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Effect of mangiferin isolated from *Salacia chinensis* regulates the kidney carbohydrate metabolism in streptozotocin-induced diabetic rats

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ABSTRACT

Objective: The present investigation was to evaluate the possible anti-diabetic effect of mangiferin from *Salacia chinensis (S. chinensis)* on the activities of kidney carbohydrate metabolic enzymes in chemically induced diabetic rats. **Methods:** Diabetes was induced by streptozotocin (STZ) in adult male rats, as a single intraperitoneal injection at a dose of 55 mg/kg body weight. The STZ-induced diabetic rats were treated by mangiferin and glibenclamide (positive control drug) for 30 days. At the end of the experiment, the rats were sacrificed and carbohydrate metabolic enzyme activities were analyzed in the kidney. **Results:** Diabetic control rats showed a significant increase in the level of fasting blood glucose and also increase the activities of carbohydrate metabolic enzymes in kidney on successive days of the experiment as compared with their basal values. Daily oral administration of mangiferin showed a significant decrease in the blood glucose when compared to diabetic control. The anti-hyperglycemic effect was obtained with the dose of 40 mg/kg b.wt. In addition, treatment of mangiferin shows alteration in kidney carbohydrate metabolic enzymes including gluconeogenic enzymes like glucose-6-phosphatase and fructose-1,6-disphosphatase. These results were comparable with positive control drug, glibenclamide. **Conclusions:** The results obtained in this study provide evidence of the anti-diabetic potential of mangiferin, mediated through the regulation of carbohydrate key metabolic enzyme activities.

1. Introduction

Diabetes mellitus is a metabolic disease, characterized by hyperglycemia resulting form insulin action/secretion together with impaired metabolism of glucose, lipids and proteins[1,2]. The total number of people with diabetes according to the International Diabetes Federation (IDF), the global prevalence of diabetes is predicted to grow from 366 million in 2011 to 552 million by 2030[3]. The raise in the incidence of diabetes mellitus is due to changing lifestyle, dietary patterns, and low birth weight has contributed to the increased diabetic ratio around world wide[4].

Currently, there are many oral anti-hyperglycemic agents for clinical use and having characteristic profiles of severe unwanted effects such as liver toxicity, diarrhea etc[5,6]. Management of diabetes without any side effects is still a challenge to the biomedical application. This leads to increasing demand for natural products with anti-diabetic property and without adverse effects. Indian traditional medicines belong to one of the richest medicinal systems among those available in the world. Different parts of Indian medicinal plants have been found to be successfully used to manage diabetes and some of them have been evaluated and their active compounds were also isolated. As a result, the search for more efficient and safer anti-diabetic agents has continued to be an important active research zone. Based on World Health Organization (WHO) recommendation, hypoglycemia agents of plant origin are very essential for use from the traditional medicine[7].

Salacia chinensis is a straggling shrub and widely distributed in India, Sri Lanka, Burma, Thailand, Indo-china, China and Malaysia[8]. It's member of Hippocrateaceae family. One of the active phytochemical present in the Salacia species is mangiferin, a xanthone glucoside[9]. Mangiferin is a naturally occurring xanthone glucoside (2-C- β -D-gluco-pyranosyl-1, 3, 6, 7- tetrahydroxyxanthone) with a molecular weight: 422.35. Mangiferin is recommended to treat immuno-deficiency diseases such as diabetes, hepatitis, arthritis,

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cardiac and mental disorders[10]. The mangiferin have multiple advantageous properties like, antidiabetic, antihyperlipidemic, antiatherogenic and antioxidant properties without causing hypoglycemia would be of greater therapeutic benefit in the treatment of diabetes[11]. The pharmacology of the mangiferin is gaining increased attention in the recent years owing to its modulatory functions on oxidative mechanisms in various disorders[12,13,14,15].

To the best of our knowledge there is no detailed study have been carried out regarding the regulation of kidney carbohydrate metabolizing enzymes in streptozotocin induced diabetic rats treated by mangiferin. Therefore, objective of the present study was designed to investigate the potential role of mangiferin from *S. chinensis* on glucose utilization pathways on STZ induced diabetic rats.

2. Materials and methods

2.1. Chemicals

Streptozotocin was procured from Sigma Chemicals, St. Louis, MO, USA, stored at −4 ℃ and protected from light. All other chemicals used for all experiments were of analytical grade.

2.2. Plant Material

The roots of *S. chinensis* were collected from Veenangaputtu, Karumpakkam, Thangal and Kurumpuram, Puducherry, India. The plant was characterized/identified with taxonomist and has been deposited in Centre for Advanced Studies in Botany (voucher specimen no: 778), University of Madras, Chennai, India.

2.3. Isolation of mangiferin

The solvent extraction, isolation and characterization of mangiferin from the *S. chinensis* were carried out according to the standard method from our previous studies[16]. The isolation of mangiferin compound was done by column chromatography and purity of mangiferin was confirmed through high performance liquid chromatography. The mangiferin purity was confirmed with authentic sample, which was procured from Sigma Aldrich Company (St. Louis, MO, U.S.A.).

2.4. Experimental Animal

Adult, male rats of Wistar strain weighing around 150-200g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India and they were housed individually in clean, sterile, polycarbonate cages in an animal house with 12 hr day-night cycle at a temperature of 22±2 ℃ and 45-60% of humidity. They were fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), and allowed to drink free access of water. The animal experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No. 02/004/06).

2.5. Induction of Experimental Diabetes

Experimental diabetes was induced in 12 hr fasted rats by administering single intraperitonial injection of a freshly prepared streptozotocin solution (55mg/kg b.wt) in 0.1M cold citrate buffer (pH4.5)[16]. Control animals were injected with citrate buffer alone. The rats were allowed to drink 5% glucose solution overnight to overcome the drug-induced effect of hypoglycemia. Animals with a fasting blood glucose range of 250 to 300 mg/dl were selected for further experiment.

2.6. Experimental Design

The experimental rats were divided into four groups each comprising a minimum of six rats each as given below. Mangiferin and glibenclamide were dissolved in water and orally administered daily up to 30 days to experimental groups of rats using intragastric tube.

Group 1: Control rats

Group 2: Diabetic control rats

Group 3: Diabetic rats treated with mangiferin (40mg/kg b.wt/day)[16]

Group 4: Diabetic rats treated with glibenclamide (600 μg/kg b.wt/day)[17].

At the end of the experimental period, all the rats were deprived of food overnight and anesthetized and sacrificed by cervical dislocation. Blood was collected in heparinised tubes and used for the estimation of various biochemical parameters. In addition, tissues were also collected for the determination of carbohydrate metabolic enzymes.

2.7. Biochemical estimations

The blood glucose level was quantified by the method of O-toludine by Sasaki et al.[18]. The plasma insulin was estimated by radio immuno assay kit procured from Stat Diagnostics (Linco Research Inc.), Mumbai, India.

Activity of hexokinase was assayed by the method of Brandstrup et al.[19]. Lactate dehydrogenase was assayed according to the method of King[20] and Glucose-6-phosphatase assayed by Koide and Oda method[21]. Pyruvate kinase was assessed by Pogson and Denton method[22]. Activities of fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase were assayed by the methods of Gancedo and Gancedo[23] and Ells and Kirkman[24] respectively.

2.8. Statistical Analysis

All data were expressed as mean±standard deviation for six rats in each group and statistical significance evaluated by one way analysis of variance (ANOVA) followed by least significant difference (LSD) test by SPSS software package version 16.0. In this present study, p-values of less than 0.05 were considered to specify statistical significant.

3. Results

3.1. Basic biochemical parameters

The blood glucose and plasma insulin in control and experimental groups of rats are revealed in Table 1. There was significant alteration in the levels of blood glucose and plasma insulin in diabetic rats, when compared with control rats. The decreased level of blood glucose and increased level of plasma insulin were observed in mangiferin and glibenclamide treated diabetic rats, when compared with diabetic rats. The mangiferin and glibenclamide treated diabetic rats significantly altered the levels of blood glucose and insulin.

Table 1

The levels of blood glucose and plasma insulin in control and experimental groups of rats

Groups	Blood glucose (mg/dl)	Plasma insulin (μU/ml)
Control	93.71±4.94	15.44±1.03
Diabetic control	288.29±21.06[a*]	4.95±0.33[a*]
Diabetic+mangiferin	102.25±7.21[b*]	12.76±0.83[b*]
Diabetic+glibenclamide	99.28±5.98[c*]	13.68±0.71[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.

3.2. Carbohydrate metabolizing enzymes

The activities of hexokinase, lactate dehydrogenase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase in kidney of control and experimental groups of rats are presents in Table 2 and Figure 1. The activities of hexokinase, lactate dehydrogenase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase were significantly altered in the kidney of STZ induced diabetic rats. Oral administration of mangiferin as well as glibenclamide recovered the activities of these enzymes to near control in diabetic rats.

Table 2

Activities of carbohydrates metabolizing enzymes in kidney of control and experimental groups of rats

Groups	Hexokinase	Lactate dehydrogenase	Pyruvate kinase
Control	142.76±9.27	473.18±31.22	8.95±0.23
Diabetic control	288.38±21.34[a*]	692.36±51.23[a*]	21.78±0.83 [a*]
Diabetic+mangiferin	157.12±10.36[b*]	493.14±33.04[b*]	9.75±0.24[b*]
Diabetic +glibenclamide	164.32±11.00[c*]	512.26±34.83[c*]	10.24±0.27[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. The enzyme activities are expressed as: i)Hexokinase - μ moles glucose-6-phosphate formed/h/mg of protein. ii)Lactate dehydrogenase - μ moles of pyruvate formed/h/mg of protein. iii)Pyruvate kinase - μ moles of pyruvate formed/min/mg of protein.

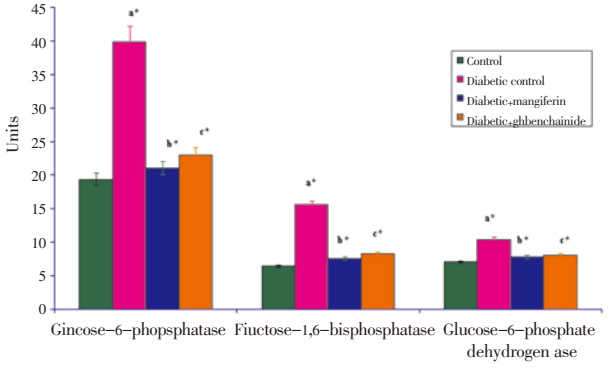

Figure 1: Activities of carbohydrates metabolizing enzymes in the kidney of control and experimental groups of rats

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: Glucose-6-phosphatase and fructose-1,6-phosphatase- μ moles of phosphate liberated/h/mg of protein; Glucose-6-phosphate dehydrogenase- μ moles of NADPH formed/min/mg of protein.

4. Discussion

The hyperglycemia induced by STZ in animal is considered a good experimental model for the preliminary screening of hypoglycemic agents[25]. The STZ caused stable diabetic condition in many animal species during experiments[26], and it also resembles with pathological status found in human diabetes[27]. In the present study also STZ effectively induced diabetes in rats.

Diabetes is a chronic disorder of metabolism of carbohydrates, protein and fat due to relative deficiency of insulin secretion and varying degrees of insulin resistance. They are causing severe complications including blindness, cardiac and kidney diseases. Diabetes is one of the most important clinical and public health problems in the world today[28,29]. Insulin deficiency may be due to inadequate secretion or diminished tissue response to insulin in the

complex pathway of hormone action, which might lead to disturbance in the metabolism of the carbohydrate, fat, and protein[30,31].

An insufficient release of insulin, that leads high blood glucose namely hyperglycemia. The treatment of medicinal plant extract to the STZ-induced diabetic rats, that activated the β-cells and granulation return to normal, like to be insulinogenic effect[32]. The glibenclamide treated STZ-induced diabetic rats showed a decrease in blood glucose level[33]. The previous reports are consistent with that of this study .The decreased level of blood glucose and increased level of plasma insulin were observed in present study (Table. 1), which indicated that mangiferin stimulates insulin secretion from the remnant β-cells or regenerated β-cells[16].

Hexokinase play a key role in the glucose homeostasis maintenance through catalyzes the phosphorylation of glucose to glucose-6-phosphate[34]. In the STZ induced diabetic animals, the insulin level was decreased that leads to increased hexokinase activity. The mangiferin and glibenclamide treated diabetic rats were significantly decreased the activity of hexokinase that may lead to activation of glycolysis and increase the utilization of glucose for energy production[16].

The role of pyruvate kinase is catalyzes the conversion of phosphoenol pyruvate to pyruvate. The alteration of hexokinase activity might be anticipated to affect the glucose metabolism and energy production. In STZ induced diabetic rats, the pyruvate kinase activity was significantly increased due to decreasing in the glucose utilization. The mangiferin and glibenclamide treated diabetic rats were decreasing the activity of pyruvate kinase that may increase the utilization of glucose. The finding suggested that the mangiferin was improving the glucose metabolism by increase the utilization of glucose[16]. Therefore mangiferin treatment improved the activities of hexokinase and pyruvate kinase helps us to understand the increasing of glucose utilization.

In anaerobic glycolysis, lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate. The lactate dehydrogenase system reflects the NAD+/NADH ratio indicated by the lactate/pyruvate ratio of hepatocyte cytosol. The activity of LDH was increased in diabetes, that due to the glucose-stimulated insulin secretion[35]. The mangiferin and glibenclamide treated diabetic rats were reversible to near normal LDH activity. This may be regulation of NAD+/NADH ratio by glucose oxidation.

The final step of glucose production is catalyzes by glucose-6-phosphatase in kidney and liver. Glucose-6-phosphatase and fructose-1,6-bisphosphatase are the important enzymes in regulating of gluconeogenic pathway. Glucose-6-phosphatase plays an important role in glucose homeostasis in liver and kidney[36]. During the diabetic condition, fructose-1,6-bisphosphatase catalyses the hydrolysis of fructose-1,6-bisphosphate into fructose-6-bisphosphate in the gluconeogenesis[37]. In the kidney tissues, glucose-6-phosphatase and fructose-1, 6-bisphosphatase were increased during the diabetic condition. The activities of glucose-6-phosphatase and fructose-1, 6-diphosphatase were increased in liver and kidney, which might result in decrease of glycolytic flux. Both the enzymes activities were normalized in the diabetic rats due to the treatment of insulin or pancreas transplantation[38]. The oral administration of mangiferin as well as glibenclamide was significantly decreasing the gluconeogenic enzymes activities in STZ-induced diabetic rats.

The activity of glucose-6-phosphate dehydrogenase was increased in kidney of diabetic rats, which might lead to renal hypertrophy[39]. The significant changes in glucose-6-phosphate dehydrogenase lead to effect on cell growth and cell death[40]. The activity of glucose-6-phosphate dehydrogenase was increased in diabetes that might result in modulating the functioning of HMP shunt and the production of reducing equivalents such as NADH and NADPH. In the mangiferin treated diabetic rats, glucose-6-phosphate dehydrogenase activity was brought back to near normal, This indicates that improvement in formation of NADPH, favoring lipogenesis and a substitute channel to dispose excess glucose via the HMP pathway.

In conclusion, our results showed that mangiferin possessed anti-hyperglycemic activity in streptozotocin-induced diabetic rats due to increased insulin secretion and the alterations in the carbohydrate metabolism. Based on this study, we can conclusively affirm that the mangiferin phytocompound from *S. chinensis* possess antidiabetic activity. It is representing as a good candidate for alternative medicine in the management of diabetes mellitus. Further research is required to find out the exact mechanism of this compound for its anti-diabetogenic properties.

Conflict of interest

We declare that we have no conflict of interest.

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