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ANTIGLYCATION AND ALDOSE REDUCTASE ACTIVITY OF *SWIETENIA MAHAGONI* LEAF EXTRACT

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ABSTRACT

Aldose reductase is a prime target enzyme for the control of retinopathic complications along with controlling glycation of proteins in diabetic subjects. *Swietenia mahagoni* is a traditional medicinal plants used by folklore to treat various disorders such as hypertension and diabetes. *Swietenia mahagoni* leaf methanol extract was assayed for in-vitro aldose reductase inhibition and antiglycation properties. The extract showed 30% inhibition inhibition on aldose reductase activity and 53.65±2.5% inhibition on glycation and the antiglycation property increased with increase in glucose concentration. The anti-glycation potential was more than amino guanidine a standard used in antiglycation agent.

KEYWORDS: *Swietenia mahagoni*, antiglycation, retinopathy, diabetes, aldose reductase.

1. INTRODUCTION

Diabetic Mellitus (DM) is one of the leading causes of death across the globe particularly in developing world. DM commonly referred as diabetes, is a group of metabolic disorder in which there is high blood sugar levels for a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst and increased hunger. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced.[1]

The convesion of glucose to sorbitol by Aldose reductase (ALR) was 1st identified by Hers in 1956 [1], in the seminal vesicles where glucose is converted into fructose to provide an energy source for sperm. The accumulation of sorbitol by the increased actvity of ALR is responsible for diabetic complication such as cataract formation in eyes. ALR require NADPH as its co-enzyme, so in the cell the NADPH level wiil be decreased with the increase ALR activity. This decrease in the level of NADPH will affect the glutathione(GSH) and nitric oxide (NO) production, that is glutathione and NO synthesis will decrease because NADPH is also required as coenzyme for NO-synthase and GSH-reductase. NO is a powerfull vasodilator, the decreased level of NO will affect the blood flow. Glutathione have the anioxidant property, it is a powerfull reducing agent. The decreased level of GSH will cause oxidative stress and this will leads to the hemolysis. The accumulated sorbitol in the cells will cause osmatic imbalance which results in damage to the cells, which eventually leads to diabetic cataract formation because the exess sorbitol in cell have the difficulty to diffuse across the cell membranes. The accumulation of sorbitol in cell will leads to osmatic swelling, changes in membrane permeability and also oxidative stress resulting in tissue injury.

Since the Aldose reductase (ALR) is the 1st and rate limiting enzyme in the polyol pathway, the enzyme has been a potential target for anti-retinopathic drug design; therefore the inhibition of aldose reductase has been an attractive approach to the prevention and treatment of diabetic eye complications.[2] Even the literature shows inhibition of ALR can be effective in the prevention of some diabetic complications including cataract, retinopathy, nephropathy and neuropathy.

2. MATERIALS AND METHODS

2.1 Collection and preparation of samples

The leaf of S.mahagoni plant was collected from the Mahajana college campus. The collected leaves of plant were thoroughly washed under running water to remove adhering dirt and other foreign particles, dried overnight at 50 °C, powdered, passed through 60 mesh sieve and stored in air tight container at 4 °C till further use.

2.2 Preparation of methanolic extract of S.mahagoni

The methanolic extract of the sample was prepared by extracting powder material with methanol in a mechanical shaker for 24 h. Filtered to separate insoluble component and the filtrate was freeze dried. The brown colored methanol extract obtained after freeze drying was used in all in-vitro.

2.3 ALR inhibition

i. ALR extraction from chicken eye

Crude Aldose reductase enzyme solution was prepared from chicken lenses as described by Haraguchi et al.[3] with some modifications. The 20 fresh chicken eye balls procured from the slaughterhouse are kept frozen at -20°C until the day of the experiment. The lenses were removed by lateral incision of the eye, washed with cold distilled water and kept cold. The lenses were deposited in a cold glass tube and the homogenized using a Teflon pestle in 3 volumes of cold 135 mM phosphate buffer (pH 7.0), containing 10 mM b-mercaptoethanol. The homogenate was centrifuged at 13,369 RPM for 15 min. The supernatant fluid was placed in a plastic 50 mL conical centrifuge tube and the protein in this fluid was then precipitated, by addition, drop by drop, of a solution 75 % $(NH_4)_2SO_4$. The pellet obtained by centrifugation was dissolved in the same buffer and used as enzyme preparation.

ii. Estimation of protein concentration of crude ALR extraction

The protein concentration was estimated by using the biuret method. A known concentration albumin (5 mg/ml) used as standard protein solution. Different conc. of the standard solution (0.2ml to 1ml) was taken in different test tubes and the known volume of the test solution (0.2ml to 1ml) was also taken in the separate test tube. Add the distilled water to all the test tubes including unknown up to the final volume of 2ml. then add 4ml of bi-uret reagent (4.25g of potassium sodium tartarat + 2.5g of KI + 4g of NaOH dissolve in 500ml of distilled water) to all the test tubes. Incubating all the test tubes for 30 min at room temperature. After the incubation period take the absorbance of all test tubes at 540nm using colorimeter. Plot the graph of absorbance of standard protein against conc. of standard protein solution to get the standard curve, using this curve as reference to calculate the conc. of unknown sample (ALR extraction) using the following formula;

$$\text{conc. of unknown} = \frac{\text{O.D. of standard} - \text{O.D. of unknown}}{\text{O.D. of standard}} \times \text{conc. of standard.}$$

iii. ALR inhibition assay

The bioassay procedure with some modifications was followed according to Haraguchi (1996) [4]. Bioassay solutions of plant extracts were prepared by dissolving 10 mg/ml of concentration in methanol. The reaction mixture was prepared at 25 °C, with a total volume of 1.8 mL, containing 50 mM Na-phosphate buffer (pH 6.2), 0.125 mM NADPH, 400 mM $LiSO_4$, and 0.5ml of enzyme preparation and 3 mM $_{dl}$-glyceraldehyde as a substrate with or without plant extract. The reaction was initiated by addition of NADPH and incubated for 45 min. Absorbance was read at 340 nm at the beginning and at the end of the reaction. 1M $NaHCO_3$ was added at the end of the incubation period. A negative control was prepared using methanol in phosphate buffer (pH 6.2). The bioassays were run in triplicate. At the end, the inhibitory activity of the extract was calculated using the following formula

$$\%ALRI = \frac{\text{Abs (Neg. Ctrl)} - \text{Abs. (Extract)}}{\text{Abs (Neg cntrl)}} \times 100$$

2.4 Anti-glycation assay

The method of matsuura (2002) [5] was followed with minor modifications. The experiment was carried out with the final reaction volume of 1.5ml. The reaction mixture consisted of 0.75ml of BSA (mg/ml) and various concentrations of 1M glucose (0.03ml, 0.06ml, 0.09ml, 0.12ml and 0.15ml) and various concentrations of extract the rest of the volume was made up with the Phosophate buffered saline (PBS) (pH 7.3) to 1.5 ml. Aminoguanidine (AG) was used as a standard inhibitor. The reaction mixtures were incubated at 60 °C for 3 day with a pinch of sodium Azide (antimicrobial agent). After the incubation period the reaction mixtures were transferred to the separate eppendorf tubes, 0.02ml of TCA was added (100% W/V) to precipitate the glycated BSA and the sediment was further settled by centrifuging at 6000 RPM for 4-5 min at 4 °C. The supernatant was discarded and the pellet was re-dissolved in the alkaline PBS. Absorbance was read at 350 nm in a spectrophotometer. A control was also run without adding the inhibitors. Percent inhibition was calculated using the following formula.

$$\%\ \text{inhibition} = \frac{(\text{abs.of control} - \text{abs.of sample})}{\text{abs.of control}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Protein concentration

From the standard graph, the protein content in the ALR extract was 1.25 mg/mL **(Figure 1).**

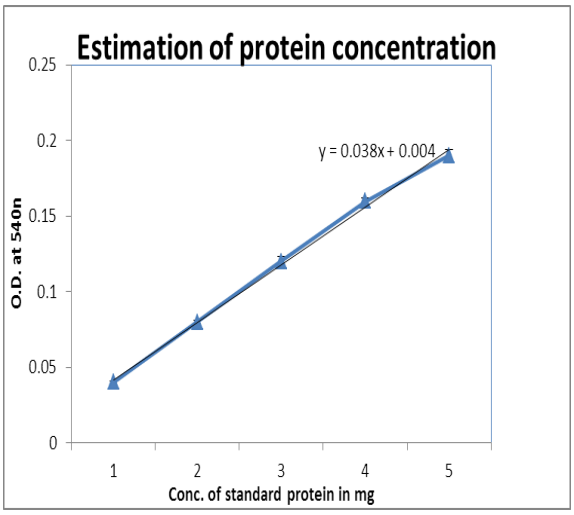

Figure: 1. Protein standard graph

3.2 ALR inhibition assay

The Figure. 2 shows the inhibitory activity of ALR by the various concentrations of the leaf extract of S.

mahagoni.The extract showed a maximum of 30% inhibition.

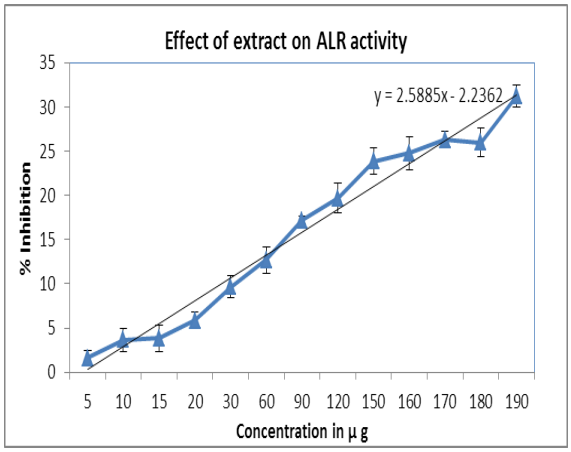

Figure: 2. Inhibitory activity the extract on ALR (in %).

3.3 Antiglycation

The effect of the extract on the inhibition of BSA glycation is presented in the Tables 1-5. From the results it is evident that the anti-glycation activity of the sample increases with the increase in extract concentration and also with the increase in glucose concentration.

Table: 1. Antiglycation activity of the extract in 100 mM glucose concentration

Sl.No.	Conc. of extract in µg/ml	% inhibition	AG (5Mm)
1	45	42.56±0.8	23.93±0.6
2	90	45.69±1.3	
3	150	50.04±1.9	

AG-Amino guanidine *The values presented are the mean of triplicate experimental values.

Table: 2. Antiglycation activity of the extract in 200 mM glucose concentration

Sl.No.	Conc. of extract in µg/ml	% Inhibition	% inhibition by standard AG (5Mm)
1	45	43.26±2.0	26.34±0.7
2	90	46.58±1.5	
3	150	50.36±1.3	

AG-Amino guanidine *The values presented are the mean of triplicate experimental values.

Table: 3. Antiglycation activity of the extract in 300 mM glucose concentration

Sl.No.	Conc. of extract in µg/ml	% Inhibition	% inhibition by standard AG (5Mm)
1	45	46.21±1.7	26.98±0.6
2	90	49.66±1.2	
3	150	52.39±1.5	

AG-Amino guanidine *The values presented are the mean of triplicate experimental values.

Table: 4. Antiglycation activity of the extract in 400 mM glucose concentration

Sl.No.	Conc. of extract in µg/ml	% Inhibition	% inhibition by standard AG (5Mm)
1	45	48.54±1.9	28.63±1.1
2	90	50.36±1.3	
3	150	53.65±2.5	

AG-Amino guanidine *The values presented are the mean of triplicate experimental values.

Table: 5. Antiglycation activity of the sextract in 500 mM glucose concentration

Sl.No.	Conc. of sample in µg	% Inhibition	% inhibition by standard AG (5Mm)
1	45	50.36±1.6	30.97±1.3
2	90	53.65±1.0	
3	150	58.86±1.8	

AG-Amino guanidine *The values presented are the mean of triplicate experimental values.

4. DISCUSSION

Elevated glucose level in the plasma leads to various pathophysiological conditions which will ultimately leads to developments of secondary complications of diabetes. The glycated proteins lead to microvascular complications in the long run.[6] Hence controlling of glycation of the proteins is a primary target to control the secondary complications associated with microvascular systems such as nephropathy and neuropathy. All over the world there is an immense amount of research is being carried out for the drugs which have anti-glycation potential. Thousands of plants have been screened for the anti-diabetic and anti-glycation properties. *Swietenia mahagoni* is a medicinal plant used worldwide to treat various ailments. The plant has been investigated for various pharmacological activities and has found to be promising pharmacological agent.[7,8,9,10] The present study is an extension of such an effort to screen *Swietenia mahagoni* for its anti-glycation and aldose reductase potential. The study utilizes the methods which simulate the physiological conditions where the glycation of proteins happen. Results show that the plant has anti-glycation potential which was comparable to the standard anti-glycating agent Amino guanidine. The activity can be attributed to the presence of notable amount of polyphenols, saponins and flavanoids in the extract.

Prolonged hyperglycemia leads to channeling of glucose into polyol pathway culminating in the production of soirbitol. Sorbitol changes the osmolar balance inside the cell leading to osmotic damage of the cell, and also use of NADPH for the pathway results in depletion of the NADPH thus resulting in oxidative stress.[11] Thus controlling flux through polyol pathway is a potential target for the control of sorbitol biogenesis. Aldose reductase is a rate limiting enzyme for the pathway hence aldose reductase inhibition is a primary target to control flux of glucose into polyol pathway.

The traditional medicine plant S. mahagoni is being used from thousands of years in various medicinal practices. Recent studies identified the phytoconstituents such as cyclomahagenol a tetracyclic triterpene, limonoids, swiemahogins, swietenin swietenolid in the leaves of the plant and their medicinal value. In this present study the values in the table-5 represent that the leaf extract of S. mahagoni has significant amount of polyphenols, saponins and flavanoids, which may attribute to the anti-glycation and aldose reductase potential of the plant extract. There is need for seperation and characterization of bio-active principles. The data obtained from this study is a preliminary support for the aldose reductase inhibition and anti-glycation activity of the plant extract thus it forms a platform for further investigations involving ex-vivo and in-vivo support for the validation of the present observation.

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