Antihyperglycemic and Insulin Secretagogue Activities of Abrus precatorius Leaf Extract

Balekari Umamahesh, Ciddi Veeresham

Department of Pharmacognosy, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India

ABSTRACT
Aim: Abrus precatorius leaves methanolic extract (APME) was evaluated for in vivo antihyperglycemic activity and in vitro insulinotropic effect. Materials and Methods: In vivo antihyperglycemic and insulin secretagogue activities were assessed in streptozotocin-induced diabetic rats by oral administration of APME (200 mg/kg body weight [bw]) for 28 days. In vitro insulin secretion mechanisms were studied using mouse insulinoma beta cells (MIN6-β). In vivo body weight and blood glucose and in vivo and in vitro insulin levels were estimated. Results: In diabetic rats, APME treatment significantly restored body weight (26.39%), blood glucose (32.39%), and insulin levels (73.95%) in comparison to diabetic control rats. In MIN6-β cells, APME potentiated insulin secretion in a dependent manner of glucose (3–16.7 mM) and extract (5–500 μg/mL) concentration. Insulin secretagogue effect was demonstrated in the presence of 3-isobutyl-1-methyl xanthine, glibenclamide, elevated extracellular calcium, and K+ depolarized media. Insulin release was reduced in the presence of nifedipine, ethylene glycol tetra acetic acid (calcium blocking agents), and diazoxide (potassium channel opener). Conclusion: The study suggests that APME antihyperglycemic activity might involve the insulin secretagogue effect by pancreatic beta cells physiological pathways via K+‑ATP channel dependent and independently, along with an effect on Ca2+ channels. Key words: Calcium, diabetes, glibenclamide, insulin secretagogue, K+‑ATP channel, mouse insulinoma beta cells

SUMMARY
• Abrus precatorius leaves methanolic extract (APME) showed a significant anti hyperglycemic and insulin secretagogue activities in streptozotocin induced diabetic rats. Also demonstrated a potent in vitro insulin secretagogue effect in mouse insulinoma beta cells (MIN6-β)
• APME treatment significantly restored body weight (26.39%), reduced blood glucose (32.39%) and enhanced circulatory insulin levels (73.95%) in diabetic rats
• APME demonstrated glucose and extract dose dependent insulin secretion
• Insulin secretagogue effect was demonstrated in the presence of 3-isobutyl-1-methyl xanthine, glibenclamide, elevated extracellular calcium, and K+ depolarized media. Insulin release was reduced in the presence of nifedipine, ethylene glycol tetra acetic acid (calcium blocking agents), and diazoxide (potassium channel opener)

INTRODUCTION

Abrus precatorius (AP) Linn. is a widely used medicinal plant from the family of Fabaceae. In traditional medicine, the parts of the plant have been reported for use in treatment of many diseases. The aerial parts of the plant used for leukorrhea, gonorrhea, diarrhoea, and dysentery. Roots and leaves of the plants are known for their use as emetic, anthelmintic, sweetening, astringent, and alexiteric agent. The seeds of the plant are used for sciatica, shoulder stiffness, paralysis, leukoderma, ulcers, wounds, alopecia, asthma, bronchitis, tubercular glands, stomatitis, hyperdipsia, and fever.[1,2] Leaves of the AP have shown antioxidant,[3] anticancer,[4] mast cell stabilizing, and antiallergic activities.[5] Although the seeds of the plants were scientifically well explored for medicinal uses, the scarce studies were conducted on leaves of the plant. Thus, keeping in view of these medicinal properties of the plant, the present study was undertaken for the 1st time on AP leaves methanolic extract (APME) to investigate the antidiabetic and insulinotropic effect along with its mechanisms involved using streptozotocin (STZ)‑induced diabetic rat model and mouse insulinoma beta cells (MIN6‑β).

MATERIALS AND METHODS

Materials
Methanol, glucose, STZ, Dulbecco’s modified Eagle’s medium (DMEM), fetal calf serum, glutamine, penicillin, streptomycin, diazoxide, 3-isobutyl-1-methylxanthine (IBMX), calcium chloride, potassium chloride (KCl), ethylene glycol tetra acetic acid (EGTA), and

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Plant material and extraction
Leaves of AP were collected from the local fields at Warangal, Telangana, India, and authenticated by Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal, India. The cleaned leaves were shade dried. Cold percolation method was used for the exhaustive extraction of the dried leaves with methanol. The APME was filtered and concentrated under reduced pressure and freeze drying.

Animal studies
Male Wistar rats (180–200 g) were obtained from Sanzyme Ltd., (Hyderabad, India), housed at 25°C and relative humidity of 45–55% under a natural light-dark cycle with unrestricted access to food and water. Throughout the experimental period, the rats were fed with balanced pellet diet. The experimental protocol was approved by the Institutional Animal Ethics Committee and performed in accordance with the guidelines of the Committee for Control and Supervision of Experimentation on Animals, Government of India on animal experimentation.

Acute toxicity studies of the extract were conducted on five groups (n = 6) of 12 h fasted rats. Extract at a dose of 0.25, 0.5, 1.0, and 2.0 g/kg bw was administered to extract treated four different groups and vehicle alone (carboxy methyl cellulose [CMC] 0.5%; 1 ml/kg bw) was administered to control group. During 24 h observation period, animals were monitored for behavioral changes and mortality due to APME acute toxicity.[30]

Oral glucose tolerance test (OGTT) was conducted in normal male Wister rats for selecting APME study dose.[35] Five groups (n = 6) of overnight fasted rats were taken. Before experiment, blood glucose concentration of each animal was determined by glucose oxidase method. Then, all the animals were administered with glucose at a dose of 2 g/kg bw. Groups A and E animals were administered with glucose alone and glibenclamide (5 mg/kg bw), respectively. APME at a dose of 50, 100, and 200 mg/kg bw was given, respectively, to the Groups B, C, and D by oral gavage. Blood samples from rats were collected at 0, 30, 60, 90, and 120 min after glucose loading and analyzed for blood glucose.[36]

Antihyperglycemic and insulin secretagogue activity of the extract was studied using STZ-induced diabetic rats. In rats, experimental diabetes was induced by a single intraperitoneal administration of freshly prepared solution of STZ (50 mg/kg bw) in 0.1 M citrate buffer (pH 4.5). To prevent STZ-induced hypoglycemia, animals were allowed to drink 10% glucose solution for 48 h. Seven days after diabetes induction, animals were tested for diabetes and animals with a blood glucose levels above 250 mg/dL were used as diabetic animals in the experiment.

In the experiment, a total of 48 rats (8 normal; 40 STZ diabetic rats) were used and divided into four groups (n = 8). Naive animals were treated as Group 1; Group 2 consisted of diabetic control rats that were treated with vehicle alone orally (CMC 0.5%, 1 ml/kg bw); Groups 3 and 4 were treated with APME (200 mg/kg bw) and standard drug, glibenclamide (5 mg/kg bw), respectively. During the study period of 4 weeks, rat blood samples were withdrawn from the retro-orbital plexus and centrifuged at 4000 rpm for 20 min using cooling centrifuge at 4°C and separated plasma samples were stored at –20°C until further analysis. For all the rats, on the first (0th) day and last (28th) day of the study, body weight, plasma glucose, and plasma insulin levels were evaluated. On 7th and 15th days of treatment, body weight and plasma glucose levels were determined. Rat insulin ELISA kit (Crystal Chem, Inc., Illinois, USA) was used for determination of plasma insulin concentrations.

Bioassay for insulin secretion
The β-cell insulin secretion assay was conducted as already described with brief modifications.[35] Insulin secretion assay was conducted in 96-well plates seeded about 30,000 MIN6-β cells per well. APME effect on insulin secretion activity of MIN6-β cells was evaluated at submaximal effective concentrations of glucose, control and APME. The cells were incubated for 60 min with only KRB (naive) and KRB with glucose to emulate a basal (3 mM) and hyperglycemic (11.1 mM) environment. The dose-dependent effect of APME on insulin secretion was evaluated at a dose range of 5–1000 μg/mL, and a submaximal dose (500 μg/mL) was selected for the study. The standard insulin secretagogue glibenclamide (10 μM) was used in the study. Unless stated, the experiment was conducted at 11.1 mM glucose concentration. The possible insulin secretagogue mechanisms of the extract were assessed using diazoxide (0.5 mM) (β-cell K+ -ATP channel opener), isobutyl methyl xanthine (100 μM) (IBMX, phosphodiesterase inhibitor), and glibenclamide. Role of K+ -ATP channel and calcium on insulin secretion was assessed in the presence of calcium chloride (1.28 mM), EGTA (1 mM, calcium chelator), nifedipine (20 μM, calcium channel blocker), and KCl (30 mM, depolarizing concentration). Supernatant from each well was collected after incubating for 60 min. The collected supernatants were centrifuged at 4000 g for 5 min at 4°C. The clear supernatants were stored at –20°C until further analysis of insulin by ELISA.

Statistical analysis
All the data are expressed as mean ± standard deviation. One-way or two-way analysis of variance was used upon suitability to perform the statistical analysis of all the data. The significance of difference was assessed using the Dunnett’s post hoc test or Bonferroni test. P < 0.05 was considered to be statistically significant. GraphPad Prism (Graph Pad Software, San Diego, CA, USA) was used for all statistical analyses.

RESULTS
Animal studies
In in vivo acute toxicity studies, the APME treatment at tested doses did not show any toxicity in Wister rats. During the 24 h period of observation, no significant changes in the behavior, visible toxicity signs, and mortality were observed in the extract-treated groups. As shown in Table 1, the blood glucose levels of all the groups were comparable with each other. Thirty minutes after glucose loading, an

In vitro studies using mouse insulinoma beta cells
MIN6-β cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 10,000 units/mL of penicillin, and 10 mg/mL of streptomycin at 37°C and 5% CO2. APME stock solution was prepared by dissolving extract in dimethyl sulfoxide (DMSO) and further diluted with Krebs-Ringer buffer (KRB) to prepare working solutions. The final concentration of the DMSO was not >0.1% (v/v) in the media, to maintain normal incubation environment.

Cell viability assay
(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay)
Cell viability assay was performed according to Adam et al., with brief modifications.[35] MTT assay was performed by seeding 30,000 MIN6-β cells per well in 96-well plates and taken for the study after reaching 90% confluence. APME cell viability was studied over a concentration range of 5–1000 μg/mL and standard drug, glibenclamide (5–100 μM/mL). Cell viability was evaluated using MTT (5 mg/mL) and read for absorbance at 570 nm using Multiskan Ex microplate reader (Thermo Scientific, USA).
apparent increase in the blood glucose levels was observed. In comparison with naive group, a significant lowering in the 1st h blood glucose levels was observed in APME (200 mg/kg bw) group, in contrast with both the 50 and 100 mg/kg bw treated groups; this response was comparable with standard drug, glibenclamide. However, intragroup blood glucose levels were decreased significantly at all the time points of treatment compared to glucose levels at 30 min.

The time course changes in the body weight of the STZ-induced diabetic rats with the treatment of APME (200 mg/kg bw) and glibenclamide (5 mg/kg bw) are presented in Figure 1. During 28 days study period, diabetic control animals continuously lost their weight until end day of the study by 22.49% (142.09 ± 3.25 g), in contrast with naive animals (183.32 ± 3.28 g). Compared with diabetic control rats, APME- and glibenclamide-treated diabetic rats significantly (P < 0.001) restored body weights to 176.59 ± 3.57 g and 179.79 ± 4.22 g, respectively. Over the 28 days study period, a significant (P < 0.001) increase in the blood glucose levels was observed in the diabetic control rats (364.06 ± 5.37 mg/dL) than the naive animals (75.24 ± 3.52 mg/dL) [Figure2]. When compared with diabetic control, APME and glibenclamide treatment to diabetic rats resulted in a significant (P < 0.001) decline in the blood glucose levels to 185.39 ± 6.86 mg/dL and 174.63 ± 6.62 mg/dL, respectively.

On the initial day of the study, serum insulin levels of the diabetic animals (0.34 ± 0.02 ng/mL) were significantly (P < 0.001) less than the naive animals (0.64 ± 0.03 ng/mL). During the study period, the serum insulin levels in the diabetic control rats were continuously decreased. In comparison with the 1st day of the study, by end day, the treatment of APME and glibenclamide significantly restored plasma insulin levels by 73.95% (0.59 ± 0.01 ng/mL) and 89.25% (0.62 ± 0.04 ng/mL), respectively. These plasma insulin levels of treated animals were significant (P < 0.001) to diabetic control animals [Figure 3]. Thus, the treatment of APME to diabetic rats restores body weight, blood glucose, and plasma insulin levels to near normal values.

**In vitro studies using mouse insulinoma beta-cells**

APME at 5–1000 μg/mL concentration range and glibenclamide at dose range (5–100 μM) have not shown any effect on the cell viability [Table 2]. Therefore, glibenclamide and APME were used at the selected doses for the *in vitro* studies on MIN6-β cells.

The APME has shown dose-dependent effect on insulin release at tested dose range of 5–1000 μg/mL under basal (3 mM) and hyperglycemic (11.1 mM) conditions [Figure 4]. However, the insulin release at higher concentration was declined than the insulin release at APME 500 μg/mL. Thus, a submaximal dose of 500 μg/mL was used in further studies. Hyperglycemic conditions potentiated insulin release by 1.7 folds (3.33 ± 0.23 ng/mL vs. 1.98 ± 0.14 ng/mL) to the basal level. Over the APME dose ranges, the increase in insulin release was significant (P < 0.001) in weighing against to insulin release at control under both the conditions. In hyperglycemic conditions, a significant (P < 0.001) enhancement in insulin release was observed compared to basal condition at the same extract concentration. Figure 5 shows the glucose-dependent (3–16.7 mM) manner of APME (500 μg/mL) on insulin release from MIN6-β cells. The presence of APME at 3, 6, 11.1, and 16.7 mM of glucose concentrations had shown 6.07, 7.88, 8.03, and 7.19 folds of insulin release than the respective controls. A significant (P < 0.001) increment in the insulin release was observed by the extract than the control at respective glucose concentrations.

The possible mechanisms of insulin secretagogue activity of the APME were evaluated using insulin release modulators [Figure 6], i.e., IBMX, glibenclamide, and diazoxide. APME treatment significantly increased (P < 0.001) insulin release by the MIN6-β cells in the presence of IBMX (34.183 ± 0.64 ng/mL), glibenclamide (33.605 ± 0.55 ng/mL), compared to APME treatment (26.721 ± 0.62 ng/mL) alone. This insulin secretagogue effect of the APME is significantly higher than the insulin release in glucose control (3.328 ± 0.72 ng/mL, P < 0.001). The enhancement in insulin release by APME was significantly decreased in the presence of diazoxide (12.82 ± 0.26 ng/mL, P < 0.001); this indicates that the rise in insulin release was a result of K⁺-ATP channel blockade but not due to the MIN6-β cell damage.

The role of K⁺-ATP channel on the insulin release by APME was tested in the presence of depolarizing concentration of KCl (30 mM). In these conditions, a significant rise in insulin release was observed by APME (32.553 ± 1.12 ng/mL, P < 0.001). Indeed, the potentiation in insulin release was decreased by diazoxide. This indicates that other than depolarization mechanisms are also involved in insulin secretion by APME. The effect of calcium (Ca²⁺) on insulin release was estimated in elevated Ca²⁺ levels by added calcium chloride (1.28 mM) to incubating media, calcium inhibitory conditions by EGTA (1 mM, calcium chelator) and nifedipine (20 μM, calcium channel blocker). In comparison with APME control, the rise in Ca²⁺ levels significantly enhanced insulin secretagogue effect of APME by

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**Table 1: Oral glucose tolerance test of *Abrus precatorius* leaves methanic extract at different doses**

|               | 0 min      | 30 min     | 60 min     | 90 min     | 120 min    |
|---------------|------------|------------|------------|------------|------------|
| Naive         | 75.26±4.04 | 131.59±6.11| 101.26±5.2 | 89.59±4.43 | 83.97±5.20 |
| Glibenclamide (5 mg/kg) | 70.35±4.51 | 104.17±4.3 | 86.35±3.38 | 76.56±3.79 | 70.29±3.23 |
| APME (50 mg/kg) | 81.42±3.1  | 139.64±5.34| 105.79±4.03| 94.52±4.67 | 89.26±3.4  |
| APME (100 mg/kg) | 81.51±4.01 | 128.08±3.35| 100.34±2.49| 91.31±4.21 | 88.29±2.21 |
| APME (200 mg/kg) | 79.86±4.06 | 118.4±3.35 | 90.38±3.47 | 89.65±4.88 | 81.38±3.53 |

Values indicate mean±SD. Data were analyzed by two-way ANOVA followed by Bonferroni test (n=6). *P<0.001, **P<0.01 as compared to naive animals at respective time points. APME: *Abrus precatorius* leaves methanic extract; SD: Standard deviation; ANOVA: Analysis of variance.
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45.44% (38.862 ± 1.02 ng/mL, *P* < 0.001). This calcium-dependent effect was verified by diminished insulin values in the presence of EGTA (24.828 ± 0.65 ng/mL, *P* < 0.001) and nifedipine (27.349 ± 0.87 ng/mL, *P* < 0.001) [Figure 7]. Therefore, insulin secretagogue effect of APME is significantly affected by Ca^{2+} influxes and enhanced Ca^{2+} concentration.

DISCUSSION

Drugs from plant origin are considered as the safe and potential therapeutic agents in the treatment of diabetes. Medicinal plants may act through a range of mechanisms; therefore, the present study was performed for an assessment of antihyperglycemic activity and its mechanisms of action of APME. This work is the first of its kind for the demonstration of APME antidiabetic mechanisms of action.

APME was not shown any toxic effect at the given doses in acute toxic studies. A dose of 200 mg/kg bw was found effective in OGTT studies in glucose-loaded rats. Administration of APME (200 mg/kg bw) to STZ-induced diabetic rats has confirmed the antihyperglycemic activity by reducing hyperglycemia in diabetic rats. However, the APME has also improved body weight and insulin levels in the APME-treated diabetic rats. This enrichment signifies the tissue protective effect of APME from diabetic stress by glycemic control and structural protein synthesis. In addition, the antihyperglycemic activity of APME would be an effect of enhanced circulatory insulin levels by activation of viable beta cells. APME treatment attributed reduction in hyperglycemia, and enhanced insulin levels were comparable to the effect of standard insulin secretagogue antidiabetic drug, glibenclamide. However, medicinal plants with antidiabetic activity are reported for potentiated insulin release. Therefore, APME antidiabetic activity could be a result of insulin secretagogue effect.

Table 2: Mouse insulinoma 6 beta-cell viability assay of glibenclamide and *Abrus precatorius* leaves methanolic extract at different doses

| Concentration | Percentage of viability | APME (μg/mL) | Percentage of viability |
|---------------|-------------------------|--------------|-------------------------|
| 1             | 97.84±2.56              | 5            | 99.72±1.15              |
| 5             | 96.8±1.45               | 50           | 99.14±1.60              |
| 50            | 96.32±1.57              | 500          | 98.28±2.87              |
| 100           | 95.8±1.18               | 1000         | 97.85±1.91              |
| Control       | 100.05±0.08             | Control      | 99.98±0.18              |

Effect of glibenclamide and APME on viability of MIN6-β cells. The values were expressed as mean±SD (*n* = 6). SD: Standard deviation; MIN6-β: Mouse insulinoma 6 beta; APME: *Abrus precatorius* leaves methanolic extract

**Figure 2:** Effect of *Abrus precatorius* leaves methanolic extract (200 mg/kg) and standard drug glibenclamide (5 mg/kg) on blood glucose levels in streptozotocin-induced diabetes model in rats (Data were analyzed by two-way analysis of variance followed by Bonferroni test (*n* = 6); **P** < 0.001 as compared to naive animals, ** ***P** < 0.001 as compared to diabetes control group at respective time points)

**Figure 3:** Effect of *Abrus precatorius* leaves methanolic extract (200 mg/kg) and standard drug glibenclamide (5 mg/kg) on plasma insulin levels in streptozotocin-induced diabetes rats on 0th and 28th day of treatment (Data were analyzed by two-way analysis of variance followed by Bonferroni test (*n* = 6); **P** < 0.001 as compared to naive animals, ** ***P** < 0.001 as compared to diabetes control group at respective time points)

**Figure 4:** Effect of *Abrus precatorius* leaves methanolic extract (5–1000 μg/mL) on insulin release from mouse insulinoma beta cells upon incubation for 60 min. ** ###P** < 0.001 respective to control at basal condition. ** ***P** < 0.001 relative to control at hyperglycemic condition. **@@@P** < 0.001 compared with insulin levels at basal and hyperglycemic conditions at the same extract concentration

**Figure 5:** Effect of *Abrus precatorius* leaves methanolic extract (500 μg/mL) on insulin release at different glucose levels (0–16.7 mM) from mouse insulinoma beta cells upon incubation for 60 min. ** ***P** < 0.001 relative to control condition. Graph insert shows insulin released at blank (0 mM glucose) with similar axes titles of main graph
in vitro leaf extract in streptozotocin induced leaves on milk induced. Chin J Nat Med Pharmacognosy Research, channel blocking; this results into Ca\(^{2+}\) influx to aid in insulin release. In contrary, diazoxide, a K⁺-ATP channel opener reduces insulin release. Insulin-releasing effect of APME was significantly decreased in the presence of diazoxide; this signifies the role of K⁺-ATP channel in APME’s effect. In addition, the extract has also demonstrated K⁺-ATP channel independent effect by enhancing insulin release from β cells under KCl (30 mM) depolarizing conditions. Therefore, K⁺-ATP channel has a significant role in the insulin-releasing mechanism of APME. Role of cAMP in insulin secretion by APME was displayed by means of enhancing insulin release in the presence of IBMX. The potentiated insulin release might be an effect of enhanced intracellular cAMP.\(^{[17]}\)

The calcium-dependent effect of APME was demonstrated by studies with calcium rich extracellular media, nifedipine, and EGTA. Treatment of APME augmented insulin release from β cells than the normal media. This dependence was also evident by reduced insulin secretion in the presence of nifedipine and EGTA. These suggest that APME exhibits its insulin secretagogue effect through common pathways.\(^{[16,17]}\)

**CONCLUSION**

The present study showed that APME exerts antidiabetic activity in STZ-induced diabetic rat model through insulin secretagogue effect on β cells via physiological pathways. Therefore, APME could be a natural remedy with insulinotropic effect in diabetes.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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