Research Article

Study on the effect of piperine on gluconeogenic enzymes in the liver of type-2 diabetic rats

B. Sachin Aditya, J. Selvaraj*, V. Vishnu Priya, R. Gayathri

ABSTRACT

Aim: The study was designed to assess the effect of piperine on gluconeogenic enzymes in high-fat diet-induced type-2 diabetic rats. **Experimental Design:** Adult male Wistar rats were used and divided as Group I: Control; Group II: Type-2 diabetic rats; Group III: Type-2 diabetic rats treated with piperine (40 mg/kg body weight [b.wt]/day, orally for 30 days); and Group IV: Type-2 diabetic rats treated with metformin (50 mg/kg b.wt/day, orally for 30 days). After 30 days of treatment, animals were anesthetized; liver tissues were dissected out and used for the assessment of gluconeogenic enzymes such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) **Results:** In diabetic animals, glucose-6-phosphatase and PEPCK activity were significantly raised ($P < 0.05$) compared with control animals. Oral administration of the piperine reduced the activity of these enzymes ($P < 0.05$) to that of the control and standard drug metformin. **Conclusion:** The present study shows that piperine possesses antidiabetic activity by reducing gluconeogenic enzymes in the liver.

KEY WORDS: Glucose-6-phosphatase, Phosphoenolpyruvate carboxykinase, Piperine, High-fat diet, Type-2 diabetes

INTRODUCTION

Diabetes mellitus is not a single disease, but a group of metabolic disorders affecting a huge number of populations in the world and is mainly characterized by chronic hyperglycemia, resulting from defects in insulin secretion (Type I) or its action (Type II). It is predicated that the number of diabetic persons in the world could reach up to 366 million by the year 2030.[1] The prolonged exposure to chronic hyperglycemia in diabetes leads to the development of complications of cardiovascular, renal, neurological, and visual systems.[2] The frequency of this disorder is on the rise globally, is likely to hit 300 million by 2025 with India projected to have the largest number of diabetic cases.[3] Although several oral antidiabetic therapies such as biguanides (phenformin and metformin), sulfonylureas (glibenclamide and glipizide), and thiazolidinediones are widely used along with insulin for the treatment of diabetes mellitus, there are certain limitations due to high cost and side effects such as development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity, and impaired renal function.[4,5]

The recommendations of the World Health Organization on the use of alternative medicines for treating diabetes provide an impetus for research in this area.[6] The center of attention of research in diabetes includes discovering newer antidiabetic agents and isolating the active compounds from herbal sources that have been recognized to have antidiabetic properties as have been described in ancient texts. Herbs have accelerated the global efforts to harness and harvest medicinal plants having multiple beneficial effects. Some pre-clinical and clinical studies have confirmed their hypoglycemic effect and actions like repair of β-cells of islets of Langerhans.[7]

Piper nigrum (family Piperaceae) is a valuable medicinal plant. It is one of the most commonly used spices and considered as "The king of spices" among various spices. Black pepper is grown in many tropical regions such as Brazil, Indonesia, and India. *P. nigrum* is commonly known as Kali Mirch in Urdu and Hindi, Pippali in Sanskrit, Milagu in Tamil, and Pepper corn, White pepper, Green pepper, and Black pepper in English. It has more than 1000 species and is a widely

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Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

***Corresponding author:** J. Selvaraj, Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600 077, Tamil Nadu, India. E-mail: jselvaendo@gmail.com

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used spice. It is valued for its distinct biting quality attributed to piperine and its isomers.[8] It contains major pungent alkaloid piperine which is known to possess many interesting pharmacological actions. It has been extensively evaluated for its antidepressant,[9] anticonvulsant,[10] antioxidant,[11,12] antimutagenic,[13] hepatoprotective,[14] endocrine,[15] and several other activities. Oral administration of *Piper longum* dried fruits has shown significant anti-hyperglycemic, antioxidant, and antilipid peroxidative effects in hyperglycemic rats.[16] It has also been accounted that piperine enhances the bioavailability of several synthetic and natural drugs such as sulfadiazine, streptomycin, rifampicin,[17] pyrazinamide, isoniazid, nateglinide,[17] propranolol,[18] curcumin, and boswellic acid.[19] Moreover, it has recently been reported that piperine exerts anti-adipogenesis effect by activating PPAR-c receptors[20,21] which play a crucial role in insulin sensitization.[22] The current study was designed to assess the effects of piperine on gluconeogenic 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 ± 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 were made diabetic (type-2) by a single intraperitoneal injection of streptozotocin (35 mg/kg body weight [b.wt]), 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.

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 b.wt/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 b.wt), liver, skeletal muscle, and adipose tissue will be excised and used for the assay of various parameters.

Activity of Glucose-6-Phosphatase

Procedure

A 100 µl of this enzyme solution was added to a mixture containing 3 ml of tris-buffer and 1 ml of glucose-6-phosphate solution. This was incubated at 37°C for 5 min.[23] To this, 900 µl of trichloroacetic acid was added and mixed well. It was incubated at 25°C for 5 min and centrifuged at 1600 × g for 10 min. A 2 ml of supernatant was taken and to this 1 ml of ammonium molybdate solution was added and incubated for 10 min at room temperature. Then, 0.4 ml of aminonaphthalenesulfonic acid was added. After 20 min incubation, this was measured at 640 nm. Standard of various concentrations was also prepared. The standard graph was drawn and the phosphate liberated was calculated accordingly. The results are expressed as µmoles of orthophosphate liberated/min/mg protein.

Activity of Phosphoenolpyruvate Carboxykinase (PEPCK)

Procedure

A 100 µl of enzyme solution was added to 800 µl ATP assay solution and final volume is made up to 1 ml with distilled water.[23] This reaction mixture was mixed well and change in absorbance was recorded for approximately 5 min at 340 nm. Blank solution was also prepared and the absorbance was recorded. ΔA340 nm/min was calculated for both test and blank solution. The activity is expressed as U/g protein.

Statistical Analysis

The data were be subjected to statistical analysis using one-way analysis of variance (ANOVA) 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 Activity of Gluconeogenic Enzymes in the Liver of Type-2 Diabetic Adult Male Rat

The activity of gluconeogenic enzymes (PEPCK and G-6-Pase) were drastically elevated in diabetic animals when compared to control. Administration of piperine significantly reduced these enzyme activities as that of standard drug metformin [Figures 1 and 2].

DISCUSSION

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.[24] The glycogen metabolic enzymes, glycogen synthase, and glycogen phosphorylase are reciprocal to each other. In the present study, the activity of glucose-6-phosphatase and PEPCK was significantly increased in type-2 diabetic rats. Treatment with piperine brought down hyperglycemic enzymes near to normal and values were comparable to that of metformin suggesting that piperine plays an important role in controlling gluconeogenic enzymes in the liver. In this regard, Arcaro *et al.* (2014)[24] reported that piperine significantly reduced hepatic gluconeogenesis when it was coadministered with curcumin.

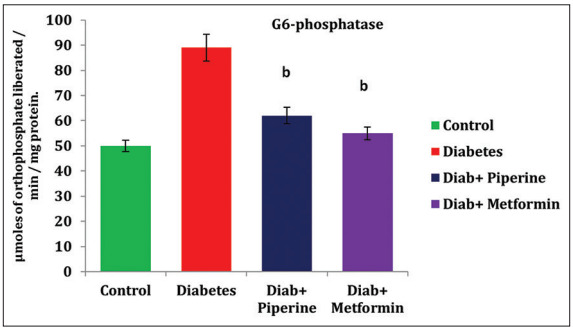

Figure 1: Effect of piperine on glucose-6-phosphatase activity in the liver of type-2 diabetic adult male rats. Each value represents Mean ± SEM of six animals ($n = 6$). Significance at $P < 0.05$, a – compared with control; b – compared with diabetic control

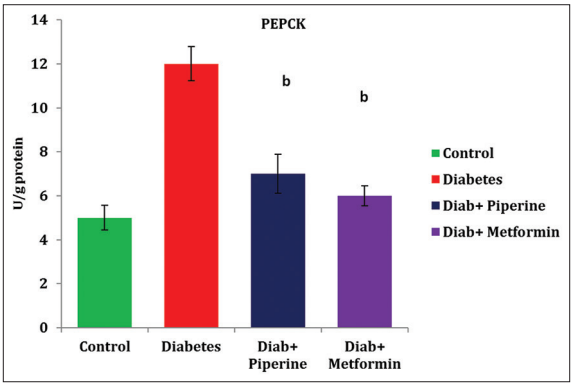

Figure 2: Effect of piperine on glucose phosphoenolpyruvate carboxykinase activity in the liver of type-2 diabetic adult male rats. Each value represents Mean ± SEM of six animals ($n = 6$). Significance at $P < 0.05$, a – compared with control; b – compared with diabetic control

CONCLUSION

The present study shows that piperine possesses antidiabetic activity by reducing gluconeogenic enzymes in the liver. Further studies on the molecular mechanism of the action of piperine are warranted.

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