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Natural mixed carotenoid complex (CaroRite™) on macular pigment optical density (MPOD), and central foveal thickness (CFT), a randomised, double-blind, placebo-controlled study

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Abstract
Background: The antioxidant properties of carotenoids may play a crucial role in mitigating the risk of macular degeneration by effectively absorbing harmful blue light, thereby highlighting the importance of improving Macular Pigment Optical Density (MPOD) and Central Foveal Thickness (CFT). Clinical investigations have demonstrated that scavenging free radicals using carotenoids localized in the macula may yield significant benefits for ocular health. The structural arrangement of the fovea is pivotal for determining the deposition of macular pigments within the retina. The quantity, quality, type of delivery, and duration of carotenoid intake through dietary sources or supplements can greatly influence absorption by the intestinal wall and subsequent delivery to the eye.
Objective: To further understand the role of carotenoids in improving MPOD and CFT, this study aimed to assess the impact of CaroRite supplementation (lutein, zeaxanthin, meso-zeaxanthin, alpha carotene, beta carotene, and lycopene-natural mixed carotenoids) on MPOD and its correlation with CFT.
Study Design & Method: A total of 93 subjects were randomized in a double-blinded, placebo-controlled, parallel, three-arm study, which was followed for 180 days. Post-study data were subjected to advanced statistical analysis to assess the efficacy of CaroRite™ versus placebo and lutein and zeaxanthin alone on MPOD and its relationship with CFT. Results: CaroRite™ ($p=0.0001$) and lutein and zeaxanthin ($p=0.0009$) showed statistically significant improvements in MPOD levels compared to baseline. In contrast, the placebo arm ($p=0.6179$) did not show any significant changes in MPOD levels compared to baseline. CaroRite™ increased the MPOD by approximately 50.53%, whereas lutein and zeaxanthin increased the MPOD by approximately 46.91%. CaroRite™ showed a marginally higher positive correlation between MPOD and CFT than lutein and zeaxanthin, while the placebo did not show any positive correlation.
Conclusion: CaroRite™ showed a statistically significant increase in MPOD compared with placebo. Compared to lutein and zeaxanthin, CaroRite™ increased the MPOD by a larger amount, although the difference was not statistically significant. Further studies are needed to explore the relationship between the added carotenoids in scavenging free radicals and their possible mechanisms of action in protecting against macular degeneration.

Keywords: Macular pigment optical density, macular assessment profile, central foveal thickness, lutein, zeaxanthin and mixed carotenoids

Introduction

Digital screens, whether big or small, have become an omnipresent part of modern society, making them an inescapable facet of daily life. This ubiquity has raised concerns regarding its potential impact on ocular health. Ocular health depends heavily on the macula, which is located in the inner retina of the eye. The macula is highly susceptible to degeneration owing to aging, genetic predisposition, environmental toxicity, inflammatory conditions, and other factors. This gradual degeneration often manifests as diminished visual acuity, which can eventually lead to significant vision impairment [1].

Age-related macular degeneration (AMD) is commonly observed among geriatric patients. However, recent trends have shown an alarming increase in the diagnosis of macular degeneration in younger individuals, indicating an epidemiological shift [2]. Macular degeneration in the younger population is primarily attributed to significant damage caused by blue light [2]. Blue light, with its short wavelength, is prevalent in the LED segments of various electronic screens such as advanced computers and smartphones. Prolonged exposure to blue LED light significantly increases reactive oxygen species (ROS) in the retinal pigment epithelium (RPE) cells, leading to macular degeneration. The degeneration of macular cells results in visible clinical symptoms, which can be easily identified by a clinician and may lead to the loss of visual acuity and vision impairment, ultimately having a long-lasting impact on eye health.

Green leafy vegetables and other nutrient sources rich in carotenoids protect the retina and macula from damage. Of the 600 carotenoids found in nature in fruits and vegetables, only lutein, zeaxanthin, and meso-zeaxanthin are directly deposited in high quantities in the retina (macula) of the eye. However, several other carotenoids present in nutrient-rich sources have been shown to improve overall health. Unfortunately, the human body cannot naturally produce carotenoids that are needed for eye protection. Therefore, obtaining daily amounts of carotenoids through diet or nutritional supplements is essential to maintain good eye health. These carotenoids are believed to function as LED blue-light filters, and the antioxidants present can protect the retina from damaging influences that are thought to play a

role in the pathogenesis of age-related macular degeneration.

Carotenoids function as antioxidants; among them, the primary carotenoids (α-carotene, β-carotene, and β-cryptoxanthin) serve as precursors of vitamin A, which significantly influences retinoid signaling levels. A review of the literature has established significant correlations between MPOD and dietary intake of carotenoids, particularly lutein and zeaxanthin, in the general adult population. However, there remains a gap in the literature regarding the relationship between MPOD and other dietary carotenoids, such as α-carotene, β-carotene, and lycopene, that are not primarily associated with macular health.

This study was conducted to evaluate the effect of CaroRite supplementation on MPOD and CFT levels, comparing it with lutein, zeaxanthin, and a placebo. This investigation was prompted by recent findings suggesting a correlation between MPOD and α-carotene [3]. Furthermore, this study aimed to establish the effect of CaroRite™ on the relationship between Macular Pigment Optical Density (MPOD) and Central Foveal Thickness (CFT) in healthy adult subjects [9].

Materials & methods

This was a randomized, double-blind, placebo-controlled, parallel, and three-arm clinical study. A total of 93 subjects were assigned to one of the three groups: 35 subjects each in the two test product groups and 23 subjects in the placebo group, based on randomization codes, for a duration of 180 days. The study was conducted at Narayana Nethralaya Eye Hospital, Near ISKCON Temple, 121/C, Chord Road, 1st R Block, Rajaji Nagar, Bangalore (CDSCO registration number ECR/187/Inst/Kar/2013/RR-19) and Shetty's Hospital, Plot No. 11 & 12, 12th "F" Main Road, Kaveri Nagar, Bommanahalli, Bangalore (CDSCO registration number ECR/918/Inst/KA/2017), which received ethics committee approval to conduct the study. The following treatment procedures were implemented during the study:

- **Treatment 1:** Subjects received lutein (5 mg) and zeaxanthin (1 mg). This was administered twice daily to the soft gel capsules (35 subjects).

- **Treatment 2:** Subjects received CaroRite™ (Lutein 5 mg & Zeaxanthin 1 mg + Natural Mixed Carotenoids 5.5 mg, comprising α-carotene, β-carotene, and lycopene). This was administered twice daily in soft gel capsules (n = 35).

- **Treatment 3:** (placebo): Subjects received sunflower oil twice daily in soft gel capsules (n = 23)

The active supplements (lutein 5 mg and zeaxanthin 1 mg), CaroRite™ (lutein 5 mg and zeaxanthin 1 mg + Natural Mixed Carotenoids 5.5 mg), and placebo were visually identical to each other. Supplements and placebo (provided by Bio-gen Extracts Private Limited, Bangalore, India) were contained in identical opaque, sealed bottles with labels that were visually identical, except for the randomization code. Each bottle contained instructions for taking two capsules daily with a meal. Compliance with the intervention was monitored through telephone calls and by counting the pills from bottles returned by the participants during the study visits.

Men and women aged 18–65 years (both inclusive) who consented to participate in the study were enrolled. Eligible subjects were those who were able and willing to follow all study-related instructions and had no prior exposure to lutein or zeaxanthin formulations, or any previous intravitreal injections. Females of childbearing age are required to use standard methods of contraception. Subjects had MPOD levels between 0.2 and 0.6%, and an average neuroepithelium thickness at the fovea centralis of 250 µm.

Exclusion criteria included pregnant or lactating women, those planning to become pregnant, individuals less than six months postpartum, and those with a history of uncontrolled or unstable medical conditions or clinical allergy/hypersensitivity to the study products. Additionally, individuals with a history of participating in a clinical trial in the past three months, or with ocular pathologies such as glaucoma or age-related macular degeneration, as well as those with renal disorders, hepatic disorders, diabetes mellitus, hypercholesterolemia, unstable medical conditions, or a history of non-compliance as assessed by the clinical investigator, were excluded from the study.

The following parameters were assessed: visual acuity using a Snellen eye chart, refraction tests, and retinal tomography (OCT). Specific ophthalmic assessments included Macular Pigment Optical Density (MPOD) and Central Foveal Thickness (CFT). All study subjects underwent a complete blood count (including red blood cells, white blood cells, platelets, differential leukocyte count, hemoglobin, and packed cell volume) as well as measurements of serum creatinine, blood urea, SGOT, SGPT, alkaline phosphatase (ALP), and total protein as part of routine blood analysis. Additionally, all participants underwent routine urine analysis and a urine pregnancy test for females of childbearing age.

All statistical analyses were conducted using SAS version 9.4. Descriptive statistics (N, mean, standard deviation, median, minimum, and maximum) were provided for quantitative data. A paired t-test was used to analyze changes in MPOD from baseline within each treatment group. ANCOVA was used to assess the impact of treatment versus placebo on Macular Pigment Optical Density (MPOD) and Central Foveal Thickness (CFT), accounting for potential confounders. The ANCOVA model included baseline data (difference between visit 4 and screening) as the dependent variable, treatment as the independent factor, and baseline measurements as covariates. Pearson's correlation coefficient was calculated to examine the association between the MPOD and CFT measures. Statistical significance was set at the 5% level ($P < 0.05$).

Results & discussion

The average age of the participants was approximately 30.7 years, with 27 females and 66 males in the study population. Except for two subjects (2.2%), all other participants were non-smokers and none had a history of alcohol use or abuse (Table 1). The average height of the participants was approximately 165.7 cm, with a mean weight of 62.0 kg at the screening visit, which increased to 62.9 kg at visit 4. Similarly, the mean BMI increased slightly from 23 at visit 4 to 22.48 at the screening visit 4 (Table 2). These changes were not statistically significant and did not affect the study products.

Table 1: Demographics of the subject population in the study

Parameter/Statistics	Test 1	Test 2	Placebo	Total
N	35	35	23	93
Age (Years)				
Mean (SD)	33.1 (10.94)	30.2 (9.39)	27.8 (8.52)	30.7 (9.92)
Gender, N (%)				
Female	10 (28.6)	11 (31.4)	6 (26.1)	27 (29.0)
Male	25 (71.4)	24 (68.6)	17 (73.9)	66 (71.0)

Table 2: Baseline characteristics of the subject population in the study

Parameter/Statistics	Visit	Test 1	Test 2	Placebo	Total
N		35	35	23	93
Height (cm)	Visit_1	165.89 ±4.97	165.43± 4.93	165.85± 5.59	165.71± 5.06
	Visit_4	165.95± 4.94	165.45± 4.94	165.85± 5.59	165.74± 5.057
Weight (kg)	Visit_1	62.3± 7.79	61.9± 5.67	61.7± 7.34	62.0± 6.87
	Visit_4	63.3± 7.24	62.6± 5.79	62.7± 7.49	62.9± 6.73
BMI (kg/m^2)	Visit_1	22.52± 2.08	22.89 ± 1.79	21.81 ± 1.55	22.48 ± 1.88
	Visit_4	23.03 ± 1.80	23.16± 1.83	22.73 ± 1.44	23.00± 1.72

Table 3: Descriptive statistics for efficacy parameters Macular Pigment Optical Density (MPOD) average of left & right eye

Treatment	Number (N)	Visit 1 (Mean±SD)	Visit 4 (Mean±SD)	p-value$ (Within treatment)	p-value# (Between treatment)
Test 1	35	0.364±0.1124	0.516±0.2708	0.0009*	0.0029* (Test 1 vs. Placebo)
Test 2	35	0.354±0.1186	0.493±0.1850	0.0001*	0.0061* (Test 2 vs. Placebo)
Placebo	23	0.378±0.1537	0.362±0.2307	0.6179	N/AP

***Statistically significant; N/AP:** Not Applicable

$ p-value (within treatment) was estimated for Test 1, Test 2, and Placebo separately using a paired t-test for the difference in MPOD from baseline to Visit-4.

p-value (between the treatments) for each treatment comparison [Test 1 vs. Placebo & Test 2 vs. placebo] was estimated using ANOVA analysis considering treatment as a fixed effect.

Table 4: Descriptive statistics for efficacy parameters Central Foveal Thickness (CFT) average of left & right eye

Treatment	Number (N)	Visit 1 (Mean±SD)	Visit 4 (Mean±SD)	p-value$ (Within treatment)	p-value# (Between treatment)
Test 1	35	237.6±22.82	243.3±22.94	0.0156 *	0.9306 (Test 1 vs. Placebo)
Test 2	35	235.9±22.76	245.4±23.23	0.0031*	0.4755 (Test 2 vs. Placebo)
Placebo	23	236.7±23.33	243.0±24.61	0.1054	N/AP

***Statistically significant; N/AP:** Not Applicable

$ p-value (within treatment) was estimated for Test 1, Test 2, and Placebo separately using a paired t-test for the difference in CFT from baseline to Visit-4.

p-value (between the treatments) for each treatment comparison [Test 1 vs. Placebo & Test 2 vs. placebo] was estimated using ANCOVA analysis considering the difference in CFT from baseline to Visit-4 as the dependent variable, treatment as fixed effect, and baseline CFT as a covariate.

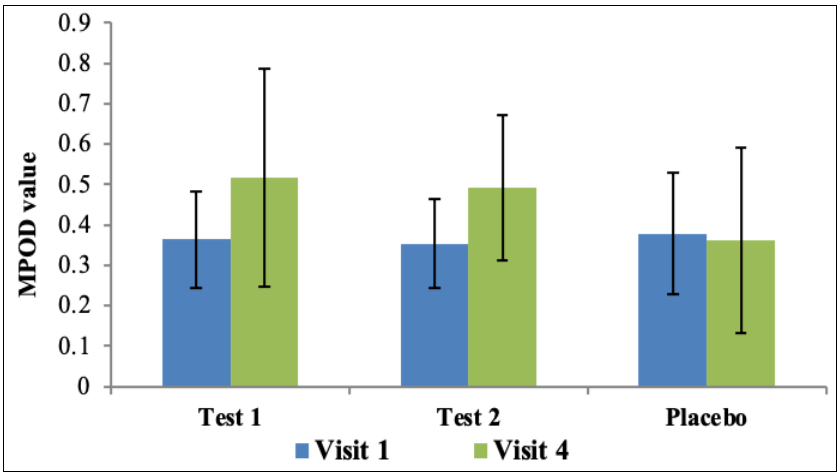

Fig 1: Error bar graph for outcome of Macular Pigment Optical Density (MPOD) across treatment settings

MPOD (Macular Pigment Optical Density)

Lutein and zeaxanthin, carotenoid pigments from the xanthophyll subclass, are present at high concentrations in the retina, especially in the macula. They function as filters that protect the macula from blue light and act as antioxidants and free-radical scavengers to reduce oxidative stress-induced damage. Observational and interventional studies have suggested that lutein and zeaxanthin may reduce the risk of various eye diseases, particularly late forms of age-related macular degeneration (AMD). *In vitro* and *in vivo* studies have shown that they can protect ocular cells against oxidative damage. In this study, the MPOD was evaluated across all three treatment groups. The mean MPOD values showed statistically significant changes compared to baseline (visit 1), with p-values of 0.0009 for Test Product 1 (lutein 10 mg and zeaxanthin 2 mg) and 0.0001 for Test Product 2 - CaroRite™ (lutein 10 mg and zeaxanthin 2 mg + Natural Mixed Carotenoids comprising α-carotene, β-carotene, and lycopene). In contrast, the placebo group did not show statistically significant changes compared to baseline, with a p-value of 0.6179 (Table 3).

Central Foveal Thickness (CFT)

The Central Foveal Thickness (CFT) refers to the thickness of the retina at the fovea, the central part of the macula, which is the area with the highest visual acuity. Changes in CFT can indicate various retinal conditions such as macular edema, age-related macular degeneration (AMD), and diabetic retinopathy. In this study, changes in the average CFT values (μm) of both eyes from visits 1 to 4 across the three treatment groups were assessed. An increase in CFT values was observed in the two treatment groups, with Test 1 showing a p-value of 0.0156 and Test 2–CaroRite™ showing a p-value of 0.0031, as detailed in Table 4.

Both Test Product 1 and CaroRite™ produced statistically significant improvements in the measured CFT values compared with the baseline. However, the placebo group did not show statistically significant changes compared to baseline, with a p-value of 0.1054.

Table 5: Correlation analysis (change in MPOD from baseline to Visit 4 vs. change in CFT from baseline to Visit 4)

Treatment	Variable	Simple Statistics (Mean±SD)	Pearson Correlation Coefficient
Test 1	CFT (Change from Baseline to Visit 4)	5.73±13.30	r=0.11 p-value=0.51
	MPOD (Change from Baseline to Visit 4)	0.15±0.25	
Test 2	CFT (Change from Baseline to Visit 4)	9.41±17.49	r=0.13 p-value=0.46
	MPOD (Change from Baseline to Visit 4)	0.14±0.18	
Placebo	CFT (Change from Baseline to Visit 4)	6.27±17.81	r=-0.008 p-value=0.97
	MPOD (Change from Baseline to Visit 4)	0.02±0.15	

An increase in macular pigment size is typically associated with an increase in the thickness of the central or macular fovea. Van Der Veen (2009) [5] confirmed a significant positive correlation between MPOD and central foveal thickness (CFT) [5]. As MPOD levels increased, CFT values also showed a positive increase, which supports the hypothesis of this study. Although all individuals possess some level of these pigments in their retina, the concentrations of retinal carotenoids in the fovea can vary significantly among individuals.

This wide individual variability raises questions about potential functional consequences. However, non-macular carotenoids derived from dietary sources may also influence macular carotenoids. Two major non-exclusive hypotheses regarding the function of macular pigment have been proposed. The "protection hypothesis" suggests that macular pigment may reduce the cumulative damage from light and oxygen, potentially delaying the development of eye diseases. The "acuity hypothesis" posits that macular pigments could enhance visual resolution by absorbing short-wavelength light, which is more prone to scattering and poor focus. These hypotheses were supported by the observed changes in MPOD levels and their proportional relationship with CFT, as detailed in Table 5.

In the current study, participants who consumed CaroRite™, lutein, and zeaxanthin exhibited significantly higher MPOD levels, whereas those in the placebo group showed no increase. Since humans cannot synthesize macular pigments, all carotenoids must be obtained from dietary sources, which may include both macular and non-macular carotenoids. Our study also found that MPOD levels increased in conjunction with retinal thickness over the central 1 mm fovea, supporting previous research that reported a positive association between MPOD and CFT at an eccentricity of 0.5 °, as measured by OCT in healthy subjects. However, the association between MPOD and CFT is limited to the central fovea and does not persist at eccentricities of 1 °or 2 °.

Sandberg *et al.* [6] also reported an increase in MPOD levels with increasing CFT in patients with retinitis pigmentosa without cystoid macular edema. Zheng *et al.* [8] found a positive correlation between MPOD and CFT in Chinese children aged 6–12 years. In contrast, Nolan *et al.* [4] found no relationship between MPOD and CFT but observed a positive and significant correlation between MPOD and foveal width. They suggested that other personal characteristics might influence the relationship between MPOD and retinal thickness.

Many observational and interventional studies suggest that lutein and zeaxanthin may reduce the risk of various eye diseases, particularly advanced forms of age-related macular degeneration (AMD). Both in vitro and *in vivo* studies have indicated that these carotenoids can protect ocular cells from oxidative damage. Clinical research has shown a close spatial relationship between cone photopigments and macular pigment distribution. There is growing evidence that macular pigment is crucial for vision in healthy individuals, and that a decline in MPOD, whether due to age or occupational factors, may result from inadequate uptake or excessive depletion of retinal carotenoids from storage sites. Free serum retinal carotenoids function as filters, shielding the macula from blue light and acting as antioxidants and free radical scavengers, thereby reducing oxidative stress-induced damage.

Different regions exhibit varying food patterns, particularly in terms of lipid intake, which may contribute to the differences in MPOD levels in relation to CFT levels. Generally, fat-soluble antioxidants, including mixed

carotenoids, are absorbed in duodenal mucosal cells with the help of dietary fats and are transported exclusively by lipoproteins. Studies have shown that cholesterol and lutein can segregate from the saturated lipid regions on cell membranes and accumulate into unsaturated phospholipids, forming carotenoid-rich domains.

Conclusion

In summary, the current study assessing the efficacy of CaroRite™ demonstrated significant increases in Macular Pigment Optical Density (MPOD) and highlighted a pronounced impact on Central Foveal Thickness (CFT). A positive correlation was observed in the CaroRite™ group compared with subjects administered lutein and zeaxanthin and those administered a placebo. These findings suggest that CaroRite may have a beneficial effect on macular health, potentially through its carotenoid constituents. However, further research is necessary to validate these observations through biochemical or larger clinical studies. Future research should aim to elucidate the underlying mechanisms responsible for these outcomes and explore the broader implications of dietary interventions on ocular health. Such efforts will enhance our understanding of the relationship between nutrition and eye function, guiding clinical practices and public health recommendations.

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