Evaluation of Antidiabetic Activity of *Ipomoea Aquatica* Fractions in Streptozotocin Induced Diabetic in Male Rat Model

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Abstract: *Ipomoea aquatica* (IA) is a common green leafy vegetable consumed in many parts of the world. The plant is considered as a good antidiabetic herbal plant. The present work was designed to investigate the oral hypoglycaemic activity of *Ipomoea aquatica* fractions in streptozotocin induced diabetic male rats. The male rats with average weight 200-220 g were divided into four groups (n=6), control, diabetic (induced with a single dose of streptozotocin (50 mg/kg body weight), T-1 and T-2 as treated diabetic with the two fractions of *Ipomoea aquatica* (IA6-1 and IA9-2), respectively. Biochemical evaluation of blood glucose, serum insulin and C-peptide were carried out. Histological examination of islets of Langerhans was done for cellular population changes. The results revealed that the oral consumption of two fractions of *I. aquatica* for 15 days, effective significantly reduced the fasting blood sugar level of streptozotocin-induced diabetic rats (p < 0.05). The percent changes were a 51% and 31% decrease in the serum glucose concentration of the diabetic rats when treated with the plant fractions of IA6-1 and IA9-2, respectively. Most biochemical parameters tested returned to nearly normal levels. Histologically, islets area and normal cell population were preserved in treated animals with superior results in T-1 and T-2 groups. In conclusion, fractions IA6-1 and IA9-2 showed potential antidiabetic effects when given orally to diabetic rats. The mechanism of action requires further investigation using both molecular and immunohistochemical methods investigation of both insulin secreting cells and insulin receptors. Future clinical trials could be tried using volunteers to confirm the present results so could be marketed as a supplement for diabetic patients.

Keywords: *Ipomoea aquatica*; Hypoglycemic activity; α-Amilase; C-peptide; Insulin.

1 Introduction

Diabetes mellitus (DM) is chronic metabolic disorders that affect human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins [1,2]. DM is characterized by the impaired insulin secretion by the islet of Langerhens of pancreas. This increases the blood glucose level and which in turn damages tissues and organs under chronic conditions [3]. Currently, DM is growing global health concern, where World Health Organization (WHO) predicted an estimated future number of 366 million affected individuals would be equivalent to a diabetes prevalence of 5% in 2030 [4]. The incidence and prevalence of diabetes are increasing, especially in developing and newly industrialized countries. About 90% of all cases of diabetes that found in developed and developing countries are noninsulin-dependent diabetes mellitus, which known also as type-2 diabetes (T2D), or adult-onset diabetes. These diagnoses are typically in adults more than 30 years of age [5], and are usually characterized by postprandial hyperglycemia, an abnormal rise in blood sugar following a meal [6]. T2D is complicated by several factors integral to the disease process such as insulin resistance, hyperinsulinemia, impaired insulin secretion, reduced insulin-mediated glucose uptake and utilization [7]. For a long time, C-peptide was considered as an important component in the biosynthesis of insulin, but otherwise believed to possess minimal biological activity [8]. C-Peptide is produced in beta-cells in the pancreas, and secreted into the blood stream. Therefore, C-Peptide is
an excellent parameter for evaluating pancreatic β-cells function.

Due to the existing synthetic drugs have many limitations, developing the new drugs is still urgently needed [9], a great progress has been made in the treatment of diabetes using oral hypoglycemic agents. Therefore, many efforts have been made to search for other effective and safe enzyme inhibitors from natural materials in order to control diabetes [10], and also to develop physiological functional food to treat diabetes [11]. Recently, the medicine from plants is drawing ever-increasing attention worldwide, due to their low toxicity and few side-effects [12]. Furthermore, World Health Organization has recommended the use of traditional plant for diabetes treatment, which they are considered to be excellent candidates for oral therapy [13]. The plant kingdom possesses a wide variety of natural substances that have antidiabetic action, and have few or no documented side effects. A number of medicinal plants extracts have showed significant hypoglycemic properties [14,15]. Despite of the fact that there are several herbal medicines are available in the market, search for much effective drug of plant origin will continue [3]. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, which are frequently implicated as having antidiabetic effect [16]. A number of isolated terpenes revealed beneficial effects in diabetes [17]. In previous study of [18], some isolated terpenes were possessed both anti-hyperglycemic effects in glucose-fasted rats as well as insulin stimulating effects in INS-1 cells. Flavonoids are well-known for their widespread biological activities including anti-diabetic activity [19,20]. Several studies have been performed to explore their potential antidiabetic properties [21,22]. Flavonoids curb the glucose level, reduce plasma cholesterol and triglycerides significantly, as well as increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets [23,24].

*Ipomoea aquatica* Forssk. (Convolvulaceae), is an aquatic or semi-aquatic edible herb [25]. *I. aquatica* (IA) is used traditionally against various disorders, such as diabetes, liver malfunction, constipation, as well as in the treatment of arsenic and heavy metal poisoning [25,26]. Moreover, according to previous studies in [27-29], the authors clearly suggested an oral hypoglycemic effect of *I. aquatica* in normoglycemic and diabetic rats. Other study showed the extract of plant leafs can be used to reduce blood sugar levels [28,30]. Literature surveys indicated the occurrence of significant amounts of phenolic compounds, flavonoids, saponins, β-carotene and ascorbic acid in *I. aquatica* [25]. The phytochemical screening analyses of *I. aquatica* fractions demonstrated the presence of several phytochemical constituents, such as, triterpenes, steroids and cardiac glycosides in the lipophilic fractions, as well as phenolic, flavonoid, alkaloid and saponin compounds in the hydrophilic fractions. The presences of these bioactive compounds might contribute to illustrate the antidiabetic effect of this plant as will be observed in the present study. Despite the widespread use of *I. aquatica* as a green leafy vegetable, which it is considered a delicacy in Chinese cuisine and is supposed to possess insulin-like principles according to traditional Sri Lankan medicine, studies describing its therapeutic effects are considered stingy. A few numbers of studies were carried out to investigate antihyperglycemic action and/or oral hypoglycemic activity for whole parts or leaves of the *I. aquatica* plant, whereas no studies for plant fractions were executed. Hence, the aim of this work is to investigate the antidiabetic properties supported by histological evidence of single dose of streptozotocin (STZ) on pancreas of a male rat in order to evaluate the possible role of *I. aquatica* fractions in reversing the diabetic effect of STZ. In this study, two fractions (dichloromethane-ethyl acetate and ethyl acetate-methanol) were selected in order to explore the oral hypoglycemic activity of these fractions and their capability to treat diabetic rats which were induced using STZ, which these fractions are containing triterpenes and flavonoids.

## 2 Materials and Methods

### 2.1 Chemicals and apparatus

Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, USA), Streptozotocin (STZ) was obtained from MP Biomedicals (Illkirch-Graffenstaden, France). The α-amylase was assayed using spectrophotometer from UviLine 9400 (Mainz, Germany), whilst insulin and C-peptide were assayed using automated ELISA from Tecan (Männedorf, Switzerland).

### 2.2 Plant material, extraction and sample preparation

The plant of *I. aquatica* was collected from Darfur (West of Sudan). The fresh plant material was dried at room temperature and grinded using mechanical grinder to obtain a fine powder. The extraction was achieved by maceration with methanol (100%) for 24 h. Later on, the extract was filtered and the solvent removed using rotatory evaporator (40 °C) to get a crude extract. After that, the crude extract was then fractionated by vacuum liquid column chromatography on silica gel at room temperature. The column was eluted with different solvent composition, such as n-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), and finally washed by 100% of distilled water, gradually increasing the degree of polarity to get fifteen major fractions. All fractions were evaporated to dryness under...
reduced pressure using rotatory evaporator and stored in a freezer at −20 °C prior to use.

2.3 Animals grouping and experimental design

Adult albino male rats, weighing 210 ± 10 g, were purchased from Animal House, Faculty of Science, Sohag University, Sohag, Egypt. The animals were acclimated for 1 week before experiments were performed. All animals were housed 6 per cage under environmentally controlled conditions of light (12-light/dark cycle, lights on at 8:00 a.m.) and temperature (24 ± 1 °C) with free access to food and tap water. The animals were randomly divided into four groups of six animals of each as follows: Group (1) was kept as normal control (G-1) or non-diabetic administered with 2% DMSO. However, group (2) was served as diabetic groups (G-2), (3) and (4) were served as diabetic groups (T-1 and T-2) and were treated by given of two I. aquatica fractions, IA6-1 (DCM:EtOAc, 75:25, v/v) and IA9-2 (EtOAc:MeOH, 50:50, v/v) with orally at dose of 200 mg/kg body weight for two weeks. This work was carried out in accordance with the guidelines of Sohag University for animal use and approved by Ethics and Animal Care Committee.

2.4 Induction of diabetes

Diabetes was induced through a single intraperitoneal (IP) injection of a freshly prepared STZ (Sigma-Aldrich, USA) solution in ice cold citrate buffer (0.1 M, pH 4.5) at a dose of 50 mg/kg body weight to the overnight fasted rats [31]. The blood glucose levels (BGL) of rats were measured after 72 hrs of STZ administration and the rats showing blood glucose level > 250 mg/dl were considered as diabetic rats and were used for the experiment.

2.5 Measurement of fasting blood glucose (FBG) and body weight (BW)

The FBG level of each animal in normal and diabetic groups was monitored on several days, 0, 7 and 15. The FBG level was measured using One Touch Glucometer (company name and country) for drop of blood which collected from the tip of tail vein of each rat. On the other hand, initial and final body weights were record as well as the percentages of change (%) in FBG level and body weights were calculated.

2.6 Collection of blood for biochemical parameters

After 15 days, all rats of each group were sacrificed and blood samples for biochemical analysis were collected from the heart into plain tubes. Serum was separated by centrifugation at 3000 rpm for 10 min, transferred into eppendorf tubes and stored in the −20 °C freezer prior to use for various biochemical parameters analyzes.

2.7 Assessment of biochemical parameters (Biochemical analysis)

The α-amylase as well as the C-peptide and insulin in the serum were measured using different suitable kits. All assessment assays and kits were performed in accordance with the manufacturers’ instructions and protocols (section 2.7.1 and 2.7.2).

2.7.1 Determination of serum α-amylase

Amylase was carried out using a colorimetric method with amylase kit (Monsano, Italy) and according to the described method in [32].

2.7.2 Determination of insulin and C-peptide

The serum insulin level and C-peptide were assayed with an ELISA kit (Lise-Meitner-Straße, Germany) and (Canoga Park, UK), respectively. The insulin level was performed according the method described in [33], whereas, the C-peptide assay was achieved according to the protocol in Ref. [34].

2.7.3 Histological study

After collection of blood for biochemical analysis, the animals from all groups were sacrificed under deep ether anesthesia, the abdomen was opened and pancreas was dissected carefully and fixed in 10% neutral buffered formalin and processed routinely for Hematoxylin and Eosin (H&E) stained 5 micron paraffin sections. The slides were examined and photographed and islets area and cell population were compared in different groups.

2.8 Statistical analysis

The data of body weight, fasting blood glucose (FBG) values and biochemical parameters were expressed as mean value with standard error of mean (M ± SEM). Determinations were obtained from six animals per group and the differences were examined using one-way analysis variance (ANOVA), followed by post hoc Dunnett’s test. All statistical analyses
were calculated using the software GraphPad Prism® Software version 5.00 (San Diego, CA). Statistical significance was accepted at p values < 0.05.

3 Results and Discussion

Plants have been effectively prescribed for the treatment of many diseases including diabetes mellitus. There are few studies dealing with the antidiabetic effect of *I. aquatica* extracts although the plant was classified as good antidiabetic herbal plant. The present study aims to investigate antidiabetic properties of *I. aquatica* fractions whilst all previous reports were focused only on plant extracts. To achieve that some biochemical parameters, such as FBG, α-amylase, insulin and C-peptide were tested in order to assess the oral hypoglycemic activity of IA fractions. Loss of weight has been known as one of the diabetic mellitus symptoms [35,36]. As shown in Table 1 and Fig. 1, the results indicated significantly decreased (p < 0.05) in the final body weight of diabetic treated groups compared to control group (1) with percent of Change (%), -13.63 and -26.39 of group (2) and (3), respectively.

Fig. 1. Effect of two IA fractions on body weight after STZ induced diabetic rats.

Fig. 2. Effect of two IA fractions on Blood Glucose Level after STZ induced diabetic rats.

The results illustrated improvement of weight loss of treated group (T-1) more than treated group (T-2) with approximate 50% which indicating higher response in T-2 group. In addition, loss of body weight associated with diabetes may be due
Table 1: Effect of IA methanol extract on body weight of control and STZ diabetic rats. (Values are expressed as Mean±SEM, n=6 in each group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th></th>
<th></th>
<th>Percent of Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>206.67±3.33</td>
<td>242.50±3.82</td>
<td>17.35±0.77</td>
<td></td>
</tr>
<tr>
<td>T-1 (200 mg/kg)</td>
<td>208.33±4.01</td>
<td>180.00±5.63</td>
<td>-13.63±1.87</td>
<td>a***</td>
</tr>
<tr>
<td>T-2 (200 mg/kg)</td>
<td>215.00±2.24</td>
<td>158.33±4.22</td>
<td>-26.39±1.54</td>
<td>a***</td>
</tr>
</tbody>
</table>

a When compared to normal control group, * significant at p < 0.05, where the significance was performed using One-way ANOVA followed by post hoc Dunnet’s test.

Table 2: Effect of IA methanol extract on FBG of control and STZ diabetic rats. (Values are expressed as Mean±SEM, n=6 in each group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Fast blood glucose level (mg/dl)</th>
<th></th>
<th></th>
<th>Percent of Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day (0)</td>
<td>Day (7)</td>
<td>Day (15)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>97.83±2.60</td>
<td>96.17±3.24</td>
<td>99.67±2.11</td>
<td>3.03±1.02</td>
</tr>
<tr>
<td>T-1 (200 mg/kg)</td>
<td>533.00±39.96***</td>
<td>378.17±39.49***</td>
<td>265.33±48.66***</td>
<td>-51.49±6.75***</td>
</tr>
<tr>
<td>T-2 (200 mg/kg)</td>
<td>302.00±11.57***</td>
<td>419.67±34.21***</td>
<td>212.50±47.81***</td>
<td>-31.88±13.57*</td>
</tr>
</tbody>
</table>

a When compared to normal control group, * significant at p < 0.05, # non-significant at p < 0.05, where the significance was performed using One-way ANOVA followed by post hoc Dunnet’s test.

Table 3: Biochemical investigation of amylase, C-peptide and insulin. (Values are expressed as Mean±SEM, n=6 in each group)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>T-1 (200 mg/Kg)</td>
<td>T-2 (200 mg/Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum α-Amylase (U/l)</td>
<td>1137.59±261.84</td>
<td>1580.39±224.19#</td>
<td>1618.40±224.61#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-Peptide (ng/ml)</td>
<td>0.15±0.02</td>
<td>0.12±0.01#</td>
<td>0.15±0.02#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>2.03±0.38</td>
<td>2.07±1.55#</td>
<td>3.40±0.96#</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a When compared to normal control group, # non-significant at p < 0.05, where the significance was performed using One-way ANOVA followed by post hoc Dunnet’s test.

To increased muscle wasting and loss of tissue proteins [37]. Furthermore, the deficiency of insulin in the diabetic rats led to decreased amino acids at the level of protein synthesis as reported in [38,39]. Insulin deficiency result in lipolysis in adipose tissue and protein breakdown [40]. Biochemical studies revealed that the intraperitoneal injection of STZ to normal rats was effectively induced diabetes and reflected by significant elevation (p < 0.05) of blood glucose level when compared to the control group (G-1) as shown in Table 2 and Fig. 2. The diabetic rats were treated for 15 days with two I. aquatica fractions of IA6-1 and IA9-2 (200 mg/Kg BWt) and the results revealed the reduction of blood glucose from 533 to 265 (mg/dl) and from 302 to 212 (mg/dl) with percentage of change -51.49 and -31.88 of tearted groups (T-1) and (T-2), respectively (Table 2 and Fig. 2). IA9-2 fraction was indicated the presences of phytochemical comounds like flavonoids whilst terpenoids and steroids were found to be in IA6-1. In the present study, potent effect of IA6-1 fraction compared to IA9-2 fraction in reducing blood glucose level was observed. Regarding α-amylase, insulin and C-peptide in table 3 and Figs. 3-4, the results showed that there was no significant differences in these parameters at p < 0.05 in treated group with IA6-1 and IA9-2 when compared to control group after two weeks of treament.
The observed hypoglycemic effect of the *I. aquatica* extract was previously reported and was suggested to be through retardation of sugar adsorption in the small intestine [29]. Furthermore, *I. aquatica* plant is rich in dietary fibers and the presence of such substances in the extract may be responsible for the observed effect [41]. A similar results were reported in [42]. However, the exact mechanism by which this plant brings about of blood glucose reduction in both human and rats was not yet known. Literature dealt with antidiabetic action of the plant extract put the posibility of enhanced insulin secratation, an increase in peripheral glucose uptake or decrease glycomeogenesis and inhibited release of counter regulatory hormones, such as cortisol, glucagon, and also growth hormome [43-45]. Histological study showed that injection of streptozotocin results in decrease of area occupied by islets cells with marked decrease in cell population whereas the remaining cells showed vascular degeneration and degeneration of nuclei Fig. 5. Similar results were reported in animals models of diabetes [46]. Administration of *I. aquatica* fractions effectively preserved cellular components of pancreatic islet Fig (5), which was most probably due to antioxidant activity protect islets cells from STZ induced oxidative stress [47]. In the present study, IA6-1 fraction which has terpenoid compounds was found to have higher or potent effect than the other fraction of IA9-2 which has flavonoid compounds. Novel terpenoids–type quinones isolated from Picanthus angolensis was proved to have potential utility in treatment of type 2 diabetes via enhancing glucose uptake by cells while no effect on insulin level [48]. More further studies were needed to investigate the mechanism by which those compund can control hperglycemic status.
Fig. 5. Sections from rat pancreas to show Langerhans islets of: a. control group (G-1) islets (dotted circle) with normal cell population (dotted arrows); b. diabetic group (G-2), showing marked decrease in islets area (dotted circle). Islets cells showed vacuolation (stars) and marked degeneration, shrinkage and decrease in cell population (dotted arrows). Notice presence of mononuclear inflammatory cells (white arrows); c. treated diabetic group (T-1), showing preservation of islets area and cell population (dotted arrows); d. treated diabetic group (T-2), showing more or less normal islets and cell population (H&E stain).

4 Conclusion

To the best of our knowledge, this paper is the first to evaluate the oral hypoglycemic activity of *I. aquatica* fractions while all other studies were focused only on plant crude extracts. The present work aims to compare the oral hyoglycemic activity of two frations of *I. aquatica* methanolic extract, which the first one contains terpenoids while the other contains flavonoids. The results of FBG showed significantly decreased of glucose level in diabetic treated rats when treatment with the two fractions for two weeks by means that these fractions are ameliorate the glucose level. However, the results of the pervious test showed higher improvement in diabetic treated group that treated with fraction which containing terpenoids than the other fraction which containing flavonoids. Therefore, fraction of DCM:EtOAc (75:25, v/v) is considered more potent antidote for diabetes than the other fraction of EtOAc:MeOH (50:50, v/v). On the other side, the results of other biochemical parameters like α-amylase, insulin and C-peptide exhibited that there were no significant differences between diabetic treated groups and control normal group. Moreover, the biochemical findings have been confirmed through the histological examination of sections taken from rat pancreas of each group. For future work, further investigations are required to explain and understand the mechanism of the hypoglycemic activity of *Ipomoea aquatica* fractionations, as well as the isolation and identification of the bioactive substances which may be responsible about this activity will be necessary.
References


