In-vitro Study of *Tamarindus Indica* Linn. Against Naphthalene-Induced Cataractogenesis by Using Isolated Goat Lenses

Srikanth Merugu\(^1\), Veeresh B\(^2\), Manjula E\(^4\), Swathi P\(^3\), Bhanuchender RK\(^3\), Ramaraju S\(^3\), Vishnu M\(^1\)

\(^1\)Shri jagadish Prasad Jhabermal Tibrewala university, Jhunjhunu, Rajasthan, India
\(^2\)Department of pharmacology, G.Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad,
\(^3\)Guru Nanak institutions, Technical campus, school of pharmacy, Ibrahimpatnam, RR Dist, Hyderabad, (A.P.), India
\(^4\)Sri Indu Institute of Pharmacy, mangalpally, RR dist, Hyderabad, (A.P.), India

Abstract:

Goat lens are incubated in TC-199 medium containing naphthalene metabolite solution to evaluate the role of naphthalene in the induction of (or) formation of naphthalene induced cataract. Goat lenses are incubated in TC-199 medium with naphthalene metabolite solution, which is renewed till 72h for every 24h. Lenses remain clear during the initial 24h but form shell like opacity around the nucleus by 48h. Opacification becomes more peripheral and wide spread after 72h. Naphthalene oxidized in liver to epoxide and then converted into naphthalene dihydrodiol (stable), which on reaching eye gets converted enzymatically to dihydroxynaphthalene, which auto oxidizes to 1,2-naphthoquinone H2O2. It alkylates proteins, glutathione and aminoacids which generate free radicals. The protein levels are decreased and increased levels of end metabolites like malondialdehyde (MDA) and lipid hydroperoxidase (LH) are noticed which make lens opaque. This suggests the aforementioned changes in the levels of proteins, MDA and LH emphasizes the role of naphthalene in the induction or formation of cataractous lens.

Keywords: Cataract, Naphthalene, Vitamin E, Crystalline lens, *Tamarindus indica* Linn. Naphthalene dihydrodiol.

INTRODUCTION:

A cataract is a clouding or opacity that develops on the clear crystalline lens of the eye or in its envelope, varying in degree from slight to complete opacity and obstructing the passage of light through the lenses. Cataract derived from latin “Cataracta” meaning “waterfall”.

Types of cataract: ¹

- Age related cataract.
- Cortical cataract
- Immature senile cataract (IMSC)
- Mature senile cataract (MSC)

*Corresponding Author
Srikanth M
• Hyper mature senile cataract (HMSC)
• Senile nuclear cataract
• Congenital cataract
• Sutural cataract
• Lamellar cataract
• Zonular cataract
• Total cataract

SIGNS AND SYMPTOMS:
Cataract becomes more opaque.
Loss of visual acuity
Shadows and color vision are less vivid.
Affected eye will have an absent red reflex.
Symptoms are similar to ocular citrosis

CAUSES:
➢ Cataracts develop for a variety of reasons, including long-term exposure to UV light, μ wave radiation.
➢ Secondary effects of diseases such as diabetes, hypertension and old age or trauma; they are usually a result of denaturation of lens protein (causes due to decreased levels of glutathione level in lenses, which is responsible for recrystallisation of protein layers)\(^4\).

NAPHTHALENE INDUCED CATARACTOGENESIS\(^3\)
Naphthalene is a bicyclic aromatic hydrocarbon.

- Chemical formula - C10H8.
- Molecular weight - 128.16.
- Colour - White.
- Odour - Aromatic.

- Solubility - Water-insoluble and soluble in 20% methanol.
- State - Solid at room temperature with a vapour pressure of 0.087mm Hg.
- Chemical structure –

Proposed Pathways for Metabolism of Naphthalene and Its Metabolites

Tissue culture-199 medium is used for the pre-incubation of isolated goat lens. Stock solution of naphthalene dihydrodiol is prepared in 20% methanol at 2.5*10\(^{-3}\)M concentration. The stock solution is diluted at 1:100 ratios and final osmolarity of solution is 295-300 osmol.\(^15\)

Isolated goat lens are incubated in TC-199 medium containing naphthalene metabolite solution. Medium is renewed daily till 72h.\(^5\)

PLANT PROFILE:
Name - Tamarindus indica Linn
Family - Fabaceae
Vernacular names - Amli, Chintachettu, Amlika.
Biological source - Dried leaves of Tamarindus indica Linn.
Parts used - Leaves.
Chemical constituents - Leaves containing flavanoid C-glycosies.
Uses - Leaves are applied to reduce Inflammatory swellings and other Eye diseases like cataract.\(^7\)
MATERIALS AND METHODS:

- **Preparation of extraction**
  The fresh leaves of *Tamarindus indica* Linn. were 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 50-60 °C.

- **Drugs and chemicals**
  Naphthalene, Vitamin E, Alkaline copper solution, Bovine serum albumin, Thiobarbituric acid, Thio chloro acetic acid, Hcl, Butylated hydroxy toluene, Xylenol orange, Ammonium ion sulphate, Methanol, Sulphuric acid. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

- **Experimental protocol**
  A total of 30 Isolated goat lenses were used and divided into the following 5 groups (n = 6) in each group.

  - **Group I**: Artificial aqueous humor alone (Solvent control)
  - **Group II**: Naphthalene 55 mM alone (Negative control)
  - **Group III**: Plant extract (500 µg/ml) + Naphthalene 55 mM
  - **Group IV**: Plant extract (1000 µg/ml) + Naphthalene 55 mM
  - **Group V**: Vitamin E (750 µg/ml) + Naphthalene 55 mM (Standard drug)

- **In vitro lens culture**:
  Fresh goat eyeballs were collected from slaughterhouse, immediately after slaughter and transported to the laboratory and temperature is maintained at 4°C. 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, NaH(PO₄)₂ 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.

- **Preparation of lens homogenate and biochemical evaluation**
  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).
Fig-1 Pictures of isolated goat lenses before and after incubated for 72h in artificial aqueous humor
RESULTS:

Table: 1 Effect of *Tamarindus indica* Linn. Hydromethanolic leaf extract on lens protein, MDA and LH in control and experimental groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/ml)</th>
<th>Protein (mg/dl)</th>
<th>MDA (min/mg)</th>
<th>LH (n moles/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent control</td>
<td>-</td>
<td>10.26</td>
<td>2.30</td>
<td>1.36</td>
</tr>
<tr>
<td>Negative control</td>
<td>55Mm naphthalene</td>
<td>2.18</td>
<td>5.28</td>
<td>4.06</td>
</tr>
<tr>
<td>Plant extract</td>
<td>100µg/ml</td>
<td>5.86</td>
<td>4.26</td>
<td>3.23</td>
</tr>
<tr>
<td>Plant extract</td>
<td>200µg/ml</td>
<td>7.20</td>
<td>3.25</td>
<td>3.22</td>
</tr>
<tr>
<td>Vitamin-E</td>
<td>50µg/ml</td>
<td>8.16</td>
<td>3.02</td>
<td>2.18</td>
</tr>
</tbody>
</table>

Figure: 2 Comparison of control groups with plant groups

Figure: 3 Comparison control groups with standard & plant groups

Figure: 4 Comparison of control, standard groups with plant groups using curves
Conclusion:
The study suggests that the leaf extract of *Tamarindus indica* posse’s anticataract and oxidant activities, which might be helpful in preventing or slowing the progress of cataract. Further investigations on the isolation and identification of active components in the leaves may lead to chemical entities with potential for clinical use in prevention and treatment of cataract.

References: