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Anti-inflammatory activity of lycopene isolated from *Chlorella marina* on carrageenan-induced rat paw edema

Authors:
Renju GL and Muraleedhara Kurup G.

Institution:
Department of Biochemistry, University of Kerala, Trivandrum, India.

Corresponding author:
Muraleedhara Kurup G.

Email:
gmkbio@gmail.com.

Phone:
+919447251408.

Fax:
91-471 2308078.

Web Address:
http://jresearchbiology.com/documents/RA0329.pdf.

ABSTRACT:

Even though role of lycopene (all-*trans*) in controlling inflammation was reported, lycopene (*cis* and all-*trans* 40:60) isolated from green algae *Chlorella marina* was not reported so far. In this present study inflammation was induced in male *Sprague dawley* rats and edema was produced acutely by injecting 0.1 ml of carrageenan into the plantar region of the right hind paw of the rats subcutaneously. Intra peritoneal administration of algal lycopene (AL) at the dose of 10 mg/kg b.wt showed maximum (83%) inhibition on paw edema. The anti- inflammatory effect was significantly ($P< 0.05$) higher in rats fed with algal lycopene when compared to the standard drug voveran (71%) and all- *trans* tomato lycopene (TL) (63%). Carrageenan induced rats showed elevated levels of cyclooxygenase (COX) and lipoxygenase (LOX) activities in monocytes. Myeloperoxidase (MPO) in serum, C- reactive protein (CRP) and ceruloplasmin activity in plasma was also high in carrageenan induced rats when compared to normal. Lycopene from *Chlorella marina* showed significant effect in reducing the above parameters to that of the standard drug while tomato lycopene showed less effect when compared to algal lycopene. Therefore algal lycopene from *Chlorella marina* would be recommended for the treatment of anti-inflammatory disorders.

Keywords:
Microalgae, *Chlorella marina*, lycopene, anti-inflammation.

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Journal of Research in Biology

INTRODUCTION

Inflammation is a response which protects and heals the host tissue after infection or injury. (Nathan, 2002). However, it is frequent that the inflammatory response to several insults erroneously leads to the damaging of normal tissues. Prostaglandin-E2 is generated from arachidonic acid by the enzyme cyclooxygenase (COX) at sites of inflammation in substantial amounts and can mediate many of the pathologic features of inflammation (Serhan and Levy, 2003). One of the early cellular events in inflammation is the margination of leukocytes, primarily neutrophils and this can be measured by myeloperoxidase activity (Goulet *et al*., 1994).

Currently, non steroidal anti-inflammatory drugs (NSAIDs) were used for inflammatory diseases. Even though this drugs transiently suppresses inflammation, but their long term use cause ulceration in the gastrointestinal tract and renal morbidity (James and Hawkey, 2003). However research focused on finding newer drugs with pharmacological actions without side effects.

Several antioxidants have been reported to have anti-inflammatory and anti-arthritic activities (Maxwell *et al*., 2006). In the present study a culturable marine edible algae *Chlorella marina* was selected to evaluate the anti-inflammatory activity of lycopene. Generally tomatoes are the source of lycopene, but it has many disadvantages (Shi and Le mague, 2000). The content of lycopene in tomato is very less and the configuration of lycopene is all-*trans*. Even though lycopene from algae has been reported (Ishikawa and Abe, 2004), no attempt has been made so far for the commercialization of algal lycopene. It can be seen that marine sources especially algae are the least exploited for their bioactive molecules (Pinky and Goswai, 2012). Work in our laboratory has shown that the lycopene content in algae is comparatively high, when compared to tomato lycopene. The most interesting observation was that algal lycopene contain cis-configuration (5-*cis*, 9-*cis*, 13- *cis* and 15-*cis*). Recently it has been reported that the cis form of lycopene is more biologically active than the *trans* form (Stahl and Sies, 1996).

MATERIALS AND METHODS

Chemicals

Lycopene, carrageenan, linoleic acid, Histopaque, arachidonic acid other fine chemicals were purchased from Sigma, St. Louis, MO, USA. Diclofenac sodium (Voveran) was obtained from Novartis, India. Salt and vitamin mixtures were purchased from Merck, Germany. All other chemicals and reagents were purchased from Sisco Research Laboratory Pvt.Ltd (SRL), India, and were of analytical grade.

Algal source

Marine algae *Chlorella marina* Butcher was collected from the Vizhinjam coast of Kerala, located at Latitude 08° 22' North Longitude. 76° 59' East on the south west coast of India and was cultured under laboratory conditions. The microalgae were identified by the botanist (Dr. G. Valsaladevi, Department of botany) and a voucher specimen (No. KUBH 5812) has been deposited in the Department of Botany, University of Kerala, India.

Culture medium

Walne's medium (1970) was used as a basal medium for the cultivation of *Chlorella marina*. 5 g /L glucose was added to the basal medium. Flasks were incubated at 25°C with continuous illumination. The pH was adjusted to 7.5. Nicotine (10 µM/ L) was sterilized by autoclaving and was added to 5 days old cultures for the production of lycopene.

Biomass harvest

Chlorella marina cells were grown in suspension cultures up to 30 to 40 days. The cells were harvested at stationary phase by withdrawing the cultures in 50 ml polypropylene tubes and centrifuged at 5000 rpm for

10 minutes. Removed the medium and the pellets were freeze dried, weighed and stored under nitrogen at -20°C.

Isolation of lycopene from Chlorella marina (AL) and analysis

Harvested biomass (5g dry weight) was suspended with 5 ml of 80% cold acetone and kept overnight under 4°C for better and easy recovery of carotenoids. The mixtures were vortexed for 2 minutes and centrifuged at 5000 rpm for 20 minutes. After repeated extractions (4 times), the supernatants were pooled and the colorless cell pellets were discarded. The extracts were dried over anhydrous sodium sulphate and reduced to a minimum volume by evaporating the solvents using N_2 stream. The crude extracts were kept for further separation of carotenoids in amber colored containers under nitrogen at -20°C. All operations were done at subdued light under nitrogen atmosphere. The absorbance in the solvent phase was quantified by spectrophotometric method at 470 nm as described by Lichtenthaler (1987).

Isolation of all-*trans* lycopene from tomato (TL)

Tomatoes obtained from the local market, Trivandrum, India were used. The all-*trans* lycopene from tomato was extracted and evaluated according to the procedure of Fish *et al*., (2002).

Determination of lycopene by HPLC

Lycopene extracted from algal cells and tomatoes were determined by HPLC method at 450 nm as described by Shaish *et al*., (1992). HPLC analysis of lycopenes were performed using a silia chrom® column (250 x 4mm + 5 x 4, NCLIOSIL 100-5-C18 5.0µm), K 1001 type pump and the UV detector type of K 2600, Germany. Elution was performed isocratically with methanol: acetonitrile (9:1) v/v at a flow rate of 1 ml min^{-1}. A UV detector with a wavelength of 450 nm was employed. Lycopene (95%) obtained from Sigma chemicals were used as standard. The retention time was recorded and peak areas of standards and tests were noted on each run and used for calculation of concentrations of different fractions. All samples were injected in duplicate.

Experimental animals

Male *Sprague Dawley* rats with the average body weight of 150- 200 g of the same breed were selected for the study. These animals were housed in the department animal house and provided standard pellet diet and water ad libitum and maintained with temperature at 25 ± 1°C, humidity (55-60%) and photoperiod (12:12 h) light and dark cycle. Experimental procedures conducted on rats were approved by the Animal Experiment Committee (218/CPCSEA) for animal care of Kerala University according to Government of Indian law on animal use and care.

Induction of acute inflammation-Carrageenan induced rat paw edema

Carrageenan-induced rat paw edema assay was conducted according to the procedure as described by Winter *et al*., (1962). Five groups of six rats were treated as AL and TL with doses 10 mg/kg and reference drug Voveran, a Diclofenac sodium preparation (20 mg/kg) were given orally and intraperitoneally (i.p), 1 h before the injection of carrageenan. Control rats were given 0.1 ml 1% carrageenan. Inflammation was induced by 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the right hind paw after 1 hour. The volume of the right paw was measured by paw edema meter before and after injection in the third and fifth hour. The paw edema and inhibition was calculated by the equation: Activity= 100 - (100 × average drug treated/average for control).

Treatment Protocol and Experimental Design in Acute Inflammation

Edema was induced on rat right hind paw by aponeurosis injection of 0.1ml of 1% carrageenan in 0.9% saline. The experimental groups consisted of 30 rats were divided in to five groups.

Group I: control (received saline only),

Group II: carrageenan alone

Group III: carrageenan + algal lycopene (AL groups, 10 mg/kg *i.p*)

Group IV: carrageenan + tomato lycopene (TL groups, 10 mg/kg *i.p*)

Group V: carrageenan + Voveran (VOV groups, 20 mg/kg *i.p.*)

At the end of third hour, the animals were sacrificed by euthanasia. Blood was removed to ice cold containers for various biochemical analyses.

Activity of Cyclooxygenase (COX) and Lipooxygenase (LOX) in Peripheral Blood Mononuclear Cells (PBMC)

Mononuclear cells were isolated the procedure described by Radhika *et al*., (2007). Cox activity was measured by the method of Shimizu *et al*., (1984). 15-LOX activity was determined by the method of Axelrod *et al*., (1981).

Biochemical analysis

Serum myeloperoxidase (MPO) activity was measured by Mullane *et al*., (1985). CRP in plasma was determined by using Immunoturbidometric kit (Diasys Diagnostics, Germany). Ceruloplasmin was estimated by the method of Ravin (1961). Protein was determined by the methods of Lowry *et al*., (1951).

Statistical analysis

The Statistical package for social sciences (SPSS/PC+), version 11.5 (SPSS Inc; Chicago. IL, USA) was used to analyze the results for statistical significance using one-way ANOVA followed by Duncan's test. P value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Sub plantar injection of carrageenan into the foot of rats caused a time-dependent increase in paw volume. The localized inflammatory response as evidenced visually by the edema reached a maximum intensity at third hour after carrageenan induction and this maximal effect was seen until the fifth hour. Administration of AL and TL has showed significant effects in decreasing carrageenan-induced paw edema. Algal lycopene showed maximum edema inhibition compared to all-*trans* tomato lycopene and drug. AL exhibited 70% and 83% edema inhibition at third/fifth hours, respectively. This effect was comparable to the reference drug Voveran which exerted 54% and 71% edema inhibition at third and fifth hour, respectively. TL showed 51% and 63% edema inhibition at third and fifth hour after carrageenan induction (Figure. 1).

COX activity in PBMC was significantly ($p<0.05$) increased in carrageenan treated rats when compared to control rats (Figure. 2). Treatment with AL showed significant ($p<0.05$) decrease in COX activity when compared to carrageenan induced rats. Prostaglandin is formed by the interaction of two distinct but related enzymes, COX-1 and COX-2 and plays an important role in promoting the signs and symptoms of inflammation (Otterness and Bliven, 1985; Ibegbulem *et al*., 2012). The activity of COX in PBMC was decreased ($p<0.05$) in AL treated group when compared to TL and voveran treated group. Reduction of paw swelling and decreased activity of COX showed the immunological protection rendered by the algal lycopene. These results showed the anti-inflammatory potential of the AL.

The activity of 5-LOX and 15-LOX in PBMC was significantly ($p<0.05$) increased in carrageenan induced rats when compared to normal rats (Figure.3 and 4). Algal lycopene treatment significantly reduced ($p<0.05$) in 5-LOX and 15-LOX activity, when compared to CII rats. The effect was significantly higher ($p<0.05$) than TL and drug treated groups. Lipoxygenases are a family of key enzymes in the biosynthesis of leukotrienes that are postulated to play an important role in the pathophysiology of several inflammatory diseases (Henderson, 1994; Yamamoto, 1992). In the normal situation, cellular leukotriene production is suppressed by selenium dependent peroxidases (Werz *et al*., 1997). On receiving

inflammatory stimuli, leukotriene production is elicited through the arachidonic acid cascade, causing micro vascular injury, vasoconstriction and production of pro-inflammatory cytokines (Peskar, 1991). Studies have shown that LOX and leukotrienes have a profound role in carrageenan-induced inflammation (Henderson, 1994; Gamache *et al*., 1986). In the carrageenan-induced inflammation model, AL significantly reduced carrageenan-induced 5-LOX and 15-LOX activities in mononuclear cells, indicating decreased leukotriene production and hence a protective effect.

MPO activity in serum was significantly increased ($p<0.05$) in carrageenan induced rats when compared to normal group (Table 1). Treatment with AL showed significant decrease ($p<0.05$) in MPO activity when compared to carrageenan induced rats. The MPO activity was significantly decreased when compared to TL and drug treated groups. The activity of MPO is a marker of neutrophil infiltration (Bradley, 1982), and was found to be significantly increased in the paw tissue of carrageenan-induced rats. AL significantly decreased ($p<0.05$) the elevated MPO activity, an indicator of neutrophil in inflamed paws, suggesting that inhibition of neutrophil infiltration might be another mechanism by which AL achieves its anti-inflammatory effect.

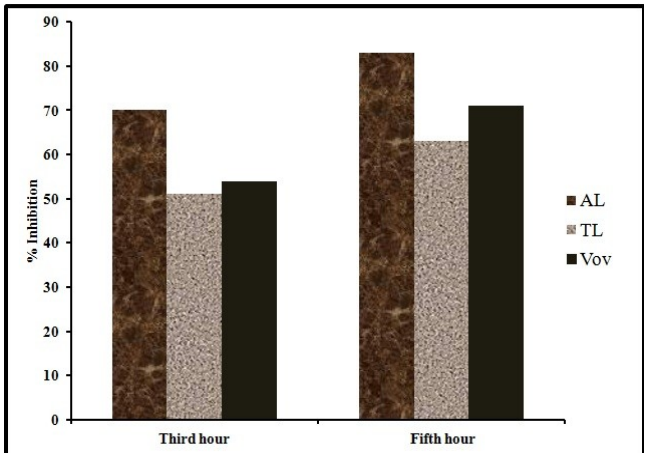
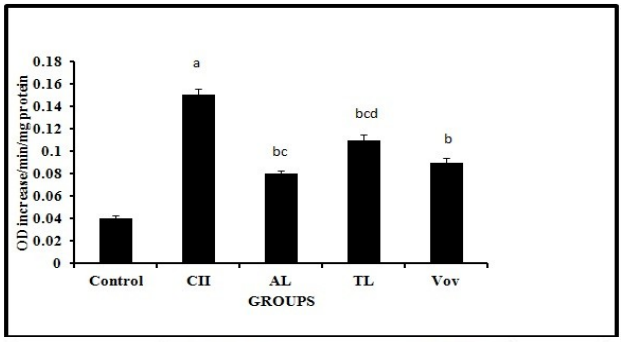


Figure 1: Effect of algal lycopene on carrageenan-induced paw edema in normal and experimental rats.

Table 1 also shows the variations in serum CRP and ceruloplasmin level in the test animals compared to control. Serum CRP and ceruloplasmin levels were significantly increased ($p<0.05$) in carrageenan induced rats when compared to normal rats. Supplementation with AL significantly decreased ($p<0.05$) the serum CRP and ceruloplasmin levels when compared to carrageenan induced rats. The levels of CRP and ceruloplasmin were decreased significantly ($p<0.05$), when compared to TL and Voveran treated groups. C-reactive protein is an acute phase protein that has been identified as an important biomarker for various inflammatory, degenerative, and neoplastic diseases. Elevated levels of CRP have been found in the blood during virtually all diseases associated with active inflammation or tissue destruction, particularly in patients with rheumatoid arthritis (Pepys and Hirschfield, 2003; Kushner, 1991). In our study the increased levels



COX	Sum of Squares	df	Mean Square	F
Between Groups	.039	4	.010	71.958
Within Groups	.003	25	.000	
Total	.042	29		

Figure 2: Effect of algal lycopene on activity of COX in PBMC of normal and experimental rats COX activity is expressed as an optical density increase (OD increase) per mg protein per minute. Values are expressed as mean ± SEM of six rats in each group.

[a] – Statistical difference of Control group with CII group when $p < 0.05$.

[b] – Statistical difference of CII group with group AL, TL and VOV when $p < 0.05$.

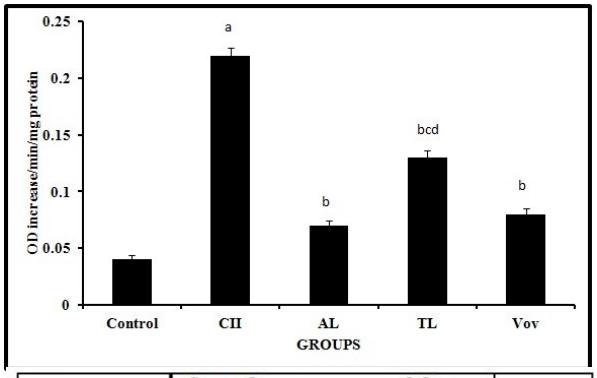
[c] – Statistical difference of VOV group with group AL and group TL when $p < 0.05$.

[d] –Statistical difference of TL group with AL when $p < 0.05$.

of CRP level was found to be significantly decreased in algal lycopene treatment when compared to TL and Voveran treatments.

The serum protein, ceruloplasmin is a powerful free radical scavenger that oxidizes iron from the ferrous to ferric state. Ceruloplasmin levels increase under conditions leading to the generation of oxygen products such as the superoxide radical and hydrogen peroxides (Revnic, 1995). Serum ceruloplasmin level was significantly increased in carrageenan induced rats when compared to normal rats. Treatment with AL showed significant decrease in the concentration of ceruloplasmin. The increased levels of ceruloplasmin in carrageenan induced rats could be decreased significantly on treatment with algal lycopene when compared to TL and standard drug Voveran might be having a protective response against free radical mediated lipidperoxidation.

Lycopene from edible marine microalgae *C. marina* showed higher anti-inflammatory activity than all-*trans* tomato lycopene and standard drug Voveran. These effects might be due to the presence of two isomeric form of lycopene (*cis* and all-*trans*) in the microalgae. Reports available indicate that the *cis*-lycopene has a high antioxidant potential when compared to all-*trans* lycopene (Stahl and Sies 1992; Clinton *et al*., 1996). Algal lycopene isolated from *C. marina* could reduce cell influx, oedema formation



5-LOX	Sum of Squares	df	Mean Square	F
Between Groups	.121	4	.030	144.084
Within Groups	.005	25	.000	
Total	.126	29		

Figure 3: Effect of algal lycopene on activity of 5- LOX in PBMC of normal and experimental rats

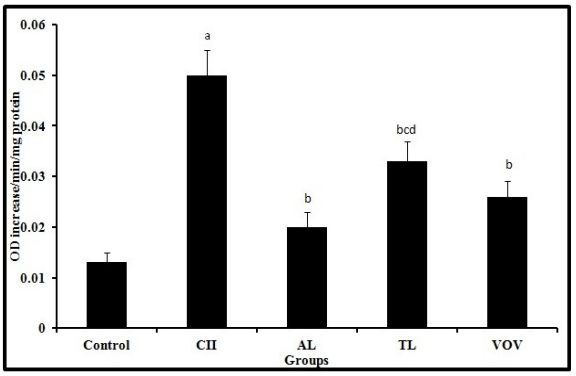
5-LOX activity is expressed as an optical density increase (OD increase) per mg protein per minute. Values are expressed as mean ± SEM of six rats in each group.

[a] – Statistical difference of Control group with CII group when p < 0.05.

[b] – Statistical difference of CII group with group AL, TL and VOV when p < 0.05.

[c] – Statistical difference of VOV group with group AL and group TL when p < 0.05.

[d] –Statistical difference of TL group with AL when p <0.05.



15-LOX	Sum of Squares	df	Mean Square	F
Between Groups	.006	4	.002	17.068
Within Groups	.002	25	.000	
Total	.008	29		

Figure4: Effect of algal lycopene on activity of 15- LOX in PBMC of normal and experimental rats

15-LOX activity is expressed as an optical density increase (OD increase) per mg protein per minute. Values are expressed as mean ± SEM of six rats in each group.

[a] – Statistical difference of Control group with CII group when p < 0.05.

[b] – Statistical difference of CII group with group AL, TL and VOV when p < 0.05.

[c] – Statistical difference of VOV group with group AL and group TL when p < 0.05.

[d] –Statistical difference of TL group with AL when p <0.05.

Table 1: Levels of CRP, Ceruloplasmin in plasma and MPO in serum of experimental animals.

Groups	MPO (µm/min/mg)	CRP (mg/ml)	Ceruloplasmin (mg/dl)
Control	5.85 ± 0.37	22.0 ± 1.24	0.10 ± 0.006
CII	20.52 ± 1.11[a]	97.71 ± 3.80[a]	0.34 ± 0.014[a]
AL	7.45 ± 0.37[bc]	45.94 ± 2.01[bc]	0.19 ± 0.007[bc]
TL	13.75 ±0.48[bcd]	80.64 ±3.18[bcd]	0.28 ± 0.016[bcd]
VOV	9.74± 0.39[b]	56.89 ± 2.42[b]	0.22 ± 0.010[b]

Values are expressed as mean ± SEM of six rats in each group.
a – Statistical difference of Control group with CII group when p < 0.05.
b – Statistical difference of CII group with group AL, TL and VOV when p < 0.05.
c – Statistical difference of VOV group with group AL and group TL when p < 0.05.
d –Statistical difference of TL group with AL when p <0.05.

Table 2: Statistical table of Myeloperoxidase in one way ANOVA followed by Duncan's test

MPO	Sum of Squares	df	Mean Square	F
Between Groups	827.642	4	206.911	91.002
Within Groups	56.842	25	2.274	
Total	884.485	29		

Where df is degrees of freedom, F is F- ratio.

Table 3: Statistical table of CRP in one way ANOVA followed by Duncan's test

CRP	Sum of Squares	df	Mean Square	F
Between Groups	20984.623	4	5246.156	121.093
Within Groups	1083.082	25	43.323	
Total	22067.705	29		

Where df is degrees of freedom, F is F- ratio.

Table 4: Statistical table of CRP in one way ANOVA followed by Duncan's test

Ceruloplas-min	Sum of Squares	df	Mean Square	F
Between Groups	.196	4	.049	59.148
Within Groups	.021	25	.001	
Total	.217	29		

Where df is degrees of freedom, F is F- ratio.

and release of mediators associated with inflammatory condition, and therefore has the potential to be used as an anti-inflammatory agent. Further studies are in progress to evaluate the molecular mechanism of its anti-inflammatory activity.

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REFERENCES

Axelrod B, Cheesebrough TM and Laakso S. 1981. Lipoxygenase from soybean. *Methods.Enzymol*; 71:441-453.

Bradley PP, Priebat DA, Christensen RD and Rothstein G. 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol*; 78:206-209.

Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ and Erdman JW. 1996. *Cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer. Epidemiol. Biomar. Preven*; 5 (10): 823–833.

Fish WW, Perkins Veazie P and Collins JK. 2002. Extraction of lycopene from tomato paste. *J. Food. Compo.Analy.*, 15: 309–317.

Gamache DA, Povlishock JT and Ellis EF. 1986. Carrageenan induced brain inflammation. Characterization of the model. *J Neurosurg*., 65 (5): 679-685.

Goulet JL, Snouweart JN, Latour AM, and Coffman TM. 1994. Altered inflammatory responses in leukotriene-deficient mice. *Proc. Natl. Acad. Sci*; 91 (26):12852–12856.

Henderson WR Jr. 1994. The role of leukotrienes in inflammation. *Ann. Intern .Med*; 121(9): 684-697.

Ibegbulem CO, Egbung GE, Okoro, KK, Kalu NN, Nwaogyu LA, and Igwe Ko. 2012. Hypothesized biochemical modes of action of palm oils used in ethno-medicine. *J. Res. Biol*; 2(6): 596–601.

Ishikawa E and Abe H. 2004. Lycopene accumulation and cyclic carotenoid deficiency in heterotrophic *Chlorella* treated with nicotine. *J. Ind. Microbiol. Biotechnol*; 31: 1367–5435.

James MW and Hawkey CJ. 2003. Assessment of non-steroidal anti-inflammatory drug (NSAID) damage in the human gastrointestinal tract. *Br. J. Clin. Pharmacol*; 56 (2): 146 -155.

Kushner I. 1991. C-reactive protein in rheumatology. Arth. Rheum; 34, 1065–1068. **Lichtenthaler Hk. 1987.** Chlorophylls and carotenoids: pigments of photosynthetic Biomembrane. *Methods. Enzymol*; 147: 350-382.

Lowry OH, Rosebrough NJ, Farr AC and Randall RJ. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem*; 193:265–267.

Maxwell SR, Payne RA, Murray GD and Webb DJ. 2006. Selectivity of NSAIDs for COX-2 and cardiovascular outcome. *Br. J. Clin. Pharmacol*; 62: 243-245.

Mullane KM, Kraemer R and Smith B. 1985. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischaemic myocardium. *J. Pharmacol.Methods;* 14 (3): 157-167.

Nathan C. 2002. Points of control in inflammation. *Nature*, 420 (6917): 846-852

Otterness IG and Bliven ML. 1985. Laboratory models for testing non steroidal anti-inflammatory drugs: Non-steroidal Anti-inflammatory Drugs, Ed.by J.G.Lombardino Wiley.Newyork. 220: 111-114.

Pepys MB and Hirschfield GM. 2003. C-reactive protein: a critical update. *J .Clin .Invest*; 111: 1805-1812.

Peskar BM. 1991. Role of leukotriene C4 in mucosal damage caused by necrotizing agents and Indomethacin in rat stomach. *Gastroenterol*; 100 (3): 619–626.

Pinky Baruah and Goswai UC. 2012. Characterization of carotenoid pigments in amphibian. *J. Res. Biol*; 2: 114 -118.

Radhika A, Jacob SS and Sudhakaran PR. 2007. Influence of oxidatively modified LDL on monocyte-macrophage differentiation. *Mol. Cell .Biochem*; 305:133 -143.

Ravin H A. 1961. An improved colorimetric enzymatic assay of ceruloplasmin. *J. Lab. Clin. Med*; 58: 161-168.

Revnic F. 1995. The significance of Serum Ceruloplasmin in Diagnosis of Rheumatoid Arthritis. *Toxicol. Lett*; 78: 70-70.

Serhan CN and Levy B. 2003. Success of prostaglandin E2 in structure–function is a challenge for structure-based therapeutics. Proc. Natl. Acad. Sci. 100:8609-8611.

Shaish A, Ben Amotz and Avron M. 1992. Biosynthesis of β carotene in D*unalialla*. *Methods. Enzymol*; 213: 439-444.

Shi J and Le Mague M. 2000. Lycopene in tomatoes: chemical and physical properties affected by food Processing. *Critical Reviews in Food. Sci. Nutr*; 40: 1–42.

Shimizu T, Kondo K and Hayaishi O. 1984. Role of prostaglandin endoperoxides in the serum thiobarbituric acid reaction. *Arch. Biochem. Biophys*; 206:271–276.

Stahl W and Sies H. 1992. Uptake of lycopene and its geometrical isomers is greater from heat Processed than from unprocessed tomato juice in humans. *J. Nutr*; 122 (11): 2161-2166.

Stahl W and Sies H. 1996. Lycopene: a biologically important carotenoid for humans? *Arch. Biochem. Biophy*; 336, 1-9.

Walne PR. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria*, and *Mytilis*. *Fish. Invest*; 26: 1–62.

Werz O, Schneider N, Brungs M, Sailer E, Safayhi H and Ammon H. 1997. A test system for leukotriene synthesis inhibitors based on in vitro differentiation of human leukemic cell lines HL-60 and Mono Mac 6 Naunyn Schmiedebergs *Arch .Pharmacol*; 356 (4): 441-445.

Winter CA, Risley EA and Nuss GW. 1962. Carrageenan-induced edema in hind paws of the rats as an assay for anti-inflammatory drugs. *Proc. Soc. Exp .Biol. Med*; 111:544-547.

Yamamoto S. 1992. Mammalian lipoxygenases: molecular structures and functions. *Biochim. Biophys. Acta*; 1128 (1-2): 117–131

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