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Antidiabetic Effects of Fenugreek Alkaliod Extract in Streptozotocin Induced Hyperglycemic Rats

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Abstract: *Background:* This study was undertaken to investigate the effect of alkaloid extract of fenugreek dried seeds (*Trigonella foenum-graecum* L.) on blood glucose, serum insulin, serum lipid profile and lipid peroxidation in addition to histoliogical and histochemical study of liver and kidney in streptozotocin induced diabetic albino rats. *Methods:* Alkaloid extract of fenugreek dried seeds was tested in streptozotocin induced hyperglycemic rats. It was administered orally (dose chosen according to LD50) for 21 days. Its effect on blood glucose, serum insulin, lipids (total cholesterol, triglycerides, HDL and LDL) and lipid peroxides (thiobarbituric acid reactive substances (TBARS) and nitric oxide) were studied in diabetic rats. In addition to histoliogical and histochemical study of their liver and kidney. *Result:* Treatment with alkaloid extract of fenugreek dried seeds, resulted in a significant reduction of blood glucose and increase in serum insulin. The herbal preparation also resulted in a significant decrease in serum lipids and lipid peroxide formation and helps to recover the pathological effects of diabetes on liver and kidney of streptozotocin induced diabetic rats. *Conclusion:* We suggest here that the mode of action of fenugreek may be caused by their contents of alkaloids through reducing the increased blood glucose level, thereby preventing hyperglycemia during diabetes and reducing lipid profile to almost normal and suppressing the oxidative stress together with converting liver and kidney pathology caused by diabetes to normal pattern.

Key wards: fenugreek alkaloid- diabetes- hyperlipedemia-experimental animal.

INTRODUCTION

Despite the great efforts that have been made in the understanding and management of diabetes, the disease and disease related complications are increasing unabated[1]. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease[2].

Many traditional treatments have been recommended in the complementary and alternative system of medicine for treatment of diabetes mellitus[3]. Diabetes mellitus is syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired metabolism of glucose and other energy-yielding fuels such as lipids and protein[4].

The mechanism of most of the herbals used to treat diabetes has not been defined[5]. It has been attributed that the antihyperglycemic effect of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels[6,7].

Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects. But little is known on the specific modes of action of these plant drugs or herbal formulation used for treating diabetes[8]. Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicine are important[9]. Plant drugs[10] and herbal formulation[11] are frequently considered to be less toxic and more free from side effects than synthetic one.

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Experimental diabetes in animals has provided considerable insight into the physiologic and biochemical derangement of the diabetic state. Many of this derangement were in the form of significant changes in lipid metabolism and structure[12]. These structural changes are clearly oxidative in nature and are associated with development of vascular disease[13].

In diabetic rats, increased lipid peroxidation was also associated with hyperlipidemia[14]. During diabetes, a profound alteration in the concentration and composition of lipids occurs. Liver and kidney are important for glucose and lipid homeostasis, they participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. Thus it is expected to have changes in liver and kidney during diabetes[15].

This study was done on streptozotocin induced diabetic rats to evaluate the role of fenugreek alkaloid extract in being an essential cause for the antidiabetic and antihyperlipidemic effects of fenugreek seeds.

MATERIALS AND METHODS

Plant Material and Extraction: The Alkaloid of fenugreek was prepared as follows: Fenugreek was purchased from the local traders, 100g of oven-dried (45 °C) seeds of plant sample was macerated with 70% methanol for 5 days. The filtrate was dried, concentrated to dryness in vacuum and weighed. The concentration of extracted alkaloids was 2.74% isolated according to Harborne[16].

Chemicals Used: Streptozotocin STZ (Sigma,USA), Ethanol,Chloroform,Ether (BDH,England).

Animal Experiments:

Experimental Design: Male albino rats weighing 150-200 g were used for this study. Rats were caged under controlled temperature 20-24°C and 12 h light/dark cycle.they were fed with standard laboratory chow and water ad libitum.

For induction of diabetes, rats were kept on fasting prior to streptozotocin injection.On the day of administration,STZ was freshly dissolved in 50mM sodium citrate (pH 4.5) solution containing 150 mM NaCl and subcutaneous injection was given at the dosage of (60mg/kg b.w.). Blood glucose concentration was checked by the glucose oxidase method[17]. After 3 days of STZ injection. The animals with glucose concentration exceeding 200mg /dl were considered diabetic.

Rats were divided into 3 groups 8 rats in each group.

Group I: normal control rats
Group II: diabetic control rats
Group III: diabetic rats received fenugreek alkaloids (50mg/kg b.w orally).

The dose was chosen according to its LD50.

Samples Collection: Blood was collected retro-orbitally from the inner canthus of the eye under ether anesthesia using capillary tubes[18]. After 21 days of the experiment, blood samples were collected in cleaned and fresh vials containing sodium fluoride, serum and plasma were separated in centrifuge at 3000 rpm for 5 minutes.

Biochemical Analysis: Serum blood glucose levels were estimated by glucose oxidase method[17]. Serum insulin levels were determined by Biosource –INS-EASIA according to Temple *et al*.[19]. Serum Triglycerides, Cholesterol,HDL,LDL levels were measured according to Allian *et al*.[20] and Friedewald *et al*.[21].

Also serum Malondialdehyde (MDA), an end product of unsaturated fatty acid peroxidation, which can react with thiobarbituric acid (TBA) to form coloured complex thiobarbituric acid reactive substances (TBARS) was measured. Lipid peroxidaton (LPO) was measured by the method of Yagi *et al*.[22] and expressed as µmol of MDA conjugate formed. Nitric oxide levels were assayed by the method of Griess reaction according to Corats and wakeid[23].

Statistical Analysis: The data for various biochemical parameters were expressed as mean ± SD and compared using t-test. Values were considered statistically significant when $p < 0.05$. Statistics were done using SPSS for windows version 10.

The Histological Study: After blood sampling for the biochemical analysis, the animals were sacrificed, quickly dissected and small slices of liver and kidney were taken and fixed in 10% formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 6 µm in thickness were prepared and stained with Haematoxylin and Eosin[24] then examined under microscopy.

The Histochemical Studies: Periodic acid-Schiff method was applied for visualization of the polysaccharide material[25].

RESULTS AND DISCUSSIONS

Results: The effects of fenugreek extract on fasting blood glucose and serum insulin is presented in table 1. Mean fasting blood glucose in the diabetic untreated group (control positive) was 280±8.33 mg/dl after 21 days of induction of diabetes. In the normal health group this value was 76±2.59 mg/dl. In comparison with the positive control group, the group which consumed fenugreek extract showed significantly lower mean fasting blood glucose 141.83±9.04 mg/dl (P<0.05).

Table 1: Effect of fenugreek alkaloid extract on fasting blood glucose, serum insulin levels in different groups studied of experimental animals 21 days after induction of diabetes.

Groups	Fasting blood glucose (mg/dl)	Serum insulin (µU/ml)
Normal	76 ±2.59	10.53±0.66
Diabetic control	280±8.33*	4.17±0.17*
Diabetic + fenu greek extract	141.83±9.04*	7.27±0.6**

* p<0.05 between normal and diabetic control and between diabetic control and diabetic with fenugreek alkaloid extract

Similarly, mean values of serum insulin in the control positive group was 4.17±0.17 µU/ml. In normal health group it was 10.53±0.66 µU/ml. In comparison with the positive control group, the group which consumed fenugreek extract showed significantly higher mean serum insulin 7.27±0.6 µU/ml (P<0.05).

Table 2 presented the effects of fenugreek alkaloid extract on lipid profile; mean serum total cholesterol in normal heath group was 98.5±2.1 mg/dl. It was significantly elevated in the positive control group 140.33±3.2 mg/dl compared to normal group (P<0.05).Consumption of fenugreek extract significantly lowered serum total cholesterol to 107.83±2.2 mg/dl (P<0.05).

The same effect was noticed with triglycerides, consumption of fenugreek extract significantly reduced the elevated mean serum triglycerides from 154.33±6.7 mg/dl to 111.83±3.3 mg/dl (P<0.05) in the positive control group compared to 97.5±2.5 mg/dl (P<0.05) in normal health group.

Effect of induction of diabetes resulted in reduction of HDL to 35.7±1.38 mg/dl compared to 42.1±0.87 mg/dl in normal health group but this difference was insignificant. While, on LDL the induction of diabetes resulted in significant increase of its value to 54.0±2.7 mg/dl compared to normal health group 35.5±0.9 mg/dl (P<0.05). Consumption of fenugreek extract significantly elevated LDL level to 47.16±2.1 mg/dl (P<0.05) and reduced LDH to 44.5±1.9 mg/dl compared to positive control group.

Diabetes is known to disturb the oxidative balance of the body leading to oxidative stress. This was confirmed in our study, the mean levels of oxidizing parameters studied (TBARS and Nitric oxide) were significantly elevated in the positive control group (0.506±0.07 µmol/L and 45.00±7.07 µmol/L respectively) compared to normal health group (0.296±0.05 µmol/L and 9.00±5.22 µmol/L respectively) with (P<0.05) as shown in table 3. Consumption of fenugreek extract significantly reduced TBARS to 0.25±0.06 µmol/L (P<0.05) and Nitric oxide to 40.5±7.14 µmol/L compared to positive control group.

Histological Results:

Liver: The liver of control rats appeared to be divided into the classical hepatic lobules; each was formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The cell cords were separated by narrow blood sinusoids (Fig. 1-A).

The histopathological examination of liver of diabetic rats showed periportal necrosis of the hepatocytes near the portal areas, dilated and congested portal vessels as well as areas of inflammatory cell infiltration (Fig. 1-B).

Examination of liver tissue of diabetic rats treated with fenugreek alkaloid indicated that the hepatic lobules appeared more or less like control (Fig. 1-C).

Kidney: Examination of the kidney of the control rats revealed normal glomeruli with thin glomerular basement membranes, normal cellularity and patent capsular space surrounding proximal and distal tubules. (Fig. 2-A).

Light microscopy of the kidney sections of diabetic rats showed an increase in the mesangial cell and matrix of the glomeruli and hyalinization of the arterioles (Fig. 2-B).

Examination of kidneys of the diabetic rats that treated with fenugreek alkaloid indicated that kidneys appeared more or less as control (Fig. 2-C).

Histochemical results:

Liver: Examination of liver sections of control rats stained with periodic acid Schiff's (PAS) technique showed the abundance of glycogen in the form of purple granules and particles at one side of the cytoplasm leaving the other one almost devoid of such material in the hepatocytes. The nuclei of the hepatocytes gave negative PAS reaction indicating the absence of glycogen. The hepatocytes at the peripheral

Table 2: Effect of fenugreek alkaloid extract on serum cholesterol, triglycerides, HDL and LDL levels in different groups studied of experimental animals 21 days after induction of diabetes

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal	98.5±2.1	97.5±2.5	42.1± 0.87	35.5± 0.9
Diabetic control	140.33±3.2*	154.33±6.7*	35.7±1.38	54.0±2.7*
Diabetic + fenugreek extract	107.83±2.2*	111.83±3.3*	47.16±2.1*	44.5±1.9

* p<0.05 between normal and diabetic control and between diabetic control and diabetic with fenugreek

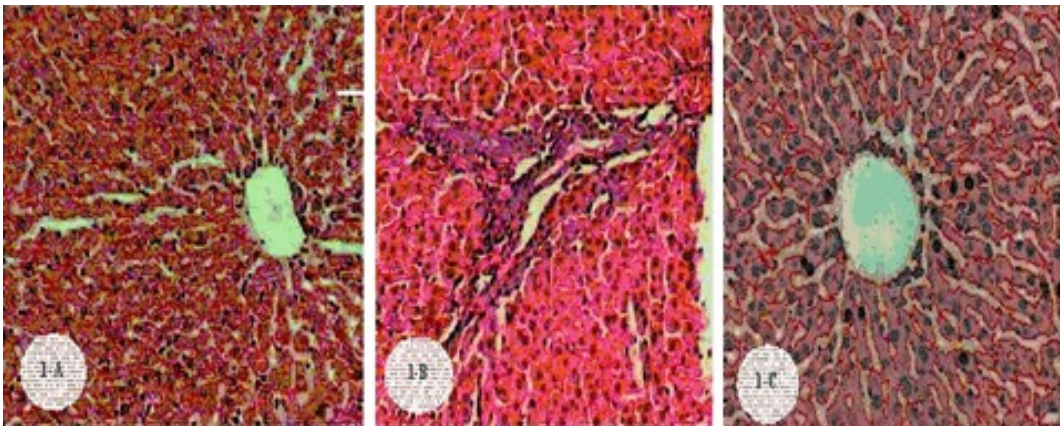

Fig. 1: Photomicrographs of liver show A): Liver that exhibits the normal structure in control rat, B): Liver of diabetic rats show a portal tract with dilated and congested vein. Notice, the periportal necrosis of the hepatocytes that surrounded the portal area that associated with inflammatory infiltration, and C): diabetic rats treated with alkaloid of fenugreek show the architecture of the hepatic lobule that appears more or less like control. (H & E X 150)

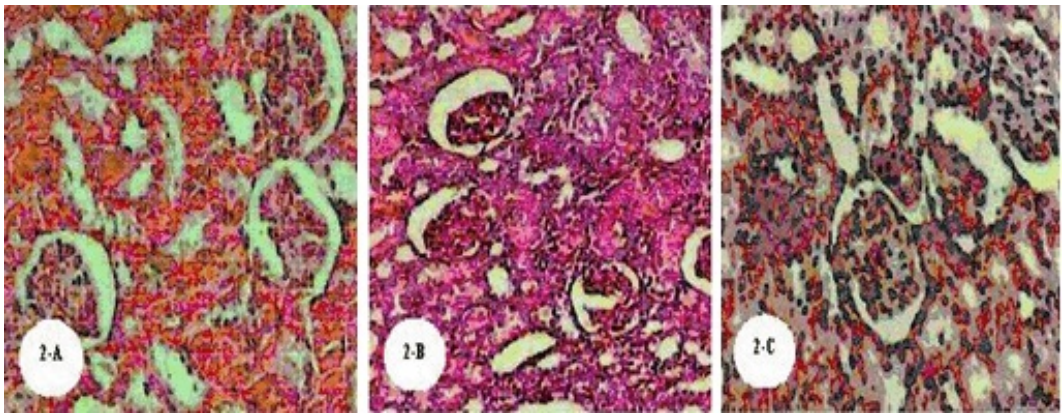

Fig. 2: Photomicrographs of kidney show A): kidney of the control rats revealed normal structure of the glomeruli and proximal and distal convoluted tubules, B): kidney of diabetic rats show an increase in the mesangial cell and matrix of the glomeruli and hyalinization of the arterioles, and C): kidneys of the diabetic rats that treated with alkaloid of fenugreek indicated the structure appear more or less as control. (H & E X 150)

regions appeared markedly rich with glycogen particles than pericentral ones (Fig. 3-A).

The histochemical examination of liver of diabetic rats showed pericentral depletion of the PAS +ve materials (Fig. 3-B).

In the liver of diabetic rats treated with fenugreek alkaloid, the polysaccharides appeared more or less like control with the exception of some cells (Fig. 3- C)

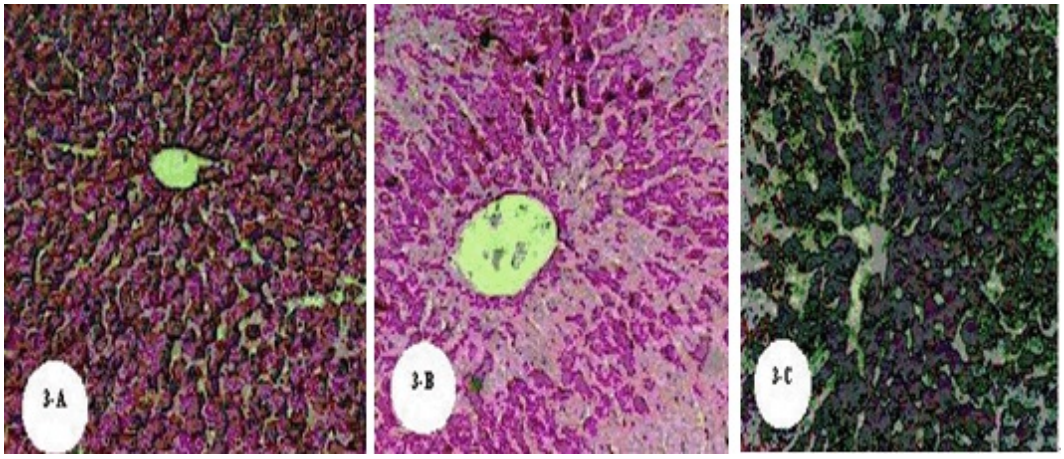

Fig. 3: Photographs of sections of the liver show the polysaccharides A): control rat showing the normal distribution, the glycogen particles accumulated at one side of the cytoplasm of hepatocytes leaving the other side almost devoid of such material. B): liver of diabetic rats showed pericentral depletion of the PAS +ve materials, C): liver of diabetic rats treated with alkaloid of fenugreek shows the polysaccharides appear more or less like control with the exception of some cells (PAS X 150).

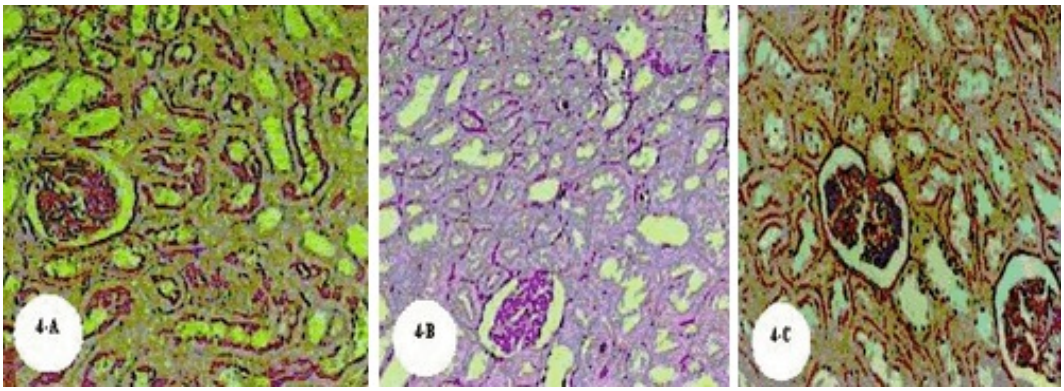

Fig. 4: Photographs of a section of the kidney show the polysaccharides A): Kidney of control rats showed the presence of polysaccharides in the form of PAS positive materials in the parietal and visceral walls of the Bowman's capsule, capillaries of the glomeruli, the basement membrane of the proximal and distal convoluted tubules and the brush border of the proximal convoluted tubules, B) kidney of diabetic rats show an increase in the PAS +ve material in the mesangial cell and matrix of the glomeruli. The basement membranes of the proximal and distal convoluted tubules appear thicker, and C): kidneys of the diabetic rats that treated with alkaloid of fenugreek show the polysaccharides that appear more or less as control (PAS X150).

Kidney: Kidneys of control rats showed the presence of polysaccharides in the form of PAS positive materials in the parietal and visceral walls of the Bowman's capsule, capillaries of the glomeruli, basement membrane of the proximal and distal convoluted tubules and the brush border of the proximal convoluted tubules (Fig. 4-A).

Light microscopy of the kidney sections of diabetic rats showed an increase in the PAS +ve material in the mesangial cell and matrix of the glomeruli. The basement membranes of the proximal and distal convoluted tubules appear thicker as compared with the control one (Fig. 4-B). Examination of kidneys of the diabetic rats treated with fenugreek alkaloid indicated that the polysaccharides of kidneys appeared more or less as control (Fig. 4-C).

Discussion: Fenugreek (Trigonella Foenum-graecum) is one of the oldest herbs known originating in the

Table 3: Effect of fenugreek alkaloid extract on TBARS and Nitric oxide levels in different groups studied of experimental animals 21 days after induction of diabetes

Groups	TBARS (μmol/L)	Nitric oxide (μmol/L)
Normal	0.296±0.05	9.00±5.22
Diabetic control	0.506±0.07*	45.00±7.07*
Diabetic + fenu greek extract	0.25±0.06*	40.5±7.14

* p<0.05 between normal and diabetic control and between diabetic control and diabetic with fenugreek

Mediterranean region and Asia[26]. Its seeds were highly praised for their beneficial uses in ancient Egypt and India and later among the Greeks and Romans[27]. As Fenugreek spread around the Mediterranean, ancient physicians learned that its seeds being esteemed as a remedy for a wide variety of conditions[28].Externally, the seeds may be applied as a paste to treat abscesses, boils, ulcers and burns, or used as a douche for excessive vaginal discharge. Internally, the nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia. They are also helpful in lowering fever as it is equal to quinine. The seeds' soothing effect makes them of value in treating gastritis and gastric ulcers. They are used to induce childbirth and to increase breast-milk production. Fenugreek is also thought to be antidiabetic and to lower blood cholesterol levels[29].

Seeds of fenugreek have been shown to have multiple benefits in patients with diabetes such as reduction of blood sugar and its complications[30,31]. Many earlier studies[32,33,34]. whether using the whole seeds [32] or extracts[33] showed that fenugreek seeds decreased fasting blood sugar levels in animals. The later studies[35,36] confirmed the hypoglycemic effects of fenugreek seeds on type 1 and type 2 diabetics but the mechanism of action is not fully understood.

At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycaemia by the use of biguanides, thiazolidinediones, sulphonylureas, D-phenylalanine derivatives, meglitinides and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes[37,38]. Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. However, only a few have been subjected to detailed scientific investigation due to a lack of mechanism-based available *in vitro* assays[39]. Fenugreek (*Trigonella foenum-graecum* L., Leguminosae), as mentioned before is one of the oldest medicinal plants, its aqueous extracts of seeds and leaves of fenugreek have been shown to possess hypoglycaemic activity and are nontoxic[40].

Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans); 20-30% proteins high in lysine and tryptophan; 5-10% fixed oils (lipids); pyridine-type alkaloids, mainly trigonelline (0.2-0.36%), choline (0.5%), gentianine and carpaine; the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin; free amino acids, such as 4-hydroxyisoleucine (0.09%); arginine, histidine and lysine; calcium and iron; saponins (0.6-1.7%); glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin); cholesterol and sitosterol; vitamins A, B_1,C and nicotinic acid; coumarin compounds and 0.015% volatile oils (n-alkanes and sesquiterpenes)[41].

Our results showed that oral administration of fenugreek alkaloid for 21 days effectively controlled hyperglycemia. Maintenance of normoglycemia, normalization of serum lipid profile and suppression of oxidative stress, all these prevents the onset of microvascular complications and also delays progression of complications in diabetes. The fenugreek alkaloids maintain the blood glucose to normoglycemia during diabetes, which acts as an essential trigger for both liver and kidney to revert to their normal metabolic homeostasis. The liver and kidney exhibits numerous morphological and functional alterations during diabetes[12]. In the present study, the histological and histochemical examination of liver of diabetic rats showed periportal necrosis of hepatocytes near the portal areas with dilated and congested portal vessels as well as areas of inflammatory cell infiltration and pericentral glycogen depletion. Light microscopy of kidney sections of diabetic rats showed an increase in mesangial cell and matrix of glomeruli with increase in glycogen deposition and hyalinization of arterioles with thickened basement membranes of proximal and distal convoluted tubules.

During diabetes liver shows decrease in weight due to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis, which is the outcome of lack of insulin and/or cellular glucose in liver cells. There is, however, an increase in kidney weight due to glucose over-utilization and subsequent enhancement in glycogen synthesis, lipogenesis and protein synthesis[42]. These changes may lead to serious microvascular renal complications, which involve a series of metabolic changes in the pathogenesis of diabetic nephropathy[43]. Our results indicated that treatment of diabetic rats with fenugreek alkaloids significantly prevented the alteration in liver and kidney weight and pathology with the return to their normal texture in agreement with previous studies[43,44].

It is well established that in severe diabetes a catabolic response develops in tissues, such as the liver, muscle and adipose tissue, with the prevalence of catabolic over anabolic processes. However, in other tissues, such as the kidneys, the reverse may be true. The diabetic kidney is characterized by some metabolic alterations that entail enhanced protein synthesis (PS). Accordingly, in the kidneys of some animals with experimental diabetes, the PS has been reported to be significantly elevated[45]. These data are consistent with the in vivo evidence of the increased synthesis of the glomerular basement membrane in experimental diabetes[46] and with the hypothesis of an "overutilization of glucose" by the kidneys – a very well known phenomenon that may favor diabetic microangiopathy [47]. On the other hand, some observations suggest that the most important change in the metabolism of the basement membrane of kidney is its reduced degradation (and not its increased synthesis)[46]. The behavior of renal PS contrasts with the severe reduction in PS that is known to occur with diabetes mellitus in other insulin-sensitive tissues, such as the liver, muscle and adipose tissue[45]. It is not known with certainty which factors may cause the increase in PS that occurs in the diabetic kidney. Both hormonal (insulin, growth hormone, sex hormones, growth factors and others) and metabolic factors (hyperglycemia, plasma fatty acids, amino acids and others) may be involved. Among the metabolic factors, hyperglycemia has attracted attention as a factor that may play an important role[47].

Stimulation of kidney PS may contribute to explain the increase in the synthesis of glycoproteins (and therefore of the basement membrane) as well as the renal hypertrophy that occurs early in diabetes[46].

In diabetes, reactive oxygen species (ROS), several immunomodulatory factors and chronic inflammatory states can contribute to insulin resistance and liver injury. Recently, several reports have at least partially elucidated the cellular and molecular mechanisms underlying this inflammatory response[48]. Nuclear factor-κB (NF-κB) mediates induction of proinflammatory cytokines implicated in insulin resistance such as interleukin-1, interleukin-6 and TNF-α and IKK-β. These findings indicate that NF-κB-dependent inflammatory mediators produced in hepatocytes, such as TNF-α, are most likely to act in a paracrine manner to down modulate insulin sensitivity in the liver and to favor liver injury[49]. This explains the presence of inflammatory reaction reported in liver and kidney of diabetic rats.

The plasma lipid level is usually raised during diabetes and presents a risk factor for the coronary heart disease[50]. Lowering the plasma lipid levels through dietary or drug therapy appears to be associated with a decrease in the risk of vascular disease[51]. We have shown here an increase in the plasma total lipids, triglycerides and total cholesterol in streptozotocin diabetic rats. This increase may be a result of increased breakdown of lipids and mobilization of free fatty acids from the peripheral depots. Since insulin inhibits the hormone-sensitive lipases, the latter becomes active in the absence of insulin. Other hormones such as glucagon and catacholamines, known to increase during diabetes, compound the effect by stimulating lipolysis[52]. The lipid profile in liver and kidney tissues also showed an increase in level - a research item, which was not included in our present study. The increase in kidney lipid level during diabetes appears to be due to increased glucose flux and reducing equivalents leading to enhanced over all biosynthetic pathways. However, the increase in hepatic lipid level is not due to *de novo* synthesis and may be due to increased uptake from the portal system as shown earlier[53]. The ability of fenugreek alkaloids treatment to reduce blood serum lipids including total lipids, triglycerides and total cholesterol have been reported in earlier studies[54,44]. We report here the marked prevention in the alteration of lipid profile by a treatment of fenugreek alkaloids to diabetic animals after 21 days of diabetes induction. There could be two possibilities for the prevention of alteration of lipid profile. Firstly, that the rate of lipogenesis is normalized by fenugreek alkaloids in a way similar to the effect of insulin on the lipid metabolism. Secondly, it could be due to achievement of normoglycemia where there was no further degradation of already accumulated lipid for otherwise glucose starved cells. Fenugreek alkaloids have also been shown to stimulate the hepatic lipogenic enzymes[43]. During diabetes, lipogenesis is decreased while lipolysis is increased in the hepatic tissue[56], which is the outcome of underutilization of glucose resulting in increased lipolysis and stimulation in the activities of gluconeogenic enzymes[53,43]. In kidney, an over-utilization of cellular glucose occurs through elevated activities of glycolytic and NADP-linked lipogenic enzymes[56, 43].

Though, extensive work has been undertaken to work out the mechanism by which fenugreek could be exerting its effects, is still not very clear. However, plausible hypothesis that may be involved in the therapeutic action of fenugreek can be considered here. Fenugreek may exert its therapeutic effect through its alkaloids content by modulation of insulin secretion. Madar and Thorne[57] attributed it to dietary fibers

present in the fenugreek seeds, which help in the management of metabolic abnormalities associated with diabetes such as peripheral insulin resistance and lipid abnormalities. Petite *et al.*[58] and Yoshikawa *et al.*[59] reported the isolation of furostanol saponins called trigoneoside Ia, Ib, IIa, IIb, IIIa, IIIb; glycoside and trifoenoside A. They claimed that these saponins are the active principles owing to their hypoglycemic effects. It has also been demonstrated in some studies that *Trigonella* seed delayed gastric emptying and caused inhibition of glucose transport as the seed contain around 50% pectin that forms a colloid suspension when hydrated and can decrease rate of gastric emptying and slow carbohydrate absorption[60]. Sauvaire *et al.,*[54] and Broca *et al.,*[61] have demonstrated evidences of insulinotropic and antidiabetic properties of 4-hydroxyisoleucine isolated from fenugreek seeds in glucose-dependent manner. They suggested that antidiabetic effect of 4-hydroxyisoleucine was, at least in part, from direct pancreatic beta cell stimulation.

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is potent inhibitor of lipolysis. Since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids[48]. During diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids in to the circulation. Increased fatty acids concentration also increases the β-oxidation of fatty acids, producing more acetyl CoA and cholesterol during diabetes[62]. Hyperglycemia was reported to generate reactive oxygen species and to attenuate antioxidant mechanisms, creating a state of oxidative stress and endothelial injury[63]. Mesangial cells, cultured in high-glucose conditions, had significantly reduced levels of glutathione compared with those grown in normal glucose conditions, which was accompanied by reduced gene expression of the rate-limiting enzyme involved in de novo synthesis of glutathione, elevated levels of intracellular malondialdehyd and increased mRNA of extracellular matrix proteins, fibronectin and collagen IV[64]. Addition of antioxidants to high glucose levels caused a significant reversal of fibronectin and collagen IV gene expression, suggesting an etiologic link between oxidative stress and increased extracellular membrane protein synthesis[65]. Thus, oxidative stress could be one of the important mediators of vascular complications in diabetes, including nephropathy. Some other data provide evidence that reactive oxygen species, generated by glucose metabolism, may act as integral signaling molecules under high glucose levels as in other membrane receptor signaling[62]. The production of nitric oxide (NO), a catabolic product of L-arginine, in kidney cortical slices was evaluated in male Sprague-Dawley rats in the presence of normal or high glucose levels by Silvia *et al.,* [47]. Their results suggest that increased NO synthetase activity, rather than altered substrate availability, may be the principal factor underlying increased NO synthesis in diabetic kidneys.

Our results show increased lipid peroxidation (TBARS and nitric oxide) in serum of diabetic control group. Previous studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats[66]. This may be because the tissues contain relatively high concentration of easily peroxidizable fatty acids. Liver during diabetes, showed a relatively severe impairment in antioxidant capacity than kidney. The kidney exhibits a characteristic pattern of changes during diabetes[67]. The increase in oxygen free radicals in diabetes could be primarily due to increase in blood glucose levels, which upon autoxidation generate free radicals and secondarily due to the effects of the diabetogenic agent (streptozotocin or alloxan)[68]. In diabetes, hypoinsulinaemia increases the activity of the enzyme, fatty acyl coenzyme, coenzyme A oxidase, which initiates β-oxidation of fatty acids resulting in lipid peroxidation. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzymes. Lipid peroxidation products (lipid radicals and lipid peroxide) are harmful to the body cells and are associated with atherosclerosis and brain damage[69]. In our study administration of fenugreek alkaloids resulted in significant antioxidant activity. Previous studies done on germinated fenugreek reported the antioxidant activity of fenugreek seeds but they ought this activity partly to the presence of flavonoids and polyphenols[65,70].

In Conclusion: We suggest here that the mode of action of fenugreek may be caused by their contents of alkaloids through reducing the increased blood glucose level, thereby preventing hyperglycemia during diabetes and reducing lipid profile to almost normal and suppressing the oxidative stress together with converting liver and kidney pathology caused by diabetes to normal pattern.

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