



Antihyperglycaemic Effect of Tetrahydrocurcumin and Pterostilbene: Effect on Key Metabolic Enzymes of Carbohydrate Metabolism in Streptozotocin Induced Diabetes

Pidaran Murugan^{1*}

¹*Assistant professor of Biochemistry, Centre for Distance and Online Education, Department of Biochemistry, Bharathidasan University, Tiruchirappalli - 620024. Tamil Nadu, India.

*Corresponding author's E-mail: manomuruganphd@gmail.com & pmpranithmurugan18@gmail.com

Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 14 Nov 2023	<p>This study is to assess the glucose lowering activity of tetrahydrocurcumin (THC) and pterostilbene (PTS), in diabetes induced rats by streptozotocin (STZ) and nicotinamide. Diabetic rats were treated THC (80 mg/kg body weight) and PTS (40 mg/kg body weight) for 45 days, after which activities of hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, sorbitol dehydrogenase in liver and glycogen content in liver and muscle were assayed. The activities of gluconeogenic enzymes were significantly increased, whereas the activities of hexokinase, glucose-6-phosphate dehydrogenase and glycogen were significantly decreased in diabetic control rats. Both THC and PTS were able to restore the altered enzyme activities to almost near normal levels. THC was more effective than PTS. Our results indicate that administration of THC and PTS to diabetic animals normalizes blood glucose and causes marked improvement of altered carbohydrate metabolic enzymes during diabetes. The THC administration showed more effective than PTS and metformin.</p>
CC License CC-BY-NC-SA 4.0	<p>Keywords: Tetrahydrocurcumin, Pterostilbene, Metformin, Carbohydrate Metabolic Enzymes, Diabetes Mellitus.</p>

1. Introduction

Diabetes is huge accruing disorders in developed and developing countries that can be characterized by increased in blood and urine sugar. Hyperglycemia is having in prolonged uncontrolled conditions that can be affect in normal body metabolism finally results in diabetic complications of morbidity and mortality (Urzúa et al., 2012). Overall, 285 million people affected by diabetes in 2010 and it will be increased about 439 million in 2030 (Chang et al., 2013). A condition of diabetes is defect in insulin utilization and increases in blood glucose. Diabetic patients have found altered metabolic status due to the prolonged alterations of gluconeogenesis (Ahmed et al., 2012). Currently available antidiabetic mostly is having side effects or economically high (Chitra et al., 2010). Therefore, all new researchers are continues finding of antihyperglycemic agents from natural sources such as plants and their products because they might not have side effect and less cost (Murugan, 2022 a,b; Murugan, 2023 a,b).

Diabetes mellitus is becoming pandemic and despite the recent upsurge in new drugs to treat and prevent the condition, its prevalence continues to soar. Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease related complications are increasing unabated. Parallel to this, recent developments in understanding the pathophysiology of the disease process have opened up several new avenues to identify and develop novel therapies to combat the diabetic plaque (Tiwari and Madhusudana Rao, 2002).

Therefore, as the disease is progressing unabated, there is a need of identifying indigenous natural resources in order to procure them and study in detail their potential on different newly identified targets in order to develop them as new therapeutics (Li et al. 2004). Currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs. The belief that natural medicines are much safer than synthetic drugs has gained popularity in recent years and lead to tremendous growth of phytopharmaceutical usage (Bhattaram et al. 2002).

Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances. *Pterocarpus marsupium* has been used for many years in the treatment of diabetes mellitus (Warrier et al., 1995). PTS was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium* (Maurya et al., 1984). It is suggested that PTS might be one of the principal anti-diabetic constituents of *Pterocarpus marsupium* (Manickam et al., 1997). An aqueous extract of heartwood of *P. marsupium* has been tested clinically and found to be effective in non-insulin dependent diabetes mellitus patients (ICMR, 1998).

THC was one of the major colourless metabolite of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin (Murugan et al., 2008). Curcumin was rapidly metabolized during absorption from the intestine, yielding THC (Murugan and Pari, 2005; Murugan and Pari, 2006), which had shown the strongest antioxidant activity among all curcuminoids (Murugan and Pari, 2007a,b). THC thought to play a pivotal role in protecting the cell membrane against lipid peroxidation, which exhibits its protective effect by means of β -diketone moieties and phenolic hydroxyl groups (Murugan and Pari, 2006; Murugan and Pari, 2007c). Several studies in experimental animals indicated that THC also prevents cancer, protect the inflammation, atherosclerotic lesions and hepatotoxicity (Pari and Murugan, 2004; Murugan, 2023c, d).

2. Materials And Methods

Drugs and chemicals

THC and PTS was a gift provided by Sabinsa Corporation, USA. All other chemicals and biochemicals were of analytical grade.

Induction of diabetes

Non-Insulin dependent diabetes mellitus was induced (Masiello et al., 1998) in overnight fasted rats by a single intraperitoneal injection of 65 mg/kg STZ, 15 min after the intraperitoneal administration of 110 mg/kg of nicotinamide. STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl will be used for the study.

Experimental design

In the experiment, a total of 30 rats (24 diabetic surviving rats, 6 normal rats) will be used. The rats will be divided into six groups of 6 each, after the induction of STZ diabetes. The duration of experiment is 45 days.

Group 1: Normal rats.

Group 2: Diabetic control rats.

Group 3: Diabetic rats given THC (80mg / kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Murugan, 2021a, b).

Group 4: Diabetic rats given PTS (40 mg / kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Murugan and Sakthivel, 2021).

Group 5: Diabetic rats given metformin (500 mg / kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Soon & Tan 2000).

At the end of 45 days, the rats were deprived of food overnight and blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose, hemoglobin and glycosylated hemoglobin. Plasma was separated for the assay of insulin. Liver was dissected out, washed in ice-cold saline, patted dry and weighed.

Analytical methods

Blood glucose and plasma insulin

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (Lott and Turner, 1975). Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany).

Hemoglobin and glycosylated hemoglobin

Hemoglobin was estimated by the method of Drabkin and Austin (1932). Glycosylated hemoglobin (HbA1C) was estimated by the method of Sudhakar Nayak and Pattabiraman (1981), modified by Bannon (1982). Saline-washed red cells were treated with water for lysis and incubated at 37°C for 15 min, and oxalate/HCl solution was then added and mixed. The filtrate was heated in a boiling water bath for 4 h, cooled with ice-cold water, treated with 40% Trichloroacetic acid (TCA), and again centrifuged at 1000 g for 10 min. The supernatant obtained was then heated with 80% phenol and H₂SO₄ and the color developed was read at 480 nm after 30 min.

Glycogen

Tissue glycogen was extracted and estimated by the method of Morales et al. (1973). 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 and a drop of 1 M ammonium acetate 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 reprecipitated with alcohol and 1 M 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 640nm against reagent blank treated in a similar manner. Standard glucose solution was also treated similarly. The glycogen content was calculated from the amount of glucose present in the sample and was expressed as mg/g tissue.

Assay of carbohydrate metabolic enzymes

activity of hepatic hexokinase

Hexokinase activity was assayed by the method of Brandstrup et al. (1957). The reaction mixture in a total volume of 5.3 ml contained the following viz., 1 ml of glucose solution, 0.5 ml of ATP solution, 0.1 ml of magnesium chloride solution, 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. The 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 removed to the tubes containing 1 ml of 10% TCA that was considered as zero time. A second aliquot was removed after 30 min incubation at 37°C. The protein precipitate was removed by centrifugation and the residual glucose in the supernatant was estimated by the method John and Turner (1975) as described previously.

Activity of hepatic glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase was assayed by the method of Ellis and Kirkman (1961). The incubation mixture contained 1 ml of buffer, 0.1 ml of magnesium chloride, 0.1 ml of NADP⁺, 0.5 ml of phenazine methosulphate, 0.4 ml of the dye solution and the requisite amount of the enzyme extract. The mixture was allowed to stand at room temperature for 10 min to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of glucose-6-phosphate. The absorbance was read at 640nm against water blank at one min intervals for 3-5 min in a UV spectrophotometer. The activity of the enzyme was calculated in units by multiplying the change in OD/min by the factor 6/17.6, which is the molar extinction co-efficient of the reduced co-enzyme.

Activity of hepatic glucose-6-phosphatas

Glucose-6-phosphatase was assayed according to the method of Koida and Oda (1959). Incubation mixture contains 0.7 ml of citrate buffer, 0.3 ml of substrate and 0.3 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 1 h. Addition of 1 ml of 10% TCA to the reaction tubes terminated the reaction of the enzyme. The suspension was centrifuged and the phosphorus content of the supernatant was estimated by the method of Fiske and Subbarow (1925). The supernatant was made up to 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 680nm.

activity of hepatic fructose-1, 6-bisphosphatase

Fructose-1,6-bisphosphatase was assayed by the method of Gancedo and Gancedo (1971). The assay mixture in a final volume of 2 ml contained 1.2 ml of buffer, 0.1 ml of substrate, 0.25 ml of magnesium chloride, 0.1 ml of potassium chloride solution, 0.25 ml of EDTA solution and 0.1 ml of enzyme homogenate. The incubation was carried out at 37°C for 15 min. The reaction was terminated by the

addition of 10% TCA. The suspension was centrifuged and the supernatant was used for phosphorus estimation by the method of Fiske and Subbarow (1925). The supernatant was made upto known volume. To this 1 ml of ammonium molybdate was added followed by the 0.4 ml of ANSA. The blue colour developed after 20 min was read at 680nm.

activity of hepatic sorbitol dehydrogenase

Sorbitol dehydrogenase, (SDH; EC. 1.1.1.14) was assayed by the method of Ulrich (1974). An aliquot of liver homogenate was treated with 0.2 ml 12 mM and 1.6 ml 0.2 M triethanolamine buffer, pH 7.4. The reaction was started after 30 min by treatment with 0.3 ml 4 M D(-)fructose. Absorbance was determined with a Systronics UV-visible spectrophotometer (Dubai, UAE) at 60-s intervals for 5- 8 min at 365 nm.

3. Results and Discussion

Changes in body weight, blood glucose and plasma insulin

The changes in body weight and food intake in normal and experimental rats are presented in table 1. The body weights in THC and PTS treated group significantly improved at the end of the experimental period when compared with diabetic control group. Table 1 also shows the level of blood glucose and insulin in control and experimental animals. The level of blood glucose was significantly increased whereas the plasma insulin was significantly decreased in STZ diabetic control rats.

Table 1. Changes in body weight, blood glucose and plasma insulin in normal and experimental rats

Groups	Body weight (g)		Fasting Blood Glucose (mg/dl)	Plasma insulin (μU/ml)
	Initial	Final		
Normal	196.54 ± 6.12	225.45 ± 5.78	94.98 ± 6.33a	12.16 ± 0.95a
Diabetic control	225.21 ± 6.42	179.41 ± 5.83 [†]	285.42 ± 7.25b	3.85 ± 0.25b
Diabetic + THC (80 mg/kg)	200.45 ± 5.45	218.54 ± 6.45*	105.25 ± 7.45c	10.15 ± 0.41c
Diabetic + PTS (40 mg/kg)	195.45 ± 5.65	211.25 ± 6.41*	110.20 ± 7.25d	9.02 ± 0.42d
Diabetic + Metformin (500 mg/kg)	190.45 ± 7.25	204.71 ± 6.54*	123.02 ± 7.65e	8.45 ± 0.2e

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Diabetic control was compared with normal, [†] p<0.001.

Experimental groups were compared with diabetic control, * p<0.001.

Hemoglobin and glycosylated hemoglobin

Table 2 shows the level of total haemoglobin, glycosylated haemoglobin, and urine sugar of different experimental groups. The diabetic control rats showed a significant decrease in the level of total haemoglobin and significant increase in the level of glycosylated haemoglobin. Oral administration of THC and PTS to diabetic rats significantly restored total haemoglobin and glycosylated haemoglobin levels. In the case of normal rats, the level of haemoglobin and glycosylated haemoglobin remained unaltered. The effect of THC was more prominent compared with PTS and metformin.

Table 2. Effect of THC and PTS on the levels of haemoglobin, glycosylated haemoglobin and urine sugar in normal and experimental rats

Groups	Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)	Urine sugar
Normal	12.35 ± 0.62a	0.27 ± 0.02a	Nil
Diabetic control	8.21 ± 0.41b	0.73 ± 0.04b	+++
Diabetic + THC (80 mg/kg)	11.18 ± 0.49c	0.32 ± 0.02c	Nil
Diabetic + PTS (40 mg/kg)	10.12 ± 0.45d	0.37 ± 0.03d	Nil
Diabetic + Metformin (500 mg/kg)	9.70 ± 1.12e	0.44 ± 0.02e	Trace

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at p <0.05 (DMRT).

+++ , > 2% sugar, ++; -2% sugar; +, -1%

Glycogen

Table 3 represents the effect of THC and PTS on glycogen content in liver and muscle of normal and experimental animals. In diabetic controls, hepatic and skeletal muscle glycogen content was decreased significantly as compared to non-diabetic controls. Treatment with THC and PTS significantly increased the hepatic and skeletal glycogen. The effect of THC was better than PTS and metformin.

Table 3. Effect of THC and PTS on the levels of liver and muscle glycogen in normal and experimental rats

Groups	Glycogen (mg/g tissue)	
	Liver	Muscle
Normal	35.29 ± 1.64a	6.61 ± 0.24a
Diabetic control	19.54 ± 1.56b	3.66 ± 0.17b
Diabetic + THC (80 mg/kg)	30.35 ± 1.58c	5.96 ± 0.210c
Diabetic + PTS (40 mg/kg)	27.31 ± 1.52c	5.55 ± 0.23c
Diabetic + Metformin (500 mg/kg)	25.52 ± 1.31d	5.12 ± 0.13d

Values are given as mean ± SD from 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

Carbohydrate metabolic enzymes

Table 4 depicts the activities of carbohydrate metabolizing enzymes in liver of normal and THC and PTS treated diabetic rats. The activities of enzyme hexokinase and glucose-6-phosphate dehydrogenase was found to be decreased whereas the activities of sorbitol dehydrogenase and gluconeogenic enzymes: glucose-6-phosphatase, fructose-1,6-bisphosphatase and sorbitol dehydrogenase were significantly increased in diabetic control rats. THC and PTS administration to diabetic rats significantly reversed the above changes when compared to diabetic control rats. The THC administration showed more effective than PTS and metformin.

Table 4. Effect of THC and PTS on the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1, 6- bisphosphatase and sorbitol dehydrogenase in normal and experimental rats

Groups	Normal	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + PTS (40 mg/kg)	Diabetic + Metformin (500 mg/kg)
Hexokinase (units/g protein)	146.65 ± 8.25a	108.37 ± 6.46b	130.32 ± 5.51c	139.21 ± 5.41d	123.61 ± 6.78e
Glucose-6-phosphate dehydrogenase (x 10 ⁴ mIU / mg protein)	4.57 ± 0.15a	2.14 ± 0.10b	3.97 ± 0.15c	3.75 ± 0.15d	3.45 ± 0.11e
Glucose- 6-phosphatase (units/2/ mg protein)	0.15 ± 0.02a	0.25 ± 0.02b	0.17 ± 0.01c	0.19 ± 0.01d	0.21 ± 0.01e
Fructose-1,6- bisphosphatase (units/3/ mg protein)	0.37 ± 0.03a	0.58 ± 0.03b	0.40 ± 0.02c	0.43 ± 0.02d	0.46 ± 0.03e
Sorbitol dehydrogenase (units/4/ g protein)	4.25 ± 0.31a	8.41 ± 0.60b	5.21 ± 0.40c	5.88 ± 0.41d	6.31 ± 0.47e

Values are given as mean ± SD from 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

1 - µmoles of glucose phosphorylated/min

2 - µmoles of Pi liberated/min

3 - µmoles of Pi liberated/hour

4 - 1 unit of sorbitol dehydrogenase = the amount of enzyme that produces a change in absorbance of 0.01/min

Statistical analysis

Values were expressed as the mean ± SD and the significance of the differences between mean values were determined by oneway analysis of variance (ANOVA) coupled with Duncan's Multiple Range Test (DMRT), taking $p < 0.05$ as significant (Duncan, 1957).

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago (Ahmed, 2002). In 1936, the distinction between type 1 and type 2 DM was clearly made. Type 2 DM was first described as a component of metabolic syndrome

in 1988. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. Type 2 DM results from interaction between genetic, environmental and behavioral risk factors (Abdulfatai et al., 2012)

The liver has an important function in maintaining glucose levels within the physiological limits. In contrast to muscle and fatty tissue, insulin does not directly regulate the uptake of glucose by the liver. Insulin takes part in regulation of glucose metabolism in the liver by stimulating glycolysis, glycogen synthesis and inhibition of gluconeogenesis (Liptakova et al. 2002). In an attempt to gain an insight into the underlying biochemical mechanism on the action of THC and PTS, we assayed the key hepatic enzymes, hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1, 6-bisphosphatase and sorbitol dehydrogenase in liver of normal and experimental rats, as liver being the main organ responsible for maintaining glucose homeostasis of the blood.

Diabetes mellitus is associated with a marked reduction in the level of liver glycogen. In the present study, hepatic and skeletal muscle glycogen content was reduced significantly in diabetic controls. Welihinda and Karunanayake (1986) also reported that hepatic and muscle glycogen is reduced significantly in diabetic control rats as compared to non-diabetic control rats. The reduced glycogen content has been attributed to the reduction in the activity of glycogen synthase and increase in the activity of glycogen phosphorylase (Pari and Murugan, 2005). Administration of THC and PTS prevented the depletion of glycogen content but could not normalize it. This prevention is due to stimulation of insulin release from β -cells (Murugan, 2023a) or due to insulin mimetic activity of some component of plant resulting in direct peripheral glucose uptake.

The key enzyme in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate. It causes initial phosphorylation of glucose after it diffuses into liver cells. Once phosphorylated, the glucose is temporarily trapped inside the liver cells and its diffusion is blocked (Bopanna et al. 1997). Hexokinase insufficiency in diabetic rats can cause decreased glycolysis and decreased utilization of glucose for energy production (Vats et al. 2003). In our present study hexokinase activity was decreased in diabetic control rats. Decrease in hepatic hexokinase has been reported in STZ diabetic rats (Pari and Murugan, 2005; Murugan, 2023b,c). Administration of THC and PTS to diabetic rats enhanced the hexokinase activity and suggests greater uptake of glucose from blood by liver cells and increased glycolysis. In addition, THC and PTS may regulate the activity of hexokinase by maintaining the critical levels of glucose-6-phosphate needed to be catabolized to produce ATP and NAD.

The activity of glucose-6-phosphate dehydrogenase, the first regulatory enzyme of pentose phosphate pathway was found to be decreased in the diabetic control rats. Glucose-6-phosphate dehydrogenase has been reported to be decreased in the diabetic state (Panneerselvam and Govindaswamy, 2002). The decrease in the activity of this enzyme in diabetic condition may result in the diminished functioning of hexose mono phosphate shunt (HMP shunt) and thereby the production of reducing equivalent such as NADH and NADPH and increased oxidative stress finally leading to diabetic complications (Zhang and Moller, 2000). In our study, administration of THC and PTS increased the activity of glucose-6-phosphate dehydrogenase considerably.

Gluconeogenic enzymes, glucose-6-phosphatase, the key enzyme in the homeostatic regulation of blood glucose levels, catalyze the terminal step in gluconeogenesis and glycogenolysis and the enzyme is mainly found in the gluconeogenic tissues liver and kidney, where it plays a major role in glucose production (Nordlie and Sukalski, 1985) and the other enzyme, fructose-1,6-bisphosphatase, catalyzes one of the irreversible step in gluconeogenesis and serves as a site for the regulation of the process. The activities of these gluconeogenic enzymes were significantly increased in the liver of diabetic control rats. Increased activity of glucose-6-phosphatase in diabetic rats enhances the synthesis of fats from carbohydrates i.e., lipogenesis (Bopanna et al. 1997) and finally contribute to increased levels of glucose in blood. Increased hepatic glucose production in diabetes mellitus is associated with impaired suppression of the gluconeogenic enzyme fructose-1, 6-bisphosphatase. Activation of gluconeogenic enzymes is due to state of insulin deficiency since under normal condition insulin function as a suppressor of gluconeogenic enzymes.

Administration of THC and PTS significantly depressed the activities of gluconeogenic enzymes in diabetic rats. The effect of THC and PTS may be primarily modulating and regulating the activities of the two gluconeogenic enzymes, either through regulation of cAMP or any other metabolite activation/inhibition of glycolysis/gluconeogenesis. The redox state of the liver cell is highly reduced in diabetes together with the changes in the energy state (Gupta et al. 1999). ATP levels are lowered in

the cytosol and could be the energy source for the higher levels of gluconeogenesis in diabetic liver. Other metabolites especially the substrates for the glucose-6-phosphatase and fructose-1, 6-bisphosphatase, glucose-6-phosphate and fructose-1, 6-bisphosphate are known to increase in the liver during diabetes due to inhibition of hexokinase, the main regulatory glycolytic enzyme (Pari and Murugan, 2005). The level of plasma insulin was found to be increased significantly in diabetic rats treated with THC and PTS, which may be a consequence for the significant reduction in the level of gluconeogenic enzymes. The reduction in the activities of gluconeogenic enzymes can result in the decreased concentration of glucose in blood.

The conversion of glucose to sorbitol, which is catalyzed by aldose reductase, in the presence of NADPH, may relate to the protection against oxidative stress and abnormalities in NO action. NADPH levels are diminished by elevated polyol pathway flux, impairing the glutathione redox cycle (Inouye et al. 1998), which is an important cellular protection mechanism against oxygen derived free radicals (OFR). The OFRs are markedly increased in diabetes; if not scavenged they cause damage to the vascular endothelium and neutralize NO (Inouye et al. 1998).

SDH catalyzes the conversion of sorbitol to fructose in the presence of NAD. Evidence has shown that the activity of SDH was elevated in diabetic rats, leading to increased availability of fructose and that fructose was 10-fold better substrate than glucose for glycosylation (Brownlee, 1992). In the present study, an increase in the activity of SDH in the liver of diabetic control rats was observed. Increased activity of SDH in STZ and nicotinamide diabetic rats has been reported by Pari and Murugan (2005). As the concentration of glucose in the liver goes up in diabetic rats, more glucose is converted to sorbitol. The observed elevation in the activity of SDH in diabetic rats may have been due to the increased availability of sorbitol. SDH activity was found to be significantly reduced on treatment with THC and PTS. The effect produced by THC and PTS may be due to decrease in blood glucose by increased activity of plasma insulin, which may prevent glucose to sorbitol conversion.

Administration of THC and PTS significantly decreased the activities of gluconeogenic enzymes in diabetic rats. The level of plasma insulin was found to increase significantly in diabetic rats with THC and PTS; which may be consequence for the significant reduction in the level of gluconeogenic enzymes. The reduction in the activities of gluconeogenic enzymes can result in the decreased concentration of glucose in blood. Administration of THC and PTS to normal rats also resulted in a significant increase in the level of hexokinase and a significant decrease in the activities of gluconeogenic enzymes glucose-6-phosphatase and fructose-1, 6-bisphosphatase and sorbitol dehydrogenase, which may result for the significant decrease in the level of blood glucose.

4. Conclusion

THC and PTS has beneficial effects on glucose concentration as well as sequential metabolic correlation between increased glycolysis, decreased gluconeogenesis, increased hydrogen shuttle reactions. It suggests the possible biochemical mechanisms through which THC and PTS regulates glucose homeostasis in diabetic condition. The effect of THC was better than PTS and metformin.

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