

Alcohol Extract of *Wattakaka volubilis* (L.F) Stapf Root Inhibits Aldose Reductase to Prevent Diabetes Associated Cataract Formation in Rats

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ABSTRACT

Aim: The aim of this study was to investigate the aldose reductase inhibition and anti-cataract property of *W. volubilis* root extract in rats with streptozotocin induced diabetes mellitus. **Methods:** Diabetes was induced by a single intraperitoneal injection of streptozotocin at the dose of 45 mg/kg. Diabetic animals were treated with glimepiride (0.5 mg/kg) and alcohol extract at 100 and 200 mg/kg for 38 d. Lens were isolated and certain markers were estimated. **Results:** Treatment of diabetic animals with alcohol extract of *W. volubilis* root inhibited aldose reductase activity ($p < 0.001$) and sorbitol formation in the lens. Additionally, a reduction in the levels of MDA, GSH, SDH and LDH in extract treated groups was observed. There was an overall decrease in the severity of cataract progression. **Conclusion:** The result provided evidence that alcohol extract of *W. volubilis* root prevents the development of diabetic cataract in rats.

Key words: *Wattakaka volubilis*, Diabetes mellitus, Cataract, Aldose reductase, Polyol pathway.

INTRODUCTION

Diabetes mellitus is a multifactorial disease characterized by hyperglycemia,¹ lipoprotein abnormalities,² raised basal metabolic rate³ and defective reactive oxygen species scavenging enzymes.⁴ Factors leading to hyperglycemia include decreased insulin secretion, decreased glucose utilization and increased production of glucose. This metabolic dysregulation associated with Diabetes mellitus causes secondary pathophysiologic changes in multiple organ system that imposes a tremendous burden on the individual with diabetes. Diabetic complications arise due to glycosylation damage to structural and functional proteins and reflect chronic failure to maintain blood glucose homeostasis. Other complications such as diabetic nephropathy, retinopathy, neuropathy and cardiomyopathy also prevail as a result of hyperglycemia.⁴ Among all the complications associated with this disease, diabetic cataract may be the

most common microvascular complication. The risk of developing diabetic cataract or other microvascular complications depends on both the duration and the severity of hyperglycemia.⁵ Aldol reductase (AR) activation is one of the most important mechanisms responsible for the development of diabetic cataract.

Studies suggest that compounds which inhibit AR could be effective in the treatment of diabetic complications. Synthetic aldose reductase inhibitors (ARIs) are associated with many deleterious side effects and have low penetrability to target tissue.⁶ Hence a number of studies are on to tap natural sources of ARIs which could be useful in the management of diabetic complications. In this study, we have investigated the potential of *Wattakaka volubilis* root extract

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for its aldose reductase inhibition potential and prevention of diabetic cataract in animals.

Wattakaka volubilis commonly known as green wax flower and cotton milk plant is a tall, woody climber with densely lenticellate and pustular branches.⁷ It is widely used in Ayurveda as one of the alternative sources of the drug Murva. The accepted botanical source of Murva is *Marsdenia tenacissima* (Roxb.) Moon.⁸ Murva is useful as analgesic, anthelmintic, antimutagenic, anticancer and antibacterial.⁹ *W. volubilis* is traditionally used for various disorders. Plant juice is used as sternutatory, roots and tender stalks are used as emetic, purgative and expectorant.⁸ Leaves are used to relieve rheumatic pain, cough, fever and cold.¹⁰ Roots are used for lithiasis.¹¹ The roots and leaves are used for skin diseases, diabetes, cough, jaundice, poison bites and for blood disorders.¹² Leaf in paste form along with pepper is taken for dyspepsia, boils and abscesses.^{12,13} Leaf juice is reported to cure sprain;¹⁴ the plant useful for scorpion and snake bites.¹⁵ Paste of bark is taken with milk for urinary problems and leaf powder in milk is taken for diabetes.¹⁶

Many phytoconstituents have been isolated from *W. volubilis* such as steroids, triterpenoids, phenolic compounds and flavonoids from root;¹⁷ β -sitosterol; aglycone drevogenin A; 9, 12 – octadecadienoic acid; quinic acid; 1, 2 – benzenedicarboxylic acid diisooctyl ester; 5, 7 – dihydroxy – 6, 8 – dimethoxyflavone; N-[4-bromo-n-butyl]-2-piperidinone and digitoxose also from the root;¹⁷ β -sitosterol, pregnane glycosides and kaempferol from bark.¹⁸ HPTLC evaluation of methanol extract of leaf of *W. volubilis* revealed the presence of oleonic acid and urolic acid.¹⁹ Polyoxypregnane glycosides, volubiloside A, B and C were isolated from the flowers;²⁰ a triterpenoid ether, multiflor-7-en-12, 13-ether and a new multiflor-7-en-12 α -ol was identified in *W. volubilis*.²¹ Glycosides dregeosides H, Dp1, Da1, Gp1, Ga1 and two biosides were isolated from the hydrolysate of glycosides from roots¹⁸ and seven glycosides dregeosides Ap1, Ao1, Aa1, A11, C11, Kp1 and Kal, Drevogenin D were isolated from the seeds and characterized as 3 β , 11 β , 12 β , 14 β , 20 ξ -pentahydroxypregn-5-ene.²²

Various pharmacological studies have also been reported on *W. volubilis* such as antiurolithiatic¹¹ and antipyretic activities²³ in root; anti-diabetic, anti-hyperlipidemic and antioxidant properties in leaf;²⁴ wound healing²⁵ and antibacterial property in root, stem and leaf.²⁶

Hypoglycaemic activity in Murva²⁷ and antidiabetic activity of *W. volubilis* leaf²⁴ have been reported. On the basis of these reports, the present work has been

undertaken to determine the efficacy of *W. volubilis* root extract to prevent cataract formation in diabetic rats.

MATERIALS AND METHODS

Collection of plant material and preparation of ethanol extract

The plant material was collected from Kalakkad forests, Tirunelveli district, Tamil Nadu in flowering condition during March 2009. Identification, authentication and preparation of Herbarium specimen were done following standard protocols.²⁸⁻³¹ Herbarium specimen (031) was deposited in the herbarium at the Department of Pharmacognosy, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bangalore.

Extraction with 95% v/v ethanol was carried out by Soxhlation method. The concentrated and dried extract (yield 5.67% w/w) was suspended in 2% w/v acacia and used for the pharmacological studies.

Phytochemical analysis

Preliminary phytochemical screening³² was performed for the detection of different constituents present in the extract. Chromatographic studies were carried out.^{33,34} HPTLC studies³⁵ were carried out using Camag HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12bit CCD camera for photo documentation, controlled by Win CATS- 4 software were used. All the solvents used were of HPTLC grade obtained from MERCK. Chloroform: methanol (8:2) was used as the mobile phase.

Animals

Albino Wistar rats of either sex bred and reared in the animal facility of our institution were used in the study. CPCSEA (Committee for the Purpose of Control on Experiments in Animals) guidelines were followed for the maintenance of experimental animals. Prior approval was obtained from the Institutional Animal Ethics Committee of the institution (IAEC certificate no. MSRCP/ M-41/ 2014).

Acute toxicity studies³⁶

A limit test was performed as per test guide line 423 of OECD, using a dose of 2000 mg/kg. After dosing, animals were observed closely for 24 h for any kind of toxic symptoms. They were under close monitoring for the following 48 h and subsequently up to 14 d.

Induction of diabetes mellitus³⁷

Diabetes was induced by single intraperitoneal injection of streptozotocin (STZ) in 0.1 mol/L chilled citrate buffer, pH 4.5, at the dose of 45 mg/kg. After injection,

animals were allowed to drink 5% glucose solution overnight.³⁸ The development of hyperglycemia in rats was confirmed by fasting serum glucose estimation 72 h post STZ injection, wherein the animals were fasted over night before blood withdrawal by puncturing retro orbital plexus. The rats with fasting serum glucose level ≥ 180 mg/dL were considered diabetic and were included in the study.

The diabetic rats were divided into 4 groups of 6 animals in each group as follows: Group II (Positive control) consisted the vehicle treated (Acacia 2% w/v p.o) diabetic rats; Group III (Standard) included Glimpiride treated (0.5 mg/kg, b.w, p.o) diabetic rats; Group IV and Group V respectively consisted of the alcohol extract 100 and 200 mg/kg treated diabetic rats. Non diabetic rats were assigned as the normal control group (Group I).

Treatments were continued once daily for 21 d. On the 22nd day, fasting blood glucose was measured in all the animals.³⁹ Treatment was continued from the 22nd day onwards for further 15 d to evaluate the effects of the extract on diabetes induced cataract. At the end of the treatment, animals were sacrificed with excess of ether anesthesia and the lens was isolated by posterior approach. Lens homogenate was prepared in chilled phosphate buffer pH (7.4) for the estimation of AR,⁴⁰ MDA,^{41,42} GSH,^{43,44} SOD,^{43,45} catalase,^{43,46} proteins,^{47,48} sodium and potassium⁴⁹ calcium,⁵⁰ Lactate Dehydrogenase (LDH),⁵¹ Sorbitol Dehydrogenase⁵² and sorbitol.⁵³

RESULTS

Preliminary phytochemical analysis indicated the presence of glycosides and saponins in the alcohol extract. HPTLC fingerprint of the alcohol extract of *W. volubilis* root was obtained at wavelengths 254, 366 and 425 nm. Figure 1 represents the HPTLC fingerprint of the alcohol extract at 254 nm.

The dose of 2000 mg/kg was found to be safe in female Wistar rats. Streptozotocin 45 mg/kg produced significant ($p < 0.001$) increase in blood glucose levels compared to the normal control animals. Diabetic rats treated with 100 and 200 mg/kg of the alcohol extract showed significant lowering of serum glucose levels. Results were statistically significant ($p < 0.001$) (Figure 2). Aldose reductase and MDA levels were found to be significantly decreased ($p < 0.001$) in the animals treated with the extract when compared with the positive control. GSH levels in lens of positive control animals were significantly ($p < 0.001$) lower than in normal rats. An elevation ($p < 0.001$) in the GSH levels were observed in the standard and extract treated groups.

The positive control animals showed a significant ($p < 0.001$) decrease in the SOD and catalase activity in the lens homogenate. The extract treated and the glimepiride treated groups showed significant increase ($p < 0.001$) in the level of these antioxidant enzymes.

SDH activity in the lens of STZ diabetic rats was significantly ($p < 0.001$) higher than the SDH activity in normal rats. A decrease in the SDH activity in the lens homogenate of extract and glimepiride treated groups in comparison with the positive control was observed.

LDH activity in the lens of STZ diabetic rats was 110.04 ± 3.03 IU/L of tissue which was significantly ($p < 0.001$) higher than the LDH activity in normal rats (56.16 ± 2.92 IU/L). LDH activity in the lens of STZ diabetic rats administered with alcohol extract at a dose of 100 and 200 mg/kg and glimepiride (0.5 mg/kg) was found to be significantly lower ($p < 0.001$) than that of diabetic control. There was a significant increase in the levels of sodium, potassium, calcium, total protein,

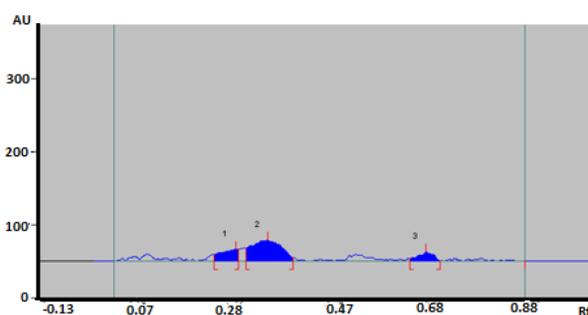


Figure 1: HPTLC fingerprint of alcohol extract of *W. volubilis* root at 254 nm.

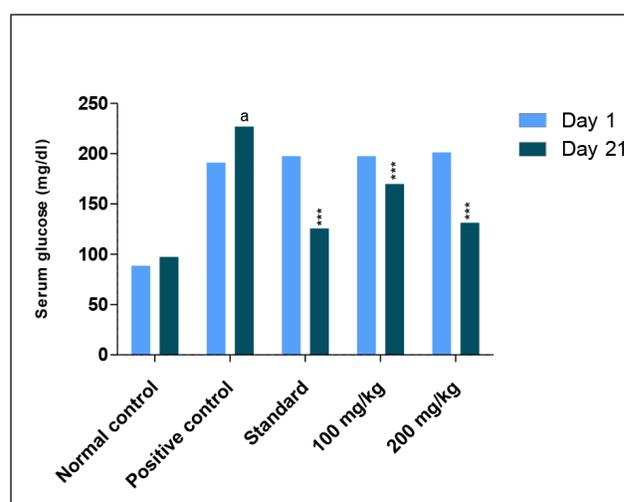


Figure 2: Effect of alcohol extract of *W. volubilis* root on serum glucose levels.

Values are expressed as Mean \pm SEM; n=6. One-way ANOVA: p value found to be 0.0001; Tukey-Kramer multiple comparisons test: ^a $p < 0.001$ in comparison with Normal control; ^{***} $p < 0.001$ in comparison with the Positive control.

Table 1: Effect of alcohol extract of *W. volubilis* root on lens parameters.

Parameters	Normal control	Positive control	Standard (Glimepiride)	Alcohol extract (100 mg/kg)	Alcohol extract (200 mg/kg)
AR activity (nmol/100mg)	0.95 ± 0.03	1.96 ± 0.02 ^a	1.43 ± 0.04 ^{***}	1.00 ± 0.03 ^{***}	0.8 ± 0.04 ^{***}
MDA (nmol/100mg)	0.36 ± 0.02	1.39 ± 0.15 ^a	0.74 ± 0.04 ^{***}	0.61 ± 0.02 ^{***}	0.60 ± 0.02 ^{***}
GSH (nmol/100mg)	51.22 ± 0.32	30.06 ± 1.50 ^a	43.34 ± 0.28 ^{***}	44.29 ± 0.36 ^{***}	46.00 ± 0.25 ^{***}
SOD activity (U/mg of tissue)	3.31 ± 0.04	0.81 ± 0.05 ^a	2.64 ± 0.04 ^{***}	2.82 ± 0.03 ^{***}	2.90 ± 0.04 ^{***}
Catalase (U/mg of tissue)	0.79 ± 0.02	0.17 ± 0.01 ^a	0.61 ± 0.01 ^{***}	0.67 ± 0.01 ^{***}	0.70 ± 0.02 ^{***}
Sodium (%)	0.15 ± 0.01	0.22 ± 0.12 ^a	0.08 ± 0.01 ^{***}	0.08 ± 0.01 ^{***}	0.07 ± 0.01 ^{***}
Potassium (%)	0.023 ± 0.003	0.098 ± 0.004 ^a	0.058 ± 0.003 ^{***}	0.061 ± 0.003 ^{***}	0.056 ± 0.003 ^{***}
Calcium (mg/dl)	6.10 ± 0.20	11.41 ± 0.40 ^a	7.40 ± 0.12 ^{***}	7.76 ± 0.11 ^{***}	7.34 ± 0.13 ^{***}
SDH (U/mg of solid)	0.37 ± 0.002	0.055 ± 0.001 ^a	0.055 ± 0.003	0.040 ± 0.002 ^{***}	0.031 ± 0.001 ^{***}
Sorbitol (µg)	0.71 ± 0.09	3.9 ± 0.08 ^a	2.0 ± 0.08 ^{***}	1.21 ± 0.08 ^{***}	1.05 ± 0.08 ^{***}
LDH (IU/L)	56.16 ± 2.92	110.04 ± 3.03 ^a	98.50 ± 3.00 [*]	70.60 ± 2.13 ^{***}	66.91 ± 2.50 ^{***}
Protein (mg/dl)	7.92 ± 0.21	2.63 ± 0.07 ^a	6.18 ± 0.12 ^{***}	10.63 ± 0.17 ^{***}	11.38 ± 0.14 ^{***}

Values are expressed as Mean ± SEM; n=6. One-way ANOVA: p value found to be 0.0001; Tukey-Kramer multiple comparisons test: *p < 0.05 in comparison with normal control; *p < 0.05 in comparison with the positive control, ***p < 0.001 in comparison with the positive control.

sorbitol in the lens homogenate of positive control ($p < 0.001$). Treatment of diabetic rats with the alcohol extract (100 and 200 mg/kg) and glimepiride showed significant improvement in these abnormalities (Table 1).

DISCUSSION

The study was conducted to investigate the role of *W. volubilis* root in the prevention of diabetic cataract. A single intraperitoneal injection of STZ at a dose of 45 mg/kg produced significant hyperglycemia without any mortality. The major clinical signs of DM like polyphagia, polydipsia, polyuria and body weight reduction were observed in all the animals. The extract was found to be safe with 2000 mg/kg as shown by the acute toxicity studies. Based on this result, 100 and 200 mg/kg of the extract were selected for further pharmacological studies. Diabetic patients are predisposed to many problems associated with their disease. Among the entire complications, diabetic cataract, a major microvascular disease is the most common chronic complication of diabetes.⁵⁴ Cataract, characterized by cloudiness or opacification of the eye lens, is the leading cause of blindness all over the world.⁵⁵ Cataractogenesis is one of the earliest secondary complications of DM particularly in rodents. Although the pathogenesis of development of diabetic cataract is unknown, a number of pathways arising as a result of hyperglycemia may be accounted for the development of cataract. Various mechanisms have been proposed to explain the pathophysiology of diabetic complications. These mainly include oxidative stress, increased polyol pathway or osmotic stress, increased formation of advanced glycation end products, activation of

protein kinase C and increased hexosamine pathway flux.⁵⁶ Among all these pathways, polyol pathway is very widely studied.

Aldehyde reductase is the main enzyme involved in polyol pathway. It is the first and rate limiting enzyme of this pathway.⁵⁷ Under normoglycemic conditions, AR has a minor role in glucose metabolism but its activity is significantly enhanced during diabetes.^{58,59} In hyperglycaemic condition, AR catalyses conversion of excess glucose to sorbitol in insulin independent tissues like lens. AR catalysed formation of excess polyols are considered as one of the mechanisms leading to diabetic cataract.⁶⁰ Hence compounds that inhibit AR activity could be effective in the treatment of diabetic cataract.

In the present study, STZ diabetic rats exhibited higher aldehyde reductase activity and which was normalised by both doses of *W. volubilis* root extract. The normalisation of aldehyde reductase activity can be well correlated with the improved biochemical status and prevention of cataract. Similarly, inhibition of SDH and LDH activities was also observed which further indicated the possible protective effect of the extract against cataract formation. Studies have indicated that intracellular accumulation of polyol leads to collapse and liquefaction of lens fibres, leading to opacity of lens. In this study, treatment with the extract decreased the sorbitol concentration in the lens which may be accounted for the delay in development of cataract.⁶¹

A decrease in GSH, increase in lipid peroxidation and altered activities of antioxidant enzymes were observed in various types of cataract, including diabetic cataract.⁴⁹ All these above mentioned changes were also seen in the present study and was normalised by *W. volubilis* extract.

Further, the accumulation of carbonyl groups in proteins is attributed to oxidative damage and is thought to contribute to general protein dysfunction as seen in diabetic control.⁴⁹ Treatment groups showed a substantial increase in the total protein content of lens which indicates that the extract prevented the decrease in total protein content. All of these indicate that *W. volubilis* extract has anti cataract potential in Diabetes mellitus. Preliminary phytochemical screening revealed the presence of glycosides and saponins. Whether these phytoconstituents have contributed to observed effects, needs to be investigated further.

CONCLUSION

This study suggests that alcohol extract of *W. volubilis* root is effective against diabetes and diabetic cataract. However, further studies are needed to elucidate the mechanism(s) of action and to know the active principle/s involved in producing these effects.

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CONFLICT OF INTEREST

The authors declare no conflicts of interests

ABBREVIATIONS

AR: Aldose Reductase; **ARIs:** Aldose Reductase Inhibitors; **b.w:** Body weight; **CPCSEA:** Committee for the Purpose of Control on Experiments in Animals; **d:** Day; **dL:** Deciliter; **DM:** Diabetes Mellitus; **GSH:** Glutathione; **h:** Hour; **HPTLC:** High Performance Thin Layer Chromatography; **IAEC:** Institutional Animal Ethics Committee; **IU:** International Units; **kg:** Kilogram; **L:** Litre; **LDH:** Lactate Dehydrogenase; **MDA:** Malondialdehyde; **mg:** Milligram; **nm:** Nanometer; **OECD:** Organisation for Economic Co-operation and Development; **p.o:** Per oral; **SDH:** Sorbitol Dehydrogenase; **SOD:** Superoxide Dismutase; **STZ:** Streptozotocin; **W. volubilis:** *Wattakaka volubilis*.

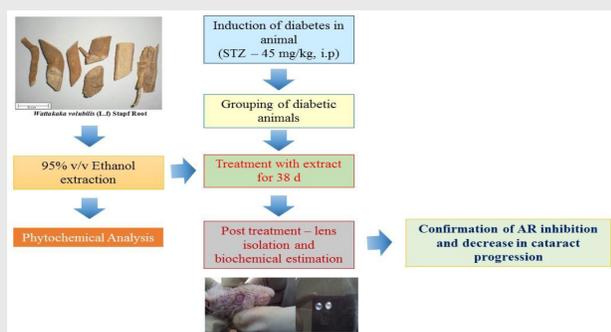
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PICTORIAL ABSTRACT



SUMMARY

- The present study investigated the anticataract potential of *W. volubilis* root extract against STZ induced diabetic rats. Ability of extract to inhibit aldose reductase and other markers in rat lens was determined. Treatment of diabetic animals with the alcohol extract of *W. volubilis* root showed significant aldose reductase inhibition and also decreased cataract progression.

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