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NATURAL DRUGS

HYPOGLYCEMIC EFFECTS OF *CITRULLUS COLOCYNTHIS* ROOTS

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Abstract: The aim of this study was to examine the effect of root of *C. colocynthis* on the biochemical parameters of normal and alloxan-induced diabetic rats. Diabetes mellitus was induced by intraperitoneal (120 mg/kg b.w.) injection of alloxan monohydrate for three days and the animals showing blood glucose level in the range of 175–300 mg/dL were selected for study. The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated on 3rd, 5th and 7th day after the start of the experiment. On day 7, blood was collected by cardiac puncture under mild ether anesthesia. Aqueous extract of roots of *Citrullus colocynthis* showed significant reduction in blood sugar level (58.70%) when compared with chloroform (34.72%) and ethanol extracts (36.60%) ($p < 0.01$). The aqueous extracts showed improvement in parameters like body weight, serum creatinine, serum urea and serum protein as well as lipid profile and also restored the serum level of bilirubin total, conjugated bilirubin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transminase (SGPT) and alkaline phosphatase (ALP).

Keywords: *Citrullus colocynthis*, alloxan, bilirubin total, conjugated bilirubin, SGOT, SGPT, ALP

Diabetes mellitus is a disease in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by insulin resulting in elevation of fasting and postprandial blood glucose levels (1).

The *Citrullus colocynthis*, also known as bitter apple, bitter cucumber, egusi, is a viny plant mainly found in Mediterranean Europe, Asia, Turkey, Nubia, Trieste, Egypt, Iran, Pakistan, Afganistan, India and North Africa. It mainly contains glycosides; cucurbitacins B, E, I and cucurbitacin E-2 glucoside, citrullol, alkaloids, resin and gums. It is a proven antioxidant, antimicrobial, antimalarial, hepatoprotective, antispermatogenic and carcinogenic. The fruits are traditionally used against poisonous bites of dogs, snake bites and also used as an enema (2–4).

The plant subjected for the current research work had been used traditionally as antidiabetic, the fruit is scientifically proved as antidiabetic; therefore, it was thought interesting to evaluate the antidiabetic profile of the selected plant part (root), which has not yet been scientifically undertaken.

EXPERIMENTAL

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 sample 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 (60–80°C) and then successively extracted with benzene, chloroform, ethyl alcohol and water. All the extracts were filtered, pooled and concentrated under reduced pressure using rotavapor (Buchi, USA.)

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Preliminary phytochemical analysis

The preliminary phytochemical screening of extract of *Citrullus colocynthis* gave 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 (180–200 g) and Swiss albino mice (25–30 g) of either sex were used for the study. The animals were kept under standard conditions temperature 23 ± 2°C, relative humidity 55 ± 10% and 12 h light/dark cycle. The animals were maintained under standard pellet diet and water *ad libitum* in 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 performed according to institution animal ethical committee (IACE) (Approval No. BBDNITM/IAEC/CLEAR/09/2009).

Acute toxicity determination

The acute toxicity study was carried out in adult Swiss albino mice by "fix dose" method according to OECD (Organization for Economic

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.

Table 2. Effect of *Citrullus colocynthis* on body weight.

Group	Initial weight (g)	Final weight (g)
I	162.47 ± 3.98	179.67 ± 3.42
II	165.24 ± 6.74	125.87 ± 6.67
III	164.34 ± 6.45	158.23 ± 4.98
IV	170.54 ± 7.60	142.21 ± 3.77
V	166.56 ± 6.88	153.91 ± 4.65
VI	172.87 ± 5.66	149.19 ± 5.67

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.

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 5. Effect of *Citrullus colocynthis* on serum creatinine, serum urea and serum protein.

Group	Serum creatinine (mg/dL)	Serum urea (mg/dL)	Serum protein (g/dL)
I	0.48 ± 0.92	24.31 ± 1.18	7.54 ± 0.37
II	1.45 ± 0.04	54.56 ± 1.20	4.05 ± 0.45
III	0.52 ± 0.13**	30.25 ± 1.34**	6.92 ± 0.32**
IV	0.63 ± 0.34*	48.69 ± 1.32*	6.05 ± 0.51**
V	0.61 ± 0.22*	45.32 ± 1.45**	6.10 ± 0.428**
VI	0.57 ± 0.80**	32.45 ± 1.30**	6.47 ± 0.34**

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

Table 6. Effect of *Citrullus colocynthis* on liver function tests.

Group	Serum bilirubin total (mg/dL)	Serum conjugated bilirubin (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	Alkaline phosphatase (IU/L)
I	0.28 ± 0.03	0.14 ± 0.05	123.08 ± 15.43	88.75 ± 7.65	205.39 ± 5.76
II	1.24 ± 0.07	0.47 ± 0.06	272.34 ± 14.27	158.45 ± 6.78	293.56 ± 11.23
III	0.34 ± 0.05**	0.10 ± 0.04**	156.34 ± 15.24**	98.67 ± 7.43**	222.34 ± 11.54**
IV	1.12 ± 0.08**	0.24 ± 0.07*	198.30 ± 18.43*	134.31 ± 5.78*	246.10 ± 13.20*
V	0.79 ± 0.06**	0.17 ± 0.05**	136.20 ± 17.42**	113.21 ± 4.65**	242.20 ± 13.25*
VI	0.34 ± 0.03**	0.14 ± 0.02**	131.10 ± 13.65**	112.44 ± 4.67**	228.30 ± 12.87**

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

Co-operation and 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).

Effects of *Citrullus colocynthis* root 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 III–V 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 colocynthis* root extracts on alloxan-induced diabetic rats

Male Wistar rats (180–200 g) were made diabetic by *i.p.* injection of 120 mg/kg b.w. of alloxan monohydrate in sterile normal saline. The rats were maintained 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 determined to confirm the development of diabetes (175–350 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 IV–VI, respectively, at a dose of 200 mg/kg, orally (12, 13). The blood glucose concentrations 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. On day 7, blood was collected by cardiac puncture under mild ether anesthesia. The inference was made by comparing blood glucose level, body weight, serum creatinine, serum urea, serum creatinine, serum triglycerides, serum total cholesterol, SGOT, SGPT, conjugated bilirubin, and alkaline phosphatase (ALP) with positive control (metformin) and negative control (alloxan treated) group (14, 15).

Statistical analysis

All data were represented as the mean ± SEM. Statistical comparison of data was made by means of one way ANOVA using Dunnett's test; $p < 0.05$ was considered as significant (16).

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 extractives and water soluble extractives are 11.70% and 26.70%, respectively. Phytochemical screening showed the presence of glycosides (saponin glycosides), triterpenoids, alkaloids, flavanoids and resins.

The acute oral toxicity study of *Citrullus colocynthis* showed 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 colocynthis* showed significant blood glucose lowering effect in the glucose tolerance 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 fasting blood sugar levels of diabetic rats is shown in Table 3. Administration of alloxan (120 mg / kg, *i.p.*) leads to 1.5 fold elevation of fasting blood glucose levels. One week of daily treatment of chloroform, ethanol, and aqueous extracts of *Citrullus colocynthis* lead to a dose-dependent fall in blood sugar levels by 25% to 50%. 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 rat's 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 significantly prevented this reduction in body weight.

The effect of extracts of *Citrullus colocynthis* in 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 colocynthis* showed maximum reduction of 58.70% ($p < 0.01$) compared to chloroform and ethanol extracts, which reduced 34.72% and 36.60%, 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 colocynthis* 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 levels 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 colocynthis* showed 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 significant reversal in the levels as compared to diabetic rats. The aqueous extract of roots of *Citrullus colocynthis* showed a more decreasing capacity, a significant 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 phosohatase (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 investigation is necessary to determine the exact phytoconstituents responsible for antidiabetic effect.

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