

# ANTIDIABETIC EFFECT OF PETROLEUM ETHER EXTRACT OF *CITRULLUS COLOCYNTHIS* FRUITS AGAINST STREPTOZOTOCIN-INDUCED HYPERGLYCEMIC RATS

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The study was undertaken to investigate the effect of petroleum ether extract of *Citrullus colocynthis* fruits on lowering the blood glucose levels and thiobarbituric acid reactive substances (TBARS) in Streptozotocin induced Diabetic albino rats. Oral administration of two different doses (300&500 mg/kg p.o) of *Citrullus colocynthis* fruit extract exhibited a significant reduction in blood glucose level in diabetic rats. In addition, PECC significantly reduces TBARS levels when compared to diabetic control groups. Glibenclamide (0.5 mg/kg) was used as reference drugs. Based on the results in our study, we speculate that the petroleum ether extract of *Citrullus colocynthis* fruits might be promising for the development of a standardized phytomedicine for the treatment of diabetes mellitus.

*Key words:* Antihyperglycemic, *Citrullus colocynthis*, Haemoglobin, Glycosylated Haemoglobin, TBARS.

## INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism, which affects a large number of people throughout the world and more so in India (Sunil, 2009). Even though it is a non communicable disease it is considered to be one of the five leading causes of death world wide. About 100 million people around the world have been diagnosed with diabetes and by the year 2010 it is projected that 251 million people will have the disease. Recently the search for appropriate antihyperglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine, to natural products that may be better treatment than currently used drugs (Kandasamy, 2006). Also the pharmaceutical drugs are either too expensive or have undesirable side effects. Treatment with sulphonylureas and biguanides are also associated with side effects. However, for a number of reasons, complementary medicine has grown in popularity in recent years. Many indigenous Indian medicinal plants have been found to be useful to successfully manage

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diabetes and some of them have been tested and their active ingredients isolated. *Citrullus colocynthis* also known as bitter apple is a desert plant of the family Cucurbitaceae naturally adapted to arid environments. It is a non-hardy, herbaceous perennial vine, branched from the base. Originally from tropical Asia and Africa, it is now widely distributed in the Saharo-Arabian phyto geographic region in Africa and the Mediterranean region (Kaleem, 2006). Each plant produces 15-30 round fruits, about 3-4 inches in diameter, green with undulate yellow stripes, becoming yellow all over when dry. Seeds are small (1-4 inch or less in length) smooth and brownish when ripe. The fruits are widely used medicinally, especially for stomach pains the pulp, because of its content of glucosides such as colocynthin, it is an effective cathartic and laxative (Dan, 1998). A number of plant secondary metabolites including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids have previously been reported from this plant (Galal, 1997, Tehila 2007, Gulcan 2006). The present study was designed to test the acute and subacute antihyperglycemic effect petroleum ether extract of *Citrullus colocynthis* fruits on Streptozotocin induced Diabetic rats.

#### MATERIALS AND METHODS

**Plant Material.** *Citrullus colocynthis* fruits were collected from Irumbulikurichi, Perambalur district and authenticated by G.V.S. Murthy, botanical survey of India (BSI), southern circle, Coimbatore, Tamilnadu, India. The obtained fruits are well dried in shadow and powdered.

##### **Preparation of *Citrullus colocynthis* Petroleum Ether Extract**

The fruit were chopped to small pieces and dried in shade. The dried fruit was powdered and a weighed quantity of the powder (790 g) was subjected to hot solvent extraction in a Soxhlet apparatus using Petroleum ether at a temperature range of 60-70° C. Before and after every extraction the marc was completely dried and weighed. The extract was concentrated to dryness at 40° C under reduced pressure in a rotary vacuum evaporator. The Petroleum ether fruit extract of *Citrullus colocynthis* yielded brown semi-solid residue, weighing 7.0 g (7.0%).

**Phytochemical Screening.** The preliminary Phytochemical screening of crude extract of *Citrullus colocynthis* was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols (Kokate, 2006).

**Experimental Animals.** The experiments were carried out on adult albino rats (100-150g) 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 given free access to food and water, except during the test period. All experiments were performed at the same time of the day and during the light period. Each group consists of a 6 animals/dose and all the animals were

used only once. The experimental protocols were approved by an institutional animal ethics committee (IAEC) and conducted according to the CPCSEA guidelines for the use and care of experimental animals, New Delhi, India.

**Toxicity Evaluation Test.** The adult albino mice of either sex, 6-8 week old with an average weight of 120-180g, were used for the study. To determine the toxicity, a single oral administration of petroleum ether extract of *Citrullus colocynthis* fruits in different doses (100, 200, 300, 400, 500, 600 and 700 mg/kg) were administered to different groups of animals. Mortality and general behavior of the animals were observed periodically for the next 48 hrs.

**Induction of Diabetes.** The animals fasted over night and diabetes were induced by a single intraperitoneal injection of freshly prepared STZ (Sigma Chemicals, USA) (55 mg/kg b.w) in 0.1 M citrate buffer (pH 4.5) (Sekar, 1990). The control group received an equivalent amount of normal saline. 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. They were kept for the 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 350 mg/dL were considered as diabetic and used for this research work.

#### **Experimental Design**

##### **ACUTE AND SUB ACUTE ANTI HYPERGLYCEMIC STUDY OF PECC.**

The animals were divided into 5 groups. The mentioned groups were treated orally as follows:

- Group I – Normal Saline,
- Group II – STZ induced Diabetic control,
- Group III – STZ +Glibenclamide (0.5 mg/kg)
- Group IV – STZ+PECC (300 mg/kg)
- Group V – STZ+PECC (500 mg/kg)

**DETERMINATION OF BLOOD GLUCOSE LEVELS.** For acute study, blood samples were collected by tail vein of the rats and the blood glucose determinations were carried out at intervals of 0, 1, 3, 5 and 7 hours by using a single touch glucometer (Ascensia ENTRUST, Bayer) based on glucose oxidase method. For Sub acute study, the above mentioned treatment schedule was followed for the respective group of animals for 14 days. Body weight changes were measured for overnight fasted animals on the 0, 7<sup>th</sup> and 14<sup>th</sup> day of study. The blood samples were collected via tail vein on 0, 7<sup>th</sup> days but on 14<sup>th</sup> day all animals were sacrificed according to CPCSEA Guidelines for animal care, New Delhi, India to estimate blood glucose levels using single touch glucometer (Ascensia ENTRUST, Bayer) based on glucose oxidase method (Trinder, 1969).

**DETERMINATION OF BLOOD HAEMOGLOBIN AND GLYCOSYLATED HAEMOGLOBIN AND TBARS.** The levels of haemoglobin and glycosylated haemoglobin and TBARS (the product of glucose oxidation in blood)

were determined by previously described methods (Drabkin, 1932, Nayak, 1981, Satoh 1978).

**DATA AND STATISTICAL ANALYSIS.** Blood glucose levels were expressed in mg/dl as the mean  $\pm$  SEM. The data were statistically analyzed using One-way ANOVA with Dunnetts Multiple comparison test. The values of  $p < 0.05$  were considered as significant.

## RESULTS

**Phytochemical Analysis.** Freshly prepared extracts were subjected to a preliminary Phytochemical screening test for various constituents. This revealed the presence of Tannins, carbohydrates, terpenes, saponins, flavonoids and alkaloids, glycosides.

**Effect of PECC on Body Weight of Stz Induced Diabetic Rats.** Table 1 shows that there was a significant reduction in body weight observed with diabetic rats when compared to control groups. Administration of PECC significantly improved body weight of diabetic animals in a dose dependent and time dependent manner.

Table 1

Effect of petroleum ether extract of *Citrullus colocynthis* fruits on body weight of STZ induced diabetic rats

Treatment and Groups	Dose (mg/kg)	Changes in body weight (g)		
		Day 0	7 <sup>th</sup> day	14 <sup>th</sup> day
Control (Normal Saline)	5 mL/kg	151.91 $\pm$ 0.76	157.05 $\pm$ 0.59	177.21 $\pm$ 0.4
STZ + Diabetic control	55	150.01 $\pm$ 0.48	132.17 $\pm$ 0.44	103.19 $\pm$ 0.52
STZ + Glibenclamide	55+0.5	151.61 $\pm$ 0.48	145.48 $\pm$ 0.33**	149.85 $\pm$ 0.26**
STZ + PECC	55+300	154.29 $\pm$ 0.68	141.34 $\pm$ 0.31**	144.29 $\pm$ 0.64**
STZ + PECC	55+500	156.34 $\pm$ 0.71	144.25 $\pm$ 0.98**	148.95 $\pm$ 0.35**

Values are mean  $\pm$  SEM, n = 6 when compared with diabetic control \* $p < 0.05$ , \*\* $p < 0.01$ .

### Antihyperglycemic Activity

**ACUTE EFFECT OF PECC FRUITS ON BLOOD GLUCOSE LEVEL IN STZ INDUCED DIABETIC RATS.** Table 2 showed the results of the effect of two different doses (300 mg/kg and 500 mg/kg) of *Citrullus colocynthis* fruit extract in diabetic rats. There were no significant decreased blood glucose levels seen up to 2

hrs of extract administration, but after 3 hrs, a significant dose dependent decrease in blood glucose levels was observed. So the oral administration of PECC produced a gradual, time dependant significant reduction in blood glucose levels in STZ induced diabetic rats.

Table 2

Effect of acute treatment of petroleum ether extract of *Citrullus colocynthis* fruits on STZ induced diabetic rats

Treatment and Groups	Blood Glucose Concentration (mg/dL)				
	0 <sup>th</sup> hr	1 hr	3 hr	5 hr	7 hr
Control	84.23±0.57	86.46±0.21	85.24±0.41	85.71±0.42	86.31±0.76
Diabetic Control	411.92±0.41	413.87±0.37	421.88±0.63	422.48±0.84	426.01±0.27
Glibenclamide	261.73±0.63	263.3±0.66	262.84±0.74**	198.02±0.17**	167.54±0.20**
PECC	412.56±0.33	413.54±0.40	397.39±0.26**	372.06±0.25**	294.47±0.54**
PECC	411.36±0.58	412.28±0.64	392.47±0.33**	357.80±0.77**	287.04±0.21**

**Dose:** Control – 5ml/kg, Diabetic Control – 55mg/kg, Glibenclamide – 0.5mg/kg, PECC – 300, 500 mg/kg. Values are mean ± SEM, n = 6 when compared with diabetic control \**p* < 0.05, \*\**p* < 0.01.

Table 3

Effect of sub acute treatment of petroleum ether extract of *Citrullus colocynthis* fruits on STZ induced diabetic rats

Treatment and Groups	Dose (mg/kg)	Blood Glucose Concentration (mg/dL)		
		0 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
Normal Saline	5 mL/kg	84.14±0.98	85.05±0.57	85.34±0.52
STZ +Diabetic control	55	378.15±3.09	381.25±0.86**	398.21±0.40**
STZ + Glibenclamide	55+0.5	271.63±0.32	186.07±0.41**	152.42±0.61**
STZ + PECC	55+300	380.73±1.21	239.99±0.57**	181.59±0.32**
STZ + PECC	55+500	373.63±0.86	236.20±1.02**	163.50±0.30**

Values are mean ± SEM, n = 6 when compared with diabetic control \**p* < 0.05, \*\**p* < 0.01.

Table 4

Effect of PECC on Hb, HbA<sub>1c</sub> and TBARS in the blood of diabetic rats

Treatment and Groups	Dose (mg/kg)	Total Hb (g/dL)	Glycosylated Hb (Hb%)	TBARS (mmol/mg protein)
Control (Normal Saline)	5 mL/kg	15.41±0.24	7.40±0.26	0.81±0.03
STZ + Diabetic control	55	10.52±0.33	12.36±0.20	1.44±0.02
STZ + Glibenclamide	55+0.5	14.06±0.11**	8.11±0.21**	0.90±0.01**
STZ + PECC	55+300	14.30±0.15**	7.34±0.25**	0.82±0.01**
STZ + PECC	55+500	15.32±0.35**	8.17±0.16**	0.60±0.01**

Values are mean ± SEM, n = 6 when compared with diabetic control \**p* < 0.05, \*\**p* < 0.01.

SUB ACUTE EFFECT OF PECC FRUITS ON BLOOD GLUCOSE LEVEL IN STZ INDUCED DIABETIC RATS. Table 3 showed that significant decreased blood glucose levels were observed on 7<sup>th</sup> day after oral administration of PECC on diabetic rats. Further significant reduction of blood glucose level was observed on 14<sup>th</sup> day which indicates the potential of antihyperglycemic activity of PECC.

EFFECT OF PETROLEUM ETHER EXTRACT OF *CITRULLUS COLOCYNTHIS* FRUITS ON BLOOD HAEMOGLOBIN AND GLYCOSYLATED HAEMOGLOBIN AND TBARS. The diabetic rats showed a significant decrease in the levels of total haemoglobin and a significant increase in the level of glycosylated haemoglobin when compared to control groups (Table 4). Administration of petroleum ether extract of colocynthis to diabetic rats restored the total haemoglobin and glycosylated haemoglobin levels (*P* < 0.01).

In our study, TBARS levels in all diabetic groups were found to be higher than in normal groups (Table 4). Petroleum ether extract of *Citrullus colocynthis* fruits treated groups showed lower levels of TBARS in the blood than diabetic control rats.

## DISCUSSION

In recent years, various plant extracts have been claimed to be useful for the treatment of diabetes mellitus. According to earlier studies, plant extracts cause antihyperglycemic effect by promoting regeneration of beta cells or by protecting the pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action or its effect beta cells to release insulin and activate the insulin receptors to absorb the blood sugar (Jadhav 2009).

The present investigation reveals that petroleum ether extract of *Citrullus colocynthis* has shown significant pharmacological activity towards lowering blood glucose in diabetes. The decreased level of haemoglobin in diabetic rats is mainly due to the increased formation of HbA<sub>1c</sub>. During Diabetic Mellitus, the excess glucose present in blood reacts with Hb to form HbA<sub>1c</sub>. HbA<sub>1c</sub> is used as a marker for estimating the degree of protein glycation in Diabetes Mellitus. Administration of PECC to diabetic rats reduced the glycosylation of Hb and thus reduces the level of HbA<sub>1c</sub> thereby increasing the level of Hb. Since blood glucose is susceptible to oxidation, hyperglycemia causes excessive ROS production, which in turn leads to high TBARS levels in tissues (Dash, 2000). These TBARS concentrations, in diabetic rat blood, were also lowered by both doses of PECC in our study. Moreover, from Phytochemical investigation we found that major chemical constituents of the extracts were terpenes, alkaloids, glycosides and saponins. On the basis of the above results, we speculate that PECC may also have brought about antihyperglycemic action through stimulation of  $\beta$ -cells of islets of langerhans to release more insulin and this effect may be due to its constituents like saponins, flavonoids and glycosides.

In conclusion, the present studies indicated a significant antihyperglycemic effect with petroleum ether extract of *Citrullus colocynthis* fruits and support its traditional usage in the treatment of diabetes. Further investigations are needed to elucidate the exact mechanisms of the antihyperglycemic effect of PECC.

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