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Original Article

Insulinomimetic Effect of Citrullus Colocynthis Roots in STZ Challenged Rat Model

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Abstract

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from either defect in insulin secretion, insulin action, or both. Postprandial hyperglycemia is a prime characteristic of diabetes mellitus and has been a focus in the therapy for diabetes. One of the therapeutic approaches which involve decreasing hyperglycemia aims at lowering blood glucose by decreasing insulin resistance, raising insulin sensitivity at the tissues and inhibiting the carbohydrate absorbance in the intestine. Plants contain different chemical constituents with potential for insulin mimetic action, decrease in insulin resistance and of α-amylase inhibitory activity may be used as therapeutic agents in the treatment of diabetes mellitus. The present study investigates the hypoglycemic activity and insulinomimetic action of aqueous and ethanolic extracts of Citrullus colocynthis by invitro α-amylase enzyme inhibition and by histopathological studies in streptozotocin (STZ) induced diabetic rats. Male Wistar rats weighing about 180- 250g were taken and divided into fifteen groups. Diabetes was induced by giving streptozotocin (STZ) (30-50mg/kg) intraperitoneally. Rats that showed blood glucose levels > 250mg/dl were selected for the study. Metformin (45mg/kg) was given as standard oral hypoglycemic agent. The aqueous and ethanolic extracts of Citrullus colocynthis (AECC and EECC) at 100mg/kg, 200mg/kg and 300mg/kg were administered to the normal and diabetic rats. The invitro α – amylase inhibitory activity was done by spectrophotometric method. Blood glucose levels were measured by glucose oxidase method. Estimation of glycogen in the lever was carried out with anthrone method. Liver and pancreas were isolated and subjected to histopathological studies. Serum insulin was monitored through chemiluminescence assay. Oral administration of both extracts showed significant inhibition of α-amylase enzyme in-vitro and decrease in blood glucose also. Glycogen and Insulin levels too were found to increase in extract treated groups which attributed for its insulinomimetic activity.
The findings revealed that Citrullus colocynthis possess a very strong anti-hyperglycemic potential justifying the use of the drug for the treatment of diabetes mellitus.

Keywords: Streptozotocin, Diabetes, Alpha-amylase, Insulin, Citrullus colocynthis, Metformin.

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1. Introduction

Diabetes mellitus is a disorder characterized by hyperglycemia resulting deficiency of insulin secretion by pancreas, ineffectiveness of produced insulin, or both [1]. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population [2].

Diabetes mellitus is classified into four groups: 1) type 1 diabetes mellitus; 2) type 2 diabetes mellitus; 3) diabetes mellitus due to other specific mechanisms or diseases; and 4) gestational diabetes mellitus (GDM) [3]. Diet, exercise, and weight loss are non-pharmacological therapeutic methods to treat diabetes. Pharmacological therapy includes drugs such as tolbutamide, metformin, and glyburide [4]. Gene therapy is another method to treat diabetes [5]. The final way to treat diabetes is through the use of medicinal herbs [6]. Increased use of alternative medicines has recently attracted the attention of many researchers around the world [6]. Before the discovery of insulin and anti-diabetic drugs, patients were treated with herbs and traditional treatments [7]. Some of these herbs were recommended by physicians or taken by patients as supplements [8].

Herbal medicines are the oldest remedies known to mankind [9]. Anti diabetic activity is not attained by lowering blood glucose levels alone but there should be supportive mechanisms for effective treatment. Supportive mechanisms suggested would increase in the serum insulin levels, insulin sensitizing effect and decrease in insulin resistance. One therapeutic approach which may prove to be beneficial for treatment of diabetes is to decrease the post-prandial hyperglycemia. This can be achieved by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes in the digestive tract. The ∝– glucosidase enzymes such as α-amylase are responsible for the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a marked decrease in the rate of glucose absorption thereby blunting the post prandial plasma glucose rise [10]. Examples of such inhibitors which find application in the clinical practice for management of diabetes are acarbose, miglitol and voglibose [11]. However, these drugs are known to be associated with various gastrointestinal side effects such as abdominal pain, flatulence, and diarrhea in the patients [12][13]. Therefore, it is the need of time to identify and explore the

amylase inhibitors from natural sources having fewer side effects. These plants may be useful as a source of new compounds for the development of oral hypoglycemic drugs or as a simple dietary supplement therapy [14]. Herbal ingredients such as terpenoids, alkaloids, flavonoids, and phenolic had been reported for their anti-diabetic properties [15]. Therefore, the present study evaluated the hypoglycemic activity, insulomimemtic, and invitro α-amylase inhibition properties of *Citrullus colocynthis* root in streptozotocin induced diabetic rats.

Citrullus colocynthis belonging to the family of Cucurbitacae is one of the ancient plants in the world, which is used in the traditional system for various ailments. It is a tree found in Mediterranean Basin and Asia, is distributed among the west coast of Northern Africa eastwards through Sahara until India. It is commonly known as bitter apple, bitter cucumber. The whole plant has many medicinal properties as anti-inflammatory, anti-candidial, and bacterial,as well as anti-oxidant and free radical scavenging activity etc.

The plant subjected to the current research work had been used traditionally as anti-diabetic, the fruit is scientifically proved as antidiabetic; therefore, it was thought interested to evaluate the antidiabetic profile of the selected plant part (root) in stretozotocin challenged rats which has not yet been scientifically undertaken.

2. Materials and Methods

2.1. Plant Material

The plant material was collected from Tirupati district, Andhra Pradesh, India. Dr. K.Madhava Chetty, Professor in the Department of Botany Sri Venkateshwara University, Tirupati, confirmed the botanical identity. A voucher specimen has been deposited in the department of botany, Shadan college of Pharmacy, Hyderabad (1487).

2.2. *Preparation of Extract*

The shade dried material (root) of *Citrullus colocynthis* was crushed, powdered and exhaustively defatted by petroleum ether (60°C-80°C) and then successively extracted with 90% ethanol using soxhalet apparatus by hot percolation method and with water by maceration method. This extraction process was repeated for three times. All the extracts were filtered, pooled, and concentrated under

Table 1. List of phytochemical constituents present in both the extracts.

Chemical Constituents	**Test Performed**		***C.colocynthis***
Alkaloids	Dragendroff's test	+	+
Carbohydrates	Molisch test	+	+
Glycosides	Liebermann Burchard test	-	+
Flavinoids	Shinoda test	+	+
Saponins	Froth test	+	+

reduced pressure using Rota vapor (Buchi, USA). The percentage yield of the dried extract was 15% of the initial raw material.

2.3. Preliminary Phytochemical Screening

Both the extracts were subjected to phytochemical analysis for the presence of various phytochemical constituents like carbohydrates, resins, saponins, steroids, anthraquinones, alkaloids, *etc*. (Table 1) [16].

2.4. Animals

Wistar male albino rats weighing 180-200gms were used for the study. The animals were procured from National Institute of Nutrition, Hyderabad, and housed into group of six animals per cage. Animal Protocol was approved by IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for Purpose of Control and Supervision on Experiments on Animals) through its reference no: IAEC/SVCP/2011/008, dated: 26/7/11. Animals were kept for acclimatization at an ambient temperature of 24°C with a relative humidity 45–55%, 12:12 hours dark and light cycle. The animals had free access to food (standard Cho pellet diet) and water *ad libitum.*

2.5. Drugs

Streptozotocin was procured from Sisco Research laboratories pvt. Ltd. Mumbai-93, India. Batch No: (T-835796), Metformin was a gift sample from Ranbaxy pvt. Ltd., Hyderabad.

2.6. Acute Oral Toxicity Study

The acute toxicity studies for the root extracts of *Citrullus colocynthis* (aqueous and ethanolic) were done according to the OECD guidelines No. 423. The extracts of the plant i.e, EECC and AECC did not cause any adverse effects and mortality up to a dose level of 2000mg/kg body weight. All the test animals survived for 14 days without showing any toxic symptoms. The LD_{50} was therefore greater than 2000mg/kg in rats. Hence the pharmacological studies of these extracts were carried out with three graded doses i.e, with the lowest dose of 100, 200 and a maximum dose of 300mg/kg body weight for *Citrullus colocynthis* were selected for the present study.

2.7. Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection of a freshly prepared Streptozotocin (STZ) solution (Dose: 30-50mg/kg) in citrate buffer 0.1 M, pH 4.5 to overnight fasted rats. Diabetes was identified by polydipsia, polyuria and by measuring blood glucose levels 48 h after injection of STZ. Animals that did not develop more than 250 mg/100 ml of blood glucose levels were rejected [17].

2.8. Experimental Design

The animals were divided into fifteen groups of 6 animals each.

Group I: Normal untreated rats (Control)

Group II: Diabetic control (STZ)

Group III: Diabetic rats given with metformin (50 mg/kg) (o)

Group IV: Normal rats given with aqueous root extract (AECC) (100mg/kg) (o)

Group V: Normal rats given with aqueous root extract (AECC) (200mg/kg) (o)

Group VI: Normal rats given with aqueous root extract (AECC) (300mg/kg) (o)

Group VII: Normal rats given with ethanolic root extract (EECC) (100mg/kg) (o)

Group VIII: Normal rats given with ethanolic root extract (EECC) (200mg/kg) (o)

Group IX: Normal rats given with ethanolic root extract (EECC) (300mg/kg) (o)

Group X: Diabetic rats given with aqueous root extract (AECC) (100mg/kg) (o)

Group XI: Diabetic rats given with aqueous root extract (AECC) (200mg/kg) (o)

Group XII: Diabetic rats given with aqueous root extract (AECC) (300mg/kg) (o)

Group XIII: Diabetic rats given with ethanolic root extract (EECC) (100mg/kg) (o)

Group XIV: Diabetic rats given with ethanolic root extract (EECC) (200mg/kg) (o)

Group XV: Diabetic rats given with ethanolic root extract (EECC) (300mg/kg) (o)

The animals of group I were given with 0.9% saline and served as control and group II served as diabetic control, group III served as standard, groups IV, V, VI, VII, VIII, IX are normal rats treated with aqueous root extract of *Citrullus colocynthis* (AECC) and ethanolic root extract of *Citrullus colocynthis* (EECC) at the doses of 100mg/kg, 200mg/kg, 300mg/kg respectively. Groups X, XI, XII are diabetic rats treated with aqueous root extract of *Citrullus colocynthis* (AECC), groups XIII, XIV, XV are diabetic rats treated with ethanolic root extract of *Citrullus colocynthis* (EECC) at the doses of 100 mg/kg, 200 mg/kg, 300 mg/kg respectively for a period of 15 days.

2.9. Oral Glucose Tolerance Test

Serum glucose was estimated on the 11th day at 0, 1, 2, 4, 6, and 8 hrs after administration of test drugs, by Accu-chek Glucometer. At the end of the experimental period, the blood was withdrawn by retro orbital puncture and centrifuged. The fasting serum insulin was estimated by Maglumi auto

analyzer (MAGLUMI 1000), Fully auto Chemiluminescence Immunoassay (CLIA) analyzer and Alpha-amylase activity was estimated by Ranbaxy assay method in auto analyzer (HITACHI 917) at 405nm, at Jeeva Life Sciences Lab, Uppal, Hyderabad.

2.10. Hypoglycemic Activity

Diabetes was induced by an intraperitoneal injection of freshly prepared STZ (30-50mg/kg) in rats. 15 groups of six animals in each group were used. The OGTT was performed in overnight fasted (18hrs) animals. After overnight fasting a 0 minutes blood sample (0.2ml) was taken from each rat in the different groups. Test drugs were administered orally in 0.25% carboxymethylcellulose and standard drug metformin was also administered orally in diabetic rats. Glucose solution (2g/kg) was administered orally 30 minutes after the administration of extracts. Blood samples were taken at 0 minutes, 30 minutes, 60 minutes, 90 minutes and 120 minutes after glucose administration. All the blood samples were collected with potassium and sodium fluoride solution for the estimation of blood glucose [18].

2.11. Estimation of Liver Glycogen

Liver sample was dissected out from 16 hrs fasted rat and digested in hot 30% KOH. Liver glycogen was precipitated with alcohol and the precipitate was dissolved in 10% TCA. The sample was processed for centrifugation to sediment the proteins, after centrifugation supernatant was precipitated once again with alcohol. After suitable dilution of the sediment with water, estimation of liver glycogen was carried out with anthrone reagent [19].

2.12. Histopathological Studies

For histopathological study, on the 16th day animals from all groups were anaesthetized with mild ether anesthesia and dissected. The whole pancreas and liver were excised out from each animal and washed with ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formalin fixative solution for histological studies. The samples were sent for evaluation to diagnostic lab (Jeeva Labs, Uppal, Hyderabad). After fixation using Bouin's solution tissues were embedded in paraffin, solid sections were cut at 5 µm using standard microtome and the sections were stained with haematoxylin and eosin [20]. The slides so prepared were than examined by pathologist and the pictures were clicked with the help of a binocular microscope fixed with a camera.

2.13. Statistical Analysis

The results of the estimation were reported as Mean ± SEM. Student's t-test was applied when two groups amongst were compared. The values were considered significant when $p < 0.05$, $p < 0.001$, $p < 0.0001$. Statistical calculations were done using Graph Pad Prism.

Table 2. Effect of *Citrullus colocynthis* root extract on Oral Glucose Tolerance Test in normal and diabetic rats.

Groups	Treatment	0 mins	30 mins	60 mins	90 mins	120 mins
I	Normal	81.0 ±0.763	102.6 ±2.036	96.51 ±1.46	91.6 ±1.46	80.71 ±1.59
II	Diabetic Control	[a]296.83 ±4.43	[a]273.40 ±2.98	[a]296.7 ±3.35	[a]294.35 ±2.04	[a]270.08 ±1.54
III	Metformin+D	[a]283.91 ±2.36	[a]262.63 ±2.30	[a]245.34 ±3.23	[a]171.76 ±2.53	[a]129.41 ±2.72
IV	N+AECC 100mg/ml	79.10 ±0.85	84.86 ±1.82	83.23 ±1.00	80.67 ±0.69	79.51 ±1.69
V	N+AECC 200mg/ml	89.80 ±1.32	81.64 ±1.408	82.68 ±1.24	79.36 ±2.030	77.94 ±1.308
VI	N+AECC 300mg/ml	85.96 ±1.52	83.41 ±1.505	81.67 ±1.77	80.74 ±0.683	77.09 ±1.501
VII	N+EECC 100mg/ml	81.15 ±2.57	79.97 ±0.871	78.26 ±1.801	77.94 ±1.221	78.33 ±1.51
VIII	N+EECC 200mg/ml	82.98 ±0.846	80.54 ±0.55	82.68 ±0.68	78.44 ±0.933	71.47 ±0.801
IX	N+EECC 300mg/ml	82.03 ±0.424	81.70 ±0.368	77.87 ±1.55	77.98 ±0.39	74.56 ±0.62
X	D+AECC 100mg/ml	[NS]297.54 ±4.035	[NS]272.83 ±2.47	[a]257.15 ±2.72	[a]179.10 ±1.93	[a]160.82 ±1.720
XI	D+AECC 200mg/ml	[NS]288.07 ±1.48	[NS]269.42 ±2.205	[a]188.74 ±2.09	[a]172.44 ±2.08	[a]149.26 ±1.640
XII	D+AECC 300mg/ml	[NS]293.47 ±2.36	[NS]271.76 ±1.83	[a]184.37 ±1.23	[a]159.08 ±0.936	[a]139.76 ±0.841
XIII	D+EECC 100mg/ml	[NS]290.06 ±2.72	[NS]276.59 ±1.168	[b]267.43 ±1.55	[b]182.62 ±2.05	[b]167.76 ±1.109
XIV	D+EECC 200mg/ml	[NS]283.16 ±0.95	[NS]277.73 ±3.36	[b]199.78 ±2.69	[b]177.66 ±2.38	[b]157.78 ±1.437
XV	D+EECC 300mg/ml	[NS]287.08 ±2.60	[NS]270.69 ±4.83	[b]181.18 ±1.54	[b]161.27 ±1.92	[b]140.82 ±1.33

Values were reported as Mean ± SEM. Each group contain six animals. Diabetic control compared with Normal [a]$p<0.0001$; Diabetic+Metformin compared to diabetic control group, [a]$p<0.0001$; Diabetic+AECC and Diabetic+EECC compared to diabetic control, [a]$p<0.0001$, [b]$p<0.001$.

3. Results and Discussion

The major therapy option in diabetes mellitus is lifestyle management besides exercise, weight control and medical nutrition therapy [21]. Patients with type I diabetes always require treatment with exogenous insulin. For Type II diabetes, treatment options begin with diet modification and lifestyle

interventions but often oral hypoglycemic agents or insulin or both are required as the disease progresses [22]. Although insulin has been designated an essential drug by WHO, it is not yet universally accessible to all those who need it in the majority of countries of the world. Continuous access to insulin remains a major problem in many developing countries [23]. Presently, there is a growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) and insulin for the treatment of diabetes mellitus [24].

Insulin plays a major biochemical role in stimulating the uptake of glucose by different cells of the body for the production of energy. As hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus, the role of insulin resistance and its sequel is gaining prominence. It is vital for understanding the role of insulin across a wide range of physiological processes and the influences on its synthesis and secretion. Numerous mechanisms of action have been proposed for plant extracts. Some hypotheses relate to their effects on the activity of pancreatic β-cells or increase of insulin sensitivity or the insulin like activity of plant extracts.

Several animal experimental models have been in use to evaluate hypoglycemic activity such as alloxan monohydrate, streptozotocin (STZ), etc. STZ is a nitrosourea compound produced by Streptomyces achromogens, which induces DNA strand breakage in β – cells causing diabetes mellitus. There is no spontaneous revision with STZ and it is also observed that 90% of rats is becoming diabetic [25]. On comparison of diabetic rats with the normal rats, there was an increase in blood glucose levels significantly. It showed that STZ produced the diabetogenic response in Wistar strain rats [26]. In the present study, STZ significantly induced hyperglycemia.

3.1. Phytochemical Screening

The list of phytochemical constituents present in both the aqueous and ethanolic extracts of *Citrullus colocynthis* are listed in tabl 1.

Preliminary phytochemical screening showed the presence of alkaloids, saponins, flavonoids, tannins, carbohydrates, glycosides, proteins and amino acids. Saponins are glycosides of triterpenes, steroids or alkaloids. Previous studies have demonstrated the hypoglycemic activity of triterpenoid glycosides [27, 28], hence the hypoglycemic activity exhibited by *Citrullus colocynthis* may be due to the prescence of saponins.

3.2. *Acute Oral Toxicity Study*

The extracts did not produce any signs of toxicity when given in doses up to 2000 mg/kg by oral route. Hence, for further studies 1/10th of the maximum tolerated dose, 200mg/kg dose of the extracts was selected for the study.

3.3. Oral Glucose Tolerance Test (OGTT)

Administration of streptozotocin (30-50 mg/kg, intra peritoneal) led to elevation of

fasting blood glucose levels, which was maintained over a period of 3 weeks. OGTT was performed in all the rats from group I to group XV (n=6). Table 1 shows the results of oral glucose tolerance test. All the drug treated (AECC and EECC) groups at 100mg/kg, 200mg/kg and 300mg/kg doses in diabetic rats showed a significant reduction in blood glucose values at 60, 90 and 120 minutes ($p<0.0001$) respectively when compared to the diabetic control group. Results of OGTT of *C. colocynthis* root extracts are shown in table 2.

3.4. α-Amylase Inhibitory Activity

Inhibition of the mammalian alpha amylase enzyme in the intestine would delay the degradation of starch and oligosaccharides to monosaccharaides before they can be absorbed. This would decrease the absorption of glucose and consequently reduce postprandial blood glucose levels [29].

These α- amylase inhibitors are also called as starch blockers as they prevent or slow the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugars [30].

In the present study α- amylase inhibitory activity of the roots of *Citrullus colocynthis* was investigated. The inhibitory effect of aqueous and ethanolic extracts was analysed. Both the extracts displayed a significant inhibition of α- amylase enzyme in a concentration dependent manner. Table 3 shows the percentage inhibition of AECC and EECC of *Citrullus colocynthis* at different concentrations. The percentage inhibition of α-

Table 3. Alpha amylase inhibitory activity of *Citrullus colocynthis* root extracts.

Groups	**Concentrations ug/ml**	**%inhibition**	**IC_{50}**
Acarbose	10	5.39±0.02	
	20	12.62±1.32	
	40	26.31±2.62	35ug/ml
	60	44.02±0.42	
	80	59.30±1.31	
	100	75.06±2.63	
AECC	10	12.22±0.004	
	20	22.30±0.005	
	40	41.62±1.36	
	60	62.31±2.42	45ug/ml
	80	73.42±1.62	
	100	78.60±0.009	
EECC	10	8.10±1.32	
	20	15.62±1.81	
	40	33.32±3.62	
	60	50.45±2.71	48ug/ml
	80	66.08±0.002	
	100	72.93±0.12	

amylase by the extracts of *C. colocynthis* was studied in a concentration range of 10-100ug/ml. The IC_{50} of AECC and EECC were 45ug/ml and 48ug/ml respectively. The activity of the extracts was compared with the enzyme control acarbose which exhibited an IC_{50} 35ug/ml. This indicates inhibition of amylase activity and utilization of starch substrate. Thus, this study confirms that the roots of *C. colocynthis* can mitigate postprandial hyperglycemia.

3.5. Anti Hyperglycemic Activity

On administration of different doses of *Citrullus colocynthis* daily at 100mg/kg, 200mg/kg, 300mg/kg for a period of 10 days

Table 4. Blood glucose levels in normal, aqueous and ethanolic extracts of Citrullus colocynthis treated experimental groups.

Groups	Treatment	0hr	1hr	2hr	4hr	6hr	8hr
I	Normal control	95.3 ±2.3	86.31 ±2.11	97.62 ±3.11	84.31 ±0.02	85.12 ±1.61	82.02 ±1.32
II	Diabetic Control	[a]250 ±1.60	[a] 267.8 ±2.4	[a]291.76 ±3.01	[a] 300 ±2.10	[a] 301 ±2.02	[a] 305 ±1.60
III	D+Metformin	[a]291.7 ±2.08	[a]261 ±0.32	[a]155 ±3.62	[a]140 ±2.16	[a]135 ±1.36	[a]127 ±1.02
IV	N+AECC(100mg/kg)	105.34 ±1.23	93.03 ±1.62	84.58 ±2.30	80.92 ±2.01	78.51 ±1.60	77.09 ±1.27
V	N+AECC(200mg/kg)	100 ±2.03	96 ±2.32	90 ±3.10	87 ±3.14	84 ±1.02	80 ±1.36
VI	N+AECC(300mg/kg)	101.09 ±0.06	95.48 ±0.07	87.41 ±2.16	81.60 ±1.23	77.91 ±0.08	74.08 ±3.06
VII	N+EECC(100mg/kg)	98 ±1.32	85 ±1.26	80 ±1.67	77 ±0.07	75 ±0.06	73 ±2.08
VIII	N+EECC(200mg/kg)	99.87 ±1.32	85.09 1.26±	80.54 ±1.67	77.83 ±0.07	75.04 ±0.06	80.13 ±2.12
IX	N+EECC(300mg/kg)	100.08 ±0.03	91.96 ±0.06	89.93 ±3.01	80.25 ±2.54	78.37 ±1.47	75.61 ±1.06
X	D+AECC(100mg/kg)	292.06 ±2.62	[a]286.87 ±1.31	[a]270.82 ±3.41	[a]260.51 ±2.02	[a]257.09 ±1.31	[a]245.70 ±2.91
XI	D+AECC(200mg/kg)	300.09 ±3.21	[a]285.65 ±0.62	[a]208.73 ±2.62	[a]172.42 ±3.12	[a]155.03 ±2.08	[a]141.92 ±2.03
XII	D+AECC(300mg/kg)	290.94 ±1.31	[a]275.50 ±0.82	[a]197.69 ±1.08	[a]160.05 ±2.96	[a]133.93 ±1.86	[a]130.76 ±1.08
XIII	D+EECC(100mg/kg)	298.87 ±0.62	[a]284.21 ±1.31	[a]260.98 ±1.02	[a]251.75 ±2.08	[a]248.87 ±2.07	[a]232.16 ±3.02
XIV	D+EECC(200mg/kg)	295.30 ±0.82	[a]280.64 ±1.61	[a]200.05 ±1.08	[a]182.65 ±3.61	[a]173.35 ±2.31	[a]152.23 ±0.08
XV	D+EECC(300mg/kg)	293 ±0.34	[a]273.08 ±2.32	[a]188.52 ±2.03	[a]153.70 ±2.60	[a]137.76 ±1.82	[a]129.40 ±1.62

The values are expressed as mean ± S.D. Each group consists of six animals, values were reported as Mean±SEM. Diabetic control compared with Normal [a]p<0.0001; Diabetic+Metformin compared to diabetic control group, [a]p<0.0001; Diabetic+AECC and Diabetic+EECC compared to diabetic control, [a]p<0.0001, [b]p<0.001.

and on the 11th day the results obtained were evaluated. At the same time hypoglcemic effectof AECC and EECC were also studied in normal rats. Evaluation of hypoglycemic activity of aqueous and ethanolic root extracts of C.colocynthis in group IV to IX was compared to normal rats and observed that there is no significant decrease in blood glucose levels and was also observed that there was no difference in glucose levels in all groups from group IV to IX when copared to group I, i.e. the values were almost to be more or less same. Hypoglycemic activity of different extracts of *C.colocynthis* roots was evaluated, the extract treated diabetic groups were compared to diabetic control group. When AECC (100, 200, 300mg/kg) extract treated group was compared diabetic group there was significant reduction ($p<0.0001$) in glucose levels at 1st ,2nd ,4th ,6th ,8th hrs. Similarly, EECC (100, 200, 300mg/kg) extract treated group was compared to diabetic control; there was a reduction in blood glucose levels significantly ($p<0.0001$). The extract treated diabetic groups were also compared to standard drug treated group.The glucose levels in extract treated group AECC and EECC at 300mg/kg were 130±1.08,129±1.62 respectively found very near to the metformin treated standard group. Daily treatment with various concentration of *C.colocynthis* root extract led to a decrease in blood glucose levels. The serum glucose levels of the *C.colocynthis* root extract treated groups were significantly reduced ($p<0.0001$) especially from the 1hrs onward when compared to the

Table 5. Effect of *Citrullus colocynthis* root extracts on insulin levels in normal and diabetic extract treated groups.

Groups	Treatment	Dose (mg/kg)	Insulin levels
I	Normal	---	7.82±0.82
II	Diabetic Control	----	[a]3.50±0.21
III	Metformin+D	50mg/kg	[a]10.32±1.02
IV	N+AECC	100 mg/kg	[NS]8.02±1.06
V	N+AECC	200 mg/kg	[NS]8.50±3.02
VI	N+AECC	300mg/kg	[NS]8.00±2.10
VII	N+EECC	100 mg/kg	[NS]7.35±1.34
VIII	N+EECC	200mg/kg	[NS]7.62±2.62
IX	N+EECC	300 mg/kg	[NS]7.92±0.83
X	D+AECC	100 mg/kg	[b]7.15±0.73
XI	D+AECC	200 mg/kg	[a]7.80±0.50
XII	D+AECC	300 mg/kg	[b]8.00±1.23
XIII	D+EECC	100 mg/kg	[a]7.86±0.28
XIV	D+EECC	200 mg/kg	[a]8.00±0.30
XV	D+EECC	300 mg/kg	[b]8.31±1.21

Values were reported as Mean±SEM. Diabetic control compared with Normal [a]$p<0.0001$; Diabetic+Metformin compared to diabetic control group, [a]$p<0.0001$; Diabetic+AECC and Diabetic+EECC compared to diabetic control, [a]$p<0.0001$, [b]$p<0.001$, normal group treated with AECC and EECC root exracts are compared to normal control, NS-non significant.

diabetic control group. Both the herbal extracts AECC and EECC treated groups were proven that they lowered blood glucose levels significantly. The glucose lowering effect of C.colocynthis can be seen in table 4.

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type I diabetes, the cause is an absolute deficiency of insulin secretion. In the other much more prevalent category, type II

Liver **Pancreas**

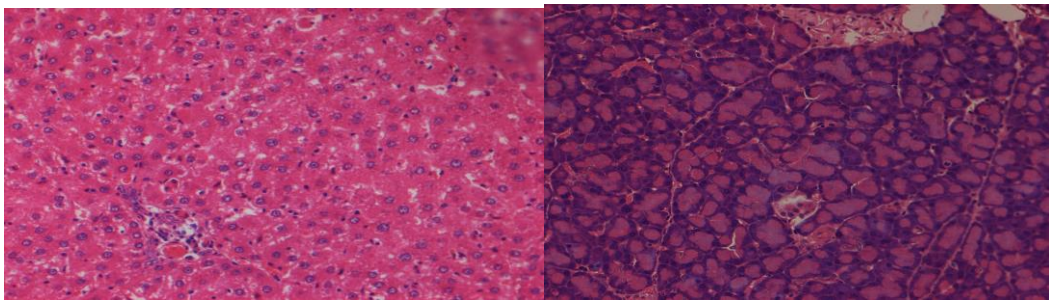

Figure 1a. Effect of *Citrullus colocynthis* root extracts - AECC &EECC in normal rats on liver and pancreas.

Hepatocytes appeared normal in entire liver. Acinar cells population appeared normal in non glandular region of pancreas.

Liver **Pancreas**

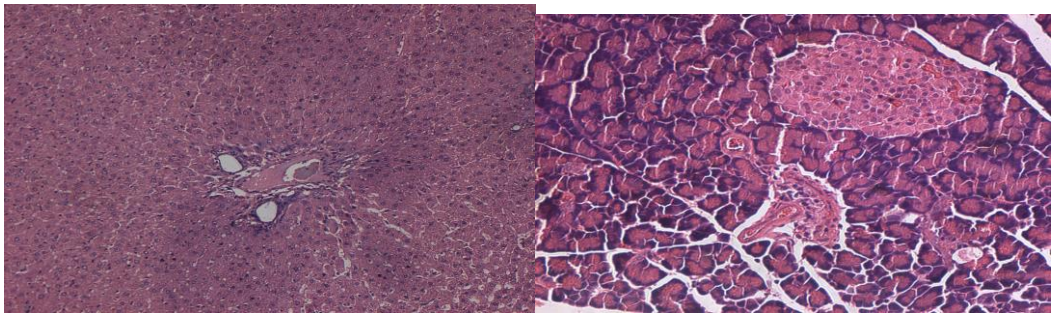

Figure 1b. Effect of standard drug –Metformin in diabetic rats on liver and pancreas.

Portal region of liver appeared normal. Beta cells in islets of glandular pancreas appeared normal.

Liver **Pancreas**

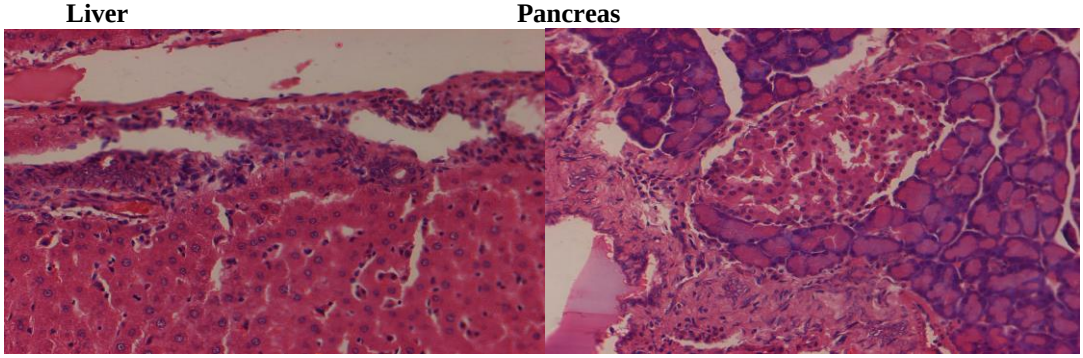

Figure 1c. Effect of STZ in STZ- induced diabetic rats on liver and pancreas.

- Moderate to severe hyperplasia of biliary epithelial cells noticed in the portal region of liver. Moderate degeneration of islets cells in glandular pancreas.

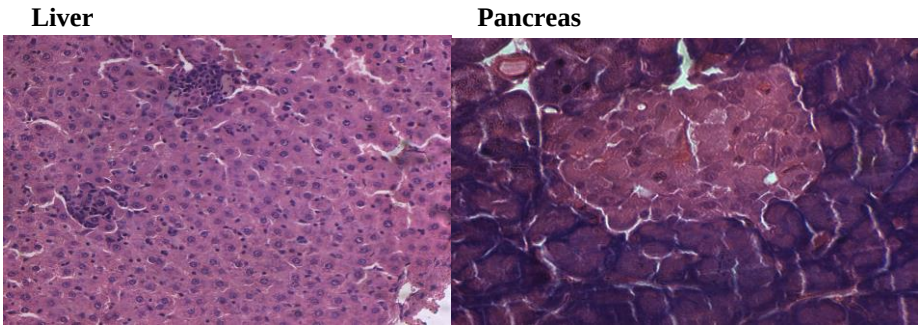

Figure 1d. Effect of Aqueous extract of Citrullus colocynthis (AECC) in diabetic rats on liver and pancreas.

Multiple foci of inflammation along with infiltration of lymphocytes noticed in centrilobular region of liver – arrow. Most of the beta cells appeared normal and no degenerative changes noticed

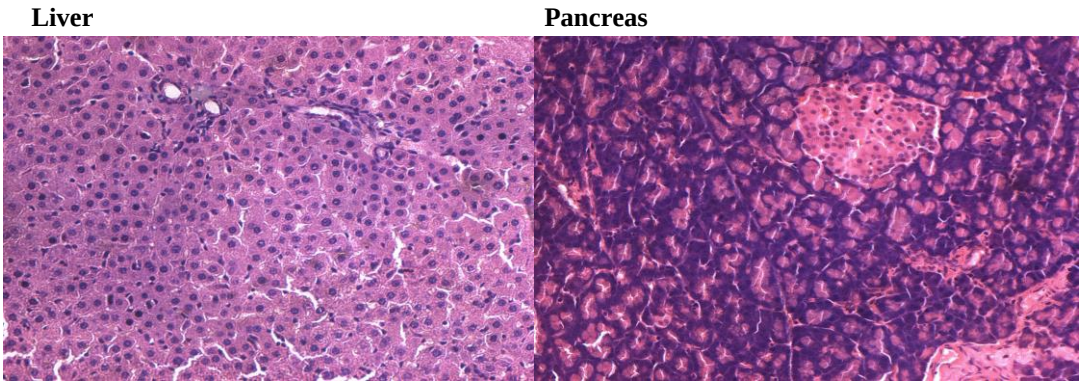

Figure 1e. Effect of Ethanolic extract of *Citrullus colocynthis* (EECC) in diabetic rats on liver and pancreas.

Completely the hepatocytes appeared normal. Glandular pancreas is showing active proliferation stage of beta cells – arrow

diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretary response [31].

Insulin levels were also measured in all the groups. In the diabetic control the insulin levels were 3.50±0.21 and in standard drug treated i.e. Metformin treated group the serum insulin levels were 10.32±1.02, where as in extract treated group AECC at the doses 200mg/kg, 300mg/kg the insulin levels shown a significant raise in 7.80±0.50 and 8.00±1.23 respectively. At the same doses EECC treated group also have shown significant raise in insulin levels 8.00 ±0.31 and 8.31±1, ($p<0.0001$) when compared to the diabetic control. Table 5 shows the effect of *C.colocynthis* on insulin levels in various groups.

Some plants' extracts are reported to exert hypoglycemic action by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin [32]. While others act through extra

Table 5. Effect of *Citrullus colocynthis* root extracts on insulin levels in normal and diabetic extract treated groups.

Groups	Treatment	Dose (mg/kg)	Insulin levels
I	Normal	---	7.82±0.82
II	Diabetic Control	----	[a]3.50±0.21
III	Metformin+D	50mg/kg	[a]10.32±1.02
IV	N+AECC	100 mg/kg	[NS]8.02±1.06
V	N+AECC	200 mg/kg	[NS]8.50±3.02
VI	N+AECC	300mg/kg	[NS]8.00±2.10
VII	N+EECC	100 mg/kg	[NS]7.35±1.34
VIII	N+EECC	200mg/kg	[NS]7.62±2.62
IX	N+EECC	300 mg/kg	[NS]7.92±0.83
X	D+AECC	100 mg/kg	[b]7.15±0.73
XI	D+AECC	200 mg/kg	[a]7.80±0.50
XII	D+AECC	300 mg/kg	[b]8.00±1.23
XIII	D+EECC	100 mg/kg	[a]7.86±0.28
XIV	D+EECC	200 mg/kg	[a]8.00±0.30
XV	D+EECC	300 mg/kg	[b]8.31±1.21

Values were reported as Mean±SEM. Diabetic control compared with Normal [a]p<0.0001; Diabetic+Metformin compared to diabetic control group, [a]p<0.0001; Diabetic+AECC and Diabetic+EECC compared to diabetic control, [a]p<0.0001, [b]p<0.001, normal group treated with AECC and EECC root exracts are compared to normal control, NS-non significant.

Table 6 Effect of aqeous and ethanolic root extracts of *Citrullus colocynthis* on liver homogenate –glycogen content.

Groups	**Treatment**	**Dose mg/kg**	**Glycogen content**
I	Normal		36.20±1.72
II	Diabetic control		[a]7.56±0.83
III	Metformin	50	[a]28.31±2.32
IV	N+AECC	100	[NS]37.37±0.52
V	N+AECC	200	[NS]38.27±0.32
VI	N+AECC	300	[NS]36±05±0.12
VII	N+EECC	100	[NS]37.00±1.32
VIII	N+EECC	200	[NS]37.60±0.72
IX	N+EECC	300	[NS]38.32±0.16
X	D+AECC	100	[NS]9.1±0.50
XI	D+AECC	200	[a]15.32±1.30
XII	D+AECC	300	[b]18.61±2.62
XIII	D+EECC	100	[NS]10.60±2.32
XIV	D+EECC	200	[a]17.42±1.60
X	D+EECC	300	[a]20.08±1.82

pancreatic mechanisms by inhibition of hepatic glucose production [33] or corrections of insulin resistance [34]. The extracts of *C. colocynthis* could have utilised one of the above mechanism in exerting its antidiabetic effect.

3.6. Histopathological Studies

The histopathological studies of liver and pancreas were done in normal, diabetic, and extract treated groups. The normal histological liver section shows the well arranged cells and clear central vein. Pancseas also depicted normal population of islets. In the diabetic group, it shows the complete destruction of

hepatocytes degeneration of central vein, fatty degeneration and also shows the damaged islets of pancreas. Histopathological changes are restored near to normal in the *C.colocynthis* treated diabetic groups. Figure1 (a, b, c, d, e) depicts histopathological changes in normal, diabetic control, standard group, AECC and EECC treated groups.

These studies evidently show that the adminstration of AECC and EECC has potential to increase the viablity of hepatocytes and pancreatic β islets and hence improve glycogen synthesis and increase the insulin secretion coresspondingly.

3.7. Liver Glycogen Estimation Results

Liver glycogen was estimated through the anthrone reagent. The importance of the liver in the regulation of carbohydrate metabolism is recognised by its ability to store carbohydrates in the form of glycogen (glycogenesis) and to release them in he form of glucose (glycogenolysis) when needed. The glycogen levels in diabetic control group were significantly low 7.56±0.83 when compared with that of normal control 36.20±1.72 ($p<0.001$). Following the administration of AECC and EECC, raised the liver glycogen levels to 15.32±1.30, 18.61±2.62 and 17.42±1.60, 20.08±1.82 respectively at a dose of 200, 300mg/kg significantly ($p<0.0001$) when compared to diabetic control groups which are shown in shown in table 6. Increase in the glycogen levels by *C.colocynthis* root extracts could therefore have been achieved by stimulation of insulin release.

In a study conducted by Sebbagh N *et al*. it is proved that *Citrullus colocynthis* enriched with oil has beneficial effects by restoring pancreatic beta cell mass in the STZ-induced diabetes rat model [35]. In another study by Abdel Hassun et al conducted shown that the aqueous extract of *Citrullus colocynthis* rind showed hypoglycemic and antihyperglycemic effects in normal and alloxan induced diabetic rats[36]. Another study also proved that the aqueous extract of Citrullus colocynthis increased plasma levels of alanine amino transferase (ALT), alkaline phosphate (ALP), asparate aminotransferase (AAT), gamma glutamyl transferase (GGT) and lactic dehydrogenase (LDH) [37]. It was also proved that the *Citrullus colocynthis* were evaluated for estimation of biochemical parameters like SGOT, SGPT, ALP in alloxan induced diabetic rats and found beneficial effects [38].The previous and current research evidences says that the antidaibetic effect of *C.colocynthis* root in streptozotocin induced diabetic rats can be attributed to the presence of the phytoconstituents. The probable mechanisms responsible for hypoglycemic action of the extracts choosen may be due to inhibitory effect of glucose metabolism i.e. alpha amylase inhibition, lowering blood glucose levels by raise in insulin sensitivity and decrease in insulin resistance and increase in insulin secretion.

4. Conclusion

In conclusion, our study shown that the hypoglycemic action of *Citrullus colocynthis* roots has beneficial effects on blood glucose level which might be mediated through its α-amylase inhibitory activity as well as insulinomimetic action. Further, molecular studies and isolation of the active component of the extract are highly warranted to elucidate the exact mechanism of action of the plant.

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