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Sinapic Acid, a Naturally Occurring Carboxylic Acid Derivative Ameliorates Hyperglycemia in High Fat Diet-Low Dose STZ Induced Experimental Diabetic Rats

R. NITHYA[1], S. SUBRAMANIAN[2]

[1]Dept of Biochemistry, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India.

[2]Associate Professor, Dept of Biochemistry, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India,
E-mail: subbus2020@yahoo.co.in.

Abstract: Diabetes mellitus is characterized by the disturbances of carbohydrate, lipid and protein metabolism, with a high risk of morbidity and mortality from primary as well as secondary complications. Though drugs are plenty for the treatment of diabetes, none is found to be ideal due to the undesirable side effects associated with long term treatment. Hence, search for novel drugs especially from plant origin continues. The aim of this study is to evaluate the effect of sinapic acid, a naturally occurring carboxylic acid widely distributed in edible plants such as berries, kiwis and plums, in high fat diet- low dose streptozotocin induced diabetic rats. Oral administration of graded doses of sinapic acid for various time intervals to control as well as diabetic group of rats revealed the non toxic nature of sinapic acid. Oral administration of sinapic acid at a concentration of 25mg\kg b.w for 30 days significantly decreased the levels of fasting blood glucose, glycosylated haemoglobin and improved the levels of plasma insulin. The altered levels of liver glycogen along with the activities of glycogen synthase and glycogen phosphorylase were reverted back to near normal after treatment with the sinapic acid. The data obtained suggested that sinapic acid is nontoxic and possess significant antidiabetic properties.

Keywords: Diabetes Mellitus, Sinapic Acid, High Fat Diet, Streptozotocin, Fasting Blood Glucose, Plasma Insulin.

I. INTRODUCTION

Diabetes mellitus is considered as one of the main threats to human health in the 21st century. In developing countries, the prevalence of diabetes is alarmingly increasing. According to World Health Organization (WHO), around 70 million people suffering from diabetes mellitus (David et al., 2010). Changes in human behaviour and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide (Zimmet et al., 2001). Diabetes mellitus, a chronic disorder is associated with long term complications including retinopathy, neuropathy, nephropathy and angiopathy. Diabetes mellitus is considered to be a major risk for cardiovascular disorders namely ischemic heart disease, cerebral stroke and peripheral artery disease leading to increased mortality (Kristova et al., 2008). Experimentally induced diabetes in animals has provided considerable insight into the physiological and biochemical derangements of the diabetic state. Significant changes in lipid metabolism have also been reported in diabetes (Holman and Turner, 1991). Concurrently, liver and kidney that participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids, and triglycerides, are also severely affected during diabetes (Akram et al., 2011). Streptozotocin (STZ) is a broad spectrum antibiotic and alkylating genotoxic agent with potent diabetogenic properties (LeDoux et al., 1986).

Induction of experimental diabetes in rats using STZ is reliable and provides a convenient model to study the efficacy of hypoglycaemic agents (Ar'Rajab and Ahren, 1993; Brenne et al., 2003). STZ is specifically toxic to pancreatic β-cells involving uptake by glucose transporter 2 (GLUT-2). STZ impairs glucose oxidation and decreases insulin biosynthesis and secretion (Nukatsuka et al., 1990). It also generates reactive oxygen species (ROS), which contribute to DNA fragmentation and evokes other deleterious changes in β-cells (Takasu et al., 1991; Fukudome et al., 2008). When injected into adult rats, STZ can cause Type-1 DM with severely elevated blood glucose levels; however, when administered to neonatal rats, the neonates develop Type-2 DM (Takada et al., 2007). High fat diet low dose STZ induced diabetic animals are in a state of polyuria, increased water intake, dehydration, weight loss, muscle wasting and increased food intake which closely resembles human type 2 diabetes (Samarghandian et al., 2012). The therapeutic management of DM with minimal side effects remains a clinical challenge. There is growing interest in the potential use of medicinal plants as an alternative treatment for diabetes as these are commonly cheaper, less toxic and with fewer side effects (Nissen and Wolski, 2007).

In recent years, flavonoids have attracted the interest of researchers because they show promise of being power antioxidants that can protect the human body from free radicals and against oxidative stress. Sinapic acid is a naturally occurring carboxylic acid. It is a member of the phenylpropanoid family. Sinapic acid is a cinnamic acid derivative which possesses 4- hydroxy-3, 5-dimethoxy cinnamic acid with molecular formula $C_{11}H_{12}O_5$ and molecular weight 224.21g/mol. It is widely distributed in edible plants such as cereals, nuts, oil seeds and berries (Shahidi and Naczk, 2004). Sinapic acid is a major free phenolic acid present in rape seed meal, with the majority found in the esterified form of sinapine (Krygier et al., 1982). Sinapic acid has demonstrated potent antioxidant capacity and its efficiency is higher than ferulic acid and sometimes comparable to that of caffeic acid (Niwa et al., 1999; Nenadis et al., 2007). In the absence of systemic studies in the literature, the present study was aimed to evaluate the anti-hyperglycemic effect of sinapic acid in HFD- Low dose STZ induced type 2 diabetes in Wistar albino rats.

II. MATERIALS AND METHODS

A. Experimental Animals

Male albino Wistar rats (160-180g) were purchased 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. The experimental rats were maintained in a controlled environment (12:12 h light/dark cycle and temperature (30 ± 2 °C).The rats were acclimatized for one week before starting the experiments.

B. High Fat Diet Fed Streptozotocin Induced Diabetes

The rats were divided into two dietary regimens by feeding either normal or high fat diet (HFD) for the initial period of two weeks (Srinivasan et al., 2005). The ingredients and chemical composition of the HFD was followed as before reported. After two weeks of dietary manipulation, the groups of rats fed with HFD were injected intraperitoneally with a low dose of STZ (35 mg/kg b.w) dissolved in 0.1M cold citrate buffer, pH 4.5). One week after STZ injection, the rats were screened for blood glucose level. Rats having fasting blood glucose (FBG) >250mg/dl that exhibited random hyperglycaemia and glycosuria were selected for the experiment. The rats were allowed to continue to feed on the respective diets until the end of the experiments.

Experimental Design: The rats were divided into 4 groups each group comprising of 6 rats.
Group 1: Control rats.
Group 2: Diabetic rats (HFD-Low dose STZ (35 mg/kg bw).
Group 3: Diabetic rats treated with sinapic acid (25 mg/kg bw).
Group 4: Diabetic rats treated with metformin (200 mg/kg bw).

Sinapic acid was dissolved in 0.2% dimethylsulfoxide (DMSO) and administrated to rats orally using an intragastric tube daily for a period of 30 days.

C. Sample Collection

After 30 days of treatment, the animals were fasted over night and then sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifugation. The liver and kidney were carefully removed, weighed and washed in ice-cold saline. The liver, kidney and muscle were sliced into pieces and homogenized in an appropriate buffer (pH 7.0). The homogenates were centrifuged at 3000 rpm for 10 min at 0°C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

D. Intraperitoneally Insulin Tolerance Test

At the end of the experimental period, fasting blood samples were withdrawn through retro-orbital bleeding from the control and experimental groups of rats. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after the intraperitoneal administration of a bolus of insulin (2 unit/kg b.w). All the blood samples were collected with EDTA for the determination of glucose by using glucose oxidase peroxidase/diagnostic enzyme kit (Span Diagnostic Chemicals, Surat, India) and the analysis was performed according to the manufacturer's instructions.

E. Biochemical Parameters

Fasting blood glucose level was estimated according to the method of Sasaki et al., (1972). Plasma insulin was assayed using the Ultrasensitive ELISA kit for rat insulin (Linco Research, St Charles, MO, USA). Glycosylated hemoglobin (HbA1c) levels were estimated according to the method of Nayak and Pattabiraman, (1981).

F. Estimation of Hepatic Glycogen Level

The glycogen level of the liver was measured by the anthrone method as demonstrated in previous studies (Carroll et al., 1956). The concentrations of liver glycogen were expressed as mg of glycogen per wet weight.

G. Assay of Key Enzymes of Carbohydrate Metabolism

A portion of the liver tissue was dissected out, washed immediately with ice-cold saline and 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 assay of glycogen, glycogen synthase (Leloir and Goldemberg, 1962) and glycogen phosphorylase (Cornblath et al., 1963).

H. Statistical Analysis

The results were expressed as mean ± S.E.M of six rats per group and statistical significance was evaluated by one-way analysis of variance(ANOVA) using SPSS (version 16) program followed by LSD. Values were considered statistically significant when $p < 0.05$.

III. RESULTS AND DISCUSSION

Table I shows the effect of sinapic acid on the levels of fasting blood glucose, plasma insulin and glycosylated haemoglobin (HbA1c) in HFD low dose STZ induced diabetic rats. The level of blood glucose and HbA1c was found to be significantly elevated in diabetic rats as compared with normal control. Oral administration of sinapic acid to diabetic rats significantly improved the altered levels.

The levels of plasma insulin were moderately decreased in HFD low dose STZ induced diabetic rats. Diabetic rats treated with sinapic aid as well as metformin showed improved insulin level. Type 2 diabetes mellitus is the most common form of the metabolic disorder which is caused by impaired insulin secretion paralleled by a progressive decline in β-cell function and chronic insulin resistance (Lupi and Del Prato, 2008). The development of an ideal model for type 2 diabetes that would closely reflect the natural history and metabolic characteristics of human T2DM is very challenging. Hyperglycemia is the essential feature of diabetes mellitus resulting in oxidative stress mediated tissue damage contributing to diabetes and its associated complications (Zhang et al., 2010). Sinapic acid administration augments insulin stimulated glucose uptake into the peripheral tissues which is evident from intraperitoneal insulin tolerance test. It is also evident that sinapic acid acts as insulin sensitizer likely due to enhanced glucose uptake in the main target organs. The levels of plasma insulin were moderately decreased in HFD low dose STZ induced diabetic rats.

Though the level was not decreased more, the insulin level in HFD low dose STZ diabetic rats could not facilitate glucose uptake due to insulin resistance. There was an upgraded insulin sensitivity and a rise in insulin level in sinapic acid treated diabetic rats compared to the diabetic control rats suggesting that sinapic acid exhibit significant insulin sensitization activity as well as improvement in the glucose homeostasis probably due to improved pancreatic ß-cell function which is evident from improved plasma insulin level. Administration of sinapic acid to diabetic rats decrease blood glucose level and this was accompanied by arise in plasma insulin concentration. The levels of blood glucose, glycosylated haemoglobin was found to be decreased in sinapic acid treated rats indicating the beneficial role of sinapic acid in upholding glucose homeostasis. Table II represents the levels of glycogen content in liver tissue of control and experimental groups of rats. The level of glycogen content was reduced in diabetic rats whereas treatment with sinapic acid as well as metformin to diabetic groups of rats restored the level of glycogen in liver tissue.

The liver is the vital organ of metabolism and plays an important role in maintaining normal blood glucose levels via its ability to store glucose as glycogen and hydrolyze glycogen to glucose (Atsuo et al., 2011). The glycogen level and the activity of glycogen synthase (GS) are responsiveness to insulin signalling are all reduced in diabetes (Chang, 1972; Kaslow et al., 1979; Groop et al., 1989; Thorburn et al., 1990). Fig.1 represents the levels of glycogen synthase content in liver tissues control and experimental groups of rats. The level of glycogen synthase content was reduced in diabetic rats, whereas treatment with sinapic acid as well as metformin to diabetic groups of rats restored the level of glycogen synthase in liver tissue. Carbohydrate metabolism is severely impaired in the hyperglycemic state and is characterized by decreased glucose uptake, changes in glycogen synthesis, and insulin resistance. The expression and activity of the enzyme glycogen synthase, which is considered to play an important regulatory role in glucose metabolism, are affected by diabetes and glucose intolerance (Rao et al., 1995; Gannon and Nuttall, 1997). GS activity was significantly lower in rats fed the high fat diet with low dose STZ incomparison to controls. The administration of sinapic acid restored the level of glycogen synthase in sinapic acid treated rats.

Fig.2 represents the activity of glycogen phosphorylase content in liver tissue of control and experimental groups of rats. The level of glycogen phosphorylase content was increased in diabetic rats, whereas treatment with sinapic acid as well as metformin to diabetic groups of rats altered the level of glycogen phosphorylase in liver tissue. Glycogen phosphorylase (GP), the main regulatory enzyme in the liver responsible for the control of blood glucose levels. Glycogen phosphorylases catalyze the breakdown of glycogen to glucose-1-phosphate for glycolysis (Rath et al., 2000).There is an increased level of glycogen phosphorylase activity seen in diabetic rats. In the present study, the administration of sinapic acid to diabetic rats improved the glycogen content and normalized the altered activities of glycogen metabolizing enzymes in both the liver tissues, which is due to improved glucose utilization and storage.

TABLE I: Effect of Sinapic Acid on Intraperitoneally Insulin Tolerance Test In Experimental Groups of Rats

Group	Blood glucose (mg/dl)	Plasma insulin(µU/ml)	HbA1c (Hb%)
Control	85.50±5.62	15.21±0.25	5.03±0.23
Diabetic	272.31±14.28	10.33±0.46	12.12±0.44
Diabetic + SA	135.14±5.22	12.11±0.29	7.11±0.33
Diabetic rats+ metformin	121.15±6.77	14.44±0.43	6.29±0.22

Units: mg/dl for blood glucose, % hemoglobin for HbA1c, µU/ml for plasma insulin. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. Values are statistically significant at $P<0.05$. The results were compared with Control rats, Diabetic rats

TABLE II: Level of Glycogen Content in Liver Tissues of Control and Experimental Groups of Rats

Group	Liver Glycogen(mg glucose/g tissue)
Control rats	49.52±3.43
Diabetic rats	19.52±2.16
Diabetic rats + SA	41.06±3.78
Diabetic rats + metformin	36.83±3.62

Units are expressed as: µmoles of Pi liberated/h/mg of protein for glycogen and Values are given as mean ± S.E.M 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: control rats; diabetic control rats. Values are statistically significant at $*P<0.05$.

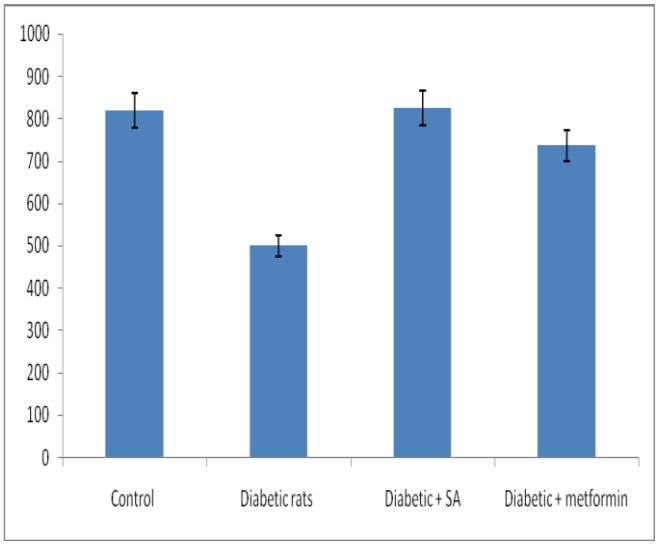

Fig.1. Effect of sinapic acid on the levels of glycogen synthase in the experimental group of rats.

Units are expressed as: µmol of UDP formed/h/mg protein for glycogen synthase. Values are given as mean ± S.D for groups of six rats in each. One-way ANOVA followed by post hoc test LSD was used for statistical analysis. Denotes significant difference in comparing with control at $P < 0.05$.

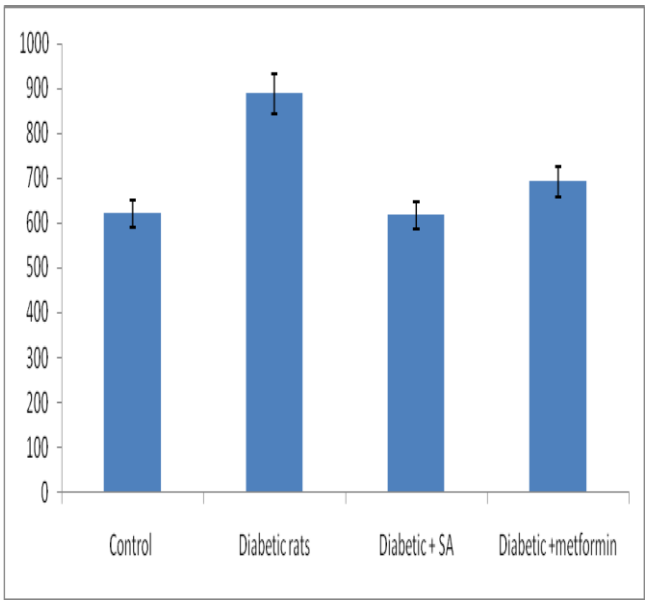

Fig.2.Effect of sinapic acid on the levels of glycogen phosphorylase in the experimental group of rats.

Units are expressed as: µmol of UDP formed/h/mg protein for glycogen phosphorylase. Values are given as mean ± S.D for groups of six rats in each. One-way ANOVA followed by post hoc test LSD was used for statistical analysis. Denotes significant difference in comparing with control at $P < 0.05$.

IV. CONCLUSION

In conclusion, the present study revealed that sinapic acid possesses a potential antihyperglycemic effect, through an increase in insulin production associated with subsequent increase in the activity of hepatic glycogen, glycogen synthase and decrease in the activity of glycogen phosphorylase.

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