Research Article

FISETIN, A BIOFLAVONOID AMELIORATES HYPERGLYCEMIA IN STZ-INDUCED EXPERIMENTAL DIABETES IN RATS

Sriram Prasath G, Subramanian S*
Department of Biochemistry, University of Madras, Guindy Campus, Chennai 600 025, India.
***Corresponding author's E-mail:** subbus2020@yahoo.co.in

Accepted on: 20-11-2010; **Finalized on:** 15-01-2011.

ABSTRACT

Fisetin, a tetrahydroxy flavone is found to be present in appreciable amounts in strawberries, onion, apple, cucumber and persimmon is known for its potential antioxidant properties. Flavonoids are useful in the treatment of free radical mediated as well as other diseases. The present study was hypothesized to evaluate the hypoglycemic efficacy of fisetin in streptozotocin (STZ) - induced experimental diabetes in rats. The diabetic rats orally treated with fisetin (10 mg/kg b.w/day) for 30 days resulted in significant ($p < 0.05$) decrease in 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 levels of plasma insulin, C peptide and hemoglobin were increased in diabetic rats treated with fisetin. The results are comparable with glyclazide, an oral standard hypoglycemic drug. The present findings indicate that fisetin could be considered as an effective therapeutic agent and /or adjunct for the treatment of diabetes mellitus.

Keywords: Fisetin, hypoglycemic, STZ, glyclazide.

INTRODUCTION

Diabetes Mellitus is an endocrine disorder characterized by altered glucose homeostasis leading to derangements in the carbohydrate metabolism, protein and lipid metabolism, resulting from partial or complete deficiency in insulin synthesis or due to peripheral resistance to insulin action [1]. The prevalence of diabetes is strongly associated with a sedentary lifestyle and high calorie-nutrition and obesity [2]. Oral hypoglycemic drugs used for the treatment of diabetes such as sulfonylureas, biguanides, α- glucosidase inhibitors, thiazolidenediones and insulin leads to undesirable side effects. Hence search for alternative drugs from plant origin would be more effective and pose no side effects. Plant derived phytochemicals such as flavonoids have been reported to exert beneficial effects in diseases such as cancer, diabetes, cardiovascular diseases and neurogenerative disorders. The mechanism through which flavonoids exert their effects remains unclear. However, from the earlier studies it is evident that their antioxidant activity is explanation for cellular response [3, 4].

Flavonoids are naturally occurring polyphenolic phytoconstituents. They are present in fruits, vegetables, tea, wine grains[5]. Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. Flavonoids exhibit a multitude of biological activities such as antioxidant, antibacterial, antiinflammatory, antiallergic, vasodilatory, anticarcinogenic, immune-stimulating, antiviral, and estrogenic effects, as well as being inhibitors of several enzymes such as phospholipase A2, cyclooxygenase, lipoxygenase, glutathione reductase, and xanthine oxidase[6]. Due to their abundance in dietary products and their potential beneficial pharmacological and nutritional effects, the flavonoids are of considerable interest for drug as well as health food supplement.

Fisetin (3, 3', 4', 7 tetrahydroxy flavone), a major plant flavonoid[7] has been reported to down regulate glycogenolysis and gluconeogenesis invitro[8]. It also exerts wide properties such as anticancer[9], as well as inhibition of angiogenesis[10], antiallergic[11], antithyroid effects[12]. Many invitro studies have been performed to study the activities of fisetin. In the presence of fisetin, the formation of glycated hemoglobin is inhibited to certain extent[13]. However, these results were typically based on *in vitro* studies. Very few reports were available on animal models. Plant derived flavonoids have better potential to be used as a therapeutic agent against diabetes. However no systematic studies were found on the effect of fisetin on experimental animal models of diabetes. Hence, the present study was aimed to evaluate the hypoglycemic efficacy of bio-flavonoid fisetin in STZ induced experimental diabetes in rats.

MATERIALS AND METHODS

Experimental Animals

Male albino Wistar rats weighing (160-180g) were purchased from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The rats were housed in polypropylene cages lined with husk. The rats were fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water. The experimental rats were maintained in a controlled environment (12:12 h light/dark cycle and temperature (30 ± 2°C). The experiments were designed and conducted in strict accordance with the ethical

norms approved by Ministry of Social justices & Environment, Government of India and Institutional Animal Ethical Committee guidelines [IAEC NO: 01/079/09]. The rats were acclimatized for one week before starting the experiments.

Acute toxicity studies and dosage fixation studies.

Acute toxicity studies on fisetin were performed in control rats. Graded doses of the fisetin was dissolved in 10% DMSO were administered orally and the animals were observed for 2 weeks following administration. Change in body weight gain, food consumption, fluid intake and psycho-motor activities were clearly monitored.

Dosage fixation studies were carried out by administering graded doses of fisetin (10, 20, and 30 mg/kg body weight) to determine the dose-dependent effect in STZ-induced diabetic rats. We found that 10 mg/kg b.w of fisetin significantly ($p < 0.05$) decreased plasma glucose levels. It was found that fisetin showed maximum hypoglycemic efficacy at a concentration of 10 mg/kg body weight administered orally for 30 days. Hence, the dosage schedule was fixed as 10 mg/kg body weight/rat/day for 30 days.

Induction of experimental diabetes

Rats were fasted overnight and experimental diabetes was induced by intraperitoneal injection of streptozotocin (STZ) with a single dose of 50 mg/kg body weight. STZ was dissolved in freshly prepared 0.1 M cold citrate buffer pH 4.5[14]. Control rats were injected with the 10% DMSO alone. Because STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, STZ-treated rats were provided with 10% glucose solution after 6 h for the next 24 h to prevent hypoglycemia[15]. No adverse effects were observed. After 3 days for development and aggravation of diabetes, rats with moderate diabetes (i.e., blood glucose concentration >250 mg/dL) that exhibited glycosuria and hyperglycemia were selected for the experiment.

Experimental protocol

The animals were divided into four groups, comprising a minimum of six animals in each group as follows:

Group 1 - Control rats receiving 10% DMSO orally.

Group 2 - STZ induced diabetic rats receiving 10% DMSO orally.

Group 3 - Diabetic rats treated with fisetin (10 mg/kg b.w/day) dissolved in 10% DMSO orally for 30 days.

Group 4 - Diabetic rats treated with glyclazide (5 mg /Kg. b.w/day) in aqueous solution orally for 30 days.

The Change in body weight gain in all groups of rats was recorded at regular intervals. After 30 days of treatment, rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected in heparinized tubes.

Oral Glucose Tolerance Test

At the end of the experimental period, a fasting blood sample was collected from all the groups of rats to perform oral glucose tolerance test, rats were fasted for 12 h before the test and 2 g/kg glucose solution was administered orally[16]. Blood samples were taken by severing the tip of the tail 1 h before and at 0.5, 1, 1.5 and 2 h after glucose administration. Blood glucose was determined using ortho toluidine reagent.

Biochemical parameters

Whole blood was used for glucose [17] and urea[18] estimation. Plasma was separated and used for insulin and C-peptide assay using radioimmunoassay (RIA) kit for rats (Linco Research, Inc., USA). Levels of hemoglobin and glycosylated hemoglobin were estimated according to methods of Drabkin and Austin[19]; Nayak and Pattabiraman[20] respectively. Plasma was used for protein assay[21]. Urine sugar was detected using urine strip. Serum was used for the determination of creatinine[22] uric acid[23], Aspartate transaminase(AST), Alanine transaminase(ALT) and Alkaline phosphatase (ALP) were assayed by the method of King [24, 25].

Statistical analysis

All the grouped data were statistically evaluated with SPSS 19.00 software. Hypothesis testing methods included one-way analysis of variance followed by least significant difference test. A value of $p<0.05$ was considered to indicate statistical significance. All results are expressed as mean ± standard deviation (SD) for six rats in each group.

RESULTS

Acute toxicity studies (data not shown) using graded doses of the fisetin revealed no signs and symptoms such as restlessness, respiratory distress, diarrhea, convulsions, and coma up to the dosage of 50 mg/kg b.wt.

Figure 1: Changes in body weight gain of control and experimental groups of rats.

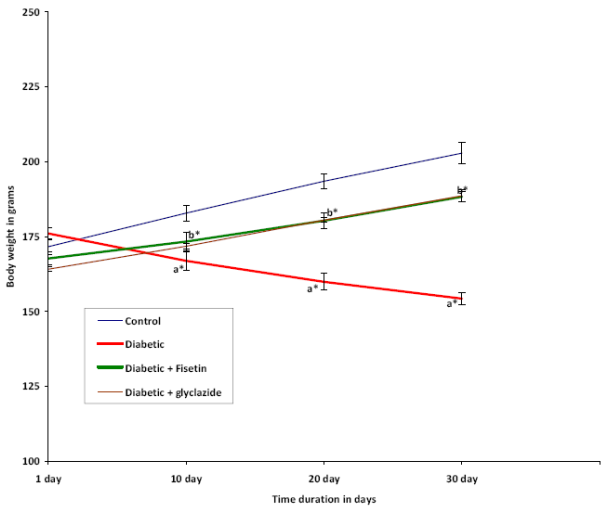

Figure 1 shows the changes in body weight in control and experimental groups of rats. A concomitant increase in body weight was observed in the control group of rats. The body weight was significantly ($p < 0.05$) decreased in STZ-induced diabetic rats when compared to control rats. Oral administration of fisetin as well as glyclazide to STZ-induced diabetic rats significantly ($p < 0.05$) increased the body weight to near normalcy.

Results are expressed as mean ± S.D. [n=6]. One-way ANOVA followed by post hoc test LSD.*p<0.05. The results were compared with [a]control; [b]Diabetic control. [c]Diabetic + Glyclazide.

Figure 2: Effect of fisetin on the blood glucose level in control and experimental groups of rats receiving an oral glucose challenge.

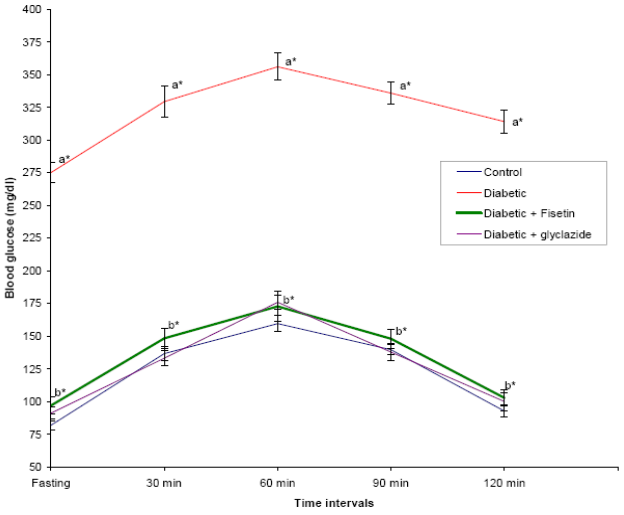

Figure 2 shows the changes on glucose tolerance curve in control and experimental groups of rats. The blood glucose level in the control rats increased to a peak value at 60 min after glucose intake and decreased to near normal level at 120 min. In STZ-induced diabetic rats the peak increase in blood glucose concentration was observed after 60 min and remained high over the next 60 min. Oral administration of fisetin as well as glyclazide on STZ-induced diabetic rats showed significant ($p < 0.05$) decrease in blood glucose concentration at 60 and 120 min when compared with diabetic control suggesting the glucose lowering properties of fisetin as well as glyclazide.

Results are expressed as mean ± S.D. [n=6]. One-way ANOVA followed by post hoc test LSD.*p<0.05. The results were compared with [a]control; [b]Diabetic control. [c]Diabetic + Glyclazide.

Effect of fisetin on the levels of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin, C peptide and urine sugar of control and experimental groups of rats

The levels of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin and urine sugar of control and experimental groups of rats are shown in Table 1. Control rats did not show any significant variation in the blood glucose throughout the experimental period. Administration of STZ led to over 3-fold elevation of blood glucose levels, which was maintained over the experimental period. In experimental rats there was a significant elevation in the levels of blood glucose and glycosylated hemoglobin during diabetes, while the levels of hemoglobin insulin and c peptide were decreased when compared with the control group of rats. Upon oral administration of fisetin and glyclazide, these levels were found to be similar to those of normal rats and the effect was more distinct in the group of rats treated with fisetin. Urine sugar present in diabetic rats was found to be drastically controlled by the rats treated with fisetin and glyclazide.

Effect of fisetin on the levels of total proteins, blood urea, serum uric acid and serum creatinine of control and experimental groups of rats

Table 2 shows the levels of total proteins, blood urea, serum uric acid and plasma creatinine of control and experimental groups of rats. The level of total protein was found to be decreased in STZ induced diabetic rats. The levels of blood urea, serum uric acid and serum creatinine were found to be elevated in STZ induced diabetic rats. These biochemical markers were reverted back to near normalcy upon the oral administration of the flavonoid, fisetin.

Effect of fisetin on serum transaminases and alkaline phosphatase

The activities of AST, ALT and ALP in the serum of control and experimental groups were presented in Table 3. A significant ($p<0.05$) elevation in the levels of AST and ALT were noted in serum of STZ induced diabetic rats. Oral administration of fisetin brought down the activity of AST, ALT and ALP to near normal in serum of diabetic rats.

Table 1: Effect of fisetin on the levels of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin, C peptide and urine sugar of control and experimental groups of rats.

Groups	Blood glucose (mg/dl)	Hemoglobin (g/dl)	Glycosylated hemoglobin (% Hb)	Plasma insulin (µU/ml)	C peptide (µU/ml)	Urine sugar
Control	81.75 ± 3.03	14.40 ± 0.67	6.05 ± 0.55	15.91 ± 0.84	262.96 ± 9.56	Nil
Diabetic control	285.41 ± 9.45[a*]	8.71 ± 0.53[a*]	13.21 ± 0.56[a*]	5.05 ± 0.39[a*]	138.93 ± 6.56[a*]	+++
Diabetic+Fisetin	101.51± 4.32[b*]	12.21 ± 0.48[b*]	7.28 ± 0.33[b*]	10.98 ± 0.93[b*]	205.93 ± 4.48[b*]	Nil
Diabetic+Glyclazide	92.85 ± 2.96[b*]	12.23 ± 0.49[b*]	6.65 ± 0.55[b*]	13.23 ± 0.43[b*]	232.45 ± 4.28[b*]	Nil

+++ indicates more than 2% sugar. Results are expressed as mean ± S.D. [n=6]. One-way ANOVA followed by post hoc test LSD.*p<0.05. The results were compared with [a]control; [b]Diabetic control. [c]Diabetic + Glyclazide

Table 2: Effect of fisetin on the levels of total proteins, blood urea, serum uric acid and serum creatinine of control and experimental groups of rats.

Groups	Total protein (g/dl)	Blood urea (mg/dl)	Serum uric acid (mg/dl)	Serum creatinine (mg/dl)
Control	8.86 ± 0.56	22.38 ± 2.20	2.61 ± 0.53	0.53 ± 0.07
Diabetic control	6.33 ± 0.39 [a*]	45.73 ± 5.16 [a*]	5.31 ± 0.67 [a*]	1.20 ± 0.31 [a*]
Diabetic + Fisetin	7.25 ± 0.30 [b*]	29.33 ± 1.82 [b*]	3.08 ± 0.30 [b*]	0.66 ± 0.07 [b*]
Diabetic + Glyclazide	7.75 ± 0.58 [b*]	30.65 ± 2.93 [b*]	2.70 ± 0.22 [b*]	0.63 ± 0.08 [b*]

Results are expressed as mean ± S.D. [n=6]. One-way ANOVA followed by post hoc test LSD.*p<0.05. The results were compared with [a]control; [b]Diabetic control.

Table 3: Effect of fisetin on serum transaminases and alkaline phosphatase

ENZYMES	Control	Diabetic control	Diabetic + Fisetin	Diabetic + Glyclazide
Serum AST	75.73 ± 5.57	129.38 ± 7.49 [a*]	91.51 ± 3.96 [b*]	83.30 ± 4.04 [b*]
Serum ALT	20.03 ± 1.84	42.41 ± 3.01 [a*]	24.56 ± 2.86 [b*]	21.93 ± 1.56 [b*]
Serum ALP	74.18 ± 3.30	145.98 ± 5.10 [a*]	84.43 ± 4.28 [b*]	82.31 ± 2.41 [b*]

Enzyme activities are expressed as: AST and ALT - μmoles of pyruvate/h/mg of protein;
ALP - μmoles of phenol liberated/min/mg of protein.
Results are expressed as mean ± S.D. [n=6]. One-way ANOVA followed by post hoc test LSD.*p<0.05. The results were compared with [a]control; [b]Diabetic control.

DISCUSSION AND CONCLUSION

Diabetogens such as alloxan and streptozotocin are widely used in the diabetic research to induce diabetes in experimental animals. However, STZ induced diabetes is similar to humans in many features[26]. Streptozotocin (N-nitro derivative of glucosamine) is a cytotoxin specific to the pancreatic beta cells in mammals [27]. STZ injection leads to the degeneration of the beta cells and is irreversible when compared to alloxan[28]. The mechanism behind is that STZ is preferentially uptaken by pancreatic beta cell via GLUT2 transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD^+ and ATP. Enhanced ATP dephosporylation after STZ treatment supplies substrate for xanthine oxidase resulting in the formation of superoxide radicals and also NO moiety is liberated from STZ leading to the destruction of B cells by necrosis[29].

Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting[30] and due to catabolism of tissue proteins[31] leading to significant reduction in the body weight gain of diabetic rats, which was observed in the present study. The reduction in body weight of diabetic rats might have occurred as a result of catabolism of structural proteins due to scarcity of carbohydrate as energy source[32]. Weight loss during diabetes is mainly related to urinary glucose excretion because cells become unable to utilize glucose. Another factor is the osmotic diuresis resulting in hyperosmotic dehydration [33]. A significant increase in the body weight was observed in diabetic rats administered with fisetin which could be due to the protective effect of the flavonoid in controlling muscle wasting and protein turn over and may also be due to the improvement in insulin secretion from the pancreatic beta cells and glycemic control.

The oral glucose tolerance test (OGTT) measures the body's ability to utilize glucose, the body's main source of energy. OGTT is a test of immense value in favor of using fasting plasma glucose concentration to facilitate the diagnosis of diabetes mellitus. It is a more sensitive measure of early abnormalities in glucose regulation than fasting plasma glucose or glycosylated hemoglobin[34]. Impaired glucose tolerance reflects hepatic gluconeogenesis and reduced uptake of glucose from blood by the insulin dependent tissues[35]. The impaired glucose tolerance observed in STZ induced diabetic group of rats were altered to near normal by the treatment with fisetin which proves the insulin stimulatory effects of fisetin from remnant beta cells [36].

Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus[37]. STZ induced diabetes leads to beta cell necrosis that is caused due to diabetic oxidative stress. As a result of beta cell necrosis insulin deficiency predominates resulting in repression of glycolytic enzyme and expresssion of gluconeogenic enzyme which promotes gluconeogenesis in liver, and decreased utilization of glucose by the peripheral tissues contributes to hyperglycemia[38]. The elevated blood glucose level observed in the diabetic rats was significantly decreased in fisetin treated rats suggesting insulin secretory effect of fisetin from the remnant b cells.

Glycosylated hemoglobin is a standard biochemical marker in assessment of diabetes. The HbA1c concentration reflects the patient's average plasma glucose over the previous several weeks making it useful

in assessing diabetic control[39]. A high glucose concentration has been found to lead to the glycosylation of amino groups of lysine residue in proteins. Non-enzymatic glycosylation of protein occurs by direct reaction between reducing sugars and aminogroups in protein. This condition favors reduction in the level of total hemoglobin and elevation in glycosylated hemoglobin, which is directly proportional to blood glucose[40]. Diabetic rats showed higher levels of glycated hemoglobin indicating their poor glycemic control. Oral administration of fisetin to diabetic rats decreased the level of glycosylated hemoglobin and increases the total hemoglobin concentration. Since the glycosylation of protein is an oxidation reaction, flavonoids should be able to prevent this reaction as they are considered as effective antioxidants. It has been reported that fisetin inhibits glycosylation of hemoglobin in vitro[13]. Several researchers have demonstrated that flavonoids attenuate hyperglycemia and there is reduced non-enzymatic glycation of proteins in animals[41].

In the present study, we have observed a significant decrease in the levels of insulin and C-peptide in streptozotocin-induced diabetic rats. C-peptide and insulin are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations [42]. The measurement of both C-peptide and insulin levels have been reported to be a valuable index of insulin secretion rather than insulin alone. In diabetes mellitus, insulin deficiency is manifested in a number of biochemical and physiological alterations. Insulin is synthesized from its precursor proinsulin. C-peptide promotes insulin action at low hormone concentration and inhibits it at high hormone levels suggesting a modulatory effect by C-peptide on insulin signaling[43]. C-peptide has insulinomimetic effects on its own by activating insulin receptor and increases glycogen synthesis and amino acid uptake. Oral administration of fisetin improves the insulin secretion and C peptide level which showed the insulin secretory effect of fisetin.

Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentration of urea in blood[44] and also diabetic oxidative stress induces elevation of the levels of urea, uric acid and creatinine, which are considered as significant markers of renal dysfunction[45]. Administration of fisetin significantly decreased the level of blood urea in STZ induced diabetic rats.

Uric acid is the chief end product of purine catabolism. In diabetes, due to the repression of glycolytic enzyme, glucose is channeled into pentose phosphate pathway resulting in increased availability of ribose 5 phosphate which leads to increased production of PRPP ultimately resulting in high concentration of uric acid in blood. Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid as well as in activity of xanthine oxidase[46]. Oral administration of fisetin decreased the level of uric acid which could have been possible due to its free radical scavenging activity.

Serum creatinine concentration is widely interpreted as a measure of the glomerular filtration rate (GFR) and is used as an index of renal function in clinical practice[47]. Creatinine concentration is the variable used to detect treatment related toxic effects of compounds on the kidney in experimental rats. In the present investigation there is a significant elevation in the levels of creatinine in STZ-induced diabetic rats. Oral treatment with fisetin for 30 days significantly reduced the serum creatinine level.

Aspartate transaminase (AST) and alanine transaminase (ALT) are the enzymes associated with the conversion of amino acids to ketoacids. They are pathophysiological marker enzymes used to assess tissue damage. The observed increase in the activities of ALT, AST, and ALP in serum of diabetic rats may primarily be due to leakage of these enzymes from liver cytosol into bloodstream as a consequence of the hepatotoxic effect of STZ[48]. The rise in serum activities of these enzymes which were formed in the liver and released into the bile is a sign of abnormal secretory function of the liver. As a result, cytoplasmic enzymes such as AST and ALT pass into blood plasma and their activities in serum increase. Restoration of normal levels of these enzymes after treatment with fisetin indicates normal functioning of liver. These results indicate the non toxic and tissue protective nature of fisetin.

The results of the present study clearly indicate that fisetin normalizes hyperglycemia by improving the plasma insulin level thereby maintains the glucose homeostasis. Thus it can be concluded that fisetin could be used as a therapeutic hypoglycemic agent in the treatment of diabetes mellitus.

REFERENCES

1. Scheen AJ, Drug treatment of non-insulin-dependent diabetes mellitus in the 1990s. Achievements and future developments, Drugs, 54(3) 1997, 355-368.
2. King H, Aubert RE, Herman WH, Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections, Diabetes Care, 21(9), 1998, 1414-1431.
3. Rice-Evans C, Flavonoid antioxidants, Current Medicinal Chemistry, 8, 2001, 797–807.
4. Spencer JPE, Schroeter H, Crossthwaithe AJ, Kuhnle G, Williams R J, Rice-Evans C, Contrasting influences of glucuronidation and O-methylation of epicatechin on hydrogen peroxide- induced cell death in neurons and fibroblasts, Free Radical Biology and Medicine, 31, 2001, 1139–1146.
5. Hertog MGL, Hollman PCH, van de Putte B, Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices, Journal of

Agricultural and Food Chemistry, 41,1993,1242–1246.

6. Rice-Evans CA, Miller NJ, Paganga G, Structure–antioxidant activity relationships of flavonoids and phenolic acids, Free Radical Biology and Medicine, 20, 1996, 933–956.
7. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, and Kinae N, Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration, Journal of Nutrition, 130, 2000, 2243–2250.
8. Constantin RP, Constantin J, Pagadigorria CL, Ishii-Iwamoto EL, Bracht A, Ono Mde K, Yamamoto NS, The actions of fisetin on glucose metabolism in the rat liver, Cell Biochemistry and Function, 28(2), 2010, 149-158.
9. Suh Y, Afaq F, Khan N, Johnson JJ, Khusro FH, Mukhtar H, Fisetin induces autophagic cell death through suppression of mTOR signaling pathway in prostate cancer cells, Carcinogenesis, 31(8), 2010,1424-1433.
10. Fotsis T, Pepper MS, Montesano R, Aktas E, Breit S, Schweigerer L, Rasku S, Wähälä K, Adlercreutz H, Phytoestrogens and inhibition of angiogenesis, Bailliere's clinical endocrinology and metabolism, 12(4), 1998, 649-666.
11. Cheong H, Ryu SY, Oak MH, Cheon SH, Yoo GS, Kim KM, Studies of structure activity relationship of flavonoids for the anti-allergic actions, Archives of Pharmacal Research, 21(4),1998,478-480.
12. Divi RL, Doerge DR, Inhibition of thyroid peroxidase by dietary flavonoids, Chemical Research in Toxicology, 9(1), 1996,16-23.
13. Sengupta B, Swenson J. Properties of normal and glycated human hemoglobin in presence and absence of antioxidant, Biochemical and Biophysical Research Communications, 2005, 334(3):954-959.
14. Rakieten N, Rakieten ML, Nadkarni MV, Studies on the diabetogenic action of streptozotocin (NSC-37917), Cancer Chemotherapy Reports, 29, 1963, 91-98.
15. Fischer LJ, Rickert DE, Pancreatic islet cell toxicity, CRC Critical Reviews in Toxicology, 3, 1975, 231-263.
16. Joy KL, Kuttan, R, Anti-diabetic activity of Picrorrhiza kurroa extract. Journal of Ethnopharmacology 167, 1999, 143–148.
17. Sasaki T, Matsy S, Sonae A, Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for the blood glucose estimation, Rinsh kagaku 1, 1972, 346-353.
18. Natelson S, Scott ML, Beffa C, A rapid method for the estimation of urea in biologic fluids, American Journal of Clinical Pathology, 21(3), 1951, 275-281.
19. Drabkin DL, Austin JH, Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood, The Journal of Biological Chemistry, 98, 1932, 719-733.
20. Nayak SS, Pattabiraman TN, A new colorimetric method for the estimation of glycosylated hemoglobin, Clinica Chimica Acta, 109(3), 1981, 267-274.
21. 21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin phenol reagent, The Journal of Biological Chemistry, 193(1) 1951, 265-275.
22. Brod J, Sirota JH, The renal clearance of endogenous creatinine in man, The Journal of Clinical Investigation, 27(5), 1948, 645-654.
23. Caraway WT. Determination of uric acid in serum by a carbonate method, American Journal of Clinical Pathology, 25(7), 1955, 840-845.
24. King J, The transaminases: alanine and aspartate transaminases, In: Practical Clinical Enzymology (Ed.) Van D. Nostrand Co., London, 1965a, 363-395.
25. King J,The hydrolases-acid and alkaline phosphatases, In: Practical clinical enzymology. (Ed.) Van D. Nostrand Co., London, 1965b, 199-208.
26. Hayashi K, Kojima R, Ito M, Strain differences in the diabetogenic activity of streptozotocin in mice, Biological and Pharmaceutical Bulletin 29, 2006, 1110-1119.
27. Weiss RB, Streptozocin: a review of its pharmacology, efficacy, and toxicity, Cancer Treat Rep, 66(3) 1982, 427-438.
28. Ikebukuro K, Adachi Y, Yamada Y, Fujimoto S, Seino Y, Oyaizu H, Treatment of Streptozotocin-induced diabetes mellitus by transplantation of islet cells plus bone Marrow cells via portal vein in rats, Transplantation, 73, 2002, 512-518.
29. Szkudelski T, The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas, Physiological Research, 50(6), 2001, 537-546.
30. Swanston-Flat, S.K., C. Day, C.J. Bailey and P.R. Flatt, Traditional plant treatment for diabetes: Studies in normal and streptozotocin diabetic mice, Diabetologia, 33, 1990, 462-464.
31. Chatterjea MN, Shinde R, Text Book of Medical Biochemistry, Jaypee Brothers Medical Publishers, New Delhi 2002, 317.
32. Pepato MT, Migliorini RH, Goldberg AL, Kettelhut IC, Role of different proteolytic pathways in

degradation of muscle protein from streptozotocin-diabetic rats, American Journal of Physiology, 271(2 Pt 1) 1996, E340-7.

33. Kaplan SA, Lippe BM, Brinkman CR, Davidson MB, Geffner ME, Diabetes mellitus, Annals of Internal Medicine, 96(5), 1982, 635-49.
34. Singleton JR, Smith AG, Russell JW, Feldman EL, Microvascular complications of impaired glucose tolerance, Diabetes, 52(12), 2003, 2867-2873.
35. Robertson RP, Estimation of beta-cell mass by metabolic tests: necessary, but how sufficient? Diabetes, 56(10), 2007 2420-2424.
36. Hii CS, Howell SL, Effects of flavonoids on insulin secretion and 45Ca2^{+} handling in rat islets of Langerhans, Journal of Endocrinology, 107(1), 1985,1-8.
37. Luzi L, Pancreas transplantation and diabetic complications, The New England Journal of Medicine, 339, 1998, 115-117.
38. Giugliano D, Ceriello A, Paolisso G, Oxidative stress and diabetic vascular complications, Diabetes Care 19, 1996, 257–267.
39. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A, Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus, The New England Journal of Medicine, 295(8), 1976, 417-420.
40. Al-yassin D, Ibrahim K, A minor haemoglobin fraction and the level of fasting blood glucose, J Faculty Med, 23, 1981,373-80.
41. Anjaneyulu M, Chopra K, Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats, Clinical and Experimental Pharmacology and Physiology, 31(4) 2004, 244-248.
42. Steiner DF, On the role of the Proinsulin C-peptide, Diabetes, 27, 1, 1978, 145-148.
43. Grunberger G, Qiang X, Li Z, Mathews ST, Sbrissa D, Shisheva A, Sima AA, Molecular basis for the insulinomimetic effects of C-peptide, Diabetologia,44(10), 2001,1247-1257.
44. Asayama K, Nakana T, Uchida N, Hayashibe H, Dobashi K, Nakazawa S Serum antioxidant status in streptozotocin-induced diabetic rat. Hormone and Metabolic Research ,26, 1994, 313-315.
45. Almdal TP, Vilstrup H, Effects of streptozotocin-induced diabetes and diet on nitrogen loss from organs and on the capacity of urea synthesis in rats, Diabetologia, 30(12), 1987, 952-956.
46. Anwar MM, Meki AR, Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin, Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 135(4), 2003, 539- 547.
47. Perrone RD, Madias NE, Levey AS, Serum creatinine as an index of renal function: new insights into old concepts, Clinical Chemistry, 38(10), 1992, 1933-1953.
48. El-Demerdash FM, Yousef MI, El-Naga NI, Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats, Food and Chemical Toxicology, 43(1), 2005, 57-63.

****