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# EFFECT OF PAPRIKA CRYSTALS ON AGE RELATEDMACULAR DEGENERATION- A CELL CULTURE STUDY

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## ABSTRACT

Carotenoids are responsible for the color of chilly. In the present study, the carotenoids were separated from chilly as paprika crystals, and developed a formulation to enhance its bioavailability. A comparative cell culture study was conducted on the effect of paprika crystal carotenoids on age related macular degeneration. The current study proves efficacy of the new formulation.

**KEYWORDS:** *Paprika crystals, AMD, ARPE-19 cells, TBARS assay*

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## INTRODUCTION

Age Related Macular Degeneration (AMD) is a common medical eye condition and a leading cause of vision loss among people over fifty[1]. It is the damage in macula, a small spot near the center of the retina which is needed for sharp, central vision.AMD can be of two types: dry (atrophic) or wet (exudative)[2]. High blood pressure, oxidative stress, deficiency of vitamins, blue light exposure, obesity etc. can also be the reasons for AMD, other than ageing[3]. Carotenoids are antioxidant tetraterpenoids, which are the key factors in the prevention mechanisms of the body. The carotenoids in macula are involved in the filtration of blue wavelengths of sunlight and the reduction of free radicals near the retinal area, which are harmful to eye cells[4]. Lutein and zeaxanthin are the carotenoids present in retina, which protect the eye cells from photo oxidative damage[5]. The chronic exposure of blue light causes reduction in cone density and cone sensitivity. It is known to be the major cause of AMD[6]; zeaxanthin can prevent the AMD by absorption of blue light[6]. The mechanism of transportation, involving membrane receptor proteins is common for carotenoids and cholesterol. One genomic study reveals that, the AMD due to high cholesterol levels can be eliminated by carotenoid intake, as the carotenoids will camouflage as cholesterol while intake, and thus reduce the intake of cholesterol and other lipids[7]. The singlet oxygen and Reactive Oxygen Species (ROS) which are generated in the retina, as the result of metabolic activities and photochemical reactions also contribute to Ocular disease; there also, it has been proved that the short wavelength blue light causes more photochemical injury[8]. The antioxidant properties of *trans*-capsanthin and zeaxanthin will reduce photo degradation due to singlet oxygen and will inhibit the growth of ROS[9].*trans*- capsanthin will also protect the retinal neurons from oxidative stress and inflammation. Thus, *trans*- capsanthin and zeaxanthin have been associated with reduced risk of development of AMD[10]. The structures of the major carotenoids in paprika crystals, *trans*-capsanthin and Zeaxanthin are as shown below

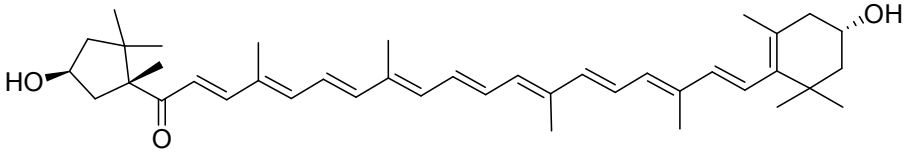



**Figure 1**
***Structure of trans-capsanthin***

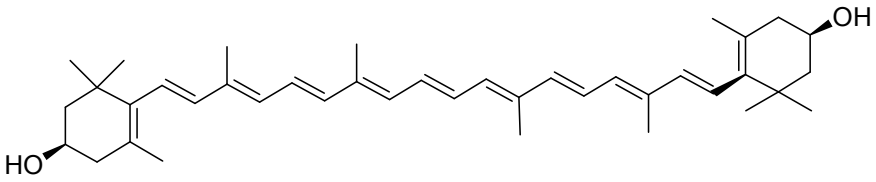



**Figure 2**
***Structure of Zeaxanthin***

In the present study, an effective formulation of the active carotenoids, with an increased bioavailability was developed.The water solubility and the bio-availability of the carotenoids was increased by the encapsulation with *beta*-Cyclodextrin. Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. The carotenoids can be entrapped in cyclodextrin lipophilic cage, hence the carotenoids-cyclodextrin matrix thus have more bio-availability, solubility and stability[9]. The protective effect of paprika crystals (PC) in the reduction of cytotoxic oxidative stress induced by $H_2O_2$ and lipid peroxidation levels in Human Retinal Pigment Epithelium (ARPE-19) cells are tested *in vitro* by TBARS assay.

## MATERIALS AND METHODS

Byadgi chilly was collected from J J Patil, Byadgi, India. All the solvents and chemicals required for the synthesis was collected from Spectrochem Pvt. Ltd., Mumbai. The solvents and chemical for HPLC, and LCMS analysis were collected from Merck.

***Preparation of test sample***

The paprika crystals were prepared in-house by the solvent extraction of paprika, from chilly pods, *Capsicum annuum* L. (family: *Solanaceae*) and subsequent repeated purification by crystallization followed by drying. The Paprika crystals were tested for color value by spectrophotometer, and purity of *trans*-Capsanthin and Zeaxanthin by HPLC. Paprika crystals were encapsulated with *beta*-cyclodextrine, in a ratio of 50:50.

***Color value of paprika crystal by spectrophotometer***
Accurately weighed paprika crystals were dissolved in acetone, and measured the absorbance at 462nm. Color value was calculated as CU.

***HPLC analysis method for carotenoids***
A normal phase HPLC system was used, which consists of shim-pack CLC-SIL(M),15cm.SHIMADZU column, solvent delivery pump (nexera LC-30AD) and UV-VIS detector (SPD-20A UFLC, 248nm). The isocratic mobile phase consisted of hexane: acetone (80:20). A flow rate of 1.5ml/min was given and the run time given was 25 minutes. The detections were made at 474nm.The retention time of zeaxanthin was 8.87, and that of trans-capsanthin was 14.18. Standards of *trans*- capsanthin and zeaxanthin were prepared of different concentrations and checked the linearity.

***Preparation of samples for HPLC***
Accurately weighed sample was dissolved in the solvent mixture of - Hexane: acetone: absolute alcohol: toluene. After shaking it for 5 minutes, added 2ml of 40% methanolic KOH, and placed it in a 56°C water bath for 10 minutes. Added 30ml of hexane, shaken it for 1 minute. Made up to the mark with 10% $Na_2SO_4$ solution and shaken vigorously for 1 minute. Kept it in dark place for 10-20 minutes until upper phase became clear. The clear solution was taken and, removed the moisture by means of $Na_2SO_4$, filtered and injected.

***Study plan***
The cell culture study was conducted at Aurous Health Care Research and Development India Private Limited. Prior to the study, basic cytotoxic assessments were performed for the solvents and test item- paprika, and were found to be nontoxic. Human retinal pigment epithelial (ARPE-19) cells were cultured and used for the assay. Age related Macular Degeneration condition was mimicked in the in vitro level by inducing oxidative stress to the ARPE - 19 cell lines with hydrogen peroxide. ARPE-19 cells were trypsinized using trypsin–EDTA solution. The cells were then incubated at 37°C with 5% CO2 under controlled humidified atmosphere for 24 h. Paprika crystals were made stock solution in DMSO and made different concentrations with DMEM-HAMSF-12 culture media. Then the media was removed and cells were treated with the test item, paprika, of different concentrations. After 2 hrs, they were treated with hydrogen peroxide, and incubated for 24 h at 37°C. Simultaneously, ARPE-19 cells without any treatment and with only the addition of hydrogen peroxide (400 µM) also were maintained. The level of oxidative stress was identified by measuring the levels of lipid peroxidation using Thiobarbituric Acid Reducing Substances (TBARS) assay as described by Jentzsch *et al*[11].

## RESULTS AND DISCUSSION

**T**he paprika crystals were found to have 700230 CU, by spectrophotometer. As per the HPLC results, *trans*-capsanthin content was 78.37% and zeaxanthin content was 16.83%. HPLC chromatogram for Paprika crystals is given below

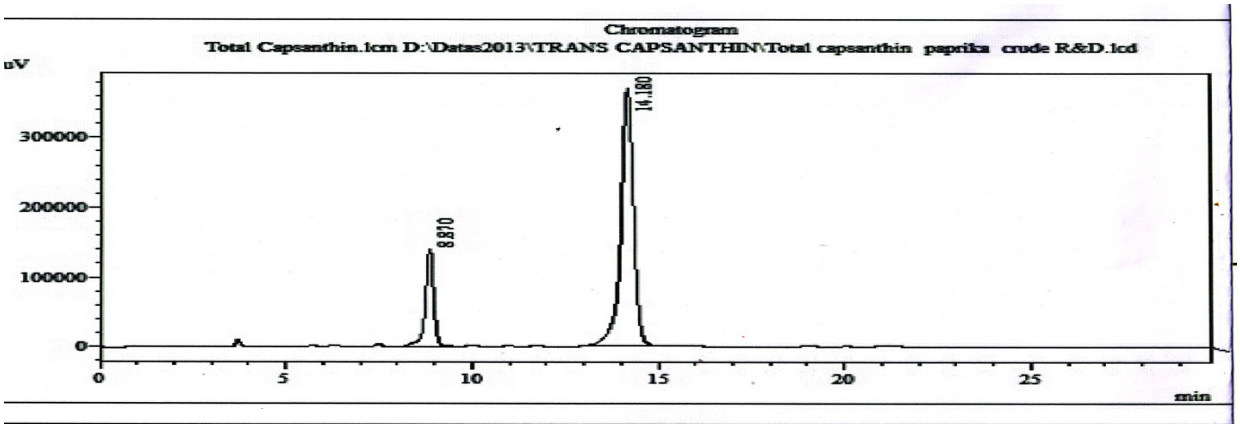



**Figure 3**
***HPLC chromatogram of Paprika crystals***

***ARS assay results are given in the below table***

**Table 1**
***TBARS Assay - Protecting Property of Paprika Crystals***

| Experimental Groups | $OD_{535nm}$-Blank | µM MDA Equivalents |
|---|---|---|
| Control | 0.0040 | 1.688 |
| $H_2O_2$ (400 µM) | 0.0505 | 21.308 |
| PC (1 µg/ml) + $H_2O_2$ (400 µM) | 0.0050 | 2.110 |
| PC (3 µg/ml) + $H_2O_2$ (400 µM) | 0.0050 | 2.110 |
| PC (5 µg/ml) + $H_2O_2$ (400 µM) | 0.0050 | 2.110 |

From the data, it is inferred that the addition of $H_2O_2$ had induced lipid peroxidation in ARPE-19 cells through the generation of oxygen free radicals upto 21.3µM MDA (Malondialdehyde) equivalents. It is proved that, the treatment with paprika crystals prevented the effect of $H_2O_2$ induced oxidative stress and lowered the MDA equivalents up to 2.1µM. All concentrations of paprika crystals significantly brought down the level of lipid perodixation as evident from TBARS Assay results. The low concentration as 1 µg/ml dose itself lowered the peroxidation considerably, but dose dependent effects were not observed in the levels of reduction of lipid peroxidation.

***The graphical representation of the above results is shown below***

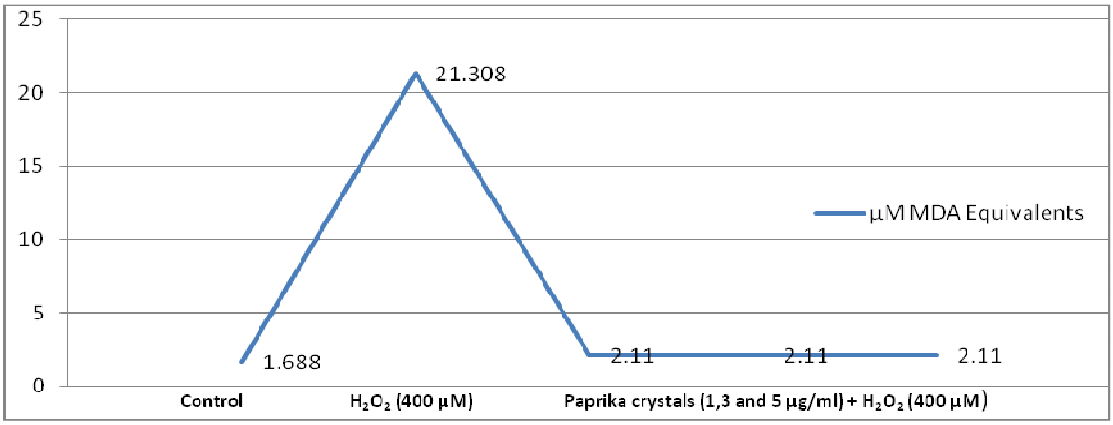
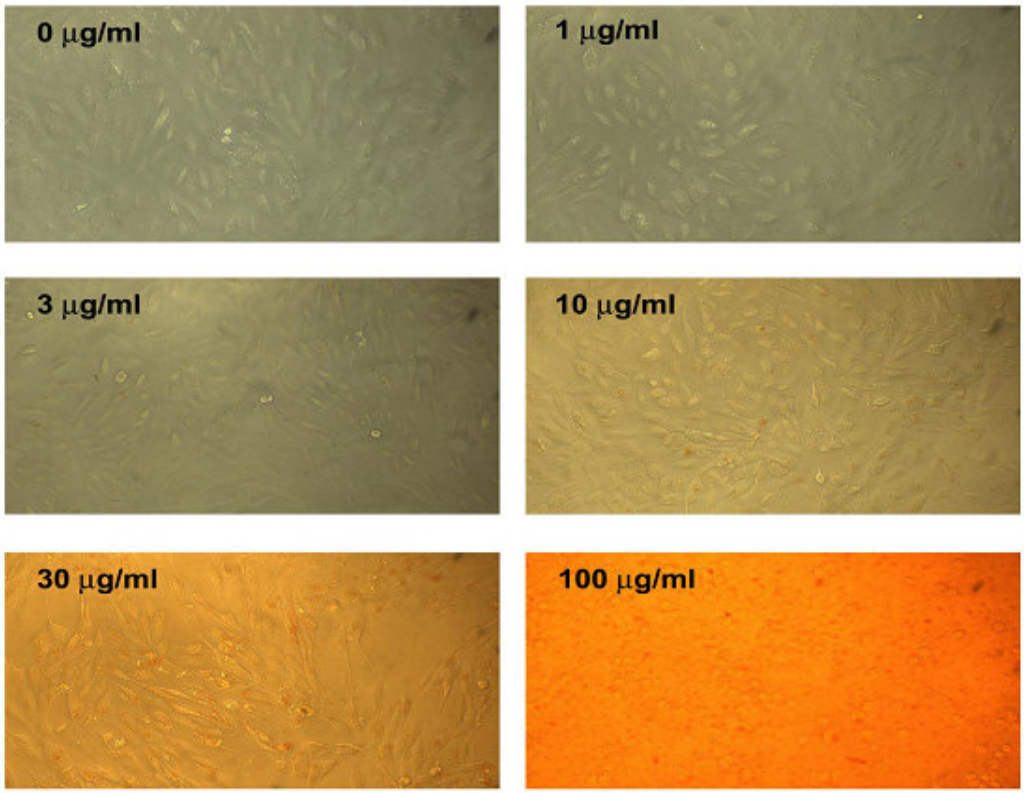



**Figure 6**
***TBARS Assay - Protecting Property of Paprika Crystals***

The images of ARPE -19 Cells after 48 hrs post-treatment with different concentrations of paprika crystals formulation are given below:




## CONCLUSION

The intention of the current cell culture study was to assess the property of Paprika Crystals formulation to protect/treat retinal cells that are affected with Age Related Macular Degeneration. The study proved that Paprika Crystals have the potential to protect/treat retinal cells affected with Age Related Macular Degeneration. So the supplements rich in paprika carotenoids will be a wise choice for avoiding/ treating AMD.

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