

Full Length Research Paper

## ***In vivo* hypoglycemic and alloxan induced antidiabetic activity of *Xeromphis uliginosa* Retz**

Mohammad Firoz Khan<sup>1\*</sup>, Zahirul Islam Khan<sup>1</sup>, Md. Rakib Uddin<sup>1</sup>, Mohammad S. Rahman<sup>2</sup> and Mohammad A. Rashid<sup>2</sup>

<sup>1</sup>Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh.

<sup>2</sup>Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh.

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***Xeromphis uliginosa* Retz. is an indigenous plant of Bangladesh. Traditionally this plant is used to treat many diseases. In order to explore the medicinal quality of this plant systematically, the crude methanol extract of roots was screened for antidiabetic activity in rats model. The antidiabetic action was determined by using oral glucose tolerance test (OGTT) and alloxan induced antidiabetic test. In OGTT, both doses (200 and 400 mg/kg body weight) of methanol extract reduced the blood glucose significantly ( $p < 0.05$ ) after 1 h on administration and continued to remain lower up to 3 h. However, the extract significantly ( $p < 0.05$ ) attenuated the blood glucose level in diabetic rats at a dose of 500 mg/kg body weight which was comparable to the standard drug used (glibenclamide).**

**Key words:** *Xeromphis uliginosa*, antidiabetic, blood glucose.

### INTRODUCTION

Plants have been used as a source of medicine by man since ancient times globally (Bargali et al., 2003). In the beginning, these were the main source of the folk or ethnomedicine (Parihaar et al., 2014). Subsequently, in Bangladesh, a considerable amount of this traditional knowledge was formulated and documented into an organized system of medicine parallel to the modern medicine. The use of plants as the principal form of medicine is increasing throughout the developed world. In fact, about 80% population of developing countries still utilizes traditional medicines derived from herbs for their health care (Bargali and Shrivastava, 2002; Shrivastava

and Bargali, 2005). As Bangladesh has numerous plants, proper scientific evaluations are required to explore the potentiality of these plants for treating various diseases (Rahmatullah et al., 2010; Banglapedia, 2012; Ashraf et al., 2014).

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (World Health Organization (WHO), 1999). Diabetes is associated with long-term micro- and macro-vascular complications and is widely recognized as a leading cause of mortality and morbidity (Hossain et al., 2007).

\*Corresponding author. E-mail: firoz@sub.edu.bd.

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The number of people with diabetes is increasing day by day due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity (Wild et al., 2004). To achieve glycemic control, therapeutic agents like insulin, sulfonylureas, biguanides and thiazolidinedione derivatives etc are used. However, on chronic usage most of these agents produced several side effects including hypoglycemic coma, insulin resistance, hyper-sensitivity, abdominal pain, anorexia and metallic test (Sharma and Kumar, 2011; Chaudhary, 2001). Moreover, it is difficult to afford and use these medicines for prolonged period as the cost and treatment failure rate are high. Until the time insulin was invented, this disorder was managed principally by using medicinal plants due to their low cost, easy accessibility and less side effects (Sharma and Kumar, 2011). There are numerous traditional medicinal plants reported to have hypoglycemic properties such as *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Vinca rosea* (Nayantara), *Momordica charantica* (Bitter ground) and *Ocimum santum* (Tulsi) (Sharma and Kumar, 2011; Grover et al., 2002).

*Xeromphis uliginosa* Retz. is an underutilized plant of the family Rubiaceae. The plant is distributed in dry and moist deciduous forests. It is native to Bangladesh, India, Sri Lanka and Thailand. The ethnic communities use the various parts of the plant as a vegetable and curing various illness like cholera, diarrhoea, dysentery, eye complaints, pimples, diuretic, tonic properties, and biliousness amongst others. The unripe fruit acts as astringent. Bark powder with egg, turmeric and calcium is used for bone fracture healing (Srivastava and Pandey, 2013). The stem bark of *Helicteres isora* Linn. along with that of *Xeromphis uliginosa* and a whole plant of *Bacopa monnieri* Wettst. are used to treat colds and coughs. A decoction of wood is used in the treatment of diabetes mellitus (Khare, 2004; Chuakul et al., 2011; Kirtikar and Basu, 1933).

Since this plant has important medicinal properties, the present study has been undertaken as part of our regular research program (Ara et al., 2006; Begum et al., 2010; Dey et al., 2014), and we, herein, report the hypoglycemic and antidiabetic properties of the roots of *X. uliginosa* for the first time.

## MATERIALS AND METHODS

### Plant

The roots of *X. uliginosa* were collected on August, 2013 from Tangail, Bangladesh and a voucher specimen has been deposited at Bangladesh National Herbarium, Mirpur, Dhaka for future reference.

### Extraction and fractionation

The collected roots were sun dried for several days and then oven dried for 24 h at 40°C to facilitate grinding. The powdered roots

(565 g) of *X. uliginosa* was extracted with 2.4 L methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator at reduced temperature (40 to 45°C) and pressure. The concentrated methanol extract was used for different biological screenings.

### Animals

Evan's rats of both sexes, weighting 150 to 200 g, bred in the animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh were used for the experiments. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55 to 65%, room temperature 25.0 ± 20°C, and 12 h light dark cycle). The animals were fed with standard diet (ICDDR, B formulated) and had free access to tap water but were fasted 12 h prior to each experiment. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental rats.

### Drugs

The drugs and chemicals used in this study include glucose solution (10%), glibenclamide (Square Pharmaceuticals Ltd., Bangladesh), dimethyl sulphoxide (DMSO, Merck Chemicals Ltd., Germany) and alloxan (Sigma Aldrich, Germany).

### Oral glucose tolerance test (OGTT) in normal rats

The OGTT was performed by the method described by Durschlag et al. (1996). Here, the lowering of blood glucose level of the experimental animals was measured by tail tipping method. Rats were divided into 4 groups of 3 rats each. The control group received 1% Tween 80 in normal saline (10 ml/kg body weight), the standard group received glibenclamide (10 mg/kg body weight) and the experimental groups received crude extract of 200 and 400 mg/kg body weight. In the evaluation of the hypoglycemic effect of the crude methanol extract of *X. uliginosa*, the blood glucose level of the experimental animals was measured at zero hour using a glucometer (Bioland G-423 S). Then the control, standard and methanolic crude extract (200 and 400 mg/kg body weight) were administered orally to the experimental animals with the help of feeding needle. At 1st, 2nd and 3rd hour after administration, the blood glucose level of the experimental animals was measured to observe the hypoglycemic effect of the test samples relative to control and standard groups.

### Alloxan Induced Antidiabetic activity

The alloxan induced antidiabetic activity was evaluated by the method described by Semwal et al. (2008) and Ahmed et al. (2010). Rats were divided into 3 groups of 4 rats each.

Group I: Normal saline treated control (20 ml/kg body weight).  
 Group II: Alloxan treated control (150 mg/kg body weight i.p.).  
 Group III: Alloxan (150 mg/kg body weight i.p.) + Standard drug, Glibenclamide (10 mg/kg body weight).  
 Group IV: Alloxan (150 mg/kg body weight i.p.) + Methanol extract of *X. uliginosa* (500 mg/kg body weight).

The root extracts, glibenclamide (10 mg/kg body weight) and normal saline were administered with the help of feeding cannula.

**Table 1.** Effect of methanol extract of *X. uliginosa* on oral glucose tolerance test.

| Group                                  | Doses     | Blood glucose level (mmol/L) |             |             |             |
|--|-----------|------------------------------|-------------|-------------|-------------|
|  |           | 0 h                          | 1 h         | 2 h         | 3 h         |
| 1% Tween 80 in normal saline (Control) | 10 ml/kg  | 5.9 ± 0.6                    | 7.3 ± 0.7   | 6.4 ± 0.6   | 5.6 ± 0.4   |
| Glibenclamide (standard drug)          | 10 mg/kg  | 6.0 ± 0.5                    | 3.5 ± 0.3** | 2.3 ± 0.2** | 2.5 ± 0.1** |
| Roots extract of <i>X. uliginosa</i>   | 200 mg/kg | 5.9 ± 0.4                    | 5.9 ± 0.5*  | 4.6 ± 0.5   | 4.1 ± 1.0   |
|  | 400 mg/kg | 5.6 ± 0.5                    | 5.6 ± 0.8*  | 4.6 ± 0.7   | 3.9 ± 1.1   |

All values are expressed as mean ± SEM; n=4, \*p < 0.05, \*\*p < 0.01, significant compared to control.

**Table 2.** Effect of roots extracts of *X. uliginosa* in alloxan induced diabetic rats.

| Groups  | Dose<br>(ml/kg body weight) | Blood glucose level (mmol/L) |            |            |           |           |           |             |           |
|---|-----------------------------|------------------------------|------------|------------|-----------|-----------|-----------|-------------|-----------|
|   |                             | Days                         |            |            |           |           |           |             |           |
|   |                             | 0                            | 1          | 2          | 3         | 4         | 5         | 6           | 7         |
| 1% Tween 80 in normal saline (Normal Control)   | 20                          | 6.5 ± 0.9                    | 5.7 ± 0.5  | 6.2 ± 0.3  | 5.1 ± 0.3 | 6.3 ± 0.7 | 5.2 ± 0.5 | 5.0 ± 0.9   | 4.4 ± 0.5 |
| 1% Tween 80 in normal saline (Diabetic Control) | 20                          | 6.0±0.2                      | 17.4±1.0   | 17.3 ± 1.0 | 16.1±1.0  | 15.8±1.0  | 15.5±0.4  | 15.5±1.0    | 14.7±0.4  |
| Glibenclamide (standard drug)                   | 10                          | 5.9±0.1                      | 13.1±0.5*  | 6.8±0.3**  | 6.8±0.2** | 3.6±0.4** | 3.8±0.3** | 4.3±0.4**   | 4.2±0.3** |
| Roots extract of <i>X. uliginosa</i>            | 500                         | 6.1±0.3                      | 17.2 ± 0.5 | 13.6±0.8*  | 11.9±0.8* | 11.1±1.0* | 4.4±0.3** | 7.1±0.1.5** | 6.3±1.5** |

All values are expressed as mean ± SEM; n = 4, \*p < 0.05, \*\*p < 0.01, significant compared to control diabetic control.

In this method, Group I to III animals were allowed to fast for 12 h. Diabetes was induced by injecting intraperitoneally a freshly prepared solution of alloxan (150 mg/kg) in normal saline after base line glucose level determination. The alloxan treated animals were allowed to feed over night to overcome drug induced hyperglycemia. After 48 h blood glucose content was measured from the tail vein by using a glucometer (Bioland G-423 S). When the diabetic model rats was established with blood glucose level above 11.1 mmol/L, the animals was selected for the study. The blood glucose level was tested in 0, 1, 2, 3, 4, 5, 6 and 7 days after the oral administration of glibenclamide and methanol extracts.

#### Statistical analysis

The values are presented as mean ± standard error of mean (SEM) and one way analysis of variance (ANOVA) was used to determine a significant difference between the

control group and experimental groups. A p-value of < 0.05 was considered to be statistically significant.

#### RESULT

The methanol extract of *X. uliginosa* was subjected to assay for OGTT at doses of 200 and 400 mg/kg body weight and alloxan induced antidiabetic activity at a dose of 500 mg/kg body weight. The extract of *X. uliginosa* when administered orally at 200 and 400 mg/kg body weight exhibited significant glucose lowering effects when compared to control. The glucose lowering effects was found to be dose dependant. However, maximum effect was seen at the dose of 400 mg/kg body weight and was comparable

with the standard drug (glibenclamide) (Table 1). Single intraperitoneal administration of alloxan monohydrates (150 mg/kg body weight) led to elevation of blood glucose level. The methanol extract of *X. uliginosa* showed significant (indicate p-value) antidiabetic property at a dose of 500 mg/kg body weight. The antidiabetic effects of methanol extract and Glibenclamide on blood sugar levels on diabetic rats was significant. However, the antidiabetic effect of root extract was comparable with the standard drug (Table 2).

#### DISCUSSION

Treatment of diabetes with the compound that has no side effects is still difficult in the field of medical

system.

As a result, nowadays demand of natural products which have significant antidiabetic activity with fewer side effects is increasing rapidly. Alloxan causes diabetes through its ability to destroy the insulin producing beta cells of the pancreas (Lenzen and Panten, 1988, Oberley, 1988). In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis (Jorns et al., 1997, Ledoux et al., 1986). The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells (Szkudelski, 2001).

According to the earlier studies, plant extracts cause anti-hyperglycemic effect by promoting regeneration of  $\beta$  cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Anti-hyperglycemic effect may also be caused by the effect of plant extract on  $\beta$  cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption (Jadav et al., 2009). It has been published in the literature that the plant extracts have antioxidant potential, flavonoids and tannins (Hossain et al., 2014; Srivastava and Pandey, 2013). Presence of flavonoids and tannins in the extracts is known to possess antidiabetic activity (Sharma et al., 2010). In our present study, the exhibited antidiabetic activity of the plant extract may be due to the presence of similar phytoconstituents.

## Conclusion

The methanol extract was very effective in diabetes management which indicates that the plant has potential antidiabetic property. The present study justifies the use of this plant in diabetes mellitus. Further extensive studies are required to isolate the bioactive compounds and to explore the underlying mechanisms to treat these diseases.

## Conflict of interests

The authors did not declare any conflict of interest.

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