EFFECTS OF METHANOLIC LEAF EXTRACT OF JUSTICIA GENDARUSSA ON ALLOXAN INDUCED DIABETIC MICE AND BRINE SHRIMP NAUPLII

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ABSTRACT

Justicia gendarussa is an important medicinal plant and many of its traditional uses have been scientifically proved. Here we examined the anti-hyperglycemic property of the methanolic leaf extract of Justicia gendarussa on alloxen induced diabetic mice at two doses (200, 400 mg/kg b.wt., i.p) in the twelve hours treatment period. Cytotoxic effect of the extract on brine shrimp nauplii was also observed and compared in presence and absence of light at different concentrations (25, 50, 100, 200, 400 and 600 μg/ml). The effect of extract on diabetic mice was profound by gradual reduction of the blood glucose level throughout the treatment periods. We found maximum 52.75% (*P<0.05) reduction of the blood glucose level at dose of 400 mg/kg at 12th hour and that for metformin was 56.17% at 150 mg/kg dose. LC₅₀ value of the extract under light was about 500 μg/ml and under dark it was 249.93 μg/ml. So, cytotoxic effect was observed higher in dark condition compare to in presence of light. The results suggested that the extract possess significant antihyperglycemic activity in alloxen induced diabetic mice and as having no significant cytotoxic effect, it may be beneficial for management of diabetic.

KEYWORDS: Diabetes, alloxan, nauplii, cytotoxicity, methanol, J. gendarussa.

INTRODUCTION

The number of diabetic patients in Bangladesh is increasing at an alarming rate. About 5.7 million people in the country are suffering from the disease, reports Bangladesh Sangbad.
Sangstha (BSS). The number of diabetic patients is annually growing at a rate of three percent in the country and if the present rate continues, the number of diabetics will double to 10.4 million by 2030 (Imam T et al., 2012). Despite the presence of known antihyperglycemic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antihyperglycemic and anti-hyperlipidemic remedies (Shukla R et al., 2000; Shukla R et al., 2000; Huang TH et al., 2005; Islam MS et al., 2011). More than 400 plant species having hypoglycemic activity have been available in literature and they contain substances which take alternative and safe effect on DM (Oliver-Bever B et al., 1986; Roy K et al., 2005).

*Justicia gendarussa* Burm (Family-Acanthaceae, English name, Black adusa) is an evergreen shrub growing in moist environment in Bangladesh, India, China, Malaysia, Indonesia, Sri Lanka and Philip-pines. The plant has folkloric use in the treatments of chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases, muscle pain, lumbago, headache, earache, hemiplegia, hair growth promotion, leucoderma, asthma, antiseptic, haemo-static, nasal bleeding, bone fracture, injuries and fever (Ahmad FB et al., 2003; Anonymous 1959; Das AK et al., 2008; Dolui AK et al., 2004; Madhu V et al., 2009; Rahman AHMM et al., 2012; Zheng X et al., 2009). Various researchers have been reported that *J. gendarussa* possess antiangiogenic effect, antioxidant and hepatoprotective potential, antifungal activity, anti-bacterial activity anti-arthritic potential, antiinflammatory and analgesic activities, antinocicep-tive activity, antisickling activity, anthelmintic activities, Larvi-cidal and adulticidal activity and in vitro HIV type 1 reverse transcriptase inhibitory activity (Periyanayagam K et al., 2009; Krishna KL et al., 2010; Sharma KK et al., 2011; Sudhanandh VS et al., 2012; Paval J et al., 2009; Jothimanivannan C et al., 2010; Ratnasooriya WD et al., 2007; Mpiana PT et al., 2010; Saha MR et al., 2012; Senthilkumar N et al., 2009; Woradulayapinij W et al., 2005). *J. gendarussa* has been found to contain alkaloids, triterpe-noids, tannins, justicin, steroids, and flavonoids, gendarusin A and B, stigmasterol, lupeol and 16 hydroxylupeol (Prajogo BEW et al., 2007; Izzah Z et al., 2010; Thomas DT et al., 2010; Uddin MR et al., 2011).

The literature survey revealed that there are no scientific studies carried out regarding the antihyperglycemic and cytotoxic activities of methanol extract of *Justicia gendarussa* leaf.
substantiate its therapeutic claim. Hence, in the present study, the methanolic extract of the leaf was examined for evaluating its antihyperglycemic and cytotoxic properties.

MATERIALS AND METHODS

Collection of leaf

The matured leaf of the plant was collected from Chittagong, Bangladesh during the month of October-November, 2010. The sample was identified by experts of Dept. of Botany, University of Chittagong, Bangladesh and a voucher specimen (accession no. 34728) for this collection has been deposited in Bangladesh National Herbarium, Dhaka, Bangladesh.

Preparation of extract

About 600 gm of the matured leaf were cut into small pieces with the help of a knife. These small pieces were dried for 7-10 days and finally kept in an electric oven for 72 hours at 40°C. After complete drying, the dried pieces were then pulverized into a coarse powder with the help of a grinding machine. In cold extraction the coarse powder was submerged in methanol. Flat bottom 2.5 litter reagent bottle were used for this purpose which were kept at room temperature and allowed to stand for 10 days with occasional shaking and stirring. When the solvent become concentrated, the liquid methanol content were filtered through cotton and then through filter paper (Whatman filter paper no. 1) Then the solvents were allowed to evaporate. Thus the highly concentrated and crystalline crude extracts were obtained.

Experimental Animal

A total number of 25 male Swiss Albino mice weighing about 20-25 gm, age 4 weeks were purchased from animal’s house of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Prior to commencement of the experiment, all the mice were acclimatized to the new environmental condition for a period of one week. During the experimental period the mice were kept in a well ventilated animal house at room temperature of 25°C and maintained with natural 12 hour light and dark cycle. Animals were allowed free access to drinking water and pellet diet, collected from ICDDR, B Dhaka. All animal experiments were performed in accordance with NIH guidelines.

Drugs

Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India and used for the induction of diabetes. The active drug, metformin hydrochloride was collected from Square Pharmaceuticals Ltd., Pabna plant. All other chemicals used were of analytical grade.
**Preparation of Dose**

Diabetes was induced with a single dose of freshly prepared alloxan (100 mg/kg b. w., i.p.) in sterile saline water. 200 and 400 mg/kg (b. w.) extracts were used for testing antihyperglycemic activity and all doses were administered intraperitoneally after dissolving in 5% dimethyl sulfoxide (DMSO) vehicle. Standard drug metformin was injected in the same route at the dose of 150 mg/ kg b. w.

**Antihyperglycemic Test**

In the experiment, a total 25 Swiss Albino mice about 20-25 gm; 4-6 weeks were used and divided randomly into five groups (five mice in each group). Treatment was done for 24 hrs as follows

Group I: Normal control mice (Vehicle treated).

Group II: Diabetic control (Received alloxan 100 mg/kg b. w, i.p.).

Group III: Diabetic mice given metformin (150 mg/kg b. w, i.p).

Group IV: Diabetic mice given extract (200 mg/kg b. w, i.p.).

Group V: Diabetic mice given extract (400 mg/kg b. w, i.p.).

Group II – V received a single dose of alloxan (100 mg/kg i.p.) after overnight fasting. Group-I received only 5% DMSO as normal control group and Group-II was diabetic control group, which did not receive either metformin, or plant extract. Metformin and extract were injected intraperitoneally to the respective groups after 24 hours of alloxan injection and blood samples were analyzed for blood glucose content at 0, 1, 3, 6, and 12 hours respectively using a glucometer kit (Accu-Check active, Roche Diagnostic GmbH, Mannheim, Germany). Fasting blood glucose levels of 13 to 15 mmol/L were considered as diabetic and included in the study.

**Brine shrimp lethality bioassay**

The cytotoxicity assay was performed on brine shrimp (*Artemia salina*) nauplii using the Meyer method (Meyer BN et al., 1982). The dried cysts of the brine shrimp were collected from an aquarium shop (Katabon, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) with strong aeration for a 48-h light/dark cycle to mature shrimp called nauplii. The test sample (extract) was prepared by dissolving extract in DMSO (not more than 50 μl in 5 ml of solution) plus sea water (3.8% NaCl in water) to attain concentrations of 25, 50, 100, 200, 400 and 600 μg/ml. A vial containing 50 μl of DMSO diluted to 5 ml was used as a control. Standard vincristine sulfate was used as the positive control. The matured shrimp
were then applied to each of the experimental vials and the control vial. After 24 h, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial was counted. From these data, the percent (%) mortality of the brine shrimp nauplii was calculated for each concentration using the following formula: % mortality = (Nt / N0) × 100, where Nt = the number of killed nauplii after 24 h of incubation and N0 = the number of total nauplii transferred. The median lethal concentration (LC50) was then determined from concentration versus mortality curve.

Statistical Analysis
The experimental data are presented as the means ± SEM. The differences between the groups were considered as significant at *P<0.05 by student’s T-test and Tukey’s test using GraphPad Prism version 4.00 for Windows.

RESULTS
Antihyperglycemic Test
The effect of single intraperitoneal injection of methanol extract of J. gendarussa leaf on blood glucose levels in normal and diabetic mice are shown in Table 1 and Figure 1. Following a 12 hours post alloxan injection, all diabetic mice exhibited hyperglycemia, which ranged between 13.27±1.64 and 14.83±0.55 mmol/L while normal control mice showed a normal blood sugar level of about 6 mmol/L. After treatment, the blood glucose levels were decreased both in positive control and test control groups. Maximum reduction observed at 12th hour which were 41.76 and 61.79 % for 200 and 400 mg/kg b.w. respectively. Standard drug metformin reduced 66.91% blood glucose level at the dose of 150 mg/kg in the treatment period. So, the extract showed better antihyperglycemic activity at higher dose than 200 mg/kg.

Table 1. Antihyperglycemic effect of leaf extract of J. gendarussa on alloxan induced diabetic mice
*P<0.05 indicates significant activity comparing with diabetic control group. Each group contains five mice. Here values are given as mean ± SEM.

![Figure 1](image1.png)

**Figure 1.** Effect of methanol extract of *J. gendarussa* leaf in lowering FBG on alloxan induced diabetic mice compared with normal and diabetic mice (*P<0.05)*.

**Brine shrimp lethality bioassay**

In this test, the toxicity of extract was studied by measuring the effect of different concentrations ranges from 25-600 µg/ml on the nauplii. The extract showed concentration dependent cytotoxicity under both light and dark conditions but percentage of mortality was higher in dark condition. LC$_{50}$ value (499.99 µg/ml) under dark was almost double.

![Figure 2](image2.png)

**Figure 2.** Cytotoxic effect of the extract under light and dark.
DISCUSSIONS

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting various organs in the body (El-Hilaly J et al., 2007). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs (Lyra R et al., 2006).

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. According to World Health Organization, about 80% of the world’s population presently uses phytotherapy for some aspect of primary health care system (WHO 2008). There are many pharmaceutical products which are available in modern medical treatment have a long history of use as herbal remedies, including aspirin, opium, digitalis and quinine. A large number of world’s population who live in developing countries cannot take the benefits of modern pharmaceuticals as those are very expensive. Hence, phytotherapy is still a popular means of primary healthcare for which people bear a little or no cost. In addition to the use in the developing world, phytotherapy is used in the industrialized nations by alternative medicine practitioners such as naturopaths. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. Approximately 25 percent of modern drugs used in the United States have been derived from plant origins. So, research on phytotherapy has got great momentum in recent years to find out noble pharmaceuticals.

Our present study revealed that methanol of leaf extract of *J. gendarussa* has significant effect in lowering fasting blood glucose level in alloxan and glucose induced diabetic mice. Metformin showed maximum reduction of blood glucose level at twelve hour and at the same time maximum reduction was obtained for extract in alloxan induced mice. Blood sugar levels were then raised slightly for both extract and metformin treated mice group till observation probably due to loss of their duration of action. In glucose induced diabetic mice, a gradual declination of blood sugar level were observed in all treatment groups throughout the reading period. The extract showed dose dependent antihyperglycemic activity that is better antihyperglycemic effect was obtained at higher doses. So, the leaf extract has considerable hypoglycemic activity considering the blood sugar level in standard and diabetic control group. Further comprehensive pharmacological and phytochemical investigations are
needed to elucidate the exact chemical compounds responsible for antihyperglycemic activity, cytotoxic activity and their mode of action.

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Conflict of interest statement
We declare that we have no conflict of interest.

REFERENCES
3. Das AK, Dutta BK, Sharma GD Medicinal plants used by different tribes of Cachar district, Assam. Ind J Tradil Know, 2008; 7(3): 446-54.


