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Research Article

Evaluation of Hepatoprotective and Antioxidant Activity of Astaxanthin - A Lipid-Solid Dispersions Formulation against Acetaminophen Induced Liver Injury in Rats

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ABSTRACT
Astaxanthin, a carotenoid was separated from crude extracts of *Villorita cyprinoides* a black water Clam. Astaxanthin was formulated by lipid-solid dispersions techniques where astaxanthin was dispersed in the lipid matrix consisting of gelucire and compritol at a ratio of 1:2. The astaxanthin: excipient ratio was maintained at 0.5:1.The prepared astaxanthin formulation (ASF 10) was evaluated for the hepato-protective activity against acetaminophen (2g/kg/b.wt/p.o suspended in 0.5% CMC) induced liver injury in Wistar rats. Hepatoprotective activities were measured by diagnostic marker enzymes such as AST, ALT, ALP, bilirubin, albumin and total protein in serum; anti oxidant activities were measured in liver homogenates *viz.*, LPO, GSH, GPx, GST, SOD and CAT. ASF 10 treatment results in significant hepatoprotective effect by normalising the activity of serum enzymes, bilirubin and proteins. ASF 10 treatment also significantly altered the antioxidant levels in liver homogenates and these effects of ASF 10 were comparable to that of standard drug Silymarin. The results suggest that ASF 10 have potential therapeutic value in the treatment of some liver disorders in *albino* (Wistar) rats by its own antioxidant defence mechanism.

Keywords: Acetaminophen, Anti oxidants, ASF 10, Carotenoids, Hepatoprotective activity

INTRODUCTION

Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Liver damage is the wide spread pathology which in most cases involves oxidative stress and is characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma[1]. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [2]. Free radical initiated auto oxidation of cellular membrane lipids can lead to cellular necrosis and is now accepted to be important in connection with a variety of pathological conditions[3]. Reactive oxygen species (ROS), from both endogenous and exogenous, are implicated in the etiology of several degenerative diseases, such as coronary artery diseases, stroke, rheumatoid arthritis, diabetes, Peptic ulcers and cancer[4]. It is well known that, free radicals are the reactive species derived from them cause damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Mammalian cells possess elaborate defence mechanisms for radical detoxification. Key metabolic steps are super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which destroy toxic peroxides. Some non-enzymatic molecules including thio-redoxin, thiols and disulfide bonding play important roles in antioxidant defence systems[5].

So far, more than 600 carotenoids have been identified, of which 60 of them occur in our daily diet, and 20 can be detected in the blood. Carotenoids are substances which give colour from yellow to red in both plants and animals which could become a new weapon to prevent and treat these diseases. Among them carotenoids from marine sources such as, lycopene, β-carotene, lutein, zeaxanthin, tunaxanthin, astaxanthin and canthaxanthin were under investigation for their therapeutic and antioxidant actions[6, 7]. Acetaminophen is a widely used antipyretic and analgesic agent which produces acute liver damage if overdoses are consumed. Acetaminophen is mainly metabolized in liver by glucuronide and sulfate conjugates[8]. However, the hepatotoxicity of acetaminophen has been attributed to the formation of toxic metabolites when a part of acetaminophen is activated by hepatic Cytochrome P-450, to a highly reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI)[9]. Carotenoids are characterized by high activity against reactive oxygen species and free radicals. High consumption of fruits and vegetables is associated with low risk of life style modified diseases, which is attributed to the antioxidant vitamins and other phytochemicals. Carotenoids acts as antioxidants through

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Table: 1. Effect of ASF 10 on serum parameters.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/100ml)	Albumin (g %)	Total Protein (mg/dL)
Group I (Normal control)	56.30± 0.315	25.21± 0.496	97.10± 0.194	0.89± 0.008	3.79± 0.14	5.24± 0.035
Group II (Acetaminophen control)	141.02 ±1.278***	64.11± 2.287***	394.13± 4.211***	2.17± 0.025***	1.43± 0.25***	3.16± 0.068***
Group III (ASF 10 group)	52.24± 1.24***	21.26± 0.45***	92.45± 2.153***	0.81± 0.04**	3.87± 0.08**	5.01± 0.03***
Group IV (Standard Drug)	50.14± 1.254***	20.73± 0.547***	90.42± 4.354***	0.84± 0.37***	3.74± 0.12***	5.10± 0.045***

*Values are mean ± SEM; n=6 in each group. Percentage inhibition/elevation compared to control. Group III and IV were compared with Group II; Group II was compared with Group I. Values are statistically significant at ** P< 0.01;*** P< 0.001.*

a free radical mechanism by quenching singlet oxygen and oxidizing species in the prevention of cellular damages[10]. The antioxidant activity of astaxanthin was reported to be ten times stronger than that of other carotenoids, viz., zeaxanthin, lutein, canthaxanthin and carotene. Hence, the present study was designed to evaluate the hepatoprotective activity of astaxanthin in a lipid-solid dispersions formulation in experimental rats.

Preparation of astaxanthin formulation (ASF 10): Astaxanthin was separated from crude extracts of *Villorita cyprinoides*, a black water Clam. In detail, the astaxanthin was formulated by lipid-solid dispersions techniques where astaxanthin was dispersed in the lipid matrix consisting of gelucire [Gelucire are polyethylene glycol glycerides composed of mono-, di- and triglycerides and mono- and diesters of polyethylene glycol (PEG)] and compritol (an innovative hot-melt coating agent for prolonged-release drug formulations) at a ratio of 1:2. The astaxanthin dispersed lipid matrix was dissolved in the DMSO and obtained a clear solution. The astaxanthin: excipient ratio was maintained at 0.5:1 which resulted in assay 92%; saturation solubility 263±15.4 (µg/ml) were selected (ASF 10) for the hepatoprotective activity in rats.

Experimental animal: The institutional animal ethics committee (Register No.160/1999/CPCSEA), Annamalai University, Annamalai Nagar, India; approved the experimental design. *Albino* (Wistar) female rats of 150-200g (weight) were used for the study. Animals were housed in well ventilated room (temperature 23±2^{o}C, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals".

Acetaminophen-Induced Liver Damage in Rats (Acute Model): Four groups (I - IV), each comprising of six albino (Wistar) male rats of 150-200g were selected. Group I served as control and received orally gelucire and compritol at a ratio of 1:2 (1 ml each) for seven days (pH 7.4 - phosphate buffer). Groups III rats received oral dose 5 mg/kg ASF10 respectively for 7 days. Group IV Rats received oral dose of Silymarin (25mg/Kg body wt) for seven days. Acetaminophen at a dose of 2gm/Kg body wt/ p.o was administered on the 8th day to all animals in groups of II, III, and IV. After 48 hrs administration of acetaminophen dosing the rats were sacrificed by cervical decapitation under Xylazine + Ketamine (16 + 100 mg/kg i.m.), prior to biochemical analysis, each hepatic tissue (100 mg/ml buffer) was homogenized in 50 mM phosphate buffer (pH 7.0); the homogenate was then centrifuged at 10,000 rpm for 15 min and the supernatant obtained was used for biochemical analysis.[11-16]. Blood samples were collected *via* abdominal aorta puncture, the separated serum were used for the determination of diagnostic marker enzymes such as AST, ALT, ALP, bilirubin, albumin and total protein levels in Secomam semi auto analyzer.[17-20].The results were expressed as mean ± SEM; differences in mean were estimated by means of ANOVA followed by "Dunnet's post hoc" test.

DISCUSSION

The present study was performed to assess the hepatoprotective activity of astaxanthin in a Lipid-solid dispersions formulation (ASF 10) in rats against acetaminophen as hepatotoxin to prove its claims in folklore practice against liver disorders. Acetaminophen a widely used antipyretic and analgesic drug produces acute liver damage if accidental overdoses are consumed. The covalent binding of N-acetyl p- benzoquinoamine, an oxidation product of acetaminophen, to sulphydryl groups of proteins resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier[21]. Hepatic cells appear to participate in a variety of enzymatic metabolic activities and acetaminophen produced marked liver damage at the given doses as expected[22].

In the assessment of liver damage by acetaminophen, the determination of enzyme levels such as AST, ALT is largely used. The elevated levels of serum enzymes are

Table: 2. Results of tissue enzyme analysis – Liver

Groups/ Parameters	LPO	GSH	GP_X	GST	SOD	CAT
Group I (Normal control)	1.14± 0.05	4.11± 0.12	2.76± 0.12	1194± 88	5.22± 0.32	8.12± 0.21
Group II (Acetaminophen control)	2.38± 0.13**	2.04± 0.14**	1.41± 0.07**	629± 56**	2.11± 0.04***	3.45± 0.09***
Group III (ASF 10 group)	1.78± 0.14**	4.18± 0.11**	2.45± 0.04**	1118± 22**	4.94± 0.24**	7.87± 0.21***
Group IV (Standard Drug)	1.11± 0.03***	4.23± 0.09***	2.67± 0.11**	1158± 45***	5.45± 0.12***	8.57± 0.08***

Values expressed: Levels of lipid peroxides (LPO) – nmol malondialdehyde released/mg protein; Reduced glutathione (GSH) – µmol (oxidized min-1 mg-1 protein);Glutathione 38peroxidise (GPx) – nmol (oxidized min-1 mg-1 protein);Glutathione-S transferase (GST)- µmol (1-chloro-2, 4-dinitrobenzene conjugate formed min-1 mg-1 protein);Catalase (CAT) – nmol (H_2O_2 decomposed min-1 mg-1);Superoxide dismutase (SOD) - (one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation).

*The values of tissue LPO, GSH, GP_X, GST, SOD and CAT values of Group III, IV were compared with Group II; Group II was compared with Group I; *** = P<0.001 highly significant **= P<0.01 moderately significant.*

indicative of cellular leakage and loss of functional integrity of cell membrane in liver[23]. Astaxanthin formulation (ASF 10) treatment resulted in decreased levels of AST, ALT in rat serum. Serum ALP and bilirubin level on other hand are related to the function of hepatic cell[24]. In the present study, treatment with ASF 10 significantly reduced the levels of ALP and bilirubin which is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Effective control of ALP and bilirubin levels points towards an early improvement in the secretary mechanism of the hepatic cell. ASF 10 formulation also significantly increased the decreased level of albumin and protein which preserves the structural integrity of the hepatocellular membrane.

Earlier studies have proved that lipid peroxidation has been postulated as being the destructive process in liver injury due to acetaminophen administration[25]. Lipid peroxide levels were significantly increased in acetaminophen intoxicated rats were revealed in our study. And also it was observed that decrease in GSH, GPx, GST, SOD and CAT activity in liver tissue during administration of acetaminophen [26-29]. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical induced cellular damage. Review of literature proved that enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms[30]. SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. Glutathione functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals and maintenance of membrane protein thiols and as a substrate for GPx and GST[31]. Deficiency of GSH within living organisms can lead to tissue disorder and injury. Acetaminophen intoxication produces significant depletion of excessive production of free radicals that resulted in the oxidative stress, which leas to damage to biomolecules.

Silymarin is a known hepatoprotective compound obtained from *Silybum marianum* is reported to have a protective effect on plasma membrane of hepatocytes and possess multiple mechanisms of actions against different hepatotoxic agents. ASF 10 treatments resulted in protection of liver against acetaminophen administration by its own antioxidant defence mechanism. These results are significantly comparable with silymarin treatment groups.

CONCLUSION

From the results it was concluded that the hepatoprotective and antioxidant effect of astaxanthin formulation (ASF 10) may be due to its antioxidant and free radical scavenging properties. In conclusion, the results of this study demonstrate that astaxanthin formulation (ASF10) has a potent hepatoprotective action upon acetaminophen induced hepatic damage in rats.

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