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Antidiabetic and aldose reductase inhibitory potentials of *Land caltrops* aqueous extract in streptozotocin-nicotinamide induced diabetic rats

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ABSTRACT

Introduction: Diabetic retinopathy is a late stage complication in diabetic patients and one which dramatically affects quality of life. Persistent hyperglycemia results in sorbitol accumulation due to increased activity of aldose reductase (AR), which leads to changes in membrane permeability and leakage of glutathione (GSH) from the lens which in turn results in the development of cataract and retinopathy. Hence, the present study was designed to assess the effect of *Tribulus terrestris* on AR activity and GSH level in diabetic rat lens, random blood glucose, hemoglobin A1c (HbA1c) and insulin.

Methods: Diabetes mellitus was induced by intra-peritoneal (i.p) injection of streptozotocin-nicotinamide (STZ-NA). Animals were divided into 5 groups including normal controls (NC) treated with saline, untreated diabetic controls (DC), *T. terrestris* (150 and 300 mg/kg) and glibenclamide (500 µg/kg) treated diabetic rats. After 16 weeks of treatment, the rats were sacrificed, the lens was removed through posterior approach and homogenate was prepared for AR activity estimation. The lens tissue homogenate was prepared in normal saline for the estimation of GSH. Blood glucose was estimated by glucometer, HbA1c by nephelometry and insulin by ELISA kit.

Results: AR activity was significantly reduced ($P<0.004$) in *T. terrestris* (both doses) treated groups compared to untreated diabetic controls. GSH levels were found significantly higher ($P<0.005$) in treated groups than the ones in diabetic controls. Glucose, HbA1c and insulin were significantly improved ($P<0.004$) in plant extract treated groups when compared to untreated diabetic rats.

Conclusion: *Tribulus terrestris* aqueous extract may be useful as AR inhibitor. It also has antioxidant and antidiabetic activities and thereby might be capable of controlling the hyperglycemia induced tissue damage.

Implication for health policy/practice/research/medical education:
The aqueous extract of *Land caltrops* may be able to control the hyperglycemia and delay the diabetic complications of type 2 diabetes. Therefore, its use in diabetic subjects is suggested.

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Introduction

Diabetic cataract or retinopathy has been considered to be one of the major long-term complications of diabetes (1). The risk of cataract development is enhanced by diabetes mellitus (2). Various biochemical pathways are involved in the pathogenesis of diabetic cataract such as formation of advanced glycated end products, polyol pathway and oxidative stress (3). Aldose reductase (AR) is the first and rate-limiting enzyme of the polyol pathway. Hyperglycemia causes the activation of AR which leads to increased formation of sorbitol in insulin-insensitive tissues such as lens, nerve and retina of diabetic patients (4). Sorbitol accumulation increases osmotic stress and swelling, which may damage the tissues. Excess polyols synthesis due to enhanced AR activity is considered as one of the major mechanisms resulting in diabetic cataract (5).

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Excessive free radical generation leading to oxidative stress is another well-known mechanism involved in the formation of cataract. Oxidative stress results in alteration in the intracellular antioxidant levels such as glutathione (GSH), superoxide dismutase (SOD) and catalase (6). In diabetes mellitus, excess glucose is moved to polyol pathway, where AR converts glucose to sorbitol by utilizing NADPH. Since NADPH is also required for generation of some antioxidants (such as GSH), excessive depletion of NADPH by AR in polyol pathway leads to impairment in intracellular antioxidant defense (7). Antioxidants are responsible for protection of the cells and tissues against oxidative injury. Thus, inhibition of AR pathway and increase in GSH in lens could be one of the strategies to prevent diabetic cataract.

Literatures have revealed that cataract advancement can be prevented by using plant extracts, which are high in flavonoids and phenols with their hypoglycemic and AR inhibitory actions (8). Plant extracts such as *Ocimum sanctum, Curcuma longa* and *Azadirachta indica* have been tested recently for *in vivo* AR inhibitory & anti-cataract activities (9-11).

Tribulus terrestris is one of the most commonly used plants (common name - Caltrop or Gokshura, Gokharu, belonging to the family Zygophyllaceae) which is widely distributed throughout India. It is used as an aphrodisiac (12), diuretic & anthelmintic (13) remedy and in the treatment of edema, ocular infections & abdominal distention. Hypolipidemic (14), hypoglycemic (15) and antioxidant activities (16) have been reported with aqueous extract of *T. terrestris* dry fruits.

Whether the additional antioxidant property along with antidiabetic activity of *T. terrestris* aqueous extract can prevent or slow down the development of diabetic cataract is not well studied. Therefore, the current study was planned to evaluate the beneficial effects of *T. terrestris* dry fruit extract in diabetes rat lens.

Materials and Methods

Plant collection

Tribulus terrestris dry fruit aqueous extract was procured from Himalaya Health Care, Bangalore, India (Batch number: NG451865). The extract was authenticated in the Department of Botany, St Aloysius College, Mangaluru, Karnataka, India.

Chemicals and drugs

Streptozotocin and nicotinamide were bought from Himedia Drug Company, India and glibenclamide from Cipla Company, Mumbai, India.

Animals

The study was approved by Institutional Animal Ethics Committee, Kasturba Medical College, Mangalore, Manipal University, Karnataka. Albino Wistar strain, either gender rats weighing 100±5 g were procured from Central Animal House, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Karnataka, India. All animals were fed in an animal facility with a 12 to 12 hours light-dark cycle and were given the standard rat chow and water ad libitum (17).

Diabetes induction

Diabetes mellitus was induced in adult rats by administering nicotinamide (NA) and streptozotocin (STZ). Animals received intraperitoneal administrations of nicotinamide - 25 mg/kg dissolved in normal saline 15 minutes before administration of STZ - 50 mg/kg. 0.1M citrate buffer was used to dissolve STZ (pH 4.5) (18). Only the rats having a blood glucose above 250 mg/dL were chosen and divided into groups.

Acute toxicity studies

OECD guidelines were followed for conducting the study. *T. terrestris* aqueous extract was administered through oral route by using feeding tube at doses of 300, 600, 1200 and 2400 mg/kg body weight (4 groups & each group 6 animals). Animals were closely observed for 24 hours. No mortality was seen with higher dose but animals were paranormally active after 4 hours of drug administration. Maximum tolerated dose (MTD) of *T. terrestris* aqueous extract was 2400 mg/kg.

Experimental design

Thirty animals were divided into five following groups (n =6) and oral administration of plant extract started on 7th day after streptozotocin-nicotinamide (STZ-NA) injection and continued for 16 weeks.

Group I- Normal controls treated with saline (NC)
Group II- Untreated diabetic controls (DC)
Group III- Diabetic rats treated with glibenclamide (500 µg/kg of b.w) (DM+Glib500)
Group IV- Diabetes rats treated with *T. terrestris* (150 mg/kg of b.w) (DM+TT150)
Group V- Diabetes rats treated with *T. terrestris* (300 mg/kg of b.w) (DM+TT300)

Determination of AR activity in the rat lens

The lens AR activity was measured according to the method of Hayman and Kinoshita with slight modification (19). AR activity was studied using glyceraldehyde as substrate and NADPH as co-enzyme. In short, the reaction mixture was prepared at 25°C, with a total volume of 2.1 mL, containing 1.6 mL 135 mM Na^+ - K^+ phosphate buffer of pH-7.0, 0.1 mL lithium sulphate (1.2796 g/50 mL in 135mM phosphate buffer of pH 7.0), 0.1 mL D-L –glyceraldehyde (3.6 mg/50 mL in 135mM phosphate buffer of pH 7.0). The centrifugate (0.2 mL) was mixed and the reaction was initiated by adding 0.1 mL freshly prepared NADPH (2 mg/mL in 135mM phosphate buffer

of pH 7.0). Absorbance was measured, at every 30 seconds intervals for 3 minutes, at 340 nm in a double beam spectrophotometer. The decrease in the absorbance of the solution at 340 nm gave a measure of decrease in the concentration of NADPH which acted as the coenzyme for the action of AR on glyceraldehyde. Similar calculations were done for the blank as well. Total protein estimation in lens supernatant was done by Lowry's method (20). The activity of AR was expressed as units of activity per mg of protein in the lens homogenate.

Reduced glutathione estimation in lens

The concentration of reduced GSH was estimated by using 5,5'- dithiobis-2-nitro benzoic acid (DTNB) (21). The lens homogenate was prepared in 0.9% saline and centrifuged at a speed of 3000 rpm for 10 minutes and supernatant was taken as test. Two milliliters of it was taken and 3 mL precipitating solution was added, mixed well and allowed to stand for 5 minutes. It was then centrifuged and 1 mL supernatant was taken, 4 mL phosphate solution and 0.5 mL DTNB reagent were added. The test was based on the yellow colour development when DTNB mixed to the test solution and that was measured at 412 nm. GSH concentration was expressed in µmol/g lens tissue.

Statistical analysis

The data was expressed as mean ± standard deviation (SD). To find the *P* value between two groups Mann-Whitney U test was used. SPSS version 20 was used for analysis. *P* value < 0.05 was considered as significant.

Results

Effect of *T. terrestris* on AR activity and GSH level in rat lens

AR activity in normal control group was found 0.271±0.087 mU/mg protein in lens. AR activity in diabetic control group was 1.06±0.10 and significantly increased ($P=0.004$) when compared to normal control group. As shown in Table 1, AR activity was significantly reduced ($P=0.004$) in *T. terrestris* treated diabetic rats in both 150 mg/kg and 300 mg/kg groups compared to the disease control group.

Increased levels of GSH were seen in diabetes rats treated with 150 and 300 mg/kg groups when compared to the disease control group. GSH levels in different groups are shown in Table 1. Mann-Whitney U test analysis showed significant increase ($P=0.01$) in groups treated with *T. terrestris* or glibenclamide compared to diabetic controls.

Tribulus terrestris effect on glycemic profile

Diabetic control group displayed elevated blood glucose ($P=0.001$), HbA1c ($P=0.004$) and insulin levels ($P=0.004$) when compared to normal control group. Sixteen weeks of treatment with *T. terrestris* in diabetic animals significantly reduced the random blood glucose ($P=0.002$), HbA1c ($P=0.003$) & insulin levels ($P=0.004$) (Table 2).

Discussion

In polyol pathway AR enzyme was found to be the crucial parameter playing a major role in cataractogenesis in diabetes. Excess polyols synthesis due to AR activation leads to sorbitol accumulation & lens opacification (22). Previous reports suggest that plant extracts are effective in preventing diabetes-induced lens opacification through an inhibition of AR activity & reducing sorbitol accumulation in eye (9). Furthermore, some flavonoids and polyphenols are found to be effective in AR inhibition (8). A few plant materials such as root of *Salacia oblonga* (23), *Salviae miltiorrhiza*, *Glycyrrhiza uralensis*, *Radix*

Table 1. Aldose reductase activity and reduced glutathione in lens homogenates of various groups

Parameters	Normal control	Diabetic control	Diabetes + Glibenclamide 500 µg/kg	Diabetes + *T.terrestris* 150 mg/kg	Diabetes + *T. terrestris* 300 mg/kg
Aldose reductase activity (mU/mg protein)	0.27 ± 0.08	1.06 ± 0.10	0.38 ± 0.16**	0.50 ± 0.04**	0.49 ± 0.07**
Reduced glutathione in lens (µmol/g)	0.28 ± 0.07	0.04 ± 0.03	0.19 ± 0.02**	0.18 ± 0.08**	0.25 ± 0.07**

Values are expressed as Mean ± SD, (n=6 in each group). ** $P < 0.01$ significantly different compared to disease control group.

Table 2. Random blood glucose, HbA1c and insulin in various groups

Parameters	Normal control	Diabetic control	Diabetes + Glibenclamide 500 µg/kg	Diabetes + *T.terrestris* 150 mg/kg	Diabetes + *T. terrestris* 300 mg/kg
Random blood glucose (mg/dL)	121.17±8.65	479.67±20.04	151.67±10.32 **	199.67±20.87 **	186.17±14.62 **
HbA1c (%)	4.04±0.26	7.53±0.25	4.74±41.36 **	5.54±0.42 **	5.28±0.53 **
Insulin (ng/mL)	12.75±5.65	26.82±2.24	15.97±1.38 **	11.31±2.16 **	9.521±1.51 **

Values are expressed as Mean ± SD, (n=6 in each group). ** $P < 0.01$ significantly different compared to disease control group.

astragali and puerarin (24) are believed to have AR inhibiting activity. These plants are rich in bioflavonoids, which are reported to reduce the over activity of AR. Supporting previous studies, the present study with *T. terrestris* dry fruit aqueous extract treatment effectively delayed the development of diabetic cataract in STZ-NA induced diabetes animals by suppressing the AR activity and increasing antioxidant status in the diabetic rat lens. Additionally, *T. terrestris* treatment successfully controlled the glycemic profile such as blood glucose, HbA1c and insulin in diabetic rats. Saponins present in *T. terrestris* may be responsible for the observed beneficial effects. The present study provides a solid support to the hypothesis that the use of AR inhibitors may be an effective strategy in the management of diabetic cataract.

Studies have documented that oxidative stress is a common underlying mechanism for the development of cataract. Strengthening of the antioxidant defenses of the lens has been shown to prevent or delay the diabetes induced cataract. *T. terrestris* was found to have significant antioxidant activity. A study by Amin et al reported that *T. terrestris* 2 g/kg oral administration for 30 days in diabetic animals increased the peripheral utilization of glucose and significantly restored GSH levels in liver when compared with untreated diabetic controls (25). In our earlier studies the antioxidant potential of *T. terrestris* has been correlated with the presence of sufficient phenols and significant DPPH free radical scavenging activity (16). In the present study, *T. terrestris* treatment for 120 days in diabetic rats significantly ($P<0.05$) improved the levels of GSH and prevented lens opacification when compared to untreated diabetic control rats.

Hyperglycemia can induce oxidative stress via several mechanisms including glucose auto-oxidation, formation of advanced glycated end products and activation of polyol pathway. It is already known that hyperglycemia induced polyol pathway activation increases the intracellular overload of sorbitol and leads to ocular lesions. Hence, the plant for the present study has been selected on the basis of its reported antidiabetic property. According to previous reports ethanolic extract of *T. terrestris* significantly ($P < 0.05$) decreased the blood glucose levels in a STZ induced diabetes animal model. The antidiabetic diabetic efficacy of plant extract was similar to the group treated with glibenclamide (26). In the present study *T. terrestris* dry fruit aqueous extract and glibenclamide significantly reduced the blood glucose and HbA1c levels in diabetes rats.

In diabetes mellitus, insulin resistance plays a major role in the progression of diabetic complications. *T. terrestris* treatment has been shown to attenuate HbA1c and improve the insulin sensitivity. In a study, two days old diabetic neonates were treated after 6 weeks with 50 mg/kg hydro-alcoholic extract of *T. terrestris* for 8 weeks. *T. terrestris* treatment reduced the blood glucose level in treated groups (27). Lamba *et al* reported that *T. terrestris* with 100 mg/kg dose for 30 days significantly ($P < 0.05$) decreased the fasting blood glucose and HbA1c levels in diabetic rats (28). In present study the positive effects of *T. terrestris* on diabetic retinopathy might be attributed to its AR inhibitory activity, anti-oxidant and anti-diabetic activities.

Conclusion

Our data suggest that aqueous extract of *T. terrestris* possesses potential antidiabetic and AR inhibitory activity at both doses. *T. terrestris* aqueous extract may be useful as AR inhibitor. It also has antioxidant and antidiabetic activities and thereby might be capable of controlling the hyperglycemia induced tissue damage in diabetic subjects.

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Authors' contributions

The ideology and design of the study were done by PAM, AH and BV. The experimental procedures were done by BV under the guidance of PAM. The statistical data and interpretation of the results were done by BV, AH and PAM. All the authors contributed to preparation of the manuscript and agreed with publication of the final proof.

Conflict of Interests

No conflict of interest associated with this work.

Ethics considerations

Authorization for the use of laboratory animals was obtained from Institutional Animal Ethics Committee (IAEC) of Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Karnataka, India, meeting held on 14/06/2013. All experimental procedures conform to the guiding principles for research as recommended by "Guide for the Care and Use of Laboratory Animals" (NIH publication 86-23 revised 1985).

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