

## ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC ACTIVITY OF SEDIMENTAL EXTRACT OF *TINOSPORA CORDIFOLIA* IN STREPTOZOTOCIN INDUCED TYPE 2 DIABETES

R.KANNADHASAN<sup>a,\*</sup> AND S.VENKATARAMAN<sup>b</sup>

Department of Pharmacology, School of Pharmaceutical Sciences, Vels University, Pallavaram, Chennai 600117, Tamil Nadu, India.  
Email: ramkhannasolutions@gmail.com, svraman2007@yahoo.com

Received: 1 Feb 2012, Revised and Accepted: 10 March 2012

### ABSTRACT

To study the Type 2 diabetes mediated obesity and their resurrection with SETc using preclinical evaluation. A study kept for OGTT, RBGT with SETc in normal and diabetic animals and the resulting time and dose dependant reduction in BGC were recorded *in vivo*. The effect of test drug among the gluconeogenic and glycolytic enzymes and the status of liver and gastromuscular muscle glucose intake were measured *in vitro*. Supporting studies in regard of obesity were assessed with their biochemical parameters. From the OGTT and RBGT, the blood glucose concentration of SETc (1000mg/kg/p.o.) treated diabetic animals were found to lie in the range near to the normal control ( $p < 0.05$ ) and maintenance of plateau therein. Furthermore, 30 days treatment with SETc (1000mg/kg/p.o.), triggers the glycolytic enzymes and restores the liver and muscle glycogen levels as evidenced from its assay *in vitro*. The prolonged therapy with SETc showed its positive impregnation over biochemical parameters and that particular with high index of HDL-C and possible reduction in LDL-C levels. Finally on conclusion, 30 days treatment of SETc (1000mg/kg/p.o.) on diabetic subjects was proven for its efficacy and clearly establishes the antidiabetic activity with antiobesity body built.

**Keywords** Sedimental extract of *Tinospora cordifolia*, Type 2 diabetes, Glycogen storage, Obesity.

### INTRODUCTION

Diabetes mellitus or type 2 diabetes (formerly called non-insulin-dependent diabetes mellitus (NIDDM), or adult-onset diabetes) is a disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency<sup>1</sup>. While it is often initially managed by increasing exercise and dietary modification, medications are typically needed as the disease progresses. Traditionally considered a disease of adults, type 2 diabetes is increasingly diagnosed in children in parallel to rising obesity rates.

In sort of clinical experience with blood glucose monitoring, its maintenance was found to be less predictive. So, preclinical investigation with glucose metabolism and improvement of liver glycogen storage was found to be quite worthy. Similarly, the lipid profile pertaining to obese in diabetics was put forth in aspect to study the high risk of low density lipoprotein levels in the circulation, a major criterion of cardiovascular diseases<sup>2</sup>.

In recent years, herbs are being effectively tried in a variety of pathophysiological states. As diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It was a great opportunity to study and reveal the properties of herbal medicine in diabetes and abnormalities in serum lipids with micro and macro vascular complications, which are the major causes of morbidity and death in diabetic subjects.

In accordance with the recommendations (WHO, 1980) an investigation of the hypoglycemic agents of plant origin used in the traditional medicine seems important. Indian traditional medicine is one of the richest medicinal systems among those available around the world. Different species have been reported to have antidiabetic and antioxidant properties. These medicinal plants were used in the ancient Indian system of medicine like ayurvedha, siddha for the treatment of Madhu meha (Diabetes mellitus) from time immemorial. In the present study one such reputed medicinal plant by name *Tinospora cordifolia* was selected for the scientific evaluation for its antidiabetic and antihyperlipidemic activities in experimental rats.

### MATERIALS AND METHODS

#### Preparation of Sedimental extract of *Tinospora cordifolia* (SETc)

*Tinospora cordifolia* collected from Irulars Tribal Women Welfare Society (ITWWS), Thandarai, Thirukazhukundram, a southern forest

region of Tamil nadu, India. The stem portions were cut into small pieces (around 1cm), and shade dried. The pharmacognosital identification was done by Plant Anatomy research Centre, Chennai. A specimen of the plant was kept in the Department of Pharmacology, C.L. Baid Metha college of Pharmacy, Chennai (Specimen No. CLBMCP/102/2005).

The dried pieces of *Tinospora cordifolia* stem, 2 kg was grounded to a coarse powder and soaked in 1000ml of distilled water for a period of 24 hrs, until the active portion to settle down. Successive decanting of upper layer in a separate container (devoid of cell debris) was evaporated to dryness and this portion is considered as water soluble portion. The sediment layer was washed for 2-3 times with fresh distilled water and admixed with water soluble extract in the ratio of 3:1 to get the final sedimental extract of *Tinospora cordifolia* (SETc) for further screening. So yield 1000mg/kg/p.o., of SETc was used for the animal studies, as dose fixed from effective dose finding procedure<sup>3</sup>.

#### Chemical and Reagents

All chemicals and reagents were purchase from the local markets, Chennai. Sigma-Aldrich Laboratories, Mumbai; SRL Laboratories, Delhi and S.D.Fine chemicals, Mumbai

#### Instruments

Ascensia one touch glucometer and strips (Code.no: 3110; 3112; 3110), were used for glucose estimation. Biochemical parameters were analyzed with institutional Clinical chemistry analyzer, Metrolab 1600DR-K with respective ready available kits. All the analytical instruments and surgical equipments were of standard manufacturers.

#### Animals

Male Sprague dawley rats (200-250gm) were purchased from King's Institute, Guindy, Chennai. All rats were randomly selected, segregated and acclimatized for a period of 1 week with 12hr day light and 12 hours dark cycle, food and water ad libitum.

#### Induction of Diabetes

Animals (n=45) were fasted for 16 hours followed by intra peritoneal injection with freshly prepared Streptozotocin (STZ ) 45mg/ml of 0.01 m citrate buffer, pH 4.5 and the diabetic state was assessed by measuring the non-fasting blood glucose concentration

48 hours of post STZ injection. Only rats with blood glucose levels  $\leq$  200mg/dl were selected and used for further experimentation<sup>4</sup>.

#### Effect of SETc in Normoglycaemic and STZ induced diabetic rats

This study was carried out in accordance with the method proposed by Gosh et al<sup>5</sup>. Male Sprague dawley rats (200-250gm) were separated and segregated into 2 groups of 6 animals each. Group I served as Normoglycaemic i.e. normal rats receiving SETc (1000mg/kg/p.o.) alone while group II will be serving as comparative control of diabetic rats treated with SETc (1000mg/kg/p.o.). Blood glucose concentration was recorded in regular interval 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup>, and 24<sup>th</sup> hour using one touch glucometer.

#### Oral glucose tolerance test (OGTT)

OGTT were performed at regular intervals of 1<sup>st</sup>, 15<sup>th</sup> and 25<sup>th</sup> days in diabetic rats by following the method of Musabayne et al<sup>6</sup>. Diabetic rats were randomly divided into 6 groups of 6 rats each. The rats were dosed daily by gavage with CMC, standard drug & SETc extract for 30 days for respective groups. Group 1 served as a normal control receiving 0.5% CMC and group 2 served as diabetic control and group 3 & 4 will be serving as the test and standard receiving SETc 1000mg /kg/p.o. and Glibenclamide (600 $\mu$ g/kg/p.o.), respectively.

The rats in all groups were loaded with glucose (1g/kg/p.o.), 30min after administration of respective drugs on 1<sup>st</sup>, 15<sup>th</sup> and 25<sup>th</sup> days of drug treatment. Blood samples were examined for its fasting glucose level just prior to glucose administration and at intervals of 30, 60, 90 and 120 min after the glucose loading with the aid of one touch glucometer. The periodical body weight differences of the individual animals were also measured during the test period.

#### Random Blood Glucose Tolerance (RBGT)

The effect of SETc on random blood sampling at regular intervals of 4<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> days in diabetic rats were performed by the method of Santha Kumari et al<sup>7</sup>.

The animals with respective drug treatment used for OGTT performance were subjected to random blood glucose screening at 4<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day with usual drug treatment. Random glucose concentrations were determined with regular time interval without removal of any feed or water. Blood glucose concentration was measured in mg/dl using one touch glucometer. Simultaneously a record on body mass index (BMI) i.e. body weight (gm) and surface area (cm), the diameter of the belly were made.

On the day 30, all fasted rats were sacrificed by decapitation; blood was collected directly from the carotid artery for various biochemical parameters. The abdomen was cut opened and various tissues were isolated devoid of adhering tissues. Skeletal muscle, Liver, Kidney, Pancreas, Heart, and Gonads was dissected out and a portion of the former 3 were homogenized separately with respective buffer solutions to study the enzyme levels on glucose metabolism and glycogen content in the liver and skeletal muscle. The remaining samples were kept preserved to study the chemical induced stress from its micro anatomical changes observed for its antioxidant study in future.

#### Effect of SETc on Glucose metabolism in Liver and Kidney

##### Gluconeogenic Enzyme Studies: Assay of Glucose-6-phosphatase (Glucose-6-phosphate: phosphohydrolase)

Glucose-6-phosphatase was assayed by the method of King J<sup>8</sup>. Incubate the mixture in total volume of 0.1ml containing 0.3ml of citrate buffer (0.1M; pH 6.5), 0.5ml of Substrate - Glucose-6-phosphate (0.01M) and 0.2ml of Extract. Incubate it for 37°C for 60min and added 1ml of 10% TCA and Centrifuged. Inorganic phosphate was measured by following the method of Fiske and Subbarow<sup>9</sup>.

The supernatant was removed and aliquots of Standards were made upto 5.0ml with water. To this added 10ml of Ammonium molybdate (2.5% in 3NH<sub>2</sub>SO<sub>4</sub>) and 0.5ml of ANSA and mixed well. A blue colour developed which is observed at room temperature for 20min. The

absorbance was measured at 620nm. The activity of Glucose-6-phosphate was measured as nmoles of P liberated/ mg/ protein at 37°C.

##### Assay of Fructose 1, 6-diphosphatase

Assay of fructose-1, 6-diphosphatase was followed by the method of Gancedo and Gancedo<sup>10</sup>. 2.0ml of final volume is made by adding 1.2ml of 0.1M Tris HCl with 0.1ml of substrate - Fructose 1,6-diphosphate (0.05M), 0.25ml of 0.1M MgCl<sub>2</sub>, 0.1ml of 0.1M KCl, 0.25ml of 0.001M EDTA and 0.1ml of enzyme. This mixture was incubated at 37°C for 25min. To this, added 1.0ml of 10% TCA and centrifuged.

The inorganic phosphate was measured by following the method by Fiske and Subbarow<sup>9</sup> as mentioned above.

##### Glycolytic Enzyme studies: Assay of Hexokinase

The assay procedure was followed by the method by Branstrup et al<sup>11</sup>. 5.0ml of final volume was prepared by adding 2.5ml of Tris HCl buffer (0.1M), 1.0ml of substrate (glucose 0.005M), 0.5ml of ATP(0.72M), 0.1ml of MgCl<sub>2</sub> (0.05M), 0.1ml of NaF (0.5M), 0.4ml of KH<sub>2</sub>PO<sub>4</sub> (0.0215M) and 0.4ml of KCl (0.1M) and this reaction mixture was incubated for 37°C for 15 min. The reaction is initiated by adding 0.1ml of enzyme. 1.0ml of the reaction mixture was withdrawn immediately (0min) into the tubes containing 1.0ml of 10%TCA. 1.0ml of aliquot of the above reaction mixture was taken after 30min incubation which was mixed to a separate set of tubes containing 1.0ml of 10%TCA. All the tubes were centrifuged. Glucose content of the supernatant was estimated by Sasaki et al<sup>12</sup>. 0.1ml of blood was mixed with 1.9ml of TCA and the protein gets precipitated which was then centrifuged. 1.0ml of supernatant solution was removed and added 4.0ml of o-toluidine and kept in a water bath for 15min until green color develops, which was then observed at 640nm. The Standard glucose was also run simultaneously.

Enzyme activity was expressed as nmoles of Glucose-6-phosphate formed/min/mg protein under incubation condition.

##### Muscle and liver glycogen content of normal, diabetic and diabetic rats treated with SETc after 30 days of antidiabetic study

Glycogen content in gastrocnemius muscle and liver were assayed by the method followed by Nicholas et al<sup>13</sup>.

**Preparation of Anthrone reagent:** A solution containing 0.05% anthrone, 1% thiourea and 72% by volume of H<sub>2</sub>SO<sub>4</sub> were made up to 120ml and kept ready for use.

**Glucose Standard:** 2ml of saturated benzoic acid solution containing 0.1mg of glucose standard were made.

1ml of 10% homogenate of the respective samples of muscle and liver were made with 5% TCA and kept separately. To each of this 1ml of homogenate sample added 5ml of 95 % ethanol and kept incubated at 37-40° C for a period of 3 hours until the glycogen gets precipitated. After precipitation the samples were centrifuged at 3000 rpm for 15 min.

The test tubes were inverted for 10mins until the pellets of glycogen gets dried. To this added 2 ml of distilled water, 10ml of anthrone reagent and cooled. This is then boiled for a period of 15mins, cooled and the absorbance read at 620nm. The results were interpreted with standard glucose using the same procedure.

##### Total lipid profile

Biochemical parameters were assessed in blood sample of respective groups using institutional Clinical chemistry analyzer. LDL and VLDL Cholesterols were estimated by substituting the following equation of (i) Friedwald<sup>14</sup> and (ii) Peter Wilson<sup>15</sup> respectively. The equations were as follows:

$$(i) \text{ LDL-C} = \text{Total cholesterol} - \text{HDL-C} - 0.20 (\text{TG})$$

$$(ii) \text{ VLDL-C} = 0.20 (\text{TG})$$

##### Statistical analysis

Statistical analyses were done using Graph pad prism software, Version 5.0. For OGTT, RBGT and BMI Two way ANOVA followed by

Bonferroni's post test comparing all columns were used and for Gluconeogenic, glycolytic enzyme level, liver and muscle glycogen content, One way ANOVA following Tukey's multiple comparison test were made and the values are expressed as mean ± SEM (n=6).

**RESULTS**

**Normoglycemic study on non diabetic and diabetic rats treated with SETc**

Normoglycemic study is indicated to study the drug capacity in alteration of blood sugar levels in normal rats. The SETc (1000 mg/kg/p.o.), treatment with normal non diabetic rats showed a normal decrease in the level of blood glucose concentration (p=ns) from the administration point to end of the study (120 min).

However, in diabetic rats the SETc treatment showed BG value near to the normal value after 60<sup>th</sup> min of drug administration and thereafter maintained a plateau (Figure 1).

**Oral Glucose Tolerance Test (OGTT)**

The OGTT was performed at intervals of 1<sup>st</sup>, 15<sup>th</sup> and 25<sup>th</sup> day of SETc treatment (1000 mg/kg/p.o.) and results are depicted in Table 2.

The SETc (1000 mg/kg/p.o) treated group showed its onset of action after 30<sup>th</sup> min of glucose loading (i.e. 60<sup>th</sup> min after drug administration) tested on regular interval of days and maintained to near normal values of blood glucose concentration (p=ns). On day 15 and 25, the blood glucose level of the SETc treated groups maintained BG at 60-120 min after drug administration as compared with that of the standard and normal control (p=ns).

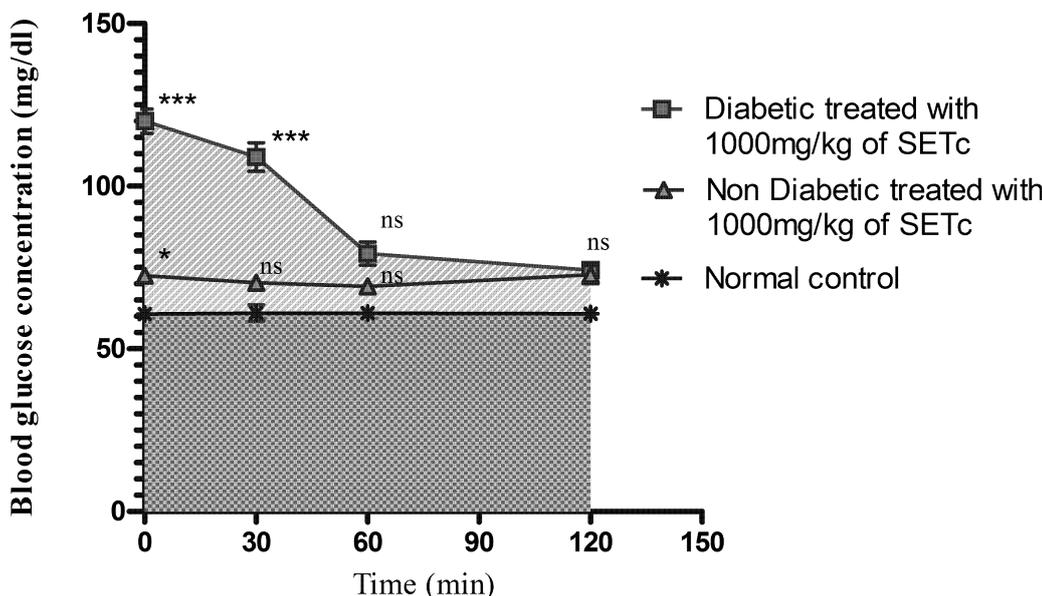


Fig. 1: showing the Normoglycemic effect of SETc in normal, non diabetic and diabetic rats

Table 2: Oral Glucose Tolerance Test (OGTT) in normal and diabetic rats treated with SETc

Days	Groups	Treatment	Blood glucose Concentration (mg/dl)				
			Fasting	OGTT at regular intervals (min)			
				0 min	30 min	60 min	120min
Day 1	Normal Control	0.5% CMC	67.670 ± 3.169	79.170 ± 2.880	107.300 ± 1.801	86.170 ± 1.887	67.670 ± 2.860
	Diabetic Control	0.5% CMC	121.3 ± 3.989 a*	111.8 ± 2.182 a*	296.5 ± 8.782 a*	384.3 ± 10.47 a*	380. ± 7.000 a*
	Test Drug	1000mg/kg of SETC	124.000 ± 4.000 a*, b <sup>ns</sup>	117.000 ± 4.359 a*, b <sup>ns</sup>	107.500 ± 3.519 a <sup>ns</sup> , b*	111.700 ± 2.765 a <sup>@</sup> , b*	115.200 ± 4.672 a*, b*
Day 15	Standard Drug	600 µg/kg of Glibenclamide	131.700 ± 5.506 a*, b <sup>ns</sup> , c <sup>ns</sup>	114.700 ± 3.537 a*, b <sup>ns</sup> , c <sup>ns</sup>	92.670 ± 7.437 a <sup>ns</sup> , b*, c <sup>ns</sup>	89.830 ± 5.498 a <sup>ns</sup> , b*, c <sup>#</sup>	76.000 ± 6.899 a <sup>ns</sup> , b*, c*
	Diabetic Control	0.5% CMC	139.200 ± 3.790 a*	136.300 ± 3.018 a*	330.700 ± 15.090 a*	435.000 ± 17.000 a*	402.500 ± 12.770 a*
	Test Drug	1000mg/kg of SETC	126.000 ± 2.000 a*, b <sup>ns</sup> , d <sup>ns</sup>	147.000 ± 4.000 a*, b <sup>ns</sup>	108.200 ± 5.718 a <sup>ns</sup> , b*	96.000 ± 4.155 a <sup>ns</sup> , b*	106.000 ± 3.000 a*, b*
Day 25	Standard Drug	600 µg/kg of Glibenclamide	153.300 ± 6.354 a*, b*, c <sup>@</sup>	133.200 ± 3.683 a*, b*, c <sup>ns</sup>	93.500 ± 6.339 a <sup>ns</sup> , b*, c <sup>ns</sup>	88.330 ± 3.471 a <sup>ns</sup> , b*, c <sup>ns</sup>	76.000 ± 6.899 a <sup>ns</sup> , b*, c*
	Diabetic Control	0.5% CMC	159.200 ± 3.311 a*	154.700 ± 2.789 a*	308.500 ± 7.903 a*	408.000 ± 7.137 a*	449.200 ± 9.123 a*
	Test Drug	1000mg/kg of SETC	111.000 ± 3.435 a*, b*, d <sup>ns</sup>	101.800 ± 4.126 a <sup>#</sup> , b*	101.300 ± 2.836 a <sup>ns</sup> , b*	111.700 ± 2.765 a <sup>@</sup> , b*	106.800 ± 4.813 a*, b*
	Standard Drug	600 µg/kg of Glibenclamide	138.200 ± 4.778 a*, b <sup>ns</sup> , c <sup>@</sup> , e*	128.000 ± 3.933 a*, b <sup>ns</sup> , c <sup>@</sup>	95.500 ± 8.508 a <sup>ns</sup> , b*, c <sup>ns</sup>	91.330 ± 5.818 a <sup>ns</sup> , b*, c <sup>#</sup>	71.500 ± 3.374 a <sup>ns</sup> , b*, c*

n = 6; Values are expressed as mean ± S.E.M. using Two way ANOVA followed by Bonferroni Multiple Comparison method. p<0.05 is considered as statistically significant. \* = p<0.001; @ = p<0.01; # = p<0.05; ns = non significant.

**Random Blood Glucose Test (RBGT)**

The RBGT was performed in treatment animals in non fasting condition at days of 4<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> and the results are shown in Table 3, and it was observed that the SETc treated diabetic rats showed its onset of action after 60<sup>th</sup> min of drug administration and maintain a plateau even after for a period ≤ 2hrs (p=ns). Starting BG

of the drug treatment at the 0 min showed a significant increase in the blood glucose level as compared with that of the normal control (p<0.001).

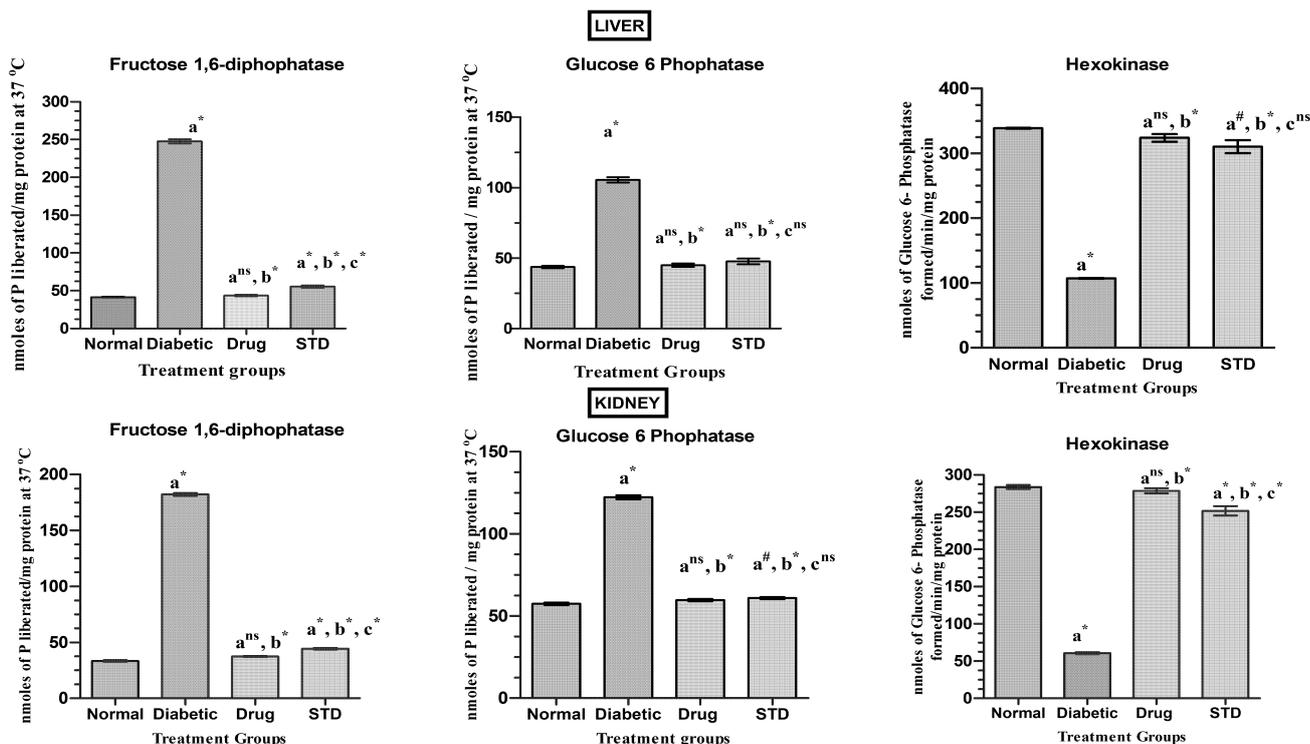
Continuous SETc administration for the period of 20 and 30 days, the 0 minute blood sample showed a significant reduction in the BG level as compared with that of untreated diabetic control.

**Table 3: Random Blood Glucose Test (RBGT) in normal and diabetic rats treated with SETc**

Days	Groups	Treatment	Blood glucose Concentration (mg/dl) RBGT at regular intervals (min)				
			0 min	30 min	60 min	120 min	720 min
Day 4	Normal Control	0.5% CMC	76.67 ± 4.529	75.17 ± 5.062	75.00 ± 4.082	76.33 ± 2.801	73.50 ± 4.530
	Diabetic Control	0.5% CMC	266.5 ± 17.68 a*	277.7 ± 6.922 a*	269.7 ± 17.14 a*	272.3 ± 10.89 a*	274.3 ± 12.98 a*
	Test Drug	1000mg/kg of SETC	263.5 ± 11.76 a*, b <sup>ns</sup>	144.2 ± 6.215 a*, b*	86.00 ± 3.109 a <sup>ns</sup> , b*	85.67 ± 1.994 a <sup>ns</sup> , b*	204.2 ± 16.64 a* b*
Day 20	Standard Drug	600 µg/kg of Glibenclamide	292.0 ± 6.703 a*, b <sup>ns</sup> , c <sup>ns</sup>	128.0 ± 6.186 a*, b*, c <sup>ns</sup>	78.83 ± 4.826 a <sup>ns</sup> , b*, c <sup>ns</sup>	78.00 ± 3.317 a <sup>ns</sup> , b*, c <sup>ns</sup>	221.0 ± 11.94 a*, b*, c <sup>ns</sup>
	Diabetic Control	0.5% CMC	282.0 ± 13.56 a*	281.2 ± 4.269 a*	282.7 ± 10.93 a*	285.8 ± 6.140 a*	286.3 ± 9.861 a*
	Test Drug	1000mg/kg of SETC	184.5 ± 4.403 a*, b*, d*	138.0 ± 6.748 a*, b*	82.83 ± 2.212 a <sup>ns</sup> , b*	82.50 ± 3.784 a <sup>ns</sup> , b*	186.7 ± 7.627 a*, b*
Day 30	Standard Drug	600 µg/kg of Glibenclamide	182.7 ± 9.614 a*, b*, c <sup>ns</sup>	131.0 ± 7.698 a*, b*, c <sup>ns</sup>	75.33 ± 1.856 a <sup>ns</sup> , b*, c <sup>ns</sup>	78.67 ± 3.190 a <sup>ns</sup> , b*, c <sup>ns</sup>	176.3 ± 7.817 a*, b*, c <sup>ns</sup>
	Diabetic Control	0.5% CMC	309.2 ± 10.02 a*	301.2 ± 11.41 a*	308.0 ± 6.962 a*	313.3 ± 12.93 a*	313.7 ± 13.19 a*
	Test Drug	1000mg/kg of SETC	167.8 ± 4.833 a*, b*, d*	122.7 ± 5.226 a*, b*	74.00 ± 2.569 a <sup>ns</sup> , b*	72.50 ± 3.722 a <sup>ns</sup> , b*	175.2 ± 8.211 a*, b*
	Standard Drug	600 µg/kg of Glibenclamide	177.2 ± 5.443 a*, b*, c <sup>ns</sup> , e <sup>ns</sup>	130.7 ± 2.692 a*, b*, c <sup>ns</sup>	75.17 ± 1.778 a <sup>ns</sup> , b*, c <sup>ns</sup>	81.33 ± 2.333 a <sup>ns</sup> , b*, c <sup>ns</sup>	172.8 ± 4.028 a*, b*, c <sup>ns</sup>

n = 6; Values are expressed as mean ± S.E.M. using Two way ANOVA followed by Bonferroni Multiple Comparison method.

p<0.05 is considered as statistically significant. \* = p<0.001; @ = p<0.01; # = p<0.05; ns = non significant.



n = 6; P<0.05 is considered as significant. Values are expressed as mean ± S.E.M using One Way ANOVA followed by Tukey's Multiple Comparison method. a = Normal control Vs Diabetic control, Test drug and Standard; b = Diabetic control Vs Test drug and Standard; c = Test drug Vs Standard. \* = p<0.001; @ = p<0.01; # = p<0.05; ns = Non Significant.

**Fig. 2: Showing the effect of SETc over gluconeogenic and glycolytic enzymes after 30 days treatment period**

### Effect of SETc on Glucose metabolism: Gluconeogenic and glycolytic enzymes

The activities of gluconeogenic enzymes (glucose 6-phosphatase and fructose-1, 6-diphosphatase) and glycolytic enzyme (hexokinase) in the liver and kidney of control and experimental animals are depicted in Figure 2. Treatment with SETc (1000mg/kg/p.o.) elevated the activity of hexokinase in the liver and kidney as compared with that of the diabetic control ( $p < 0.001$ ) and reaches near to the normal values. In this case, the SETc treated rats were showed its significant reduction in these two enzymes (glucose-6-phosphatase, fructose-1,6-diphosphatase) in liver and kidney as compared with that of the diabetic control ( $p < 0.001$ ) and reaches near to the normal values after SETc treatment as shown (Fig. 2).

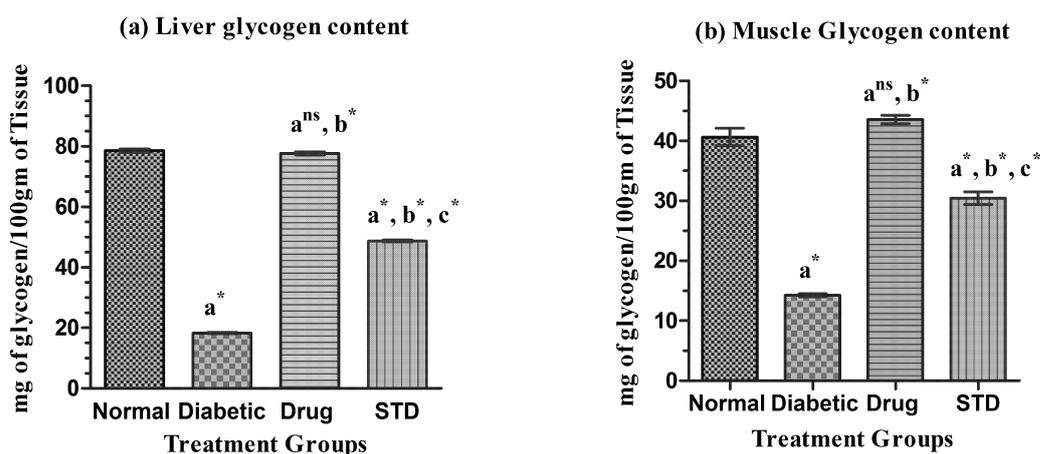
### Effect of SETC on Liver and Muscle glycogen in Normal and STZ induced diabetic rats

Glycogen content of skeletal muscles and liver was markedly decreased in diabetes (Table 3.a & b). Administration of SETc to

diabetic animals increased the activity of hexokinase in liver and kidney as compared with that of the diabetic control ( $P < 0.001$ ).

### Total lipid profile

From the table 4, it was observed that there was an increase in total cholesterol, triglycerides, LDL and VLDL cholesterol levels in diabetic control as compared with that of the normal ( $p < 0.001$ ). In the present study, the test drug SETc (1000mg/kg) was found to have the property to reduce the total cholesterol and triglyceride level near to the normal and there was a significant increase in the HDL-C and more significant decrease ( $P < 0.001$ ) in LDL-C, when compared with that of the diabetic control, which was then further proved by the BMI data. The result showing that there was no change in the body weight of the animal gaining HDL-C with less diameter of belly ( $p = ns$ ) rather than those of diabetic animals gained less weight and increased belly diameter ( $p < 0.001$ ) on 30th day observation of the study (Fig. 4).



$n = 6$ ;  $P < 0.05$  is considered as significant. Values are expressed as mean  $\pm$  S.E.M using One Way ANOVA followed by Tukey's Multiple Comparison method.

a = Normal control Vs Diabetic control, Test drug and Standard.

b = Diabetic control Vs Test drug and Standard.

c = Test drug Vs Standard.

\* =  $p < 0.001$ ; @ =  $p < 0.01$ ; # =  $p < 0.05$ ; ns = Non Significant.

Fig. 3: showing the effect of SETC on liver and muscle glycogen in normal and STZ induced diabetic rats

Table 4: Total Lipid profile after 30 days in normal and diabetic rats treated with SETc

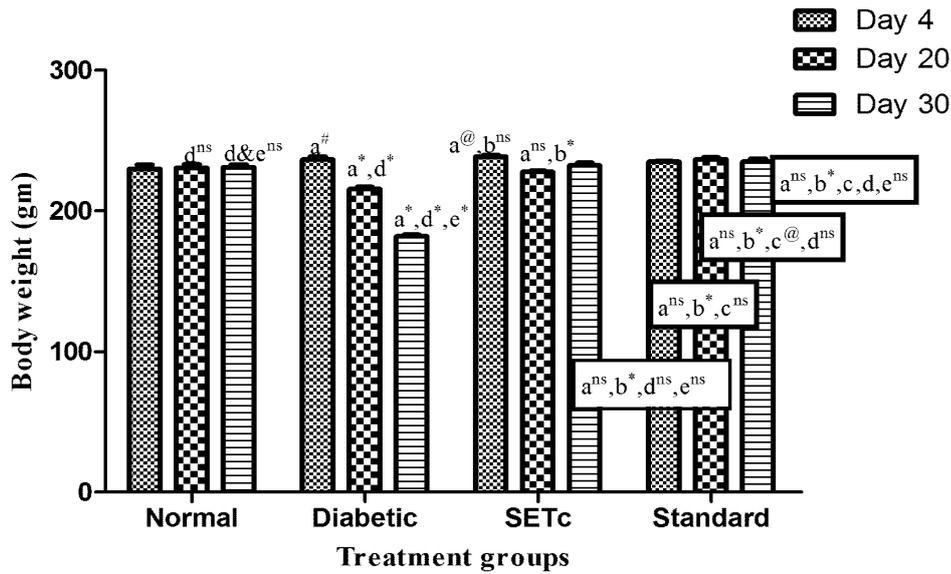
Groups	Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL - C (mg/dl)	LDL - C (mg/dl)	VLDL - C (mg/dl)
Normal control	0.5% CMC	79.67 $\pm$ 1.383	107.8 $\pm$ 1.707	35.38 $\pm$ 1.327	22.73 $\pm$ 2.055	21.56 $\pm$ 0.3391
Diabetic control	0.5% CMC	129.2 $\pm$ 4.324 a*	131.7 $\pm$ 2.697 a*	26.61 $\pm$ 1.362 a@	73.97 $\pm$ 4.727 a*	26.41 $\pm$ 0.5700 a*
Test Drug	1000mg/kg of SETC	77.03 $\pm$ 1.796 a <sup>ns</sup> , b*	102.2 $\pm$ 2.152 a <sup>ns</sup> , b*	35.93 $\pm$ 1.865 a <sup>ns</sup> , b*	20.65 $\pm$ 3.209 a <sup>ns</sup> , b*	20.45 $\pm$ 0.4302 a <sup>ns</sup> , b*
Standard Drug	600 $\mu$ g/kg of Glibenclamide	97.61 $\pm$ 2.101a*, b*, c*	115.1 $\pm$ 2.065 a <sup>ns</sup> , b*, c@	30.80 $\pm$ 0.9900 a <sup>ns</sup> , b <sup>ns</sup> , c <sup>ns</sup>	44.59 $\pm$ 2.236 a*, b*, c*	23.04 $\pm$ 0.4047 a <sup>ns</sup> , b*, c@

$n = 6$ ; Values are expressed as mean  $\pm$  S.E.M. using One way ANOVA followed by Tukey's Multiple Comparison method.

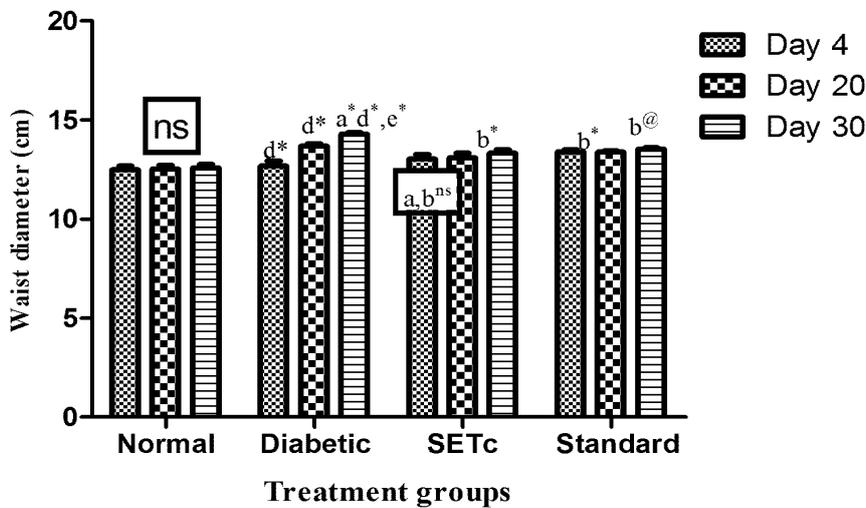
$p < 0.05$  is considered as statistically significant.

\* =  $p < 0.001$ ; @ =  $p < 0.01$ ; # =  $p < 0.05$ ; ns = non significant.

**Body weight (gm) at regular intervals in normal and SETc treated diabetic rats**



**Surface area of waist diameter (cm)**



n = 6; Values are expressed as mean ± S.E.M. using Two way ANOVA followed by Bonferroni's Multiple Comparison method. p<0.05 is considered as statistically significant.

- a = normal control Vs diabetic control, test drug and standard.
- b = diabetic control Vs test drug and standard.
- c = test drug Vs standard.
- d = day Vs day 20 & 30
- e = day 20 Vs day 30

\* = p<0.001; @ = p<0.01; # = p<0.05; ns = non significant.

**Fig. 4: showing the Body mass index of normal and SETc treated diabetic rats**

**DISCUSSION**

It is well documented that STZ destroys the beta cells of the pancreas and causes hyperglycemia in rats. This is because streptozotocin causes a massive reduction in insulin release by the destruction of b-cells of the islets of langerhans and thereby induces hyperglycemia<sup>16</sup>. Any reversal of STZ induced experimental hyperglycemia by a molecule without affecting the normal glycemc index of test

subjects<sup>17</sup>; protect the subjects from hypoglycemic shock, which is an established side effect of orally effective sulfonyl urea drugs. Normoglycemia study revealed the test drug's capacity to lower blood glucose level without producing alarming low BG level to the state of hypoglycemic shock and within the physiological norms.

Theoretically, blood glucose level after glucose loading depends on insulin secretion, glucose utilization, intestinal glucose absorption

and intestinal motility<sup>18</sup>, thereafter additional hypoglycemic responsive mechanisms may be due to stimulation of insulin secretion, glucose utilization as well as inhibition of intestinal glucose absorption and intestinal motility, or combination of one or other. In present study, the diabetic animals treated with the test drug SETc (1000mg/kg/p.o.) was found to show its well tolerable antihyperglycemic index after glucose load. And the same in the random study, continuous administration of SETc 1000mg/kg/p.o., with regular diet and on the 20<sup>th</sup> and 30<sup>th</sup> day of observation, the 0 minute blood sample showed a significant reduction in the BG level as compared with that of untreated diabetic control may be through possible potentiating of pancreatic insulin from the  $\beta$ -cell of islets or due to enhanced transport of blood glucose to the peripheral tissues. This leads to the maintenance of BGC at a plateau range.

Liver is the main site for glycolysis, a process where glucose is degraded and gluconeogenesis, where glucose is synthesized from lactate, amino acids and glycerol. A part of this was also processed in the secondary metabolizing organ, the kidney. Those are the two important complementary events that balance the glucose load in our body<sup>19</sup>. STZ induced hyperglycemia results in imbalance of these enzymes responsible for glucose utilization by the body. Hexokinase is the prime enzyme catalyzing glucose phosphorylation. The first step in glycolysis<sup>20</sup> is severely impaired during diabetes<sup>21</sup>. Impairment of hexokinase activity suggests the impaired oxidation of glucose via glycolysis leading to its accumulation resulting in hyperglycemia. Hexokinase is universally present in cells of all types. Hexokinase catalyzes the conversion of glucose to glucose 6-phosphate and plays a central role in the maintenance of glucose homeostasis. The hexokinase activity was found to be decreased in diabetic rats which may be due to insulin deficiency (insulin stimulates and activates hexokinase). SETc may stimulate insulin secretion, which may activate hexokinase, thereby increasing utilization of glucose leading to decreased blood sugar levels<sup>22</sup>.

Fructose-1,6-diphosphatase and glucose-6-phosphatase are important regulatory enzymes in gluconeogenesis. In diabetic animals the enzyme levels were observed to increase<sup>23</sup>. The increased activities of glucose 6-phosphatase and fructose 1, 6-diphosphatase in liver and kidney of the Streptozotocin induced diabetic rats may be due to insulin insufficiency. As reported earlier, the insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose 1, 6-diphosphatase, phosphoenol pyruvate carboxykinase and pyruvate carboxylase<sup>24</sup>. This action of SETc may be attributed to increased insulin secretion, which is responsible for the repression of the gluconeogenic key enzymes. The Sedimental Extract of *Tinospora cordifolia* (SETc) exhibited antidiabetic activity by enhancing the peripheral utilization of glucose by correcting the impaired liver and kidney glycolysis and by limiting its gluconeogenic formation similar to insulin.

The decrease in glycogen content of liver and skeletal muscle observed in the present study is probably due to the lack of insulin in the diabetic state. This prevention of glycogen depletion in the liver and muscles might possibly be due to stimulation of insulin release<sup>25</sup>. In the liver, this above enzyme is an important regulator of glucose storage and disposal<sup>26</sup>.

The extract induced decrease in the concentration of blood glucose in Streptozotocin treated rats may be the result of increased glycolysis. This is in agreement with the previous studies on *Catharanthus roseus*<sup>27</sup>, *Gymnema sylvestre*<sup>28</sup>, the activity of gluconeogenic enzyme glucose-6-phosphatase is enhanced during diabetes<sup>29</sup>. During the SETc treatment days, the extract-induced lowering of the blood glucose also might be due an increase in liver and muscle glycogen content. This may be due to mobilization of blood glucose towards liver and muscle glycogen reserve.

The levels of triglycerides and total cholesterol in the serum of the diabetic rats had been reported to be greatly elevated<sup>30, 31</sup>. Elevation of serum lipids in diabetes indicates either the defective removal or overproduction (or both) of one or more lipoproteins<sup>32</sup>. Statement from the BMI clearly shows the deposition of HDL-C in the adipose tissue, so that changes in the belly diameter appears to be normal but as in case of diabetic untreated rats suffered from reduction of

HDL-C due to metabolic imbalance and further deposition of low weight cholesterol in the adipose tissue (LDL-C). A significant increase in HDL-cholesterol level and a reduction in LDL-cholesterol level observed in the treated animals is an added advantage, so it gives a strong belief that the extract can reduce the cardiovascular risk factors which contribute to high percentage mortality in diabetic subjects<sup>33</sup>.

The overall reports concludes that the SETc (1000mg/kg/p.o.) treatment, does not affect the normal glycemic index of the normal rats but significantly decreased the elevated blood glucose levels in diabetic rats and onset was seen within 30 min of drug treatment. Prolonged administration of SETc has got better antidiabetic therapeutic effect in fasting and random glucose estimation in diabetic rats. Oral GTT test of SETc treatment in diabetic rats showed a therapeutic equivalence to that of glibenclamide. SETc administration does not affect the gluconeogenic enzyme levels, however significant effect was observed in the increase of glycolytic enzyme levels. Liver and muscle glycogen storage levels were increased in the SETc treated animals when compared to non treated diabetic rats. In support, the biochemical parameter particular of increased beneficiary HDL-C with less localized low density lipoproteins clearly establishes the antihyperlipidemic activity with non obese abdomen and reduced risk of cardiovascular disorders.

Furthermore, the OGTT study with SETc for 30 days of chronic treatment showed a reversal in blood glucose concentration which enlightens an idea to study the persistence of antioxidant principles and their protection of beta cells against STZ induced oxidative stress in the future.

**Abbreviation** BGC Blood Glucose Concentration, RBGT Random blood glucose test, TG Triglycerides.

#### REFERENCES

1. Kumar V, Fausto N, Abbas AK et al. Pathologic Basis of Disease. 7<sup>th</sup>edn. Saunders publishers: Philadelphia; 2005, pp: 1194-1195.
2. Kornelis JJ Van Hateren, Gijs WD Landman, Nanne K et al. The Lipid Profile and Mortality Risk in Elderly Type 2 Diabetic Patients: A Ten-Year Follow-Up Study (ZODIAC-13). Plosone 2009; 4(12), pp: 1-6.
3. R.Kannadhasan. Antidiabetic and antioxidant activities of water extract of *Tinospora cordifolia* in streptozotocin induced diabetes in rats. Doctor of Philosophy. Thesis, Faculty of Pharmacy, MAHER University, Chennai, Tamilnadu, India (2010).
4. Soon YY, Tan BKH. Evaluation of Hypoglycemic and Antioxidant activities of *Morinda officinalis* in STZ-induced diabetic rats. Singapore Med J 2002; 43(2), pp: 77-85.
5. Gosh R, Sharatchandra K, Rita S, Thocho IS. Hypoglycaemic activity of *Ficus hispida* in normal and diabetic albino rats. Indian J Pharmacol 2004; 36(4), pp: 222-225.
6. Musabayane CT, Mahlela N, Shode FO, Ojewole JAO. Effect of *Syzygium cordatum* (Hochst.) [Myrtaceae] leaf extract on plasma glucose and hepatic glycogen and epatic glycogen in streptozotocin-induced diabetic rats. Journal of Ethnopharmacol 2005; 97, pp: 485-490.
7. Santhakumari P, Prakasam A, Pugalendi KV. Modulation of oxidative stress parameters by Piper betle leaf in streptozotocin diabetic rats. Indian Journal of Pharmacology 2003; 35, pp: 373-378.
8. King J. The transferases-alanine and aspartate amino transaminases. In: Van D (ed.), Practical Clinical Enzymology. London: Nostrand company Ltd; 1965b, pp: 191-208.
9. Fiske CH, Subbarow Y. The colorimetric determination of Phosphorous. J Biol Chem 1925; 66 (2), pp: 375 - 400.
10. Gancedo JM, Gancedo C. Fructose-1, 6- diphosphatase, phosphofructokinase and glucose-6- phosphate dehydrogenase. Achiv fur Mikrobiologie 1971; 76, pp: 132 - 138.
11. Branstrup N, Krik JE, Bruni C. The hexokinase and phosphoglucosomerase activities of aorta and pulmonary artery tissue in individuals of various ages. J Gerontol 1957; 12, pp: 166 -171.

12. Sasaki T, Matsuy S, Sanae A. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose determination. *Rinsho Kagaku* 1972; 1, pp: 346-350.
13. Nicholas VC, Robert WL, Joseph HR. The determination of glycogen in liver and muscle by use of anthrone reagent. *J Biol Chem* 1955; 28, pp: 583-593. Online source available at [www.jbc.org](http://www.jbc.org).
14. Friedewald WT, Levy RI, Fredrikson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. *Clin Chem* 1972; 18, pp: 499-502.
15. Peter Wilson W, Robert AD, Robert GJ, William CP. Estimation of Very Low density Lipoprotein Cholesterol from data on Triglyceride concentration in plasma. *Clin Chem* 1981; 27, pp: 2008-2010.
16. Siyem D, Syngai G, Khup PZ, Khongwir BS, Kharbuli B, Kayang H. Hypoglycemic effects of *Potentilla fulgens* L.in normal and alloxan diabetic mice. *J Ethnopharmacol* 2002; 83, pp: 55-61.
17. Palmar AM, Thomas CR, Gopaul N et al. Dietary antioxidant supplementation reduces lipid peroxidation but impairs vascular function in small mesentric arteries of the streptozotocin diabetic rats. *Diabetologia* 1998; 41, pp: 148 - 152.
18. Peungvicha P, Thirawarapan SS, Watanabe H. Possible mechanism of hypoglycemic effect of 4-hydroxybenzoic acid, a constituent of *Pandanus odoratus* root. *Japanese J Pharmacol* 1998; 78(3), pp: 395-398.
19. Bhavapriya V, Govidasamy S. Biochemical studies on the hypoglycemic effect *Aegle marmelos* (Linn). *Correa Ex Roxb.* in streptozotocin induced diabetic rats. *Indian Drugs*. 2000; 37(10), pp: 474-477.
20. Vestergoard H. Studies of gene expression and activity of hexokinase, phosphofructokinase and glycogen synthetase in human skeletal muscle in states of altered insulin stimulated glucose metabolism. *Dan Med Biol* 1999; 46, pp: 13-34.
21. Sato T, Magata K, Koga N. Defect of an early event of glucose metabolism in skeletal muscle of the male OLETE rat, an NIDDM model. *Biochem Biophys Res Commun* 1998; 245(2), pp: 378-381.
22. Ragavan B, Krishnakumari S. Antidiabetic effect of T.arjuna bark extract in alloxan induced diabetic rats. *Ind J Clin Biochem* 2006; 21(2), pp: 123-128.
23. Arathi G, Sachdanandam. Therapeutic effect of *Semecarpus* and *Cardium* Linn, nut milk extract on carbohydrate metabolizing and mitochondrial TCA cycle and respiratory chain enzymes in mammary carcinoma rats. *J Pharm Pharmacol* 2003; 55, pp: 1283-1290.
24. Murray RL, Granner DK, Mayes PA, Rodwell VW. *Harper's Biochemistry*, 25<sup>th</sup> ed, Appleton and Lange Stanford Connecticut; 2000.
25. Chakrabarti S, Biswas TK, Rokeya B et al. Advanced studies on the hypoglycemic effect of *Caesalpinia bonducella* in type 1 and type 2 diabetes in long evans rats. *J Ethnopharmacol* 2003; 84, pp: 41 - 46.
26. O'Doherty RM, Lehman DL, Telemaque potts S, Newgard CB. Metabolic impact of glucokinase overexpression in liver: Lowering of blood glucose in fed rats is accompanied by hyperlipidemia. *Diabetes* 1999; 48, pp: 2022-27.
27. Singh SN, Vats P, Suri S et al. Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 2001; 76, pp: 269-277.
28. Shanmugasundaram KR, Paneerselvam C, Samudram P, Shanmugasundaram ERB. Enzyme changes and glucose utilization in diabetic rabbits: the effect of *Gymnema sylvestre*. *J Ethnopharmacol* 1983; 7, pp: 205-234.
29. Vijayvargia R, Kumar M, Gupta S. Effect of *Enicostemma littorale* Blume on alloxan -induced diabetes mellitus in rats. *Indian J Exp Biol* 2000; 38, pp: 781-784.
30. Mak DHF, Li PC, Poon MKT, Ko KM. Alteration in tissue glutathione antioxidant system in streptozotocin-induced diabetic rats. *Mol Cell Biochem* 1996; 62, pp: 153-158.
31. Wittenstein B, Klein M, Finckh B, Ullrich K, Kohlschutter A. Plasma antioxidants in pediatric patients with glycogen storage disease, diabetes mellitus, and hypercholesterolemia. *Free Radical Biology and Medicine* 2002; 33, pp: 103-110.
32. Akula A, Kota MK, Gopisetty SG. Biochemical, histological and echo- cardiographic changes during experimental cardiomyopathy in STZ-induced diabetic rats. *Pharmacological Research* 2003; 48, pp: 429 - 435.
33. Barnett HA, O'Gara G. *Diabetes and the heart*. Clinical Practice Series (UK): Churchill Livingstone. Edinburgh; 2003, pp: 11-32.