

ORIGINAL RESEARCH ARTICLE

Efficacy Of Naringin On Hepatic Enzymes Of Carbohydrate Metabolism In Streptozotocin - Nicotinamide Induced Type2 Diabetic Rats

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ABSTRACT

Dietary antioxidant compounds such as bioflavonoid may offer some protection against the early stage of diabetes mellitus and the development of complications. This study was aimed to evaluate the effect of naringin on blood glucose and glycosylated hemoglobin level; hepatic glucose regulating enzyme activities; glycogen concentration and plasma insulin level in normal and streptozotocin (STZ)-nicotinamide induced diabetic rats. To induce non-insulin dependent diabetes mellitus (NIDDM), single intraperitoneal injection of 45 mg/kg STZ, 15 min after the intraperitoneal administration of 110mg/kg of nicotinamide. Diabetic rats were orally administered with naringin (20, 40, and 80 mg/kg body weight) for 6 weeks on glucose and plasma insulin was determined. Administration of naringin at 80 mg/kg significantly decreases blood glucose and increase insulin. Based on these data, the higher dose 80 mg/kg naringin was selected as effective dose for further study. Treatment with naringin resulted in a significant reduction of glycosylated hemoglobin and an increase in total hemoglobin level. Hepatic glucokinase activity and glucose-6- phosphate dehydrogenase were significantly increased whereas the activity of glucose-6-phosphatase and fructose 1,6 bisphosphatase were decreased in diabetic rats administered with naringin. Furthermore, plasma insulin was inversely correlated with the blood glucose level. The current results suggest that naringin can play an important role in preventing the progression of hyperglycemia, partly by increasing hepatic glycolysis and glycogen concentration.

Key Words: Naringin; Antidiabetic; Carbohydrate Metabolic Enzymes; Glycogen .

INTRODUCTION

Diabetic mellitus (DM) is characteristic by hyperglycemic due to disturbance in the group of metabolism of carbohydrates, fat and protein, resulting from defects in insulin secretion action or both^[1-2]. Currently there are over 150 million diabetics worldwide and this number is likely to increase 300 million or more by the year 2025 due to increase in sedentary lifestyle, consumption of energy rich diet, and obesity^[3]. The insulin insensitivity and insulin deficiency in several other animal models of type 2 diabetes mellitus lead to a decrease in blood glucose utilization by the liver, the muscles and the adipose tissue and to an increase in hepatic glucose production^[4]. Glucose homeostasis involves the co-ordinate regulation of several metabolic pathways, including gluconeogenesis and glycolysis, which is due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response^[5]. Recently there has been a growing interest in hypoglycemic agents from

natural products, especially those derived from plants^[6]. Because plant source are usually considered to be non-toxic, with fewer side effects than synthetic sources. Flavonoids cannot be produced by the human body and have taken in through the daily diet^[7]. Naringin (4',5,7-trihydroxy flavanone 7-rhamno-glucoside) is a bioflavonoid derivative of grape fruit and related citrus species. Currently there is much interest in the usefulness of citrus fruits because of their intake appears to be associated with reduced risk of certain chronic diseases and increased survival^[8]. Naringin has been reported to have several pharmacological properties such as antimicrobial, antimutagenic, anticancer, anti-inflammatory, cholesterol lowering, free radical scavenging and antioxidant effects^[9-10]. The present study was designed to evaluate the influence of naringin on blood glucose concentration and the activities of hepatic key enzymes in normal and streptozotocin-nicotinamide induced diabetic rats.

MATERIALS AND METHODS

Chemicals

All the biochemicals and chemicals used in this experiment were from Sigma, St.Louis, M.O. USA. The chemicals were of analytical grade.

Animals and diet

Male Albino Wistar rats weighing 200-220g body weight were obtained from the Central animal house, Rajah Muthiah Institute of Health Science, Annamalai University, were used in this study. The rats were fed on pellet diet (Hindustan lever, Mumbai, India) and water *ad libitum*. The animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian council of Medical Research, Hyderabad, India, and the study was approved by the ethical committee (Vide. No. 582, 2008), Annamalai University.

Induction of Diabetes Mellitus

Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Non-insulin dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 45 mg/kg streptozotocin, 15 min after the i.p administration of 110 mg/kg of nicotinamide [11]. Hyperglycemia was confirmed by the elevated levels of blood glucose were determined at 72 h. The animals with blood glucose concentration more than 250mg/dl will be used for the study.

Experimental Procedure

In the experiment, a total of 36 rats (24 diabetic surviving rats 12 normal rats) were divided into six group of six rats in each: Group 1: Normal rats; Group 2: Normal rats given naringin 80 mg/kg body weight; Group 3: Diabetic control rats; Group 4: Diabetic rats given naringin 20 mg/kg body weight; Group 5: Diabetic rats given naringin 40 mg/ kg body weight; Group 6: Diabetic rats given naringin 80 mg/kg body weight for 6 weeks.

At the end of experimental period, the rats were deprived of food overnight, and blood was collected into potassium oxalate and sodium fluoride tubes for assay of blood glucose, hemoglobin and glycosylated hemoglobin. Plasma was separated for the assay of insulin. Liver was dissected out, washed in ice-cold saline, patted dry and weighed.

Determination of plasma glucose, insulin, hemoglobin and glycosylated hemoglobin

The level of plasma glucose was estimated spectrophotometrically using commercial diagnostic kit (Agappe Diagnostics Pvt. Ltd., India) Trinder [12]. Plasma insulin level was assayed by enzyme linked immunosorbent assay kit (ELISA) (Boehringer Mannheim kit). Hemoglobin and glycosylated hemoglobin was estimated by diagnostic kit (Agappe Diagnostic Pvt. Ltd., India) Bisse and Abragam [13].

Determination of carbohydrate metabolic enzymes

Glucose-6-phosphatase was assayed by the method of Koide and Oda [14]. Incubation mixture contained 0.7 ml of citrate buffer, 0.3 ml of substrate, and 0.3 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 1 h. Addition of 1ml of 10% TCA to the reaction tubes terminated the reaction of the enzyme. The suspension was centrifuged and the phosphorus content of the supernatant of tissue homogenate was estimated by the method of Fiske and Subbarow [15]. The supernatant was adjusted to known volume. To this 1ml of ammonium molybdate was added followed by 0.4 ml of ANSA. After 20 min the absorbance was read at 680 nm.

Fructose-1,6-bisphosphatase activity was measured by the method of Gancedo and Gancedo [16]. The assay mixture in a final volume of 2 ml contained 1.2 ml of Tris-HCl buffer (0.1 M, pH 7.0), 0.1 ml of substrate (0.05 M), 0.25 ml of magnesium chloride (0.1 M), 0.1 ml of potassium chloride (0.1 M), 0.25 ml of EDTA (0.001 M) and 0.1 ml of liver homogenate. The incubation was carried out at 37°C for 15 min. The reaction was terminated by adding 1 ml of 10% TCA. The suspension was centrifuged and the supernatant was used for phosphorus determination by the method of Fiske and Subbarow [15] as described previously.

Hepatic hexokinase activity was assayed by the method of Brandstrup *et al* [17]. The reaction mixture in a total volume of 5.3 ml contained the following, 1ml of glucose (5mM) solution, 0.5 ml of ATP (0.072M) solution, 0.1 ml of magnesium chloride (0.05M) solution, 0.4 ml of potassium dihydrogen phosphate (0.0125 M), 0.4 ml of potassium chloride (0.1 M), 0.4 ml of sodium fluoride (0.5M) and 2.5 ml of Tris-HCl buffer (0.01 M, pH 8.0). The mixture was pre-incubated at 37 °C for 5min. The reaction was

initiated by the addition of 2ml of tissue homogenate. 1ml of the reaction mixture was immediately transferred to the tubes containing 1ml of 10% TCA that was considered as zero time. A second aliquot was removed and deproteinised after 30 min incubation at 37°C. The protein precipitate was removed by centrifugation and the residual glucose in the supernatant of tissue homogenate was estimated by the method of Trinder [12] as described previously.

Glucose-6-phosphate dehydrogenase was assayed by the method of Ellis and Kirkman [18]. The incubation mixture contained 1ml of Tris-HCl buffer (0.05 M, pH 7.5), 0.1 ml of magnesium chloride (0.1 M), 0.1 ml of NADP⁺ (0.1 M), 0.5 ml of phenazine methosulphate, 0.4 ml of the dye solution and the requisite amount of the enzyme extract. The mixture was allowed to stand for 10 min at room temperature to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of glucose-6-phosphate. Absorbance of the sample was read at 640nm against water blank at 1-min intervals for 3–5 min. The enzyme was expressed in units by multiplying the change in OD/min by the factor 6/17.6, the molar extinction co-efficient of the reduced co-enzyme.

Determination of glycogen

Tissue glycogen was extracted and estimated by the method of Morales *et al.* [19] the alkali extract of the tissue was prepared by digesting 50 mg of fresh tissue for 15 min with 3 ml of 30% potassium hydroxide solution in a boiling water bath. The tubes were cooled and mixed with 5 ml of absolute alcohol and a drop of 1 M ammonium acetate to precipitate glycogen and then placed in the freezer overnight for complete precipitation. Glycogen was collected by centrifugation for 20 min at 2000 g. the precipitate was dissolved in distilled water with the aid of heating and again the glycogen was reprecipitated with alcohol/1 M ammonium acetate and centrifuged. The final precipitate was dissolved in saturated ammonium chloride solution; 4 ml of anthrone reagent was added by cooling the tubes in an ice bath. The tubes were shaken well, covered with marble caps, and heated for 20 min in a boiling water bath. After cooling, the absorbance was read at 640 nm against a reagent blank treated in a similar manner. A standard glucose solution was also treated similarly. The glycogen content was calculated from the amount of glucose present in the sample and expressed as

mg/g tissue. Protein was determined by following the method of Lowry *et al* [20].

Statistical Analysis

The data for various biochemical parameters were analyzed for the significance of the difference between mean values were determined using analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a statistically software package (SPSS for Windows, V.13.0, Chicago, USA). Results were presented as mean ± S.D., p < 0.05 were considered as statistically significant [21].

RESULTS

Changes in blood glucose and plasma insulin

Table 1 shows the effect of treatment with the naringin on blood glucose and plasma insulin levels. The concentration of blood glucose was significantly increased whereas the plasma insulin was significantly decreased in diabetic rats. Administration of naringin significantly reversed the changes in dose dependent manner. Naringin at a dose of 80 mg/kg body weight showed a highly significant effect compared with 20 and 40 mg/kg body weight. Based on these data, the higher dose of 80 mg/kg body weight naringin was selected for further study.

Table 1. Changes in the levels of blood glucose and plasma insulin in normal and experimental rats

Groups	Glucose (mg/dl)		Insulin (µU/ml)
	Initial	Final	
Normal control	72.51 ± 6.96 ^a	74.89 ± 6.63 ^a	14.72 ± 1.08 ^a
Normal + naringin (80 mg/kg)	80.48 ± 7.06 ^a	66.42 ± 6.51 ^a	14.85 ± 1.09 ^a
Diabetic control	310.24 ± 27.07 ^b	395.19 ± 32.85 ^b	7.39 ± 0.43 ^b
Diabetic + naringin (20 mg/kg)	292.21 ± 24.95 ^b	184.92 ± 15.23 ^c	8.52 ± 0.56 ^c
Diabetic + naringin (40 mg/kg)	296.68 ± 24.24 ^b	163.19 ± 13.71 ^d	9.68 ± 0.61 ^d
Diabetic + naringin (80 mg/kg)	305.58 ± 26.36 ^b	119.38 ± 10.79 ^e	11.98 ± 0.72 ^e

Values are mean ± SD for 6 rats in each group. ^{a-e} In each column, means with different superscript letter differ significantly at p < 0.05 (DMRT).

Effect of naringin on hemoglobin and glycosylated hemoglobin

Table 2 shows the concentration of total hemoglobin, glycosylated hemoglobin and urine sugar of normal and experimental rats. The diabetic rats showed a significant decrease in the

concentration of total hemoglobin and a significant increase in the concentration of glycosylated hemoglobin. Administration of naringin tends to bring these parameters towards near normal. The administration of naringin to normal rats showed a not significant lowering blood glucose and increasing plasma insulin and also the levels of hemoglobin and glycosylated Hb remains unaltered.

Table 2. Effect of naringin on the levels of hemoglobin, glycosylated hemoglobin and urine sugar in normal and experimental rats

Groups	Hemoglobin (g/dl)	Glycosylated Hemoglobin (mg/gHb)	Urine sugar
Normal control	11.78 ± 0.74 ^a	0.40 ± 0.03 ^a	Nil
Normal + naringin (80 mg/kg)	11.83 ± 0.80 ^a	0.38 ± 0.03 ^a	Nil
Diabetic control	6.91 ± 0.59 ^b	0.82 ± 0.06 ^b	+++
Diabetic + naringin (80 mg/kg)	10.27 ± 0.71 ^c	0.47 ± 0.04 ^c	Nil

Values are mean ± SD for 6 rats in each group. ^{a-c} In each column, means with different superscript letter differ significantly at $p < 0.05$ (DMRT). +++, > 2% sugar

Table 3. Effect of naringin on the activities of carbohydrate-metabolizing enzymes in normal and experimental rats

Groups	Normal control	Normal + naringin (80 mg/kg)	Diabetic control	Diabetic + naringin (80 mg/kg)
Gluconeogenic enzymes				
Glucose-6-phosphatase (μ moles of Pi liberated / min/ mg protein)	0.187 ± 0.016 ^a	0.181 ± 0.015 ^a	0.278 ± 0.021 ^b	0.219 ± 0.017 ^c
Fructose-1, 6-bisphosphatase (μ moles of Pi liberated / min/ mg protein)	0.385 ± 0.032 ^a	0.372 ± 0.031 ^a	0.621 ± 0.045 ^b	0.427 ± 0.029 ^c
Glycolytic enzyme				
Hexokinase (μ moles of glucose phosphorylated / min / g protein)	146.07 ± 10.35 ^a	149.56 ± 10.59 ^a	103.65 ± 8.13 ^b	132.29 ± 9.21 ^c
Hepatic shunt enzyme				
Glucose-6-phosphate dehydrogenase (x 10 ⁻⁴ mIU/mg protein)	4.43 ± 0.25 ^a	4.55 ± 0.27 ^a	2.81 ± 0.13 ^b	3.34 ± 0.19 ^c

Values are mean ± SD for 6 rats in each group. ^{a-c} In each rows, means with different superscript letter differ significantly at $p < 0.05$ (DMRT).

Effect of naringin on glycogen

The effect of naringin on glycogen content in liver and muscle of normal and experimental rats is presented in **Table 4**. There was a significant reduction in liver and muscle glycogen of diabetic rats. Treatment with naringin significantly increased the concentration of hepatic and skeletal glycogen.

DISCUSSION

Type2 diabetes mellitus identifies patients who do not require insulin treatment to remain alive. The two metabolic defects characterizing type2 diabetes mellitus are 1. dearrangement of insulin secretion that is delayed or is insufficient

Effect of naringin on carbohydrate metabolic enzymes

Table 3 shows the change in the activities of glucose-6-phosphatase, fructose – 1 , 6 – bisphosphatase, hexokinase and glucose-6-phosphate dehydrogenase in the liver of normal and experimental rats. The activities of hepatic gluconeogenic enzyme were significantly increased, whereas hexokinase and glucose-6-phosphate dehydrogenase were significantly decreased in experimental diabetic rats. Oral administration of naringin to diabetic rats reversed the changes in the activities of these hepatic enzymes. Oral administration of naringin to normal rats resulted in a not significant decrease in hepatic gluconeogenic enzymes and also the activities of hexokinase and glucose -6-phosphate dehydrogenase were not altered significantly compared with normal rats.

relative to glucose load and 2. Inability to peripheral

Table 4. Effect of naringin on the levels of liver and muscle glycogen in normal and experimental rats

Group	Glycogen (mg/g tissue)	
	Liver	Muscle
Normal control	32.84 ± 2.26 ^a	6.15 ± 0.53 ^a
Normal + naringin (80 mg/kg)	34.25 ± 2.67 ^a	6.21 ± 0.51 ^a
Diabetic control	20.07 ± 1.71 ^b	3.18 ± 0.26 ^b
Diabetic + naringin (80 mg/kg)	29.36 ± 2.15 ^c	5.56 ± 0.41 ^c

Values are mean \pm SD for 6 rats in each group.^{a-c} In each column, means with different superscript letter differ significantly at $p < 0.05$ (DMRT).

tissues to responds to insulin-called insulin resistance^[22]. β -cell defect and insulin resistance are essential features of non-insulin dependent diabetes mellitus, and both features are the focus of intensive investigation^[23]. Major characteristics of type2 diabetes include impaired utilization of glucose and resistance to the ability of insulin to stimulate glucose uptake and disposal in tissues^[24]. Naringin at a dose of 80mg/kg body weight showed a highly significant effect compared with 20 and 40mg/kg body weight. The protective effect of naringin may be connected with the normalization of hyperglycemia; it is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes,

Flavonoids, glycosides stimulate the secretion of insulin in β -cells of pancreas^[25]. In glucose - loaded animals, it is possible that the compound may act by potentiation the pancreatic secretion or increasing glucose uptake. On the basis above evidence it is possible that the presence of glycosides and tannins are responsible for the activity^[26].

In general, increased hepatic glucose production, plus decreased hepatic glycogen synthesis and glycolysis, are the major symptoms in type 2 diabetes that result in hyperglycemia^[27]. Hepatic glucokinase is the most sensitive indicator of the glycolytic pathway in diabetes and its increase can increase the utilization of blood glucose for glycogen storage in the liver^[28]. In the current study, the effect of naringin in experimental diabetic rats increased hepatic glucokinase. Also, hepatic glycogen reserves are important for whole-body glucose homeostasis and are markedly low in the diabetic state^[29-31]. In the current study, the hepatic glycogen concentration was significantly higher in the naringin compared with the control group.

The gluconeogenic enzyme glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis^[32]. These seem to be the consequence of the high glucose-6-phosphatase activities in a diabetic state^[33-35]. Glucose-6-phosphate dehydrogenase activity was decreased in diabetic state can result in the diminished functioning of the pentose phosphate pathway and thereby the production of reducing equivalent

such as NADH and NADPH^[36-37]. In the current study, the administration of naringin considerably increased the activity of glucose-6-phosphate dehydrogenase and decreases the activity of glucose-6-phosphatase, while the decrease in plasma glucose concentration causes the activation of the pentose phosphate pathway, inactivation of the sorbitol pathway and consequently an increase in the NADPH level^[38]. Hepatic glucose production is raised in diabetic state is associated with the impaired suppression of the gluconeogenic enzyme fructose 1,6-bisphosphatase. Gluconeogenic enzyme is activation is due to the state of insulin impairment because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes^[39].

Chronic insulin deficiency and insulin insensitivity are the major causes of the decreased hepatic glucose utilization and increased glucose production in several animal models of type 2 diabetes, because insulin decreases the hepatic glucose output by activating glycogen synthesis and glycolysis, and by inhibiting gluconeogenesis^[4]. In the present study, the level of plasma insulin indices of insulin secretion in the experimental diabetic rats of naringin were significantly higher than those in the control group may have been mediated via the stimulation of insulin secretion in the β -cells, because intact and specific insulin-positive cells were confined to the pancreatic islet β -cells, regardless of naringin^[40].

In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including hemoglobin and β -crystalline of lens^[41]. Glycosylated hemoglobin was found to increase in the patients with diabetes mellitus, and the amount of increase is directly proportional to the fasting blood glucose level^[42]. During diabetes, the excess glucose present in the blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased in diabetic rats^[43]. Administration of naringin prevents a significant elevation in Glycosylated hemoglobin thereby increasing the level of total hemoglobin in diabetic rats.

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen

phosphorylase. Since STZ causes selective destruction of β -cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin for influx of glucose^[31]. That the administration of naringin prevented the depletion of glycogen content but could not normalize it is due to the stimulation of insulin release from β -cells by naringin.

CONCLUSION

The data present here indicate that the protective effect of naringin in hyperglycemic conditions may be associated with an intensification of glucose uptake by peripheral tissues, which seemingly mediated via elevated glycolysis and hepatic glycogen concentration resulting from the effect of glucokinase. In particular, naringin regulated gluconeogenic enzymes suggests the possible biochemical mechanism through which glucose homeostasis are regulated.

REFERENCES

1. Georg P, Ludvik B. Lipids and Diabetes. *J Clin Basic Cardiol* 2000; 3: 159-162.
2. Nyholm B, Porksen N, Juhl CB, Gravholt CH, Butler PC, Weeke J, Veldhuis S, Pincus S, Schimtz O. Assessment of insulin secretion in relatives of patients with type2 (non-insulin-dependent) diabetes mellitus: evidence of early beta-cell dysfunction. *Metabolism* 2000; 49: 896-905.
3. Yajnik CS. The insulin resistance epidemic in India: fetal origins, later lifestyle, or both? *Nutr Rev* 2001; 59:1-9.
4. McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. *Science* 1992; 258:766-770.
5. Reaven GM. Role of insulin-resistance in human diseases. *Diabetes* 1998; 37: 1597-607.
6. Sabu MC, Smitha K, Ramadasan K. Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *J Ethnopharmacol* 2002; 83: 109-116.
7. Ali MM, El Kader MA. The Influence of Naringin on the Oxidative State of Rats with Streptozotocin-Induced Acute Hyperglycemia. *Z Naturforschung* 2004; 59: 726-733.
8. Chen H, Ward MH, Graubard BI. Dietary patterns and adenocarcinoma of the esophagus and distal stomach. *Am J Clin Nutr* 2002; 75: 137-144.
9. Jagetia GC, Reddy TK. Modulation of radiation induced alteration in the antioxidant status of mice by naringin. *Life Sci* 2005; 77: 780-794.
10. Rajadurai M, Stanely Mainzen Prince P. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in wistar rats. *Toxicol* 2007; 230: 178-188.
11. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes* 1998; 47: 224-9.
12. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969; 6: 24-27.
13. Bisse E, Abragam EC. New less temperature sensitive, microchromatographic method for the separation and quantitation of glycosylated haemoglobin using a non cyanide buffer system. *J Chromatogr* 1985; 344: 81-91.
14. Koida H, Oda T. Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. *Clinica Chimica Acta* 1959; 4: 554-561.
15. Fiske CH, Subbarow J. The colorimetric determination of phosphorus. *J Biol Chem* 1925; 66: 375-400.
16. Gancedo JM, Gancedo C. Fructose-1,6-diphosphatase, phospho-fructokinase and glucose-6-phosphate dehydrogenase from fermenting and non fermenting yeasts. *Arch Microbiol* 1971; 76: 132-138.
17. Brandstrup N, Kirk JE, Bruni C. The hexokinase and phosphoglucoisomerase activities of aortic and pulmonary artery tissue in individuals of various ages. *J Gerontol* 1957; 12: 166-171.
18. Ellis HA, Kirkman HN. A Colorimetric method for assay of erythrocyte glucose-6-phosphate dehydrogenase. *Proc Soc Exp Biol Med*, 1961; 106: 607-609.
19. Morales MA, Jabbagy AJ, Tenenzi HP. Mutations affecting accumulation of

- glycogen. Neurospora News Lett 1973; 20: 24-25.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
 21. Duncan BD. Multiple range tests for correlated and heteroscedastic means. Biometrics 1957; 13: 359-364.
 22. Arulmozhi DK, Veeranjanyulu A, Bodhandar SL. Neonatal streptozotocin-induced rat model of type 2 diabetes mellitus: A glance. Indian J pharmacol 2004; 36: 217-221.
 23. Murugan P, Pari L. Antioxidant effect of tetrahydrocurcumin in streptozotocin-nicotinamide induced diabetic rats. Life Sci 2006; 79: 1720-1728.
 24. Shulman GI. Cellular mechanisms of insulin resistance. J Clin invest 2000; 106: 171-176.
 25. Hii CST, Howell SL. Effect of flavonoids on insulin secretion and Ca²⁺ handling in rats islets of Langerhans. J Endocrinol 1985; 107: 1-8.
 26. Chauhan NS, Dixit VT. Antihyperglycemic activity of the ethanolic extract of *Curculigo orchioiodes* Gaertn. Pharmacog Mag 2007; 12, 237-240.
 27. Jung UJ, Lee MK, Jeong KS, Choi MS. The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db mice. J Nutr 2004; 134: 2499-2503.
 28. Iynedjian PB, Gjinovci A, Renold AE. Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats. J Biol Chem 1988; 263: 740-744.
 29. Hornbrook KR. Synthesis of liver glycogen in starved alloxan diabetic rats. Diabetes 1970; 19: 916-923.
 30. Migliorini RH. Early changes in the levels of liver glycolytic enzymes after total pancreatectomy in the rat. Biochimica et Biophysica Acta 1971; 244: 125-128.
 31. Whitton PD, Hems, D.A. (1975). Glycogen synthesis in the perfused liver of streptozotocin-diabetic rats. Biochemical 1975; 150: 153-165.
 32. Mithievre G, Vidal G, Zitovn C, Miriasian C. Glucose-6-phosphatase mRNA and activity are increased to the same extent in liver and kidney of diabetic rats. Diabetes 1996; 45: 891-896.
 33. DeFronzo RA. The triumvirate: beta cell, muscle, liver. A collusion responsible for type2 diabetes. Diabetes 1988; 37: 667-687.
 34. Reaven GM. Banting Lecture 1988. Role of insulin resistance in human disease. Nutr 1997; 13: 64-66.
 35. Guignot L, Mithieux G. Mechanisms by which insulin associated or not with glucose, may inhibit hepatic glucose production in the rat. Am J Physiol Endocrinol Metabol 1999; 277: 984-989
 36. Weber G, Convery HJH. Insulin: Inducer of glucose-6-phosphate dehydrogenase. Life Sci 1996; 5: 1136-1146.
 37. Saraswathi Pannerselvam R, Govindasamy S. Effect of sodium molybdate on carbohydrate metabolizing enzymes in alloxan induced diabetic rats. J Nutr Biochem 2002; 13: 21-26.
 38. Sinclair AJ. Free radical mechanisms and vascular complications of diabetes mellitus. Diabetes Rev 1993; 2: 7-10.
 39. Pari L, Murugan P. Effect of tetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats. J Basic clin physiol pharmacol 2005; 16: 257-274.
 40. Jung UJ, Lee MK, Park YB, Kang MA, Choi MS. Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. Int J Biochem Cell Biol 2006; 38: 1134-1145.
 41. Asgary S, Nader GA, Sarraf-zadegan N, Vakili R. The inhibitory effects of pure flavonoids on in vitro protein glycosylation. J Herb Pharmacother 2002; 2: 47-55.
 42. Goodarzi MT, Zal F, Malakooyi M, Safari MR, Sadeghian S. Inhibitory activity of flavonoids on the lens aldose reductase of healthy and diabetic rats. Acta Medica Iranica 2006; 44: 41-45.
 43. Sheela CG, Augusti KT. Antidiabetic effect of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Indian J Exp Biol 1992; 30: 523-526.