

## ***In vivo*, Acute, Normo-Hypoglycemic, Antihyperglycemic, Insulinotropic Actions of Orally Administered Ethanol Extract of *Citrullus colocynthis* (L.) Schrab Pulp**

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**Abstract: Problem statement:** *Citrullus Colocynthis* (L.) Schrab (cucurbitaceae family) (Handal) is a fruit commonly known as bitter apple or bitter cucumber. Traditionally, *Citrullus colocynthis* has been used for the treatment of diabetes. In particular, the acute effect and rout of administration of ethanol extract of the seedless pulp *in vivo* remains untested. We investigated the effect of different routs of administration and their hypoglycemic and insulinotropic effects of ethanol/water (20/80 V/V) extract of the dried seedless pulp of *Citrullus colocynthis* (L.) Schrab on normal and alloxan-induced diabetic rats. **Approach:** Rats were divided into two groups, normal and diabetic rats. Non-diabetic rats were fasted for 18 h before the beginning of the experimental procedure. About 250  $\mu$ L of normal saline (i.e., vehicle) was administered to control groups either i.p. (Group I) or orally (Group III) using modified feeding canula. As 250  $\mu$ L pulp extract was administered i.p. (Group II) or orally (Group IV). Five min, after the administration of the extract or vehicle, an Intraperitoneal Glucose Tolerance Test (IPGTT) was conducted (1.5 g glucose/kg) on groups I, II, III and IV, but not Groups V or VI. Alloxan-induced diabetic rats were fasted for 6 h before the beginning of the experimental procedure. They were divided into control (Group V) and treated groups (Group VI), each of 6 rats. As 250  $\mu$ L of the extract (equivalent to 300 mg kg<sup>-1</sup>) was administered orally to alloxan treated-group, while 250  $\mu$ L of the vehicle was administrated orally to the animals of control group. Serum samples were collected at 0, 1, 2, 3, 4 and 6 h following the treatment and were used for glucose and insulin determination. **Results:** Oral, but not intraperitoneal (i.p.) administration of ethanol extract (300 mg kg<sup>-1</sup>) resulted in acute, significant ( $p < 0.05$ ) and time-dependent changes in rat serum glucose and insulin levels in both normal and alloxan-induced diabetic rats. In extract-treated alloxan-free rats, a maximum of 32.9% reduction in serum glucose levels was observed following Intraperitoneal Glucose Tolerance Test (IPGTT) using glucose-oxidase assay. A maximum reciprocal increase of 59.5% in serum insulin levels following IPGTT was determined by ELISA. Further, in alloxan/ethanol extract-treated group, a maximum of 31% reduction in serum glucose levels as well as 370.2% increase in serum insulin levels following IPGTT were observed. **Conclusion:** These results demonstrated that oral administration of the ethanol extract of the dried seedless pulp of *Citrullus colocynthis* had normo-hypoglycemic (i.e., in normal rats), antihyperglycemic as well as insulinotropic actions in alloxan-induced diabetic rats.

**Key words:** Alloxan, ethanol extract, *Citrullus colocynthis*, hypoglycemic, insulinotropic, pulp

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### **INTRODUCTION**

Diabetes Mellitus (DM) is a common endocrine disease, possibly the world's fastest growing metabolic

disease. DM can be 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

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protein metabolism and an increased risk of cardiovascular diseases<sup>[1]</sup>. Knowledge of its heterogeneous phenotypes is on the rise<sup>[2]</sup>. Diabetes is recognized as one of the leading causes of morbidity and mortality in the world; about 2.5-7% of the world's population are diagnosed with diabetes mellitus<sup>[3]</sup>. Despite of the significant effect of anti-hyperglycemic drugs and insulin sensitizers, there remains side effects that necessitate finding other alternatives.

Medicinal plants provide such valuable therapeutic alternative Reaven *et al.*<sup>[4]</sup>. In spite of the fact that insulin has become one of the most important therapeutic drugs for diabetes, efforts are ongoing to find insulin substitutes from other sources. In fact, aside from classical chemically prepared antihyperglycemics, the use of traditional medicinal plants with hypoglycemic effect has recently gained popularity world wide. More than 400 traditional plant treatments for Diabetes Mellitus (DM) have been reported, but only a small number of these have received scientific and medical evaluation<sup>[5,6]</sup>.

*Citrullus Colocynthis* (L.) Schrab (cucurbitaceae family) (Handal) is a fruit commonly known as bitter apple or bitter cucumber, found in Sudan, Iran and India and in the deserts<sup>[7]</sup>. The dried pulp of *Citrullus Colocynthis* has been used for constipation, edema, bacterial infections, cancer and diabetes<sup>[8-11]</sup>. A. Recently, the antioxidant effects and the effect of the aqueous extract of the pulp on kidney and liver functions were reported<sup>[12]</sup>. Nevertheless, to date, the scientific scrutiny of *Citrullus colocynthis*, is insufficiently documented and warrants systematic analysis. In particular, the acute effect of ethanol extract of the seedless pulp in vivo remains untested. In the present study, the effects of orally administered *Citrullus colocynthis* seedless pulp ethanol extract on serum glucose and insulin levels in normal and alloxan induced diabetic-rats were investigated.

## MATERIALS AND METHODS

**Collection of the plant:** Fresh *Citrullus colocynthis* fruits were collected from the city of Aqaba in the southern province of Jordan. Mature black seeds were separated manually from the pulp of the fruits. Then, the pulp was dried and minced with a grinder (Muleinex) into a powder in preparation for extraction.

**Preparation of the extract:** The pulp powder from individual *Citrullus colocynthis* (250 g) was extracted three times at room temperature with 100 mL of water/ethanol mixture (80/20, v/v) for 6 h each round<sup>[17]</sup>. Ethanol-soluble portions were pooled from

the 300 mL filtrate in hot oven (45-50°C). The oven dried ethanol extract (12 g) was dissolved in freshly prepared normal saline (0.9%) to a final stock solution (1200 mg mL<sup>-1</sup>), which was used later to administer 250 µL (300 mg kg<sup>-1</sup>) of the extract to individual rats in various treatment groups.

**Animals:** White albino rats of both sexes (230-250 g) were obtained from the animal house at the Faculty of Medicine in Jordan University of Science and Technology. Rat handling was in compliance with Ethical Guidelines of University Committee on Animal Resources. The rats were housed in standard metal cages (5 rats/cage) and were fed a stock diet (i.e., carbohydrate free) containing 50 wheat, 21 corn, 20 soybean, 8% concentrated proteins and a 1% a mixture of salts, vitamins and dicalcium phosphate. Water was supplied *ad libitum*. These rats were kept at room temperature (22°C) at all times.

**Preparation of diabetic rats:** In order to achieve steady state levels of serum glucose, normal rats were fasted for 18 h, while diabetic rats for 6 h prior to the administration of the extract<sup>[12]</sup>. Alloxan monohydrate (Sigma, USA) dissolved in tap water (i.e., pH<6.0) was administered i.p. to 18 h fasted-rats at a dose of 150 mg kg<sup>-1</sup>. Alloxan protocol was executed 48 h before the onset of the experimental procedure and was followed by the measurement of serum glucose and insulin levels<sup>[12,13]</sup>. Diabetic rats those were defined as having a serum glucose in the range of 13.9-20.8 m mol L<sup>-1</sup> (250-375 mg dL<sup>-1</sup>), while normal non diabetic rats were defined as those having serum glucose in the range of 6-7.6 m mol L<sup>-1</sup> (108-137 mg dL<sup>-1</sup>)<sup>[12]</sup>.

## Experimental procedure:

**Non-diabetic rats (Fig. 1):** Rats in this study were divided into different treatment groups 6 rats each and were labeled as follow:

Non-diabetic rats were fasted for 18 h before the beginning of the experimental procedure. 250 µL of normal saline (i.e., vehicle) was administered to control groups, either i.p. (Group I) or orally (Group III) using modified feeding canula. 250 µL pulp extract was administered i.p. (Group II) or orally (Group IV). Five min, after the administration of the extract or vehicle, an Intraperitoneal Glucose Tolerance Test (IPGTT) was conducted (1.5 g glucose/kg) on Groups I-IV, but not Groups V or VI (diagram). 500 µL serum samples were collected from waken rats using substernal heart pricking of each and every rat at 0, 15, 30, 45 and 60 min following glucose administration.

Table 1: Treatment protocol for the Control and Experimental groups of normal and diabetic rats

Figure	Group No.	i.p. Admin (250 $\mu$ L)	Oral admin (250 $\mu$ L)	Extract (250 $\mu$ L) (300 mg kg)	Alloxan (150 mg kg <sup>-1</sup> )	Vehicle (0.9% Saline)	IPGTT* (1.5 g kg <sup>-1</sup> )
1	I (Control)	+	-	-	-	+	+
	II (Experimental)	+	-	+	-	-	+
2 and 3	III (control)	-	+	-	-	+	+
	IV (Experimental)	-	+	+	-	-	+
4 and 5	V (control)	-	+	-	+	+	-
	VI (Experimental)	-	+	+	+	-	-

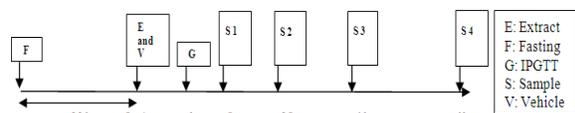


Fig. 1: Experimental procedure in normal non-diabetic rats

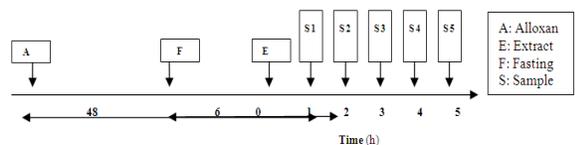


Fig. 2: Experimental procedure in alloxan-induced diabetic rats

The 500  $\mu$ L serum samples were immediately transferred on ice, centrifuged at 5000 rpm at RT for 2 min and kept on ice until glucose and insulin assays were performed. Thereafter, 150  $\mu$ L of the serum was immediately analyzed for glucose and insulin levels.

**Alloxan-diabetic rats (Fig. 2):** Alloxan-induced diabetic rats were fasted for 6 h before the beginning of the experimental procedure. They were divided into control (Group V) and treated groups (Group VI), each of 6 rats. As 250  $\mu$ L of the extract (equivalent to 300 mg kg<sup>-1</sup>) was administered orally to alloxan treated-group, while 250  $\mu$ L of the vehicle was administered orally to the animals of control group. Serum samples were collected at 0, 1, 2, 3, 4 and 6 h following the treatment and were used for glucose and insulin determination.

**Determination of serum glucose and insulin levels:** Glucose was determined in serum obtained from collected serum samples by the glucose oxidase method (Trinder Method) using Labkit (Chronolab, USA). Insulin was measured in serum using double antibody ELISA, using Medgenix-Ins-ELISA kit (Biosource Europe SA).

**Statistical analysis:** Data are given as the mean  $\pm$  SEM. Student's t-test was used to determine if the difference observed among various treatment groups at individual time points was significant.

## RESULTS

In the absence of IPGTT, the experimental dose of the extract was empirically determined through a dose response-curve (50-350 mg kg<sup>-1</sup>) on serum glucose levels (data not shown). At 300 mg kg<sup>-1</sup> glucose steady state fasting serum levels dropped from 118.7 $\pm$ 3.8-64.5 $\pm$ 4.8 mg dL<sup>-1</sup>. Lower doses were unproductive, while 350 mg kg<sup>-1</sup> was lethal in rats. In the presence of IPGTT, 500 mg kg<sup>-1</sup> reduced serum glucose levels from (257 $\pm$ 5.6-155 $\pm$ 3.4), an effect similar to that observed at the dose of 300 mg kg<sup>-1</sup> without IPGTT, while 600 mg kg<sup>-1</sup> was found lethal. In order to avoid sublethal toxicity, while observing pharmacological effect, the dose of 300 mg kg<sup>-1</sup> was chosen.

A preliminary comparison of the individual effect of the peel, pulp or seeds portions of *Citrullus colocynthis* on blood glucose levels showed that only the pulp but not the peel (rind) or the seeds exhibited marked hypoglycemic effect on serum glucose levels (data not shown). Interestingly, the ethanol extract of the pulp portion of *Citrullus colocynthis* exhibited hypoglycemic effects; one on the steady state normoglycemic levels (Fig. 4) and the second on the hyperglycemic serum glucose levels in diabetic rats (Fig. 6).

### The effect of i.p. administration of the crude extract on serum glucose levels in normal non-diabetic rats:

The time course of glucose levels of fasted Group I and Group II rats following i.p. administration of the extract are shown in Fig. 3. The time course shows that rats in both groups had normoglycemic glucose levels at the onset of the experiment (Fig. 3). Both groups showed gradual time-dependent increase in serum glucose levels following IPGTT. Typical of normal physiological response to IPGTT, maximum serum glucose levels were reached at 15 min (110.5 $\pm$ 8.0) and returned to normal in about one hr. The administration of the extract (300 mg kg<sup>-1</sup>) i.p. did not significantly alter IPGTT-induced elevation of serum glucose (111.0 $\pm$ 7.7 mg dL<sup>-1</sup>) in Group II when compared to Group I (Table 2). The effect of oral administration of the crude extract on serum glucose and insulin levels in normal non-diabetic rats.

Table 2: Serum glucose and insulin levels as well as change in their respective values following administration of ethanol extract of the pulp portion of *Citrullus colocynthis* to normal and alloxan-treated rats

Comparison groups	No. Groups		Serum glucose					Serum insulin				
			(A) initial* level (mg dL <sup>-1</sup> )	(B) **level of maximum change (mg dL <sup>-1</sup> )	Δ of (A and B) (mg dL <sup>-1</sup> )	Δ of (B) (mg dL <sup>-1</sup> )	Δ of (B) (%)	(A) initial* level (μU L <sup>-1</sup> )	(B) ** level of maximum change (μU L <sup>-1</sup> )	Δ of (A and B) (μU L <sup>-1</sup> )	Δ of (B) (μU L <sup>-1</sup> )	Δ of (B) (%)
C1	1	I (Normal control) IP	110.5±8.0	200.2±17.0	+89.7	↓6.3	↓3.1	-				
	2	II (Normal + Extract) IP	111.0±7.7	206.5±20.1	+95.5							
C2	3	III (Normal control) ORAL	134.8±15.4	225.8±32.5	+91.0	↓74.3	↓32.9	19.5±2.4	55.8±5.2	+36.3	↑33.2	↑59.5
	4	IV (Normal +Extract) ORAL	124.3±7.0	151.5±6.9	+27.2			18.3±1.6	89.0±4.1	+70.7		
C3	5	V (Diabetic control) ORAL	362.5±4.4	362.5±5.5	+0.0	↓112.3	↓31.0	3.1±0.8	3.7±0.6	+0.6	↑13.7	↑370.3
	6	VI (Diabetic + Extract) ORAL	360.2±2.9	250.2±3.1	-110			4.1±0.5	17.4±0.3	+13.3		

\*Initial level is the determination at t = 0 min \*\*Minimum/Maximum level is the determination at t = 15 min for C1 and C2 groups and t = 3 h for C3 groups in glucose comparisons and t = 2 h for C3 groups in insulin comparisons

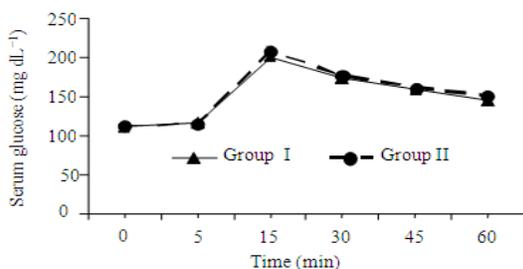


Fig. 3: The effect of i.p. administration of pulp extracts on serum glucose levels in Normal rats. Group II received 300 mg kg<sup>-1</sup> *Citrullus colocynthis* intraperitoneally followed by IPGTT (1.5 g glucose/kg), whereas Group I received vehicle only (0.9% saline). The extract was given at -5 min and glucose measured at 5, 15, 30, 45 and 60 min time points. No significant difference was detected

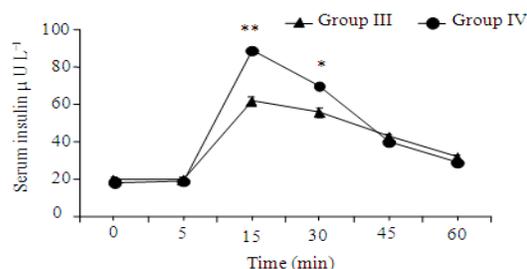


Fig. 5: The effect of oral administration of pulp extract on serum insulin levels in Normal rats. Group IV received 300 mg kg<sup>-1</sup> *Citrullus colocynthis* extract orally followed by IPGTT, whereas Group III received vehicle only (0.9% saline). The extract was given at -5 min. and glucose measured at 5, 15, 30, 45 and 60 min time points. (\*\*p<0.001; \*p<0.05)

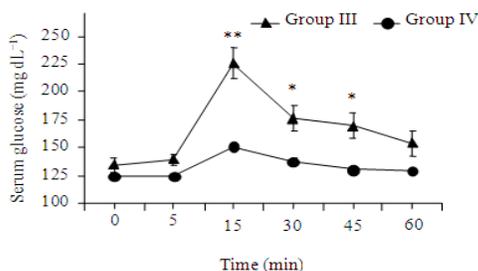


Fig. 4: The effect of oral administration of pulp extract on serum glucose levels in Normal rats. Group IV received 300 mg kg<sup>-1</sup> *Citrullus colocynthis* extract orally followed by IPGTT, whereas Group III received vehicle only (0.9% saline). The extract was given at -5 min. and glucose measured at 5, 15, 30, 45 and 60 min time points. (\*\*: p<0.001; \*: p<0.05)

The time course of serum glucose levels (Fig. 4) of fasted rats of Groups III and IV as well as serum insulin levels (Fig. 5) are shown following oral administration of the pulp extract (300 mg kg<sup>-1</sup>).

Serum glucose in control animals (Group III) reached maximum levels after 15 min (225.5±32.5 mg dL<sup>-1</sup>) and returned to normal in about 1 h. Group IV, normoglycemic rats with orally administered pulp extract, had 151.5±6.9 mg dL<sup>-1</sup> serum glucose levels 15 min following the IPGTT. Group IV essentially maintained normoglycemic glucose levels throughout the time course at 15, 30, 45 and 60 min. A significant reduction in serum glucose levels (32.9%) in extract-treated rats was observed and reached maximum levels at 15 min time point.

The time course for insulin serum levels (Fig. 5) for Groups III and IV revealed peaks of 55.8±5.2 and 89.0±4.1 μU L<sup>-1</sup>, respectively. Peak time of insulin coincided with that of glucose. Close examination of Fig. 5 revealed sustained elevation in insulin levels up to 45 min, which was reciprocal to the rapid drop in glucose levels from 15-30 min. This is a characteristic normal response to IPGTT-induced hyperglycemic state in normal rats. Insulin levels returned to steady state

levels approximately one hour later. Treatment with extract (Group IV) resulted in a significant ( $p < 0.001$ ) 59.5% increase in insulin levels ( $89.0 \pm 4.1 \mu\text{U L}^{-1}$ ) when compared to Group III ( $55.8 \pm 5.2 \mu\text{U L}^{-1}$ ). Further, insulin levels of Group IV rats declined thereafter in a similar manner to Group III and returned to steady state levels by 60 min time point.

**Effect of oral administration of the extract on serum glucose and insulin levels in alloxan-induced diabetic rats:**

The time course for serum glucose (Fig. 6) and insulin (Fig. 7) were determined in alloxan/extract-treated rats (Group VI) versus alloxan/vehicle-treated controls (Group V). IPGTT step was eliminated in this protocol because unlike normoglycemic rats of Groups III and IV, rats in Groups V and VI had a hyperglycemic steady state serum glucose levels, thus were risk-free of glucose-depletion following extract administration at  $300 \text{ mg kg}^{-1}$ . Figure 6 shows that Group V rats were indeed diabetic as evidenced by the hyperglycemic steady state levels of glucose ( $361.3 \pm 3.8 \text{ mg kg}^{-1}$ ; average of glucose levels in all groups at 0 time point). The oral administration of *Citrullus colocynthis* pulp extract ( $300 \text{ mg kg}^{-1}$ ) produced a gradual, time-dependent and significant ( $p < 0.001$ ) (i.e., from  $360.2 \pm 2.9 \text{ mg kg}^{-1}$  to  $250.2 \pm 3.1 \text{ mg dL}^{-1}$ ) decrease in glucose levels (Group VI). Glucose levels exhibited a significant ( $p < 0.001$ ) maximum 31% reduction from baseline 3 h post extract administration ( $250 \text{ mg dL}^{-1}$ ). Glucose levels returned to the initial fasting hyperglycemic steady state levels in about 6 h. As a positive control, Glibenclamide ( $600 \text{ mg kg}^{-1}$ ) was administered under the same conditions and resulted in a significant decrease in serum glucose levels from control diabetic of  $325 \pm 6.3$ – $128 \pm 5.2 \text{ mg dL}^{-1}$  (61%).

The time course curves of insulin levels (Fig. 7) for Groups V and VI revealed a significant peak ( $p < 0.001$ ; 370.3% of baseline above control group) at 2 h post extract administration which inversely correlated with the decrease in glucose serum levels (Fig. 7). Insulin levels gradually declined and returned to baseline in about 6 h.

A meta analysis of the percent change in glucose and insulin in normal and in alloxan-treated rats revealed the following: First, a 32.9% reduction in normal rats' glucose was brought about, in whole or in part, by a 59.5% increase in insulin. Second, a 31.0% reduction in alloxan-treated rats' glucose, was brought about by 370.3% increase in insulin.

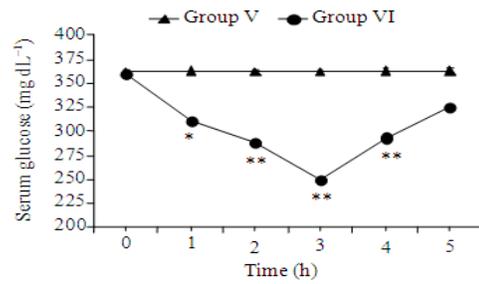


Fig. 6: The effect of oral administration of pulp extract on serum glucose levels in alloxan-treated rats. Group VI, fasted for 6h, received  $300 \text{ mg kg}^{-1}$  *Citrullus colocynthis* extract orally followed by IPGTT, whereas Group V received vehicle only (0.9% saline). The extract was given at -5 min. and glucose measured at 1, 2, 3, 4, 5 and 6 h time points. (\*\*:  $p < 0.001$ ; \*:  $p < 0.05$ )

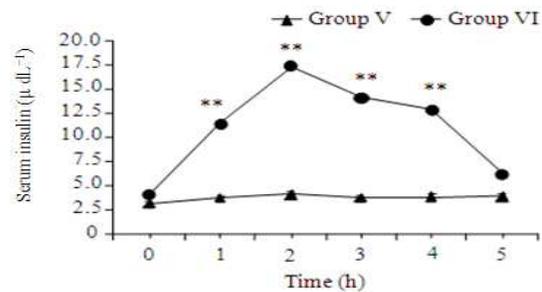


Fig. 7: The effect of oral administration of pulp extract on serum insulin levels in alloxan-treated rats. Group VI, fasted for 6 h, received  $300 \text{ mg kg}^{-1}$  *Citrullus colocynthis* extract orally followed by IPGTT, whereas Group V received vehicle only (0.9% saline). The extract was given at -5 min and glucose measured at 1, 2, 3, 4, 5 and 6 h time points. (\*\* $p < 0.001$ )

**DISCUSSION**

The literature on *Citrullus colocynthis* is quiet heterogeneous, in particular, the portion of the fruit being evaluated for physiological and toxic effects, solvent used for extraction, dosage of the lyophilized extract administered, route of administration and acute or chronic effect of the extract. This heterogeneity though motivated by delineating the therapeutic potential(s) of this fruit, it limits comparisons of various findings. Indeed, the differential therapeutic and/or toxic actions of the extracts from various parts of this fruit ought to be center stage of investigations.

In this cursory study, we wanted to investigate two hypotheses: First is that ethanol extract from the pulp portion of *Citrullus colocynthis* has a hypoglycemic effect and Second is that the pulp's extract acts, at least in part, via increasing serum insulin levels. Present data demonstrate that the extract from the pulp portion of the fruit, exhibits a significant hypoglycemic effect at 300 mg kg<sup>-1</sup> (Fig. 4 and 6) and that it acts via increasing insulin levels (Fig. 5 and 7, Table 2). Furthermore, the hypoglycemic effect is evident under both alloxan induced-diabetic hyperglycemic state (Fig. 6), as well as under normoglycemic state (Fig. 4; Table 2). This later observation warrants consideration when determining later on appropriate therapeutic dosing.

Technical considerations in our study included the following: collection and extraction of fresh samples of *Citrullus colocynthis*, optimizing the extract dosage, selecting appropriate route of administration, verification of alloxan-induced hyperglycemia and accurate and reliable determination of glucose and insulin in serum samples. Dose response curve showed that 300 mg kg<sup>-1</sup> was the optimal dose for two reasons, the First being 350 mg kg<sup>-1</sup> (in our hands) and 400 mg kg<sup>-1</sup> (published) were found either detrimental or hepatotoxic<sup>[14]</sup> and the Second is that 300 mg kg<sup>-1</sup> of the extract lowered blood glucose to a critical level of 64.5±4.8 mg dL<sup>-1</sup>. It is well established that at or below 60 mg dL<sup>-1</sup> serum glucose levels, which 350 mg kg<sup>-1</sup> induced, a physiological response is stimulated and it includes the release of cortisol, epinephrine, growth hormone and/or glucagon which collectively cooperate to correct serum glucose levels via stimulating glycogenolysis, gluconeogenesis, lipolysis and protein breakdown. To circumvent this interference, even at our lower dose of choice 300 mg kg<sup>-1</sup>, we decided to assess the effect of the extract following the administration of IPGTT. Alloxan-induced diabetic rats are known to become hyperglycemic, thus did not exhibit this problem. IPGTT step was eliminated from the protocol performed on diabetic rats. Further, in our lab, the pulp extract exerted its effect when administered orally but not intraperitoneally suggestive of an IP-mediated inactivation of the extract active ingredient(s) or the presence of structural and transport-dependent unmet requirements for the action of the active ingredient(s)<sup>[15]</sup>. As such, oral administration did assure the bioavailability of the extract in the main circulation (i.e., compare Fig. 3 and 4). This appears to be in contrast to a recent report by Dehghani and Panjehshahin<sup>[16]</sup> who have used IP route to report on the toxic effects of *Citrullus colocynthis* at 400 mg kg<sup>-1</sup> of an extract of the whole fruit. However, no statements

were made by the authors regarding changes in serum glucose levels whether in normal or hyperglycemic alloxan-treated rats.

Alloxan treatment increased the steady state glucose levels from 120.2±14.0-361.3±3.8 mg dL<sup>-1</sup>, a 202% increase. Also, it is noteworthy that baseline insulin levels in normal rats were 529% above their counterpart in alloxan-treated rats (Group V), thus confirming establishing a diabetic rat model.

Glucose and insulin levels were determined in normal rat groups over a period of 60 min, while in alloxan-treated groups over a period of 6 h, because of the hyperglycemic state (361.3±3.8 mg kg<sup>-1</sup>) of the later group. The time course selection was proven accurate as maximum changes in glucose and insulin levels were recorded midway at 15 min in a 60 min time course and 3 h in a 6 h time course, respectively. In regards, to alloxan treatment, alloxan is a cytotoxin that has been found to induce free radical generation<sup>[17]</sup> and cause irreversible, semi-complete and selective destruction of β cells of the islets of Langerhans<sup>[18]</sup>. Baseline glucose levels (361.3±3.8 mg kg<sup>-1</sup>) alongside trace levels of serum insulin (3.1±0.8 μU L<sup>-1</sup>) in alloxan-treated rats demonstrated diabetic rats were established<sup>[13]</sup>. These results show a highly significant decrease in glucose levels (p<0.01) and a highly significant increase in insulin levels (p<0.001) (Table 2) during the first 4 h of the extract administration.

The data presented here strongly argue that the hypoglycemic effect of the extract in the normoglycemic rats (Fig. 4), as well as the antihyperglycemic effect in alloxan-treated rats (Fig. 6) were mediated via insulin, since the surge in insulin levels preceded or coincided with the maximum reduction in glucose levels observed in respective groups (Fig. 5 and 7). These findings are clinically significant. In fact, a study just published by<sup>[19]</sup>, reports on the first short term clinical trial using extract of *Citrullus colocynthis* showing initial promising results. We maintain that the optimal benefits of *Citrullus colocynthis* awaits addressing the therapeutic potential of its different parts in sufficient depth.

The percent increase in insulin and decrease in glucose levels in normal rats were 59.5 and 32.9%, respectively; that is a 2:1 ration, while the percent increase in insulin and decrease in glucose levels in diabetic rats were 370.3 and 31%, respectively; that is an 11.9:1 ration. Therefore, in diabetic rats, the extract was quiet effective in elevating serum insulin levels to 17.4±0.3 μU L<sup>-1</sup> and reducing glucose levels to 250.2±3.1 mg dL<sup>-1</sup>. The desired final glucose levels of

120.2±14.0 mg dL<sup>-1</sup> can possibly be achieved by isolating and purifying the hypoglycemic ingredient(s) present in this ethanol extract of the pulp. This, we suspect, will increase the benefit-to-side effects ratio.

Chemical analysis studies of *Citrullus colocynthis* are currently focused on isolating, identifying and characterizing individual compounds present in the fruit in an attempt to map the reported therapeutic effects to a specific compound(s). Various chemical extractions from the seeds<sup>[20]</sup>, pulp<sup>[21]</sup>, peel<sup>[14]</sup> or whole fruit<sup>[22]</sup> were chemically characterized revealing elaborate chemical composition. We believe that these massive efforts can even be more fruitful if directed to a portion(s) of the fruit with well established and verified physiological effect, which is still not well studied to date. In fact, this was the impetus behind our study, which clearly revealed the dose-dependent, hypoglycemic, antihyperglycemic, insulinotropic effects of the ethanol extract from the pulp portion alone. Despite of our observations reported here and the recent isolation of the antioxidants flavinoids<sup>[21,23]</sup> from the pulp portion, there remains an essential need to closely assess dose-dependent as well as, portion-dependent hepatotoxicity of this fruit.

*Citrullus colocynthis* has been shown to contain H<sub>2</sub>O, protein, saponin, carbohydrates, fibers, Ca<sup>+2</sup> and phosphate<sup>[10]</sup>. Saponin extraction of the rind of this fruit exhibited a hypoglycemic effect<sup>[22]</sup>. In fact, bioactive saponins and glycosides have been isolated and characterized from the whole fruit; however, not all isolated moieties have been fully characterized in terms of function<sup>[22]</sup>. In regards to the hypoglycemic effect of the fruit under normoglycemic state, this observation can be due to insulin-dependent inhibition of liver gluconeogenesis, inhibition of glycogenolysis and/or insulin-insensitive enhancement of peripheral metabolism of glucose. Finally, acute and chronic toxicity studies of the ethanol extract of the pulp portion of *Citrullus colocynthis* in rats including biochemical profile, histochemistry and histopathology, are still at large and require due attention, we are currently working on it on our labs.

### CONCLUSION

The current study reports on the physiological effects of the ethanol extract of the pulp portion of *Citrullus colocynthis*. The extract exhibits hypoglycemic effect on the steady state normoglycemic levels, as well as antihyperglycemic effect on steady state hyperglycemic levels in diabetic rats. These physiological actions were mediated, at least in part, via an increase in insulin.

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