Original Research Article

Antiglycation and fatty acids profiling in response to Phyocyanin extracted from *Chlorophyta Ulva lactuca* algae loaded on albumin nano-particles (ULANP) in diabetic rats.

Abstract

Aim: This study evaluated the effect of Phyocyanin extracted from *Chlorophyta Ulva lactuca* algae loaded on albumin nano-particles (ULANP) on diabetic rats . **Materials and methods:** Fifty albino rats were divided into 5goups. GPI: control and GPII: rats were injected with alloxan (75 mg /kg) *i.p* for six consecutive days for induction of diabetes. This group was subdivided into 4 subgroups: GP IIa: (Untreated diabetic): GP IIb: rats were given with ULANP (100 mg/kg).GP IIc: Rats were given ULANP (200 mg/kg) *i.p*. GP IId: Rats were given insulin (100 unit/ day). Serum NO, interleukin-6 glucose, AGEs and fatty acids profile was determined. **Results:** Analysis of ULANP by FTIR showed the characteristic band (2100cm-1~ 3700 cm-1) that is indicated mainly from -COO, – CO and conjugated double bond. These bonds showed spectral bands peak 2985 cm-1and 2860 cm-1, 2986cm-1. Administration of ULANP in diabetic rats exerted an anti-inflammatory by lowering NO and IL-6 levels and hypoglycemic effects by decreased glucose and reduced AGEs levels. In addition, ULAPN lowered percent of saturated fatty acids while elevated unsaturated fatty acids percent. **Conclusion:** It was concluded that, ULAPN is a promising effective anti-inflammatory and hypoglycemic agent compared with other therapeutic agents with lower site effects.

Key words: Antiglycation- fatty acids - phyocyanin - *Chlorophyta Ulva lactuca* algae nano-particles - rats.

1. Introduction

Diabetes mellitus is considered as the most medical problem worldwide. Most researches focus on the reduction of prevalence of the condition by early predication or minimize its complications that increase morbidity and mortality [1]. Metabolomics is a new research trend to discover different metabolites in different cases (normal and diseases) in biological fluids to use as diagnostic or prognostic or follow up therapeutic protocol [2]. This is allowing an accurate and rapid identification about that disease. Metabolomic strategies present several practical advantages, high throughput and fully automated [3]. Metabolomics profiling of diabetes relate to two different stages of the disease in order to see whether metabolomics profiling might be an early diagnostic and prognostic biomarkers for diabetic. The life styles and food type could affect the metablomic profiling and hence can be monitored.

Nanoparticles syntheside from natural products are of pharmaceutical importance as they will impact human life. *Chlorophyta Ulva lactuca* algae was found to produce a wide variety of bioactive compounds. Antioxidant, antiproliferation and food supplemts. These active compounds like phyocanin have been characterized to have pharmacological and ecological importance [4].

Phyocyanin is a water soluble pigment present in different types of algae. It is an accessory pigment for chlorophyll. All phycobiliproteins are water-soluble, so they possess biological activities as anticancer and antioxidant. The Saudi coast line in Red Sea contains different species of marine algae [5]. However; there are a few studies on the biological effects of the marine algae in this region. This study was designed to explore ant- diabetic of the *Ulva lactuca* algae extract effect of *Chlorophyta Ulva lactuca* algae loaded on albumin nano-particles (ULANP) as promising drug for chemotherapy. In this study we determined serum

fatty acids profile that could be used as a biomarker for the chronic disease but substantially for those which are behind or associated with the disease.

2. Materials and Methods

Samples of Chlorophyta *Ulva lactuca* algae was collected at depths of about 25–150 cm from Red Sea at Jeddah. Samples were identified by Stuff member of marine biology department at King Abdulaziz University, stored in -20°C till analysis.

2.1. Preparation of algae extract

The Chlorophyta *Ulva lactuca* samples were air-dry at room temperature, and was grounded to powder with glass homogenizer. Hundred grams of the sample was extracted by 500 ml methanol for 4 hours at 65°C, then, evaporated by rotary vacuum pump. The residue was washed with distilled water and stored at -20°C till use [6].

2.2. Preparation and characterization of of *Ulva lactuca* algae extract loaded on albumin nanoparticles (ULANP).

Ulva lactuca algae extract loaded on albumin nanoparticles (ULANP) was prepared by dissolving 10 mg of extract in 4 ml of 10 mM NaCl with continous stirring, then add ethanol dropwise until the solution become turbid. 100 µl of glutaraldehyde (10%) was added to enhance particle cross linking with stirring the suspension for 24 hrs at 4 °C. The obtained nanoparticles was ultrafilterated by centrifugation (25000×g, 10 min), 5 times and the pellet were dispersed in 10 mM NaCl at pH values of 7.5 . Each redispersion step was performed in an ultrasonication bath [7].Nanoparticles will be characterized by Infrared spectra (FT-IR/NICOLET–ESPN670).

2.3. Identification of methanol algae extract by GC/MS

Methanol extract of algae was dissolved in hexane 95 % and identified by GC/MS 5975 (Agilent, CA, USA) system including Agilent 7890 A gas chromatography. In hexane solvent: weight approximate 1.0 mg of extracted algea samples and dissolved in 1 mL of hexane in 2 ml amber LC vials and were kept at -10 °C until analysis via gas chromatography mass spectrometry (GC-MS)

2.4. Experiment animals design

Handling of animals study was done according to KAU reglations, Jeddah. Fifty male albino rats were included in this study divided into 5 group I: control and Group II injected alloxan (75 mg /kg) i.p. for six consecutive days for induction of diabetes . This group was subdivided into: GP IIa: (Untreated diabetic): GP IIb: rats was give orally with L-ULANP (100 mg/kg).GP IIc: Rats was given with ULANP (200 mg/kg) ip. GP IId: Rats was given insulin (100 unit/ day). At the end of the experiment (6 weeks). Blood was collected directly from all groups. Serum was used for the determination of NO and interleukin-6 level by using ELISA kit and free fatty acids metabolites by Gas chromatography/ mass spectrum.

2.5 Gas chromatography mass spectrometry

Agilent GCMS 5975 (Agilent, CA, USA) system including Agilent 7890 A gas chromatography equipped with Agilent 5975C-VL MSD mass spectrometer with Agilent 7693 A automatic liquid sampler was used for analysis of plant extracts. The compound was identified via both electron impact ionization mass spectrum and modified retention indexes (programmed temperature n-alkane based retention index).

2.6. Statistical analysis.

Results were statistically analyzed using SPSS version 21, one-Way ANOVA, Post Hoc, LSD to compare between groups in the same time interval.

3.Results

Chemical analysis of methanol extract of ***Ulva lactuca*** algae by GC-MS reported in fig 1. The GC-MS analysis revealed the presence of saturated and unsaturated fatty acids. The major (Phyocyanin). Fig 2 showed a Analysis of ULANP by FTIR showed the characteristic band (2100cm-1~ 3700 cm-1) that is indicated mainly from -COO, – CO and conjugated double bond. These bonds showed spectral bands peak 2985 cm-1and 2860 cm-1, 2986cm-1.

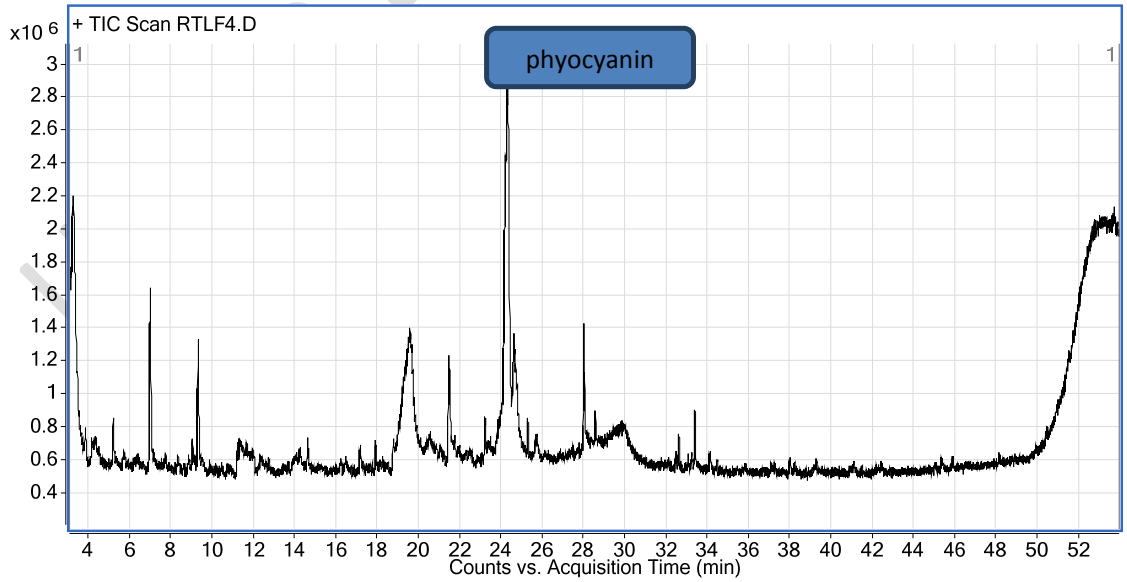

Fig. 1 GC/MS analysis of MEC showed that, measure RI at 1290 and reference RI at 1287 . This signal is specific for phyocyanin.

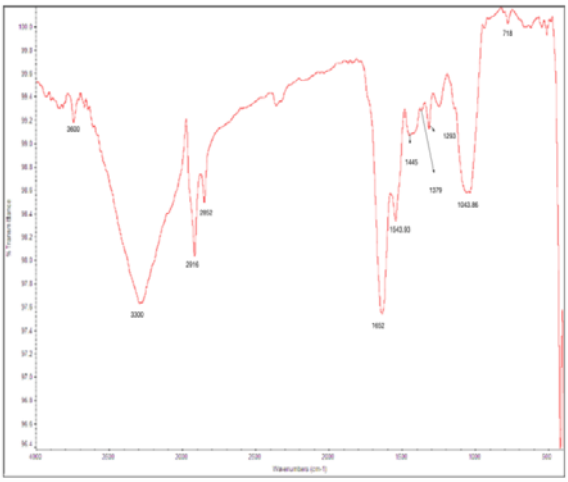

Fig. 2.FTIR spectrum for phyocyanin extract has transmittance maxima at 1652 .

Results in table 1 revealed that, the levels of inflammatory mediators NO and IL-1 were significantly elevated in comparison with control group ($p<0.01$ and <0.05) respectively. Treatment with ULANP (100 or 200 mg/kg) resulted in a significant reduction of their levels ($p<0.001$), the effect was dose depended. In addition, in diabetic group, increased formation of AGEs in comparison with to control group. While treatment with ULANP (100 or 200 mg/kg) showed a significant reduction of glucose and AGEs levels compared with untreated group.

Diabetic rats showed a significant increase in blood glucose and AGEs levels compared with the control group ($p<0.001$). Treatment of diabetic with either ULANP (100 or 200 mg/kg) resulted in a significant decrease in blood glucose and AGEs compared with the untreated diabetic animals ($p<0.001$) (Table1). The hypoglycemic effect exerted by ULANP (100 or 200 mg/kg) was dose dependent and better than that induced by insulin ($p<0.01$).

The identification of individual fatty acids were carried out by GC-MS and presented in Table (1). The peaks related to different fatty acids at different retention times (RT) were shown in Figure (1), some of these peaks were detected and identified, while others about (3.43%) were unidentified. Results in Table (1) showed that 11 fatty acids were identified and detected in the oil extracted from borage seeds, some of these fatty acids were saturated represented (13.26 % of total fatty acids) as Lauric, palmitic, stearic and arachidic acid, and the most predominant saturated fatty acid was palmitic acid (7.64%). Seven unsaturated fatty acids were detected and identified including palmitoleic, oleic, linoleic, γ-linolenic, brassidic, erucic and nervonic acids, total unsaturated fatty acids represented (83.31 % of total fatty acids composition). The unsaturated linoleic acid represented the majority of total fatty acids composition (34.23%) followed by γ-linolenic (24.79 %) and oleic acid (

Table 1. Levels of serum NO,IL-6, Glucose and AGEs in all studied groups (Mean ±SD).

Groups	NO µ mol/dl	IL-6 µg /dl	Glucose mg/dl	AGEs µg/mg protein
Group I Mean ± S.D.	40.7 ± 3.8	12 ± 1.6	80 ± 1.7	11 ± 1.54
Group IIa Mean ± S.D.	262 ± 8 [a]	123.6 ± 11 [a]	312 ± 35 [a]	73 ± 6.8 [a]
Group IIb Mean ± S.D.	152 ± 9 [a, b]	99 ± 9 [a, b]	231 ± 22.4 [a, b]	42 ± 2.6 [a, b]
Group IIc Mean ± S.D.	63 ± 8 [a, b]	65 ± 3.1	149 ± 5.6 [a, b]	31 ± 2.0 [a, b]
Group IId Mean ± S.D.	71 ± 9	45± 2.2	101 ± 6.6	29 ± 1.3

([a] p value, all groups vs control; b : p value, treated vs untreated) .

Table (2): Serum fatty acids methyl esters as a percentage of total fatty acids in different studied groups.

Common Name	RT (min)	GPI	GPIIa	GPIIb	GPIIc	GPIId
Palmitic acid	29.66	9.64	13.64	7.64	7.64	7.64
Palmitoleic acid	30.15	8.25	14.25	6.25	6.25	6.25
Stearic acid	30.46	5.08	13.08	3.08	3.08	3.08
Oleic acid	31.08	15.21	10.23	14.23	14.23	14.23
Linoleic acid	32.58	35.10	22.23	34.23	34.23	34.23
γ- Linolenic acid	34.11	22.79	14.79	24.79	24.79	24.79
Arachidic acid	37.10	3.4	2.4	1.4	1.4	1.4
Pentadecanoic acid	29.66	3.64	4.5	8.21	7.11	6.14
Palmitoleic acid	30.15	8.25	14.25	6.25	6.25	6.25
heptadecanoic acid	31.46	7.18	14.01	11.00	9.23	8.1

4. Discussion

Metabolomics is an emerging technology increasingly popular in epidemiology to capture broad-based information about large sample sets [8,9]. It was found that, the prepared ULANP exert potent hypoglycemic effect by lowering blood glucose level and AGEs in diabetic rats compared with untreated group. The treatment is dose dependent and approximately similar to insulin. the current study investigated the role of ULANP against protein glycation in diabetic rats. It was found that, these compounds reduced release of inflammation mediators, thereby delaying the progression to diabetic complications [10-14]. The action of these compounds on protein glycation (AGEs) is related to their suppression of free radicals release, they inhibit the oxidation and the subsequent formation of AGEs. This is due to presence of phyocyanin, it is being to be no toxic for human use. This anti-inflammatory agents used as traditional medicine for many diseases.

In current study, diabetic rats showed elevated levels of SFAs and reduced USFAs compared with control group. Rats treated with ULANP (100 or 200 mg) reversed this results . This is in accordance with previous study. The evidence of an association between saturated fatty acids (SFAs) and diabetes are discordant. The relationship between nine SFAs in the plasma with the risk of diabetes was investigated. The study revealed that even-chain SFAs (palmitic acid 16:0 and stearic acid 18:0) were positively correlated with the incidence of diabetes while odd-chain SFAs (pentadecanoic acid 15:0 and heptadecanoic acid 17:0) and longer-chain SFAs (arachidic acid 20:0, behenic acid 22:0, tricosanoic acid 23:0, and lignoceric acid 24:0) were inversely associated with diabetes. Obviously different SFAs have a differential correlation with metabolic risk of diabetes [15-20]. The metabolomic analysis observed that total SFAs, including palmitic acid (C16:0) and stearic acid (C18:0) and monounsaturated fatty acids (MUFAs), were significantly decreased in individuals exposed to an energy-restricted diet for the 8-week in overweight and obese older adults. Furthermore, palmitoleic acid (C16:1) was found to be a negative predictor of change in body fat loss. Total polyunsaturated fatty acids (PUFAs) significantly decreased, although the overall total amounts of PUFAs did not affected

[21-25]. The most specific significant association with diabetes is the variation in the of oleic and linoleic acids.

Conclusion:

It was concluded that, ULAPN is a promising effective anti-inflammatory and hypoglycemic agent compared with other therapeutic agents with lower site effects.

5.Acknowledgment

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