International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.10, pp 186-195, 2017

In vitro anti-inflammatory activity of microencapsulated and non-encapsulated astaxanthin

V. Suganya*, V.Anuradha, M. Syed Ali, P. Sangeetha and P. Bhuvana

Department of Biochemistry, Mohamed Sathak College of arts & science, Sholinganallur, Chennai, Tamil Nadu, India.

Abstract : Encapsulated astaxanthin along with non-encapsulated astaxanthin was evaluated for its anti-inflammatory activity by *invitro* methods. *In vitro* anti-inflammatory activity such as inhibition of albumin denaturation, membrane stabilization by heat induced method, proteinase inhibitory action, HRBC membrane stabilization were determined at different concentration. Standard drug aspirin, Diclofenacsodium was used as positive control. In albumin denaturation at concentration 500 µg/ml ME 4 showed highest inhibition with 94.40 ± 0.208 % than standard (91.84 ± 0.115 %) and other samples. The highest activity was showed by ME 4 (91.15%) followed by ME 3 (90.30%), ME 2 (90.18%), ME 1 (89.04%) and non-encapsulated astaxanthin (88.41%) at concentration 500 µg/ml than standard drug (87.84%) in membrane stabilization test. In other two method also the test sample ME 4 exhibit better activity than other test and standard.

Keywords : Anti-inflammatory, HRBC method, membrane stabilization, albumin denaturation.

Introduction

Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of more number of proliferative factors (like Vasoactive, chemotactic) at different stages and there are many targets for anti-inflammatory activity. Their respective tissue injury is a type of inflammatory response suppressed by glucocorticoids and this is the basic clinical uses and also it interferes with several steps in the inflammatory response. Numbers of corticoids are only palliative; do not act on the inflammation instead of they favor spread of infections capacity of defensive cells to kill microorganisms is impaired at the same time interfere with healing and scar formation. The alternate use of corticoids is hazardous other than the corticosteroids the NASIDs are also used to treat inflammation. The main mechanism of action of the NASIDs is the inhibition of prostaglandin (PG) synthesis or preferential or selective COX-2 inhibition. Due to the inhibition of prostaglandin (PG) synthesis it may produce toxic effects like bleeding, inhibition of platelet function, gastric mucosal damage, asthma and anaphylactic reactions may cause some individuals.[1]

Astaxanthin was first isolated in 1938 from American lobster. It is a xanthophyll carotenoid of predominantly marine origin, with potent antioxidant and anti-inflammatory effects demonstrated in both experimental and human studies.[2]Astaxanthin is chemically3,30-dihydroxy-β, β -carotene-4, 40-dionea naturals pigments which has been widely used in feed as colorant approved by US FDA for specific use in animal and fish food.[3] However, it isa powerful Antioxidant; it is 10 times more capable than other carotenoids.[4] However the carotenoids are biosynthesized by plant, certain bacteria, and fungi etc.

Astaxanthin was found to inhibit the expression and formation of the aforementioned pro-inflammatory mediators and cytokines in both lipopolysaccharide-stimulated cells and primary macrophages. Astaxanthin suppressed the serum levels of NO, prostaglandin E2, tumor necrosis factor and interleukin-1 beta in lipopolysaccharide-administrated mice. Astaxanthin inhibited NF-kappaB activation as well as NO synthase promoter activity in lipopolysaccharide-stimulated cells. NF-KappaB has a seminal role in immunity, as it activates pro-inflammatory genes encoding for NO synthase, tumor necrosis factor- and several interleukins. Astaxanthin inhibits inflammatory mediator production by blocking NF-kappaB activation and as a consequent suppression of Ikappa B kinase activity and I kappa B-alpha degradation.[5]

In present study, the encapsulated and non-encapsulated astaxanthin was assayed for *in vitro* anti-inflammatory activity by different methods such as inhibition of albumin denaturation, membrane stabilization test using heat induced hemolysis, protein inhibitory action and HRBC membrane stabilization method.

Materials and Methods

A. Microencapsulation of astaxanthin using different agents

Astaxanthin purchased from Rudra Bio ventures Pvt Ltd, Bangalore was encapsulated using four different agents by ionotropic gelation method. In first method, microencapsulated astaxanthin was prepared by using sodium alginate and calcium chloride.[6,7,28] In second method, microencapsulated astaxanthin was prepared using sodium alginate and chitosan.[8] In third method, chitosan – Tripolyphosphate was used to produce microencapsulated astaxanthin.[9,10] In fourth method, liposome encapsulated astaxanthin was carried out by the method followed by [11]. The *invitro* anti-inflammatory activities were performed for both microencapsulated and non – encapsulated astaxanthin (non-encapsulated astaxanthin). The detailed procedures were given below.

B. *Invitro* anti-inflammatory activity

I. Inhibition of albumin denaturation

Method of [12,13]wasfollowed with minor modifications. The reaction mixture was consisting of test samples (100 – 500 µg/ ml) and1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 51°C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug of varied concentration 100 – 500 µg/ ml. The experiment was performed in triplicate.

Percent inhibition of protein denaturation was calculated as follows:

% inhibition = (Abs control –Abs sample) X 100/ Abs control
Where Abs control is the absorbance of the DPPH radical+ solvent,
Abs sample is the absorbance of DPPH radical+ sample extract/standard.

II. Membrane stabilization test

Preparation of Red Blood cells(RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000 rpm for10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.[13,14]

Heat induced hemolysis

The reaction mixture (2 ml) consisted of 1 ml of test solution and 1 ml of10% RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug in the concentration ranges from 100 – 500 µg/ ml. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at2500 rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates.[13,14]

Percent of HRBC membrane stabilization or protection was calculated as follows:

% of protection = (100 – O.D of drug treated sample – O.D of control) X 100

III. Proteinase inhibitory action

The test was performed according to the modified method of [13,15] 2 ml of reaction mixture containing0.06 mg trypsin, 1 ml 20 mMTrisHCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 – 500 µg/ ml) were mixed together. The mixture was incubated at 37^{o}C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70%perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. Aspirin was taken as standard drug (100 – 500 µg/ ml). The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

% of proteinase inhibitory = (Abscontrol –Abssample) X 100/ Abscontrol

IV. HRBC Membrane Stabilization Method

The human red blood cell (HRBC)membrane stabilization method was used for the study of *in vitro* anti-inflammatory activity. The blood was collected from healthy human volunteer under aseptic conditions who was not taken any Non-Steroidal Anti-inflammatory Drugs for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate,0.5% citric acid and 0.42% NaCl). It was centrifuged at 3,000 rpm and the packed cells were washed with isosaline (Nacl, pH 7.2) and a 10%suspension was made. To 0.5ml of test samples, 1ml of phosphate buffer (0.15 M, pH 7.4), 2 ml hyposaline (0.36% NaCl) and0.5 ml of HRBC suspension were added. The solution was incubated at 37°C for 30min and centrifuged at 3,000 rpm for 20 min. The content of the supernatant solution was absorbed spectrophotometrically at 560 nm.[16]Control was taken without the test sample. Diclofenac (100 - 500 µg/ml) was used as reference standard.[14,17,18]

Percentage (%) of protection = [100 – OD of the drug treated sample/ OD of the control] × 100

Result and Discussion

***In vitro* anti-inflammatory activity**

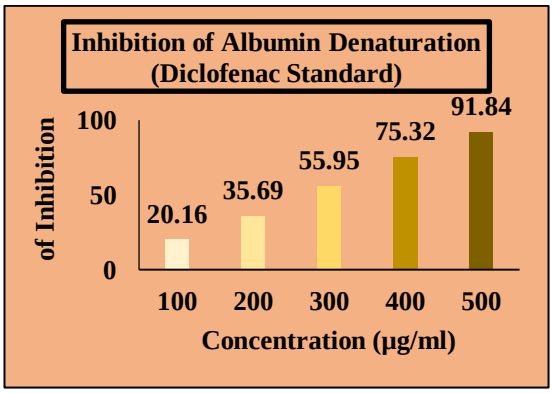
1. Inhibition of albumin denaturation

Table 1 Anti-inflammatory activity of standard Diclofenac sodium by albumin denaturation

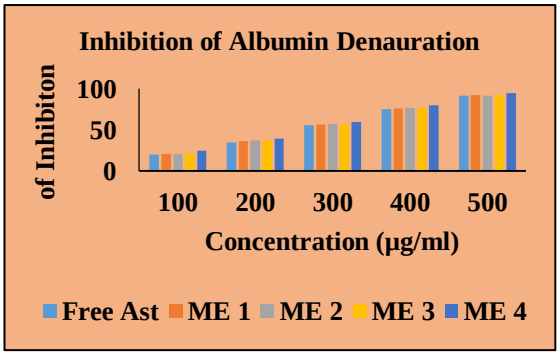
Content	Concentration (µg/ml)	Mean ± S.D Percentage	IC 50 Values
Blank	-	0	268.348
S1	100	20.16 ± 0.058	
S2	200	35.69 ± 0.100	
S3	300	55.95 ± 0.866	
S4	400	75.32 ± 0.100	
S5	500	91.84 ± 0.115	

Table 2 Inhibition of albumin denaturation percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Mean ± SD Percentage	ME 1 Mean ± SD Percentage	ME 2 Mean ± SD Percentage	ME 3 Mean ± SD Percentage	ME 4 Mean ± SD Percentage
100	19.86 ± 0.581	20.75 ± 0.379	21.24 ±0.100	21.53 ± 0.100	24.78 ± 0.681
200	35.20 ± 0.608	36.28 ± 0.436	36.97 ± 0.208	37.36 ± 0.115	39.92 ± 0.153
300	55.56 ± 0.058	56.34 ± 0.173	57.13 ± 0.100	57.62 ± 0.100	60.08 ± 0.265
400	74.93 ± 0.208	75.81 ± 0.289	76.50 ± 0.058	77.19 ± 0.577	80.14 ± 0.153
500	91.54 ± 0.153	92.04 ± 0.115	91.94 ± 0.100	92.72 ± 0.115	94.40 ± 0.208
IC 50 Values	270.408	265.713	262.660	260.024	245.035



Graph 1 Inhibition of albumin denaturation by Diclofenac sodium standard drug



Graph 2 Inhibition of albumin denaturation of encapsulated and non-encapsulated astaxanthin

During inflammation, lysosomal hydrolytic enzymes are released which causes damages of the surrounding organelles and tissues with attendance variety of disorders.[19] Various methods were employed to screen and study drugs, chemicals, herbal preparations that exhibit anti-inflammatory properties or potentials. In the present study, astaxanthin was encapsulated and its anti-inflammatory activity were determined.

Denaturation of proteins is a well-documented cause of inflammation. The ability of protein denaturation by encapsulated and non-encapsulated astaxanthin was studied. Test samples showed very effective in inhibiting heat induced albumin denaturation along with standard Diclofenac (**Table 1** and **Table 2**)

Inhibition of albumin denaturation by test samples and standard was plotted in **Graph 1** and **Graph 2.** Standard Diclofenac sodium showed 20.61% at concentration 100 µg/ml and 91.84% at concentration 500 µg/ml. It was compared with the test samples non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4. Maximum inhibition at 100 µg/ml was observed by the sample ME 4 (24.78%) followed by ME 3 (21.53%) and ME 2(21.24%). Inhibition of albumin denaturation by non-encapsulation astaxanthin (19.86%) and ME 1

(20.75%) was slightly lower than other samples. At concentration 500 µg/ml ME 4 possessed maximum activity of 94.40% than ME 3 (92.72%), ME 2 (91.94%), ME 1 (92.04%) and non-encapsulation astaxanthin (91.54%)**.** IC 50 values of test samples such as non-encapsulation astaxanthin, ME 1, ME 2, ME 3 and ME 4 were founded to be 270.408, 265.713, 262.660, 260.024 and 245.035 µg/ml which is similar to standard drug (268.348 µg/ml).

2. Membrane stabilization test: Heat induced hemolysis

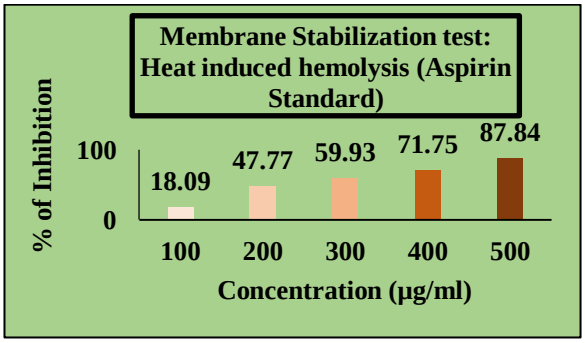
Table 3 Anti-inflammatory activity of standard aspirin by membrane stabilization test: heat induced hemolysis

Content	Concentration (µg/ml)	Mean ± S.D Percentage	IC 50 Values
Blank	-	0	256.716
S1	100	18.09± 0.153	
S2	200	47.77± 0.100	
S3	300	59.93± 0.200	
S4	400	71.75± 0.100	
S5	500	87.84± 0.115	

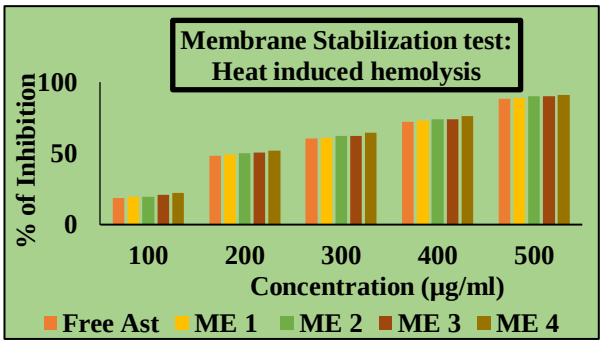
Table 4Membrane stabilization test: Heat induced hemolysis percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Mean ± SD Percentage	ME 1 Mean ± SD Percentage	ME 2 Mean ± SD Percentage	ME 3 Mean ± SD Percentage	ME 4 Mean ± SD Percentage
100	18.89± 0.058	19.52± 0.578	19.58± 0.058	20.89± 0.058	22.43± 0.100
200	48.52± 0.115	49.14± 0.578	50.29± 0.200	50.51± 0.200	52.00± 0.115
300	60.45± 0.058	61.19± 0.153	62.39± 0.153	62.56± 0.153	64.61± 0.058
400	72.43± 0.058	73.12± 0.058	74.03± 0.058	74.20± 0.058	76.14± 0.100
500	88.41± 0.058	89.04± 0.058	90.18± 0.100	90.30± 0.100	91.15± 0.100
IC 50 Values	252.501	248.460	243.652	240.361	230.276

The mechanism of anti-inflammatory activity was studied through stabilization of RBCs membrane. All the samples were effectively inhibiting the heat induced hemolysis. Thus, the results shows that the samples inhibit the release of lysosomal content of neutrophils at the site of inflammation. The test samples inhibited the heat induced hemolysis of RBCs at varies concentration from 100 – 500 µg/ml (**Table 3**and**Table4**).At concentration 500 µg/ml the maximum inhibitions 91.15% from ME 4 followed by ME 3 (90.30%) and ME 2(90.18%) was obtained. The aspirin standard drug showed the maximum inhibition of 87.84%. When compared to other samples, inhibition of88.41% was observed from non-encapsulated astaxanthin followed by ME 1(89.04%) which was plotted in **Graph 3** and **Graph 4.**IC 50 values of each samples were minimum in range which possess 252.501 µg/ml (non-encapsulated astaxanthin), 248.460 µg/ml (ME 1), 243.652 µg/ml (ME 2), 240.361 µg/ml (ME 3) and 230.276 µg/ml (ME 4).



Graph 3 Membrane stabilization test: Heat induced hemolysis by aspirin standard drug



Graph 4 Membrane stabilization test: Heat induced hemolysis by encapsulated and non-encapsulated astaxanthin

3. Protein inhibitory action

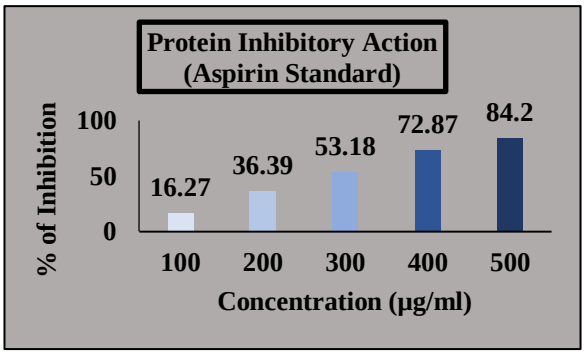
Anti-inflammatory activity of encapsulated and non-encapsulated astaxanthin was done by protein inhibitory action at different concentration in the range of 100 – 500 µg/ml is shown in the **Table 5** and**Table 6**. Results of the present investigation revealed that astaxanthin exhibited significant anti-inflammatory property which increases with increase in concentration of both test samples and standard drug

Table 5 Anti-inflammatory activity of standard drug aspirin by protein inhibitory action

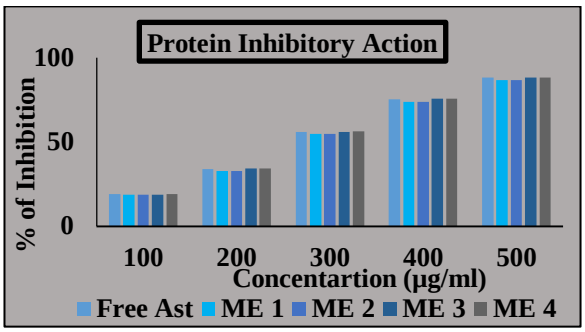
Content	Concentration (µg/ml)	Mean ± S.D Percentage	IC 50 Values
Blank	-	0	285.018
S1	100	16.27± 0.100	
S2	200	36.39± 0.058	
S3	300	53.18± 0.153	
S4	400	72.87± 0.100	
S5	500	84.20± 0.529	

Table 6 Protein inhibitory action percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Mean ± SD Percentage	ME 1 Mean ± SD Percentage	ME 2 Mean ± SD Percentage	ME 3 Mean ± SD Percentage	ME 4 Mean ± SD Percentage
100	19.17± 0.100	18.79± 0.058	18.50± 0.100	18.64± 0.100	18.88± 0.100
200	33.87± 0.058	32.59± 0.100	32.69± 0.058	34.06± 0.058	34.25± 0.058
300	55.74± 0.153	54.74± 0.058	54.84± 0.100	56.02± 0.058	56.26± 0.058
400	75.28± 0.100	73.77± 0.100	73.86± 0.100	75.43± 0.100	75.57± 0.058
500	87.95± 0.058	86.67± 0.058	86.72±0.058	88.09± 0.100	88.24± 0.058
IC 50 Values	275.404	281.282	281.296	275.326	274.228



Graph 5 Protein inhibitory action by aspirin standard drug



Graph 6 Protein inhibitory action by encapsulated and non-encapsulated astaxanthin

Percentage of inhibition at concentration 100 µg/ml by protein inhibitory action was founded to be maximum by standard drug aspirin (16.27%) when compared with test samples. At 100 µg/ml the percentage of inhibition by non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 was 19.17%, 18.79%, 18.50%, 18.64% and 18.88% respectively. (**Graph 5** and **Graph 6**).

At concentration 500 µg/ml, it was founded that the maximum activity was showed by standard drug was 84.20% which is lower than the test samples ME 4 (88.24%), ME 3 (88.09%) and non-encapsulation astaxanthin (87.95%). The percentage of inhibition by ME 1 (86.67%) and ME 2 (86.72%) was founded to be similar. Thus, in protein inhibitory action the standard drug shows high anti-inflammatory activity than the all test samples.

4. HRBC membrane stabilization method

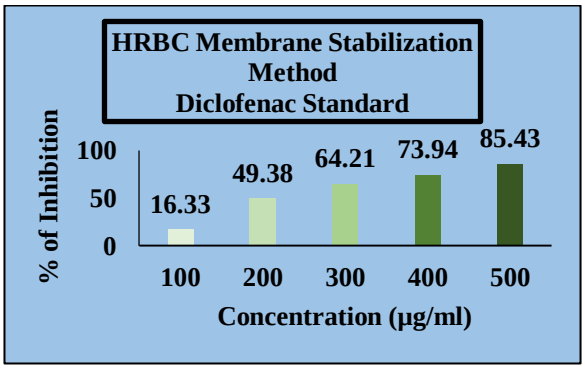
The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by encapsulated and non-encapsulated astaxanthin to predict the anti-inflammatory activity.

Table 7 Anti-inflammatory activity of Diclofenac standard by HRBC membrane stabilization method

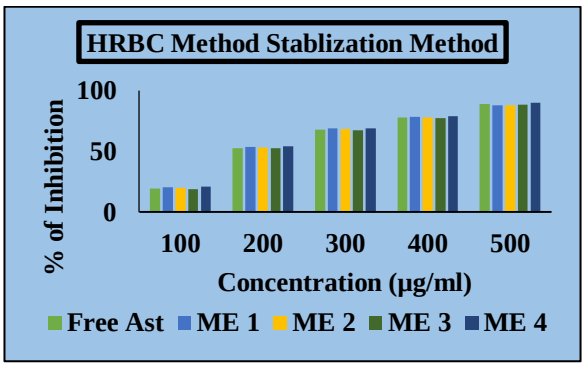
Content	Concentration (µg/ml)	Mean ± S.D Percentage	IC 50 Values
Blank	-	0	251.720
S1	100	16.33± 0.200	
S2	200	49.38± 0.110	
S3	300	64.21± 0.153	
S4	400	73.94± 0.058	
S5	500	85.43± 0.058	

Table 8 HRBC membrane stabilization method percentagefor different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Mean ± SD Percentage	ME 1 Mean ± SD Percentage	ME 2 Mean ± SD Percentage	ME 3 Mean ± SD Percentage	ME 4 Mean ± SD Percentage
100	19.07± 0.100	20.18± 0.100	19.60± 0.115	18.62± 0.153	21.10± 0.058
200	52.78± 0.100	53.63± 0.058	53.04± 0.115	52.45± 0.115	54.08± 0.115
300	67.93± 0.100	68.78± 0.200	68.26± 0.153	67.69± 0.100	68.91± 0.058
400	77.79± 0.153	78.51± 0.100	77.93± 0.100	77.34± 0.100	78.84± 0.115
500	88.90± 0.100	88.24± 0.200	87.72± 0.058	88.57± 0.153	90.01± 0.100
IC 50 Values	231.414	226.286	229.808	233.758	222.574



Graph 7 HRBC membrane stabilization method by Diclofenac standard drug



Graph 8 HRBC membrane stabilization method by encapsulated and non-encapsulated astaxanthin

In present investigation, the HRBC membrane stabilization method showed that the ME 4 (90.01%) possess maximum activity followed ME 3 (88.57%), non-encapsulated astaxanthin (88.09%) and ME 1 (88.24%). ME 2 showed slight decreased in activity which exhibit 87.72%. When compared to all the test samples the standard drug Diclofenac exhibit lower activity of 85.43% at concentration 500 µg/ml. the values were entered in **Table 7**and**Table 8** and the diagrammatic representation was plotted in **Graph 7** and **Graph 8**.

The overall result of the present investigation, showed that, both encapsulated and non-encapsulated astaxanthin possessed high anti-inflammatory activities. Thus, the studies were confirmed by other findings. Scientific studies have confirmed the fact that free radicals are the underlying cause of any health problem. Free radicals seem to be the cause of inflammation in the Joints and cause pain in the joints. With rheumatoid arthritis it is an immune rejection in joints and the first immune response of the body is to generate free radicals. Nutritional therapy with astaxanthin is an effective way in controlling inflammatory conditions. Studies have shown that astaxanthin as an antioxidant pigment contains specific anti-inflammatory properties and through this antioxidant property, astaxanthin seems to be more effective in these conditions as this carotenoid help in neutralizing the free radicals and minimize the oxidative damage in the joints.

Astaxanthin is showing benefits as an anti-inflammatory [21] by inhibiting the production of the pro-inflammatory mediators.[527] studies, demonstrated a dose dependent anti-inflammatory effect of astaxanthin by suppression of nitric oxide (NO), prostaglandin E2 (PGE2) and tumor necrosis factor (TNF-) production by directly blocking nitric oxide synthase (NOS) enzyme activity. [22] also reported that carrageenan induced swelling of the paw of rats fed with astaxanthin was significantly lower than that of control. This explains the anti-inflammatory role of astaxanthin. [23,24]reported that dietary astaxanthin was found to help fight symptoms of ulcer disease from *Helicobacter pylori* which causes inflammation of gastric tissues. [25] reported that astaxanthin is effective in protection against gastric lesions induced by the use of non-steroid anti-inflammatory drugs such as naxopen. [26] reported that suppression of T-cell activation makes astaxanthin as effective as commonly used antihistamines and hence may have a role in novel antiasthmatic formulations. The anti-inflammatory activity observed in the scientific studies was attributed to antioxidant effect and oxygen scavenging activity of astaxanthin.[5]Astaxanthin has been shown to be 550 times stronger than vitamin E in singlet oxygen quenching.[21] Thus, encapsulated and non-encapsulated astaxanthin has been founded to have good anti-inflammatory activity.

Conclusion

The present study shows that free and encapsulated astaxanthin possess potent invitro anti-inflammatory activity when determined by all the four invitro methods. It is the initial scope of the study to focus on the effect of astaxanthin modified by different encapsulation methods on inflammation. Our earlier preliminary study proved the quenching effect of astaxanthin in free form and encapsulated form on free radicals and lipid peroxidation. Thus, our present study suggests astaxanthin in encapsulated forms to be a potent active drug for diseases associated with inflammatory disorders and in relieving the symptoms of pain.

References

1. Tripathi, KD. Essentials of Medical Pharmacology, V edn. Jaypee brother's medical publishers (P) Ltd, New Delhi. 2004: 257-259.
2. Robert Fassett, G and Jeff Coombes, S.Astaxanthin: A Potential Therapeutic Agent in Cardiovascular Disease. Marine drugs. 2011, 9: 447-465.
3. Shan Qin, et al. The accumulation and metabolism of Astaxanthin in Scenedesmusobliquus. Process bioavailability. 2008, 43: 795-802.
4. Uma Nath Ushakumari and Ravi Ramanujan. Isolation of Astaxanthin from marine yeast and study of its pharmacological activity. International current Pharmaceuticals. 2013, 2(3): 67-69.
5. Lee, SJ, et al. Astaxanthin inhibits nitric oxide production and inflammatory gene expression by suppressing I kappa B kinase-dependent NF-kappa B activation. Molecules and Cells. 2003, 16(1): 97-105.
6. Lin, SF, et al. Improving the Stability of Astaxanthin by Microencapsulation in Calcium Alginate Beads. PloS One. 2016, 11(4): 1-10.
7. Park, SA, et al. The effects of particle size on the physiochemical properties of optimized astaxanthin-rich Xanthophyllomycesdendrorhous- loaded microparticles. LWT-Food Science and Technology. 2014, 55: 638-644.
8. Krasaekoopt, W, et al. Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT- and conventionally treated milk during storage. Food Science and Technology. 2006,39:177-183.
9. Phathanee Thamaket and Patcharin Raviyan. Preparation and physical properties of carotenoids encapsulated in chitosan cross linked triphosphate nanoparticles. Food and Applied Bioscience Journal. 2015, 3 (1): 69-84
10. Yangchao, L, et al. Preparation and characterization of zein/chitosan complex for encapsulation of alpha tocopherol and Its*in vitro* controlled release study. Colloids and Surfaces B: Biointerfaces. 2011, 85: 145-152.
11. Chiu, CH, et al. Improved Hepatoprotective Effect of Liposome-Encapsulated Astaxanthin in Lipopolysaccharide-Induced Acute Hepatotoxicity. International Journal of Molecular sciences. 2016, 17: 1-17.

12. Mizushima, Y and Kobayashi, M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology. 1968, 20: 169- 173.
13. Sakat, S, et al. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Inn. International Journal of Pharmacy and Pharmaceutical Sciences. 2010, 2(1): 146- 155.
14. Govindappa, M, et al. Phytochemical screening, antimicrobial and in vitro anti-inflammatory activity of endophytic extracts from Loranthus sp. Pharmacognosy Journal. 2011, 3 (25): 82-90.
15. Oyedepo, OO and Femurewas, AJ. Anti-protease and membrane stabilizing activities of extracts of Fagrazanthoxiloides, Olaxsubscorpiodes and Tetrapleuratetreptera. International Journal of Pharmacognosy. 1995, 33: 65- 69.
16. Vadivu, R and Lakshmi, KS. In vitro and In vivo anti-inflammatory activity of leaves of Symplocoscochinchnensis (Lour) Moore ssplaurina. Bangladesh Journal of Pharmacology. 2008, 3: 121-124.
17. Gandhisan, R, et al. Anti-inflammatory action of Lanneacoromandelica HRBC membrane stabilization. Fitoterapia. 1991, 62: 81-83.
18. Prasanth, NV, et al. Evaluation of in vitro and in vivo anti-inflammatory activity of Garcinia Combogia L. International Journal of Pharmacy and Pharmaceutical Sciences. 2013, 5(2): 263-264.
19. Sadique, J, et al. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterpia. 1989, 60: 525.
20. Oghami,K,et al. Effects of astaxanthin on lipopolysaccharide induced inflammation in vitro and in vivo. Investigative Ophthalmology and VisualScience.2003,44(6): 269-2701.
21. Shimizu, N, et al. Carotenoids as single oxygen quenchers in marine organisms. Fisheries Science. 1996, 62: 134-137.
22. Kurashige,M,et al. Inhibition of oxidative injury of biological membranes by astaxanthin. Physiology Chemistry and Physics and Medical NMR. 1990,22: 27-38.
23. Bennedsen,M,et al. Treatment of H. pylori infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. Immunology Letter.1999,70: 185-189.
24. Wang,WH,et al. Mechanism of apical K+ channel modulation in principal renal tubule cells. Effect of inhibition of basolateral Na+ - K+ ATPase. Journalof physics A: General Physics.1993,101: 673-694.
25. Kim, JH, et al. Protective effects of astaxanthin on naxopen-induced gastric antral ulceration in rats. European Journal of Pharmacology.2005,514(1): 53-59.
26. Mahmoud, FF, et al. In vitro effects of astaxanthin combined with ginkgolide B on T lymphocyte activation in peripheral blood mononuclear cells from asthmatic subjects. Journal of Pharmacology and Science. 2004,94: 129-136.
27. Ohgami,K,et al. Effects of astaxanthin on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. Invest Ophthalmol Vis Sci. 2003,44(6):2694-701.
28. Suganya, V and Asheeba, ST. Microencapsulation of astaxanthin using ionotropic gelation method isolated from three crab varieties. International Journal of Current Pharmaceutical Research. 2015, 7(4): 96-99.

*****