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Evaluation of novel and superior formulation CaroTexTM developed by Biofusion Technology

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ABSTRACT

As Per WHO, vitamin A deficiency is a systemic disease affecting cells, organs throughout the body. The gradual depletion of vitamin A stores results in xerophthalmia, night blindness, xerosis etc., reversed by vitamin A therapy. Beta-Carotene, a carotenoid, is the precursor of vitamin A, which cannot be synthesized by the human body. Hence, beta carotene formulation has gathered considerable attention in the healthcare industry. Hydrophobicity of Beta carotene being a major challenge in the formulation, Zeushygia Life Sciences Pvt. Ltd (Telangana India) developed a novel beta carotene formulation, CaroTex, with enhanced bioavailability. The present study assesses the bioavailability of CaroTex in a rodent model. The animals were divided into 5 groups, Normal Control, Vehicle Control, Standard beta Carotene, Comparator and CaroTex. As rats efficiently convert beta carotene into vitamin A, high dose of beta-carotene (50mg/kg) was given orally for 7 consecutive days. Beta carotene, its metabolic product retinal were measured in rat liver. It was observed that beta carotene concentration in rats fed with CaroTex was about 1.70, 2.55 times higher than Comparator and control respectively whereas the concentration of retinal was the same in all groups. It is evident from this study that novel formulation technology (BioFusion Technology) of CaroTex reflects in relatively higher concentration levels of beta carotene.

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INTRODUCTION

The properties of carotenoids of being potential immunostimulant and antioxidant have inculcated interest in researchers, formulation scientists and peers all over the world (Hosotani and Kitagawa, 2003). Beta -Carotene is a source of vitamin A, which is essential for good vision and eye health, a strong immune system, and healthy skin. It is also a strong antioxidant, protecting the body against reactive oxygen species and hence from the damage due to free radicals. Thus it could be helpful against various diseases in which the presence of free radicals is deleterious, like cardiovascular diseases, chronic inflammation and cancer. It also has found its place in the cosmetic industry as a colouring agent or as

an agent for sun protection.

Beta –Carotene, due to its high hydrophobicity is insoluble in aqueous systems, hence having poor uptake in the body (De Paz *et al.*, 2013). Therefore, formulating beta carotene in a suitable drug delivery system with improvement in bioavailability becomes a challenge.

CaroTex (A ZeusHygia LifeSciences Pvt. Ltd. formulation of Beta-carotene) is produced with proprietary BioFusion technology of ZeusHygia's, which is available in various forms like oils, emulsions, powders and beadlets which demonstrates superior bio availability, stability and wide application ranges like dietary supplement in the form of soft gels, tablets and two-piece hard capsules, fortified foods and beverages, source of vitamin A in multivitamin premixes. This BioFusion technology enables to encapsulate natural betacarotene as well as nature-identical betacarotene in the same free-form found in common fruits and vegetables with unique biopotentiator with encapsulation technology. Betacarotene gets encapsulated in food-grade carriers and forms free-flowing, uniform, nano to the micro-particulate structure. It is possible by employing BioFusion technology to encapsulate the bio active in the smallest, absolutely homogeneous units in the form of dry and/or liquid product forms.

In this technique, a poorly soluble active is dispersed in highly soluble hydrophilic excipients with the combination of bio-potentiator, which enhances the rate of dissolution. Spray drying technique yields solid dispersion or solid solution (molecular level matrix system) product. Presence of the active in the submicron state improves wettability and formation of high free energy amorphous forms of solid dispersion which contribute towards solubility enhancement. Biopotentiator helps in enhancing bio-accessibility and bio-efficacy of a particular activity with which it is combined, without any typical pharmacological activity of its own at the dose used. It may increase the absorption of active in the GIT. The novel formulation CaroTex was further evaluated in a rodent model to assess its bio availability.

Rodents very efficiently convert beta -carotene into vitamin A (Duszka *et al.*, 1996; Goodman *et al.*, 1966). Therefore rodents need to be fed with significant quantities of Beta-carotene for it to be absorbed and quantified (Hosotani and Kitagawa, 2003; Lakshman *et al.*, 1989). Earlier studies show the accumulation of Beta-carotene in rat tissue, in a dose-dependent manner, the liver being the foremost storage site. Beta carotene is metabolized by two enzymes namely beta carotene 15, 15'-dioxygenase and Beta-carotene 9', 10' oxygenase-2 invertebrates. The enzyme Beta-carotene 9', 10' oxygenase-2 cleaves the carotenoid eccentrically which yields apo-10'-carotenals and ionones. Whereas the enzyme beta carotene 15, 15'-dioxygenase converts provitamin A carotenoids into vitamin A by a central oxidative cleavage yielding retinal, which is stored in liver (Hosotani and Kitagawa, 2003; Lakshman *et al.*, 1989; Roth *et al.*, 1977; Zhang *et al.*, 2017; Gugger *et al.*, 1992). Hence, in the present study liver was chosen as the organ to scrutinize the concentrations of Beta-carotene and retinal.

MATERIALS AND METHODS

All chemicals procured from SD Fine Chemicals LTD (Mumbai, India). The solvents for analysis of the samples were of HPLC grade. All-trans retinal obtained from Biotech India Pvt.Ltd., sunflower oil obtained from AAKKamani, Mumbai (Batch Number SFO F/01 2017), Standard beta carotene obtained from Xi'an Xin Sheng Bio-Chem Co., Ltd, China (Batch Number 170318).

Animals and treatment

Healthy adult male Wistar rats with a body weight around 230g were obtained from Bombay Veterinarian College (BVC), Mumbai, India. The rats were housed at 22 ± 2°C and relative humidity 60 ± 5% with 12 h light-dark cycle in the animal house at Institute of Chemical Technology (ICT), Mumbai, India. Animals were fed with Standard commercial laboratory chow and supplied with purified water ad libitum. The experiment was conducted in accordance with the study protocol approved by the Institutional Animal Ethics Committee (ICT/IAEC/2017/P27), Mumbai. The rats were acclimatized for 7 days and randomly divided into five experimental groups with six animals in each group. The groups were; Normal Control, Vehicle Control, Standard Beta-carotene Formulation, Comparator Formulation and CaroTex Formulation. Animals in Normal Control did not receive any treatment; animals in the Vehicle control group received Sunflower oil (the vehicle for beta carotene dose preparation); animals in Standard Beta-carotene group received 50mg/kg oral dose of 95% Standard beta carotene; animals in Comparator formulation group received 50mg/kg oral dose of 30% beta carotene; animals in Test formulation group received 50mg/kg oral dose of CaroTexcontaining 30% beta carotene. The animals (Vehicle Control, Standard Beta-carotene, Comparator and CaroTex) received once a day dose for seven consecutive days. On the eighth day, animals were sacrificed by CO_2 asphyxiation and liver samples removed, rinsed

with phosphate-buffered saline (PBS) and stored at -80°C.

Rat Liver Homogenate

The rat liver tissues isolated after sacrifice were homogenized using Remi Homogenizer (RQT-127-A) with 0.1% BHT (Butylated Hydroxy Toluene) in HPLC grade methanol (tissue/BHT ratio; 1g/5ml) and stored at -80°C until analysis (Goodman *et al.*, 1966).

HPLC Analysis

On the day of analysis 1ml of saturated potassium hydroxide (KOH) solution was added to 1ml of liver homogenate in a 15ml test tube. The solution was mixed and saponified by heating at 70ºC for 30 min. The solution allowed to cool and transferred to a clean 15ml centrifuge tube. 5ml n-hexane was added to the centrifuge tube and the contents were shaken vigorously for 10 min to allow extraction of beta carotene in n-hexane and then centrifuged at 500g for 10min. The supernatant (n-hexane) was separated with the help of micropipette and transferred to another centrifuge tube. The liver homogenate was again subjected to extraction with 5ml of n-hexane and the procedure repeated twice more. All three extracts were combined and evaporated under a stream of nitrogen gas. The residue after evaporation was reconstituted in 200μl of mobile phase {Acetonitrile: Ethyl Acetate (40:60)}. All samples were prepared in triplicate. The HPLC system used was of JASCO Corporation equipped with an auto sampler and photodiode array detector. The chromatograms obtained were analyzed by JASCO ChromNAV version 1.19.01 software. The HPLC method was developed in house using Syncronis C_{18} column (4.6 mm X 150 mm; 5μ). The mobile phase flow rate for analysis was kept at 1ml/min. The analysis was carried out at ambient auto sampler and column temperature, with the detector wavelength at 450nm for beta carotene and 365nm for all-trans retinal.

RESULTS AND DISCUSSION

HPLC Chromatograms for beta carotene and Retinal

Chromatograms for Beta-carotene and retinal dissolved in n-hexane as a standard solution are shown in Figure 1 and Figure 2 respectively.

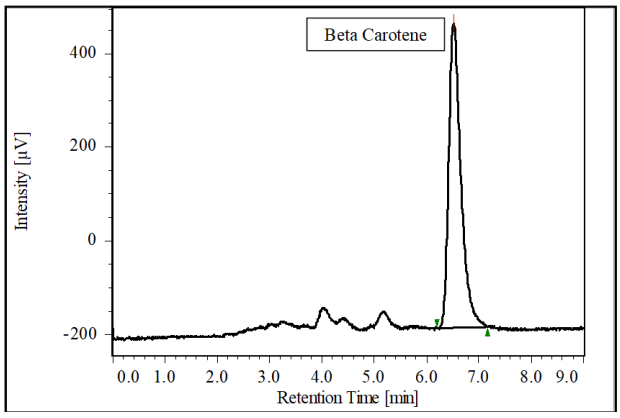

Figure 1: Beta Carotene HPLC Peak in Standard Solution

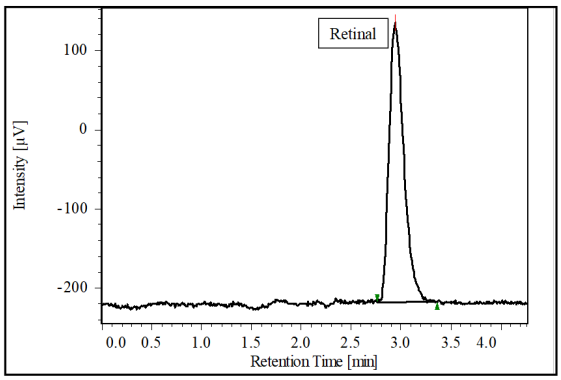

Figure 2: Retinal HPLC Peak in Standard Solution

Standard Curves

To identify the effect of tissue matrix, known amounts of the standard that is beta carotene and all trans retinal were added into the blank tissue homogenate. The coefficient of standard solution and calibration curve slopes are represented in Table 1.

Least squares regression analysis was used to establish the linearity. The calibration curve slopes were identical for the standard solution and liver tissue homogenate spiked with the standard. The linear concentration ranged from 5 to 30μg/ml for both beta carotene and retinal. The lowest detection limits were 0.15μg/ml and 2μg/ml for beta carotene and retinal respectively for 50μl injection volume, the chromatogram for the same is shown in Figure 3.

Levels of Beta-carotene and Retinal

The concentration of beta carotene in the liver sample for Normal Control, Vehicle Control, Standard beta carotene group, Comparator formulation group and CaroTex formulation group was found to be 1.27, 1.28, 2.42, 1.92 and 3.23 μg/ml whereas retinal was found to be 3.80, 3.88, 3.70. 3.85 and 3.95 μg/ml respectively.

Almost 65% of beta carotene gets converted to retinyl esters (Wang *et al.*, 1991; Parvin and Sivakumar, 2000). Retinyl esters have the physiological function in the body like maintaining healthy

Table 1: Linearity of Beta–Carotene and Retinal

Compound		Linear Regression	r
Beta Carotene	Standard Solution	y = 3542.x - 8214	0.996
	Rat Liver Homogenate	y = 36534x – 42845	0.995
Retinal	Standard Solution	y = 38026x - 1955.1	0.997
	Rat Liver Homogenate	y = 3787.5x – 12643	0.997

Table 2: Compiled result for Beta-carotene

Liver Homogenate Sample for each Animal	Normal Control (Concentration μg/ml)	Vehicle Control (Concentration μg/ml)	Standard beta carotene (Concentration μg/ml)	Comparator Formulation (Concentration μg/ml)	CaroTex Formulation (Concentration μg/ml)
1	1.2	1.2	2.3	1.8	2.5
2	1.3	1.3	2.2	1.9	3.7
3	1.2	1.3	2.3	2.1	3
4	1.3	1.3	2.4	1.8	3.3
5	1.3	1.3	2.7	2	3.5
6	1.3	1.3	2.6	1.9	3.4

Table 3: Compiled result for Retinal

Liver Homogenate Sample for each Animal	Normal Control (Concentration μg/ml)	Vehicle Control (Concentration μg/ml)	Standard beta carotene Formulation (Concentration μg/ml)	Comparator Formulation (Concentration μg/ml)	CaroTex Formulation (Concentration μg/ml)
1	3.9	4	3.7	4	4
2	3.8	3.8	3.7	3.9	3.8
3	3.7	3.8	3.7	3.9	3.7
4	3.7	4	3.7	4	3.9
5	3.9	3.9	3.7	4	3.8
6	3.8	3.8	3.7	3.9	3.7

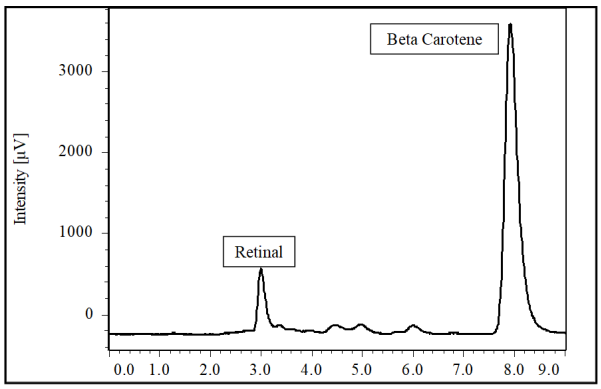

Figure 3: Retinal and Beta Carotene HPLC Peak in rat liver homogenate

teeth, skeletal and soft tissue, mucus membranes, and skin. Thus, the conversion of beta carotene into retinyl esters is extremely important for physiological activities and the stores of beta carotene are important for this conversion (Barua and Olson, 2000). In the study, the CaroTex formulation indicates significantly higher levels of beta carotene stores in the liver, with p-value <0.0001 indicating higher bioavailability, in comparison to the standard beta carotene and comparator formulation as shown in Figure 4.

Data expressed as mean ±SEM of 6 values. Comparison between normal control, vehicle control, Standard beta carotene formulation, Comparator formulation and CaroTex formulation were made by using

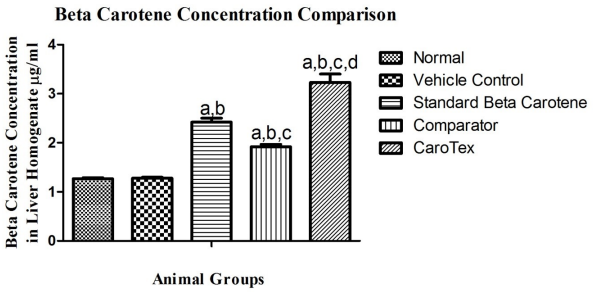

Figure 4: Concentration of beta-carotene in rat tissue

one-way ANOVA followed by Bonferroni's Multiple Comparison Test.

'**a**' represents statistical difference (p<0.0001)from normal control group.

'**b**' represents statistical difference (p<0.0001)from vehicle control group.

'**c**' represents statistical difference from Standard beta carotene formulation group when compared with CaroTex formulation group (p<0.0001) and when compared with Comparator formulation group (p<0.001).

'**d**' represents statistical difference (p<0.0001)from Comparator formulation group.

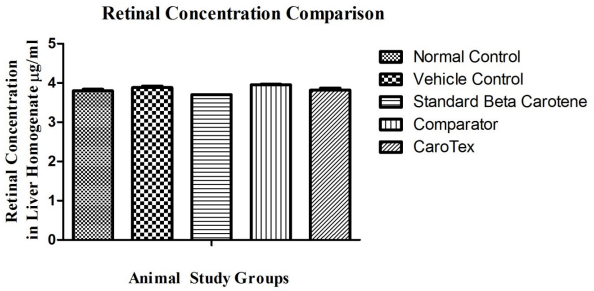

Figure 5: Concentration of retinal in rat tissue

Data expressed as mean ±SEM of 6 values. Comparison between normal control, vehicle control, Standard beta carotene formulation, Comparator formulation and CaroTex formulation were made by using one-way ANOVA followed by Bonferroni's Multiple Comparison Test.

The retinal level in all the groups including the control as well as vehicle control group is similar, as shown in Figure 5. Comparison between normal control, vehicle control, Standard beta carotene formulation, Comparator formulation and CaroTex formulation were made by using one-way ANOVA followed by Bonferroni's Multiple Comparison Test. This is because the conversion of Beta carotene to retinyl esters will occur only when the body needs it. This conversion is high in Vitamin A-deficient rats . Since we used normal healthy wistar rats, the levels of retinal are similar in all groups.

Rats need to be fed with significantly higher quantities of beta carotene to be absorbed as such. Since rats efficiently convert beta carotene into vitamin A.The concentration of beta carotene in the liver of the study animals when dosed continuously for 7 days is significantly higher (p<0.0001) for the CaroTex formulation group in comparison to the Standard beta carotene and comparator formulation groups, as indicated in Table 2.

Since the animals used in the study were healthy without vitamin A deficiency and were kept on a complete diet there was no need-based conversion of carotenoids to Vitamin A, thus enhancing liver stores of Beta-carotene. The higher concentration of Beta-carotene in the liver samples was observed only in the CaroTex formulation group. This could be due to the formulation attributes itself. The 50mg/kg beta carotene dosed daily to the animals is very high and hence gets stored in the animal liver directly. Since all the animals received the same treatment the higher beta carotene in CaroTex formulation animal group explains the superiority of the formulation technology used to develop the novel beta carotene formulation. The Beta-carotene concentration levels of CaroTex in rat species is higher by 68.23% compared to comparator brand of Beta-carotene. Carotex shows about 1.70 & 2.55 times higher concentration than Comparator and control respectively as shown in Figure 6.

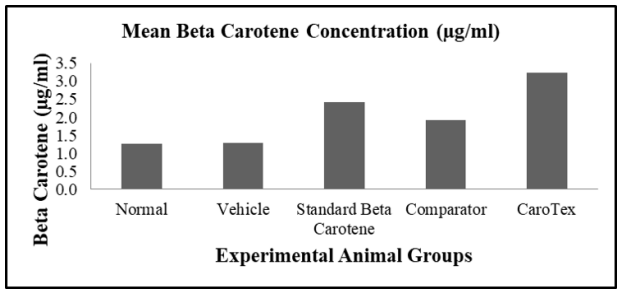

Figure 6: Comparison of mean beta carotene levels for all experimental groups

Hence the CaroTex formulation is better bioavailable in comparison to the other two formulations as depicted in Figure 7. Therefore, the new formulation developed using Biofusion technology by ZeusHygia Lifesciences Pvt. Ltd. is superior in comparison to the Comparator formulation as it is better bio available as demonstrated by the animal study.

The level of retinal in all the groups is the same, as shown in Table 3, irrespective of the superiority of the CaroTex formulation. This could be explained

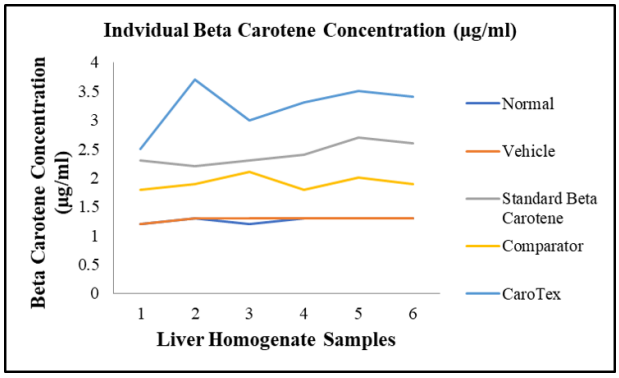

Figure 7: Comparison of individual beta carotene concentrationfor all experimental groups

by the fact that beta carotene is converted to retinal only in a deficient state. Since the animals used were normal healthy and non-deficient in vitamin A, the retinal level remains unchanged in all groups.

CONCLUSION

Considering the results of the present study, the novel formulation CaroTex would be a superior supplementation option in the pool of vitamin A supplements available.

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