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Toxicity studies on *Trigonella foenum-graecum* L. seeds used in spices and as a traditional remedy for diabetes

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SUMMARY

Acute (24 h) and chronic (90 days) oral toxicity studies on the ethanol extract of *Trigonella foenum-graecum* Leguminosae (L.) seeds were carried out. Acute dosages were 0.5, 1.0 and 3 g/kg while chronic dosage was 100 mg/kg per day of the extract. All morphological, biochemical, haematological and spermatogenic changes, in addition to mortality, body weight changes and any change in vital organs were recorded. Histopathological investigations were done on vital organs. Growth arrest in the treated animals was observed. The treated mice gained no significant weight during chronic treatment while there was a significant gain in body weight of the control group mice. Biochemical studies revealed a significant decrease in blood sugar levels of fenugreek treatment groups while haematological parameters remained comparable to the control. In the treatment, male group there was a significant decrease in weight of testes as compared to the control. There was a marginal weight gain in kidney weight of mice after chronic treatment as compared to the control. Fenugreek chronic treatment caused a highly significant spermatotoxic effects in male mice.

Key words: Fenugreek; Acute toxicity; Chronic toxicity studies; Slowness of growth; Blood glucose lowering potential; Spermatotoxic effect

INTRODUCTION

Trigonella foenum-graecum Leguminosae, (L.) commonly called 'fenugreek', is an annual herb, used in traditional medicine of different countries worldwide (Chopra *et al.*, 1956; Gruenwald *et al.*, 2000; Barnes *et al.*, 2002; Malviya *et al.*, 2010). Fenugreek seeds have an aromatic odor and characteristic bitter taste. Fenugreek is used for fragrance in spices and is known to possess various medicinal properties (Hussein, 1985; Dweck, 1997; Beutler and DerMarderosian 2002; Shabbeer *et al.*, 2009). Its seeds are claimed to have antidiabetic, mucilaginous, demulcent, laxative, nutritive, expectorant and orexigenic properties (Karawya *et al.*, 1980; Duke, 2001; Barnes *et al.*, 2002). The seeds have been documented as a traditional herbal treatment for diabetes in different countries (Khosla *et al.*, 1995; Abdel-Barry *et al.*, 1997; Hunt *et al.*, 2000; Haddad *et al.*, 2001).

Fenugreek seeds were also used traditionally in the treatment of dyspepsia, gastritis and convalescence, and topically for furuncolosis, myalgia, lymphadenitis, gout, wounds, inflammations and leg ulcers (Duke, 2001; Beutler and Marderosian 2002; Malviya *et*

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al., 2010). The crude infusion of the fenugreek seeds is used as tonic, carminative, antispasmodic (Duke, 2001), antidiabetic (Shani *et al.*, 1974; Puri *et al.*, 2002; Mondal *et al.*, 2004), antioxidant (Thirunavukkarasu *et al.*, 2003; Dixit *et al.*, 2005), cytoprotective (Pandian *et al.*, 2002), hepato-protective (Kaviarasan *et al.*, 2007) anti-inflammatory (Vyas *et al.*, 2008; Malviya *et al.*, 2010), antimalarial control (Khater and Shalaby, 2008; Palaniswamy *et al.*, 2008); antifungal (Haouala *et al.*, 2008), antineoplastic (Sur *et al.*, 2001; Amin *et al.*, 2005), anti thyroid hormone (Kar and Tahiliani, 2003), immunostimulant (Bin-Hafeez *et al.*, 2003), hypocholesterolaemic (Valette *et al.*, 1984; Narender *et al.*, 2006), diuretic and as a remedy against kidney stones (Ahsan *et al.*, 1989; Rohini *et al.*, 2009). Several such claims were confirmed experimentally. Various pharmacologically active compounds such as alkaloids (Shani *et al.*, 1974; Duke, 2001; Zhao *et al.*, 2003; Satheeshkumar *et al.*, 2010); flavonoids (Adamska and Lutomski, 1971; Shang *et al.*, 1998); tannin like phenolic compounds (Duke, 2001); polyphenols (Kaviarasan *et al.*, 2008); steroids (Taylor *et al.*, 1997); saponins (Dawidar *et al.*, 1973; Pasich *et al.*, 1983; Gupta *et al.*, 1986; Yoshikawa *et al.*, 1997; Sauvaire *et al.*, 1998; Murakami *et al.*, 2000; Raju *et al.*, 2004; Yang *et al.*, 2005); dioscin; free amino acids (Duke, 2001); an unusual amino acid 4-hydroxyisoleucine (Haefelé *et al.*, 1997; Sauvaire *et al.*, 1998; Haeri *et al.*, 2009); coumarin derivatives (Khurana *et al.*, 1982); lipids (Beutler and Der Marderosian, 2002); phospholipids (Xu *et al.*, 1992); mucilaginous fibers (Srichamroen *et al.*, 2009); vitamins; and minerals have been isolated from fenugreek seeds (Farnsworthand Marles, 1995; Duke, 2001). Some of the steroidal saponins isolated from fenugreek seeds, were found to stimulate growth-hormone release in rat pituitary cells (Shim *et al.*, 2008), while higher phenol and polyphenols contents of fenugreek controlled oxidative damage (Kaviarasan *et al.*, 2007; Dixit *et al.*, 2008; Kaviarasan *et al.*, 2008).

As regards the toxicity of fenugreek treatment is concerned, in some patients rhinorrhea, wheezing, facial angioedema, and fainting was reported (Patil *et al.*, 1997; Faeste *et al.*, 2009). It was interesting to notice that in certain ethnic groups, use of fenugreek seeds as food and herbal remedy, caused myopathy that was confirmed in ruminants (Egyed and Shlosberg, 1983). Fenugreek seeds are known to contain trypsin inhibitors cheymotrypsin inhibitors, and acetycholinesterase inhibitors (Duke, 2001; Beutler and Marderosian, 2002; Satheeskumar *et al.*, 2010). Treatment with debitterized fenugreek powder (10% in diet) failed to induce any toxicity and showed no effect either on the daily food intake or on growth (Muralidhara *et al.*, 1999). Some studies also showed possible anti-fertility activity induced by fenugreek seeds (Kassem *et al.*, 2006). However, no details are available in the scientific literature about the toxic potential of fenugreek seed extract upon chronic treatment.

Several crude drugs were previously reported to possess significant toxicity (Shah *et al.* 1989; Qureshi *et al.*, 1990; Shah *et al.*, 1998; Al-Ashban *et al.*, 2005). Keeping in view the worldwide common use of fenugreek seeds in traditional medicine, and as food and spice, and the absence of reports on its adverse effects on chronic treatment, the present study was undertaken to investigate the toxicity of this popular natural drug after acute and chronic treatment in mice following World Health Organization (W.H.O.) protocol (WHO, 1967).

MATERIALS AND METHODS

Plant extract

The fenugreek seeds used in the current toxicity studies were collected from Riyadh region (Saudi Arabia) and identified by the Plant Taxonomy Unit of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia where the herbarium specimens (Voucher specimen # 15853) were kept on record. The powdered seeds were extracted with 95% ethanol and later the

solvent was evaporated at low pressure. The ethanol free extract was kept in refrigerator and used in the present investigations following ethical guidelines.

Animal stock

Swiss albino mice (home bred) aged 6 - 7 weeks, weighing about 22 - 26 g, and fed on Purina Chow diet and water *ad libitum* were used in the present study. The animals were maintained under controlled temperature, humidity, and automated 12-h light/dark cycle (Shah *et al.*, 1989).

Acute toxicity

A total of 20 mice were randomly allotted to one control and 3 treatment groups. The drug in each case was suspended in 0.1% CMC. The suspended extract was administered orally in three doses, namely 0.5, 1.0, and 3 g/kg body weight. The toxic symptoms observed were autonomic responses, motor activity and CNS excitation, etc. The animals were observed for 24 h for all signs of toxicity and mortality (Shah *et al.*, 1989; Al-Ashban *et al.*, 2005). Acute treatment with 0.5 g/kg was found to cause significant lowering in blood glucose levels in the treatment groups as compared to the control; hence, this dose was selected as the pharmacologically active dose.

Chronic toxicity

A total of 30 male and 30 female mice were randomly allotted to the control and the extract-treated groups separately (15 male and 15 female animals separately in each group). The fenugreek seed extract was given in drinking water (Shah *et al.*, 1989). The dose selected was 100 mg/kg/day, which is 1/5 of the pharmacologically active dose (Shah *et al.*, 1989; Al-Ashban *et al.*, 2005). The treatment was continued for a period of 3 months following W.H.O. protocol (W.H.O. 1967). The animals were observed for all external general symptoms of toxicity, body weight changes, and mortality daily up to the end of the experiment to analyze the impact of treatment. The average pre- and post-treatment body weights, vital organ weights, and viscera of the chronically treated animals were compared with the control group. The blood was analyzed for white blood cells (WBC), red blood cells (RBC), and haemoglobin using Contraves Digicell 3100H (Zurich). The blood biochemistry was performed by using Bohringer kits (Al-Ashban *et al.*, 2005).

Furthermore, the chronically treated male animals were also analyzed for spermatogenic dysfunction using the sperm abnormality test, which is considered a reliable parameter for assessing germ cell mutagenicity and carcinogenicity (Bruce and Wyrobek, 1975; Wyrobek *et al.*, 1983). The caudae epididymides and the vas deferens from the same animals were dissected out and transferred to a centrifuge tube containing 3 ml Krebs Ringer bicarbonate buffer as described earlier (Al-Ashban *et al.*, 2005). The sperm suspension was filtered through an 80 μm silk mesh to remove tissue fragments and 0.5 ml of 1% eosin Y was added to each tube. The contents were thoroughly mixed and the slides were made by placing one drop of the solution on a slide and spread by three passes of another slide. Coded slides were examined for the following abnormalities of the sperm head: amorphous, flat head, microcephali, megacephali and swollen achrosome (Bruce and Wyrobek, 1975; Wyrobek *et al.*, 1983).

Histopathological procedures

Tissue samples of liver, heart, testis, spleen, lungs, and kidney were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Using an American Optical Rotary Microtome, sections of thickness about 5 μm were cut and stained with haematoxylin and eosin. These were examined under the microscope for histopathological changes (Al-Ashban *et al.*, 2005).

Statistical analysis

The different parameters studied were subjected

to statistical analyses by the Chi-square test or student's *t*-test.

RESULTS

Effect of acute treatment

No alarming signs of toxicity except mild increase in respiration and excitation were seen in the mice treated with 3 g/kg dose of fenugreek extract. No mortality was observed up to 3 g/kg dose level during the acute toxicity test, indicating that the extract to be less toxic in the given dose levels (Al-Ashban *et al.*, 2005).

Effects of chronic treatment

During chronic toxicity studies one male mice developed fore-limb inflammation at the end of the 30-days of treatment. The same male mice also developed alopecia at the end of 40 days. Two more male mice developed inflammation in the fore-limbs and hind limbs during 40-60 days of treatment. In the survival studies (Table 1). the lethality in the treatment groups was significantly higher ($P < 0.05$) as compared to the control group.

Effects on body weight

During the chronic treatment with fenugreek extract, the body weight gain was arrested in both male and female treatment groups (Table 2). as compared to control group. The slowness of growth was evident by body weights and was significant at $P < 0.05$ and $P < 0.01$ in male and female mice, respectively.

Effect on water intake

There was a significant increase ($P < 0.05$) in water intake of animals in both male and female control groups. However, increase in water intake in the treatment groups was not significant indicating toxic effect due to weight arrest caused by the treatment (Table 3).

Effects on vital organ weights

In the present study, prolonged treatment for 90 days had minimal effect on organ indices of animals. The observed increase in weight of kidneys in the treatment groups was statistically non-significant, however, in the male treatment group there was a significant decrease ($P < 0.05$) in the weight of testes as compared to the control (Table 4).

Table 1. Quantitative data on the mortality induced in mice on chronic treatment with ethanol extract of fenugreek seeds (*T. foenum graecum*)

Treatment and dose 100 mg/kg (3 months)	N		Mortality						Total dead animals		Lethality (%)	
			0 - 30 days		31 - 60 days		61 - 90 days		M	F	M	F
	M	F	M	F	M	F	M	F				
Control	15	15	0	1	0	0	0	0	0	1	0	10
Fenugreek	15	15	1	0	2	1	2	2	5	3	33*	20

*$P < 0.05$ (Chi-square test). M = male mice, F = female mice

Table 2. Effect of chronic oral treatment with fenugreek seeds extract on body weight of mice[a]

Treatment and dose 100 mg/kg (3 months)	Pre-treatment average body weight ± S.E.		Post-treatment average body weight ± S.E.[b]	
	Male	Female	Male	Female
Control (water)	25.2 ± 1.0	24.2 ± 1.2	34.0 ± 0.9**	29.5 ± 1.5*
Fenugreek	25.5 ± 1.4	25.1 ± 1.8	26.1 ± 1.4*[c]	25.2 ± 1.5*[c]

Significant relative to pre-treatment values: *$P < 0.05$, **$P < 0.01$ (Student's *t*-test). [a]Fifteen male and fifteen female mice were used in each group. [b]The average weight was calculated based on the number of surviving animals. [c]Post-treatment average body weight of the treatment group was also compared with the control group. *$P <$ 0.001 (Student's *t*-test).

Table 3. Effect of chronic oral treatment with fenugreek seed extract on the water intake of mice.

Treatment and dose 100 mg/kg (3 months)	Pre-treatment average daily water intake ml ± S.E.		Post-treatment daily average water intake ml ± S.E.[b]	
	Male	Female	Male	Female
Control (water)	3.9 ± 0.3	3.8 ± 0.2	6.4 ± 0.2*	6.1 ± 0.4*
Fenugreek	4.1 ± 0.4	3.9 ± 0.3	5.2 ± 0.3	4.5 ± 0.5

Significant relative to pre-treatment values: *$P < 0.05$, (Student's *t*-test)

Table 4. Effect of chronic oral treatment with fenugreek seeds extract on organ weights (per 100 g body weight) of mice

Treatment and dose 100 mg/kg(3 months)	Average organs weight (per 100 g body weight).						
	Heart	Lungs	Liver	Kidney	Spleen	Testis	Seminal vesicles
Control	0.45 ± 0.02	0.69 ± 0.03	5.25 ± 0.25	1.45 ± 0.05	0.53 ± 0.07	0.67 ± 0.02	0.86 ± 0.14
Fenugreek	0.46 ± 0.03	0.80 ± 0.23	5.21 ± 0.15	1.68 ± 0.09	0.52 ± 0.11	0.55 ± 0.05*	0.80 ± 0.12

*$P < 0.05$ (Student's *t*-test). The tabular values represent the mean ± S.E.M. of five randomly selected animals.

Table 5. Effect of chronic oral treatment with fenugreek seeds extract on the haematological parameters in mice

Treatment and dose (100 mg/kg, 3months)	WBC × 10^3(N/ml)	RBC × 10^6(N/ml)	Hemoglobin(%)
Control	5.26 ± 0.65	7.85 ± 0.22	11.94 ± 0.45
Fenugreek (*T. foenum graecum*)	6.74 ± 0.77	7.69 ± 0.24	12.21 ± 0.58

$P > 0.05$ (Student's *t*-test). The tabular values represent the mean ± S.E.M. of five randomly selected animals.

Effects on hematological and biochemical parameters

All the hematological parameters of both male and female mice remained within normal range and were comparable to the control groups after chronic treatment (Table 5).

On the other hand, the biochemical studies revealed a significant decrease ($P < 0.05$) in blood sugar levels and increase in ALT/GPT levels of animals in the treatment groups as compared to the control (Table 6).

Acute treatment with 0.5 and 1.0 g/kg body weight in mice induced a significant ($P < 0.001$) fall in blood glucose levels after 6 h of treatment as compared to the control. The blood glucose lowering effect was also observed in animals after chronic treatment (Table 7).

Effects on sperm morphology

Treatment of male mice with fenugreek alcohol extract during the current study for 90 days clearly increased ($P < 0.01$) sperm morphological abnormalities as compared to the respective control group (Table 8). The indices screened for the morphological abnormalities, namely, swollen achrosomes, amorphous, microcephali, megacephali, rotated head and flat head showed significant increase in all these indices as compared to the control, indicating spermatotoxic properties of fenugreek seeds extract.

Table 6. Effect of chronic oral treatment with fenugreek seeds extract on the biochemical parameters in mice

Parmaters	Control	Fenugreek
Blood glucose (mg/dL)	80.00 ± 2.14	49.50 ± 3.91**
ALT/GPT (U/L)	21.59 ± 6.85	27.50 ± 4.98*
AST/GOT (U/L)	58.97 ± 6.49	56.77 ± 7.61
CK-MB	137.36 ± 17.50	134.86 ± 11.50
Creatinine (μmol/L)	27.58 ± 4.86	29.27 ± 3.66
Urea (μmol/L)	8.10 ± 1.99	9.39 ± 1.45

*$P < 0.05$, **$P < 0.01$, (Students *t*'-test). Five animals were used in each group. Treatment groups were compared with the control.

Table 7. Effect of fenugreek seeds extract on blood glucose levels of mice in acute and chronic regimen

Group No.	Treatment	Blood glucose level (mg/dl; Mean ± SE)		
		Day 0	6 hours	Day 90
1	Control	76.9 ± 1.71	80.0 ± 1.95	80.0 ± 2.14
2	Fenugreek / Acute 0.5 g/kg	ND	55.9 ± 4.40*	ND
3	Fenugreek / Acute 1 g/kg	ND	50.0 ± 4.90*	ND
4	Fenugreek 100 mg/kg/day (3M)	ND	ND	49.5 ± 3.91*

ND = not done; M = months. 5 animals were used in each group. Treatment groups were compared to control at respective time points. Students' *t*-test. *P < 0.001

Table 8. Effect of fenugreek seeds extract on the epididymal spermatozoa after chronic treatment in mice.

Treatment	Total sperms screened	Percent sperm head abnormalities (Mean ± S.E.)						Abnormal sperms(%)
		Swollen achrosome	Amor-phous	Micro-cephali	Mega-ceph-ali	Rotated head	Flat head	
Control	4934	0.50 ± 0.09	0.40 ± 0.09	0.06 ± 0.04	0.06 ± 0.04	0.23 ± 0.06	0.04 ± 0.02	1.31 ± 0.26
Fenugreek	5603	2.81 ± 0.88**	2.07 ± 0.16**	0.09 ± 0.03*	0.49 ± 0.09*	1.15 ± 0.23**	0.25 ± 0.08*	3.98 ± 1.70**

*P < 0.05, **P < 0.01 (Students' *t*-test).

DISCUSSION

During the current acute toxicity test, no alarming signs of toxicity were seen except mild increase in respiration and excitation in the animals treated with the highest dose of 3 g/kg. None of the mice died up to 3 g/kg dose level. Histopathological investigations proved the visceral condition and all vital organs to be normal and comparable to the control. Our results are in agreement with an earlier report documenting LD50 for fenugreek extract to be 5 g/kg dose level (Muralidhara *et al.*, 1999). There was a significant decrease in blood glucose level of mice during acute treatment. However, all hematological indices remained unchanged up to 3 g/kg dose treatment as compared to the control.

During chronic toxicity studies, two male mice developed fore-limb inflammation and alopecia, while two other male mice developed inflammation in their fore-limbs as well as hind limbs in the treatment groups as compared to the control. These findings clearly indicated the prolonged fenugreek treatment to be toxic. However, in the female treatment group there were no toxicity symptoms. In the survival studies after fenugreek

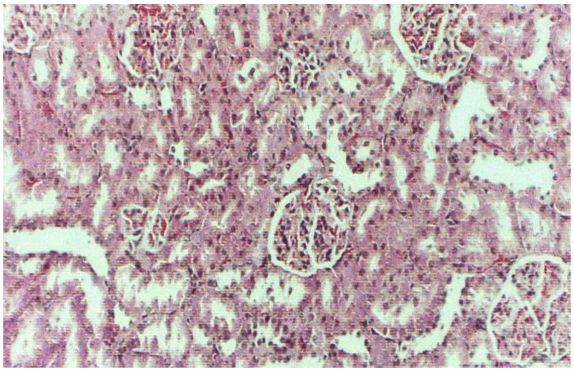

Fig. 1. Showing a control group mice kidney with normal appearance (Haemtoxylin-Eosin). Original magnification × 100.

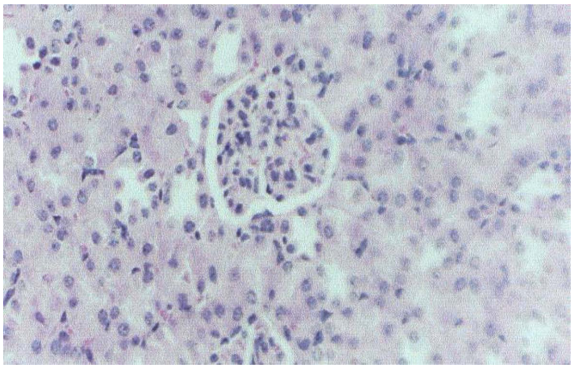

Fig. 2. Showing no change in kidney of an animal after chronic treatment with fenugreek seeds extract. (Haemtoxylin-Eosin). Original magnification × 100.

treatment for 90 days (Table 1). the lethality was significant in the last two months of treatment. In control group, one female mouse died during of 0-30 days of treatment. However, in the treatment groups, one male mouse died during 0-30 days of treatment; while two male and one female mouse died during 31-60 days of treatment. During 61-90 days of treatment, two more male and two female mice died. These results evidently verified the toxic potential of fenugreek seeds extract on prolonged treatment.

During the current chronic regimen of treatment with fenugreek extract, the slowness of growth was evident by body weights and was significant being $P < 0.05$ and $P < 0.01$ in male and female mice respectively as compared to the control. Similarly, the water intake was also affected. Our findings are not in full agreement with the observations of earlier studies (Bin-Hafeez *et al.*, 2003; Shim *et al.*, 2008). However, our results are supported by a report, where fenugreek treatment caused slowness in growth of animals (Muralidhara *et al.*, 1999).

In our present study, the treatment protocol was extended to 90 days. The weight arrest observed in the treatment groups may be attributed to some fenugreek chemical constituents, such as protodioscin (Hibasami *et al.*, 2003), galactomannan (Hannan *et al.*, 2007; Srichamroen *et al.*, 2009) which possess such properties and to the reduction of intestinal glucose up take caused by fenugreek treatment (Al-Habori *et al.*, 2001, Srichamroen *et al.*, 2009). In addition, fenugreek is known to posses hypocholesterolemic and hypotriglyceridmeic properties (Valette *et al.*, 1984; Ribes *et al.*, 1987; Al-Habori and Raman, 1998; Narender *et al.*, 2006) which might also contribute to weight arrest.

Our findings are further supported by an earlier report where both oral and parenteral administration of fenugreek crude seed saponins for 21 days, lead to depress body weight in Hisex-type chicks (Nakhla *et al.*, 1991). It is worth mentioning that dietary fiber fraction of fenugreek seeds was found to inhibit carbohydrate digestion and absorption (Beutler and DerMarderosian, 2002; Vats *et al.*, 2003; Hannan *et al.*, 2007) resulting in growth arrest. Our results add support to all earlier reports where slowness of growth was recorded in animals treated with fenugreek.

In the present study, prolonged treatment of animals caused a significant reduction ($P < 0.05$) in weight of testes and some increase in the weight of kidneys that was statistically insignificant ($P > 0.05$). All other vital organs showed no significant changes as compared to the control. In contrast, in an earlier study, fenugreek treatment caused only reduction in weights of some vital organs and the authors ascribed no specific reason (Muralidhara *et al.*, 1999). In another study fenugreek treatment induced no changes in the weights of vital organs (Bin-Hafeez *et al.*, 2003). Similarly, fenugreek treatment for 30 days was also found to induce no significant decrease in the absolute weights of liver and kidneys (Kar and Tahiliani, 2003). However, treatment with fenugreek crude saponins, caused epithelial degeneration of renal tubules (Nakhla *et al.*, 1991).

The mild increase observed in weight of the kidneys in current study, could possibly be attributed to the prolonged treatment protocol and to a probable accumulation of some of fenugreek extract constituents. Since the literature review, did not provide any such reference for comparison, the possibility of kidney weight increase by fenugreek treatment, could not be excluded (Ahsan *et al.*, 1989; Rohini *et al.*, 2009). To rule out the controversy, histopathological studies were conducted (Microphotograph 1, Microphotograph 2) which confirmed all the vital organs of the animals including kidneys, to be normal and comparable to the vital organs of mice in the control groups. Biochemical studies also revealed no significant differences in creatinine and urea levels of animals in the treatment groups as compared to the control. Thus confirming fenugreek treatment to be devoid of any toxic

effects on kidneys of mice after chronic treatment.

After chronic treatment with fenugreek, all the hematological parameters remained within normal range and were comparable to the control groups. Our results are in agreement with the earlier findings (Muralidhara *et al*., 1999), which described hematological constants like WBC, RBC and hemoglobin to remain in normal range after prolonged treatment with de-bitterized fenugreek powder in rats, and proved that fenugreek chronic treatment was devoid of hematological toxicity.

In the present study, the biochemical parameters revealed a significant decrease in blood sugar levels of mice in the treatment groups as compared to the control. Our findings are in agreement with the earlier reports in the scientific literature; showing significant fall in blood glucose levels both in the normal as well as diabetic animals when treated with fenugreek seeds extract (Ghafghazi *et al*., 1977; Khosla *et al*., 1995; Abdel-Barry *et al*., 1997; Ajabnoor and Tilmisany, 1998; Al-Habori and Raman, 1998). The hypoglycemic effect observed during the current toxicity studies on fenugreek seeds and the results of the earlier investigations add support to the traditional claim and medicinal use of fenugreek seeds by diabetic patient in different countries (Haddad *et al*., 2001; Beutler and Der Marderosian, 2002; Otoom *et al*., 2006).

Fenugreek seeds were reported to exhibit antidiabetic effect by changing of glucose and lipid metabolizing enzyme activities (Raju *et al*., 2001; Beutler and Der Marderosian, 2002; Hannan *et al*., 2007). However, the soluble dietary fiber fraction of fenugreek seeds was shown to exert antidiabetic effects mediated through inhibition of carbohydrate digestion and absorption, and enhancement of peripheral insulin action (Ali *et al*., 1995). Another report suggested that the hypoglycemic effect of fenugreek chemical constituents might be mediated through stimulation of insulin synthesis and/or secretion from the cells of Langerhans (Puri *et al*., 2002). The hypoglycemic effect of fenugreek treatment was slow and sustained, and without any risk of developing severe hypoglycemia (Ali *et al*., 1995; Ajabnoor and Tilmisany, 1998). Data from the literature further suggested that fenugreek treatment has been used to ameliorate 1-thyroxine-induced hyperglycemia in rats (Tahiliani and Kar, 2003).

The results of our current study further added support to the claimed hypoglycemic potential of fenugreek treatment (Beutler and DerMarderosian, 2002; Hannan *et al*., 2007; Srichamroen *et al*., 2009) which might be mediated through the inhibition of intestinal glucose uptake by galactomannan (Srichamroen *et al*., 2009); delaying the carbohydrate metabolism (Vats *et al*., 2003; Hannan *et al*., 2007). Furthermore, other contributing factor might the chemical constituents found in the defatted seed fractions (Ribes *et al*., 1987); alkaloid rich fraction (Shani *et al*., 1974; Puri *et al*., 2002), and compounds such as diosgenin (Pasich *et al*., 1983; Farnsworth and Marles, 1995), trigonelline (Mishkinsky *et al*., 1967), coumarin (Khurana *et al*., 1982), nicotinic acid (Duke, 2001), and 4-hydroxyisoleucine (Narender *et al*., 2006; Haeri *et al*., 2009) isolated from fenugreek seeds extract.

Fenugreek seeds were earlier reported to possess antioxidant activity (Dixit *et al*., 2005, 2008) and the hepatoprotective effect of fenugreek in chronic alcoholism, was attributed to polyphenols isolated from its seeds (Kaviarasan *et al*., 2008). However, during current study, there was a significant increase ($P < 0.05$) in ALT/GPT levels of animals in the treatment groups, which indicated an adverse effect on liver by prolonged use of fenugreek. Some of the fenugreek chemical constituents possessing anti-inflammatory (Vyas *et al*., 2008; Malviya *et al*., 2010), anticancer and antineoplastic effects (Sur *et al*., 2001; Raju *et al*., 2004; Amin *et al*., 2005; Shabbeer *et al*., 2009) may be held responsible for such activity. However, histopathological investigations revealed that the liver of animals in the treatment groups was normal and comparable to the control.

During current study, fenugreek chronic treatment significantly decreased ($P < 0.05$) weight of testes in the male treatment group and significantly increased ($P < 0.01$) the sperm morphological abnormalities as compared to the control. In the present study, on average, the percentage of abnormal sperms increase, in the treatment group, was up to 3.98 (± 1.70) percent; while in the control male mice percent abnormal sperm were 1.31 (± 0.26)%. Noxious stimuli like chemicals and radiation are known to induce detrimental genotypic changes in the spermatogenic cells (Bruce and Wyrobek, 1974; Shah *et al.*, 1989). The mouse sperm morphology assay, has been used to characterize mutagenic properties of a wide variety of chemicals (Bruce and Wyrobek, 1974; Wyrobek *et al.*, 1983). Special Foodstuff formulated for people with diabetes, containing fenugreek extract with 40% 4-hydroxyisoleucine, was earlier found to possess no genotoxic properties (Muralidhara *et al.*, 1999; Flamming *et al.*, 2004; Dixit *et al.*, 2008). However, the damage induced in the sperm morphology during the current study, reflected genetic damage in the male germ cells, either due to small deletions or point mutations (Bruce and Wyrobek, 1974; Wyrobek *et al.*, 1983), or protein abnormality (Brinkworth *et al.*, 1987; Flamming *et al.*, 2004).

Several anticancer compounds were earlier reported to be germ cell toxicant/mutagen (Bruce and Wyrobek, 1974; Wyrobek *et al.*, 1983). The spermatotoxic properties of fenugreek observed in the present study may be attributed to some of its chemical constituents possessing antineoplastic (Sur *et al.*, 2001), anti-leukemic (Hibasami *et al.*, 2003), antifertility (Kassem *et al.*, 2006), and anticancer potential (Raju *et al.*, 2004; Amin *et al.*, 2005; Shabbeer *et al.*, 2009).

Based on the results of present study, it is suggested that special caution must be taken when fenugreek seeds and/or their extracts are added to special foodstuff formulated for people with diabetes. Furthermore, the results of the current study provide basic information about the toxicity of fenugreek seeds that might be helpful in planning future pre-clinical experiments on this potent natural drug.

It is also worth mentioning that in our earlier experiments on herbal drugs with antidiabetic properties, for example: *Artemisia abyssinica* (Qureshi *et al.*, 1990), *Teucrium polium* (Al-Ashban *et al.*, 2005), and now *Trigonella foenum-graecum*, all were found to possess spermatotoxic activity and further studies are warranted on this issue.

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