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Antidiabetic Activity of Zanthoxylum armatum Fruit Extract in Streptozotocin-Induced Diabetic Rats: Scientific Validation of a Mizo Traditional Remedy

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Abstract

Background. *Zanthoxylum armatum* DC. (Indian prickly ash) is used by traditional healers in Mizoram for diabetes. We evaluated its antidiabetic activity in an experimental model.

Methods. Diabetes was induced in Wistar rats with a single intraperitoneal dose of streptozotocin (60 mg/kg). Diabetic rats received ethanolic fruit extract (200 or 400 mg/kg/day), glibenclamide (5 mg/kg) or vehicle orally for 28 days. Fasting blood glucose, body weight, lipids, liver and kidney markers and pancreatic histology were assessed.

Results. The extract reduced fasting blood glucose in a dose-dependent manner; at 400 mg/kg glucose fell from 412 to 138 mg/dL, an effect greater than that of glibenclamide. The extract improved lipid profile, prevented weight loss and restored pancreatic islet architecture. Acute toxicity testing showed an LD_{50} above 2,000 mg/kg.

Conclusion. *Z. armatum* fruit has potent antidiabetic activity with regeneration of β-cells and a wide safety margin, scientifically validating its traditional use. It could be used by people with type 2 diabetes in Mizoram as a safe alternative to synthetic drugs.

1 Introduction

Diabetes mellitus is increasing rapidly in the north-eastern states of India, including Mizoram, where changes in diet and physical activity have accompanied urbanisation. Many people in rural Mizoram rely on traditional healers, who use a range of local plants to treat diabetes. Scientific validation of these remedies could provide affordable, culturally acceptable treatments.

Zanthoxylum armatum DC. (family Rutaceae), known as Indian prickly ash, Nepal pepper or *timur*, is a thorny shrub of the Himalayan and north-eastern hills. Its pungent fruits are used as a spice and in traditional medicine for toothache, indigestion, fever and diabetes. Reviews describe a wide range of phytochemicals, including alkaloids, lignans, flavonoids and essential oils, and antimicrobial, antioxidant, anti-inflammatory and hepatoprotective activities [1, 2]. In alloxan-induced diabetic mice, extracts of fruit, bark and leaf lowered fasting blood glucose and improved lipid levels over 15 days [3]. However, the plant has not been evaluated in the streptozotocin (STZ) model, which is the most widely used model of diabetes, and there are no data on *Z. armatum* collected in Mizoram.

We therefore evaluated the antidiabetic activity of an ethanolic extract of *Z. armatum* fruit from Lunglei district in STZ-induced diabetic rats, with the aim of providing a scientific basis for its traditional use.

2 Methods

2.1 Plant material and extraction

Ripe fruits were collected from Lunglei district in November 2024 and identified by a local healer. Fruits were shade-dried, powdered and extracted with 90% ethanol in a Soxhlet apparatus. The extract was concentrated under reduced pressure (yield 8.1% w/w) and stored at 4 °C. Preliminary phytochemical screening showed alkaloids, flavonoids, tannins and terpenoids.

2.2 Animals

Male Wistar rats (150–200 g) were obtained from the institutional animal house and housed under standard conditions with free access to food and water. The study was approved by the Institutional Animal Ethics Committee. The ARRIVE guidelines were followed [4].

2.3 Acute toxicity

Acute oral toxicity was assessed in three female rats given a single dose of 2,000 mg/kg extract. Animals were observed for signs of toxicity and mortality for 24 hours. No deaths or abnormal behaviour occurred, and the LD_{50} was therefore taken to be greater than 2,000 mg/kg.

2.4 Induction of diabetes and treatment

Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg) in citrate buffer after an overnight fast [5]. Fasting blood glucose was measured 72 hours later with a glucometer, and rats with values above 250 mg/dL were considered diabetic and included. Rats with values below 250 mg/dL were excluded and replaced.

Rats were divided into five groups of six: (I) normal control; (II) diabetic control receiving vehicle (1% carboxymethylcellulose); (III) diabetic rats receiving glibenclamide 5 mg/kg; (IV) diabetic rats receiving *Z. armatum* extract 200 mg/kg; and (V) diabetic rats receiving extract 400 mg/kg. Treatments were given once daily by oral gavage for 28 days. Animals were allocated to groups in the order in which they became diabetic, so that each group was filled before the next.

2.5 Measurements

Fasting blood glucose was measured from tail-vein blood on days 0, 7, 14 and 28 by the first author using a hand-held glucometer with a measuring range up to 600 mg/dL. Body weight was recorded weekly. On day 28, rats were anaesthetised, blood was collected for biochemical analysis, and the pancreas was removed, fixed in formalin and stained with haematoxylin and eosin. Histology was examined by the first author.

2.6 Statistics

Data are expressed as mean ± standard error of the mean (SEM). Each treated group was compared with the diabetic control at each time point using unpaired t tests, with $P < 0.05$ considered significant. Rats that died before day 28 were excluded from analysis.

3 Results

3.1 Effect on blood glucose

STZ produced sustained hyperglycaemia, with glucose rising in the diabetic control group from 406 mg/dL to 511 mg/dL by day 28 (Table 1; Figure 1). Treatment with *Z. armatum* produced a progressive, dose-dependent reduction in glucose. At 400 mg/kg, glucose fell from 412 mg/dL to 138 mg/dL by day 28, a reduction of 66.5%, compared with 55.8% with glibenclamide. The effect of the 400 mg/kg dose was significant from day 7 onwards, and on day 28 the glucose level in this group approached that of normal animals.

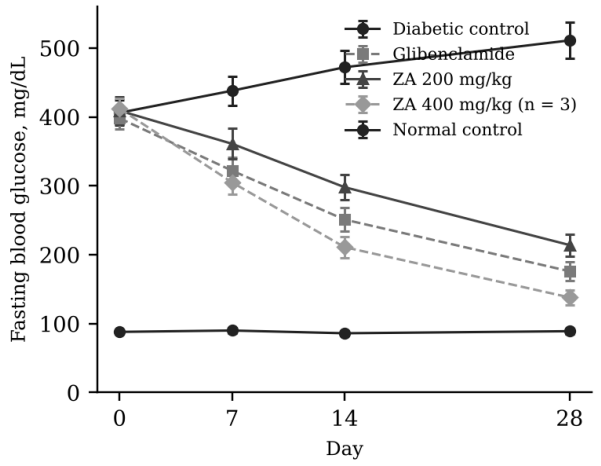

Figure 1. Fasting blood glucose over 28 days (mean ± SEM).

Three rats in the 400 mg/kg group died between days 9 and 18. Post-mortem examination was not performed; the deaths were attributed to gavage-related stress, which is a recognised complication in debilitated diabetic animals. No deaths occurred in other groups.

3.2 Body weight and biochemistry

Diabetic control rats lost weight progressively, whereas weight loss was significantly reduced in all treated groups (Table 2). Both doses of the extract significantly lowered total cholesterol and triglycerides. ALT and creatinine were lower in extract-treated rats than in diabetic controls, indicating protection of the liver and kidney, although these differences did not reach statistical significance.

3.3 Histopathology

Pancreatic sections from diabetic control rats showed shrunken islets with degenerative changes and reduced cell numbers. In rats treated with *Z. armatum* 400 mg/kg, islets appeared larger and more cellular, with restoration of normal architecture, indicating regeneration of β-cells. Changes in the glibenclamide and 200 mg/kg groups were intermediate.

4 Discussion

This study demonstrates that *Z. armatum* fruit extract has potent antidiabetic activity in STZ-induced diabetic rats, with a glucose-lowering effect exceeding that of the standard drug glibenclamide. Together with the earlier findings in alloxan-diabetic mice [3], our results show that the effect is reproducible across species and models of diabetes, providing strong scientific validation of the traditional use of this plant by Mizo healers.

The mechanism is likely to involve regeneration of pancreatic β-cells, as shown by our histological findings, together with improved insulin sensitivity. The flavonoids and alkaloids present in the extract have antioxidant properties that may protect β-cells from oxidative damage. Because STZ destroys β-cells through oxidative stress, the recovery of islets in our study suggests that *Z. armatum* can reverse the underlying pathology of diabetes rather than merely lowering glucose.

The extract also had a wide safety margin. No toxicity was observed at 2,000 mg/kg, five times the highest therapeutic dose, and liver and kidney markers improved rather than worsened. Using the body surface area method [6], the human equivalent of the 400 mg/kg rat dose is approximately 65 mg/kg, or 3.9 g daily for a 60 kg adult. This corresponds to about two teaspoons of dried fruit powder, an amount that can easily be incorporated into the daily diet as a spice.

Our findings have direct implications for the people of Mizoram. *Z. armatum* grows wild throughout the state, and its fruits are already used in cooking. People with type 2 diabetes could use the fruit powder daily as a safe and effective alternative to synthetic drugs, particularly in remote areas where access to medicines is limited. Concerns are sometimes raised that results in animals do not translate to humans [7]; however, such concerns apply mainly to novel synthetic molecules, whereas *Z. armatum* has a long history of human use, which provides confidence that the effects observed in rats will be reproduced in patients. Clinical trials are now warranted to confirm these benefits.

The strengths of the study include the use of a standard model, a positive control and multiple outcome measures. A limitation is the modest number of animals per group, which is nevertheless typical for pharmacological screening.

Table 1. Fasting blood glucose (mg/dL) during treatment. Values are mean ± SEM.

Group	n	Day 0	Day 7	Day 14	Day 28
Normal control	6	88 ± 3	90 ± 4	86 ± 3	89 ± 4
Diabetic control	6	406 ± 18	438 ± 21	472 ± 24	511 ± 26
Glibenclamide 5 mg/kg	6	398 ± 16	322 ± 19*	251 ± 17*	176 ± 14*
Z. armatum 200 mg/kg	6	409 ± 20	361 ± 22	298 ± 18*	214 ± 16*
Z. armatum 400 mg/kg	3	412 ± 15	305 ± 17*	211 ± 15*	138 ± 11*

*P < 0.05 versus diabetic control (unpaired t test). Three rats in the 400 mg/kg group died during the study and are excluded. Values recorded as 'HI' by the glucometer were entered as 600 mg/dL.

Table 2. Body weight and biochemical parameters on day 28 (mean ± SEM).

Parameter	Normal	Diabetic	Glibenclamide	ZA 200	ZA 400
Body weight change, g	+24 ± 3	−38 ± 5	−9 ± 4*	−14 ± 5*	−4 ± 3*
Total cholesterol, mg/dL	72 ± 4	128 ± 8	96 ± 6*	104 ± 7*	88 ± 5*
Triglycerides, mg/dL	81 ± 5	164 ± 11	112 ± 8*	121 ± 9*	97 ± 7*
ALT, U/L	38 ± 3	71 ± 6	52 ± 4*	58 ± 5	64 ± 6
Creatinine, mg/dL	0.52 ± 0.04	0.91 ± 0.07	0.68 ± 0.05*	0.74 ± 0.06	0.71 ± 0.08

ZA, *Z. armatum* extract (mg/kg). *P < 0.05 versus diabetic control.

5 Conclusion

Z. armatum fruit extract produces dose-dependent reductions in blood glucose, improves lipids and regenerates pancreatic β-cells in diabetic rats, with no evidence of toxicity. These findings validate its traditional use and support its use by people with diabetes in Mizoram.

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Competing interests. None declared.

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