



## Research Article

### ANTI-OXIDANT STUDY OF *CITRULLUS COLOCYNTHIS* ROOTS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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#### ABSTRACT

**Objective:** The main objective is to investigate anti-oxidant activity of ethanolic and aqueous extract of *Citrullus colocynthis* (EECC & AECC) in streptozotocin –induced diabetic rats. **Methods:** Acute toxicity study of EECC and AECC was carried out in rats to determine its dose for further study. Oral glucose tolerance test was performed to evaluate EECC and AECC on elevated blood glucose levels. Diabetes was induced in rats by administration of streptozotocin (STZ) (45mg/kg) and it was confirmed after 48hrs after induction. Both the extracts of *Citrullus colocynthis* was given intraperitoneally to the diabetic rats up to 15 days and blood glucose levels were estimated after 10 days. On the 15<sup>th</sup> day of the experiment, rats were sacrificed for the anti-oxidant estimation in liver homogenate. **Results:** Acute toxicity of EECC and AECC did not show toxicity and death up to dose of 2000mg/kg. Both the extracts at 200 and 300mg/kg doses significantly ( $p < 0.001$ ) reduced blood glucose levels in OGTT. Both the doses of EECC and AECC treatment significantly ( $p < 0.001$  &  $p < 0.001$ ) showed free radical scavenging action with DPPH, NO and Reducing power assay in-vitro and in-vivo when compared with STZ treated rats were found to be very close to the standard Ascorbic acid. **Conclusion:** From the above results it was concluded that the plant extract is having the ability of managing hyperglycemia and complications of diabetes in STZ induced diabetic rats. Hence the plant may be considered as one of the source for the isolation of new oral anti-hyperglycemic agent.

**Keywords:** Diabetes mellitus, Streptozotocin, Oral glucose tolerance test, Hyperglycemia.

#### INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder due to absolute or relative lack of insulin and characterized by hyperglycemia in the postprandial and or fasting state, mainly associated with ketosis and protein wasting in severe condition. Free radicals derived from oxygen have been implicated in the pathophysiology of various diseases including diabetes mellitus. Moreover, also, evidence suggested that diabetes induced changes in the activities of antioxidant in various tissues.<sup>1</sup> The free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated endogenously through various chemical reactions during normal metabolism. Our body continuously encounters the hazard effects of above mentioned oxidants (ROS & RNS). They can be also produced upon acquaintance to various exogenous sources like organic solvents, pesticides, air pollution, tobacco smoke and radiations<sup>2</sup>. The free radicals/oxidants induced damage of various organelles is protected by antioxidants (chemical species) inhibiting the oxidative chain reactions<sup>3</sup>.

The dietary antioxidants ( $\beta$ -carotene, ascorbic acid,  $\alpha$ -tocopherol, uric acid, & glutathione) and some chemical messengers (angiotensin & estrogen), superoxide dismutase) are well recognized defense systems available in our body for detoxification of free radicals.<sup>4</sup>

Due to some internal and/or external factors as well as in diseased conditions like diabetes our body antioxidant defense system weakens which leads to delay in elimination of oxidants there by resulting in their accumulation in different tissues/organs. The oxidative stress arises due to the imbalance between the production of oxidants (ROS and RNS) and antioxidants<sup>5</sup>. The free radicals possess an unpaired electron in their outermost orbital, they are highly unstable and readily attack other molecules to obtain a stable electronic configuration thereby damaging almost all types of biomolecules including DNA, proteins as well as lipids<sup>6</sup>. Due to the excessive levels of these oxidants may cause mild to severe oxidative stress occurs leading to worsening of diseases such as diabetes, coronary heart disease, hypertension, atherosclerosis and cancer<sup>7</sup>.

In Ayurveda and Siddha medicines, there are a number of Indian medicinal plants, which have found to be useful to successfully manage diabetes. Advantage of traditional medicinal plants is no or lesser adverse effects with multiple therapeutic actions due to the presence of different bioactive compounds<sup>8</sup>. In this study our focus was to check the in vitro and in vivo antioxidant status of herb during diabetes obtained from the pollution free environment from higher altitudes. Quite surprisingly the herb showed good results with all the experiments performed for evaluating antioxidant potential. This herb would prove beneficial for the reduction of

oxidative stress generated by the formation of free radicals. Deficiency of Superoxide Dismutase (SOD) Catalase (CAT) enzymes and Lipid Peroxides (LPO) deficiency are important factors in the development of diabetic complications in vivo. Many other substances have been proposed to act as anti-oxidants in vivo. They include  $\beta$ -carotene, other carotenoids, xanthophyll's, metallothioein, taurin and its precursors, creatinine, polyamines, retinol, flavonoids, and other phenolic compounds of plant origin<sup>9</sup>.

The *Citrullus colocynthis*, also known as bitter apple, bitter cucumber, egusi, is a viny plant mainly found in Mediterranean Europe, Asia, Turkey, Nubia, Trieste, Egypt, Iran, Pakistan, Afghanistan, India and North Africa. The preliminary phytochemical analysis of EECC and AECC revealed presence of tannins, flavonoids, saponins and phenolic compounds. It is well known that phyto chemical constituents from this group were reported for many pharmacological actions including antidiabetic activity.

Moreover up to date literature research revealed that there is no scientific report on *C. colocynthis* plant to supports its use in the treatment of diabetes. Hence, objective of the present study is to investigate Antioxidant potential of ethanolic and aqueous extracts of *C. colocynthis* in STZ induced diabetic rats.

## MATERIAL AND METHODS

### Plant Material Collection

Plant *Citrullus colocynthis* was collected and procured from Tirumala Hills, Tirupathi, Andhra Pradesh during the month of July-Aug and it was identified and authenticated by Dr. K. Madhava Chetty, Professor in the Department of Botany, Sri Venkateshwara University, Tirupati.

### Preparation Of Plant Extracts

*Citrullus colocynthis* roots were shade dried and were crushed, powdered and exhaustively defatted by petroleum ether (60°C-80°C) and then successively extracted with ethanol and water. All the extracts were filtered, pooled and concentrated under reduced pressure using Rota vapor (Buchi, USA).

### Preliminary Phytochemical Analysis

The preliminary phytochemical screening of extract of *Citrullus colocynthis* gave positive tests for Alkaloids, Flavonoids, Carbohydrates, Saponin, Steroids, Tannins, Triterpenoids and Steroids.

### Drugs And Chemicals

Streptozotocin was purchased from Sisco Research laboratories Pvt Ltd. Metformin was a gift sample from Ranbaxy Pvt. Ltd Punjab India. Antioxidants kits were purchased from Himedia Pvt Ltd., Mumbai. Other chemicals and reagents used in the study were analytical grade.

### Animals

Healthy Male Wistar rats (180-250gms) are used to study the in vivo antioxidant activity. The animals were procured from National Institute of Nutrition (NIN), Hyderabad and housed into group of

six animals per cage maintained at 24°C±1°C with relative humidity 45-55% and 12:12 hour's dark and light cycle. The animals had free access to food (standard chow pellets) and water ad libium. The Experiments were carried out after obtaining permission from Institutional Animal Ethical Committee (Approval number: IAEC/SVCP/2011/007), Sri Venkateshwara College of Pharmacy, Hyderabad and Telangana (Dated 26/7/11).

## EXPERIMENTAL PROCEDURES

### IN VITRO ANTIOXIDANT ACTIVITY

#### DPPH Radical Scavenging Activity

The free radical scavenging activity of *Citrullus colocynthis* was measured by 1, 1- diphenyl-2-picryl-hydrazil (DPPH) according the method of Sreejayan method.<sup>10</sup> 1ml of different concentrations 50,100,200,300,400 and 500  $\mu$ g/ml of the test extract and standard were taken in different test tubes. To this, add 3ml of methanolic solution of DPPH and incubated at 37°C for 20 min. The absorbance was measured at 517nm on a spectrophotometer (UV-spectrophotometer). Ascorbic acid is used as standard antioxidant agent. The concentration of the test extracts required to decrease the initial concentration by 50% (IC<sub>50</sub>) was calculated.

#### Reducing Power Assay

A spectrophotometric method<sup>11</sup> was used for the measurement of reducing power. For this 2.5ml of each of the extracts was mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (10mg/ml). The mixture was incubated at 50°C for 20min, then rapidly cooled and mixed with 2.5ml of 10% of trichloroacetic acid then centrifuged at 6500rpm for 10 min. An aliquot of (2.5ml) of supernatant was diluted with distilled water (2.5ml) and then ferric chloride (0.5ml, 0.1%) was added and allowed to stand for 10min. The absorbance was recorded spectrophotometrically at 700nm. Ascorbic acid is taken as standard for comparison at a concentration range of 50 $\mu$ g/ml-500 $\mu$ g/ml. The concentration of the test extracts required to decrease the initial concentration by 50% (IC<sub>50</sub>) was calculated.

#### Nitric oxide scavenging activity

At physiological pH, sodium nitroprusside in aqueous solution spontaneously generates nitric oxide, producing nitrite ions by interacting with oxygen which can be determined by Griess reagent. In the present investigation, Nitrite ions reacting with Griess reagent forms a purple azo dye. In the presence of test extract, the number of nitrite formation was decreased. The degree of decreased intensity of purple azo dye formation will influence the scavenging property of the sample, the absorbance was measured at 540nm. 0.5ml of extracts at various concentrations 50,100,200,300,400 and 500  $\mu$ g/ml were mixed with 2ml of 5mM sodium nitroprusside in 0.5ml phosphate buffer saline (pH 7.4-1ml) and the mixture was incubated at 25°C for 150 min. From this mixture, 0.5ml of the Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% H<sub>3</sub>PO<sub>4</sub>) was added<sup>12</sup>. The absorbance was measured at 540 nm with a spectrophotometer. The experiment was performed in triplicates. The concentration of the test extracts required to decrease the initial concentration by 50% (IC<sub>50</sub>) was calculated.

### 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.

### 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 Acu-chek Glucometer. At the end of the experimental period, the blood was withdrawn by retro orbital puncture and centrifuged. And the studies was conducted at Jeeva Life Sciences Lab, Uppal, Hyderabad.

### IN VIVO ANTIOXIDANT STUDY

#### Induction Of Diabetes Mellitus

Diabetes mellitus (DM) was induced in overnight fasted wistar rats by a single intraperitoneal (i.p) injection of 35 mg/kg of streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5). The fasting blood glucose levels were checked after 3 days. On day 14, animals having a blood glucose level higher than 250 mg/dL were considered as diabetic and selected for the experiment. After induction of diabetes all the animals were kept in laboratory on normal diet<sup>13</sup>.

#### Experimental design for the assessment of antioxidant property

After induction of diabetes, the selected animals are divided into fifteen groups each group consists of six animals. The grouping details are:

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

- Group I: Normal untreated rats (Control)
- Group II: Diabetic control (STZ)
- Group III: Diabetes 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)

Animals of group I were given with 0.9% saline and served as control and groups 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* 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, and on 15th day blood was collected by retro-orbital sinus puncture.

The Fasting blood glucose (FBS) and Post-Prandial glucose (PLBS) levels were estimated by Glucose-oxidase method

On day 16<sup>th</sup>, overnight fasted animals of all groups received respective treatments and after 1 h, all animals were anesthetized and killed by cervical dislocations to dissect out liver. They were washed immediately with ice-cold saline to remove blood, kept at -70 °C for the estimation of antioxidants levels until the completion of study<sup>14</sup>.

#### Tissue Supernatant Preparation For LPO, CAT And SOD Assay

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris-HCl buffer (0.025M, pH 7.4) using homogenizer. The homogenate obtained was centrifuged at 5000rpm for 10 mins and supernatant was collected and used to assay the LPO, CAT and SOD activities.

#### Estimation Of Lipid Peroxidation (LPO), Catalase (CAT) Super Oxide Dismutase (SOD) Antioxidant System

Lipid peroxidation is based on the reaction of Malondialdehyde with thiobarbituric acid to form Thiobarbituric acid reactive substances (TBARS), which gives a pink colour with *Citrullus colocynthis* at 540 nm. Catalase (CAT) is determined by Aebi method<sup>15</sup> and super oxide dismutase (SOD) activity was determined colorimetrically by the method of Kono<sup>16</sup>

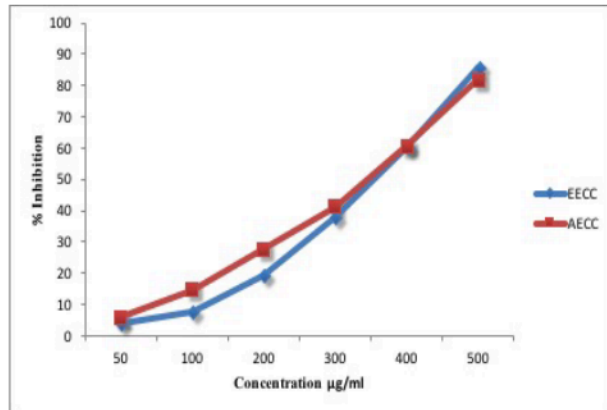
#### Statistical Analysis

All the data were expressed as mean  $\pm$  SEM and were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons using prism Graph pad version 5.0 and values of P<0.05 were considered statistically significant.

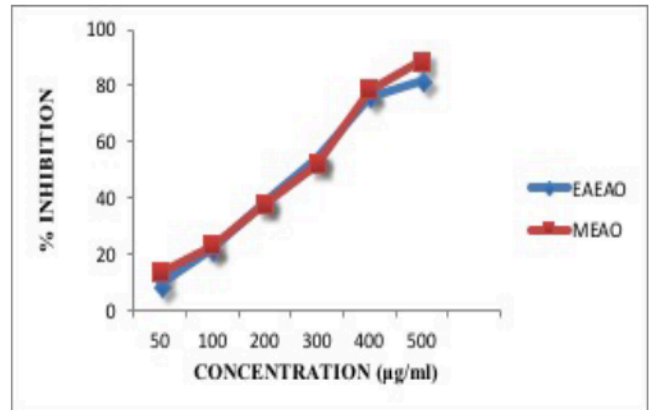
**Table: 1** Percentage inhibition of extracts of *Citrullus colocynthis* in DPPH free radical scavenging assay with IC<sub>50</sub> values

Groups	Concentrations (µg/ml)	% Inhibition	IC <sub>50</sub>
Ascorbic acid	50	6.14±0.17	295µg/ml
	100	13.75±0.20	
	200	32.06±0.30	
	300	51.13±0.44	
	400	67.86±0.20	
	500	91.78±0.40	
EECC	50	4.16±0.19	<sup>a</sup> 326µg/ml
	100	7.82±0.41	
	200	19.4±0.97	
	300	38.13±0.80	
	400	60.35±1.08	
	500	85.98±0.51 <sup>a</sup>	
AECC	50	6.00±0.08	<sup>a</sup> 353µg/ml
	100	14.83±0.40	
	200	27.63±0.82	
	300	41.24±0.42	
	400	60.53±1.08	
	500	81.90±0.86 <sup>a</sup>	

<sup>a</sup>P<0.0001 considered as significant; compared with corresponding Standard.



**Figure: 1** Effect of % inhibition of extracts of *Citrullus colocynthis* in DPPH assay



**Figure: 2** Effect of percentage inhibition of extracts of *Adenium obesum* in NO assay

**Table: 2** Absorbance of different extracts of *Citrullus colocynthis* and Ascorbic acid at various concentrations in Reducing Power Assay

Concentration (µg/ml)	EECC	AECC	Ascorbic acid
50	0.20±0.005 <sup>a</sup>	0.24±0.005 <sup>a</sup>	0.34±0.002
100	0.25±0.003 <sup>a</sup>	0.36±0.005 <sup>a</sup>	0.53±0.003
200	0.93±0.003 <sup>a</sup>	0.75±0.005 <sup>a</sup>	1.12±0.002
300	1.06±0.004 <sup>a</sup>	1.05±0.005 <sup>a</sup>	1.61±0.001
400	1.34±0.003 <sup>a</sup>	1.33±0.005 <sup>a</sup>	2.01±0.005
500	1.96±0.003 <sup>a</sup>	1.85±0.003 <sup>a</sup>	2.48±0.005

**Table:3 Percentage inhibition of extracts of *Citrullus colocynthis* in NO scavenging assay with IC<sub>50</sub> values**

Groups	Concentrations (µg/ml)	% Inhibition	IC <sub>50</sub>
Ascorbic acid	50	10.02±0.15	255µg/ml
	100	18.82±0.25	
	200	30.48±0.60	
	300	53.81±0.40	
	400	73.08±0.21	
	500	93.98±0.37	
EECC	50	15.93±0.22	<sup>a</sup> 285µg/ml
	100	31.46±0.55	
	200	42.15±0.96	
	300	56.09±0.91	
	400	65.13±0.85	
	500	79.29±1.39 <sup>a</sup>	
AECC	50	10.92±0.72	<sup>a</sup> 250µg/ml
	100	20.64±0.38	
	200	40.55±0.47	
	300	60.05±1.01	
	400	80.62±1.01	
	500	93.48±0.78 <sup>a</sup>	

<sup>a</sup>P<0.0001 considered as significant; compared with corresponding Standard**Table 4: Effect of *Citrullus colocynthis* root extract on OGTT in normal and diabetic rats**

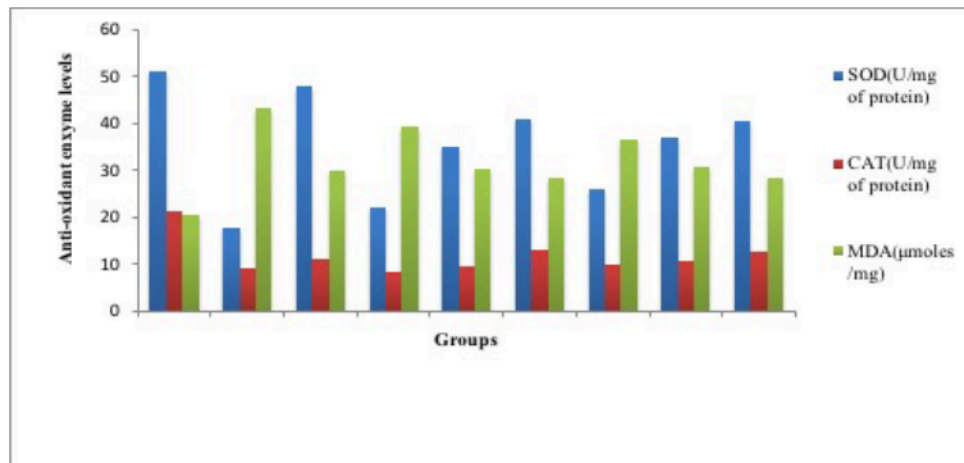
OGTT of <i>Citrullus Colocynthis</i>						
Blood glucose levels mg/ml						
Groups	Treatment	0 mins	30 mins	60 mins	90 mins	120 mins
I	Normal	81.0 ±0.763	102.6 ±2.036	96.51 ±1.46	91.6 ±1.46	80.71 ±1.59
II	Diabetic Control	296.83 ±4.43	273.40 ±2.98	296.7 ±3.35	294.35 ±2.04	270.08 ±1.54
III	Metformin+D	283.91 ±2.36	262.63 ±2.30	245.34 ±3.23	171.76 ±2.53	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	297.54 ±4.035	272.83 ±2.47	257.15 ±2.72	179.10 ±1.93	160.82 ±1.720
XI	D+AECC 200mg/ml	280.07 ±1.48	269.42 ±2.205	188.74 <sup>a</sup> ±2.09	172.44 <sup>a</sup> ±2.08	149.26 <sup>a</sup> ±1.640
XII	D+AECC 300mg/ml	293.47 <sup>a</sup> ±2.36	271.76 ±1.83	184.37 <sup>a</sup> ±1.23	159.088 <sup>a</sup> ±0.936	139.76 <sup>a</sup> ±0.841
XIII	D+EECC 100mg/ml	290.06 ±2.72	276.59 ±1.168	267.43 ±1.55	182.62 ±2.05	167.76 ±1.109
XIV	D+EECC 200mg/ml	283.16 ±0.95	267.73 ±3.36	199.78 <sup>a</sup> ±2.69	177.66 <sup>a</sup> ±2.38	157.78 <sup>a</sup> ±1.437
XV	D+EECC 300mg/ml	287.08 ±2.60	260.69 ±2.83	181.18 <sup>a</sup> ±1.54	161.27 <sup>a</sup> ±1.92	140.82 <sup>a</sup> ±1.33

**Table 5 : Effect of *Citrullus colocynthis* on blood glucose levels in control and experimental rats**

Groups	Treatment	FBS mg/dl	PLBS mg/dl
I	Normal	95.6 ±2.32	135.5 ±2.56
II	Diabetic Control	299.04 ±3.61	292.3 ±3.16
III	Metformin+D	282.41 ±4.64	155.16 ±1.310
IV	N+AECC 100mg/ml	88.5 ±2.35	90.14 ±2.62
V	N+AECC 200mg/ml	86.32 <sup>a</sup> ±1.05	92.45 <sup>a</sup> ±3.80
VI	N+AECC 300mg/ml	90.15 <sup>a</sup> ±1.40	94.82 <sup>a</sup> ±0.892
VII	N+EECC 100mg/ml	90.32 ±3.21	25.61 ±0.642
VIII	N+EECC 200mg/ml	89.62 ±2.51 <sup>a</sup>	96.32 ±0.182 <sup>a</sup>
IX	N+EECC 300mg/ml	91.41 ±0.93 <sup>a</sup>	92.15 ±0.143 <sup>a</sup>
X	D+AECC 100mg/ml	280.83 ±1.61	185.41 <sup>b</sup> ±1.82
XI	D+AECC 200mg/ml	282.0 ±2.32 <sup>a</sup>	175.69 ±2.08 <sup>a</sup>
XII	D+AECC 300mg/ml	279.0 ±0.65 <sup>a</sup>	165.83 ±1.81 <sup>a</sup>
XIII	D+EECC 100mg/ml	275 ±1.60	189.32 <sup>b</sup> ±1.23
XIV	D+EECC 200mg/ml	278 ±2.34 <sup>a</sup>	177.52 ±2.42 <sup>a</sup>
XV	D+EECC 300mg/ml	281.0 ±1.82 <sup>a</sup>	165.0 ±0.89 <sup>a</sup>

**Table : 6 Effect of extracts of *Citrullus colocynthis* on SOD, CAT and MDA in STZ-induced diabetic rats**

Groups	Treatment and Dose (mg/kg)	SOD (U/mg of protein)	CAT (U/mg of protein)	MDA (µ moles/mg)
I	Normal	51.1 ±1.44	21.34±1.40	16.4±1.79
II	Diabetic Control (DC)	17.6 ±1.52 <sup>a</sup>	9.00±0.670 <sup>a</sup>	43.30±1.28 <sup>a</sup>
III	D+Metformin(50)	47.8±1.56	11.11±0.653	20.93±0.86
IV	D+EECC(100)	22.08±1.02	8.53±0.93	39.24±0.56 <sup>b</sup>
V	D+EECC(200)	34.92±0.62 <sup>a</sup>	9.60±2.61 <sup>b</sup>	30.50±1.23 <sup>b</sup>
VI	D+EECC(300)	40.76±0.70 <sup>b</sup>	13.08±1.32 <sup>a</sup>	28.36±1.28 <sup>b</sup>
VII	D+AECC(100)	25.92±1.62 <sup>b</sup>	9.80±0.36 <sup>b</sup>	36.78±0.31 <sup>a</sup>
VIII	D+AECC(200)	36.92±0.62 <sup>a</sup>	10.60±2.61 <sup>b</sup>	30.80±0.25 <sup>a</sup>
IX	D+AECC(300)	40.42±1.42 <sup>a</sup>	12.61±0.92 <sup>a</sup>	28.50±0.30 <sup>a</sup>



**Figure. 3 Effect of *Citrullus colocynthis* on SOD, CAT, MDA levels**

## RESULTS

### Effects Of EECC And AECC On DPPH Radical Scavenging Activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is the extensively used rapid and genuine in vitro assay used for free radical reducing capacity of new compounds or drugs. It is a free radical with purple color having nitrogen carrying an unpaired electron which can be quenched by an antioxidant or hydrogen donating antioxidant and as such transform it to yellow colored complex DPPH-H. The change of color from purple to yellowish indicates the scavenging ability. The extracts of *Citrullus colocynthis* tested against DPPH stable radicals spectrophotometrically, which reveals that the radical scavenging activity of EECC shows excellent antioxidant ability by, improved with the increasing concentration of the extract. At a concentration of 500 µg/ml of EECC and AECC extracts percentage of inhibition was found to be 85.98% (IC<sub>50</sub>, 326 µg/ml) and 81.90% (IC<sub>50</sub>, 353 µg/ml) respectively. However, the scavenging activity of ascorbic acid at the same concentration was found to be 91.78% (IC<sub>50</sub>, 295µg/ml).

### Effects Of EECC And AECC On Reducing Power Assay

In this in vitro assay Fe<sup>3+</sup>- Fe<sup>2+</sup> formation was checked by using extracts as electron donors for reducing Fe<sup>3+</sup>/ ferricyanide complex to Fe<sup>2+</sup> form, Which can be accomplished by Perl's Prussian blue formation and is measured at 700 nm. In the present study, the reducing power of the EECC was found to be unresolved and gradually increase in direct proportion to the increasing concentrations of the extract. The reducing power of a 500 µg/ml concentration of the EECC was found to be 98.25 (IC<sub>50</sub>, 250µg/ml), which was relatively more marked than that of standard ascorbic acid 91.55 (IC<sub>50</sub>, 274 µg/ml), while the AECC shows relatively less reducing power 88.31 (IC<sub>50</sub>, 275 µg/ml) than the ethanolic and standard.

### Effects Of EECC And AECC On Nitric Oxide Levels

The generation of nitric oxide from sodium nitroprusside was effectively reduced by the AECC. The nitric oxide generation at 500 µg/ml concentration of the AECC was found to be significant 93.48 (IC<sub>50</sub>, 250µg/ml), which was relatively more similar to that of standard ascorbic acid 93.98 (IC<sub>50</sub>, 255µg/ml), while the EECC shows significant reduction nitric oxide generation 72.29 (IC<sub>50</sub>, 285µg/ml) but less than the aqueous and standard .

### ORAL GLUCOSE TOLERANCE TEST

In rats, diabetes was induced by using STZ at a dose of (30-50mg/kg), where blood glucose levels were >250mg/dl that indicated the induction of diabetes and the results were evaluated. The acute oral toxicity study of *Citrullus colocynthis* showed no mortality rate up to 2000mg/kg.

OGTT was performed in all the rats from group I to group XV (n=6). Table 4 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<0.001) respectively when compared to the diabetic control group.

Table 5 shows the levels of blood glucose levels i.e., FBS and PLBS in control and experimental animals. Diabetic rats showed a significant increase in blood glucose compared to corresponding control rats. Following the oral administration of aqueous and ethanolic extracts of *Citrullus colocynthis* (200mg/kg, 300mg/kg) PLBS levels significantly decreased (p<0.0001) in diabetic treated group when compared to diabetic control group. In the present study, metformin was used as a standard oral hypoglycemic agent, which showed significant reduction in postprandial blood glucose as compared to diabetic rats.

The STZ treated rats produced marked hyperglycemia as significant (P<0.001) elevation in blood glucose level as compared to control rats. Administration of AECC and EECC in STZ induced diabetic rats at the dose of 200 and 300 mg/kg produced significant (P<0.001 & P<0.001) and dose dependent fall in blood glucose levels when compared with STZ treated rats. Metformin is taken as standard anti-diabetic drug. The results were compared to metformin treated group also. It is observed that the reduction in the blood glucose levels in AECC and EECC treated groups were almost same as in metformin treated group.

### IN VIVO ANTIOXIDANT PROPERTIES OF EECC AND AECC

#### Effects Of EECC And AECC On Lipid Peroxidation

In the present study, the TBARS levels in diabetic rats were significantly (P<0.001) increased than normal control rats which supported the occurrence of lipid peroxidation. Administration of EECC (100, 200 & 300 mg/kg) reduced the TBARS level in diabetic rats significantly (P<0.01) and AECC (100, 200 & 300 mg/kg) reduced the TBARS level in diabetic rats significantly (P<0.001) when compared with diabetic control.

#### Effects Of EECC And AECC On SOD Levels

In this study, diabetic rats showed significant (P<0.001) decrease of the SOD levels when compared with normal control rats 17.6±1.52. However, administration of EECC (200 & 300 mg/kg) and AECC (200 and 300 mg/kg) in diabetic rats significantly (P<0.001) increased the SOD levels when compared with diabetic control rats. Also, AECC dose (100 & 200 mg/ kg) and EECC its lower dose (100 mg/kg) showed significant (P < 0.001 and P < 0.05) effect respectively. This action may be owing to blood glucose reduction ability of AECC and EECC in diabetic rats and hence anticipation of glucose oxidation, thereby falling superoxide anion formation and restoration of the SOD level.

#### Effects Of EECC And AECC On CAT Levels

In our study, CAT levels in diabetic rats were significantly (P<0.001) reduced when compared with normal control rats which confirms the oxidative stress and free radicals production after the induction of diabetes by STZ (Table 6). The AECC (100, 200 & 300 mg/kg) and EECC (200 & 300 mg/kg) treatment to the diabetic rats exhibited significant (P<0.001) increases in CAT levels. Administration of EECC 100 & 200 mg/kg to the diabetic rats increased CAT levels significantly (P<0.01). The restoration of CAT levels by both extracts may be due to decrease the formation of hydrogen peroxide via inhibition of glucose oxidation by control of the blood glucose level in diabetic rats. The results are shown in Table 6 and Figure 3.

## DISCUSSION

Medicinal plants are widely used by many people to treat acute and chronic diseases. They have different therapeutic actions due to the presence of secondary metabolites like alkaloids, steroids, terpenoids, flavonoids, tannins, etc. in addition, medicinal plants are important sources of antioxidants that are reported to relieve many terrible diseases like neurodegenerative, cardiovascular diseases and cancer by augmenting free radical scavenging<sup>17</sup>.

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 specially induces DNA strand breakage in  $\beta$  -cells causing diabetes mellitus. As there is no spontaneous revision with STZ and it is also observed that 90% of rats is becoming diabetic<sup>18</sup> [28]. When there was 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<sup>19</sup> [29]. In the present study, STZ significantly induced hyperglycemia.

Acute toxicity study of both the extracts demonstrated that two different doses of *Citrullus colocynthis* were non-toxic throughout the experiment. The lethality was found to be zero in the groups of *Citrullus colocynthis* extracts. Phytochemical investigation of AECC and EECC reveals the presences of flavonoids, saponins and tannins and phenolic compounds. It is well known that certain flavonoids exhibit hypoglycemic activity and pancreas beta cell regeneration ability<sup>20</sup>. These principle phytoconstituents are known to be bioactive for the management of diabetes. In our present study the AECC and EECC produced significant antihyperglycemic activity at a dosage of 200 and 300mg/kg when compared with STZ treated rats.

A lot of diseases are triggered by imbalance between the oxidative stress and anti-oxidative defense as such it is likely to limit the damage and inhibit the progress of many diseases by antioxidant defense supplements. This study has assessed the *Citrullus colocynthis* extracts with numerous biological parameters and was compared with known antioxidant ascorbic acid. The enzymatic and non-enzymatic methods under in vitro and in vivo circumstances were employed for the amendment of oxidative stress by herbal extracts. DPPH assay is one of the important assays for determining the anti-oxidant activity of herbal extracts. DPPH is an unchanging free radical at room temperature and its drop or to receive an electron or a hydrogen radical from antioxidants is determined by determining the reduction in its absorbance values at 517 nm. DPPH radical scavenging activity of *Citrullus colocynthis* extracts were compared with standard ascorbic acid. The mechanism of reducing power assay of antioxidants or polyphenolic compounds leads to the reduction of ferric form of iron to ferrous form and as such changes the color of the solution to different colors from green to blue that is governed by on the reducing capability of the compounds<sup>21,22</sup>.

Nitric oxide is a powerful pleiotropic inhibitor of biological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in different biological systems including neuronal messenger, vasodilation and antimicrobial and anti-tumor activities.<sup>23</sup> Nitric oxide is an unstable free radical involved in many biological processes which is accompanying with several diseases.

It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over production is an important goal.<sup>24</sup>

The main goal of the present study is to evaluate the beneficial effect of EECC and AECC on antioxidant status in STZ-induced diabetic rats. The exaggerated free radical production through STZ-mediated experimental diabetes resulted in the raised levels of lipid peroxides and hydro peroxides by oxidative degradation of polyunsaturated fatty acids. These are uneven, cytotoxic and highly reactive, leading to free radical damage to proteins and DNA and finally cause various diabetes mediated difficulties. The degree of tissue damage influenced by free radicals on the balance between free radical generation and the endogenous antioxidant defense mechanism.<sup>25</sup> The most widely used biomarker to explore the oxidative injury on lipids is TBARS a major lipid peroxidation product. It can react with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification.<sup>26</sup> In STZ-diabetic rats increase in TBARS levels was observed in compared with their respective normal controls. However, treatment with *Citrullus colocynthis* extracts to the diabetic rats significantly deteriorated back the TBARS levels to normal values which show the anti-lipid peroxidative property of *Citrullus colocynthis* extract in experimental diabetes. Many studies have shown lower antioxidant and improved peroxidative status in diabetes mellitus<sup>27</sup>.

The SOD, CAT enzymes that destroy the peroxides play a significant role in providing antioxidant defenses to an organism. CAT is involved in the removal of H<sub>2</sub>O<sub>2</sub>, and SOD acts to dismutase superoxide radicals to H<sub>2</sub>O<sub>2</sub><sup>28</sup>. The functions of all enzymes are interrelated and lowering of their activities effects in the accumulation of lipid peroxides and amplified oxidative stress in diabetic rats<sup>29</sup>. Free radicals are formed disproportionately in diabetes by glucose auto oxidation, thus result in development of complications in diabetes.

$\beta$ -cell is particularly sensitive to damage by free radicals because of their low level of free radical scavenging enzymes that leads to hyper glycaemic condition. Reduced oxidative stress due to reduced hyperglycemic status in diabetic condition had been observed in experimental animals following the administration of certain natural compounds.<sup>30</sup> Administration of AECC & EECC significantly reduced TBARS and increased SOD, CAT in diabetic rats. The action of both the extracts had restored the altered antioxidant enzymes in STZ-induced diabetic rats indicates its free radical scavenging potential and hence it has the ability to prevent diabetic associated complications.

## CONCLUSION

The present study clearly concluded that aqueous and ethanol extracts of *Citrullus colocynthis* possess ability to control blood glucose in diabetes. It's free radical scavenging property has potential to prevent diabetic associated complications. Our current study suggests the beneficial use of *Citrullus colocynthis* in the treatment of diabetes mellitus.

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