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Antioxidant potential of meso-zeaxanthin a semi synthetic carotenoid

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ABSTRACT

Semi synthetic carotenoid meso-zeaxanthin was evaluated for its antioxidant potential *in vitro* and *in vivo*. Meso-zeaxanthin was found to scavenge superoxide radicals, hydroxyl radicals and inhibited *in vitro* lipid peroxidation. Concentrations needed for 50% inhibition (IC_{50}) were 27.0, 3.5 and 3.2 μg/ml, respectively. It scavenged 2,2-azobis-3-ethylbenzthiozoline-6-sulphonic acid and 2,2-diphenyl-1-picryl hydrazyl radicals and IC_{50} were 46.5, 6.25 μg/ml, respectively. It also scavenged nitric oxide radicals and IC_{50} was found to be 2.2 μg/ml. Oral administration of meso-zeaxanthin inhibited superoxide radicals generated in macrophages by 25.2%, 50.1% and 67.2% at doses of 50, 100 and 250 mg/kg b.wt., respectively. One month oral administration of meso-zeaxanthin to mice significantly increased catalase, superoxide dismutase, glutathione and glutathione reductase levels in blood and liver. Levels of glutathione peroxidase and glutathione-S-transferase were also found to be increased in the liver, in a dose dependent manner. These results showed that meso-zeaxanthin has significant antioxidant activity *in vitro* and *in vivo*.

1. Introduction

Free radical production is ubiquitous in all respiring organisms, and is enhanced in many disease states. Most free radicals in biological systems are derivatives of oxygen (reactive oxygen species, "ROS") but there are also derivatives of nitrogen (reactive nitrogen species, "RNS"). Oxidative and nitrosative stress occurs when the rate of production of ROS and RNS exceed the antioxidant capability of the cell. In such cases, ROS/RNS interact with and modify the cellular proteins, lipids and DNA, which results in altered cell function (Singh & Agarwal, 1995). Although free radicals are formidable weapons in the arsenal of our immune system, they have been implicated in the etiology of various diseases including inflammation, cancer, Alzheimer's disease, ischaemic reperfusion injury and a myriad of other disease conditions. The link between free radicals and disease processes led to considerable research to develop non-toxic drugs that can scavenge the free radicals. Several plant extracts and products have been shown to possess significant antioxidant potential (Sabu & Kuttan, 2003).

Carotenoids are red, yellow and orange coloured natural fat soluble pigments found in plants and have been reported to have a number of biological actions, like antioxidant activity, immuno enhancement, inhibition of mutagenesis and transformation, and regression of premalignant lesions (Krinski, 1993). Zeaxanthin [(3R,3′R)-β,β-carotene-3,3′-diol] and lutein [(3R,3′R,6′R)-β,ε-carotene-3,3′-diol] are the non-provitamin A carotenoids, commonly found in green and yellow vegetables. These xanthophyll carotenoids are the predominant carotenoids found in human retina and their concentration is greatest in the fovea centralis of the macula lutea, where they constitute the macular pigment and serve to decrease the risk of age-related macular degeneration (Beatty, Boultan, Henson, Koh, & Murray, 1999) and to protect the retinal pigment epithelium against a photo oxidative damage initiated in part by light absorption (Broekmans et al., 2002; Snodderly, 1995). There is good evidence that this protection is mainly due to their antioxidant properties (Zhang, Cooney, & Bertran, 1991).

Recently a third carotenoid, meso-zeaxanthin [(3R,3′S)-β,β-carotene-3,3′-diol] was found to be effective in the ageing macula to maintain its structural density (Bone, Landrum, Alvarez-Correa, Etienne, & Ruitz, 2003). Meso-zeaxanthin resides directly over the centre of the macula, where light is focused and where the strongest need for hazardous actinic blue light protection exists (Landrum, Bone, Moore, & Gomez, 1999). It is startling that the retina accumulates only the xanthophylls meso-zeaxanthin, zeaxanthin and lutein, whilst not even traces of other carotenoids are found in this tissue (Landrum & Bone, 2001) (Fig. 1).

Unlike lutein and zeaxanthin, meso-zeaxanthin is not found in the diet, and is undetectable in the blood serum. But this carotenoid is of such importance to the eye that it is exclusively synthesised there from ingested lutein. If taken as a supplement, meso-zeaxanthin is absorbed into the blood stream and effectively increases macular pigment levels (Bone et al., 2003). In the present work, we have investigated the antioxidant potential of

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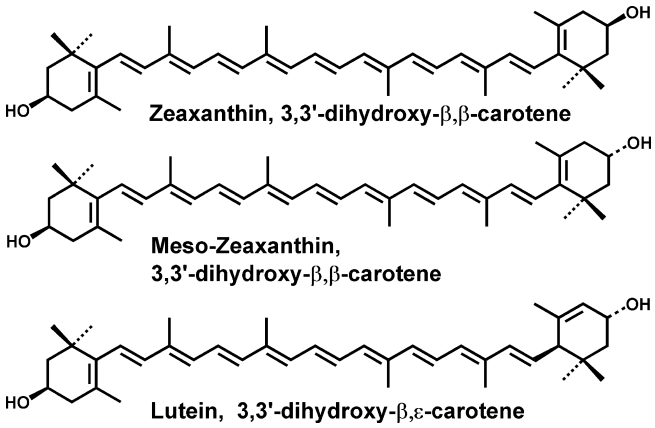

Fig. 1. Structures of carotenoids present in the macula of eye.

meso-zeaxanthin *in vitro* as well as *in vivo* and compared with zeaxanthin, the known antioxidant.

2. Materials and methods

2.1. Chemicals and reagents

Nitroblue tetrazolium (NBT), glutathione, glutathione oxidised (GSSG), nicotinamide adenine dinucleotide phosphophosphate reduced (NADPH) and 5-5′dithiobis (2-niotrobenzoic acid) (DTNB) were purchased from Sisco Research Laboratories Pvt. Ltd., (Mumbai, India), 2,2-diphenyl-1-picryl hydrazyl (DPPH·) and 2,2-azobis-3-ethylbenzthiozoline-6-sulphonic acid (ABTS·) were purchased from Sigma Aldrich (St. Louis, USA), phorbol-12-myristate-13-acetate (PMA) was a gift from Dr. Allen Conney, USA. All other chemicals and reagents used were of analytical grade.

2.2. Drugs

Zeaxanthin and meso-zeaxanthin were supplied by Omni Active Health Technologies Pvt. Ltd., Mumbai. In order to get a uniform suspension of zeaxanthins for *in vitro* studies, zeaxanthin and meso-zeaxanthin powders were dissolved in hexane (10 mg/10 ml) and 10 μl of triton X 100 was added and further evaporated to dryness and made up the volume to 10 ml with distilled water. For *in vivo* studies, a 5% suspension was prepared in sunflower oil.

2.3. Animals

Male Swiss albino mice (20–25 g) were used in the study. They were purchased from Small Animal Breeding Station, Mannuthy, and Kerala, India and were housed in well ventilated cages under controlled conditions of light and humidity and provided with normal mouse chow (Sai Durga Food and Feeds, Bangalore, India) and water *ad libitum*. All the animal experiments were done as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India, and implemented through the Institutional Animal Ethical Committee of the Research Centre.

2.4. A comparative study on in vitro antioxidant activities of zeaxanthin/meso-zeaxanthin

2.4.1. Determination of superoxide radical scavenging activity

Superoxide radical scavenging activity was determined by the NBT reduction method (Mc Cord & Fridovich, 1969). The reaction mixture contained EDTA (6 μM) NaCN (3 μg), riboflavin (2 μM), NBT (50 μM), various concentrations of carotenoids-zeaxanthin and meso-zeaxanthin (3–70 μg) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent lamp for 15 min, and the optical density was measured at 560 nm before and after illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and experimental tubes.

2.4.2. Determination of hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compound for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The hydroxyl radical attacks deoxyribose, which results in thiobarbituric acid reacting substance (TBARS) formation (Elizabeth & Rao, 1990). The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1.0 mM), ascorbic acid (0.1 mM), KH_2PO_4–KOH buffer (20 mM, pH 7.4) and various concentrations of carotenoids (0.1–5 μg) in a final volume of 1 ml. The reaction mixture was incubated for 1 h at 37 °C. Deoxyribose degradation was measured as TBARS and percentage inhibition was calculated (Ohkawa, Oshishi, & Yagi, 1979).

2.4.3. Determination of inhibition of lipid peroxidation

Reaction mixture (0.5 ml) containing rat liver homogenate (0.1 ml, 25% w/v) in Tris–HCl buffer (40 mM, pH 7.0), KCl (30 mM), ferrous ion (0.16 mM) and ascorbic acid (0.06 mM) were incubated for 1 h at 37 °C in the presence (0.1–20 μg) and absence of zeaxanthin and meso-zeaxanthin. The lipid peroxide formed was measured by TBARS formation (Ohkawa et al., 1979). Incubation mixtures (0.4 ml) were treated with sodium dodecyl sulphate (SDS; 8.1%, 0.2 ml), thiobarbituric acid (TBA, 0.8%, 1.5 ml) and acetic acid (20%, 1.5 ml, pH 3.5). The total volume was then made up to 4 ml with distilled water and kept in a water bath at 100 °C for 1 h. After cooling, 1 ml of distilled water and 5 ml of *n*-butanol and pyridine (15:1 v/v) were added and vortexed. After centrifugation, the absorbance of the organic layer was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compound with those of the control, not treated with the extract.

2.4.4. Determination of DPPH· radical scavenging activity

In this method, a commercially available and stable free radical DPPH? soluble in methanol was used. DPPH· in its radical form has an absorption peak at 515 nm, which disappeared on reduction by an antioxidant compounds (Aquino et al., 2001). Different concentrations of carotenoids (0.5–25 μg) were added to 1.5 ml of freshly prepared DPPH· solution (0.25 g/l in methanol). Absorbance was measured at 515 nm; 20 min after the reaction was started. The percentage inhibition of $DPPH^+$ in the reaction medium was calculated by comparing with the control.

2.4.5. Determination of ABTS· radical scavenging activity

ABTS· radical scavenging activity of the extract was determined using ferryl myoglobin/ABTS protocol (Alzoreky & Nakahara, 2001). The stock solutions and 500 μM ABTS diammonium salt, 400 μM myoglobin (MbIII), 740 μM potassium ferricyanide, and 450 μM H_2O_2 were prepared in phosphate-buffered saline (PBS; pH 7.4). Metmyoglobin was prepared by mixing equal amounts of myoglobin and potassium ferricyanide solutions. The reaction mixture (2 ml) contained ABTS (150 μM), MbIII (2.25 μM), varying concentrations of zeaxanthin and meso-zeaxanthin (5–50 μg) and PBS. The reaction was initiated by adding 75 μM H_2O_2 and lag time in seconds was recorded before absorbance of $ABTS^+$ at 734 nm began to increase.

2.4.6. Determination of nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent (Green et al., 1982). Stock solution (10 mM) of sodium nitroprusside was prepared in PBS (pH 7.4). Various concentrations of carotenoids (0.1–4 μg) and sodium nitroprusside (1 mM) in PBS in a final volume of 3 ml were incubated at 25 °C for 150 min. After incubation, 0.5 ml of the solution was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during the diazotisation of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm. The percentage inhibition of nitric oxide was calculated by comparing with the control.

2.4.7. Ferric reducing antioxidant power assay (FRAP)

The Ferric reducing ability was measured at low pH (Benzie & Strain, 1996). The FRAP reagent contained 2.5 ml 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) solution, 2.5 ml ferric chloride solution (20 mM) and 25 ml acetate buffer. Freshly prepared FRAP reagent (900 μl) was mixed with various concentrations of sample (10–100 μg) and incubated at 37 °C for 15 min. Read absorbance at 595 nm against distilled water. Values are expressed as milli moles of ferrous chloride formed.

2.5. Determination of the effect of meso-zeaxanthin on PMA-induced superoxide radical generation in peritoneal macrophages

Male Swiss albino mice (4–6 weeks) weighing 20–25 g were used for the study. Animals were divided into four groups (three animals/group). All the animals were injected (i.p) with sodium caseinate (5%) to elicit macrophages. Group I was kept as control. Group II, III and IV were treated with single dose of meso-zeaxanthin (50, 100 and 250 mg/kg b.wt., respectively). On the fifth day after 1 h of drug administration, peritoneal macrophages elicited by sodium caseinate were activated *in vivo* by injecting PMA (100 mg/animal). After 3 h, peritoneal macrophages were harvested. The effect of meso-zeaxanthin on the inhibition of superoxide generation in the macrophages was measured by inhibition in the reduction of NBT to formazan by the method of Dwivedi, Verna, and Ray (1992). The percentage inhibition was determined by comparing the absorbance values of untreated and treated animals.

2.6. Determination of in vivo antioxidant activity of meso-zeaxanthin

Twenty-four Swiss albino male mice were divided into 4 groups of 6 animals and they were treated orally with meso-zeaxanthin dissolved in sunflower oil at different doses for 30 days.

- Group I: Normal.
- Group II: Control treated with Sunflower oil (0.2 ml).
- Group III: meso-zeaxanthin – 100 mg/kg b.wt.
- Group IV: meso-zeaxanthin – 250 mg/kg b.wt.

At the end of the experiment, animals were sacrificed, and blood was collected by heart puncture and liver was excised and washed in ice-cold Tris–HCl buffer (0.1 M, pH 7.4), and cytosolic samples of liver homogenate were prepared by centrifugation at 10,000 rpm for 30 min at 4 °C.

Estimation of the total protein was carried out by the method of Lowry, Rosenbrough, Farr, and Randall (1951). Hemoglobin was estimated by the cyanmethemoglobin solution using Drabkin's method (Drabkin & Austin, 1932). The following parameters were assayed in both blood and liver to assess the oxidative stress. Superoxide dismutase (SOD) activity was measured by the NBT reduction method of Mc Cord and Fridovich (1969). Catalase activity was estimated by the method of Aebi (1974) by measuring the rate of decomposition of hydrogen peroxide at 240 nm. Glutathione (GSH; γ-glutamyl cysteinyl glycin**e**) activity was assayed by the method of Moron, Depierre, and Manner Vick (1979), based on the reaction with DTNB. The assay of glutathione peroxidase (GPX) was carried out by the method of Hafeman, Sundae, and Houestra (1974) based on the degradation of H_2O_2 in the presence of GSH. Glutathione reductase (GR) activity was measured by the method of Racker (1955), where the amount of reduced form of NADP consumed during the conversion of GSSG to GSH was estimated. The method of Habig, Pabst, and Jakoby (1974) was followed to assay the activity of glutathione-S-transferase (GST) based on the rate of increase in conjugate formation between GSH and 1-chloro-2,4-dinitrobenzene (CDNB).

2.7. Statistical analysis

The values were expressed as mean ± standard deviation (SD). Statistical evaluation of the data was done by one way ANOVA followed by Dunnet's test (post-hoc) using Instat 3 software package.

3. Results and discussion

*3.1. **In vitro** antioxidant activities of zeaxanthin and meso-zeaxanthin*

Both zeaxanthin and meso-zeaxanthin were found to scavenge superoxide, hydroxyl radicals and inhibited tissue lipid peroxidation *in vitro* in a concentration dependent manner (Fig. 2). The concentrations of zeaxanthin and meso-zeaxanthin needed for scavenging (IC_{50}) of superoxide generated by photo reduction of riboflavin were found to be different and meso-zeaxanthin was found to be superior to zeaxanthin as concentration needed for 50% inhibition was half that of zeaxanthin. However it was found that zeaxanthin was found to be a better scavenger for hydroxyl radical generated by Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fenton reaction). Similarly zeaxanthin was found to be better inhibitor of lipid peroxidation compared to that of meso-zeaxanthin. Stable free radicals such as DPPH$^{\cdot}$ and ABTS$^{\cdot}$ were effectively scavenged by zeaxanthin and meso-zeaxanthin. In the case of DPPH$^{\cdot}$, meso-zeaxanthin was found to be superior to zeaxanthin however, ABTS$^{\cdot}$ radical was found to be scavenged much better with zeaxanthin. Nitric oxide, another free radical in biological system, which is produced during oxidative stress has a major role in disease causation, was also found to be scavenged by both these carotenoids. The IC_{50} values for zeaxanthin was again found to be lower than meso-zeaxanthin. The ferric reducing activities of zeaxanthin and meso-zeaxanthin were found to be almost similar, 0.2 and 0.23 mM, respectively. In summary the free radical scavenging activities of meso-zeaxanthin was found to be very much similar to that of zeaxanthin even though there are some minor variations. However the IC_{50} values in both cases were significantly low compared to other known antioxidants (Table 1).

3.2. Effect of meso-zeaxanthin on PMA-induced superoxide radical generation

The effect of meso-zeaxanthin on *in vivo* superoxide scavenging was determined by PMA-induced superoxide generation method. Superoxide radical generated during the activation with PMA in sodium caseinate-induced macrophages was found to be scavenged after oral administration of meso-zeaxanthin in a concentration dependent manner. The percentage inhibition was 25.2%, 50.1% and 67.2% for 50, 100 and 250 mg/kg b.wt., respectively.

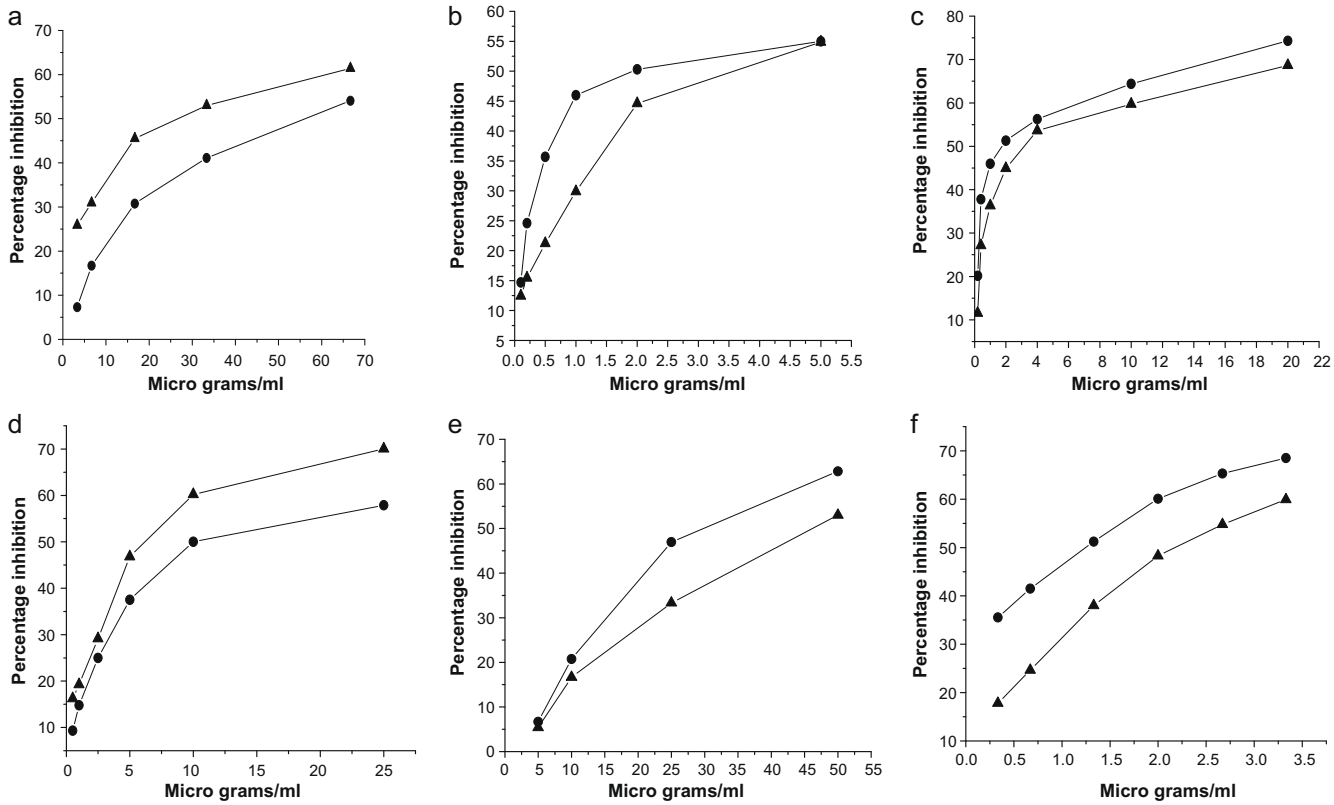

Fig. 2. *In vitro* free radical scavenging activities of zeaxanthin and meso-zeaxanthin. ▲, meso-zeaxanthin; ●, zeaxanthin. (a) Superoxide radical scavenging activity, (b) Hydroxyl radical scavenging activity, (c) Inhibition of lipid peroxidation, (d) DPPH· radical scavenging activity, (e) ABTS· radical scavenging activity and (f) Nitric oxide radical scavenging activity.

Table 1
Comparison of free radical scavenging activities of zeaxanthin and meso-zeaxanthin with other antioxidants.

	IC_{50} values			
	Zeaxanthin	Meso-zeaxanthin	Vitamin C	Trolox
Superoxide radical scavenging activity	56	27	>2936	–
Hydroxyl radical scavenging activity	2	3.5	1742	–
Inhibition of lipid peroxidation	1.8	3.2	493	–
DPPH radical scavenging activity	10	6.25	14	13
ABTS radical scavenging activity	30	46.5	1.2	0.65
Nitric oxide radical scavenging activity	1.25	2.2	–	–

IC_{50} – concentration needed for 50% inhibition.

Table 2
Effect of meso-zeaxanthin administration on antioxidant systems in blood.

Treatment	Catalase (k/g Hb)	Superoxide dismutase (U/g Hb)	Glutathione (nmol/ml)	Glutathione reductase (U/mg protein)
Normal	101.1 ± 8.5	547.1 ± 69.7	32.7 ± 2.8	2.5 ± 0.41
Vehicle control	89.6 ± 16.2[ns]	550.38 ± 88[ns]	43 ± 5.1[ns]	2.59 ± 0.49[ns]
100 mg/kg b.wt.	134.04 ± 5.5[b]	645 ± 39.9[ns]	46 ± 1.65[ns]	3.5 ± 0.24[a]
250 mg/kg b.wt.	180 ± 21.7[b]	812.4 ± 32.9[b]	62.4 ± 4.5[b]	5.58 ± 0.5[b]

[ns] Not significant.
[a] $p < 0.01$.
[b] $p < 0.001$.

3.3. Effect of meso-zeaxanthin administration on antioxidant enzymes and glutathione

Effect of meso-zeaxanthin on the antioxidant enzymes in the blood and serum of mice after given for a period of thirty days is shown in Table 2. Catalase was found to be significantly increased in animals treated with meso-zeaxanthin ($p < 0.001$). SOD was also found to be significantly elevated in 250 mg/kg b.wt. group ($p < 0.001$). GR in the serum was found to be significantly increased in 100 mg/kg b.wt. ($p < 0.01$) and 250 mg/kg b.wt. ($p < 0.001$)

Table 3
Effect of meso-zeaxanthin administration on antioxidant systems in liver.

Treatment	Catalase (U/mg protein)	Superoxide dismutase (U/mg protein)	Glutathione peroxidase (U/mg protein)	Glutathione (nmol/ml)	Glutathione reductase (nmol of NADPH consumed/min/mg protein)	Glutathione-S-transferase (nmol/mg protein)
Normal	5.82 ± 1	1.01 ± 0.1	6.65 ± 1.5	11.83 ± 3	58.44 ± 8	39.07 ± 10
Vehicle control	5.23 ± 0.9[ns]	1.079 ± 0.1[ns]	7.24 ± 0.6[ns]	9.33 ± 2[ns]	64.1 ± 13[ns]	33.17 ± 7[ns]
100 mg/kg b.wt.	56.2 ± 10[b]	8.64 ± 2[a]	1.1 ± 0.1[ns]	8.8 ± 1.06[ns]	16.33 ± 1[c]	71.9 ± 15[ns]
250 mg/kg b.wt.	96.54 ± 14[c]	12.3 ± 3[c]	1.38 ± 0.03[a]	11.96 ± 1[c]	19 ± 2.4[c]	108.08 ± 26[b]

[ns] Not significant.
[a] $p < 0.01$.
[b] $p < 0.005$.
[c] $p < 0.001$.

groups. Similarly GSH level was found to be significantly elevated ($p < 0.001$) in the blood of animals treated with 250 mg/kg b.wt. of meso-zeaxanthin.

The effect of meso-zeaxanthin on the antioxidant enzymes in mice liver after treatment for 30 days is given in Table 3. Catalase was significantly increased in 100 mg/kg b.wt. ($p < 0.01$) and 250 mg/kg b.wt. ($p < 0.001$). SOD and GPX were found to be increased significantly in 250 mg/kg b.wt. group. GSH level increased significantly in both 100 and 250 mg/kg b.wt. groups. GR activity was significantly increased in 250 mg/kg b.wt. ($p < 0.005$) treated group. GST level was found to be increased significantly in both 100 and 250 mg/kg b.wt. ($p < 0.005$ and $p < 0.001$) groups.

The human body has several mechanisms to counteract damage by free radicals and other reactive oxygen species. These act on different oxidants as well as in different cellular compartments. One important line of defence is a system of antioxidant enzymes including SOD, catalase, GPX and GR. SOD is a metalloprotein, converts two superoxide radicals into hydrogen peroxide and O_2. To eliminate H_2O_2, before the Fenton reaction which can create highly reactive hydroxyl radicals, organisms use catalase – a homotetrameric ferri heme containing enzyme and/or GPX – a selenium-dependent enzyme. The Km value for GPX is lower than that for catalase, and hence GPX is considered most important in physiological conditions. GSH abundant in most cells, is an important substrate for GPX and GST and also act by quenching free radicals. GST is GPX like enzyme and its function is to eliminate various hydroperoxides. GR is a member of the disulphide oxidoreductase family, catalyses the NADPH-dependent reduction of glutathione disulphide (GSSG) to GSH.

The second line of defence against free radical damage is the presence of antioxidants. An antioxidant is a molecule stable enough to donate an electron to a free radical and neutralise its damaging effect. Although about 4000 antioxidants have been identified, the best known are vitamin E, vitamin C and the carotenoids. Carotenoids are two different types. One that yield vitamin A and others do not. Due to conjugated polyene structure, carotenoids have significant antioxidant activity. Role of β-carotene in the cancer prevention has been extensively studied (Hoyoku, 1998).

Carotenoid meso-zeaxanthin used in the current study effectively scavenged superoxide, hydroxyl and nitric oxide radicals *in vitro*. These radicals are generated inside the body during the normal metabolism or in presence of xenobiotics. The stable free radicals DPPH· and ABTS· were also found to be scavenged by meso-zeaxanthin. Meso-zeaxanthin also scavenged the superoxide generated *in vivo* after the administration of phorbol esters in the mice. Natural functions of the carotenoids are dependant on the individual physical and chemical structures of the molecules. *In vitro* experiments indicate that zeaxanthin is a more potent antioxidant than lutein (Kim, Nakha nishi, Itagaki, & Sparrow, 2006). In one study quenching of singlet oxygen by zeaxanthin was approximately twice as effective as quenching by lutein (Cantrell, Mc Garvey, Truscott, Rancan, & Bohm, 2003). The reason is presumable due to the extended conjugation of zeaxanthin compared with lutein. Meso-zeaxanthin, a stereo isomer of zeaxanthin, shares these electronic features with zeaxanthin and therefore should possess the same antioxidant potential as zeaxanthin. It has also been reported that in association with a zeaxanthin binding protein, the pi-isoform of glutathione-S-transferase, meso-zeaxanthin provides slightly better protection against lipid membrane oxidation than zeaxanthin (Bhosale & Bernstein, 2005).

The level of antioxidant enzymes like catalase, SOD and GSH were significantly increased in both blood and liver of meso-zeaxanthin treated groups, in a dose dependent manner. GR, GPX and GST activity were also found to be significantly increased in the liver of meso-zeaxanthin treated groups. All these results show that carotenoid meso-zeaxanthin has a profound effect on the antioxidant defence system both *in vitro* and *in vivo*. In addition, meso-zeaxanthin can be considered as "pure" antioxidant because it exhibits little or no pro oxidative behaviour, even at high carotenoid concentration and at high oxygen tension (Martin et al., 1999).

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