Research Article

Beneficial Effects of *Citrullus Colocynthis* Seeds Extract Studied in Alloxan-induced Diabetic Rats

B. Lakshmi[1], V. Sendrayaperumal[1], S. Subramanian[1*]
[1]Department of Biochemistry, University of Madras, Guindy Campus, Chennai, India
*Corresponding author's E-mail: subbus2020@yahoo.co.in

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ABSTRACT

Citrullus colocynthis (Cucurbitaceous), commonly known as 'bitter apple' possess a wide range of pharmacological activities such as antimicrobial activity, antioxidant and antiinflammatory activity. In the present study we have evaluated the hypoglycemic, hypolipidemic and antioxidant properties of *Citrullus colocynthis* seeds extract in alloxan-induced experimental diabetic rats. The phytochemical screening of the *Citrullus colocynthis* ethanolic seeds extract revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, phenols, saponins and glycosides. The effect of oral administration of *Citrullus colocynthis seeds* extract (250 mg/kg b.w.) on the levels of biochemical parameters were determined in experimental groups of rats. The altered levels of biochemical parameters in the diabetic rats were significantly reverted back to near basal values by the administration of *Citrullus colocynthis seeds* extract for 30 days. The level of glycogen content was improved upon the extract treatment. The altered activities of serum aminotransferases and alkaline phosphatase were restored to normalcy. The levels of lipid peroxides in the plasma and pancreatic tissues of diabetic rats were elevated significantly and were normalized by the administration of *Citrullus colocynthis seeds* extract. The activities of pancreatic, hepatic enzymatic antioxidants and the levels of plasma non-enzymatic antioxidants were markedly declined in the diabetic rats. Upon treatment with *Citrullus colocynthis seeds* extract to diabetic rats, decreased levels were elevated to near normal values. The altered levels of lipid profile were reverted back to near normalcy upon the extract treatment. The results of the study indicate that *Citrullus colocynthis seeds* extract possesses antidiabetic, antioxidant and antilipidemic activity. The results are comparable with gliclazide, an oral standard hypoglycemic drug. The phytochemicals present in the *Citrullus colocynthis seeds* extract may account for the observed pharmacological properties.

Keywords: *Citrullus colocynthis*, antidiabetic, antioxidant, antilipidemic, alloxan.

INTRODUCTION

Diabetes mellitus is an endocrine disorder characterized by persistent hyperglycemia that result from defects in insulin secretion, or its action, or both[1]. The global increase in the prevalence of diabetes is due to population growth, aging, urbanization and an increase of obesity and physical inactivity. Roughly 80% of people with diabetes are in developing countries, of which India and China share the larger contribution[2]. The use of plants for medicinal purposes is as old as our civilization. The World Health Organization (WHO) estimates that 80 percent of the world's population presently uses herbal medicine for some aspect of primary health care. In the developing world, herbal medicine is used in industrialized nations by alternative medicine practitioners such as naturopaths.

One such traditionally used medicinal plant is *Citrullus colocynthis*. *Citrullus colocynthis* (Cucurbitaceous), commonly known as 'bitter apple'. The plant has been used to treat constipation, Diabetes, oedema, fever, jaundice, leukaemia, bacterial infections, and cancer and used as an abortifacient[3]. *Citrullus colocynthis* Schrad is a member of the family Cucurbitaciae. This plant grows widely in the Arabian and Sahara deserts and in Sudan and it was introduced by Arabs in the middle ages to Saapain and Cyprus[4]. *Citrullus colocynthis* possess a wide range of pharmacological activities such as antimicrobial activity[5], antiinflammatory activity[6], anesthetic activity[7]. In the absence of systemic reports an attempt has been made to assess the antidiabetic, antioxidant and hypolipidemic potentials of *Citrullus colocynthis* extract in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

Citrullus colocynthis seeds were collected from Tiruvallur District, Tamil nadu. The plants were identified and authenticated by a taxonomist and a voucher specimen was deposited at the Department of Botany, University of Madras,Chennai.

Preparation of Plant extract

The *Citrullus colocynthis* seeds were dried at room temperature and powdered in an electrical grinder, which was then stored in an airtight container at 5°C until further use. The powdered root was delipidated with petroleum ether (60 - 80° C) for overnight. It was then filtered and soxhlation was performed with 95% Ethanol. Ethanol was evaporated in a rotary evaporator at 40 – 50° C under reduced pressure (yield 18.4g).

Experimental Animals

Male albino Wistar rats (150-180g) were purchased from Tamilnadu Veterinary and Animal Sciences University, MADAVARAM, Chennai. The rats were housed in polypropylene cages lined with husk and maintained in Animal house, Department of Biochemistry. It was

renewed every 24 hours. The rats were fed with commercial pelleted rats chow (VRK Nutritional Solutions, Maharashtra, India) and had free access to water. The experimental rats were maintained in a controlled environment (12:12 hours light/dark cycle) and temperature (30 ± 2°C). The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines for the investigation of experimental pain in conscious rats. The rats were acclimatized for one week before starting the experiments.

Preliminary Phytochemical Screening

The ethanolic extract of *Citrullus colocynthis* seeds were subjected to preliminary phytochemical screening [8].

Induction of Diabetes Mellitus

Diabetes was induced by single intraperitoneal injection of alloxan monohydrate dissolved in sterile normal saline at a dose 120 mg/Kg, after 18 hours fasting to induce hyperglycemia. After 1 hour alloxan administration, the animals were fed on standard pellets and water *ad libitum.* Rats were supplied with 5% glucose solution for 48 hours after alloxan injection in order to prevent severe hypoglycaemia. After 1 week time for the development and aggravation of diabetes, the rats with moderate diabetes having persistent glycosuria and hyperglycemia (Blood Glucose range of above 250 mg/dL) were considered as diabetic rats and used for the experiment. The treatment was started on the eighth day after alloxan injection and this was considered as first day of treatment.

Experimental Design

The rats were grouped into 4 groups, comprising of 6 rats in each group as follows:

Group I: Control Rats (Water and food *ad libitum*).

Group II: Alloxan induced diabetic Rats.

Group III: Diabetic rats treated with *Citrullus colocynthis seeds* extract (250 mg/Kg body weight/rat/day) in aqueous solution orally for 30 days.

Group IV: Diabetic Rats treated with gliclazide (5mg/Kg body weight/day) in aqueous solution orally for 30 days.

During the experimental period, body weight and blood glucose levels of all the rats were determined at regular intervals. At the end of the experimental period, the rats were fasted over night, anaesthetized, and sacrificed by cervical decapitation. The blood was collected with or without anticoagulant for plasma or serum separation respectively.

Preparation of tissue homogenate

The liver and pancreatic tissues were excised, rinsed in ice- cold saline. Known amount of the tissues were homogenized in Tris–HCl buffer (100 mM, pH 7.4) at 4°C, in a Potter–Elvehjem homogenizer with a Teflon pestle at 600 rpm for 3 min. The homogenate was then centrifuged at 12,000-×g for 30 min at 4°C. The supernatant was collected as tissue homogenate, which was used to assay various parameters.

Biochemical Estimations

Blood glucose level was estimated by the method of glucose oxidase/peroxidase as described by Trinde[9] and urea by Natelson et al.[10]. Plasma was separated and used for insulin assay using ELISA kit for rats. Levels of hemoglobin and glycosylated hemoglobin were estimated according to methods of Drabkin and Austin[11] and Nayak and Pattabiraman[12], respectively. Plasma was used for protein assay [13] and serum for determination of creatinine[14] and uric acid[15]. Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were assayed by the method of King et al.[16, 17]. For the estimation of glycogen, the extraction was carried out by the method of Morales et al. (1973)[18]. The pancreatic tissue homogenate was then centrifuged at 5000g to remove cellular debris and supernatant was used for the determination of lipid peroxides and enzymatic antioxidants.

Lipid peroxides were determined using thiobarbituric acid reactive substances by the method of Ohkawa et al.[19] Levels of vitamin C, vitamin E, ceruloplasmin and glutathione (GSH) were determined by the methods of Omaye et al,[20] Desai[21], Ravin[22], Sedlak and Lindsay[23], respectively. Enzymatic antioxidants such as superoxide dismutase[24], catalase[25], glutathione peroxidase[26] in pancreatic supernatant.

Oral Glucose Tolerance Test (OGTT)

At the end of the experimental period, a fasting blood sample was collected from all the groups of rats to perform oral glucose tolerance test, rats were fasted for 12 h before the test and 2 g/kg glucose solution was administered orally. Blood samples were taken by severing the tip of the tail 1 h before and at 30, 60, 90 and 120 minutes after glucose administration. Blood glucose was determined using ortho toluidine reagent.

Lipid profile

Plasma was used for the estimation of lipid profile. Cholesterol content was estimated by the method of Parekh and Jung[27]. Triglyceride was estimated by the method of Rice[28]. HDL Cholesterol fraction was separated by the precipitation techniques of Burstein and Scholnick[29] and the cholesterol content was determined by method of Parekh and Jung[27]

RESULTS

Table 1 shows the qualitative analysis of phytochemical in the ethanolic extract of *Citrullus colocynthis* seeds. The phytochemical screening of the *Citrullus colocynthis* ethanolic seeds extract revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, phenols, saponins and glycosides.

Table 1: Phytochemical screening of *C. colocynthis seeds extract*

Phytoconstituents	Inference
Alkaloids	+
Flavonoids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Tannins	+
Phytosterol	+
Triterpenoids	+
Anthraquinones	-
Phenols	+

The results presented in table 2, shows the changes of body weight in control and experimental group of rats. Diabetic rats exhibited reduction in body weight; However, oral administration of the seeds extract to diabetic rats showed a significant improvement in body weight. Similar observation was also noted in the diabetic rats treated with gliclazide.

Table 3 shows the changes in the levels of blood glucose, after oral administration of glucose (2g/ kg) in control and experimental rats. The data of OGTT revealed that the blood glucose value in control rats reach peak at 60 minutes after the oral glucose load and gradually return backs to normal levels after 120 minutes. In diabetic control rats, the peak increases in blood glucose concentration was observed after 60 minutes and stayed high over the next 60 minutes. Treatment with *Citrullus colocynthis* seeds extract showed definite lower peak blood glucose values, 60 minutes after glucose load also gives lower values almost at the end of 120 minutes.

Table 2: Effect of *C. colocynthis* seeds extract on changes in body weight of experimental groups of rats after 30 days treatment.

Groups	Body weight (g)	
	Initial	Final
Control	168.12 ± 3.54	202.82 ± 5.32
Diabetic	169.32 ± 4.75	147.09 ± 7.15*
Diabetic + *C. colocynthis* extract	163.19 ± 3.05	179.56 ± 5.91@
Diabetic + gliclazide	165.44 ± 4.92	185.26 ± 5.96@

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 4 depicts the effect of *C. colocynthis* seeds extract on the levels of blood glucose, plasma insulin, hemoglobin, glycosylated hemoglobin, and urine sugar in the experimental groups of rats. The elevated levels of blood glucose, glycosylated hemoglobin in the diabetic group of rats were reverted to near normal level by the administration of *Citrullus colocynthis* seeds extract. Conversely, the decreased levels of plasma insulin, hemoglobin in diabetic group of rats were elevated by the administration of *Citrullus colocynthis* seeds extract to diabetic rats for 30 days. Urine sugar which is present in the diabetic group of rats was absent in *Citrullus colocynthis* seeds extract as well as gliclazide treated diabetic group of rats.

Table 3: Effect of *C. colocynthis seeds extract* on the blood glucose level (mg/dl) in the experimental groups of rats receiving an oral glucose load.

Groups	Fasting	30 min	60 min	90 min	120 min
Control	91.15 ± 6.18	148.21 ± 8.54	174.26 ± 15.17	130.69 ± 13.05	100.45 ± 10.15
Diabetic	266.19 ± 19.06*	304.83 ± 24.06*	391.28 ± 27.41*	350.14 ± 24.84*	317.13 ± 21.26*
Diabetic + *C. colocynthis* extract	153.61 ± 11.84@	184.18 ± 17.14@	235.80 ± 21.94@	190.97 ± 20.02@	141.21 ± 14.99@
Diabetic + gliclazide	136.24 ± 10.19@	171.54 ± 16.45@	227.45 ± 19.19@	174.94 ± 14.49@	128.87 ± 12.41@

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 4: Effect of *C. colocynthis seeds extract* on the levels of blood glucose, plasma insulin, hemoglobin, glycosylated hemoglobin, and urine sugar in the experimental groups of rats.

Groups	Glucose (mg/dl)	Insulin (µU/ml)	Hemoglobin (g/dl)	Glycosylated Hemoglobin (%)	Urine sugar
Control	98.49 ± 10.12	15.85 ± 2.65	14.55 ± 2.26	6.47 ± 1.52	Nil
Diabetic	298.18 ± 21.36*	5.64 ± 1.12*	10.28 ± 1.78*	13.11 ± 2.59*	+++
Diabetic + *C. colocynthis* extract	146.52 ± 12.84@	10.61 ± 2.35@	12.94 ± 2.39@	8.10 ± 1.89@	Nil
Diabetic + gliclazide	123.12 ± 16.37@	12.06 ± 1.76@	13.19 ± 2.04@	7.83 ± 2.02@	Nil

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 5: Effect of *C. colocynthis seeds extract* on the levels of liver and muscle glycogen content in the experimental groups of rats.

Groups	Glycogen (mg glucose/g tissue)	
	Liver	Skeletal muscle
Control	40.06 ± 3.62	7.82 ± 0.83
Diabetic	18.44 ± 2.38*	3.67 ± 0.45*
Diabetic + *C. colocynthis* extract	32.61 ± 3.88@	5.26 ± 0.44@
Diabetic + gliclazide	30.74 ± 2.93@	5.67 ± 0.92@

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 6: Effect of *C. colocynthis* seeds extract on the levels of protein, urea, creatinine and uric acid in plasma of experimental groups of rats.

Groups	Protein (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	7.91 ± 2.04	27.64 ± 2.15	0.98 ± 0.11	2.45 ± 0.78
Diabetic	5.71 ± 1.27*	50.44 ± 5.12*	2.09 ± 0.19*	4.81 ± 1.77*
Diabetic + *C. colocynthis* extract	6.89 ± 1.05@	30.89 ± 4.76@	1.29 ± 0.10@	3.19 ± 1.34@
Diabetic + gliclazide	7.06 ± 1.56@	35.77 ± 3.81@	1.21 ± 0.13@	3.42 ± 1.68@

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 7: Effect of *C. colocynthis seeds extract* on the activity of AST, ALT and ALP in the serum of experimental groups of rats

Groups	AST	ALT	ALP
Control	66.82 ± 6.34	18.48 ± 2.46	84.51 ± 8.96
Diabetic	122.79 ± 12.68*	47.28 ± 4.61*	154.26 ± 18.94*
Diabetic + *C. colocynthis* extract	91.17 ± 10.19@	23.92 ± 3.07@	100.05 ± 11.98@
Diabetic + gliclazide	82.62 ± 8.59@	21.93 ± 2.38@	101.64 ± 14.91@

The enzyme activities are expressed as: AST and ALT µmoles of pyruvate liberated /h/mg of protein; ALP µmoles of phenol liberated/min/mg of protein. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 8: Effect of *C. colocynthis seeds extract* on the level of TBARS in plasma, pancreas, liver and kidney of experimental groups of rats.

Groups	TBARS		
	Plasma	Pancreas	Liver
Control	4.08 ± 0.79	40.14 ± 4.83	1.77 ± 0.39
Diabetic	8.22 ± 1.71*	78.44 ± 9.56*	4.38 ± 0.71*
Diabetic + *C. colocynthis* extract	5.39 ± 1.26@	58.38 ± 6.95@	2.50 ± 0.41@
Diabetic + gliclazide	5.12 ± 1.08@	56.03 ± 7.63@	2.75 ± 0.40@

Units: mM/100 g in tissues; nM/ml in plasma. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 9: Effect of *C. colocynthis seeds extract* on the activity of enzymatic antioxidants in pancreas of experimental groups of rats.

Groups	SOD	Catalase	GPx	GSH
Control	5.38 ± 1.45	15.62 ± 2.04	6.37 ± 1.01	26.12 ± 2.95
Diabetic	1.59 ± 0.61*	5.73 ± 1.38*	3.09 ± 0.42*	12.51 ± 1.73*
Diabetic + *C. colocynthis* extract	3.91 ± 0.97@	12.05 ± 1.67@	4.73 ± 0.55@	20.98 ± 2.22@
Diabetic + gliclazide	4.01 ± 0.88@	13.12 ± 1.78@	5.38 ± 0.94@	21.09 ± 2.61@

Activity is expressed as: 50% of inhibition of epinephrine autooxidation/min/mg of protein for SOD; µmoles of hydrogen peroxide decomposed/min/mg of protein for catalase; µmoles of glutathione oxidized/min/mg of protein for GPx; mg/100 g tissue for GSH. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 10: Effect of *C. colocynthis seeds extract* on the activity of enzymatic antioxidants in liver of experimental groups of rats.

Groups	SOD	Catalase	GPx	GSH
Control	16.06 ± 2.37	71.91 ± 8.20	11.36 ± 1.09	38.19 ± 5.62
Diabetic	5.09 ± 0.62*	31.26 ± 3.62*	3.74 ± 0.69*	20.29 ± 2.83*
Diabetic + *C. colocynthis* extract	10.75 ± 1.14@	62.54 ± 5.31@	8.15 ± 1.01@	30.68 ± 4.18@
Diabetic + gliclazide	12.02 ± 1.21@	65.61 ± 5.24@	9.12 ± 1.16@	32.25 ± 4.56@

Activity is expressed as: 50% of inhibition of epinephrine autooxidation/min/mg of protein for SOD; µmoles of hydrogen peroxide decomposed/min/mg of protein for catalase; µmoles of glutathione oxidized/min/mg of protein for GPx; mg/100 g tissue for GSH.
Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 11: Effect of *C. colocynthis seeds extract* on the levels of vitamin C, vitamin E, ceruloplasmin and GSH in plasma of experimental groups of rats.

Groups	Vitamin C	Vitamin E	Ceruloplasmin	GSH
Control	1.36 ± 0.14	0.65 ± 0.06	12.19 ± 1.72	30.64 ± 3.80
Diabetic	0.52 ± 0.04*	0.30 ± 0.02*	5.16 ± 0.99*	13.98 ± 3.05*
Diabetic + *C. colocynthis* extract	0.95 ± 0.09@	0.54 ± 0.09@	9.92 ± 1.36@	23.96 ± 2.97@
Diabetic + gliclazide	1.03 ± 0.15@	0.58 ± 0.04@	10.31 ± 1.78@	24.15 ± 3.16@

Units: mg/dl. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 12: Effect of *C. colocynthis seeds extract* on the levels of plasma lipid profile of experimental groups of rats.

Groups	Total cholesterol	Triglycerides	LDL	HDL
Control	75.47 ± 14.04	58.61 ± 8.79	45.28 ± 5.71	27.99 ± 2.95
Diabetic	182.61 ± 17.84*	146.28 ± 12.99*	149.52 ± 12.47*	16.85 ± 2.17*
Diabetic + *C. colocynthis* extract	99.15 ± 10.18@	74.26 ± 9.72@	75.14 ± 6.19@	22.64 ± 2.86@
Diabetic + gliclazide	102.54 ± 15.86@	91.57 ± 10.32@	80.12 ± 7.38@	20.67 ± 2.92@

Units: mg/dl. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 5 depicts the level of liver and muscle glycogen content in control and experimental group of rats. The significant decrease in liver and muscle glycogen content were observed in diabetic rats when compared with normal control rats and the level was brought back nearer to normal by oral administration of *Citrullus colocynthis* seeds extract as well as gliclazide.

The effect of oral administration of *Citrullus colocynthis* seeds extract on the levels of total protein, urea, uric acid and creatinine are presented in Table 6. The altered levels of these parameters were reverted back to near normalcy upon the treatment with the seeds extract.

Table 7 depicts the level of serum enzymes such as AST, ALT and ALP in normal control and experimental group of rats. The increased levels of these enzymes were reverted back to near normalcy upon the treatment with the seeds extract as well as gliclazide.

The level of TBARS in plasma, pancreas and hepatic tissues of control and experimental group of rats are presented in table 8. Diabetic rats showed marked increase in TBARS when compared with control rats. Upon treatment of *Citrullus colocynthis* seeds extract as well as gliclazide to the diabetic rats showed significant decrease in the levels of TBARS when compared with diabetic rats.

Table 9 and 10 shows the level of antioxidant enzymes such as SOD, Catalase, glutathione peroxidase and reduced glutathione in pancreatic and liver tissues respectively in normal control and experimental group of rats. A significant decrease in the level of antioxidant enzymes was observed in alloxan induced diabetic rats. Upon treatment with ethanolic extract of *Citrullus colocynthis* seeds as well as gliclazide to alloxan induced diabetic rats restored the level of antioxidant enzymes to normal.

The levels of non enzymatic antioxidant such as Vitamin E, Vitamin C, Ceruloplasmin and reduced glutathione in plasma of control and experimental group of rats are shown in Table 11. The diminished levels of non-enzymatic antioxidants in the diabetic group of rats were significantly improved to near normal values by the oral administration of *Citrullus colocynthis* seeds extract as well as gliclazide, after 30 days of treatment.

Table 12 depicts the level of total cholesterol, triglycerides and lipoproteins (LDL and HDL) levels of normal control and experimental group of rats. The elevated levels of lipid and lipoproteins (LDL) and reduced

level of HDL cholesterol was observed in diabetic rats than normal control and the level was restored back nearer to the normal value was achieved by administration of *Citrullus colocynthis* seeds extract as well as gliclazide.

DISCUSSION

Animal models of diabetes such as genetically derived, Nutrition induced, and chemically induced have been used extensively in diabetes research. Alloxan, a β-cytotoxin, induces diabetes through selective destruction of pancreatic beta cells which results in a decrease of insulin secretion[30]. Gliclazide, used as reference antidiabetic drug in this study, is a member of sulphonylureas. It has been proposed that sulphonylureas produce their hypoglycemic effect by increasing the release of insulin from pancreatic beta cells[31].

In recent years, various plant extracts have been claimed to be useful for the treatment of diabetes mellitus. Earlier reports suggests that the plant extracts cause antihyperglycemic effect by promoting regeneration of beta cells or by protecting the pancreas from destruction, by restricting glucose as well as by promoting unrestricted endogenous insulin action or its effect on beta cells to release insulin and activate the insulin receptors to absorb blood sugar[32].

The medicinal values of plant lie in bioactive phytochemical constituents that produce definite physiological actions on the human body. These bioctive phytochemical constituents in medicinal plants include alkaloids, flavonoids, phenolic compounds, tannins, anthracine derivative and essential oil[33]. There is a need that the medicinal plants be evaluated for phytochemistry so as to determine the potential of this indigenous source of medicines. More than 900 different phytochemicals have been found in plants and more will be discovered. These protective plant compounds are in emerging area of nutrition and health of new research reported every day.

The phytochemical screening of the *Citrullus colocynthis* ethanolic seeds extract revealed the presence of alkaloids, flavonoids, tannins, carbohydrates. The literature suggests that the phytochemicals present in seeds are flavonoids, alkaloids, glycosides, saponins, phyto-sterols, steroids, proteins and triterpenoids[34]. These chemical compounds were speculated to account for the observed pharmacological effect of the extract.

Alloxan induced diabetes is characterized by severe loss in body weight and this was also observed in the present study. The increase in body weight noticed in uncontrolled diabetes might be the result of protein wasting due to under utilization of carbohydrate as an energy source. Diabetes causes drastic changes in body weight. Similarly a significant reduction in body weight has been observed in the present study. The plant extract altered the body weight changes in diabetic rats and the observation showed the beneficial effect *of Citrullus colocynthis* extract on experimental diabetes.

Diabetes mellitus is characterized by impaired glucose tolerance due to low secretion of insulin. This is manifested by elevated blood glucose level and glycosuria, which may be accompanied by changes in lipid and protein metabolism. In control rats, the peak increase in blood glucose concentration was observed after one hour and two hours when compared with diabetic control rats[35]. The glucose tolerance effect was more pronounced after a two hour interval. When the diabetic rats were challenged with an oral glucose load, the blood glucose level reached a peak at 60 minutes and gradually decreased to pre-glucose load level around 120 minutes, the present study revealed that oral administration of the ethanolic extract of *Citrullus colocynthis* seeds improved the glucose tolerance in alloxan – induced diabetic rats.

Diabetes mellitus is characterized by decreased glucose tolerance due to low secretion of insulin or its action. This is manifested by elevated blood glucose levels and glucosuria, which may be accompanied by changes in lipid and protein metabolism. Shanie et al., (1974)[36] have reported the presence of biologically important phytochemicals responsible for the medicinal properties of herbal plants. Oral administration of *Citrullus colocynthis* seeds extract to alloxan induced diabetic rats resulted in activation of beta cells and granulation returns to normal insulinogenic effect. Since the percentage fall in plasma glucose level was different in models with varying intensity of hyperglycemia it implies that the anti hyperglycemic effect of plant is based on the dose of diabetogenic agent and on the degree of β- cell destruction[37] (Grover et al., 2000). The hypoglycemic activity of *Citrullus colocynthis* extract was compared with gliclazide, a standard drug. The blood glucose levels were significantly decreased in the extract treated diabetic rat. A single dose of the plant extract could markedly reduce the blood glucose level in diabetic rats indicating the hypoglycemic potential of the extract. Urine sugar which was present in the diabetic groups of rats was found to be absent in the rats treated with extract indicating the improved glucose homeostasis.

Glycosylated hemoglobin is considered as a gold-standard marker for accurate and reliable measurement of fasting glucose, which is strongly associated with level of ambient glycemia during a 3-month period and indicates the degree of glycation of proteins. Chronic hyperglycemia results in glycosylation in which excess glucose nonenzymatically reacts with hemoglobin to form glycosylated hemoglobin. The rate of glycosylation is proportional to the concentration of blood sugar at the peak of glucose tolerance curve correlates with glycosylation[38] and with the improvement of glycemic control. In the present study, the effect of administration of *Citrullus colocyinthis* extract tend to bring the altered levels of hemoglobin and glycosylated hemoglobin indicating the improved glycemic control.

Glycogen is the stored form of glucose. In food, the carbohydrate breaks down and absorbs it as glucose. Liver plays a crucial role in the regulation of glucose homeostasis and is capable of synthesizing and degrading glycogen in its parenchyma cells. Regulation of glycogen metabolism *in vivo* is multifunctional and glycogen synthase and phospharylase plays a major role in the above process[39]. Muscle is generally believed to represent the principal site of insulin resistance in type 2 diabetes. Once taken up by the muscle, glucose can be oxidized to carbon dioxide, converted to lactate, which is released into the blood, or stored as glycogen or fat. In the present study, oral administration of *Citrullus colocynthis* seeds extract to the diabetic rats restored the level of glycogen by means of decreasing the activity of glycogen phosphorylase and increasing the activity of glycogen synthase.

Dighe et al (1984)[40] reported that accelerated proteolysis of uncontrolled diabetes occurs as a result of deranged glucagon mediated regulation of CAMP formation in insulin deficiency. Oral administration of *Citrullus colocynthis* extract to diabetic rats significantly inhibit proteolysis caused by insulin deficiency and thus increased the level of plasma protein to near normal levels. The levels of protein, urea and creatinine were brought back to near normal upon treatment with *Citrullus colocynthis* extract probably by decreasing proteolysis.

Uric acid, one of the major endogenous water-soluble antioxidants of the body, has been thought to be a metabolically inert end product of purine metabolism[41]. Elevated levels of serum uric acid are due to either an increase in uric acid production or a decrease in its excretion. It is a biomarker for the development of diabetic complications. The levels of serum uric acid in diabetes induced rats were found to increased *Citrullus colocynthis* extract treatment for the diabetic rats significantly ameliorated the toxic effects of alloxan as indicated by the restoration of serum uric acid levels.

The activities of serum transaminase and alkaline phosphatase were found to be significantly increased in the diabetic rats. The enzymes directly associated with the conversation of aminoacid to keto acid are aspartate transaminase and alanine transaminase. Various researchers observed the elevated levels of this transaminase in diabetic rat serum, liver and kidney tissue[42]. The elevated levels of serum, liver and kidney AST and ALT in diabetes induced animal demonstrated hepatic cellular dysfunction. Upon treatment with *Citrullus colocynthis* extract, the AST and ALT levels reached near normal status which indicates the non toxic as well as organ protective role of the extract.

Alkaline phosphatase is a non specific hepatic marker enzyme, which is utilized for hydrolysis of various ester orthophosphates under alkaline condition. It reflects the pathophysiological alterations in bile acid synthesis[43]. In the present study, elevated levels of serum ALP were noted in alloxan-induced diabetic rats and similar results have been reported by many workers[44, 45]. *Citrullus colocynthis* extract treatment of the diabetic rats significantly reduced the toxic effects of alloxan as indicated by the normalized serum ALP levels.

Free radicals and associated oxidative stress have been implicated in the pathological changes in diabetes mellitus. Persistent hyperglycemia causes increased production of oxygen free radicals such as superoxide ($O_2^{\cdot-}$), hydrogen peroxides (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) through auto-oxidation of glucose[46] and non-enzymatic protein glycation. These highly reactive species exert their cytotoxic effect on membrane phospolipid and cause a wide spectrum of cell damage, including lipid per oxidation, inactivation of antioxidant enzymes, alterations in the intracellular oxidation-reduction state and DNA damage[47].

Diabetes is usually accompanied by increase production of free radicals or impaired antioxidant defenses. Thiobarbituric acid reactive substances (TBARS) are commonly measured as a direct marker of oxidative stress; they are formed from breakdown products during the oxidation of unsaturated fatty acids by reactive oxygen species' attack. Lipid peroxides, hydroperoxides and protein carbonyls are the secondary products of oxidative stress and are unleashed as a result of the toxic effect of reactive oxygen species produced during lipid peroxidation in diabetes[48]. There are several reports in the literature demonstrated the elevated levels of lipid peroxides, hydroperoxides and protein carbonyls in the hepatic tissues of experimental diabetic model[49]. This normalization may be accomplished by the antioxidant and free radical quenching nature of seeds extract.

The pancreatic β cells confine extremely low levels of enzymatic antioxidants such as SOD, catalase, GPx and GST, it is very crucial to avert any extreme oxidative stress that may overwhelm the limited antioxidative defense capacity of the β cells. However, this low defense can be easily overwhelmed in hyperglycemia mediated oxidative stress[50]. Supraphysiological glucose not only engenders excessive reactive oxygen species, but also attenuates antioxidative machinery through glycation of enzymatic antioxidants. Chronic hyperglycemia resulted in the decreased antioxidant competence both in liver as well as pancreatic tissues. Oral administration of the seeds extract to the diabetic rats restored the impaired activities of the enzymatic antioxidants in liver and pancreas indicating the antioxidant potential of the seeds extract which may significantly reduce the prevailing oxidative stress.

The antioxidant vitamins may ameliorate diabetes mellitus especially as a protective factor against free radical generation[51]. Vitamin E is also responsible for protecting PUFA against lipid peroxidation and its deficiency in diabetes may be due to their exhaustion during detoxification of free radicals produced by membrane lipid peroxidation[52]. The declined levels of

vitamins C and E and reduced glutathione were due to the increased production of free radicals. However, oral administration of *Citrullus colocynthis* seeds extract to diabetic group of rats showed a significant increase in levels of these non-enzymatic antioxidants, thereby suggesting the free radical scavenging potential of *Citrullus colocynthis* seeds extract which in turn may be responsible for its antioxidant property.

Diabetes is associated with the profound alterations in the lipid and lipoprotein profile as well as an increased risk of premature atherosclerosis, coronary insufficiency and myocardial infarction[53]. The serum lipid profile may provide an additional tool for monitoring the degree of severity and control of diabetes mellitus. The increased level of serum lipids in diabetic subjects is mainly due to the increased mobilization of free fatty acids from peripheral deposits[54]. The impairment of insulin secretion results in enhanced mobilization of lipids from the adipose tissue to the plasma. In the present study, increased level of serum and tissue lipids were brought back to almost upon treatment with *Citrullus colocynthis* extract indicating the hypolipidemic action of the extract.

CONCLUSION

The result of the present study indicates that the *Citrullus colocynthis* seeds extract has hypoglycemic, hypolipidemic and antioxidant activities. The possible mechanism of the antidiabetic action of *Citrullus colocynthis* may be through inhibitory effect of glucose absorption, increased incorporation of circulating glucose as hepatic glycogen and enhanced secretion of insulin. Extraction, isolation and identification of molecules responsible for the observed beneficial effects of *Citrullus colocynthis* seeds may provide a better understanding mechanism of the action of seeds in maintaining normoglycemia in experimental diabetes.

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