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Evaluation of the Leaves of *Ipomoea aquatica* for its Hypoglycemic and Antioxidant Activity

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Abstract:
The present study was accomplished to explore the hypoglycemic and antioxidant activity of methanolic extracts of the leaves of *I .aquatica.* The animals used to test the antidiabetic activity were *Swiss albino* mice of 7-8 weeks, average weight 20-30 gm of both male and female sexes. Twenty four mice were randomly grouped into four where there were two drug groups, one control and one standard control group. Metformin was used as standard in the testing of hypoglycemic activity. Different doses as 200 mg/kg and 400 mg/kg body weight of extract were used during the course of study. Both of the doses showed potent hypoglycemic activity in comparison with the control ($P<0.001$). But more activity was observed incase of 400 mg/kg body weight dose. Incase of antioxidant effect, the extract showed potent free radical scavenging activity with IC_{50} value of 4.4 µg/ ml. Ascorbic acid was used as standard with IC_{50} value of 15.83 µg/ ml.

Key words: Diabetes mellitus (DM), *Ipomoea aquatica*, 1, 1-diphenyl-2 picrylhydrazyl (DPPH), streptozocin, hypoglycemia.

INTRODUCTION:

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves [1]. It is now become an epidemic, with a worldwide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS [2]. In recent years, a great scientific research interest for the understanding of the etiopathogenesis of diabetes mellitus (DM) and the ultimate development of definitive curative and/or prophylactic options in its management have been accelerated [3-4]. Drug management of DM without associated untoward effect has also remained a challenge for orthodox medical practice. This has necessitated exploration and screening of medicinal plants with acclaimed therapeutic efficacies in DM management as recommended by the WHO Expert Committee on DM [5-6].

Most of the plants prescribed for diabetes mellitus (DM) are not edible and therefore the studies on edible plants which have a hypoglycaemic effect would be of great value in the dietary management of the disease [7-8]. Leafy vegetables are extensively investigated as a newer source of natural antioxidants and other bioactive compounds of human health benefits [9]. Therefore the aim of the present study is to explore the hypoglycemic and antioxidant activity of *I. aquatica* that is an edible vegetable of Bangladesh.

Ipomoea aquatica belongs to the family Convolvulaceae is an aquatic plant that is found abundantly in muddy stream banks, freshwater pond and lakes. It is a trailing vine with milky sap. *I. aquatica* is commonly consumed in Cameroon and other countries as green leafy vegetable [10-11]. The leaves contain the adequate amount of amino acids like aspartic acids, glycine, alanie etc. in accordance with the WHO recommended pattern for an ideal dietary protein. This can be compared with the conventional food crops such as soybeans or whole egg indicating its potential for utilizing as food supplements.

I. aquatica is one of the richest sources of carotenoids and chlorophylls [12]. An extensive study on the identification of these pigments was carried out using high performance liquid chromatography (HPLC) revealed that it contains 12 components [9]. The leaves contain adequate quantities of most of the essential amino acids, eg aspartic acid, glycine, alanine and leucine, in accordance with the WHO-recommended pattern for ideal dietary protein [13]. This is comparable to conventional foodstuffs such as soybean or whole egg, indicating the potential of *I. aquatica* for utilisation as a food supplement [14].

The medicinal value of *I. aquatica* was notable from the ancient science of Indian medicine (Ayurveda) and homeopathy. At that time, extracts of *I. aquatica* leaves were administered orally to alleviate antioxidant related disorders. [15] The plant is also used effectively against nosebleed and high blood pressure [16-17]. Further, its leaf extract can be used to reduce blood sugar levels [18-19] and as an antibiotic against *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* [20]. The floral buds are used as an anthelmintic [21].

MATERIALS AND METHODS:

Experimental animals and their Management

Swiss-albino mice aged 7-8 weeks, average weight 20-30 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition for one week for acclimatization after their purchase and fed ICDDR, B formulated rodent food and water ad labium. They were housed individually in cages and were kept at constant room temperature (25.0 ± 3.0ºC), humidity 35-60% and 12 hours light and 12 hours dark cycle. Excreta were removed from the cages on every day. The animals were divided into four groups having 6 mice in each group.

Preparation of the plant extract:

The fresh leaves of *Ipomoea aquatica* were collected during the month of September 2009 from the area of Munshigonj district in Dhaka division. About 200 g of dried, ground separate parts of the plant were soaked in 1.5 L of 98% methanol for 5-7 days, stirring every 18 h using a sterilized glass rod, separately. The final extracts were passed through No. 1 Whatman filter paper (Whatman Ltd., UK). The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4°C for further use.

Induction of diabetes mellitus and measurement of plasma glucose:

Diabetes was induced by a single intraperitoneal (i.p.) injection of 100 ml of sterile phosphate buffered solution (PBS—pH 7.4) containing streptozotocin (STZ) (65 mg kg^{-1}), (Zanosar, Pharmacia & Upjohn, ON, Canada) and after 4 days the hyperglycemia was established [22]. Glucose concentration was measured in a blood sample obtained from tail puncture, with a glucometer (One touch Ultra). Only animals that had a blood glucose concentration higher than 20 mM 4 days after treatment with STZ were used for the study [22]. Control rats were injected with PBS only.

Antioxidant Activity (DPPH Free Radical Scavenging Activity) of Methanolic Extract:

The antioxidant activity of flower extract was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay [23-25]. The free radical scavenging capacity of the methnolic extract of different parts of the plant *Ipomoea aquatica* Forsk was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude methanolic extract of different parts of the plant *Ipomoea aquatica* Forsk was mixed with 95% methanol to prepare the stock solution (10mg/100mL). The concentration of extract of different parts of *Ipomoea aquatica* solution was 10 mg /100 ml or 100µg/ml. From stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes & by serial dilution with methanol and was made the final volume of each test tube up to 10 ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing extract of different parts of *Ipomoea aquatica* Forsk (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml) and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10mg/100mL or 100µg/ml) of extract of different parts of *Ipomoea aquatica* Forsk. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation-

% DPPH radical-scavenging
= [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] x 100

Then % inhibitions were plotted against respective concentrations used and from the graph (Graph 1) IC_{50} was calculated.

Statistical analysis:

The value of glucose (mmol/l) was expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's t-test. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 18) was applied for the analysis of data. Differences between groups were considered significant at $P < 0.05$, 0.001 levels.

RESULTS AND DISCUSSION:

The methanolic extract of the leaves of *Ipomoea aquatica* exhibited potent hypoglycemic activity and it was dose dependant. Both of the doses (200 mg/ kg and 400 mg/ kg) reduced blood glucose level and it was statistically highly significant ($P< 0.001$) in comparison with control group. But the magnitude of reduction was higher in 400 mg/ kg body weight dose (Table 1). In case of antioxidant activity, it showed potent antioxidant activity with IC_{50} value of 4.4 µg/ ml (Graph 1).

Streptozocin is a broad-spectrum antibiotic that is obtained from *Streptomyces achromogenes*. It is suggested that streptozocin possess diabetogenic properties mediated by pancreatic beta cell destruction, this compound has been widely used to induce diabetes in experimental animals [26]. Several evidence suggesting that it induces oxidative stress [27]. Oxidative stress is the result of relative overload of oxidants i.e. reactive

Graph 1: Free radical scavenging activity of methanolic extract of the leaves of *Ipomoea aquatica*.

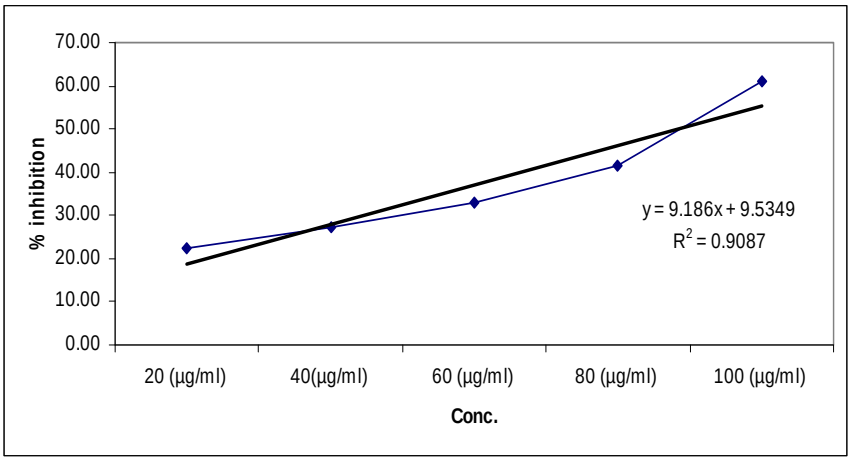

Table 1: Hypoglycemic effect of methanolic extract of the leaves of *Ipomoea aquatica*.

Group of rats	Number of mice in each group	1st week	3rd week
		Glucose (mmol/ l)	Glucose (mmol/ l)
200 mg/ kg body weight	6	22.87 ± 3.04	7.35 ± 0.55 ***
400 mg/ kg body weight	6	25.41 ± 2.60	6.85 ± 0.55 ***
Standard control (metformin)	6	20.80 ± 3.16	5.63 ± 0.557 ***
Control	6	20.38 ± 0.35	25.34 ± 1.43

Values are given as mean ± SEM, (n = 6); *: p<0.05, ***: p<0.001, Dunnet test as compared to diabetic control

oxygen species. The observed hypoglycemic effect of *I. aquatica* as previously reported may be due in part to the retardation of sugar absorption in the small intestine [28]. *I. aquatica* is rich in dietary fibres and the presence of such substances in the extract may be responsible for the observed effect [29]. Similar results have been reported by Bnouham *et al.*, 2003 [30]. However it is also reported by Malalavidhane *et al.,* 2000a that the hypoglycaemic effect of this plant is not entirely due to dietary fibers and some other active component is present [31]. The mechanism(s) by which this plant brings about a reduction in blood sugar level of rats or humans is not known. A previous study has indicated that the oral hypoglycaemic activity exerted by this plant extract is comparable to that of tolbutamide [32]. The antidiabetic action of *I. aquatica* may be due to enhanced insulin secretion or due to increase in preipheral glucose uptake, decreases gluconeogensis and inhibited release of counter-regulatory hormones i.e., cortisol, glucagon, and growth hormone, are possibilities which may be considered [33-35].

Antioxidants have been reported to exert beneficial effects on pancreatic β-cell function by preventing or delaying β-cell dysfunction due to glucose toxicity [36]. Diabetes is strongly co-related with oxidative stress induction [37]. The observed antioxidant activity of *I. aquatica* is due to the presence of glycoside, 7-O-β-D-glucopyranosyl-dihydroquercetin-3-O- α-D-glucopyranoside as reported by K Nagendra *et al.,* 2005. [38] Besides polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity [39]. The phenolic compounds may contribute directly to antioxidative action [40]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when up to 1.0 g is daily ingested from a diet rich in fruits and vegetables [41]. The antioxidative activities observed can be attributed both to the different mechanisms exerted by different phenolic compounds and to the synergistic effects of different compounds.

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