ANTIDIABETIC PROPERTY OF METHANOL EXTRACT OF B. ALBA (FRUITS ALONG WITH SHOOTS) AND ITS RELATIONSHIP WITH THE ANTIOXIDANT PROPERTY

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ABSTRACT

Diabetes is one of the most life threatening diseases of the present world. Among many causes excessive oxidative reactions are notable of them. The present study was designed to evaluate the comparative studies on antidiabetic and antioxidant properties of B. alba. Antidiabetic study was carried out on experimental animal model (Swiss albino mice) for 120 minutes treatment and significant blood sugar decline was observed (16.94 to 7.94 mM/L) at the dose of 250 mg B. alba extract and (20.0 to 7.30 mM/L) at 500 mg dose compared to control group whereas metformin was taken as a reference standard. Antioxidant property was done through free radical scavenging method. Methanol extract of B. alba showed IC₅₀ 496.32 (µg/ml). Where, Ascorbic acid was used as a standard with IC₅₀ of 4.78 (µg/ml).

Key words: Antidiabetic, Alloxan, Metformin, antioxidant, DPPH, free radical, B. alba

INTRODUCTION

Diabetes, a metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. According to WHO in 2014, 9% of adults 18 years and older had diabetes. 1.5 million deaths worldwide occurs in 2012 due to diabetes and more than 80% of diabetes deaths occur in low- and middle-income countries.[1-3]

In particular, type 2 diabetes mellitus (T2DM) is the most encountered form of diabetes, accounting for more than 80% of the total cases of diabetes [4-6]. Glucose metabolism disturbances are major factors leading to diabetes. The insulin released by the pancreatic β-cells is the hormone responsible for glucose homeostasis [7,8]. Insulin stimulates hepatocytes, myocytes, and adipocytes to uptake glucose from the circulatory system. Depending on need, glucose can either be used as an energetic source by glycolysis, or alternatively, stored as glycogen inside muscle or liver cells. The inappropriate utilization of insulin leads to insulin resistance, which is characterized by the inability of cells to respond to normal levels of circulating insulin [4,5], thus leading to the occurrence of the disease.
Medicinal plants are always a tremendous source of drugs. Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols etc. Traditional medicine refers to health practices, knowledge and beliefs incorporating plants, animals and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. Over the years, medicinal plants have been found useful in the treatment and management of various health problems. Traditional medicine is undoubtedly a reliable alternative approach to health care delivery in the metropolis because it is cheap, easily accessible and efficacious. [9]

There are many plant species available all over the world which has been used for the multi beneficial activities. Bangladesh, India and China are the major countries that are rich in many of the medicinal plant species. In spite of millions of chemically synthesized drug for a number of diseases; natural products of plant origin has got its own importance and has remained the most important source of new drugs. One such medicinal herb is Basella alba.

Beside this it is the major cause of adult blindness, kidney failure, neuropathy, heart attack and stroke. It also characterize by excessive disturbance of carbohydrate, protein and lipid metabolism, thickening of capillary basement membrane throughout the body leading to microangiopathy, macroangiopathy and long term complications which effects eye, kidneys, nervous system and circulatory system.[9]

Table 1: Effect of the methanolic extract of the fruits and shoots of Basella Alba L. on oral glucose tolerance test in diabetic mice

| Group     | Group I | Group II | Group III | Group IV  | Group V  |
|-----------|---------|----------|-----------|-----------|----------|
| Time      |         |          |           |           |          |
| 0 min     | 5.8±0.36| 20.6±0.26| 23.6±0.20***| 16.94±0.43***| 20.0±0.36 |
| 30 min    | 5.7±0.32| 23.4±0.47| 14.2±0.25***| 15.87±0.31***| 16.3±0.25***|
| 60 min    | 5.8±0.52| 19.7±0.21| 10.2±0.35***| 12.28±0.25***| 14.3±0.40***|
| 90 min    | 7.7±0.25| 17.2±0.27| 8.7±0.45***| 10.05±0.27***| 10.9±0.28***|
| 120 min   | 5.7±0.22| 16.6±0.45| 5.2±0.33| 7.94±0.56| 7.3±0.58 |

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Taxonomy of the plant

Kingdom : Plantae
Phylum : Magnoliophyta
Class : Magnoliopsida
Order : Caryophyllales
Family : Basellaceae
Genus : Basella
Species : alba

Basella alba is a wildly cultivated, cool season vegetable with climbing growth habit. It is a succulent, branched, smooth, twining herbaceous vine, several meters in length. Stems are purplish or green. Leaves are fleshy, ovate or heart-shaped, 5 to 12 cms long, stalked, tapering to a pointed tip with a core data base. Spikes are axillaries, solitary, 5-29 cm long. Fruit is fleshy, stalk less, ovoid or spherical, 5-6 mm long, and purple when mature. Mainly leaves and stems are used for the medicinal purpose. [13]

Basella alba has been used for many of its useful product from ancient times. Daily consumption of Basella alba has a positive effect on total-body vitamin A stores in men. [14] The paste of root of red B. alba along with rice washed water is taken in the morning in empty stomach for one month to cure irregular periods by the rural people of Orissa, India. Leaves of B. alba is used for the treatment of hypertension by Nigerians in Lagos, and malaria in Cameroonian folk medicine. The plant has been reported for its antifungal, anticonvulsant, analgesic, anti-inflammatory and androgenic activities and for the treatment of anemia. The leaves of B. alba are traditionally used in ayurveda system of medicine to bring sound refreshing sleep when it is applied on head about half an hour before bathing. [12] A paste of the root is applied to swellings and is also used as a rubefacient. Sap is applied to acne eruptions to reduce inflammation. Decoction of leaves used for
its mild laxative effects. Pulped leaves applied to boils and ulcers to hasten suppuration. Sugared juice of leaves is useful for catarrhal affections. Leaf-juice mixed with butter, is soothing and cooling when applied to burns and scalds. In Ayurveda, it is used for hemorrhages, skin diseases, sexual weakness, ulcers and as laxative in children and pregnant women. The plant is febrifuge, its juice is a safe aperient for pregnant women and a decoction has been used to alleviate labour. It is also an astringent and the cooked roots are used in the treatment of diarrhea. The leaf juice is a demulcent, used in cases of dysentery. [14] This plant serves as a Thai traditional vegetable. The fruit provides dark violet color for food colorant. Basella mucilage has been used in Thai traditional medicine as topical application for irritant, bruise, ringworm and laboring. Stem and leaves are used as mild laxative, diuretic and antipyretic. [15] In India, it has been used for antirpruritis and burn [16], and has been used in Bangladesh for acne and freckle treatment. [17] The Ayurvedic treatment in India has been used B. alba leaves and stem for anticancer such as melanoma, leukemia and oral cancer. [18] Root and leaves has been used for the removal of after birth, stomach pains and increase milk production. [19] Basella alba is administered orally for the treatment of anal prolapsed or hernia. Ground leaves of Basella alba are rubbed on the human hand to introduce the whole preparation into the animal vagina every morning for the treatment of sterility. [20] The leaf juice is used in Nepal to treat dysentery, catarrh and applied externally to treat boils. The mucilaginous qualities of the plant make etc. The purplish sap from fruits is used as a colouring agent in pasteries and sweets. [21] Basella alba has been used for the treatment of Anemia in women, coughs, cold (leaf with stem), cold related infections. [22] Maceration is taken orally for infertility, pelvic inflammatory disease, orchitis, epididymitis, threatened abortion, spurious labour. [23] Leaves are used in constipation, poultice for sores, urticarial and gonorrhoea. It is also used in poultice local swellings, intestinal complaints etc. [24] The mucilaginous liquid obtained from the leaves and tender stalks of plants is popular remedy for headaches. [25] Species of the plant called Basella rubra has been scientifically investigated to possess hypoglycemic activity in streptozotin-induced diabetes in rats [26] and aqueous leaf extract of of B. alba has antidiabetic effect in alloxan-induced diabetes rats. [27] Aside this, information about anti-diabetic effects of B. alba fruits and shoots are acanty. This study was carried out to evaluate the anti-diabetic effect of aqueous extract of B. alba fruits and shoots in alloxan induced albino rats.

PLANT MATERIALS
The fresh fruits and shoots of Basella Alba L. were collected from Kawran Bazar, Dhaka. The plant materials were identified and authenticated by the Chief Herbarium officer of the Dept. of Botany,

MATERIALS AND METHOD

Fig. 1: Effect of the methanolic extract of the fruits and shoots of Basella Alba L. on oral glucose tolerance test in diabetic mice.
Animals
A total of 20 male, eight week-old Swiss albino mice (27-30g) purchased from Jahangirnagar University, Dhaka, Bangladesh, were used in the study. The animals were kept in cages under standard environmental conditions (25± 2°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University, Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute. After randomization into various groups, the rats were acclimatized for a period of 2 weeks in the environment before the initiation of the experiment. The cages were cleaned and washed daily. All procedures involving the use of animals in the study compiled with the guiding principle for research involving animals as recommended by the declaration of Helsinki and the guiding principles in the care and use of animals. [28] University of Rajshahi. The collected plant parts (fruits and shoots) were washed into tap water and shaded dried after which they are reduced into fine powder by grinding. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

PREPARATION OF PLANT EXTRACT
About 300 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 1500 ml of 90% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by anapiece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract wasobtained. Fine powder of the fruits and shoots of *Basella Alba* L are dissolved in 90% Methanol and then evaporation the solvent.

Drugs and Chemicals
The standard drug, Metformin hydrochloride was the generous gift samples from Beximco Pharmaceuticals Ltd of Bangladesh. Blood samples analyzed for blood glucose content by using OK meter Match glucose test meter (Hsinchu, Taiwan). Alloxan, DPPH (1,2-diphenyl-2-picrylhydrazyil), Ascorbic acid and other solvents were collected from laboratory of Bangladesh University. All chemicals that were used in the research were analytical graded.

PREPARATION OF STANDARD DRUG
5gm of Metformin were obtained from Beximco pharmaceuticals. Then the powder was dissolved in 25.70 ml of distilled water to get the stock solution of 19.20 g/dl.

Induction of Diabetes mellitus
The animal were weighted and injected via intraperitoneal route: 90 mg/kg of Alloxan dissolved in normal saline using insulin syringes. Diabetes mellitus was confirmed after 72 hr of alloxan injection by testing the fasting glucose level in the blood obtained from the tail vein of the animals using glucometer and glucose test strip. The result of the blood glucose measurement by glucometer correlates excellently well with the result obtained from the laboratory methods. [29] The accuracy of the test result was confirmed by the use of glucose test kit.

EXPERIMENTAL DESIGN
Twenty (20) Swiss albino mice were grouped into five (5) different groups containing 4 mice in each group. All the groups were kept into different cages. The groupings of the mice were done by as follows:

1. **Group I:** Normal Control group, they were neither induced with diabetes nor given any treatment throughout the experiment.
2. **Group II:** Diabetic Control, these rats were induced diabetes but not given any form of treatment throughout the experiment procedure.
3. **Group III:** Diabetic Standard (Standard Group, Metformin HCl, 100 mg/kg), the rats were induced with diabetes and treated with a standard drug (Metformin) at a dose of 100mg/kg.
4. **Group IV:** Diabetic Extract (Extract Group 250 mg/kg), the rats were induced with diabetes and treated with low dose of B. alba fruits and shoots extracts.
5. **Group V:** Diabetic Extract (Extract Group 500 mg/kg), the rats were induced with diabetes and treated with higher dose of B. alba fruits and shoots extracts.

ADMINISTRATION OF DRUGS
Both Metformin and B. alba were administered via the oral route with the aid of an oropharyngeal cannula. The rats were handled appropriately to restrict movement and prevent trauma to the mice during drug administration. Measurement of fasting blood glucose level
All mice received 1gm /kg glucose, after 1 hour of feeding of extract and/ or drug and three more blood samples were collected at 30, 60, 90 and 120 minutes intervals and blood glucose level was...
estimated in all the experiments by using glucometer.

**ANTIOXIDANT ACTIVITY**

In-vitro Antioxidant DPPH (1,2-diphenyl-2-picrylhydrazyl) radical scavenging activity[30] 0.1 mL of various concentrations of sample was mixed with 0.4 mL of 0.3 mM DPPH reagent prepared in methanol. Mixture were shaken and kept into dark at room temperature for 30 min. absorbance of the reaction mixture was taken at 517 nm. DPPH free radical scavenging activity was calculated by the following formula:

\[
\% \text{ of scavenging} = \frac{A_{\text{absorbance of control}} - A_{\text{absorbance of sample}}}{A_{\text{absorbance of control}}} \times 100
\]

**STATISTICAL ANALYSIS**

All analyses tests were done three times to get most reliable data. Experimental data were presented as mean ± SEM. For graphical analysis Microsoft Excel 2010 were used. For statistical evaluation IBM-SPSS software version 20 was utilized. By using the Dunnett test significant differences (**P-value<0.001**) between the means were determined.

**RESULTS**

**Effect of B. alba fruits and shoots extract and metformin on fasting oral glucose tolerance test in alloxan induced mice**

The results of this experiment are shown in table 1. Diabetes mellitus was induced at the beginning of experiment at 72 hr, which represents the values after induction. Mice with fasting glucose level 10 mM/L were considered as having diabetes mellitus.[31] The results show that mice show no effect on fasting blood glucose in Normal group (Group I). There were sustained hyperglycemic conditions in diabetic control group (Group II). The fasting blood glucose level is significantly higher in group II, II, IV & V than that of group I throughout the experiment. The mean fasting blood glucose level in IV & V after 60 min of administration was significant (19.7±0.21, 10.67±0.25, 14.3±0.40 mM/L) compared to group I. there were no statistical difference in group I and group II mice throughout the experiment. In group III mice where standard drug were used, the fasting blood glucose level remains in controlled limit within the time.

As depicted in Table 1, fasting blood glucose level in group III, IV & V were significantly decreased compared to group II. The decreases lasted throughout the experimental procedure. Experimental data were presented as mean ± SEM. For statistical evaluation IBM-SPSS software version 20 was utilized. By using the Dunnett test significant differences (**P-value<0.001**) between the means were determined where n=03

**DPPH RADICAL SCAVENGING ACTIVITY**

Free radical causes huge damage to the several parts of the body organ. Scavenging of these radicals is an important strategy to treat several diseases including diabetes. For free radical scavenging activity, DPPH radical scavenging model is well accepted. DPPH is a stable colored free radical which loses its color when free radicals are scavenged. For methanolic extract of B. alba the percentage of free radical scavenging activity increased in dose dependent manners. As the concentration increased the scavenging activity also increased. The IC50 was found at the concentration 496.32 (µg/ml). Here, Ascorbic acid was used as a standard with IC50 of 4.78 (µg/ml).

**DISCUSSION**

Antidiabetic effect of B. alba fruits and shoots methanolic extract

| Concentration (µg/ml) | 1      | 5       | 10     | 50      | 100     | 500     |
|----------------------|--------|---------|--------|---------|---------|---------|
| % of inhibition      |        |         |        |         |         |         |
| Ascorbic acid        | 20.92±1.21 | 51.36±1.42 | 63.43±1.13 | 81.26±2.01 | 88.22±1.56 | 92.15±1.87 |
| Methanolic Extract of B. alba | 12.04±0.94 | 16.22±1.11 | 19.25±1.03 | 24.66±1.29 | 28.07±1.56 | 51.32±1.98 |
The antidiabetic effects of *B. alba* fruits and shoots methanol extract and Metformin on alloxan induced diabetic mice was examined in this study. The healthy normal group has normal fasting blood glucose level. Therefore, this showed that normal mice chow has no effect on the fasting blood glucose level. The increase in fasting blood glucose level above 10 mM/L in groups II, III, IV and group V, which were significant when compared to healthy control group confirmed induction of diabetes mellitus in the test mice. Diabetic mice treated with *B. alba* fruits and shoots methanolic extract significantly lower fasting blood glucose level compared to diabetic control group. The anti-hyperglycemic effect of *B. alba* fruit and shoots was noticed at two different levels for two different doses. It was found that hyperglycemic effect of *B. alba* fruit and shoots shows dose depended activity. The activity increased with higher dosage than lower dosage. The activity also shows time depended criteria. After administration of drug it absorb in the gastrointestinal tracts of the mice. With increasing time presence of extract also increases in the blood stream. So after administration the blood glucose level decreases time to time. At 30 min after administration the blood glucose level were found 15.9±0.31 mM/L and 16.3±0.25 mM/L for lower and higher concentration respectively. But after 120 min of administration the decreasing trend continues & the blood glucose level were found 7.94±0.56 mM/L & 7.3±0.58 mM/L L for lower and higher concentration respectively. The decrease of fasting blood glucose level may be due to the presence of antioxidant activities of *B. alba*. Because *B. alba* is well known for its antioxidant activities. [31] We also know free radical can damage most of the body organs including pancreatic beta cells which are involved in insulin production. [32-35] the antioxidant can prevent oxidative damage of the beta cell thus increase the secretion of the insulin and by this way it can be useful in controlling blood glucose level. Other reasons which may account for the reduction of fasting blood glucose levels observed may possibly include inhibition of glucose absorption, increase sensitivity of receptors to insulin and stimulation of peripheral glucose uptake. Although present finding suggested that methanolic extract of fruits and shoots of *B. alba* shows antidiabetic property. Although no specific compound yet been found, but the research is still going on.

### Antioxidant effect of *B. alba* fruits and shoots methanolic extract

Free radicals are formed disproportionately in diabetic patients mainly by glucose oxidation and non-enzymatic glycation of proteins. Beside this the subsequent oxidative degradation of glycated proteins is also thought to be responsive phenomena. Abnormally high levels of free radicals are formed disproportionately in diabetic patients mainly by glucose oxidation and non-enzymatic glycation of proteins. Beside this the subsequent oxidative degradation of glycated proteins is also thought to be responsive phenomena. Abnormally high levels of free radicals are formed disproportionately in diabetic patients mainly by glucose oxidation and non-enzymatic glycation of proteins. Beside this the subsequent oxidative degradation of glycated proteins is also thought to be responsive phenomena. Abnormally high levels of free radicals are formed disproportionately in diabetic patients mainly by glucose oxidation and non-enzymatic glycation of proteins. 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radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of most cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. From the above the data it is found that the extracts of fruits and shoots of the B. alba not only possesses anti-diabetic properties but also anti-oxidant properties. The IC50 of extracts of fruits and shoots of the B. alba was 496.32 µg/ml.

To sum up, based on the result of the current work, the methanolic extracts of fruits and shoots of the B. alba has anti-diabetic effect on alloxan induced diabetic mice. At dose 500 mg/kg the anti-diabetic effect of the methanolic extracts of fruits and shoots of the B. alba is comparable to that of metformin-treated diabetic mice. Beside this the methanolic extracts of fruits and shoots of the B. alba also possesses mild antioxidant activity. Thus consumption of B. alba fruits and shoots as a vegetable should be encouraged to manage or treat diabetes mellitus.

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