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# Hypoglycemic effects of *Potentilla fulgens* L. in normal and alloxan-induced diabetic mice

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## Abstract

Tap roots of *Potentilla fulgens* L. traditionally chewed along with betel nut (*Areca catechu*) and betel leaves (*Piper betel*), are commonly used by local practitioners for various types of ailments. The crude methanolic extract of the roots was tested for its effects in normoglycemic and alloxan-induced diabetic mice. Hypoglycemic activity was observed to be dose- and time- dependent. The extracts 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 by 63%, while in normal mice a 31% reduction was observed 24 h after the effective dose of extract was administered. Further, in the diabetic mice a prolonged anti-hyperglycemic action was observed where glucose levels was, found to be significantly low (79%) when compared with control even on the third day. Glucose tolerance was also improved in both normal and diabetic mice. The results were compared against those of insulin, glibenclamide, metformin, and the probable mechanism of action is discussed.

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**Keywords:** Alloxan; Glibenclamide; Metformin; NIDDM; OGTT; *Potentilla fulgens*

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## 1. Introduction

Numerous plants have been reported to possess hypoglycemic activity (Bailey and Day, 1989; Handa, 1991; Ivory et al., 1989; Swanston-Flatt et al., 1989, 1990; Nagaraju and Rao, 1990; Rastogi and Mehrotra, 1993; Marles and Farnsworth, 1994). Different mechanisms are used by plants for reducing blood sugar levels. There are plants, which exhibit properties similar to the well known sulfonylurea drugs like glibenclamide, effecting hypoglycemia in normal animals by stimulating insulin release from pancreatic  $\beta$ -cells, besides reducing hepatic clearance of insulin hormone (Ivorra et al., 1988; Davis and Granner, 1996), while others act like the biguanides specifically, metformin (Hermann et al., 1994; De Fronzo et al., 1995; Stumvoll et al., 1995)

which, although anti-hyperglycemic, does not effect hypoglycemia in the normal state (Bailey et al., 1985). Metformin-like activity of plants (Zhang and Tan, 2000a,b), augments insulin action by increasing the number of glucose transporters, inhibits gluconeogenesis, reduces absorption from the intestine but increases glucose metabolism in the liver (Kessler et al., 1975; Wilcock and Bailey, 1990; Bailey, 1992). An extract of *Ganoderma lucidum*, exerts its hypoglycemic activity by functioning as a  $\beta$ -blocker, inhibiting the effects of catecholamines, which are known to promote gluconeogenesis and glycogenolysis (Kimura et al., 1988). Other plants are also reported to show insulin sparing action (Kumar and Agusti, 1994).

*Potentilla fulgens* L. of the Rosaceae family, commonly found at higher altitudes (1500–2000 m MSL) of Khasi Hills, Meghalaya, India has been used as folk remedy for a variety of ailments, including diabetes mellitus. Traditionally, pieces of roots are chewed along

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with betel, composed of raw areca nut (*Areca catechu*), locally called *Kwai*, and betel leaf (*Piper betel*).

Literature surveys have yielded scanty information on the pharmacological properties of *P. fulgens* vis-à-vis diabetes. The present investigation was, therefore, undertaken to study the effects of root extracts of *P. fulgens* on fasting blood glucose and glucose tolerance in normal as well as alloxan-induced diabetic mice. The results were compared with those of insulin, glibenclamide and metformin to provide some insight into the mechanism of action.

## 2. Materials and methods

### 2.1. Chemicals

Alloxan was procured from Sigma 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 E. Merck and Hi-media, India.

### 2.2. Experimental animals

Healthy, adult female 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 controlled conditions with temperature maintained at 22 °C on a 12-h light:12-h dark cycle and were fed balanced mice feed obtained from Amrut Laboratory, Pune, India

### 2.3. Preparation of root extracts

*P. fulgens* L. was collected from Shillong peak area of Meghalaya. A voucher specimen was deposited in the herbarium of the Department of Botany, North Eastern Hill University, Shillong (voucher number 464). The root samples were separated, weighed, washed, shredded and sun-dried. It was then powdered, homogenized and repeatedly extracted with 10 vol. of aqueous-methanol solution (1:4) (Harborne, 1998). The mixture was filtered and the filtrate evaporated to dryness at 40 °C in a Buchi rotatory vacuum evaporator. The dried mass obtained was used for the investigation. The yield of methanolic extract (w/w from dried starting material) was 7.76%. Prior to use, weighed powder was dissolved in 2% ethanol and kept on a boiling water bath for 10 min, cooled and centrifuge at low rpm for 10 min. The clear supernatant was used for further study.

### 2.4. Administration of extracts to normal mice

Animals were divided into three test and one control group, each group comprising a minimum of six mice ( $n = 6$ ). The crude extract in varying doses ranging from 150 to 650 mg/kg b.w. was administered to the test group by intraperitoneal injection and glucose level was monitored at different time intervals up to 5 days following the extract administration. The control groups received only 2% ethanol 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 4 weeks after administration of the extract.

### 2.5. Toxicity studies

Normoglycemic animals 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.

### 2.6. Preparation of diabetic mice

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 more than 3–4-fold increased in their blood sugar levels were considered diabetic and used for further tests.

### 2.7. Administration of extract to alloxan-induced diabetic mice

Alloxan-induced diabetic mice were administered (i.p) the extract at varying doses (150–450 mg/kg b. w.) and the blood glucose levels was measured at varying time intervals up to a period of 5 days. All animals treated were observed for behavioral changes like polydipsia, polyphagia and polyurea.

### 2.8. Oral glucose tolerance test

Normal or alloxan-diabetic mice, fasted overnight but provided water ad libitum, were administered the test samples (orally or intraperitoneally) one and a half hour prior to the oral glucose load of 2 gm/kg b.w. Glucose concentration was measured before administration and subsequently at 30, 60 and 120 min after the glucose load. A control group received only the glucose load.

### 2.9. 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 (Ivorra et al., 1988). The blood samples so collected were analyzed for glucose levels employing glucostix with the glucometer (Ames) and also *O*-toluidine reagent (Sigma Bulletin, 1980).

### 2.10. Statistical analysis

Student's *t*-tests were used for determining the levels of significance between the control and the test values. Results are expressed as mean  $\pm$  S.E.M.

## 3. Results

### 3.1. Normal mice

Blood glucose levels of the normal mice receiving the crude extract (i.p) at varying doses were observed to show significant reduction, in a time- and dose-dependent manner (Fig. 1). A hypoglycemic effect was observed at all doses used (150–650 mg/kg b.w.). The effect was significant at 2, 4, 6 and 24 h. At the dose of 150 mg/kg b.w. a marked reduction of glucose level was observed 2 h after extract administration with glucose level 65% ( $P < 0.001$ ) from that of control but was normal at 24 h. At 450 mg/kg b.w. a comparatively more pronounced reduction was observed with the glucose level 56% ( $P < 0.01$ ) from that of control at 2 h. Notably, hypoglycemia was more prolonged at the higher dose and glucose level was 68% compared with that of control even after 24 h ( $P < 0.01$ ). The higher dose of 650 mg/kg b.w. was found to be toxic with severe hypoglycemia at 2 and 4 h with glucose level at 31 and 33%, respectively, of the control (Fig. 1). Mice, however, did not survive beyond 6 h at this dose after administration.

### 3.2. Alloxan-induced diabetic mice

Varying doses of the methanolic extract administered (i.p) to diabetic mice also elicit marked and prolonged anti-hyperglycemic action in a dose-dependent manner similar to that observed in normal mice (Fig. 2). Anti-hyperglycemic effect was observed at 4, 6 and 24 h with 150 mg/kg b.w. resulting in the glucose level being reduced to 65% ( $P < 0.01$ ), 54% ( $P < 0.001$ ) and 69% ( $P < 0.001$ ) respectively. At the dose of 450 mg/kg b.w. glucose levels was observed to be 58% ( $P < 0.01$ ), 73% ( $P < 0.01$ ), 50 ( $P < 0.001$ ) and 37% ( $P < 0.001$ ) of the control at 2, 4, 6 and 24 h, respectively. The higher dose

of 650 mg/kg b.w. was not used as it is toxic to normal mice.

### 3.3. Oral glucose tolerance test

Administration of the crude extract (450 mg/kg b.w.) either intraperitoneally or orally one and a half hour prior to glucose load showed improved glucose tolerance in normal mice. The magnitude of effect varied with the mode of administration. (Fig. 3). As shown the oral mode improved glucose tolerance at 60 min bringing down glucose level to 77% of the control, while the i.p route was more effective at 30, 60 and 120 min with glucose level 81, 79 and 64% respectively from that of control. Glucose level measured 24 h after extract administration also exhibit pronounced reduction in glucose to 41% of the control in mice administered via i.p route only. It may be mentioned that either mode of administration resulted initially in hyperglycemia followed by gradual reduction after the second hour (data not shown).

Glucose tolerance in alloxan-induced diabetic mice (Fig. 4) exhibited a similar pattern to that of normal mice, with the oral route improving glucose tolerance at 30 min whereas, i.p mode improved glucose tolerance at all the time intervals measured. As with normal mice i.p administration showed a more pronounced effect, with glucose levels dropping to 41 ( $P < 0.001$ ), 59 ( $P < 0.01$ ) and 76% ( $P < 0.01$ ) from that of control at 30, 60 and 120 min. At 24 h the magnitude of reduction was much closer to insulin where the glucose level was significantly reduced to 34% ( $P < 0.01$ ) 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 650-mg/kg b.w. resulted in severe hypoglycemia followed by death within 6 h after administration of extract.

## 4. Discussion

Non-insulin dependent diabetes mellitus (NIDDM) condition, which is common amongst diabetic subjects, is characterized by reduced circulating concentration of insulin, poor insulin sensitivity or insulin resistant, poor glucose tolerance resulting in high sugar in plasma. Hyperglycemia condition per se impairs insulin secretion (Davis and Granner, 1996). The results clearly show that the extracts of *P. fulgens* exert significant hypoglycemic and anti-hyperglycemic effects in normal and alloxan-induced diabetic mice. The magnitude of this reduction was found to be dependent on the dose, time and the mode of administration.

In normal mice, hypoglycemic action of the extract was observed to be dose-dependent, with prolonged hypoglycemia at the higher doses. The lower dose (150

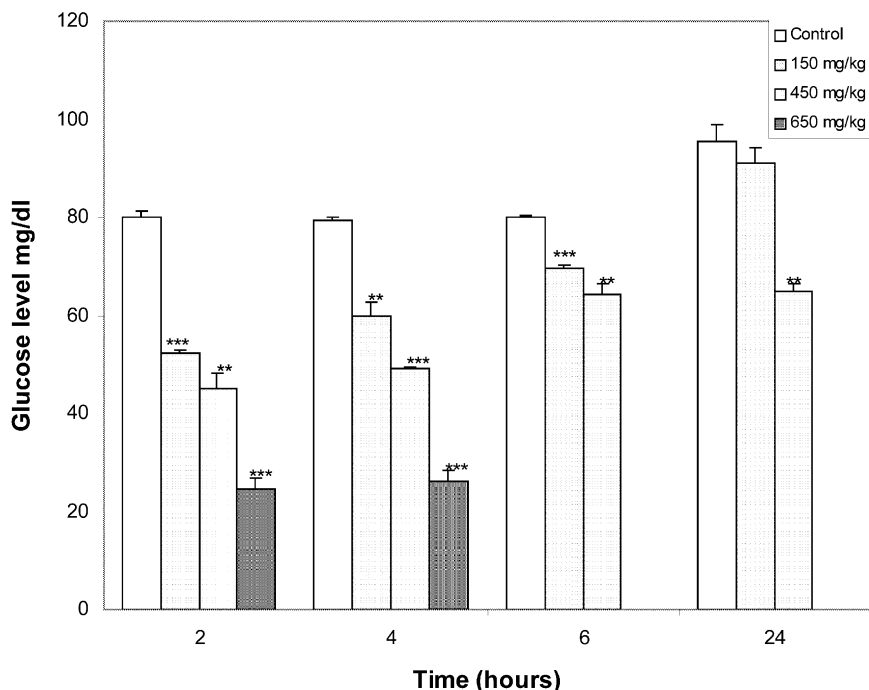


Fig. 1. Effect of varying doses of crude extract on fasting blood glucose level in normal mice assayed at different time intervals. Values are expressed as mean  $\pm$  S.E.M. (\*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ ).

mg/kg b. w.) is probably countered by the homeostatic regulatory mechanism which normalizes after 6 h. However, at higher dosage, the hypoglycemic action of the extract causes pronounced hypoglycemia, followed by death at the dose of 650 mg/kg b.w. At this juncture we cannot rule out other toxic effects of the crude extract.

In alloxan-induced diabetic mice, where  $\beta$ -cells are partly compromised, higher doses of the extract (450 mg/kg b.w.) were more potent. Notably, this effect was significantly prolonged and glucose level was observed to be low even on the third day (79% of the control) with no contraindications (Data not shown). The more pronounced effect of the extract in alloxan-induced

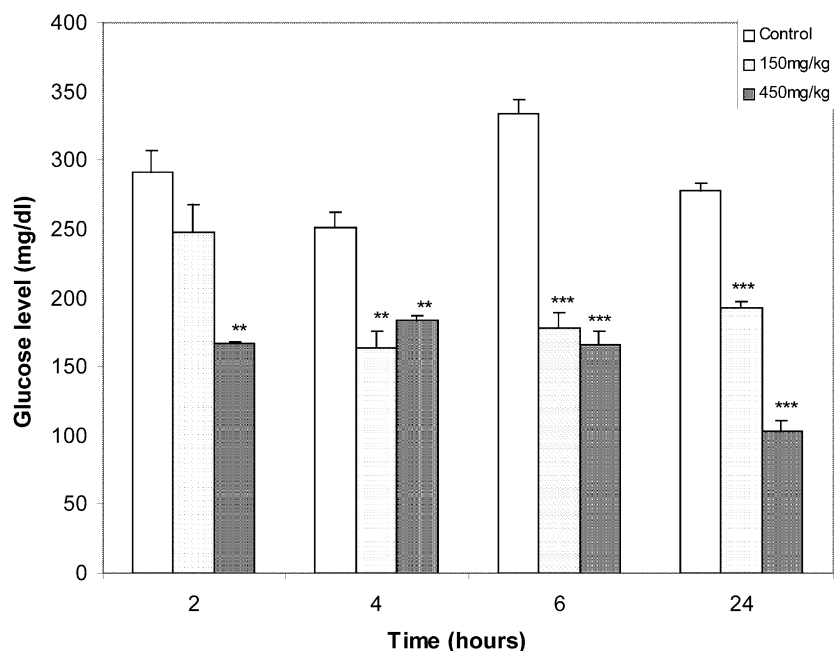


Fig. 2. Effect of varying doses of crude extract on fasting blood glucose level in alloxan-induced diabetic mice, assayed at different time intervals. Values are expressed as mean  $\pm$  S.E.M. (\*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ ).

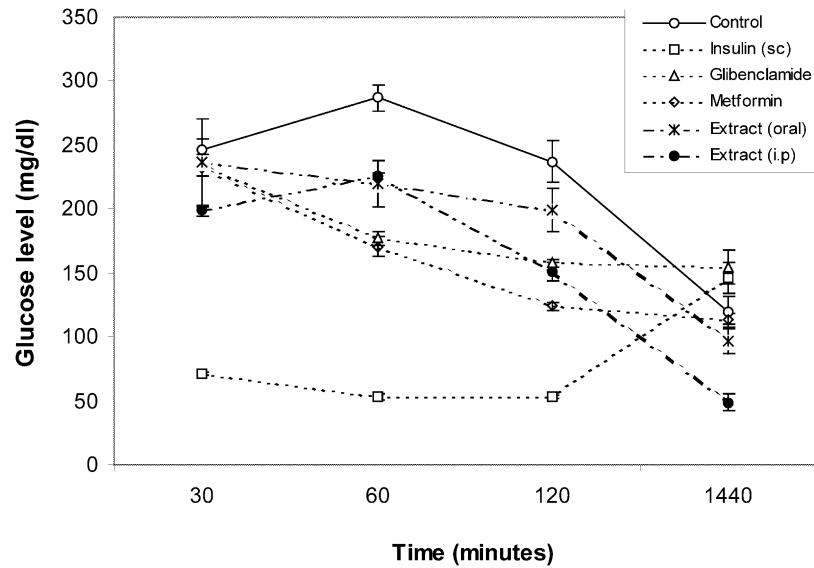


Fig. 3. Glucose tolerance test in normal mice administered with crude extract (450 mg/kg), assayed at different time intervals. Values are expressed as mean  $\pm$  S.E.M. (sc-subcutaneous, ip-intraperitoneal).

diabetic mice may possibly be due to the limited or compromised action of insulin in diabetic condition, and conversely a greater and more direct role of the hypoglycemic principle present in the extract. Several hypoglycemic principles have been reported which effects hypoglycemia at 24 h (Hikino et al., 1985; Akhtar and Ali, 1985; Takahasi et al., 1985).

Glucose tolerance in normal and alloxan-induced diabetic mice was improved on oral or i.p administration of the extract. The extract while comparable to the oral hypoglycemic agents differing only slightly in magnitude, contrasted sharply from insulin at these time intervals. However, after 24 h, the level of glucose dropped to a value which is 73% (oral) and 34% (i.p)

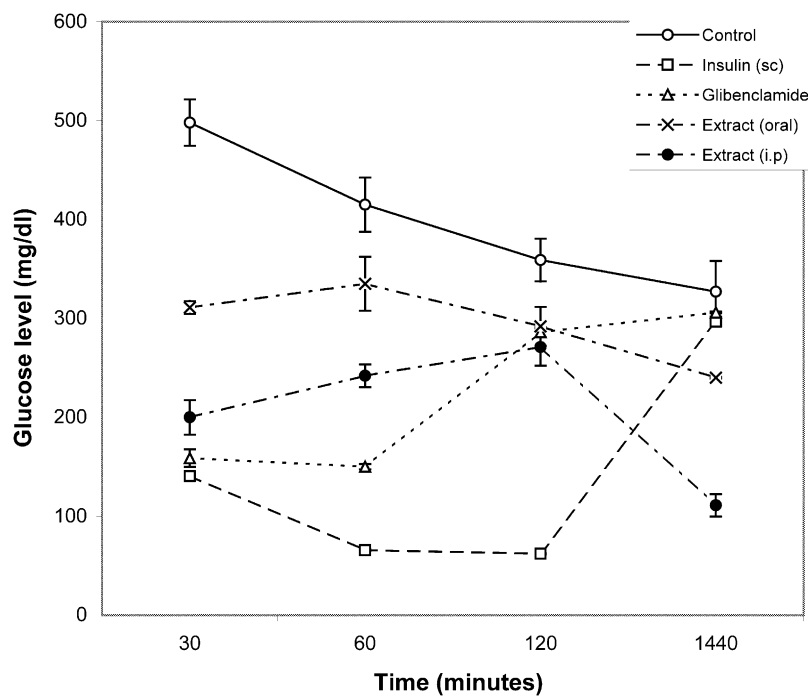


Fig. 4. Glucose tolerance test in alloxan-diabetic mice administered with crude extract (450 mg/kg), assayed at different time intervals. Values are expressed as mean  $\pm$  S.E.M. (sc-subcutaneous, ip-intraperitoneal).

from that of the control and thus, comparable to insulin. Further, i.p administration was more effective and the effects are comparable to glibenclamide (Figs. 3 and 4). While it is likely that the extract has metformin type of action as the oral route was found to be slightly effective vis-à-vis hypoglycemia, however, unlike the *P. fulgens* extract, these drugs are known to have no hypoglycemic effects in normal animals (Zhang and Tan, 2000b; Bailey, 1992). Further, the prolonged hypoglycemic effects observed even after 24 h contrasted from the positive controls used. Thus, while the results of glucose tolerance indicate that the effects of the extract may be comparable to glibenclamide within the 120 min time intervals, the magnitude of reduction was much closer to insulin at 24 h where the glucose level was found to be significantly reduced to 41 and 34% of the control in normal and diabetic mice, respectively. This contrasted sharply from insulin and the oral hypoglycemic drugs

From the results, it may also be postulated that at least more than one hypoglycemic principle may be present. One acting via the oral route implying metformin type of activity and the other effective via the intraperitoneal route exhibiting a more marked and prolonged hypoglycemic activity strongly indicating glibenclamide type of action. Glibenclamide is a well-known insulin secretagogue that is active only in mild alloxan-induced diabetes (Gilman et al., 1981). Other probable factors could be a more direct insulin-like effect as reported for *Cuminum nigrum* (Akhtar and Ali, 1985) and *Momordica charantia* (Day et al., 1990) with a longer half-life, or even as a  $\beta$ -receptor antagonist (Kimura et al., 1988) cannot also be ruled out.

In conclusion, *P. fulgens* may be added to the growing list of hypoglycemic and anti-hyperglycemic plants. Most likely it exerts multiple effects involving both pancreatic and extrapancreatic 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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