Research Article

Study on the effect of piperine on carbohydrate metabolic enzymes on experimental diabetic rats

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ABSTRACT

Background: Type II diabetes mellitus is an increasingly prevalent disorder worldwide. India is reported to be the diabetic capital of the world. The contemporary treatment for type II diabetes involves insulin and drug therapies. The conventional drugs used are not able to cope to the multifactorial exhibit of type II diabetes. The administration of medicinal plants either alone or in addition to conventional drugs has been shown to address the causes of type II diabetes at various levels and improve the quality of diabetic patients. **Aim:** The present study was aimed at assessing the effect of piperine on carbohydrate metabolizing enzymes in experimental diabetic rats. **Materials and Methods:** Adult male albino rats of Wistar strain 150–180 days old with 180–120 g of body weight were randomly divided into four groups of six rats each: Group 1: Normal rats, Group 2: Type 2 diabetic rats, Group 3: Type 2 diabetic rats treated with piperine (40 mg/kg for orally for 30 days), and Group 4: Type 2 diabetic rats treated with metformin 50 mg/kg orally for 30 days. **Results:** The activity of glucokinase and glycogen synthase was reduced conversely the activity of glycogen phosphorylase and glycogen-6-phosphatase which were increased significantly ($P < 0.05$). Oral administration of piperine significantly normalized ($P < 0.05$) altered levels of these enzymes to that of control and standard drug metformin level. **Conclusion:** Our present findings conclude that piperine possesses hypoglycemic activity through the regulation of carbohydrate metabolic enzymes. Further studies on the possible role of piperine on insulin signaling molecules are warranted.

KEY WORDS: Carbohydrate metabolic enzymes, High-fat diet, Piperine, Type-2 diabetes

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders affecting an alarming number of people in the world and is mainly characterized by chronic hyperglycemia or high blood glucose, resulting from defects in insulin secretion (type I) or its action (type II). The World Health Organization has noted that the number of diabetics has risen to a startling 422 million in 2014. Recent results from the International Diabetes Federation Diabetes Atlas which has collected data 255 high-quality data sources published between 1990 and 2018, representing 138 countries state that by 2030, the diabetics in the world would be 578 million and 700 million by 2045. High prevalence is especially seen in low- and middle-income countries. Age, obesity, family history, and sedentary lifestyle are some of the causes of type II diabetes.

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Different treatment strategies such as drugs and insulin are available. Some of them are oral anti-diabetic drugs – biguanides (phenoformin and metformin), sulfonylureas (glibenclamide and glipizide), thiazolidinediones, disaccharide inhibitors (acarbose), meglitinides (repaglinide), good laboratory practice analogs (exenatide), amylin analogs (pramlintide), DPP-IV inhibitors, and insulin. Bioavailability, high cost, and side effects (development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity, impaired renal function, flatulence, diarrhea, etc.)[1-4] are some of the limitations that result using these conventional treatments.

Although conventional Western medicine is in practice, alternative traditional practices for health have been widely accepted and practiced from time immemorial. Some of the reasons for its widespread acceptance throughout the world are naturalness, very minimal or no side effect, low cost, and easy availability. In addition, most of the bioactive compounds used in conventional medicine are plant products.

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Alarcon-Aguilar *et al*. (2002)[5] stated that around the world, 1200 plants are used to treat diabetes.

Some pre-clinical and clinical studies have confirmed that some medicinal plants have hypoglycemic effect and actions like repair of β-cells of islets of Langerhans.[6] *Gymnema sylvestre*, *Momordica charantia*, *Ficus benghalensis*, *Tinospora crispa*, *Pistacia atlantica*, *Syzygium jambolanum*, *Allium sativum*, *Cephalandra indica*, *Azadirachta indica*, *Andrographis paniculata*, *Aloe vera*, *Ocimum sanctum*, *Piper nigrum*, *Piper longum*, etc., are some of the plants which have been proved to have antidiabetic effect. Unlike conventional medicine which has one particular site of action, herbs are able to act at multiple sites such as inhibiting alpha-amylase, reducing insulin resistance, and protecting and repairing pancreatic beta-cell.

Pepper is one of the common household spices used in cooking. A recent study has reported the hypoglycemic activity of piperine in normal mice.[7] Piperine, the active alkaloid derived from *P. nigrum* and *P. longum*, gives the pungent taste to pepper and belongs to the family Piperaceae. This chief alkaloid and active ingredient of *P. nigrum* has been extensively explored for its antidepressant,[8] anticonvulsant,[9] antioxidant,[10] antimutagenic,[11] hepatoprotective,[12] anti-inflammatory properties,[8] and several other activities. In addition to these characters, piperine is also said to be antidiabetic in nature. Piperine derivatives have been reported to act as peroxisome proliferator-activated receptor gamma agonists[13]and as bioenhancers in lowering blood glucose. In combination with other plant species such as *A. sativum*, *Zingiber officinale,* and *Capsicum frutescens*, piperine has been shown to exhibit antidiabetic effect. The leaf[14] and root extracts[14] of pepper showed antioxidant and antihyperlipidemic activity alongside its glucose-lowering effect. The study was designed to assess the effect of piperine on carbohydrate metabolic enzymes in high-fat diet- and sucrose-induced type-2 diabetic rats.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in the present study were molecular and analytical grade, and they were purchased from Sigma Chemical Company, St. Louis, MO, USA; MP Biomedicals (India) Pvt., Ltd., Mumbai, India; and Sisco Research Laboratories, Mumbai, India; nicotine was purchased from Sigma Chemicals Company, USA.

Animals

Animals were maintained as per the National Guidelines and Protocols approved by the Institutional Animal Ethical Committee (IAEC No: BRULAC/SDCH/SIMATS/IAEC/07-2019 / 028). Healthy adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing 180–200 g (100 days old) were used in the present study. Animals will be housed in polypropylene cages under specific humidity (65% ±5%) and temperature (21°C ±2°C) with constant 12 h light and 12 h dark schedule at Biomedical Research Unit and Lab Animal Center, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai – 600 077. They will be fed with a standard rat pellet diet (Lipton India, Mumbai, India), and clean drinking water will be made available *ad libitum.*

Induction of Type 2 Diabetes

Adult male rats will be made diabetic (type-2) by a single intraperitoneal injection of streptozotocin (35 mg/kg body weight), after feeding the animals with high-fat diet containing 3% of cholesterol, 1% of cholic acid, 30% of coconut oil, 66% of standard rat feed, and 30% of sucrose feeding through drinking water (25%) for 30 days. The low dose of streptozotocin is given to generate a slight trauma to beta cells of pancreas to mimic the chronic hypoinsulinemic insulin-resistant condition (Balaji *et al*., 2012).

Experimental Design

The animals were divided into four groups each consisting of six animals: Group-I: Normal control rats fed with normal diet and drinking water; Group-II: Type-2 diabetic rats induced by high-fat diet; Group-III: Diabetic (type-2) rats treated with piperine (40 g/kg bodyweight/day), orally for 30 days; and Group-IV: Diabetic (type-2) rats treated with metformin (50 mg/kg body weight/day), orally for 30 days. Two days before sacrifice, control and experimental animals were subjected to oral glucose tolerance test after overnight fasting. At the end of treatment, animals were anesthetized with sodium thiopentone (40 mg/kg body weight), Liver, skeletal muscle, and adipose tissue will be excised and used for the assay of various parameters.

Assessment of Carbohydrate Metabolic Enzymes

Standard protocols were used for the assay of glucokinase,[15] glucose-6-phosphatase,[16] glycogen synthase,[17] as well as glycogen phosphorylase[18] by spectrophotometric analysis.

Statistical Analysis

The data were be subjected to statistical analysis using one-way analysis of variance and Duncan's multiple range test to assess the significance of individual variations between the control and treatment groups. In Duncan's test, significance was considered at the level of $P < 0.05$.

RESULTS

Effect of Piperine on Glucose Metabolic (glucokinase) and Gluconeogenic Enzyme (glucose 6-phosphatase) Activity Diabetic Rats

The activity of glucokinase was drastically decreased in diabetic rats. Conversely, glucose-6 phosphatase activity was found to be significantly raised in the diabetic rats ($P < 0.05$). Treatment with piperine restored the altered levels of these enzyme activities to the level of normal control rats [Figure 1a and b].

Effect of Piperine on Activity of Glycogen Metabolizing Enzymes in Diabetic Rats

The activity of enzyme glycogen synthase which involves in glycogenesis was found to be significantly reduced ($P < 0.05$) in diabetic rats. Whereas, the activity of glycogen phosphorylase that involves in glycogenolysis was significantly increased in diabetic rats. These activities were partially restored to normal control rats and metformin treated diabetic rats by piperine administration [Figure 2a and b].

DISCUSSION

The present study was undertaken to identify antidiabetic activity of piperine on insulin signaling molecule in high-fat diet- and sucrose-induced type II diabetic rats. The excess glucose is stored in the form of glycogen chiefly in the liver and skeletal muscles. Cells employ insulin to stimulate glycogen synthase and inhibit glycogen phosphorylase, which will, in turn, promote glycogen synthesis while inhibiting the glycogen breakdown, respectively. Although hyperglycemia is primarily caused by reduced glycolysis and hepatic glycogen storage with an increased hepatic glucose production, a complex of various enzymes involved in the glucose storage and glycogen degradation pathway tightly regulates these processes. Glycolysis is initiated by the key enzyme glucokinase, an insulin-dependent enzyme, which phosphorylates glucose to glucose-6-phosphate.[19] In the present study, the levels of glucokinase were reduced in high-fat diet-induced type-2 diabetes which reflects on the reduced glucose utilization by the tissues. However, the treatment with piperine treatment raised the levels of this rate-limiting enzyme of glycolysis, proportionally to the levels of insulin. This confirms the improvement of glucokinase levels due to the increase in the insulin levels which stimulated better glucose utilization by the hepatic tissues.

The glycogen metabolic enzymes, glycogen synthase, and glycogen phosphorylase are reciprocal to each

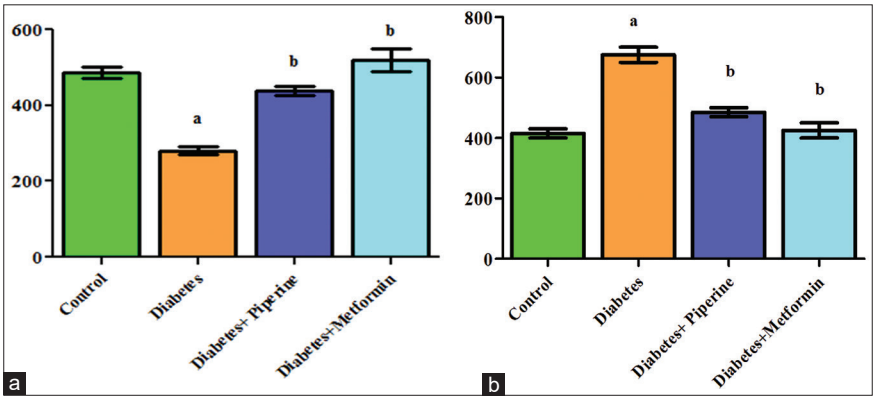

Figure 1: (a and b) Effect of novel on glucokinase and glucose-6-phosphatase in high-fat diet-induced in type-2 diabetic rats and compared with metformin. Each bar represents mean±SEM of six animals. Significance at $P < 0.05$, a – compared with control, b – compared with control, diabetic control

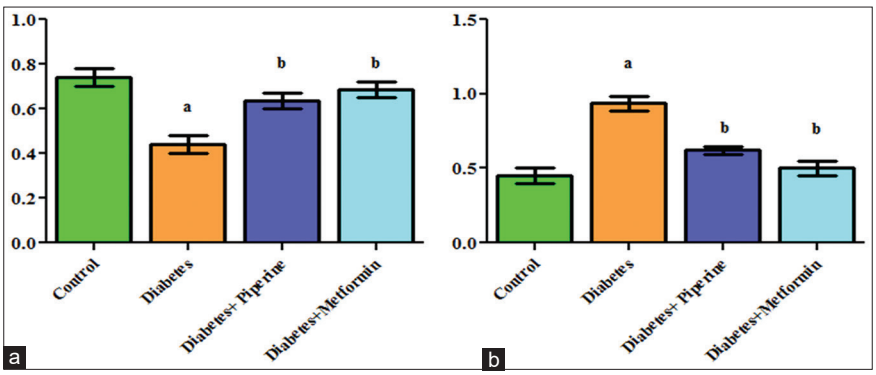

Figure 2: (a and b) Effect of novel on glycogen synthase and glycogen phosphorylase in high-fat diet-induced type-2 diabetic rats compared with metformin. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$, a – compared with control, b – compared with control, diabetic control

other. The activity of glycogen phosphorylase and glucose-6-phosphatase was significantly increased with concomitant decrease in the activity of glycogen synthase activity which was observed in high-fat diet-induced type-2 diabetic rats. Storage level of hepatic glycogen content was also decreased significantly in type-2 diabetic rats compared to control rats. Treatment with piperine brought down hyperglycemic enzymes near to normal and values were comparable to that of metformin. Antidiabetic activity of piperine and black pepper has been shown to have antidiabetic activity in animal models as well as *in vitro* studies.[20,21]

CONCLUSION

The results of the above experiments have concluded that piperine regulates both glycogen metabolic and gluconeogenic enzymes in the liver. Hence, it can be used for the management of type-2 diabetes as a natural compound. Further studies on the possible role of piperine on insulin signaling molecules are warranted.

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