Superior Bioavailability of a Novel Lutein and Zeaxanthin Formulation in Healthy Human Subjects

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ABSTRACT

Introduction: Lutein (L) and zeaxanthin (Z) are carotenoids that are found in the macula of the human eye and are known to improve visual functions. However, poor bioavailability of supplemental L and Z poses a challenge to achieving significant benefits after consumption. We developed a novel patented formulation of L and Z (Ocusorb®) and demonstrated the improved bioavailability in a pharmacokinetic clinical study.

Methods: Ninety adult human volunteers were recruited in this randomized, double-blind, parallel, comparative bioavailability study. Volunteers were randomly assigned to receive single dose of 10 mg lutein and 2 mg zeaxanthin from test (LZO) or reference (LZC) formulations after breakfast. Blood samples were collected pre-dose at -48, -24, and 0 h and at 2, 4, 6, 8, 10, 12, 16, 20, 24, 48, and 72 h postdose. Serum concentrations of L and Z were quantified by using a validated HPLC method. The LZO and LZC formulations were compared for L and Z on the basis of C_{max}, AUC_{0-72}, and AUC_{0-t}.

Results: All 90 subjects completed the study. The LZO group demonstrated significantly higher levels of L and Z in serum at several time points as compared to LZC group. The LZO group showed significantly higher bioavailability for lutein (2.5 times higher C_{max}, 2.9 times higher AUC_{0-72}, and 3.2 times higher AUC_{0-t}) and zeaxanthin (1.8 times higher C_{max}, 2.2 times higher AUC_{0-72}, and AUC_{0-t}) as compared to the LZC group. No safety issues were reported.

Conclusion: The study results show superior bioavailability of lutein and zeaxanthin from our novel LZO formulation as compared to LZC. The enhanced bioavailability from the LZO formulation can be advantageous for individuals looking to quickly improve their L and Z status and enhance their vision performance.

Trial Registration: http://ctri.nic.in/. Identifier: CTRI/2019/11/022082.

Keywords: Absorption; Bioavailability; Carotenoids; Lutein; Macular pigment optical density; Zeaxanthin

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Key Summary Points

Lutein (L) and zeaxanthin (Z), known as macular carotenoids, are found in the macula of the human eye and are known to improve visual functions.

Poor bioavailability of supplemental L and Z poses a challenge to achieve significant benefits after consumption.

The study was conducted to evaluate the bioavailability of L and Z from a novel formulation known as Ocusorb® in comparison to the market sample.

Ocusorb® demonstrated superior bioavailability for lutein—2.5 times higher C_{max}, 2.9 times higher AUC_{0-72}, and 3.2 times higher AUC_{0-t} compared to the market sample.

Ocusorb® demonstrated superior bioavailability for zeaxanthin—1.8 times higher C_{max}, 2.2 times higher AUC_{0-72}, and AUC_{0-t} compared to the market sample.

Enhanced bioavailability from Ocusorb® can be beneficial for individuals looking to quickly improve their L and Z status and enhance their vision performance.

INTRODUCTION

Lutein and zeaxanthin (L and Z) are nutritionally important carotenoids found in several fruits and vegetables. As a result of selective accumulation in the macula of the human retina, L and Z are also known as macular carotenoids [1, 2]. L and Z have antioxidant properties and are known to absorb high energy blue light and protect the retina in the eye. The benefits of L and Z have been extensively researched for their roles in ocular, cognitive, and skin health [1, 3–5]. L and Z accumulate in the macula of the human eye and enhance visual performance [3, 5–11]. L and Z have also been shown to reduce the risk of age-related macular degeneration (AMD) [6, 7]. Choroidal neovascularization (CNV) plays a critical role in the pathogenesis of AMD, and in vivo animal models for neovascular AMD demonstrated significant suppression of the inflammatory process associated with CNV in response to lutein supplementation [12]. Regular intake of carotenoid-rich food has been associated with the reduced risk of several chronic diseases like photosensitivity diseases, cataracts, AMD, and cardiovascular diseases [13, 14].

L and Z are isomers with identical chemical formulas. Lutein occurs as a single stereoisomer, while zeaxanthin occurs as a mixture of three stereoisomers, namely (3R,3′R)-zeaxanthin, (3R,3′S)-zeaxanthin, and (3S,3′S)-zeaxanthin. L and Z from the diet reach plasma and subsequently the macula where lutein is also converted to *meso*-zeaxanthin. Thus, the macular carotenoids responsible for macular pigment optical density (MPOD) are lutein, zeaxanthin, and *meso*-zeaxanthin at an approximate ratio of 2:1:1 in the retina [15]. Humans cannot synthesize lutein and (3R,3′R)-zeaxanthin and are dependent on dietary sources such as spinach, corn, lettuce, green peas, kale, pepper, orange, zucchini, and egg yolk to support adequate L and Z status [16, 17]. However, the intake of these fruits and vegetables are below the recommendations and dietary L and Z consumption is very low in most parts of the world. Inadequate dietary intakes of L and Z resulting in low serum levels of L and Z are associated with low macular pigment density and increased risk of AMD [18]. Supplementation with L and Z can help to support adequate L and Z status [19–21] and have been clinically demonstrated through increased serum levels of L and Z as well as higher deposition in the retina [3, 10]. However, considerable inter-individual variation in serum concentrations and macular pigment density has been reported following supplementation with L and Z [10]. Factors that affect the absorption of carotenoids, such as the matrix of the formulation and the form (i.e., free versus esterified) of the carotenoids determine the amount of L and Z achieved in serum after supplementation [4, 11]. Moreover, L and

Z have poor bioavailability due to poor stability and solubility of the crystalline form in an aqueous medium. Hence, there has been considerable effort to develop L and Z formulations with improved stability and enhanced bioavailability to achieve improved health benefits. Various methods that have been used to develop formulations for L and Z include dispersion of crystals in edible oils, encapsulation with biopolymers [22], or mechanochemical complexation [23–26].

Here we report a novel formulation of L and Z (LZO) with improved bioavailability (patent filed WO 2021/074763 A1) using an optimized marigold and paprika extract as a source of L and Z respectively at a 5:1 ratio as found in nature in the form of natural dietary sources [27]. We used a micronization process to reduce the particle size followed by formation of an oil suspension in medium chain triglyceride (MCT) oil along with a combination of functional oils that helps to improve absorption from the small intestine. We evaluated the bioavailability of LZO in healthy human volunteers after single oral dose as compared to commercially available formulation (LZC) with L and Z at a similar dose.

METHODS

The LZO formulation (Ocusorb®) was made using excipients like sunflower lecithin, olive oil, mixed tocopherol, thyme oil, linseed oil, and lime peel extract oil dissolved in MCT oil followed by addition of micronized marigold/paprika oleoresin mixture. This finished formulation was used for preparation of the study capsules. The final preparation was analyzed by HPLC using reference standard to ensure each gram of final product contains 80% of lutein and 16% of zeaxanthin. Each capsule (LZO) contained 10 mg of lutein and 2 mg of 3′R,3R-zeaxanthin. The reference group utilized LZC capsules which contained a blend of commercially available sample of lutein 20% and zeaxanthin 14% to obtain the dose of 10 mg lutein and 2 mg RR-zeaxanthin per capsule.

Study Design and Procedures

This was a double-blind, balanced, randomized, two-treatment, single-period, single-dose, parallel, comparative bioavailability study in healthy, adult, human subjects under fed conditions. The study was initiated after obtaining written approval from an institutional ethics committee, Maarg Independent Ethics Committee (EC registration Number ECR/77/Indt/AP/2013/RR-19), Secunderabad, India on 18 November 2019. The study was carried out in compliance with the requirements of the Indian Council of Medical Research (ICMR) ethical guidelines, International Council for Harmonization (ICH) Guidance on Good Clinical Practice (E6R2), and Declaration of Helsinki. The study was registered with the Clinical Trials Registry of India (CTRI/2019/11/022082).

Informed consent was voluntarily obtained from every participant before enrolling for the study. Subjects were randomly assigned in a 1:1 ratio to receive either LZO or LZC. The randomization schedule was generated by a non-study assigned, independent expert ensuring the treatment balance using SAS® statistical software, version 9.4. Staff who were involved in the investigational product-related activities were not involved in the study-related activities to ensure double blindness of the study.

Subjects stayed at the study center for a total of 6 days, 3 days before dosing and 3 days after dosing. During their stay, subjects were provided standard meals to control lutein and zeaxanthin in the diet. Subjects fasted overnight for at least 10 h and then received a high-fat, high-calorie breakfast comprising of 900 kcal with 57% fats, 27% carbohydrates, and 16% proteins the next day morning and 30 min later were administered a single oral dose of LZO or LZC containing 10 mg L or 2 mg Z along with 240 ± 2 mL of water. The subjects were dosed in a parallel manner to either LZO or LZC in the single period. The study product was administered to the subjects in a sitting posture and they remained in a sitting posture for the first 2 h (except at the time of vitals, natural and medical emergencies) unless clinically indicated. Thereafter, the subjects were allowed to

engage only in normal activities while avoiding severe physical exertion. Blood test, vitals, and medical examinations were done for safety analysis.

Fourteen blood samples of 1×6 mL were collected from each subject. Three pre-dose blood samples were collected at 48, 24, and 0 h before dosing to establish baseline levels of lutein and zeaxanthin. Post-dose blood samples were collected at 2, 4, 6, 8, 10, 12, 16, 20, 24, 48, and 72 h. The blood sample collection and analysis were carried out under yellow monochromatic light because of the light sensitivity of lutein and zeaxanthin. After sample collection, samples were placed at room temperature for up to 30 min (time required for proper clot formation) and then centrifuged at 4 °C and 4000 rpm for 10 min to separate serum. The samples were placed in a deep freezer maintained at $- 70\,°C \pm 10\,°C$ until completion of serum sample analysis. A bioanalytical method for the estimation of lutein and zeaxanthin in human serum was developed and validated as per regulatory guidelines. Lutein and zeaxanthin were extracted from human serum by a liquid–liquid extraction method. Astaxanthin was used as internal standard for both lutein and zeaxanthin. The separation was achieved using a Kromasil 100-5SIL 250 × 4.6 mm, 5 μm with hexane/ethyl acetate (55:45 v/v) as mobile phase. The measured concentrations for each subject for all the time points were calculated against the calibration curve prepared with known standards. The retention time was 5.85, 6.48, and 3.90 min for lutein, zeaxanthin, and astaxanthin, respectively. Lutein and zeaxanthin were detected at wavelength of 456 nm and their concentrations in human serum samples were quantified using LabSolution 6.86 SP2 with a Shimadzu HPLC–photodiode array detection (PDA) system. The method was linear and validated over a concentration range of 20.92 to 10,434.15 ng/mL for lutein and 20.05 to 9998.85 ng/mL for zeaxanthin.

Study Population and Inclusion/Exclusion Criteria

A total of 90 healthy adult human subjects were enrolled in the study in accordance with the inclusion and exclusion criteria listed in Table 1.

Statistical Analysis

A sample size of 41 subjects per arm (82 subjects for both arms) was sufficient considering the difference in means and standard deviation with a 0.05 as alpha, two-sided significance level, and 90% as power. However, considering the 10% dropouts due to non-compliance or due to personal reasons, 90 subjects were randomized and dosed.

Study conduct and statistical analysis were performed at AnaCipher Clinical Research Organisation, Hyderabad using the SAS system for windows, version 9.4 (SAS institute Inc., Cary NC, USA). Pharmacokinetics parameters (C_{max}, $AUC_{0–72}$, $AUC_{0–t}$, T_{max}, and $t_{1/2}$) were determined from the serum lutein and zeaxanthin concentrations by using Phoenix WinNonlin Version 8.2.

The arithmetic mean, minimum, maximum, standard deviations, and geometric mean were reported for the pharmacokinetic parameters of untransformed data. The geometric means were reported for $AUC_{0–72}$, $AUC_{0–t}$, and C_{max} of log-transformed data.

The log-transformed pharmacokinetic parameters ($AUC_{0–72}$, $AUC_{0–t}$, and C_{max}) were analyzed using an analysis of variance (ANOVA) model with the main effects of treatment. A 5% level of significance was used throughout the analysis. Each analysis of variance included calculation of least-square means, adjusted differences between treatment means, and the standard error associated with these differences.

For the pharmacokinetic parameters ($AUC_{0–72}$, $AUC_{0–t}$, and C_{max}), 90% confidence intervals for the ratios of LZO and LZC averages were calculated using the ANOVA output from the analysis of the log-transformed data.

The 90% confidence intervals for the ratio of geometric least squares means between the

Table 1 Inclusion and exclusion criteria for subject selection

Inclusion criteria

   Healthy, adult, human subjects within the age range of ≥ 21 to 55 years

   Body mass index between 20 and 27 kg/m^2

   Voluntary participation

   Subjects avoiding lutein and zeaxanthin—yellow and green-rich vegetables and fruits such as mangoes, tangerines, oranges, asparagus, broccoli, butternut squash, cilantro, collards, orange pepper, parsley, papaya, peas, pistachio, romaine lettuce, scallions, zucchini, kale, *Brassica oleracea*, spinach, carrots, corn, tomatoes, nectarines, peaches, etc.—during the study period and restricted to ≤ 1–2 servings per day within 2 weeks before dosing

   Subjects willing to exclude eggs from diet 2 weeks prior to dosing and during the study period

   Non-smokers and non-alcoholics

   Given written informed consent to participate in the study

   Absence of disease markers of HIV 1 & 2, hepatitis B & C virus

   Absence of significant disease or clinically significant abnormal laboratory values on laboratory evaluation, medical history, physical examination, and systemic examination during the screening

   A normal 12-lead ECG

   A normal chest X-ray (PA view) done within past 6 months

   Compliance with the requirement of the entire protocol

Exclusion criteria

   Institutionalized subjects

   History or evidence of hypersensitivity to lutein or zeaxanthin

   History or evidence of chronic diseases such as diabetes, hypertension, liver disorders, kidney disorders, pulmonary disease or infections, cardiovascular disease, pancreatic disease, infectious diseases, dermatological problems, arthritis, and any cancer

   History of hypo- and hyperthyroidism

   Presence of alarm signs or symptoms, including fever, gastrointestinal bleeding, unintentional weight loss, anemia, dysphagia, or abdominal mass

   History of milk, gluten allergies, or other known food intolerances and or any food allergies

   History of significant systemic diseases, seizures, psychiatric disorders, neurological disorders, depression, mental illness, or allergic rash

   Habit of consuming high caffeine (more than 5 cups of coffee or tea/day) consumption

   History of difficulty with donating blood or difficulty in accessibility of veins

   History of addiction to any recreational drug or drug dependence

   Donation of blood (1 unit or 350 mL) within 90 days prior to study check-in

   Participation in any clinical study within the past 90 days

Table 1 continued

Receipt of any prescription drugs or over-the-counter drugs (e.g., cold preparations, antacid preparations, and natural products used for therapeutic benefits) within 30 days prior to screening

Current use of vitamin or multivitamin supplements providing carotenoids (e.g., supplements including lutein/zeaxanthin, beta-carotene, lycopene, or beta-cryptoxanthin)

History of dehydration from diarrhea, vomiting, or any other reason within a period of 24 h prior to check-in of study period

An unusual or abnormal diet within 48 h prior to check-in of study period, for whatever reason, e.g., because of fasting due to religious reasons

Received pharmacological agents known to significantly induce or inhibit drug metabolizing enzymes within 14 days of the start of the study

Consumption of food and beverages containing xanthine (chocolates, tea, coffee, or cola drinks) for at least 48 h prior to check-in of study period

Consumed grapefruit and grapefruit-like citrus fruit (mosambi/sweet lime) or juice within the 7 days prior to check-in of study period

Positive results for drugs of abuse (benzodiazepines, opioids, amphetamines, cannabinoids, cocaine, and barbiturates) in urine during the check-in of study period

Use of macular carotenoids supplementation, omega-3 fatty acids, alpha-lipoic acid, or fatty acid supplements within 14 days of the start of the study

Current use or a history of use (i.e., within 60 days of the screening visit) of lutein and/or zeaxanthin supplements

Positive results for alcohol breathe analysis during the check-in of study period

formulations were calculated, for log-transformed pharmacokinetic parameters—C_{max}, AUC_{0-72}, AUC_{0-t} for lutein and zeaxanthin.

The p values were calculated using the PROC GLM (ANOVA) output from the analysis of the log-transformed data. Probability values less than 0.05 denote statistically significant differences between LZO and LZC.

Safety assessments included monitoring of adverse events, physical examination, vital signs measurements, and laboratory assessments.

RESULTS

Ninety male subjects completed the study with 100% compliance (Fig. 1). Out of the 90 randomized subjects, 45 subjects were allocated to the LZO group and 45 subjects to the LZC group. The demographic characteristics are provided in Table 2. The mean age of subjects was 29.96 ± 6.28 years in the LZO group and 31.00 ± 5.41 years in the LZC group. The mean body mass index (BMI) was 23.76 ± 2.30 in the LZO group and 23.55 ± 2.08 in the LZC group. There was no statistically significant difference between the two groups for age, and BMI at the baseline. Female subjects did not participate in the study because of social reasons associated with long-term housing at the study center.

The analysis of pharmacokinetic parameters is presented for lutein and zeaxanthin in Table 3.

RESULTS FOR LUTEIN

The LZO group showed significantly ($p < 0.05$) higher lutein serum concentrations (> 2-fold) at
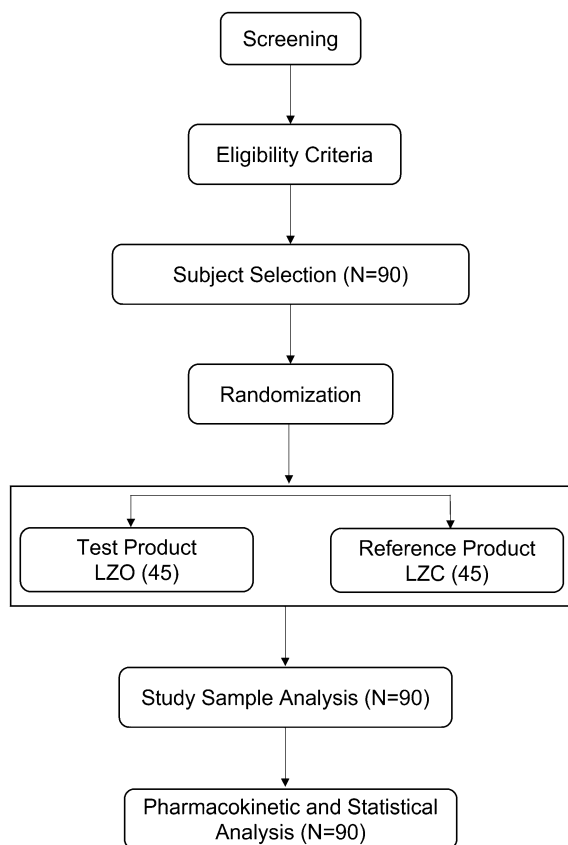
Fig. 1 Flowchart of this randomized, double-blind, comparative bioavailability study of lutein and zeaxanthin formulations

all time points i.e., 2 to 72 h post dose as compared to the LZC group (Fig. 2a).

The maximum serum concentration (C_{max}) was significantly ($p < 0.05$) higher for LZO as compared to LZC (219.15 vs. 87.01 ng/mL; 90% CI 191.97–330.44%). Similarly, the total systemic exposure measured as area under the curve (AUC) was significantly ($p < 0.05$) higher for LZO as compared to LZC represented by AUC_{0-72} (8499.89 vs. 2892.09 h·ng/mL; 90% CI 217.33–397.46%) and AUC_{0-t} (8264.02 vs. 2622.97 h·ng/mL; 90% CI 224.50–442.17%), respectively.

The LZO group showed significantly ($p < 0.05$) higher absorption for lutein based on 2.5 times higher C_{max}, 2.9 times higher AUC_{0-72}, and 3.2 times higher AUC_{0-t} levels compared to the LZC group. (Table 3, Fig. 3).

The median T_{max} value for LZO was 20 h and LZC was 16 h for Lutein.

RESULTS FOR ZEAXANTHIN

The LZO group showed significantly ($p < 0.05$) higher serum zeaxanthin concentrations (> 1.5-fold) from 4 to 72 h post dose as compared to the LZC group (Fig. 2b).

The maximum serum concentration (C_{max}) was significantly ($p < 0.05$) higher for LZO as compared to LZC (56.95 vs. 32.55 ng/mL; 90% CI 140.70–217.50%), respectively. Similarly, the total systemic exposure measured as area under the curve (AUC) was significantly ($p < 0.05$) higher for LZO when compared to LZC represented by AUC_{0-72} (1929.93 vs. 861.65 h·ng/mL; 90% CI 159.64–314.26%) and AUC_{0-t} (1817.29 vs. 814.17 h·ng/mL; 90% CI 154.35–322.79%), respectively.

The LZO group showed significantly ($p < 0.05$) higher absorption for zeaxanthin with 1.8 times higher C_{max}, 2.2 times higher AUC_{0-72}, and 2.2 times higher AUC_{0-t} levels as compared to the LZC group (Table 3, Fig. 4).

Table 2 Demographic characteristics of the subjects

Parameters	Test product (LZO), N = 45			Reference product (LZC), N = 45		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Age	29.96 ± 6.28	21	43	31.00 ± 5.41	21	43
BMI	23.76 ± 2.30	20.05	26.97	23.55 ± 2.08	20.12	26.93
Gender	Male—45	–	–	Male—45	–	–
	Female—0			Female—0		

N total number of subjects in the specified group, *SD* standard deviation

Table 3 Results of baseline-corrected pharmacokinetic parameters for lutein and zeaxanthin

Pharmacokinetic parameters	Baseline-corrected arithmetic mean ± SD		Geometric least squares mean		LZO/LZC ratio (%)	90% confidence interval (%)		p value log transformed	LZO/LZC (fold difference)
	Test product (LZO), N = 45	Reference product (LZC), N = 45	Test product (LZO), N = 45	Reference product (LZC), N = 45					
Lutein									
C_{max}[a] (ng/mL)	284.53 ± 208.04	112.51 ± 75.70	219.15	87.01	251.86	191.97	330.44	< 0.0001*	2.52
AUC_{0-72}[a] (h·ng/mL)	11,270.98 ± 8593.10	4012.05 ± 3047.83	8499.89	2892.09	293.90	217.33	397.46	< 0.0001*	2.94
AUC_{0-t}[a] (h·ng/mL)	11,134.91 ± 8555.32	3912.93 ± 3117.76	8264.02	2622.97	315.06	224.50	442.17	< 0.0001*	3.15
T_{max}[a,d] (h)	20.00 (4.00–72.00)	16.00 (6.00–48.00)	–	–	–	–	–	–	–
$T_{1/2}$[b] (h)	92.24 ± 83.25	91.34 ± 167.70	–	–	–	–	–	–	–
Zeaxanthin									
C_{max}[a] (ng/mL)	64.77 ± 39.73	38.86 ± 26.11	56.95	32.55	174.93	140.70	217.50	< 0.0001*	1.75
AUC_{0-72}[a] (h·ng/mL)	2434.31 ± 1733.51	1270.31 ± 1218.34	1929.93	861.65	223.98	159.64	314.26	0.0002*	2.24
AUC_{0-t}[a] (h·ng/mL)	2451.02 ± 1740.97	1276.91 ± 1228.25	1817.30	814.17	223.21	154.35	322.79	0.0005*	2.23
T_{max}[a,d] (h)	20.00 (0.00–72.00)	24.00 (0.00–72.00)	–	–	–	–	–	–	–
$T_{1/2}$[c] (h)	64.85 ± 59.10	251.73 ± 417.74	–	–	–	–	–	–	–

SD standard deviation

*p < 0.05

[a] N = 45 for LZO and N = 45 for LZC

[b] N = 26 for LZO and N = 23 for LZC

[c] N = 12 for LZO and N = 10 for LZC

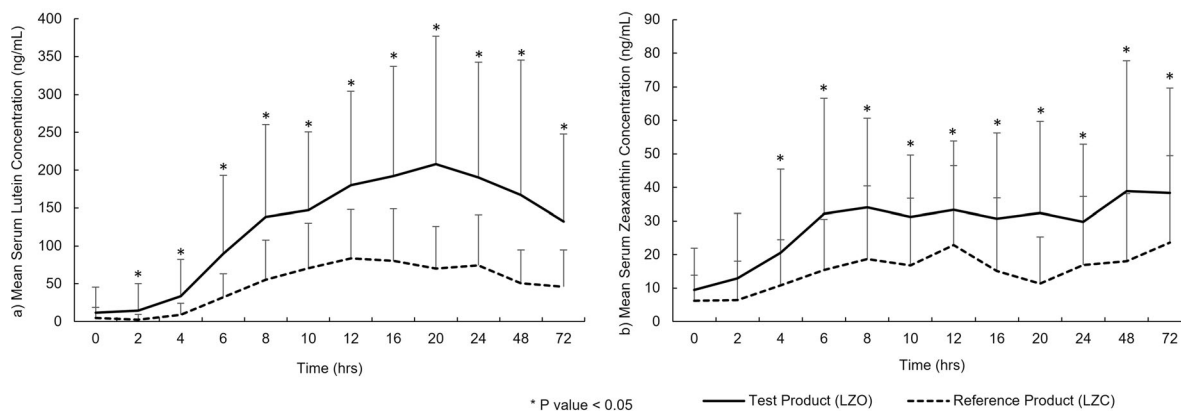[d] Median (minimum−maximum)

Fig. 2 Lutein and zeaxanthin serum levels versus time. The LZO group showed significantly ($p < 0.05$) higher serum lutein levels (> 2-fold) at all time points i.e., 2–72 h post dose (**a**) and higher serum zeaxanthin levels (> 1.5-fold) from 4 to 72 h post dose (**b**) as compared to the LZC group
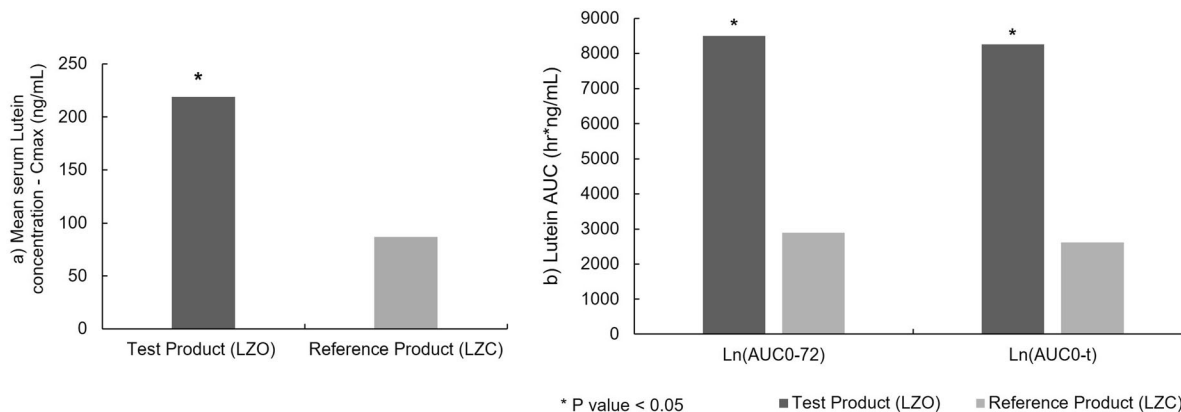

Fig. 3 Pharmacokinetic parameters of lutein in serum for test product (LZO) and reference product (LZC). **a** Mean serum lutein maximum concentration (C_{max}). **b** Lutein area under the curve (AUC) serum concentration versus time for 0–72 h (AUC_{0-72}) and 0 to last measurable concentration (AUC_{0-t}). C_{max}, AUC_{0-72}, and AUC_{0-t} were significantly ($p < 0.05$) higher for LZO compared to LZC

The median T_{max} for LZO was 20 h and LZC was 24 h for Zeaxanthin.

The study product was well tolerated and did not show any adverse events.

DISCUSSION

L and Z, also known as macular carotenoids, support visual performance and provide protection from ocular diseases such as AMD and cataract [1, 2, 28, 29]. Multiple human pharmacokinetic studies of L and Z have shown that intake of L and Z through diet or supplementation increases circulating levels of L and Z in the body leading to enhanced deposition in the macula of the eye. However, L and Z in the natural form have low bioavailability after oral intake due to their poor stability and inefficient absorption in the intestine leading to varying plasma concentrations [30]. In the current study we report a highly bioavailable formulation of L and Z that demonstrated significant
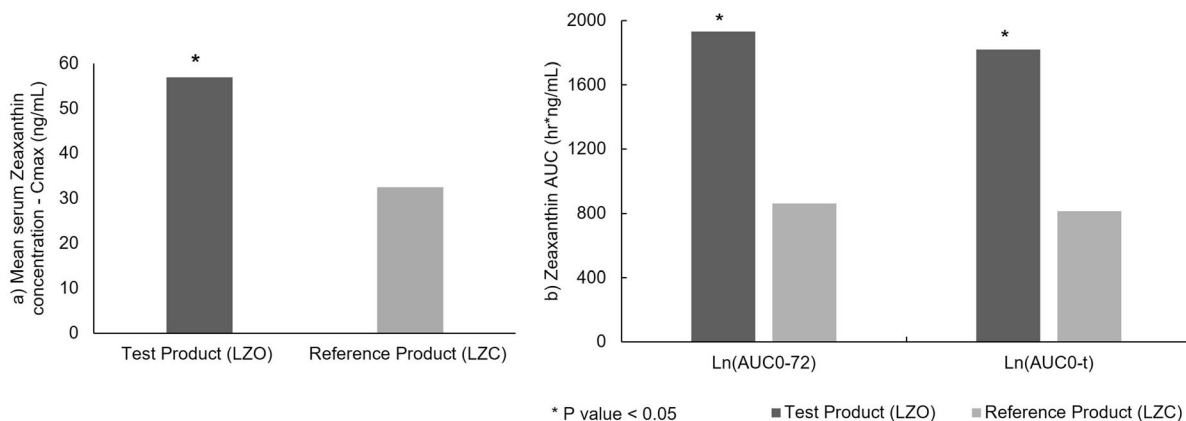
Fig. 4 Pharmacokinetic parameters of zeaxanthin in serum for test product (LZO) and reference product (LZC). **a** Mean serum zeaxanthin maximum concentration (C_{max}). **b** Zeaxanthin area under the curve (AUC) serum concentration versus time for 0–72 h (AUC_{0-72}) and 0 to last measurable concentration (AUC_{0-t}). C_{max}, AUC_{0-72}, and AUC_{0-t} were significantly ($p < 0.05$) higher for LZO compared to LZC

improvement in absorption leading to increased serum levels after a single oral dose.

We observed a significant ($p < 0.05$) increase in serum lutein (> 2-fold) and zeaxanthin (> 1.5-fold) concentrations in the case of the LZO formulation at all time points measured post dose as compared to the LZC group. The maximum serum lutein concentration (C_{max}) for LZO was 219.15 ng/mL which was 2.5 times higher and statistically significant ($p < 0.05$) as compared to LZC (C_{max} 87.01 ng/mL). Similarly, maximum serum zeaxanthin concentration was 1.8 times higher with a statistically significant ($p < 0.05$) increase (C_{max} 56.95 ng/mL) in the case of LZO as compared to LZC (C_{max} 32.55 ng/mL). Further area under the curve (AUC) measurements for both AUC_{0-72} and AUC_{0-t}, which is the total analyte exposure across time, significantly increased absorption for both L and Z in the case of LZO as compared to the LZC formulation. The LZO group showed 2.9 times higher AUC_{0-72} and 3.2 times higher AUC_{0-t} levels for lutein and 2.2 times higher AUC_{0-72} and AUC_{0-t} levels for zeaxanthin as compared to the LZC group.

The time taken for lutein to reach its highest concentration in serum (T_{max}) was 20 h in the case of LZO as compared to 16 h in the case of LZC. Similarly, T_{max} for zeaxanthin was 20 h in the case of LZO and 24 h for LZC. Overall, we observed an increased serum L and Z levels in the case of the LZO formulation as a result of faster and better absorption after supplementation which may aid better accumulation in the macula and enhance eye protection. Increased serum L and Z levels are known to increase the MPOD and directly improve visual performance [31].

Several studies have reported variable bioavailability of carotenoids due to the type of formulation matrix used, form of the bioactives (free versus esterified), and effects of other nutrients [32]. We micronized marigold and paprika oleoresin (source of L and Z, respectively) to reduce the particle size which in turn is known to improve dissolution rate and enhance oral bioavailability [33, 34]. Lutein in free form is not very stable and hence we used an oil suspension of medium chain triglycerides (MCT). MCT oil is considered as a healthy fatty acid, known to enhance permeability of intestinal mucosal membrane [35], improve absorption of lutein [36], and widely used in the nutraceutical industry [35, 37]. Further, we also incorporated multiple functional oils in the formulation that are known to improve absorption in the gut [38–40]. We believe that the cumulative effect of reduced particle size and combination of excipients used in the formulation improved the bioavailability of L and Z in the LZO formulation.

Both free and esterified lutein have been used to study single-dose, pharmacokinetic (PK) properties in human subjects [41–43]. Although variables such as intestinal absorption, metabolism, and serum clearance of carotenoids have an impact on circulating serum levels, the dose of L and Z used is also directly correlated with serum levels achieved, with an increased dose resulting in higher serum concentration [41]. Further, at a higher dose, the free form of lutein is better absorbed than lutein esters leading to increased plasma lutein levels [27, 44]. In the current study we observed increased circulating levels of L and Z owing to our improved formulation which is significant as this is achieved without increasing the dose. Higher circulating levels of L and Z are known to increase MPOD levels leading to improved visual protection and performance. The National Eye Institute sponsored the AREDS2 clinical study to evaluate the protective effects of carotenoids on progression of eye diseases. The study reported that 5-year supplementation of 10 mg lutein and 2 mg zeaxanthin leads to 18% reduction in AMD progression [29]. Other studies reported that increased dose of L and Z supplementation leads to increased plasma circulating levels of L and Z and improved MPOD [32] with improved health benefits such as improved contrast sensitivity, disability glare performance, and photo stress recovery [45, 46].

A limitation of the study was that female subjects did not participate because of social reasons associated with long-term housing at the study center. However, gender-specific differences in blood concentrations of L and Z have been reported to be very minimal [47–49].

CONCLUSIONS

L and Z protects the retina from blue light and provides antioxidant and anti-inflammatory support to retina and choroidal blood vessels that supply blood to the macular region of the retina [12]. Increased intake of L and Z not only increases the MPOD in the macula [28, 32] leading to improved visual acuity, sensitivity, and glare [29, 30, 50–52] but also reduces the risk of AMD [28, 53–59]. We developed an improved formulation of L and Z with significantly improved oral absorption leading to higher blood levels of L and Z which may further help in increased MPOD levels. L and Z supplements are generally considered to be safe [32] and are well tolerated. The enhanced bioavailability of this proprietary formulation can be advantageous for individuals looking to quickly improve their L and Z status and enhance their vision performance. Further, long-term human studies that evaluate MPOD and other related eye health benefits should help in accurate prediction of long-term benefits of our improved formulation.

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Disclosures. Abhijeet Morde, Muralidhara Padigaru, Deshanie Rai, and Mohan Lal are employees of OmniActive Health Technologies. All named authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Compliance with Ethics Guidelines. Institutional ethics approval was obtained from Maarg Independent Ethics Committee (EC registration Number ECR/77/Indt/AP/2013/RR-19), Secunderabad, India. This study was conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with the International Conference on Harmonization (ICH), Good Clinical Practice (GCP) Guidelines, as well as in strict compliance with the "The New Drugs and Clinical Trial Rules-2019", the Ministry of Health and the Government of India, India GCP guidelines released by Central Drugs Standard Control Organization, India, at all stages of the trial for adherence to protocol and compliance with ethical and regulatory guidelines. The EC was duly apprised of the progress and updates of the trial at regular intervals as per prescribed guidelines.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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