Journal of
APPLIED
BIOMEDICINE

J Appl Biomed
7: 151–159, 2009
ISSN 1214-0287

ORIGINAL ARTICLE

Antidiabetic properties of *S*-allyl cysteine, a garlic component on streptozotocin-induced diabetes in rats

Ganapathy Saravanan[1, 2], **Ponnusamy Ponmurugan**[1, 3], **Gandhipuram Periasamy Senthil Kumar**[2], **Thatchinamoorthi Rajarajan**[4]

[1]Research and Development Centre, Bharathiyar University, Coimbatore, Tamil Nadu, India
[2]Department of Biochemistry, Centre for Biological Science, K.S.R. College of Arts and Science, Thokkavadi, Tiruchengode, Tamil Nadu, India
[3]Department of Biotechnology, K.S.R. College of Technology, Thokkavadi, Tiruchengode, Tamil Nadu, India
[4]Department of Biochemistry, School of Biotechnology, SASTRA University, Thanjavore, Tamil Nadu, India

Received 14th May 2009.
Revised 24th June 2009.
Published online 8th July 2009.

Summary
The present study was carried out to investigate the hypoglycaemic effect of S-allyl cysteine (SAC), a garlic component, on some biochemical parameters of STZ induced diabetic rats. STZ induced diabetic rats were treated with SAC at two different doses (100 mg/kg b.w. and 150 mg/kg b.w.) for 45 days. Treatment with SAC significantly decreased the levels of blood glucose, glycosylated hemoglobin, blood urea, serum uric acid, serum creatinine, and diminished activities of pathophysiological enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). The antihyperglycaemic nature of SAC is also evidenced from the improvement in the levels of plasma insulin and haemoglobin. Further, the results are comparable with glyclazide, an oral standard drug. A 150 mg/kg b.w. dose produced a better effect than a 100 mg dose. Thus, the present findings suggest that SAC may be considered as an effective therapeutic agent for the treatment of diabetes mellitus.

Key words: streptozotocin; *Allium sativum*; S-allyl cysteine; diabetes mellitus

Abbreviations

SAC	S-allyl cysteine
STZ	streptozotocin
AGE	aged garlic extract
AST	aspartate transaminase
ALT	alanine transaminase
ALP	alkaline phosphatase
b.w.	body weight

✉ Ganapathy Saravanan, Department of Biochemistry, Centre for Biological Science, K.S.R. College of Arts and Science, Thokkavadi, Tiruchengode, Tamil Nadu, India, 637215
💻 saravana_bioc@rediffmail.com
☎ +91 984-395-4422

INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by defects in insulin secretion, insulin action, or both (ADA 2009). The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025 and it has been predicted that the major burden will occur in developing countries (Kim et al. 2006). Studies

conducted in India in the last decade have highlighted that not only is the prevalence of diabetes high but also that it is increasing rapidly in the urban population (Ramachandran et al. 2002). It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025.

In the modern system of medicine, there is still no satisfactory effective therapy available to cure diabetes mellitus. Although insulin therapy is used for the management of diabetes mellitus there are several drawbacks to its use, which include insulin allergy, insulin antibodies, lipid dystrophy and autoimmunity (Defronzo et al. 1982). Additionally, pharmaceutical drugs such as sulfonylurea and biguanides are used for the treatment of diabetes but these are either too expensive or have undesirable side effects or contraindications (Rang et al. 1991). Alternative strategies to the current modern pharmacological therapy of diabetes mellitus are urgently needed (WHO 2002), because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of modern therapies for many rural populations in developing countries. To overcome these consequences medicinal plants are being investigated for possible use in the treatment of diabetes.

Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease (Ueda et al. 2002, Kumar et al. 2007). Consumption of garlic (*Allium sativum*, Liliaceae) has been reported to have many medicinal properties including antidiabetic and anti lipidemic activities (McKenna et al. 2002). Garlic contains a variety of effective compounds that exhibit antioxidant (Augusti and Sheela 1996) as well as hypotensive activities (Banerjee and Maulik 2002). S-allylcysteine, a sulfur containing amino acid derived from garlic, has been reported to possess antioxidant (Herrera-Mundo et al. 2006), anti-cancer (Chu et al. 2007), antihepatotoxic (Nakagawa et al. 1989) and neurotrophic activity (Moriguchi et al. 1997). Although garlic has been used for remedies and as a food for more than 1 000 years, most people used garlic based on their experiences without any knowledge and relationships between biological activities and compounds of garlic or its transformation products (Block 1992). The present study was aimed to investigate the hypoglycaemic effect of S-allyl cysteine, a garlic component on some biochemical parameters of STZ induced diabetic rats compared to a control group. The effects produced by these treatments are compared with standard drug Glyclazide.

MATERIALS AND METHODS

Animals

Male Wister rats of body weight 150–180 g were used in this study. The animals were maintained in The Animal house, Sastra University, Thanjavore, India and fed on standard pellet diet (AMRUT, Pune, India) and water *ad libitum*. The protocol of this study was approved by Institutional ethical committee of Sastra University.

Chemicals

SAC (99%) was purchased from LGC Prochem, Bangalore, India. Streptozotocin was purchased from Himedia, Bangalore, India. All other chemicals used were of analytical grade.

Induction of diabetes

Diabetes was induced (Kaleem et al. 2006) in overnight fasted adult Wister strain albino male rats weighing 150–180 g by a single intraperitoneal injection of 55 mg/kg streptozotocin. Streptozotocin was dissolved in citrate buffer (pH 4.5). Hyperglycaemia was confirmed by elevated glucose levels (above 250 mg/dl) in blood, determined at 72 h and then on day 7 after injection.

Experimental design

After the successful induction of experimental diabetes, the rats were divided into five groups each comprising a minimum of six rats.

Group 1: Control rats.

Group 2: Diabetic control rats.

Group 3: Diabetic rats administered with SAC (100 mg/kg b.w./rat) in aqueous solution orally for 45 days.

Group 4: Diabetic rats administered with SAC (150 mg/kg b.w./rat) in aqueous solution orally for 45 days.

Group 5: Diabetic rats administered with glyclazide (5 mg/kg b.w./rat) in aqueous solution orally for 45 days (Pulido et al. 1997).

These doses of SAC were determined from a preliminary dose-response study evaluating the effects of 50 mg, 100 mg and 150 mg doses on fasting serum glucose in diabetic rats [Low dose (50 mg) data is not shown].

Body weight and blood glucose level measurements were conducted periodically. At the end of the experimental period, the rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with or without anticoagulant for plasma or serum separation respectively.

Oral glucose tolerance test

At the end of the experimental period, fasting blood samples were taken from all the groups of rats. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after the administration of a glucose solution at a dosage of 2 g/kg b.w. (Joy and Kuttan 1999).

Biochemical estimations

Fasting blood glucose was estimated by the O-toluidine method (Sasaki et al. 1972). Haemoglobin and glycosylated haemoglobin were estimated according to the methods of Drabkin and Austin (1932) and Nayak and Pattabiraman (1981) respectively and the plasma protein was determined according to the method of Lowry et al. (1951). Blood urea (Natelson et al. 1951) serum creatinine (Brod and Sirota 1948) and uric acid (Caraway 1963) were also assessed. A plasma insulin assay was carried out using an enzyme linked immunosorbent assay kit (ELISA, Boehriger, Mannheim, Germany). The activities of pathophysiological enzymes such as serum aspartate transaminase, serum alanine transaminase and serum alkaline phosphatase were assayed (King 1965a, b).

Statistical analysis

All the results were expressed as the mean ± S.D. for six animals in each group. All the grouped data were statistically evaluated with SPSS 10.0 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test at the significance level 2 α=0.05.

RESULTS

Figure 1 presents the oral glucose tolerance test of the control and treated animals. The blood glucose level in the control rats was elevated to a maximum value at 60 min after glucose load and declined to near basal levels at 120 min, whereas, in STZ induced diabetic rats, the peak increase in blood glucose level was noticed even after 60 min and remained high over the next 60 min. Supplementation with SAC as well as glyclazide to diabetic rats elicited a significant decrease in blood glucose level at 60 min when compared with untreated diabetic rats.

Table 1 shows the level of body weight development, blood glucose and plasma insulin in control and experimental animals. There was a significant elevation in blood glucose level with a significant decrease in plasma insulin levels and body growth rate in STZ induced diabetic rats, compared with the control rats. Administration of SAC and glyclazide tended to bring blood glucose, plasma insulin and body growth rate towards near normal levels.

Table 2 summaries the level of haemoglobin and glycated haemoglobin levels in control and experimental animals. A significant reduction in the level of haemoglobin and a concomitant increase in the level of glycated haemoglobin were observed in STZ diabetic rats and these levels were normalized after treatment with SAC and glyclazide.

Table 3 shows the activities of serum AST, ALT and ALP in control and experimental groups of rats. There was a significant elevation in serum AST, ALT and ALP in STZ diabetic rats when compared with the control rats. Oral treatment of SAC and glyclazide tended to bring AST, ALT and ALP towards near normal levels.

Table 4 presents the level of serum total protein, blood urea, serum uric acid and serum creatinine in the control and experimental groups of rats. There was a significant elevation in blood urea, uric acid and creatinine with a significant decrease in total protein in STZ diabetic rats when compared with the control rats. Administration of SAC and glyclazide tended to bring urea, uric acid and creatinine towards the near normal range.

DISCUSSION

The STZ-induced diabetic rat is one of the animal models of human diabetes mellitus. Diabetes arises from the destruction of pancreatic β-cells, causing degranulation and reduction of insulin secretion (Zhang and Tan 2000). STZ-induced diabetes is characterized by a severe loss in body weight (Chen and Ianuzzo 1982). The decrease in body weight in diabetic rats shows that the loss or degradation of structural proteins is due to diabetes, and structural proteins are known to contribute to the body weight (Rajkumar and Govindarajulu 1991). The present study demonstrated that oral administration of SAC for 45 days shows an antihyperglycaemic effect in STZ-induced diabetic rats. When diabetic rats were treated with SAC, the weight loss was recovered. The capability of SAC to protect body weight loss seems to be related to its ability to reduce hyperglycaemia.

The fundamental mechanism underlying hyperglycaemia in diabetes mellitus involves the overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by tissues (Latner 1958) and studies have shown that

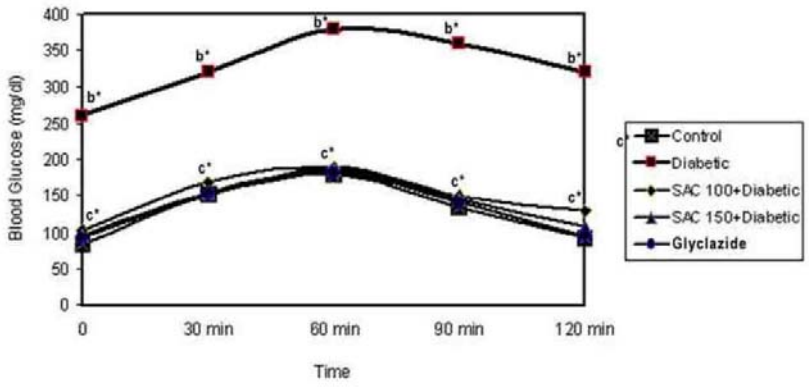

Fig. 1. **Effect of SAC and glyclazide on glucose tolerance in control and laboratory rats.** Symbols as in Table 1.

Table 1. **Effects of SAC on body weight development, blood glucose, plasma insulin and urine sugar in control and experimental animals.**

Groups	Body weight (g)		Blood glucose (mg/dl)	Plasma insulin (μU/ml)	Urine sugar
	Initial	Final			
Control	164.24 ± 2.57	180.74 ± 3.47	84.13 ± 0.33	16.10 ± 0.61	Nil
Diabetic control	170.14 ± 2.45^{b*}	146.17 ± 2.68^{a*}	261.11 ± 3.45^{b*}	4.98 ± 0.25^{a*}	+++
Diabetic + SAC (100 mg)	165.63 ± 1.98^{c*}	160.74 ± 3.54^{b*}	100.21 ± 3.97^{c*}	12.15 ± 0.36^{b*}	+
Diabetic + SAC (150 mg)	165.85 ± 2.17^{c*}	162.81 ± 3.27^{b*}	94.17 ± 2.95^{c*}	12.73 ± 0.31^{b*}	Nil
Diabetic + glyclazide	163.94 ± 2.64^{c*}	164.10 ± 2.79^{b*}	90.3 ± 2.1^{c*}	13.21 ± 0.45^{b*}	Nil

Values are mean ± S.D, n = 6
[b] Significantly different from control
[c] Significantly different from diabetic control

Table 2. **Effects of SAC on haemoglobin and glycosylated haemoglobin in control and experimental animals.**

Groups	Haemoglobin (g/dl)	Glycosylated haemoglobin (% Hb)
Control	14.23 ± 0.38	6.31 ± 0.23
Diabetic control	7.87 ± 0.57^{b*}	13.17 ± 0.19^{b*}
Diabetic + SAC (100 mg)	11.54 ± 0.28^{c*}	7.93 ± 0.14^{c*}
Diabetic + SAC (150 mg)	12.63 ± 0.31^{c*}	7.52 ± 0.12^{c*}
Diabetic + glyclazide	13.15 ± 0.39^{c*}	7.10 ± 0.11^{c*}

Symbols as in Table 1

Table 3. **Effects of SAC on serum AST, ALT and ALP in control and experimental animals.**

Groups	AST	ALT	ALP
Control	82.31 ± 0.51	21.15 ± 0.91	71.03 ± 0.98
Diabetic control	115.63 ± 2.83^{b*}	43.57 ± 1.32^{b*}	138.31 ± 1.93^{b*}
Diabetic + SAC (100 mg)	88.17 ± 1.28^{c*}	25.51 ± 0.78^{c*}	79.82 ± 1.05^{c*}
Diabetic + SAC (150 mg)	85.71 ± 0.98^{c*}	23.91 ± 0.65^{c*}	76.17 ± 0.88^{c*}
Diabetic + glyclazide	83.61 ± 1.17^{c*}	22.72 ± 0.71^{c*}	74.57 ± 0.61^{c*}

Symbols as in Table 1
Enzyme activities are expressed as: AST and ALT µmoles of pyruvate/h/mg/ of protein; ALP µmoles of phenol liberated/min/mg of protein.

Table 4. **Effects of SAC on serum total protein, blood urea, creatinine and uric acid in control and experimental animals.**

Groups	Total protein (g/dl)	Blood urea (mg/dl)	Serum creatinine (mg/dl)	Serum uric acid (mg/dl)
Control	8.91 ± 0.16	22.57 ± 0.78	0.49 ± 0.06	2.27 ± 0.06
Diabetic control	4.13 ± 0.1^{b*}	45.11 ± 0.83^{b*}	1.10 ± 0.08^{b*}	5.73 ± 0.13^{b*}
Diabetic + SAC (100 mg)	6.85 ± 0.12^{c*}	28.64 ± 0.64^{c*}	0.65 ± 0.05^{c*}	3.15 ± 0.05^{c*}
Diabetic + SAC (150 mg)	7.53 ± 0.14^{c*}	26.91 ± 0.58^{c*}	0.60 ± 0.04^{c*}	2.95 ± 0.04^{c*}
Diabetic + glyclazide	7.88 ± 0.13^{c*}	24.38 ± 0.61^{c*}	0.55 ± 0.02^{c*}	2.60 ± 0.03^{c*}

Symbols as in Table 1

the level of blood glucose is elevated in STZ-induced diabetic rats. Hence, in the present study, we observed an increased level of blood glucose. In the oral glucose tolerance test, oral administration of SAC significantly reduced the blood glucose level in glucose-loaded rats at 30, 60, 90 and 120 min. The hypoglycaemic potency has been attributed to the sulphur compound (Augusti and Sheela 1996). The mechanism of the hypoglycaemic action probably involves direct or indirect stimulation of insulin secretion (Carson 1987). Further, it is also suggested that S-allyl cysteine may have the possible mechanism of sparing insulin from -SH inactivation by reacting with an endogenous thiol containing molecule such as cysteine and glutathione. The SAC treatment might enhance glucose utilization because it significantly decreased the blood glucose level in glucose-loaded rats. It may be due to the restoration of a delayed insulin response, or to the inhibition of intestinal absorption of glucose. This could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells or its release from bound insulin.

Hyperglycaemia is the clinical hallmark of poorly controlled diabetes, which is known to cause protein glycation, also known as non-enzymatic glycosylation (Zhang and Swaan 1999). It has been reported that various proteins, including haemoglobin, albumin, collagen, low-density lipoprotein, a crystalline of lens and fibronectin, undergo non enzymatic glycation in diabetes (Koyama et al. 1998, Kumar et al. 2005). In long term diabetes, the glycosylated form of haemoglobin has an altered affinity for oxygen and this may be a factor in tissue anoxia (Bunn et al. 1979, Yiping et al. 2004). Glycosylated haemoglobin is found to be significantly increased in diabetic animals and the amount of this increase is directly proportional to the fasting blood glucose level (Koening et al. 1978, Peters et al. 1996). The level of total haemoglobin is found to be decreased in the diabetic group and this may be due to the increased formation of glycosylated haemoglobin. This was well correlated with earlier studies, which reported that there was a decrease in the level of haemoglobin in experimental diabetic rats (Shirwaikar et al. 2006). The increase in the level of haemoglobin in animals given SAC may be due to the decreased level of blood glucose.

The increase in the activities of plasma AST, ALT and ALP indicated that diabetes may be induced due to liver dysfunction. Ohaeri (2001) also found that the liver was necrotized in STZ-induced diabetic rats. Therefore, an increase in the activities of AST, ALT and ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al. 1993) which gives an indication of the hepatotoxic effect of STZ induced diabetic rats. These manifestations are a consequence of a metabolic alteration, with an increase of glyconeogenesis and of cetogenesis and/or of hepatic lesions that occur in diabetic animals (Mori et al. 2003). On the other hand, treatment of the diabetic rats with SAC and glyclazide caused a reduction in the activity of these enzymes in serum, compared to the mean values of the diabetic group, and consequently may alleviate liver damage caused by STZ-induced diabetes. Our results are also in line with a previous report (El-Demerdash et al. 2005).

The decrease in total protein concentrations in the serum of diabetic rats may be ascribed to (i) a decreased amino acid uptake (Garber 1980) (ii) a greatly decreased concentration of a variety of essential amino acids (Brosnan and Man 1984) and (iii) an increased conversion rate of glycogenic aminoacid to CO_2 and H_2O (Mortimore and Manton 1970). Administration of SAC and glyclazide maintained the protein level near normal.

Urea is the main end product of protein catabolism in the body. An enhanced breakdown of both liver and plasma proteins leads to the accumulation of urea nitrogen in experimental diabetes (Green and Miller 1960). Increased peripheral release of nitrogenous substances and increased hepatic elimination of urea nitrogen are due to the alterations in nitrogen homeostasis. Thus, the observed negative nitrogen balance may be due partly to changes occurring within the hepatocytes (Almadal and Vilstrup 1988). The oral administration of SAC and glyclazide to diabetic rats significantly decreased the altered levels of blood urea suggesting a prophylactic role for SAC and glyclazide in protein metabolism.

Increased protein glycation in STZ-induced diabetes was reported to be associated with increased muscle wasting and thereby, an increased release of purines. The elevated levels of purine in diabetes are reported to be the main source of uric acid, in addition to xanthine oxidase activity (Anwar and Meki 2003). Elevated levels of serum uric acid are due either to an increase in uric acid production or a decrease in its excretion (Modan et al. 1987). There is accumulating evidence that increased oxidative stress is closely related to diabetes and its vascular complications (Baynes 1991). Thus, the elevated levels of circulating uric acid levels may be an indicator that the body is trying to protect itself from the deleterious effects of free radicals by increasing the products of endogenous antioxidants, such as uric acid. Interestingly, uric acid prevents oxidative modification of endothelial enzymes and preserves the ability of endothelium to mediate vascular

dilatation in the face of oxidative stress (Becker 1993). In the present study, the increased levels of serum uric acid observed in diabetic rats were restored to near normal by the administration of SAC and glyclazide indicating the free radical scavenging activity of SAC and glyclazide.

Creatinuria occurs in any condition associated with extensive muscle breakdown as in starvation and poorly controlled diabetes mellitus (Ganong 1995). The serum creatinine concentration may vary based on a number of factors including diet composition, muscle mass and gender. Serum creatinine values also depend on the ability of the kidney to excrete creatinine. An elevation in creatinine usually occurs simultaneously with an increase in blood urea nitrogen. Creatinine concentration is often used as a variable not only to assess impairment of kidney function but also as a clinical end point to detect the treatment related toxic effects of compounds on the kidney in experimental animals (Travlos et al. 1996). In the present study, the oral treatment with SAC and glyclazide for 45 days significantly reduced the serum creatinine level. Therefore, it may be concluded that the previous changes in serum creatinine concentrations strongly suggested impairment of kidney function in diabetes.

In conclusion, the observed effects of SAC on reversing the adverse effect of hyperglycaemia provides an insight into the pathogenesis of diabetic complication and may be used to advantage in therapeutic approaches. Further biochemical and pharmacological investigation are underway to elucidate the precise mechanisms and site of action of this drug.

ACKNOWLEDGEMENT

The authors are grateful to The Management of K.S.R. Institutions, Tiruchengode for encouraging this research work and the authors are also thanking to The Management of Nantha College of Pharmacy, Erode for providing animals.

REFERENCES

ADA: Diagnosis and Classification of Diabetes Mellitus. American Diabetes Association. Diabetes Care 32:S62–S67, 2009.

Almadal TP, Vilstrup H: Strict insulin treatment normalizes the organic nitrogen contents and the capacity of urea-nitrogen synthesis in experimental diabetes in rats. Diabetologia 31:114–118, 1988.

Anwar MM, Meki AMR: Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin. Comp Biochem Physiol 135:539–547, 2003.

Augusti KT, Sheela CG: Antiperoxide effect of S-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. Experientia 52:115–120, 1996.

Banerjee SK, Maulik SK: Effect of garlic on cardiovascular disorders: a review. Nutr J 1:4–8, 2002.

Baynes JW: Role of oxidative stress in development of complications in diabetes. Diabetes 40:405–412, 1991.

Becker BF: Towards the physiological function of uric acid. Free Radic Biol Med 14:615–631, 1993.

Block E: The organosulfur chemistry of the genus *Allium*: implications for the organic chemistry of sulfur. Angew Chem Int Ed Engl 31:1135–1178, 1992.

Brod J, Sirota JH: The renal clearance of endogenous "creatinine" in man. J Clin Invest 27:645–654, 1948.

Brosnan JT, Man KC: Interorgan metabolism of aminoacids in STZ induced diabetic rats. Am J Physiol 244:151–158, 1984.

Bunn HF, Shapiro RM, McManus M, Garrick L, McDonald MJ, Gallop PM, Gabbay KH: Structural heterogeneity of human haemoglobin A due to nonenzymatic glycosylation. J Biol Chem 254:3892–3898, 1979.

Caraway WI: Uric acid. In Seligson D (ed.): Standard Methods of Clinical Chemistry, Vol. 4, Academic Press, New York 1963, pp. 239–247.

Carson JF: Chemistry and biological properties of onion and garlic. Food Res Intern 3:71–103, 1987.

Chen K, Ianuzzo CD: Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. Can J Physiol Pharmacol 60:1251–1256, 1982.

Chu Q, Lee DT, Tsao SW, Wang X, Wong YC: S-allylcysteine, a water-soluble garlic derivative, suppresses the growth of a human androgen-independent prostate cancer xenograft, CWR22R, under *in vivo* conditions. BJU Int 99:925–932, 2007.

Defronzo RA, Hendelkar R, Simonson D: Insulin resistance is prominent feature of insulin dependent diabetes. Diabetes 31:795–801, 1982.

Drabkin DL, Austin JH: Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J Biol Chem 98:719–733, 1932.

El-Demerdash FM, Yousef MI, Abou El-Naga NI: Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol 43:57–63, 2005.

Ganong WF: Review of Medical Physiology, 17th ed. Lange Med. Publ., Los Angeles, USA 1995, p. 86.

Garber AJ: The impact of STZ- induced diabetes mellitus on cyclic nucleotide regulation of muscle amino acid metabolism in the rat. J Clin Invest 65:478–487, 1980.

Green M, Miller LL: Protein catabolism and protein synthesis in perfused livers of normal and alloxan-diabetic rats. J Biol Chem 235:3202–3208, 1960.

Herrera-Mundo MN, Silva-Adaya D, Maldonado PD, Galvan-Arzate S, Andres-Martinez L, Perez-De La Cruz V, Pedraza-Chaverri J, Santamaria A: S-allylcysteine prevents the rat from 3-nitropropionic acid- induced hyperactivity early markers of oxidative stress and mitochondrial dysfunction. Neurosci Res 56:39–44, 2006.

Joy KL, Kuttan K: Antidiabetic activity of *Picrorrhiza kurroa* extract. J Ethnopharmacol 67:143–148, 1999.

Kaleem M, Asif M, Ahmed Q, Bano B: Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin induced diabetic rats. Singapore Med J 47:670–675, 2006.

Kim SH, Hyun SH, Choung SYM: Antidiabetic property of cinnamon extract on blood glucose in db/db mice. J Ethnopharmacol 104:119–123, 2006.

King J: The hydrolases-acid and alkaline phosphatases. In Van D (ed.): Practical Clinical Enzymology, Nostrand Co., London 1965a, pp. 199–208.

King J: The transaminases: alanine and aspartate transaminases. In Van D (ed.): Practical Clinical Enzymology, Nostrand Co., London 1965b, pp. 363–395.

Koening RJ, Peterson CM, Jones R: Correlation of glucose regulation and haemoglobin A1C in diabetes mellitus. N Engl J Med 295:417–420, 1978.

Koyama I, Yakushijin M, Goseki M: Partial breakdown of glycated alkaline phosphatases mediated by reactive oxygen species. Clin Chim Acta 275:7–41, 1998.

Kumar G, Sharmila Banu G, Ganesan Murugesan A, Rajasekara Pandian M: Antihyperglycaemic and antiperoxidative effect of *Helicteres isora* L. bark extracts in streptozotocin-induced diabetic rats. J Appl Biomed 5:97–104, 2007.

Kumar PA, Haseeb A, Suryanarayana P, Nasreen Z, Ehtesham G, Bhanuprakash R: Elevated expression of aA- and aB crystallins in streptozotocin-induced diabetic rat. Arch Biochem Biophys 444:77–83, 2005.

Latner A: Clinical Biochemistry. Saunders, Philadelphia 1958, p. 48.

Lowry OH, Rosebrough J, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.

McKenna DJ, Jones K, Hughes K, Humphrey S: Botanical Medicines, the desk reference for majorherbal supplements, Second Edition. The Haworth Herbal Press, New York 2002, p. 28.

Modan M, Halkin H, Karasik A, Lusky A: Elevated serum uric acid a facet of hyperinsulinaemia. Diabetologia 30:713–718, 1987.

Mori DM, Baviera AM, De Oliveira Ramalho LT, Vendramini RC, Brunetti IL, Pepato MT: Temporal response pattern of biochemical analytes in experimental diabetes. Biotechnol Appl Biochem 38:183–191, 2003.

Moriguchi T, Matsura H, Kodera Y, Itakura Y, Katsuki H, Saito H, Nishiyama N: Neurotrophic activity of organosulfur compounds having a thioallyl group on cultured rat hippocampal neurons. Neurochem Res 22:1449–1452, 1997.

Mortimore GE, Manton CE: Inhibition of insulin of valine turnover in liver. J Biol Chem 245:2375–2383, 1970.

Nakagawa S, Kasuga S, Matsuura H: Prevention of liver damage by aged garlic extract and its components in mice. Phytother Res 3:50–53, 1989.

Natelson S, Scott ML, Beffa C: A rapid method for the estimation of urea in biological fluids. Am J Clin Pathol 21:275–281, 1951.

Navarro CM, Montilla PM, Martin A, Jimenez J, Utrilla PM: Free radicals scavenger and antihepatotoxic activity of *Rosmarinus*. Planta Med 59:312–314, 1993.

Nayak SS, Pattabiraman TN: A new colorimetric method for the estimation of glycosylated hemoglobin. Clin Chim Acta 109:267–274, 1981.

Ohaeri OC: Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. Biosci Rep 21:19–24, 2001.

Peters AL, Davidson MB, Schrige DL, Hasselblad VA: Clinical approach for the diagnosis of diabetes mellitus: an analysis using glycosylated haemoglobin levels. Metaanalysis research group on the diagnosis of diabetes using glycated haemoglobin levels. J Am Med Assoc 276:1246–252, 1996.

Pulido N, Suarez A, Casanova B, Romero R, Rodriguez E, Rovira A: Gliclazide treatment of

streptozotocin diabetic rats restores GLUT4 protein content and basal glucose uptake in skeletal muscle. Metabolism 46:10–13, 1997.

Rajkumar L, Govindarajulu P: Increased degradation of dermal collagen in diabetic rats. Indian J Exp Biol 29:1081–1083, 1991.

Ramachandran A, Snehalatha C, Viswanathan V: Burden of type 2 diabetes and its complications-the Indian scenario. Curr Sci 83:1471–1476, 2002.

Rang HP, Dale M, Ritter JKI: The endocrine system pharmacology. In Rang HP, Dale M, Ritter JKI (eds.): Pharmacology, Longman Group. Ltd, London 1991, pp. 505–508.

Sasaki T, Matsy S, Sonae A: Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsho Kagaku 1:346–353, 1972.

Shirwaikar A, Rajendran S, Barik R: Effect of aqueous bark extract of *Garaga pinnata* Roxbin streptozotocin-nicotinamide induced type II diabetes mellitus. J Ethnopharmacol 107:285–290, 2006.

Travlos GS, Morris RW, Elwell MR, Duke A, Rosenblum S, Thompson MB: Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13-week toxicity studies in rats. Toxicology 107:17–29, 1996.

Ueda H, Kaneda N, Kawanishi K, Alves SM, Moriyasu M: A new isoflavone glycoside from *Ceiba pentandra* (L). Gaertner. Chem Pharm Bull (Tokyo) 50:403–404, 2002.

WHO: WHO launches the first global strategy on traditional medicine. WHO/38, Press Release 2002.

Yiping JIA, Ramasamy S, Wood F, Alayash A, Rifkind JM: Crosslinking with O-raffinose lowers oxygenaffinity and stabilizes haemoglobin in a noncooperative T-state conformation. Biochem J 384:367–375, 2004.

Zhang EY, Swaan PW: Determination of membrane protein glycation in diabetic tissue. AAPS PharmSci 20:1–7, 1999.

Zhang XF, Tan BK: Antihyperglycaemic and anti-oxidant properties of *Andrographis paniculata* in normal and diabetic rats. Clin Exp Pharmacol Physiol 27:358–363, 2000.