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RESEARCH ARTICLE

Intestinal absorption of astaxanthin and fucoxanthin: Role peroxisome proliferator-activated receptor gamma and scavenger receptor B1 and modulation by selected peroxisome proliferator-activated receptor gamma ligands

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ABSTRACT

Background: Non-provitamin A carotenoids astaxanthin (AX) and fucoxanthin (FX) are bioactive molecules that are abundant in seafood. Although their dietary intake is beneficial, only around 10% of ingested carotenoids are absorbed. Recent studies have speculated the role of redundant lipid transporters such as scavenger receptor B1 (SRB1), in carotenoids transport, and this SRB1 transporter is under the control of nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPARγ). **Aims and Objectives:** This study aims at investigating the role of SRB1 and its transcriptional regulation by PPARγ, under the influence of selected PPARγ agonists (conjugated linoleic acid [CLA] and pioglitazone [PGZ]) in intestinal uptake of AX and FX. **Materials and Methods:** AX and FX were extracted from shrimp carapace and the seaweed *Padina tetrastomatica*, purified, and purity were assessed. The carotenoids are then gavaged for 14 days, and along with CLA or PGZ. On the 15th day, the animals were sacrificed and tissues were analyzed for carotenoid uptake (liver and serum), protein expression (PPARγ and SRB1) in enterocytes by western blotting and gene expression (PPARα, PPARγ, peroxisome proliferator-activated gamma coactivator 1α, SRB1, and sterol regulatory element-binding protein 1) by quantitative polymerase chain reaction. **Results:** The results of the study indicate an increase in intestinal uptake, when coadministered with PPARγ agonists (AXCLA>AXPGZ>AX and FXCLA>FXPGZ>FX), signifying a role of PPARγ in the process. Protein expression of PPARγ in comparison with the control in AXCLA increased (1.4 folds); AX and AXPGZ groups had no change. FX and FXPGZ treatments exhibited half the expression, which was restored in FXCLA (1.1 folds). The SRB1 was decreased in AX and did not change in FX group (0.3 and 1.0 folds, respectively). Although AXCLA group exhibited a decrease in SRB1 expression, when compared to control (0.57 folds), there was an increase in comparison with the AX group. There was a similar increase in FXCLA group (1.3 folds). PGZ treatment did not show any significant change in SRB1 expression either with AX or FX (AXPGZ-1.2 and FX PGZ 1.0 folds). Similarly, gene expression of PPARγ and SRB1 increased in AX (2.0 and 1.57), CLA (2.4 and 3.9), and AXCLA groups (4.9 and 3.0 folds), respectively, with an additive effect in AXCLA. **Conclusion:** These results indicate that the absorption of carotenoids is modulated by the PPARγ agonists CLA and PGZ, by altering the protein and gene expression of PPARγ and SRB1, also the carotenoids themselves influence these markers.

KEY WORDS: Astaxanthin; Fucoxanthin; Peroxisome Proliferator-Activated Receptors; Scavenger Receptor B1; Bioavailability

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INTRODUCTION

Carotenoids are one among the most widespread class of pigments in nature, synthesized only by photosynthetic organisms and some fungi. They are required by animals for normal physiological functions as they are the major antioxidants;[1] in plants, they act as accessory pigments in harvesting energy from light and also protect cells against photosensitization and inhibit the destructive effect of reactive oxygen species. Similarly, in animals, dietary carotenoids scavenge reactive oxygen species generated during cellular respiration and in conditions like inflammation[2] besides contributing protection against ultraviolet (UV) radiation in the eyes and skin. Although more than 675 naturally occurring carotenoids are known, only around 20 can be found in the human body. The main sources of carotenoids are fruits, vegetables, leaves, and seafood.

Astaxanthin (AX) (3,3'- dihydroxy β, β carotene 4 4''- dione) and fucoxanthin (FX) (3,5'-dihydroxy-8-oxo-6',7'-didehydro-5,5',6,6',7,8-hexahydro-5,6-epoxy-β,β-caroten-3'-yl acetate) are the major xanthophylls carotenoids found in marine organisms such as microscopic algae (phytoplankton) and brown seaweeds (macroscopic algae of *Phaeophyceae*), respectively. Although being non-provitamin A carotenoids, these are more potent antioxidants in comparison with the provitamin A carotenoids.[3] This enhancement is attributed to the substituted functional groups in the β-ionone ring of backbone structure. Specifically, the 4 and 4' keto groups in AX augment the catalytic property of 3, 3' hydroxyl groups. The presence of this conjugated carbonyl moiety enhances the stability of the trapped radical by decreasing its tendency for continued chain propagation reaction. Furthermore, AX positions itself parallelly in the lipid bilayer of biological membranes, exposing the substituted ring on both sides and the central chain will be embedded in the hydrophobic core of the membrane. This positioning is the key factor that makes AX a major protector of lipid peroxidation in membranes. Similarly, FX possesses an epoxy group on the one ring and on the other ring an allene group, 3'OH is acetylated and C8 on the polyene chain is hydroxylated and tautomerized to keto group; these functional groups are responsible for an enhanced reactivity toward the reactive oxygen species, and also responsible for its color and polarity. Hence, these carotenoids are being studied recently and are attributed to anticancerous, antiobesity, and anti-inflammatory activities.[4] However, their mechanism of action remains elusive.

Despite their vital role, the bioavailability of carotenoids is very poor and only 10–20% of the total carotenoids in the food material are bioavailable. Carotenoids are lipid soluble, and this is the major cause of their limited bioavailability. They undergo the same intestinal absorption process as dietary fat and must be solubilized in the digestive fluid through several steps before they are assimilated by intestinal epithelial cells.[5] Intestinal absorption of carotenoids depends on several dietary and non-dietary factors including level and origin of dietary fat, a number of carotenoids, digestion of food, and the presence of antioxidants or dietary fibers.[6] All the major works to date are focused on the role of dietary factors on the bioavailability of carotenoids. These studies have taken account of only passive diffusion as the mode carotenoid uptake. However, recent studies show that at least a part of carotenoid transport is facilitated and many lipids transporters such as scavenger receptor B1 (SRB1) and Niemann-Pick C1-like 1 (NPC1L1) are known to participate in the process.[7,8] This is due to the lipophilic nature of carotenoids, and hence, they follow the same pathway as that of dietary lipids. This fact makes the absorption of these two classes of nutrients intertwined and complicated. The factors that influence lipid absorption also have an impact on carotenoid absorption. The lipid themselves influence the bioavailability of carotenoid[9] and the lipid transporters such as SRB1 NPC1L1 and ATP binding cassette are all reported to involve in transporting carotenoids.[10]

Dietary lipids are also reported to compete with carotenoids in the intestine.[11] Furthermore, the lipid transporters that are thought to transport carotenoids such as SRB1 are known to be under the regulation of peroxisome proliferator-activated receptor gamma (PPARγ).[12] PPARγ is a nuclear transcription factor which acts as a ligand-activated modulator in regulating the gene expression of a wide array of enzymes, transporters, and other proteins involved in lipid/energy metabolism,[13] differentiation and development of tissues such as adipose and intestine and hence has an established role in pathogenesis of metabolic syndrome and cancer.

MATERIALS AND METHODS

Materials

Brown algae *Padina tetrastomatica* was collected off the Eastern Indian coast. Shrimp exoskeleton was purchased from the local market. Ezetimibe, pioglitazone (PGZ), and conjugated linoleic acid (CLA) were purchased from a local pharmacist. High-performance liquid chromatograph (HPLC) grade acetonitrile, hexane, methanol, and dichloromethane were purchased from Sisco Research Lab (Mumbai, India). Vitamins, minerals, cellulose, dextrin choline bitartrate, and methionine were purchased from HiMedia (Mumbai, India) and were of high purity food grade. Cornstarch and peanut oil were obtained from a local supermarket. All other chemicals used were of AR grade.

Extraction and Purification of Carotenoids

AX was extracted and purified from the exoskeleton of shrimp (*Pneus indica*) by the procedure previously described by Ravi and Baskaran[15] with slight modification. In brief, fresh shrimp carapace (100 g) was washed with fresh water and dried in the shade on an absorbent filter paper.

The dried carapace was ground to a coarse powder using a mixer grinder. This carapace powder was extracted by homogenization with cold hexane:isopropanol (9:1). For each extraction, the conical flask containing the exoskeleton was shaken at 100 strokes/min at 4°C for 2 h. The pooled extract was filtered using Buchanan filter, evaporated to dryness using a flash evaporator (Buchi, Switzerland) at 30°C and re-dissolved in 30 mL acetone:methanol (1:3 v/v). This extract was saponified with 10 mL 30% (w/v) methanolic KOH, incubated at RT for 45 min in the dark. The resulting mixture was partitioned in hexane:water (10:1 v/v) to remove soap and excess alkali. The hexane phase containing AX was evaporated to dryness by flash evaporation at 30°C and re-dissolved in 5 mL of hexane. The concentration and purity of the sample were determined by a colorimeter and HPLC, respectively.

FX was extracted and purified from the Indian brown seaweed, *Padina tetrastromatica*.[14] In brief, fresh *P. tetrastromatica* (100 g) was washed with freshwater and dried at 38 ± 2°C in a drier (Kilburn-024 E, Mumbai, India). The dried seaweed was ground to a fine powder using a mixer grinder, and powder was extracted by homogenization with cold acetone (4 times). For each extraction, the conical flask containing the seaweed was shaken at 100 strokes/min at 4°C for 2 h. The pooled extract (400 mL) was filtered using Buchanan filters, evaporated to dryness using a flash evaporator (Buchi, Switzerland) at 30°C and re-dissolved in methanol (100 mL). The extract was partitioned in methanol:water:hexane (10:1:10, v/v/v) and washed several times with hexane. The lower methanol/water phase was subjected to extraction thrice by diethyl ether (200 mL). The diethyl ether phase containing FX was evaporated to dryness by flash evaporation at 30°C and re-dissolved in 5 mL of hexane. This was applied on to open glass column (45 cm length, and 3 cm diameter) packed with silica gel (mesh size 60–120) previously equilibrated with hexane. Chlorophylls and carotenoids other than FX were eluted with hexane (300 mL) followed by 250 mL of hexane:acetone (9:1, v/v). The FX rich fraction was eluted with 200 mL of hexane:acetone (4:1, v/v). The extract was evaporated to dryness using a flash evaporator at 30°C. The residue was re-dissolved in methanol (3 mL) and used for purification. The extract was purified by preparatory HPLC (LC-8A, Shimadzu, Japan) using TSK-gel ODS 80Ts column (10 × 250 mm, Tosoh) with methanol (100%) as mobile phase (4 mL/min). The volume of extract injected to HPLC was 3 mL. The purity of FX was checked by HPLC.[15]

Mixed micelles served as the medium of administration wherein the carotenoids as well as other molecules to be fed were dispersed. These mixed micelles facilitate in dispersing the compounds to homogeneity and as a common base for both hydrophobic and hydrophilic molecules. The phosphate-buffered saline (pH 7.4, 200 µl) was subsequently added with mono-oleoylglycerol (2.5 mM), oleic acid (7.5 mM), sodium taurocholate (12 mM), and cholesterol (0.5 mM), and each dissolved before the addition of the next component by vigorous shaking at 40°C until the solution is clear.[9]

Animal Experiments

Animal experiments were carried out in compliance with the guidelines of the Institutional Animal Ethical Committee. Weanling male Wistar rats (n = 45), weighing 30 ± 5 g were housed in individual stainless steel cages in the institute animal house facility at room temperature (28 ± 2°C). Rats were acclimatized with commercial chow for a week and later fed with AIN 93-G diet for 2 weeks,[16] and a 12-h Light/Dark cycle was maintained, and the rats had free access to food and water. Then, the animals were grouped randomly into nine groups with five animals each. Two groups were gavaged for 14 days with AX and FX each, and four groups received either CLA or PGZ, two each with both the carotenoids dispersed in mixed micelle (0.1 mg in 0.2 mL micelle solution/rat/day) and two groups that received either CLA or PGZ without carotenoid served as positive control. Similarly, one group was given only mixed micelle and served as a baseline. Animals were sacrificed on the 15th day, and tissues were collected for further analyses. At the termination of the experiment, rats in each group were sacrificed by cardiac puncture mode, anesthetized in a CO_2 chamber and blood was drawn from the heart into heparinized (0.2 mg/mL blood) tubes for plasma separation. Liver adipose, brain, and intestine were sampled and washed with ice-cold saline (0.9%). Samples were processed under a dim yellow light, in an ice bath (<5°C) to minimize isomerization and oxidation of carotenoids. The plasma was separated from the blood into clean tubes by centrifugation (Remi India Ltd., Mumbai) at 2000 g for 15 min at 4°C.

Extraction of Carotenoids from Plasma and Tissue Samples

AX was extracted from the plasma and liver homogenates as per Raju *et al*., 2007.[17] Similarly, FX extraction was done with a variation in the procedure of Ravi and Baskaran[15] plasma preparation, tissue homogenization, and microsomes preparation were the same as described earlier.[18] Plasma (0.8 mL) was diluted with 3 mL of dichloromethane:methanol (1:2; v/v) containing 2 mM a-tocopherol, mixed for 1 min using a vortex mixer, followed by the addition of 1.5 mL hexane. The mixture was centrifuged at 5000 g for 15 min at 4°C, and the upper hexane-dichloromethane layer was withdrawn. The extraction was repeated for the lower phase twice using dichloromethane:hexane (1:1.5; v/v). The pooled extract was evaporated to dryness under a stream of nitrogen, redissolved in dichloromethane:methanol (2:1; v/v) and analyzed by HPLC.

HPLC Analysis of Carotenoids

AX and FX in plasma, liver homogenate extracts were quantified by HPLC (LC-10Avp; Shimadzu, Kyoto, Japan) equipped

with photodiode array detector (SPD-M20A Shimadzu). All the components were separated on a Phenomenex C18-RP column (250 mm × 4.6 mm; 5 mm), isocratically eluting (1 mL/min) with acetonitrile:dichloromethane:methanol (60:20:20; v/v/v) containing 0.1% ammonium acetate as mobile phase. AX and FX were monitored at 450 and 474 nm using Shimadzu Class-VP version 6.14SP1 software. The peak identities were confirmed by comparing characteristic UV-visible spectra with authentic standards and quantified by comparing their peak areas [Figure 1].[15]

Protein Analysis and Western Blotting

Protein content in the samples was measured by the method of Lowry *et al*. (1951). Freshly prepared Lowry's reagent (0.98 mL of 2% sodium carbonate, 0.01 mL of 2% copper sulfate, and 0.01 mL of 2% sodium potassium tartar ate) was vortexed (1 mL) with the diluted sample (1 mL) and allowed to stand for 10 min, followed by the addition of 100 µl of 1N Folin–Ciocalteu's phenol reagent and immediately vortexed. The samples were allowed to stand for 30 min at room temperature, and the absorbance was read at 690 nm. The protein in the samples was quantified based on the absorbance obtained in a linear graph obtained for known concentrations of standard bovine serum albumin (BSA).

Vertical gel electrophoresis was used to separate proteins in the intestinal scrapings. The resolving gel consisted of 10% acrylamide, comprising 2 mL of polyacrylamide (29.2 g acrylamide and 0.8 g bisacrylamide) 2.4 mL water, 1.5 mL running gel buffer (18.6 g tris in 100 mL, pH 8.8), and 60 µL of 15% ammonium persulfate (APS) and 15 µL TEMED to form the gel. Similarly, 4% acrylamide served as the stacking gel (0.35 polyacrylamide, 0.5 mL stacking gel buffer [3.06 Tris in 50 mL, pH 6.8], APS, and TEMED).[19] A known concentration of protein from the intestinal scrapings (50 µg) was dissolved in sample buffer (8 mL stacking gel buffer, 2 mL glycerol, 200 mg sodium dodecyl sulfate, 0.5 mL β-mercaptoethanol, and a pinch of bromophenol blue, made to 10 mL) and was loaded in the stacking gel and subjected to 100 V electrical power supply until they reached resolving gel after which 150 V supply was given. After the completion of the run, the gel was sandwiched with polyvinylidene difluoride membrane, activated in methanol for 1 min, in between filter paper stack and the blotting cassette (Biorad), dipped in the transfer buffer (3 g

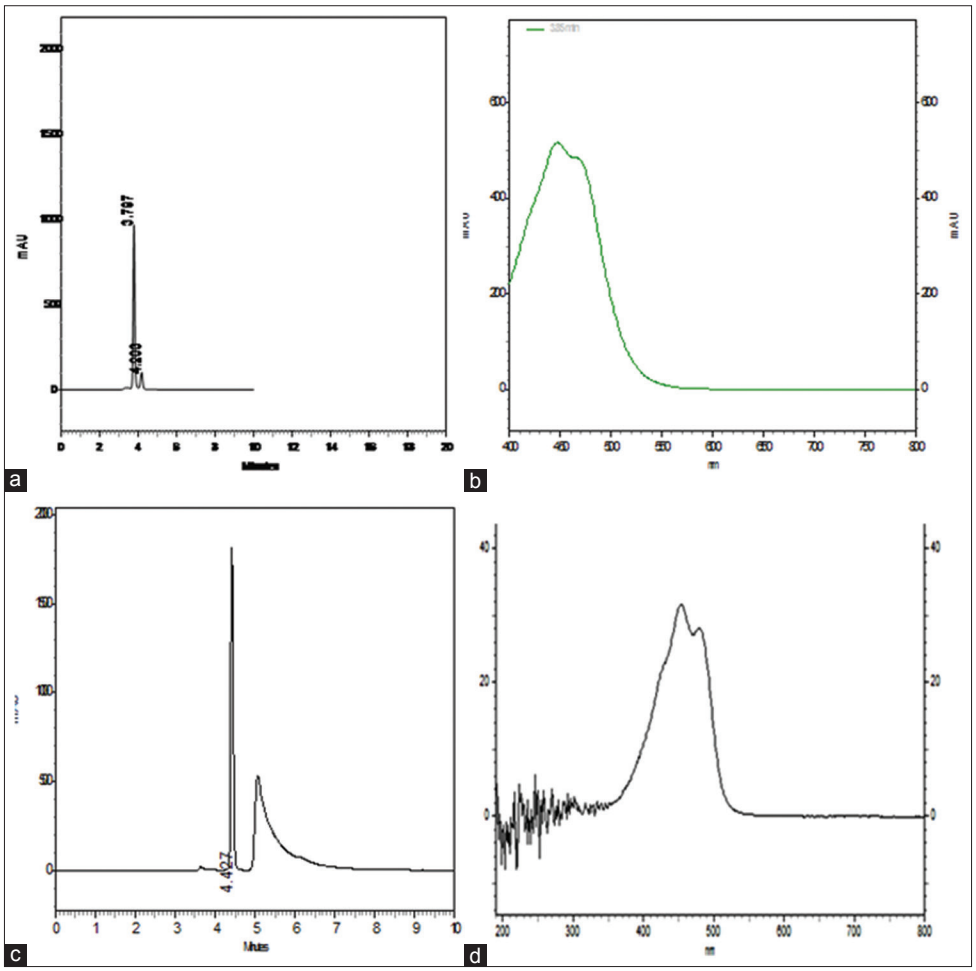

Figure 1: High-performance liquid chromatograph chromatographs showing characteristic peaks of (a) fucoxanthin (retention time 3.8 min) and (b) spectrum (absorption maxima at 474 nm) and (c) astaxanthin (RT 4.42 min) and (d) its spectrum (absorption maxima at 448 nm)

Tris and 4 g glycine dissolved in 900 mL water and 100 mL methanol) and subjected to 200 mA electrical power supply for 2 h at 4°C. The successful transfer of protein bands on the membrane was confirmed with Ponceau staining, and thoroughly washed in water. The membrane was treated with 5% BSA solution at 4°C overnight to block the free area of the membrane followed by primary and secondary antibody treatments with three intermittent washes with Tris-buffered saline (pH 8)-Triton X100 (1%). The horseradish peroxidase conjugated secondary antibody was detected using the chemiluminescent product formation under the blot reader (Syngene G box). The intensity of the band was measured with the software provided with the instrument to express the differences in the protein expression (densitometric units/μg protein).

Gene Expression Studies by Quantitative Polymerase Chain Reaction (PCR)

Total RNA from the intestinal scrapings was isolated using TRI reagent (Sigma), and later cDNA was prepared by with the cDNA Kit (Applied Biosystems) and SYBR green master mix was obtained from Roche, all the protocols were carried out according to the instructions provided by the suppliers. The primers were designed using the primer blast. The relative expression of these genes was calculated by the comparative ΔΔct values obtained after the quantitative PCR amplification.

Statistical Analysis

Statistical analysis was done by one-way ANOVA followed by Tukey's test with $P \leq 0.05$. The fold of expression in western blotting and PCR was in comparison with micelle (vehicle) control, and the significance was calculated against only carotenoids (AX or FX) group versus treatments (CLA and PGZ).

RESULTS

Carotenoid Uptake

The concentration of both the carotenoid in both liver and serum increased along with treatment (CLA and PGZ) as compared to only carotenoid fed groups. Among the treatments, AXCLA group showed a higher concentration of AX in both plasma and liver [Figure 2]. This is more nonpolar than FX. It is known that intestinal uptake of carotenoids increases with dietary lipids. Nidhi and Baskaran[20] have reported that the absorption of lutein was highest with coconut oil and olive oil which contain lauric and oleic acid (82%) as the major constituents, respectively. However, linoleic acid (LA) is the next abundant constituent (3.5–21%), and this vital polyunsaturated fatty acids are found in all major vegetable oils under human consumption such as sunflower safflower and peanut oils. CLA is a geometric isomer of

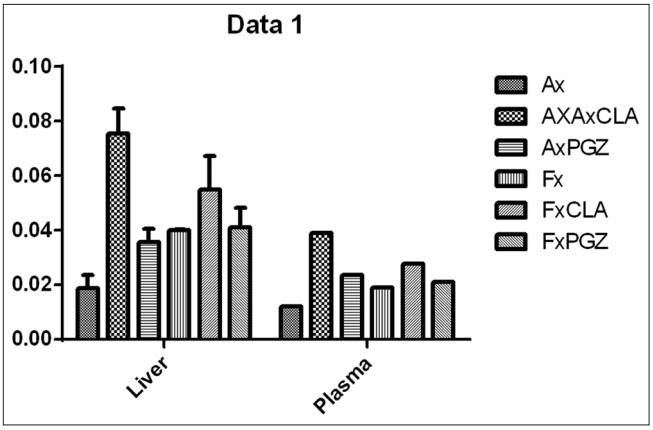

Figure 2: Graph showing the concentration of astaxanthin and fucoxanthin in liver (mg/g tissue) and plasma (mg/dL), respectively, in the treatment groups

LA formed either due to heat treatment as in safflower oil extraction or in ruminants by gut microbiota.[21] Furthermore, CLA and PGZ may have a role in increasing the facilitated transport of the carotenoids.

Western Blotting

As compared to the control, there was an increase in the expression of PPARγ in AXCLA group (1.4 folds), whereas only AX and AXPGZ had no change. On the contrary, carotenoid FX showed a decrease in PPARγ expression by half as compared to control, as also FXPGZ treatment. However, the PPARγ expression was restored in FXCLA group (1.1 folds), as in control. This result shows that CLA treatment has increased PPARγ protein expression. The expression of SRB1 was decreased in AX and did not change in FX group (0.3 and 1.0 folds, respectively) in comparison with the control group. Although the AXCLA group exhibited a decrease in SRB1 expression when compared to control (0.57 folds), there was an increase in comparison with AX group; similarly, there was an increase in FXCLA group (1.3 folds). The PGZ treatment did not show any significant change in SRB1 expression either with AX or FX in comparison with control (AXPGZ-1.2 and FX PGZ 1.0 folds) (Figure 3a and 3b). These results suggest that the treatment with CLA was potent in enhancing the intestinal protein expression of PPARγ and SRB1. Furthermore, since the absorption of carotenoids is high in CLA group, this can imply that the CLA has contributed to the higher carotenoid bioavailability by PPARγ and SRB1 mediated facilitated transport.

Quantitative PCR

When the effect of PPARγ agonists used in the study (CLA and PGZ), on the genes involved in carotenoid/lipid absorption were analyzed, the treatments showed an increase in the gene expression PPARγ and SRB1 [Figure 4]. The PPARγ expression was decreased in AX group but in

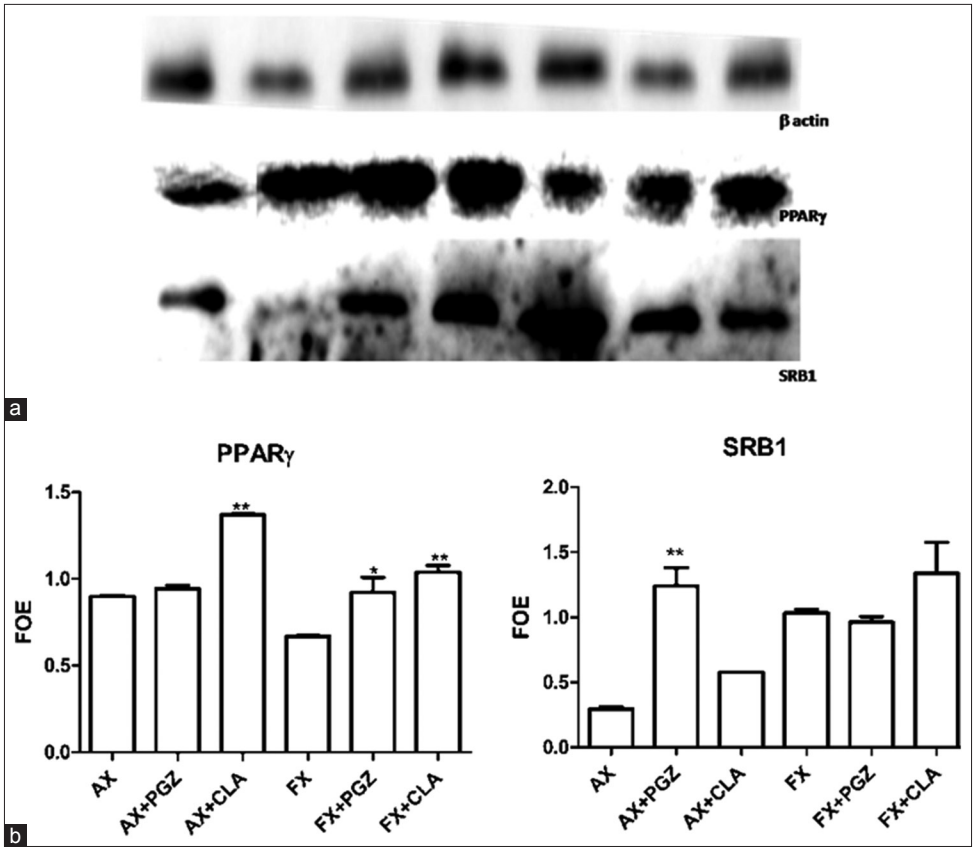

Figure 3: (a) Western blot image and (b) the graph representing the band intensity (densitometric units per μg protein) for expression of peroxisome proliferator-activated receptor gamma and scavenger receptor B1 in the intestine of rats with treatments 1. Control, 2. Astaxanthin (AX), 3. AX+conjugated linoleic acid (CLA), 4. AX+pioglitazone (PGZ), 5. Fucoxanthin (FX), 6. FX+CLA, 7. FX+PGZ

combination with the agonists, the gene expression was further upregulated. (AX-2.8 folds, AXCLA 4.9 folds, and AXPGZ 4.3 folds, respectively, in comparison with control). FX also enhanced the expression of PPARγ (1.8 folds), and the treatments had the additive effect (3.8 and 1.8 folds, respectively, in FXCLA and FXPGZ groups). Similarly, the SRB1 gene, which is under the influence of PPARγ, exhibited comparable results. Both the carotenoids had a nominal effect; wherein there was an increase of 1.5 fold (AX and FX group) in the gene expression. CLA alone had 3.9 fold increase in SRB1gene expression and for PGZ treatment it was 2.8 folds. Coadministration of PPARγ agonists had an enhancing effect (3.0 folds in AXCLA and 1.3 folds in AXPGZ), whereas there was no significant variation in coadministration of PPARγ agonists with FX. PPARa gene expression was increased in both the carotenoid groups (1.2 and 7.6 folds, respectively, in AX and FX groups). The PPARγ agonists had only a marginal increase (CLA - 1.4 and PGZ -1.5 folds). Carotenoids exhibited the increase in gene expression even when coadministered with CLA (AXCLA – 4.0 and FXCLA – 2.3 folds), rather marginal increase can be seen in PGZ combination groups (AXPGZ - 1.5 and FXPGZ 1.9 folds). Both the carotenoids also increased the peroxisome proliferator-activated gamma coactivator 1α (PGC1α) gene expression (AX – 4.5 and FX – 3.3). Among the drug, only groups PGZ had a pronounced enhancement compared to CLA (4.9 and 1.3 folds, respectively); similarly, AXPGZ group showed 6.2 fold, AXCLA group showed 2.8, FXCLA 4.2 and FXPGZ 3.8 fold increase in PGC1α gene expression. The sterol regulatory element-binding protein 1 (SREBP1) gene was downregulated in all the groups in comparison with the control. The SREBP1 gene is a gene involved in intestinal lipid synthesis and absorption. PGC1α is a coregulator which is involved in activating PPARγ. Any modulation on either the expression or activity of these gene products will have implication in lipid/energy homeostasis along with carotenoid absorption.

DISCUSSION

In the present study, it has been observed that (i) the natural agonist of PPARγ, CLA has a positive impact on intestinal absorption of carotenoids studied,[22] (ii) coadministration of carotenoids with PPARγ agonist enhanced SRB1 expression by an increase in PPARγ expression, and (iii) this is further confirmed from the gene expression study wherein carotenoid treatment alone has a negative effect and on CLA administration the gene expression of PPARγ and SRB1 improve.

Carotenoid coadministered with CLA has shown the higher uptake and accumulation in plasma and liver compared

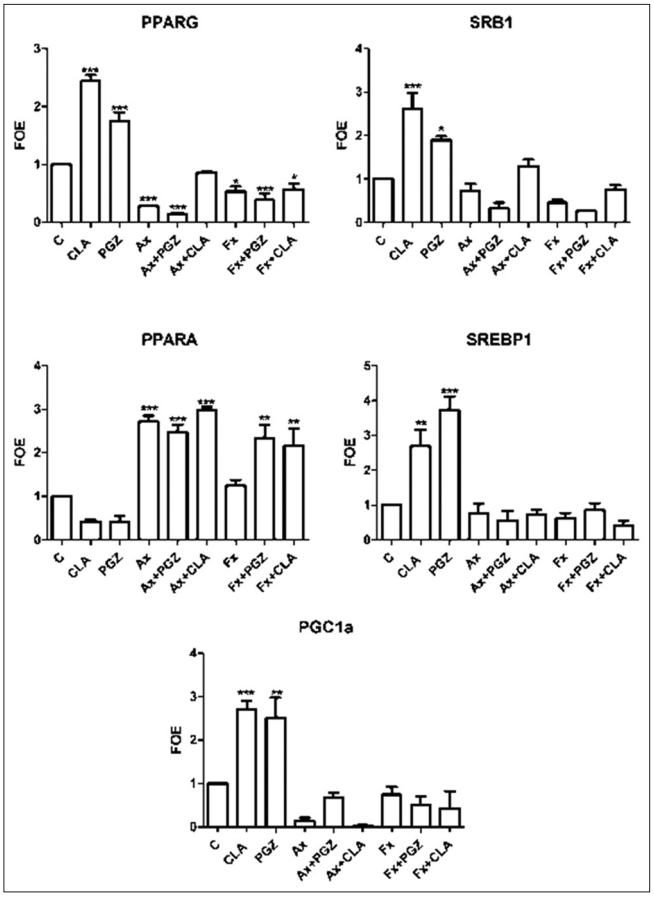

Figure 4: Graphical representation of the change in gene expression (in folds); by quantitative polymerase chain reaction analysis (in comparison with vehicle control/mixed micelle)

to carotenoid alone or with PGZ. This can be due to both physical and physiological effects exerted by CLA. CLA being a fatty acid helps in micellization of the carotenoids,[20,23] and thereby help in passive absorption. Furthermore, as seen in this study, CLA upregulates protein and gene expression of PPARγ and SRB1, that are responsible for enhanced facilitated uptake. Furthermore, both PPARγ upregulation in the intestine is known to be helpful in maintaining the structural and functional integrity, maintenance of healthy gut microbiota, and reducing inflammation. This observation is in line with previous investigations.[24] Furthermore, PGZ used in the study enhanced the uptake of carotenoids through PPARγ upregulation.[25,26]

Although studies have shown an antagonistic activity of AX,[27] there can be an increase in the protein and gene expression. Ravi and Baskaran[28] have shown that FX treatment has increased the protein expression of PPARγ. Furthermore, in many studies, the anti-inflammatory and anticarcinogenic effects of these carotenoids are reported to be by upregulation of PPARγ.[26,28] Although being the agonist of PPARγ, both CLA and PGZ had an influence on gene and protein expression of it.[29] Ahmed *et al*.[12] have shown that SRB1 is regulated by PPARγ, and this SRB1 has a role in the transport of carotenoids in the intestine and Caco 2 cells.[30]

CONCLUSION

Carotenoids are lipophilic essential micronutrients and enhancing their bioavailability is a challenge. This study helps in understanding the ways in which this can be accomplished and the mechanism behind it. This can be helpful in managing health hazards involving oxidative stress, such as metabolic syndrome and cancer since these are natural and abundant dietary components which also enhance the sensory property of the food they are present in, carotenoids make the safe and affordable nutraceuticals.

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