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Original Article

Antihyperlipidemic and Antioxidant Potential of *Citrullus colocynthis* fruits on HFD-Induced Hyperlipidemic and STZ-Induced Diabetic rats

Jayaraman Rajangam [1, *], A J M Christina [2], S Pushpa Latha[1], Narahari N Palei [3]

[1,*] Department of Pharmacology, Faculty of Pharmacy, Sree Vidyanikethan College of Pharmacy –Tirupati, 517501. Andhra Pradesh, India.

[2]Department of Pharmacology, Ahalia School of Pharmacy Ahalia Health, Heritage & Knowledge Village,Kozhippara,Palakkad,Kerala-678557,India.

[1] PG Scholar, Department of Pharmacology, Sree Vidyanikethan College of Pharmacy –Tirupati, 517501. Andhra Pradesh, India.

[3] Department of Pharmaceutics, Sree Vidyanikethan College of Pharmacy,Tirupati- 517501, India.

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ABSTRACT

Citrullus colocynthis is claimed in traditional medical practice as cathartic and laxative and also used for diabetic conditions. A number of plant secondary metabolites including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids have previously been reported from this plant. The objectives of this study to test the antihyperlipidemic and antioxidant Potential of ethanol extract of *Citrullus colocynthis* fruits on hyperlipidemic and diabetic rats. Albino rats were made hyperlipidemic, diabetic with high fat diet (HFD) and streptozotocin (STZ) respectively. Consequently, ethanol extract of *Citrullus colocynthis* (EECC) were administered orally for 30 days and their plasma glucose, serum lipid profiles, lipid Peroxidation (LPO), Superoxide dismutase (SOD), Catalase (CAT) levels were estimated by using standard procedures. Treatment with the extract EECC (200 and 400 mg/kg p.o) significantly ($p<0.01$) reduced the elevated levels of blood glucose in diabetic and serum lipid levels in both hyperlipidemic and diabetic rats were observed. Also significant improved levels of SOD, CAT and there was significant reductions in the elevated level of LPO were observed in both hyperlipidemic and diabetic rats. From this study, the ethanol extract of *citrullus colocynthis* fruits has proved not only to be an effective hypoglycemic agent, but also possesses significant ($p<0.01$) antihyperlipidemic and antioxidant properties against HFD induced hyperlipidemic and STZ induced diabetic albino rats.

Keywords: *Citrullus colocynthis*, streptozotocin (STZ), High fat diet, Antihyperlipidemic activity, Antioxidant potential.

Corresponding author *
Jayaraman Rajangam
Professor & Head
Department of Pharmacology,
Sree Vidyanikethan College of Pharmacy
Tirupati, 517501. Andhra Pradesh, India
E-mail: jayaraam81@gmail.com

1. INTRODUCTION

The main causative factor for atherothrombotic diseases is the disturbances occurring in lipid metabolism. Though there are a large class of antihyperlipidemic drugs used in the

treatment, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effects. Hence efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases [1]. Besides Hyperglycaemia, the levels of plasma lipids are usually raised in diabetes mellitus causing a risk factor for coronary heart disease. Accumulation of lipids in diabetes is mediated through a variety of disarrangements in metabolic and regulatory processes, especially insulin deficiency, thereby rendering the diabetic patient more prone to hypercholesterolemia and Hypertriglyceridemia. Patients with diabetes mellitus have an increased risk for coronary artery disease due to hyperglycemia, hypertension, dyslipidemia, and other risk factors like Oxidative stress is reported to be increased in patients with diabetes mellitus. Accumulating evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of diabetes mellitus. Reactive oxygen species generated in the cells are scavenged by antioxidant enzymes. Moreover, diabetes also induces changes in the tissue content and activity of the antioxidant enzymes[2].

Citrullus colocynthis is a desert plant from family Cucurbitaceae family and widely found in tropical areas. Every plant commonly produces around 15-30 round shaped fruits. 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 [3].A number of plant secondary metabolites including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids have previously been reported from this plant [4-5].By considering the phytoconstituents and and traditional usage, The present study was designed to test the antihyperlipidemic and antioxidant Potential of *Citrullus colocynthis* fruits on hypelipidemic and diabetic models in rats.

2. MATERIALS AND METHODS

Plant material

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

Preparation of ethanol extract from *Citrullus colocynthis*

The shade dried and chopped fruits were powdered and a weighed quantity of the powder (790 g) was subjected to hot solvent extraction in a Soxhlet apparatus using ethanol at a temperature range of 70-80°c.The obtained extract was concentrated to dryness at 40°c under reduced pressure in a rotary vacuum evaporator. The ethanol extract of *Citrullus colocynthis* fruits yielded brown semi-solid residue, weighing 7.0g (7.0%).

Phytochemical evaluation

The preliminary Phytochemical screening were carried out by following standard conventional protocols in order to ascertain the presence of various phytoconstituents[6].

Experimental animals

All the experimental procedures were performed on adult albino rats of weighing about 120-180g of either sex. They were housed in a normal room temperature of 25 ± 1^0C and relative humidity of about 45-55%. A 12:12 light/dark cycle was maintained throughout the study. They were given free access to food and water 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/KMRET/Pharm/06/12) and conducted according to the CPCSEA guidelines for the use and care of experimental animals, New Delhi, India.

Toxicity evaluation test

An acute oral toxicity study was performed according to the OECD guidelines for the testing of chemicals, Test No. 423 (OECD, 2001; acute oral toxicity acute toxic class method. Wistar rats (n= 3) of either sex were selected by a random sampling technique for the acute toxicity study. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. Extract was administrated orally in increasing dose up to 2000mg/kg.

Blood glucose measurement

The blood glucose level was measured by using Accu-Check one touch glucometer (Roche Diabetic Care,India). The glucose level was measured on regular basis but only initial and final values were shown in the current study [7].

Preparation of high fat diet (HFD)

High fat diet was prepared by using 1% cholesterol (w/w), 5% hydrogenated fat and 0.2% cholic acid (w/w) were well mixed with the finely- ground commercial diet [8-9].

Induction of hyperlipidemia

Experimental animals were made hyperlipidemic by feeding with high cholesterol fat diet (HFD) 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 [10].

Induction of diabetes

A single i.p injection of freshly prepared streptozotocin (STZ) (Sigma,USA) (55mg/kg b.w) in 0.1M citrate buffer (p^H 4.5) were used to induce the diabetes in overnight fasted animals. The control group received equivalent amount of normal saline [11]. The rats were treated with 20% glucose solution after 6 hr of STZ administration and then 5% glucose solution for next 24 hours 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 200mg/dl were considered as diabetic and used for the study.

Study Design

Antihyperlipidemic and Antioxidant study of EECC fruits on Hyperlipidemic rats.

I : Control (0.9% Nacl, 5ml/kg b.w.p.o)
II : High Fat diet (HFD)
III : HFD + Atorvastatin (10mg/kg)
IV : HFD + EECC (200mg/kg)
V : HFD + EECC (400mg/kg)

Antihyperlipidemic and Antioxidant study of EECC fruits on Diabetic rats.

I : Control (0.9% Nacl,5ml/kg b.w.p.o)
II : STZ (55mg/kg b.w.i.p)
III : STZ + Glebenglamide (5mg/kg)
IV : STZ + EECC (200mg/kg.p.o)
V : STZ + EECC (400mg/kg.p.o)

Blood Collection and separation of serum samples

Experimental animals were treated with extracts of *citrullus colocynthis* (200 & 400 mg/kg p.o) started on the same day of feeding with HFD and continued for next 30 days. Body weight of the animals in all groups was recorded periodically. On the 31st day with overnight fasting animals were sacrificed by cervical dislocation method and the blood samples were collected for serum separation. Separated serum samples were stored at 20^0 C for further biochemical estimations.

Biochemical parameters

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) whereas serum lipid profiles were determined by using semiautoanalyzer (Agappe, Kerala, India) with commercially available assay kits according to the described standard methods [12-17].

***Invivo* antioxidant status.**

The respective liver tissues were removed and washed immediately with ice-cold saline to remove as much as blood possible and homogenated (5% w/v) in cold potassium phosphate buffer (50 mM, pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 3000 rpm for 10 min, using Remi C-24 refrigerated centrifuge. The obtained supernatant was used for the estimation of Superoxide dismutase (SOD) and lipid peroxidation (LPO), Catalase (CAT) [18-20].

Statistical Analysis

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

3. RESULTS

Phytochemical investigations: Results of the Phytochemical screening revealed that the presence of carbohydrates, terpenes, saponins, flavonoids and alkaloids, glycosides, steroids and sterols etc.

Antiyperlipidemic activity

Table 1 depicts the values of serum lipid profile in control, HFD and EECC treated groups of hyperlipidemic rats. Serum total cholesterol, LDL-C, VLDL-C, and TG levels were increased significantly after 30 days of high fat diet whereas concurrent administration of EECC showed a significant dose dependant reduction of ($p<0.05$) serum total cholesterol, LDL-C, VLDL-C, and TG levels when compared with HFD rats. Further, there is a marked rise in HDL level were noticed when compared to HFD animals.

Table 1: Effect of Treatment of EECC on serum lipid profile of Hyperlipidemic rats.

Treatment	Lipid profiles (mg/dl)				
	TC	TG	LDL	HDL	VLDL
Control	79.34 ± 0.37	60.40 ± 1.02	103.71±1.34	42.38 ± 4.46	62.92±0.76
HFD	308.17 ± 0.61	122.29±0.84	217.92± 2.95	32.66 ± 1.32	95.33±1.90
HFD+ATOR	95.79± 0.69**	82.52±2.44**	96.08± 17.19**	52.17± 0.81**	71.18±0.92**
HFD+EECC 200	141.28±2.58**	116.44±2.49**	123.24±1.36**	35.29 ± 1.73*	91.40±2.59*
HFD+EECC 400	113.48±4.59**	102.30±3.62**	114.70±5.47**	38.72±3.39**	83.22±3.41**

Values are mean ± SEM for six animals, Values are statistically significant at *p<0.05, **p<0.01

Atorvastatin, EECC treated hyperlipidemic groups were compared with cholesterol treated groups.

Table 2 shown the values of serum lipid profile of control, STZ induced diabetic and EECC extract treated groups.Results demonstrate that, serum total cholesterol, TG, LDL-C and VLDL-C levels were increased significantly in STZ induced diabetic groups whereas concurrent administration of EECC caused a significant dose dependant reduction ($p<0.05$) in the levels of serum total cholesterol, LDL-C, VLDL-C, and TG levels when compared with STZ induced diabetic rats.But surprisingly, there was a marked rise of HDL were observed in EECC treated animals in a dose dependant manner.

Effect of EECC on blood glucose level

Table 3 depicts the values of fasting blood glucose levels (Initial and final values).Before administration of STZ, fasting blood glucose level in all animals was within normal range but after 72hr treatment with STZ, the fasting blood glucose level was significantly elevated in the range of 250-350mg/dl and it was significantly ($p<0.05$) reduced by 30 days treatment with EECC.

Table 2: Effect of Treatment of EECC on serum lipid profile of STZ induced diabetic rats

Treatment	Lipid Profiles (mg/dl)				
	TC	TG	LDL	HDL	VLDL
Control	72.33 ± 0.55	74.32±1.43	44.79±1.60	43.38 ± 4.46	32.44±1.10
STZ Treated	110.34±1.36	145.83±1.86	87.62±.10	37.66 ± 1.32	85.38±1.61
STZ + Gliben	80.64±0.69**	82.89±2.24**	50.43±1.73**	52.17±0.81**	43.03±0.95**
STZ +EECC 200	103.46±2.37*	97.29±2.75**	75.59±3.75**	39.38 ± 2.42*	68.56±3.42**
STZ + EECC 400	86.62±2.73**	85.48±4.33**	59.62±2.56**	42.71±2.48**	50.33±2.81**

Values are mean ± SEM for six animals, Values are statistically significant at *p<0.05, **p<0.01

Glibenclamide, EECC treated diabetic groups were compared with STZ induced diabetic groups.

Table 3: Effect of EECC on blood glucose levels in STZ induced diabetic rats.

Groups	Blood glucose (mg dl)	
	Initial	Final
Control	80.27±3.23	84.46±1.29
STZ Induced diabetic	284.47±12.0	321.91±1.79
STZ + Glibenclamide	278.31±4.6	118.64±0.59***
STZ +EECC 200	263.46±7.47	182.82±0.99**
STZ +EECC 400	268.43±5.49	130.64±1.43**

Values are mean ± SEM for six animals, Values are statistically significant at *p<0.05, **p<0.01

Glibenclamide, EECC treated diabetic groups were compared with STZ induced diabetic groups.

Antioxidant activity

Fig 1: depicts the levels of LPO, SOD, CAT of hyperlipidemic rats. There was elevation in the levels of LPO and this elevation was significantly reduced in dose dependant manner by administration of EECC and atorvastatin for the period of 30 days. Furthermore, the levels of SOD, CAT were significantly improved by EECC when compared with HFD groups.

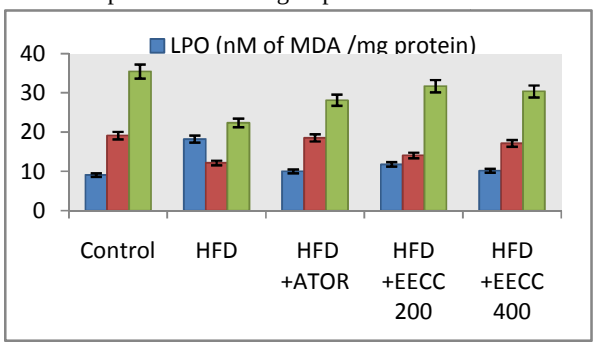

Fig 1: Effect of EECC on antioxidant status of HFD induced Hyperlipidemic rats.

Values are mean ± SEM for six animals, Values are statistically significant at *p<0.05, **p<0.01

Atorvastatin, EECC treated hyperlipidemic groups were compared with cholesterol treated groups.

Fig 2: shows the levels of LPO, SOD, and CAT of diabetic rats. There was significant reduction in the level of SOD, CAT and elevated levels of LPO were observed in STZ induced diabetic groups. The elevated levels of LPO, ALT and AST were significantly reduced in dose dependant manner by the concurrent administration of EECC for 30 days. Also the decreased levels of SOD, CAT were significantly ($p<0.05$) improved by EECC when compared with diabetic rats.

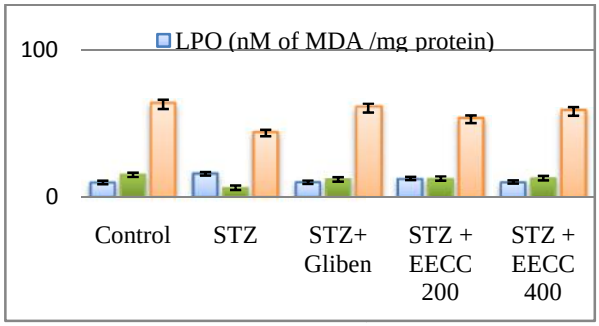

Fig 2: Effect of EECC on antioxidant status of STZ induced diabetic rats.

Values are mean ± SEM for six animals, Values are statistically significant at *p<0.05, **p<0.01

Glibenclamide, EECC treated diabetic groups were compared with STZ induced diabetic groups.

4. DISCUSSION

As per the data's, exogenous administration of cholesterol and high fat diets (HFD) may leads to elevation of various parameters like total cholesterol, triglycerides and LDL etc in various hyperlidemic animal models. The same trends were noticed in the present study whereas treatment with EECC or atorvastatin altered this elevation to different degrees. The lipid profiles were increased in hyperlipidemic groups whereas the increase was altered in some extent in atorvastatin treated animals. The same trend was noticed in a dose-dependent manner with EECC treated animals but the significant one being with 400 mg/kg dose (Table 2).the obtained results suggesting beneficial modulatory influence on cholesterol metabolism and turnover possibly by increased reverse cholesterol transport from peripheral organs to liver.

Table 3 shows the total cholesterol, TG, LDL-C and HDL-C, were significantly higher in diabetic rats and this possibly due to the increased mobilization of free fatty acids from peripheral deposits, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids [23]. Further, it has been reported that diabetic rats treated with insulin shows normalized lipid levels [24]. Thus, the results indicate that EECC shows insulin-like action by virtue of its lipid lowering levels.

It is suggested that hyperlipidemia increases the level of lipid peroxidation in the serum of rats fed with high cholesterol diet.The present study explored the effect of antioxidant capacity on the metabolism of blood lipid of rats

fed an high fat diet.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 EECC to high fat diet-fed rats prevented 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.

The ability of LDL-cholesterol to form lipid peroxides was found specifically responsible for the atherogenesis in diabetic patient's .Oral administration of *Citrullus colocynthis* extract normalized these effects, possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues.

The present study, the oral treatment of EECC 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 [21, 22]. EECC may also have brought about hypoglycaemic action through stimulation of surviving ß-cells of islets of Langerhans to release more insulin.

5. CONCLUSION

In conclusion, results of the present study demonstrate that EECC has on antidiabetic effect, which is evidenced by reduction of blood glucose and antihyperlipidemic effect, which is evidenced by the reduction of TC, TG, LDL-C, VLDL-C 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. Administration of EECC decreased lipid peroxidation indicating antioxidant like activity which alleviates oxidative stress. From these results it can be concluded that EECC contains active component which decreases serum lipid profile and enhances the antioxidant levels in cholesterol fed hyperlipidemic and STZ induced diabetic rats which deserves further investigation in order to elucidate the exact mechanism of aforesaid effects.

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