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The effects of Lutein and Zeaxanthin (Lute-gen®) supplementation, with and without natural mixed carotenoids on Central Foveal Thickness and its relationship with Macular Pigment Optical Density in healthy adult subjects: a randomized, double-blind, placebo-controlled study

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Abstract

The fovea is a specialised part of the retina which accumulates macular carotenoids. Isomeric carotenoids like lutein, zeaxanthin and meso-zeaxanthin with antioxidant properties are the principal components of macular pigment (MP) accounting for the yellowish colour of the macular area of the retina. MP blocks short wavelength (blue) light that would otherwise damage photoreceptors and pigment epithelial cells causing irreversible changes to the eye architecture. The concentration of MP peaks at the fovea and is visible on the inner and outer plexiform layers of the parafoveal area, thereby protecting retinal damage, which is measured through the relationship between CFT (central foveal thickness) and MPOD (macular pigment optical density).

The objective of this clinical study was to assess the effect of Lutein and Zeaxanthin (Lute-gen®) supplementation, with and without natural mixed carotenoids on central foveal thickness, and its relationship with macular pigment optical density (MPOD) in healthy adult subjects. A total of 93 subjects were randomized in a double-blind, placebo-controlled, parallel, three-arm study which was followed up for 180 days. Data obtained post the study period was subjected to advanced statistical analysis to determine the efficacy of Lutein and Zeaxanthin (Lute-gen®) with and without natural mixed carotenoids on central foveal thickness (CFT) and its relationship with MPOD to determine the physiological status of the eye health. Lutein and Zeaxanthin (Lute-gen®) supplementation, with natural mixed carotenoids (P=0.003) and without natural mixed carotenoids (P=0.012) showed statistically significant improvements in the CFT levels when compared to baseline. On the other hand, the placebo arm (P=0.11) did not show any significant change in CFT levels when CFT measurements were performed on both the eyes. Our study showed a significant positive correlation between MPOD and CFT suggesting MPOD is strongly correlated with CFT. These studies on the relationship between MPOD and CFT can contribute to a stronger understanding on the relationship between scavenging radicals and antioxidant activities of lutein, zeaxanthin and other mixed carotenoids on eye health.

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

Introduction

Macular pigment (MP) is composed of three dietary carotenoids, lutein, zeaxanthin and meso-zeaxanthin. It is mainly present at the nerve fibre layers and ganglion cell layers of the retina, with peak concentrations in the central fovea. MP levels peak at the central foveal area (predominantly found in the retina), which is measured through central foveal thickness (CFT). Earlier it was thought that the degradation of the macula located in the inner retina was due to aging, genetic factors, toxicity, inflammation, etc., resulting in decreased visual acuity and severe loss of vision. [7,8] However, current trends show that younger people are also diagnosed with macular degeneration due to exposure to blue light, which could be due to extensive usage of electronic devices at work and/or for leisure. Blue light has a short wavelength incorporated in the LED of various electronic screens such as computers and smart phones. When one is exposed to high-energy blue light on a continuous basis, reactive oxygen species (ROS) are increased in retinal pigment epithelium (RPE) cells, leading to macular degeneration[9]. These degenerated macular cells lead to clinical symptoms which can be recognized and affect vision and acuity. Unfortunately, these conditions are irreversible and may have a long-lasting impact on eye health. Literature suggests that MP functions as a blue-light quencher through antioxidation and protects the retina from the damaging influences of blue light transmitted through modern communication devices. Dihydroxy-carotenoids are proposed to be selectively deposited in the central fovea (retina), wherein they filter out light in the potentially harmful region of the visible spectrum (~400–500 nm)[6]. Antioxidant activities of these carotenoids are proposed to contribute towards the preservation of visual sensitivity, resolution and protection against eye diseases [1-5].

It is found that MP architecture declines exponentially from the fovea which houses macular carotenoids and is the main cause of age-related macular degeneration. Atypical MPOD spatial profiles have been observed in approximately 20% of subjects containing a secondary pigment peak/ring or central

dip here collectively termed the "foveal macular pigment dip" (FMPD). Increased prevalence of atypical central dip profiles is a further risk factor for AMD, and has been associated with age, smoking, certain ethnicities, and intake of dietary carotenoids. Isomeric carotenoids with antioxidant properties are the principal macular pigment components at the centre of the fovea with an ability to quench the triplet state of photosensitizers and singlet oxygen, to react with free radicals, and to retard the peroxidation of membrane phospholipids. Therefore, measuring CFT and its relationship with MPOD may suggest the physiological status of eye health.

This clinical study was to assess the effect of Lutein and Zeaxanthin (Lute-gen®) supplementation, with and without natural mixed carotenoids on the relationship between central foveal thickness and macular pigment optical density in healthy adult subjects.

Materials & Methods

Subject randomization

The study was a randomized, double-blind, placebo-controlled, parallel, three-arm clinical study. A total of 93 subjects were randomized by a clinical coordinator who had no data collection responsibilities. A set of numerical codes were generated in SAS software that corresponded with either the active supplements or the placebo. The codes were placed in an opaque envelope, and a unique code was drawn for each participant. Of the 93 participants or subjects who were randomized, 23 participants were randomized into the placebo group, and 35 participants each were randomized into the active supplement groups. Treatment break ups with details are as follows:

Treatment 1 – subjects received Lute-gen® (Lutein 5 mg + Zeaxanthin 1 mg). This was administered twice daily in soft gel capsules (35 subjects).

Treatment 2 – subjects received Lute-gen® + Natural Mixed Carotenoids (Lutein 5 mg + Zeaxanthin 1 mg + Natural Mixed Carotenoids 5.5 mg). This was administered twice daily in soft gel capsules (35 subjects)

Treatment 3 (Placebo) – subjects received sunflower oil twice daily in soft gel capsules (23 subjects)

The active supplements Lute-gen® (Lutein 5 mg + Zeaxanthin 1 mg) and Lute-gen® + Natural Mixed Carotenoids (Lutein 5 mg + Zeaxanthin 1 mg + Natural Mixed Carotenoids 5.5 mg), and the placebo were visually identical to the placebo. Supplements and placebos (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 on the label and contained instruction for two capsules, daily to be taken from the bottle with a meal. Compliance to the intervention was monitored by telephone calls and pill counts from bottles returned by the participants during study visits.

Ethics

The tenets of the Declaration of Helsinki and ICMR guidelines on ethics in human research were always adhered to during the study. All the participants issued written and verbal informed consent prior to the study enrolment, and consent documents were administered by trained study personnel. Narayana Nethralaya Eye Hospital, Near ISKCON temple, 121/C, Chord Road, 1st R Block, Rajaji Nagar, Bangalore (CDSCO vide 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 vide registration number ECR/918 /Inst/KA/2017) approved all study-related documents and procedures prior to study initiation, and all study personnel received training in ethics principles and procedures in human subject's research.

Inclusion Criteria

Subjects or participants who met all the following criteria were included in the study:

1. Men and women aged over 18 to 65 years, both inclusive.
2. Able and willing to follow all study-related instructions
3. Subjects who are naïve to previous Lutein or Zeaxanthin formulations administration and to any previous intravitreal injections.
4. Females of child-bearing age should be willing to use standard methods of contraception
5. Must be willing to give written informed consent and comply with the study procedures.
6. Subjects' complete blood count parameters to be within clinically acceptable range by Investigator
7. Subjects with MPOD level between 0.2 – 0.6
8. Subjects with average thickness of neuroepithelium at fovea centralis of 250µm.

Exclusion Criteria

Subjects or participants who met any one of the following criteria were excluded from the study:

Pregnant/lactating women and women who are planning to get pregnant, or less than six months post-partum.

1. History of any uncontrolled and or unstable medical illness.
2. Clinical history of allergy/hypersensitivity to the study products.
3. Recent (within the last 3 months) participation in a clinical trial
4. Subjects having other ocular pathologies e.g. glaucoma or age-related macular degeneration as assessed by the Investigator.
5. Subjects having systemic diseases like renal disorder, hepatic disorder, diabetes mellitus, hypercholesterolemia.
6. Subjects with unstable medical conditions
7. Not willing to follow study restrictions
8. Subjects otherwise judged by the investigator or sub-investigator to be inappropriate for inclusion in the study.
9. No previous Lutein and other antioxidants replacement therapy.
10. Subjects with a history of difficulty of swallowing capsules.

Efficacy evaluation

Efficacy assessments including general ophthalmic assessments were carried out for visual acuity tests that included Snellen eye chart, refraction test and retinal tomography (OCT). Specific ophthalmic assessments were carried out for CFT stratus optical coherence tomography (OCT)3 (Carl Zeiss Meditec Inc, Dublin, CA, USA) following the manufacturer's protocol. Nolan *et al.* (15) details the measurement of CFT and intersection of scans to determine the same. The aforesaid protocol was considered

for the CFT values and measurement. MPOD was measured psychophysically using heterochromatic photometry. About nine trials were completed both centrally, at 30-min of eccentricity along the horizontal meridian of the temporary retina, and parafoveally at 7° of eccentricity. These trials were completed using a test stimulus that consisted of a waveband peaking at 460 nm (strongly absorbed by MP) that alternated in counterphase with a reference waveband peaking at 570nm. Data collection was limited to skilled coordinators.

Statistical analysis

All statistical analyses were performed using SAS version 9.4. Descriptive statistics (N, mean, standard deviation, median, minimum, and maximum) were provided for quantitative data. Paired t-test was used for the analysis of MPOD change from baseline data within the treatment. ANCOVA was evaluated for the impact of treatment vs. placebo confounders on MPOD and central foveal morphology parameters. ANCOVA model had baseline data (difference from visit 4 and screening) as dependent variable, treatment as independent factor and baseline as covariate. Pearson's correlation coefficient was calculated to examine the association between central foveal thickness parameters and MPOD measures. Statistical significance was accepted at 5% level of significance ($p< 0.05$).

Results & Discussion

The average age of the participants was 30.7 years and there were about 27 (female) and 66 (male) subjects in the study population. All the subjects except 2 (2.2%) were non-smokers, and none of them had any history of alcohol use/abuse (Table 1). The average height of the participants was 165.7 cm with a mean weight of 62.0 kgs on screening visit. However, it was 62.9 kg on visit 4. Similarly, the mean BMI slightly increased to 23 on visit 4 from the mean value of 22.48 during the screening visit. These changes are statistically insignificant and are not related to the study products under investigation.

Table 1: Demographics of subject population in the study.

Parameter/Statistics	Test 1	Test 2	Placebo	Total
Age (Years)				
N	35	35	23	93
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 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± 4.939	165.45±4.944	165.85±5.591	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 (Central Foveal Thickness Average of Left & Right Eye)

Treatment	Visit 1		Visit 4		p-value$ (Within treatment)	p-value# (Between treatment)
	N	Mean ±SD	N	Mean ±SD		
Test 1	35	237.6±22.82	35	243.3±22.94	0.0156 *	0.9306 (Test1 vs. Placebo)
Test 2	35	235.9±22.76	35	245.4±23.23	0.0031*	0.4755 (Test2 vs. Placebo)
Placebo	23	236.7±23.33	23	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 & Placebo separately using paired t-test for the difference in CFT from baseline to Visit-4 # p-value (between treatment) 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 dependent variable, Treatment as fixed effect and baseline CFT as covariate.

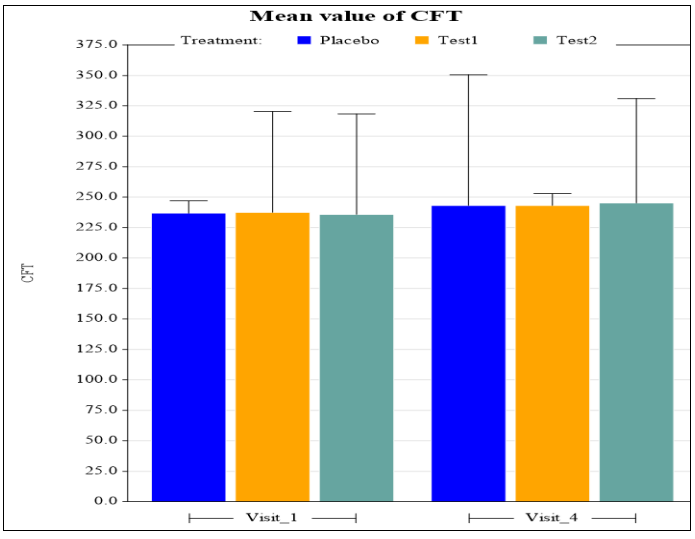

Fig 1: Error bar graph for outcome of Central Foveal Thickness (CFT) across treatment settings

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

Treatment	Variable	Simple Statistics		Pearson Correlation Coefficient
		N	Mean ± SD	
Test 1	CFT (Change from Baseline to Visit 4)	35	5.73±13.30	r=0.11 p-value=0.51
	MPOD (Change from Baseline to Visit 4)	35	0.15±0.25	
Test 2	CFT (Change from Baseline to Visit 4)	35	9.41±17.49	r=0.13 p-value=0.46
	MPOD (Change from Baseline to Visit 4)	35	0.14±0.18	
Placebo	CFT (Change from Baseline to Visit 4)	23	6.27±17.81	r=-0.008 p-value=0.97
	MPOD (Change from Baseline to Visit 4)	23	0.02±0.15	

Central Foveal Thickness (CFT)

In the eye, a tiny pit located in the macula of the retina that provides the clearest vision of all is called the fovea. Only in the fovea are the layers of the retina spread aside to let light fall directly on the cones, the cells that give the sharpest image. This is also called the central fovea or fovea centralis. In the present study, changes in the average CFT values (µm) of both eyes from visit 1 to visit 4 across the three treatment groups were observed. An increase in CFT values was seen in the two treatment groups that included subjects dosed with Test 1 (p=0.0156) and Test 2 (p=0.003) as shown in Table 3.

In this study, CFT was measured across the three treatment groups and the observed mean CFT values were compared with baseline CFT values. These measured CFT values are depicted in the Figure 1 where Test product 1 (Lutein 10 mg + Zeaxanthin 2 mg), p=0.0156 and for Test product 2 (Lutein 10 mg + Zeaxanthin 2 mg + Natural Mixed Carotenoids), p=0.0031. Both the Test products 1 and 2 produced statistically significant improvements in measured CFT values compared to the baseline. However, there was no statistical significance for the placebo when compared with the baseline, p=0.1054. However, Test Product 2 (Lutein 10 mg + Zeaxanthin 2 mg+ Natural Mixed Carotenoids) was statistically more significant in improving mean CFT values from baseline compared to both Test product 1 and placebo.

Correlation between MPOD & CFT

With an increase in the macular pigment, it is expected that the central or macular fovea also increases in its thickness. Van Der Veen (2009) confirms positively significant correlation between MPOD and central foveal thickness [10]. In the current study there is a positive correlation between MPOD and CFT for both the test products. With increase in MPOD levels there is a positive increase in CFT values and this is in line with the hypothesis of the study. These changes are considered to be directly proportional to the changes in the levels of CFT to MPOD as shown in the Table 4.

There is increasing evidence that MP is important for vision in normal subjects and that age-related or occupational induced decline in MPOD must be attributable to either inadequate uptake or excessive depletion of the retinal carotenoids from the storage sites.[11] Free serum retinal carotenoids act as filters, protecting the macula from blue light, and as resident antioxidants and free radical scavengers reduce oxidative stress-induced damage. Many observational and interventional studies have suggested that lutein and zeaxanthin may reduce the risk of various eye diseases, especially late forms of AMD [12]. *In vitro* and *in vivo* studies indicate that they could protect various ocular cells against oxidative damage [2]. There are clinical studies that demonstrated a close spatial relationship between cone photopigment and MP distribution [6, 7, 14].

One of the recognized specialties of the primate fovea is the existence of a yellow macular pigment (MP) that is composed of the hydroxy-carotenoids that include lutein and zeaxanthin. Although it appears that all humans have some quantity of these pigments within their retina, the foveal concentrations of the retinal carotenoids tend to vary quite dramatically. This wide individual variability has prompted questions regarding possible functional consequences. At least two major nonexclusive hypotheses regarding the function of MP have been proposed. The "protection hypothesis" has received the most attention and is based on the possibility that MP could reduce the cumulative effects of damage due to light and oxygen and retard the development of age-related eye disease. The "acuity hypothesis" states that MP could improve visual resolution by absorbing short-wave light, which is easily scattered and poorly focused. Lutein and zeaxanthin could improve human visual performance through both acute optical effects at the site of the retina and by maintaining the health and functional integrity of the retina and crystalline lens [12].

Conclusion

The study showed that carotenoids (Lute-gen®) with or without mixed carotenoids were more significant in comparison to placebo in improving the CFT levels as reported in previous studies [10, 13, 14]. Increased CFT levels in the retina may have benefits, by reducing the risk of occupational-induced macular degeneration or age-related macular degeneration (AMD) by providing protection against oxidative damage and protecting eyes against rays of blue-light. Increased CFT levels compared to the baseline or in comparison with placebo demonstrate consumption of carotenoids has some effect on the MP.

The study showed a significant positive correlation between MPOD and CFT suggesting MPOD is strongly correlated with CFT. These relationships are directly proportional and in line with the previously reported studies [10, 11, 14]. However, according to this study, the treatment group with mixed carotenoids was highly significant in comparison to the treatment group without mixed carotenoids. Mixed carotenoids do have a role beyond the combination of Lutein and Zeaxanthin in the overall management of eye health. These studies on the relationship between MPOD and CFT can contribute to understanding the relationship between scavenging radicals and the antioxidant activities of lutein, zeaxanthin and other mixed carotenoids in eye health.

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