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Effects of hydro-ethanol extract of *Citrullus colocynthis* on blood glucose levels and pathology of organs in alloxan-induced diabetic rats

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PEER REVIEW

Peer reviewer

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Comments

This is a good study since it actually fills a gap in knowledge. The methodology is standard and presentation of results is adequate. The results are interesting and support the traditional usage of the *C. colocynthis* seed extract in the treatment of diabetes mellitus.

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ABSTRACT

Objective: To evaluate the differential effects of ethanol extraction of *Citrullus colocynthis* (*C. colocynthis*) on the blood glucose concentration and pathology of pancreas, liver, lungs, kidney and gastrointestinal tract in the alloxan induced diabetes in rats.

Methods: Diabetes mellitus was induced in 20 adult female Albino rats, using intraperitoneal injection of 120 mg/kg alloxan. The diabetic rats were randomly assigned into two equal groups. The first group was treated with the extract of *C. colocynthis* seed (300 mg/kg) and the rats of the second group, as an untreated diabetic group, received ordinary diet. Ten non diabetic rats remained as a normal control group.

Results: The results of this study indicate that *C. colocynthis* was able to reduce blood glucose significantly compared with the control diabetic group ($P < 0.05$). Histopathologically, alloxan resulted in severe necrotic changes in the pancreatic islets, especially in the central area of the islets. Tissue sections of the pancreas in the treated rats demonstrated enhanced regeneration of B cells and increased size of pancreatic islets. Liver of the treated diabetic rats revealed significant improvement of the hepatic tissue compared to those of the untreated diabetic rats.

Conclusions: The present study indicated a significant anti-hyperglycemic effect of *C. colocynthis* seed and supported its traditional usage in treatment of diabetes mellitus.

KEYWORDS

Diabetes mellitus, *Citrullus colocynthis*, Histopathological change, Pancreatic islets

1. Introduction

Diabetes mellitus is a common endocrine disease that is defined as a group of metabolic diseases characterized by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action or both, causing impaired carbohydrate, lipid and protein metabolism and increased risk of cardiovascular diseases[1]. It is a growing health concern worldwide. Increase in sedentary lifestyle, consumption of energy-rich diets, and obesity are some of the factors resulting the rise in the number of diabetics[2]. Diabetes is recognized as one of the leading causes of

morbidity and mortality in the world. While about 2.5–7% of the world's population has been diagnosed with diabetes mellitus, it is still expected to increase in future[2]. Despite the significant effect of the anti-hyperglycemic drugs and insulin sensitizers, side effects such as hypoglycemia at higher dose administration[3], low oral bioavailability due to degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, and poor permeability across the intestinal epithelium[4], make it necessary to find other alternatives. This leads to an increasing demand for more study about the natural products with anti-diabetic activity and fewer side effects.

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Herbal therapy has been used in patients with insulin-dependent and non-insulin-dependent diabetes, diabetic retinopathy, diabetic peripheral neuropathy, and other consequences of this metabolic disease^[3]. The herbal drugs are prescribed widely because of their effectiveness, fewer side effects and relatively low cost and the traditional plant medicines are used throughout the world for a range of diabetic presentations^[5]. *Citrullus colocynthis* (Cucurbitaceae) (*C. colocynthis*), commonly known as “bitter apple”, “colosynth”, “vine-of-sodom” and “tumba” is a well-known fruit traditionally used as an anti-diabetic medication in the Mediterranean countries^[6]. The ingredients of this plant may act on blood glucose through different mechanisms; some of them may have insulin’s activity^[7]. Oxidative stress plays an important role in the chronic complications of diabetes mellitus. Hyperglycemia is involved in the generation of oxygen-free radicals^[8]. *C. colocynthis* contains large amounts of phenolics and flavonoids that have antioxidant activity^[9]. This plant is used in folk medicine by people in rural areas as a purgative, antirheumatic, and as a remedy for skin infections. The plant contains cucurbitacins A, B, C and D, α -aelaterin, and probably other constituents^[10]. The plant *C. colocynthis* was claimed to possess hypoglycemic property and additionally contain some toxic effects as reviewed from various literatures^[11–14].

Therefore, the purpose of the present study was to examine the influence of the oral administration of *C. colocynthis* on the blood glucose concentration together with the pathological changes in pancreas, liver, kidneys, heart, lungs, stomach, large and small intestines and spleen of the alloxan-induced diabetic rats.

2. Materials and methods

2.1. Animals

Adult female Albino rats [(200±20) g] were obtained from the university animal house. The animals were maintained under standard environmental condition at (23±1) °C, with (55±5)% humidity and a 12 h light/dark cycle and maintained with free access to water and *ad libitum* standard diet (70% carbohydrates, 25% protein, 5% lipids).

2.2. Induction of diabetes by alloxan in rats

Diabetes was induced in 20 rats by single intraperitoneal injection of 120 mg/kg of alloxan tetrahydrate (10%) (Sigma, St. Luis, MO, USA). The rats were fasted for 12 h before and 12 h after injection of alloxan. The range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be toxic and result in loss of many animals^[15]. This dose was selected according to Mansour *et al.* and Sheweita *et al.* studies^[16,17]. Each rat of the normal control group was injected with the same amount of normal saline. The rats with blood glucose above 250 mg/dL, as well as with polydipsia and polyurea, which last for at least 3 d, were selected for the experiment.

2.3. Preparation of the *C. colocynthis* seed extract

C. colocynthis fruits were obtained from the local market. The black seeds of *C. colocynthis* were separated manually from the pulp of the dried fruit and were ground into powder. The powder was extracted by 1 L of hydro-ethanol mixture (80/20, v/v) for 8 h. This step was repeated for four times. The filtrate was pooled and concentrated under vacuum at a temperature, not exceeding 60 °C^[18]. The alcoholic extract was stored at –20 °C until being used.

2.4. Experimental design

The 30 rats of the present experiment were assigned into the following three groups:

Group 1: Normal control group. The rats of this group were administrated with 1 mL normal saline.

Group 2: Untreated diabetic group. Diabetes was induced in the animals of this group by a single intraperitoneal injection of 120 mg/kg, 10% alloxan tetrahydrate. These rats were then left untreated and received ordinary diet.

Group 3: Treated diabetic group. Diabetes was induced in the animals of this group by single intraperitoneal injection of 120 mg/kg, 10% alloxan tetrahydrate. The rats of this group were then daily treated for 12 d by oral route, using an intragastric tube, with *C. colocynthis* seed extract (1 mL/kg body weight, equivalent to 300 mg/kg).

2.5. Measurement of blood glucose

The blood glucose was measured every day until the end of the experiment. Blood was collected from the tail of the animals after 12 h fasting by a glucometer Easy Gluco (Ames, Korea). Accuracy of the glucometer was checked by the Orthotolidine method.

2.6. Histopathological evaluation

Fifteen days after diabetes induction and at the end of the 12 day’s treatment, the animals of all groups were euthanized by xylazin (5 mg/kg) and ketamin HCl (40 mg/kg). The study was approved by the local Ethics Committee of our faculty, in accordance with the ethics standards of “Principles of Laboratory Animal Care”.

Appropriate tissue samples were collected from pancreas, liver, kidneys, heart, lungs, stomach, large and small intestines and spleen were then fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μ m thickness, and stained with hematoxylin-eosin staining for light microscopic examination. The sections were quantitatively (morphometric) and qualitatively (morphologic) evaluated. For quantitative analysis the following factors were considered. (a) Volume density of the islets in 100 microscopic fields was determined to evaluate the proportion of the islets’ tissue to total tissue sections; (b) volume density of the B cells in the islet tissue was obtained by counting approximately 50 parts of the islet and determining the B cells to total islet cells; (c) percent of B cells of 5 islets of each tissue section from an animal and totally 50 islets of each group were counted; (d) number of

islets per square millimeter was calculated; (e) The average area of the islets was determined by measuring the diameter of 5 islets in each section and totally of 50 islets in each group was measured.

2.7. Statistical analysis

Descriptive statistics including mean, standard error, median, minimum and maximum were calculated for all variables. The One-way ANOVA followed by Tukey post-hoc test were used for comparison of different parameters. The data were analyzed by SPSS software, version 16 and differences of $P < 0.05$ were considered significant.

3. Results

3.1. Effect of *C. colocynthis* extract on the blood glucose

The effect of the *C. colocynthis* extract on the blood glucose in the diabetic rats has been shown in Figure 1. The blood glucose concentration in the untreated diabetic rats was significantly higher at all intervals after intraperitoneal administration of alloxan in comparison to those of the normal rats ($P < 0.05$). While the glucose concentration of the untreated rats remained high at all intervals, administration of *C. colocynthis* seed extract at doses of 300 mg/kg body weight tended to bring the blood glucose significantly toward normal values from Day 3 onwards, while the normal rats did not exhibit any significant alterations in their blood glucose concentration during the course of the study.

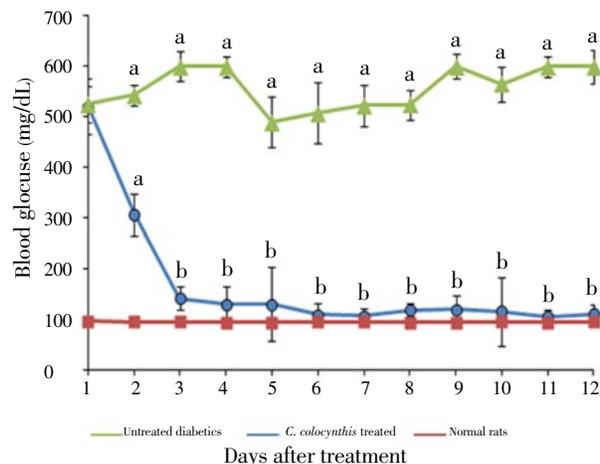


Figure 1. Comparative effects of treatment with *C. colocynthis* ($n=10$) on blood glucose concentration in the alloxan induced diabetic rats with nondiabetics ($n=10$) and untreated diabetics ($n=10$) rats.

Different letters in each serum sampling period show significant differences between the groups ($P < 0.05$). Values are presented as mean \pm SD.

Table 1

Effects of the *C. colocynthis* extract on the histomorphometric parameters of the pancreas of the diabetic rats.

Group	Volume density of B cells in islets	Volume density of islets	Percent of B cells	Volume density of B cells in pancreas	Number of islets (per mm ²)
Untreated diabetic	8.20 \pm 2.24 ^b	0.95 \pm 0.04 ^b	3.85 \pm 1.50 ^b	0.090 \pm 0.004 ^b	42.60 \pm 4.45 ^b
Normal control	58.72 \pm 6.80 ^a	1.90 \pm 0.22 ^a	32.55 \pm 2.95 ^a	1.250 \pm 0.340 ^a	80.00 \pm 6.72 ^a
Treated diabetic	15.25 \pm 3.65 ^b	1.20 \pm 0.24 ^b	10.50 \pm 1.45 ^b	0.300 \pm 0.020 ^b	52.00 \pm 4.41 ^b

Means within a column with different superscript letters denote statistically significant differences ($P < 0.05$).

3.2. Histopathological findings

In the untreated diabetic rats, alloxan resulted in severe necrotic changes of the pancreatic islets, particularly the cells in the center of the islets. Nuclear changes such as pyknosis, karyorrhexis, karyolysis, disappearance of the nucleus and in some places, residues of the destroyed cells were visible. The relative reduction in the size and number of the islets especially those around the central vessel together with severe reduction in the beta cells were demonstrated in these animals (Figure 2A). While the pancreas of the treated diabetic rats showed increased size of pancreatic islets, having cells with hyperchromatic nucleus and regeneration of the B cells in the sections stained with haematoxylin and eosin (Figure 2B).

The results of the histomorphometric study are summarized in Table 1. Although number/mm² of the pancreatic islets, volume density of the islets, volume density of the B cells in pancreas, presence of B cells and volume density of the B cells in the pancreatic islets in the treated rats showed some improvement, but these parameters in both the treated and untreated diabetic groups were still significantly lower than those of the normal group. While the histologic sections of the pancreas of the treated animals showed marked regeneration of the B cells, but there was no significant difference between the histomorphometry of the treated and untreated diabetic rats at this stage. The pancreas of the normal control rats had normal structure.

The histopathologic sections of the liver of the untreated diabetic rats showed degenerative changes in the hepatocytes represented by disorganization of the hepatic cords, congestion of the central veins with mild hepatocellular necrosis and the sinusoids were infiltrated by mild nonspecific inflammatory cells. The hepatocytes of the untreated rats showed morphological change such as pyknosis, karyorrhexis, chromatolysis and cytoplasmic vacuolization (Figure 2C). However, the liver of the treated diabetic rats revealed significant improvement in the structure of the hepatic tissue compared to those of the untreated diabetic ones and except the presence of a few mildly degenerated hepatocytes around the central vein of the treated rats, some cytoplasmic vacuoles, other hepatocytes and portal and sinusoidal areas were almost normal. In addition, there was no evidence of hemorrhages, inflammatory cells infiltration or parenchymal cell necrosis in the livers of the treated diabetic rats (Figure 2D). The liver of the normal control rats had normal structure.

The kidneys of the normal control rats had normal structure and the proximal and the distal convoluted

tubules, renal corpuscles, glomerulus and glomerular capsule had normal architecture. Microscopic examination of the kidneys of the treated and untreated diabetic rats showed mild tubular necrosis with moderate degenerative and necrotic changes in the glomerular epithelium and diffused interstitial and glomerular haemorrhages.

No lesions were found in the heart, lungs, stomach, large and small intestine and spleen of either normal control, treated or untreated diabetic rats.

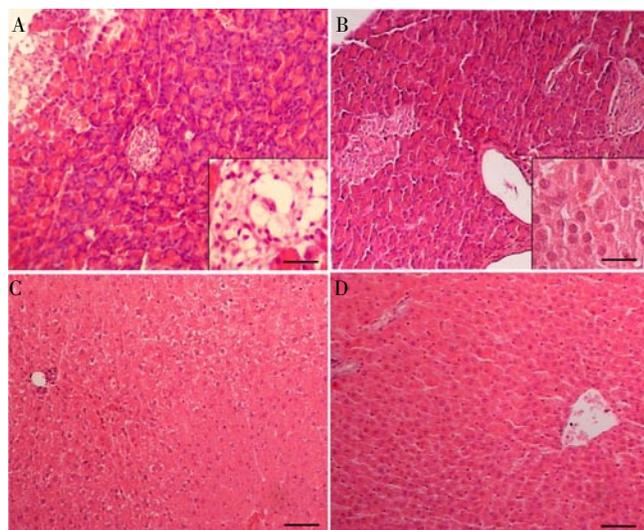


Figure 2. Histological sections of pancreas and liver from the untreated diabetic rats and diabetic rats treated with *C. colocyntis* seed extract 300 mg/kg.

A: The pancreas section from an untreated diabetic rats, B: Pancreas section from a treated diabetic rat; C: Liver section from an untreated diabetic rat; D: Liver section from a treated diabetic rat. Scale bar=50 μ m.

4. Discussion

The results of the present study showed that daily oral administration of 300 mg/kg ethanol extract of *C. colocyntis* for 12 d decreased the blood glucose concentrations into normal range in the alloxan-induced diabetic rats. The present findings are supported by those reported by Ziyat *et al.* and Aburjai *et al.* who confirmed the anti-diabetic properties of *C. colocyntis* extract^[19,20]. Consistent with the findings of the present study, Abdel-Hassan *et al.* showed that the *C. colocyntis* extract had a hypoglycemic effect in rabbit and they attributed this effect to the saponin and glycosidic components present in this plant^[11]. Mukherjee *et al.* also stated that saponins are bioactive compounds present naturally in many plants and known to possess potent hypoglycemic activity^[3]. Khoshvaghti and Hamidi (2011) showed that daily oral administration of a 100 mg/kg capsule of *C. colocyntis* powder for 8 d decreased the serum glucose concentration to normal range in the alloxan-induced diabetic dogs^[21]. Similar to the findings of the present study, Dallak *et al.* stated that oral administration of

C. colocyntis pulp extract (300 mg/kg) produced a gradual, time-dependent and significant decrease in glucose concentrations of the diabetic rats, and effectively increased the insulin concentration of the serum^[11]. Consistent with the findings of the present study Huseini *et al.* showed that oral administration of 300 mg/kg/day of the extract of *C. colocyntis* produced a significant effect on the glucose profile in human with type II diabetes^[22].

The antidiabetic effect of *C. colocyntis* extract could be linked to more than one mechanism, because it has been stated that this plant not only stimulate the beta cells and subsequently facilitate release of insulin but it also participates in activation of the insulin receptors too^[23]. It has been stated that the anti-diabetic action of *C. colocyntis* is probably due to enhanced insulin secretion, decreased gluconeogenesis, and inhibited release of counter-regulatory hormones including cortisol, glucagon, and growth hormone^[24].

On the other hand, it has been suggested that the mechanism responsible for the serum glucose lowering effect of *C. colocyntis* is attributed to an inhibitory effect of glucose absorption, an increased incorporation of circulating glucose as hepatic glycogen or an enhanced secretion of insulin^[24,25]. Nmila *et al.* reported that the seed extract of *C. colocyntis* has an insulintropic effect, evaluated *in vitro* in the isolated rat pancreas and isolated rat islets in the presence of 8.3 mmol/L glucose, which may, at least, partly account for their anti-diabetic actions^[26].

Decrease in the serum concentration of glucose toward normal value in the treated animals could be related either to the partial regeneration or preservation of the pancreatic B-cell mass by *C. colocyntis* in rats. The histopathologic sections of the pancreas of the treated animals in the present study showed an increase in the size of the islets, with hyperchromic nucleus and regeneration of the B cells. These findings reveal that the anti-hyperglycemic effect of *C. colocyntis* is not only through the insulin-like substances such as saponin present in this plant or activation of the insulin receptors, but this plant is able to increase the number of the B cells in the islets of Langerhans which results in a higher production of insulin. Sebbagh *et al.* showed that 16 d after administration of this seed extract, glycaemia in the streptozotocin-induced diabetes in rats was reduced and stated that this effect could be related to the partial regeneration or preservation of the pancreatic B-cell mass after streptozotocin treatment in rats^[6].

The liver of the untreated rats in the present study showed disorganization of the hepatic cords, vacuolization of the cytoplasm together with pyknotic changes in the nuclei of the hepatocytes. Following *C. colocyntis* intake, in the present study, the livers of the treated rats showed, more or less, an improvement in the histological architecture with persistence of the cytoplasmic vacuoles in some hepatocytes that could be attributed to the residual adverse effect of

the diabetic affliction. Liver, an insulin-dependent organ, has an essential role in glucose and lipid homeostasis. Several mechanisms are implicated in the pathogenesis of the functional and morphological alterations of the liver of diabetic patients[27].

There is a growing concern about the hepatotoxicity of the herbal remedies[28]. It has been shown that higher doses of *C. colocynthis* seed is toxic and the dose should be selected with a great concern. Diwan *et al.* reported that high concentration of saponin present in the elevated doses of *C. colocynthis* resulted in histopathologic changes of the liver and finally mortality of the animals[29]. Sheep which were fed with higher doses of the fresh *C. colocynthis* fruits and leaves (0.2–10.0 g/kg) showed signs of poisoning[14]. Doses of 10 g/kg of *C. colocynthis* from 1 day to 2 weeks caused death in goats[30]. Acute toxic colitis and reversible infertility have been claimed as the other side effects of higher doses of this plant[31,32]. Concentrations up to 100 µg/mL of *C. colocynthis* extract have been found free of hepatotoxic effect but higher concentrations seem to have a dose-dependent degrees of hepatotoxicity[33]. The histopathologic findings showed that the dose used in the present study was safe and did not have adverse toxic effects on the liver, lungs, heart, kidneys, small and large intestine and spleen.

In conclusion, the present study indicated a significant antihyperglycaemic effect of *C. colocynthis* seed. The pancreases and livers of the treated rats showed an improvement in their histological architecture. These results support the traditional usage of the *C. colocynthis* seed extract in the treatment of diabetes mellitus.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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Comments

Background

Diabetes mellitus is recognized as one of the leading causes of morbidity and mortality in the world. While about 2.5 to 7 percent of the world's population has been diagnosed with diabetes mellitus, it is still expected to increase in future. Despite the significant effect of the anti-

hyperglycemic drugs and insulin sensitizers, some side effects lead to an increasing demand for more study about the natural products with anti-diabetic activity and fewer side effects.

The herbal drugs are prescribed widely because of their effectiveness, fewer side effects and relatively low cost. *C. colocynthis* is a well-known fruit traditionally used as an anti-diabetic medication in the Mediterranean countries. This plant was claimed to possess hypoglycemic property and additionally contain some toxic effects as reviewed from various literatures. Therefore, the purpose of the present study was to examine the influence of the oral administration of *C. colocynthis* on the blood glucose concentration together with the pathological changes in some organs.

Research frontiers

Studies are being performed in order to indicate a significant antihyperglycaemic effect of *C. colocynthis* seed.

Related reports

The present findings are supported by those reported by Ziyat *et al.* (1997) and Aburjai *et al.* (2007) who confirmed the anti-diabetic properties of *C. colocynthis* extract. Consistent with the findings of the present study, Abdel-Hassan *et al.* (2000) showed that the *C. colocynthis* extract had a hypoglycemic effect in rabbit and they attributed this effect to the saponin and glycosidic components present in this plant. Consistent with the findings of the present study Huseini *et al.* (2009) showed that oral administration of 300 mg/kg/day of the extract of *C. colocynthis* produced a significant effect on the glucose profile in human with type II diabetes.

Sheep which were fed with higher doses of the fresh *C. colocynthis* fruits and leaves (0.2–10.0 g/kg) showed signs of poisoning. Doses of 10 g/kg of *C. colocynthis* from 1 day to 2 weeks caused death in goats. This result is not agreement with the present findings.

Innovations & breakthroughs

The present study indicated a significant antihyperglycaemic effect of *C. colocynthis* seed (300 mg/kg). The pancreases and livers of the treated rats showed an improvement in their histological architecture.

Applications

The herbal drugs can prescribe widely because of their effectiveness, fewer side effects and relatively low cost throughout the world for a range of diabetic presentations.

Peer review

This is a good study since it actually fills a gap in knowledge. The methodology is standard and presentation of results is adequate. The results are interesting and support the traditional usage of the *C. colocynthis* seed extract in the treatment of diabetes mellitus.

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