Acute Toxicity, Hypoglycemic and Antihyperglycemic Effect of Ethanolic Extract of Citrullus Colocynthis L. Seeds in Normal and Streptozotocin-Induced Diabetic Rats.

Azzi Rachid , Lahfa Farid Boucif, Mezouar Dounia, Benmehdi Houcine, and Djaziri Rabah

Abstract — The present study was carried out to evaluate acute toxicity and the potential hypoglycemic and antihyperglycemic activity of the ethanolic extract of Citrullus colocynthis seeds (ECc) in normal and streptozotocin diabetic rats.

For acute toxicity test, rats were treated intraperitoneally with graded doses (20 to 300mg/kg body weight) of ECc. Rats were observed for signs of toxicity and LD50 was determined.

Antidiabetic activity of intraperitoneal injection of 20mg/kg b.w of ECc was assessed in normal and in diabetic rats.

The acute toxicity study of ethanolic glycosides cucurbitacins extracts from the seeds of colocynth (Citrullus colocynthis) reported an LD50 of 166 mg/kg b.w.

Also, the injection of 20mg/kg b.w (i.p) of ECc has antihyperglycemic effect in normal and diabetic rats. For 3 hours, it corrects blood glucose levels STZ- induced diabetic rats about 28.65%. This effect persists during 21 days after injection of extract with levels of reduction values of 40.74%.

Keywords — acute toxicity, hypoglycemic, antihyperglycemic, Citrullus colocynthis L. Wistar rats.

I. INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

According to the recent data from International Diabetes Federation (IDF), the number of people affected with diabetes worldwide has increased dramatically over recent years. In 2013, 382 million people had diabetes; this number is expected to rise to 592 million by 2035 [2].

A. Plant material and extraction

The pharmacological agents currently used for the treatment of type 2 diabetes include sulfonylurea, biguanide, thiazolidinedione and α-glycosidase inhibitors. These agents, however, have restricted usage due to several undesirable side effects and fail to significantly alter the course of diabetic complications [3].

Traditional plant medicines are used throughout the world for a range of diabetic presentations. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost [4].

Ethnobotanical information collected in several regions of the world estimate that more than 1123 plant species, more than 725 genera belonging to 183 families, are used for their hypoglycemic and anti-hyperglycemic properties [5-7].

Algeria has a rich heritage of medicinal plants of wide diversity, which are used by the local population and the traditional healers for the treatment of several diseases including diabetes [8, 9].

Citrullus colocynthis (family of Cucurbitaceae) commonly known as colocynth, bitter apple, is one of the plants with a long history of anti-diabetic use in traditional medicine [10].

The colocynth, originally from tropical Asia and Africa, is now widely distributed in the Saharo-Arabian phytogeographic region in Africa and the Mediterranean region [11].

The present study was undertaken to evaluate the potential hypoglycemic and anti hyperglycemic activity of the ethanolic extract of Citrullus colocynthis seeds (ECc) in two different animal models, normal and streptozotocin diabetic rats.

II. MATERIAL AND METHODS

A. Plant material and extraction

The fruits of the colocynth (Citrullus colocynthis L. Schard); family of Cucurbitaceae; are harvested at maturity during the month of September 2011 in the region of Ain Safra, Wilaya of Naama, (western Algeria). Mature black seeds were selected and pulverized.

The dried seeds (100 g) were defatted with 750 ml petroleum ether (40–60°C) for 3h in a Soxhlet apparatus. Fifty grams of the seed defatted powder was suspended with
80% ethanol (200mL) and heated to boil under reflux for 6h. The decoction obtained was centrifuged, filtered, and concentrated on a rotary evaporator at 40-50°C to give a residue (yield = 1.37% w/w).

For assuring stability, the residue was stored at −20°C until used.

During experiment the crude ethanolic extract (ECc) was diluted with saline solution (Nacl 0.9%) just before administration to animals.

**B. Animals**

Male Wistar rats (Rattus norvegicus var. albinus) (aged 2-3 months, weighing 150–250 g) were obtained from the Department of Biology, faculty of Natural Sciences and Life Sciences of the Earth and the Universe, University Tlemcen (Algeria). The animals were kept under standard environmental conditions (22 ± 2°C); 12:12 h dark/light cycle.

Water and industrialized dry food (O.N.A.B. Remchi, Tlemcen, Algeria) were made available ad libitum.

**C. Preliminary acute toxicity testing**

Sixty rats randomly divided into 6 groups of 10 rats each were used to evaluate the acute intraperitoneal (i.p) toxicity of ECc extract. Five groups were respectively treated intraperitoneally with graded doses (20, 75, 100, 200 and 300mg/kg body weight (b.w)) of ECc extract, while the remaining group received the vehicle (1 ml/100g b.w saline solution).

Rats were observed over a period of 24h for signs of toxicity, such as changes in behavior and death. The intraperitoneal LD50 was calculated using the method of Miller and Tainter (1944) [12].

**D. Antidiabetic activity of ECc**

Antidiabetic activity of ethanolic extract of Citrullus colocynthis seeds (ECc) was assessed in normal, glucose loaded hyperglycemic (oral glucose tolerance model) and streptozotocin-induced diabetic rats.

In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

**Hypoglycemic activity of ECc in normoglycemic fasted rats**

Twenty five normal rats were randomly divided into five groups (n = 5/group). These rats were injected intraperitoneally (1 ml/100g b.w) with saline solution (Nacl 0.9%) (Control) or different doses of ECc, in the following manner:

- Group 1 (RTN) : Normal control rats treated with saline solution;
- Group 2 (RN20): Normal rats treated with ECc (20 mg/kg b.w);
- Group 3 (RN75): Normal rats treated with ECc (75 mg/kg b.w);
- Group 4 (RN100): Normal rats treated with ECc (100mg/kg b.w);
- Group 5 (RN200): Normal rats treated with ECc (200mg/kg b.w).

Blood was collected by a puncture of tail-vein, before and after the treatments (0, 1, 2 and 3h) for estimation of blood glucose.

**Induction of experimental diabetes**

Diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) (Sigma–Aldrich, no 242-646-8) solution 55 mg/kg b.w. in ice-cold acetate buffer (0.1M, pH 4.5) to overnight-fasted rats [13]. After 72h of STZ injection, the animals with fasting glycemias higher than 200 mg/dl and with signs of polyuria and polydipsia were considered to be diabetic and included in the study.

**Antihyperglycemic activity of ECc in STZ-induced diabetic fasted rats**

In the experiment, a total of 30 rats (10 normal rats; 20 STZ-diabetic rats) were used. The rats were divided into 3 groups of 10 each.

- Group 1(RTN): Normal control rats treated with saline solution;
- Group 2(RTD): Diabetic control rats treated with saline solution;
- Group 3(RDE): Diabetic rats treated with ECc (20 mg/kg b.w).

Animals in the control group (RTN and RTD) received a single i.p injection of saline solution (Nacl 0.9%). The test group of animals (RDE) was treated by ECc with a single i.p injection dose of 20 mg/kg b.w.

In the short term, blood was collected by a puncture of tail-vein, before and after the treatments (0, 1, 2 and 3 h) for estimation of blood glucose.

Antihyperglycemic effects of ECc, were sought during three weeks, after i.p injection of 20mg/kg b.w, for one dose per week, in rats described previously.

Blood glucose was measured using a Gucometer reader (One Touch Ultra, ICON, USA) on blood collected by a puncture of tail-vein.

Glucose levels are expressed in mg/dl and changes in blood glucose levels are expressed in percentage (%) compared to ‘G0’ indicates the initial glycemias in which the treatment commenced.

Percentage glycemias change was then calculated using the following formula:

\[
\% \text{ Glycemia}= \left[\frac{(\text{final glycemia}−\text{initial glycemia})}{\text{initial glycemia}}\right]×100
\]

**E. Statistical analysis**

The results were expressed by the means±standard deviation.

The statistical difference was determined by Student’s t test. The values were considered significantly different when the P-value was less than 0.05 in comparison to baseline values (starting values).
III. RESULTS

A. Preliminary acute toxicity

Rats in the groups given 200 and 300mg/kg b.w (i.p) of the ECc extract showed behavioral changes and manifested clinical signs such as hypo activity, reduced response to external stimuli, hyperventilation, tremors, coma and death. The onset/severity of the clinical signs was dose related as rats given the highest dose (300 mg/kg b.w (i.p)) of ECc extract exhibited the most severe signs and a quicker onset of signs of toxicity.

Mortality was 100% in the group given 300 mg/kg mg/kg b.w, 70% in the group given 200mg/kg and 10% in the group given 100mg/kg mg/kg b.w of the ECc extract. Rats given 75mg/kg b.w manifested only transient hypo activity with no mortality and rats in the groups given 20mg/kg b.w (i.p) showed no signs of toxicity and no mortality.

According to the linear regression line shown in Fig. 1, the calculated intraperitoneal LD50 of ECc extract was 158.49mg/kg b.w (Fig. 1).

B. Hypoglycemic activity of ECc in normoglycemic fasted rats

Table 1 shows the dose dependent lowering effects of i.p administration of ECc on blood glucose level in normoglycemic fasted rats.

ECc, at the dose of 20 and 75 mg/kg b.w (i.p), did not exhibit significant hypoglycemic effect in fasted normal rats after 3h of administration; but after the same duration, ECc at the dose of 100 and 200 mg/kg B.w (i.p), shows significant hypoglycemic effects (p<0.0001) in normal rats compared to control (RTN). This reduction is about 49% compared to the basal blood glucose time G0 (0min) (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>G0 (mg/kg b.w)</th>
<th>1h</th>
<th>2h</th>
<th>3h (% glycemia)</th>
<th>Day 21 (% glycemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTN</td>
<td>87±9</td>
<td>86±2</td>
<td>84±10</td>
<td>89±9</td>
<td>77±4 (11.49%)</td>
</tr>
<tr>
<td>RDE</td>
<td>338±65</td>
<td>367±46</td>
<td>350±32</td>
<td>362±35 (+7.08%)</td>
<td>294±28 (-13.02%)</td>
</tr>
<tr>
<td>RN20</td>
<td>20</td>
<td>93±4</td>
<td>115±12</td>
<td>94±16</td>
<td>44 (1.08%)</td>
</tr>
<tr>
<td>RN75</td>
<td>100</td>
<td>75±0.12</td>
<td>77±13</td>
<td>59±21(+) 39±14(-)</td>
<td>48.27%</td>
</tr>
<tr>
<td>RN100</td>
<td>200</td>
<td>76±0.09</td>
<td>59±7(+) 44±5(-)</td>
<td>38±7(+) 49.47%</td>
<td></td>
</tr>
</tbody>
</table>

Values were expressed as mean± SEM, n = 5 in each group; ^ (p<0.05) ; * (p<0.01) ; # (p<0.001) ; $ (p<0.0001) by comparison with G0; ^ (p<0.01) ; * (p<0.001) ; $ (p<0.0001) compared to RTN.

C. Antihyperglycemic activity of ECc in STZ-induced diabetic fasted rats

Evaluation of antihyperglycemic activity of intraperitoneal administration of ethanolic extract of Citrullus colocynthis seeds (20mg/kg b.w) on blood glucose level in normal and STZ-induced diabetic fasted rats was shown in Table 2.

Compared to the baseline glucose level (0 min), there was no significant change in the blood glucose level in normal and diabetic control rats (RTN and RTD).

But, in experimental rats group (RDE), diabetic rats treated with ECc (20 mg/kg b.w (i.p)), very significant reduction (p < 0.0001) in glucose level, compared to the baseline glucose level and diabetic control (RTD), was observed at 3h and after 21 days of treatment, with values of 28.64 % and 40.74% reductions, respectively (Table 2).

IV. DISCUSSION

This study was carried out in order to elucidate the influence of intraperitoneal injection of 20mg/kg b.w of ethanolic extract of Citrullus colocynthis seeds (ECc) on fasting blood glucose levels in normal and STZ-induced diabetic rats.
A result of the acute toxicity obtained in this study in this study has shown that the ECc extract has low acute toxicity, with intraperitoneal LD50 values of 158.49mg/kg b.w in rats Wistar. According to Hodge and Sterner (1949) [14], ECc would be considered as moderately toxic.

The severity of toxicity was positively proportional with increase in ECc doses, this indicate that toxicity was due to the presence of toxic components like saponine and alkaloids that induce toxicity according with the dose.

*C. colocynthis* toxicity at high doses has been reported in experimental studies in animals and in humans [15-19]. According Dehghani and Panjehshahin (2006), a single daily dose of ethanolic extract of *C. colocynthis* (50, 100, 200, 400 mg/kg) administered intraperitoneally, for 14 days, can have toxic effects on liver cells which may induce hepatocyte necrosis and liver fibrosis. These effects were dose dependent [20].

STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents [21].

The intraperitoneal injection of streptozotocin at the dose of 55 mg/kg into rats was characterized by polydipsia, polyuria, weight loss and hyperglycemia. The elevated level of blood glucose observed after 72h of streptozotocin induction confirmed the diabetic state in rats which may be attributed to the selective cytotoxic effect of streptozotocin on the beta cells [13, 22].

Results of this study demonstrated that injection of 20mg/kg b.w (i.p) of ethanolic extract of *Citrus colofynthis* seeds (ECc) has antihyperglycemic effect in normal and STZ-induced diabetic rats.

For 3 hours, it corrects blood glucose levels in STZ-induced diabetic rats of about 28.65%. This effect persists during 21 days after injection of extract with values of 40.74%.

In addition, we noted a hypoglycemic effect in normal rats treated to intraperitoneal injection of over 100 mg / kg b.w.of ECc extract.

Although, the hypoglycemic effect of *C. colocynthis* fruit has also been reported in experimental studies [23-27].

Recently, Oryan et al., (2014) showed that daily oral administration of 300 mg/kg ethanol extract of *C. colocynthis* for 12 day decreased the blood glucose concentrations into normal range in the alloxan-induced diabetic rats [28].

The mechanism underlying the glucose lowering effect of *C. colocynthis* is not clear. One study indicated that *C. colocynthis* had an insulin tropic effect on isolated pancreatic islets [29]. Another study showed that *C. colocynthis* inhibited the toxic effect of streptozotocin on pancreatic cells in rats [24, 30]. These two effects may suggest a role in the production and protection of pancreatic cells in rats from harmful metabolic products produced during hyperglycemic conditions [31].

On the other hand, it has been suggested, probably, that the mechanism responsible for the serum glucose lowering effect of *C. colocynthis* are attributed to an inhibitory effect of glucose absorption, an increased incorporation of circulating glucose as hepatic glycogen, an increases in peripheral glucose uptake, an decreased gluconeogenesis or an enhanced secretion of insulin [25].

V. CONCLUSION

The present study indicated that the intraperitoneal injection of 20mg/kg b.w. of ethanolic extract of *Citrus colofynthis* seeds induced a significant antihyperglycemic effect in normal and STZ- diabetic rats. However, higher doses (> 100mg/kg b.w) of this extract cause a risk of severe toxicity and hypoglycemia.

Our results give support to the traditional use of *C. colocynthis* and antiabetic herbal medicine. The exact mechanism(s) by which the extract induced these effects remains to be investigated.

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