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Hypo and Antihyperglycaemic Effect of *Citrullus colocinthis* L. Seeds in Normal and Streptozotocin-Induced Diabetic Rats

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Citrullus colocinthis L. is used in Algerian folk medecine for the treatment of diabetes mellitus. Intra peritoneal administration of the aqueous extract of the seed (1.25 g/kg body weight) to streptozotocin-induced diabetic rats produced a significant reduction of blood sugar level in the long term, while the same extract produced no alteration of glycaemia in normal rats in the short term. The aqueous extract of this plant seems to have maximal adverse effect and high LD_{100} value.

Key Words: *Citrullus colocinthis* L., Hypoglycaemic, Glucose, Extracts, Streptozotocin.

INTRODUCTION

Through the ages, plants have been used as a source of drugs that were administered empirically to sick people. Some of these plants and herbs had been applied for treatment of diabetes mellitus[1]. *Citrullus colocinthis* L. which is known in Arabic language as 'Hantal' is a perennial herbaceous vine, indigenous to warm and desert regions of Africa and Asia where it is occasionally cultivated. This plant was well known by Greeks, Romans and Arab physicians[2]. It is an old drug used in Muslim and Hindu medicine.

Several workers reported that *C. colocinthis* possesses various activities such as antiinflammatory[3,4] and antitumoral[5]. The fruits are widely used medicinally, especially for stomach pains. The pulp, because of its content of glucosides, such as colocynthin, is a drastic hydragogue, cathartic and laxative[6,7]. The fruits were exported as a laxative from Gaza Strip to Europe in the early 20th century[8]. On the other hand the ingestion of cathartic fruit can have many undesired effects, including changes in the colon with other

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laxative abuse[9]. The seeds are edible and when ground provide a rude bread for the desert Bedouins[10]. In the oriental Morocco, *C. colocinthis* was one of 38 plants cited treat diabetes[11]. The present ethnopharma-cological survey revealed that *C. colocinthis* is used by diabetics to two forms: (i) In bath: a fresh fruit is placed under feet until the patient feels his saliva becomes bitter. (ii) Oral administration of seeds: generally one to two seeds per day are swallowed.

The present study showed that the aqueous extract of seeds possesses an antihyperglycaemic effect in streptozotocin-induced diabetic rats, whereas the same extract induces no variation in plasma glucose in normal rats. Obviously, further studies aiming at clarifying the pharmacological effects of *C. colocinthis* are needed and that is why this study represents an attempt to: (a) document the antidiabetic effect of the plant and to test if the seeds actually contain any antihyperglycaemic agent, (b) examine the activity of the aqueous extract on normal and diabetic rats, (c) explore the antihyperglycaemic effect by enzymatic dosage, (d) determine the serum glucose, triglycerides and ketonuria when the extract is injected intraperitoneally (i.p.) and (e) determine the lethal dose of aqueous extract of seeds in normal rats.

EXPERIMENTAL

C. colocinthis was collected from Bechar region (Bidandou) in autumn and was dried at 25 °C. A voucher specimen of the plant was identified and authenticated at the Laboratory of Botany at the Biology Institute. The dried plant was then separated into roots, leaves, stems, barks, seeds and pulp. Then each part was ground by an electrical mill mesh and the powdered parts were stored separately in nylon bags in a deep freeze until the time of use.

Preparation of extracts: To prepare an aqueous extract, a suspension of seeds 50 g in 100 mL of distilled water was magnetically stirred at 45 °C for 6 h. The residue was removed by filtration, centrifuged at 500 g × 5 min and the extract evaporated to dryness at low temperature (< 40 °C) under reduced pressure in a rotary evaporator to give yellowish-brown material 3.25 g. The residue was dissolved and kept in brine[12].

Preparation of streptozotocine: Streptozotocine (65 mg/kg of body weight) was dissolved in an aqueous NaCl solution (0.9 %) and injected intraperitoneally (i.p.).

Animals used: The present tests were achieved on 92 female and male adult Wistar rats (250 ± 70 g).

All the animals were housed in an air-conditioned animal room at 25 °C and fed with equilibrated food and water *ad libitum*. They were divided in three groups.

Group I: (n = 36 rats), for determination of lethal dose (LD_{100}).

Group II: (n = 24 rats), for long term evaluation effect of *C. colocinthis* treatment of normal and diabetic rats (47 d).

Group III: (n = 32 rats), for short term evaluation effect of *C. colocinthis* treatment of normal and diabetic rats (4 h).

Collection of blood samples: Blood samples were collected by needle puncture from the retro-orbial sinus. This technique eliminates the need for the anaesthetic agents which affect biochemical parameters. Blood was collected in a test-tube containing heparin and centrifuged (1200 g × 15 min). The plasma used for glucose and lipids assays was stored at -25 °C.

Analytical techniques: Plasma glucose[13] is measured by enzymatic colorimetric method using a test kit (Prochima, Algeria). Rats were considered diabetic when glycosurea was positive[14]. The blood glucose was measured by glucose oxidase method[15].

Statistical analysis: Data are expressed as the average ± standard error (SEM). Student's t-test of significance was used to evaluate the data[16].

Determination of LD_{100}: Different doses of the aqueous extract of the seeds parts of the plant were administered intraperitoneally to eight lots (n = 4 rats each). The ninth lot served for control and was administered physiological water (NaCl 0.9 %) for 2 h. They were kept in transparent plastic cages at 25 °C. Mortality was noted after 24 h.

RESULTS AND DISCUSSION

Table-1 shows that the lethal dose was 12.5 g/kg when the extract was administered by intraperitoneal injection in normal rats, whereas the other doses prepared by dilution of the mother solution presented no toxicity at all.

TABLE-1
LETHAL DOSE IN RATS FED WITH 12.5 g/kg OF AQUEOUS EXTRACT OF SEED PART OF *C. colocinthis*

Lot's no.	Average weight (g)	Dose (g/kg)	Mortality (%)
1	271.25 ± 13.67	0.02	0
2	296.50 ± 12.63	0.12	0
3	247.00 ± 05.92	0.25	0
4	277.25 ± 11.24	1.25	0
5	275.00 ± 11.56	2.50	0
6	257.50 ± 08.56	5.00	0
7	187.33 ± 01.32	7.50	0
8	211.75 ± 17.49	12.50	100
9	293.50 ± 12.13	NaCl (0.90 %)	0

The rats that were fed with amounts of 0.02, 0.12, 0.25 and 1.25 g/kg of *C. colocinthis* aqueous extract react well after treatment, but rats that were fed with amounts of 2.5, 5 and 7.5 g/kg showed a faintness accompanied by a loss of equilibrium after 2 h the injection, as compared to witnesses that were injected physiological water. The lethal amount of *C. colocinthis* is assessed to 12.5 g/kg concentration. In order to avoid any risk of toxicity in the rats, we have limited the amount of the extract to 1.25 g/kg.

After determination of the lethal dose, we have tested the activity of the aqueous extract of seeds at short (4 h) and long time (47 d). The results presented in Fig. 1 show that administration of 1.25 g/kg of an aqueous extract of seeds to diabetic rats caused a significant reduction ($p < 0.05$) in plasma sugar after 3 h treatment and became highly significant ($p < 0.01$) 4 h later. It is suggested that *C. colocinthis* has an antihyperglycaemic effect which appears significant starting from the after 3 h treatment and increased up to 4 h.

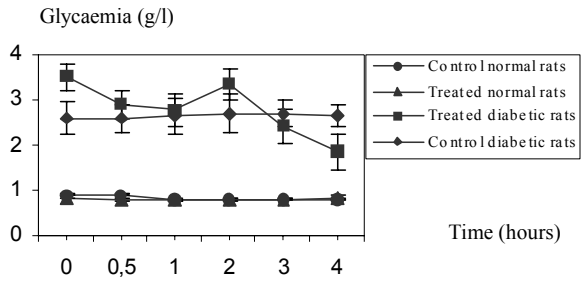

Fig. 1. Blood sugar level in rats intra peritoneal injected with 1.5 g/kg of *C. colocinthis* aqueous extract of seeds

On the other hand, it is observed that the administration of the same extract to normal rats did not produce any significant change on the level of glycaemia after 4 h treatment[17]. According to these results, it seems that 1 g/kg of an aqueous gross extract of *C. colocinthis* seeds have an antihyperglycaemic activity on diabetic rats in the short term and no effect on the plasma sugar of normal rats at all.

Then, the long-term effect (47 d) of *C. colocinthis* seeds aqueous extract was tested on normal and streptozotocine-induced diabetics rats. The results obtained are presented in Fig. 2:

*lot 1: A significant rise in the plasma sugar level at the beginning of the test was observed that reached a maximum at the end of the first week, then decreased gradually from the third week and finally stabilized in the fifth week of experiment, to reach the normal values[18-21].

*lot 2: Three phases in present results were distinguished:

First phase: (0-7 d) corresponds to injection of the streptozotocin where it has been noted an important continuous increase of blood sugar level.

Second phase: (7-11 d), after 4 d injection of *C. colocinthis*, the blood sugar level decreased in a very significant ($p < 0.01$) way compared to the witnesses having experimental diabetes and continued to decrease one week after injection.

Third phase: (29-47 d) which corresponds to the remission of the diabetes in these animals: Indeed, we have noted five weeks later that the animals progressively reached their normal plasma sugar.

*In third lots rats: The plasma sugar level did not undergo any significant variation during the treatment by *C. colocinthis*. It was always close to the normal values.

*In fourth lots rats: It did not reveal any variation of plasma sugar level during the experiment (0.7; 1.2 g/L).

Finally, it was observed that no significant variation of the glycaemia in normal rats. Besides, on diabetic rats, no significant difference of glycaemia on batch treated by the *C. colocinthis* compared to the witnesses diabetic-induced by streptozotocine was observed until after 24 h the first injection. However, it is noted a significant reduction of sugar level in the rats treated by the *C. colocinthis* after 4 d the first injection which continued until the 5th week after which it reached normal values for the two batches. In addition it is again noted that stopping injections of *C. colocinthis* extracts in the animals showed an immediate increase in the glycaemia 2 weeks later and then decreased the 3rd week.

The long-term evolution of the glycemia of the diabetic rats treated during 47 d per injection of aqueous crude extracts of *C. colocinthis* is indicated in the Fig. 2.

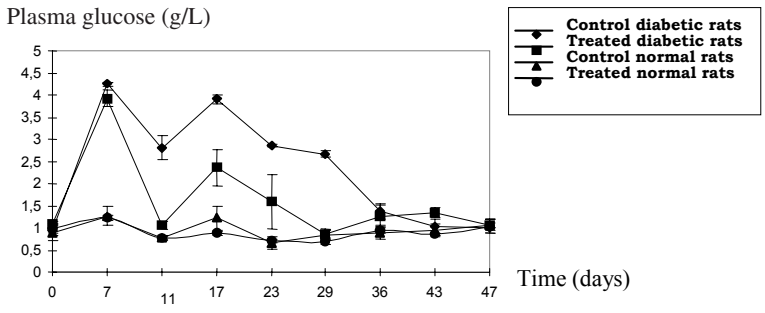

Fig. 2. Blood sugar level in rats intraperitoneal injected with 1.25 g/kg of aqueous extract of seeds.
lot 1: diabetic normal rats; lot 2: treated diabetic rats
lot 3: treated normal rats; lot 4: control normal rats

In comparison to the untreated witness diabetic rats, the hyperglycaemia decreases significantly in the first week after the injection of crude extracts of coloquinte and is maintained until the end of the experimentation to reach quasi normal values of glycaemia towards the 30th day.

Recently, there have been some studies concerning the use of *C. colocinthis* as source of antidiabetic drugs. This study involved a comprehensive procedure to elucidate this point. In order to explain the anti-hyperglycaemic effect of the aqueous extract of seeds and the mixture of the seeds and pulp, several authors[22,23] observed in their studies on local plants extracts.

1. The dose of the aqueous extract is important since rats were healthy.

2. Contra regulation phenomenon which appears just after the injection of the aqueous extract of *C. colocinthis* can be due to the intervention of the α-pancreatic cells that secrete the glucagon hormone responsible for the hyperglycaemic effect.

Concerning the results given in Fig. 2, intra-peritoneal injection of 65 mg/kg of streptozotocin in rats (lot 1and 2) caused an elevation of blood glucose levels (4.2 g/l) during 7 d and apparition of triglycerides in the blood with glycosuria and a traces of ketonuria. These results are compared to those of lots 3 and 4 where it is noted a low variation of blood sugar levels and no appearance of ketonuria and glycosuria. For lot 2, the seed aqueous extract of *C. colocinthis* produced a significant reduction of blood sugar levels in comparison with control lot 1 (Fig. 2). The antihyperglycaemic effect of this plant lasted many days in comparison with others plants such as *Eucalyptus globulus* and *Urtica dioica*[17,24]; *Ammi visnaga, Erythraea centaurium* and *Thymus ciliatus*[25]; *Salicia reticulata*[26]; *Sclerocarya birrea*[24] and *Artemisia herba alba*[12,27]; *Caralluma attenuata*[28]; *Momordica cymbalaria*[29]; *Alpinia galanga rhizome*[30]; *Euphrasia officinale*[18]; *Ocimum gratissimum*[31] which showed an hypoglycaemic activity at short term evaluation (3 to 24 h).

According to this study on the hypoglycaemic effect of *C. colocinthis* two hypotheses are set forth:

1. *C. colocinthis* induces Langerhans β-cells to regenerate or produce hyperactivity for the secretion of insulin by the remaining β-cells. It is supposed that the *C. colocinthis* stimulates and produces a hypersecretion of insulin after 4 d injection. This hyperactivity was confirmed by present results. Thus, it is observed an immediate effect corresponding to the increase of blood sugar level to 2.35 ± 0.42 g/L, when we stopped the treatment with *C. colocinthis*. Chakravarthy *et al.*[22] reported that the intraperitoneal injection of extract of the *Pterocarpus marsupium* increases the synthesis of insulin and DNA.

2. *C. colocinthis* potentiates the peripheric effects of insulin. The anti-hyperglycaemic effect of *C. colocinthis* reported by many patients is evidenced

by biological tests in streptozotocin-induced diabetic rats. So this study proves that the seed of *C. colocinthis* contains antihyperglycaemic agent(s) that may affect not only the blood sugar levels but also the triglycerides and ketonuria rates. Precocious disappearance of glycosuria in diabetic and treated rats by *C. colocinthis* might be caused by the decrease of sugar in the blood.

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