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COMPARATIVE SINGLE DOSE ORAL PHARMACOKINETICS STUDIES OF LUTEIN FORMULATIONS IN MALE WISTAR ALBINO RATS

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ABSTRACT

The bioavailability of Lutein solubilized in essential oil of Ajowan (A group) and suspension in vegetable oil (A1 group) were evaluated following a single dose in two groups of male rats (n=8). The bioavailability measured over 48 hrs showed that the mean plasma Lutein levels in the A1 group were significantly lower than those of the other group.

KEY WORDS

Lutein, Bioavailability, Antioxidant and Single dose.

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INTRODUCTION

Lutein and Zeaxanthin (Figure No.1) are a Xanthophyll carotenoid found in plants, particularly in leaves, fruits and vegetables. In the human body, Lutein and Zeaxanthin have been found in blood, eye, skin, adipose fat tissue, skin and brain. Lutein and its isomers viz. R, R - Zeaxanthin and R, S - zeaxanthin (meso - zeaxanthin) are present exclusively in the various parts of eye, specifically in the retina and lens. The key biological functions of Lutein are its ability to filter light from photo-toxic damage to the tissues of the eye and fight against free radicals in the retina. The protective effect is due in part, to the reactive oxygen species quenching ability of these carotenoids. Earlier

studies in our laboratory proved that, antioxidant proteins isolated, purified and characterized from dietary components, such as Turmeric (*Curcuma longa*), Curry leaves (*Murraya Koeneigii*) and Lutein isolated from Methi leaves effectively inhibited ROS-induced lipid peroxidation and DNA damage (Dinesha and Leela Srinivas, 2011, Ningappa et al., 2008, Smitha et al, 2009, Shalini and Srinivas L., 1987, Srinivas and Shalini, 1991, Sujatha and L Srinivas, 1995, Thammannagowda et al., 2010). Further, Lutein and Zeaxanthin are more stable to decomposition by pro-oxidants than are other carotenoids such as beta-carotene and lycopene. zeaxanthin is the predominant pigment in the fovea, the region at the center of the macula. The quantity of zeaxanthin gradually decreases and the quantity of Lutein increases in the region surrounding the fovea, and Lutein is the predominant pigment at the outermost periphery of the macula. Lutein, Z and M Z have been strongly implicated in protection against eye diseases. Fruits, vegetables and egg yolk are the most important sources of Lutein and RR-zeaxanthin. The desired level of intake of Lutein by an adult from food source appears to be low (less than 2 mg/day) compared with what is considered optimal (10-20 mg/day) for disease prevention and maintenance of eye health. The ability to increase the amount of macular pigment by dietary supplementation with Lutein has been demonstrated (Landrum et al, FASEB. J, 10, A242, 1996). The reduced vision function due to cataract and the adult blindness due to Age Related Macular Degeneration can be substantially controlled by consuming fruits and vegetables and dietary supplements containing Lutein and zeaxanthin. The present study reports comparative plasma profile of Lutein after administration of specified quantity of Lutein formulated in essential oil of Ajowan and in vegetable oil.

MATERIALS AND METHODS

Deionised water, ethyl alcohol (USP grade), and triethylamine (TEA). Hexane, Dichloromethane, Methanol, Acetronitrile, used were of HPLC grade. Shimadzu LC-20APHigh pressure liquid chromatography (HPLC) system coupled to a photodiode array detector M20 PDA, Auto sampler SIL -20AHT and Shimadzu UV-Vis spectrophotometer were used for the carotenoid analysis. Lutein sample and standard were obtained from Omni Active health Technologies Ltd. Essential oils of Ginger, Ajowan, Cassia, Lemon Grass, pepper, celery, cumin, palmarosa and Turmeric sourced from Kancor Ingredients Ltd, Cochin. Canthaxanthin were from Sigma Aldrich.

Lutein formulation in essential oil

Lutein oil samples were prepared by agitating Lutein (74.5% purity) with essential oil at 80 ^{0}C followed by filtration. The precipitate formed after cooling was filtered again and the clear solution obtained was kept for stability study in amber coloured bottles. The stability results are given in the Table No.1.

It was observed that Lutein solubilised in essential oil of Ajowan did not show any degradation and was hence used for the supplementation study.

5 gm of Lutein crystals (74.50%) blended with 30 gm of sunflower oil was ground well in a mortar and pestle. To this additional 65 gm of sunflower oil was added and homogenised well. The material showed 3.63 % Lutein.

Supplementation study

Animals were housed in groups of three and two per cage in compliance with committee for the purpose of care and supervision of experiments in animals (CPCSEA) guidelines. Proper lighting was provided. The room temperature was maintained 17 to 24°C and relative humidity was maintained 40-70%. Sterile RO water was provided *ad libitum*, to each animal in polypropylene bottles with stainless steel sipper tubes. No contaminants are known to be present in the water at levels that would be expected to interfere with the results of this study. Study animals were acclimatized to their housing for 5 days prior to first day of dosing. Male Wistar albino rats aged 6 - 8 weeks and weighing around 160 to 200 g were used for the experimentation. Animals were fasted overnight with free access to water. Overnight fasted animals were administered the test

substance (Lutein solubilised in Ajowan oil and Lutein oil suspension in vegetable oil) by oral gavage with a recommended dose in a recommended vehicle as mentioned above. With mild anaesthesia, blood samples (300 µL) were collected by retro-orbital route at pre-dose and post-dose. Feed were given to animals 3 h post-dose.

Blood samples were collected in pre-labeled centrifuge tubes containing anticoagulant (K_2EDTA-2 mg/mL of blood). Blood samples were centrifuged at 3000 rpm at 4°C for 10 minutes and the corresponding plasma samples were harvested into pre-labeled tubes and were stored at -80°C until analysis.

Measurement of Lutein in serum

Mobile Phase (Acetonitrile: Methanol: Dichloromethane 6: 2: 2 v/v/v), Exactly 600 mL of Acetonitrile, 200 mL of Methanol and 200 mL of Dichloromethane were added to a reagent bottle, mixed well and sonicated. This solution was stored at room temperature and used within three days from the date of preparationn.

Dilution solvent (Methanol: Water 80:20 % v/v)

Exactly 800 mL of methanol and 200 mL of milli-Q-water were added to a reagent bottle, mixed well, and sonicated. This solution was stored at room temperature and used within seven days from the date of preparation.

Preparation of Lutein stock solution

About 1.00 mg of Lutein was weighed accurately and transferred into a 2.0 mL volumetric flask and dissolved in methanol. The volume was made up with methanol to obtain 500 µg/mL stock solution.

Internal standard stock solution

About 1mg of Canthaxanthin was weighed accurately and transferred into a 1.0 mL volumetric flask and dissolved in methanol. The volume was made up with methanol to obtain an approximately 1000 µg/mL stock solution.

Protein Precipitation Method for Plasma Samples

100 µL of plasma sample and 50 µL of Internal Standard solution were added to the centrifuge tube (50 µg/mL solution of canthaxanthin) and vortexed. 400 µL of Acetonitrile were added and vortexed for 1 minute. The samples were centrifuged at 4500 rpm for 10 minutes at 10°C. The supernatant organic layer was separated and then transferred into the auto sampler vials and placed in the auto sampler of HPLC system. Intersil ODS -3V C18 250 x 4.6 mm, 5 µm, mobile phase (acetonitrile: Methanol: Dichloromethne 6:2:2 v/v/v), flow rate 0.7 mL/min. Injunction volume 50 µL, Auto sampler temperature 10°C ± 4°C, Detection 446 nm.

The concentrations of the calibration curve standards were plotted against analyte to internal standard peak area ratio using a weighted least squares regression analysis using $1/x^2$ for Lutein as the weighting factor. The concentrations of quality control samples were calculated using the linear equation (y=mx + c) from the calibration curve and by solving the equation for x. Where, y = peak area ratio.m = slope of the calibration curve. x = concentration of a drug. c = y-axis intercept of the calibration curve.

RESULTS AND DISCUSSION

The serum Lutein response of the Lutein formulation in Ajowan oil (sample A) and Lutein formulation in vegetable oil (Sample A1) are presented in Table No.1 and 2. The mean Lutein concentration in A group at 2hrs was 21.51 ng/ml and it lowered to 12.33 ng/ml after 8 hrs and down to below base line after 24 hrs reflecting very low serum concentration. The mean serum concentration in group A1 (formulated in vegetable oil) was 1.18 ng/ml after 2 hrs and increased to 6.12 at 4 hrs and lowered to 2.25 ng/ml at 8 hrs and it to base line after 24 hrs.

The serum Lutein concentration comparison of two groups against time is presented in Figure No.2. The absorption of the Lutein from Ajowan oil blend was greater in most of the animals.

The implications of Lutein in its functional biological role are too well known and it emphasizes its nutritional significance. However, there is not much information on the various factors that need to be considered regarding its bioavailability from diet. The present study shows that the vehicles available for its absorption are much more critical while assessing the importance of dietary sources of

Lutein. If the bioavailability of Lutein could simply be enhanced at least 4-5 fold by mere selection of an appropriate vehicle such as Ajowan oil used in the present study it would go a long way in realizing the full potential of dietary sources of Lutein. While we have examined only two vehicles for Lutein dispersion, perhaps more involved studies involving different methods of dietary Lutein formulations could be evaluated. Such studies might eventually be capable of making appropriate recommendations to fully utilize the potential of dietary sources of Lutein for health.

Table No.1: Stability study data of Lutein formulations in essential oil

S.No	Essential oil	Lutein % 0 day	Lutein % 15 days	Lutein % 30 days	Lutein % after 30 days	Remarks
1	Ginger oil	0.816	-	-	-	Not qualified
2	Cassia oil	1.510	0.567	0.236	84.37%	high degradation
3	Ajowan oil	3.618	3.60	3.612	-	Not degradation
4	Lemon Grass oil	1.93	1.55	1.567	18.80%	Precipitation and degradation observed
5	Pepper oil	0.53	-	-	-	not qualified
6	Celery oil	1.27	0.875	-	-	not qualified
7	Cumin oil	1.81	1.59	1.58	12.70%	Precipitation on storage
8	Palmarosa oil	1.42	1.19	0.95	35.9	Precipitation on storage
9	Turmeric oil	0.88	-	-	-	not qualified

Table No.2: PK study data of sample A and A1

Group	Single dose oral pharmacokinetics (PK) study of Sample A in male Wistar albino Rats (20 mg/kg b.w.)										
	Plasma Concentrations of Lutein (ng/mL)										
	Time (h)	**R001**	**R002**	**R003**	**R004**	**R005**	**R006**	**R007**	**R008**	**Mean**	**STDV**
Blank	**0.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.000**
G-1	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.000**
	2.00	9.99	2.71	8.04	2.89	5.99	66.55	8.39	4.90	**13.68**	**21.519**
	3.00	4.19	8.99	25.52	7.95	39.72	59.36	7.88	24.89	**22.31**	**19.319**
	4.00	7.55	6.59	48.54	7.66	39.72	23.59	11.39	34.73	**22.47**	**16.673**
	6.00	8.90	17.90	12.89	18.61	37.78	12.56	4.65	6.58	**14.98**	**10.462**
	8.00	39.09	10.68	6.45	0.00	13.01	8.17	9.67	0.00	**10.88**	**12.338**
	24.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.000**
	48.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.000**

Single dose oral pharmacokinetics (PK) study of Sample A1 in male Wistar albino Rats (20 mg/kg b.w.)

Group	Plasma Concentrations of Lutein (ng/mL)										
	Time (h)	**R001**	**R002**	**R003**	**R004**	**R005**	**R006**	**R007**	**R008**	**Mean**	**STDV**
Blank	**0.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.000**
G-1	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.000**
	2.00	5.64	2.20	4.60	2.44	3.22	3.69	2.54	3.95	**3.54**	**1.184**
	3.00	8.21	5.00	5.01	3.60	3.93	4.59	2.55	5.66	**4.82**	**1.680**
	4.00	18.06	8.75	5.60	2.80	10.84	21.52	11.67	12.39	**11.45**	**6.125**
	6.00	10.52	4.33	4.24	10.12	3.17	9.03	5.69	9.03	**7.02**	**2.962**
	8.00	0.00	3.74	3.23	3.36	2.57	3.96	6.39	7.33	**3.82**	**2.256**
	24.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.000**
	48.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.000**

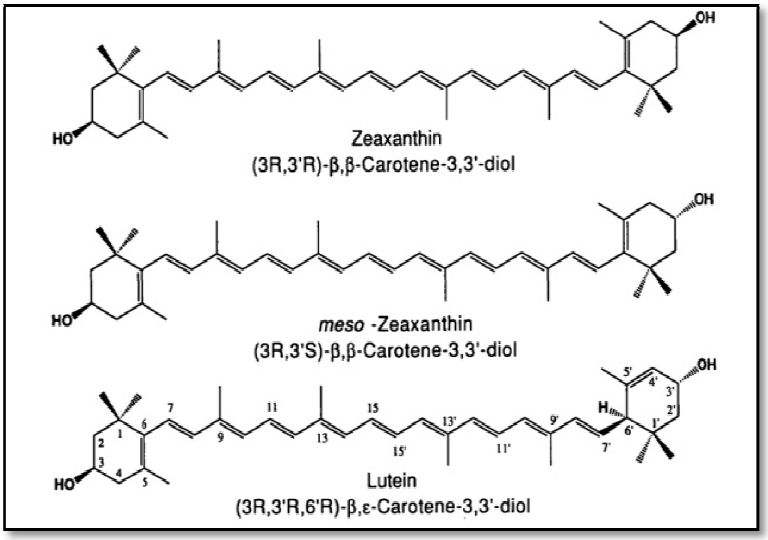

Figure No.1: Chemical structure of Lutein, Zeaxanthin and meso-Zeaxanthin

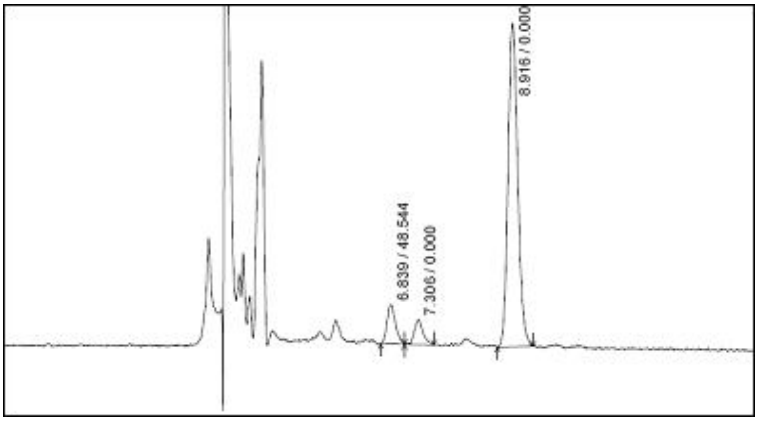

Figure No.2: HPLC chromatogram of Lutein sample a after 3hrs of supplementation

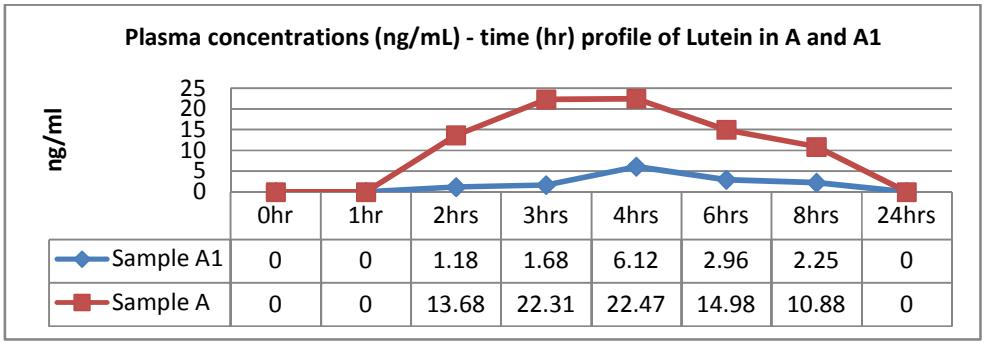

Figure No.3: Oral PK study - Plasma cconcentrationss (ng/mL) - time (hr) profile of Lutein in Sample A1 (suspension in veg. oil) and A

CONCLUSION

The implications of Lutein in its functional biological role are too well known and it emphasizes its nutritional significance. However, there is not much information on the various factors that need to be considered regarding its bioavailability from diet. The present study shows that the vehicle available for its absorption is much more critical while assessing the importance of dietary sources of Lutein. If the bioavailability of Lutein could simply be enhanced at least 4-5 fold by mere selection of an appropriate vehicle such as Ajowan oil used in the present study it would go a long way in realizing the full potential of dietary sources of Lutein . While we have examined only two vehicles for Lutein dispersion, perhaps more involved studies involving different methods of dietary Lutein formulations could be evaluated. Such studies might eventually be capable of making appropriate recommendations to fully utilize the potential of dietary sources of Lutein for health.

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