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Polyphenols of *Cassia tora* leaves prevents lenticular apoptosis and modulates cataract pathology in Sprague-Dawley rat pups

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ABSTRACT

Cataract is a leading cause of visual impairment worldwide with multifactorial etiology and is a significant global health problem with increasing prevalence with age. Currently, no pharmacological measures are discovered to prevent and treat cataract and a significant number of epidemiological studies have suggested the potential role of antioxidants in the prevention of cataract by scavenging free radicals and preventing lens protein derangement and lenticular cell damage. The main goal of the present study is to evaluate *Cassia tora* leaves; an edible leafy vegetable employed in Ayurvedic and Chinese system of medicine for eye rejuvenation in preventing selenite-induced cataract in rat pups and to identify the active components that produce the effect. ECT pre-treatment effectively restored both enzymatic and metabolic antioxidant levels, membrane integrity and reduced metal accumulation and thus down-regulate epithelial cell death. Gene expression studies also confirmed these findings. ESI–MS analysis of ECT revealed the presence of chrysophanol, emodin, kaemferol, quercetin, stigmasterol and isoquercetin. The study suggests the possible role of *C. tora* in alleviating cataract pathology and presence of many anthraquinones and flavonoids. As it is an edible plant, the incorporation of these leaves in daily vegetables might prevent or delay the onset and maturation of cataract.

1. Introduction

Cataract is a global health problem and accounts half of blindness worldwide [1]. It is a vision impairment condition in which the natural crystalline lens of the eye becomes opaque which causes a gradual and painless loss of sight. The pathogenesis of most cataracts is believed to be the result of multiple factors acting over many years. The balance between the production and catabolism of oxidants by cells and tissue is essential for maintenance of the biologic integrity of the tissue and this is regulated by the antioxidant mechanism. The imbalance between pro-oxidants and antioxidants gives rise to cellular oxidative stress, which plays an important role in the pathogenesis of cataract. This hypothesis is supported by studies that examined the anticataractogenic effect of different nutritional and physiological antioxidants [2]. Oxidative stress resulting in protein and lipid modifications, loss of protein function, ion imbalance, the formation of protein aggregates of high molecular mass etc. and all these which decreases the transparency of lens and resulting in lens opacities. As cataract is a protein oxidation disease, the opacity is irreversible and the only strategy for its treatment is the surgical removal of the opaque lens and replacement with an artificial one. However, the expense of surgery, post operative complications etc. still made this a social burden. Hence exploring a pharmacological measure to prevent or delay cataract pathology is of great interest.

Plants have been the basis of traditional medicines throughout the world and according to World Health Organization, 80% of world's population rely on traditional medicine therapy using plant extracts and their active components for their primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects [3]. Nutraceuticals or functional food based therapy is a novel modality for affording protection against diseases through food sources with further health profit in addition to the basic nutrition. *Cassia tora* Linn. (Family: Caesalpiniaceae) is an edible, annual herb, growing as a wasteland rainy season weed. The whole plant is reputed for its medicinal values and the leaves are found to be used in eye ailments, leprosy, ring worm, flatulence, dyspepsia, cough, bronchitis and cardiac disorders in the Ayurvedic system of medicine [4]. The present work is an extension of our previous

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studies on the reduction of lens protein and lipid modifications by *C. tora* leaves on selenite cataract [5,6] (Sreelakshmi and Abraham, 2015, 2016) and the study is carried out to investigate the potential of *C. tora* leaves in lens epithelial cell death.

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

C. 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 is was partitioned successively using petroleum ether, ethyl acetate, butanol and water. Each fraction was concentrated again and the bulk of the antioxidant activity (radical scavenging capacity) was showed by ethyl acetate fraction of *C. tora* (ECT). It was dissolved in phosphate buffered saline (prepared in sterile water) for the animal experiments.

2.3. 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 procedures

A dose response study was carried using ECT at different concentrations (1, 2.5, 5 and 10 μg/g body weight) against selenite-induced cataract. The minimal effective dose was fixed as 5 μg/g body weight by measuring the activities of Superoxide dismutase, (SOD) Catalase (CAT) and Calcium ATPase (Results not shown). The rat pups were grouped into four with 6 pups in each group.

Group I Control

Group II Control + 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 [7] while ECT was administered by gastric intubation from the 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. The animals were euthanized by sodium pentothal injection on the 15th day for stress markers NF kappa B (NF κ B) and early growth response protein 1 (Egr 1) and on the 30th day for other parameters. Lenses were extracted through posterior approach.

2.5. Analytical procedures

2.5.1. Biochemical parameters

Total phenolic content of ECT was determined using the Folin-Ciocalteu method [8]. Selenium, copper and iron concentrations in the lens samples were assayed by atomic absorption spectroscopy (Shimadzu) after digestion in nitric acid/perchloric acid mixture (5:1) operated with a slit width of 0.5 nm, with the wavelengths set at 190.6 nm for selenium, 324.8 nm for copper and 248.3 nm for iron using specific standards. DNA fragmentation in the lens was evaluated by the methods of [9], SOD was assayed by the method of [10], CAT by the method of [11], Vitamin C by the method of [12], Vitamin A and E by the methods of [13] and [14] respectively, Na^+ K^+ ATPase activity by the method of [15], lens protein was fractionized by the method of [16] and protein values by the method of [17]. Indirect ELISA was performed according to the method of [18] using specific primary antibodies and HRP conjugated secondary antibody.

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 [19]. RT-PCR and PCR amplifications were carried out using kits from Thermo Scientific, India. The primer sequences used are listed in Table 1.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. Electrospray ionization mass spectrometric (ESI–MS) analysis

ESI–MS analysis of column fractions was performed using an Agilent 1100 LC/MSD ion trap MS (Agilent, Palo Alto, CA, USA) equipped with an ESI interface. Nitrogen was used as a nebulizing gas at a pressure of 50 psi at 10L/min, a temperature of 35 °C, and a capillary voltage of −4 kV. ESI–MS analyzes were carried out in the positive-ion mode with the scan range m/z 100–600.

Table 1
Rat specific PCR primer sequences.

Genes	Forward primer	Reverse primer
NFκB	5′ACCTGAGTCTTCTGGACCGCTG3′	5′CCAGCCTTCTCCCAAGAGTCGT3′
egr1	5′CTTCAGTCGTAGTGACCACCTTACC3′	5′CGTGGAGTTGACCAGAAAGTCTGTAC3′
bad	5′CAGTGATCTGCTCCACATTC3′	5′ TCCAGCTAGGATGATAGGAC3′
bax	5′CTGCAGAGGATGATTGCTGA3′	5′GATCAGCTCGGGCACTTTAG3′
caspase 3	5′GGACCTGTGGACCTGAAAAA3′	5′TACCCCACTCCCAGTCATTC3′
bcl_2	5′GCTACGAGTGGGATACTGG3′	5′GTGTGCAGATGCCGGTTCA3′
β actin	5′TCCTGTGGCATCCATGAAACTAC3′	5′AGCACTGTGTTGGCATAGAGGTC3′