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CYTOPROTECTIVE EFFECTS OF *MORINDA CITRIFOLIA* (NONI) ON CULTURED LENS EPITHELIAL CELLS

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ABSTRACT: Oxidative stress is one of the factor that initiating development of cataract and describes the events leading to lens opacification, generally cataract is a clouding of the lens of the eye that will affect the vision either partially or completely. Proteins that are present in the lens begin to break down and the lens becomes cloudy. This will be avoided by taking external food supplements that are rich in antioxidants. *Morinda citrifolia* fruit (Noni) juice is rich in antioxidants. This food supplement with lot of nutraceuticals support good health and diminish the effects of aging. To investigate the involvement of Noni in protection against oxidative stress, chick lens epithelial cells were subjected with hydrogen peroxide (100 µM H_2O_2) over a time course, with and without pre-treatment of Noni fruit extract and commercial Noni. Na^+K^+ATPase enzyme estimation was performed to determine the role of Noni juice and Noni extract. Our study revealed that Noni juice and extract preserved physiological functions of chick and human lens epithelial cells when subjected to oxidative stress.

INTRODUCTION: Oxidative stress is one of the risk factors for cataract formation. It describes that oxidative stress is an imbalance between oxidants and antioxidants [1]. Cataracts are the main cause of human blindness, responsible for 48 % of the total cases of blindness [2]. Understanding the patho-physiology of cataract formation is important not only to advance the state of medical knowledge but also for public health purposes [3 - 5]. Apoptosis of lens epithelial cells by various factors can cause cataract formation [6].

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It was found that people at high risk of developing advanced stages of cataract formation, lowered their risk by 25 percent when treated with a high-dose combination of Vitamin C, Vitamin E, beta-carotene and zinc. In the group, which includes people with intermediate cataract formation in one eye but not the other eye, the nutrients reduced the risk of vision loss by about 19 percent [7 - 15].

***Morinda citrifolia*:** *Morinda citrifolia* Linn. is commonly known as Indian mulberry or Noni, has been used in tropical regions as food and folk medicine. The recent use of Noni as a dietary supplement is reported to have a broad range of therapeutic effects, including anti-bacterial, anti-viral, antifungal, antitumor, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune enhancing effects. Noni is rich with vitamin a, beta carotenoids, vitamin e, vitamin C,

vitamin B complex, with all trace minerals like Ca, Mg, K, Zn, Molybdenum and many more flavonoids. These ingredients present in Noni have made it the most powerful antioxidant [16 - 20]. The high antioxidant property of Noni helps to prevent the formation of cataract [21 - 22]. Our recent reports suggest that Noni fruit juice and extract have anti-apoptotic activity in oxidative stress induced lens epithelial cells. The present paper depict the cyto-protective activity of Noni.

MATERIALS AND METHODS:

Lens Organ Culture: The lenses were isolated from chickens brought from slaughter houses. The eyes were removed and the lenses were carefully dissected by a posterior approach. Lens Epithelial Cells (LECs) were separated from each of the dissected lenses by incubating in 1X Trypsin-EDTA solution for 1-2 minutes at 37 °C. 0.5×10^6 cells were cultured in a 6-well culture plate containing 1.5 mL minimal essential medium (MEM199, M-3769; Sigma) containing 10 % FBS for 6 days. Transparent lenses were selected for experimentation. All chick lens experiments (n=6) were performed in the MEM 199 containing 26 mM $NaHCO_3$ as buffer. The MEM 199 was prepared with ion-exchange double-distilled water, sterilized by filtration through 0.22-µm filter with a pH adjusted to 7.4.

Oxidative Stress Induction in the Lens Epithelial Cells: 100 µM H_2O_2 (Sigma) concentrations were used in this investigation. Commercially available Noni and Noni fruit extract (Hexane) were made to final volume of 100 µL and diluted to appropriate concentration and added to the culture medium as required. The cells were washed twice in PBS and the growth medium was replaced and further incubated for 0 h, 3 h, 6 h and 12 h.

This method was used for the subsequent examination with and without the pre-treatment of commercially available Noni and Noni fruit extract. Commercially available Noni and Noni fruit extract treatment to cell cultures involved a 2 hour pre-incubation followed by continued administration of the commercially available Noni and Noni extract in the presence of H_2O_2. Everyday 1 mL of the Fresh medium was replaced. **Fig. 1**. Represents chick lens treated with commercially available Noni and Noni extract.

Na^+K^+ATPase Pump Assay: Cells were induced with H_2O_2 along with Noni Extract (Hexane) and Divine Noni. ATP Assay was performed by standardized protocol of Na^+K^+ATPase [23 - 24]. Assay of inorganic phosphorous sodium potassium dependent adenosine triphosphate (Na^+K^+ATPase) (ATP Phosphohydrolase). Na^+K^+ATPase was assayed according to the method described by Bonting, 1970.

Reagents Used:

1. Tris HCl buffer (92mM, pH 7.5), 11.13 gm of tris dissolved in 900 mL of distilled water and the pH was adjusted to 7.5 mL with 1M HCl. The resulting solution was diluted to 1000 mL with distilled water.

2. $MgSO_4$ solution (5 mM): 1.232 gm of MgSO4 dissolved and made up to 1000 mL with distilled water.

3. KCl solution (5 mM): 0.372 gm of KCl was dissolved in 400 mL of distilled water and the final volume was made up to 1000 mL with distilled water.

4. NaCl solution (60 mM): 3.231 gm of NaCl was dissolved in 400 mL of distilled water and the final volume was made up to 1000 mL with distilled water.

5. EDTA solution (0.1 mM): 0.372 gm of EDTA was dissolved in 400 mL of distilled water and the final volume was made up to 1000 mL with distilled water.

6. ATP solution (40 mM): 0.220gm of ATP was dissolved in 4 mL of distilled water the final volume was made up to 10 mL with distilled water.

7. TCA (10 % w/v): 10 gm of TCA was dissolved in sufficient quantity of distilled water final volume was made up to 100 mL with distilled water.

1 mL of Tris HCl buffer and 0.2 mL of each of magnesium sulphate, sodium chloride, potassium chloride, EDTA, ATP, were added to test tube containing 0.2 mL of homogenate. The mixture was incubated at 36 °C for 15 mins. The reaction was arrested by addition of 1 mL of 10 % TCA, mixed well and centrifuged. The phosphorus content of the supernatant was estimated. **Fig. 2**, **3**. Represents estimation of NaKATPase pump activity (inorganic phosphate group) in wild-and broilertype chick lens epithelial cells.

Phosphorus Content of the Supernatant was Estimated by Modified Metol Method: After incubation the absorbance of standard (S) and test (T) were measured against blank (B) with Ultraviolet - Visible spectrophotometer at 680 nm. The % weight of inorganic phosphate (Pi) was calculated using the following formula;

$$\% \text{ Weight of Pi} = \frac{\text{OD of Test}}{\text{OD of Standard}} \times 5$$

Culturing of Human Lens Epithelial Cells: Same procedure was followed as described in previous experiments except in the case of human lens epithelial cells 15 % FBS Conc was found to be suitable for maintenance of Human lens epithelial cells. **Fig. 4** Represents human lens capsules obtained from cataract patients. **Fig. 5** Represents Human lens treated with commercially available Noni and Noni fruit extract.

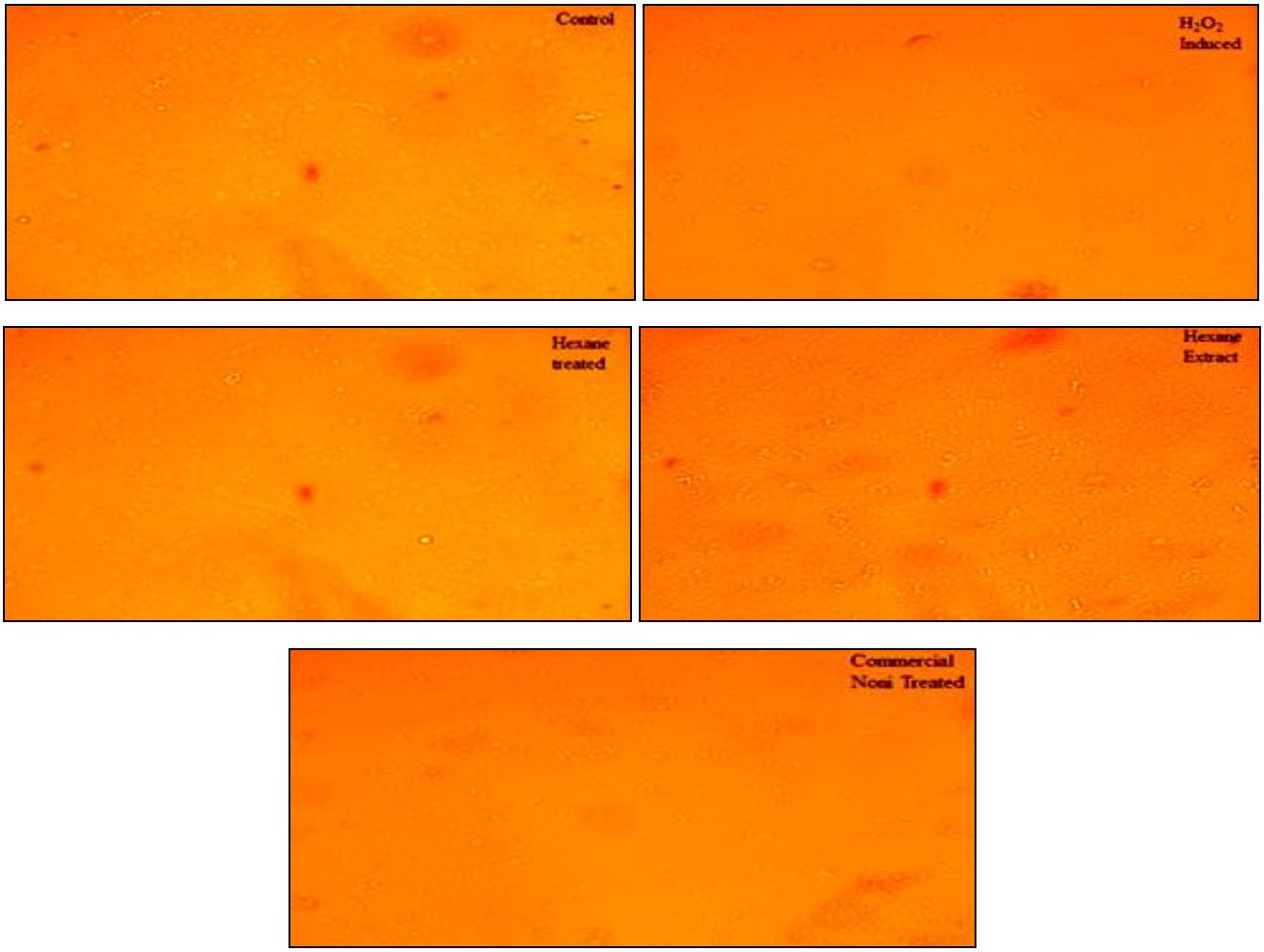

FIG. 1: CHICK LENS TREATED WITH COMMERCIALLY AVAILABLE NONI AND NONI EXTRACT

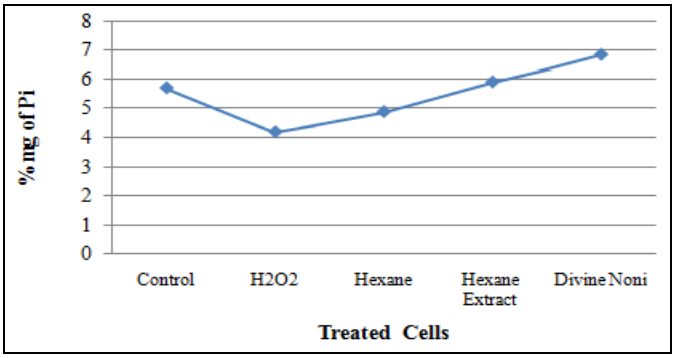

FIG. 2: ESTIMATION OF NaKATPase PUMP ACTIVITY (INORGANIC PHOSPHATE GROUP) IN WILD-TYPE CHICK LENS EPITHELIUM

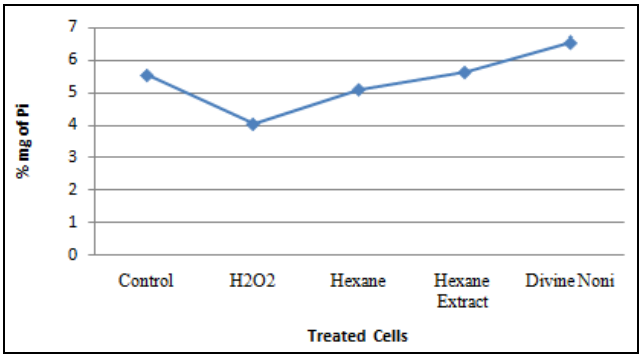

FIG. 3: ESTIMATION OF NaKATPase PUMP ACTIVITY (INORGANIC PHOSPHATE GROUP) IN BROILER CHICK LENS EPITHELIUM

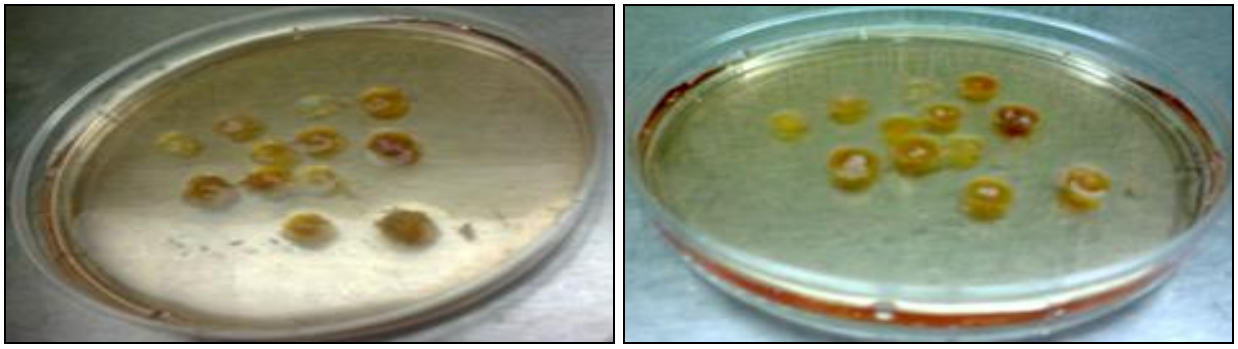

FIG. 4: HUMAN LENS CAPSULES OBTAINED FROM CATARACT PATIENTS. BOTH THE PICTURES SHOW THE SAMPLE SIZE FOR CELL CULTURE AND ANALYSIS

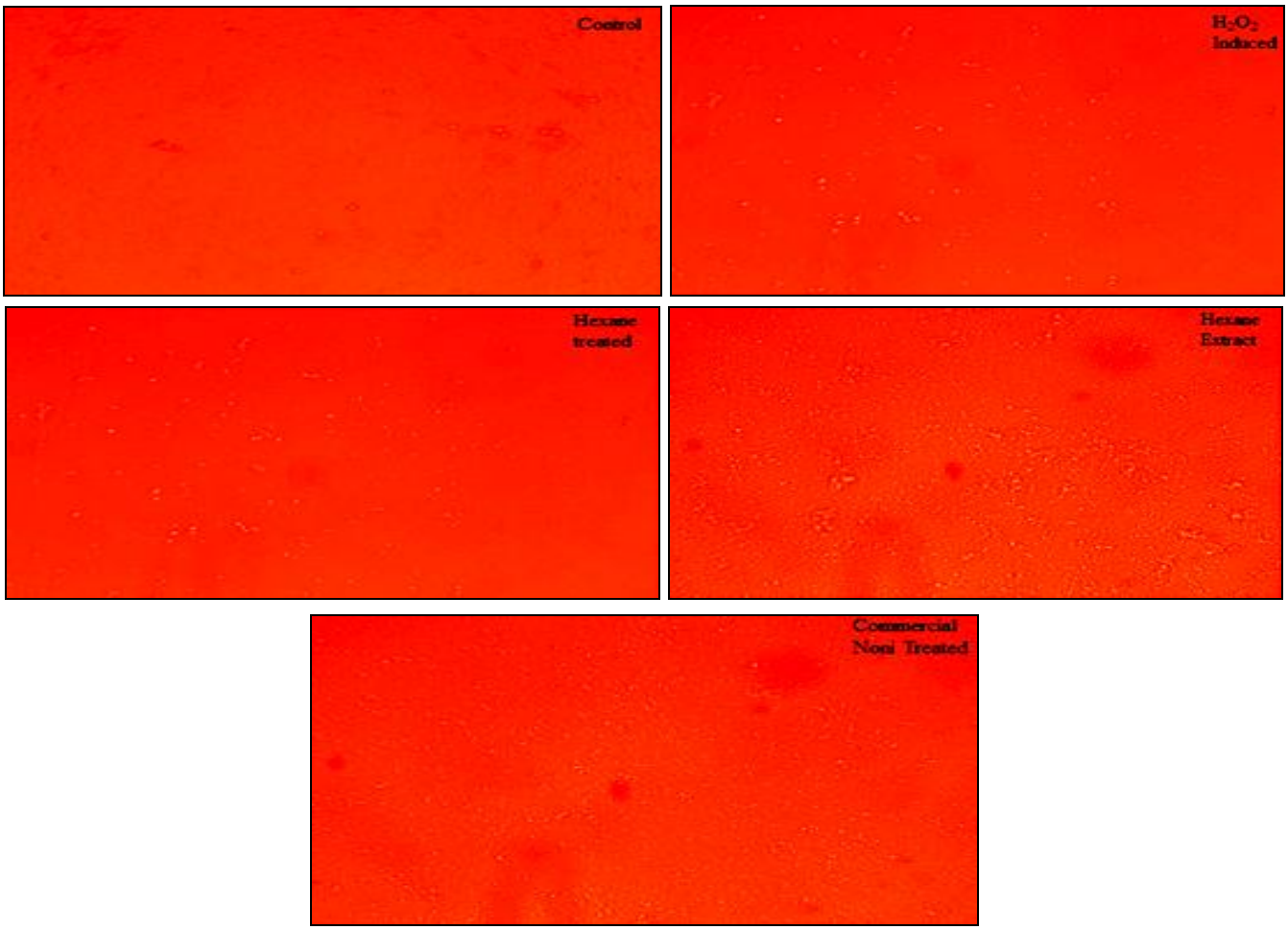

FIG. 5: HUMAN LENS TREATED WITH COMMERCIALLY AVAILABLE NONI AND NONI FRUIT EXTRACT

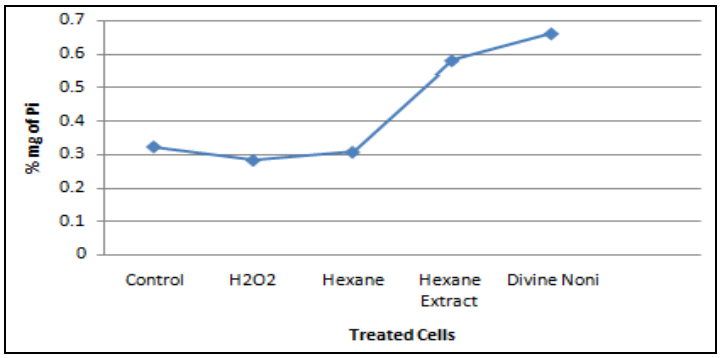

FIG. 6: CYTOPROTECTIVE EFFECT OF *MORINDA CITRIFOLIA* (NONI) FRUIT EXTRACT ON HUMAN CATARATOUS LENS EPITHELIAL CELLS

Fig. 6 Represents Estimation of NaKATPase pump activity (inorganic phosphate group) on human cataratous lens epithelial cells

CONCLUSION: The present study reports that commercial Noni juice and Noni fruit extract would protect chicken lens as well as human Cataractous lens epithelial cells, which was shown in *in-vitro* oxidative stress model. Divine Noni contains excellent nutraceuticals which are having scavenging activity as well as protecting activity of lens proteins. The viability of human cataractous lens epithelium has been maintained due to the cyto-protective effects of Noni juice and extract and hence may be beneficial in the treatment of cataract.

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CONFLICT OF INTEREST: Nil

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