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The effects of lutein and zeaxanthin (Lute-gen®) supplementation, with and without natural mixed carotenoids on macular pigment optical density in healthy adult subjects: A randomized, double-blind, placebo-controlled study

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Abstract
Levels of the macular xanthophylls lutein, zeaxanthin and other carotenoids have been associated with slower progression of age-related macular degeneration potentially through their action by quenching reactive oxygen species, scavenging blue light and thereby protecting the eye from photo-oxidative damage. 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 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. Subjects who met the inclusion criteria underwent general physical and ophthalmic examinations. Further, study specific macular pigment optical density (MPOD) was assessed through macular assessment Profile (MAP) test. Data obtained post study period were subjected to advanced statistical analysis to determine efficacy of Lutein and Zeaxanthin (Lute-gen®) with and without natural mixed carotenoids on macular pigment optical density (MPOD). Lutein and Zeaxanthin (Lute-gen®) supplementation, with natural carotenoids (P=0.0009) and without natural mixed carotenoids (P=0.0001) showed statistically significant improvements in the MPOD levels when compared to baseline from the last visit. On the other hand, the placebo arm (P=0.6179) did not show any significant change in MPOD levels. It can be concluded that higher levels of MPOD may reduce the risk of age-related macular degeneration (AMD) and provide necessary protection against oxidative damage and blue-light.

Keywords: macular pigment optical density, macular assessment profile, optical coherence tomography, lutein, zeaxanthin and ANCOVA

Introduction

Age-related macular degeneration (AMD) is the leading cause of legal blindness, and its prevalence is likely to increase because of increasing longevity. Pathogenesis of AMD due to oxidative retinal injury remains poorly understood and the mechanisms believed to be due to causative factors like genetic factors, cumulative light damage, free radical injury, and hemodynamic processes is yet to be completely understood [1, 3, 4, 5]. However, there are increased number of studies to relate role of dietary habits and consumption patterns and the pathogenesis of eye diseases. Macular pigment, because of its absorbance spectrum and its pre-receptor location is an effective filter of damaging blue light that causes photo-oxidative retinal injury such as macular degeneration. Dihydroxy-carotenoids are proposed to be selectively deposited in the 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 to preservation of visual sensitivity, resolution and protect against eye diseases.

Denature of macula located in the inner retina due to aging, genetic factors, toxicity, inflammation, etc., results in decreased visual acuity and severe loss of vision. Literature shows most of the patients with macular degeneration were mostly correlated to age related macular degeneration (AMD) [7, 8]. However, current trends show that young people are diagnosed with macular degeneration due to exposure of 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 long lasting impact on eye health. Clinical studies have reflected that a positive relation between

lutein/ zeaxanthin consumption with serum lutein/ zeaxanthin concentrations and MPOD exists. MPOD was significantly and positively associated with both reported dietary intakes, and serum lutein and zeaxanthin concentrations. MPOD assessments may prove to be an additional biomarker for non-invasive lutein and zeaxanthin assessment. Consequently, it has been hypothesized that macular pigment protects against AMD. Isomeric carotenoids that include lutein and zeaxanthin, are the principal macular pigment components which peak at the center of the fovea. Both are powerful antioxidants with the ability to quench the triplet state of photosensitizers and singlet oxygen, to react with free radicals, and to retard the peroxidation of membrane phospholipids. It is found in literature that external consumption of carotenoids can protect the macular pigment from the deleterious free radicals which would have important health care implications.

Macular pigment is mainly present at the nerve fiber layers and ganglion cell layers of the retina with peak concentrations in the fovea composed of dietary carotenoids that includes lutein, zeaxanthin, and many other known and unknown carotenoids. It is thought to function as a blue-light filter and antioxidant, and protects the retina from damaging influences that are thought to play a role in the pathogenesis of macular degeneration. This clinical study was to assess the effect of Lutein and Zeaxanthin (Lute-gen®) supplementation, with and without natural mixed carotenoids on macular pigment optical density in healthy adult subjects.

Materials and Methods

Subject randomization

This 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 placebo group, and 35 participants each were randomized into the active supplement group. 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 placebos was visually identical to the active supplement. 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 enrollment, 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:

1. Pregnant /lactating women and women who are planning to get pregnant, or less than six months post-partum.
2. History of any uncontrolled and or unstable medical illness.

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

Efficacy evaluation

Efficacy assessment 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 Central Foveal Thickness (CFT) and MPOD values. MPOD were 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^{o} 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. Heterochromatic photometry is the gold standard for measuring MPOD and has been used previously in participants with poor ocular health (e.g., With cataract and age-related macular degeneration).

Safety evaluation

Safety evaluation included complete blood count (Red Blood Cells, White Blood Cells, Platelets, Differential Leukocyte count, Hb, PCV). Serum creatinine, blood urea, SGOT, SGPT, Alkaline Phosphatase (ALP) and total Protein. Routine Urine Analysis and urine pregnancy test (for females of childbearing age) were carried out. All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” were captured on the appropriate CRF. Information collected included event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which included MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study were documented appropriately regardless of relationship. All AEs were followed till adequate resolution.

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 analysis of MPOD change from baseline data within 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 and 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.

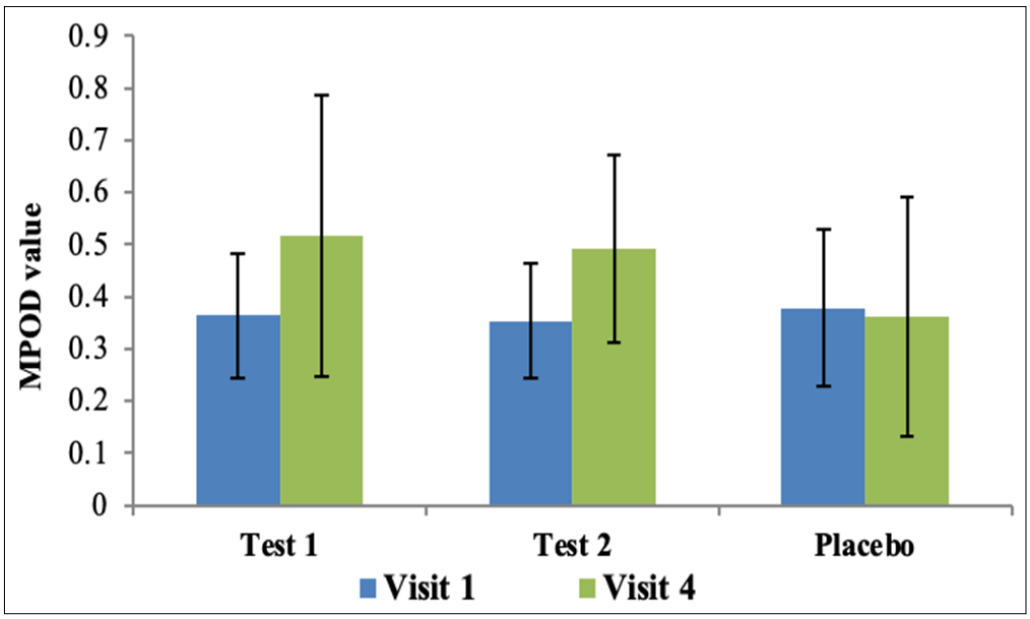
All the subjects except 2 (2.2%) were non-smokers, and none of them had any history of alcohol use/abuse (Table 1). The average height of subjects was 165.7 cm with a mean weight of 62.0 kgs on screening visit. However, it was 62.9 kg on visit 4. Similarly, mean BMI slightly increased to 23 on visit 4 from mean value of 22.48 in screening visit. These changes are statistically insignificant and 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



Values are expressed as mean± SD through error bars.

Fig 1: Improvement in MPOD levels in subjects treated with Lute-gen® test and placebo between Visit 1 to Visit 4.

Table 3: Improvement in MPOD levels in subjects treated with Lute-gen® test and placebo between Visit 1 to Visit 4.

Treatment	MPOD				P-value$^{\$}$ (Within treatment)	P-value$^{\#}$ (Between treatment)
	N	Visit 1	N	Visit 4		
Test 1	35	0.36±0.11	35	0.52±0.27$^{\$*}$	0.0009*	0.0034* (Test 1 vs. Placebo)
Test 2	35	0.35±0.12	35	0.49±0.19$^{\$*}$	0.0001*	0.0075* (Test 2 vs. Placebo)
Placebo	23	0.38±0.15	23	0.36±0.23	0.6179	N/AP

Values are expressed as mean ± SD. *p<0.05 when compared within the treatment groups from Visit 1 to Visit 4; #p<0.05 when compared between the treatment groups against placebo [Test 1 vs. Placebo & Test 2 vs. placebo].

Lutein and zeaxanthin, two carotenoid pigments of the xanthophyll subclass, are present in high concentrations in the retina, especially in the macula [11]. They work as a filter protecting the macula from blue light and as a resident antioxidant and free radical scavenger to 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]. In this study, MPOD was studied across all the three treatment groups. The mean MPOD values are statistically significant (Fig 1) in treatment groups when compared with baseline (Visit 1), at P=0.0009 for Test product 1 (Lutein 10 mg + Zeaxanthin 2 mg) and P=0.0001for Test product 2 (Lutein 10 mg + Zeaxanthin 2 mg + Natural Mixed Carotenoids), whereas it has not reached a statistically significant value when compared with baseline, at P=0.6179 for Placebo (Table 3).

The central portion of the retina or macula is responsible for optimal spatial vision. Macular pigment (MP) is a generic term used to describe the yellow pigment composed principally of the three isomeric carotenoids lutein, zeaxanthin and meso-zeaxanthin, which accumulate in the macula. There is increasing evidence that MP is important for vision in normal subjects. The age-related or occupational induced decline in MP optical density must be attributable to either inadequate uptake or excessive depletion of the retinal carotenoids. The decline of MP optical density with increasing age may simply reflect loss of photoreceptors and their axons in which lutein, zeaxanthin and other carotenoids. There are studies which demonstrated close spatial relationship between cone photopigment and MP distribution.

One of the recognized specialities of the primate fovea is the existence of a yellow macular pigment (MP) that is composed of the hydroxy-carotenoids that includes lutein and zeaxanthin. Although it appears that all humans have some quantity of these pigments within their retina, foveal concentrations 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" that 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 health and functional integrity of the retina and crystalline lens. Since oxidative damage seems to be an important factor for the development and exacerbation of some retinal diseases, the postulated protective role of MP in some disorders [12].

Conclusion

The study showed that carotenoids (Lute-gen®) with or without mixed carotenoids were more significant in comparison to placebo in improving the MPOD levels. Increased MPOD levels may benefit 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 bule-light. Further research will understand the relationship between MPOD and central foveal thickness[10] and how this can contribute to understand relationship between scavenging radicals and antioxidant activity of lutein, zeaxanthin and other mixed carotenoids in eye health.

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