



Preclinical evaluation of anticataract activity of different fractions isolated from methanolic extract of whole plant of *Hygrophila auriculata* on isolated goat lens: By *in-vitro* model

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ABSTRACT

In the present study investigate effect of different fractions isolated from the methanolic extract of whole plant of *Hygrophila auriculata* on cataract induced by glucose. Goat eye lens were divided into five groups. Group I lens were incubated in artificial aqueous humor with glucose concentration 5.5 mM (normal control). Group II lens were incubated with glucose concentration 55 mM (toxic control). Group III, IV and V lens incubated with glucose concentration 55 mM were incubated along with fraction A (4mg/ml), fraction B (4mg/ml) and fraction F (4mg/ml) and subjected to photographic evaluation for opacity. The grades of opacity was 0, +++, +, ++ and +++ in group I, II, III, IV and V, respectively. Fraction A showed potent prevention on *in vitro* glucose induced cataract. Thus, the goat lens model could be used for testing of various anticataract agents.

Key words: Cataract, artificial aqueous humor, lens, *Hygrophila auriculata*

INTRODUCTION

Vision loss due to cataract is related to risk factors including malnutrition, sunlight, smoking, hypertension, aging, and diabetes [1]. Progression of cataracts results in opaque eye lens leading to poor or complete vision loss [14]. Decrease in antioxidant enzyme activities in the cataractous lens points to the importance of antioxidant enzymes in the prevention of oxidative damage to the lens and the subsequent development of cataract [3]. *Hygrophila auriculata* (K. Schum) Heine (syn.) *Asteracantha longifolia* Nees, Acanthaceae are described in ayurvedic literature as Ikshura, Ikshugandha and Kokilasha "having eyes like the Kokila or Indian Cuckoo". The plant is widely distributed throughout India, Srilanka, Burma, Malaysia and Nepal [4]. The whole plant has medicinal properties and it is being used in Ayurveda for various ailments like jaundice, hepatic obstruction, rheumatism, inflammation, pain, diuretic, aphrodisiac, spermatorrhea [5] and in the treatment of dropsy, scanty urine and ascites [6,7]. The plant is known to possess hypoglycemic activity in human subjects [8], antitumor [9], haematinic [10], anti-nociceptive [11], hepatoprotective [12] free radical scavenging and lipid peroxidation [13] activities. The present investigation was to evaluate *in vitro* effect of different fractions isolated from the methanolic extract of whole plant of *Hygrophila auriculata* the development of cataract in goat eye lens model.

EXPERIMENTAL SECTION

2.1 Collection of plant material

The Whole plant of *Hygrophila auriculata* was collected from the deciduous forest of Thirumala in Andhra Pradesh State, India. Samples were authenticated by Dr. Madhavi setti, Department of Botany, Sri Venkateswara University. The Whole plant of *Hygrophila auriculata* were sorted, cleaned and air-dried at room temperature for one week.

These were ground to powder using the laboratory Hammer mill. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

2.2 Preparation of extracts

The powdered materials of *Hygrophila auriculata* (whole plant) were extracted successively with hexane, chloroform and methanol (95%) in Soxhlet apparatus, each for 18 hours. The extracts were concentrated to dryness in Rota evaporator till free from the solvents and obtained yield was respectively 1.58 g/kg, 2.85 g/kg, 18.85 g/kg and 28.41g/kg.

2.3 Isolation of fractions

Thin-layer chromatography method was carried out using silica gel aluminum plate 60F-254, 0.5mm (TLC plates, Merck). The solvent system used for TLC was Petroleum ether/chloroform (9:1). The spots of both marker and extract were applied and the plates were developed and dried with help of a hair dryer. The spots were visualized in UV light and 10% of H₂SO₄ in methanol. The methanolic extract was subjected to column chromatography (silica gel 60-100) for further purification. The column was equilibrated for one hour with petroleum ether at flow rate 5 ml/min. The sample was (2 g dissolve in methanol) was loaded on to the column and five fractions were collected using different eluents (Petroleum ether (two fraction; white powder and another white sticky material), Petroleum ether : Chloroform (9:1) one fraction; green sticky material, Chloroform : Methanol (9:1) one fraction yielded sticky dark brown material, Chloroform : Methanol (9:2) one fraction yielded sticky dark brown material). The yield of five fractions was fraction A 140 mg/g, fraction B 40 mg/g, fraction C 18.8 mg/g, fraction D 32 mg/g and fraction E 20 mg/g (w/w) respectively. From above, fractions C was excluded from pharmacological studies due to low yield. Later two fractions (fraction D and E) were pooled into one fraction (fraction F) based upon their chromatogram on TLC plates. All three fractions were (fraction A,B and F) checked for their anti cataract activity by invitro model on goat lens.

2.4 Phytochemical analysis

Phytochemical analysis of fractions was carried out by different methods [14].

2.5 Lens Culture

A Fresh goat eyeballs were obtained from the slaughter house and immediately transported to the laboratory at 0-4 °C. The lens were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5mM, MgCl₂ 2 mM, NaHCO₃ 0.5 mM, NaH₂ PO₄ 0.5 mM, CaCl₂ 0.4 mM and glucose 5.5 mM) at room temperature and maintain pH 7.8 by addition of NaHCO₃). Ceftriaxone dose 5mg/ml added to the culture media to prevent bacterial contamination

2.6 Induction of *in-vitro* Cataract [15]

Glucose at a concentration of 55 mM was used to induce cataracts. At high concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes over hydration and oxidative stress [16]. This generates cataractogenesis. A total of 30 lens were used for the study. These lens were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM served as normal control and 55 mM served as toxic control) for 72 hours.

2.7 Study Design and Groups

Goat lenses were divided into five groups of six lens each and incubated as follows:

- Group I : Glucose 5.5 mM (normal control).
- Group II : Glucose 55 mM (toxic control).
- Group III : Glucose 55 mM + Fraction A 4 mg/ml.
- Group IV : Glucose 55 mM + Fraction B 4 mg/ml.
- Group V : Glucose 55 mM + Fraction F 4 mg/ml.

2.8 Morphological and Photographic Evaluation

Lenses were placed on a wired mesh with the posterior surface touching the mesh, The pattern of mesh number of squares clearly visible through the lens was observed to measure lens opacity. The degree of opacity was graded as follows:

- 0 : Absence
- +
- ++ : Presence of diffuse opacity
- +++ : Presence of moderate diffuse opacity
- ++++ : Presence of extensive thick opacity

RESULTS**3.1 Phytochemical analysis**

S. No	Fraction	Presence of phytochemicals
1	A	Terpinoids
2	B	Terpinoids, Sterols
3	F	Terpinoids

3.2 Lens morphology in vitro/ photographic evaluation

All six lenses in group I remained transparent whilst all six lens in group II developed dense opacities (figure 1). The opacity progressively increased towards the centre with complete opacification by 72 hours.

Fractions at 4 mg/ml effectively retarded the development of opacity compared to group II. The grades of opacity was 0, +, ++, +++ and ++++ in group I, II, III, IV and V respectively.



Normal (5.5mM of glucose)



Toxic control (55 mM Glucose)



Incubate with Fraction A 4mg/ml



Incubate with Fraction B (4 mg/kg)



Incubated with Fraction F (4mg/kg)

DISCUSSION

Oxidative stress is an important factor in the development of cataracts and the use of antioxidants [17] may be advocated in patients to delay or prevent the formation of cataract. Earlier studies shown that the methanolic extract of *Hygrophila auriculata* whole possess significant anti oxidative effect against STZ induced stress on rats [13]. In present study, fraction A obtained from the methanolic extract of *Hygrophila auriculata* whole plant has produced maximum protection against cataract, compare to other fractions. The phytochemical analysis of fraction A revealed the presence of valuable terpenoids (Table). They have a broad range of biological activities. They function has a powerful anti oxidants and some are reported to have anti diabetic activity [18].

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