The Effects of Haematococcus Pluvialis Astaxanthin on Streptozotocin-Induced Diabetes in Rats

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***Abstract*—We evaluated the effects of Haematococcus pluvialis astaxanthinon on streptozotocin-induced diabetes and its mechanisms in rats. Method: Adult male SD rats were supplied. The rats were randomly divided into six groups (12/group): the control group, the model group, Metformin hydrochloride group and Haematococcus pluvialis low, medium and high dose groups. All groups rats, except control group, were modeled to diabetes by injecting Streptozotocin (STZ). Metformin hydrochloride group (metformin (0.285g/kg BW). The blood samples of rats were collected and the studying targets including anti-inflammatory and antioxidant capacity were measured. Results: compared to the model group, The body weight as well as water intake and diet intake were significantly inhibited. Activity of antioxidant enzymes, included SOD and GSH-PX were increased significantly. MDA content were decreased significantly. Blood glucose were decreased significantly. The levels of Cytokines TNF-α, IL-1β were decreased significantly. The levels of TC and TG were decreased significantly and the levels of HDL were increased significantly but the levels of LDL were decreased significantly .Conclusion: Haematococcus pluvialis astaxanthin attenuated streptozotocin-induced diabetes in rats. The possible mechanisms at least include both an anti-inflammatory effect and improvement of antioxidant capacity.**

***Index Terms*—haematococcus pluvialis, astaxanthin, blood glucose, diabetes; rat**

I. Introduction

Diabetes are metabolic disorders characterized by dysregulation of blood glucose levels. The hyperglycemia that occurs in diabetes increases the production of reactive oxygen species (ROS) and depletes cellular antioxidant defense capacities, resulting in enhanced oxidative stress. Inflammation may also play a key role in the development and progression of diabetes [1]-[3]. Astaxanthin (AST) is one of xanthophy family. They could scavenge ROS to be powerful biological antioxidants and anti-inflammatory agents. AST is a more potent antioxidant than other carotenoids and present in many organisms especially rich in Haematococcus pluvialis. [4]-[8] In this study, we evaluated the potential protective effects of Haematococcus pluvialis astaxanthinon against diabetes-induced damage in Streptozotocin (STZ)-induced diabetic rats. We examined the effects of Haematococcus pluvialis astaxanthinon on the production of oxidative stress mediators, the levels of Cytokines TNF-α, IL-1β in the Blood serum. [9] - [10]

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II. Materials and Methods

A. Plant Materials and Reagents

Haematococcus pluvialis astaxanthin(The astaxanthin content was 2%) was purchased from Hangzhou Xinwei Low-carbon Technology RD Co (Hangzhou. China); Streptozotocin was purchased from Sigma-Aldrich Company (St. Louis, MO,USA); Kits for SOD, MDA, GSH-Px, IL-1β, TNF-α, Insulin were procured from Nanjing Jiancheng Crop(Nanjing,China).

B. Animals

Sprague–Dawley (SD) rats (n=72, 180±20 g, Male, Certificate No. SCXK2012-0001) were supplied by the Breeding Center of the Institute of Experimental Animals (Beijing, China). The rats were housed in an air-conditioned room (temperature 22 ± 2℃,relative humidity of 50-60%,12 h light/dark cycle) and were allowed free access to tap water and standard laboratory chow. Before conducting experiments, animals were acclimatized to laboratory conditions for 3 days.

C. Induction of Diabetes and Experimental Design

Adult male Sprague–Dawley (SD) rats were supplied by the Breeding Center of the Institute of Experimental Animals (Beijing, China). The rats were housed in an air-conditioned room (temperature 22 ± 2 °C), relative humidity of 50–60%, 12 h light/dark cycle) and were allowed free access to tap water and standard laboratory chow. Before conducting experiments, animals were acclimated to laboratory conditions for 3 days. The rats were randomly divided into six groups (12/group): the control group, the model group, Metformin hydrochloride group and Haematococcus pluvialis low, medium and high dose groups. All groups, except control group, had been modeled by injecting Streptozotocin (STZ) hormone with the dose of 35mg/kg bw after having been fed with high fat diet for 4 weeks, then, to

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keep feeding 4 weeks. Haematococcus pluvialis groups were respectively administered by gavagethe appropriate doses of Haematococcus pluvialis (50mg/kg, 100mg/kg and 200mg/kg) for 4w. For the Metformin hydrochloride group was fed with high fat diet yet and with metformin intervention simultaneously (0.285g/kg BW).

D. Assessment of Blood Glucose Levels

Determination of glucose content in serum of rats with glucose assay kit, operating according to the kit instructions, the principle of glucose production in red quinoneimine glucose oxidase and peroxidase under the action of the maximum absorption peak at 505nm

E. Assessment of Blood MDA SOD and GSH-Px Levels

Malondialdehyde (MDA), as a marker for free radicals-induced lipid peroxidation, was estimated by reaction with thiobarbituric acid. Briefly, 0.1ml serum and aqueous solution of thiobarbituric acid. After heating at 95℃ for 40min, the red pigment produced was extracted with nbutanol- pyridine mixture and estimated by the absorbance at 532 nm; Superoxide Dismutase (SOD) can clear free radical, protect cell damage, and indirect reaction organism's ability to clear free radical. The principle is that the production of superoxide anion radicals by xanthine and xanthine oxidase reaction system, the oxidation of nitrite formation in light amine, chromogenic agent under the action of purple, with a visible light spectrophotometer to measure the absorbance when measured in samples containing SOD, while the specific inhibitory effects on superoxide anion free radical. The formation of nitrite reduction colorimetric determination tube absorbance value is lower than the control values of absorbance, calculated by the formula in the sample to be tested the activity of SOD; Glutathione peroxidase (GSH-Px) is an important enzyme that catalyzes the decomposition of hydrogen peroxide in the body, which can protect the structure and function of cell membrane. The principle is that GSH-Px can promote hydrogen peroxide and reduced glutathione reaction of water and oxidized glutathione, through the determination of the enzymatic reaction of reduced glutathione depletion can be obtained enzyme activity.

F. Assessment of Blood TG, HDL, LDL Levels

The serum packed in EP tubes, two of each tube, respectively. Serum automatic analyzer for the determination of TG, HDL-C, LDL-C used to be checked. One spare tube, -20 C refrigerator freezing.

G. Assessment of Blood TNF-α, IL-1 β and Lnsulin by ELISA

The levels of TNF-a, IL-1β and Insulin in the colon samples were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instruction, respectively. Briefly, cytokine molecules of the specimen bind to monoclonal antibody pre-coated wells and react with enzyme-conjugated secondary antibody. The chroma of color and the concentration of the cytokine molecules of sample were positively correlated.The optical densities were measured at 450nm using microplate reader (Biochrom Asys, UK).

H. Statistical Analysis

Results were expressed as mean±S.E.M. (standard error of mean) and statistical comparisons were carried out using one-way analysis of variance(ANOVA), followed by Duncan's Multiple-Rangetest which was used for multiple comparisons between groups.All the statistical analyses were carried 5 out using SPSS Version12.0 (SPSS Inc., Chicago, IL).The minimal level of significance was identified at $p<0.05$.

III. Results

A. Haematococcus Pluvialis Astaxanthin Relieved the Symptoms of Diabetes

During the test, The rats in normal group were in good condition, shiny fur; n the model group, dark coat, a hair loss phenomenon, bradykinesia, listlessness, slow weight gain; compared with the model group, rat mental state of haematococcuspluvialis intervention group is better

The body weight of Haematococcus pluvialis astaxanthin group rats decreased insignificantly compared to diabete rats. (data not be presented). There was a significant difference in water intake and diet intake between Haematococcus pluvialis astaxanthin group and diabete rats as well as in blood glucose ($p<0.01$) (Table I).

TABLE I. The Influence of Haematococcus Pluvialis Astaxanthin on Water Intake, Diet Intake and Blood Glucose

Treated groups	n	water intake (g/one)	diet intake (ml/one)	blood glucose (mmol/L)
Normal	12	22.51±1.19	42.65±0.21	6.22±0.53
model	12	32.76±0.19**	45.9±0.28**	11.73±0.70**
AST-L	12	31.81±1.41##	45.45±0.09	10.37±0.98
AST-M	12	27.55±0.72##	45±0.28	9.47±1.69##
AST-H	12	25.53±0.61##	43.05±0.64##	8.82±1.13##
Hypoglycemic drug	12	25.4±0.28##	45.55±0.49	8.59±1.08##

*P<0.05 VS Normal group; # P<0.05 VS Model group

B. Effect of Haematococcus Pluvialis Astaxanthin on MDA, SOD, GSH-Px

Activity of antioxidant enzymes, included SOD and GSH-PX were increased significantly in Haematococcus pluvialis astaxanthin groups (100mg/kg and 200mg/kg dose). MDA content were decreased significantly in Haematococcus pluvialis astaxanthin groups (100mg/kg and 200mg/kg dose) (Table II).

C. Effect of Haematococcus Pluvialis Astaxanthin on TG, HDL, LDL Levels of Blood

The levels of TC and TG were decreased significantly in Haematococcus pluvialis astaxanthin groups (100mg/kg and 200mg/kg dose). The levels of HDL were

increased significantly and the levels of LDL were decreased significantly in Haematococcus pluvialis astaxanthin groups (100mg/kg and 200mg/kg dose) (Table III).

TABLE II. EFFECT OF HAEMATOCOCCUS PLUVIALIS ON T-SOD, GSH-PX AND MDA OF SERUM (U/ML)

Treated groups	n	SOD	MDA	GSH-Px
Normal	12	250.42±4.54	5.09±0.62	314.01±14.06
model	12	208.91±9.87**	10.56±0.98**	268.8±7.98**
AST-L	12	232.45±7.54##	8.48±0.58##	273.33±10.71
AST-M	12	239.89±9.46##	7.48±0.83##	296.78±8.88##
AST-H	12	247.45±6.96##	7.37±0.69##	304.39±5.29##
Hypoglycemic drug	12	229.46±8.88##	8.21±0.99##	287.18±6.18##

*P＜0.05 VS Normal group; # P＜0.05 VS Model group

TABLE III. EFFECT OF HAEMATOCOCCUS PLUVIALIS ON BLOOD LIPID (MMOL/L)

Treated groups	n	HDL	LDL	TG
Normal	12	1.07±0.15	0.33±0.06	0.63±0.12
model	12	0.89±0.04	0.66±0.05	1.72±0.25
AST-L	12	0.86±0.16	0.62±0.19	1.08±0.34
AST-M	12	0.98±0.09	0.57±0.09	0.78±0.15
AST-H	12	1.06±0.23	0.47±0.11	0.64±0.11
Hypoglycemic drug	12	0.81±0.12	0.65±0.13	1.09±0.2

*P＜0.05 VS Normal group; # P＜0.05 VS Model group

D. *Effect of Haematococcus Pluvialis Astaxanthin on TNF-α, IL-1β*

levels of TNF-a and IL-1β cytokines were shown in Table IV. The levels of TNF-α and IL-1β were significantly elevated (P＜0.01) in the diabetes samples of animals treated with STZ. Blood levels of these cytokines were significantly reduced in rats treated with ASX. These observations indicated that ASX can modulate the inflammatory cytokines and to mitigate STZ diabetes(Table IV).

TABLE IV. EFFECT OF HAEMATOCOCCUS PLUVIALIS ON IL-1Β, TNF-Α OF SERUM (U/ML)

Treated groups	n	IL-1β	TNF-α
Normal	12	28.83±1.8	155.46±14.34
model	12	36.24±1.67**	264.17±8.29**
AST-L	12	32.51±1.12##	217.22±19.26##
AST-M	12	32.38±1.49##	183.31±21.61##
AST-H	12	20.59±1.94##	162.79±16.83##
Hypoglycemic drug	12	33.46±0.86#	237.69±31.03

*P＜0.05 VS Normal group; # P＜0.05 VS Model group

IV. DISCUSSION

Diabetes, called Xiao ke disease in traditional Chinese medicine, is a metabolic diseases induced by inadequate insulin secretion absolutely or relatively, which results in the disorders of sugar, fat and proteins directly and of vitamin, water and electrolyte metabolic secondary, and breaks oxidation balance. There are 4 types diabetes generally in clinic, but type1 and type2 occur frequently [10]-[11]. Until now, diabetes mechanism is not clear. Although combination treatment including dietary therapy, movement therapy and drug treatment used for diabetes in clinic, but mostly drug is used control blood glucose level, reduce the symptoms of diabetes, and delay further deterioration of diabetes complications [12]. So researchers turn to the natural plant that control blood glucose effectively, hope to control blood glucose level.

In this study, we established a model of type 2 diabetes mellitus (T2DM), which is a combination of high fat and high sugar diet and drug induced. Drugs including Streptozotocin (STZ), four oxygen pyrimidine, Alloxan Monohydrate, which is the most commonly used STZ. Streptozotocin (STZ) has a similar glucose results of the side chain, the islet B cell error identification, and enter the cell, and Streptozotocin (STZ) toxicity groups have toxic effects on islet B cells, B cell damage, studies showed that B cell apoptosis plays a very important role for in the development of diabetes [13], [14]. Studies have indicated that, high fat and high sugar fed rats, but rats appear insulin resistance, while intraperitoneal injection of STZ can induce a successful model of type 2 diabetes mellitus (T2DM).

Under normal circumstances, the oxidation and antioxidation in the body is in a dynamic equilibrium, when a certain reason causes the increase of free radicals and the decrease of antioxidant capacity, which will lead to the production of various pathological changes [15]-[18]. The latest research supports, type 2 diabetes mellitus (T2DM) is a chronic low-grade inflammatory state of the hypothesis. Many studies have indicated that the incidence of type 2 diabetes mellitus (T2DM) is often accompanied by a decrease in the antioxidant enzyme activity of the body and an increase in the concentration of inflammatory factors. IL-1 beta and TNF- alpha is a proinflammatory cytokine, is involved in a wide range of human tissue damage, edema and other pathological process, IL-1 beta and TNF- alpha in vivo inflammatory reaction state of two of the most sensitive inflammatory factor; MDA is a degradation product of polyunsaturated fatty acids with peroxide. Strong toxicity and lipoprotein after crosslinking, therefore MDA as main indicators of lipid peroxidation in vivo measure, to a certain extent reflects the degree of oxidative damage to cells; SOD can eliminate free radicals, protect cell injury, indirect reaction induced free radical scavenging ability; GSH-Px is a kind of important hydrogen peroxide catalyzed by widespread the body of the decomposition enzyme, can protect the structure and function of cell membrane integrity function.

The results obtained in the present experiment reveal that AST markedly attenuates the inflammatory response to STZ-induced experimental diabetes, which is considered a model validated to find drugs potentially active in this disease. This is evidenced by increased body weight,, lowered MDA, IL-1β and TNF-α levels, increased SOD, GSH-Px, and insulin. Thus, The

mechanism at least involves anti-inflammatory effect and improve the typical symptoms of diabetes.

REFERENCES

[1] P. Richard, P. Shin, T. Beeson, *et al.*, "Quality and cost of diabetes mellitus care in Community Health Centers in the United States," *Plos One*, vol. 12, no. 10, p. e0144075, 2015.

[2] B. Liu, S. Liu, Y. Chen, *et al.*, "Clinical and research tatus of islet transplantati On for type 1 diabetes in China,"*Joumal of Clinical Rehablitative Tissue Engineering Research*, vol. 15, no. 44, pp. 8346-8349. 2011.

[3] W. Wang, W. P. McGreevey, C. Fu, *et al.*, "Type 2 diabetes mellitus in China: a preventable economic burden," *Am. J. Manag. Care*, vol. 15, pp. 593-601, 2009.

[4] J. Nuovo, X. Yang, *et al.*, "Changes in management mode for type 2 diabetes," *Academic Journal of Second Military Medical University*, vol. 33, no. 3, pp. 324-327, 2012.

[5] C. Marciniak, X. Marechal, D. Montaigne, *et al.*, "Cardiac contractile function and mitochondrial respiration in diabetes-related mouse models," *Cardiovasc Diabetol*, vol. 13, pp. 118, 2014.

[6] P. Zimmet, K. G. Alberti, and J. Shaw, "Global and societal implications of the diabetes epidemic," *Nature*, vol. 414, pp. 782-787, 2001.

[7] R. T. Lorenz and G. R. Cysewski, "Commercial potential for Haematococcus microalgae as a natural source of astaxanthin," *Tibtech*, vol. 18, pp. 160-167, 2000.

[8] Y. K. Lee and S. Y. Ding, "Cell cycle and accumulation of astaxanthin in Haematococcus Lacusiris," *Journal of Phycology*, vol. 30, no. 3, pp. 445-449, 2004.

[9] T. G. Polotow, C. V. Vardaris, A. R. Mihaliuc, *et al.*, "Astaxanthin supplementation delays physical exhaustion and prevents redox imbalances in plasma and soleus muscles of Wistar rats ," *Nutrients*, vol. 12, no. 6, pp. 5819-5838, 2014.

[10] S. Ursoniu, A. Sahebkar, M. C. Serban, *et al.*, "Lipid profile and glucose changes after supplementation with astaxanthin: a systematic review and meta-analysis of randomized controlled trials," *Arch. Med. Sci.*, vol. 2, no. 11, pp. 253-66, 2015.

[11] M. M. Ciccone, F. Cortese, M. Gesualdo, *et al.*, "Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care," *Mediators Inflamm*, vol. 2013, pp. 782137, 2013.

[12] S. Bhuvaneswari, B. Yogalakshmi, S. Sreeja, *et al.*, "Astaxanthin reduces hepatic endoplasmic reticulum stress and nuclear factor-kappa B-mediated inflammation in high fructose and high fat diet-fed mice," *Cell Stress Chaperones*, vol. 2, no. 19, pp. 183-91, 2014.

[13] J. Xu, H. Gao, L. Zhang, *et al.*, "A combination of flaxseed oil and astaxanthin alleviates atherosclerosis risk factors in high fat diet fed rats," *Lipids Health Dis*., vol. 13, pp. 63, 2014.

[14] P. Bjornstad, D. Cherney, and D. M. Maahs, "Early diabetic nephropathy in type 1 diabetes: New insights," *Curr. Opin. Endocrinol. Diabetes Obes.*, vol. 4, no. 21, pp. 279-286, 2014.

[15] M. Boukhris, S. D. Tomasello, F. Marza, *et al.*, "Coronary heart disease in Postmenopausal women with type II diabetes mellitus and the impact of estrogen replacement therapy: A narrative review," *Int. J. Endocrinol.*, vol. 2014, pp. 413920, 2014.

[16] R. Lupi, F. Dotta, L. Marscella, *et al.*, "Prolonged exposure to free fatty acids has cytosatatic and pro-apototic effects on human panereasnc islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramde pathway and Bcl-2 redulated," *Diabetes*, vol. 51, pp. 1437, 2002.

[17] E. Leinonnen, E. Hurt-Camejo, O. Wiklund, *et al.*, "Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes," *Atherosclerosis*, vol. 166, no. 2, pp. 387-394, 2003.

[18] S. A. Ateer, A. L. Scarim, K. T. Chambers, *et al.*, "Interleukin-I stimulates beta-cell necrosis and release of the immunological adjuvant HMGB1," *Plos Med*., vol. 3, no. 2, p.17, 2006.

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