Beneficial effects of mangiferin isolated from *Salacia chinensis* on biochemical and hematological parameters in rats with streptozotocin-induced diabetes

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Abstract: *Salacia chinensis* L. is a traditional Southeast Asian herbal medicine and used in the treatment of diabetes. To investigate the antidiabetic properties of mangiferin from *Salacia chinensis* and its beneficial effect on toxicological and hematological parameters in streptozotocin induced diabetic rats. Mangiferin was orally treated with the dose of 40 mg/kg body weight/day for 30 days to diabetic rats. Biochemical (blood glucose, uric acid, urea and creatinine), toxicological (AST, ALT and ALP) and hematological parameters (red and white blood cells) and their functional indices were evaluated in diabetic treated groups with mangiferin and glibenclamide. Mangiferin treated diabetic rats significantly ($p<0.05$) lowered the level of blood glucose, in addition, altered the levels of biochemical parameters including urea, uric acid, and creatinine. Toxicological parameters including AST, ALT and ALP were also significantly reduced after treatment with mangiferin in diabetic rats. Similarly, the levels of red blood, white blood cells and their functional indices were significantly improved through the administration of mangiferin. Thus, our results indicate that mangiferin present in *S. chinensis* possesses antidiabetic properties and nontoxic nature against chemically induced diabetic rats. Further experimental investigations are warrant to make use of its relevant therapeutic effect to substantiate its ethno-medicinal usage.

Keywords: Diabetes, hematological parameters, mangiferin, *Salacia chinensis*, streptozotocin.

INTRODUCTION

Diabetes mellitus is a syndrome, primarily considered as a loss of glucose homeostasis because of the insulin action and/or secretion or both. Current epidemiological data represented that there are more than 150 million people in the world affected by diabetes mellitus and probably, increases up to 300 million or even more in 2025 (Wild *et al*., 2004). Based on the World Health Organization (WHO) report, diabetes is an ever-growing endocrine disorder. All parts of global population are affected by diabetes whereas higher in Asia and Europe countries (World Diabetes Foundation [WDF], 2010). Specific concern for this rise based on the epidemiological parameters which include sedentary lifestyle, intake of energy rich diet, and obesity (American Diabetes Association, 2010).

Diabetes has been treated with standard drugs which act as hypoglycemic components and/or insulin production modulators (Ogbonnia *et al*., 2008). Type 2 diabetes is treated with sulfonylurea and metformin whereas they are not frequently maintain the blood glucose level within the normal range (Senthilvel *et al*., 2006). These drugs are limited pharmacokinetic activities, even though when effective glycaemic control is achieved, the use of these drugs is restricted by their pharmacokinetic properties, secondary failure proportions and accompanied adverse effects (Egwim, 2005). Meanwhile the remedy is lifelong therapeutic agents lack of adverse property should be appreciated and one among the approach is the use of alternative system of medicine comprising herbal products (Arulselvan and Subramanian, 2008). Hence, there is a great need for a search of a suitable, inexpensive and safe blood glucose reducing effective remedy for diabetes and also useful to avoid adverse effects from presently available oral anti-diabetic drugs.

Traditional drugs of numerous ethnic societies and epidemiological records are primary source to find out a natural products or their active ingredient for the prevention or remedy of chronic illnesses (Ribeiro and Salvadori, 2003). Nowadays looking for nontoxic and effective medicinal plants, those having antioxidant and anti-diabetic properties are very essential for the treatment of chronic metabolic syndrome including diabetes.

Mangiferin is a xanthone glucoside and an active phytochemical present as a principal constituent of *Salacia chinensis* (Periyar selvam *et al*., 2009). *Salacia chinensis* Linn. (Hippocrateaceae) roots are used in indigenous system of medicine. Mangiferin is also termed C-glucosyl xanthone and 2-β-D-glucopyranosyl-1,3,6,7-

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tetrahydroxy xanthone. Mangiferin have more therapeutic attention because of its strong anti-oxidant (Ghosh *et al*., 2011), anti-diabetic (Miura *et al*., 2001; Sellamuthu *et al*., 2012), and anti-apoptotic properties (Campos-Esparza *et al*., 2009). Mangiferin also act as immunomodulatory drug by inhibiting NFκB activation and sequence of pro-inflammatory cytokines (Leiro *et al*., 2004). Xanthone have the capacity to control the genes expression which involved a main role in the regulating apoptosis and inflammation.

The objective of the current study was to investigate the possible beneficial effect of mangiferin from *S. chinensis* on streptozotocin induced changes in levels of various toxicological and hematological parameters indices in diabetic rats.

MATERIALS AND METHODS

Chemicals

The streptozotocin was procured from Sigma Chemicals; St. Louis, MO, U.S.A. Solvents for all biochemical assays were obtained from SD fine chemicals Ltd., India. The analytical graded chemicals were used for all the experiments.

Plant material

The roots of *S. chinensis* were harvested from Veenangaputtu, Karumpakkam, Thangal and Kurumpuram, Puducherry, India. Plant identification was done by plant taxonomist (Prof. K. Murugesan, Centre for Advanced studies in Botany, University of Madras) and specimen (778) has been deposited in CAS in Botany, University of Madras, Tamilnadu, India.

Extraction and isolation of mangiferin

The extraction and isolation of mangiferin from the root of *S. chinensis* was done by Periyar Selvam *et al*. (2009) method. Purification of mangiferin compound was performed by column chromatography and purity of mangiferin was confirmed through high performance liquid chromatography. The authentic mangiferin was procured from Sigma Aldrich Company (St. Louis MO, U.S.A.), when compared with authentic sample; the isolated mangiferin was closely resembled with authentic purity that was found to be 99.4% (w/w) and its molecular weight: 422.35 (Periyar selvam *et al*., 2009) (Data not shown).

Experimental animals

Male adult Wistar rats (around 150-200/g) were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India and were housed in polycarbonate cages (animal house) at standard optimized temperature of 22 ± 2˚C and humidity of 45-60%. They were fed with commercial pelleted rats food (Hindustan Lever Ltd., Bangalore, Karnataka, India), and allowed to free access the water *ad libitum*. The pre-clinical animal experiments were carried out according to the standard ethical norms which was approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No.02/004/06).

Induction of experimental diabetes

Diabetes mellitus (moderate type 1) was induced by intraperitonial streptozotocin injection (single dose 55 mg/kg b.wt) in 0.1M cold citrate buffer (pH 4.5) in a volume of 1 ml/kg b.wt to the overnight fasted rats (Periyar Selvam *et al*., 2009). The citrate buffer alone was injected to control rats. They were access to drink 5% glucose solution overnight to overcome the initial drug-induced hypoglycemia (Arulselvan and Subramanian, 2007). Hyperglycaemia was confirmed 7 days after STZ-induction via determination of blood glucose concentration. Diabetic induced animals with a fasting (16 hr) blood glucose range of 250 to 300 mg/dl were selected for this animal experiment.

Experimental design

In the experiment, a total of 24 rats (6 normal rats and 18 streptozotocin-induced diabetic rats) were used and divided into four groups of six each.

Group 1: Control rats (Normal Control)

Group 2: Diabetic control rats

Group 3: Diabetic rats treated with mangiferin (40 mg/kg b.wt/day) in aqueous solution orally for 30 days (Periyar Selvam *et al*., 2009)

Group 4: Diabetic rats treated with 0.6 mg/kg b.wt/day of glibenclamide (Arulselvan *et al*., 2006).

After end of the total experiment period (30 days), the rats were deprived of food overnight and anesthetized and sacrificed through cervical dislocation. Blood was collected in heparinised tubes for estimate various biochemical and hematological parameters.

Biochemical estimations

Fasting blood glucose was assessed by O-toluidine method (Sasaki *et al*., 1972). Blood urea (Natelson *et al*., 1951), serum creatinine (Brod and Sirota, 1948), and uric acid (Caraway, 1963) were estimated via specific standardized methods. The activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were performed according to the method of King (1965a and 1965b).

Determination of hematological parameters

Hematological parameters including red blood cells (RBCs), white blood cells (WBCs), and platelets from blood samples were assessed with an auto analyzer (MISPA-EXCEL, Japan).

STATISTICAL ANALYSIS

All data were expressed as mean ± standard deviation for six animals in every group. The data were statistically evaluated with Statistical Package for Social Scientists (SPSS) software package version 16.0 Hypothesis testing methods comprised of one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. Values were statistically considered as significant at $P<0.05$.

RESULTS

Basic biochemical parameters

Blood glucose, urine sugar, urea, creatinine and uric acid in control and experimental groups are revealed in tables 1 and 2. There was significant change in the levels of blood glucose, urea, creatinine, uric acid and urine sugar were observed in mangiferin and glibenclamide administered diabetic rats, while compared with untreated diabetic rats. The urine sugar was normalized in mangiferin and glibenclamide treated diabetic rats, due to the regularization of blood glucose level. The mangiferin and glibenclamide treated diabetic rats significantly prevented the elevated kidney functional parameters including urea, creatinine and uric acid levels.

Table 1: The levels of blood glucose and urine sugar in control and experimental groups of rats

Groups	Blood glucose (mg/dl)	Urine sugar
Control	84.92±5.60	Nil
Diabetic control	252.27±18.92[a*]	+++
Diabetic+mangiferin	98.73±6.51[b*]	Nil
Diabetic+glibenclamide	95.62±6.11[c*]	Nil

Table 2: The levels of urea, serum creatinine and uric acid of control and experimental groups of rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	19.15± 0.88	0.85± 0.04	2.64± 0.06
Diabetic control	38.11± 2.09[a*]	3.62± 0.20[a*]	5.27± 0.18[a*]
Diabetic+ mangiferin	19.85± 0.93[b*]	0.97± 0.05[b*]	2.95± 0.07[b*]
Diabetic+ glibenclamide	20.76± 0.95[c*]	1.26± 0.05[c*]	2.98± 0.08[c*]

Data were given as mean ± standard deviation for six animals in each group. One way ANOVA followed by *post hoc* test LSD. Values are statistically significant at *P<0.05.
[a]Diabetic control rats were compared with control rats.
[b]Mangiferin treated diabetic rats were compared with diabetic control rats.
[c]Glibenclamide treated diabetic rats were compared with diabetic control rats.
+++ - Above 2% of sugar.

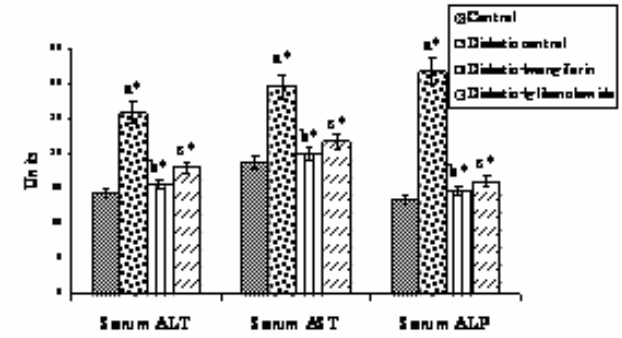

Fig. 1: Activities of ALT, AST and ALP in the serum of control and experimental groups of rats
Data were given as mean ± standard deviation for six animals in each group. One way ANOVA followed by post hoc test LSD. *P<0.05.
[a]Diabetic control rats were compared with control rats.
[b]Mangiferin treated diabetic rats were compared with diabetic control rats.
[c]Glibenclamide treated diabetic rats were compared with diabetic control rats.
Units: AST and ALT - µmoles of pyruvate/h/mg of protein; ALP - KA Units/l

Activities of ALT, AST and ALP in the serum of control and experimental groups are illustrates in fig. 1. There was a significant increase in the activities of ALT, AST and ALP in serum of diabetic rats when compared to that of untreated diabetic rats. The activities of ALT, AST and ALP were decreased in the mangiferin and glibenclamide treated diabetic rats, which were nearly equivalent to that of control rats.

Hematological parameters

The hematological parameters such as red blood corpuscles (RBC), white blood corpuscles (WBC) and platelets are represents in fig. 2. The content of RBC and WBC were significantly reduced in streptozotocin induced diabetic rats, while compared to control rats. The content of platelets was significantly elevated in diabetic rats when compared to untreated diabetic rats. The levels of these parameters were nearly comparable to that of control rats after administration with mangiferin and glibenclamide.

DISCUSSION

Streptozotocin is used as a well known chemical agent to induce experimental diabetes mellitus by specific cytotoxicity effect on pancreatic β-cells. Streptozotocin was employed to chemically induce diabetes in experimental animal models and affects the endogenous insulin release/action or both and as a result increases fasting blood glucose level (Nastaran, 2011). In this present study, we used well known anti-diabetic drug

glibenclamide for comparison of potential therapeutic effect of mangiferin. Glibenclamide is a standard anti-diabetic drug that stimulates insulin secretion from β-cells of islets (Bedoya *et al*., 1996).

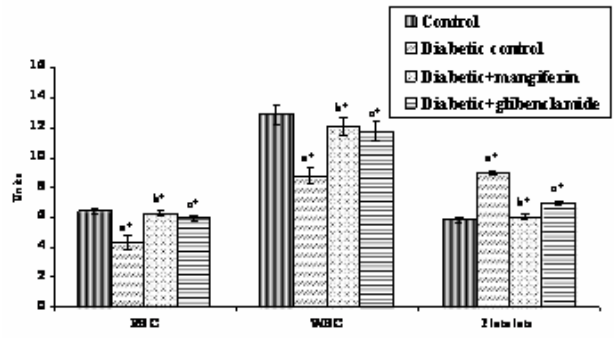

Fig. 2: The levels of RBC, WBC and platelets in control and experimental groups of rats.

Data were given as mean ± standard deviation for six animals in each group. One way ANOVA followed by post hoc test LSD. *P<0.05.

[a]Diabetic control rats were compared with control rats.

[b]Mangiferin treated diabetic rats were compared with diabetic control rats.

[c]Glibenclamide treated diabetic rats were compared with diabetic control rats.

Units: RBCX 10^6mm-3; WBCX 10^6mm-3; Platelets X 10^6mm-3

The intraperitoneal administration of mangiferin exhibited anti-diabetic, anti-hyperlipidemic and anti-atherogenic properties in diabetic rats. It also showed the development in oral glucose tolerance in glucose-load normal rats without causing hypoglycemic (Muruganandan *et al*., 2005). In the present study, it was observed that due to oral administration of mangiferin, 40 mg/kg of b.wt/day for 30 days exhibited significant anti-diabetic activity without any considerable toxicity.

Blood glucose level was considerably lowered in the glibenclamide treated STZ-induced diabetic rats. *Momorodica charantia* seed extract treatment activates the β-cells and granulation returns to normal insulinogenic effect in mild streptozotocin induced diabetic rats (Kedar and Chakrabarti, 1982). The previous reports are consistent with that of present study. The declined level of blood glucose was observed in present study, which indicated that mangiferin stimulates insulin secretion from the remnant or regenerated β-cells (Periyar Selvam *et al*., 2009).

Increases in the levels of kidney functional markers such as urea, uric acid, and creatinine may have been due to STZ-induced metabolic disturbances, and renal dysfunction. Indication of non-protein nitrogenous substances levels are always used as potential markers for the evaluation of renal dysfunction (Arulselvan *et al*., 2006; Almadal and Vilstrup, 1988).

Urea is the central end product of protein catabolism in the living system. The diabetic animal manifested a negative nitrogen stability related to improved proteolysis in muscles and other vital tissues, which is associated with dropping the protein synthesis and increasing the blood urea level (Rannels *et al*., 1997). Modification in nitrogen homeostasis status might leads to elevated hepatic dismissal of urea nitrogen and elevated peripheral release of nitrogenous constituents. In diabetes, insulin treatment leads to normalized nitrogen contents through metabolism of urea synthesis (Almadal and Vilstrup, 1988). The treatment of mangiferin to diabetic rats significantly reduced the level of blood urea suggesting that prophylactic nature in protein metabolism.

Uric acid is key endogenous water-soluble antioxidants of the human body and metabolic end product of purine metabolism. Protein glycation was elevated in streptozotocin induced diabetes which related to increased muscle wasting and, thereby, raises the release of purines. The level of uric acid was significantly increased in diabetic condition due to the excessive production of purine and activity of xanthine oxidase via the metabolic pathway. Uric acid is further degraded to allantoin via the urate oxidase (uricase) enzyme in the majority of mammals and this is easily metabolized and excreted from the body in the urine (Anwar and Meki, 2003; Kutzing and Firestein, 2008). Increase the levels of uric acid is one among the risk factor of cardiovascular illness (Maxwell and Brainsma, 2001).

In the current study, uric acid level was increased in streptozotocin induced diabetic rats, might be due to disturbance in metabolic activity that increased activities of lipid peroxidation, triglyceride and cholesterol (Madianov *et al*., 2000). The mangiferin and glibenclamide treated diabetic rats showed near-normal level of uric acid. Therefore, the increased level of uric acid may be diagnostic indicator of protection of body against harmful properties of free radicals through endogenous antioxidants (uric acid) and this result suggests that antioxidant nature of mangiferin (table 2).

Creatinine is a derivative of creatine and phosphocreatine these are considered as an energy storage compounds in muscle. The variable concentration of creatinine is not only used to evaluate the deficiency of kidney function, whereas used to detect, treatment associated toxic properties of compounds/drugs in the kidney of experimental rats (Travlos *et al*., 1996). Creatinine was increased in diabetes patients, which might leads to renal dysfunction (Mulec *et al*., 1990). Mangiferin and glibenclamide treated diabetic rats were significantly reduced the level of creatinine, which suggested that mangiferin treatment produced beneficial effects to kidney in diabetic rats (table 2).

The liver plays an essential role in the maintenance of various metabolism especially glucose metabolism. AST, ALT and ALP in serum were increased owing to outflow of those enzymes from the liver cytosol into the blood stream (Navarro *et al*., 1993) that was a sign of hepatotoxic nature of streptozotocin. The hepatic impairment of streptozotocin was confirmed through histopathological changes in the liver and also elevate in the activities of liver marker enzymes (Ghosh and Suryawansi, 2001).

The present findings indicate that significant increases in activities of AST and ALT of streptozotocin diabetic rats which are concomitant with the Arulselvan *et al*. (2006). The elevation of these enzymes may also reflect the damage of the hepatic cells. On the other hand, other researchers have postulated that diabetes could induce defects in sarcolemmal enzymatic activities (Micheal *et al*., 1985) which lead finally to such effects.

Increase of protein catabolism is accompanied by gluconeogenesis and urea formation in diabetic condition that may be reason for raise of transaminases in vital tissues. Nanbora *et al*. (1990) also observed that the elevated activities of ALT and AST in the liver of diabetic mice were thought to be consistent with their greater need for gluconeogenic substrates. The increased activities of ALP and ACP in serum were related to the liver damage, observed in alloxan-induced diabetic rats (Dutt and Sarkar, 1993). The serum ACP and ALP activities were higher in streptozotocin induced diabetic rats (Mori *et al*., 2003). The serum ALP activities were increased in the liver and released into the bile, which might lead to loss of the secretory function of the liver (Baraona *et al*., 1980).

Excessive release and absorption of ALP activity from the intestine was due to the possible reason for increased serum ALP activity in diabetic rats (Navarro *et al*., 1993). ALP activity is considered as good indication on the hepatotoxic nature of streptozotocin. AST, ALT and ALP activities were increased in serum and their activities were decreased in the liver may be owing to the discharge of these enzymes in the liver. AST, ALT and ALP activities were return back to near normal in mangiferin treated streptozotocin induced diabetic rats which indicated the defensive and non-toxic nature of the phytocompound, because ALP level acts as indicator of liver function (fig. 1).

Evaluation of various haematological parameters could be used to reveal the deleterious effect of different foreign compounds including phyto-extracts on the blood constituents of experimental animals. They are also used to determine possible alterations in the levels of bio-molecules such as enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs (Magalhaes *et al*., 2008).

In diabetic condition, incidence of anemia is due to increase in the non-enzymatic glycosylation of RBC membrane proteins that related with increase of glucose level. The RBC has shortened life span during diabetes, because of the non-enzymatic glycosylation of red cell membrane proteins. The mangiferin treated diabetic rats showed nearly normal RBC count.

The level of RBC in the diabetic animals were drastically reduced which may be attributed to the infections on the body systems. This result agrees with report of Baskar *et al*. (2006) who reported anti-diabetic property of *Rubia cordifolia* (root extract) in streptozotocin induced diabetic rats. The changes of this hematological parameter are well known to cause anaemic condition in human (Balasubraimanian *et al*., 2009).

Following phytocompound, mangiferin administration, the level of RBC and its related indices were appreciably improved especially at 40 mg/kg bwt. This gives an indication that the mangiferin can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is an important glycoprotein hormone which induces stem cells (bone marrow) to create the red blood cells (Ohlsson and Aher, 2006). These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood.

Streptozotocin is a well-known chemical that suppresses the immune system by damaging WBC and certain organs in the body. The intraperitoneal injection of streptozotocin into rats significantly reduced the WBC count and its related indices. The reduction of these parameters could be correlated to suppression of leukocytosis from the bone marrow which may account for poor defensive mechanisms against infection (Oyedemi *et al*., 2010). The white blood cell count and its related indices were significantly restored to near normal after oral administration of mangiferin.

Platelet aggregation ability has been shown in diabetic patient with long term poor glycaemic control due to lack or deficiency of insulin (Jarald *et al*., 2008). Rising of platelet count has create two major complication, one is elevate the clotting or defense beside bleeding and other one is rise the insulin resistance which leads to undesired effect against cardiovascular function. Platelet count was raised in non-obese diabetic patients that may autonomous presumption of insulin resistance (Taniguchi *et al.*, 2003). If platelet count was elevated to two fold that leads to risk factor of cardiovascular function. During the cancer, increased of platelet was bring adverse prognosis in both animal trial and patients (Kaushansky, 2009). The mangiferin and glibenclamide treated diabetic rats showed the normal level of WBC and platelet that prove the normoglycemic efficacy of the drug (fig. 2).

CONCLUSION

The findings of the present investigation obviously specify that oral administration of mangiferin from *S. chinensis* to diabetic rats significantly lower the blood glucose level. Oral administration of mangiferin had a similar effect when compared to glibenclamide in reducing the alteration in various biochemical, toxicological and hematological parameters in streptozotocin induced diabetic rats. This oral administration of mangiferin could be used as an effective anti-diabetic agent.

REFERENCES

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

American Diabetes Association (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **34**: 62-69.

Anwar MM and Meki AMR (2003). Oxidative stress in streptozotocin-induced diabetic rats: Effects of garlic oil and melatonin. *Comparative Biochem. Physiol.,* **135**: 539-547.

Arulselvan P, Senthilkumar GP, Sathish Kumar D and Subramanian S (2006). Anti-diabetic effect of *Murraya koenigii* leaves on streptozotocin induced diabetic rats. *Pharmazie.,* **61**: 874-877.

Arulselvan P and Subramanian SP (2007). Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic beta-cells in experimental diabetes in rats. *Chem. Biol. Interact.,* **165**: 155-164.

Arulselvan P and Subramanian S (2008). Ultrastructural and biochemical abnormalities in the liver of streptozotocin-diabetic rats: Protective effects of *Murraya koenigii. J. Pharmacol. Toxicol.,* **3**: 190-202.

Balasubraimanian T, Lal MS, Mahananda S and Chatterjee TK (2009). Antihyperglycaemia and antioxidant activities of medicinal plant *Stereospermum suaveolens* in streptozotocin-induced diabetic rats. *J. Diet. Suppl*., **6**: 227-251.

Baraona E, Pikkarainen P, Salapuro M, Finkelman F and Lieber CS (1980). Acute effects of ethanol on hepatic protein synthesis and secretion in the rat. *Gastroentrol*, **79**: 104-111.

Baskar R, Bhakshu LM, Bharathi GV, Reddy SS, Karuna R, Reddy GK and Saralakumari D (2006). Antihyperglycemic activity of aqueous root extract of *Rubia cordifolia* in streptozotocin-induced diabetic rats. *Pharm. Biol*., **44**: 475-479.

Bedoya FJ, Solano F and Lucas M (1996). N-monomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. *Experientia*, **52**: 344-347.

Brod J and Sirota JH (1948). The renal clearance of endogenous "creatinine" in man. *J. Clin. Invest*, **27**: 645-654.

Campos-Esparza MR, Sánchez-Gómez MV and Matute C (2009). Molecular mechanisms of neuroprotection by two natural antioxidant polyphenols. *Cell Calcium*, **45**: 358-368.

Caraway WI (1963). Uric acid. D. Seligson (Ed.), Standard Methods of Clinical Chemistry, Vol. 4, Academic Press, Pp.239-247.

Dutt P and Sarkar AK (1993). Alterations in rat intestinal sucrose and alkaline phosphatase activities in alloxan induced experimental diabetes. *Indian J. Biochem. Biophys*., **30**: 177-180.

Egwim E (2005). Hypoglycaemic potencies of crude ethanolic extracts of Cashew roots and unripe Pawpaw fruits in guinea pigs and rats. *J. Herb. Pharmacother*, **5**: 27-34.

Ghosh J, Das J, Manna P and Sil PC (2011). The protective role of arjunolic acid against doxorubicin induced intracellular ROS dependent JNK-p38 and p53 mediated cardiac apoptosis. *Biomaterials*, **32**: 4857-4866.

Ghosh S and Suryawansi SA (2001). Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. *Indian J. Exp. Biol*., **39**: 748-759.

Jarald E, Joshi SB and Jain DC (2008). Diabetes and herbal medicines. *Iran. J. Pharmacol. Ther*., **7**: 97-106.

Kaushansky K (2009). Determinants of platelet number and regulation of thrombopoiesis. *Hematology*, **1**: 147-152.

Kedar P and Chakrabarti CH (1982). Effects of bittergourd (*Momordica charantia*) seed and glibenclamide in streptozotocin induced diabetes mellitus. *Indian J. Exp. Biol*., **20**: 232-35.

King J (1965a). *The transaminases: Alanine and aspartate transaminases.* Practical clinical enzymology. Van Nostrand Reinhold Co., London, Pp.363-395.

King J (1965b). The hydorlases-acid and alkaline phosphatases. Practical clinical enzymology. Van Nostrand Reinhold Co., London, Pp.199-208.

Kutzing MK and Firestein BL (2008). Altered uric acid levels and disease states. *J. Pharmacol. Exp. Ther.,* **324**: 1-7.

Leiro J, Arranz JA, Yanez M, Ubeira FM, Sanmartin ML and Orallo F (2004). Expression profiles of genes involved in the mouse nuclear factor-kappa B signal transduction pathway are modulated by mangiferin. *Int. Immunopharmacol*., **4**: 763-778.

Madianov IV, Balabolkin MI, Markov DS and Markova TN (2000). Main causes of hyperuricemia in diabetes mellitus. *Ter. Arkh*., **72**: 55-58.

Magalhaes P, Appell H and Duarte J (2008). Involvement of advanced glycation end products in the pathogenesis of diabetes complication: The protective role of regular physical activity. *Eur. Rev. Aging Phys. Act*., **5**: 17-29.

Maxwell AJ and Brainsma KA (2001). Uric acid is closely related to vascular nitric oxide activity. *JAM Coll. Cardiol*., **38**: 1850-1858.

Micheal A, Cros G, EL MC, Nell JH and Serrano JJ (1985). Cardiac adenylate cyclase activity in diabetic rats after 4 months of diabetes. *Life Sci*., **37**: 2067-2075.

Miura T, Ichiki H, Hashimoto I, Iwamoto N, Kato M, Kubo M, Ishihara E, Komatsu Y, Okada M, Ishida T and Tanigawa K (2001). Antidiabetic activity of a xanthone compound mangiferin. *Phytother. Res*., **8**: 85-87.

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

Mulec H, Johnson SA and Bjorck S (1990). Relation between serum cholesterol and diabetic nephropathy. *Lancet*, **335**: 1537-1538.

Muruganandan S, Srinivasan K, Gupta S, Gupta PK and Lal J (2005). Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. *J. Ethnopharmacol*., **97**: 497-501.

Nanbora S, Tanaka K, Koide H, Tanaka T and Hayashi T (1990). Changes on levels of B6 vitamin and aminotransferase on the liver of diabetic animals diabetes. *Res. Clin. Pract*., **9**: 109-114.

Nastaran JS (2011). Antihyperglycaemia and antilipidaemic effect of *Ziziphus vulgaris* L on streptozotocin induced diabetic adult male Wistar rats. *Physiol. Pharmacol*., **47**: 219-223.

Natelson S, Scott ML and Beffa C (1951). A rapid method for the estimation of urea in biologic fluids. *Am. J. Clin. Pathol*., **21**: 275-281.

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

Ogbonnia SO, Odimegwu JI and Enwuru VN (2008). Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of *Treculia africana* Decne and *Bryophyllum pinnatum* Lam. and their mixture on streptozotocin (STZ)-induced diabetic rats. *Afr. J. Biotech*., **7**: 2935-2939.

Ohlsson A and Aher SM (2006). Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst. Rev*., 19: CD004863.

Oyedemi SO, Yakubu MT and Afolayan AJ (2010). Effect of aqueous extract of *Leonotis leonurus* (L) R. Br leaves in male Wistar rats. *Hum. Exp. Toxicol*., **29**: 377-384.

Periyar Selvam S, Balu PM, Sathiyamoorthi P and Murugesan K (2009). Antihyperglycemic effect of mangiferin in streptozotocin induced diabetic rats. *J. Health Sci*., **55**: 206-214.

Rannels DE, Mckee EE and Morgan HE (1997). Action of insulin. In: Biochemical Actions of Hormones, Vol. IV (Litwack G., Ed). Academic Press, Pp. 135-195.

Ribeiro LR and Salvadori DMF (2003). Dietary components may prevent mutation related diseases in humans. *Mutat. Res*., **544**: 195-201.

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

Sellamuthu PS, Arulselvan P, Muniappan BP, Fakurazi S and Kandasamy M (2012). Influence of mangiferin on membrane bound phosphatases and lysosomal hydrolases in streptozotocin induced diabetic rats. *Lat. Am. J. Pharm.*, **31**: 1013-1020.

Senthilvel G, Jegadeesan M, Austin A, Thirugnanasambanthan P, Mayisurem E and Balasubramanian M *et al*., (2006). Effect of a poly-herbal formulation (Diarun plus) on Streptozotocin induced experimental diabetes. *Int. J. Trop. Med*., **1**: 88-92.

Taniguchi A, Fukushima M, Seino Y, Sakai M, Yoshii S and Nagasaka S *et al*. (2003). Platelet count is independently associated with insulin resistance in nonobese Japanese type 2 diabetic patients. Metabolism: *Clin. Exp*., **52**: 1246-1249.

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

Wild S, Roglic G, Green A, Sicree R and King H (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, **27**: 1047-1053.