3). But the values were still in the normal range. Treatment with GII 50 mg/kg bw for 15 days brought the lipids of the moderately diabetic rabbits further down. But in the severely diabetic rabbits the lipid parameters were brought down by GII only slightly but the values were closer to but still above normal for total lipids and TC, but TG was in the normal range. So the treatment was given for 30 days and the lipid values of the treated severely diabetic rabbits (Table 4) all came back to normal. Treatment of the severely diabetic rabbits for 30 days with GII 50 mg/kg bw per day brought down in the liver total lipids from 62.1 ± 6 to 33.1 ± 4 (47% fall), TC from 4.1 ± 0.4 to 2.8 ± 0.2 mg/g tissue (32%) and TG from 6.6 ± 0.5 to 2.0 ± 0.2 mg/g tissue (70% fall). All these three lipids of the liver in the treated severely diabetic rabbits were in the normal range. The fall of 70% in TG in the liver was the highest. In the heart lipids even after 30 days treatment

Table 2 Effect of GII (50 mg/kg bw) treatment for 15 days on the liver total lipids, TC and TG in the diabetic rabbits

	Total lipids (mg/g)	TC (mg/g)	TG (mg/g)
Normal	28.2 ± 4	2.7 ± 0.4	2.1 ± 0.3
Sub diabetic untreated	26.7 ± 4	2.9 ± 0.4	2.2 ± 0.3
Sub diabetic treated	27.3 ± 4	2.7 ± 0.3	2.4 ± 0.4
Change after treatment	+5%	−7%	+8%
	Not significant for all the parameters		
Moderately diabetic untreated	56.7 ± 5	4.7 ± 0.4	5.8 ± 0.4
Moderately diabetic treated	28.7 ± 4	2.6 ± 0.4	2.1 ± 0.2
Change after treatment	−49%	−45%	−59%
	Significance of all is $P < 0.01$		
Severely diabetic untreated	58.4 ± 5	4.9 ± 0.4	5.8 ± 0.5
Severely diabetic treated	41.0 ± 4	3.1 ± 0.3	2.4 ± 0.2
Change after treatment	−30%	−37%	−59%
	Significance of all is $P < 0.01$		

Table 3 Effect of GII (50 mg/kg bw) treatment for 15 days on the heart total lipids, TC and TG in the moderately and severely diabetic rabbits

	Total lipids (mg/g)	TC (mg/g)	TG (mg/g)
Normal	23.1 ± 2.4	1.8 ± 0.31	4.8 ± 0.2
Moderately diabetic untreated	24.9 ± 0.3	2.2 ± 0.3	5.1 ± 0.3
Moderately diabetic treated	23.1 ± 0.2	1.5 ± 0.3	4.1 ± 0.2
Change after treatment	−7%	−32%	−20%
Severely diabetic untreated	25.2 ± 4	2.2 ± 0.3	5.2 ± 0.2
Severely diabetic treated	24.7 ± 2	2.0 ± 0.2	4.3 ± 0.2
Change after treatment	−2%	−9%	−17%

Table 4 Effect of 30 days treatment with GII (50 mg/kg bw/day) on the liver and heart lipids (mg/g tissue) in the severely diabetic rabbits

	Normal	Untreated (mg/g)	Treated (mg/g)	Change after treatment (%)
Liver total lipids	28.2 ± 4	62.1 ± 6	33.1 ± 4	−47
TC	2.7 ± 0.4	4.1 ± 0.4	2.8 ± 0.2	−32
TG	2.1 ± 0.3	6.6 ± 0.5	2.0 ± 0.2	−70
Heart total lipids	23.1 ± 2.4	24.7 ± 3	25.6 ± 0.3	+4
TC	1.8 ± 0.31	1.97 ± 0.2	2.07 ± 0.2	+5
TG	4.8 ± 0.2	4.3 ± 0.4	4.1 ± 0.3	−4

with GII there was not much change. Thus 30 days treatment with GII of severely diabetic rabbits normalized the liver lipids but did not have any effect on heart lipids.

The glycogen content of the liver and muscle in the moderately and severely diabetic rabbits after 15 days of administration of GII (50 mg/kg bw) were determined and the results are shown in Table 5. The glycogen content of the liver decreased from the normal value of 27.1 ± 3 mg/g tissue to 9.1 ± 2 mg/g tissue in the untreated moderately diabetic rabbits and to 9.6 ± 1 mg/g tissue in the untreated severely diabetic rabbits. Treatment for 15 days with GII (50 mg/kg bw) brought up liver glycogen to normal (28.0 ± 4 mg/g tissue, 207% increase) in the moderately diabetic rabbits. But in the severely diabetic rabbits there was increase to 21.6 ± 4 mg/g tissue (125% increase) which was still less than normal value. Even after 30 days treatment with GII, there was not much change in the liver and the increase in glycogen was only to 22.1 ± 4 mg/g tissue (130% increase) which was still less than normal. In the muscle the glycogen content of the normal rabbits was 14.0 ± 3 mg/g tissue which decreased to 6.8 ± 1 mg/g tissue in the untreated moderately diabetic rabbits and to 5.1 ± 1 mg/g tissue in the untreated severely diabetic rabbits. The treatment with GII for 15 days increased the muscle glycogen to 11.1 ± 1 mg/g tissue in the moderately diabetic rabbits (63% increase) and to 10.7 ± 1 mg/g tissue (109% increase) in the severely diabetic rabbits. Since the increase in the muscle glycogen content was below the normal value after 15 days treatment with GII, the severely diabetic rabbits were treated for 30 days with the same dose of GII. But even after 30 days treatment with GII there was only very slight increase in the glycogen content to 22.1 ± 4 mg/g in the liver (130%) and in the muscle to 12.9 ± 1 mg/g tissue (153%) of the severely diabetic rabbits and the values were still below the normal range. This means that even after prolonged treatment with GII for 30 days glycogen content did not return to normal. Perhaps higher dose is necessary.

Table 5 Effect of administration of GII (50 mg/kg bw) on the liver and muscle glycogen in the moderately and severely diabetic rabbits

Glycogen (mg/g)	Liver	Muscle
Normal	27.1 ± 3	14.0 ± 0.3
Moderately diabetic untreated	9.1 ± 2	6.8 ± 1
Moderately diabetic treated for 15 days	28.0 ± 4	11.1 ± 1
Change	+207%	+63%
Severely diabetic untreated	9.6 ± 1	5.1 ± 1
Severely diabetic treated for 15 days	21.6 ± 4	10.7 ± 1
Change	+125%	+109%
Severely diabetic treated for 30 days	22.1 ± 4	12.9 ± 1
Change	+130%	+153%

Then enzymes of glucose utilization namely hexokinase, glucokinase, pyruvate kinase, malic enzyme, enzymes of gluconeogenesis, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, enzymes of polyol pathway sorbitol dehydrogenase, aldose reductase, antioxidant enzymes superoxide dismutase, glutathione reductase, glutathione peroxidase, enzymes of liver and kidney function tests alkaline phosphatase, serum glutamate pyruvate transaminase (SGPT) and some liver and kidney function parameters serum bilirubin, serum creatinine and blood urea were estimated.

The activities of the enzymes of glycolysis, hexokinase and glucose specific enzyme glucokinase in the subdiabetic, moderately diabetic and severely diabetic rabbits are given in Table 6.

In the untreated subdiabetic rabbits hexokinase activity was slightly decreased in the liver and muscle but the values are still within the normal range. In the kidneys it was not affected. Treatment with GII 50 mg/kg bw increased the values closer to normal. But in the untreated moderately and severely diabetic rabbits, hexokinase content decreased to about 0.46–0.47 u/g in the liver and 1.12–1.0 u/g in the muscle. But treatment with GII increased only the hexokinase of the moderately diabetic rabbits value to normal but in the muscle of the moderately diabetic and in the liver and muscle of the severely diabetic rabbits there was increase in the values, which were, however, still not normal. But glucokinase which is a more specific enzyme for glucose metabolism which was present only in the liver decreased in the subdiabetic rabbits (50% fall) to 0.68 ± 0.04 u/g from the normal value of 1.7 ± 0.07 u/g tissue. In the moderately diabetic and severely diabetic rabbits the fall was much more to 0.32 ± 0.03 and 0.35 ± 0.03 u/g tissue which means a fall of approximately 80%. But treatment with GII 50 mg/kg bw for 15 days brought up glucokinase values to normal range of 1.68–1.70 u/g tissue.

The enzymes of glucose utilization pyruvate kinase and malic enzyme were also studied and the results are given in Table 7. With pyruvate kinase there was not much difference between the controls and the subdiabetic untreated and treated rabbits in the liver, muscle and kidneys. In the moderately diabetic rabbits there was no significant change in the kidneys. But in the moderately diabetic and severely diabetic untreated rabbits liver there was decrease of pyruvate kinase to 19.4 ± 1.0 and 17.2 ± 1 u/g tissue respectively, when compared with the normal value of 25.9 ± 2 u/g tissue (Table 7). But on treatment with GII (50 mg/kg bw) there was increase in the liver pyruvate kinase of 35% and 46% in the moderately diabetic (26.2 ± 2 u/g) and severely diabetic (25.1 ± 2 u/g) rabbits. In the muscle there was no change in the pyruvate

Table 6 Effect of GII (50 mg/kg bw) administration for 15 days on hexokinase and glucokinase in the liver, muscle and kidneys of the subdiabetic, moderately diabetic and severely diabetic rabbits

Hexokinase (u/g tissue)	Liver	Muscle	Kidneys
Normal	0.7 ± 0.05	2.7 ± 0.2	0.9 ± 0.06
Subdiabetic untreated	0.62 ± 0.05 (9% fall from normal)	2.46 ± 0.1 (9% fall from normal)	0.9 ± 0.05 (no change)
Subdiabetic treated (change% from untreated)	0.67 ± 0.04 (+8%)	2.67 ± 0.1 (+8.5%)	0.9 ± 0.04 (no change)
Moderately diabetic untreated	0.46 ± 0.05 (34% fall from normal)	1.12 ± 0.12 (55%) fall from normal	0.9 ± 0.04 (no change)
Moderately diabetic treated (change% from untreated)	0.64 ± 0.04 (+39%)	1.54 ± 0.13 (+38%)	1.0 ± 0.04 (+11%)
Severely diabetic Untreated	0.47 ± 0.03 (34% fall from normal)	1.0 ± 0.1 (63% fall from normal)	0.77 ± 0.03 (14% fall from normal)
Severely diabetic treated (change% from untreated)	0.59 ± 0.04 (+25%)	1.47 ± 0.15 (+47%)	0.93 ± 0.05 (+21%)

Glucokinase (u/g tissue)	Liver
Normal	1.7 ± 0.07
Subdiabetic untreated	0.68 ± 0.04 (60% fall from normal)
Subdiabetic treated (change% from untreated)	1.76 ± 0.08 (+159%)
Moderately diabetic untreated	0.32 ± 0.03 (81% fall from normal)
Moderately diabetic treated (change% from untreated)	1.68 ± 0.07 (+425%)
Severely diabetic untreated	0.35 ± 0.03 (79% fall from normal)
Severely diabetic treated (change% from untreated)	1.70 ± 0.08 (+386%)

Table 7 Effect of 15 days treatment of GII on the pyruvate kinase in the liver, muscle and kidneys, malic enzyme in the liver and kidneys of the subdiabetic, moderately and severely diabetic rabbits and glucose-6-phosphate dehydrogenase in the liver of the moderately and severely diabetic rabbits

Pyruvate kinase (u/g)	Liver	Muscle	Kidneys
Normal	25.9 ± 2	28.9 ± 2	12.2 ± 0.6
Subdiabetic untreated	24.8 ± 3	28.8 ± 9	12.1 ± 0.7
Subdiabetic treated (change% from untreated)	25.9 ± 2 (+4%)	27.1 ± 1 (-5%)	12.2 ± 0.6 (+1%)
Moderately diabetic untreated	19.4 ± 1 (22% fall from normal)	18.3 ± 1 (37% fall from normal)	12.2 ± 0.8 (No change)
Moderately diabetic treated (change% from untreated)	26.2 ± 2 (+35%)	28.1 ± 2 (+54%)	12.3 ± 0.7 (+1%)
Severely diabetic untreated	17.2 ± 1 (36% fall from normal)	21.9 ± 0.9 (24% fall from normal)	10.0 ± 1 (18% fall from normal)
Severely diabetic treated (change% from untreated)	25.1 ± 2 (+46%)	27.1 ± 1 (24%)	11.9 ± 0.8 (+19%)

Malic enzyme (u/g)	Liver	Kidneys
Normal	1.10 ± 0.07	1.62 ± 0.1
Subdiabetic untreated	1.08 ± 0.08 (no change)	1.11 ± 0.09 (32% fall from normal)
Subdiabetic treated (change% from untreated)	1.12 ± 0.04 (+4%)	1.06 ± 0.08 (−5%)
Moderately diabetic untreated	0.59 ± 0.04 (46% fall from normal)	1.04 ± 0.07 (36% fall from normal)
Moderately diabetic treated (change% from untreated)	1.09 ± 0.08 (+85%)	0.99 ± 0.08 (−5%)
Severely diabetic untreated	0.58 ± 0.04 (47% fall from normal)	1.01 ± 0.08 (39% fall from normal)
Severely diabetic treated (change% from untreated)	1.01 ± 0.07 (+74%)	1.07 ± 0.08 (+6%)

kinase in the untreated and GII treated subdiabetic rabbits. There was decrease to 18.3 ± 1 u/g (37% fall) and 21.9 ± 0.9 u/g tissue (24% fall) of muscle pyruvate kinase in the untreated moderately and severely diabetic rabbits. After treatment with GII for 15 days there was 54% increase of the pyruvate kinase in the moderately diabetic rabbits to 28.1 ± 2 u/g and 24% increase to 27.1 ± 1 u/g tissue in the severely diabetic rabbits. In the kidneys of the subdiabetic and moderately diabetic rabbits pyruvate kinase did not change in the untreated as well as treated rabbits when compared with normal rabbits (Table 7). But in the untreated severely diabetic rabbits it decreased to 10.0 ± 1 u/g tissue when compared to normal value of 12.2 ± 0.6 u/g tissue. But on treatment with GII for 15 days it returned to normal range (11.9 ± 0.8 u/g tissue) by an increase of 19%. The action of GII could bring affected pyruvate kinase to normal value.

Malic enzyme (Table 7) in the liver of the untreated subdiabetic rabbits was altered only slightly to 1.08 ± 0.8 u/g) but considerably reduced in the untreated moderately and severely diabetic rabbits to 0.59 ± 0.04, 0.58 ± 0.04 u/g tissue respectively compared to normal value of 1.10 ± 0.07 u/g liver. Treatment with GII (50 mg/kg bw) for 15 days brought the levels of malic enzyme back to normal. But in the kidneys this enzyme was reduced even in the subdiabetic (1.11 ± 0.09 u/g, 32% fall) along with moderately diabetic (1.04 ± 0.07 u/g, 36% fall) and severely diabetic (1.01 ± 0.08 u/g, 39% fall) rabbits when compared with normal enzyme value of 1.62 ± 0.1 u/g kidneys. Treatment for 15 days with GII did not bring about much change in the malic enzyme activity of the kidneys (Table 7) of the subdiabetic, moderately and severely diabetic rabbits. In fact there was slight further reduction in the content of these enzymes after GII treatment in the subdiabetic and moderately diabetic rabbits. Therefore treatment with GII at the same dose for 30 days was tried. Liver malic enzyme came back to normal even in the severely diabetic rabbits. But there was only very slight improvement in the malic enzyme of the kidneys of the subdiabetic and moderately diabetic rabbits (results not shown in the table). The reduced malic enzyme (1.01 ± 0.8 u/g tissue) of the kidneys of the severely diabetic rabbits was not affected implying that GII 50 mg/kg bw did not have much improvement on decreased renal malic enzyme of the kidneys even after 30 days treatment. This means that higher dose may be necessary or the drug has no effect on kidneys malic enzyme in only severe diabetes. Of course 30 days treatment with GII still kept hexokinase and pyruvate kinase in the normal range. This implies that since 15 days treatment brought them to normal and prolonged treatment for 30 days did not have any adverse side effects on these enzymes.

The gluconeogenic enzymes glucose-6-phosphate dehydrogenase and glucose-6-phosphatase were also studied.

The glucose-6-phosphate dehydrogenase (Table 8) was considerably reduced in the liver of both the untreated moderately and severely diabetic rabbits to 79.6 ± 6 and 83.3 ± 6 u/g tissue respectively when compared to normal value of 141.4 ± 4 u/g tissue. Treatment with GII showed very good improvement in the moderately diabetic (46% increase) and significant improvement (19% increase) in the severely diabetic rabbits to 116.5 ± 9 and 99.7 ± 8 u/g tissue respectively. But the values are still slightly below normal. Glucose-6-phosphatase was considerably elevated (Table 8) in the liver of both the moderately and severely diabetic rabbits to 70.1 ± 4 and 66.6 ± 4 u/g tissue compared to normal value of 38.3 ± 3 u/g tissue. Treatment with GII for 15 days at a dose of 50 mg/kg bw brought down the value in moderately diabetic (17% fall) and severely diabetic rabbits (11% fall) to 58.1 ± 4 and 59.4 ± 4 u/g tissue respectively. The treated values are still slightly above normal. Treatment for 30 days would perhaps bring the values of both the enzymes to normal.

The effect of GII on the enzymes of polyol pathway was also studied (Table 9).

The sorbitol dehydrogenase in the liver of both the untreated and GII (50 mg/kg bw) treated subdiabetic diabetic rabbits was not affected much. But in the liver of the untreated moderately diabetic and severely diabetic rabbits the enzyme value increased to 9.9 ± 0.8 and 10.7 ± 0.7 u/g respectively and GII treatment brought it down to normal in the moderately diabetic rabbits to 9.3 ± 0.5 (by 6%). In the severely diabetic rabbits, after GII treatment there was some fall (−7%) but the enzyme was still 9.9 ± 0.6 u/g which was slightly above the normal value. In the kidneys the sorbitol dehydrogenase was not affected in the untreated and treated subdiabetic rabbits. But in the kidneys of the untreated moderately and severely diabetic rabbits there was increase to 8.0 ± 0.6 and 8.6 ± 0.8 u/g tissue respectively and GII treatment brought the values to normal in the moderately diabetic rabbits (6.8 ± 0.5 u/g, 15% fall) and in the severely diabetic rabbits to 7.2 ± 0.6 u/g (16% fall) which was very slightly higher than the normal value.

Aldose reductase in the liver of the untreated and treated subdiabetic rabbits did not change from the normal value (Table 9). But in the liver of the untreated moderately and severely diabetic rabbits the enzyme value increased to 0.33 ± 0.04, 0.33 ± 0.05 u/g tissue and after GII treatment the enzyme values came back to normal. But in the kidneys, aldose reductase increased from a normal value of 0.21 ± 0.4 u/g tissue to 0.26 ± 0.06, 0.27 ± 0.06 and 0.30 ± 0.08 u/g tissue in the subdiabetic, moderately and severely diabetic rabbits. But after treatment with GII (50 mg/kg bw) for 15 days, the values came down to normal in all the three groups of diabetic rabbits.

Since the sorbitol dehydrogenase did not come to normal after 15 days treatment with GII in the severely diabetic rabbits, treatment with GII was prolonged for 30 days in these rabbits. Then the sorbitol dehydrogenase and aldose reductase values increased in 30 days to 10.7 ± 0.7 and 0.28 ± 0.07 u/g in the kidneys of the severely diabetic treatments, but after treatment the enzyme values were 9.8 ± 0.7 and 0.26 ± 0.03 u/g respectively. In the liver the values of sorbitol dehydrogenase and aldose reductase increased to 8.6 ± 0.8 and 0.35 ± 0.08 u/g after 30 days and treatment with GII brought the values down to 6.5 ± 6 (24% fall) and 0.21 ± 0.5 u/g (40% fall) respectively.

Effect of GII administration on the antioxidant enzymes in all the three types of diabetic animals was studied. In diabetes, there is oxidative damage and tissue injury leading to the increase in the free radicals to cause chronic complications [11]. This affects the status of antioxidant enzymes to counteract the damage caused by free radicals

Table 8 Effect of 15 days administration of GII (50 mg/kg bw) on glucose-6-phosphate dehydrogenase and glucose-6-phosphatase in the liver of the moderately and severely diabetic rabbits

Glucose-6-phosphate dehydrogenase (u/g)	Value	
Normal	141.4 ± 4	
Moderately diabetic untreated	79.6 ± 6 (−44% fall from normal)	
Moderately diabetic treated (change% from untreated)	116.5 ± 9 (+46%)	
Severely diabetic untreated	83.3 ± 6 (−41% fall from normal)	
Severely diabetic treated (change% from untreated)	99.7 ± 8 (+19%)	
Glucose-6-phosphatase (u/g tissue)	**Value**	**% change from treated**
Normal	38.3 ± 3	
Moderately diabetic untreated	70.1 ± 4 (+83% increase from normal)	
Moderately diabetic treated	58.1 ± 4	−17
Severely diabetic untreated	66.6 ± 4 (+74% increase from normal)	
Severely diabetic treated	59.4 ± 4	−11

Table 9 Effect of GII (50 mg/kg bw) treatment for 15 and 30 days on the enzymes of polyol pathway in the liver of the subdiabetic, moderately diabetic and severely diabetic rabbits

	Kidneys	Liver
Sorbitol dehydrogenase (u/g tissue)		
Normal	6.6 ± 0.6	9.4 ± 0.6
Subdiabetic untreated	6.8 ± 0.6	9.1 ± 0.6
Subdiabetic treated (15 days)	6.5 ± 0.5 (−4%)	9.3 ± 0.7 (+2%)
Moderately diabetic untreated	8.0 ± 0.6	9.9 ± 0.8
Moderately diabetic treated (15 days)	6.8 ± 0.5 (−15%)	9.3 ± 0.5 (−6%)
Severely diabetic untreated	8.6 ± 0.8	10.7 ± 0.7
Severely diabetic treated (15 days)	7.2 ± 0.6 (−16%)	9.9 ± 0.6 (−7%)
Severely diabetic untreated (30 days)	10.7 ± 0.7	8.6 ± 0.8
Severely diabetic treated (30 days)	9.8 ± 0.7 (−9%)	6.5 ± 6 (−24%)
Aldose reductase (u/g tissue)		
Normal	0.21 ± 0.4	0.29 ± 0.05
Sub diabetic untreated	0.26 ± 0.06	0.29 ± 0.04
Sub diabetic treated (15 days)	0.23 ± 0.04 (−12%)	0.28 ± 0.03 (−4%)
Moderately diabetic untreated	0.27 ± 0.06	0.33 ± 0.04
Moderately diabetic treated (15 days)	0.22 ± 0.08 (−15%)	0.27 ± 0.03 (−18%)
Severely diabetic untreated	0.30 ± 0.08	0.33 ± 0.05
Severely diabetic treated (15 days)	0.23 ± 0.07 (−23%)	0.28 ± 0.07 (−15%)
Severely diabetic untreated (30 days)	0.28 ± 0.07	0.35 ± 0.08
Severely diabetic treated (30 days)	0.26 ± 0.03	0.21 ± 0.05 (−40%)

[12]. Out of the three antioxidant enzymes (Table 10), superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase, there was no change in the activity of glutathione reductase in the subdiabetic, moderately diabetic and severely diabetic rabbits and the values were similar to the normal value of glutathione reductase of 6.7 ± 0.2 and 5.8 ± 0.2 (u/g) in the liver and kidneys (not shown in the tables). Superoxide dismutase was not affected in the subdiabetic rabbits both in the liver and kidneys (Table 10). But in the liver of the moderately diabetic untreated rabbits, the SOD fell down from the normal value of 236.4 ± 22 to 185.2 ± 14 u/g in the untreated moderately diabetic and to 172.7 ± 17 u/g tissue in the severely diabetic untreated rabbits. But after treatment with GII (50 mg/kg bw) for 15 days, the SOD increased by 21% to 221.9 ± 15 u/g tissue which is near normal value in the liver of the moderately diabetic rabbits. But in the liver of the treated severely diabetic rabbits,

Table 10 Effect of 15 days treatment with GII 50 mg/kg bw on the antioxidant enzymes in the subdiabetic, moderately diabetic and severely diabetic rabbits

	Liver	Kidneys
Super oxide dismutase (u/g tissue)		
Normal	236.4 ± 22	123.2 ± 12
Sub diabetic untreated	233.3 ± 21	123.9 ± 18
Sub diabetic treated	221.7 ± 23 (−5%)	120.9 ± 16 (−2%)
Moderately diabetic untreated	185.2 ± 14	137.7 ± 0.9
Moderately diabetic treated	221.9 ± 15 (+21%)	126.4 ± 17 (−8%)
Severely diabetic untreated	172.7 ± 17	137.4 ± 18
Severely diabetic treated	246.6 ± 19 (+43%)	130.4 ± 16 (−5%)
Glutathione peroxidase (u/g tissues)		
Normal	23.1 ± 2	6.3 ± 0.5
Sub diabetic untreated	22.8 ± 2	6.6 ± 0.4
Sub diabetic treated	22.6 ± 2	6.8 ± 0.4
Moderately diabetic untreated	16.6 ± 1	6.5 ± 0.5
Moderately diabetic treated	21.7 ± 2 (+31%)	6.6 ± 0.4
Severely diabetic untreated	16.2 ± 1	10.2 ± 1
Severely diabetic treated	23.5 ± 2 (+45%)	8.9 ± 0.8 (−13%)

the value increased by 43% to 246.6 ± 19 u/g liver which is still slightly above the normal level. In the kidneys, there was not much change in the untreated and treated subdiabetic rabbits. But in the untreated moderately and severely diabetic rabbits kidneys there was increase of SOD to 137.7 ± 0.9 and 137.4 ± 18 u/g. In the moderately diabetic rabbits GII treatment for 15 days brought down the activity of SOD to near normal value of 126.4 ± 17 u/g kidneys (8% fall). But in the treated severely diabetic rabbits the superoxide dismutase came down only very slightly (5% fall) to 130.4 ± 16 u/g tissue. So treatment with GII was given for 30 days which brought it down to 126.4 ± 16 u/g tissue which was almost normal value. So this indicates that treatment for 30 days can bring the antioxidant enzyme SOD to normal value.

Glutathione peroxidase was not affected much in the subdiabetic rabbits in both the liver and kidneys and in the moderately diabetic rabbits in the kidneys (Table 10). But the glutathione peroxidase was decreased in the liver of both the untreated moderately and severely diabetic rabbits to 16.6 ± 1 and 16.2 ± 1 u/g liver from the normal value of 23.1 ± 2 u/g tissue. Both the values returned to normal after 15 days treatment with GII 50 mg/kg bw daily once. In the kidneys however, there was increase in the glutathione peroxidase of the untreated severely diabetic rabbits to 10.2 ± 1 u/g when compared to the normal value of 6.3 ± 0.5 u/g kidneys.

Treatment with GII for 15 days decreased the value to 8.9 ± 0.8 u/g tissue (13% fall) which was still above the normal value (Table 10). So the treatment was tried for 30 days. The results in the Table 11 show that after treatment for 30 days there was slight improvement to 7.6 ± 0.7 u/g kidneys (26% fall) which were still above normal. Perhaps longer treatment or higher dose are necessary. In the liver even after 15 days treatment the values were normal and after 30 days they continued to be normal indicating that there were no adverse effects of GII treatment for 30 days.

Histopathological study of the liver, pancreas and kidneys of the three groups of the untreated and treated diabetic rabbits was carried out. In the liver of the untreated and treated subdiabetic rabbits no abnormal histopathological changes were seen. In the liver of the untreated moderately diabetic rabbits also there were no changes except in one animal in which vacuolated hepatic parenchymal cells were seen and chromatin was pushed to one side (not shown in figure). With the administration of GII at a dose of 50 mg/kg bw for 30 days these histopathological changes were reversed. In the liver of the untreated severely diabetic rabbits there was fatty infiltration, cysts formation in the periportal area and vesicular appearance of parenchymal cells of the liver with prominent basophilic nucleus. Some of these changes are shown in Fig. 1a. Treatment with GII at a dose of 50 mg/kg bw for 30 days improved the above changes and the hepatic tissues appeared histologically normal (Fig. 1b).

In the pancreas of the untreated severely diabetic rabbits there was heavy fatty infiltration in the exocrine portion of the tissues and islet cells could not be seen (Fig. 2a). But in the GII (50 mg/kg bw) treated group the fatty infiltration disappeared and the islet cells could be seen (Fig. 2b). This explains why there was 74% improvement in the fasting plasma glucose and plasma insulin levels in the GII treated severely diabetic rabbits. In the untreated moderately

Table 11 Effect of GII treatment for 30 days on the superoxide dismutase and glutathione peroxidase

	Liver	Kidneys
Super oxide dismutase untreated	172.7 ± 17	137.4 ± 8
Super oxide dismutase treated	218 ± 16 (+26%)	126.4 ± 16 (−7%)
Glutathione peroxidase untreated	16.2 ± 1	16.2 ± 1
Glutathione peroxidase treated	23.0 ± 2 (+42%)	7.6 ± 0.7 (−26%)

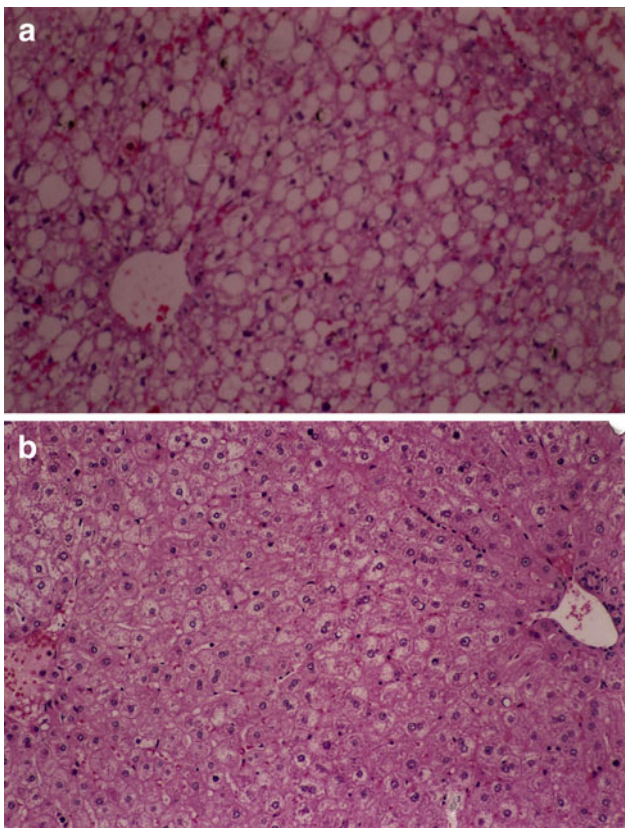

Fig. 1 **a** Fatty changes in the liver of an untreated severely diabetic rabbit. H & E stain ×200. **b** Histologically normal liver of a treated severely diabetic rabbit. H & E stain ×200

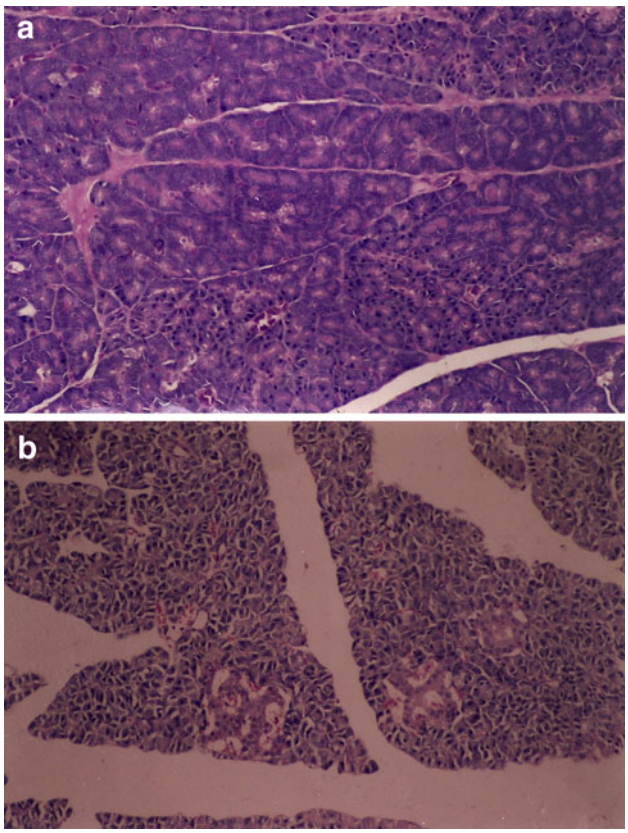

Fig. 2 **a** Fatty infiltration in the pancreatic tissue of an untreated severely diabetic rabbit. H & E stain ×200. **b** Histologically normal pancreatic tissue of a severely diabetic rabbit, treated for 30 days. Islet cells are clearly visible. H & E stain ×400

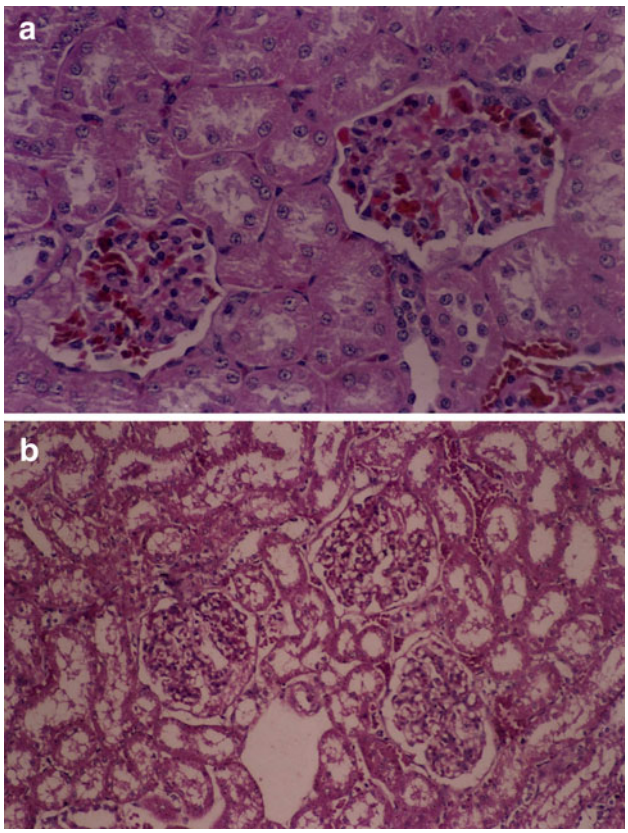

Fig. 3 **a** Vacuolated cells in the renal tubules and hyalinized material in the glomeruli in kidney of an untreated severely diabetic rabbit. H & E stain ×400. **b** Histologically normal kidney of a severely diabetic rabbit, treated for 30 days. H & E stain ×200

diabetic rabbits the fatty infiltration was less and was removed after treatment with GII. In the subdiabetic rabbits there was no change before and after treatment.

In the kidneys of the untreated severely diabetic rabbits, proliferation of the small blood vessel walls, focal areas of inflammation with mononuclear cell infiltration and fibrosed glomeruli were observed. In a few animals hyalanized material was present in the glomeruli and the renal tubules. Some of the changes are shown in Fig. 3a. After treatment with GII for 30 days all the pathological changes disappeared with the exception of pyelonephritic changes (Fig. 3b). Thus GII treatment arrested the progression of nephropathy and brought about improvement in the severely diabetic rabbits. In the subdiabetic and moderately diabetic rabbits no pathological changes were observed except in one moderately diabetic rabbit in which subcapsular scarring was seen. In the GII treated moderately and subdiabetic rabbits no pathological changes were seen. This shows that GII can reverse the histopathological changes seen in the severely diabetic and in few moderately diabetic rabbits.

The liver and kidney function tests namely alkaline phosphatase, serum glutamate pyruvate transaminase, serum bilirubin, creatinine, total proteins and urea were found to be within the normal range after treatment with GII for 30 days in the subdiabetic, moderately diabetic and severely diabetic rabbits (values not shown in tables). This indicates that GII does not have any adverse effects on the liver, kidneys and any other part of the body and is safe.

Discussion

It is well known that in diabetes mellitus there will be elevation of serum and tissue lipids as a complication. Treatment with GII (50 mg/kg bw), a purified product from the water extract of fenugreek seeds for 15 days in the subdiabetic and moderately diabetic and 30 days in the severely diabetic rabbits brought down all the elevated serum lipids TC, TG and (LDL + VLDL)C to normal. It increased serum HDLc which decreased in diabetes (Table 1). This implies that longer treatment time of 30 days is necessary to bring down the serum lipids in severely diabetic rabbits. Total lipids did not change much in the liver of the subdiabetic rabbits but increased in the liver of the moderately diabetic rabbits to 56.7 ± 5 mg/g

tissue and in the liver of the severely diabetic rabbits to 58.4 ± 5 mg/g tissue (Table 4). Treatment with GII for 15 days in the moderately diabetic and 30 days in the severely diabetic rabbits brought the lipid parameters down to normal values. Similar changes in the elevated TC and TG values of the diabetic rabbits were brought down to normal after 15 and 30 days treatment with GII in the moderately and severely diabetic rabbits respectively. But there were no significant changes in the heart tissue of the diabetic animals when compared to the values of normal animals (Table 3). Thus GII can effectively control the altered lipid parameters seen in the diabetic animals.

The glycogen content of the liver and muscle decreased in the untreated moderately and severely diabetic rabbits. Treatment with GII (50 mg/kg bw) for 15 days brought the liver glycogen content to the normal level in the moderately diabetic rabbits. In the severely diabetic rabbits there was good improvement in the liver glycogen content after 30 days treatment with GII, but the values of glycogen were still less than normal implying that longer or higher dose treatment may be necessary. In the muscle, the glycogen content decreased in the moderately and severely diabetic rabbits. But even after treatment for 30 days the glycogen values were still less than normal. This might mean that higher dose of GII may be necessary for the liver and muscle glycogen to return to the normal value.

About the enzymes of glycolysis, hexokinase and pyruvate kinase were not much affected in the subdiabetic rabbits (Tables 6 and 7). But glucokinase which is a more specific enzyme for glucose utilization was decreased by 60% to 0.68 ± 0.04 mg/g tissue from the normal value of 1.7 ± 0.07 mg/g tissue even in the subdiabetic rabbits. Treatment with GII brought the glycogen value to normal. In the moderately and severely diabetic rabbits, hexokinase was reduced by 34 and 33% in the liver and 55 and 63% in the muscle (Table 6). But decrease was more in the case of the glucose specific enzyme glucokinase to 81% in the moderately diabetic and to 79% in the severely diabetic rabbits. Treatment with GII for 15 days brought the activities of both of these enzymes, hexokinase and glucokinase, to normal. In the kidneys there was no change in hexokinase not only in the subdiabetic but also in the moderately diabetic rabbits. There was only a slight fall of 14% in the severely diabetic rabbits which was brought to normal with GII treatment. As with hexokinase, pyruvate kinase in the liver and muscle did not change much in the subdiabetic rabbits (Table 7). But pyruvate kinase decreased by 18% to 19.4 ± 1 and by 34% to 17.2 ± 1 u/g tissue in the liver of the untreated moderately and severely diabetic rabbits. Treatment with GII restored the activity to normal (Table 7). In the muscles also the pyruvate kinase decreased by 37% in the untreated moderately diabetic and by 24% in the severely diabetic untreated rabbits but restored to normal with 15 days of GII treatment. The stimulation of hexokinase, glucokinase and more so pyruvate kinase in the moderately and severely diabetic rabbits means that GII is diverting glucose through glycolysis and probably tricarboxylic acid cycle also. This effect could be pancreatic through insulin or extrapancreatic in the tissues of the moderately and severely diabetic animals.

The other enzyme of glucose metabolism, malic enzyme (Table 7) was not affected much in the liver of the subdiabetic rabbits but reduced considerably (46%) in the liver of the untreated moderately and severely diabetic rabbits and treatment with GII restored the activity to normal (Table 7). But in the kidneys, there was reduction of malic enzyme even in the untreated subdiabetic rabbits (32%) besides more or less the same reduction in the untreated moderately (36%) and severely diabetic rabbits (37%). Treatment with GII (50 mg/kg bw) for 15 days did not have any significant improvement in the malic enzyme activity. Even 30 days treatment did not have any change in the malic enzyme activity (results not shown). The reason for GII treatment having effect on the liver malic enzyme activity and not having effect on the kidney malic enzyme activity is to be investigated. One reason could be that the function of kidneys is different from that of the liver.

GII treatment for 15 days brought down slightly the gluconeogenic enzyme glucose 6-phosphatase to 58.1 ± 4 and 59.4 ± 4 u/g from the elevated values of 70.1 ± 4 and 66.6 ± 4 u/g tissue respectively in the moderately and severely diabetic rabbits. After 30 days treatment at the same dose the values came down slightly further but did not come to normal level. Perhaps longer treatment or higher dose would bring it down to normal level. Same thing was true in the case of glucose 6-phosphate dehydrogenase which was decreased in moderately (79.6 ± 6 u/g tissue) and severely diabetic (83.3 ± 6 u/g tissue) rabbits. But treatment with GII could bring it to 116.5 ± 9 u/g tissue in the moderately diabetic rabbits (+46%) and to 99.7 ± 8 u/g tissue in the severely diabetic rabbits in which there was only partial recovery (+19%). So the tendency to improve glucose 6-phosphatase and glucose 6-phosphate dehydrogenase by GII treatment in 30 days is there (results in the tables show only after 15 days treatment but not after 30 days) but longer treatment or higher dose is necessary for these two enzymes to be brought to normal level.

The enzymes of polyol pathway, sorbitol dehydrogenase and aldose reductase increased in the untreated diabetic rabbits. In the kidneys of the untreated moderately (8.0 ± 0.6 u/g) and severely diabetic rabbits (8.6 ± 0.8 u/g) the increase was by 21 and 30% respectively. But after treatment with GII they were brought back to normal range. Among the antioxidant enzymes, glutathione reductase was not affected

much (not shown in the table) in the untreated and treated moderately and severely diabetic rabbits. But the superoxide dismutase and glutathione peroxidase were not affected in the subdiabetic rabbits, but reduced in the liver and increased in the kidneys of the untreated moderately and severely diabetic rabbits when compared to normal animals. GII treatment brought the values to normal range in the moderately diabetic rabbits liver and kidneys by 15 days treatment. But in the severely diabetic rabbits after 15 days of treatment with GII the values of SOD improved both in the liver and kidneys, but the values were slightly higher than normal. Glutathione peroxidase which decreased in the liver in the untreated moderately and severely diabetic rabbits was brought up to normal with GII treatment for 30 days. But in the kidneys the enzyme increased only in the untreated severely diabetic but not in the moderately diabetic rabbits. After 30 days treatment with GII the value came down but only slightly above normal. This indicates that GII treatment has favourable effect on the enzymes of polyol pathway but longer treatment or higher dose is necessary for bringing down this enzyme to normal value.

The fact that all the liver and kidneys parameters and enzymes were normal in the GII treated animals even for 30 days shows that the GII treatment is absolutely safe and free from any side effects. The histopathological examination of the tissues revealed that GII treatment corrected most of the abnormalities seen in the liver, pancreas and kidneys of the untreated diabetic animals. Regeneration of the pancreas is an interesting improvement by GII. This indicates that GII treatment brings down blood glucose, glycosylated haemoglobin, lipids and improves utilization of glucose by stimulating serum insulin levels and stimulates the enzymes of glycolysis. It also improved tissue lipids by their utilization and by decreasing the activity of gluconeogenic enzymes. It also restored the altered glycogen levels and antioxidant enzymes.

Thus GII purified from fenugreek seeds has got good potential as an antidiabetic drug for use in diabetes for intermittent therapy and with reasonably good mechanism of action.

Conclusions

Treatment with GII purified from fenugreek seeds for 15 days in the subdiabetic and moderately diabetic rabbits and 30 days in the severely diabetic rabbits (i) decreased the elevated serum lipids TC, TG, (LDL + VLDL)C and increased the decreased (HDL)C (ii) decreased the elevated liver and heart total lipids, TC and TG (iii) increased the decreased liver and muscle glycogen (iv) increased the decreased hexokinase, glucokinase, pyruvate kinase, malic enzyme, glucose-6-phosphate dehydrogenase, superoxide dismutase and glutathione peroxidase, (v) decreased the increased glucose-6-phosphatase, sorbitol dehydrogenase and aldose reductase.

Results thus show that the GII treatment for 15 days in the subdiabetic and moderately diabetic rabbits and 30 days in the severely diabetic rabbits corrected the alterations in the serum and tissue lipids and the enzymes of glycolysis, gluconeogenesis, glycogen metabolism and antioxidant enzymes.

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