Beneficial effects of *Annona squamosa* extract in streptozotocin-induced diabetic rats

Kaleem M, Medha P, Ahmed Q U, Asif M, Bano B

ABSTRACT

Introduction: The present study investigated the possible therapeutic effects of *Annona squamosa* (*A. squamosa*) extract on certain biochemical markers in streptozotocin (STZ)-induced diabetes mellitus in rats.

Methods: The effects of an aqueous extract of *A. squamosa* leaves on blood glucose, insulin, C-peptide, albumin, albumin/globulin ratio, urea, uric acid and creatinine and the activities of diagnostic marker enzymes aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase were examined in the plasma, liver and kidney tissues of control and experimental groups.

Results: Oral administration of *A. squamosa* (300 mg/kg) aqueous extract to diabetic rats for 30 days significantly reduced blood glucose, urea, uric acid and creatinine, but increased the activities of insulin, C-peptide, albumin, albumin/globulin ratio and restored all marker enzymes to near control levels.

Conclusion: The present results shown that *A. squamosa* extract has an antihyperglycaemic effect and consequently may alleviate liver and renal damage associated with STZ-induced diabetes mellitus in rats.

Keywords: *Annona squamosa*, blood glucose, diabetes mellitus, insulin

Singapore Med J 2008;49(10):800-804

INTRODUCTION

Diabetes mellitus (DM) is metabolic disorder of multiple aetiologies characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1] Globally, the estimated incidence of DM and projection for year 2010, as given by International Diabetes Federation is 239 million.[2] DM is grossly reflected by profound changes in protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs.[3] Increased urea nitrogen production in DM may be accounted for by enhanced catabolism of both liver and plasma proteins.[4] Management of DM without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use natural products with antidiabetic activity, because insulin and oral hypoglycaemic drugs have undesirable side effects.[5] Medicinal plants are a good source of natural antioxidants believed to exert their effect by reducing the formation of the final active metabolite of the drug-induced systems or by scavenging the reactive molecular species to prevent their reaching a target site.[6-8] It has been documented that several medicinal plants show their hypoglycaemic effects associated with a significant alteration in the activity of liver hexokinase,[9] glucokinase.[10] In addition, Bopanna et al[9] and Eskander et al[11] demonstrated that the administration of several herb extracts could restore the changes in the activities of serum enzymes, like alkaline phosphatase (ALT), acid phosphatase and transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Phytochemicals isolated from plant sources have been are used for the prevention and treatment of cancer, heart disease, DM, and high blood pressure.[12]

Annona squamosa (A. squamosa) L. (Family: Annonaceae), commonly known as custard apple, is cultivated throughout India, mainly for its edible fruit. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has antifertility, antitumour and abortifacient properties.[13-18] Ethanolic extracts of leaves and stem are reported to have an anticancerous activity.[19] The aqueous leaf extract has also been reported to ameliorate hyperthyroidism,[20] which is often considered as a causative factor of DM.[21] The tribes and villagers of the Aligarh district[17] and Chotanagpur division[18] in India extensively use the young leaves of *A. squamosa* along with the seeds of *Piper nigrum* for the management of DM. In our previous study, we have demonstrated the antidiabetic effect of *A. squamosa* in streptozotocin

Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, India

Kaleem M, MSc, PhD
Research Associate

Medha P, MSc, PhD
Research Scholar,

Bano B, MSc, MPhil, PhD
Professor

Department of Medicine, JN Medical College

Asif M, MSc, DMLT
Senior Technical Assistant

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia, Kuantan 25200, Malaysia

Ahmed QU, MSc, PhD
Assistant Professor

Correspondence to:
Prof Bilqees Bano
Tel: (91) 944 510 6286
Fax: (91) 571 270 2758
Email: bilqeesbano@gmail.com

Table I. Effect of treatment with *A. squamosa* leaf extract for 30 days on plasma parameters of control and experimental groups of rats.

Parameter	Control	Diabetic	Diabetic + Insulin	Diabetic + *A. squamosa*
Blood glucose (mg/dL)	91.0 ± 6.3	287 ± 7.2*	93.4 ± 5.5*	97.5 ± 4.5*
Plasma insulin (μU/ml)	16.0 ± 0.80	6.25 ± 0.55*	12.5 ± 0.75*	11.9 ± 0.70*
C-peptide (pmol/L)	260.2 ± 12.5	155.4 ± 10.1*	227.7 ± 9.8*	242.1 ± 10.7*
Protein (g/dL)	7.56 ± 0.95	4.40 ± 0.80*	7.20 ± 0.68*	6.98 ± 0.55*
Albumin (g/dL)	3.90 ± 0.45	1.92 ± 0.28*	3.62 ± 0.16*	3.41 ± 0.24*
A/G ratio	1.06 ± 0.18	0.77 ± 0.15*	1.01 ± 0.21*	0.94 ± 0.25*
Urea (mg/dL)	24.6 ± 1.8	38.0 ± 2.7*	22.5 ± 1.6*	25.8 ± 1.4*
Uric acid (mg/dL)	1.10 ± 0.2	2.0 ± 0.1*	1.18 ± 0.08*	1.42 ± 0.06*
Creatinine (mg/dL)	0.95 ± 0.08	2.15 ± 0.25*	1.20 ± 0.09*	1.39 ± 0.05*

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at *$p < 0.05$.
Diabetic rats were compared with control rats; *A. squamosa*-treated diabetic rats were compared with diabetic rats; insulin-treated diabetic rats were compared with diabetic rats.

(STZ)-induced DM in rats.[22] Therefore, the present study aimed to examine the influence of oral administration of *A. squamosa* extract on the levels of some biochemical parameters and the activities of some enzymes in plasma, liver and kidney of STZ-induced DM in rats.

METHODS

STZ was purchased from Sigma Chemical Company (St Louis, MO, USA). All the other chemicals used were of analytical grade and were purchased from commercial sources. The young leaves of the plant *A. squamosa* were collected from The Survey of Medicinal Plant Unit, Regional Research Institute of Unani Medicine, Aligarh, India. Identification of the samples was done by using standard botanical monographs. They were further confirmed with the Department of Botany, Aligarh Muslim University, Aligarh, India, and a voucher specimen (HB 546) was deposited in the department herbarium. The aqueous extract was prepared by cold maceration of 250 g of the shade dried leaf powder in 500 ml of distilled water and allowed to stand overnight and boiled for 5–10 min till the volume was reduced by half. The solution was then cooled, filtered, concentrated, dried *in vacuo* (yield 36 g) and the residue stored in a refrigerator at 2–8°C for subsequent experiments

Male albino Wistar rats, weighing about 150–180 g obtained from Central Animal House, JN Medical College, Aligarh Muslim University, Aligarh, India, were used for the present investigations. The animals were maintained on standard rat feed supplied by Hindustan Lever Ltd, India. The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC). The animals were fasted overnight and DM was induced by a single intraperitoenal (i.p.) injection of freshly prepared STZ (55 mg/kg body weight of rats) in 0.1 M citrate buffer (pH 4.5).[23] The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dL on the third day after STZ injection. The treatment was started on the fourth day after STZ injection and this was considered as the first day of treatment. The treatment was continued for 30 days.

The rats were divided into four groups comprising eight animals in each group as follows:

Group 1: Control rats given only buffer.

Group 2: Diabetic controls.

Group 3: Diabetic rats treated with protamine-zinc insulin i.p. injection (6 units/kg body weight of rats /day).[24]

Group 4: Diabetic rats treated orally with *A. squamosa* (300 mg/kg body weight /day) in aqueous solution for 30 days.

At the end of the experiment, blood was collected into heparinised tubes, and the plasma and serum were separated by centrifugation. The liver and kidney were quickly removed, washed in ice-cold, isotonic saline and blotted individually on ash-free filter paper, and the organ weights were measured. The tissues were then homogenised in 0.1 M Tris–HCl buffer, pH 7.4. The homogenate was used for the estimations of proteins, enzymes, and other parameters. Blood glucose, urea, uric acid and creatinine were estimated using a commercial diagnostic kit (Ranbaxy Laboratories, New Delhi, India). Plasma insulin (Boerhringer Mannheim kit, Germany) and C-peptide (Packard, USA) were determined using a radioimmunoassay kit. The albumin and globulin contents were estimated by the method described by Reinhold.[25] The enzymes, AST, ALT and ALP, were assayed by the method of King and Armstrong[26] and γ-glutamyl transpeptidase (γ-GT) was assayed by the method of

Table II. Effect of treatment with *A. squamosa* leaf extract for 30 days on AST, ALT, ALP and γ-GT activities in plasma, liver and kidney of control and experimental groups of rats.

Groups	Control	Diabetic	Diabetic + Insulin	Diabetic + *A. squamosa*
Plasma				
AST	74.4 ± 6.93	112.1 ± 6.42*	83.2 ± 2.70*	84.5 ± 3.45*
ALT	32.6 ± 2.2	64.2 ± 4.60*	37.5 ± 3.15*	41.0 ± 2.52*
ALP	76.6 ± 4.72	138.1 ± 6.41*	85.7 ± 5.32*	90.5 ± 4.75*
γ-GT	13.6 ± 1.20	25.5 ± 2.84*	17.4 ± 1.62*	17.8 ± 1.80*
Liver				
AST	754.0 ± 16.0	960.4 ± 21.3*	744.7 ± 14.7*	760.0 ± 12.9*
ALT	907.3 ± 16.2	1241.6 ± 19.4*	935.5 ± 13.0*	999.2 ± 19.7*
ALP	0.16 ± 0.02	0.30 ± 0.04*	0.22 ± 0.01*	0.24 ± 0.02*
γ-GT	3.44 ± 0.36	5.67 ± 0.54*	3.65 ± 0.34*	3.73 ± 0.24*
Kidney				
AST	785.0 ± 12.8	745.5 ± 14.0*	788.2 ± 10.8*	775.7 ± 8.90*
ALT	841.4 ± 18.8	808.2 ± 20.8*	830.7 ± 15.3*	821.6 ± 17.1*
ALP	0.22 ± 0.03	0.43 ± 0.06*	0.30 ± 0.03*	0.33 ± 0.02*
γ-GT	2.71 ± 0.30	5.66 ± 0.36*	3.01 ± 0.24*	3.25 ± 0.16*

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at *$p < 0.05$.
Diabetic rats were compared with control rats; *A. squamosa*-treated diabetic rats were compared with diabetic rats; insulin-treated diabetic rats were compared with diabetic rats.
Units of measurement (per L) for AST and ALT: μmol of pyruvate liberated/hr; ALP: μmol of phenol liberated/min; γ-GT: mol of p-nitro aniline liberated/min.

Rosalki and Rau.[27] The protein content in the plasma, liver and kidney were estimated by the method of Lowry et al.[28] All spectrophotometric measurements were carried out in a Camspec UV-visible (Camspec M330B, UK) spectrophotometer. All the grouped data was statistically evaluated via the Statistical Package for Social Sciences version 7.5 (SPSS Inc, Chicago, IL, USA). Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant differences test. p-values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for eight animals in each group.

RESULTS

A significant increase in the level of blood glucose, a decrease in plasma insulin and C-peptide were observed in diabetic rats when compared to control rats. Administration of *A. squamosa* (300 mg/kg) and insulin to diabetic rats significantly decreased the level of blood glucose, increased plasma insulin and C-peptide to near control level. Table I demonstrates the levels of protein, plasma albumin and albumin/globulin ratio in control and STZ-diabetic rats. The level of protein in plasma was found to be reduced in diabetic animals ($p < 0.05$) when compared to control animals. The lowered level of protein, after *A. squamosa* treatment, increased to near control. The levels of albumin and albumin/globulin ratio in plasma were decreased in diabetic animals. These lowered levels of plasma albumin and albumin/globulin ratio level were restored significantly in *A. squamosa*-treated diabetic rats.

Urea, uric acid, and creatinine levels were significantly elevated in STZ-DM in rats ($p < 0.05$) when compared to control animals. Oral administration of *A. squamosa* extract for 30 days significantly lowered urea, uric acid and creatinine levels in STZ-diabetic rats. Table II shows the activities of AST, ALT, ALP and γ-GT in plasma, liver and kidney of control and STZ-diabetic rats. The activities of these enzymes were found to be significantly increased ($p < 0.05$) in the plasma and liver of diabetic rats. In the kidney of diabetic animals, the activities of ALP and γ-GT were increased, while the activities of AST and ALT were not altered. Oral administration of *A. squamosa* for 30 days resulted in the near normalisation of the activities of AST, ALT, ALP and γ-GT in the plasma, liver and kidney of diabetic rats.

DISCUSSION

Type I DM is a chronic disease characterised by high blood glucose levels due to an absolute or relative deficiency of circulating insulin levels. Although various types of oral hypoglycaemic agent are currently available along with insulin for treating DM, there is a growing interest in herbal remedies due to the side-effects associated with the existing therapeutic hypoglycaemic agents.[29,30] The present investigation indicates the hypoglycaemic and protective effects of *A. squamosa* leaves in the liver and kidney of STZ-diabetic rats. We have observed a significant decrease in blood glucose in *A. squamosa*-treated diabetic rats, when compared with diabetic control rats. The optimum dosage (300 mg/kg) was standardised and confirmed by a previous study with significant hypoglycaemic and antihyperlipidemic activity.[22] The possible mechanism of *A. squamosa*

hypoglycaemic action may be through potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to the peripheral tissue. This was clearly evidenced by the increased level of insulin in diabetic rats treated with *A. squamosa*. In this context, a number of other plants have also been reported to have hypoglycaemic and insulin release stimulatory effects.[31,32]

C-peptide and insulin are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels have been reported to be a valuable index of insulin secretion rather than insulin alone.[33] In this study, the plasma C-peptide and insulin levels were significantly higher in the *A. squamosa* than in the DM group. Although the reduction of plasma glucose in these two groups was small, it was significant. Reduction in plasma total protein and albumin level was observed in diabetic rats and this is consistent with the results obtained by Bakris[34] and Tuvemo et al.[35] The decrease in protein and albumin may be due to microproteinuria and albuminuria, which are important clinical markers of diabetic nephropathy,[36] and/or may be due to increased protein catabolism.[37] The results of the present study demonstrated that the treatment of diabetic rats with the aqueous extract of *A. squamosa* caused a noticeable elevation in the plasma total protein and albumin levels as compared with their normal levels. Such improvement of serum protein and albumin was previously observed after the oral administration of *Balanites aegyptiaca (B. aegyptiaca)* to experimentally diabetic rats.[38] It has been established that insulin stimulates the incorporation of amino acids into proteins.[37]

The plasma levels of urea, uric acid and creatinine levels were measured, as DM also causes renal damage due to abnormal glucose regulation, including elevated glucose and glycosylated protein tissue levels, haemodynamic changes within the kidney tissue, and increased oxidative stress.[39] The STZ-induced diabetic rats exhibited significantly higher plasma urea, uric acid and creatinine levels compared to the DM group. However, the *A. squamosa* supplement lowered these plasma values to a control range. A significant elevation in serum creatinine and urea levels indicate an impaired renal function of diabetic animals.[40] Thus, it would appear that the *A. squamosa* leaves supplement lowered the plasma urea, uric acid and creatinine levels by enhancing the renal function that is generally impaired in diabetic rats. These results are in agreement with other previous studies on the mesocarp extract of *B. aegyptiaca*,[38,41] and herbal formulation D-400.[42]

The increase in the activities of plasma AST, ALT and ALP indicated that DM may induce hepatic dysfunction. The enzymes directly associated with the conversion of amino acids to keto acids are AST and ALT, and are increased in the diabetic condition. Begum and Shanmugasundaram also reported an increase in the activities of AST and ALT in the liver of diabetic animals.[43] Treatment with *A. squamosa* or insulin normalised these enzyme activities. Similarly, increased activities of AST and ALT in the diabetic liver were also reported by Jorda et al.[44] The increased protein catabolism accompanying gluconeogenesis and urea formation that are seen in the diabetic state might be responsible for the elevation of these tissue transaminases. The rise in the activity of ALT is due to hepatocellular damage and is usually accompanied by a rise in AST.[45] This might be the reason for the elevated activities of these enzymes, which were brought back to near normal value by *A. squamosa* treatment. This result shows the normalising effects of *A. squamosa* on hepatocellular damage and suppression of gluconeogenesis.

Elevated activity of ALP was observed in STZ-diabetic rats. Prince et al have also reported increased ALP activity in experimentally diabetic rats.[32] The increased activity of this enzyme in plasma may be a result of diabetes-induced damage to the tissues. *A. squamosa* treatment restored the activity of this enzyme to near normal by reducing its induction in DM. γ-GT catalyses the transfer of the γ-glutamyl group from γ-glutamyl peptides to another peptide or L-amino acids or to water. The assay of γ-GT is a helpful adjunct in detecting hepatic damage. A highly significant elevation in the activity of γ-GT was observed in plasma, liver and kidney of STZ-induced diabetic rats. This is in accord with earlier investigations,[46] wherein a dramatic increase in γ-GT expression was found in the liver of diabetic rats. Elevated activity of γ-GT in plasma takes place as a result of hepatic induction of the enzyme. In addition, hepatocellular damage or cholestasis may also contribute to the elevation in the activity. Increased activity of γ-GT in STZ-induced diabetic rats was lowered to near normal by *A. squamosa* treatment that indicates the possible prevention of necrosis by *A. squamosa* treatment.

In conclusion, *A. squamosa* leaves extract lowered blood glucose with a simultaneous increase in the plasma insulin and C-peptide levels. In addition, *A. squamosa* extract could influence protein metabolism and marker enzymes in STZ-induced diabetic rats. Phytochemical studies showed that the *A. squamosa* contained a high amount of favonoids like rutin and hyperoside.[15,16] Longer duration studies of *A. squamosa* and its isolated compounds on chronic models are necessary to develop a potent antidiabetic drug.

REFERENCES

1. Baquer NZ, Gupta D, Raju J. Regulation of metabolic pathways in liver and kidney during experimental diabetes: effects of antidiabetic compounds. Indian J Clinical Biochem 1998; 13:63-80.
2. Gandhi HR. Diabetes and coronary artery disease: importance of risk factors. Cardiol Today 2001; 1:31-4.
3. Almdal TP, Vilstrup H. Effect of streptozotocin-induced diabetes and diet on nitrogen loss from organs and the capacity of urea synthesis in rats. Diabetologia 1987; 30:952-6.
4. Jorda A, Gomez M, Cabo J, Grisolia S. Effect of streptozotocin-diabetes on some urea cycle enzymes. Biochem Biophys Res Commun 1982; 106:37-43.
5. Kameswara Rao B, Appa Rao CH. Hypoglycemic and antihyperglycemic activity of alternifolium Walp. Seed extracts in normal and diabetic rats. Phytomed 2001; 8:88-93.
6. Shanmugasundaram ERB, Rajeswari G, Baskaran K. Use of Gymnema sylvestre leaf extract in the control of blood glucose in insulin-dependent diabetes mellitus. J Ethnopharmacol 1990; 30:281-94.
7. Kaleem M, Kirmani D, Asif M, Ahmad QU, Bano B. Biochemical effects of Nigella sativa L seeds on diabetic rats. Indian J Exp Biol 2006; 44:745-8.
8. Kaleem M, Sheema, Sarmed H, Bano B. Protective effects of Piper nigrum and Vinca rosea in alloxan-induced diabetic rats. Indian J Physiol Pharmacol 2005; 49:65-71.
9. Bopanna KN, Kannan J, Godgil S, Balaraman R, Rathod SP. Antidiabetic and antihyperlipidemic effects of neem seed kernel powder on alloxan-diabetic rabbits. Indian J Pharmacol 1997; 29:162-72.
10. Kumari K, Mathew BC, Augusti KT. Antidiabetic and hypolipidemic effect of S-methyl cysteine sulfoxide isolated from Allium cepa Linn. Indian J Biochem Biophys 1995; 32:49-54.
11. Eskander EF, Jun HW, Ibrahim KA, Abdelal WE. Hypoglycaemic effect of a herbal formulation in alloxan-induced diabetic rats. Egypt J Pharm Sci 1995; 36:253-70.
12. Waltner-Law ME, Wang XL, Law BK, et al. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem 2002; 277:34933-40.
13. Asolkar LV, Kakkar KK, Chakre OJ. Glossary of Indian Medicinal Plants with Active Principles. New Delhi: Publication and Information Directorate, 1992: 72-3.
14. Vohora SB, Kumar I, Naqvi S. Phytochemical, pharmacological, antibacterial and anti-ovulatory studies on Annona squamosa. Planta Medica 1975; 28:97-100.
15. Yoganarasimhan SN. Medical Plants of India–Tamil Nadu. Vol II. Bangalore: International Book Publisher, Print Cyper Media, 2000: 48-62.
16. Seetharaman TR. Flavonoids from the leaves of Annona squamosa and Polyalthia longifolia. Fitoterapia 1986; 57:189-98.
17. Atique A, Iqbal M, Ghouse AKM. Use of Annona squamosa and Piper nigrum against diabetes. Fitoterapia 1985; 56:190-2.
18. Topno KK. Plants used by tribals of Chotanagpur against diabetes. Botanica 1997; 47:99-101.
19. Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BB. Screening of Indian plants for biological activity: part-II. Indian J Exp Biol 1969; 7:250-62.
20. Sunanda P, Anand K. Possible amelioration of hyperthyroidism by the leaf extract of Annona squamosa. Current Science 2003; 84:1402-4.
21. Williams JB. Adverse effects of thyroid hormones. Drugs Aging 1997; 11:460-9.
22. Kaleem M, Asif M, Ahmad QU, Bano B. Antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin induced diabetic rats. Singapore Med J 2006; 47:670-5.
23. Sekar N, Kanthasamy S, William S, Subramanian S, Govindasamy S. Insulinic actions of vanadate in diabetic rats. Pharmacol Res 1990; 22:207-17.
24. Gupta S, Kataria M, Gupta PK, Murganandan S, Yashroy RC. Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. J Ethnopharmocol 2004; 90:185-9.
25. Reinhold J. Determination of serum total protein, albumin and globulin fractions by the biuret method. In: Varley H, Gowen Lock AH, Bell M, eds. Practical Clinical Biochemistry. Vol I. 5th ed. London: William Heinemann, 1980: 45-7.
26. King KJ, Armstrong AL. Calcium, phosphorus and phosphatase. In: Varley H, ed. Practical Clinical Biochemistry. 4th ed. New Delhi: CBS Publishers, 1988: 457-61.
27. Rosalki SB, Rau D. Serum γ-glutamyl transpeptidase activity in alcoholism. Clin Chim Acta 1972; 39:41-7.
28. Lowry OH, Rosenbrough NJ, Farr AL, Randall RL. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:264-75.
29. Holman RR, Turner RC. Oral agents and insulin in the treatment of NIDDM. In: Pickup J, Williams G, eds. Textbook of Diabetes. Oxford: Blackwell, 1991:467-9.
30. Kameswara R, Giri B, Kesavulu MM, Apparao C. Herbal medicine in the management of diabetes mellitus. Manphar Vaidhya Patrika 1997; 1:33-5.
31. Pari L, Uma Maheswari J. Antihyperglycaemic activity of Musa sapientum flowers: effect on lipid peroxidation in alloxan-induced diabetic rats. Phytother Res 2000; 14:136-8.
32. Prince PSM, Menon VP, Pari L. Effect of Syzigium cumini extracts on hepatic hexokinase and glucose 6-phosphatase in experimental diabetes. Phytother Res 1997; 11:529-31.
33. Doda RF. Diabetes mellitus, In: Kaplan LA, Amadeo JP, eds. Clinical Chemistry. St Louis: Mosby Year Book, 1996: 613-41.
34. Bakris GL. Diabetic nephropathy. What you need to know to preserve kidney function. Postgrad Med 1997; 93:89-94.
35. Tuvemo T, Ewald U, Kobboh M, Proos LA. Serum magnesium and protein concentrations during the first five years of insulin-dependent diabetes in children. Acta Paediatr Suppl 1997; 418:7-10.
36. Mauer SM, Steffes MW, Brown DM. The kidney in diabetes. Am J Med 1981; 70:63-6.
37. Almdal JP, Vilstrup H. Strict insulin therapy normalizes organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. Diabetologia 1988; 31:114-8.
38. Mansour HA, Newairy AA. Amelioration of impaired renal function associated with diabetes by Balanites aegyptiaca fruits in streptozotocin-induced diabetic rats. J Med Res Inst 2000; 21:115-25.
39. Aurell M, Bjorck S. Determination of progressive renal disease in diabetes mellitus. Kidney Int 1992; 41:38-42.
40. Shinde UA, Goyal RK. Effect of chromium picolinate on histopathological alterations in STZ and neonatal STZ diabetic rats. J Cell Mol Med 2003; 7:322-9.
41. Saeed A, Ibrahim N, Bashandy S, El-Gengaihi S. Saponin of Balanites aegyptiaca Del fruits and biological evaluation. Bull Fac Pharm 1995; 33:105-9.
42. Dubey GP, Dixit SP, Singh A. Alloxan-induced diabetes in rabbits and effect of a herbal formulation D-400. Indian J Pharmacol 1994; 26:225-6.
43. Begum N, Shanmugasundaram KR. Transaminases in experimental diabetes. Arogya J Health Sci 1978; 4:116-22.
44. Jorda A, Gomez M, Cabo J, Grisolia S. Effect of streptozotocin diabetes on some urea cycle enzymes. Biochem Biophys Res Commun 1982; 106:37-43.
45. Mohan Rao GM, Morghom LO, Kabar MN, Benmohamud BM, Ashibani K. Serum glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) levels in diabetes mellitus. Indian J Med Sci 1989; 5:118-22.
46. McLennan SV, Heffernan S, Wright L, et al. Change in hepatic glutathione metabolism in diabetes. Diabetes 1991; 40:344-8.