



ANTIBIABETIC EFFICACY OF ELLAGIC ACID IN STREPTOZOTOCIN-INDUCED DIABETES MELLITUS IN ALBINO WISTAR RATS

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ABSTRACT

The objective of the study was to evaluate the effect of ellagic acid on plasma glucose, insulin and C-peptide, blood hemoglobin and glycosylated hemoglobin, glycogen and carbohydrate metabolic enzymes in experimental diabetes in albino wistar rats. Diabetes was induced by intraperitoneal injection of streptozotocin (45mg/kg) to albino wistar rats. Ellagic acid (50 mg/kg and 100 mg/kg) dissolved in 0.2% dimethyl sulfoxide was administered orally to diabetic rats using an intragastric tube daily for a period of 35 days. A significant increase in plasma glucose, blood glycosylated hemoglobin and hexokinase activity and decrease in plasma insulin and C-peptide, blood hemoglobin, glycogen (liver and muscle) and the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney were observed in STZ-induced diabetic rats. Oral administration of ellagic acid restored all these biochemical parameters to near normal. Our present study clearly revealed that ellagic acid possesses potent antihyperglycemic effect in STZ-induced diabetic rats.

Keywords: Ellagic acid, Streptozotocin, Diabetes, Carbohydrate metabolic enzymes, Insulin and C-peptide.

INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia due to disturbance in the group of metabolism of carbohydrates, fat and protein, resulting from defects in insulin secretion, action or both^{1,2}. Currently there are over 150 million diabetics worldwide and this number is likely to increase due to increase in sedentary lifestyle, consumption of energy rich diet and obesity³. In modern medicine, there is still no satisfactory effective therapy available to cure diabetes⁴.

Therefore, it has become necessary to search for an economically and therapeutically effective treatment, especially for usage in developing and under-developed countries. Many indigenous medicinal plants have been found to be useful for the successful management of diabetes⁵.

Streptozotocin (STZ) is well known for its selective pancreatic islet cell toxicity and has been extensively used for the induction of diabetes mellitus in animals⁶. Streptozotocin induced diabetes is a well-documented model of experimental diabetes. Previous reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used⁷. STZ-induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia. STZ is a pancreatic β -cell toxin that induces rapid and irreversible necrosis of β -cells⁸.

Recently there has been a growing interest in hypoglycemic agents from natural products, especially those derived from plants. Plant sources are usually considered to be non-toxic, with fewer side effects than synthetic sources. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms.

Unlike primary metabolites, absence of secondary metabolites does not result in immediate death but rather in long-term impairment of the organism's survivability, fecundity or aesthetics or perhaps in no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses⁹.

Flavonoids are a class of water-soluble plant pigments. Flavonoids are classified into isoflavones, anthocyanidins, flavans, flavonols, flavones and flavanones. Some of the best-known flavonoids include genistein in soya and quercetin in onions¹⁰. Flavonoids also include hesperidin, rutin, citrus flavonoids and a variety of other supplements¹¹. While they are not considered essential nutrients,

some flavonoids support health by strengthening capillaries and other connective tissue and some function as anti-inflammatory, antihistaminic and antiviral agents¹². Flavonoids are found in a wide range of foods. For example, flavanones are in citrus, isoflavones in soya products, anthocyanidins in wine and bilberry and flavans in apples and tea¹³. Flavonoids cannot be produced by the human body and have taken in through the daily diet¹⁴.

Ellagic acid is a polyphenol antioxidant found in numerous fruits and vegetables including blackberries, raspberries, strawberries, cranberries, walnuts, pecans, pomegranates, wolfberry and other plant foods. Ellagic acid was discovered by Braconnot in 1831. The antiproliferative and antioxidant properties of ellagic acid have spurred preliminary research into the potential health benefits of ellagic acid consumption.

The highest levels of ellagic acid are found in raspberries. In plants, ellagic acid is present in the form of ellagitannin. Ellagic acid contains antioxidant, anti-mutagen and anti-cancer properties¹⁵. The present study was thus designed to evaluate the influence of ellagic acid on biochemical parameters and the activities of carbohydrate metabolic key enzymes in normal and STZ-induced diabetic rats.

MATERIALS AND METHODS

Experimental animals

Female albino wistar rats (150-200 g) obtained from Venkateswara Enterprises, Bangalore were used in this study. They were housed in polypropylene cages (47cm x 34cm x 20cm) lined with husk. It was renewed every 24 hours under a 12:12 hour light: dark cycle at around 22^o C and had free access to water and food.

The rats were fed on a standard pellet diet (Pranav Agro Industries Limited., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fiber, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolizable energy of 3600 kcal. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drug and chemicals

Streptozotocin (STZ) was purchased from Himedia Laboratories Private Limited, Mumbai. Ellagic acid was purchased from Sigma-Aldrich, St. Louis, USA. Glucose, hemoglobin and glycosylated hemoglobin kits were purchased from Agappe diagnostics, Kerala,

India. Insulin and C-peptide kits were obtained from Monobind Inc, Lake Forest, CA 92630, USA. All other chemicals used in the study were of analytical grade.

Induction of experimental diabetes

Streptozotocin was used to induce diabetes mellitus in normoglycemic female albino wistar rats. A freshly prepared solution of STZ (45mg/kg body weight) in 0.1M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1ml/kg body weight to overnight fasted rats. After 48 hours of STZ administration, rats with moderate diabetes having glycosuria and hyperglycemia were selected for the experiment ¹⁶.

Experimental design

A total of 36 rats were used in the present investigation. The animals were randomly divided into 6 groups of 6 rats in each group.

Group	1:	Normal control rats
Group	2:	Normal rats + Ellagic acid (50 mg/kg)
Group	3:	Normal rats + Ellagic acid (100 mg/kg)
Group	4:	Diabetic control rats
Group	5:	Diabetic + Ellagic acid (50 mg/kg)
Group	6:	Diabetic + Ellagic acid (100 mg/kg)

Ellagic acid was dissolved in 0.2% dimethyl sulfoxide and administered to rats orally using an intragastric tube daily for a period of 35 days.

Sample collection

At the end of the treatment period, all rats were fasted for 12 hours and sacrificed by cervical decapitation. The blood was collected into heparinized tubes and plasma was separated by centrifugation and used for biochemical analysis. Liver and kidney were dissected out, washed in ice-cold physiological saline, patted dry and weighed. The tissues were then homogenized in 0.1M Tris-HCl buffer, pH 7.4. The homogenate was used for the estimation of carbohydrate metabolic enzymes.

Biochemical estimations

Plasma glucose, insulin, C-peptide, blood total hemoglobin and glycosylated hemoglobin were estimated using commercial kits. Glycogen content in liver and muscle was estimated by the method of Wieland ¹⁷. Hexokinase, glucose-6-phosphatase and fructose-1, 6-bisphosphatase were assayed by the method of Brandstrup ¹⁸, Koide and Oda ¹⁹ and Gancedo and Gancedo ²⁰ respectively.

Statistical analysis

Results were expressed as mean \pm SD for six rats in each experimental group. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) 9.05 software. The data were analyzed using one-way analysis of variance (ANOVA) and group means were compared with Duncan's Multiple Range Test (DMRT). P-values < 0.05 were considered as significant.

RESULTS

Effect of ellagic acid on plasma glucose, insulin and C-Peptide

Table 1 shows the effect of ellagic acid on the levels of plasma glucose, insulin and C-peptide in normal and STZ-induced diabetic rats. Rats induced with STZ, showed a significant (p<0.05) increase in the level of plasma glucose and decrease in the levels of plasma insulin and C-peptide as compared to normal rats. Oral administration of ellagic acid for a period of 35 days significantly (p<0.05) decreased the level of plasma glucose and increased the levels of plasma insulin and C-peptide in STZ-induced diabetic rats.

Effect of ellagic acid on hemoglobin and glycosylated hemoglobin

The levels of hemoglobin and glycosylated hemoglobin in normal and STZ-induced diabetic rats are presented in Table 2. The diabetic rats showed a significant (p<0.05) decrease in the level of

hemoglobin and a significant (p<0.05) increase in the level of glycosylated hemoglobin when compared to normal rats. Oral administration of ellagic acid in STZ-induced diabetic rats reversed the changes in the levels of hemoglobin and glycosylated hemoglobin to near normal.

Effect of ellagic acid on liver and muscle glycogen

The effect of ellagic acid on liver and muscle glycogen content of normal and STZ-induced diabetic rats are depicted in Table 3. A significant (p<0.05) reduction in liver and muscle glycogen was observed in STZ-induced diabetic rats as compared to normal rats. Treatment with ellagic acid significantly (p<0.05) increased the concentration of liver and muscle glycogen when compared with untreated diabetic rats.

Effect of ellagic acid on carbohydrate metabolic enzymes

Table 4 and 5 illustrates the effect of ellagic acid on carbohydrate metabolic enzymes in liver and kidney of normal and STZ-induced diabetic rats. The activity of hexokinase was significantly (p<0.05) decreased in liver, whereas the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase were significantly (p<0.05) increased in the liver and kidney of diabetic rats when compared with normal rats.

Oral administration of ellagic acid significantly (p<0.05) increased the activity of hexokinase in liver and decreased the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney of STZ-induced diabetic rats when compared with diabetic controls.

Table 2: Effect of ellagic acid on the levels of hemoglobin and glycosylated hemoglobin in normal and STZ-induced diabetic rats

Groups	Hemoglobin (g/dl)	Glycosylated hemoglobin (mg/g Hb)
Normal control	15.62 \pm 0.5 ^a	6.74 \pm 0.30 ^a
Normal + Ellagic acid (50mg/kg)	15.72 \pm 0.9 ^a	6.73 \pm 0.25 ^a
Normal + Ellagic acid (100mg/kg)	15.66 \pm 0.5 ^a	6.78 \pm 0.2 ^a
Diabetic control	7.42 \pm 0.03 ^b	11.66 \pm 0.35 ^b
Diabetic + Ellagic acid (50mg/kg)	11.51 \pm 0.64 ^c	9.53 \pm 0.29 ^c
Diabetic + Ellagic acid (100mg/kg)	13.61 \pm 0.12 ^d	7.80 \pm 0.5 ^d

Each value is mean \pm S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 3: Effect of ellagic acid on liver and muscle glycogen content in normal and STZ-induced diabetic rats

Groups	Liver glycogen (mg/g tissue)	Muscle glycogen (mg/g tissue)
Normal control	54.43 \pm 1.32 ^a	9.613 \pm 0.99 ^a
Normal + Ellagic acid (50mg/kg)	55.75 \pm 4.68 ^a	9.566 \pm 0.97 ^a
Normal + Ellagic acid (100mg/kg)	55.28 \pm 4.65 ^a	9.650 \pm 1.09 ^a
Diabetic control	31.25 \pm 3.12 ^b	4.848 \pm 0.51 ^b
Diabetic + Ellagic acid (50mg/kg)	44.50 \pm 2.10 ^c	7.208 \pm 0.58 ^c
Diabetic + Ellagic acid (100mg/kg)	49.65 \pm 2.28 ^d	8.580 \pm 0.24 ^d

Each value is mean \pm S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 1: Effect of ellagic acid on plasma glucose, insulin and C- Peptide in normal and STZ-induced diabetic rats

Groups	Glucose (mg/dl)	Insulin(μ U/ml)	C-Peptide(ng/ml)
Normal control	85.35 \pm 4.4 ^a	17.61 \pm 0.60 ^a	6.89 \pm 1.61 ^a
Normal + Ellagic acid (50mg/kg)	84.45 \pm 5.1 ^a	17.21 \pm 1.20 ^a	7.23 \pm 1.54 ^a
Normal + Ellagic acid (100mg/kg)	83.24 \pm 4.73 ^a	17.04 \pm 0.66 ^a	6.04 \pm 1.20 ^a
Diabetic control	279.33 \pm 6.40 ^b	9.20 \pm 0.50 ^b	1.56 \pm 0.08 ^b
Diabetic + Ellagic acid (50mg/kg)	154.00 \pm 5.70 ^c	14.11 \pm 1.40 ^c	2.64 \pm 0.58 ^c
Diabetic + Ellagic acid (100mg/kg)	112.66 \pm 4.7 ^d	15.37 \pm 1.30 ^d	3.73 \pm 0.62 ^d

Each value is mean \pm S.D. for six rats in each group.

Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 4: Effect of ellagic acid on the activity of hexokinase in liver of normal and STZ-induced diabetic rats

Groups	Hexokinase(Unit ^A /h/mg protein)
Normal control	0.946 \pm 0.07 ^a
Normal + Ellagic acid (50mg/kg)	0.956 \pm 0.16 ^a
Normal + Ellagic acid (100mg/kg)	0.958 \pm 0.19 ^a
Diabetic control	0.348 \pm 0.01 ^b
Diabetic + Ellagic acid (50mg/kg)	0.769 \pm 0.05 ^c
Diabetic + Ellagic acid (100mg/kg)	0.859 \pm 0.06 ^d

A - μ moles of glucose phosphorylated

Each value is mean \pm S.D. for six rats in each group.

Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 5 Effect of ellagic acid on the activities of gluconeogenic enzymes in liver and kidney of normal and STZ-induced diabetic rats

Groups	Glucose-6-phosphatase (Unit ^B /min/mg protein)		Fructose-1,6-bisphosphatase (Unit ^C /h/mg protein)	
	Liver	Kidney	Liver	Kidney
Normal control	0.182 \pm 0.01 ^a	0.183 \pm 0.03 ^a	0.445 \pm 0.06 ^a	0.594 \pm 0.16 ^a
Normal + EA (50mg/kg)	0.174 \pm 0.02 ^a	0.175 \pm 0.02 ^a	0.435 \pm 0.05 ^a	0.469 \pm 0.06 ^a
Normal + EA (100mg/kg)	0.186 \pm 0.01 ^a	0.163 \pm 0.01 ^a	0.146 \pm 0.06 ^a	0.421 \pm 0.06 ^a
Diabetic control	0.383 \pm 0.03 ^b	0.383 \pm 0.02 ^b	0.929 \pm 0.09 ^b	2.629 \pm 0.56 ^b
Diabetic + EA (50mg/kg)	0.296 \pm 0.02 ^c	0.280 \pm 0.01 ^c	0.810 \pm 0.05 ^c	1.881 \pm 0.05 ^c
Diabetic + EA (100mg/kg)	0.243 \pm 0.04 ^d	0.220 \pm 0.02 ^d	0.589 \pm 0.04 ^d	1.410 \pm 0.03 ^d

EA - Ellagic acid

B - μ moles of inorganic phosphorous liberated

C - μ moles of inorganic phosphorous liberated

Each value is mean \pm S.D. for six rats in each group.

Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

DISCUSSION

Diabetes mellitus is a chronic disease characterized by high blood glucose levels due to an absolute or relative deficiency of circulating insulin levels. In the present study, diabetic rats exhibited a significant increase in plasma glucose level. This result is in consistent with other studies in rats ²¹.

The increased glucose level might be due to the fact that STZ causes notable reduction in insulin release by the destruction of pancreatic β -cells. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in STZ-induced diabetic rats ²². We have observed a significant decrease in glucose level in ellagic acid treated diabetic rats when compared with non-treated diabetic rats. The possible mechanism of hypoglycemic action may be through potentiation of pancreatic secretion of insulin from beta cells of islets or due to enhanced transport of blood glucose to the peripheral tissue ²³.

Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurement of both insulin and C-peptide levels has been reported to be a valuable index of insulin secretion rather than insulin alone ²³. C-peptide and insulin levels were significantly decreased in STZ-induced diabetic rats due to the

destruction of β -cells of pancreas there by inhibiting insulin release.

Oral administration of ellagic acid significantly increased the levels of plasma insulin and C-Peptide in STZ-induced diabetic rats when compared with diabetic control rats. Flavonoids stimulate the secretion of insulin from β -cells of pancreas. In hyperglycemic animals, it is possible that, ellagic acid may act by potentiation of pancreatic secretion or increasing glucose uptake.

The decreased level of total hemoglobin observed in diabetic rats might be due to the increased formation of glycosylated hemoglobin. Glycosylated hemoglobin was found to increase in uncontrolled diabetes and the increase is directly proportional to the fasting blood glucose level ²⁴. Measurement of glycosylated hemoglobin remains the standard biochemical marker for the assessment of glycemic control in patients with diabetes ²⁵.

During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin ²⁶. Oral administration of ellagic acid to STZ-induced diabetic rats reduced the formation of glycosylated hemoglobin by virtue of its normoglycemic activity. Since the level of glycosylated hemoglobin has been shown to provide an index of blood glucose concentration ²⁷, the decreased level of glycosylated hemoglobin

and the increased level of hemoglobin in treated diabetic rats showed the antihyperglycemic activity of ellagic acid.

Liver and muscle glycogen content was significantly reduced in STZ-induced diabetic rats. Glycogen is the primary intracellular storage form of glucose and its levels in various tissues are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since STZ causes selective destruction of β -cells of pancreas resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues decrease as they depend on insulin for influx of glucose²⁸. In general, increased hepatic glucose production, plus decreased hepatic glycogen synthesis and glycolysis, are the major symptoms of type 2 diabetes that result in hyperglycemia²⁹.

Glycogen synthesis in the rat liver and skeletal muscle is impaired in diabetes³⁰. Also, hepatic glycogen reserves are important for whole-body glucose homeostasis and are markedly low in the diabetic state^{28, 31}. Oral administration of ellagic acid to STZ-induced diabetic rats significantly increased the liver and muscle glycogen content by stimulating the remnant β -cells to release insulin.

In experimental diabetes, enzymes of glucose metabolism are markedly altered. In the current study, diabetic rats showed significant decrease in the activity of hepatic glucokinase and increase in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in the liver and kidney. Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes.

One such enzyme is hexokinase that catalyses the conversion of glucose to glucose-6-phosphate and plays a central role in the maintenance of glucose homeostasis³². In the liver, hexokinase is an important regulatory enzyme in the oxidation of glucose³³. Being an insulin-dependent enzyme, the hepatic hexokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the absence of insulin³⁴. This impairment results in a marked reduction in the rate of glucose oxidation *via* glycolysis, which ultimately leads to hyperglycemia. Oral administration of ellagic acid to STZ-induced diabetic rats resulted in a significant reversal in the activity of hexokinase, thereby increased the oxidation of glucose.

Glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis³⁵. Fructose-1, 6-bisphosphatase is one of the key enzymes of gluconeogenic pathway. Hepatic glucose production is raised in diabetic state and is associated with the impaired suppression of the gluconeogenic enzyme fructose 1, 6-bisphosphatase. Gluconeogenic enzyme activation is due to the state of insulin impairment because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes³⁶. Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1, 6-bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxykinase³⁷.

Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances by altering carbohydrate-metabolizing enzymes and leads to diabetes³⁸. Diabetic rats treated with ellagic acid showed significant decrease in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in the liver and kidney. Ellagic acid may primarily be modulating and regulating the gluconeogenic enzymes through regulation of cAMP or inhibition of gluconeogenesis.

In conclusion, the results of the present study indicated that ellagic acid has a beneficial effect on normalizing glucose level and carbohydrate metabolic enzymes in STZ-induced diabetic rats. This suggests the efficacy of ellagic acid in the maintenance of glucose

homeostasis and may be used as a therapeutic agent in the management of diabetes mellitus.

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