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Original Article

Evaluation of the antidiabetic property of aqueous leaves extract of *Zanthoxylum armatum* DC. using *in vivo* and *in vitro* approaches

Carey Vana Rynjah [a], Ningthoujam Neelima Devi [a], Nasheman Khongthaw [a], Donkupar Syiem [b], Suktilang Majaw [a, *]

[a] Department of Biotechnology & Bioinformatics, North-Eastern Hill University, Shillong, Meghalaya, 793022, India
[b] Department of Biochemistry, North-Eastern Hill University, Shillong, Meghalaya, 793022, India

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ABSTRACT

The present study was designed to evaluate the antidiabetic potential of the aqueous leaves extract of *Zanthoxylum armatum* DC. leaves using *in vivo* and *in vitro* approaches. For *in vivo* studies, blood glucose level was monitored at different intervals after administration of varying doses of the extract for its hypoglycemic (100–6000 mg/kg b.w.) and antihyperglycemic (250 mg/kg b.w.) effect in normoglycemic and diabetic mice. *In vitro* enzymatic inhibition activity was tested against α-amylase, α- and β-glucosidase and lipase. Additionally hydroxyl radical, hydrogen peroxide scavenging assay and phytochemical screening were also performed. Element analysis of the plant was studied by Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). The plant extract showed significant hypoglycemic and antihyperglycemic effect in normoglycemic and diabetic mice. The IC_{50} values of extract for α-amylase, β-glucosidase, lipase, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity were 7.40 mg/ml, 0.30 mg/ml, 8.35 mg/ml, 3.25 mg/ml, 9.62 mg/ml respectively and the percentage of inhibition for α-glucosidase was 79.82% at 0.8 mg/ml. *In vitro* studies were compared with their respective standards. Elemental analysis revealed the presence of essential elements such as Mg, V, Fe, Cr, Zn, Cu, Mo, Mn, K, Ca, P and Sr which are all known to play a role in regulating blood glucose. The results demonstrate that *Z. armatum* aqueous leaves extract possess antidiabetic property in both *in vivo* and *in vitro* condition.

1. Introduction

Diabetes mellitus (DM) is a group of commonly known metabolic diseases characterized by chronic hyperglycemia due to defects in insulin secretion, insulin action or both.[1] Many different therapeutic approaches are available for treating diabetes and one of the treatment includes retarding absorption of glucose by inhibiting the carbohydrate hydrolysing enzymes like amylase and glucosidases.[2–4] The human α-amylase (EC 3.2.1.1), is commonly found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into simple and absorbable sugars.[2] On the other hand, α-glucosidase (EC 3.2.1.20) and β-glucosidase (EC 3.2.1.21) are key enzymes that are located at the border brush of the small intestine which catalyses the cleavage of glycosidic bonds releasing glucose from the non-reducing end of an oligo- or polysaccharide chains.[4] Hence, inhibition of α-amylase and glucosidase enzymes will result in delaying the breakdown of carbohydrates in the small intestine thus diminishing the post-prandial blood glucose level. Further, hyperlipidaemia which is known to be associated with disturbances in the lipid metabolism have been linked to the development of obesity and diseases including diabetes. The important strategy in the prevention and treatment of hyperlipidaemia includes delaying fat digestion and absorption through gastrointestinal mechanisms such as the inhibition of pancreatic lipase (EC 3.1.1.3).[5] Therefore, the use of lipase inhibitors will result in reduced absorption of glycerides and fatty acids from the gut and consequently, less fat will be synthesised in the body.[6] Further, it has also been shown in a number of studies that DM is associated with oxidative stress, leading to an

* Corresponding author.
E-mail addresses: smajaw.nehu@gmail.com, smajaw@nehu.ac.in (S. Majaw).
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increased production of reactive oxygen species (ROS), including superoxide radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) or reduction of antioxidant defence system.[7] Antioxidants play an important role in scavenging the free radicals and protect the human body from oxidative stress.[8] Therefore, a drug with both antioxidant and antidiabetic property would be useful for the treatment of DM. Although various types of antidiabetic drugs are easily available for controlling blood glucose level however, these medications are known to be associated with undesirable effects. Thus, managing diabetes using currently available drugs devoid from side effects is still a challenge.[9] Hence, the search for more effective and safer therapeutic agents of natural origin is continuing as it is considered valuable.[9] WHO has suggested the evaluation of traditional plant therapies for DM as they are effective, nontoxic, with less or no side effects and are considered to be excellent candidates for oral therapy.[10] There are various mechanisms by which plants show antihyperglycemic activity by acting either insulinomimetic or secretagogues properties, some resemble the effect of metformin, others inhibit enzymes such as α-amylase, α-/β-glucosidase, lipase and some are due to their antioxidant potential.[11–15] Therefore, documenting and validating the efficacy of medicinal plants having antidiabetic property has increased and characterization of chemical constituents is focused in drug discovery programmes to bring a better lead molecule for diabetes treatment.[16]

Zanthoxylum armatum DC. (Rutaceae) is a small tree or large spiny shrub. It is widely distributed in India from Kashmir to Bhutan at altitudes up to 2500 m and occurs through North East India.[17] The parts of the plant such as the leaves, bark, stem, fruits and seeds are extensively used in indigenous system of medicine as a tonic, carminative, stomachic and anthelmintic.[18,19] *Z. armatum* is also commonly used in traditional practices by the Khasi tribe of Meghalaya in North-Eastern India and in neighbouring regions including South-East Asia.[20] People of Meghalaya used the aromatic fruits (local name: Jaiur) and leaves as spices for preparing traditional foods.[21] *Z. armatum* extract has shown to possessed antifungal activity, hepatoprotective activity, anti-inflammatory activity, antioxidant and antimicrobial activities.[22–25] Other genus of Zanthoxylum are also known to possessed potent antidiabetic property.[26,27] Previous studies has shown that the hydromethanolic bark extract of *Z. armatum* is also known to possessed antidiabetic property.[28] Currently, there is no scientific validation displaying the antidiabetic potential of *Z. armatum* aqueous leaves extract using *in vivo* and *in vitro* approaches. Hence, the present study was aimed to investigate the antidiabetic activity of aqueous leaves extract of *Z. armatum* in diabetic mice using the above mentioned approaches.

2. Material and methods

2.1. Chemicals

Alloxan was procured from Sigma Co., USA; Insulin from Knoll Pharmaceutical Ltd., India; Metformin from USV Ltd., India; Acarbose from Bayer Zydus Pharma, India; Orlistat from Meyer Organics Pvt. Ltd., India. Other chemicals used were of analytical grade obtained from Sisco Research Laboratories (SRL), India and Himedia, India.

2.2. Collection of plant material

Leaves of *Z. armatum* (ZA) were collected from Diengpasoh, East Khasi Hills, Shillong, Meghalaya, India. The plant was authenticated by a Herbarium curator, Dr. P. Gurung, Department of Botany, NEHU, Shillong, Meghalaya, India, with a voucher No. 11963.

2.3. Preparation of the plant extract

The leaves were properly washed, dried in oven at 40 °C and grounded. 40 g of powdered leaves dissolved in 200 ml of distilled water was filtered using Whatmann filter paper No.1. The filtrate was then evaporated using rotary evaporator and then lyophilized to dryness.[29] The lyophilized powder was stored at 4 °C for further use. The yield percentage of *Z. armatum* extract (ZAE) was 6.31%.

2.4. In vivo studies

2.4.1. Experimental animals

Female swiss albino mice, weighing about 25–30 g were obtained from Pasteur Institute, Shillong, India, and used for the study. Mice were housed in a room kept under control conditions with temperature maintained at 22 °C on a 12-h dark cycle and fed with standard mice feed. Mice were fasted overnight before performing the following experiment but given water *ad libitum*. Food was again fed after 6 h to mice during the hypoglycemic and antihyperglycemic study and after 120 min during glucose tolerance test. The experiments were conducted after the approval by the Institutional Ethics Committee (IEC) (Dated: 01.09.2014) of North-Eastern Hill University, Shillong, Meghalaya, India.

2.4.2. Lethal dose investigation

Single dose of ZAE (100–6000 mg/kg b.w.) was administered intraperitoneally (i.p.). The mice were kept under observation for 24 h. The LD_{50} was calculated using Karber[30] method with modification by Aliu, Nwude.[31] The calculation is given below:

$$\mathbf{LD_{50}} = \frac{\mathbf{c} - \{(\mathbf{a} \times \mathbf{b})\}}{\mathbf{n}} \quad (1)$$

n = total number of animal in a group.
a = the difference between two successive doses of administered extract/substance.
b = the average number of dead animals in two successive doses.
c = maximum dose.

2.4.3. Hypoglycemic activity in normoglycemic mice

The extract in varying doses ranging from 100 to 6000 mg/kg b.w. were administered to the normoglycemic mice by i.p. injection and glucose level was monitored at 2, 4, 6 and 24 h. SD check glucometer was used for measuring the blood glucose level. The control mice were given only distilled water.

2.4.4. Induction of diabetes

Alloxan monohydrate dissolved in sodium acetate buffer 0.15 M pH 4.5 at 80 mg/kg b.w. were intravenously administered to overnight fasted mice.[32] After 48 h mice showing blood glucose level above 200 mg/dl were used for the study. The control group received only the buffer.

2.4.5. Antihyperglycemic activity in diabetic mice

The extract at the dose of 250 mg/kg b.w. were administered through i.p. injection to the diabetic mice. Metformin at 250 mg/kg b.w. (MET) and insulin at 0.1 IU/kg b.w. (INS) were used as reference drugs. The blood glucose level was determined after extract/standard drugs administration at 2, 4, 6 and 24 h.[33]

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