# EFFECTS OF *POTENTILLA FULGENS* LINN. ON CARBOHYDRATE AND LIPID PROFILES IN DIABETIC MICE

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## Summary

The effects on carbohydrate and lipid profiles of the anti-diabetic plant, *Potentilla fulgens* Linn. (Rosaceae), were evaluated in alloxan-induced diabetic mice. The methanol extract of *P. fulgens* at the optimized dose of 250 mg/kg b.w. was administered to diabetic mice on alternate days for a period of one week. On the eighth day, blood samples were collected for the estimation of cholesterol, triglyceride and HDL cholesterol levels and the animals sacrificed for the assay of the glycolytic enzymes - glucokinase and hexokinase. The effects were compared against the standard drugs- metformin, glibenclamide and insulin. It was found that *P. fulgens* treatment reduced the serum cholesterol and triglyceride levels while selectively increasing hepatic hexokinase activity. It can therefore be concluded that *P. fulgens* extract exerts anti-hyperlipidemic effect and improves hexokinase activity in diabetic mice in a tissue specific manner.

**Key words:** *Potentilla fulgens*, anti-hyperlipidemic, glycolytic enzymes, metformin, glibenclamide, insulin.

## Introduction

Diabetes is a heterogeneous group of metabolic disorders which is increasing in prevalence [1]. Physiologically it is characterized by the deficiency in insulin or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders [2, 3]. Prolonged diabetes also leads to micro and macrovascular complications [4] as well as the development of lipoprotein abnormalities [5]. In the last decade more than a thousand traditional plants used for treating diabetes mellitus have been recorded, but only some of these have received scientific and medical evaluation [6, 7, 8, 9]. Many of these plants possessing anti-diabetic property are also reported to exhibit hypolipidemic activity [10, 11, 12]. Hexokinase and Glucokinase, key glycolytic enzymes which bring about the first phosphorylating steps of glucose metabolism are reduced significantly under diabetic condition [13] but are improved by some of these plants which function via extrapancreatic mechanism similar to the drug metformin [14,15]. Other plants like *Momordica charantia* [16] and leaf of *Azardirachta indica* [17] are insulin secretagogues involving the pancreas an effect similar to the hypoglycemic drug, glibenclamide [18]. The mechanism of action for some of these active principles has been described [19]. Further, the presence of insulin mimetics and insulin-like molecules has also been reported [20].

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*Potentilla fulgens* Linn. of the Rosaceae family, commonly found at higher altitudes between 1500-2000 Mean Sea Levels (MSL) of Khasi Hills, Meghalaya, India, has been used as folk remedy for a variety of ailments, including diabetes mellitus. Other species of *Potentilla* as reviewed exhibit various pharmacological properties [21]. *P. candicans* [22] is reported to inhibit aldose reductase, a polyol pathway enzyme while the polyphenols from *P. erecta* also possesses biological activity [23]. We have previously reported that *P. fulgens* root extract possesses hypoglycemic, anti-hyperglycemic and anti- tumor activities [24, 25]. In the present study we investigated the effects of the *P. fulgen*s extract on total cholesterol, triglyceride, HDL cholesterol, insulin levels and the enzymes glucokinase and hexokinase activity in alloxan-induced diabetic mice and compared against the effects of standard antidiabetic drugs metformin, glibenclamide and insulin.

## Materials and Methods

### Chemicals

Alloxan was procured from Sigma Chemical Co. (USA), cholesterol, triglyceride and HDL cholesterol test kits were from Span Diagnostic Ltd. (India), RIA kit was from Linco Res. Ltd. (Mumbai), glucokinase, glucose-6-phosphate dehydrogenase (G-6-PDH), sucrose Tris-HCL were from Sigma Co. (USA), adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADP) were from Boehringer (Germany), glibenclamide from Hoechts, insulin from Knoll Pharmaceutical Ltd., metformin from USV Limited (Maharashtra, India). Other chemicals were of analytical grade obtained from E. Merck and Hi-Media (India).

### Experimental animals

Healthy, adult Swiss albino mice of both sexes, approximately 4 months in age and 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 twelve hour light/dark cycle and were fed balanced mice feed obtained from Amrut Laboratory (Pune, India). Institutional guidelines were followed for all experiments.

### Plant material

*P. fulgens* was collected from Shillong peak area of Meghalaya and a voucher specimen (voucher no 464) was deposited and identified by herbarium curator Dr. P. B. Gurung, Department of Botany, North-Eastern Hill University, Shillong, Meghalaya.

### Extraction

Roots of *P. fulgens* were separated, weighed, washed, shredded and dried. They were then powdered and extracted with aqueous-methanol solution (1:4). The mixture was filtered and the filtrate evaporated to dryness at 40°C in a Buchi rotatory vacuum evaporator [26]. The dried mass obtained was used for the investigation. The yield of methanol extract (w/w) from dried starting material was 7.76%.

### Induction of non-insulin dependent diabetes mellitus (NIDDM)

Animals were administered alloxan monohydrate (150 mg/kg b.w., ip) prepared in acetate buffer (0.15M, pH 4.5) as described earlier [24]. The control group received only the buffer. Prior to administration, mice were fasted overnight but given water *ad-libitum*. Mice with more than a 3-4 fold increase in their blood sugar levels were considered diabetic and used for further tests.

**Experimental design**

Alloxan-induced diabetic mice were divided into one control and five test groups. Another group of normal mice receiving only 2% ethanol also served as control (Table). Each group comprised of six mice (n= 6). The test groups were administered *P.fulgens* methanol extract dissolved in 2% ethanol via oral and intraperitoneal mode [24], other groups were administered metformin (500 mg/kg b.w.), glibenclamide (10 mg/kg b.w.) and insulin (10 U/kg b.w.) as described previously [27]. The optimized dose of 250 mg/kg b.w. which was earlier standardized [24] was used for the study. The extract and the standard drugs were administered on alternate days for a period of one week and animals were sacrificed on the eighth day by decapitation.

**Table**

| | | |
|---|---|---|
| 1 | Control 1 | Normal mice untreated |
| 2 | Control 2 | Diabetic mice untreated |
| | Test groups | |
| 3 | PF(o) | Diabetic mice treated orally with 250 mg/kg b.w. of plant extract |
| 4 | PF(ip) | Diabetic mice treated intraperitoneally with 250 mg/kg b.w. of plant extract |
| 5 | MF(ip) | Diabetic mice treated intraperitoneally with 500 mg/kg b.w. of metformin |
| 6 | GB(ip) | Diabetic mice treated intraperitoneally with 10 mg/kg b.w. of glibenclamide |
| 7 | IN(sc) | Diabetic mice treated subcutaneously with 10 U/kg b.w. of insulin |

**Collection of Blood**

Blood samples were routinely collected by orbital sinus puncture using heparinized capillary glass tubes [28] for monitoring blood glucose levels to monitor development of diabetic condition. For test groups blood was collected on the eighth day using non heparinised but sterilized capillary tubes for sera preparation. The blood/serum samples so collected were used for analysis.

**Estimation of biochemical parameters**

Cholesterol, triglyceride and HDL cholesterol levels of normal, diabetic and extract treated mice were determined quantitatively by enzymatic method using cholesterol kit supplied by Span Diagnostic. Protein concentrations were determined by the dye binding method of Bradford [29] using bovine serum albumin as reference standard.

**Enzyme assay**

Tissues such as liver, adipose tissue and skeletal muscles were removed and chopped in ice-cold conditions using normal saline. Perfusion was performed for 15 minutes with two changes of normal saline. The tissues were then homogenized in 2.5 volumes of sucrose-tris (ST) buffer (0.225 M sucrose and 0.025 M Tris), pH 7.4 in a glass vessel using a potter evehjem apparatus at 0-4°C and a motor driven teflon pestle. The homogenate was centrifuged at 12 000 g for 10 min at 0-4°C and the supernatant was used for enzyme assay. Glucokinase (GCK; EC: 2.7.1.1) and Hexokinase (HK; EC: 2.7.1.2) were assayed according to Goward [30] with slight modifications.

**Insulin assayed by Radioimmunoassay (RIA)**
Serum insulin levels of normal, diabetic and diabetic mice treated with *P. fulgens* extract were assayed using the RIA kit obtained from Linco. Res Ltd. (Mumbai) and measured in a Gamma Counter.

**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 ± SEM.

## Results

**Effects of PE on total cholesterol, triglyceride and HDL-cholesterol levels**
Alloxan-induced diabetic mice treated with *P. fulgens* extract (250 mg/kg b.w.) orally and intraperitoneally reduced total cholesterol and triglyceride levels and improved HDL- cholesterol to normal levels (Fig. 1). The intraperitoneal (ip) mode, however, was found to be more effective. The levels of cholesterol and triglyceride were reduced to 72% ($p<0.001$) and 81% ($p<0.001$) respectively, while the HDL-cholesterol level was increased to 119% ($p<0.001$) from that of diabetic control. Amongst the reference drugs, insulin was the most potent, causing significant lowering of cholesterol and triglyceride levels to 57% and 77% respectively from that of diabetic control. Similarly, a significant improvement in the level of HDL-cholesterol was also observed with insulin showing an increase of 152% from that of the diabetic control.

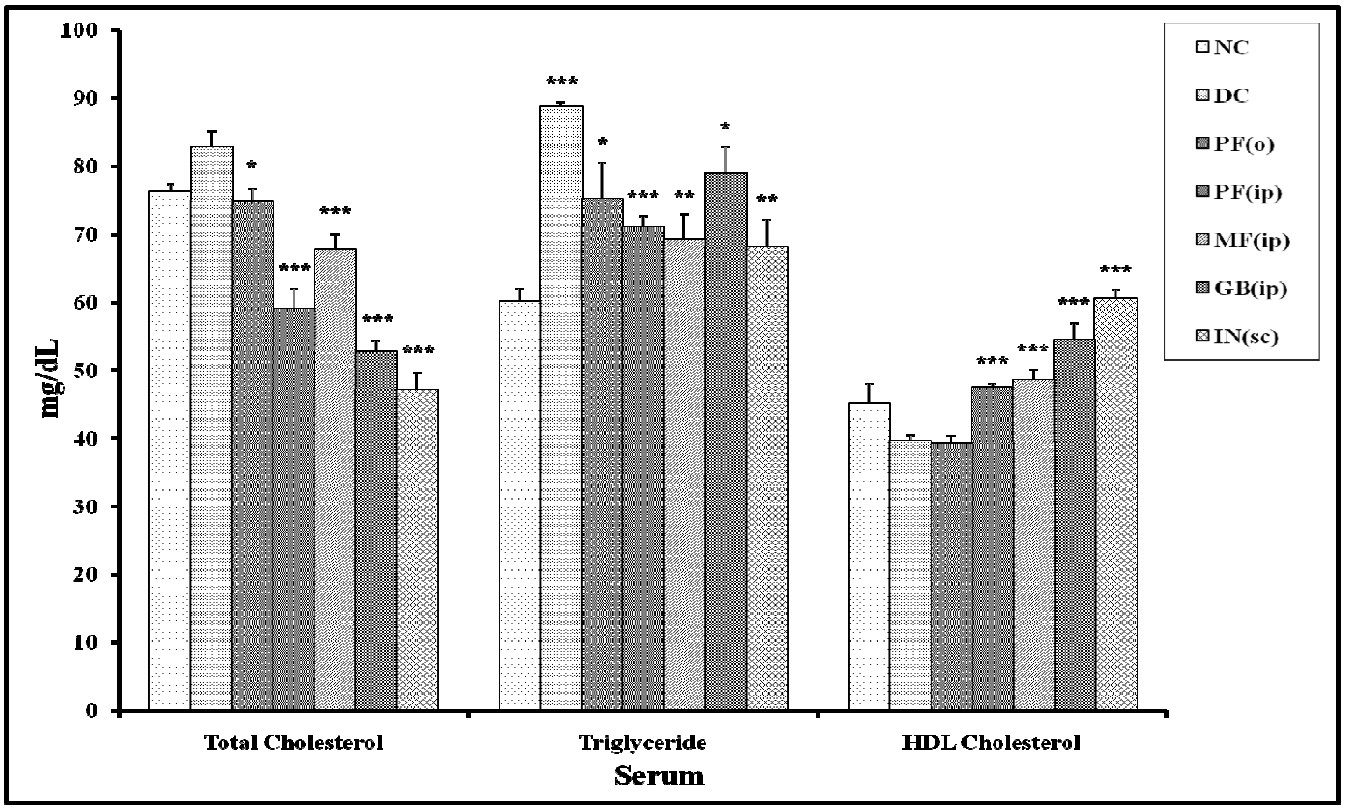


**Figure 1**: Total cholesterol, triglyceride and HDL-cholesterol levels in the serum of normal and alloxan-induced diabetic mice following administration of PF extract (250 mg/kg b.w.) via oral (o) and intraperitoneal (ip) mode. Standard drugs metformin (MF), glibenclamide (GB) and insulin (IN) were administered as mentioned in the text. Values are expressed as Mean ± SEM (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

**Selective effect of PE on glucokinase and hexokinase activities**

In diabetic mice, the glucokinase and hexokinase activities of liver and skeletal tissues were reduced from that of normal mice which served as normal control. Diabetic mice treated with the extract at a dosage of 250 mg/kg b.w., orally and intraperitoneally, did not have any influence on the GK activity. The reference drug glibenclamide also did not alter GK activity whereas metformin and insulin increased the enzyme activity (Fig 2)**.** Liver HK activity on the other hand was increased by *P. fulgens* extract to 238% ($p<0.01$) from that of the diabetic control via the intraperitoneal route. The increase was comparatively lower than the reference drugs, glibenclamide and insulin which also selectively affect only the liver hexokinase activity (Fig 2). No changes were observed in the HK activity of skeletal muscle following administration of either extract or standard drugs (Fig 2).

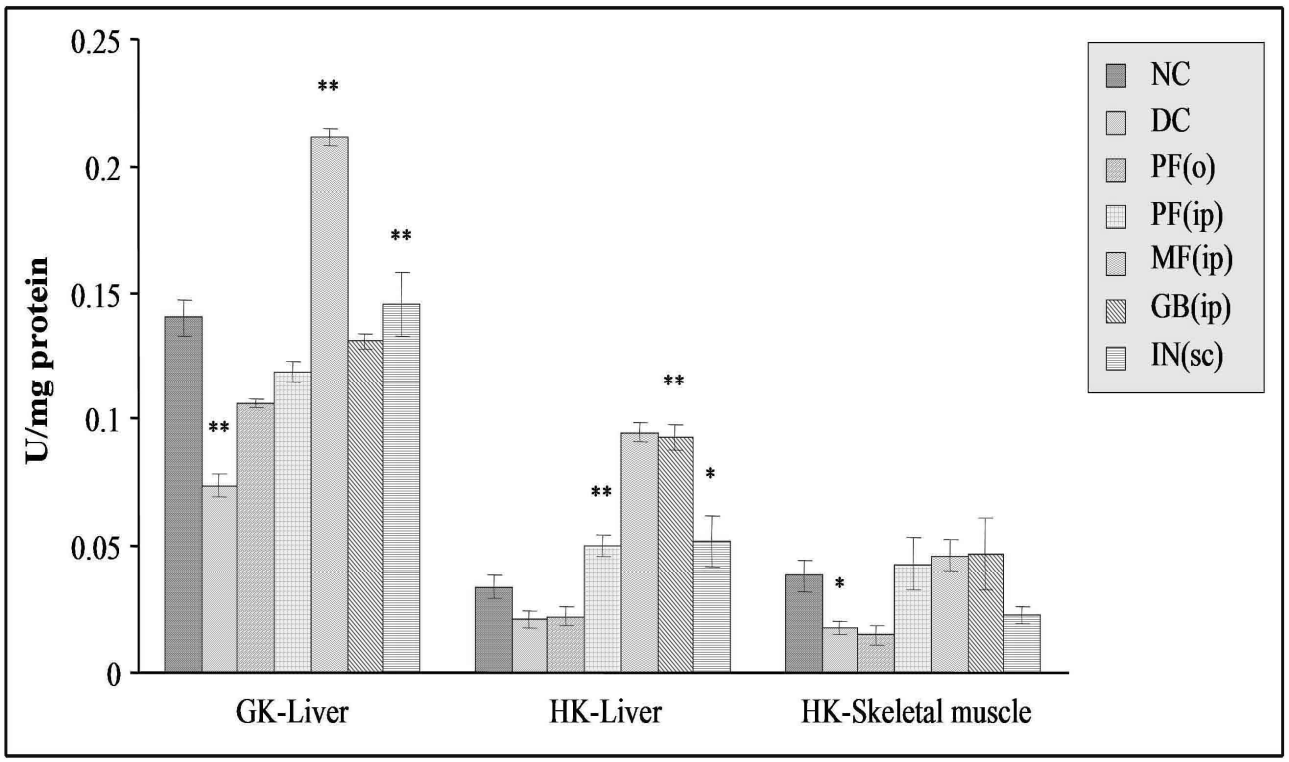


**Figure 2**: Glucokinase and Hexokinase activities in different tissues of alloxan-induced diabetic mice following administration of PF extract (250 mg/kg b.w.) via oral (o) and intraperitoneal (ip) mode. Standard drugs metformin (MF), glibenclamide (GB) and insulin (IN) were administered as mentioned in the text. Values are expressed as Mean ± SEM. (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

**PE has no influence on insulin secretion**

Serum insulin level of diabetic mice was found to be lowered to 64% ($p<0.05$) from that of normal control (Fig 3). Alloxan-induced diabetic mice treated with the extract by either route did not result in any changes in the serum insulin level. However, the reference drugs glibenclamide and insulin raised the serum insulin level to 165% and 207% respectively (Fig 3).

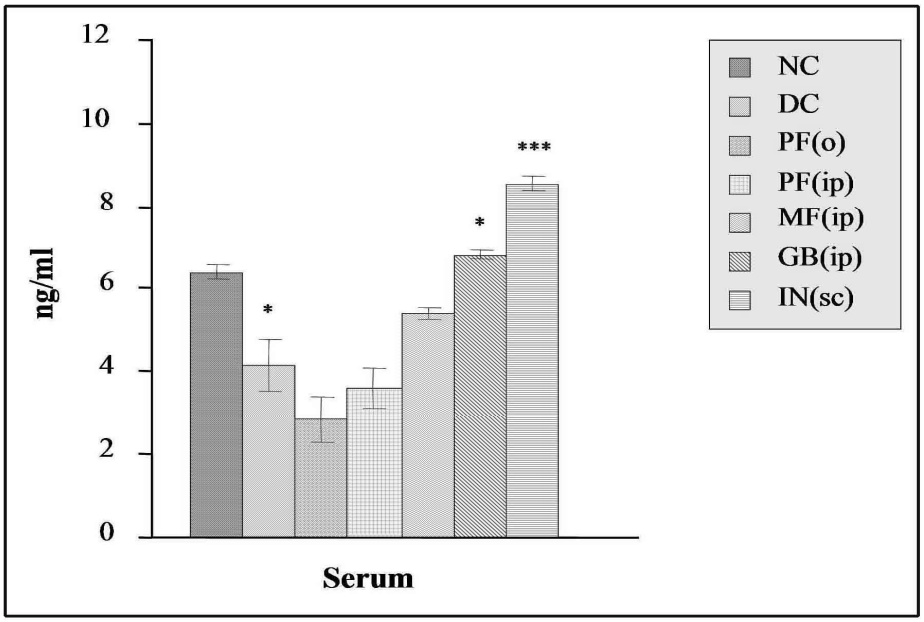


**Figure 3**: Serum insulin level of alloxan-induced diabetic mice following administration of PF extract (250 mg/kg b.w.) via oral (o) and intraperitoneal (ip) mode. Standard drugs metformin (MF), glibenclamide (GB) and insulin (IN) were administered as mentioned in the text. Values are expressed as Mean ± SEM. (*p<0.05; **p<0.01; ***p<0.001).

## Discussion

*P. fulgens* extract produced significant lowering of cholesterol and triglyceride in the serum while simultaneously elevating the serum HDL cholesterol level. The pattern and magnitude of effects of *P. fulgens* extract on the lipid profile were comparable to the standard drugs metformin and the hypoglycemic agents, glibenclamide and insulin. The lipid lowering activity can be attributed directly or indirectly to the influence on various lipid regulatory systems [10]. The observed varying responses in the lipid levels may be due to target responsiveness, specificity and sensitivity elicited by the extract within the time interval used for the study. In conformity with other studies [31], our finding shows that the liver and skeletal glucokinase and hexokinase activities were reduced in alloxan-induced diabetic mice compared to normal mice. Alloxan is reported to inhibit GK and HK activity by binding to the SH group of the enzyme inactivating it [32]. Diabetic mice treated with the extract resulted in selective response in GK and HK activities in the liver. While GK remains unchanged in the liver, HK activity was moderately elevated. A major action of insulin in the liver is to stimulate hepatic GK expression and under diabetic conditions loss of sensitivity to insulin will attenuate at least one major effect of insulin to enhance glucose utilization through the GK activity [33]. The standard drugs also elicited varied responses in liver GK and HK activity, while the skeletal HK activity was not altered by either the *P. fulgens* extract or the standard drugs. This may be understood from the homeostatic and regulatory roles of liver and that enzymes in tissues are differentially regulated. *P. fulgens* extract like glibenclamide did not alter the activity of GK in liver, while metformin did. Further, *P.fulgens* extract and glibenclamide enhanced the activity of HK in liver but no alteration was observed with metformin. Thus, while the effects of *P. fulgens* extract on muscle HK activity were comparable to glibenclamide however, as insulin concentration was unaltered insulin secretagogue action typical of sulphonylureas [3] may be ruled out. *P. fulgens* extract alteration of HK and GK activities in a tissue specific manner indicates extrapancreatic mechanism.

*P. fulgens* extract did not affect serum insulin levels of diabetic mice within the period of study indicating that its reported glucose lowering properties [24] works via mechanism not involving insulin secretion. In this respect, *P. fulgens* may be compared to the action of metformin which acts by increasing the insulin sensitivity of peripheral target tissue as reported for *Trigonella foenum graecum* [34]. Like the biguanides, *P. fulgens* extract may work via extra pancreatic means sensitizing tissues to insulin resulting in glucose lowering, inhibiting hepatic gluconeogenesis as well as improving the lipid profile [35]. Metformin, however, is known to exert no hypoglycemic effects in normal animals [11,36] and its anti-hyperglycemic action is by stimulating peripheral glucose consumption i.e. via extrapancreatic mechanism**.** In our previous studies, we have observed that *P. fulgens* crude extract decreases blood glucose in normal as well as in alloxan-induced diabetic mice [24]. It may thus be inferred that the extract possesses properties that are selectively comparable to both the sulphonyureas and biguanides. It is possible that more than one glucose lowering principle is present in *P. fulgens* extract.

## Conclusion

*P. fulgens* may be added to the ever growing list of medicinal plants with antihyperlipidemic and antihyperglycemic properties. Its hypolipidemic coupled to its anti-hyperglycemic effects offers potential pharmacological treatment for treating diabetes and diabetic dyslipidemia and therefore merits further investigation.

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