



DIVINE NONI – A POTENTIAL NUTRACEUTICAL TO PREVENT OXIDATIVE STRESS INDUCED CATARACT FORMATION IN CHICK LENS EPITHELIAL CELLS

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ABSTRACT

Oxidative stress is one of the leading causes of Cataract. Fruits of *M.citrifolia* are rich in antioxidants and many bioactive compounds. This food supplement with lot of nutraceuticals support good health. To investigate the role of “Divine NONI” in protection against oxidative stress, chick lens epithelial cells were induced with hydrogen peroxide (100µM H₂O₂) over a time course of several hours, with and without pre-treatment of “Divine NONI” and Noni fruit extract (Hexane). The Pi in the chick lens epithelial cells was estimated by performing NaK ATPase assay. The results obtained in the present investigation suggest that Noni extract and juice can preserve the viability and physiological functions of chick lens epithelial cells during oxidative stress.

Key words: Oxidative stress, Lens epithelial cells, Cataract formation, Inorganic Phosphorus (Pi), Apoptosis and “Divine NONI”

INTRODUCTION

Cataracts are the main cause of human blindness worldwide, responsible for 48% of

the total cases of blindness [1]. Understanding the pathophysiology of cataract formation is important not only to advance the state of medical knowledge but also for public health purposes [2-4]. Apoptosis of lens epithelial cells by various factors can cause cataract formation. Cataract represents a large financial burden on health-care systems, and there remains a need to develop effective therapeutic agents for the prevention or treatment of cataract [5-13].

Scientists found that people at high risk of developing advanced stages of cataract formation, a leading cause of vision loss, lowered their risk by about 25 percent when treated with a high-dose combination of vitamin C, vitamin E, beta-carotene, and zinc. In the same high risk group -- which includes people with intermediate cataract formation in one eye but not the other eye -- the nutrients reduced the risk of vision loss caused by advanced cataract formation by about 19 percent [14-21]. Noni is rich with vitamin A, beta carotenoids, vitamin E, vitamin C, vitamin E, vitamin B complex, with all trace minerals like Ca, Mg, K, Zn, Molybdenum etc, all flavonoids, and besides that Noni contain more than 150 phytonutrients. Those all ingredients being present in one fruit; has made the Noni, a most powerful antioxidant [22-27].

MATERIALS AND METHODS

Lens Organ Culture: The lenses used in this investigation were isolated from chicks 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. 0.5x10⁶ cells were cultured in a well of 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 NaHCO₃ 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. 50µM and 100µM H₂O₂ (Sigma) concentrations were used in this investigation. "Divine NONI" was made to final concentration of 50 mM and diluted to appropriate concentration in culture medium as required. Everyday 1 ml of the medium was replaced.

ATPase Assay.

After that Cells were induced with H₂O₂ along with Noni Extract (Hexane) and Divine Noni. And the ATP Assay was performed by standardized protocol of NaKATPase.

Assay of inorganic Phosphorous Sodium Potassium dependent adenosine triphosphate (Na+ K+ ATPase) (ATP Phosphohydrolase)

Procedure:

1ml of tris Hcl buffer and 0.2ml of each of magnesium sulphate, sodium chloride, potassium chloride, EDTA, ATP, were added to test tube containing 0.2ml of homogenate. The mixture was incubated at 36°C for 15 mins. The reaction was arrested by addition of 1ml of 10% TCA, mixed well and centrifuged. The phosphorus content of the supernatant was estimated [28-30].

Phosphorus content of the supernatant was estimated by Modified Metol method:

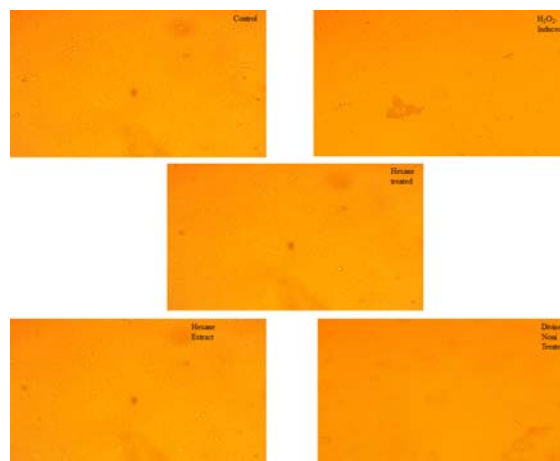
Principle: Ammonium Molybdate under acidic condition react with Phosphorus to form Phosphomolybdate complex which is reduced to blue coloured complex by metol, the

absorbance of the colour developed is proportional to the inorganic phosphorus concentration.

Method:

DIRECTIONS FOR USE ON ANALYSERS:

- Reaction Type : End point with std.
- Reaction Slope : Increasing
- Wave Length : 680 nm (red filter)
- Incubation Temp



- : Room Temperature
- Incubation Time : 5 min.
- Standard : 5 mg%
- Linearity : 15 mg%
- Unit : mg%

PROCEDURE:

Pipette into clean dry test tubes labeled Blank (B), Standard (S), and Test (T).

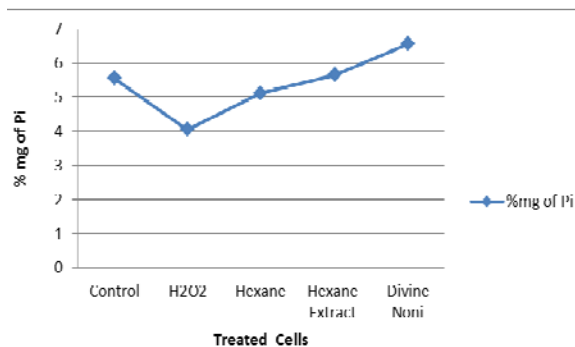
	B	S	T
Catalyst Reagent (1)	1.0 ml	1.0 ml	1.0 ml
Molybdate Reagent (2)	1.0 ml	1.0 ml	1.0 ml
Deionised Water	0.1 ml	-	-
Standard (4)	-	0.1 ml	-
Serum/Dilute urine	-	-	0.1 ml
Metol Reagent (3)	1.0 ml	1.0 ml	1.0 ml

Mixed well and incubated at room temperature for five minutes. After five minutes of incubation the absorbance of Standard(s) and Test (T) measured against Blank (B) either on a Spectrophotometer at 680 nm, within 30 minutes. And the %mg of Pi was

$$\text{Serum phosphorus in mg\%} = \frac{\text{A of Test}}{\text{A of Standard}} \times 5 (\text{Std. Conc})$$

calculated using the following formula.

Fig. 1. Chick lens treated with Divine Noni and



Noni extract
Fig: 2

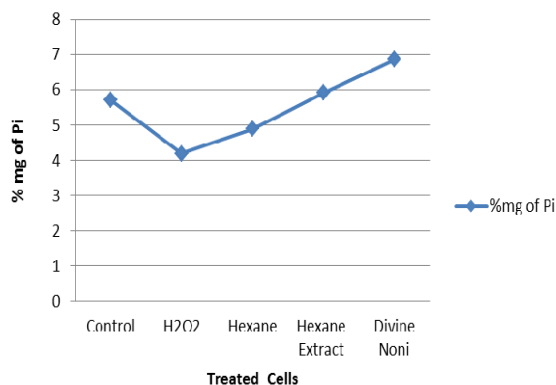


Fig: 3

DISCUSSION

The present study reports that "Divine NONI" and Noni extract was effective in protecting Chick lens epithelial cells from oxidative stress.

ACKNOWLEDGEMENTS

We are whole heartedly thankful to Dr. T. Marimuthu, Dr. Kirthi Singh and Dr. KV Peter of World Noni Research Foundation, Chennai for financial assistance for the entitled project.

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