EFFECT OF *GYMNOPETALUM COCHINCHINENSIS* ON BLOOD GLUCOSE LEVEL IN NORMAL AND ALLOXAN- INDUCED DIABETIC MICE

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Summary

Gymnopetalum cochinchinensis (LOUR) KURZ fruit is traditionally used by local practitioners of Jaintia Hills of Meghalaya for treating various types of ailments including diabetes. The aqueous extract of the fruits was tested for its hypoglycemic and anti-hyperglycemic effects in both normal and alloxan-induced diabetic mice. Hypoglycemic and anti-hyperglycemic activity was observed to be dose- and time- dependent. The aqueous extract reduced blood glucose level 2 h following administration in both normal and alloxan-induced diabetic mice. In alloxan- induced diabetic mice, blood glucose was markedly reduced to 56% from that of control at 4 h, while in normal mice the blood glucose level was 59% from that of control. Maximum reduction was observed at 6 h in alloxan-induced diabetic mice. Glucose tolerance was also improved in both normal and diabetic mice. The results were compared against those of insulin, glibenclamide, metformin, and the possible mechanism of action discussed.

Keywords: Hypoglycemic, Anti- hyperglycemic, Alloxan, Glibenclamide, Metformin, Insulin, Glucose tolerance, *Gymnopetalum cochinchinensis*.

Introduction

Globally, numerous plants have been reported to possess hypoglycemic activity [1, 2, 3, 4] including those used by local ethnic community of the North Eastern Region of India [5, 6, 7]. Different mechanisms have been proposed for reducing blood sugar levels by plants extract. Thus, the action of plants have been compared to glibenclamide, a well known sulfonylurea drug which effects hypoglycemia by stimulating insulin release [8, 9, 10]. Other plants exert action like the biguanides, specifically metformin which have been reported to enhance glucose transport, inhibit gluconeogenesis, reducing glucose absorption from the intestine and increase glucose metabolism in the liver [11, 12]. Still other plants function as β-Blocker inhibiting the effects of catecholamines, which are known to promote gluconeogenesis and glycogenolysis [13]. *Momordica charantia* has also been reported to have a role on the recovery of partially destroyed β-cell [14].

Gymnopetalum cochinchinensis (LOUR) KURZ locally known as Sawakthang is a fruit vegetable that belongs to the family of Cucurbitaceae, and grows in some parts of Meghalaya. It is now domesticated and cultivated as a curcurbitaceous crop [15]. The fruit, which is very bitter in taste, is cooked as a vegetable. It is locally believed to have medicinal values and is also known for its curative properties viz; controlling and curing certain diseases including diabetes (Type-II) and malaria. Traditionally, the fruits are generally boiled and taken, or the juice obtained after boiling are taken once a day. The fruit is said to be poisonous, though at the young stage it is edible.

Literature information on the pharmacological properties of *G cochinchinensis* is scanty. The present investigation was, therefore, undertaken to study the effects of fruit juice of *G cochinchinensis* on fasting blood glucose in normal as well as alloxan- induced diabetic mice.

Materials and methods

Chemicals

Alloxan was procured from Sigma Co. USA, while other chemicals used were of analytical grade obtained from E. Merck and Hi-media, India.

Test animals

Healthy, adult male swiss albino mice of approximately 4 months in age, weighing 20-30 g were used for the study. Mice were housed in a room kept under control conditions with temperature maintained at 22 ^{o}C on a 12-h dark cycle and were fed balanced mice feed obtained from Amrut Laboratory, Pune, India. All experiments were conducted as per the institutional ethical guidelines.

Plant Material

G. cochinchinensis was collected from Mookabeng village of Jaintia Hills, Meghalaya (Voucher No: 104002, BSI, Shillong). The specimen was submitted and identified by Dr Shanpru of Botanical Survey of India, Eastern Circle, Shillong.

Extraction

The fruit samples were washed and cut into pieces. After separating the seeds, the fruit were shredded and dried in the shade. It was then powdered, homogenized and repeatedly extracted with 10 vol. of aqueous solution [16]. The mixture was filtered and the filtrate evaporated to dryness at 40 °C in an oven. The dried mass obtained was used for the investigation. The yield of aqueous extract was 4.27 % (w/w from dried starting material). Prior to use, weighed powder was dissolved in 100 ml distilled water and kept on boiling water bath for 10 min. The clear supernatant was used for further study.

Normoglycemic studies

Experimental design

Animals were divided into three test and one control group (Table I), each group comprising a minimum of six mice (n =6). The aqueous extract in varying dose ranging from 150 to 850 mg/kg b.w. was administered to the test group by intraperitoneal injection and glucose level was monitored at different time intervals up to 48 h following the aqueous extract administration. The control groups received only distilled water being the solvent used for preparation. Food, but not water was withheld during test period not exceeding 24 h. Food, fluid intake and body weights were monitored for a week after administration of the aqueous extract [17].

Table I

The animals were divided into four groups. Each groups comprised of six mice

1	Control	Normal mice untreated
2	T-1	Normal mice treated with 150mg/kg b.w. of plant extract
3	T-2	Normal mice treated with 450mg/kg b.w. of plant extract
4	T-3	Normal mice treated with 850mg/kg b.w. of plant extract

Anti-hyperglycemic studies

Induction of non-insulin dependent diabetes mellitus (NIDDM)

Animals were administered intraperitoneally alloxan monohydrate (150 mg/kg b.w.) prepared in acetate buffer (0.15 M, pH 4.5). The control group received only the buffer. Prior to administration, mice were fasted overnight but given water ad libitum. Animals were then kept under observation for a week following administration and blood glucose levels were subsequently determined. Mice with blood glucose levels ranging between 200- 300 mg/dl were considered diabetic and used for further tests [5]

Administration of extract to alloxan-induced diabetic mice

Animals were divided into two test and one control group (Table II), each group comprising a minimum of six mice (n =6). Alloxan-induced diabetic mice were administered (i.p) the aqueous extract at two doses (150 and 450 mg/kg b.w.) and the blood glucose levels were measured at varying time intervals. The dose of 850 mg/kg b.w. was excluded as it was found to be fatal to normal mice. All animals treated were observed for behavioural changes like polydipsia, polyphagia and polyurea.

Table II

The animals were divided into three groups. Each groups comprised of six mice

1	Control	Diabetic mice untreated
2	D-1	Diabetic mice treated with 150mg/kg b.w. of plant extract
3	D-2	Diabetic mice treated with 450mg/kg b.w. of plant extract

Glucose Tolerance Test (GTT)

Experimental design

Mice were divided into one control and four test groups to study the glucose tolerance in normal (Table III A) and alloxan-induced diabetic mice (Table III B). Mice fasted overnight but provided water *ad libitum*, were administered the test samples intraperitoneally 1.5 h prior to the oral glucose load of 2gm/kg b.w. according to the method used earlier [17]. Glucose concentration was measured before administration and subsequently at 30, 60, 120, 480 and 1440 minutes after the glucose load. The control group received only the glucose load, while the reference drugs metformin [18] glibenclamide [19] and insulin [20] were administered following the respective cited method. Each group comprised of 6 mices.

Table III

(A) Glucose Tolerance Test on Normal Mice

The animals were divided into five groups. Each group comprised of six mice

1	Control	Mice given oral glucose load of 2gm/kg b.w.
2	OT-1	Mice given oral glucose load of 2gm/kg b.w. + 450 mg/kg b.w. of plant extract
3	OT-2	Mice given oral glucose load of 2gm/kg b.w.+ 10 mg/kg b.w. of glibenclamide
4	OT-3	Mice given oral glucose load of 2gm/kg b.w.+ 500 mg/kg b.w. of metformin
5	OT-4	Mice given oral glucose load of 2gm/kg b.w.+10U/ kg b.w. of insulin

(B) Glucose Tolerance Test on Diabetic Mice

The animals were divided into five groups. Each group comprised of six mice

1	Control	Mice given oral glucose load of 2gm/kg b.w.
2	OT-D1	Mice given oral glucose load of 2gm/kg b.w. + 450 mg/kg b.w. of plant extract
3	OT-D2	Mice given oral glucose load of 2gm/kg b.w.+ 10 mg/kg b.w. of glibenclamide
4	OT-D3	Mice given oral glucose load of 2gm/kg b.w.+ 500 mg/kg b.w. of metformin
5	OT-D4	Mice given oral glucose load of 2gm/kg b.w.+10U/ kg b.w. of insulin

Toxicity studies

Normoglycemic animals were administered up to a dose of 850 mg/kg b.w. and kept under observation up to 4 weeks for any signs of distress, convulsion, coma or death.

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*-tests were used for determining the levels of significance between the control and the test values. Results were expressed as mean ±S.E.M.

Results

Normal mice

Blood glucose levels of the normal mice receiving the aqueous extract (i.p.) at varying doses were observed to show significant reduction, in a time- and dose- dependant manner (Fig. 1). Hypoglycemic effect was observed at all doses used (150-850 mg/kg b.w.). The effect was significant at 2, 4, 6, 8 and 24 h. At the dose of 150 mg/kg b.w. a marked reduction of glucose level was observed with glucose levels reduced to 76 % ($P< 0.05$), 63 % ($P< 0.001$), 63 % ($P< 0.001$), 60 % ($P< 0.001$) and 63 % ($P< 0.001$) from that of the control at 2, 4, 6, 8 and 24 h respectively. At the dose of 450 mg/kg b.w., glucose levels was observed to be 57 % ($P< 0.001$), 59 % ($P< 0.001$), 58 % ($P < 0.05$), 52 % ($P < 0.001$) and 51 % ($P< 0.001$) of the control at 2, 4, 6, 8 and 24 h respectively. The higher dose of 850 mg/kg b.w. resulted in severe hypoglycemia 6 h after extract administration with glucose level being 30.48 % ($P<0.001$) from that of the control (Fig. 1). Mice however did not survive beyond 8 h at this dosage.

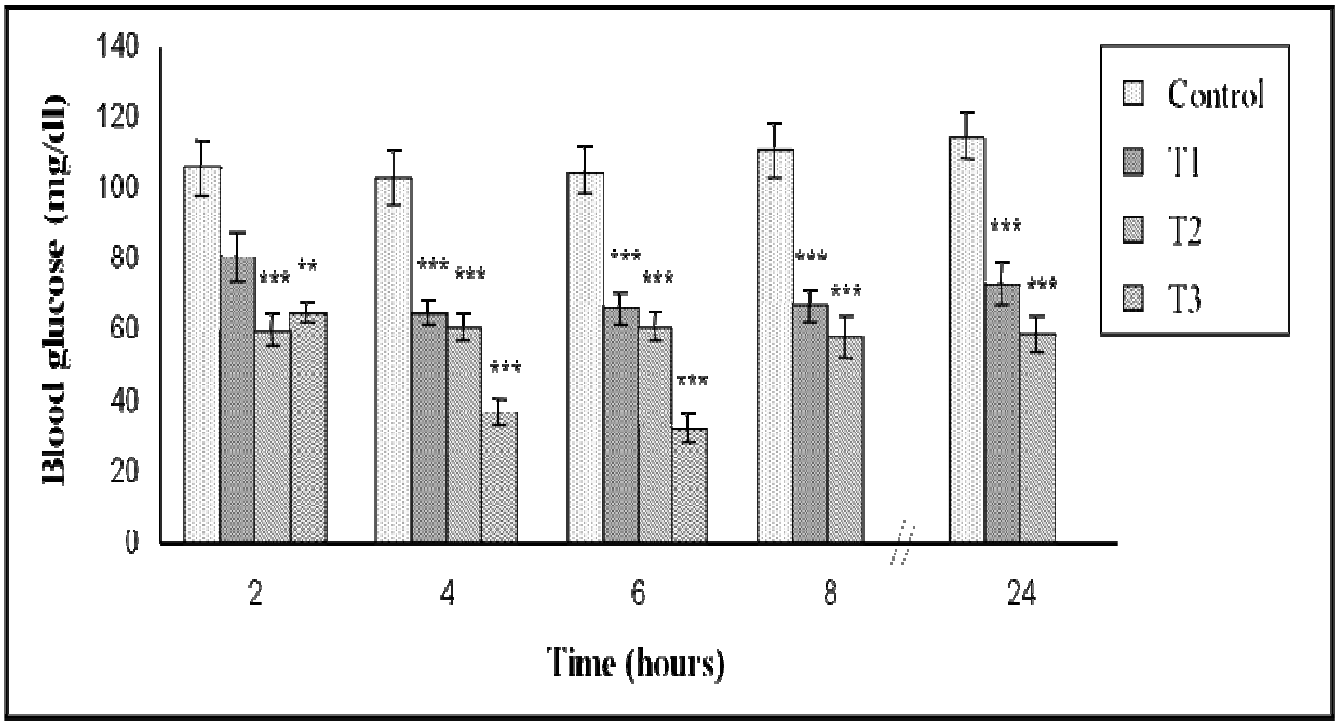

Fig 1: Effect of varying doses of crude extract on fasting blood glucose level in normal mice assayed at different time intervals. Values are expressed as mean ± SEM (*.p 0.05. **.p 0.01. ***. p 0.001)

Alloxan-induced diabetic mice

Varying doses of the aqueous extract administered (i.p.) to diabetic mice also elicited marked and prolonged anti-hyperglycemic action in a dose- and time- dependent manner similar to that observed in normal mice (Fig. 2). Anti-hyperglycemic effect was observed at all the time intervals measured. At 150 mg/kg b.w., glucose level was reduced to 67 % ($P< 0.001$), 61 % ($P< 0.01$), 74 % ($P< 0.01$), 83 % ($P< 0.05$) and 87 % (NS) from that of the control at 2, 4, 6, 8 and 24 h respectively. At the dose of 450 mg/kg b.w., glucose levels was observed to be 69 % ($P< 0.001$), 56 % ($P< 0.01$), 48 % ($P < 0.001$), 56 % ($P < 0.001$) and 66 % ($P< 0.001$) of the control at 2, 4, 6, 8 and 24 h, respectively. The higher dose of 850 mg/kg b.w. was not used as it was observed to be toxic to normal mice.

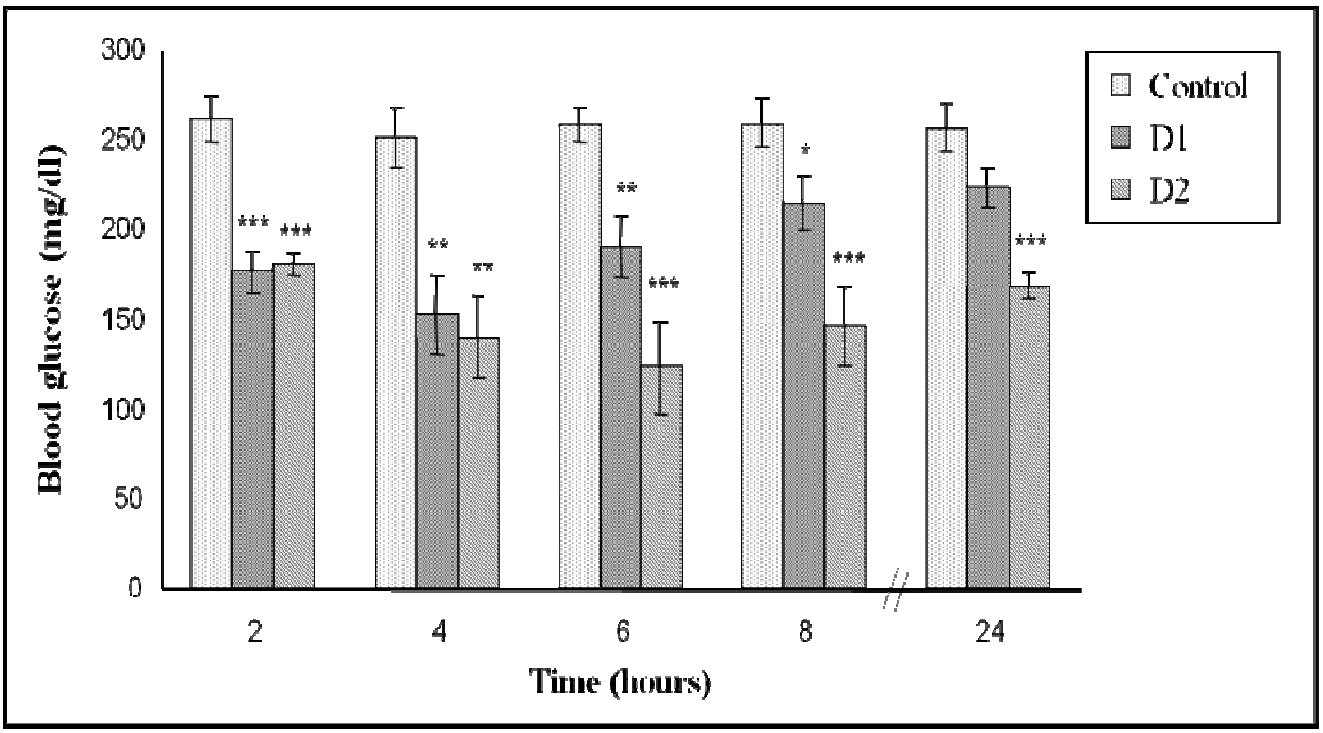

Fig 2: Effect of varying doses of crude extract on fasting blood glucose level in alloxan-induced diabetic mice. assayed at different time intervals. Values are expressed as mean ± SEM (*.p 0.05, **.p 0.01, ***.p 0.001)

Glucose Tolerance Test (GTT)

Administration of the aqueous extract (450 mg/kg b.w.) intraperitoneally one and a half hour prior to glucose load improved glucose tolerance in normal mice. The magnitude of effect varied with the mode of administration (Fig 3). As shown the i.p route improved glucose tolerance at 30, 60 and 120 min with glucose level being 68.4 %, 64 % and 58 % respectively from that of the control. Glucose level measured 24 h after extract administration also exhibit pronounced reduction with glucose level being 52 % of the control.

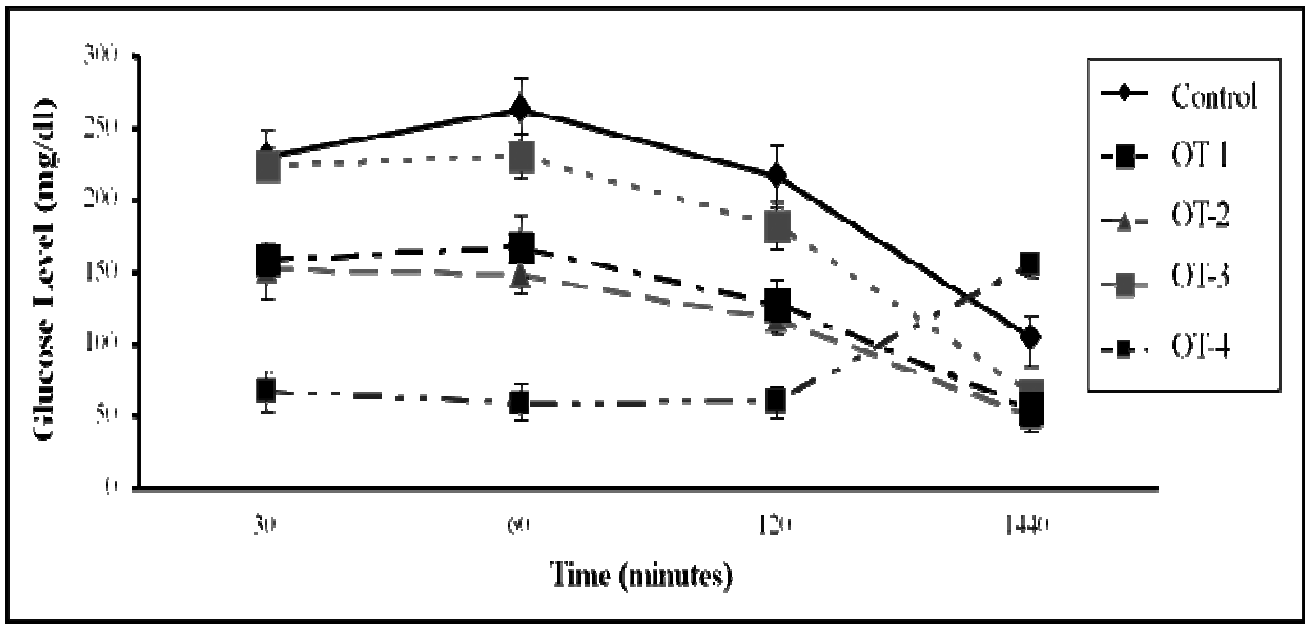

Fig 3. Glucose tolerance test in normal mice administered with extract of *G cochinchinensis* (450 mg kg) and reference drugs assayed at different time intervals. Values are expressed as mean ± S.E.M. (se-subcutaneous, ip-intraperitoneal). Experimental details are described under materials and methods.

Glucose tolerance in alloxan-induced diabetic mice (Fig 4) exhibit similar pattern to that of normal mice with the i.p mode improving glucose tolerance at all time intervals measured. Thus, glucose levels dropped to 60 % ($P < 0.02$) and 77 % ($P< 0.05$) from that of control at 60 and 120 min respectively. At 24 h, the glucose levels were still significantly low, being 36 % from that of the control.

Toxicity studies carried out on mice up to a dose of 450 mg/kg b.w. did not show any adverse effects during the 4- weeks of observation. However, doses of 850-mg/kg b.w. resulted in severe hypoglycemia in normal mice followed by death within 6 h following administration of extract.

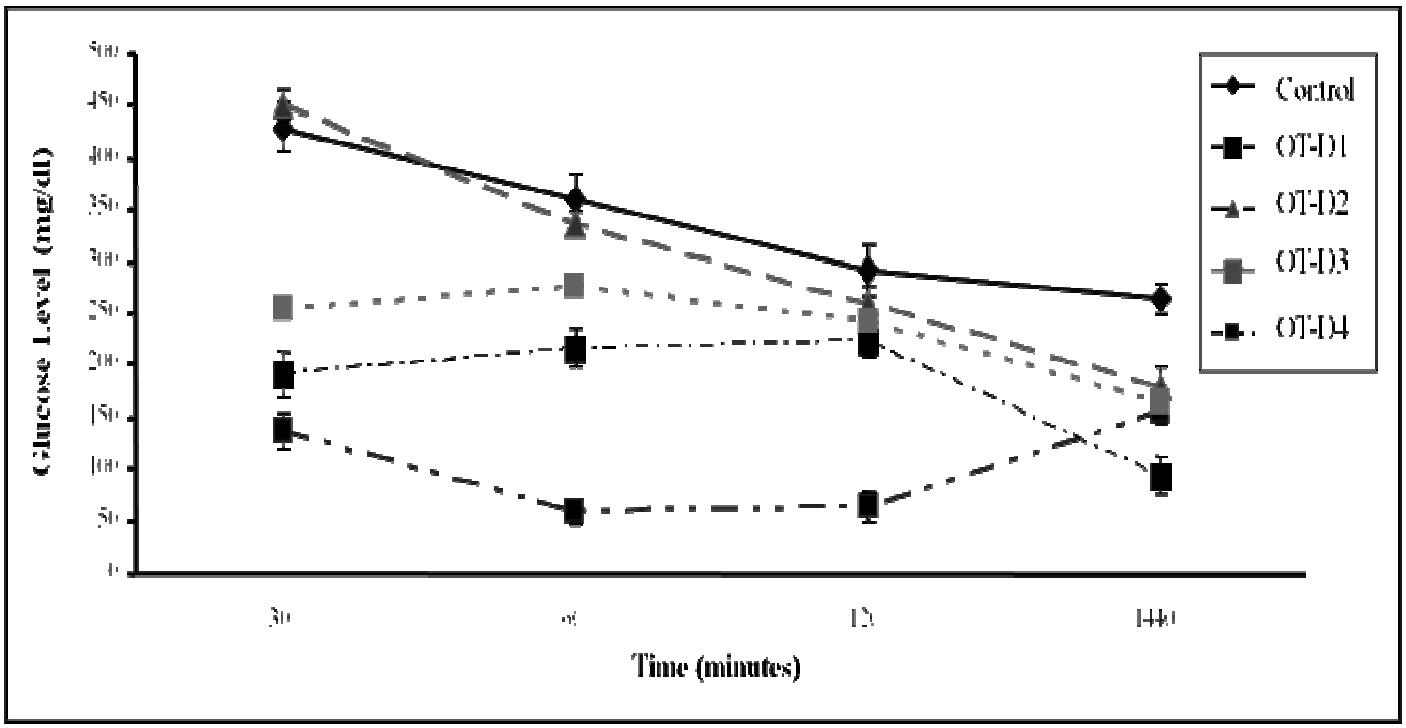

Fig 4. Glucose tolerance test in alloxan-diabetic mice administered with extract of *G cochinchinensis* (450 mg/kg) and reference drugs assayed at different time intervals. Values are expressed as mean ± S.E.M. (sc-subcutaneous, ip-intraperitoneal)
Experimental details are described under materials and methods.

Discussion

In normal mice, hypoglycemic action of the extract was observed to be dose- and time-dependent, with prolonged hypoglycemia being observed at all doses. At the highest dosage used (850 mg/kg b.w.) the extract causes pronounced hypoglycemia, followed by death. At this juncture we cannot rule out other toxic effects of the aqueous extract. In alloxan-induced diabetic mice, where β-cells are partly compromised, higher doses of the extract (450 mg/kg b.w.) were found to be effective at 6 h. Thus, at the dose of 450 mg/kg b.w. blood glucose levels of diabetic mice was reduced to normal levels at 6 h (123 mg/kg b.w.) while at 24 h it was still significantly reduced by 36 % from that of the control. Evidently, the extract contains principle(s) that causes prolonged hypoglycemic action. The results of glucose tolerance indicate that the extract is comparable to glibenclamide where the suppression of glucose peak was similar in magnitude at 30, 60 and 120 mins in both normal and alloxan-induced diabetic mice. It may be postulated that the extract has a similar effect like that of glibenclamide which is a well known insulin secretagague [22]. Other probable mechanism could be a more direct insulin like effect as reported for *Cuminum nigrum* [23] and *Momordica charanchia* [8].

In conclusion, *G cochinchinensis* may be added to the growing list of hypoglycemic and anti- hyperglycemic plants. Like other plants it may possibly exert via; both pancreatic and extra pancreatic mechanism. The marked and prolonged activity necessitates a more comprehensive chemical and pharmacological investigation to elucidate the exact mechanism and to isolate and identify its active principle (s). Its toxic effect needs to be understood within the pharmacological framework, keeping in mind that this plant is locally consumed without any reports of adverse effects.

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