



α - GLUCOSIDASE AND ALDOSE REDUCTASE INHIBITORY ACTIVITY *IN VITRO* AND ANTI-DIABETIC ACTIVITY *IN VIVO* OF *TRIBULUS TERRESTRIS* L. (DUNAL)

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ABSTRACT

Background: Inhibition of α -glucosidase and aldose reductase inhibitors plays a major role in hyperglycemia induced vascular damage. It has also been implicated as a major process affecting the glucose uptake mechanism.

Objective: Reported study was done for a comparative evaluation of α -glucosidase and aldose reductase inhibitory activity of *Tribulus terrestris* and *Curculigo orchiooides* which are well-known Ayurvedic Rasayana herbs.

Design: A total of 48 Wistar strain rats were rendered hyperglycemic by treating them with streptozotocin (75 mg/Kg body weight). Diabetic rats were treated for 30 days with 100 mg/kg body weight of ethanolic extract of *T. terrestris* (TT100) and *C. orchiooides* (CO100) once daily.

Settings: Enzymatic and other parameters *viz.* blood glucose level, glycated hemoglobin, insulin, liver glycogen and urinary albumin levels were measured using an auto analyzer. Body weight of the rats was also recorded past treatment for 30 days. *In vitro* α -glucosidase inhibition was determined using acarbose and epicatechin as controls.

Main outcome measures: Both TT and CO were effective in inhibiting α -glucosidase and aldose reductase. There was a reduction in fasting blood glucose level observed as well. Treatment with extracts could also ameliorate the loss of body weight as observed in case of hyperglycemic control group animals.

Results and conclusion: A significant ($p < 0.05$) decrease in fasting blood glucose level was observed in case of TT100 treated group similar to the one treated with glibenclamide. A 59.6 ± 2.1 % inhibition of α -glucosidase and 98.6 ± 1.1 % inhibition of aldose reductase was observed at a dose of 30 μ g in case of TT100. For CO100 the decrease in fasting blood glucose level was also significant ($p < 0.05$). A 60.4 ± 1.2 % inhibition was observed in case of α -glucosidase and 99.2 ± 1.4 %, inhibition was observed in case of aldose reductase activity. Elevation in glycated hemoglobin and liver glycogen levels were also observed in case of diabetic rats, and were similar to untreated control group animals in case of WG100 treated group.

Similar to acarbose a significantly higher α -glucosidase inhibitory action was observed with WG. The low albumin excretion rate correlated well with the protective effects of the extracts. The study provides molecular interventions for the utilization of *C. orchiooides* and *T. terrestris* in Ayurvedic system of medicine.

Keywords: α -glucosidase inhibition, aldose reductase, urinary albumin level, *Curculigo orchiooides*, *Tribulus terrestris*

INTRODUCTION

Natural products have been the main stay of treatment of various disorders in human beings^{1,2}. Numerous Ayurvedic herbal drugs have been indicated for their effectiveness in treatment of diabetes and related disorders. The advantages that herbal drug possess are numerous and multifaceted³⁻⁶. An ever increasing number of patients suffering from diabetes around the world have shifted the research focus to find safe and effective herbal alternatives. Uncontrolled hyperglycemia can lead to progressive secondary complication in diabetes such as nephropathy, neuropathy, retinopathy and cardiovascular diseases⁷. Insulin, Insulin sensitizers and insulin secretagogues, as well as enzyme inhibitors such as α -glucosidase and aldose reductase inhibitors contribute as an important therapeutic option in treatment of diabetes⁸.

Tribulus terrestris Linn, which is also known as Gokhru and *Curculigo orchiooides* Gaertn., are very popular as Rasayana in Ayurvedic system of medicine in India⁹⁻¹². In Northern India traditional healers used dried fruits of *T. terrestris* and *C. orchiooides* for the treatment of diabetic patients though it's the possible mechanism defining its activity have not been evaluated systematically. It belongs to a category of Ayurvedic drugs known as Ayurvedic Rasayana, which act by modulating the neuro-endocrin-immunological system^{10,13,14}.

Ayurvedic drugs belonging to Rasayana category have been getting a lot of attention lately for their ability to ameliorate the damages caused by oxidative overload in diabetic subjects⁵. α -glucosidase inhibition is useful for inhibiting post prandial rise in diabetic

glucose levels. It is a promising non-invasive therapy in both types of diabetes for a good control of postprandial hyperglycemia¹⁵⁻¹⁷. Similarly, inhibition of aldose reductase also appears to have an important role in treatment of cataractogenesis and nephropathy. While, aldose reductase has a low affinity for glucose under normoglycemic conditions, under hyperglycemic stress there is an accumulation of sorbitol in insulin - independent tissue occurs, leading to secondary diabetic complications like edema and ionic imbalance.¹⁸⁻²¹

In the present study ethanolic extracts of *C. orchiooides* and *T. terrestris* were tested for the effectiveness in treating streptozotocin induced hyperglycemia *in-vivo* and inhibition of α -glucosidase and aldose reductase *in vitro*.

MATERIALS AND METHODS

Dried fruits of *T. terrestris* and rhizomes of *C. orchiooides* were purchased from the local herbal market of Kanpur, India and identified by Dr P.K. Khare, Botanist, Department of Botany, Dr. H. S. Gour University, Sagar (M.P.) India. A voucher specimen no. (HL-121/09-10) has been submitted. The dried drugs (5 kg) were mechanically crushed to a coarse powder passing through sieve no.60 and retained on sieve number 80. The dried powder was fed in a Soxhlet apparatus and defatted by extraction with petroleum ether (60-80 C). The marc was collected dried in a hot air oven at 50° C and further extracted with 80% ethanol. The extract was collected and dried in a rotary vacuum evaporator (Heidolph, Germany) under reduced pressure to obtain semisolid material, which was then lyophilized to get a powder (10.2 % w/w).

Animal grouping

Animal experimentation was carried out after prior permission from the institutional ethical committee of Advance Institute of Biotech and Paramedical Sciences, Kanpur (U.P.), India. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India were adhered to during the whole experimentation.

Adult Wistar strain male albino rats aged 3-4 months and weighing between 180 ± 1 gm were used. The rats were fed with standard diet purchased from Gold Pellet, India Ltd. Animals were provided with food and water *ad libitum* and maintained at 25-28°C. The lyophilized extract was then suspended in 2% PVP solution and administered orally using metal canula. Streptozotocin was administered as aqueous solution intra peritoneally. Blood glucose level of animals was monitored 96 h after administration of streptozotocin. Male animals in group of 8 each were taken for the studies and dosing protocol for different groups were as follows.

Group I served as control and was administered vehicle only (C).

Group II: 50 mg/Kg b. w. streptozotocin intraperitoneally once (HC)

Group III: 50 mg/Kg b. w. streptozotocin intraperitoneally once and 100 mg/Kg body weight of *W. coagulans* extract orally (WC100)

Characterization of extract

Characterization and standardization of ethanolic extract of *C. orchoides* and *W. coagulans* was done using high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC). Extracts were also characterized for the total phenolic content using chemical analysis.

Yeast α -glucosidase inhibitory activity

The method of Watanabe et al. ²² was used. Yeast α -glucosidase (0.7 U) dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/l bovine serum albumin, and 0.2 g/l NaN_3 , and 5 mM *p*-nitrophenyl- α -D-glucopyranoside in the same buffer (pH 7.0) were used as an enzyme and a substrate solution, respectively. The enzyme solution (50 μ l) and 10 μ l of the test sample at various concentrations were mixed, and absorbance at 405 nm was measured using a microplate reader (Perkin Elmer, USA). After incubation for 5 min, 50 μ l of the substrate solution were added and incubated for an additional 5 min. The increase in absorbance from time zero was measured, and inhibitory activity was calculated as a percentage of the blank control. The inhibitory activities of the *C. orchoides* and *T. terrestris* extract, and acarbose (positive control), against α -glucosidase were measured at concentrations of 0.50, 0.25, 0.10, and 0.05 mg/ml. The measurements were performed in triplicate, and the IC_{50} value, i.e., the concentration of the extract that results in 50% inhibition of maximal activity, was determined.

Mammalian α -glucosidase inhibitory activity

Normal rats fasting for 20 h were sacrificed by cervical dislocation. The small intestine obtained was flushed several times with ice-cold saline. Intestine was averted and mucosa was scraped with glass slide. The obtained material was centrifuged and pellet homogenized in phosphate buffer (pH 7.0, 0.05M) containing 1% Triton X 100, further cold *n*-butanol was added to remove Triton and sample subjected to overnight dialysis. The activity was assayed with 25 mM maltose as substrate for 30 min at 37°C. Different concentration of *Piper nigrum* (dissolved in DMSO) and acarbose were used during inhibition study. Released glucose was quantified using commercial glucose oxidase assay (GOD-POD Biolab Diagnostic Pvt. Ltd.). One unit of enzyme activity (U) is defined as amount of enzyme forming μ mole of glucose per min per ml under standard conditions. Assays were performed in triplicate.

Aldose reductase inhibitory activity

Crude aldose reductase was prepared from rat lenses as described by Lee ²¹ without any further modifications. Aldose reductase inhibitory activity was assayed according to the method described by Lee and Kim ¹⁸. The incubation mixture contained 135 mM

Na,K-phosphate buffer (pH 7.0), 100 mM lithium sulfate, 0.03 mM NADPH, 0.04 mM DL-glyceraldehyde, and 50 μ l of an enzyme preparation, with or without a plant extract, at a total volume of 1.0 mL. Each plant extract was dissolved in dimethyl sulfoxide, which was found to have no effect on the enzyme activity at less than 1%. Appropriate blanks contained all of the abovementioned compounds except DL-glyceraldehyde. The reaction was initiated by adding NADPH at 37 °C and stopped upon addition of 300 μ l of 0.5 N hydrochloric acid. Subsequently, 6 N NaOH (1 mL) containing 10 mM imidazole was added, and the mixture was incubated at 60 °C for 10 min to convert NADP into a fluorescent product. The fluorescence was measured at room temperature with a spectrofluorometer (Shimadzu, Japan) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Standards of NADP (0.1-5 μ M) were treated in the same manner. All determinations were performed in triplicate. The concentration of each test sample giving 50% inhibition of the enzyme activity (IC_{50}) was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity. The protein content of the enzyme preparation was 0.021 g/L, and aldose reductase activity in the preparation was 7.38 U/L or 370.1 U/g of protein at 37 °C. The protein content of the enzyme preparation was determined using Coomassie blue reagent (Bio-Rad) according to the manufacturer's instructions, with bovine serum albumin as a standard.

Effect of treatment on body weight and blood glucose levels

Body weight of the rats was measured after 30 days of treatment in all the three groups. Blood was collected from the tail vein and blood glucose level was measured by enzyme-colorimetric test using the glucose oxidase test kit from Invitrogen, USA.

Liver glycogen, serum insulin and glycosylated hemoglobin measurement

After 30 days of treatment the rats were sacrificed by cervical decapitation and blood was collected from peripheral vein. Liver and kidney were immediately removed and washed with PBS (pH 7.4). Liver glycogen estimated by KOH method. Serum insulin was determined by ELISA using a commercial kit with no further modifications to the protocol (Biorad, India). Glycosylated hemoglobin was determined by ion exchange method (Biorad, India).

Statistical analysis

All data is expressed as mean \pm SE. Statistical significance was analyzed by student test when comparing two groups and one way analysis of variance (ANOVA) with Dunnet's test in case of multiple comparison. Significance was set at $p < 0.05$ with a confidence interval of 95%.

RESULTS

The effect of CO and TT on mammalian α -glucosidase inhibition was compared with acarbose as standard. At concentrations of 500, 250, 100, 50 and 25 μ g/ml 70.2, 51.2, 28.5, 12.1, and 5.4% inhibition of α -glucosidase *in vitro* was observed in case of *T. terrestris*. It was 71.6, 55.1, 32.5, 22.1, and 10.2% respectively at similar concentrations in case of *C. orchoides*. Acarbose showed an inhibition of 26.5, 20.1, 11.4, and 4.5% at similar concentrations.

In vitro aldose reductase inhibition was also observable in TT and CO. For TT at 20 μ g/ml there was 80% inhibition, while at 30 μ g/ml a 100% inhibition. In case of CO at 20 μ g/ml a 90% inhibition was observed and at 25 μ g/ml 100% inhibition was observed. Epicatechin was effective at a concentration of 100 μ g/ml concentration and resulted in a 100% inhibition.

The blood glucose level was < 250 mg/dl in all the groups at the start of the study. There was a moderate decrease in the blood sugar level of diabetic rats upon administration of CO100 and TT100 on 30th day of treatment. In streptozotocin and CO100 treated group it was (115.4 ± 2.01 mg/dl), in TT100 treated group it was (114.3 ± 1.07 mg/dl) compared to the streptozotocin control (233.15 ± 1.02 mg/dl) (Table 1).

After 30 days of sustained hyperglycemia a decrease in the body

weights of the male rats in streptozotocin treated group was observed ($p < 0.05$). The loss of body weight was significantly ameliorated in CO100 and TT100 treated groups and the body weight of rats after 30 days of treatment was nearly similar to the normoglycemic control group animals.

The glycated hemoglobin, kidney weight and liver glycogen levels were almost normal after 30 days of treatment. The data for all the values has been shown in Table 1.

DISCUSSION

Renal hypertrophy which was assessed as the ratio between the kidney weight and total body weight was ameliorated after 30 day treatment with CO and TT extracts. Extracts of many plants *Momordica charantia*, *Eugenia jambolana*, *Mucuna pruriens* and *Tinospora cordifolia* have shown reduction in renal hypertrophy²³. This was further validated in case of CO and TT treated animals.

Furthermore, a possible androgenic modulation via the FSH-LH cascade due to the presence of steroidal saponins in *W. coagulans* can be a contributory factor for the amelioration of lean body mass loss in diabetic rats²⁴.

Inhibition of α -glucosidase and aldose reductase is useful in treatment of both postprandial hyperglycemia and hyperinsulinaemia, and thereby in improving sensitivity to insulin. α -Glucosidase is a key enzyme in carbohydrate digestion in the small intestine^{19,20,23}. It is further helpful in reducing the stress on beta cells of the pancreas. Therefore, α -glucosidase inhibitors could delay digestion of dietary carbohydrates to reduce postprandial glucose. In fact, α -glucosidase inhibitors have become the most common oral agents used to improve postprandial hyperglycemia since their introduction^{1,8,16,24}. The only reason inhibiting the usage of synthetic analogues of α -glucosidase inhibitors is undesirable side effects, such as flatulence, diarrhea, and abdominal cramping^{18,25}. Natural products and compounds derived from natural products have been shown promise as α glucosidase inhibitors with low side effects. A few illustrated examples include *Rhus chinensis*, *Commelina communis* L., *Punica granatum*, *Saururus chinensis* Baill., *Eunonymus elatus* Linn., *Piper nigrum* Linn., *Swertia chirata* Linn. These compounds do not induce hypoglycemia and have a good safety profile, although gastrointestinal adverse effects may limit long term compliance to therapy. *C. orchoides* and *T. terrestris* could be further added to this list as well.

In conclusion, it can be stated that the ethanolic extract of *W. coagulans* acts via the modulation of enzymatic activity and can be

further evaluated as a antidiabetic medicinal plant. Further in-depth research on insulinotropic activity may add to the established molecular mechanism validating its role as a valid alternative for synthetic agents.

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