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Evaluation of lens aldose reductase inhibitory potential of fruit extracts of *Terminalia bellerica Roxb.*

Kushagra Dubey [1]*, Raghvendra Dubey [2], Revathi A Gupta [2], Arun Gupta [2]

[1] Smriti College of Pharmaceutical Education, Indore (M.P.), India

[2]Faculty of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore (M.P.), india

ABSTRACT

Aim: The aim of the research work was to carried out the antidiabitic inhibitory potential of the fruit extracts of T. Bellerica Roxb. against Aldose Reductase enzyme.

Material & Methods: The dried fruit powder of T. Bellerica Roxb was extracted by successive solvent extraction method to obtained methanolic and aqueous extract. Both the extracts were screened for *invitro* aldose reductase inhibitory activity in purified goat lens using Hayman and Kinoshita method in which decrease in NADPH concentration was estimated at 340nm using UV Visible spectrophotometer.

Result & Discussion: From the result it was observed that both the extracts inhibit AR activity, but at different extent. From dose response curve it was found that methanolic extract (ME) is more effective then aqueous extract (AE) with IC_{50} values of 25.26 ±0.23 µg/ml and 109.14 ±1.15 µg/ml respectively.

Conclusion: In the end it was concluded that among the two extracts, methanolic extract of T. Bellerica Roxb is potent in inhibiting the aldose reductase enzyme which contribute major role in the diabetes complication.

Keywords: Aldose Reductase, Goat Eye Lens, NADPH, Methanolic Extract, Aqueous extract.

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***Address for Correspondence:**

Mrs Kushagra Dubey, Associate Professor, Smriti College of Pharmaceutical Education, MR-11, Nipania, Indore (M.P.) 452001

INTRODUCTION

Diabetes mellitus is a chronic disorder of carbohydrate, lipid and protein metabolism characterized by persistent elevation of blood glucose level in the body. It is a major risk factor of cataract which is the leading cause of blindness over the world. Various pharmacological strategies are used to prevent the cataract formation, among them aldose reductase inhibitors have received much attention because of its involvement in the pathophysiology of diabetic complications including cataract. Aldose reductase is found in almost all mammalian cells, but at high levels in organs such as the cornea, lens, retina, kidney and sciatic nerves, which are affected by diabetic complications. When polyol pathway activity is increased, it causes accumulation of polyol in lens fibers, influx of water and generation of osmotic stress, osmotic swelling, changes in membrane permeability, and oxidative stress culminating in tissue injury, that's why it has got special attention in the clinical treatment of secondary complications of diabetes. Formation of sorbitol is a result of enzymatic degradation of glucose through polyol pathway which is a tissue poison whose accumulation increases osmotic pressure and may damage the tissues by causing them to swell. Since aldose reductase is localized primarily in lens epithelial cells, it is possible to prevent cataract *via* inhibition of the activity of aldose reductase[1]

Terminalia bellerica also referred to as, Beleric Myrobalan belonging to family Combretaceae is found in the Indian subcontinent, Srilanka, and South East Asia, upto 1200 meters in elevation, in a wide variety of ecologies. The phytoconstituents isolated from various parts of the plant include alkaloid, coumarin, flavones, steroids (β-Sitosterol), lignans (termilignan, thannilignan), tannins (gallic acid, ellagic acid), glycosides (fructose, sucrose, galactose), terpenoid (belleric acid and chebulagic acid), saponin (bellericoside and bellericanin). Terminalia bellerica is one such plant showing multifarious medicinal properties viz. analgesic activity, antibiofilm activity, anticancer activity, antidepressant activity, antidiabetic activity, antidiarrhoeal activity, anti-ulcer activity, immunomodulatory activity, anti-spasmodic and bronchodialatory activity, antifertility activity, antihypertensive activity, antifungal, antimicrobial activity, anti-inflammatory activity, antioxidant activity[2]. In the present study an attempt is

made for evaluation of antidiabetic potential of extracts of fruits of T. Bellerica Roxb. by using aldose reductase inhibition assay.

MATERIALS AND METHODS

Plant Materials

The fruits of T. Bellerica were purchased from herbal supplier in indore (Madhya Pradesh) and were identified by the Department of Pharmacognosy, College of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore (M.P.). The fruits were later air-dried, powdered and stored in an air-tight container for further use.

Shimadzu UV 1800 UV Visible spectrophotometer, Continue soxhlet extractor and Chemicals from Sdfine, Loba Chem and HiMedia Lab were used.

Preparation of Extracts

The dried and powdered fruits were soxhlet extracted three times with petroleum ether for 4hr at 60°C. After drying and levigation, one part of the residues were inverse flow extracted 10 times with 70% methanol for 4hr at 85°C, then were filtrated and the other part was extracted with distilled water for 48hr under reflux condition. The extracts was evaporated to dryness under vaccum and the dried extracts were screened for phytochemical analysis. [3]

Preparation of Enzyme

The lenses were quickly removed from goat eyeballs which were obtained from local abattoir soon after slaughtering. Lenses (100 to 200 g) were homogenized in 3 volumes of cold distilled water in a homogenizer and centrifuged at 10,400 RPM at 0-4°C for 15 minutes to remove insoluble material. Saturated ammonium sulfate was added to the supernatant fluid to 40% saturation. After the thick suspension had been allowed to stand with occasional stirring for 15 minutes to ensure completeness of precipitation, it was centrifuged, and the precipitate was discarded. Additional inert protein was removed by increasing the ammonium sulfate concentration to 50% saturation and centrifuging the mixture. This two-step procedure appeared to give a cleaner separation of aldose reductase activity from contaminating protein than could be achieved with a single ammonium sulfate precipitation. Aldose reductase activity was then precipitated from the 50% supernatant solution by the addition of powdered ammonium sulfate to 75'% saturation and was recovered by centrifugation. The precipitate obtained was used for the enzymatic assay.[4]

Aldose reductase inhibitory assay

For determining the aldose reductase inhibitory activity a sample cuvette was taken containing mixture of 0.3mL of enzyme extract, 0.5mL NADPH (0.104mM), 0.75mL sodium phosphate buffer (pH 6.2, 0.1M), 0.1 ml extracts (AE and ME) and 0.7 mL of deionized water. The above mixture was incubated at 30ºC for 10 min. and 0.75mL D,L-glyceraldehyde (10mM) was added to substrate and the absorbance was recorded at 340 nm for 3 minutes at 30 sec. interval. Absorbance was taken against a reference cuvette containing all components but not DL-glyceraldehyde. Quercetin was used as standard.The assay was performed in triplicate. IC_{50} value and Percentage inhibitions was calculated from a dose–response curve. [5]

RESULTS AND DISCUSSION

AR inhibitory activity was screened for methanolic and aquous extracs of fruits of T. Bellerica and it was found that both the extracts were significantly inhibit goat lens aldose reductase to various extents with IC_{50}values ranging from 25.26µg/mL to >100 µg/mL, as showed in Table 1. Highest percentage of inhibition was shown by methanolic extract [IC_{50} (25.26 ±0.23 µg/mL)] followed by aqueous extract [IC_{50} (109.14 ±1.15 µg/mL)]. From the result it was clearly indicated that the methanolic extracts is more potent inhibitor than aqueous extract.

Table 1: Qualitative phytochemical analysis of fruit extract of Terminalia bellerica

Phytochemicals	Aqueous Extract	Methanolic Extract
Alkaloids	+	+
Phenol	+	+
Amino Acid	-	-
Flavonoid	+	+
Saponin	-	-
Tannin	+	+
Quinone	-	+
Carbohydrate	-	-
Glycoside	+	+
Steroids/Terpenoids	-	-

Table 2: Aldose reductase inhibitory activity of Aqueous and Methanolic extracts of T. Bellerica

Extract of TBF	Percentage Inhibition						IC_{50}
	50 µg/ml	75 µg/ml	100 µg/ml	125µg/ml	150 µg/ml	175µg/ml	
AE	22.93±0.23	33.40±1.34	42.87±0.54	55.33±0.89	61.80±1.45	70.27±0.12	109.14 ±1.15
ME	61.24±1.23	66.36±0.56	71.48±2.34	76.60±1.89	82.72±1.09	88.84±1.89	25.26 ±0.23
Standard	72.75±0.23	78.62±0.45	84.5±1.89	90.37±0.67	99.25±0.21	105.12±0.87	20.45 ±0.67

TBF: Fruits of T. Bellerica. AE: Aqueous Extract, ME: Methanolic Extract, IC_{50}: 50% Inhibitory Concentration

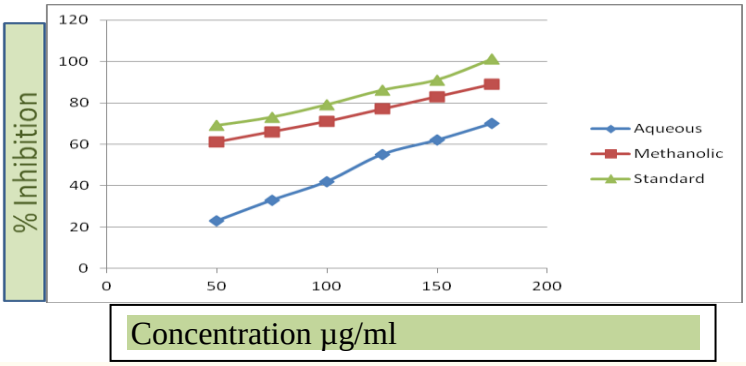

Figure 1: Graphical Plot of Aldose reductase inhibitory activity of Aqueous and Methanolic extracts of T. Bellerica

CONCLUSION

In conclusion, the present investigation suggests that the fruit of T. Bellerica showed significant aldose reductase inhibitory activity in vitro may be due to the presence of the different type of constituents. However further study is necessary to know the exact mechanism of action and the compounds responsible for the AR inhibitory activity[5]. From the result it can be concluded that the methanolic extract of fruit could be helpful in the treatment of cataract and can be used effectively for the management of diabeties complications.

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