Evaluation of antihypercholesterolemic activity of green alga *Haematococcus pluvialis* astaxanthin extract

Ashaq Hussain Rather[1], Rekha Rao[2]

[1]Algal Biotechnology Laboratory, Department of Post Graduate Studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur-482001 (M.P.), India
[2]Regional Ayurveda Research Institute for Urinary Disorders, Jammu-181123, (J&K), India

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Corresponding author: **Ashaq Hussain Rather.** Email: drashaqhrather21919@gmail.com

ABSTRACT

Introduction and Aim: *Haematococcus pluvialis* is one of the richest sources of natural astaxanthin, which is considered as a "super anti-oxidant". In the present study, we investigated the antihypercholesterolemic activity in the astaxanthin extract of green alga *H. pluvialis*.

Materials and Methods: To accomplish this, astaxanthin extracted with acetone and was separated by thin-layer chromatography. Animals were housed in separate cages under the controlled condition with temperature (22 ± 2°C) provided a 12 h light/dark cycle. Rats were divided into five groups; each group consisted of six rats. Group 1 (Normal group NC) rats were given (orally) normal standard rat feed (250 mg/kg b.w./per day). Group 2 was given high fat diet (250 mg/kg b.w./per day). Group 3 was given *H. pluvialis* astaxanthin extract (250 mg/kg b.w./per day). Group 4 was given astaxanthin extract along with a high protein diet (250 mg/kg b.w./per day) and group 5 was given astaxanthin standard, dissolved in an equal volume of liquid paraffin for 30 days.

Results: Among the treatment, astaxanthin extract was found to be an effective antihypercholesterolemic agent.

Conclusion: The present study reveals that *H. pluvialis* astaxanthin extract can be used for the different food formulations for human welfare.

Keywords: *Haematococcus pluvialis*; astaxanthin; antihypercholesterolemic; green alga.

INTRODUCTION

According to the report of the American heart association, heart diseases affect 16.3 million in the United States which is extremely related to cholesterol level (1). The main cause of heart diseases is lifestyle choices. Among the prevention of heart diseases, cholesterol regulation plays an important role. Excess consumption of lipids may result in hyperlipidemia (2), which results in an abnormal elevation in one or more of the serum lipids such as total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG). Hyperlipidemia is taken into account as a serious risk factor for cardiovascular diseases (3). Over the period, various investigation has been done in an effort to bring a novel approach, which can reduce or regulate the TC or TG levels. Among the dietary fiber (4), plant sterols (5), herbal extracts, and yeast extract (6, 7). *H. pluvialis* astaxanthin is well known for its antioxidant potential (8). In our present investigation, an effort has been made for another direction for the reduction or regulation of cholesterol. *H. pluvialis*, freshwater green microalga is considered a potent producer of astaxanthin (3, 3′-dihydroxy- β, β- carotene-4, 4′-dione; 9-11).

MATERIALS AND METHODS

Procurement of culture and cultivation of *H. pluvialis*

The *H. pluvialis* culture was procured from the culture collection of algae at the University of Texas, Austin, USA. *H. pluvialis* culture was grown in the bold basal medium (BBM; 12). The encysted red cells, rich in astaxanthin were harvested, dried in the oven at 70˚C (13).

Chemicals

All the media chemicals used for the experiments were analytical grade, obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai; HiMedia Laboratories Pvt. Ltd., Mumbai; Loba Chemie Pvt. Ltd., Mumbai. Qualigens Fine Chemicals, Mumbai and Sigma-Aldrich Chemicals, USA. The rat feed was obtained from Golden Feed, New Delhi.

Extraction of astaxanthin

Ten mg of biomass was homogenized in mortar and pestle in presence of neutral glass powder (Borosil Glass Works Ltd., India) extracted with acetone, hexane, DMSO and centrifuged at 5000 rpm for 10 minutes. The supernatant was used for the estimation of total astaxanthin. The absorbance of the extract was determined at 492 nm and the amount of the pigment was calculated according to Davies (14). Solvents were evaporated in the rotary evaporator under reduced pressure and only acetone extract was used in further study.

Animals used for the study

The institutional animal ethics committee (IAEC) approved all animal experiments. Male Albino Wistar rats, weighing 200 ±220 g were used for the study. Animals were housed in separate cages under the controlled condition with temperature (22 ± 2°C) provided a 12 h light/dark cycle. Rats were divided into groups of four consisting of six rats respectively. Group 1 (Normal group (NC*) rats were given normal standard rat feed (250 mg/kg b.w./per day). Group 2 was given high fat diet (HFD; 250 mg/kg b.w./per day). Group 3 was given *H. pluvialis* astaxanthin extract (Ast Ex; 250 mg/kg b.w./per day). Group 4 was given astaxanthin extract along with a high-fat diet (250 mg/kg b.w./per day) Group 5 was given astaxanthin standard, dissolved in an equal volume of liquid paraffin for 30 days.

Estimation of total triglycerides (TG)

Estimation of TG was done by the method of Fossat *et al.*, (15). Rats were anesthetized with diethyl ether. 1 ml of the reaction solution was taken, to this 10 µl of the serum sample was added, vortexed, and incubated for 15 minutes at room temperature. The absorbance was recorded at 546 nm

Estimation of total serum cholesterol

Estimation of serum was done by the method of Allian *et al.*, (16). 10 µl of serum sample was taken, added 1ml of the reaction solution. The contents were vortexed and incubated for 10 minutes at 20 ±2 degree. Absorbance was read against the cholesterol-reagent solution as blank at 546 nm.

Estimation of HDL cholesterol

Estimation of HDL Cholesterol was performed by the method of Lopes-Virella *et al.*, (17). 200 µl of serum was taken in tubes and incubated for 10 minutes at room temperature. Further, the tubes were subjected to centrifugation for 10 minutes at 4000 rpm and the supernatant was separated and used as a sample. 100 µl of the sample was added to the reaction solution and incubated at room temperature for 20 minutes. The absorbance was measured at 546 nm.

Estimation of creatinine

The estimation of creatinine was done by the method of Bowers (18). 500 µl of buffer solution (phosphate buffer, pH 7.0) was taken in each of the tubes, to them 200 µl serum sample was added and incubated for 5 minutes at room temperature. 500 µl coloring reagent (picric acid) was added to all tubes. O.D. was measured at 492 nm.

Histological analysis

Liver sections of rats were stained with hematoxylin and eosin and observed under 40× magnification.

Statistical Analysis

All the experimental analyses were done in triplicate. Result values were expressed as mean ± SD. The values were evaluated by one-way ANOVA and Duncan's multiple range test.

RESULTS AND DISCUSSION

Cultivation of *H. pluvialis*

H. pluvialis was grown in the bold basal medium for 20d in controlled laboratory conditions and astaxanthin was extracted with acetone, hexane, and DMSO (Fig. 1).

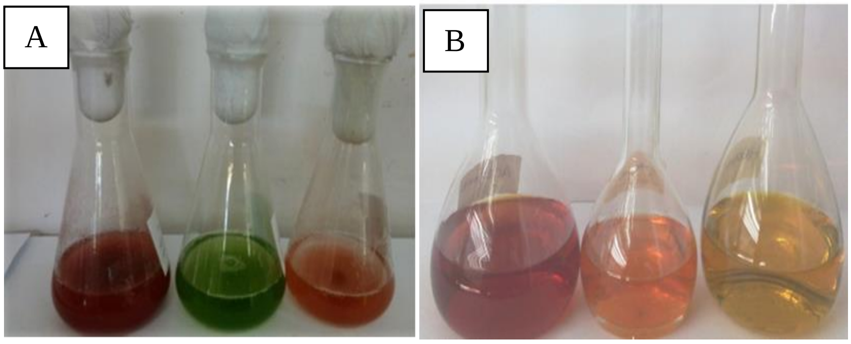

Fig. 1: Cultivation of *H. pluvialis* in controlled laboratory conditions (A) Extraction of astaxanthin with different solvents (B).

Astaxanthin separation by thin-layer chromatography

H. pluvialis, astaxanthin extracted with acetone was separated by thin-layer chromatography. The Rf values for the bands were 0.21 which corresponded in accordance with the standard astaxanthin Rf value measured on the TLC plate (Fig. 2).

Fig. 2: TLC pigment profile of astaxanthin extracted from Haematococcus cells, (A) Acetone extract. (B) Astaxanthin Standard.

Effect of HFD, Ast Ex, Ast Ex HFD on the total serum cholesterol in rats

Total serum cholesterol level in rats was measured, in experimental rats treated with HFD, Ast Ex, and Ast Ex HFD. 122% increase was observed in the HFD group whereas in Ast Ex and Ast Ex HFD the total serum cholesterol level was significantly lowered by 43% and 63% respectively when compared to NC (Table 1).

Table 1: Effect of HFD, Ast Ex, and Ast Ex HFD on the total serum cholesterol in rats.

S.No.	Treatments	Cholesterol (mg/dL)
1	NC	72.98 ± 0.84
2	HFD	160.10 ± 0.61
3	Ast Ex	103.20 ± 1.19
4	Ast Ex HFD	118.19±0.25
5	Ast standard	66.77±0.74

Values were expressed as mean ± SE, n=5. ($P \leq 0.05$).

Effect of HFD, Ast Ex and Ast Ex HFD on total glycerides in rats

The level of total triglycerides was significantly elevated by 211% in the HFD group while in the Ast Ex group and Ast Ex HFD group, the total triglycerides level was lowered by 14% and 44% respectively when the groups were compared with the NC (Table 2).

Table 2: Effect of HFD, Ast Ex and Ast Ex HFD on total triglycerides in rats.

S.No.	Treatments	TG (mg/dL)
1	NC	68.36±0.25
2	HFD	212.46±1.36
3	Ast Ex	78.28±2.16
4	Ast Ex HFD	98.24±0.25
5	Ast Standard	63.77±1.1

Effect of HFD, Ast Ex and Ast Ex HFD on HDL cholesterol in rats

The HDL level in the HFD group was increased by 57% however, it was decreased by 14.7%, and 5.7% in the Ast Ex group and Ast Ex HFD group respectively when the groups were compared with the NC (Table 3).

Table 3: Effect of HFD, Ast Ex, and Ast Ex HFD on HDL cholesterol in rats.

S.No.	Treatments	HDL cholesterol (mg/dL)
1	NC	42.46±0.37
2	HFD	18.45±0.22
3	Ast Ex	36.20±0.05
4	Ast Ex HFD	40.0±0.25
5	Ast Standard	41.0±0.27

Effect of HFD, Ast Ex and Ast Ex HFD on creatinine in rats

The creatinine level in the HFD group was increased by 50%, however, it was decreased to 22%, and 32% in the Ast Ex group and Ast Ex HFD group respectively when the groups were compared with the NC (Table 4).

Table 4: Effect of HFD, Ast Ex, and Ast Ex HFD on creatinine in rats.

S.No.	Treatments	Creatinine (mg/dL)
1	NC	0.93±0.04
2	HFD	0.46±0.01
3	Ast Ex	0.72±0.04
4	Ast Ex HFD	0.63±0.02
5	Ast Standard	0.88±0.06

An extensive study has been done previously on β-carotene (pro-vitamin-A) and vitamin E. However, in recent times there has been a shift towards the carotenoids like astaxanthin extracted from *H. pluvialis* which has additional positive features like potent quenching and anti-lipid peroxidation lacking both in β-carotene and vitamin E (8). Astaxanthin is an effective agent for the regulation of blood pressure with relation to its antioxidant ability (9). Rats fed with Ast Ex showed significantly lower cholesterol, TG as well HDL and creatinine (Table 1-4), there was noticeably less cholesterol accumulation in the liver sections of Ast Ex fed rat models compared with the HFD fed rats as seen by histological analysis (Fig. 3).

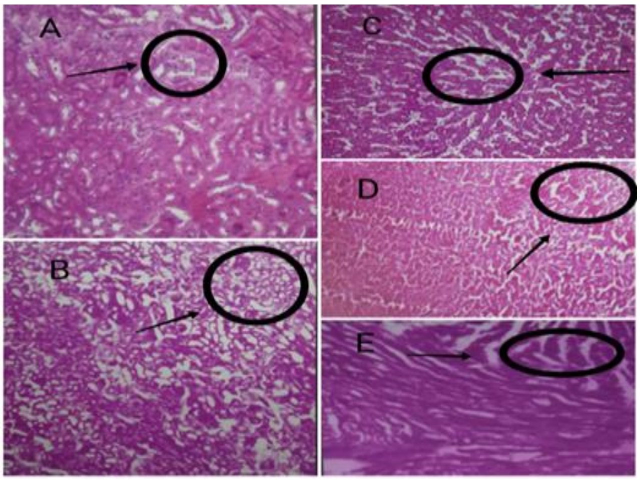

Fig. 3: Effect of HFD, Ast Ex, and Ast Ex HFD on liver sections of rat models stained with hematoxylin and eosin under 40× magnification. (A) NC (B) HFD (C) Ast Ex (D) Ast Ex HFD (E) Ast standard.

In this study, we evaluated if *H. pluvialis* astaxanthin supplementation may regulate the cholesterol or reduce in experimental rat models. Our results indicated that astaxanthin extract was found an effective factor for the regulation of cholesterol as well as triglycerides. There is limited literature available on anti-hypercholesterolemic property of algae particularly on *H. pluvialis* astaxanthin (19). Significant decreases in plasma TG and high-density lipoprotein (HDL) cholesterol, as well as HDL cholesterol, were found in type 2 diabetic patients who consumed 2 g/day of Spirulina for 2 months (20).

CONCLUSION

In conclusion, *H. pluvialis* astaxanthin extract can be used in various food formulations for a good lifestyle.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Roger, V. L., Go, A. S., Lloyd-Jones, D. M., Benjamin, E. J., Berry, J. D., Borden, W. B., *et al.,* Heart disease and stroke statistics update: a report from the American Heart Association. Circulation. 2012; 125: 2-220.
2. Oliveros, L. B., Videla, A. M., Ramirez, D. C., Gimenez, M. S. Dietary fat saturation produces lipid modifications in peritoneal macrophage of mouse. J. Nutr. Biochem. 2003; 14: 370-377.
3. Talbert, R., Hyperlipidemia DePiro Textbook of Threrapeytics. Appleton and Lange, Connecticut. 1997; 357-386.
4. Yamada, K., Tokunaga, Y., Ikeda, A., Ohkura, K., Kaku-Ohkura, S., Mamiya, S., *et al.,* Effect of dietary fiber on the lipid metabolism and immune function of aged Sprague-Dawley rats. Biosci. Biotechnol. Biochem. 2003; 67: 429-433.
5. Nigon, F., Serfaty-Lacrosniere, Beucler, I., Chauvois, D., Neveu, C., Giral. P., *et al.,* Plant sterol-enriched margarine lowers plasma LDL in hyperlipidemic subjects with low cholesterol intake: effect of fibrate treatment. Clin. Chem. Lab. Med. 2001; 39: 634-640.
6. Megalli, S., Aktan, F., Davies, N. M., Roufogalis, B. D. Phyto-preventive anti-hyperlipidemic effects of *Gynostemma pentaphyllum* in rats. J. Pharm. Pharm. Sci. 2005; 8:507-515.
7. Elwood, J. C., Nash, D. T., Streeten, D. H. Effect of high chromium brewer's yeast on human serum lipids. J. Am. Coll. Nutr. 1982; 1(3): 263-274.
8. Miki, W. Biological functions and activities of animal carotenoids. Pure and Appl. Chem. 1991; 63(1): 141-146.
9. Borowitzka, M. A., Huisman, J. M., Osbo, A. Culture of the astaxanthin- producing green alga *Haematococcus pluvialis*. Effects of nutrients on growth and cell type. J. Appl. Phycol. 1991; 3: 295-304.
10. Boussiba, S., Vonshak, A. Astaxanthin accumulation in the green alga *Haematococcus pluvialis*. Plant and Cell Physio. 1991; 32: 1077-1087.
11. Kobayashi, M., Kakizono, T., Nagai, S. Astaxanthin production by a green algal, *Haematococcus pluvialis* accompanied with morphological changes in acetate media. J. Ferment. Bioengng. 1991; 71(5): 335-339.
12. Kanz, T., Bold, H. C. In: Physiological Studies. 9. Morphological and Taxonomic Investigations of *Nostoc* and *Anabaena* in Culture. Austin Texas, University of Texas, publ. 1969; p.6924.
13. Sarada, R., Vidhyavathi, R., Usha, D., Ravishankar, G. A. An efficient method for extraction of astaxanthin from green alga *Haematococcus pluvialis*. J. of Agri. and Food Chem. 2006; 4: 7585-7588.
14. Davies, B. H. Carotenoids. In Chemistry and biochemistry of plant pigments. W. Ed, Academic Press, London 1976; pp 38-166.
15. Fossati, P., Prencipe, L., Berti, G. Enzymatic colorimetric method of determination of uric acid in serum. Clin. Chem. 1980; 26(2): 227-273.
16. Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W., Fu, P. C. Enzymatic determination of total serum cholesterol. Clin. Chem. 1974; 20: 470-475.
17. Lopes-Virella, M. F., Stone, P., Ellis, S., Colwell, J. A. Cholesterol determination in HDL separated by three different method. Clin. Chem. 1977; 23: 882 -884.
18. Larry, B. D.,Wong. E. T. Kinetic serum creatinine assays. II. A critical evaluation and review. Clin. Chem. 1980; 26(5): 555-561.
19. Fassett, R. G. Combes, J. S. Astaxanthin: a potential therapeutic agent in cardiovascular disease. Mar. Drugs. 2011; 9: 447-465.
20. Parikh, P., Mani, U., Iyer, U. Role of Spirulina in the Control of Glycemia and Lipidemia in Type 2 Diabetes Mellitus. J. Med. Food. 2001; 4: 193-199.