

ANTI-HYPERGLYCEMIC EFFECT OF ALCOHOLIC EXTRACTS OF CNIDOSCOLUS CHAYAMANSA IN EXPERIMENTAL DIABETES AND THEIR EFFECTS ON KEY METABOLIC ENZYMES INVOLVED IN CARBOHYDRATE METABOLISM

K. Kulathuran Pillai*¹, N. Chidambaranathan,² M. Mohamed Halith,² S. Jayaprakash,² and N. Narayanan³

¹Department of Biotechnology, Periyar Maniammai University, Vallam, Thanjavur, Tamil Nadu, India.

²K. M. College of Pharmacy, Uthangudi, Madurai, Tamil Nadu, India.

³Joint Director of Medical Education (Pharmacy) Directorate of Medical Education, Kilpauk, Chennai, Tamil Nadu, India.

ABSTRACT

In India the decoction of Chaya (*Cnidocolus chayamansa*) are used as a house hold remedies for diabetics. Chaya leaf is especially high in protein (5.7%), Crude fibre (1.9%), calcium 199.4% mg/100gm, potassium 7.02mg/100gm, iron 11.4mg/gm, Vitamin C 164.7 mg/100gm and carotene 0.085mg/100gm. The antihyperglycemic effect of ethanolic extracts of Chaya (*C.chayamansa*) was evaluated in diabetic rats using Alloxan monohydrate (150mg/kg) as diabetogenic agents for pilot study (4 weeks) so as to assess their effect in mild (plasma sugar more than 160mg/dl) diabetes mellitus. In the pilot study maximum reduction of 31.3% and 35.0% glucose levels was observed in animals receiving 100mg/kg and 250mg/kg per day of Ethanolic extracts of Chaya (*C.chayamansa*) after 4 weeks of treatment, respectively. The alteration in hepatic glycogen content and hepatic glucokinase, hexokinase and glucose-6-phosphate levels in diabetic rats were partially restored by Ethanolic extracts of Chaya (*C. chayamansa*).

Keywords Ethanolic extracts of Chaya, glycogen content, glucokinase, hexokinase, glucose-6-phosphate, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a metabolic disorder in which the body does not produce or properly utilize insulin. It causes disturbance in carbohydrate, protein and lipid metabolism and complications such as retinopathy, microangiopathy and nephropathy. The commonly encountered acute and late diabetic complications are already responsible for major causes of morbidity, disability and premature death in Asian countries. The

underlying process attributed to hyperglycemia ultimately result in oxidative stress, alteration in enzyme activity, protein glycosylation and several structural changes.¹ Enzyme activities of glucogenesis have been shown to increase in the glucogenolytics and lipolytic pathways. Diabetes also has been accompanied with the decrease in the enzyme activity of the glycolytics and pentose phosphate pathways.²

Despite the immense strides that have been made in the understanding and management

of diabetes and disease related complications are increasingly unabated. In spite of the presence of series of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Many traditional plant treatments for diabetes are used throughout the world and there is an increasing demand from patients to use the natural products with anti-diabetic activity.

Herbal drugs are used widely even when their biologically active compounds are unknown, because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Several plant extracts are used in the treatment of diabetes and diabetic associated hyperlipidemia, among this armamentarium, the plant, *C. chayamansa* is selected for the present study.

Chaya (*Cnidioscolus chayamansa*, Mc vaugh) belonging to the family Euphorbiaceae, shrub growing in Central American and the hot topics of southern Mexico.³ However, the plant show great adaptability to milder climates and can be found growing in northern latitudes, under dryer environments and in different solid.⁴ The leafy vegetable was consumed by Mayan Indians and is traditionally incorporate in salad an regional dishes.⁵ Chaya consumption has also become popular among Hispanic population in southern Texas Florida. The nutritional value of Chaya is very attractive when compared with spinach other common vegetable.^{6,7} The plant which is also called spinach tree, has a great potential to alleviate deficits in population of developing countries as it is rich in essential amino acid, vitamin and mineral.^{8,9,10} Unfortunately the leave have undetermined amounts of cyanogenic glycosides.⁴ However, cooking as well as other heat treatment will hydrolyze these glycosides, minimizing the risk of poisoning.⁴ Although Chaya is primarily as a food plant, it has been used therapeutically for a number of ailments such as diabetes¹¹, arteriosclerosis, gallstone and high cholesterol. It is also believed that Chaya cleans, circulatory system, stimulate lactation, improve eyesight, strengthens nails, improve digestion, and is a diuretic and laxative agent.¹²

The present investigation is undertaken to study the effect of *C. chayamansa* (CCM) extract on changes in Body weight, Plasma glucose, RBC, WBC, HB, Platelets, Hepatic glucokinase & hexokinase, Glucose-6-phosphate and Glycogen content.

MATERIALS AND METHODS

Chemicals

Alloxan monohydrate was obtained from S. D Fine. Chem. Ltd, Mumbai, India. Glipizide was obtained from Micro Labs, Hosur, India.

Selection and acclimatization of animals

Wistar strains of male albino rats weighing between 180-200g are used for this study. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water *ad libitum*. The animals were well acclimatized to the standard environmental condition of temperature ($22 \pm 5^{\circ}\text{C}$) and humidity ($55 \pm 5\%$) and 12 hr light dark cycles throughout the experimental period.

INDUCTION OF DIABETES MELLITUS

Diabetes mellitus is induced in wistar rats by single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (150mg/kg Body Weight) in physiological saline after overnight fasting for 12hr.¹³

Alloxan is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the β -cells of pancreas possibly by generating the excess reactive oxygen species such as H_2O_2 , O_2 and HO. The development of hyperglycemia in rats is confirmed by plasma glucose estimation 72 hr post alloxan injection. The rats with fasting plasma glucose level of 200-260mg/dl were used for this experiment.

Experimental procedure

IAEC, K. M. College of Pharmacy, Madurai has approved this experimental procedure. Approval No: (KKp/Ph.D/pmu/2010). In the experiment a total of 30 rats (24 diabetic surviving rats & 6 normal rats 6 in each group.) were used. Diabetes was induced in rats 3 days before starting the experiment. The rats were divided into 5 groups after the induction of alloxan diabetes.

TREATMENT PROTOCOL

Group-I: (Normal control) consist of normal rats treated with 10ml/Kg of normal saline, orally.

Group-II: (Toxic control) Diabetic control received 150mg/Kg of Alloxan monohydrate through Intra peritoneally.

Group-III: (positive control) Diabetic rat received Glipizide (10mg/Kg i.p)¹⁴ for 28 days, orally.

Group-IV: (Treatment group) Diabetic rat received low dose (100mg/Kg)¹⁵ of ethanolic extract of *C.chayamansa* daily using intra-gastric tube for 28 days.

Group-V: (Treatment group) Diabetic rat received high dose (250mg/Kg) of ethanolic extract of *C.chayamansa* daily using intra-gastric tube for 28 days.

METHODOLOGY

Sample collection

After 28 days of treatment, the blood glucose level and body weight were measured. Then blood was collected retro-orbitally under light ether anesthesia using capillary tubes. Blood was collected in fresh vials containing EDTA as anticoagulant agents and plasma was separated in a T8 electric centrifuge at 2000 rpm for 2 minutes. Then animal was sacrificed by decapitation. Liver and pancreas were immediately dissected out, washed in ice-cold saline to remove the blood and liver was used for estimation of enzyme activity while pancreas was subjected to histopathological studies.

BIOCHEMICAL ANALYSIS

Estimation of blood glucose

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method.¹⁶

Hepatic glucokinase and hexokinase activity

The part of liver for each test was perfused with ice cold 0.15M KCl and 1mM EDTA solution and homogenized twice its weight of ice cold buffer (0.01 cysteine and 1mM EDTA in 0.1 ml Tris-HCL, pH 7.4) and centrifuged for 20 min at 4°C. Glucose phosphorylation was

assayed by means of glucose 6 phosphate dependent spectrophotometric method.¹⁷

Glucose-6-phosphatase activity

The part of the liver for each test was homogenized with 40 times its weight of ice cold buffer (0.1 citrate-KOH, pH 6.5) and filtered through cheese cloth. Glucose-6-phosphatase activity was measured by phosphate release by the method Marjorie. The determination of phosphoric acid concentration in assay mixture was done calorimetrically.¹⁸

Glycogen Content

The tissue sample was digested by hot concentrated 30% KOH and treated with anthrone reagent. Glycogen content was determined calorimetrically.¹⁹

Hematological and Biochemical parameters

Blood samples were assessed for RBC, WBC, HB, and Platelets with an auto analyzer (MISPA-EXCEL, Japan).

Histopathological examination

Pancreas tissue section was fixed in 4g/L formaldehyde and embedded in paraffin. Paraffin section was then stained with Hematoxylin-eosin.²⁰ each sample was observed at 400 X magnification and scored according to the injuries.²¹

Statistical analysis

The data for different biochemical parameters were analyzed by using one way ANOVA followed by Newman Keul's multiple range test.

Table 1: Effect of body weight of normal and experimental animals in each group

Groups	Initial Body Weight (g)	Final Body Weight (g)
Group I (G 1)	202.10 ± 4.09	206.33 ± 3.10
Group II (G 2)	198.90 ± 4.70	156.40 ± 2.35 ^{*a}
Group III (G 3)	206.10 ± 4.80	220.25 ± 4.62 ^{*b}
Group IV (G 4)	216.55 ± 5.45	220.25 ± 3.90 ^{*b}
Group V (G 5)	205.50 ± 4.24	225.5 ± 3.74 ^{*b}

G1- Normal Control; G2- Diabetic Control; G3- Positive control (Glipizide);

G4- Treatment group 100mg/kg; G5- Treatment group 250mg/kg.

Values are expressed as Mean ± SEM.

Values were calculated by using one way ANOVA followed by Newman Keul's multiple range tests.

*a values were significantly different from normal control (G 1).

*b values were significantly different from diabetic control (G 2).

Table 2: Effect of treatment (4 weeks) with various doses of EECC on glucose levels (mg %) in alloxan diabetic rats

Groups	0 Day	14 th Day	28 th Day
Group I	72.6 ± 4.80	74.75 ± 4.30	9.40 ± 4.40
Group II	152.75 ± 5.55	170.20 ± 6.22 ^{*a}	215.8 ± 7.50 ^{*a}
Group III	185.8 ± 5.15	143.42 ± 4.22 ^{*b}	130.70 ± 3.66 ^{*b}
Group IV	195.20 ± 3.98	155.30 ± 4.35 ^{*b}	148.20 ± 3.45 ^{*b}
Group V	190.85 ± 4.60	158.20 ± 4.35 ^{*b}	148.20 ± 4.20 ^{*b}

G1- Normal Control; G2- Diabetic Control; G3- Positive control (Glipizide);

G4- Treatment group 100mg/kg; G5- Treatment group 250mg/kg

Values are expressed as Mean ± SEM.

Values were find out by using one way ANOVA followed by Newman

Keul's multiple range tests.

*a values were significantly different from normal control (G 1).

*b values were significantly different from diabetic control (G 2).

Table 3: Effect of EECC on glycogen content (mg/gm tissue)

Groups	Liver Tissue Glycogen Content (mg/g tissue)
Group I	39.25 ± 2.25
Group II	8.10 ± 0.45 ^{*a}
Group III	30.45 ± 1.52 ^{*b}
Group IV	22.35 ± 1.05 ^{*b}
Group V	24.80 ± 1.22 ^{*b}

G1- Normal Control; G2- Diabetic Control;

G3- Positive control (Glipizide);

G4- Treatment group 100mg/kg;

G5- Treatment group 250mg/kg.

Values are expressed as Mean ± SEM.

Values were find out by using one way

ANOVA followed by Newman

Keul's multiple range tests.

*a values were significantly different from normal control (G 1).

*b values were significantly different from diabetic control (G 2).

Table 4: Effect of EECC on enzymes involved in carbohydrate metabolism in rats

Groups	Hexokinase (µg/mg)	Glucose-6-Phosphate (µg/mg)	Glucokinase (µg/mg)
Group I	0.210 ± 0.013	0.390 ± 0.010	24.36 ± 1.38
Group II	0.092 ± 0.004 ^{*a}	0.126 ± 0.007 ^{*a}	4.88 ± 0.28 ^{*a}
Group III	0.127 ± 0.007 ^{*b}	0.30 ± 0.010 ^{*b}	14.10 ± 0.92 ^{*b}
Group IV	0.118 ± 0.005 ^{*b}	0.230 ± 0.007 ^{*b}	11.10 ± 0.50 ^{*b}
Group V	0.140 ± 0.006 ^{*b}	0.240 ± 0.008 ^{*b}	15.15 ± 0.90 ^{*b}

G1- Normal Control; G2- Diabetic Control; G3- Positive control (Glipizide);

G4- Treatment group 100mg/kg; G5- Treatment group 250mg/kg.

Values are expressed as Mean ± SEM.

Values were find out by using one way ANOVA followed by

Newman Keul's multiple range tests.

*a values were significantly different from normal control (G 1).

*b values were significantly different from diabetic control (G 2).

Table 5: Effect of EECC on hematological parameters

Groups	WBC × 10 ³ /µL	RBC × 10 ⁶ /µL	HB % gm/dL	Platelet × 10 ³ /µL
Group I	8.40 ± 0.60	6.45 ± 0.30	12.30 ± 0.60	310.40 ± 38.75
Group II	8.10 ± 0.68	6.95 ± 0.15	13.15 ± 0.40	295.45 ± 13.22
Group III	6.45 ± 0.35	6.50 ± 0.45	14.20 ± 0.35	278.40 ± 30.85
Group IV	7.10 ± 0.30	7.77 ± 0.20	12.80 ± 0.55	312.77 ± 17.90
Group V	8.74 ± 0.80	6.78 ± 0.32	11.70 ± 0.55	315.40 ± 14.35

G1- Normal Control; G2- Diabetic Control; G3- Positive control (Glipizide);

G4- Treatment group 100mg/kg; G5- Treatment group 250mg/kg.

Values are expressed as Mean ± SEM.

Values were find out by using one way ANOVA followed by

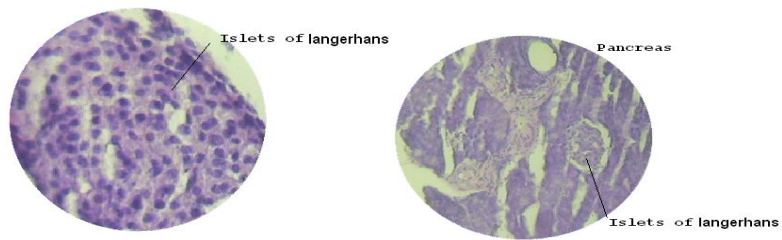
Newman Keul's multiple range tests.

Values were not significantly different from normal and diabetic control.

HISTOPATHOLOGICAL STUDY OF PANCREAS OF RATS

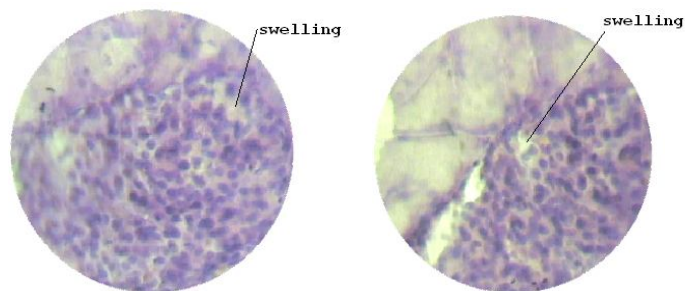
Group I: Normal Control (Saline)

Fig. 1:



Group II: Toxic Control (Alloxan monohydrate)

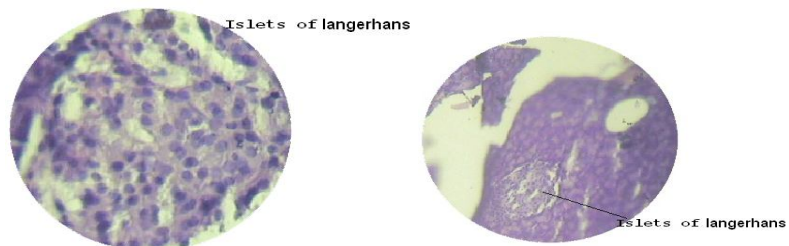
Fig. 2:



- The number of islet cells were significantly decreased, islet cells were severely swelled.

Group III: Positive Control (Alloxan monohydrate + Glipizide)

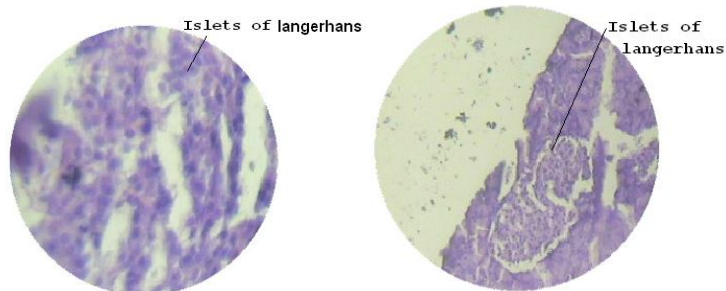
Fig. 3:



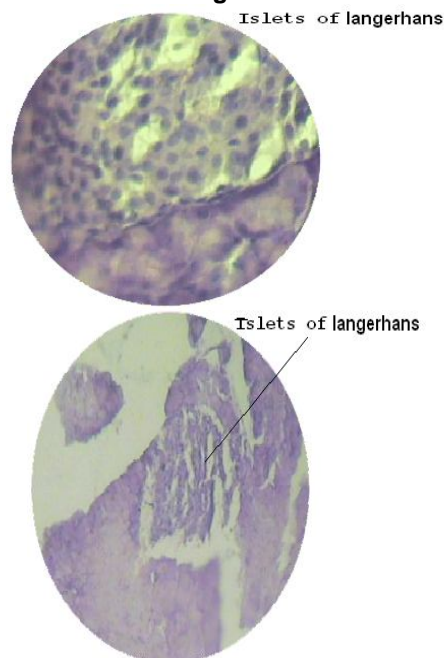
The number of islet cells were moderately decreased, islets cells were mildly swelled.

Group IV: Treatment group (Alloxan monohydrate + *EECC* 100mg/kg)

Fig. 4:



The number of islet cells were moderately decreased, islet cells were moderately swelled.

Group V: Treatment group (Alloxan monohydrate + EECC 250mg/kg)**Fig. 5:**

The numbers of islet cells were moderately decreased, islet cells were moderately swelled.

RESULTS

Table 1 shows the values of body weight of normal and experimental animals in each group. The mean body weight of diabetic rats (156.40 ± 2.35) was significantly decreased as compared to normal animals (206.33 ± 3.10).

The body weight of diabetic rats treated with ethanolic extract of *C. chayamansa* (EECC) at different doses 100 mg/kg and 250 mg/kg was significantly increased to 220.25 ± 3.90 and 225.50 ± 3.74 respectively as compared to non-treated diabetic animals. In group III treated animals also showed an increase in body weight significantly as compared to diabetic rats.

Effect of EECC on Blood Glucose Levels

In all groups prior to alloxan administration the basal level of plasma glucose of the rats were not significantly higher in the rats selected for the study. In contrast non-diabetic control remained steadily euglycemic throughout the course of study.

In pilot study (mild diabetics) the **Table 2** values show the effect of treatment of various doses of EECC at a dose of 100 mg/kg and 250mg/kg respectively on plasma glucose levels. Blood glucose level was increased significantly to 170.20 ± 6.22 and 215.8 ± 7.50 at 14th and 28th day of treatment respectively,

in the diabetic animals as compared to normal animals.

In the EECC treated groups (both doses), significant anti-hyperglycemic ($p < 0.001$) effect was evident from the 2nd week onwards, the decrease in blood sugar was maximum on completion of the 4th week in the group receiving 100 mg/kg and 250mg/kg of *C. chayamansa* respectively, whereas in group III treated animals receiving Glipizide at a dose of 10mg/kg also restored the blood sugar level near to normal range.

Effect of EECC on Glycogen Content

Glycogen content of liver tissue was estimated on the 28th day in non-diabetic control, diabetic control drug, treated group and positive control group as shown in **Table 3**. In diabetic control rat liver glycogen content decreased significantly by 79.36 % as compared to non-diabetic control. Treatment with Glipizide, EECC at two doses (100mg/kg and 250mg/kg) led to 73.39 %, 63.75 % and 67.33% increase in liver glycogen content in comparison to diabetic control.

Effect of EECC on Hepatic Enzymes

To establish diabetic, plasma glucose was determined 72hr after alloxan administration. Only those rats with over 180 mg% were included in the study. On the 28th day, hepatic enzymes Hexokinase, Glucokinase and

substrate Glucose-6-phosphate were estimated in saline control (group I), diabetic control (group II) and treatment controls (groups III, IV, V).

The result has been compiled in **Table 4**. As compared to non-diabetic control values, mean level of enzymes Hexokinase, Glucokinase and substrate Glucose-6-phosphate values decreased in diabetic control. The respective percentage decrease was 56.19%, 79.96% and 67.69% in diabetic control. Treatment with EECC at two doses (100mg/kg and 250mg/kg) for 28 days led to rise in percentage of these parameter by 22.03% and 56.03%, 45.21% and 34.28%, 67.78% and 47.5% respectively ($P < 0.001$) as compared to diabetic control. Also, treatment with Glipizide 10mg/kg for 28 days led to rise in percentage of these parameters by 27.55%, 65.39% and 58.0% respectively ($P < 0.001$) as compared to diabetic control.

Effect of EECC on Hematological Parameters

Table 5 values show the hematological parameters of group I to V treated animals. At the end of 28 days of the study period, no statistically significant differences were seen in the mean WBC and RBC counts, HB and Platelet values as compared to the non-diabetic animals.

Histopathological Study

In histopathological study, the **Fig-1** showed normal acini and normal cellular population in the islets of langerhans in pancreas of non-diabetic rats (group-I). **Fig- 2** showed extensive damage and reduced number of islets of langerhans in pancreas of diabetic rats (group-II). **Fig- 3** showed restoration of normal cellular population size of islets with hyperplasia by Glipizide (group-III). **Fig-4 and Fig-5** showed partial restoration of normal cellular population and enlarged size of β -cells with hyperplasia in EECC treated groups (group IV & group V).

DISCUSSION

Currently available drug regimens for the management of diabetes mellitus have certain drawbacks²² and further, there is a need for safer and more effective antidiabetic drugs. This study was therefore undertaken to assess anti-hyperglycemic property of *C. chayamansa* which have been reported in Ayurvedic system of medicine.

In the current study, diabetes mellitus was induced by alloxan monohydrate at a dose of 150 mg/kg i.p. Alloxan causes enormous

reduction in insulin release through the destruction of β -cells of the islets of langerhans. The mechanism of alloxan action was completely described elsewhere.^{23,24} In our study we have observed a significant increase in plasma glucose level in alloxan induced diabetic rats, whereas treatment with Glipizide (10mg/kg), Ethanolic extract of *C.chayamansa* at two different doses (100mg/kg and 250mg/kg) showed significant antihyperglycemic activity in mild hyperglycemia. In mild diabetes, the maximum percent reduction in glucose level was seen in groups receiving EECC 250mg/kg per day. This could be due to potentiation of insulin effect of plasma by increasing their pancreatic secretion of insulin from existing β -cells of islets of langerhans or its release from bound insulin. The significant and consistent antidiabetic effect of EECC in alloxan-induced diabetic rats is also due to enhanced glucose utilization by peripheral tissues. The above finding is correlated with an earlier study, which reported that the water extract of dried fruits of *Terminalia chebula*²⁵ improves glucose tolerance and bring down fasting blood glucose in diabetic rats.

The body weights were decreased in alloxan-induced diabetic rats. The administration of EECC at two doses shows increased body weight in alloxan-induced diabetic rats. The ability of EECC to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

As reported earlier²⁶ in the current study also the liver glycogen content was reduced significantly in diabetic control as compared to non-diabetic control. Treatment with EECC at two doses prevented this alteration in glycogen content of liver tissue, but could not normalize the content of glycogen of the non-diabetic control. This prevention or depletion of glycogen in liver is possibly due to either stimulation of insulin release from β -cells²⁷ or due to the insulinomimetic activity of some components of the plants resulting in direct peripheral glucose uptake.

Decreased enzymatic activity of Hexokinase, Glucokinase and substrate glucose-6-phosphate has been reported in diabetic animals resulting in depletion of liver and muscle glycogen.²⁸ The present study also had similar results. Treatment with EECC significantly increased the hexokinase, Glucokinase activity and glucose-6-phosphate level in the liver, indicating an overall increase in glucose influx thus *C. chayamansa* seems to have an overall effect of increase in glucose utilization. Studies also assess this plant extract showed no adverse effect on

hematological parameters including WBC, RBC counts, Hb and Platelets. Thus this plant extract can be presumed to be free from toxicological effects.

Histopathological studies revealed that EECC and Glipizide significantly improved the histological architecture of the islets of langerhans. The groups treated with EECC (100mg/kg and 250mg/kg) and Glipizide (10mg/kg) showed greater persistence of islets of langerhans and lesser degree of necrotic changes as compared to the untreated alloxan-induced diabetic rats.

CONCLUSION

In conclusion, the ethanolic extract of *C.chayamansa* at high dose (250mg/kg) and low dose (100mg/kg) exhibited significant anti-hyperglycemic activity in normal and alloxan-diabetic rats. This extract also showed improvement in the parameters like body weight, liver glycogen content and carbohydrate metabolizing enzymes as well as regeneration of β -cells of pancreas and so might be of value in diabetes treatment. Studies are in progress in our laboratory to elucidate the active components responsible for the blood sugar lowering effect in experimental diabetes.

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