Effect of Different Solvent Extracts of *Potentilla Fulgens* L. on Aldose Reductase and Sorbitol Dehydrogenase in Normoglycemic and Diabetic Mice

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Summary

The polyol pathway comprising of aldose reductase (AR) and sorbitol dehydrogenase (SDH), converts glucose to fructose is accelerated under elevated glucose concentration. Several biochemical features implicate the polyol pathway as a plausible and important contributor to complications of diabetes. Plant extracts are known to inhibit the enzymes of the polyol pathway. We report here the effect of different fractions of *Potentilla fulgens* on AR and SDH activity in liver, kidney and eye of normoglycemic and diabetic mice. Administration of these fractions to the normoglycemic and diabetic mice resulted in varying degree of inhibition of the AR and SDH activity in the liver, kidney and eye.

Key words: Aldose reductase, diabetes mellitus, polyol pathway, *Potentilla fulgens,* sorbitol dehydrogenase.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia along with specific long-term complications affecting the retina, kidney and nervous system [1]. Polyol pathway is one of the intracellular events that occur in the presence of high-glucose ambience [2, 3]. The resulting altered concentrations of pathway products and cofactors can cause osmotic stress through multiple mechanisms that include the generation of precursors of advanced glycation end products [4]. Aldose reductase (AR; EC 1.1.1.21), the first and rate-limiting enzyme in the pathway, reduces glucose to sorbitol using NADPH as a cofactor; sorbitol is then metabolized to

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fructose by sorbitol dehydrogenase (SDH; EC 1.1.1.14) that uses NAD^+ as a cofactor. The utilization of NADPH by AR reduces the cofactor availabilities for glutathione reductase, which is critical for the maintenance of the intracellular pool of reduced glutathione (GSH) and thus, diminishes the capability of cells to respond to oxidative stress [5]. Increased polyol pathway activity is known to alter the redox state and increased $NADH/NAD^+$ ratio reportedly results in cellular abnormalities similar to those seen during hypoxia, despite oxygen being above the hypoxic threshold [4]. This condition is referred to as pseudohypoxia associated to altered cellular redox, oxidant and osmotic stress [6]. The fructose produced by the polyol pathway can become phosphorylated to fructose-3-phosphate [5, 7], and further broken down to 3-deoxyglucosone; both compounds are powerful glycosylating agents that contributes to the formation of advanced glycation end products (AGEs) [8]. Thus, activation of the polyol pathway can initiate and affects several pathways leading to cellular damage.

Due to their implication in the development of diabetic complications, AR and SDH has been a drug target in the clinical management of secondary complications of diabetes [9-14]. Inhibition of SDH is associated with lower cytosolic $NADH/NAD^+$ and lower glyceraldehydes-3-phosphate level [15]. SDH inhibitors are shown to attenuate the diabetes-induced increase in cytosolic $NADH/NAD^+$ ratio in diabetic retina [14].

The use of herbs as source of drugs and for treatment of many chronic disorders including diabetes is gaining acceptance [16-18]. However, relatively little work has been done to study the effects of the anti-diabetic agents on secondary complications of diabetes. We have earlier reported the hypoglycemic, anti-hyperglycemic, anti-hypolipidemic, antioxidant and anti-tumor activity of the crude extract of *Potentilla fulgens* including its effect on AR and SDH [19-25]. The current study was aimed to investigate the effect of different solvent extracts of *P. fulgens* roots i.e., terpenoid & phenolic (TP), quaternary alkaloid (QA) and major alkaloid (MA) on polyol pathway enzymes in liver, kidney and eye of normoglycemic and diabetic mice.

Materials and Methods

Chemicals

Alloxan, DL-glyceraldehyde was procured from Sigma Chemical Co. (USA), D-sorbitol, nicotinamide adenine nucleotide (NAD), nicotinamide adenine dinucleotide phosphate

(NADPH) 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 old 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 Pranav Agro Industries Ltd, New Delhi. The animal treatment procedures were approved by the institutional animal ethical committee.

Preparation of different solvent extracts of *P. fulgens*

(a) The samples (roots) collected were washed, shredded, dried and weighed. It was then powdered, homogenized and extracted with aqueous-methanolic solution (1:4). The mixture was filtered and the filtrate was further fractionated as per the method outlined in Harborne [26].

(b) Terpenoid & phenolic (TP) fraction: Obtained by evaporating the filtrate (from step a) to 1/10 volume (40 °C) followed by acidification with 2M H_2SO_4 to pH 0.89. This was further, extracted with chloroform (×3). The chloroform layer was separated and evaporated to yield the TP fraction.

(c) Alkaloid fractions: The aqueous layer obtained in step (b) was basified to pH 10 with ammonia solution and further extracted with chloroform: methanol (3:1, twice). The chloroform-methanol layer was separated and evaporated to yield the major alkaloid (MA) fraction. Quaternary alkaloid (QA) fraction was obtained by evaporating the aqueous layer followed by extraction with methanol.

Prior to use, weighed powder mass was dissolved in 2% ethanol and kept on a boiling water bath for 10 min, cooled and centrifuge at low rpm (3000 rpm) for 10 min [19]. The clear supernatant was used for the study.

Experimental design-I: Administration of fractions to normal mice

In this study, mice were divided into 2 tests (each comprising of 6 animals) for determining the effects of different fractions of *P. fulgens i.e.* TP, QA and MA (dissolved in 2% ethanol)

administered via intraperitoneal (i.p.) route. In each test, the control group received 2% ethanol while the experimental groups were given 250 mg/kg body weight (b.w.) of each fraction through i.p. route. 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 AR and SDH.

Preparation of Diabetic mice

Animals were administered alloxan monohydrate prepared in acetate buffer (0.15 M, pH 4.5) via i.p. route [19]. Prior to administration, mice were fasted over night but given water ad libitum. The blood samples collected were analyzed for glucose levels employing glucostix with the blood glucometer. Mice with more than 3-4 fold increased in blood glucose were considered diabetic.

Experimental Design-II: Administration of fractions to diabetic mice

As described in experimental design-I, the respective fractions (250 mg/kg b.w.) exhibiting the effective response against AR and SDH activity in normoglycemic mice was selected and was administered to alloxan-induced diabetic mice 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 AR and SDH activity.

Tissue preparation

Weighed tissues were homogenized in 2.5 volumes of 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 for 30 min. The pellet was discarded and the supernatant was used as enzyme preparation.

Assays for AR and SDH

AR was assayed according to Wolff and Crabbe with some modifications [27]. The reaction mixture was prepared at 25ºC, in a total volume of 1 ml, containing 50 mM Na-phosphate buffer (pH 6.5), 0.2 mM NADPH, 100 µl enzyme preparation, 100 mM dl-glyceraldehyde as a substrate. The reaction was initiated by addition of NADPH. Absorbance measurements were

taken at λ340 nm. The change in absorbance per min (ΔA) was monitored spectrophotometrically. *A unit of AR is defined as the amount required to oxidize 1μmol of NADPH/min at 25°C.*

SDH was assayed according to the method of Gerlach [28]. 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. *A unit of SDH is defined as the amount required to reduce 1μmol of NAD^+/min at 25°C.*

Protein estimation

Protein concentrations were determined according to the method of Bradford using bovine serum albumin (BSA) as the standard [29].

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

P. fulgens yielded 0.78%, 17.22% and 0.2% (w/w of dried study material) of MA, QA and TP fraction respectively.

**Effect of different fractions of PF on AR and SDH activity of different tissues in normoglycemic mice**

The fractions i.e., TP, QA and MA of *P. fulgens* administered separately as a single dose of 250 mg/kg b.w. via i.p. route on normoglycemic mice caused a reduction in the level of AR activity. Among the fractions, TP fraction was more effective in lowering the activity of AR as shown in **(Figure 1)**. Thus, TP fraction lowered the activity of AR by 50% (p<0.01), 61% (p<0.01) and 41% (p<0.01) in liver, kidney and eye respectively from that of the control group.

Similarly, normoglycemic mice treated with TP, QA and MA fractions of *P. fulgens* exhibited a reduction of SDH activity with the TP fraction being potent in reducing the activity

(Figure 2). The TP fraction lowered the SDH activity by 29% (p<0.01) in liver, 36% (p<0.01) in kidney and 32% (p<0.05) in eye from that of the control group.
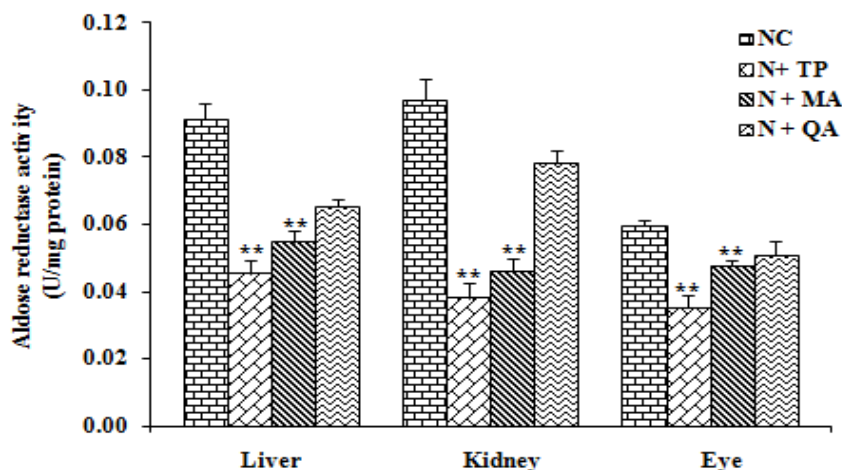

Figure 1: Effect of fractions, TP, MA and QA of *P. fulgens* on AR activity (U/mg protein) in liver, kidney and eye of normoglycemic mice. Values are represented as mean ± S.E.M. SEM(±):- Standard Error Mean, p:- level of significance at *P<0.05, **<0.01 respectively, NC:- normoglycemic control, N:- normoglycemic, TP:- terpenoid & phenolic, MA:- Major Alkaloid, QA:- Quaternary Alkaloid.
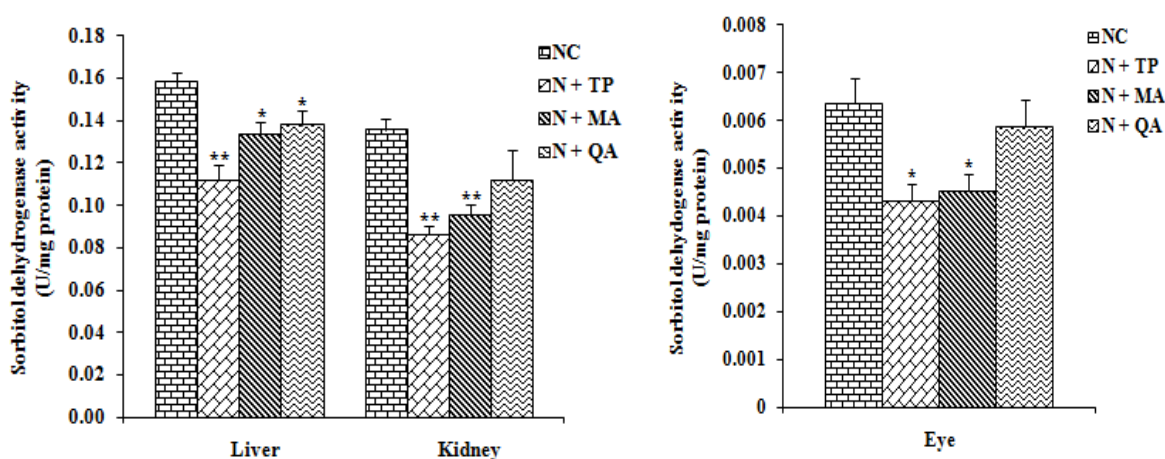
Figure 2: SDH activity (U/mg protein) in liver, kidney and eye of normoglycemic mice treated with TP, MA and QA fractions of *P. fulgens*. Mean (M) are obtained from 6 separate experiments; SEM (±):- Standard Error Mean, p:- level of significance at *P<0.05, **<0.01 respectively, NC:-normoglycemic control, N:- normoglycemic, TP:- terpenoid & phenolic, MA:-Major Alkaloid, QA:- Quaternary Alkaloid.
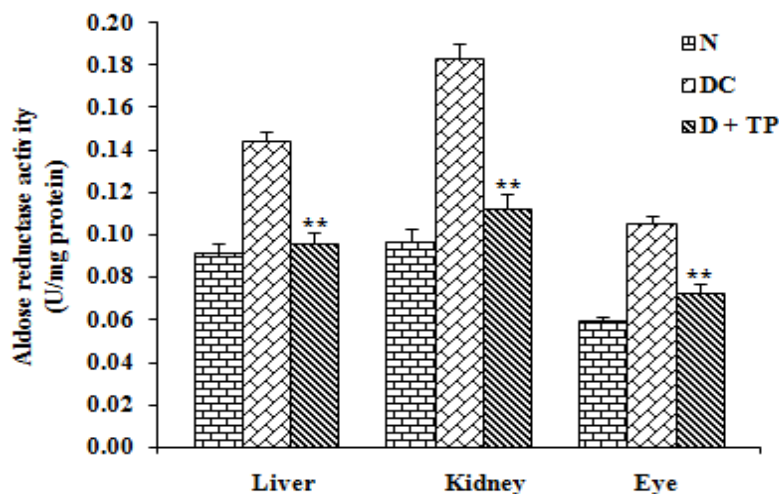
Figure 3: Effect of TP fraction of *P. fulgens* on AR activity (U/mg protein) in liver, kidney and eye of diabetic mice. Mean (M) are obtained from 6 separate experiments; SEM (±):- Standard Error Mean, p:- level of significance at *$P<0.05$, **<0.01 respectively, N:- normoglycemic, DC:- diabetic control, D:- diabetic mice, TP:- terpenoid & phenolic.
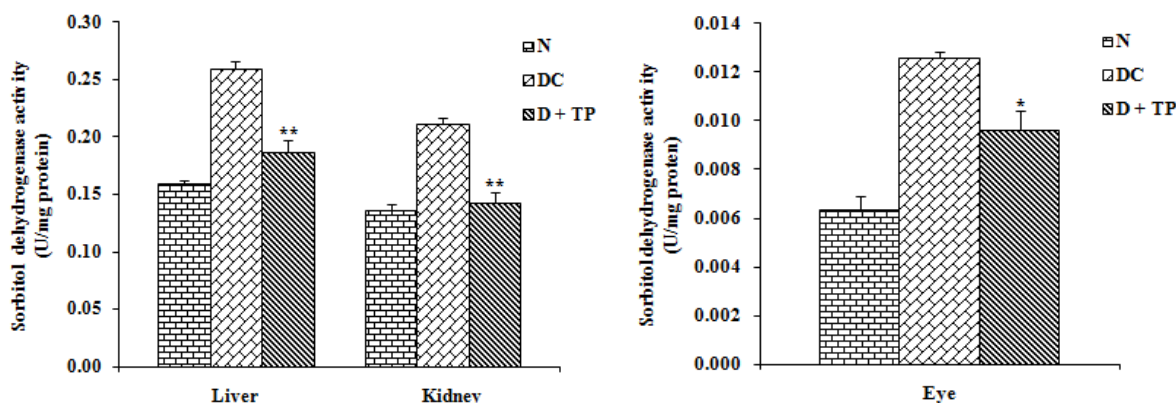
Figure 4: Effect of TP fraction of *P. fulgens* on SDH activity (U/mg protein) in liver, kidney and eye of diabetic mice. Mean (M) are obtained from 6 separate experiments; SEM (±): Standard Error Mean, p:- level of significance at *$P<0.05$, **<0.01 respectively, N:- normoglycemic, DC:- diabetic control, D:- diabetic mice, TP:- terpenoid & phenolic.

Effect of fractions of PF on AR and SDH activity in diabetic mice of 6 month old

As shown in **Figure 3**, TP fraction exerted highest inhibitory activity on AR compared to other fractions in normoglycemic mice. Hence, TP fraction was used for studying its effect in alloxan-induced diabetic mice. Administration of TP fraction to diabetic mice at the dose of 250 mg/kg

b.w. reduced the activity of AR by 34% ($p<0.01$) in liver, 39% ($p<0.01$) in kidney and 32% ($p<0.01$) in eye from that of diabetic control group. Administration of TP fraction to diabetic mice similarly caused significant lowering of SDH activity by 28% ($p<0.01$), 32% ($p<0.01$) and 23% ($p<0.05$) in liver, kidney and eye respectively from that of the diabetic control group **(Figure 4)**.

The magnitude of reduction of AR and SDH activity in both normoglycemic and diabetic mice varied in a tissue-specific manner. The extent of inhibition of AR and SDH activity in the diabetic mice was much less than that observed in normoglycemic mice comparing to earlier studies wherein it was shown that diabetes reduces the enzyme sensitivity to the inhibition of the enzyme AR and SDH [30, 31].

Conclusion

From the results, it was evident that the phenolics components of *P. fulgens* contain principles that exert inhibitory action on AR and SDH of the polyol pathway. This is also in agreement with previous reports that most of the AR inhibitors from natural sources belong to phenolic compounds [32, 33]. Polyphenolic compounds like quercetin, hesperidin have been previously reported to inhibit SDH activity [34, 35]. In conclusion, the inhibitory effect of TP fractions on the enzymes of polyol pathway offers opportunity in developing a promising drug for treating diabetes, obesity and diabetes-related complications.

Acknowledgement

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