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Original Article

IN VITRO ANTI-DIABETIC ACTIVITY OF MICROENCAPSULATED AND NON-ENCAPSULATED ASTAXANTHIN

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ABSTRACT

Objective: Diabetes is a long term condition which indicates the high blood pressure. The symptoms indicates, polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia). Many drugs has been discovered for curing diabetes. Recent studies reported that the administration of astaxanthin reduces the blood pressure in the diabetic patient. Astaxanthin is a powerful antioxidant found in wide variety of aquatic living organism which has wide applications in pharmacological studies.

Methods: *In vitro* antidiabetic study of both encapsulated and non-encapsulated astaxanthin such as DNSA method, starch-iodine color assay method and α glycosidase enzymes assay was carried out.

Results: The results of the present study indicated that both encapsulated and non-encapsulated astaxanthin shows higher antidiabetic activity in all the method. Each test samples possess the best activity when compared to standard drug acarbose.

Conclusion: The present study, it is concluded that both non-encapsulated and encapsulated astaxanthin exhibit good antidiabetic activity.

Keywords: Astaxanthin, Anti-diabetic, DNSA, Starch-iodine, Acarbose, α glycosidase

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INTRODUCTION

Diabetes is one of the major causes of premature death worldwide. Every ten second a person dies from diabetes related causes mainly from cardiovascular complications. Metabolic disease, including dyslipidemia and diabetes, constitutes a major emerging health crisis in the world. The WHO, in the 2009 report, states that high blood plasma ranked first in the list of leading global risks for mortality and accounted for 7.5 million deaths in the world in 2004 [1]. According to reports, 415 million people worldwide were diabetic in 2015, most of them suffering from Type II diabetes [2].

Diabetes, which is diagnosed based on blood plasma hyperglycemia, has been linked to lipid overload and abdominal obesity and may synergize with these conditions to promote negative clinical outcomes [3, 4]. Although the symptoms and clinical pathology and physiology of these conditions are well understood, the question of pharmacologic treatment of dyslipidemia and diabetes remains unresolved well. The marine world, due to its phenomenal biodiversity, is a rich natural resource of many biologically active compounds such as polyunsaturated fatty acids (PUFAs), sterols, proteins, polysaccharides, antioxidants and pigments. People worldwide know that marine foods participate in human health promotion. A diet rich in marine products is considered to result in a lower incidence of diabetes, cancer and obesity. To date, many of reports have also showed that bioactive compounds from marine organisms, including Fucoxanthin, Astaxanthin, Marine Collagen Peptides, Dieckol and Krill Oil, exert a positive influence on metabolic dysfunction (diabetes and obesity) [1].

Astaxanthin, a red-orange carotenoid pigment, is a biological antioxidant that naturally found in a wide variety of aquatic living organisms, such as shrimp, crab, and salmon [5]. The green micro algae *Haematococcus pluvialis* and the red yeast *Phaffi a rhodozyma* are common sources of natural astaxanthin [5]. Astaxanthin has shown various pharmacological activities, including anti-inflammatory [6, 7] and antidiabetic activities [8], as well as antioxidative effects [9-12]. Diabetes mellitus is strongly associated with oxidative stress, which can be a consequence of increased free radical production, reduced antioxidant defences or both [13]. Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic b-cells and various forms of tissue damage in patients with diabetes mellitus. It was found that astaxanthin could diminish the oxidative stress caused by hyperglycemia in the pancreatic β cells, significantly improve glucose tolerance, increase serum insulin levels, and decrease blood glucose levels, indicating that astaxanthin might exert beneficial effects on pancreatic b-cell function and could protect pancreatic b-cells against glucose toxicity by preventing the progressive destruction of these cells [8]. The main objective of the present study is to investigate the antidiabetic activity for both encapsulated and non-encapsulated astaxanthin.

MATERIALS AND METHODS

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 the first method, microencapsulated astaxanthin was prepared by using sodium alginate and calcium chloride [14, 15, 40]. In the second method, microencapsulated astaxanthin was prepared using sodium alginate and chitosan [16]. In the third method, chitosan–Tripolyphosphate was used to produce microencapsulated astaxanthin [17, 18]. In the fourth method, liposome encapsulated astaxanthin was carried out by the method [19]. These test samples (Both encapsulated and non-encapsulated astaxanthin) were used to study the antidiabetic activity using four different methods.

In vitro anti-diabetic activity

α amylase enzyme assay (DNSA method)

Starch solution (0.1% w/v) was prepared by stirring 0.1g of starch in 100 ml of 16 mmol of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent was prepared by mixing the sodium potassium tartrate solution and 3, 5 Di-nitro salicylic acid solution at 96 mmol concentration [20, 21]. The control tube contains an only reagent and the test sample in the range of 100–500 µg/ml was prepared. From this, 500 µl of sample was mixed with 500 µl of starch solution and 500 µl of alpha-amylase solution

which is incubated at 37 ° C for 10 min. The reaction was stopped by the addition of 1 ml of 3, 5 Di-nitro salicylic acids and incubated in boiling water bath for 5 min, cooled at room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water. The absorbance was measured at 540 nm [22, 23, 24]. Control was tested by replacing test sample with DMSO. The similar procedure were also followed for the standard drug Acarbose. The percentage of inhibition was calculated using the formula:

% inhibition = [(O.D. of control–O.D. of test sample)/O.D. of control] × 100

α amylase enzyme assay (Starch-Iodine color assay method)

Screening of test samples for α-amylase inhibitors was carried out according to [25, 26] with slight modification based on the starch-iodine test. Test samples of varied concentrations in 500 µL were added to 500 µL of 0.02 M sodium phosphate buffer (pH6.9 containing 6 mmol sodium chloride) containing 0.04 units of the α-amylase solution and were incubated at 37 °C for 10 min.

Then 500 µL soluble starch (1%, w/v) was added to each reaction well and incubated at 37 °C for 15 min. 1 M HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µL of iodine reagent (5 mmol I2 and 5 mmol KI). The color change was noted and the absorbance was read at 620 nm on a microplate reader. The control reaction representing 100% enzyme activity were taken. Inhibition of enzyme activity was calculated as:

% inhibition = [(O.D. of control–O.D. of test sample)/O.D. of control] × 100

α glucosidase enzyme assay

The inhibitory activity of α-glucosidase enzyme was determined by 1 ml solution of starch substrate (2 % w/v maltose or sucrose) with 0.2 M Tris buffer pH 8.0 and 1 ml of test samples in the range of 100–500 µg/ml were incubated separately for 5 min at 37 °C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35 °C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the color was measured at 540 nm [27, 20, 24]. A control experiment was done by replacing the test sample with DMSO and also for a standard drug Acarbose [28, 29, 30]. Percentage of inhibition was calculated by the formula:

% inhibition = [(O.D. of control–O.D. of test sample)/O.D. of control] × 100

α glucosidase enzyme assay (alternate method)

The α-glucosidase inhibitory activity was determined according to a modified procedure [31, 32]. Briefly, 50µl of 0.1M potassium phosphate buffer (pH6.9) was pre-incubated with50µl of reduce dglutathione (1 mgml-1), 20µl a-glucosidase (1Uml–1 in0.1M phosphate buffer, pH 6.9 and 20µl of test samples at37 °C for 10 min. After the incubation, 20µl pNPG was added and the mixture was further incubated at 37 °C for 30 min. The reaction was terminated by adding1 ml of 0.1Msodiumcarbonate. The absorbance of the samples and control were taken at 405 nm against a blank devoid of pNPG and sample. The control reaction (with 100% enzyme activity) contained buffer or DMSO instead of the irrespective samples while acarbose was used as a positive control. The inhibitory activity was calculated by using the following Equation:

% inhibition = [(O.D. of control–O.D. of test sample)/O.D. of control] × 100

Statistical analysis

The statistical analyses for all the experiments were done using Excel 2013 through the statistical formula. Experimental data were expressed as mean±SD and IC 50 values were calculated. The experiment was performed in triplicates for all the test samples.

RESULTS

***In vitro* anti-diabetic activity**

***In vitro* Alpha-amylase inhibitory activity (DNSA method).**

In the present study, astaxanthin was encapsulated using four different methods were investigated for their potential to inhibit α-amylase activity and α-glucosidase activity. Five different concentrations viz., 250, 500, 750, 1000 and 1250 µg/ml of test samples were separately tested for the inhibition of α-amylase activity and α-glucosidase activity along with standard acarbose.

Table 1: *In vitro* Alpha-amylase inhibitory activity (DNSA method) of standard drug acarbose

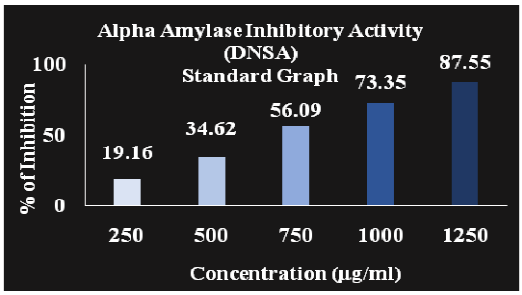
Content	Concentration (µg/ml)	mean±SD percentage	IC 50 values
Blank	-	0.00	
S1	250	32.3±0.100	690.830
S2	500	51.1±0.058	
S3	750	66.2±0.053	
S4	1000	82.9±0.100	
S5	1250	99.2±0.058	

Table 2: *In vitro* Alpha-amylase inhibitory activity (DNSA method) percentage for different concentration of test samples

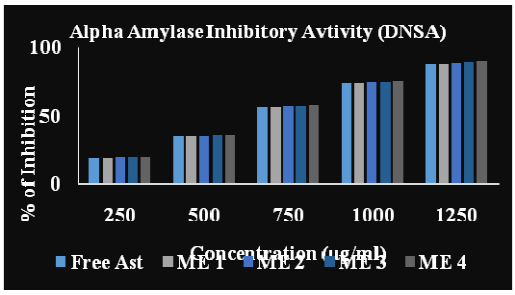
Concentration (µg/ml)	Non-encapsulated astaxanthin mean±SD percentage	ME 1 mean±SD percentage	ME 2 mean±SD percentage	ME 3 mean±SD percentage	ME 4 mean±SD percentage
250	19.53±0.115	19.37±0.100	19.79±0.058	19.95±0.058	20.32±0.100
500	35.04±0.100	34.83±0.153	35.30±0.058	35.57±0.058	36.04±0.115
750	56.46±0.058	56.31±0.058	56.83±0.100	57.10±0.115	57.57±0.115
1000	73.77±0.100	73.56±0.058	74.35±0.153	74.62±0.058	75.04±0.100
1250	87.97±0.100	87.81±0.153	88.92±0.058	89.08±0.058	98.82±0.115
IC 50 Values	685.169	687.703	679.168	675.780	669.129

Among all the test samples (both encapsulated and non-encapsulated astaxanthin) the ME 4 at 1250 µg/ml concentration, had the highest amylase inhibition of 89.82% followed by ME 2 and ME 3 with inhibition of 88.92% and 89.08% respectively. ME 1 and non-encapsulated astaxanthin showed the inhibition of 87.81% and 87.97% at concentration 1250 µg/ml. The standard drug acarbose showed the percentage of inhibition 87.55% at concentration 1250 µg/ml when compared with the test samples. The graph was represented in Graph 1 and Graph 2.

The IC 50 values were also calculated from the percentage of inhibition by each samples. The IC 50 values of standard drug acarbose was 690.830 µg/ml which is compared with test samples such as non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 that possessed 685.169 µg/ml, 687.703 µg/ml, 679.168 µg/ml, 675.168 µg/ml and 669.129 µg/ml respectively (table 1 and table 2). Thus, all the test samples showed highest α-amylase inhibition at different concentration (250 µg/ml to 1250 µg/ml) than standard drug acarbose.



Graph 1: ***In vitro*** **Alpha-amylase inhibitory activity (DNSA method) standard drug acarbose**



Graph 2: ***In vitro*** **Alpha-amylase inhibitory activity (DNSA method) for different concentration of test samples**

Table 3: ***In vitro*** **Alpha-amylase inhibitory activity (Starch-Iodine color assay method) of standard drug acarbose**

Content	Concentration (µg/ml)	mean±SD percentage	IC 50 values
Blank	-	0.00	658.755
S1	250	19.84±0.211	
S2	500	35.01±0.037	
S3	750	60.07±0.091	
S4	1000	75.28±0.042	
S5	1250	94.34±0.103	

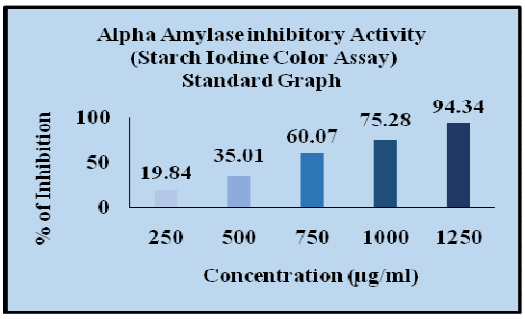
Table 4: ***In vitro*** **Alpha-amylase inhibitory activity (Starch-Iodine color assay method) percentage for different concentration of test samples**

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
250	18.37±0.115	19.20±0.093	20.53±0.113	19.84±0.141	21.17±0.191
500	32.55±0.132	36.93±0.051	34.12±0.121	33.23±0.072	35.40±0.116
750	55.10±0.051	55.64±0.003	57.61±0.213	56.23±0.063	57.56±0.053
1000	71.54±0.033	72.82±0.001	72.43±0.115	71.54±0.041	73.36±0.051
1250	84.64±0.001	85.57±0.012	85.92 ±0.043	86.66±0.004	88.08±0.158
IC 50 Values	714.438	690.224	689.056	699.113	675.573

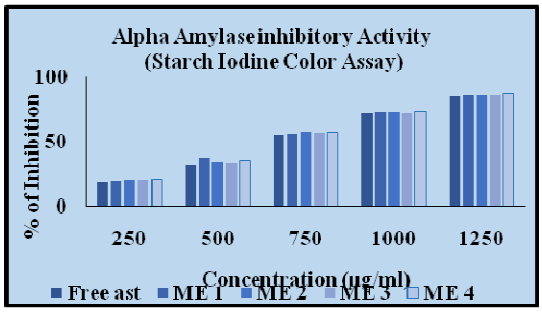
In vitro **Alpha-amylase inhibitory activity (Starch-Iodine color assay method)**

We investigated the encapsulated and non-encapsulated astaxanthin as well as standard drug acarbose for their α-amylase inhibitory activities using starch iodine color assay. The OD values were noted in table 3 and table 4.

When we compared the nonencapsulated and encapsulated astaxanthin, the maximum activity at 1250 µg/ml was exhibited by ME 4 (88.08%) followed by ME 3 (86.66%) and ME 2 (85.92%). Non-encapsulated astaxanthin and ME 1 test sample exhibit 84.64% and 85.57% which is similar to other test samples. Standard drug acarbose possessed very high inhibition of above 94.34% at concentration 1250 µg/ml which is greater than that of the test samples. At concentration 250 µg/ml the test samples such as non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 possessed 18.37%, 19.20%, 20.53%, 19.84% and 21.17% respectively. The standard exhibit maximum of 19.84% when compared with all other test samples (Graph 3 and Graph 4). The IC 50 values of standard drug, non-encapsulated astaxanthin and encapsulated astaxanthin was 658.755 µg/ml, 714.438 µg/ml, 690.224 µg/ml, 689.056 µg/ml, 699.113 µg/ml and 675.573 µg/ml respectively.



Graph 3: ***In vitro*** **Alpha-amylase inhibitory activity (Starch-Iodine color assay method) of standard drug acarbose.**



Graph 4: ***In vitro*** **Alpha-amylase inhibitory activity (Starch-Iodine color assay method) for different concentration of test samples**

***In vitro* Alpha-glucosidase inhibitory activity**

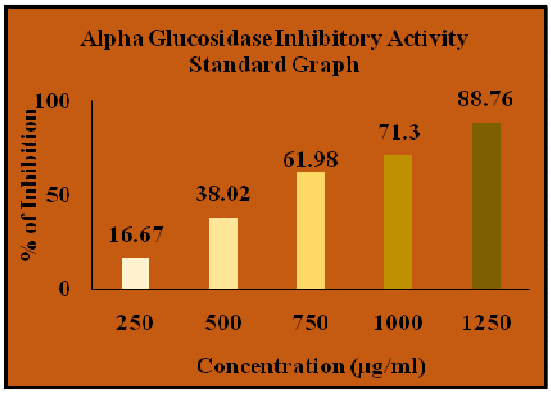
Table 5: *In vitro* Alpha-glucosidase inhibitory activity of standard drug ascorbose

Content	Concentration (µg/ml)	mean±SD percentage	IC 50 values
Blank	-	0.00	674.687
S1	250	16.67±0.158	
S2	500	38.02±0.058	
S3	750	61.98±0.208	
S4	1000	71.30±0.238	
S5	1250	88.76±0.118	

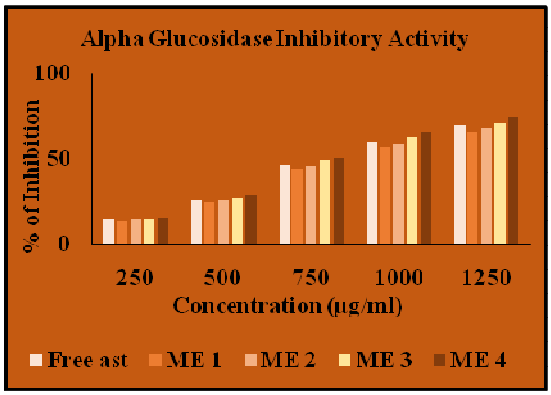
The results of the α-glucosidase are summarized in table 5 and table 6. All the test samples showed the varying effect on α-glucosidase activity. The standard drug showed maximum inhibition with the highest value of 88.76% seen at 1250 µg/ml concentration. Among the test sample, the highest value was obtained by ME 474.30% and ME 3 70.83% at concentration 1250 µg/ml. Compared to these test samples non-encapsulated astaxanthin, ME 1 and ME 2 possessed 68.96%, 64.93% and 67.46% (Graph 5 and Graph 6) respectively.

Table 6: *In vitro* Alpha-glucosidase inhibitory activity 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
250	14.79±0.178	13.44±0.113	14.37±0.167	14.79±0.153	15.22±0.123
500	25.61±0.143	24.58±0.134	25.28±0.153	26.45±0.124	28.89±0.146
750	46.54±0.243	43.73±0.234	45.60±0.145	49.20±0.153	50.23±0.126
1000	59.93±0.232	56.55±0.156	58.38±0.178	62.41±0.156	65.26±0.174
1250	68.96±0.134	64.93±0.145	67.46±0.103	70.83±0.183	74.30±0.126
IC 50 Values	869.760	923.286	889.683	838.895	802.093



Graph 5: *In vitro* Alpha-glucosidase inhibitory activity of standard drug acarbose



Graph 6: *In vitro* Alpha-glucosidase inhibitory activity for different concentration of test samples

The IC 50 values were also evaluated from the percentage of inhibition by each test samples. Test samples such as non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 showed IC 50 values of 869.760, 923.286, 889.683, 838.895 and 802.093 µg/ml along with standard drug acarbose that possessed 674.687 µg/ml.

***In vitro* Alpha-glucosidase inhibitory activity (Alternative method)**

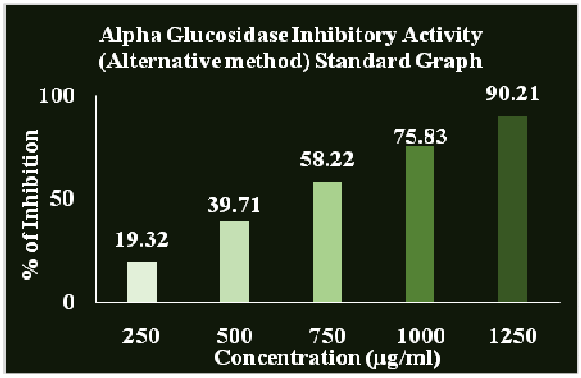
The result of *In vitro* Alpha-glucosidase inhibitory activity (Alternative method) was given in table 7 to table 8. All samples showed maximum inhibition at 1250 µg/ml and least inhibition at 250 µg/ml. At concentration 1250 µg/ml the test samples such as non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 produced 88.68%, 83.11%, 85.53%, 84.37% and 89.94% along with standard i.e. 90.21%. The least inhibition at 250 µg/ml of concentration were recorded by test samples i.e. 18.96%, 17.61%, 16.98%, 18.15% and 19.95%. The standard drug also exhibits similar percentage of inhibition 19.32%. The graph was plotted against the percentage of inhibition and concentration for both standard and test samples indicated in Graph 7 and Graph 8.

Table 7: *In vitro* Alpha-glucosidase inhibitory activity (Alternative method) of standard drug acarbose

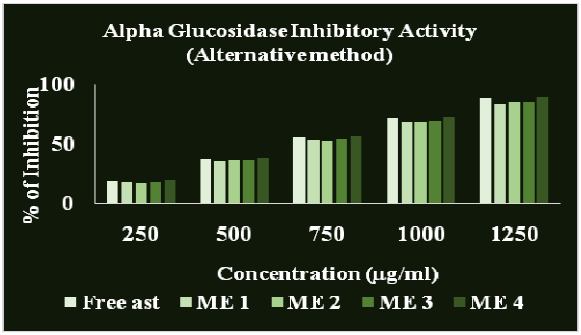
Content	Concentration (µg/ml)	mean±SD percentage	IC 50 values
BLANK	-	0.00	656.436
S1	250	19.32±0.141	
S2	500	39.71±0.017	
S3	750	58.22±0.043	
S4	1000	75.83±0.221	
S5	1250	90.21±0.107	

Table 8: *In vitro* Alpha-glucosidase inhibitory activity (Alternative method) 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
250	18.96±0.189	17.61±0.043	16.98±0.191	18.15±0.231	19.95±0.097
500	37.35±0.119	35.22±0.057	36.12±0.117	36.66±0.145	38.27±0.063
750	55.71±0.113	53.46±0.061	53.37±0.102	54.27±0.173	56.33±0.037
1000	71.97±0.210	68.55±0.113	68.46±0.134	69.72±0.029	72.60±0.183
1250	88.68±0.173	83.11±0.182	85.53±0.165	84.37±0.075	89.94±0.197
IC 50 Values	684.879	725.811	719.134	710.211	672.294



Graph 7: *In vitro* Alpha-glucosidase inhibitory activity (Alternative method) of standard drug acarbose



Graph 8: *In vitro* Alpha-glucosidase inhibitory activity (Alternative method) for different concentration of test samples

DISCUSSION

Lack of insulin affects the metabolism of carbohydrates, proteins, fat and causes significance disturbance of water and electrolyte homeostasis [33]. Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase both are important in carbohydrate digestion and glucose absorption) have leads to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with micro-and macro-vascular complications in diabetes and is more strongly associated with the risk for cardiovascular diseases than are fasting blood glucose. α-Glucosidase enzymes in the intestinal lumen and in the brush border membrane play main roles in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would, in turn, cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation [34].

Alpha-glucosidase inhibitor retards the digestion of carbohydrates and slows down the absorption. Acarbose and miglitol are a competitive inhibitor of α-glucosidases and reduces absorption of starch and disaccharides [35]. Hence one of the therapeutic approaches for reducing postprandial (PP) blood glucose levels in a patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition of these enzymes (α-amylase and α-glucosidases) reduced the high postprandial (PP) blood glucose peaks in diabetes [36]. Acarbose and Miglitol are a competitive inhibitor of α glucosidases and reduces absorption of starch and disaccharides [35]. The α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in the breakdown of starch. Synthetic inhibitor causes side effect such as abdominal pain, diarrhoea and soft faeces in the colon.

The present study reveals that both encapsulated and non-encapsulated astaxanthin effectively inhibit both α-amylase and α-Glucosidase enzymes. Our findings were compared with other research articles. The mechanisms were investigated underlying the insulin sensitivity effects of ASX in a non-genetic insulin resistant animal model. The results showed that ASX improved insulin sensitivity by activating the post-receptor insulin signalling, *i.e.* enhancing the auto phosphorylation of insulin receptor-b (IR-b), IRS-1 associated PI3-kinase step, phospho-Akt/Akt ratio and GLUT-4 translocation in skeletal muscle [37].

Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic β-cells and various forms of tissue damage in patients with diabetes mellitus. It was found that astaxanthin could diminish the oxidative stress caused by hyperglycemia in the pancreatic β-cells, significantly improve glucose tolerance, increase serum insulin levels, and decrease blood glucose levels [8]. Recently,

[38] demonstrated that astaxanthin could substantially improve insulin sensitivity through abolishing significant elevation in both glucose and insulin levels induced by a high fat plus high fructose diet in mice.

But beyond that, the effects of astaxanthin in a metabolic syndrome animal model of spontaneously hypertensive corpulent rat,the results showed that astaxanthin markedly decreased the levels of blood glucose, triglycerides and non-esterified fatty acids, and significantly increased the levels of high-density lipoprotein cholesterol and adiponectin. It is suggested that astaxanthin ameliorates insulin resistance and improve insulin sensitivity by increasing glucose uptake, and by modulating the levels of circulating adiponectin and blood lipids [39].

CONCLUSION

To date, more and more metabolic diseases have influenced in human's health and quality of life. In the last few years, there has been a growing interest in the herbal medicine in care and management of diabetes both in developing and developed countries, due to their natural origin and less side effects.

The adverse effects of current drug treatment are not always satisfactory in maintaining normal levels of blood Glucose. Hence there is continuous thirst towards discovering or identification of bioactive compounds derived from plants and marine sources with potent antidiabetic activity. In the present study, the astaxanthin in free form and encapsulated form has been found to exhibit better antidiabetic potential by inhibition of Amylase and Glucosidase. Our other studies also proved their antioxidant, radical scavenging and anti-inflammatory potential. However, further research should go in this direction in order to show new preventive and potential therapeutic strategies against diabetes and associated disorders.

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. Hang Wang, Zhi-ming Fu, Chun-chao Han. The potential applications of marine bioactive against diabetes and obesity. Am J Mar Sci 2014;2:1-8.
2. IDF Diabetes Atlas. International Diabetes Federation; 2015. Available from: http://www.idf.org/idf-diabetes-atlas-seventh-edition. [Last accessed on 14 Apr 2016]
3. Pontiroli AE, Camisasca R. Additive effect of overweight and type 2 diabetes in the appearance of coronary heart disease but not of stroke: a cross-sectional study. Acta Diabetol 2002;39:83-90.
4. Grundy SM, Bryan Brewer JH, Cleeman JI, Sidney J, Smith C, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.
5. Higuera-Ciapara I, Félix-Valenzuela L, Goycoolea FM. Astaxanthin: a review of its chemistry and applications. Crit Rev Food Sci Nutr 2006;46:185–96.
6. Kurashige M, Okimasu E, Inoue M, Utsumi K. Inhibition of oxidative injury of biological membranes by astaxanthin. Physiol Chem Phys Med NMR 1990;22:27–38.
7. Ohgami K, Shiratori K, Kotake S, Nishida T, Mizuki N, Yazawa K, *et al.* Effects of astaxanthin on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. Invest Ophthalmol Visual Sci 2003;44:2694–701.
8. Uchiyama K, Naito Y, Hasegawa G, Nakamura N, Takahashi J, Yoshikawa T. Astaxanthin protects beta-cells against glucose toxicity in diabetic db/db mice. Red Rep 2002;7:290–300.
9. O'Connor I, O'Brien N. Modulation of UVA light-induced oxidative stress by beta-carotene, lutein and astaxanthin in cultured fibroblasts. J Dermatol Sci 1998;16:226–30.
10. Iwamoto T, Hosoda K, Hirano R, Kurata H, Matsumoto A, Miki W, *et al.* Inhibition of low-density lipoprotein oxidation by astaxanthin. J Atheroscl Throm 2000;7:216–22.
11. Kang JO, Kim SJ, Kim H. Effect of astaxanthin on the hepatotoxicity, lipid peroxidation and antioxidative enzymes in the liver of CCl4-treated rats. Methods Find Exp Clin Pharmacol 2001;23:79–84.
12. Aoi W, Naito Y, Sakuma K, Kuchide M, Tokuda H, Maoka T, *et al.* Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. Antioxid Redox Signaling 2003; 5:139–44.
13. Leite MF, de Lima A, Massuyama M, Otton R. *In vivo* astaxanthin treatment partially prevents antioxidant alterations in dental pulp from alloxan-induced diabetic rats. Int Endocrinal J 2010;43:959–67.
14. Lin SF, Chen YC, Chen RN, Chen LC, Ho HO, Tsung YH, *et al.* Improving the stability of astaxanthin by microencapsulation in calcium alginate beads. PloS One 2016;11:1-10.
15. Park SA, Ahn JB, Choi SH, Lee JS, Lee HG. The effects of particle size on the physiochemical properties of optimized astaxanthin-rich Xanthophyllomyces dendrorhous-loaded microparticles. LWT-Food Sci Tech 2014;55:638-44.
16. Krasaekoopt W, Bhandari B, Deeth H. Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT-and conventionally treated milk during storage. Food Sci Technol 2006;39:177-83.
17. Phathanee Thamaket, Patcharin Raviyan. Preparation and physical properties of carotenoids encapsulated in chitosan cross linked triphosphate nanoparticles. Food Appl Biosci J 2015;3:69-84.
18. Yangchao L, Boce Z, Monica W, Liangli Y, Qin W. Preparation and characterization of zein/chitosan complex for encapsulation of alpha tocopherol and Its *in vitro* controlled release study. Colloids Surf B 2011;85:145-52.
19. Chiu CH, Chang CC, Lin ST, Chyau CC, Peng RY. Improved hepatoprotective effect of liposome-encapsulated astaxanthin in lipopolysaccharide-induced acute hepatotoxicity. Int J Mol Sci 2016;17:1-17.
20. Bhatt UP, Sati SC, Subhash Chandra, Sudhir Kumar, Amit Anthwal, Rajendra Singh, *et al.* Evaluation of *in vivo a*nd *in vitro* antidiabetic activity of *Roylea Cinerea*. Int J Pharm Sci Rev Res 2015;32:210-3.
21. Malik CP, Singh MB. Plant Enzymology and Histoenzymology, Kalyani Publishers, New Delhi; 1980. p. 278.
22. Murugesan S, Anand Babu M, Bhuvaneswari S, Kotteswari M, Thennarasan S. *In vitro* antidiabetic activity of methanolic extracts of selected marine algae. Eur J Pharm Med Res 2015;2:256-60.
23. Narkhede MB, Ajimire PV, Wagh AE, Manoj Mohan, Shivashanmugam AT. *In vitro* antidiabetic activity of *Caesalpina Digyna* (R.) methanol root extract. Asian J Plant Sci Res 2011;1:101-6.
24. Murugesan S, Bhuvaneswari S, Sivamurugan V. Evaluation of *in vitro* antidiabetic activity of red seaweed *Portieria Hornemannii* (Lyngbye) (Silva) and *Spyridia Fusiformis* (Wulfen). World J Pharma Sci 2016;4:415-9.
25. Xiao Z, Storms R, Tsang A. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. Anal Biochem 2006;351:146-8.
26. Ashok Kumar BS, Saleemulla Khan, Gopi Setty Saran, Nandeesh, Manjunath NK. *In vitro* antidiabetic activity of nisamalaki churna. Sains Malaysiana 2013;42:625-8.
27. Krishnaveni S, Theymoli B, Sadasivam S. Phenol sulfuric acid method. Food Chem 1984;15:229.
28. Andrade-Cetto A, Becerra-Jimenez J, Cardenas-Vazquez R. Alfa-glucosidase-inhibiting activity of some mexican plant used in the treatment of type-2 diabetes. J Ethnopharm 2008;116:27-32.
29. Matsuura H, Asakawa C, Kurimoto M, Mizutani J. Alpha-glucosidase inhibitor from the seeds of balsam pear (Momordica charantia) and the fruit bodies of Grifola frondosa. Biosci Biotech Biochem 2002;66:1576-8.
30. Tietz NW. In: Burtis CA, Ashwood ER. Eds. Tietz Textbook of Clinical Chemistry. Third Ed. Saunders WB; 1999. p. 750–78.
31. Kim YM, Wang MH, Rhee HI. A novel alpha-glucosidase inhibitor from pine bark. Carbohydr Res 2004;339:715–7.
32. Tonmoy Ghosh, Khushbu Bhayani, Chetan Paliwal, Rahulkumar Maurya, Kaumeel Chokshi, Imran Pancha, *et al.* Cynobacterial pigments as natural anti-hyperglycemic agents: an *in vitro* study. Front Marine Sci 2010;3:1-10.

33. Frier BM, Fisher M. Diabetes mellitus. In: Boon NA, Colledge NR, Walker BR, Hunter JAA. Ed. Davidson's principle and practice of medicine. 20th ed. (Churchill Livingstone Elsevier: Ediburgh; 2006. p. 805-45.
34. Puls W, Keup U, Krause HP, Thomas G, Hoffmeister F. Glucosidase inhibition. A new approach to the treatment of diabetes, obesity, and hyperlipoproteinaemia. Naturwiss 1997;64:536.
35. Davis SN, Granner DK. Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas. In: Brunton LL, Lazo JS, Parker KL. Ed. Goodman and Gilman's: The pharmacological basis of therapeutics. 11th ed. (McGraw-Hill Medical Publication Division: New York; 2001. p. 1706-7.
36. Conforti F, Statti G, Loizzo MR, Sacchetti G, Poli F, Menichini F. *In vitro* antioxidant effect and inhibition of alpha-amylase of two varieties of *Amaranthus caudatus* seeds. Biol Pharm Bull 2005;28:1098-1102.
37. Elumalai Arunkumar, Saravanan Bhuvaneswari, Carani Venkatraman Anuradha. An intervention study in obese mice with astaxanthin, a marine carotenoid-effects on insulin signaling and pro-inflammatory cytokines. Food Func 2012;3:120-6.
38. Bhuvaneswari S, Arunkumar E, Viswanathan P, Anuradha CV. Astaxanthin restricts weight gain, promotes insulin sensitivity and curtails fatty liver disease in mice fed an obesity-promoting diet. Process Biochem 2010;45:1406-14.
39. Hussein G, Nakagawa T, Goto H, Shimada Y, Matsumoto K, Sankawa U, *et al.* Astaxanthin ameliorates features of metabolic syndrome in SHR/NDmcr-cp. Life Sci 2007;80:522-9.
40. Suganya V, Asheeba ST. Microencapsulation of astaxanthin using ionotropic gelation method isolated from three crab varieties. Int J Cur Pharm Res 2015;7:96-9.

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