

***Citrullus colocynthis* attenuates hyperlipidemia and hyperglycemia through its anti-oxidant property against hyperlipidemic and diabetic animal models**

Jayaraman Rajangam^{1*} and A. J. M. Christina²

¹*Karpagam University, Coimbatore, TamilNadu, India*

²*A I M S T University, Malaysia*

ABSTRACT

Citrullus colocynthis-*colocynth*-*Cucurbitaceae*, is a widely used plant in traditional medical practice in many disorders, especially for diabetic, stomach pains, cathartic and laxative. Due to the scarcity of scientific reports for its traditional use, we aimed to investigate its effects in high fat (HF) induced hyperlipidemic and streptozotocin (STZ)-induced diabetic rats. Methanolic extract of *Citrullus colocynthis* (MECC-200 and 400mg/kg) were administered orally for 30 days. At the end of study, we employed biochemical estimation, which reveals that hyperlipidemic and diabetic groups were shown significant elevation in the level of serum lipid profiles, plasma glucose respectively along with the elevation of serum AST, ALT, lipid peroxides, and this elevation were significantly attenuated by treatment with MECC-200 and MECC-400. In addition, treatment with MECC-200 mg/kg and MECC-400 mg/kg were significantly improved the levels of SOD, GSH, CAT in cholesterol fed hyperlipidemic and STZ-induced diabetic rats (** $p < 0.01$). Moreover methanolic extract of *Citrullus colocynthis* fruits was screened for its free-radical scavenging effect, reducing power and Nitric oxide radical scavenging activity. Results from our study indicates, the definite radical quenching activity of the extract towards DPPH radicals, NO free radicals in comparison with ascorbic acid. In reducing power method, MECC demonstrated dose dependent antioxidant activity comparable with Ascorbic acid. Thus, it clearly indicates that *Citrullus colocynthis* might be beneficial in attenuating hyperlipidemic and diabetes conditions. Moreover the results suggested the ability of the extract to combat oxidative stress by quenching free radicals which reveals that, the attenuation due to its anti-oxidant property.

Keywords: *Citrullus colocynthis*, hyperlipidemia, hyperglycemia, high fat diet, anti-oxidant.

INTRODUCTION

The occurrence of atherothrombotic diseases are mainly due to the disturbance in lipid metabolism or elevated level of lipids. Accumulation of this elevated lipids in diabetes is mediated through a variety of derangements mechanisms in metabolic and regulatory processes, especially insulin deficiency or insulin sensitivity, thereby rendering the diabetic patient more prone to hyperlipidemia [1]. Moreover, reactive oxygen species like superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide radicals, play an important role in oxidative stress related to the pathogenesis of numerous diseases [2,3]. So data from earlier studies shows that antioxidants act as a major defense mechanism by protecting the damages caused by free radicals. Drugs or products which contains antioxidants are getting increased focus for the prevention and treatment of complex diseases like, diabetes, Alzheimer's disease and cancer, in the last three decades [4]. This has attracted a great deal of research interest in natural antioxidants. Moreover, accessibility and affordability of the medicinal plants have made them vital part of many people's life all around the world and regarding the lack of safe modern drug, evaluation of effective plants for numerous diseases has been recommended by WHO [5].

Citrullus colocynthis also known as bitter apple is a desert plant of the family Cucurbitaceae naturally adapted to arid environments and originally from tropical Asia and Africa. Each plant produces 15-30 round fruits, about 3-4 inches in diameter, green with undulate yellow stripes, becoming yellow all over when dry. The fruits are widely used medicinally, especially for stomach pains the pulp, because of its content of glucosides such as colocynthin, is an effective cathartic and laxative [6]. A number of plant secondary metabolites including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids have previously been reported from this plant [7,8]. The present study was designed to test the antihyperlipidemic and antioxidant Potential of methanolic extract of *Citrullus colocynthis* fruits on hypelipidemic and diabetic rats.

MATERIALS AND METHODS

Plant material

Citrullus colocynthis fruits were collected from Irumbulikurichi, Perambalur district, Tamilnadu, India and authenticated by G.V.S Murthy, botanical survey of India (BSI), southern circle, Coimbatore, Tamilnadu, India (BSI/SC/5/23/11-12/Tech-1759).

Chemicals

Chemicals used in this study, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich, India, ascorbic acid, phosphoric acid, naphthyl ethylene diamine dihydrochloride, are obtained from Sd Fine chemicals Ltd, India, and potassium ferri cyanide, trichloro acetic acid, sulfanilamide were obtained from Himedia, Laboratories Pvt-Ltd, India, sodium nitroprusside obtained from Qualigens fine chemicals. Atorvastatin and Glebenglamide were obtained from SUN Pharma, India as a gift sample. All reagents used in the study were analytical grade.

Preparation of *Citrullus colocynthis* methnolic extract

The chopped and shade dried fruits were powdered and passed through a 40-mesh sieve then extracted with methanol in a Soxhlet apparatus. The solvent from the methanolic extract was completely removed and concentrated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. The *Citrullus colocynthis* fruits yielded brown semi-solid residue of methanolic extract, weighing 9.0% w/w with respect to the dried starting material.

Phytochemical screening

The preliminary Phytochemical screening of crude extract of *Citrullus colocynthis* was carried out in order to ascertain the presence of its constituents by utilizing standard conventional protocols [9].

Experimental animals

The experiments were carried out on adult albino rats (120-180g) of either sex. They were housed in a quite temperature of 25±1°C and relative humidity of 45-55%. A 12:12 light/dark cycle was maintained during the experiment. They were fed with standard rat feed with water ad libitum, except during the test period. Each group consists of a 6 animals/dose and the experimental protocols were approved by institutional animal ethics committee (IAEC KMCRET/Pharm/06/2012) and conducted according to the CPCSEA guidelines for the use and care of experimental animals, New Delhi, India.

Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test (OGTT) was performed for two different doses of MECC (200 and 400mg/kg.b.w.p.o). The blood samples were obtained from tail prick method and the glucose level was measured by one touch glucometer (accu-check) at the interval of 0, 30, 60, and 120 min after the administration of extract [10]. (Only the initial and final blood glucose was shown in Table 4).

Preparation of high cholesterol diet

1% cholesterol (w/w), 5% hydrogenated fat and 0.2% cholic acid (w/w) were well mixed with the finely-ground commercial diet [11].

Experimental induction of hyperlipidemia

Hyperlipidemia was induced in animals by feeding with high cholesterol diet for 30 days and all the animals had free access to food and water ad libitum during the experimental period. The test group animals concurrently received plant extracts except for control rats every morning. At the end of 30 days, blood samples were collected from the rats in all groups for the biochemical determinations [12].

Experimental induction of induction of diabetes

The animals fasted over night and diabetes were induced by a single i.p injection of freshly prepared STZ (Sigma, USA) (55mg/kg b.w) in 0.1M citrate buffer (P^H4.5). The control group received equivalent amount of citrate

buffer [13]. Since STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were treated with 20% glucose solution after 6 hr then kept for next 24 hours on 5% glucose solution to prevent hypoglycemia. Forty eight hours after the injection of STZ, the rats were checked for fasting blood glucose levels. The animals showing fasting glucose more than 250mg/dl were considered as diabetic and used for this research work. They were treated with MECC for next 30 days.

Study Design

Hypolipidemic and Antioxidant study of MECC fruits on Hyperlipidemic rats.

- Group 1 : Vehicle (Citrate buffer or 0.9% NaCl, 5ml/kg b.w.p.o)
- Group 2 : High cholesterol diet
- Group 3 : High cholesterol diet + Atorvastatin (10mg/kg)
- Group 4 : High cholesterol diet + MECC (200mg/kg)
- Group 5 : High cholesterol diet + MECC (400mg/kg)

Hypolipidemic and Antioxidant study of MECC fruits on Diabetic rats.

- Group 1 : Vehicle (Citrate buffer or 0.9% NaCl, 5ml/kg b.w.p.o)
- Group 2 : STZ Induced Diabetic
- Group 3 : Diabetic + Glibenclamide (300µg/kg)
- Group 4 : Diabetic + MECC (200mg/kg)
- Group 5 : Diabetic + MECC (400mg/kg)

Collection of blood and analytical methods

Treatment with different compounds started on the same day of feeding with cholesterol diet and continued for 30 days. Body weight of the animals in all groups was recorded until end of the experiment (Results not shown). On the 31st day, after a fast of 12 hours, the animals were sacrificed and the blood was collected and immediately centrifuged. Serum was separated and stored at 100°C until lipid profile analysis.

Biochemical assays

Fasting blood glucose level, serum lipid profiles and biomarker enzymes were evaluated in Hyperlipidemic and diabetic rats. The blood glucose levels were estimated by one touch glucometer (Accu check). Serum levels of ALP, AST were determined according to the described standard methods [14] and serum lipid profiles were measured by standard methods by using diagnostic kits from coral clinical systems, India.

In vivo antioxidant status

The antioxidant levels were performed in liver tissue homogenate. LPO, GSH, CAT and SOD were assayed by the standard methods [15,16].

Invitro-Antioxidant activity

DPPH radical scavenging activity

The free radical scavenging activity of methanolic extract of *Citrullus colocynthis* fruits was measured by the decrease in the absorbance of methanolic solution of DPPH and this activity was measured by spectrophotometric method. 1mL of methanolic solution of extract of MECC at various concentrations (100, 100, 100, 800 and 1600 µg/mL) were mixed with 1mL of methanolic solution of DPPH (100µM). Similarly 1mL methanolic solutions of ascorbic acid (100 µg/mL) was mixed with 1mL of DPPH solution. A mixture of 1mL of methanol and 1mL of methanolic solution of DPPH (100 µM) served as control. After mixing, all the solutions were incubated in dark for 20 minutes and absorbance was measured at 517 nm [17]. The experiments were performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition.

$$\text{Scavenging \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity was measured by using Griess' reagent. 5mL of each extract solutions of different concentrations (100, 100, 100, 800 and 1600 µg/mL) in standard phosphate buffer solution (pH 7.4) were incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4) at 25°C for 5 hours. In an identical manner 5mL of ascorbic acid solution (100 µg/mL) in standard phosphate buffer solution (pH 7.4) was also incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4). Control experiments without the extract but with equivalent amount of buffer were also conducted. After incubation, 0.5mL of the incubation mixture was mixed with 0.5 mL of Griess' reagent (Sulphanilamide 1%, O-phosphoric acid 2% and naphthyl ethylene diamine dihydrochloride 0.1%) and the absorbance was measured at 546nm [18]. From

the absorbance the percent scavenging activity was calculated. The experiments were performed in triplicate and scavenging activity was expressed as percentage of inhibition.

Reducing power

2.5mL of solutions of different concentrations of the extract (100,100,100,800,1600 µg/mL) in standard phosphate buffer solution (pH 6.6) were incubated with 2.5mL of potassium ferri cyanide solution (1% w/v) at 50°C for 20 min. In an identical manner solution of ascorbic acid (100 µg/mL) was also incubated. After incubation, 2.5mL of 10% trichloro acetic acid solution was added to each tube and the mixture was centrifuged at 650 rpm for 10 minutes. 5mL of the upper layer solution was mixed with 5mL of deionized water and 1mL of ferric chloride solution (1% w/v) and the absorbance was measured at 700 nm [19].

Statistical Analysis

Values (mean ± S.E.M) were statistically analyzed using One-way ANOVA with Dunnett's test. The values of $p < 0.05$ were considered as significant.

RESULTS

From the Phytochemical screening, the obtained results revealed, the presence of Tannins, Phenol, terpenes, saponins, flavonoids, alkaloids, glycosides, steroids and sterols. Results from Table 1 shows that serum lipid profiles has increased significantly in animals treated with high fat diet (group II) for the period of 30 days. In group IV animals, administration of MECC caused a significant dose dependant reduction ($p < 0.01$) in the levels of serum total cholesterol, LDL-C, VLDL-C, and TG levels when compared with cholesterol fed control rats. Further, the level of HDL were significantly increased in MECC 200 mg/kg ($*p < 0.05$), MECC 400 mg/kg ($**p < 0.01$) treated animals in a dose dependant manner.

Table 1. Effect of Treatment of MECC for 30 days on serum lipid profile of Hyperlipidemic rats.

| Treatment | Lipid profiles (mg/dl) | | | | | |
|-----------------------|------------------------|-----------------|-----------------|----------------|----------------|-----------------|
| | TC | TG | LDL | HDL | VLDL | Phospholipids |
| Untreated | 79.34 ± 0.37 | 60.40 ± 1.02 | 103.71 ± 1.34 | 42.38 ± 4.46 | 62.92 ± 0.76 | 85.85 ± 0.81 |
| Cholesterol Treated | 308.17 ± 0.61 | 122.29 ± 0.84 | 217.92 ± 2.95 | 46.66 ± 1.32 | 95.33 ± 1.90 | 148.68 ± 0.54 |
| Cholesterol +ATOR | 95.79 ± 0.69** | 82.52 ± 2.44** | 96.08 ± 17.19** | 52.17 ± 0.81** | 71.18 ± 0.92** | 93.86 ± 0.87** |
| Cholesterol +MECC 200 | 139.18 ± 1.14** | 106.26 ± 1.13** | 129.49 ± 0.77** | 50.76 ± 0.78* | 89.47 ± 0.84* | 131.57 ± 1.06** |
| Cholesterol +MECC 400 | 110.73 ± 2.71** | 96.25 ± 1.17** | 104.59 ± 1.36** | 55.28 ± 0.80** | 83.55 ± 2.0** | 93.64 ± 2.02** |

Values are mean ± SEM for six animals, Values are statistically significant at $*p < 0.05$, $**p < 0.01$
ATOR-Atorvastatin, MECC treated hyperlipidemic groups were compared with cholesterol treated groups.

Table 2. Effect of Treatment of MECC for 30 days on serum lipid profile of diabetic rats.

| Treatment | Lipid Profiles (mg/dl) | | | | | |
|------------------------|------------------------|----------------|----------------|----------------|----------------|----------------|
| | TC | TG | LDL | HDL | VLDL | Phospholipids |
| Untreated | 72.33 ± 0.55 | 74.32 ± 1.43 | 44.79 ± 1.60 | 43.38 ± 4.46 | 32.44 ± 1.10 | 72.27 ± 0.79 |
| STZ-Treated | 110.34 ± 1.36 | 145.83 ± 1.86 | 87.62 ± 1.10 | 46.66 ± 1.32 | 85.38 ± 1.61 | 120.31 ± 1.61 |
| Diabetic+Glibenclamide | 80.64 ± 0.69** | 82.89 ± 2.24** | 50.43 ± 1.73** | 52.17 ± 0.81** | 43.03 ± 0.95** | 89.05 ± 1.51** |
| Diabetic+MECC 200 | 105.49 ± 1.94* | 99.47 ± 0.75** | 76.41 ± 0.79** | 50.76 ± 0.78* | 66.71 ± 1.62** | 96.19 ± 1.82** |
| Diabetic+ MECC 400 | 88.53 ± 0.73** | 88.39 ± 0.63** | 61.39 ± 1.22** | 57.28 ± 0.80** | 51.24 ± 0.8** | 85.00 ± 1.48** |

Values are mean ± SEM for six animals, Values are statistically significant at $*p < 0.05$, $**p < 0.01$
Glibenclamide, MECC treated diabetic groups were compared with STZ induced diabetic groups.

Table 3. Effect of Treatment of MECC for 30 days on blood glucose levels in diabetic rats.

| Groups | Blood glucose (mg dl ⁻¹) | |
|-----------------------------|--------------------------------------|-----------------|
| | Initial | Final |
| Vehicle | 80.27 ± 3.23 | 84.46 ± 1.29 |
| STZ Induced diabetic | 284.47 ± 8.57 | 321.91 ± 1.79 |
| STZ treated + Glibenclamide | 278.31 ± 4.6 | 118.64 ± 0.59** |
| STZ +MECC 200 | 283.92 ± 6.9 | 166.82 ± 0.99** |
| STZ +MECC 400 | 285.36 ± 3.09 | 127.64 ± 1.43** |

Values are mean ± SEM for six animals, Values are statistically significant at $*p < 0.05$, $**p < 0.01$
Glibenclamide, MECC treated diabetic groups were compared with STZ induced diabetic groups.
(Initial Value – After the induction of Diabetes except control groups)

As depicted in Table 3, rise in blood glucose level were observed in STZ induced diabetic animals (group II) in the range of 250-350mg/dl and it was significantly ($p < 0.01$) reduced by 30 days treatment with MECC (group IV and group V). Before treatment schedule, fasting blood glucose level in all animals was within normal range (Table 3.). After 24hr treatment with STZ, the fasting blood glucose level was significantly changed in the range of 250-285

mg/dl and it was reduced (** $p < 0.01$) significantly by 30 days treatment with methanolic extract of *Citrullus colocynthis* (Table.3)

As depicted in Table 4, there was elevation in the levels of LPO, ALT, AST in cholesterol fed group II animals and this elevation was significantly reduced by administration of MECC and Atorvastatin for the period of 30 days. Furthermore, the levels of SOD, GSH, CAT in group IV and group V were significantly improved by MECC (** $p < 0.01$) when compared with cholesterol fed groups.

Table 4. Effect of treatment of MECC for 30 days on Liver lipid peroxidation and antioxidants, Liver Marker enzymes on hyperlipidemic rats.

| Treatment | Antioxidant, Serum, ALT, AST Levels | | | | | |
|----------------------|-------------------------------------|---|---|---|---------------------|--------------------|
| | LPO (nmoles/mg protein) | SOD ($\mu\text{g}/\text{mg}$ protein) | GSH ($\mu\text{g}/\text{mg}$ protein) | CAT ($\mu\text{g}/\text{mg}$ protein) | ALT (IU/dl) | AST (IU/dl) |
| Control | 9.10 \pm 0.38 | 19.14 \pm 0.20 | 45.44 \pm 0.24 | 22.46 \pm 0.48 | 101.29 \pm 0.55 | 21.31 \pm 0.72 |
| Cholesterol/Treated | 13.26 \pm 0.45 | 12.15 \pm 0.40 | 27.77 \pm 0.64 | 35.36 \pm 0.37 | 100.82 \pm 0.43 | 66.53 \pm 1.33 |
| Cholesterol +ATOR | 10.01 \pm 0.30** | 18.57 \pm 0.28** | 42.57 \pm 0.29** | 48.16 \pm 0.50** | 104.82 \pm 0.21** | 45.09 \pm 0.38** |
| Cholest+MECC 200 | 11.80 \pm 0.20* | 14.09 \pm 0.43* | 35.20 \pm 0.43** | 37.72 \pm 0.95* | 139.15 \pm 0.23** | 61.23 \pm 0.70* |
| Cholesterol+MECC 400 | 10.18 \pm 0.25** | 17.18 \pm 0.36** | 40.82 \pm 0.28** | 42.39 \pm 0.53** | 117.97 \pm 0.23** | 49.89 \pm 0.32** |

Values are mean \pm SEM for six animals, Values are statistically significant at * $p < 0.05$, ** $p < 0.01$ Atorvastatin, MECC treated hyperlipidemic groups were compared with cholesterol treated groups.

Table 5. Effect of treatment of MECC for 30 days on Liver lipid peroxidation and antioxidants, Liver Marker enzymes on diabetic rats.

| Treatment | Antioxidant, Serum, ALT, AST Levels | | | | | |
|-------------------|-------------------------------------|---|---|---|---------------------|--------------------|
| | LPO (nM of MDA /mg protein) | SOD ($\mu\text{g}/\text{mg}$ protein) | GSH ($\mu\text{g}/\text{mg}$ protein) | CAT ($\mu\text{g}/\text{mg}$ protein) | ALT (IU/dl) | AST (IU/dl) |
| Untreated | 7.88 \pm 0.22 | 13.02 \pm 0.21 | 47.86 \pm 1.24 | 73.02 \pm 1.08 | 104.56 \pm 1.4 | 71.34 \pm 0.56 |
| STZ/Treated | 15.77 \pm 0.21 | 8.24 \pm 0.31 | 29.24 \pm 0.95 | 43.57 \pm 0.87 | 204.78 \pm 0.95 | 95.45 \pm 0.45 |
| STZ+Glibenclamide | 9.06 \pm 0.21** | 13.94 \pm 0.31** | 44.48 \pm 1.28** | 64.48 \pm 1.28** | 107.58 \pm 0.97** | 55.89 \pm 0.56** |
| Diabetic+MECC 200 | 13.57 \pm 0.18** | 11.54 \pm 0.21** | 37.38 \pm 1.80** | 52.94 \pm 0.67** | 143.49 \pm 1.41** | 62.38 \pm 0.22** |
| Diabetic+MECC 400 | 10.23 \pm 0.14** | 12.83 \pm 0.234** | 42.05 \pm 2.59** | 58.34 \pm 1.51** | 121.58 \pm 1.17** | 57.28 \pm 0.47** |

Values are mean \pm SEM for six animals, Values are statistically significant at * $p < 0.05$, ** $p < 0.01$ Glibenclamide, MECC treated diabetic groups were compared with STZ induced diabetic groups.

Results from our invitro antioxidant activity in rats was revealed that MECC scavenged free radicals in a concentration dependent manner in the models studied. Maximum percentage inhibition of DPPH radicals by the extract was about 68% at 1600 $\mu\text{g}/\text{mL}$ concentration. Standard drug ascorbic acid showed about 81% inhibition of the DPPH radicals at 200 $\mu\text{g}/\text{mL}$. (Table.6).

Table 6. Invitro Antioxidant activity of Methanolic extracts of *Citrullus colocynthis* fruits.

| Sample ($\mu\text{g}/\text{mL}^{-1}$) | % Inhibition (Mean \pm SEM) | | Absorbance (Mean \pm SEM) |
|--|--------------------------------|------------------|-----------------------------|
| | DPPH | NO | Reducing Power |
| MECC 1600 | 68.17 \pm 2.31 | 71.27 \pm 2.11 | 0.921 \pm 0.01 |
| MECC 800 | 62.67 \pm 1.63 | 56.63 \pm 0.57 | 0.69 \pm 0.05 |
| MECC 400 | 58.65 \pm 1.47 | 41.25 \pm 1.34 | 0.51 \pm 0.03 |
| MECC 200 | 53.65 \pm 1.24 | 33.72 \pm 1.55 | 0.43 \pm 0.02 |
| MECC 100 | 48.85 \pm 1.33 | 29.65 \pm 0.34 | 0.56 \pm 0.08 |
| MECC 50 | 25.91 \pm 1.37 | 18.52 \pm 0.58 | 0.26 \pm 0.06 |
| MECC 25 | 19.71 \pm 1.56 | 08.37 \pm 0.12 | 0.04 \pm 0.005 |
| Ascorbic acid 200 | 81.43 \pm 1.63 | 62.23 \pm 0.43 | 1.23 \pm 0.37 |
| Ascorbic acid 100 | 69.17 \pm 2.11 | 38.36 \pm 0.22 | 0.78 \pm 0.06 |
| Ascorbic acid 50 | 47.69 \pm 2.45 | 27.11 \pm 0.41 | 0.69 \pm 0.09 |
| Ascorbic acid 25 | 34.77 \pm 2.76 | 19.52 \pm 0.63 | 0.58 \pm 0.02 |

Values represent the mean \pm SEM: Number of readings in each group = 3

In the nitric oxide radical scavenging model, the maximum percentage inhibition of nitric oxide radicals by MECC about 71% at 1600 $\mu\text{g}/\text{mL}$ (Table.6). Ascorbic acid at 200 μg caused about 62% inhibition and the reducing power of MECC was dose dependent. The maximum absorbance of MECC at 1600 $\mu\text{g}/\text{mL}$ is comparable with ascorbic acid 100 $\mu\text{g}/\text{mL}$ (Table.6).

DISCUSSION

In the present study, Administration of exogenous cholesterol resulted in significant increase of various parameters of lipid profile in experimental animals. Treatment with MECC or Atorvastatin altered this elevation to different degrees. The lipid profiles were increased in hyperlipidemic groups whereas the increase was reduced in atorvastatin treated animals. The same trend was noticed in a dose-dependent manner with MECC (200,400 mg/kg) treated animals, the significant one being with 400 mg/kg dose (Table 1).the obtained results suggesting beneficial modulatory influence on cholesterol metabolism and turnover possibly by increased reverse cholesterol transport from peripheral organs to liver.

Hyperlipidaemia is considered as major consequence of diabetes mellitus and diabetes induced hyperlipidaemia is attributable to excess mobilization of free fatty acids from peripheral deposits [20,21].In our study, STZ induced diabetic rats showed rise in blood glucose level was accompanied with marked increase in TC,LDL-C,TG and reduction in HDL-cholesterol. Repeated oral administration MECC (200,400 mg/kg) normalized these effects. Further, it has been reported that diabetic rats treated with insulin shows normalised lipid levels[22].Thus, the obtained results indicate that MECC shows insulin-like action by virtue of its lipid lowering levels.

In addition to this,the present study, the oral treatment of MECC decreased the blood glucose levels in diabetic rats. It has been reported that using medicinal plant extract to treat STZ-induced diabetic rats results in activation of β -cells and insulinogenic effects [23].MECC may also have brought about hypoglycaemic action through stimulation of surviving β -cells of islets of Langerhans to release more insulin.

An imbalance between free radical production and antioxidant level leads to oxidative stress, which is obvious from the depressed antioxidant defense system in the high fat diet group of our study. Administration of MECC to high fat diet -fed rats reduced the buildup of oxidative stress by restoring normal activities of the enzymatic antioxidant SOD and normal levels of the non-enzymatic antioxidant GSH in the serum.

Additionally we have employed, The DPPH radical scavenging and nitric oxide radical scavenging method for direct measurement of radical scavenging and reducing power methods for indirect antioxidant measurement activity of the extract[24].Obtained results from our study indicates, the definite scavenging activity of the methanolic extract of towards DPPH radicals in comparison with ascorbic acid. Nitric oxide is a free radical produced in the mammalian cells and is involved in regulation of various physiological processes. However excess production of nitric oxide is associated with several diseases [25].Administration of Methanolic extract of *Citrullus colocynthis* has demonstrated dose dependent radical scavenging activity against NO free radicals. So MECC demonstrated good radical quenching activity against both DPPH and the nitric oxide radicals.In reducing power method as well, a dose dependent increase in absorbance is indicative that the extract is capable of donating hydrogen atoms. MECC demonstrated dose dependent antioxidant activity comparable with Ascorbic acid. In all the methods, scavenging effect of MECC increases with increasing concentration and maximum antioxidant activity was found at the dose of 1600 μ g/MI and above of MECC.

In summary, thus our findings demonstrate that MECC has antidiabetic effect, which is evidenced by reduction of blood glucose and also antihyperlipidemic effect, which is evidenced by the reduction of TC, TG, LDL-C, VLDL-C and PL, and increased HDL-C in hyperlipidemic and diabetic rats. Moreover a significant elevation in the level of lipid peroxide indicates enhanced oxidative stress in both hyperlipidemic and diabetic groups. Results from our invitro antioxidant study indicates, the definite radical quenching activity of the extract towards DPPH radicals,NO free radicals in comparison with ascorbic acid. Also in reducing power method, MECC demonstrated dose dependent antioxidant activity comparable with Ascorbic acid.moreover results from phytochemical analysis of *citrullus colocynthis* fruits indicated the presence of flavounoids , phenolic compounds.Thus, results from our priliminary studies,clearly indicates that *Citrullus colocynthis* might be beneficial in attenuating hyperlipidemic and diabetes conditions and the results suggested that the atteneuation due to its anti-oxidant property.

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