

**STUDY OF *IN VITRO* ANTICATARACT ACTIVITY OF TAMARINDUS INDICA LINN ON ISOLATED GOAT LENSES**Srikanth Merugu¹, Veeresh B², Deepa Rekulapally^{1*} and Swetha T¹¹Gurunanak School of pharmacy, Ibrahimpatnam, R. R. District, Andra Pradesh, India²G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad***Corresponding author e-mail:** deepa.reddy90@yahoo.com**ABSTRACT**

The antioxidant such as *Tamarindus indica Linn.* was subjected to prevent cataract formation in vitro on galactose induced cataract model. Goat lenses were incubated in artificial aqueous humor containing 55mM galactose (cataractogenesis) and *Tamarindus indica Linn.* extract in different concentrations kept at room temperature for 72 h. Biochemical parameters were studied in the lens homogenate, which are malondialdehyde (MDA), lipid peroxidase and proteins. Galactose-induced opacification of goat lens began 8-10 hrs after incubation and was complete in 72-80 h. Cataractous lenses showed higher MDA ($P < 0.001$), and water-soluble protein content. Lenses treated with *Tamarindus indica Linn.* extract in concentrations of 50, 75 μ g/ml showed higher protein (total proteins) content and prevented formation and progress of cataract by galactose, as evidenced by biochemical parameters.

Keywords: Antioxidant, Vitamin-E, *Tamarindus indica Linn.*, Anticataract.**INTRODUCTION**

A cataract is a clouding that develops in the crystalline lens of the eye or in its envelope (lens capsule), varying in degree from slight to complete opacity and obstructing the passage of light^[1]. It is 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^[2]. Although many cataractogenic factors have been identified, the biochemical background of cataractogenesis is still unknown. It is a multifactorial disease occurs mainly due to formation of large protein aggregates in the lens^[3]. Although a number of agents have been tried for prevention and treatment of cataract but none have proved to useful^[4]. Aldose reductase is a lens enzyme probably involved in the development of this eye problem (cataract). Aldose reductase, key enzyme of polyol pathway, catalyzes the reduction of glucose, galactose and xylose into the corresponding sugar alcohol, sorbitol, which is subsequently metabolized into fructose by sorbitol dehydrogenase. Sorbitol, an

osmolyte leads to osmotic swelling, changes in membrane permeability, leakage of glutathione and myo-inositol and perhaps even the generation of free radicals and hydrogen peroxide, primarily causing for the development of diabetic complications such as cataract, retinopathy^[5].

The use of traditional medicine is widespread and plants are large source of natural antioxidants that might serve as leads for the development of novel drugs^[6]. Some of the plants were scientifically proved for slowing the progression of cataract and some are not yet. *Tamarindus indica Linn* belonging to the family Fabaceae, it is a tree widely distributed in India. The leaves are reported to have antioxidants, and used in eye diseases^[7].

Oxidative mechanism plays an important role in biological phenomena including cataract formation. The formation of superoxide radicals in the aqueous humor and in lens, lens and its derivatization to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to formation of cataract^[8]. We took Vit-E as standard

and measure various parameters including Proteins (total proteins and water soluble proteins) and malondialdehyde (MDA), lipid hydro peroxidase in vitro on goat lenses.

MATERIALS AND METHODS

Plant: Fresh *Tamarindus indica* Linn. leaves were collected from the village Ibrahimpatnam, Rangareddy district, Hyderabad, Andhra Pradesh.

Drugs: Vit E, Penicillin and streptomycin were obtained from QT drugs & chemicals Private Ltd. Meerpet.

Extraction of Plant Material (*Tamarindus indica* Linn.): The fresh *Tamarindus indica* Linn. leaves were collected and allowed to dry (air-dry) for one week. They were powdered mechanically and sieved through No. 20 mesh sieve and stored in an airtight container until the time of use. The extraction was carried out by continuous hot percolation method using a Soxhlet apparatus. The solvent used was a mixture of methanol: water in the ratio of 7:3. About 500 g of powder was extracted with 2.5 l of solvent. The extract was concentrated to dryness under controlled temperature between 40-50 °C^[9].

Eye Balls: Goat eye balls were used in the present study. They were obtained from the slaughterhouse Hyderabad, immediately after slaughter and transported to laboratory at 0-4 degree Celsius.

Preparation of Lens Culture: The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl - 140 mM, KCl - 5 mM, MgCl₂ - 2 mM, NaHCO₃ - 0.5 mM, NaH₂PO₄ - 0.5 mM, CaCl₂ - 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 h. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination^[10]. Galactose in a concentration of 55 mM was used to induce cataract.

Inducer and treating groups^[11]: *Tamarindus indica* Linn. extract was taken into two concentrations 50 µg/ml, 75 µg/ml respectively. A total of 30 lenses were divided into following categories (n=6 in each category):

Group-I: Positive control (Artificial aqueous humor alone)

Group II: Negative control (Galactose 55 mM alone)

Group III: Standard control (Galactose 55 mM + vit E)

Group IV:

A) Inducer + plant extract (*Tamarindus indica* Linn. extract 50 µg/ml)

B) Inducer + plant extract (*Tamarindus indica* Linn. extract 75 µg/ml)

Homogenate preparation: After 72 h of incubation, homogenate of lenses was prepared in Tris buffer (0.23 M, pH 7.8) containing 0.25 X 10⁻³ M EDTA and homogenate adjusted to 10 % w/v. The homogenate was centrifuged at 10,000 G at 4°C for 1 hour and the supernatant used for estimation of biochemical parameters. For estimation of water-soluble proteins, homogenate was prepared in sodium phosphate buffer (pH 7.4)^[11].

Biochemical estimation: protein estimation was done by protein by *Lowry's method*^[12]. The degree of oxidative stress was assessed by measuring the MDA levels by *Wilbur's method*^[13]. Lipid hydro peroxidase was estimated by *Nieshus & Samnesson method, 1986*.

Photographic Evaluation: Lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of hexagons clearly visible through the lens) was observed through the lens as a measure of lens opacity.

Statistical Analysis: The data was presented as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by post hoc-Dunnett's test using Graph Pad Prism software, version 4.01.

RESULTS

Incubation of lenses with galactose 55 mM showed opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification at the end of 72 hrs. As shown in (Table 1) Galactose 55 mM treated lenses (Group-II) also showed significantly low concentrations of proteins (total proteins) in the lens homogenate (P<0.001), very high concentration of MDA (P<0.001) and high concentration of lipid hydro peroxidase (P<0.001) compared with normal lenses (Group-I). Vit E treated lenses (Group-III) and Lenses treated with *Tamarindus indica* Linn. extract (Group-IV) showed higher concentrations of proteins (total proteins) (P<0.001), lower concentration of lipid hydro peroxidase (P<0.001) and lower concentration of MDA (P<0.001) compared with Galactose 55 mM treated lenses (Group-II).

DISCUSSION

Oxidative stress has been suggested as a common underlying mechanism of cataractogenesis, Studies

are ongoing to explore the potential of antioxidant agents against cataractogenesis in various experimental models of cataract. Among these models, the galactose induced cataract is commonly used, as the model is reasonable to assume that factors initiating galactose cataracts in young rats are similar to those involved in the human galactose cataract model ^[14]. Furthermore, the galactose produces a large amount of its reduced form, galactitol, inside the lens that leads to osmotic stress. Accumulation of high concentration of polyols in the lens leads to an increase in the intracellular ionic strength resulting in excessive hydration, eventually loss of membrane integrity and leakage of free amino acids, glutathione and myo-inositol etc. ^[15]

The parameters commonly considered in cataractogenesis are malondialdehyde (MDA), lipidhydro peroxidase and proteins (total proteins). In this study MDA levels, lipidhydro peroxidase levels were significantly higher in Galactose 55 mM treated Group, compared with normal lenses Group (chart 1).

The MDA levels, lipid hydro peroxidase levels were significantly lower in vit E and *Tamarindus indica L.* extract treated groups. Vit E and *Tamarindus indica L.* extract treated groups have also been shown to increase the content of total proteins, retarding the process of cataractogenesis initiated by high galactose concentration. This proves that *Tamarindus indica L.* leaves delay the cataract formation through its anti-oxidant properties.

CONCLUSION

Our study has demonstrated that leave *Tamarindus indica L.* extract offers protection from cataract induction by reducing lens protein insolubilization and lens peroxidation and by increasing lens antioxidant status. Consequently, a reduction in lens apoptosis and epithelial proliferation occurred. The study also reports that administration of the extract after the onset of cataract may reduce cataract progression.

Table 1: Proteins (total proteins) Malondialdehyde (MDA), lipid hydro peroxidase in lens homogenate after 72 h. of incubation

Study Groups	Total proteins	Lipidhydroperoxidase	MDA
Normal lens	16±1.89	3.44±0.37	5.26±0.54
Inducer	6.32±0.56	8.13±0.85	9.20±1.23
Inducer+ standard	13.32±1.22	3.57±0.28	5.38±0.48
Inducer+ plant extract 50ug/ml	11.18±0.92	3.84±0.26	6.13±0.54
Inducer+ plant extract 75ug/ml	13.82±2.72	3.55±0.73	5.65±0.42

Values are mean ± SEM; n=6 in each P <0.01 when compared to normal control; P<0.01 when compared to glucose control (one way ANOVA followed by Dunnett's test). Protein = nmoles/min/mg , MDA = nmoles/min/mg protein, LH = nmoles/min/mg protein.

Chart 1: Comparison of Normal, Inducer and Standard control groups

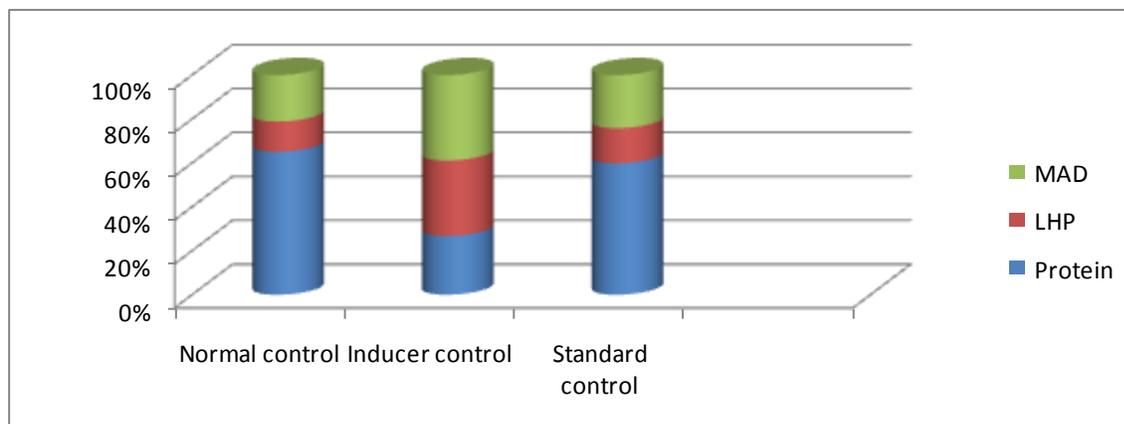


Chart 2: Comparison of Normal, Standard lens groups with plant extract treated lens groups

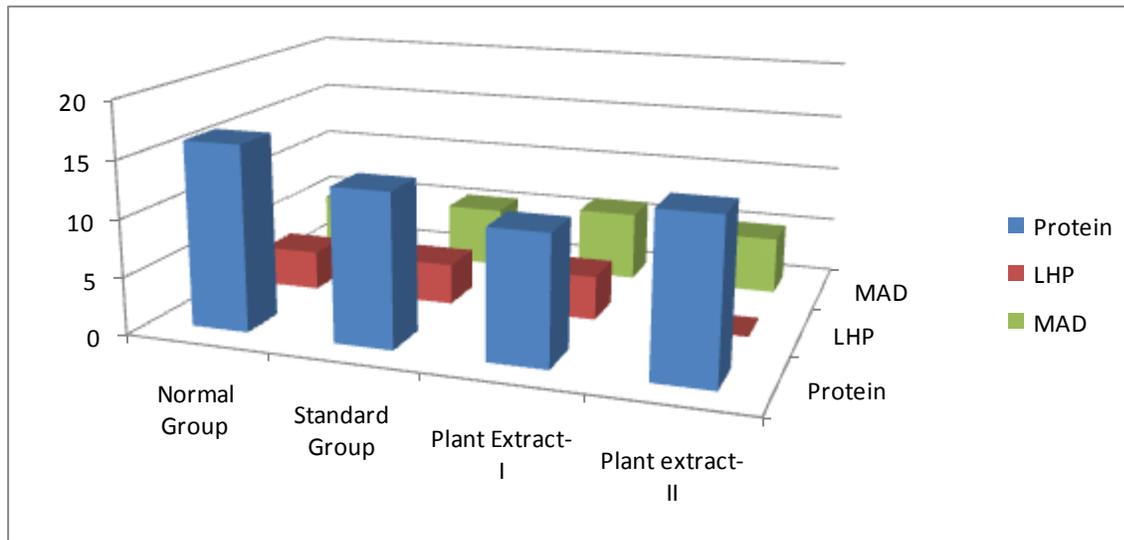


Chart 3: Comparison of inducer control group with Plant extract groups

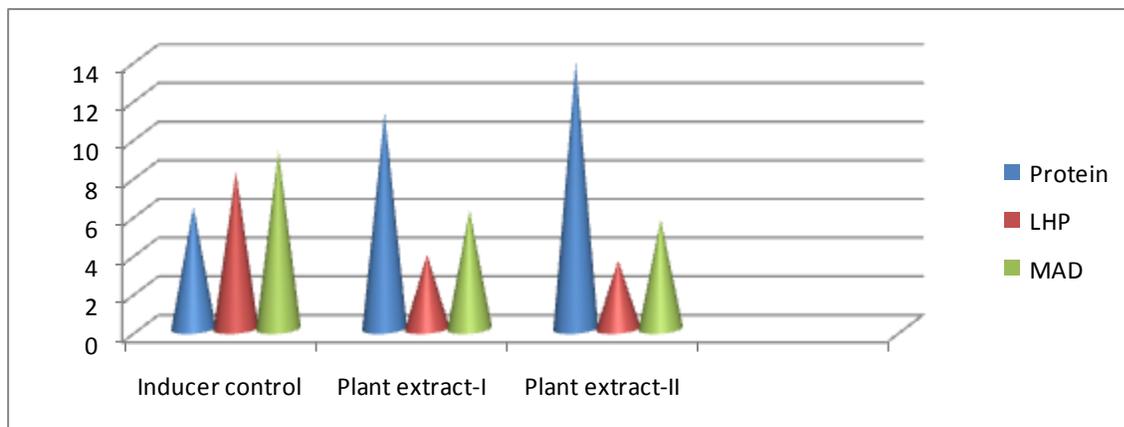


Chart 4: Comparison of control groups with standard and plant extract treated groups

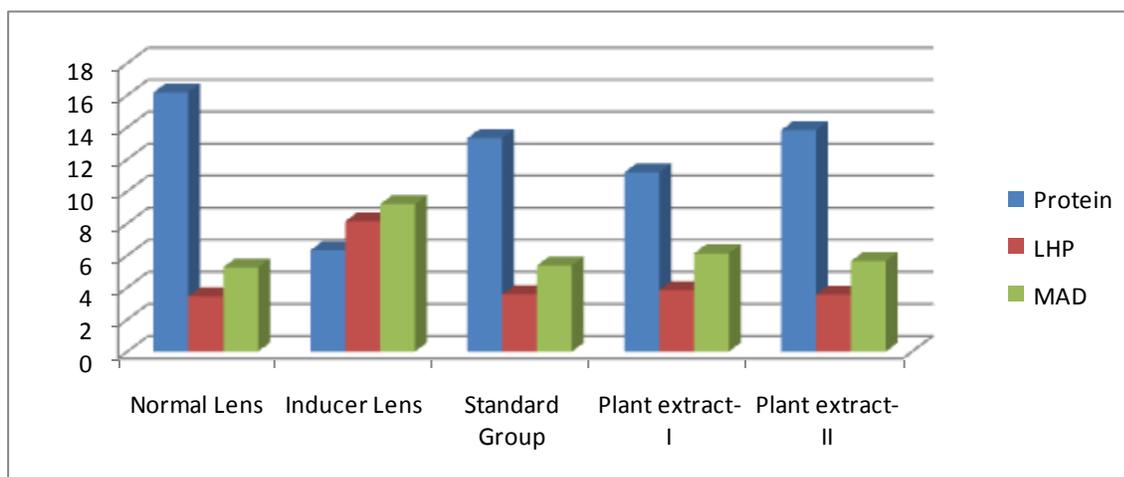
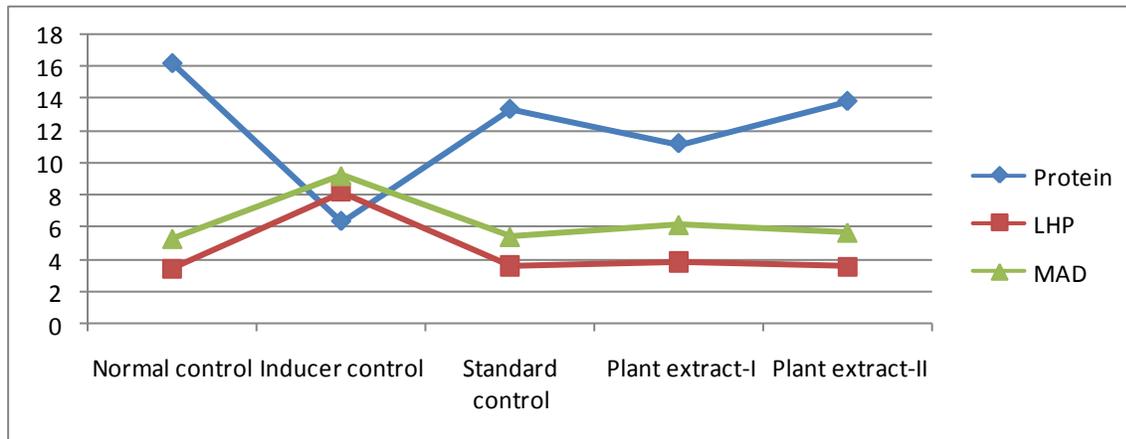


Chart 5: comparison of protein content, malondialdiyhde levels, and lipid hydro peroxidase levels in all groups.



Normal eye



Normal lens



Lens treated with Plant extract I



Lens treated with Plant extract II



Lens treated with standard



Lens treated with inducer



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