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ORIGINAL ARTICLE

Antidiabetic Effect of GII Compound Purified from Fenugreek (*Trigonella foenum graecum* Linn) Seeds in Diabetic Rabbits

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Abstract Aim is to study the antidiabetic effect of a compound GII purified earlier from the water extract of fenugreek (*Trigonella foenum graecum*) seeds by Murthy and his colleagues (patented in India and USA) in diabetic rabbits. Diabetes was induced in rabbits by injecting 80 mg/kg bw of alloxan intravenously into rabiits. Rabbits were subdivided into subdiabetic [fasting blood sugar (FBG) up to 120 mg/dl with abnormal glucose tolerance in glucose tolerance test (GTT)], moderately diabetic (FBG below 250 mg/dl) and severely diabetic (FBG above 250 mg/dl). Blood glucose and glycosylated hemoglobin (HbA1C) were estimated by procedures in the kits of Stangen Immunodiagnostics, Mumbai using, respectively, glucose oxidase method and absorbance at 415 nm. Serum insulin was estimated by the ELISA method as described in the kit of Boehringer Mannheim Immunodiagnostics, Mumbai, India. GII was found to improve blood glucose utilization in GTT and reduced FBG and HbA1C. In the present communication detailed studies were carried out with GII in the subdiabetic, moderately diabetic and severely diabetic rabbits. GII at a dose of 50 mg/kg bw per day brought down the elevated FBG levels in the untreated subdiabetic (FBG 96.6 ± 7 mg/dl), moderately diabetic (150.1 ± 14 mg/dl) and severely diabetic rabbits (427 ± 46 mg/dl) to normal in 12, 15 and 28 days of treatment. It improved serum HbA1C and insulin levels also in these rabbits. Intermittent therapy once a week for 6 weeks with GII at the same dose brought down the FBG values to normal in the subdiabetic (FBG 96.0 ± 2 mg/dl) and in the moderately diabetic rabbits to 133.0 ± 12 mg/dl. After stopping therapy of the subdiabetic and moderately diabetic rabbits whose FBG values came to normal after treatment with GII 50 mg/kg bw, the values remained normal for 1 week and showed a tendency to increase only after 15 days. If these animal studies are applicable to humans these results indicate that a diabetic person need not take GII daily when once the FBG value comes to normal or near to normal. Patients might be able to take GII only when the FBG value shows tendency to increase. So, intermittent therapy is possible with the potent product GII of the fenugreek seeds which is of a great advantage.

Keywords Fenugreek GII · Antidiabetic · Blood glucose · Glycosylated hemoglobin · Insulin

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Introduction

The use of many medicinal plants for diabetes has been mentioned in the ancient Indian system of medicine Ayurveda in India. Many scientists all over the world are working on medicinal plants useful in diabetes mellitus and related diseases. These were recently reviewed by Jung et al. [1] and by Mukherjee et al. [2].

There are reports on the use of fenugreek seed extracts and some compounds trigonelline and nicotinic acid purified from them in diabetes mellitus [3]. A compound GII (different from trigonelline, nicotinic acid and a steroid

reported earlier from fenugreek seeds by other scientists) was purified by Murthy and his colleagues [4] from fenugreek seeds and patented in India and USA. They showed that the GII compound improved glucose utilization in the glucose tolerance test (GTT) and reduced the fasting blood glucose (FBG) and glycosylatyed hemoglobin in the subdiabetic and moderately diabetic rabbits to normal value at a dose of 50 mg/kg bw, and reduced significantly but to slightly above normal blood glucose values in the severely diabetic rabbits at a higher dose of 100 mg/kg bw) [5]. In this detailed communication we report that the purified GII fenugreek compound not only improved GTT at a lower dose of 50 mg/kg bw but also reduced the FBG and glycosylated hemoglobin to normal values and also increased the serum insulin in the subdiabetic, moderately diabetic and severely diabetic rabbits.

Methods and Materials

Chemicals

All the biochemicals and chemicals were purchased from Sigma Chemical Co., St. Louis, USA and Stanger Immuno Diagnostics, Boehringer Manheim and British Drug Houses Ltd. Mumbai, in India.

Methods

Glucose and glycosylated hemoglobin (HbA1C) were estimated by the procedure given in the kits from Stangen immuno diagnostics. Glucose was converted by glucose oxidase to gluconic acid and H_2O_2. Peroxidase oxidized H_2O_2 to water and oxygen. This oxygen oxidized phenol to give a coloured compound with 4-aminophenazone which was measured at 515 nm.

For glycosylated hemoglobin whole blood was hemolysed and mixed with a cation exchange resin of the kit. The non-glycosylated hemoglobin binds to the resin leaving glycosylated hemoglobin (HbA1C) free in the supernatant. The free HbA1C was measured by its absorbance at 415 nm.

Serum insulin was estimated by the procedure in the kit from Boehringer Mannheim Immuno Diagnostics using enzyme linked immunosorbent assay (ELISA) technique. Insulin present in the serum samples binds with antiinsulin antibodies coated along the inner walls of the plastic tubes of the ELISA plate. The antiinsulin antibody—peroxidase (POD) conjugate added subsequently binds with the insulin molecules. The amount of binding is proportional to the serum insulin concentration and was measured at 420 nm by adding diammonium-2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonate as chromogen.

Isolation of the Purified Compound GII

Isolation of the purified compound GII was briefly by a method patented earlier and described in the paper by Radha Moorthy et al. [5] which was by soaking fenugreek seeds in water overnight at 4°C. The compound GII of the water extract of fenugreek seeds was purified by DEAE cellulose chromatography and subsequent gel filtration by Sephadex G100 column.

Glucose Tolerance Test

From the overnight fasted rabbits blood was withdrawn from their marginal ear vein and blood glucose was determined. This served as the fasting blood glucose (FBG) value. Immediately water was given orally. After 90 min blood was again withdrawn and glucose was determined. This served as zero hour value for GTT. Then 3 g/kg bw of glucose in water was given orally and blood was withdrawn at 1 and 2.5 h. This was the initial GTT. After 1 week the same animals were used for the effect of the drug on GTT. After drawing blood from overnight fasted rabbits 50 mg/kg bw of GII purified from fenugreek seeds was given orally. After 90 min blood was withdrawn and glucose estimated. This would give an idea of the effect of GII compound on fasting blood glucose, if any, in 90 min. Then glucose was given and GTT was conducted as described above. The advantage of this method developed by Murthy and his colleagues [6] in our laboratory was that the same animals serve as their own controls.

Induction of Diabetes

Diabetes was induced with 80 mg/kg bw of alloxan given in 0.7% sterile saline adjusted to pH 4.5 with citric acid intravenously as described earlier by Moorthy et al., and Babu et al. [5, 6]. Rabbits with stabilized diabetes (at least 1 month after alloxan injection) were used. They were classified as the subdiabetic (near normal FBG up to 120 mg/dl with abnormal GTT), moderately diabetic (FBG below 250 mg/dl) and severely diabetic (FBG above 250 mg/dl) rabbits (five in each group) depending on their FBG levels.

Results

Effect of GII on the FBG and GTT in the Subdiabetic, Moderately Diabetic and Severely Diabetic Rabbits in 90 min

As mentioned under methods, blood from the overnight fasted subdiabetic (FBG 94.8 ± 5 mg/dl), moderately diabetic (FBG 156.1 ± 9 mg/dl) and severely diabetic

Table 1 Effect of single dose (50 mg/kg bw) administration of GII compound on FBG and GTT in the subdiabetic and moderately diabetic rabbits

Diabetic status	Plasma glucose mg/dl			
	Fasting	1 h	2.5 h	AUC
Normal	90.1 ± 3	148.2 ± 5	134.6 ± 4	20,160
Subdiabetic untreated	96.1 ± 4	296.6 ± 5	270.0 ± 9	37,230
Subdiabetic treated	92.7 ± 4	220.7 ± 7	200.2 ± 4	28,260
Change after treatment	−4%	−25%	−26%	−24%
	NS	$P < 0.01$	$P < 0.01$	
Moderately diabetic untreated	150.4 ± 15	286.0 ± 18	189.1 ± 8	34,700
Moderately diabetic treated	147.3 ± 9	196.0 ± 7	170.2 ± 9	26,730
Change after treatment	−2%	−34%	−10%	−21%
	NS	$P < 0.01$	$P < 0.01$	

AUC Area under the curve of GTT, *NS* Not significant

(FBG 464.1 ± 42 mg/dl) rabbits was withdrawn. GII 50 mg/kg bw in water was given orally. After 90 min blood was again withdrawn from all the groups of animals and blood glucose was determined. There was fall in FBG of 3.7% in the subdiabetic, 4% fall in FBG in the moderately diabetic and 3.8% fall in FBG in the severely diabetic rabbits. This means that single dose administration of GII did not have any significant effect on FBG in 90 min. These results are not shown in the table. Then the effect of a single dose (50 mg/kg bw) of GII in the diabetic rabbits on GTT was tried by giving it orally. Glucose 3 g/kg bw was given and GTT was performed as described under methods in the subdiabetic and the moderately diabetic animals and blood glucose values are given in Table 1.

Results in Table 1 indicate that even though GII did not have any effect (4% fall) on the FBG of the subdiabetic treated rabbits in 90 min after giving GII, it improved GTT in them by reducing blood glucose by 25 and 26% at 1 and 2.5 h and area under the curve of GTT by 24%. In the moderately diabetic rabbits also there was no effect of GII on the FBG (2% fall) of the treated rabbits. But in GTT, GII caused reduction of blood glucose at 1 h from 286.0 ± 18 mg/dl in the untreated rabbits by 34% to 196.0 ± 7 mg/dl in the treated moderately diabetic rabbits. But at 2.5 h the reduction in the blood glucose was only 10% from 189.1 ± 8 mg/dl in the untreated group to 170.2 ± 9 mg/dl in the treated group. Area under the curve was reduced by 21% by GII treatment in the moderately diabetic rabbits. This shows that the GII compound did not have any effect on the FBG but reduced the blood glucose in GTT both in sub diabetic and moderately diabetic rabbits.

Effect of Treatment with GII on the FBG and GTT of the Subdiabetic, Moderately Diabetic and Severely Diabetic Rabbits

The effect of GII (50 mg/kg bw) daily once was tried till the elevated FBG values returned to normal range. GTT was performed every week and at the end of the treatment. It is evident from Table 2 (only values on day 0 and at the end of the experiment were given) that in the subdiabetic rabbits treated with GII for 12 days there was reduction of only 10% in the fasting blood glucose but 50% reduction of blood glucose at 1 h and 48% reduction at 2.5 h and 47% reduction in AUC during GTT. All these values were in the normal range. In the moderately diabetic rabbits, treatment with 50 mg/kg bw for 15 days reduced FBG and improved GTT. The reduction in FBG was 40% and reduction in blood glucose at 1 and 2.5 h during GTT was 48 and 41% respectively. AUC also was reduced by 38%. All these values were in the normal range. This shows that there was over all improvement in fasting blood glucose (FBG) and by 40–48% in blood glucose during GTT in the moderately diabetic rabbits. These results indicate that GII brings down in 15 days FBG only when it was elevated in the moderately diabetic rabbits but not much (only 10% fall) when it was near normal in the subdiabetic rabbits. This also means that there is no risk of hypoglycemia.

In the severely diabetic rabbits there was reduction in the FBG to near normal value in a longer time of 29 days. In the severely diabetic rabbits GTT was not tried because some of the animals die if glucose was given to them.

The results in Table 3 show that in the untreated subdiabetic rabbits on day 0 there was no increase in FBG and only slight increase of blood glucose at 1 and 2 h during GTT. But serum insulin levels (fasting and during GTT) decreased on day 0. Treatment with GII for 15 days brought down the elevated blood glucose and decreased serum insulin values to normal. In the untreated moderately diabetic rabbits on day 0 there was increase in the fasting blood glucose and decrease in fasting serum insulin when compared with the subdiabetic rabbits. During GTT at 1 and 2 h there was increase in both blood glucose and very slight increase in serum insulin. But after 15 days treatment with GII there was decrease in blood glucose from 150 ± 11.7 to 89 ± 9.8 mg/dl. After 15 days treatment

Table 2 Effect of GII compound on the FBG and GTT in the subdiabetic, moderately diabetic and severely diabetic rabbits

Diabetic status	Plasma glucose mg/dl			
	Fasting	1 h	2.5 h	AUC
Normal	90.1 ± 3	148.2 ± 5	134.6 ± 4	20,160
Subdiabetic day 0	96.6 ± 7	296.6 ± 38	270.7 ± 22	37,230
Subdiabetic day 12	86.6 ± 6	146.1 ± 11	138.2 ± 9	19,740
Percent change from day 0	−10%	−50%	−48%	−47%
Moderately diabetic day 0	156.1 ± 14	286.6 ± 39	187.1 ± 13	31,410
Moderately diabetic day 15	89.5 ± 5	148.6 ± 12	110.1 ± 8	19,350
Percent change from day 0	−40%	−48%	−41%	−38%
Severely diabetic day 0	427.8 ± 46	GTT was not done in the severely diabetic rabbits because some animals die if glucose is given		
Severely diabetic day 29	110.1 ± 11			
Percent change from day 0	−75%			

Table 3 Effect of GII (50 mg/kg bw) treatment for 15 days on blood glucose and glucose induced serum insulin levels during GTT in the subdiabetic, moderately diabetic and severely diabetic rabbits

		Fasting	1 h	2 h
1	Subdiabetic			
	Serum insulin μl/ml			
	Day 0	11.6 ± 1.4	19.8 ± 2	10.9 ± 2.1
	Day 15	15.4 ± 1.6	29.8 ± 1.1	28.4 ± 5.6
	Blood glucose mg%			
	Day 0	90 ± 5.6	220 ± 19	188 ± 12.3
	Day 15	86 ± 8.2	140 ± 16.7	108 ± 14.2
2	Moderately diabetic			
	Serum insulin μl/ml			
	Day 0	3.04 ± 0.5	4.02 ± 0.5	3.8 ± 0.4
	Day 15	8.9 ± 1.6	19.3 ± 2	16.3 ± 2
	Blood glucose mg%			
	Day 0	150 ± 11.7	246 ± 19.4	198 ± 14.3
	Day 15	89 ± 9.8	140 ± 11.6	110 ± 10.2
3	Severely diabetic			
	Serum insulin μl/ml			
	Day 0	0.10 ± 0.03	GTT not done	
	Day 30	0.115 = 0.03 (+15%)		
	Blood glucose mg%			
	FBG	427 ± 46.1		
	Day1	110 ± 11.1		
	Day 30	(−74%)		
4	Normalserum insulin blood glucose	11.6 ± 1.6	30.4 ± 2	28.2 ± 2
		90 ± 5.9	148 ± 10.1	122 ± 8.3

with GII there was increase in fasting serum insulin and during GTT at 1 and 2 h there was increase in serum insulin to normal values. In the severely diabetic rabbits GTT could not be done as mentioned earlier because some animals die after giving glucose. But the FBG came down from 427 ± 46.1 mg/dl on day 0 to 110 ± 11.1 mg/dl after 29 days treatment with GII which is only slightly above normal.

This shows that in 15 days treatment with GII blood glucose came down and serum insulin levels returned to normal in the subdiabetic and moderately diabetic rabbits and in 30 days in the severely diabetic rabbits.

Subdiabetic, moderately diabetic and severely diabetic rabbits were maintained in diabetic state for 48 days and then treated with GII 50 mg/kg bw daily once for 28 days (4 weeks). Glycosylated hemoglobin was estimated on day

Table 4 Effect of GII treatment for 4 weeks at a dose of 50 mg/Kg bw on glycosylated hemoglobin levels in subdiabetic moderately diabetic and severely diabetic rabbits

Day	HbA1C values%		
	Sub diabetic	Moderately diabetic	Severely diabetic
Day1 untreated	2.4 ± 0.2	2.7 ± 0 .3	2.5 ± 0.3
Day 48 untreated	3.05 ± 0.3	3.8 ± 0.4	4.8 ± 0.5
Percent increase	27%	38%	92%
After 4 weeks treatment with GII	2.1 ± 0.2	2.6 ± 0.2	3.1 ± 0.3
Percent change	−32%	−33%	−36%
Untreated groups after 4 weeks	3.3 ± 0.4	3.7 ± 0.3	5.2 ± .05

Table 5 Effect of GII 50 mg/kg bw once a week for 6 weeks on the FBG and glucose during GTT (mg/dl) in the subdiabetic and moderately diabetic rabbits

Day	Subdiabetic			Moderately diabetic		
	Fasting	1 h	2.5 h	Fasting	1 h	2.5 h
Before treatment	96.0 ± 2	294.3 ± 29	292.2 ± 40	133.0 ± 12	350.1 ± 32	330.2 ± 32
After treatment 1 week	99.6 ± 1	205.1 ± 20	170.2 ± 14	113.5 ± 16	310.4 ± 32	197.5 ± 19
3 weeks	90.1 ± 3.7	172.2 ± 18	140.3 ± 11	95.4 ± 7	178.9 ± 17	156.5 ± 14
6 weeks	90.6 ± 4	136.6 ± 12	122.7 ± 13	96.6 ± 7	154.8 ± 11.7	130.4 ± 11.2

1 and day 48 in the untreated state and after 4 weeks of treatment (Table 4). Both the subdiabetic and moderately diabetic rabbits returned to day 1 level from 3.05 ± 0.3 to 3.8 ± 0.4% i.e., a fall of 32 and 33%, respectively. But in the untreated rabbits the values further increased to 3.3 ± 0.4% in the subdiabetic and remained the same (3.7%) in the moderately diabetic rabbits. In the severely diabetic rabbits there was a fall of 36% from 4.8 ± 0.5 to 3.1 ± 0.3%. The treated severely diabetic rabbit values were still above day 1 value.

Thus, the treatment was very effective in the subdiabetic and moderately diabetic rabbits which brought back the HbA1C values to normal, but very good, in the severely diabetic rabbits which showed fall in HbA1C of 36%.

It was intended to see whether GII should be given every day like all drugs in current use for diabetes or intermittent therapy is possible with GII in which case it would be beneficial. Intermittent therapy was not done in severe diabetes because one should not take a chance, but bring down severe diabetes to moderate diabetes at least. So GII was given at a dose of 50 mg/kg bw once every week for 6 weeks to the subdiabetic and moderately diabetic rabbits. FBG and GTT were done once every week but values at 1, 3 and 6 weeks only were given in Table 5. From the results in plasma glucose during GTT it can be seen (Table 5) that after 6 weeks treatment with GII the fasting plasma glucose levels and blood glucose at 1 and 2.5 h during GTT came back to normal in the subdiabetic and moderately diabetic rabbits.

After treatment with GII (50 mg/kg bw) at 2.5 h during GTT even by first week, there was good fall in blood glucose in both the subdiabetic (from 292.2 ± 40 to 170.2 ± 14 mg/dl, 42% fall) and moderately diabetic (from 330.2 ± 32 to 197.5 ± 19 mg/dl, 40% fall) rabbits. FPG was unaffected in the subdiabetic rabbits but came down by 1 week from 133.0 ± 12 to 113.5 ± 16 mg/dl, (15% fall) in the moderately diabetic rabbits. By 3 weeks there was further improvement. After 6 weeks treatment, the FBG and blood glucose values during GTT came down to normal values. In order to see how long the favourable effect persisted without treatment, the animals were left without treatment and FPG was determined after 1, 2, 3 and 4 weeks. The results (not shown in the table) showed that after treatment the FPG which was normal (96.6 ± 7 mg/dl) in the moderately diabetic rabbits on day 0 increased post treatment to 106.6 ± 6, 104.2 ± 9, 108.1 ± 8 and 146.2 ± 91 mg/dl on 1, 2, 3, and 4 weeks. In the severely diabetic rabbits the FPG value was 110.7 ± 8 mg/dl on day 0 and after 1, 2, 3, and 4 weeks the values were 112.1 ± 9, 177.3 ± 16, 398.0 ± 16, and 418.7 ± 51 mg/dl, respectively. These results indicate that the FPG values were normal without treatment for almost 3 weeks in the moderately diabetic rabbits and increased by 4 weeks. In the severely diabetic rabbits the values were normal only for 1 week and showed slight increase by 2 weeks and reached diabetic values only by 4 weeks. These results also suggest that the animals (perhaps humans also) can be left for 2–3 weeks in the moderately diabetic rabbits and in the severely diabetic rabbits for 1 week without treatment safely.

In the diabetic animals the body weight was reduced by 9% in subdiabetic, 29% in moderately diabetic and 25% in severely diabetic rabbits (results not shown in the table).

But after treatment with GII the animals in the above experiments returned to the normal weight.

Discussion

The GII (50 mg/kg bw) compound purified from the water extract of fenugreek seeds did not have much effect on the FBG in 90 min but improved GTT in the normal animals. This indicates that GII does not do any harm (no hypoglycemic effect) to FBG in apparently normal or subdiabetic animals or human individuals but improves their GTT. In the subdiabetic rabbits the FBG came down to normal in 12 days with 50% reduction in blood glucose at 1 h during GTT. In the moderately diabetic rabbits, the FBG came down to normal in 15 days and the reduction in blood glucose in GTT was 48%. In the severely diabetic rabbits the FBG came down to normal in 4 weeks. GTT was not done in the untreated severely diabetic rabbits because some animals die if glucose was given to them. In the untreated subdiabetic rabbits the fasting insulin was normal but during GTT the insulin levels decreased. Treatment with GII 50 mg/kg bw restored the insulin values in GTT to normal. In the moderately diabetic rabbits, the decreased serum insulin levels in the untreated rabbits increased to normal level after treatment with GII for 15 days. There was 15% increase in the serum insulin and 74% decrease in FBG in severely diabetic rabbits and both were brought to normal values after 30 days treatment with GII. The increase in serum insulin may be due to increased synthesis or release of insulin by the pancreas or both. This indicates that GII 50 mg/kg bw treatment can effectively control FBG and serum insulin in the moderately and severely diabetic rabbits and improve GTT in the subdiabetic and moderately diabetic rabbits without affecting normal FBG in the subdiabetic rabbits (perhaps humans also). Glycosylated hemoglobin (HbA1C) also returned to normal in the subdiabetic and moderately diabetic rabbits after treatment with GII. In severe diabetes there was 35% fall in HbA1C and its values were closer to normal after 15 days treatment with GII. The increased body weight of all untreated diabetic animals decreased to normal after treatment with GII (results not shown).

In the intermittent therapy (instead of daily therapy) given once every week for 6 weeks to the subdiabetic and moderately diabetic rabbits, the FBG and blood glucose at 1 and 2.5 h during GTT came down to normal values. This proves that intermittent therapy is effective with purified GII compound in subdiabetes and moderate diabetes. Of course in severe diabetes one has to give daily treatment to reduce the severity at least till the FPG comes down to moderate diabetes stage. In order to see how long the treatment with GII would be effective after stopping treatment, GTT was performed every week for 4 weeks after stopping the treatment. It was found that moderately diabetic animals were almost normal for 2–3 weeks and FPG increased by 4 weeks. Severely diabetic animals were normal for week. They showed slight increase in blood glucose only after 2 weeks. This means that one need not take drug for 2–3 weeks in moderate diabetes and for 1 week in severe diabetes. This is a great advantage because with modern drugs daily treatment is necessary life long.

In the past many scientists including our group showed [1–3, 7–9] that many plants are useful in diabetes. Our present studies with purified GII compound from fenugreek (*Trigonella foenum graecum*) seeds showed that it is not only effective in daily therapy but useful in intermittent therapy. When GII brings down FBG to normal, the treatment can be stopped for at least 2–3 weeks in moderate diabetes and nearly 2 weeks in severe diabetes and FBG levels will remain normal. Treatment can be started again only when blood glucose levels start increasing. Thus, intermittent therapy with effective control of blood glucose and GTT in moderately and severely diabetic rabbits is a great advantage with our GII compound. To the best of our knowledge this is the only plant purified product which has the benefit of intermittent therapy.

Conclusions

In the rabbits with alloxan induced diabetes, the elevated fasting blood glucose and glycosylated hemoglobin values were brought down to normal values by treatment with GII purified from the water extract of the seeds of fenugreek seeds (*Trigonella foenum graecum*) at a dose of 50 mg/kg bw. The serum insulin values which were also less in the untreated diabetic rabbits increased to normal level after treatment with GII. An interesting feature is that intermittent therapy once a week for 6 weeks in the subdiabetic and moderately diabetic rabbits with GII at the same dose brought down the FBG values to normal. After stopping the therapy of the subdiabetic and moderately diabetic rabbits whose FBG values came down to normal after treatment with GII 50 mg kg bw, the values remained normal without treatment for 1 week and showed a tendency to increase only after 15 days. Thus, intermittent therapy is possible with the potent product GII of the fenugreek seeds which is of a great advantage.

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