Effect of *Potentilla Fulgens* L. Methanolic Extract on Sorbitol Dehydrogenase in Normal and Alloxan-Induced Diabetic Mice

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Summary

Sorbitol dehydrogenase (SDH; NAD+ oxidoreductase, EC 1.1.1.14) is a polyol pathway enzyme that catalyzes the conversion of sorbitol to fructose using NAD+ as a cofactor. The pathway comprising SDH and AR known to be elevated in diabetes and implicated in the pathogenesis of diabetic cataract and microvascular damage of the retina, kidney. We report here the tissue specific inhibition of SDH by methanolic extract of *Potentilla fulgens* L in normal and diabetic mice. The magnitude of inhibition also varied with the mode of administration.

Keywords: Diabetes mellitus, polyol pathway, *Potentilla fulgens,* sorbitol dehydrogenase

Introduction

Sorbitol dehydrogenase (SDH; NAD+ oxidoreductase, EC 1.1.1.14) an enzyme known by many names, e.g., polyol dehydrogenase, L-iditol:NAD+ oxidoreductase [1-4] and is primarily a cytoplasmic enzyme [5, 6]. SDH is second step enzyme of the polyol pathway which converts sorbitol to fructose using NAD+ as a cofactor. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia along with specific long-term complications affecting the retina, kidney and nervous system [7]. Polyol pathway is one of the intracellular events that occur in the presence of high-glucose ambience. Increased polyol pathway activity is known to alter the redox state, due to altered NADH/NAD^+ [8]. Increased NADH/NAD^+ ratio results in cellular abnormalities similar to those seen during hypoxia, despite the fact that the available oxygen is well above the hypoxic threshold. This condition is therefore referred to as pseudohypoxia leading to altered cellular redox, oxidant and osmotic stress [9].

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Long-term secondary complications are the main cause of morbidity and mortality in diabetic patients [8]. The major microvascular complications of diabetes include nephropathy, neuropathy, and retinopathy while cataract is, however, an avascular complication [10]. Several metabolic factors contribute to the dysfunction observed in diabetes which includes increased glucose flux through the polyol pathway, increased production of reactive oxygen species by the mitochondrial respiratory chain, nonenzymatic glycations, protein kinase-C activation and increased flux through the hexosamine pathway. The polyol pathway has received considerable attention [8].

The use of herbs as source of drugs and for treatment of many chronic disorders including diabetes is gaining acceptance [11-15]. However, relatively little work has been done to study the effects of the anti-diabetic agents on secondary complications of diabetes. *Potentilla fulgens* (Family: Rosaceae) roots commonly found at higher altitudes (1500-2000 m MSL) of Khasi Hills, Meghalaya, India is used as a folk remedy for a variety of ailments. *P. fulgens* activity has been reported to have hypoglycemic, anti-hyperglycemic, anti-hypolipidemic, antioxidant and anti-tumor activity [16-19]. Studies on other species of potentilla have also been reported [20]. We recently reported the inhibitory effect of *P. fulgens* on aldose reductase activity in normal and diabetic mice [21]. However, there are no reports pertaining to the inhibitory activity of *P. fulgens* against sorbitol dehydrogenase. In this study, we report the SDH inhibitory potential of methanolic extract of *P. fulgens* in normal and diabetic mice.

Materials and methods

Chemicals

Alloxan was procured from Sigma Chemical Co. (USA), D-sorbitol, nicotinamide adenine nucleotide (NAD) was from Sisco Research Laboratories (SRL), India. Other chemicals were of analytical grade obtained from E-merk and SRL, India.

Experimental Animals

Healthy, Swiss albino mice of approximately 6 months 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 with balanced mice feed obtained from Amrut Laboratory, Pune, India. The animal treatment procedures were approved by the institutional animal ethical committee.

Preparation of extracts

The roots of *P. fulgens* were separated, weighed, washed and dried. These were then powdered, homogenized and repeatedly extracted with 10 volume of aqueous-methanol solution (1:4) [22]. As earlier described [16], the mixture was filtered and the filtrate was lyophilized to dryness which was used as methanolic extract.

Toxicity Studies

Normoglycemic animals were administered via i.p. and oral route up to a dose of 450 mg/kg b.w. and kept under observation up to 4 weeks for any signs of distress, convulsion, coma or death.

Experimental design-I: Administration of extract to normal mice

In this study, mice were divided into 2 tests (comprising of 6 animals) group for determining the effects of varying dose of extracts of *P. fulgens* (dissolved in 2% ethanol) administered via i.p. and oral route respectively. In each test, the control group received 2% ethanol while the experimental groups were given (50-350 mg/kg body weight) of extract through i.p. and oral route. A feeding needle was used for oral route administration. At the end of four weeks period, animals were sacrificed by cervical dislocation and dissected carefully to remove tissues like liver, kidney and the eye balls of individual group to analyze the activity of SDH.

Preparation of Diabetic mice

Animals were administered alloxan monohydrate prepared in acetate buffer (0.15 M, pH 4.5) via i.p. route [16]. Prior to administration, mice were fasted over night but given water ad libitum. Mice with more than 3-4 fold increased in blood glucose (measured using glucostix; Ames) were considered diabetic.

Experimental Design-II: Administration of extract to diabetic mice

Alloxan-induced diabetic mice were administered the extract (250 mg/kg b.w.) on alternate days via i.p. route for a period of 4 weeks. At the end of 4 weeks, experimental animals were sacrificed by cervical dislocation and dissected carefully to remove the liver, kidney and the eye balls of individual group for subsequent analysis of SDH activity.

Assays for SDH

The tissue were homogenized in cold 0.225 M sucrose-Tris buffer (pH 7.4) and centrifuged at 9000 xg for 15 min. The supernatant was further centrifuged at 16,000 xg. The pellet was discarded and the supernatant was used as enzyme preparation. SDH was assayed according to the method of Gerlach [23]. The reaction mixture in a total volume of 3.0 ml contained 0.1 M Tris-HCl buffer (pH 9.0), 32 mM NAD^+, enzyme preparation of 50 µl and 1.1 M D-sorbitol as the substrate. The reaction was initiated by addition of NAD^+. Absorbance measurements were taken at λ340 nm. One enzyme unit was defined as the amount of enzyme consuming 1 mM of NADPH per minute under assay conditions.

Protein concentrations were determined according to the method of Bradford using bovine serum albumin (BSA) as the standard [24].

Statistical Analysis

Student's't'-tests was used for determining the levels of significance between the control and the test values. Results are expressed as mean ± S.E.M.

Results and Discussion

The effects of methanolic extract of *P. fulgens* caused no mortality up to 350 mg/kg b.w. Normal mice treated with 50 mg/kg b.w. showed no significant changes in the SDH activity of liver tissue when compared to the untreated control group. At 150 mg/kg, inhibition of SDH activity was observed with activity being 18% ($p<0.05$, i.p.) and 10% ($p<0.05$, oral) from that of the control group. At the dose of 250 mg/kg, a comparatively more pronounced reduction was observed with the activity being 34% ($p<0.01$, i.p.) and 23% ($p<0.01$, oral) respectively from that of the control group. At the higher dose of 350 mg/kg, the decreased was 37% ($p<0.001$, i.p.) and 33% ($p<0.001$, oral) respectively.

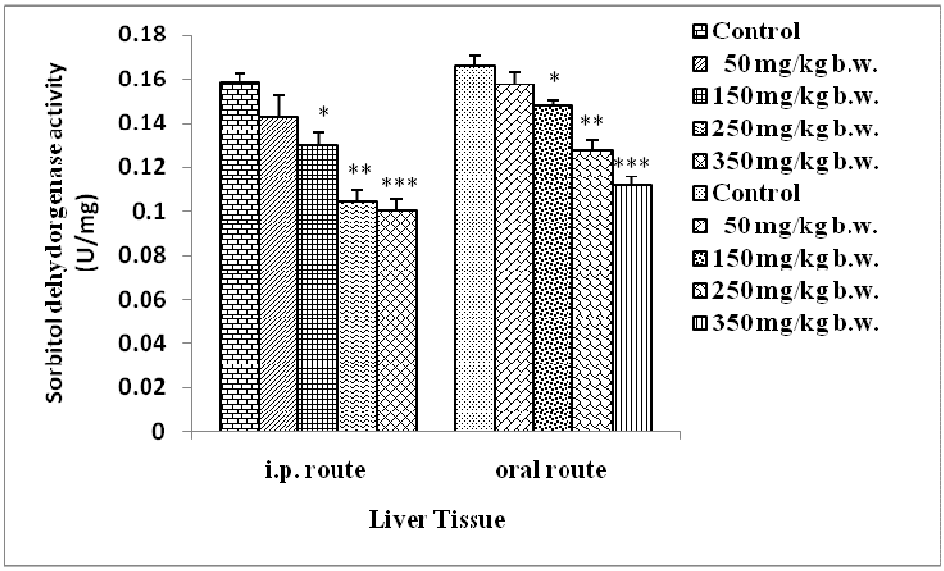

Figure 1: Effect of varying doses of methanolic extract of *P. fulgens* on the SDH activity in liver tissue of normal mice administered by i.p. and oral route. Values are represented as mean ± S.E.M. (*, $p<0.05$, **, $p<0.001$, ***, $p<0.001$).

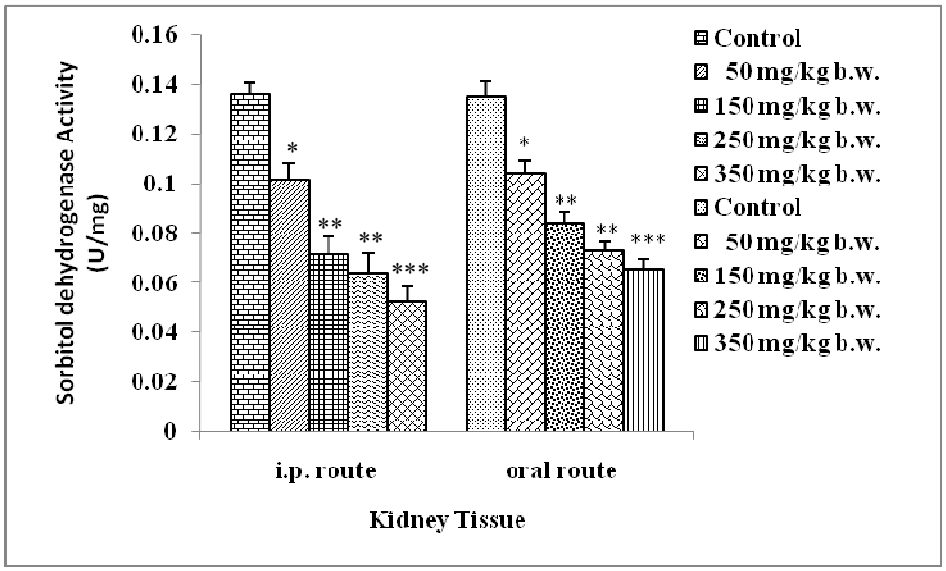

Figure 2: Effect of varying doses of methanolic extract of *P. fulgens* on the SDH activity in kidney tissue of normal mice administered by i.p. and oral route. Values are represented as mean ± S.E.M. (*, $p<0.05$, **, $p<0.001$, ***, $p<0.001$).

The kidney SDH was similarly altered. Thus, at 50 mg/kg the reduction in the activity of SDH was 25% ($p<0.01$, i.p.) and 23% ($p<0.05$, oral) respectively from that of the control group. At the dose of 150 mg/kg, the activity was further reduced to 47% ($p<0.01$, i.p.) and 37% ($p<0.01$, oral) respectively from that of the control group. The dose of 250 mg/kg reduced the SDH activity to 53% ($p<0.01$, i.p.) and 46% ($p<0.01$, oral) respectively from that of the control group (Figure 2). At the higher dose of 350 mg/kg, SDH activity was further reduced to 61% ($p<0.001$, i.p.) and 51% ($p<0.001$, oral) respectively.

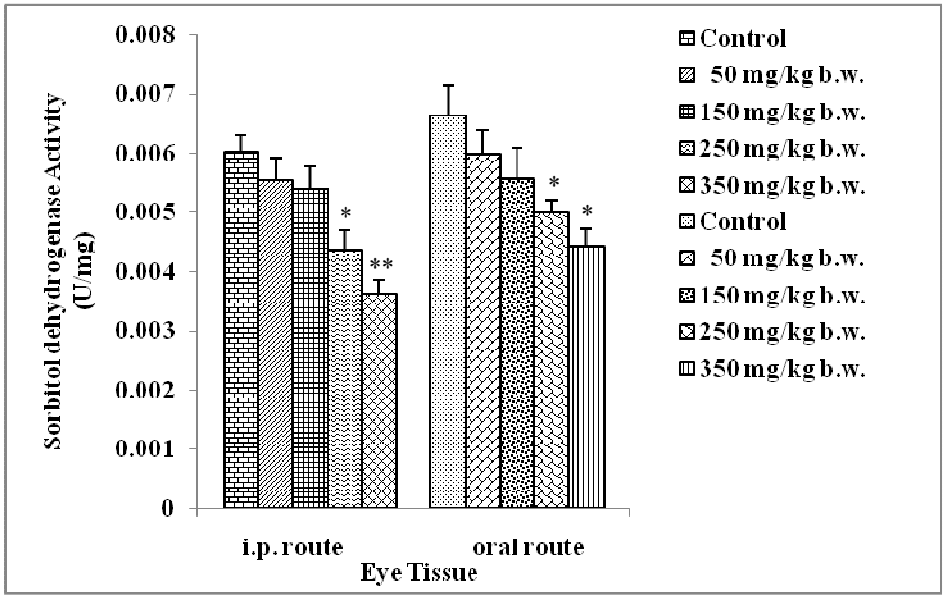

Figure 3: Effect of varying doses of methanolic extract of *P. fulgens* on the SDH activity in eye tissue of normal mice administered by i.p. and oral route. Values are represented as mean ± S.E.M. (*, $p<0.05$, **, $p<0.001$, ***, $p<0.001$).

As shown in Figure 3, administration of extract (50 mg/kg and 150 mg/kg) via oral and i.p. route did not bring any significant changes in SDH activity. At the dose of 250 mg/kg, the activity was lowered by 27% ($p<0.05$, i.p.) and 25% ($p<0.05$, oral) respectively relative to the control group. At 350 mg/kg, the activity was reduced to 40% ($p<0.01$, i.p.) and 33% ($p<0.05$, oral) respectively from that of control.

The activity of SDH in liver, kidney and eye tissue of normal mice was observed to decrease, the magnitude of which varied with increasing dose of the extract when compared to the control group. Further, the SDH activity of normal mice varied in a tissue specific manner where the SDH activity was higher in liver followed by kidney and then in eye tissue. As shown in Figure 1-3, the magnitude of effect also varied with the mode of administration. It may be mentioned that either route of administration resulted in the decreased SDH activity in liver, kidney and eye tissue from that of the control group but the i.p. route was more effective in reducing the activity of the enzyme than the oral route. Thus, SDH activity of normal mice treated with *P. fulgens* extract is tissue, dose- and route-dependent.

Table 1: A comparison of the effect of crude methanolic extract of *P. fulgens* on the SDH activity (expressed as U/mg protein) in liver, kidney and eyes tissue of normal control, diabetic control and treated (diabetic mice treated with 250 mg/kg extract as described in materials and methods) mice. Values are represented as mean ± S.E.M. (*, $p< 0.05$, **, $p< 0.01$, ***, $p<0.001$).

Tissue	Normal Control	Diabetic Control	Treated
Liver	0.1715 ± 0.005	$0.2698 \pm 0.004^{***}$	$0.1883 \pm 0.007^{***}$
Kidney	0.1306 ± 0.008	$0.2126 \pm 0.05^{**}$	$0.1303 \pm 0.004^{***}$
Eyes	0.0081 ±0.0006	$0.0125 \pm 0.0008^{*}$	$0.0095 \pm 0.0003^{*}$

SDH activity of alloxan-induced diabetic mice was significantly increased from that of the normal control. Thus, SDH activity in liver was increased by 57% ($p<0.001$), in kidney by 63% ($p<0.01$) and in eye tissue by 55% ($p<0.05$) as compared to the normal control. SDH activity of diabetic mice treated with *P. fulgens* extract (250 mg/kg b.w. via i.p. route) was significantly reduced. Thus, the SDH activity was reduced by 30% ($p<0.001$) in liver, 39% ($p<0.001$) in kidney and 24% ($p<0.05$) in eye from that of the diabetic control mice (Table 1). In the present study, an increase in SDH activity was observed in liver, kidney and eye tissues of diabetic mice. Since glucose concentrations are increased in diabetic mice, more glucose is

converted to sorbitol. The elevation in SDH activity observed in diabetic mice have been ascribed to the increased availability of sorbitol [25]. SDH activity under diabetic condtion was found to be significantly reduced after treatment with methanolic extracts of *P. fulgens*. Other plant extracts like *Cucurma longa, Scoparia dulcis* have also been reported to inhibit SDH activity in diabetic rats [25, 26]. As reported earlier, SDH over expression stimulate reactive oxygen species generation in high glucose-exposed retinal pericytes and subsequently, potentiate the cytopathic effects of glucose [27]. Therefore, inhibition of SDH activity reduces the possible effects that could arise during diabetic condition. As the plant has been shown to possess inhibitory activity against aldose reductase (AR), the first member enzyme of the polyol pathway [21], the combined inhibitory effects of this plant on the polyol pathway enzymes can prevent the formation of sorbitol and fructose as well as alterations in pyridine nucleotides metabolites contributing to tissue injury [28].

Conclusion

In conclusion, *P. fulgens* extracts exhibit significant dose-dependent inhibitory activity against SDH of liver, kidney and eye tissues of normal and diabetic mice. Given its reported glucose lowering, anti-hyperlipidemic and antioxidant activities the plant merits further scientific investigation.

Acknowledgement

We thank the UGC for sponsoring the University with Potential for Excellence Project, DST-FIST and to the Department of Biochemistry, North Eastern Hill University, and Shillong, India for providing facilities.

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