Indian Journal of Medical and Allied Research
Volume 12, Issue 4, May 2023 pp 1-7. **www.ijmar.in** ISSN: 2278-0890

A Randomised Placebo-Controlled Clinical Trial To Evaluate The Efficacy Of Marigold Extract On Cognitive Performance And Mood In Adults With Stroke And Parkinson's Disease

C A Anzar*[1], Joseph M V[1], C P Prasad[1], Vadiraj G Bharawaj[1], Prasanna Anjaneya Reddy Lebaka[1], Shobith Jagadeesh[2]

1. OLIVE LIFESCIENCES PVT LTD No.165/5, Near NH-4, Nelamangala, Bangalore – 562123, Karnataka, India
2. Clinical Research Millikatta Neuro Centre, Mallikatte, Kadri, Mangaluru, Karnataka 575002, India.

Submitted: 09-3-2023 Accepted: 10-5-2023

ABSTRACT

Objective: The purpose of this clinical study was to assess the impact of OLS-01, a proprietary formulation, on cognitive function in individuals aged 35 to 75 years who suffer from cognitive dysfunction. The study was randomized, double-blind, and placebo-controlled. Each capsule of OLS-01 contained Extract of Marigold flowers, which is equivalent to 10mg of Lutein and 1mg of Zeaxanthin. The participants received two capsules of OLS-01 per day, providing a daily dose of 20mg of Lutein and 2mg of Zeaxanthin. *Methods:* The study recruited 30 individuals who had been diagnosed with cognitive dysfunction resulting from either Parkinson's disease or stroke. The participants were block-randomized and divided into two groups, G1 and G2. Group G1 received OLS-01, while Group G2 received a placebo for a duration of five weeks. Compliance was assessed by conducting participant interviews and questionnaires. The participants underwent cognitive performance testing using the Standardized Cognitive Assessment Revised (SCAr) at the beginning of the study and at weeks 1, 2, 3, 4, and 5. *Results:* The SCAr test results showed that 46.6% of the individuals in the treatment group exhibited notable improvement in cognitive abilities. In contrast, none of the participants in the placebo group showed any improvement in cognitive function throughout the study period. *Conclusion:* The results indicate that taking the Lutein supplement formulation OLS-01 for five weeks can enhance cognitive function in people who suffer from cognitive dysfunction due to Parkinson's disease or stroke. These findings suggest that OLS-01 supplementation could be a hopeful intervention for individuals seeking to preserve and enhance their cognitive abilities. Findings of this study gives hope for further research on Cognitive benefits of OLS-01.

Keywords: Marigold Extract, Cognitive Performance, Mood In Adults, Stroke, Parkinson's Disease

INTRODUCTION

Lutein and zeaxanthin belong to the carotenoid family of fat-soluble nutrients. Lutein can be found in dark green leafy vegetables like kale and spinach, as well as in egg yolks and corn. Meanwhile, zeaxanthin is predominantly present in yellow and orange foods such as egg yolks, corn, orange capsicums, tangerines, persimmons, mandarins, and oranges (PMID: 20608883, PMID: 23571649). These nutrients are present in various parts of the body, including the eye, brain, breast, and adipose tissue. Although lutein is not the most prevalent carotenoid in our diet, it is the carotenoid with the highest concentration in human brain tissue (PMID: 23840953). Lutein and zeaxanthin together account for approximately 66 to 77% of the total carotenoid concentration in the human brain tissue, and have been identified in various areas of the brain including the hippocampus, cerebellum, and frontal, occipital, and temporal cortices (PMID: 35252311). Due to their potent antioxidant and anti-inflammatory properties, there is growing interest in their potential neuroprotective effects.

Numerous studies have explored the relationship between the dietary intake of carotenoids, such as lutein and zeaxanthin, and cognitive health. These studies have consistently found a positive association between lutein and zeaxanthin intake and cognitive function. For instance, in middle-aged women, a higher intake of lutein and/or zeaxanthin was linked with a lower risk of experiencing moderate-to-poor cognitive function (PMID: 32386230). Similarly, in older adults, higher intake of lutein and/or zeaxanthin was associated with better immediate and delayed word recall, as well as higher scores

on cognitive-based measures in adults over 60 years old (PMID: 30326796). Moreover, studies have found that higher plasma concentrations of lutein and/or zeaxanthin were related to better cognitive function in older adults, improved visual-spatial functioning in older adults, and enhanced relational memory performance in young and middle-aged adults. Additionally, macular pigment optical density (MPOD), a measure of lutein and zeaxanthin concentration in the brain, was found to be positively associated with better cognitive performance in older adults, adults with mild cognitive impairment, and adults with age-related macular degeneration (PMID: 29610850).

Stroke is a leading cause of cognitive dysfunction, including impairments in memory, attention, executive function, and language. The damage caused by stroke can disrupt neural networks and alter the structural and functional connectivity of the brain, leading to persistent cognitive deficits. Research has shown that stroke-induced cognitive dysfunction is associated with both gray matter and white matter alterations including reductions in cortical thickness, changes in brain activation patterns, and disruptions in white matter tracts (PMID: 27090751). These changes can result in deficits in various cognitive domains, including working memory, attention, and language processing. Treatment options for stroke-induced cognitive dysfunction are limited, and there is currently no universally effective intervention.

Cognitive dysfunction is a significant clinical nonmotor symptom of Parkinson's disease (PD). It encompasses a range of cognitive deficits, including mild cognitive impairment and dementia, particularly executive dysfunction and other impairments in speech, visual spatial ability, and memory. Notably, cognitive dysfunction is found to be prevalent among individuals with PD, including those who are newly diagnosed. According to recent epidemiological studies, the cumulative prevalence of Parkinson's disease dementia (PDD) over an eight-year period is as high as 78.2%. Furthermore, about 40% of PD patients at an earlier stage also exhibit mild cognitive impairment, which substantially increases their risk of developing PDD (PMID: 32269747).

Considering the reported benefits of lutein and prevalence of cognitive dysfunctions in patients with stroke and/or Parkinson’s disease, the objective of the current clinical study was to evaluate the safety and efficacy of OLS-01. OLS-01 is a product that is licenced under AYUSHas a proprietary ayurvedic medicine. Each capsule under these codes contain Extract of Marigold flowers equivalent to Lutein 10mg and Zeaxanthin 1mg. These capsules were administered twice daily which corresponds to 20mg of Lutein and 2mg of Zeaxanthin as a daily dose.

The study is designed as a double-blind randomized placebo-controlled trial, which means that neither the researchers nor the participants know who is receiving the active treatment or the placebo. The ethical clearance was obtained at Mangala Institutional Ethics Committee vide letter Ref. No. MIEC/V6.1/015 and the trial was registered at CTRI vide registration Number CTRI/2022/06/043208 The study aims to assess whether the formulation OLS-01 can improve cognitive function, as well as to identify any potential side effects or adverse reactions associated with its use.

MATERIALS AND METHODS

Trial design - The trial was a randomized, double-blind, placebo-controlled clinical study with two parallel treatment groups. Healthy individuals aged between 35 to 75 years were recruited from the community through advertising. After screening, 30 participants were enrolled in the study and block-randomized into two treatment groups G1 and G2 using a computer-based randomization model. The study arms were equally balanced regarding age and gender. To be eligible to participate, individuals had to meet specific inclusion criteria, including the ability to give informed consent, diagnosed with required disease/severity/symptoms, and be willing to comply with all trial requirements. Individuals had to meet exclusion criteria such as significant renal or hepatic impairment, scheduled elective surgery or other procedures requiring general anaesthesia during the trial, or any significant disease or disorder that could put the participants at risk or influence the result of the trial. Female participants who were pregnant, lactating, or planning pregnancy during the trial were also excluded. See supplementary information for more details

Intervention - During the intervention period, participants in the treatment group (G1) ingested two capsules daily, containing marigold extract, standardized to Lutein 10 mg and Zeaxanthin as 1 mg. Participants in the placebo group (G2) consumed capsules which were similar to the capsules provided to treatment group. The capsules were provided as sealed bottles with 60 capsules each, and stored at room temperature for the study's duration. The label on the bottles contained a comprehensive set of information such as - Manufacture date, Expiry date, Serial number assigned to the trial dose, a clear indication that it was intended solely for clinical trial purposes and not for commercial use, and a warning that it was an investigational product. Additionally, the label cautioned against taking a higher dosage, advised that it be kept away from children and stored in a cool, dry place, recommended taking the medication with water to avoid swallowing difficulties, and suggested exercising caution if experiencing any discomfort. Participants attended the investigating units at baseline, 1st 2nd 3rd 4th and 5th weeks, where they underwent cognitive testing and completed questionnaires. Compliance was measured by counting the number of returned capsules at the end of the study and by participant interviews during the scheduled clinic visits.

Cognitive tests – During the visits, participants underwent a battery of cognitive tests according to Standardized cognitive assessment revised (SCAr). SCAr provides a comprehensive evaluation of cognitive function across multiple domains. It has been used in a wide range of clinical and research settings to assess cognitive impairment in various neurological conditions, including Alzheimer's disease, Parkinson's disease, the tests assess various cognitive abilities (orientation, remote memory, digit repetition, word recall, visual memory, word finding, reading comprehension,

abstract thinking, calculation, writing, right/left orientation, verbal comprehension, delayed word recall, word recognition, copying, spatial reversal, ideometer, clock drawing, and perseveration) in a quantitative manner.

The scores were recorded across 5 visits for the participants were used as a measure to assess if there were any changes in the cognitive abilities during the intervention period. Based on these scores, the participants were assessed either as "improved" or "no improvement".

Statistical analysis: The sample size in each group for this clinical trial was 15 and the efficacy of the intervention was measured as two categorical outcomes –"improved" or "no improvement". Two tailed Fisher exact test was used to measure the statistical significance using stats package in R programming. Two tailed test was adapted to ensure the effect of intervention is scored in either direction.

OLS-01 *formulation*: The OLS-01 used for treatment group contains extract of *Tagetes erecta* (flower) standardized to Lutein 10mg & Zeaxnthin 1 mg in Oil of *Cocus nucifera* (MCT), packed into vegan capsules. For the placebo group, capsules containing MCT oil 500 mg, packed into vegan capsules were used.

RESULTS:

Participants and grouping: As described in materials and methods, 30 participants diagnosed with cognitive dysfunction due to Parkinson's disease and/or Stroke, were randomized into two groups – G1 or G2. Details of the participants, their diagnosis, age and gender distributions are as detailed in Table-1A, B, C, D, E.

Table-1A

PatientID	Diagnosis during the recruitment	Group	Age	Gender
1001	PD & Stroke	1, (OLS-19-01)	51	Male
1002	Stroke	2 , (OLS-19-02)	40	Male
1003	Stroke	2 , (OLS-19-02)	64	Male
1004	Stroke	1, (OLS-19-01)	59	Male
1005	Stroke	1, (OLS-19-01)	61	Male
1006	PD	1, (OLS-19-01)	50	Female
1007	Stroke	1, (OLS-19-01)	49	Female
1008	Stroke	2 , (OLS-19-02)	60	Male
1009	PD	1, (OLS-19-01)	38	Male
1010	PD	2 , (OLS-19-02)	53	Male
1011	PD	1, (OLS-19-01)	70	Male
1012	PD	2 , (OLS-19-02)	53	Female
1013	PD	2 , (OLS-19-02)	38	Male
1014	PD	1, (OLS-19-01)	56	Female
1015	PD	2 , (OLS-19-02)	45	Male
1016	PD	1, (OLS-19-01)	71	Male
1017	PD	1, (OLS-19-01)	65	Female
1018	Stroke	2 , (OLS-19-02)	62	Male
1019	Stroke	1, (OLS-19-01)	51	Male
1020	Stroke	2 , (OLS-19-02)	52	Female
1021	Stroke	1, (OLS-19-01)	63	Male
1022	Stroke	1, (OLS-19-01)	63	Female
1023	Stroke	1, (OLS-19-01)	65	Male
1024	PD	2 , (OLS-19-02)	58	Male
1025	PD	1, (OLS-19-01)	60	Male
1026	PD	2 , (OLS-19-02)	65	Male
1027	PD	1, (OLS-19-01)	48	Male
1028	Stroke	2 , (OLS-19-02)	41	Female
1029	Stroke	2 , (OLS-19-02)	58	Female
1030	Stroke	2 , (OLS-19-02)	63	Male

Indian Journal of Medical and Allied Research
Volume 12, Issue 4, May 2023 pp 1-7. **www.ijmar.in** ISSN: 2278-0890

Table – 1B

Gender	Counts	%
Female	9	30
Male	21	70

Table – 1C

Types of Patients recruited		
	Number	%
PD	14	46.67
PD & Stroke	1	3.33
Stroke	15	34.88

Table – 1D

Cross Table-1 (Patient categories based on Gender)		
	Female	Male
PD	4	10
PD & Stroke	0	1
Stroke	5	10

Table-1E

Cross Table-2 (Patient Groups based on Gender)		
Groups	Female	Male
G1,OLS-19-01	5	11
G2, OLS-19-02	4	10

Effect of OLS-01 *treatment on cognitive functions*: Each of the participants was administered either OLS-19-01 (group G1) or OLS-19-02 Placebo capsules (group G2) twice daily for 5 weeks. Their cognitive performances were scored based on Standardized cognitive assessment revised (SCAr) test as described in materials and methods. At the end of 5 weeks, based on the scores of the SCAr assessment test, each of the participant were assessed as 'Improved' or 'no improvement' by qualified clinicians. The results as shown in Table-2 and Figure-1.

Table-2A

PatientID	**Diagnosis during the recruitment**	**Group**	**Age**	**Gender**	**Clinician impression**
1001	PD & Stroke	1, (OLS-19-01)	51	Male	IMPROVED
1002	Stroke	2 , (OLS-19-02)	40	Male	NO IMROVEMENT
1003	Stroke	2 , (OLS-19-02)	64	Male	NO IMROVEMENT
1004	Stroke	1, (OLS-19-01)	59	Male	IMPROVED
1005	Stroke	1, (OLS-19-01)	61	Male	NO IMROVEMENT
1006	PD	1, (OLS-19-01)	50	Female	IMPROVED
1007	Stroke	1, (OLS-19-01)	49	Female	IMPROVED
1008	Stroke	2 , (OLS-19-02)	60	Male	NO IMROVEMENT
1009	PD	1, (OLS-19-01)	38	Male	NO IMROVEMENT
1010	PD	2 , (OLS-19-02)	53	Male	NO IMROVEMENT

Indian Journal of Medical and Allied Research
Volume 12, Issue 4, May 2023 pp 1-7. www.ijmar.in ISSN: 2278-0890

1011	PD	1, (OLS-19-01)	70	Male	NO IMROVEMENT
1012	PD	2 , (OLS-19-02)	53	Female	NO IMROVEMENT
1013	PD	2 , (OLS-19-02)	38	Male	NO IMROVEMENT
1014	PD	1, (OLS-19-01)	56	Female	IMPROVED
1015	PD	2 , (OLS-19-02)	45	Male	NO IMROVEMENT
1016	PD	1, (OLS-19-01)	71	Male	NO IMROVEMENT
1017	PD	1, (OLS-19-01)	65	Female	IMPROVED
1018	Stroke	2 , (OLS-19-02)	62	Male	NO IMROVEMENT
1019	Stroke	1, (OLS-19-01)	51	Male	NO IMROVEMENT
1020	Stroke	2 , (OLS-19-02)	52	Female	NO IMROVEMENT
1021	Stroke	1, (OLS-19-01)	63	Male	NO IMROVEMENT
1022	Stroke	1, (OLS-19-01)	63	Female	NO IMROVEMENT
1023	Stroke	1, (OLS-19-01)	65	Male	NO IMROVEMENT
1024	PD	2 , (OLS-19-02)	58	Male	NO IMROVEMENT
1025	PD	1, (OLS-19-01)	60	Male	IMPROVED
1026	PD	2 , (OLS-19-02)	65	Male	NO IMROVEMENT
1027	PD	1, (OLS-19-01)	48	Male	NO IMROVEMENT
1028	Stroke	2 , (OLS-19-02)	41	Female	NO IMROVEMENT
1029	Stroke	2 , (OLS-19-02)	58	Female	NO IMROVEMENT
1030	Stroke	2 , (OLS-19-02)	63	Male	NO IMROVEMENT

Table-2B

Treatment outcomes based on Gender		
	Female	Male
IMPROVED	4	3
NO IMPROVEMENT	1	8

Table-2C

Treatment outcomes in different class of patients			
	PD	PD&Stroke	Stroke
IMPROVED	4	1	2
NO IMPROVEMENT	4	0	5

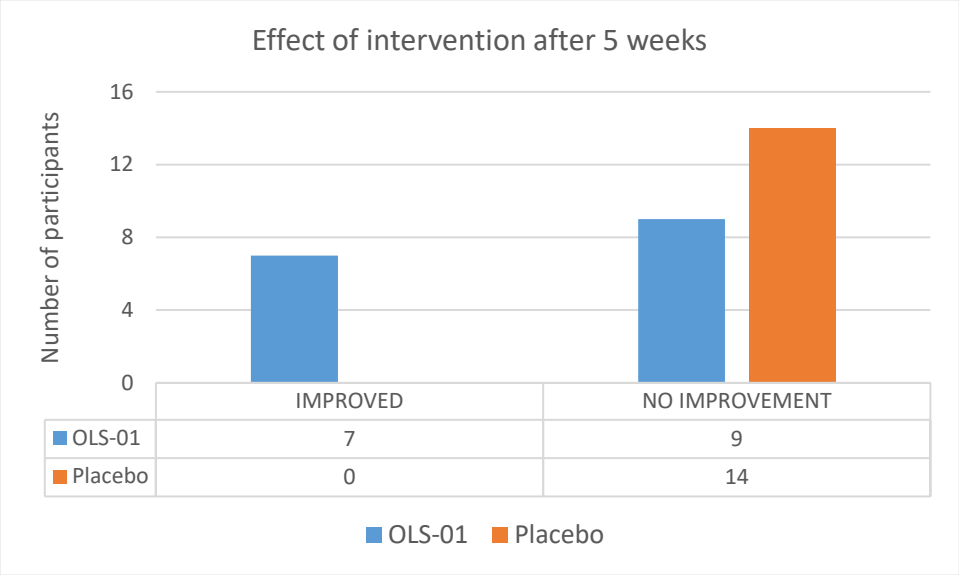

Figure 1

As evident from the Figure-1, 46% of the people treated with OLS-01 showed improvement in their cognitive performance. Considering the sample size and type of variables measured, Fishers exact test was used to score the significance of the effect of OLS-01. A two tailed Fishers exact test resulted in the p value of 0.007305, indicating a strong association between the intervention and the outcome.

Discussion and conclusion: The brain's activity plays a vital role in cognitive function, behaviour, and subjective experience. Neural networks interact to produce mental activity. Aging causes changes in neural cell assemblies leading to cognitive decline, necessitating larger portions of networks to complete the same task, slowing down dynamic cognition compared to static functions (PMID: 8759042). Neurodegenerative disorders such as Parkinson's disease, conditions such as stroke are debilitating conditions that significantly impact cognitive function. These disorders affect the nervous system, resulting in the loss of nerve cells and synapses, leading to cognitive decline. Several interventions have been proposed, including pharmaceuticals, cognitive rehabilitation, and lifestyle changes, but they often provide only modest benefits. Therefore, there is a growing interest in exploring the potential of health supplements to improve cognitive function. While several interventions have been proposed to improve cognitive function in patients with neurodegenerative disorders, there is still a significant need for safe and effective health supplements to address the cognitive decline associated with these conditions.

Lutein, a dietary antioxidant found in green leafy vegetables, has potential for maintaining brain structure by reducing chronic oxidative stress (PMID: 26566524). The brain is highly vulnerable to damage from chronic inflammation, and Lutein and Zeaxanthin (L and Z) have potent anti-inflammatory properties (PMID: 22465791). However, it remains uncertain whether such dietary components that prevent loss are effective in later life, after significant loss has already occurred.

Preliminary data suggests that supplementing L and Z in younger individuals increases systemic levels of brain-derived neural growth factor compared to a placebo (PMID: 28661438). Additionally, clinical trials have found that L and Z supplementation increases visual processing speed and reaction times in younger individuals (PMID: 25251377, PMID: 25483230). In our study, the patients belonging to debilitating conditions such as Parkinson's disease and stroke were employed to evaluate the effect of OLS-01. The study results indicate that about 46% of the patients who consumed OLS-01 showed significant improvement in cognitive functions.

Our results showed that supplementing with OLS-01 for 5 weeks, can result in significant improvements in cognitive performance compared to the placebo group. Specifically, in treatment group 46% of the patients showed significant improvement in cognitive functions such as memory, attention, and executive function. The intervention was well-tolerated, with no significant adverse effects reported.

These findings suggest that Marigold extract standardised to Lutein and Zeaxanthin, holds promise as a potential intervention for cognitive dysfunction and may improve quality of life in affected individuals.

REFERENCES

1. Al-Ahmary KM. The carotenoids of some food stuffs in Saudi Arabia. Int J Food Sci Nutr. 2010 Dec;61(8):823-8. doi: 10.3109/09637486.2010.488638. Epub 2010 Jul 8. PMID: 20608883.
2. Abdel-Aal el-SM, Akhtar H, Zaheer K, Ali R. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. Nutrients. 2013 Apr 9;5(4):1169-85. doi:

10.3390/nu5041169. PMID: 23571649; PMCID: PMC3705341.
3. Johnson EJ, Vishwanathan R, Johnson MA, Hausman DB, Davey A, Scott TM, Green RC, Miller LS, Gearing M, Woodard J, Nelson PT, Chung HY, Schalch W, Wittwer J, Poon LW. Relationship between Serum and Brain Carotenoids, α-Tocopherol, and Retinol Concentrations and Cognitive Performance in the Oldest Old from the Georgia Centenarian Study. J Aging Res. 2013;2013:951786. doi: 10.1155/2013/951786. Epub 2013 Jun 9. PMID: 23840953; PMCID: PMC3690640.
4. Lopresti AL, Smith SJ, Drummond PD. The Effects of Lutein and Zeaxanthin Supplementation on Cognitive Function in Adults With Self-Reported Mild Cognitive Complaints: A Randomized, Double-Blind, Placebo-Controlled Study. Front Nutr. 2022 Feb 17;9:843512. doi: 10.3389/fnut.2022.843512. PMID: 35252311; PMCID: PMC8891800.
5. Yuan C, Fondell E, Ascherio A, Okereke OI, Grodstein F, Hofman A, Willett WC. Long-Term Intake of Dietary Carotenoids Is Positively Associated with Late-Life Subjective Cognitive Function in a Prospective Study in US Women. J Nutr. 2020 Jul 1;150(7):1871-1879. doi: 10.1093/jn/nxaa087. PMID: 32386230; PMCID: PMC7330480.
6. Christensen K, Gleason CE, Mares JA. Dietary carotenoids and cognitive function among US adults, NHANES 2011-2014. Nutr Neurosci. 2020 Jul;23(7):554-562. doi: 10.1080/1028415X.2018.1533199. Epub 2018 Oct 16. PMID: 30326796; PMCID: PMC6467741.
7. Ajana S, Weber D, Helmer C, Merle BM, Stuetz W, Dartigues JF, Rougier MB, Korobelnik JF, Grune T, Delcourt C, Féart C. Plasma Concentrations of Lutein and Zeaxanthin, Macular Pigment Optical Density, and Their Associations With Cognitive Performances Among Older Adults. Invest Ophthalmol Vis Sci. 2018 Apr 1;59(5):1828-1835. doi: 10.1167/iovs.17-22656. PMID: 29610850.
8. Wang Y, Liu G, Hong D, Chen F, Ji X, Cao G. White matter injury in ischemic stroke. Prog Neurobiol. 2016 Jun;141:45-60. doi: 10.1016/j.pneurobio.2016.04.005. Epub 2016 Apr 14. PMID: 27090751; PMCID: PMC5677601.
9. Fang C, Lv L, Mao S, Dong H, Liu B. Cognition Deficits in Parkinson's Disease: Mechanisms and Treatment. Parkinsons Dis. 2020 Mar 24;2020:2076942. doi: 10.1155/2020/2076942. PMID: 32269747; PMCID: PMC7128056.
10. Salthouse TA. The processing-speed theory of adult age differences in cognition. Psychol Rev. 1996 Jul;103(3):403-28. doi: 10.1037/0033-295x.103.3.403. PMID: 8759042.
11. Erdman JW Jr, Smith JW, Kuchan MJ, Mohn ES, Johnson EJ, Rubakhin SS, Wang L, Sweedler JV, Neuringer M. Lutein and Brain Function. Foods. 2015 Dec;4(4):547-564. doi: 10.3390/foods4040547. Epub 2015 Oct 9. PMID: 26566524; PMCID: PMC4638416.
12. Kijlstra A, Tian Y, Kelly ER, Berendschot TT. Lutein: more than just a filter for blue light. Prog Retin Eye Res. 2012 Jul;31(4):303-15. doi: 10.1016/j.preteyeres.2012.03.002. Epub 2012 Mar 21. PMID: 22465791.
13. Bovier ER, Renzi LM, Hammond BR. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on neural processing speed and efficiency. PLoS One. 2014 Sep 24;9(9):e108178. doi: 10.1371/journal.pone.0108178. PMID: 25251377; PMCID: PMC4176961.
14. Bovier ER, Hammond BR. A randomized placebo-controlled study on the effects of lutein and zeaxanthin on visual processing speed in young healthy subjects. Arch Biochem Biophys. 2015 Apr 15;572:54-57. doi: 10.1016/j.abb.2014.11.012. Epub 2014 Dec 4. PMID: 25483230.
15. Stringham JM, Stringham NT, O'Brien KJ. Macular Carotenoid Supplementation Improves Visual Performance, Sleep Quality, and Adverse Physical Symptoms in Those with High Screen Time Exposure. Foods. 2017 Jun 29;6(7):47. doi: 10.3390/foods6070047. PMID: 28661438; PMCID: PMC5532554.