HYPOGLYCEMIC AND ANTI-HYPERGLYCEMIC ACTIVITY OF *CURCUMA AMADA* ROXB. IN NORMAL AND ALLOXAN-INDUCED DIABETIC MICE

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Summary

Curcuma amada has a long history of traditional use as a medicinal plant among the indigenous Monsang tribe of Manipur. Its rhizome has been used in treating various local ailments. The hypoglycemic and anti-hyperglycemic activity of the crude aqueous methanolic (1:4) extract of *Curcuma amada* was evaluated in normal and alloxan-induced diabetic mice. Administration of different doses (150-650 mg/kg b.w.) lowered blood glucose level in a dose- and time-dependent manner. Mild hypoglycemic activity was observed with all the administered doses, while the anti-hyperglycemic activity was found to be pronounced above the dose of 250 mg/kg b.w. No apparent toxicity was observed in any of the mice even at the dose of 650 mg/kg b.w. Glucose tolerance was also improved in both normal and diabetic mice on administration of the extract. The results were compared with those of insulin, glibenclamide and metformin which were used as reference drugs.

Keywords: *Curcuma amada*, hypoglycemic, anti-hyperglycemic, alloxan

Introduction

Diabetes is a syndrome of disordered metabolism, usually due to combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels. The incidence of diabetes mellitus is increasing at an alarming rate even in developing countries including India [1]. Prolonged diabetes leads to micro and macrovascular complications [2, 3]. Atherosclerosis develops rapidly in diabetic patients and the incidence of heart disease and ischemic heart mortality is fairly higher in people with diabetes [4, 5]. Uncontrolled diabetes has been associated with stroke, end-stage renal failure, nerve diseases, blindness and amputations. Insulin resistance, obesity and inflammation are some associated complications [5, 6].

There has been renewed interest in medicinal plant as source of drugs and many plants have been used to treat diabetes [7-10]. Several plants have provided entirely new hypoglycemic compounds such as castanospermine in *Castanospermum australe* and neomyrtillin in bilberry [11]. We have earlier reported the antidiabetic properties of *Potentilla fulgens*, *Osbeckia chinensis*, *Flemingia macrophylla*, *Albizzia lebbek* and *Gymnopetalum cochinensis* [12-16]. *Curcuma amada* (Family-Zingiberaceae) is a perennial herb cultivated as an annual with rhizomes having characteristic odour of raw mangoes. Though anti-inflammatory, antioxidant and anti-hypercholesterolemic properties of *Curcuma amada* have been reported [17, 18], so far no studies have been conducted on its hypoglycemic and anti-hyperglycemic effects. The present investigation was therefore, undertaken to study the effects of *Curcuma amada* rhizome extract on fasting blood glucose level and glucose tolerance in normal as well as alloxan-induced diabetic mice.

Materials and Methods

Chemicals
Alloxan was procured from Sigma chemicals Co. USA, glibenclamide from Hoechts, insulin from Knoll Pharmaceutical Ltd., metformin from USV limited, Maharashtra, while other chemicals used were of analytical grade obtained from Sisco Research Laboratories and Hi-media, India.

Test animals
Healthy Swiss albino mice (balb/c strain) weighing 25-30 g were used for the study. They were housed in a room kept under controlled conditions with temperature maintained at 22 ^{o}C on a twelve hour light/dark cycle and were fed with balanced mice feed obtained from Amrut Laboratory, Pune, India. Institutional Ethics Committee (IEC) guidelines on the use of animals were followed during experimentation.

Plant material
Rhizomes of *Curcuma amada* Roxb. were collected from Monsang Pantha village of Chandel district, Manipur. The specimen was identified by Dr. P.B. Gurung, herbarium Curator, Department of Botany, NEHU, Shillong, Meghalaya (voucher number: NEHU-11897).

Extraction
The rhizomes were weighed, washed, chopped into pieces and dried in the shade. It was then powdered, homogenized and repeatedly extracted with 10 volume of aqueous-methanol solution (1:4) [19]. The mixture was filtered and the filtrate evaporated to dryness at 40 ^{o}C in a Yamato-rotary vacuum evaporator. The dried mass obtained was used for the investigation. Prior to use, weighed powder was dissolve in 2% ethanol and centrifuged at low rpm for 10 min [12]. The clear supernatant was used for further study.

Normoglycemic studies

Experimental design
Following the method used in our earlier studies [12], mice were divided into one control and six test groups to study the effects of varying doses of *Curcuma amada* (CA) extract in normal (Table-I) and diabetic mice (Table-II). Each group comprised of six mice. Varying doses of CA extract ranging from 150-650 mg/kg body weight (b.w.) were administered to the test groups intraperitoneally (i.p.) and glucose level was monitored at different time intervals up to 8 h following administration of extract. The control group received only 2% ethanol, being the solvent used for preparation. Food, but not water was withheld during the test period not exceeding 8 h.

Table –I

Normal mice		Treatment
Sl. No.	Category	
1	Control	Untreated
2	N-1	150 mg/kg b.w. of CA extract
3	N-2	250 mg/kg b.w. of CA extract
4	N-3	350 mg/kg b.w. of CA extract
5	N-4	450 mg/kg b.w. of CA extract
6	N-5	550 mg/kg b.w. of CA extract
7	N-6	650 mg/kg b.w. of CA extract

Anti-hyperglycemic studies

Induction of non-insulin dependent diabetes mellitus (NIDDM)

Animals were administered alloxan monohydrate (180 mg/kg b.w., ip) prepared in acetate buffer (0.15 M, pH 4.5) on three alternate days, as described earlier [12]. The control group received only the buffer. Prior to administration, mice were fasted overnight but given water *adlibitum*. Mice with blood glucose level above 200 mg/dL were considered diabetic and used for further tests.

Table –II

Diabetic mice		Treatment
Sl. No.	Category	
1	Control	untreated
2	D-1	150 mg/kg b.w. of CA extract
3	D-2	250 mg/kg b.w. of CA extract
4	D-3	350 mg/kg b.w. of CA extract
5	D-4	450 mg/kg b.w. of CA extract
6	D-5	550 mg/kg b.w. of CA extract
7	D-6	650 mg/kg b.w. of CA extract

Administration of extract to alloxan-induced diabetic mice

Following the same experimental design (Table-II) as with normoglycemic studies, alloxan-induced diabetic mice were administered (i.p.) the test extract at varying doses (150-650 mg/kg b.w.) and the blood glucose levels were measured at varying time intervals. All animals treated were observed for behavioral changes like polydipsia and polyphagia.

Glucose Tolerance Test (GTT)

Experimental design

Mice were divided into one normal control, one diabetic control and four test groups to study the glucose tolerance in normal and alloxan-induced diabetic mice (Table-III) following administration of the effective dose of the CA extract. Normal or alloxan-diabetic mice, fasted overnight but provided water *ad libitum*, were administered the test samples intraperitoneally 2 h prior to the glucose load of 2 g/kg b.w. according to the method used earlier [12]. Glucose concentration was measured before administration and subsequently at 30, 60, 120 and 1440 min after the glucose load. The control groups received only the glucose load, while the reference drugs metformin, glibenclamide and insulin were administered as described earlier [12]. Each group comprised of six mice.

Table –III

1	NC	Normal mice given intraperitoneal glucose load of 2 g/kg b.w.
2	DC	Diabetic mice given intraperitoneal glucose load of 2 g/kg b.w.
3	OT-1	Diabetic mice given intraperitoneal glucose load of 2 g/kg b.w. + 350 mg/kg b.w. of CA extract
4	OT-2	Diabetic mice given intraperitoneal glucose load of 2 g/kg b.w. + insulin
5	OT-3	Diabetic mice given intraperitoneal glucose load of 2 g/kg b.w. + metformin
6	OT-4	Diabetic mice given intraperitoneal glucose load of 2 g/kg b.w. + glibenclamide

Toxicity studies

Normoglycemic mice were administered up to a dose of 650 mg/kg b.w and kept under observation up to 4 weeks for any signs of distress, convulsion, coma or death [20].

Collection of blood and determination of blood glucose level

Blood samples from the control and experimental mice were collected by orbital sinus puncture using heparinised capillary glass tubes [21]. The blood samples so collected were analyzed for glucose levels employing glucostix with the glucometer (Ames).

Statistical analysis

Student's t-test was used for determining the levels of significance between the control and the test values. Results are expressed as Mean ± SEM.

Results

Normal mice

The varying doses (150-650 mg/kg b.w.) of *Curcuma amada* extract administered intraperitoneally showed a time- and dose-dependant hypoglycemic effect on normal mice (Fig 1). Weak hypoglycemic effect was observed throughout the experimental period for the 150 mg/kg b.w. with maximum 17% lowering of blood glucose level found at 6 h from that of control. Similar hypoglycemic effect was observed at 250, 350 and 450 mg/kg b.w. doses till 4 h. Doses of 350 and 450 mg/kg b.w. exerted more pronounced effect with the blood glucose levels being 33% and 42%, respectively at 8 h as compared to the control. The higher doses of 550 and 650 mg/kg b.w. exerted very similar effect on the blood glucose level with blood glucose being reduced to 40% and 42%, respective from that of control at 8 h. No sign of toxicity was observed in any of the mice even at the dose of 650 mg/kg b.w.

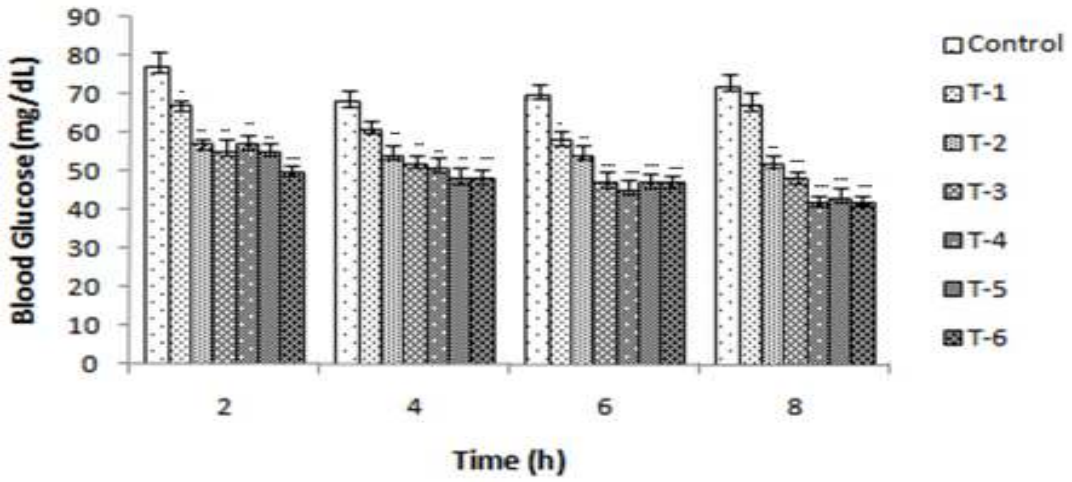

Fig 1. Effect of *Curcuma amada* extract (150-650 mg/kg b.w.) on blood glucose level of overnight fasted normal mice assayed at different time intervals. Values are expressed as Mean ±SEM (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

Diabetic mice

The intraperitoneal administration of methanolic extract of *Curcuma amada* to overnight fasted alloxan-induced diabetic mice elicited a marked and prolong anti-hyperglycemic effect (Fig. 2). The anti-hyperglycemic effect was dose- and time-dependant. Maximum reduction was observed at 8 h following extract administration for all the doses used. The reduction was 25%, 48%, 63%, 67%, 68% and 68% at 150, 250, 350, 450, 550 and 650 mg/kg b.w., respectively.

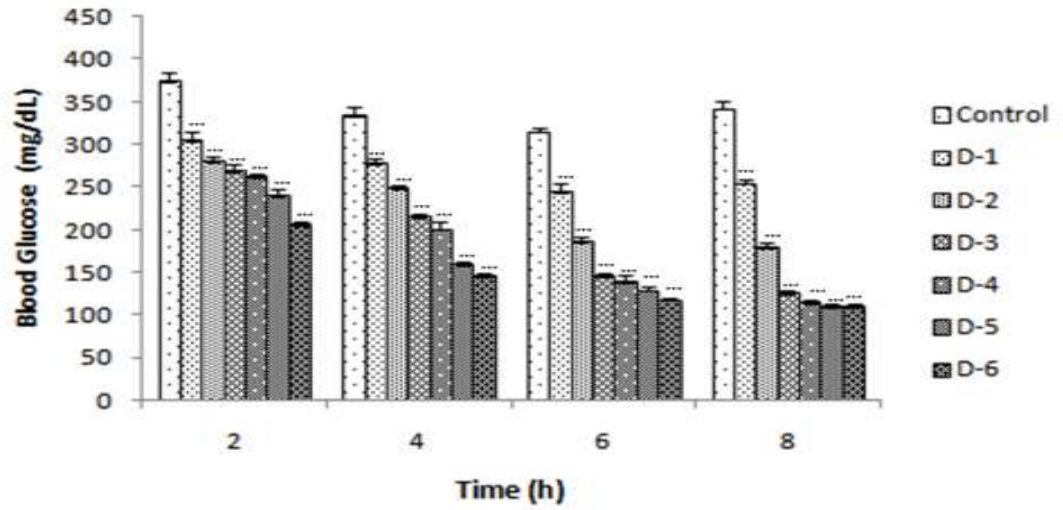

Fig 2. Effect of *Curcuma amada* extract (150-650 mg/kg b.w.) on blood glucose level of overnight fasted diabetic mice assayed at different time intervals. Values are expressed as Mean ± SEM (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

Glucose tolerance test

Intraperitoneal administration of the extract (350 mg/kg b.w.) 2 h prior to intraperitoneal glucose load showed improved glucose tolerance in the overnight fasted diabetic mice (Fig. 3). Maximum suppression of the blood glucose level was observed at 120 min after the glucose load with the glucose level being 75% from that of control. The effect of *Curcuma amada* extract is comparable with the standard oral anti-hyperglycemic drugs metformin and glibenclamide in terms of magnitude of effects and response time. The effect of insulin was more pronounced as compared to the other drugs with 95% reduction in blood glucose level from that of control observed at 60 min. The effects of *Curcuma amada* as well as the standard drugs were observed to normalize at 24 h.

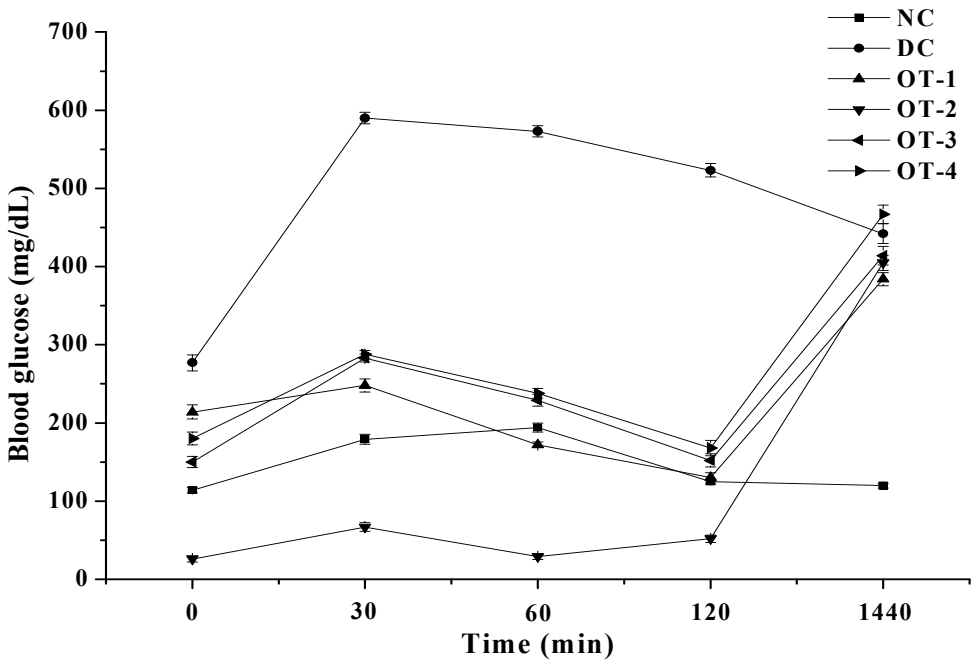

Fig 3. Glucose tolerance test in normal and diabetic mice administered with *Curcuma amada* extract (350 mg/kg b.w.), and standard drugs assayed at different time intervals. Values are expressed as Mean ±SEM.

Toxicity studies carried out on normal as well as diabetic mice up to a dose of 650 mg/kg b.w. did not show any adverse effects during the 4 weeks of observation.

Discussion

The results of the present study indicate that the aqueous-methanolic extract of *Curcuma amada* can reduce blood glucose levels in normal as well as alloxan-induced diabetic mice in a time- and dose-dependant manner. 350 mg/kg b.w. was found to be the optimum dose with no contraindications both in the normal as well as diabetic mice.

In normal mice, *Curcuma amada* extract exerted only a mild hypoglycemic effect even at a high dose of 650 mg/kg b.w. However, pronounced anti-hyperglycemic effect was observed in the extract treated diabetic mice, with blood glucose reaching normal levels. The effect therefore implies a mechanism similar to metformin which is a known anti-hyperglycemic drug but not hypoglycemic in action. In contrast to sulfonylureas, metformin, a biguanide is not an insulin secretagogue and does not cause hypoglycemia even in large doses [22-24]. Glibenclamide, a sulfonylurea derivative causes hypoglycemia by stimulating pancreatic β-cells to release more insulin, and inhibiting glucagon secretion [25]. The β-cells are partially compromised in alloxan-induced diabetic mice [26]. In a fully compromised pancreas, glibenclamide has no effect in lowering the blood glucose level. The mild hypoglycemic and prolonged anti-hyperglycemic effects observed in our study may therefore involve an extrapancreatic mechanism. Other plants such as *Cuminum nigrum* [27] and *Momordica charantia* [28] are reported to have insulin-like effects.

Glucose tolerance was significantly improved in alloxan-induced diabetic mice treated with the plant extract and standard drugs. Glucose tolerance test is indicative of the extent of intestinal glucose absorption and hepatic glucose metabolism [29, 30]. As also suggested by the hypoglycemic and anti-hyperglycemic assays, the effects of the plant extract and metformin are found to be similar in the glucose tolerance test. Interestingly, the effect of glibenclamide is also found to be very similar to that of metformin. The pancreas must have been only partially damaged in the diabetic mice leading to the stimulation of the healthy β-cells to secrete some amount of insulin by glibenclamide. Insulin exerted the best blood glucose lowering potential till 120 min after the glucose load. Though the effect of the *Curcuma amada* extract strongly suggests metformin-type of action, the mild hypoglycemic potential exerted especially by higher doses of 350 mg/kg b.w. and above could be due to the presence of more than one factors with different mechanisms of action.

In conclusion, the methanol extract of *Curcuma amada* rhizome exhibited anti-hyperglycemic and mild hypoglycemic activities in mice. As no toxic effects were associated with the plant extract even at high dose of 650 mg/kg b.w., *Curcuma amada* is very promising plant for further biochemical investigations including phytochemical studies.

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