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 (Ogbonna *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 (Senthivel *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-
Beneficial effects of mangiferin isolated from Salacia chinensis on biochemical and inflammatory markers

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, Kurumpakkam, 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 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 intraperitontional 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>84.92±5.60</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>252.27±18.92</td>
<td>+++</td>
</tr>
<tr>
<td>Diabetic+mangiferin</td>
<td>98.73±6.51*</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetic+glibenclamide</td>
<td>95.62±6.11*</td>
<td>Nil</td>
</tr>
</tbody>
</table>

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

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

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.

aDiabetic control rats were compared with control rats.
bMangiferin treated diabetic rats were compared with diabetic control rats.
cGlibenclamide 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

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.

aDiabetic control rats were compared with control rats.
bMangiferin treated diabetic rats were compared with diabetic control rats.
cGlibenclamide treated diabetic rats were compared with diabetic control rats.

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 (Baraoea 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 (Balasubramianian 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


