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Musa Paradisiaca flower extract improves carbohydrate metabolism in hepatic tissues of streptozotocin-induced experimental diabetes in rats

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ABSTRACT

Objective: *Musa Paradisiaca*, commonly known as plantains have been traditionally used for various medicinal purposes. In the absence of an ideal drug to alleviate the primary and secondary complications of diabetes mellitus, search for novel drugs without side effects, preferably from plant origin continues. Recently, we have reported the presence of biologically active phytochemicals as well as the hypoglycemic activity of *Musa paradisiaca* tepals extract in STZ induced experimental diabetes in rats. The present study was aimed to evaluate the role of tepals, an integrated part of *Musa paradisiaca* flowers on carbohydrate metabolism in hepatic tissues of experimental diabetic rats. **Methods:** Streptozotocin-induced diabetic rats were treated with ethanolic extract of tepals at a concentration of 200mg/kg body weight/day for 30 days. The levels of fasting blood glucose, plasma insulin and glycosylated hemoglobin were estimated. The activities of key enzymes in carbohydrate metabolism such as glucokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1, 6-bisphosphatase, glucose-6-phosphate dehydrogenase, glycogen synthase and glycogen phosphorylase in hepatic tissues were assayed. The levels of glycogen in hepatic tissues were also estimated. **Results:** Oral administration of *Musa paradisiaca* tepals extract significantly improved the altered levels of blood glucose, plasma insulin, glycosylated hemoglobin and modulated the activities of carbohydrate metabolizing enzymes. The glycogen content in hepatic tissues was significantly increased in diabetic rats treated with tepals extract. **Conclusions:** The results of the present study clearly indicate that the tepals extract plays pivotal role to maintain normoglycemia in diabetes by modulating the activities of carbohydrate metabolic enzymes.

1. Introduction

Diabetes Mellitus is a chronic metabolic disorder of multiple etiologies characterized by altered glucose homeostasis leading to derangements in the carbohydrate, protein and lipid metabolism, resulting from partial or complete deficiency in insulin synthesis or due to peripheral resistance to insulin action. Diabetes is rapidly emerging at an alarming rate and is considered to be one of the biggest health catastrophes in the world [1], causing significant health and economic burdens on patients and communities [2].

In 2011 there are 366 million people with diabetes, and this is expected to rise to 552 million by 2030 of the total world population [3]. Current strategies for diabetes mellitus include diet, exercise, various oral antidiabetic drugs and insulin therapy [4]. The modern drugs, insulin and other oral hypoglycemic agents such as Biguanides, sulphonylureas, α-glucosidase inhibitors have characteristic profile of adverse effects, which include hypoglycemia, frequent diarrhea, hypertension, hypercoagulability, lactic acidosis, hepatotoxicity, and dyslipidemia [5]. Hence search for novel therapeutic agents, preferably from natural sources continues.

Medicinal plants, since times immemorial have been used in virtually all cultures as a basis for primary health care and serve as a rich source of medicines in various communities. Traditionally, a number of medicinal plants have been used in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically [6]. Historically all medicinal preparations were derived from plants, either in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. More than 800 plants have been studied for their antidiabetic potentials [7]. Today a substantial number of drugs with potential pharmacological activities are developed from plants [8] which are active

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against a number of diseases. In the developed countries 25 percent of currently available medicines are based on plants and their derivatives [9]. However there is a reluctance observed in accepting herbal remedies by modern system because of the lack of proper documentation and scientific validity [10]. One such valuable medicinal plant which lacks scientific scrutiny is *Musa paradisiaca* [11].

The Plantain (Musa paradisiaca) and Banana (Musa sapientum) are monocotyledonous, triploid, perennial, succulent crops belonging to the family Musaceae. They grow prolifically in most tropical regions of the world. Morphologically, the two can be distinguished only at the fruiting stage. Plantains are longer than bananas and relatively starchy and not sweet. They are widely used as a vegetable in many recipes whereas bananas can be eaten and tastes different at every stage of development.

The cylindrical stem like portion or pseudostem of the plant is formed from the long convolute leaf sheaths and the real stem portion is the underground tuberous rhizome called a corm. The pseudo stem has a tender inner core portion which in fact, is the stalk of the inflorescence [12]. The undifferentiated slim, tubular and white colored clusters found to be present inside the heart shaped male flowers are named as tepals. Due to high bitterness and time consuming processes associated with the separation and processing, tepals are not considered for edible purpose. However tepals are used in the traditional medicine system for the treatment of various diseases including urolithiasis, ulcer and other gastrointestinal disorders. Recently, we have reported for the hypoglycemic activity of *Musa paradisiaca* flower extract [13]. In the absence of systemic reports in the literature, the present study was aimed to evaluate the role of *Musa Paradisiaca* tepals extract in the regulation of carbohydrate metabolism in hepatic tissues of streptozotocin-induced diabetes in rats.

2. Materials and Methods

2.1 Plant Material

The authentication of the plant, *Musa paradisiaca* was carried out by a Taxonomist in the Centre for Advanced Studies in Botany, University of Madras. The plantations were free from any artificial fertilization. The male flowers used in this study were harvested from a commercial plantation at Thirukazhukundram, Kanchipuram District, Tamil Nadu, India. The tepals were selectively removed from the bracts, shadow dried and powdered using a pulverizer.

2.2. Preparation of tepals extract

The powdered tepals material was defatted with petroleum ether (60-80℃) and then extracted with 95% ethanol in a Soxhlet apparatus. The solvent was evaporated under reduced pressure using rotary evaporator, which yields a black sticky residue (26.5 % w/w) with respect to powdered tepals. The tepals extract was stored in a desiccator for further investigation.

2.3. Experimental Animals

Male albino Wistar rats weighing (160-180g) were procured 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 ad libitum. The experimental rats were maintained in a controlled environment (12:12 h light/dark cycle and temperature (30±2℃)). The experiments were designed and conducted in accordance with the current ethical norms approved by Ministry of Social justices & Environment, Government of India and Institutional Animal Ethical Committee guidelines [IAEC NO:01/01/2010]. The rats were acclimatized for at least 7 days before starting the experiments.

2.4. Induction of experimental diabetes

Experimental diabetes was induced in overnight fasted rats by single intraperitoneal injection of streptozotocin (50 mg/kg) dissolved in 0.1M of cold citrate buffer (pH 4.5) [14]. Since, STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release; the animals were allowed to drink 10% glucose solution after 6 h for the next 24 h to overcome the drug-induced hypoglycemia [15]. After 48 h of STZ injection their fasting blood glucose levels were monitored and the rats that exhibited blood glucose level above 250 mg/dl were considered as diabetic [16].

2.5. Experimental design

The normal and diabetic rats were divided into four groups, comprising a minimum of six rats in each group as follows:
Group 1 - Normal control rats.
Group 2 - STZ induced diabetic rats.
Group 3 - Diabetic rats treated with tepals extract (200 mg/kg b.w/rat/day) dissolved in aqueous solution orally for 30 days.
Group 4 - Diabetic rats treated with gliclazide (5 mg /Kg. b.w/rat/day) in aqueous solution orally for 30 days.

At the end of the treatment period, the rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with and without EDTA for plasma and serum separation, respectively. Blood glucose and glycosylated hemoglobin were estimated according to the methods of Trinder et al [17] and Nayak and Pattabiraman [18], respectively. Insulin assay was performed by using commercially available ELISA kit (Crystal Chem inc. Life Technologies, India). Liver was dissected out, washed with ice-cold saline and were homogenized in 0.1M Tris-HCl buffer (pH 7.4) for the assay of key enzymes of carbohydrate metabolism. The homogenate was centrifuged at 10,000 rpm to remove the debris and the supernatant was used as enzyme source for the assays of glucokinase [19] , pyruvate kinase[20] , glucose-6-phosphatase[21] , fructose-1, 6-bisphosphatase[22] , glucose-6-phosphate dehydrogenase

[23], glycogen synthase[24] and glycogen phosphorylase[25]. A smaller portion of wet liver tissue was used for the quantification of glycogen content [26].

2.6. Statistical analysis

All the grouped data were statistically evaluated with SPSS 16.0 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.

3. Results

Table 1 depicts the effect of oral administration of extract on blood glucose, glycosylated hemoglobin and plasma insulin levels in experimental groups of rats. There was a significant elevation in the levels of blood glucose and glycosylated hemoglobin of streptozotocin induced diabetic rats as compared with control group of rats. Upon treatment with extract as well as gliclazide for 30 days, diabetic rats showed a significant decrease in the levels of blood glucose and glycosylated hemoglobin, which were comparable with control group of rats. Moreover, the diminished plasma insulin level of diabetic rats was improved significantly to near normal level by the administration with extract as well as gliclazide.

Table 1

Levels of fasting blood glucose, plasma insulin and glycosylated hemoglobin (HbA1c) in control and experimental groups of rats after 30 days experimental period.

Groups	Glucose	Insulin	HbA1c
Control	79.79±11.06	0.95±0.22	5.13±0.63
Diabetic	308.91±27.74[a]	0.38±0.07[a]	13.04±1.22[a]
Diabetic + tepals extract	131.09±17.05[b]	0.73±0.08 [b]	7.26±0.49[b]
Diabetic + gliclazide	117.37±15.60[b]	0.82±0.09[b]	6.94±0.65[b]

Units: Glucose - (mg/dl); Insulin - (ng/ml); HbA1c - (% Hemoglobin). Values are given as mean±SD for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]-control rats; [b]-diabetic rats; Values are statistically significant at $P < 0.05$.

Table 2 depicts the effect of extract on the activities of glucokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase in liver tissue of experimental groups of rats. The activities of glucokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase were significantly diminished in liver tissue of streptozotocin induced diabetic rats. Oral administration of extract to diabetic rats altered the activities of these enzymes to near normalcy in liver tissue similar to gliclazide treated rats.

Table 3 depicts the activities of glucose-6-phosphatase, fructose-1, 6-bisphosphatase, glycogen phosphorylase and glycogen synthase in liver tissue of control and experimental groups of rats. The liver tissue of diabetic rats showed a significant elevation in the activities of glucose-6-phosphatase, fructose-1, 6-bisphosphatase and glycogen phosphorylase with a concomitant decrease in the activities of glycogen synthase. The altered activities of these enzymes were reverted to near normalcy by treatment with extract as well as gliclazide in diabetic groups of rats.

Table 2

Activities of glucokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase (G6PDH) in liver tissues of control and experimental groups of rats after 30 days experimental period.

Groups	Glucokinase	Pyruvate kinase	G6PDH
Control	4.19±0.50	210.64±14.99	454.15±42.65
Diabetic	1.50±0.34[a]	108.07±12.26[a]	256.20±40.97[a]
Diabetic + tepals extract	2.76±0.62[b]	151.70±15.80[b]	345.73±59.05[b]
Diabetic + gliclazide	2.68±0.52[b]	158.03±17.37[b]	375.76±52.93[b]

Units are expressed as: mU for glucokinase and pyruvate kinase, μmoles of NADPH/min/mg of protein for glucose-6-phosphate dehydrogenase. Values are given as mean±SD for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]-control rats; [b]-diabetic rats; Values are statistically significant at $P < 0.05$.

Figure 1 depicts the level of glycogen in liver tissues of control and experimental groups of rats. The level of glycogen content was reduced in diabetic rats whereas treatment with extract as well as gliclazide to diabetic groups of rats restored the level of glycogen in liver tissues.

Table 3

Activities of glucose-6-phosphatase, fructose-1, 6-bisphosphatase, glucose-6-phosphate dehydrogenase, glycogen synthase and glycogen phosporylase in liver tissues of control and experimental groups of rats after 30 days experimental period.

Groups	Glucose-6-phosphatase	Fructose-1,6-bisphosphatase	Glycogen synthase	Glycogen phosphorylase
Control	667.28±51.39	349.47±50.35	815.17±94.75	489.31±45.91
Diabetic	1221.55±150.27[a]	976.27±110.73[a]	310.22±36.21[a]	806.82±77.21[a]
Diabetic + tepals extract	848.92±67.82[b]	586.26±67.85[b]	702.70±73.02 [b]	582.04±64.08 [b]
Diabetic + gliclazide	881.16±73.79[b]	641.26±81.32[b]	659.85±66.97[b]	591.54±55.10[b]

Units are expressed as: μmoles of Pi liberated/h/mg of protein for glucose-6-phosphatase and fructose-1,6-bisphosphatase, μ moles of UDP formed/h/mg protein for glycogen synthase and μmoles Pi liberated/h/mg protein for glycogen phosphorylase. Values are given as mean±SD for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]-control rats; [b]-diabetic rats; Values are statistically significant at $P < 0.05$.

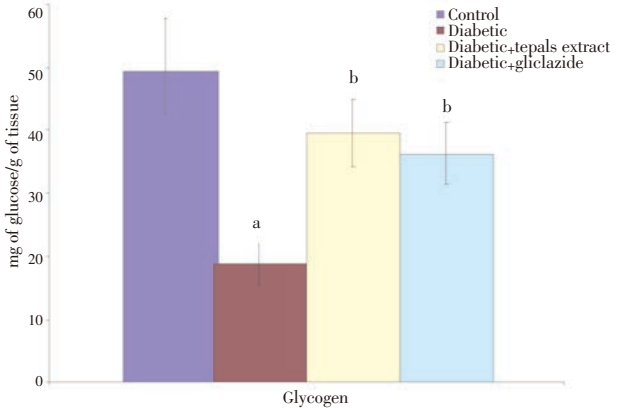

Fig.1: Level of glycogen content in liver tissues of control and experimental groups of rats after 30 days experimental period.
Values are given as mean±SD for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: a-control rats; b-diabetic rats; Values are statistically significant at $P < 0.05$.

4. Discussion

Streptozotocin is a selective β-cell genotoxicant which induces rapid onset of diabetes by generating sufficient levels of DNA adducts to cause overactivation of poly (ADP) ribose synthetase in the base excision repair [27]. The extensive poly (ADP) ribose synthetase activation results in rapid depletion of cellular NAD^+ that leads to β-cell death through necrosis. STZ-induced diabetic animal models resemble diabetes in humans by many features [28]. Several studies have indicated the detrimental effects of streptozotocin-mediated persistent hyperglycemia is a proximate cause of inexorable deterioration of β-cell function, which is mediated and complicated through the enhanced formation of reactive free radicals [29]. Therefore, in the present study we evaluated the effects of tepals extract in STZ-induced diabetic rats.

Diabetes mellitus is a chronic disease characterized by chronic hyperglycemia due to an absolute or relative deficiency of circulating insulin levels. Diabetes is also associated with augmented glucagon secretion resulting in increased hepatic glucose production [30]. Lowering blood glucose levels is the main factor in the prevention of microvascular complications. The elevated blood glucose level observed in the diabetic rats was significantly decreased in extract treated rats indicating the hypoglycemic property of tepals. Insulin regulates the activities of various enzymes involved in carbohydrate metabolism in its target organs including liver thereby maintaining glucose homeostasis. The observed increase in the levels of insulin in the extract treated groups of rats may be due to the pancreatic tissue protective or insulin stimulating effect of the tepals extract. Several reports are available in the literature implicating active phytochemicals in plants are being responsible for their antidiabetic activities [31-33].

Management and treatment of diabetes is mainly aimed at glycaemic control and international guidelines recommend reducing glycated hemoglobin (HbA1c) to 6.5-7% [34]. Persistent hyperglycemia results in glycation of hemoglobin that leads to the formation of glycosylated hemoglobin. Glycosylated hemoglobin was found to increase in uncontrolled diabetes mellitus and the increase is directly proportional to the fasting blood glucose level for about 3 months [35]. HbA1c test can be used to estimate long-term average glycemia and design dosage regimen for Diabetes Mellitus [36]. Every percentage point decrease in HbA1c, reduces the risk of microvascular complications by 35% and every 10% reduction in HbA1c is associated with 21% reduction in cardiovascular disease [37]. The observed decrease in the level of glycosylated hemoglobin in the experimental diabetic rats treated with extract indicates the improved glucose tolerance.

Glucokinase (GK) is the predominant isoenzyme in the liver and it plays a key role in glucose homeostasis. Reports on animal models and isolated hepatocytes established that hepatic GK exerts a strong impact on glucose utilization and glycogen synthesis and their activities are very low in both human and rodent diabetes. Due to its significant role in glucose sensing, GK is a potential target for new treatment strategies for the management of type 2 diabetes, as has recently been reviewed [38,39]. The observed increased in the activity of GK in tepals extract treated diabetic rats indicates the effective utilization of glucose for oxidation. The activation of GK in mice with high-fat diet-induced insulin resistance improves islet function and normalizes glucose tolerance in mice.

Pyruvate kinase (PK) is a ubiquitously expressed, key glycolytic enzyme and its altered activity during diabetic conditions could be expected to diminish the metabolism of glucose and ATP production. Hence, the observed decline in the activity of PK in the liver tissues of diabetic rats promptly responsible for the reduced glycolysis and amplified gluconeogenesis signifying that these two pathways are distorted in diabetes. Glucose-6-phosphate dehydrogenase catalyzes the rate-limiting step of the hexose monophosphate shunt and produces NADPH required for the maintenance of the levels of reduced glutathione, a non enzymatic antioxidant [40]. The observed decrease in the activity of glucose- 6-phosphate dehydrogenase in the hepatic tissues of diabetic rats suggests a decrease in metabolism via the phosphogluconate oxidation pathway. The treatment with extract showed a notable increase in GK, pyruvate kinase and glucose 6 phosphate dehydrogenase activities in hepatic tissues of diabetic rats signifying the improved utilization of glucose by the hepatic tissues.

Glucose-6-phosphatase, a key enzyme in the homeostatic regulation of blood glucose catalyzes the dephosphorylation of glucose-6-phosphate to free glucose as the final step in gluconeogenesis and glycogenolysis [41]. The activity of glucose-6-phosphatase is stimulated by cAMP and repressed

by insulin. Trinh et al., (1998) [42] have demonstrated that glucose-6-phosphatase activity impairs hepatic glucose utilization, while simultaneously enhancing hepatic glucose production. It is concluded that enhanced hepatic glucose output in STZ-induced diabetic rats probably involves dysregulation of hepatic glucose-6-phosphatase activity. Fructose-1, 6-bisphosphatase is another gluconeogenic enzyme which catalyzes the dephosphorylation of fructose-1, 6- bisphosphate to fructose-6-phosphate in the gluconeogenic pathway gluconeogenesis [43]. The increased activity of fructose-1, 6-bisphosphatase has been observed in animal models of diabetes, insulin resistance and obesity and suggests a principal role for fructose-1, 6-bisphosphatase in the gluconeogenic flux. In the present study, the reduced activities of both glucose-6-phosphatase and fructose-1, 6-bisphosphatase in hepatic tissues of diabetic rats upon oral administration of extract reveal the reduced endogenous glucose production there by tepals may play a crucial role in maintaining the fasting blood glucose level. The regulation of gluconeogenic flux by the extract might be one of the possible mechanisms for its antihyperglycemic nature.

Glycogen is the primary intracellular storable form of glucose and its quantity in various tissues is a direct manifestation of insulin activity as insulin supports intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase [44]. Glycogen synthase is a crucial and rate-limiting enzyme which catalyzes the transfer of glucose from UDP-glucose to glycogen. Glycogen phosphorylase is a rate-limiting enzyme of glycogenolysis and is regulated by phosphorylation and by allosteric binding of AMP, ATP, glucose-6-phosphate and glucose [45]. During diabetic conditions, the glycogen levels, glycogen synthase activity and responsiveness to insulin signaling are diminished and glycogen phosphorylase activity is significantly increased. Oral administration of tepals extract to diabetic rats restored the glycogen content and the activities of glycogen metabolizing enzymes demonstrating the possible role of tepals in the regulation of glycogen metabolism.

The observed decrease in plasma glucose as well as glycosylated hemoglobin levels, along with a concomitant increase in plasma insulin and hepatic glycogen levels, and modulation in the activities of carbohydrate metabolic enzymes in liver suggests that the phytoconstituents such as flavonoids, alkaloids, tannins, saponins, sterols and triterpenes present in the tepals might play a therapeutic role in maintaining glucose homeostasis.

5. Conclusion

The results of the present study clearly indicate that oral administration of tepals extract to diabetic rats increased the activities of glucokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase suggesting the effective utilization of glucose. The improved activity of glycogen synthase along with improved glycogen content and reduced activities of glucose 6 phosphatase, fructose-1,6-bisphosphatase and glycogen phosphorylase in hepatic tissues of diabetic rats treated with tepals extract reveals the reduced endogenous glucose production. Thus it can be concluded that tepals modulates hyperglycemia by attenuating key enzymes of carbohydrate metabolism in hepatic tissues of diabetic rats.

Conflict of interest statement

We declare that we have no conflict of interest.

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