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Cassia tora leaves modulates selenite cataract by enhancing antioxidant status and preventing cytoskeletal protein loss in lenses of Sprague Dawley rat pups

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ABSTRACT[1]

Ethnopharmacological relevance: Cataract is the clouding or opacity that develops in the eye's lens and is considered to be an unavoidable consequence of aging due to irreversible lens damage. Free radicals and oxidant species are reported to be the major factor responsible for the onset and pathology of cataract. No pharmacological measures are formulated to treat cataract blindness and surgical removal of the opaque lens is the only remedy till date. Boosting of antioxidant potential of the lens is proved to prevent cataract and many indigenous plants have been screened for anticataractogenic potential in the last decades. The objective of the present study was to determine whether *Cassia tora* leaves; the plant employed in traditional medicine for eye rejuvenation and ailments, can prevent cataract in neonatal rats.

Materials and methods: Cataract was induced by a single subcutaneous injection of sodium selenite at a dose of 4 μg/g body weight on the 10th day and *Cassia tora* leaves was administered orally from 8th day upto 12th day at a concentration of 5 μg/g body weight. After 30 days; lens morphology, oxidant-antioxidant equilibrium, glutathione metabolism, cytoskeletal protein/gene expressions were monitored.

Results: Lens morphology, biochemical analysis and expression studies supported the anticataractogenic effect of *Cassia tora* leaves.

Conclusion: In summary, it can be suggested that the consumption of these leaves afford protection to the lens with its antioxidant action and seems to be a new therapeutic approach against cataract by preventive protection.

1. Introduction

The visual system allows individuals to assimilate information from their surroundings and eyes are complex organs, with many parts that must work together to produce clear vision. The crystalline lens plays the major role in vision, is a transparent, layered structure that focuses light onto retina to produces neural impulses, these signals are processed in the brain and resulting in the process of seeing. The sharpness of vision depends on the clarity of the lens. Lens is encapsulated in a collagenous basement material and a single layer of cuboidal nucleated epithelial cells lie deep to the anterior capsule. As the lens is an avascular tissue, it is reliant upon the metabolically active epithelial cells for delivery of nutrients and other molecules, as well as removal of waste. At the equatorial zone epithelial cells elongate, divide and differentiate to form the regularly arranged lens fibers during which time they lose the organelles and begin synthesizing large quantities of the structural proteins called crystallins and cytoskeletal proteins make up to 90% of soluble proteins. Transparency of the lens is maintained by the proper packing of lens proteins and well defined architecture of the lens fibers. The lens is particularly vulnerable to insult due to the lack of mechanism for replacing or repairing damaged proteins and membranes in the interior of the lens (Bloemendal et al., 2004; Avila et al., 2015) that may interfere with the passage of light through it. Eventually lens has evolved antioxidant systems to defend against the toxic damage of reactive oxygen species (ROS) or free radicals, including enzymatic and metabolic antioxidants.

Cataract, the opacity of eye lens is the foremost cause of blindness globally and accounts for more than half of total blindness (Pascolini and Mariotti, 2012). Cataract is caused by the breakdown of the lens architecture results in the scattering of light entering the eye before reaching the retina. Oxidative stress is regarded as the key factor of cataractogenesis by protein oxidation, disulfide bridges, non disulfide covalent cross links, calcium

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[1] ECT – Ethyl acetate fraction of *Cassia tora* leaves.

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bridges and changes in lens epithelial cells is associated cataract formation (Takamura et al., 2003). Replacement of opaque lens with an artificial material is the current treatment for cataract, but it has its own complications and side effects (Chan et al., 2010). Therefore, significant research has been dedicated to exploring an alternative medicinal treatment for the prevention/delay of cataract.

Herbal medicine offers a virtually untapped reservoir of chemical compounds with many potential uses and renewed interest in this area as a source for new leads in drug discovery programs has been occurring in the recent past. Recognition of medicinal plants with anticataractogenic potential revolutionized the field of ophthalmologic research. *Cassia tora* Linn. (Caesalpiniaceae) is an edible herb grows in moist soil and whole part of the plant is employed for medicinal value and used to treat skin diseases, cough, hepatitis, fever, and hemorrhoids (Bhalerao et al., 2013). The leaves described in Ayurveda for having ophthalmic properties (Ali, 2008) and a rich source of anthraquinones and flavonoids (Jain and Patil, 2010). The present study was aimed to evaluate the therapeutic efficacy of *Cassia tora* leaves in oxidative stress induced cataract models.

2. Materials and methods

2.1. Chemicals

All the chemicals and biochemicals used were of analytical grade and purchased from Sigma India, SRL, Ranbaxy and Spectrochem, India.

2.2. Plant material

Cassia tora Linn. leaves were collected from Kariavattom campus, authenticated by an expert and deposited in the herbarium of Department of Botany, University of Kerala, Thiruvananthapuram, India (Accession No: KUBH 5844). The leaves were dried under shade and extracted with 80% methanol, filtered and the solvent was evaporated. The dry extract was partitioned successively using petroleum ether, ethyl acetate, butanol and water. Each fraction was concentrated again and the bulk of the antioxidant activity was shown by ethyl acetate fraction of *Cassia tora* leaves (ECT). It was dissolved in phosphate buffered saline (prepared in sterile water) for the animal experimentations.

2.3. Experimental animals

Sprague–Dawley rat pups at 8–10 days postpartum were housed along with their mother in polypropylene cages under a day/night cycle of 12 h, at 25 ± 1 °C room temperature. The rats received laboratory chow (Hindustan Lever Ltd., India) and distilled water. All ethical guidelines were followed for the conduct of animal experiments in strict compliance with the Institutional Animal Ethical Committee and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (IAEC-KU-5/2012-13, BC. AA32b).

2.4. Experimental design

The rat pups were grouped into four with 6 pups in each group.

Group I Normal

Group II Normal + ECT (5 μg/g body weight)

Group III Sodium selenite (4 μg/g body weight)

Group IV Sodium selenite (4 μg/body weight) + ECT (5 μg/g body weight)

Group III and Group IV were given a single subcutaneous injection of sodium selenite (4 μg/g body weight) on the 10th day (Ostadalova et al., 1978) while rats of Group I were administered with sterile water. ECT was administered by gastric intubation from 8th day up to 12th day at a concentration 5 μg/g body weight to Group II and IV. Cataract could be visualized from the 15th day with the help of an ophthalmoscope and later on with the naked eye. Animals were euthanized by sodium pentothal injection on the 30th day and lenses were extracted through posterior approach.

2.5. Analytical procedures

2.5.1. Biochemical parameters

Glutathione metabolism in lens were assayed;reduced glutathione (GSH) by the method of Sedlak and Lindsay (1968); gamma glutamyl cysteine synthase (GGCS) by the method of Orolowski and Meister (1971); glutathione peroxidase (GPx) by the methods of Lawrence and Burk (1976) and Agerguard and Jense (1982); glutathione reductase (GR) by the method of David and Richard (1983); glutathione S transferase (GST) by the method of Habig et al. (1974) and gamma glutamyl transferase/transpeptidase (GGT) by the method of Szasz (1969).The oxidant-antioxidant status of lens were evaluated; lipid peroxidation products malondialdehyde (MDA) by the method of Niehaus and Samuelsson (1968); conjugated dienes (CD) by the method of Recknagel and Ghoshal (1996), hydroperoxides (HP) by the method of Mair and Hall (1971); total peroxide concentration (TP) by the methods of Miyazawa et al. (1989) and Cao and Prior (1998). Ferric reducing ability of lens (FRAL) of lens was checked by the method of Benzie and Strain (1996); total antioxidant capacity (TAC) was assayed by method developed by Erel (2004) and the ratio of TP to TAC gave the OSI, an indicator of the degree of oxidative stress (Harma and Erel, 2005). Protein values were determined by the method of Lowry et al. (1951).

2.5.2. Isolation of RNA from lens and RT-PCR study

RNA was isolated from rat lens using trizol reagent (Sigma-Aldrich, St. Louis, MO, USA) as described by Chomczynski and Sacchi (1987). The RNA concentration was determined from the absorbance at 260 nm (BioPhotometer, Eppendorf AG, Hamburg, Germany). All samples had a 260/280 nm absorbance ratio 1.78 ± 0.06. RT-PCR and PCR amplifications were carried out using kits from Thermo Scientific, India. Initial PCR activation for 15 min at 95 °C was followed by 3 steps of cycling process. The primers used for filensin were 5′CCCTGGA A CAAG CTATTAAGCATG3′ (F) and 5′TTCCGGAGGTTTTCGATCTG3′(R), for phakinin were 5′ CTCCAGGCTGAGACAGAATCTTTA C 3(F)′ and 5′ TCATGCCAGTGCTTGGCA T 3′(R). β-actinprimers5′TCCTGTGGCATCCATGAAACTAC3′(F) and 5′AGCACTGTGTTGGCATAGAGGTC3′(R) were used as internal control. Each cycle consists of denaturation for 1 min at 94 °C, annealing for 1 min at 65 °C, extension for 1 min at 72 °C, repeated for 37 cycles and final extension for 10 min at 72 °C. The PCR products were electrophoresed in 1% agarose gels containing 0.05 μg/ml ethidium bromide. The mRNA expression was quantified using a phosphorimager and using the Image Quant software and the relative expression was compared and normalized to the expression of β actin in the same sample.

2.5.3. ELISA analysis of vimentin

Indirect ELISA was performed according to the method of de Echaide et al. (2005) using specific antibodies. Antigen was coated on wells, washed with blocking buffer after 3 h of incubation and incubated with primary antibodies for 3 h at room temperature. After washing, wells were treated with HRP conjugated secondary antibody and washed again. O-dianisidine, citrate phosphate buffer and hydrogen peroxide were added to the wells, kept in dark