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Effect of Citrullus colocynthis on glucose and lipid profiles

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ABSTRACT

Diabetes mellitus is a wide spread disease. Since ancient era, various drugs have been utilized as the treatment of this disease in the traditional Iranian medicine. In the present study, the effects of aqueous extract of seeds of *Citrullus Colocynthis* on serum glucose levels and lipidprofile in an animal model was evaluated. Eighteen male adult rats of Wistar strain (with average corporal mass of 200 to 250 g) were divided randomly into three groups (treatment, sham operated and control) and maintained in isolated cages. Diabetes was induced with injection of Streptozotocin (60 mg/kg, i.p.) for the treatment and sham operated groups and the control group was given an injection of normal saline. The treatment group received aqueous extract of *C. Colocynthis* seeds (200 mg/kg) via intra gastric for three weeks.

Then their blood samples were taken to be evaluated for glucose and lipids levels. According to the one way analysis of variance(ANOVA), it was verified that the administration of the extract of *C. Colocynthis* seeds promoted a significant decrease in blood levels of glucose, cholesterol and triglycerides ($p<0.01$) in diabetic rats in comparison with control group. From the results obtained in this study, it was proposed that the extract of *C. colocynthis* seeds can have significant effects on various blood glucose and lipids in diabetic rats, thus further studies are necessary to elucidate the extent and mechanism of these changes.(1)

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies [2]. Antihyperglycemic effects of these plants were attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. However, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, Flavonoids, cartenoids, etc., that arefrequently

implicated as having antidiabetic effect [3]. *Citrullus colocynthis* demonstrates multiple beneficial antidiabetic mechanisms, including modulation of carbohydrate metabolism, restoration of beta-cell integrity, insulin-releasing activity and improvements in glucose uptake utilization [4]. Moreover, it decreases gluconeogenesis and inhibits release of counter-regulatory hormones. On the other hand, it has been suggested that the mechanism responsible for the serum glucose lowering effect of *Citrullus colocynthis* were attributed to an inhibitory effect of glucose absorption ; an increased incorporation of circulating glucose as hepatic glycogen, or an enhanced secretion of insulin. Furthermore, components of Citrullus colocynthis extract appear to have structural similarities to animal insulin, as measured by electrophoresis and infrared spectrum analysis [5]. Accordingly, this study was performed to

investigate the hypoglycemic, antioxidant and hypolipidemic effect of *Citrullus colocynthis on* experimentally induced diabetic rats.(2)

EXPERIMENTAL

The Plant material The plant material (root) was collected from Kanpur dist. U.P. India. The plant material was identified and authenticated taxonomically by National Botanical Research Institute (NBRI), Lucknow. A voucher specimen of the collected sam-ple was deposited in the institutional herbarium for future reference (Voucher specimen number is NBRI/CIF/84/2009).

Preparation of extracts

The shade dried plant materials was crushed, powdered and exhaustively defatted by petroleum ether (60ñ80 C) and then successively extracted with

benzene, chloroform, ethyl alcohol and water. All the extracts were filtered, pooled and concen-trated under reduced pressure using rotavapor(Buchi, USA.)

Preliminary phytochemical analysis

The preliminary phytochemical screening of extract of Citrullus colocynthisgave positive tests for carbohydrates, resins, saponin, anthraquinone, steroids and alkaloids (5).

Physicochemical parameters (6, 7)

Physicochemical parameters of the powdered drug such as loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol and water soluble extractive values for the root of Citrullus colocynthis were performed according to the standard methods.

Test animals:

Wistar rats (180ñ200 g) and Swiss albino mice C, relative humidity 55 ± 10% and12 h light/dark cycle. The animals were maintained under standard pellet diet and water ad libitumin animal house of BBDNITM, Lucknow, India. Initial body weight of each animal was recorded and they were given seven days time to get acclimatized to the laboratory conditions.

All experiments were per-formed according to institution animal ethical com-mittee (IACE) (Approval No. BBDNITM/IAEC/ CLEAR/09/2009).

Acute toxicity determination:

The acute toxicity study was carried out in adult Swiss albino mice by ìfix doseî method according to OECD (Organization for Economic Co-operation and

Table 1. Effect of *Citrullus colocynthis* on oral glucose tolerance test in normal fasted rats.

Group	Blood glucose level (mg/dL)				
	0 min	30 min	60 min	120 min	180 min
I	56.78 ± 1.6	150.43 ± 1.8	148.46 ± 1.7	157.67 ± 1.0	141.34 ± 0.7
III	58.78 ± 0.79	134.56 ± 1.6**	122.45 ± 1.1**	108.67 ± 0.6**	98.76 ± 0.4**
IV	60.46 ± 0.88	143.10 ± 1.36*	143.21 ± 1.25**	140.23 ± 0.32**	139.10 ± 0.98*
V	59.34 ± 1.15	142.10 ± 1.87**	140.56 ± 1.10**	132.45 ± 0.61**	120.54 ± 0.32**
VI	57.47 ± 1.04	136.23 ± 2.10**	128.21 ± 1.45**	118.60 ± 0.87**	114.23 ± 0.36**

Values are the mean ± SEM; n = 6, *p < 0.05,**p < 0.01 *vs.* disease control. Group I: control, Group II: standard (metformin), Group III: disease control, Group IV: chloroform extract, Group V: ethanol extract, Group VI: aqueous extract.

Development) guideline no. 420. Test procedure with fixed dose of 2000 mg/kg b.w. was adopted. The animals were fasted overnight and next day extracts of the plant Citrullus colocynthis (suspended in 0.5% w/v sodium CMC) were administered orally at dose level 2000 mg/kg. Then, the animals were observed continuously for three hours for general behavioral, neurological, autonomic profiles and then every 30

min for next three hours and finally for mortality after 24 hours till 14 days (8, 9).

Table 4. Effect of *Citrullus colocynthis* on lipid profile.

Group	Serum cholesterol (mg/dL)	Serum triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
I	111.72 ± 14.35	85.96 ± 12.35	33.22 ± 3.56	88.45 ± 9.80
II	160.87 ± 12.45	160.67 ± 16.15	25.43 ± 5.30	198.56 ± 12.34
III	85.98 ± 13.24**	85.67 ± 14.56**	44.45 ± 3.56*	95.20 ± 7.80**
IV	104.10 ± 13.20*	102.10 ± 13.63**	41.98 ± 0.30*	141.78 ± 11.34**
V	98.32 ± 13.25**	98.32 ± 13.43*	42.31 ± 0.25*	124.56 ± 7.8**
VI	92.56 ± 11.25**	92.65 ± 11.25**	47.65 ± 4.32**	110.65 ± 12.34**

Values are the mean ± SEM; n = 6, *p < 0.05,**p < 0.01 *vs.* disease control. Groups designation see Table 1.

Table 3. Effect of *Citrullus colocynthis* on the blood glucose level in alloxan-induced hyperglycemia in rats.

Group	Blood glucose level (mg/dL)			
I	0 day	3rd day	5th day	7th day
II	86.11 ± 0.98	85.67 ± 0.58	84.68 ± 0.54	86.23 ± 0.48
III	192.34 ± 1.56	210.44 ± 0.68	232.42 ± 1.27	247.68 ± 1.24
IV	188.45 ± 1.99	156.88 ± 0.82**	125.77 ± 1.45**	104.10 ± 1.72**
V	181.18 ± 1.06	208.010 ± 0.45*	180.23 ± 1.21**	161.10 ± 1.89**
VI	183.25 ± 1.02	206.45 ± 0.25**	175.45 ± 1.32**	158.31 ± 1.41**

Values are the mean ± SEM; n = 6, *p < 0.05,**p < 0.01 *vs.* disease control. Groups designation see Table 1.

Effects of Citrullus colocynthisroot extracts on glucose tolerance in rats:

All the animals were fasted overnight before experimentation but allowed free access to water. Fasted rats were divided into five groups of six rats each. Group I served as a control and received vehicle only. Group II received metformin which was used as standard. Groups IIIñV received chloroform, ethanol and aqueous extracts respectively at a dose of 200 mg/kg b.w. as a fine aqueous suspension (suspended in 0.5% w/v sodium CMC) orally. The rats of all groups were given glucose (2 g/kg b.w., p.o.) 30 min after administration of the drug. Blood samples were collected from the tail vein just prior to glucose administration and at 30, 60, 120 and 180 min after

the glucose loading. Blood glucose levels were measured by glucometer (Accu Chek) (10, 11).

Effect of Citrullus colocynthisroot extracts on alloxan-induced diabetic rats:

Male Wistar rats (180ñ200 g) were made diabetic by i.p. injection of 120 mg/kg b.w. of alloxan mono-hydrate in sterile normal saline. The rats were main-tained on 10% glucose solution for next 24 h to prevent hypoglycemia. Three days later blood samples were drawn from tail vein and glucose levels were deter-mined to confirm the development of diabetes(175ñ350 mg/dL). The diabetic rats were divided into six groups, each containing six animals. Group I served as normal control and received vehicle only. Group II served as a disease control and received alloxan only. Group III served as a positive control and

received metformin (11.3 mg/kg), while Citrullus colocynthis chloroform, ethanol, and aqueous extracts of roots were given to groups IVñVI, respectively, at a dose of200 mg/kg, orally (12, 13). The blood glucose concen-trations of the animals were measured at the beginning of the study and the measurements were repeated on 0, 3rd, 5th and 7th day after the start of the experiment.

RESULTS AND DISCUSSION

The moisture content was 10.13%, which was not so high as to facilitate bacterial growth. The other physicochemical parameters which ascertain the quality, purity and also help in evaluating the crude drug, are the ash value, acid insoluble ash value and water soluble ash value, which were determined to be not more than 11.33% w/w, 3.5% w/w and 1%

w/w, respectively, which indicated the presence of the total foreign inorganic matter. The alcohol soluble extrac-tives and water soluble extractives are 11.70% and26.70%, respectively. Phytochemical screening showed the presence of glycosides (saponin glyco-sides), triterpenoids, alkaloids, flavanoids and resins. The acute oral toxicity study of Citrullus colo-cynthisshowed no mortality up to 2000 mg/kg. The effects of extracts of Citrullus colocynthis(2000 mg/kg b.w.) on glucose tolerance test are shown in Table 1. Citrullus colocynthisshowed significant blood glucose lowering effect in the glucose toler-ance test in 2 h. This result indicates that the test extracts of roots showed reduction of glucose level. The antidiabetic effect of the extracts on the fast-ing blood sugar levels of diabetic rats is shown in Table3. Administration of alloxan (120 mg / kg, i.p.) leads to1.5 fold elevation of fasting blood glucose levels.

One week of daily treatment of chloroform, ethanol, and aqueous extracts of Citrullus colocynthislead to a dose-dependent fall in blood sugar levels by 25% to50%. The effect of the extracts on body weight in the alloxan (120 mg / kg b.w. i.p.) induced diabetic rats is given in Table 2. In disease control ratís body weight were reduced significantly. The body weight was slightly increased in the normal control rats compared to initial body weight. The extracts (200 mg/kg b.w.) as well as metformin (11.3 mg/kg b.w.) treatment signifi-cantly prevented this reduction in body weight. The effect of extracts of Citrullus colocynthisin alloxan (120 mg/kg b.w. i.p.) induced rats is shown in Table 3. The result showed the significant difference between experimental and diabetic rats in lowering fasting blood glucose level. The aqueous extract of roots of Citrullus colocynthisshowed maximum reduction of 58.70% (p

< 0.01) compared to chloro-form and ethanol extracts, which reduced 34.72% and36.60%, respectively. The alloxan monohydrate induces diabetes mellitus in rats by selective necrotic action on the β-cells of pancreas leading to insulin deficiency, which leads to various metabolic aberrations in animals, increased blood glucose level (17), decreased protein content (18), and increased levels of cholesterol and triglycerides (19). The animals treated with test extracts showed significant results when compared with alloxan-treated group. The significant decrease in the blood glucose levels of diabetic rats treated with the extracts may be by stimulation of residual pancreatic mechanism or by probably increasing peripheral utilization of glucose. The aqueous extract of roots of Citrullus colocyn this showed significant reduction in blood sugar level when compared with standard groups ($p < 0.01$). The effect of

the extracts on diabetes induced hyperlipedemia was also studied. It was observed that due to diabetes there was an increase in the cholesterol levels as well as triglycerides levels. The HDL levels were reduced in the diabetic animals and the LDL lev-els were increased significantly. All the extracts showed a significant decrease in the cholesterol, triglyceride and LDL levels. In particular, the aqueous extract of roots of Citrullus colocynthisshowed most prominent action. It also increased the HDL levels as compared to the standard drug. (Table 4). The serum creatinine and serum urea levels were found to significantly increase in the diabetic rats. The extract treated animals showed a signifi-cant reversal in the levels as compared to diabetic rats. The aqueous extract of roots of Citrullus colocynthis showed a more decreasing capacity, a sig-nificant increase was also observed in the serum protein level (Table 5). It is well

known that the alloxan monohydrate affect the insulin deficiency, which directly affects the liver function (20). The rise in serum level of total bilirubin, conjugated bilirubin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transminase (SGPT) and alkanine phoso-hatase (ALP) have been attributed to the damaged structural integrity of the liver. Hence, the extracts were subjected to liver function tests which mediated reduction in level of bilirubin total, conjugated bilirubin, SGOT, SGPT and ALP towards normal values that may indicate the stabilization of plasma membrane as well as repair of hepatic tissue damage (Table 6). The model used to induce diabetes was alloxan-induced diabetes which is almost comparable to type I diabetes model with near β-cell destruction (21).

CONCLUSION

From the above discussion it is concluded that aqueous extracts of Citrullus colocynthis(2000 mg/kg) exhibited significant antidiabetic activity in alloxan-induced diabetic rats. These extracts also showed improvement in such parameters like body weight, lipid profile serum creatinine, serum urea and serum protein as well as enzyme levels of liver. Further inves-tigation is necessary to determine the exact phytocon-stituents responsible for antidiabetic effect.

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