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ORIGINAL ARTICLE

Modulatory Effect of Carotenoid Supplement Constituting Lutein and Zeaxanthin (10:1) on Anti-oxidant Enzymes and Macular Pigments Level in Rats

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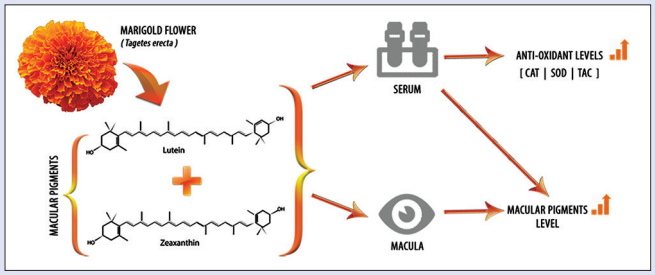
ABSTRACT

Background: Human eye is constantly exposed to different wavelengths and intensities of light. Oxidative stress results in distinct changes to retinal organs and tissues. Macular pigments (lutein and zeaxanthin), present in the central macular region, provide protection from photodamages by absorption of high energy blue light and also by virtue of their anti-oxidant activity. Ocular phototoxicity is thus prevented by our efficient anti-oxidant system, in both young and old. One of the best commercial sources of pure lutein and zeaxanthin is Marigold flowers. **Objective:** In the present study, oil-soluble dietary carotenoid supplement constituting lutein and zeaxanthin in the ratio of 10:1 was evaluated for its modulatory effect on anti-oxidant enzymes and macular pigments in the serum and macula of the Swiss albino rats. **Materials and Methods:** Male Swiss albino rats were treated with carotenoid supplement constituting lutein and trans-Zeaxanthin (10:1) at two different doses daily, under standard experimental conditions for 42 days. End of the treatment, serum and macula were collected and used for measurement of lutein and zeaxanthin levels along with anti-oxidant parameters. **Statistical Analysis Used:** Statistical differences were assessed by analysis of variance (ANOVA) followed by Dunnet's test. $P < 0.05$ was considered statistically significant. All the results were expressed as mean ± standard deviation. **Results:** The supplement exhibited significant elevation of anti-oxidant enzyme levels in treated animals in dose-dependent manner. Concomitantly, the total anti-oxidant capacity has also been found to show similar increment at the end of the study period. This study revealed significant expression of the two macular pigments investigated. **Conclusions:** Our study, therefore, provides a strong claim for the anti-oxidant effect of the oil-soluble dietary carotenoid supplement, and thus substantiates its use in the prevention of phototoxic damage to the eye on long-term supplementation.

Key words: Anti-oxidant enzymes, dietary carotenoid supplement, macular pigments in serum and macula, retinal pigment epithelium, trans-Lutein, trans-Zeaxanthin

SUMMARY

- Apart from its ornamental value, Marigold (*Tagetes erecta* L.) flowers are well known as an herbal remedy due to its antimicrobial, anti-inflammatory, and anti-oxidant activities. Epidemiological studies have implicated prolonged exposure to ultraviolet radiations & blue light and in turn oxidative stress in the pathogenesis of the majority of the eye diseases, since childhood. Studies have shown that with age a number of changes occur predisposing the retinal various organs and tissues to oxidative stress. These changes manifest in decreased levels in plasma of Vitamin C, Vitamin E, glutathione, Retinal Pigment Epithelium (RPE), Catalase (CAT), Super Oxide Dismutase (SOD), Thiobarbituric Acid Reactive Substance (TBARS), and total anti-oxidant capacity (TAC). Age- and diet-related loss of Lutein and Zeaxanthin enhance phototoxic damage to the eye, and thus supplementation of these carotenoids becomes vital for maintaining optimal eye health
- In the present study, XanMax® 2002 oil, a supplement constituting lutein and trans-Zeaxanthin, extracted from the flowers of *T. erecta*, was evaluated for its modulatory effect on anti-oxidant enzymes and macular pigments in the serum and macula of the Swiss albino rats. XanMax® 2002 oil exhibited significant elevation of anti-oxidant enzyme levels in treated animals in dose-dependent manner. Concomitantly, the TAC has also been found to show similar increment at the end of the study period. This study revealed significant expression of the two macular pigments investigated.



Abbreviations used: AMD: Age related Macular Diseases; RPE: Retinal Pigment Epithelium; CAT: Catalase; SOD: Super Oxide Dismutase; TAC: Total Antioxidant Capacity; ROS: Reactive Oxygen Species; LC-MS: Liquid chromatography-mass spectrometry; p.o.: Per Orally; CMC Carboxymethyl cellulose.

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INTRODUCTION

A major sensory organ that requires utmost care for a healthy life is the "eyes." It is one of the most susceptible organs to light damage, apart from the skin. The human eye is constantly exposed to different wavelengths and intensities of light, namely, ultraviolet (UV)-B (295–320 nm), UV-A (320–400 nm), and visible light (400–700 nm) [Figure 1]. Shorter the wavelength, greater the energy, and therefore, the potential for damage will also be greater. However, longer wavelengths which are less energetic also penetrate the eye deeply. The human eye has a unique filtering characteristic that determines the light absorbing site. Thus,

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intensity of light, its wavelength, the site of damage, chromophore, and defender systems are few of the factors that has to be considered while determining whether the light is damaging.[1]

Photochemical reaction occurs in the eye only when light is transmitted to a particular ocular tissue and further absorbed by a particular chromophore. Chromophores act as photosensitizers and are susceptible to oxidative damage.[2] Apart from the protective pigments (kynurenines and melanin) present in the eye, an efficient anti-oxidant system plays a vital role to provide protection against UV and blue light damage, in young and old. As we age, due to unhealthy lifestyle and other environmental assaults, there is a reported decrease in anti-oxidants and anti-oxidant enzymes along with increase in phototoxic chromophores in the eye. Hence, exposure to intense light either causes or aggravates photo-oxidation reaction and in turn age-related ocular diseases such as age-related macular degeneration (AMD), cataract, glaucoma among many others.[1,3]

Ocular damage can occur either through an inflammatory response or a photo-oxidation reaction.[4] Photo-oxidation reaction occurs when a chromophore absorbs light and produces harmful reactive oxygen species (ROS), namely, superoxide and singlet oxygen that damages the ocular tissues. In addition, as the eye ages, the protective chromophores become phototoxic and have the potential to produce singlet oxygen[5,6] [Figure 2]. Hence, optimal levels of anti-oxidant enzymes, namely, catalase (CAT), superoxide dismutase (SOD), and anti-oxidants, namely, lutein, zeaxanthin, Vitamin C, and Vitamin E; that serve to protect against phototoxicity and oxidative stress becomes essential.[7] Lutein and zeaxanthin are anti-oxidants that accumulate in the retina and lens of the human eye. Commonly known as macular pigment, these dietary carotenoids cannot be synthesized by mammals and must therefore be obtained from diet. One of the best commercial sources of pure lutein and zeaxanthin is marigold flowers.[8]

Marigold flowers (*Tagetes erecta* L.)

Commonly known as "Marigold," the species of Tagetes, belonging to Asteraceae family, is grown for its high therapeutic and ornamental values in many parts of the world. The bioactive extracts of Tagetes have shown to exert diverse pharmacological actions, mainly, antimicrobial, anti-inflammatory, anti-oxidant, and wound healing activities.[9] This genus is also recognized as a potential source for therapeutically active constituents, namely, Carotenoids, lutein and zeaxanthin, that are used as nutritional supplements (specially for the eye), natural food colorant, and as poultry feed additive.[9-11] Containing about 50 species of annual or perennial herbaceous plants, "Tagetes" is native to Mexico and other warmer parts of America. It is widely cultivated in other Asian countries such as India, China, Bhutan, and Nepal. The most commonly cultivated varieties of Tagetes is *Tagetes erecta* L[12-14] [Figure 3].

Scientific classification

The taxonomic classification of *T. erecta* L. is shown in Table 1.[13]

Table 1: The taxonomic classification of *Tagetes erecta* L.[13]

Kingdom	Plantae
(unranked)	Angiosperms
(unranked)	Eudicots
(unranked)	Asterids
Order	Asterales
Family	Asteraceae
Subfamily	Asteroideae
Tribe	Tageteae
Genus	Tagetes
Species	Erecta

Common names

Some common names of *T. erecta* used worldwide is given as follows:

African marigold; American marigold; Aztec marigold; big marigold; marigold; or saffron marigold – English, Gainda – Hindi, cheonsugug – Korean, wan shou ju – Chinese, tagète rose d'Inde – French, hohe Studentenblume – German, senju-giku – Japanese, maravilha – Portuguese, barchatcy prjamostojajcie – Russian, flor de muerto – Spanish, stor tagetes – Swedish (USDA, National Genetic Resource Program)

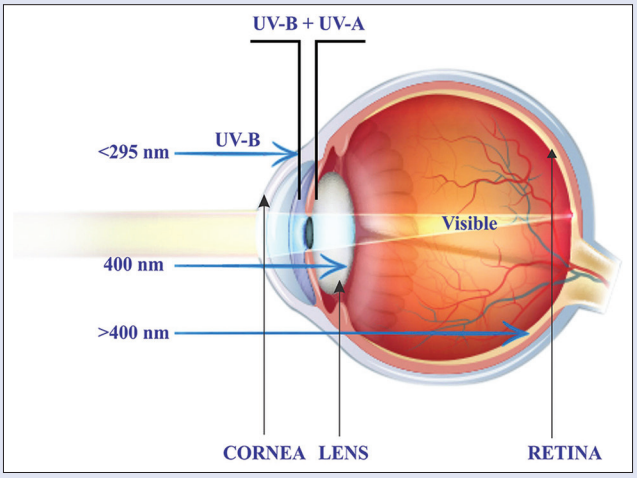

Figure 1: Wavelength transmission of the adult human eye

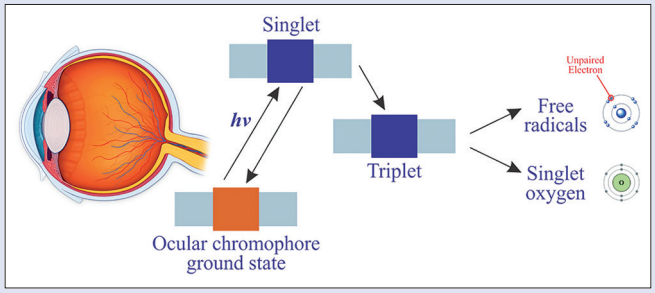

Figure 2: Photo-oxidation

Figure 3: Marigold field

The major categories of petrochemicals identified and studied extensively in Tagetes are the terpenoids, flavonoids, alkaloids, and fatty acids including the nature pigments-carotenoids.[9] Carotenoids are a class of a naturally occurring pigments present in a majority of vegetables and fruits such as orange, broccoli, spinach, kale, corn, and avocado.[15] About 40 carotenoids are known to be present in a typical human diet, among 750 carotenoids present in nature. Out of 40, only 20 are found in blood and only two from them accumulate in the retina, that is, lutein and zeaxanthin.[16-18]

Numerous epidemiological, clinical, and interventional studies have enumerated the role of lutein and zeaxanthin in human health, particularly in eye health. Lutein and zeaxanthin constitute the main macular pigment found in the yellow spot of the human retina that protects the macular from ocular damage due to blue light or if radiation, by scavenging harmful ROS. They are essential for maintaining optimal visual acuity, enhancing contrast sensitivity and in turn improving visual performance[19] [Figure 4]. Most diet does not deliver adequate amounts of lutein and zeaxanthin because we simply do not consume as many leafy vegetables. XanMax® has been specially formulated to bridge this gap and also to ensure optimal lutein and zeaxanthin supplementation, in turn, sufficient protection for healthy vision, naturally.

The product used in the present study, an oil-soluble dietary carotenoid supplement, XanMax® 2002 oil, constituting natural lutein and zeaxanthin, was obtained by extraction and purification from the flowers of *T. erecta*, Marigold flowers, using patented technology and formulated by dispersing in a vegetable oil using a proprietary technology. The proprietary technology used in formulating this product provides high degree of homogeneity, stability, and bioavailability, in turn ensuring maximum efficacy of the product.

An *in vivo* preclinical study was conducted on this dietary carotenoid supplement with an objective to determine the modulatory role as an anti-oxidant and macular pigment levels in both serum and in the macula of healthy Swiss albino rats by an oral administration at two different doses for 6 weeks.

MATERIALS AND METHODS

Chemicals and reagents

Carboxymethyl cellulose was procured from SD fine chemicals, India. Phosphate-buffered saline (PBS) was procured from Himedia Laboratories, India. Demineralized water was obtained from spectrum chemicals, India. N-Hexane and ethyl acetate were obtained from Fisher Scientific, India. All the other chemicals and reagents were of analytical grade.

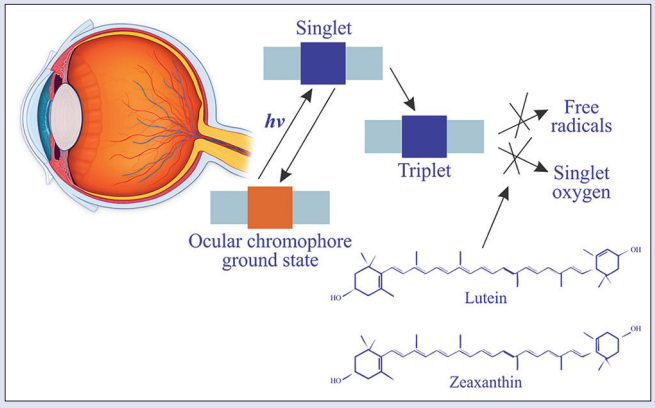

Figure 4: Mechanism of protection by lutein and zeaxanthin against photo-oxidation

Equipments

All the instruments, Weighing Balance (Citizen, Singapore), Microplate Reader (Biotech, USA), UV-Vis spectrophotometer (Systronics, India), Liquid chromatography-mass spectrometry (LC-MS) (Agilent 6130), and other laboratory equipment used for the experiments were calibrated and validated regularly.

Preparation of XanMax® 2002 oil (oil-soluble dietary carotenoid supplement constituting lutein and zeaxanthin [ratio of 10:1])

XanMax® 2002 oil is a natural carotenoid supplement constituting 20% of trans-Lutein and 2% of trans-Zeaxanthin dispersed and homogenized in sunflower oil. The natural carotenoid pigments were obtained by extraction, separation, and purification of the extract from the flowers of *T. erecta* (Marigold) using a patented process. The carotenoids suspension in sunflower oil was processed uniquely using proprietary technology to obtain a maximum bioavailability and desired efficacy in the eye health. The product XanMax® 2002 oil is a uniform homogeneous orange-red, free-flowing liquid suspension, having a narrow particle size distribution, which is largely responsible for its efficacy.

Selection and maintenance of animals

In-house bred male Swiss albino rats, weighing around 200–240 g and in the age group of 4–6 weeks were selected for the study. These were examined for their health, marked and kept for acclimatization under laboratory conditions for 7 days. Only animals without any visible signs of illness were used for the study. The selected animals were randomized and grouped manually. Animals were marked as per the group with different color indelible marker pen on the tail and housed in cages with unique number.

Husbandry

Animals were maintained under standard laboratory conditions in an air-conditioned animal room with adequate air changes per hour. The animals were provided with a light cycle of 12 h light and 12 h dark and were housed in groups of three in polycarbonate cages (approximate internal dimensions of 365 mm × 202 mm × 180 mm height) with paddy husk bedding. Animals were fed with rodent feed *ad libitum* and provided with GenPure RO water *ad libitum*.

All the animal experiments were conducted as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, Government of India. The study was implemented through the Institutional Animal Ethical Committee of Radiant Research Services Pvt. Ltd., Bengaluru, India, with the authorization from local Ethics Committee (project approval no: RR/IAEC/007-2016 dt. 09th January 2016).

Grouping and treatment

The animals were divided into four groups, each group comprising six animals were treated with placebo and test product for 42 days. Group I treated with vehicle (Carboxy Methyl Cellulose), p.o. (per orally), Group II treated with sunflower oil at 1.028 mg/kg bwt twice daily, p.o. (per orally), Group III and Group IV were treated with XanMax® 2002 oil at 0.514 and 1.028 mg/kg bwt twice daily mg/kg bwt, p.o. (per orally).

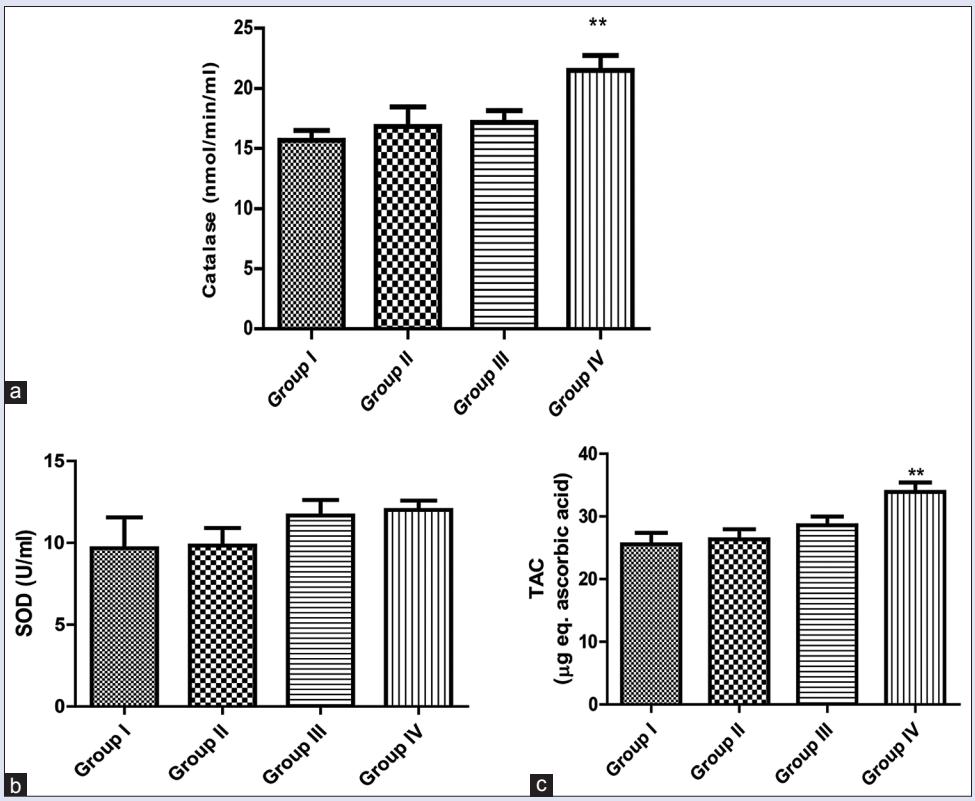

Figure 5: Modulatory effect of XanMax® 2002 oil on serum anti-oxidant levels in rats. Animals were treated with XanMax® 2002 oil for 42 days and serum anti-oxidant markers (a) Catalase and (b) superoxide dismutase were measured along with (c) total Anti-oxidant activity of the serum was estimated. Values are expressed as mean ± standard deviation for six animals in each group. $^{**}P < 0.005$ between control and treated groups

Collection of serum

On day 0 and 42, blood samples were collected from retro-orbital sinus plexus under mild anesthesia. The collected blood was allowed to clot at room temperature, followed by the separation of serum. The serum samples were processed for the biochemical analysis using standard procedures.

Isolation of macula

At the end of the treatment on day 42, the animals were sacrificed. The eye of the rats were washed with PBS and pulled out from the eye socket using forceps. The eyeball was washed with PBS and then is totally removed by cutting the optic nerve connected to it. The macula portion was carefully separated from the eyeball by cutting out the retinal epithelium and washed out with PBS again and placed in 1 ml PBS. Isolated macula was homogenized and centrifuged at 7000 rpm for 10 min. After the centrifugation, the supernatant fluid was collected to the microcentrifuge tubes.

Extraction of carotenoids from serum and macula

The serum (0.5 ml) and macula homogenates (1.0 ml) were extracted separately with n-hexane using solvent extraction method. Briefly, the samples were transferred to glass tube and 5 ml of n-hexane was added to it, mixed in a cyclomixer for 5 min, allowed to separate into two layers for 2 min, separated the upper organic layer into the other tube. This step was repeated two more times and all the organic layers were pooled together, evaporated the solvent under reduced pressure in a stream of nitrogen gas completely to yield the dried residues of the carotenoid mixture. These were further solubilized in mobile phase using sonicator for 2 min. These samples were used for LC-MS analysis.

Biochemical analysis

The serum samples were analyzed for their total anti-oxidant capacity (TAC), SOD, and CAT levels using standard kits. Standard methods were used for the analysis of respective enzyme using references.

Liquid chromatography-mass spectrometry analysis

The serum and macula homogenates were analyzed by LC-MS technique using a silica 4.6 × 250 mm column, 5 micron. The flow rate was 1.5 ml/min in an isocratic system maintained at a column temperature 25°C. The wavelength of detector maintained to 446 nm to detect the peaks and the chromatography runs using an injection volume of 500 µl for 40 min. The analysis was performed under standard instrument conditions using the mobile phase n-Hexane: Ethyl acetate (75:25).

Statistical analysis

Statistical differences were assessed by analysis of variance followed by Dunnet's test. $P < 0.05$ was considered statistically significant. All the results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

In the study, XanMax® 2002 oil established anti-oxidant activity in a dose-dependent manner by elevating the levels of anti-oxidant enzymes significantly in the high treatment dose compared to lower dose-treated animals. Treatment with the higher dose at 2.056 mg/day for 6 weeks has elevated the levels of CAT and SOD by 37.29% and 24.22%, respectively, in the serum over control group as well as placebo. Simultaneously, the TAC has increased significantly by 32.75% over the placebo and control. This indicates that the carotenoids supplement, XanMax® 2002

oil, has a profound action on the anti-oxidant defense system. Lutein and zeaxanthin, by their strong anti-oxidant effect, may reduce the oxidative damage and minimize oxidative stress [Table 2 and Figure 5]. To substantiate the effect of XanMax® 2002 oil, we have evaluated the levels of lutein and zeaxanthin in the serum and the macular protein of retina of the animals both at baseline (day 0) and on day 42. From the separated serum and macular homogenates, LC-MS analysis was done to estimate the content of lutein and zeaxanthin. Both these macular carotenoids were detected and quantified in a dose-dependent manner; however, the control and placebo group samples did not show these carotenoids in any detectable range. In the low-dose group, serum lutein was shown in only two out of six animals with a mean value of 138.9 ± 67.8 ppb, but zeaxanthin was not detected in any animal in the group. In the high-dose group, serum lutein and zeaxanthin was detected in all animals with a mean value 251.6 ± 109.4 ppb and 119.6 ± 65.5 ppb, respectively.

In the macular homogenates, the low-dose group of animals, four out of six animals, showed low level of lutein and quantified with only a mean value of 19.1 ± 4.5 ppb, but zeaxanthin was not detected and quantified in any animal in the group. However, in the high-dose group, macular lutein was detected as all animals with an appreciable mean value of 196.1 ± 119.3 ppb, where zeaxanthin expression from macula was marginal (mean value 14.7 ± 1.7 ppb) and was detected in only two out of six animals [Table 3 and Figure 6].

This study leads us to make some interesting conclusions. Treatment of XanMax® 2002 oil to Swiss albino rats at a dose level of 2.056 mg/day for 6 weeks, significantly elevated the levels of CAT, SOD, and TAC over placebo and control group. Simultaneously, this group of animals expressed significant levels of lutein and zeaxanthin from the serum, but in the macular portion, although the levels of expression of lutein were reasonably high, the level of Zeaxanthin was low.

Increasing concern exists over the adverse effects of environmental pollution on health.[20] Long-term and continued exposure to such abuses have been shown to result in severe oxidative stress to humankind leading to chronic illnesses of inflammatory nature, covering almost all organs and tissues including, the eyes. In a healthy human, the critical role of ROS in the deterioration of health and the importance of anti-oxidant defense systems to ameliorate the toxicity is significant.[21] To maintain defense against destructive oxygen species, an appropriate balance of enzymatic and nonenzymatic antioxidative defenses is necessary.[22] Besides, the natural phenomenon for the deterioration of health, namely, aging and unhealthy food habits, the dietary depletion of vitamins, minerals and carotenoids, also play a leading role in the development of chronic diseases.

Table 2: Modulatory effect of XanMax® 2002 oil on serum anti-oxidant levels in rats

Groups	SOD (units/ml)		Catalase (nmol/min/ml)		TAC (µg per equivalent of ascorbic acid)	
	0 day	42 day	0 day	42 day	0 day	42 day
Group I	10.33±2.16	9.66±4.63	13.16±0.98	15.66±2.06	22.88±3.09	25.55±4.44
Group II	9.83±1.94	9.83±2.63	13.00±2.53	16.83±3.97	21.56±2.41	26.35±3.94
Group III	10.00±1.41	11.66±2.33	12.50±2.58	17.16±2.40	21.99±2.22	28.58±3.39
Group IV	9.66±1.21	12.00±1.41	13.00±1.54	21.50±3.01**	21.47±2.61	33.92±3.63**

**$P<0.005$ between control and treated groups. Values are expressed as mean±SD for six animals in each group. SD: Standard deviation; SOD: Superoxide dismutase; TAC: Total anti-oxidant capacity

Table 3: Lutein and zeaxanthin levels in serum and macula portion of retina from rats treated with XanMax® 2002 oil

Group	Animal number	Serum				Macula			
		Lutein	Mean±SD	Zeaxanthin	Mean±SD	Lutein	Mean±SD	Zeaxanthin	Mean±SD
Group I	1	ND	ND	ND	ND	ND	ND	ND	ND
	2	ND		ND		ND		ND	
	3	ND		ND		ND		ND	
	4	ND		ND		ND		ND	
	5	ND		ND		ND		ND	
	6	ND		ND		ND		ND	
Group II	7	ND	ND	ND	ND	ND	ND	ND	ND
	8	ND		ND		ND		ND	
	9	ND		ND		ND		ND	
	10	ND		ND		ND		ND	
	11	ND		ND		ND		ND	
	12	ND		ND		ND		ND	
Group III	13	91.02	138.99±67.84	ND	ND	24.97	19.13±4.53	ND	ND
	14	186.96		ND		18.82		ND	
	15	ND		ND		13.90		ND	
	16	ND		ND		18.82		ND	
	17	ND		ND		ND		ND	
	18	ND		ND		ND		ND	
Group IV	19	168.51	251.60±109.45***	47.23	119.58±65.55***	115.62	196.16±119.28***	15.93	14.70±1.73
	20	405.90		118.08		182.04		ND	
	21	217.71		100.12		23.19		13.47	
	22	282.90		226.32		201.11		ND	
	23	328.41		106.15		330.26		ND	
	24	106.15		ND		324.72		ND	

***$P<0.0001$ between control and treated groups. Values are expressed as mean±SD for each group. Lutein and zeaxanthin values were determined from LC-MS data and expressed as ppb concentration. ND: The concentrations of lutein/zeaxanthin were not in detected range and for statistical analysis ND was considered as 0 ppb; LC-MS: Liquid chromatography-mass spectrometry; SD: Standard deviation

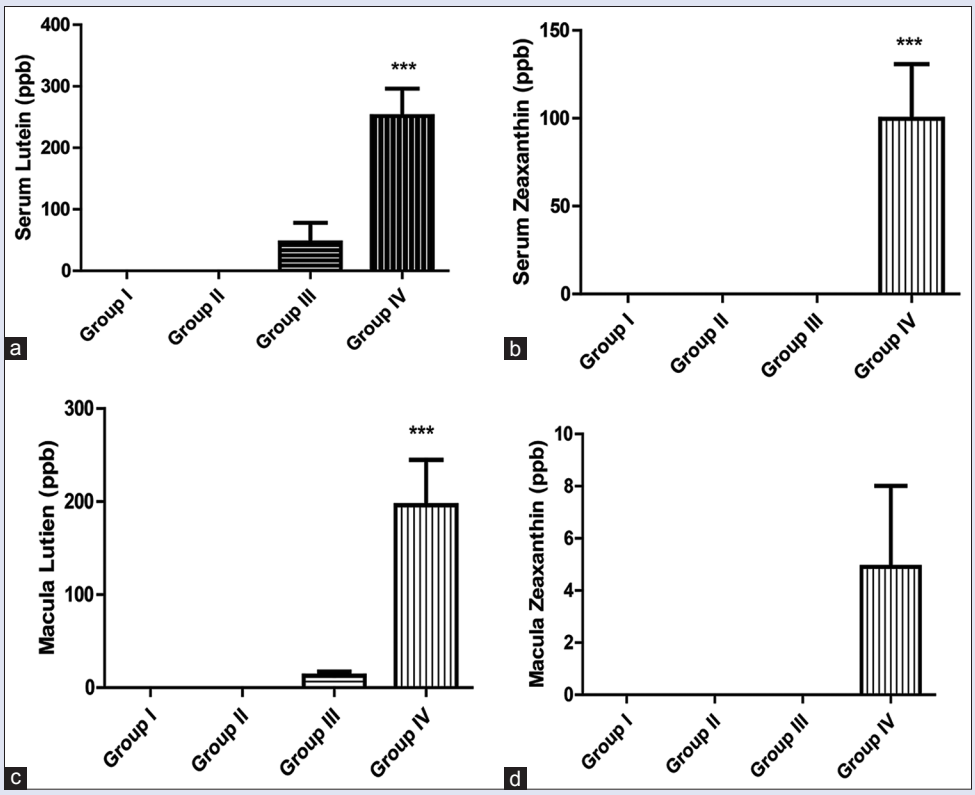

Figure 6: Modulatory effect of XanMax® 2002 oil on Lutein and Zeaxanthin levels in serum and macula portion of the retina from rats. Rats were treated with XanMax® 2002 oil for 42 days and lutein and zeaxanthin values in serum (a and b respectively), and macular homogenates (c and d respectively) were determined by liquid chromatography-mass spectrometry and expressed as ppb concentration. Values are expressed as mean ± standard deviation for each group; ***$P < 0.0001$ between control and treated groups

In the eye, the retina or more particularly, the macula, is considered as the most vulnerable site for the generation of ROS, more so, the damage is caused by the long exposure to external insults.[23,24] The first line of anti-oxidant defense systems include the anti-oxidant enzymes, namely, CAT, SOD, glutathione peroxidase, besides the other nonenzymatic anti-oxidants such as vitamins, minerals, natural polyphenols, and carotenoids. These play a key role in protecting the retinal pigment epithelium cells and photoreceptors from the damages caused to the eyes due to oxidative stress.[25,26] Studies have indicated that carotenoids, in particular, lutein and zeaxanthin, either help prevent or reduce the risk of progression of eye diseases and macular degeneration. It is believed that these two natural carotenoids block blue light from reaching the underlying retinal structures, thereby reduce the risk of light-induced oxidative damage that causes photo-oxidation and in turn macular degeneration. *In vitro* experiments with lutein indicates that it absorbs blue light at wavelengths around 450 nm with a peak absorption at 446 nm that are known to induce light-mediated damage to the retina. By acting as a blue light filter, the lens and macular pigments lutein and zeaxanthin can protect underlying retinal structures from light-induced damage.[27,28] Thus, it is observed that consumption of diets with higher levels of lutein and zeaxanthin are associated with lower incidence of eye diseases such as AMD, cataract, and diabetic retinopathy.

It is clearly observed in the present study that treatment with XanMax® 2002 oil improves the pigment and anti-oxidant enzyme levels in both serum and macula. Epidemiological research shows an inverse relationship between macular pigment levels of lutein and zeaxanthin and ocular diseases. Increased macular pigment density and anti-oxidant enzyme level have been positively linked with decreased risk and progression of ocular diseases. This, in turn, helps to substantiate the fact that increased pigment and anti-oxidant enzyme of lutein and zeaxanthin present in XanMax® 2002 oil will help in mitigating early onset of ocular diseases in young and decrease the risk of progression of ocular diseases in old, on long-term supplementation. This fact is further validated by the clinical trial carried out on XanMax® 2002 oil by the authors.

CONCLUSIONS

Deterioration of vision need not necessarily be a consequence of aging alone. Unhealthy lifestyle, smoking, alcohol consumption, prolonged exposure to blue light (due to usage of mobile phones, laptops, etc.), UV damage, diabetes, and obesity can also cause visual woes. Balanced nutrition thus becomes integral part of good eye care.

The ocular damage caused by phototoxic reactions can be prevented effectively by use of appropriate anti-oxidant quenchers. Among the various carotenoids that occur in nature, only lutein and zeaxanthin have been known to be accumulated in the macula and provide the rich and beneficial effects as anti-oxidants. Age- and diet-related loss of lutein and zeaxanthin enhance phototoxic damage to the eye, and thus supplementation of these carotenoids becomes vital for maintaining optimal eye health.

Our present study, therefore, guides us to conclude that regular supplementation of XanMax® 2002 oil (oil-soluble dietary carotenoid supplement constituting lutein and trans-Zeaxanthin [ratio of 10:1]) would help to maintain a healthy macula through deposition of optimal levels of carotenoid pigments and antioxidative enzymes.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Roberts JE. Ocular phototoxicity. J Photochem Photobiol B 2001;64:136-43.
2. Scripsema NK, Hu DN, Rosen RB. Lutein, zeaxanthin, and meso-zeaxanthin in the clinical management of eye disease. J Ophthalmol 2015;2015:865179.
3. Turner PL, Mainster MA. Circadian photoreception: Ageing and the eye's important role in systemic health. Br J Ophthalmol 2008;92:1439-44.
4. Pouya ND, Boyer DS. The Role of Inflammation in AMD. Retinal Physician; 2011. Available from: http://www.retinalphysician.com/issues/2011/september-2011/the-role-of-inflammation-in-amd.
5. Rahman T, Hosen I, Towhidul Islam MM, Shekhar HU. Oxidative stress and human health. Adv Biosci Biotechnol 2012;3:997-1019.
6. Roberts JE. Screening for ocular phototoxicity. Int J Toxicol 2002;21:491-500.
7. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. Arch Ophthalmol 2004;122:598-614.
8. Jia YP, Sun L, Yu HS, Liang LP, Li W, Ding H, *et al.* The pharmacological effects of lutein and zeaxanthin on visual disorders and cognition diseases. Molecules 2017;22. pii: E610.
9. Vasudevan P, Kahsyap S, Sharma S. Tagetes: A multipurpose plant. Bioresour Technol 1997;62:29-35.
10. Park YJ, Park SY, Valan Arasu M, Al-Dhabi NA, Ahn HG, Kim JK, *et al.* Accumulation of carotenoids and metabolic profiling in different cultivars of tagetes flowers. Molecules 2017;22. pii: E313.
11. Gupta P. Carotenoids of therapeutic significance from marigold. Nat Prod Chem Res 2014;2:e110.
12. Karwani G, Sisodia SS. *Tagetes erecta* plant: Review with significant pharmacological activities. World J Pharm Sci 2015;3:1180-3.
13. Regaswamy D, Koilpillai J. Physicochemical screening of *Tagetes erecta* Linn. Proc World Congr Eng 2014;I.
14. Shetty LJ, Sakr FM, Al-Obaidy K, Patel MJ, Shareef H. A brief review on medicinal plant *Tagetes erecta* Linn. J Appl Pharm Sci 2015;5:91-5.
15. Eisenhauer B, Natoli S, Liew G, Flood VM. Lutein and zeaxanthin-food sources, bioavailability and dietary variety in age-related macular degeneration protection. Nutrients 2017;9. pii: E120.
16. Ramirez M. Why lutein is important for the eye and the brain. OCL 2016;23:D107.
17. Bone RA, Landrum JT, Tarsis SL. Preliminary identification of the human macular pigment. Vision Res 1985;25:1531-5.
18. Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC: Retinal distribution and age study. Invest Ophthalmol Vis Sci 1988;29:843-9.
19. Koushan K, Rusovici R, Li W, Ferguson LR, Chalam KV. The role of lutein in eye-related disease. Nutrients 2013;5:1823-39.
20. Kelly FJ. Oxidative stress: Its role in air pollution and adverse health effects. Occup Environ Med 2003;60:612-6.
21. Chaitanya KV, Pathan AA, Mazumdar SS, Chakravarthi GP, Parine N, Bobbarala V. Role of oxidative stress in human health: An overview. J Pharm Res 2010;3:1330-3.
22. Leppadaman J, Atalay M. Protection against Oxidative Stress. Physiology and Maintenance. Vol. II. Available from: http://www.Eolss.net/ebooklib.
23. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 2000;45:115-34.
24. Cai J, Nelson KC, Wu M, Sternberg P Jr., Jones DP. Oxidative damage and protection of the RPE. Prog Retin Eye Res 2000;19:205-21.
25. Tokarz P, Kaarniranta K, Blasiak J. Role of anti-oxidant enzymes and small molecular weight anti-oxidants in the pathogenesis of age-related macular degeneration (AMD). Biogerontology 2013;14:461-82.
26. Frank RN, Amin RH, Puklin JE. Anti-oxidant enzymes in the macular retinal pigment epithelium of eyes with neovascular age-related macular degeneration. Am J Ophthalmol 1999;127:694-709.
27. Kijlstra A, Tian Y, Kelly ER, Berendschot TT. Lutein: More than just a filter for blue light. Prog Retin Eye Res 2012;31:303-15.
28. Roberts JE, Dennison J. The photobiology of lutein and zeaxanthin in the eye. J Ophthalmol 2015;2015:687173.