In Vitro Anticataract Activity of *Tamarindus Indica* Linn, Againest Glucose-Induced Cataractogeisins

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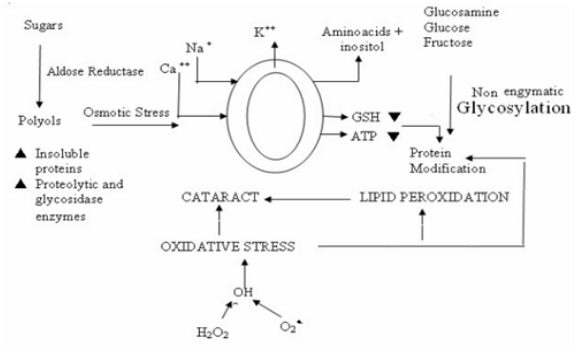
***Index Terms*— About four(minimum) key words or phrases in alphabetical order, separated by commas.**

I. INTRODUCTIO]

The crystalline lens is a biconvex, vascular, transparent structure enclosed by a capsule, a basement membrane secreted by the lens epithelium. The capsule, responsible for moulding the lens substance during accommodation, is thickest in the equatorial zone and thinnest at the posterior pole of the lens. The normal lens is transparent and any congenital or acquired opacity in the lens capsule or substance, irrespective of the effect on vision, is a cataract (Kanski, 2003).

Cataract is the opacification of lens often associated with old age and is a major complication of diabetes mellitus because higher glycosylated hemoglobin levels are significantly associated with increased risk of cataract (Langade, 2006).



Mechanisms associated with cataract

There are mainly two mechanisms involved in the development of cataract. Trypotophan which is an amino acid is present in eye lens in higher concentration. When it absorbs UV radiation, it forms N- Formyl Kynurenine. It may combine with the 3-OH Kynurenine and riboflavin which are lens photosensitizers, they absorb photons form the light and emits electrons. These electrons react with the molecular O_2 to form super oxide anion radical which reacts with Na^+ K^+ ATPase pump in eye and may cause the swelling of the eye and cause lens opacification.

II. PROCEDURE

Preparation of extraction

The fresh leaves of *Tamarindus indica* Linn are collected, dried in shade under room temperature, powdered mechanically and sieved through No. 20 mesh sieve. The finely powdered leaves were kept in an airtight container until the time of use. The extraction was carried out by continuous hot percolation method using Soxhlet apparatus. The solvent used was a mixture of methanol: water in the ratio of 7:3. About 100 g of powder was extracted with 600 ml of solvent. The extract was concentrated to dryness under controlled temperature between 40-50 ^oC.

Preparation of lens homogenate

After 72 h of incubation, homogenate of lenses (10% w/v) was prepared in Tris buffer (0.23 mM, pH 7.8) containing $0.25x10^{-3}$ M EDTA. The homogenate was centrifuged at 10,000 g for 1 h and the supernatant was used for estimation of total protein (TP), determination of the end products of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxides (LH), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione

reductase (GSSH), peroxidase (Px), and glutathione peroxidase (GPx), and the nonenzymatic antioxidant reduced glutathione (GSH).

Determination of enzymatic antioxidants Estimation of superoxide dismutase (SOD)

SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of the homogenate at 480 nm. The reaction mixture contained 150 µl of lens homogenate, 1.8 ml of carbonate buffer (30 mM, pH 10.2), and 0.7 ml of distilled water and 400 µl of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate (Kakkar *et al.,* 1984).

IV. UNITS

Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM, potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidized glutathione and 0.1 ml (10 mg/ml) of bovine serum albumin (BSA). The reaction was started by the addition of 0.02 ml of tissue homogenate with mixing and the decrease in the absorbance at 340 nm was measured for 3 min against a blank. Glutathione reductase activity was expressed as nmoles NADPHoxidized /min/mg lens protein at 30^0C (Racker, 1955).

Estimation of peroxidase (Px)

Peroxidase activity was measured spectrophotometrically by following the change in absorbance at 460 nm due to *O*-dianisidine oxidation in the presence of H_2O_2 and enzyme. Reaction mixture contained 0.2ml of 15 mM *O*-dianisidine, 0.1ml of tissue homogenate and 2.5 ml of 0.1M potassium phosphate buffer pH 5.0 and were incubated at 37^0C for 15 min and the reaction was started with the addition of 0.2 ml of hydrogen peroxide and the absorbance at 460 nm was followed against a blank, spectrophotometrically for about 3-5 min at 37^0C. Unit of enzyme activity defined as µmoles of *O*-dianisidine/min at 37^0C (Lobarzewski and Ginalska, 1995).

V. HELPFUL HINTS

Glutathione peroxidase activity was measured by the procedure of Paglia and Valentine, 1967. The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide, 0.2 ml of glutathione and 0.2 ml of supernatant incubated at 37^0C for 10 min. The reaction was arrested by the addition of 10 % TCA and the absorbance was measured at 340 nm. Activity was expressed as nmoles/min/mg lens protein.

The method was based on the reaction of reduced glutathione with dithiobisnitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. To the homogenate, 0.1 ml of 10% TCA was added and centrifuged. About 0.1 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0) and the absorbance was read at 412nm. Activity was expressed as nmoles/min/mg lens protein (Ellman, 1959).

VI. PUBLICATIONPRINCIPLES

The contents of the journal are peer-reviewed and archival. International Journal of Innovative Research in Technology publishes scholarly articles of archival value as well as tutorial expositions and critical reviews of classical subjects and topics of current interest.

Authors should consider the following points:

1) Technical papers submitted for publication must advance the state of knowledge and must cite relevant prior work.
2) The length of a submitted paper should be commensurate with the importance, or appropriate to the complexity, of the work. For example, an obvious extension of previously published work might not be appropriate for publication or might be adequately treated in just a few pages.
3) Authors must convince both peer reviewers and the editors of the scientific and technical merit of a paper; the standards of proof are higher when extraordinary or unexpected results are reported.
4) Because replication is required for scientific progress,

Papers submitted for publication must provide sufficient information to allow reader stoper form similar experiments or calculations and use the reported results. Although not everything need be disclosed, a paper must contain new, useable, and fully described information. For example, as pecimen's chemical composition need not be reported if the main purpose of a paper is to introduce a new measurement technique. Authors should expect to be challenged by reviewers if the results are not supported by adequated at a and critical details.

VII. CONCLUSION

The present thesis entitled "*In vitro* anticataract activity of *Tamarindus indica* L. against glucose-induced cataractogenesis" deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant *Tamarindus indica* L. belonging to the family Fabaceae, which is traditionally used by the local people and tribals in India for the treatment of inflammatory swelling, tumors, ringworm; useful in disease of blood, small pox, ophthalmia and other eye diseases, earache, snake-bike (Krithikar and Basu, (1981).

Cataract is one of the leading causes of visual disability often leading to blindness. It is an age-related phenomenon over and above, oxidative stress also plays an important role. The situation can be remedied surgically by extirpation of the cataractous lens. The limitations of cataract surgery have stimulated experimental cataract research in laboratory animals and epidemiological studies to determine the incidence, prevalence and risk factors for the development of cataract so as to focus on the preventive aspects of cataract (Gupta *et al*., 1997a)

Cataract was induced *in vitro* with glucose at a concentration of 55 mM in aqueous humor media and incubated for 72 h at room temperature (Langade 2006). After incubation the lens homogenate was used for the estimation of total protein (TP) content, determination of end products of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxides (LH), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSH), peroxidase (Px), glutathione peroxidase (GPx), and non-enzymatic antioxidant reduced glutathione (GSH).

Lipid peroxidation is an autocatalytic process, which is a common cause of cell deth (Bandhyopadhyay *et al.*, 1999). In order to elucidate the protective mechanism of the leaves of *Tamarindus indica*, glucose-induced goat lens was examined for lipidperoxide levels. Decomposition of lipid peroxides initiate the chain reactions that produce reactive carbonyl compounds. The by-products of lipid peroxidation are the toxic compounds malondialdehyde (MDA) and lipid hydroperoxides (LH) whose involvement in cataractogenesis has been suggested, mainly due to its cross linking ability. Lens MDA may be the result of lipid peroxidation of the lens cell membrane or may represent the consequence of its migration from the readily peroxidizable retina or from the central compartment. In our studies, glucose-induced goat lenses showed an increase in malondialdehyde and lipid hydroperoxide levels in lens. Incubation at different concentrations (100 & 200 µg/ml) of extract of *Tamarindus indica*, simultaneously with glucose (55 mM) for 72h caused a significant ($P<0.01$) decrease in the lens malondialdehyde and lipid hydroperoxides and an increase in total protein level. This effect was almost similar to the vitamin E treated group.

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