ANTI-HYPERGLYCEMIC ACTIVITY OF *IPOMOEA STAPHYLINA* LEAVES EXTRACT ON CARBOHYDRATE METABOLIZING ENZYMES AND GLYCOGEN CONTENT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract : Medicinal plants provide a new effective alternative compounds for treating hyperglycemic conditions. Recently scientific reports proved that medicinal plants provide a lot of hypoglycemic chemical compounds. Our present studies evaluate the effect of *Ipomoea staphylina* (ISEE) leaves extract on streptozotocin (STZ) induced diabetic rats. ISEE (200 mg/kg b.wt) was administered orally for 45 days to diabetic animals. Plasma glucose, insulin, carbohydrate metabolizing enzymes, glycogen content was determined in diabetic rats. The levels were restored to normal by the administration of ISEE. The results of this study revealed the antidiabetic effect of ISEE on STZ induced diabetic models. Finally, this study also proved the application of ISEE as promising alternate and complementary pharmacological agent against diabetes and its complications.

Keywords **- Diabetes, Streptozotocin, *Ipomoea staphylina*, insulin, glucose, carbohydrate metabolizing enzymes.**

I. INTRODUCTION

Diabetes mellitus (DM) is a long term disorder characterized by inherited and or pancreatic insufficiency in insulin secretion. Due to insulin insufficiency the condition hyperglycemia occurs in fasting and postprandial condition linked with ketosis and in rigorous condition loss of protein occurs (Ramakrishnan *et al*., 2017). DM is attaining a pandemic figure throughout the world and the state of affairs in developing nations is worsening day by day (Kumar *et al.,* 2010). According to the international diabetes federation (IDA) report, the number of diabetic patients is expected to increase from 171 million in year 2000 to 366 million or more by the year 2030 (IDA, 2012).

A hyperglycemia-associated mechanism induces many dysfunctions. These include increased polyol pathway flux, altered cellular redox state, increased lipid profile and accelerated reactive oxygen species (ROS) (Das Evcimen and King, 2007). Suffering from such a pandemic metabolic disease associated with hyperglycemia, patients with diabetes mellitus are known to have various changes in metabolic pathway including carbohydrate metabolism. Defects in regulation of carbohydrate metabolism and reliable 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. Persistent deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia. Gluconeogensis and glycolysis are the important mechanism for the maintenance of normoglycemic status (Ashokkumar and Pari, 2005). This presents a moving therapeutic target that requires different agents to address the different features of the disease (Bailey, 2000).

Management of diabetes with conventional treatment is not possible without adverse effect and high economic input (Srivastava *et al*., 1993). Traditional medicinal plants and their active biomolecules play a major role in the management of diabetes mellitus from the beginning of the last century (Pari and Venkateswaran, 2003). Thus a suitable antioxidant therapy would benefit in diabetes apart from traditional antidiabetic treatment. In recent years, considerable focus have been given to an intensive search for novel type of antioxidants present in plants and plant derived products for treating diabetes and related complications (Fabricant and Farnsworth, 2001; Kameswara Rao and Appa rao, 2001).

Ipomoea staphylina is a wide spread rambler belong to the family convolvulaceae. It is a persistent, timbered and tonsured hedge plant of pink colored flowers. A review of literature determines the various activities include antiulcer (Firdous and Nitesh, 2009) and anti-inflammatory, α-amylase, α-glucosidase and 5-lipoxygenase inhibition (Firdous *et al*., 2012) of *I. staphylina* (Firdous *et al*., 2016). It is a traditional medicine for treating respiratory problems, purgative, digestive disorders, anti-helmintic, and bronchitis. In advanced research of *Ipomoea staphylina* two bioactive compounds namely sitosteryl-3-O-β-Dglucoside and chiro deoxy inositol were isolated (Kota *et al*., 2013).

Till now, there is no scientific research to assess the antidiabetic activity of leaves of IS in STZ induced diabetic animals. So the present study was carried out to determine the effect of antidiabetic activity of *Ipomoea staphylina* leaves in STZ induced diabetic rats by assessing the carbohydrate metabolic enzymes in serum, muscle and kidneys of STZ induced diabetes rats.

II. MATERIALS AND METHODS

Preparation of extracts

Fresh IS leaves were collected and cleaned with distilled water and allowed to dry. Then powdered using a mechanical grinder and stored in a sterile container at 4°C until future use. 30 g of *IS* leaf powder was extracted with 200 ml of solvent ethanol at room temperature for 24 hours and filtered using Whatman No.1 filter paper to remove extractable substances, at every 3 hours interval. The combined extracts were then evaporated at 40°C to dryness. The dried extracts were stored at 4°C until further use.

Animals

Male albino wistar rats weighing approximately 160-200g were purchased and used for the study. The animals were maintained under standard conditions of humidity, temperature (25 ± 2ºC) and light (12 h light/dark). They were acclimatized to animal house conditions and were fed on a commercial pellet rat chow (AVM Cattle Feeds, Coimbatore, Tamilnadu) and water ad libitum. Experimental animals were handled according to the university and institutional Legislation, regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. All the protocol to execute this study was approved by Institutional ethical committee with the approval number (1416/PO/a/11/CPCSEA & 17.06.2013).

Albino wistar rats weighing 180-200g was overnight fasted and induced with STZ (55 mg/kg BW) by intraperitoneal (IP) injection. Citrate buffer with pH 4.5 was used for STZ preparation. The increased glucose level (>240 mg/dl) in blood determined at 72 hours and on 7th day after STZ administration confirms hyperglycemia. After induction of diabetes profitably the experimental animals were divided into four groups with six rat's minimum in each group.

Experimental design

Group I: Control rats.
Group II: Diabetic control rats.
Group III: Diabetic rats administered orally with IS ethanol extract of leaves (200 mg/kg b.w/rat) for 45 days (Pande *et al*., 2016)
Group IV: Diabetic rats administered orally with glibenclamide (600 µg / kg b.w/rat) in aqueous solution orally for 45 days (Muruganathan *et al*., 2017).

Collection of plasma or serum

Weight gain regarding body weight (data not shown) and glucose level in blood was determined randomly. At the end of the study, the animals was overnight fasted, anaesthetized and sacrificed by cervical decapitation. Blood was collected with or without EDTA for plasma or serum separation respectively.

Tissue homogenate preparation

10% homogenate of kidney and muscle were prepared with 0.1 M Tri-HCl buffer, pH 7.4. The homogenate were centrifuged at 3000 rpm for 10 min at 0°C. Supernatant was separated and used for various biochemical estimations.

BIOCHEMICAL ESTIMATIONS

Estimation of Blood glucose level

Glucose level in blood was determined by method of Sasaki *et al*., 1979. To 10 µl of sample added 1.0 ml of working standard reagent, mixed and incubated at 37°C for 15 min. The colour developed was read at 505 nm against blank containing distilled water instead of the sample. A standard was also processed similarly. The level of glucose is expressed as mg dl^{-1}.

Assay of insulin

A plasma level of insulin was determined using kits from Bio-Merieux, RCS, Lyon, France. 25 µl of the plasma was dispensed in microwells coated with anti-insulin antibody. To this, 100 µl of the enzyme conjugate was dispensed into each well, mixed for 5 sec and incubated at 25°C for 30 min. The wells were rinsed five times with washing buffer. Then, 100 µl of solution A and then 100 µl of solution B were dispensed into each well. This was incubated for 15 min at room temperature. The reaction was stopped by adding 50 µl of 2 N HCl to each well and read at 450 nm. The values are expressed as µU ml^{-1}.

Assay of hexokinase

Tissue hexokinase was assayed by the method of Lapeir and Rodnick, 2001. To 1 ml of glucose add 0.5 ml of adenosine triphosphate (ATP), 0.1 ml of magnesium chloride, 0.4 ml of potassium dihydrogen phosphate, 0.4 ml of potassium chloride, 0.4 ml of sodium fluoride and 2.5 ml of Tris-HCl buffer and this mixture was pre-incubated at 37°C for 5 min. The reaction was initiated by the addition of 2 ml of tissue homogenate. 1 ml of the reaction mixture was immediately transferred to the tubes containing 1 ml of 10% TCA that was considered as zero time. A second aliquot was removed and deproteinised after 30 min incubation at 37°C. The protein precipitate was removed by centrifugation and the residual glucose in the supernatant was estimated.

Assay of Glucose -6- phosphatase

G-6-Pase was measured by the method of Koida and Oda, 1959. To 0.7 ml of citrate buffer (0.1 mol/l, pH 6.5) add 0.3 ml of substrate (0.01mol/l) and 0.3 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 1 hour. The reaction of the enzyme was arrested by adding 1 ml of 10% TCA. The suspension was centrifuged and the phosphorus content of the

supernatant. The supernatant was made up to a known volume. To this, 1 ml of ammonium molybdate was added followed by 0.4 ml of amino naphthol sulphonic acid (ANSA). The blue colour developed after 20 min was read at 680 nm.

Assay of Fructose-1,6-bisphosphatase

Fructose-1,6-bisphosphatase activity was measured by Gancedo and Gancedo, 1971. The assay mixture in a final volume of 2 ml contained 1.2 ml of Tris-HCl buffer (0.1 mol/l, pH 7.0), 0.1 ml of substrate, 0.25 ml of magnesium chloride, 0.1 ml of potassium chloride solution, 0.25 ml of ethylene diamine tetra acetic acid (EDTA) solution and 0.1 ml of enzyme homogenate. The incubation was carried out at 37°C for 5 min. The reaction of the enzyme was arrested by the addition of 10% TCA. The suspension was centrifuged and the supernatant was used for. The supernatant was made up to a known volume. To this, 1 ml of ammonium molybdate was added followed by 0.4 ml of ANSA. The blue colour developed after 20 min was read at 680 nm.

Estimation of glycogen

Tissue glycogen was estimated by the method of Ong and Khoo, 2000. The alkali extract of the tissue was prepared by digesting 50 mg of fresh tissue with 3 ml of 30% potassium hydroxide solution in boiling water bath for 15 min. The tubes were cooled and mixed with 5 ml of absolute alcohol. A drop of 1 mol/l ammonium acetate was added to precipitate glycogen and left in the freezer overnight for complete precipitation. Glycogen was collected by centrifugation at 2000×g for 20 min. The precipitate was dissolved in distilled water with the aid of heating and again the glycogen was re-precipitated with alcohol and 1mol/l ammonium acetate and centrifuged. The final precipitate was dissolved in saturated ammonium chloride solution and 4 ml of anthrone reagent was added by cooling the tubes in an ice bath. The tubes were shaken well, covered with marble caps and heated in a boiling water bath for 20 min. After cooling, the absorbance was read at 640 nm against reagent blank treated in a similar manner.

Statistical analysis

The experimental results are expressed as means ± SD of six animals. The data were subjected to ANOVA and the significance of difference between samples means were calculated by DMRT using IRRISTAT version 3.1. P values < 0.05 were regarded as significant.

III. Results and Discussion

Results

The level of plasma glucose and insulin in normal and experimental animals was depicted in Fig 1. Increased glucose ($p<0.05$) and concomitant decrease level of plasma insulin was observed in diabetic control animals. Administration of ISEE at a dose of 200 mg/kg b.w significantly altered the level of blood glucose and insulin.

Fig. 2 showed the oral glucose tolerance test in normal and experimental diabetic animals. An augmented level of blood glucose (maximum value at 60 min) in the diabetic rats was observed after glucose load and decreased to near normal levels at 120 min, whereas, in STZ induced diabetic rats, the peak increase in blood glucose level was observed even after 60 min and remained high over the next 60 min. Supplementation with ISEE as well as glibenclamide to diabetic rats elicited a significant decrease in blood glucose level at 60 min when compared with untreated diabetic rats.

Table 1 depicts the activities of hexokinase and fructose1-6-bis phosphatase in the normal control and experimental diabetic animals. Decreased hexokinase level and concomitant increased level of fructose1-6- bis phosphatase was observed in serum, muscle and kidneys of STZ induced diabetic animals and it was normalized after ISEE treatment and glibenclamide.

Table 2 showed the activity of glucose 6-phosphatase in serum and kidney Increased level of glucose 6-phosphatase was found in diabetic animals. Administration of ISEE and glibenclamide decreased the activity of glucose 6-phosphatase ($P < 0.001$) in serum and kidneys of experimental diabetic rats.

The glycogen content in kidney and muscle in the experimental animals was represented in Table 3. Declined glycogen level in muscle and concomitant increased in level of kidneys were found in the diabetic condition. Administration of ISEE and standard significantly ($P < 0.05$) reversed the glycogen contents in the tissues to near normal.

Table 1. Effects of ISEE on Hexokinase and Fructose-1,6 bis Phosphatase in control and experimental animals

Groups	Hexokinase			Fructose-1,6 bis Phosphatase		
	Serum	Muscle	Kidney	Serum	Muscle	Kidney
Control	18.9±2.4	0.53±0.07	0.37±0.04	44.5±1.6	21.1±1.64	30.5±1.4
Diabetic	7.08±2.0^{a***}	0.34±0.02^{a***}	0.20±0.02^{a***}	63.01±2.9^{a***}	33.2±2.7^{a***}	57.7±3.1^{a***}
Diabetic+ISEE (200mg/k.g.b.w)	14.9±2.02^{b***}	0.45±0.03^{b***}	0.29±0.03^{b***}	38.4±1.2^{b***}	24.3±1.5^{b***}	34.4±3.3^{b***}
Diabetic+glibenclamide (600µg/k.g b.w)	15.9±1.5^{b***}	0.51±0.03^{b***}	0.33±0.01^{b***}	41.6±1.8^{b***}	22.5±1.5^{b***}	32.2±14.1^{b***}

Values are mean ± S.D, n = 6
[a] Significantly different from control
*[b] Significantly different from Diabetic control ***P < 0.001*
Hexokinase : µ moles of glucose phosphorylated / min/ mg of protein
Fructose-1,6 bis Phosphatase : µ moles of Pi liberated / min/ mg of protein

Table 2. Effects of ISEE on glucose 6-Phosphatase in control and experimental animals

Groups	Glucose 6-phosphatase	
	Serum	Kidney
Control	85.04±2.7	9.5±1.16
Diabetic	154.0±6.2^{a***}	24.2±2.03^{a***}
Diabetic+ISEE (200mg/k.g.b.w)	105.1±2.8^{b***}	14.4±2.9^{b***}
Diabetic+glibenclamide (600µg/k.g b.w)	89.3±2.7^{b***}	13.1±2.5^{b***}

Values are mean ± S.D, n = 6
[a] Significantly different from control
*[b] Significantly different from Diabetic control ***P < 0.001*
Glucose-6-Phosphatase: µ moles of Pi liberated / min/ mg of protein

Table 3. Effects of ISEE on glycogen content in control and experimental animals

Groups	Glycogen (mg/g wet tissue)	
	Kidney	Muscle
Control	6.7±0.6	4.6±0.3
Diabetic	3.4±0.7^{a***}	6.5±0.6^{a***}
Diabetic+ISEE (200mg/k.g.b.w)	5.4±0.7^{b**}	5.4±0.2^{b**}
Diabetic+glibenclamide (600µg/k.g b.w)	5.6±0.5^{b***}	5.2±0.2^{b**}

Values are mean ± S.D, n = 6
[a] Significantly different from control
*[b] Significantly different from Diabetic control **P < 0.01, *** P < 0.001*

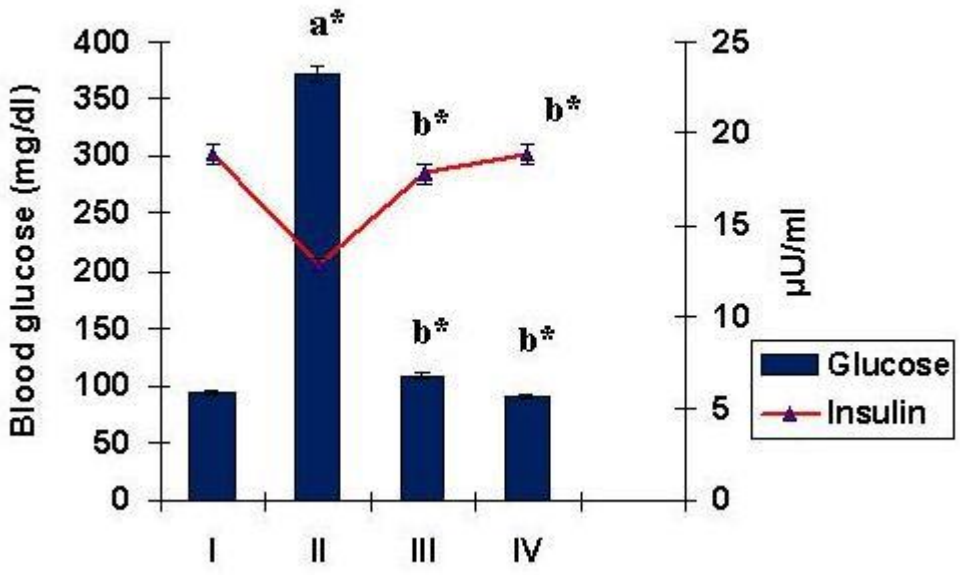

Figure 1. Effects of ISEE on blood glucose and plasma insulin in control and experimental animals

*Values are mean ± S.D, n = 6; [a] Significantly different from control; [b] Significantly different from Diabetic control *P < 0.05*

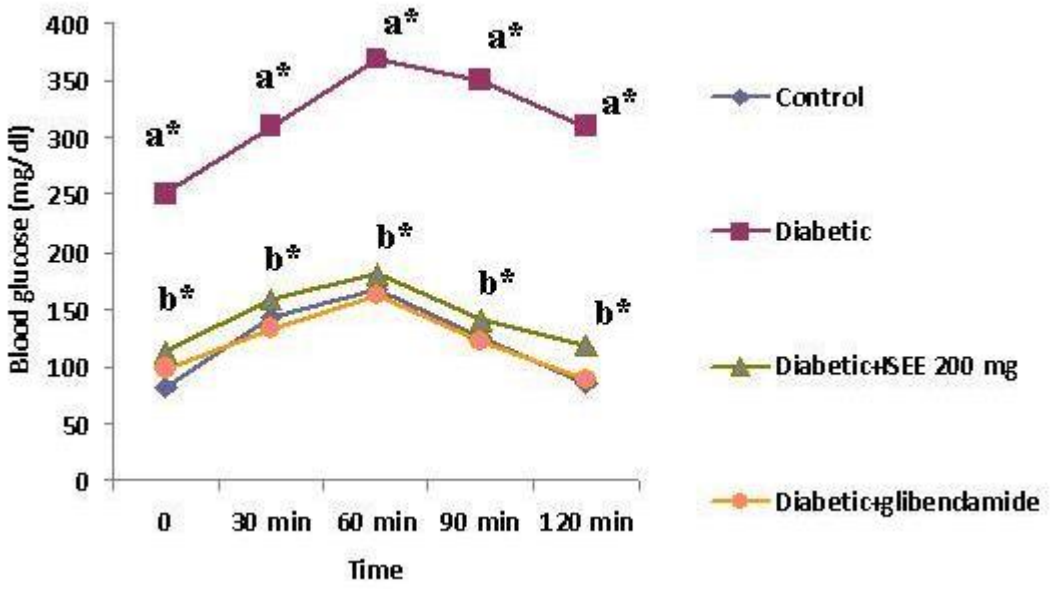

Figure 2. Effects of ISEE on glucose tolerance in control and experimental animals (after experimental period)

*Values are mean ± S.D, n = 6; [a] Significantly different from control; [b] Significantly different from Diabetic control *P < 0.05*

Discussion

Diabetes mellitus (DM) is characterized by persistent hyperglycemia due to progressive dysfunction of pancreatic β-cells which leads to decrease insulin secretion. DM occurs due to improper metabolism of carbohydrate with lack or insufficient insulin secretion. During diabetes, pancreatic islets are destructed and reduced the secretion of insulin which leads to hyperglycemia (Zhu *et al*., 2012). STZ-induced hyperglycemia in experimental rats has been commonly used one to study the effect of various hypoglycemic agents. STZ damage the pancreatic β-cells which lead to deficient insulin secretion and type 1 diabetic model (Nisha and Mini, 2013). STZ induction also resulted in a significant increase in the blood glucose level and reduction in plasma insulin level. The oral administration ISEE and the drug glibenclamide was halting the sustained hyperglycemia and reverting back the blood glucose and insulin levels to that of control animals.

Hexoinase is glucose metabolizing enzyme which play a very important role (as a glucose sensor) in secreting insulin for regulating plasma glucose levels. It is an insulin-dependent and insulin-sensitive enzyme and is almost completely inhibited or inactivated in diabetic rat liver in the absence of insulin (Gupta *et al*., 1997). There are many reports revealed the decreased enzymatic activity of hexokinase diabetic animals, resulting in depletion of liver and muscle glycogen (Laakso *et al*. 1995 and Murray *et al*., 2000). This increased activity of hexokinase can cause the increased utilization of glucose for energy production (Prince *et al*., 1997). In our study, decreased level of hexokinase was observed in serum, muscle and kidneys of STZ induced diabetic rats. Administration of ISEE and glibenclamide to STZ treated rats resulted in an increased activity of hexokinase.

Fructose 1,6-bisphosphatase, a vital key enzyme in gluconeogenic pathway, converts fructose-1,6-bisphosphate to fructose-6-phosphate and this is the essential step to perform reversal of glycolysis (Murray *et al*., 2000). Fructose 1,6-bisphosphatase is also present in liver, kidney and muscle (Tillmann *et al*., 2002). In gluconeogenesis, the irreversible step serves as a site for enzyme regulation. These enzyme activities were elevated in serum, muscle and kidney of experimental rats due to insulin deficiency which leads to hyperglycemic condition (Aoki *et al*., 1999). On administration of ISEE orally STZ induced diabetic rats significantly decreased the activities of this enzyme. This was due to the modulatory effect of ISEE against the enzyme activity by regulating cAMP or by activating metabolism of glycolysis and gluconeogenesis. Our findings are in line with Saravanan *et al*., (2009).

Glucose-6- phosphatase is main glucose regulating enzyme in tissue catalyzes end step that converts glucose-6-Phosphate to glucose and phosphate (Zhou *et al*., 2015). This enzyme plays vital role in balancing free glucose and stored glucose as glycogen. During diabetes, an increased activity of glucose-6-phosphatase was observed (Venkateswaran and Pari, 2002). Activation of glucose-6-phosphatase is due to state of insulin deficiency since under normal condition insulin function as a suppressor of glucose-6-phosphatase enzyme. In the present study, increased glucose-6-phosphatase activity in diabetic rats was observed than that of normal rats and the oral feeding of ISEE and glibenclamide markedly lowered its activity. The reduction in enzyme activity corresponded to the decrease in serum glucose as less glucose was being produced and released into the blood stream (Tahrani *et al*., 2011).

Glycogen, a stored form of glucose is synthesized by the enzyme glycogen synthase. Its amount represents the activity of insulin that supports deposition of glycogen by enhancing glycogen synthase and inhibiting glycogen phosphorylase (Pederson *et al*., 2005). Deposition of glycogen is impaired in diabetic rats (Bollen *et al*., 1998) in relation to insulin deficiency (Gannon *et al*., 1997). Diabetic rats showed a lower content of glycogen in liver and increased in kidney (Vats *et al*., 2004). Lower levels of glycogen were due to the deficiency of insulin and in liver and muscle (Kalaiarasi *et al*., 2009). In our study, kidney glycogen content was increased and this confirmed that tglucose entry under hyperglycemic state is not dependent on insulin action. This

elevated deposition of glycogen causes glycosylation. Glycogen rise in kidneys was already reported (Roden and Bernroider, 2003).On oral administration of ISEE changes the glycogen content favorably by elevating insulin action and consequently lowered glucose levels in blood.

Conclusion

Our present study determines the ISEE ability as anti-hyperglcemic effect in STZ induced diabetic rats. The effect of ISEE provides favorable effect against hyperglycemia and it was due to enhancement in the external glucose consumption, modulating glycolysis and gluconeogenesis. The beneficial effect of ISEE was comparable with standard drug, glibenclamide. Oral administration of ISEE of leaves was used for managing diabetes and prevention of diabetic complications.

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