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ORIGINAL RESEARCH

Effects of *Spirulina platensis* microalgae on antioxidant and anti-inflammatory factors in diabetic rats

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Objectives: Lipid peroxidation and hyperglycemia are common signs for diabetes. Natural antioxidants such as *Spirulina platensis* microalgae (SPM) may prevent lipid peroxidation and hyperglycemia. This study aimed to evaluate the effects of SPM on antioxidant and anti-inflammatory in diabetic rats.

Materials and methods: Sixty-four rats were divided into eight groups (n=8) and orally treated with 0, 10, 20 and 30 mg/kg body weight of SPM extract. Experimental groups included diabetic rats fed with 0 (DC), 10, 20 and 30 mg/kg SPM. Healthy rats were treated with 0 mg/kg SPM (HC), 10 mg/kg SPM, 20 mg/kg SPM and 30 mg/kg SPM. At the end of the trial, blood samples were collected and the plasma concentrations of trace minerals (TMs), biochemical parameters, and antioxidant enzymes in liver were evaluated. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), TNF-α (tumor necrosis factor-alpha) and IL-6 (interleukin-6) were evaluated.

Results: Our findings showed that diabetes significantly lowered the plasma concentration of TMs and antioxidant enzymes in liver and also increased the levels of malondialdehyde, glucose, lipid profile, AST, ALT, TNF-α and IL-6 (DC vs HC). However, an oral supplement of SPM (20 and 30 mg/kg body weight) lowered levels of malondialdehyde level, glucose, lipid parameters, AST, ALT, TNF-α and IL-6. The same levels increased the plasma contents of zinc, iron, copper and selenium and activity of antioxidant enzymes ($P<0.05$).

Conclusion: It can be concluded that diabetes decreases TM concentration and antioxidant enzymes and also increases lipid profile, glucose, AST, ALT, TNF-α and IL-6 concentrations. Inclusion of SPM supplementing (20 and 30 mg/kg body weight) increased some TMs and antioxidant enzymes. SPM may provide TMs for synthesis of antioxidant enzymes which subsequently reduce lipid profile, glucose concentration and anti-inflammatory responses.

Keywords: antioxidant enzymes, diabetes, lipid profile, plasma selenium, *Spirulina platensis*

Introduction

Diabetes mellitus is a disease with multiple etiologies which has signs such as hyperglycemia and disturbances in carbohydrate, fat and protein metabolism. These disturbances are due to deficiencies in insulin secretion, insulin activity and/or both. In the long term, diabetes can cause problems such as disturbances in various organs. Some studies have shown a relation among trace elements (TMs), diabetes mellitus[1] and changes in nutrient metabolism.[2–5] Individuals with diabetes have reported to have inflammatory cytokines.[6] Some TMs such as chromium, magnesium, vanadium, zinc, manganese, molybdenum and selenium can proteinate insulin activity.[7] Zinc modulates insulin synthesis and storage and protects the structural integrity of insulin.[3] Magnesium has

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a critical role in phosphorylation reactions of glucose and its metabolism. Magnesium is involved in insulin resistance, carbohydrate tolerance, and dyslipidemia.[8] Chromium as a trace mineral improves insulin activity through enhancing intracellular signaling.[9] TMs are essential for some activities in the body and they affect the pathogenesis of diabetes mainly by their role in peroxidation and inflammation. In addition, it was reported that diabetes markedly elevated the serum level of glucose.[10] Montilla et al[10] stated that hyperglycemia can create injuries in tissues and the brain, which subsequently may increase glucose excretion. Hypertriglyceridemia and hypercholesterolemia are the most common lipid abnormalities in diabetes,[11] and are related to glucose intolerance.[12] Oxidative stress and reactive oxygen species production can decompose lipids and cause lipid peroxidation and lipolysis. Oxidative stress is dominant when antioxidant defense systems are defaulted.[13] Previous studies have shown reduced antioxidant enzyme activity in diabetic animals compared with control animals.[14] We believe that some functional foods, eg, *Spirulina platensis* microalgae (SPM), may improve levels of some TMs.[15] SPM lipopeptides have a major role in physiological functions, eg, cytotoxic, antitumor, antiviral, antibiotic, antimalarial, antimycotic, multidrug resistance reversing, antifeedant, herbicide, and immunosuppressive factors.[16] SPM, in a dose-dependent manner, decreased the metabolic activity of functional neutrophils, showing that SPM has anti-inflammatory activity.[17] It contains more iron, magnesium, copper and zinc than other TMs. Antioxidants can help to prevent diabetes or alleviate the negative effects of diabetes on blood parameters and inflammatory phase. It is well accepted that antioxidants inhibit lipid peroxidation and subsequently alleviate harmful effects of diabetes on lipids and glucose. We hypothesized that diabetes reduces TMs, and SPM supplementing increases the required elements for synthesis of antioxidants; on the other hand, antioxidants can prevent lipid peroxidation and overproduction of pro-inflammatory cytokines. Thus, the present study was conducted to investigate the antioxidant mechanism of SPM on TMs and its effects on blood biochemical and anti-inflammatory parameters.

Materials and methods
Animals
Sixty-four male Wistar rats, 200±20 g and 2.5 months of age at the start of the trial, were kept at room temperature (25°C) and under a light cycle of 12 hours light/12 hours dark for 5 weeks. All rats had ad libitum access to a standard pellet diet (Javaneh Khorasan Company, Mashhad, Iran) and water.

The pellet composition was as follows: 20% protein, 3% fat, 4% ash and 6% fiber. All experiments were approved by the Ethical Committee of IAU University, Kerman, Iran (IAUKB-1105). The experiments were conducted in accordance with the National Institutes of Health Guide (NIH Publication No. 85–23, revised 1996) for the Care and Use of Laboratory Animals.

Pilot study
After 24 hours fasting, 36 Wistar rats were divided into four groups (each group six animals) and animals orally received 0.3 mL distilled water, and SPM extract (20, 40 and 60 mg/kg of body weight). The animals were monitored in order to evaluate the behavioral responses, toxicity signs and mortality for 24 hours. No mortality or toxicity signs were observed; thus we used levels of 10, 20 and 30 mg/kg.

Experimental treatments
Streptozotocin (STZ; Sigma-Aldrich, St Louis, MO, USA) was used in order to induce diabetes mellitus in rats, as explained by Armstrong and Al-Awadi.[19] Diabetes was induced by intraperitoneal injection of STZ (55 mg/kg BW). It was first dissolved in citrate buffer (pH 4.5, 0.1 mol/L trisodium citrate, 0.1 mol/L citric acid). Diabetes was confirmed after measurement of blood glucose levels, three days after the STZ injection. Rats with plasma glucose level higher than 250 mg/dL were considered as diabetic. Glucose level was assessed using a kit from Pars Azmoon (Tehran, Iran). After induction of diabetes, rats were divided into two sections including healthy and diabetic. Animals were orally treated with microalgae (10, 20 and 30 mg/kg of body weight). Control rats were also treated with 0.3 mL distilled water. Each 10 mg of microalgae was diluted in 2 mL distilled water and then orally administrated. Experimental treatments were as follows. Healthy rats fed with SPM at 10, 20 and 30 mg/kg of body weight were marked as SH10, SH20, and SH30, respectively. Rats treated with SPM at 10, 20 and 30 mg/kg of body weight were marked as SD10, SD20, and SD30 respectively. Two healthy control (HC) and diabetic control (DC) groups were also considered. Therefore, animals were grouped into eight groups with eight rats per group. The SPM was purchased from Ghazaye Sabze Khalij Company (Bandar Abbas, Iran). The chemical composition of SPM is presented in Table 1. Chemical analysis of the SPM was done using the Association of Official Analytical Chemists (AOAC) method.[18] Calibrations of trace minerals were performed with a series of mixtures containing graded concentrations of standard solutions of the same trace mineral.

Table 1 Chemical composition of SPM

Components	Amount
Dry matter (%)	95
Ether extract (%)	5.3
Crude protein (%)	61.8
Crude fiber (%)	9.5
Ash (%)	6.9
Ca (mg/100 g)	500
P (mg/100 g)	800
Fe (mg/100 g)	90
K (mg/100 g)	1,235
Mg (mg/100 g)	195
Cu (mg/100 g)	20
Zn (mg/100 g)	15
Se (mg/100 g)	1
Cr (mg/100 g)	1

Abbreviation: SPM, *Spirulina platensis* microalgae.

Blood samples

At 35 days of trials, all rats were anaesthetized and blood samples were collected from the tail vein in order to measure the blood biochemical parameters and TMs. The blood samples were transferred into tubes containing heparin for measurement of blood biochemical parameters and TMs, respectively. The samples were centrifuged at 2,000 g for 5 minutes and plasma was separated. Blood parameters were determined by the Pars Azmoon kit. The samples were analyzed using commercial kit (Pars Azmoon) in order to measure the triglycerides, glucose, cholesterol and low-density lipoprotein (LDL). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed by auto-analyzer apparatus (Gesan Production Srl, Campobello di Mazara (TP), Italy). The samples were evaluated for the concentration of TNF-α (tumor necrosis factor-alpha) and IL-6 (interleukin-6) by specified kits of Abcam (Cambridge, UK), as recommended by manufacturer's instructions. Levels of plasma TMs were evaluated by atomic absorption spectrophotometer. Calibrations of trace minerals were performed with a series of mixtures containing graded concentrations of standard solutions of the same trace mineral.

Activity of antioxidant enzymes and malondialdehyde (MDA)

After blood sampling, animals were anesthetized by ketamine. The liver was separated and liver tissues were washed in ice-cold 0.9% saline (w/v), then weighed and stored at –70°C. Activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) and also levels of MDA were measured as previously explained by Bukan et al.[14]

Statistical analysis

The data were analyzed by GraphPad Prism statistical software (GraphPad Software, Inc., La Jolla, CA, USA) and the data were analyzed by one-way analysis of variance (ANOVA) with Dennett's multiple comparison posttest. Treated and non-treated rats in each section were compared by Dennett's multiple comparisons posttest. Healthy rats were compared with diabetic rats in the same group by Student's *t*-test. For example, control healthy rats were compared with control diabetic rats and data are presented in tables. The results are presented as mean±SD. A value of *P*<0.05 was considered to be statistically significant.

Results

Our findings in Table 2 showed that diabetes reduced the plasma concentration of TMs (*P*<0.05: DC vs HC), but oral supplementing with SPM (20 and 30 mg/kg body weight) significantly increased the plasma concentrations of zinc, iron, selenium and copper (*P*<0.05). Treatment with SPM did not improve other TMs. The plasma concentrations of TMs were not influenced by SPM supplementing in healthy rats (*P*>0.05). Results (Tables 3 and 4) showed that diabetes significantly increased the plasma concentrations of glucose, triglycerides, cholesterol, LDL, ALT, AST, TNF-α and IL-6. However, rats treated with SPM (20 and 30 mg/kg body weight) showed the lower plasma concentrations of glucose, triglycerides, cholesterol, LDL, ALT, AST, TNF-α and IL-6 compared with DC. Our findings indicated that diabetes lowered activity of antioxidant enzymes and increased MDA levels compared with HC (*P*<0.05). Similar to the above findings, oral supplementing with SPM at levels of 20 and 30 mg/kg body weight increased activity of antioxidant enzymes and lowered MDA levels (*P*<0.05).

Discussion

Our findings showed that diabetes significantly lowered the plasma concentration of TMs. Comparing the data for HC and DC groups showed that diabetes decreases the plasma concentrations of TMs. The data for TMs were as follows: for zinc (58.50±2.07 vs 35.75±1.66), iron (28.10±2.07 vs 15.75±1.66), magnesium (48.50±3.07 vs 36.75±3.32), copper (28.38±0.74 vs 16.50±1.85), selenium (13.38±0.51 vs 8.00±0.75), and chromium (23.38±0.75 vs 18.28±0.51). Studies have shown that deficiencies in zinc and chromium levels may cause glucose intolerance and insulin resistance.[20,21] Zinc is an essential cofactor for SOD, which acts as an intracellular antioxidant enzyme. It has been suggested that pancreatic beta cells have low antioxidative enzyme activities which might be more sen-

Table 2 Effect of SPM at different levels on TMs concentration (µmol/L)

	Zinc	Iron	Magnesium	Copper	Selenium	Chromium
Groups						
HC	58.50±2.07$_a$	28.10±2.07$_a$	48.50±3.07$_a$	28.38±0.74$_a$	13.38±0.51$_a$	23.38±0.75$_a$
SH10	57.38±1.40$_a$	27.38±1.40$_a$	47.38±1.40$_a$	28.13±0.64$_a$	13.38±0.53$_a$	23.42±0.51$_a$
SH20	57.13±1.24$_a$	27.13±1.24$_a$	47.13±1.24$_a$	27.50±0.53$_a$	13.38±0.74$_a$	23.25±0.71$_a$
SH30	58.00±1.19$_a$	28.00±1.19$_a$	48.00±1.19$_a$	28.00±1.19$_a$	13.38±0.62$_a$	23.52±0.93$_a$
DC	35.75±1.66$_c$	15.75±1.66$_c$	36.75±3.32$_b$	16.50±1.85$_c$	8.00±0.75$_c$	18.28±0.51$_b$
SD10	36.75±1.38$_c$	16.75±1.38$_c$	35.25±4.86$_b$	17.25±1.28$_c$	8.20±0.71$_c$	18.78±0.42$_b$
SD20	45.38±1.91$_b$	23.75±1.90$_b$	38.50±2.07$_b$	22.50±1.30$_b$	9.75±1.28$_{bc}$	18.58±0.91$_b$
SD30	46.00±2.07$_b$	25.00±3.38$_a$	36.38±3.07$_b$	23.75±1.75$_b$	11.13±0.83$_b$	13.38±0.51$_b$
P-value	***	***	***	***	***	***

Notes: Subscripts (a–c) show significant differences among groups per column and *** shows significant differences at *P*<0.001.
Abbreviations: SPM, *Spirulina platensis* microalgae; TM, trace minerals.

Table 3 Effect of SPM at different levels on some lipid profiles and glucose concentration (mg/dL) and liver enzymes (U/L)

	Triglycerides	Cholesterol	LDL-C	Glucose	AST	ALT
Groups						
HC	38.70±3.07$_c$	70.15±8.50$_c$	32.60±5.07$_c$	135.20±11.50$_c$	98.20±11.50$_c$	85.20±7.50$_c$
SH10	39.38±2.45$_c$	68.20±7.40$_c$	34.50±3.40$_c$	141.20±12.50$_c$	97.10±10.50$_c$	83.20±5.15$_c$
SH20	37.23±2.24$_c$	69.50±6.50$_c$	35.50±2.44$_c$	139.80±10.90$_c$	98.10±10.50$_c$	82.10±7.15$_c$
SH30	38.20±2.29$_c$	67.50±5.15$_c$	38.00±3.55$_c$	135.70±9.40$_c$	99.10±11.50$_c$	84.40±9.15$_c$
DC	68.78±4.76$_a$	108.5±10.66$_a$	65.10±5.31$_a$	279.00±18.40$_a$	310.10±17.50$_a$	145.40±12.14$_a$
SD10	66.75±3.38$_a$	99.50±9.61$_a$	63.21±3.66$_a$	269.50±14.50$_a$	298.10±17.50$_a$	149.40±9.14$_a$
SD20	55.38±2.90$_b$	70.50±7.10$_b$	45.50±3.07$_b$	205.20±15.70$_b$	265.10±15.50$_b$	119.40±9.14$_b$
SD30	54.10±2.07$_b$	68.10±5.15$_b$	42.61±5.07$_b$	195.50±11.50$_b$	268.10±17.30$_b$	121.40±7.14$_b$
P-value	***	***	***	***	***	***

Notes: Subscripts (a–c) show significant differences among groups per column and *** shows significant differences at *P*<0.001.
Abbreviations: SPM, *Spirulina platensis* microalgae; TM, trace minerals; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 4 Effect of SPM at different levels on liver SOD (U/mg protein), GSH-Px (U/mg protein), CAT (K/mg protein), MDA levels (nmol/g tissue) and pro-inflammatory cytokines (pg/mL)

	SOD	GSH-Px	CAT	MDA	TNF-α	IL-6
Groups						
HC	30.50±2.25$_a$	10.50±1.50$_a$	0.34±0.05$_a$	22.50±1.50$_c$	5.10±0.50$_c$	4.50±0.40$_c$
SH10	29.45±1.45$_a$	11.20±1.40$_a$	0.33±0.06$_a$	21.50±1.70$_c$	5.30±0.60$_c$	4.80±0.50$_c$
SH20	28.25±1.25$_a$	10.50±1.50$_a$	0.34±0.02$_a$	23.50±1.90$_c$	5.20±0.15$_c$	5.10±0.50$_c$
SH30	28.80±1.30$_a$	10.80±1.10$_a$	0.35±0.05$_a$	24.10±2.40$_c$	5.10±0.4$_c$	5.50±1.00$_c$
DC	21.80±2.75$_c$	7.50±1.50$_b$	0.19±0.01$_c$	29.50±2.40$_a$	10.50±0.50$_a$	10.50±1.00$_a$
SD10	22.75±2.35$_c$	8.10±0.90$_b$	0.18±0.04$_c$	28.70±1.50$_a$	12.30±0.70$_a$	9.60±1.30$_a$
SD20	26.45±1.90$_b$	9.50±2.10$_a$	0.26±0.04$_b$	25.50±1.10$_b$	8.50±0.50$_b$	7.50±1.10$_b$
SD30	26.40±1.50$_b$	10.50±0.90$_a$	0.27±0.02$_b$	25.10±0.90$_b$	8.10±0.70$_b$	7.50±0.80$_b$
P-value	***	***	***	***	***	***

Notes: Subscripts (a–c) show significant differences among groups per column and *** shows significant differences at *P*<0.001.
Abbreviations: SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MDA, malondialdehyde; TNF-α, tumor necrosis factor-alpha.

sitive to free radicals, especially in zinc deficiency.[22] Similarly, chromium has a role as a modulator in diabetes,[23] glucose intolerance,[24] and corticosteroid-induced diabetes.[25] Magnesium is a trace element involved in glucose homeostasis, so there is a positive correlation between magnesium and glucose metabolisms. Magnesium has an important role in insulin action, and insulin stimulates magnesium uptake in insulin-sensitive tissues. Intracellular magnesium deficiency may affect the development of insulin resistance and alter the glucose entry into the cell. Magnesium is required for both proper glucose utilization and insulin signaling.[26] Metabolic alterations in cellular magnesium, which may play the role of a second messenger for insulin action, contribute to insulin resistance.[26,27] Selenium plays a main role in the antioxidant system by participation in glutathione peroxidases, thioredoxin reductase, and iodothyronine deiodinase. In view of

its important function in protection against oxidative stress, selenium was suggested to play a protective role against diabetes.[28,29] All the mentioned studies clearly state modulation of TMs in diabetes. Our findings also showed decreased levels of TMs during diabetes. As mentioned, TM deficiency influences diabetes mellitus, but TMs may help patients with diabetes mellitus. However, oral supplementing with SPM (20 and 30 mg/kg body weight) increased the plasma concentrations of zinc, iron, copper and selenium. The reason why SPM at high levels increases levels of zinc, iron, copper and selenium is unknown. SPM may help to more absorption of TMs which may increase TMs concentration in plasma.

Diabetes increased the plasma concentrations of cholesterol, triglycerides, LDL-C and glucose. Comparing the data for HC and DC shows the differences clearly. The data were as follows: for cholesterol (70.15±8.50 vs 108.5±10.66), triglycerides (38.70±3.07 vs 68.78±4.76), LDL-C (32.60±5.07 vs 65.10±5.31), glucose (135.20±11.50 vs 279.00±18.40), ALT (85.20±7.50 vs 145.40±12.14) and AST (98.20±11.50 vs 310.10±17.50). Previous reports showed that STZ markedly increased the serum level of glucose.[10] Montilla et al[10] have reported that hyperglycemia can cause injuries in tissues and brain which subsequently may increase glucose excretion. Hypertriglyceridemia and hypercholesterolemia are known as the most common lipid abnormalities in diabetes[11] and are related to glucose intolerance.[12] It is well known that oxidative stress and reactive oxygen species production can degrade lipids and cause lipid peroxidation and lipolysis. Oxidative stress contributes to many pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia and diabetes.[13] Studies have reported that diabetes increases levels of ALT and AST in serum[30] and kidney.[31] Okada et al[32] have reported that AST activity was significantly lower in diabetic rat tissues, because of inactivation of cytosolic AST in the diabetic rat tissues by a glycation reaction and/or by faulty glucose utilization in STZ induced diabetes. In contrast to our findings, Khan et al[33] have reported that dietary inclusion of microalgae did not change ALT and AST concentration in male rats. Our findings showed that higher levels of SPM decreased the plasma concentrations of cholesterol, triglycerides, LDL-C, glucose, ALT and AST. The oxygen species, as oxidants, are responsible for increased lipids and glucose. Antioxidants may interact with oxidants and thus alleviate their negative effects on lipid parameters, glucose concentration and liver enzymes. MDA is an index for lipid peroxidation and our findings showed (Table 4) that it was significantly decreased in SD20 and SD30. As Table 4 shows, oral supplementing with SPM at higher levels increased antioxidant enzymes in liver. It seems that the increased levels

of antioxidants interact with oxidants and partly alleviate negative effects of diabetes on lipids, glucose and liver enzymes. We believed that higher levels of SPM may provide essential TMs for synthesis of antioxidant enzymes, and, on the other hand, the increased levels of antioxidants decrease the plasma concentrations of glucose, lipid parameters, and liver enzymes and MDA. The role of some TMs in lowering glucose levels may be another reason for the reduced glucose, because higher levels of SPM increased some TMs. Interestingly, SPM had no significant effect on TM levels, antioxidant levels and blood biochemical parameters in healthy rats. In addition, our findings showed that SPM (20 and 30 mg/kg body weight) significantly alleviated adverse effects of diabetes on TNF-α and IL-6. These cytokines play significant roles in the pathogenesis of diabetes. It has been reported that diabetes increases levels of inflammatory cytokines.[6] Overproduction of ROS increases oxidative stress and inflammatory responses.[34] A study has shown that SPM reversed increased proinflammatory cytokines in the cerebellum, such as TNF-α and TNF-β.[17] It seems that SPM shows anti-inflammatory effects by reducing ROS. This means that SPM decreases pro-inflammatory cytokines by antioxidant properties. Our findings showed that SPM increased anti-inflammatory responses as it increased antioxidant properties, implying a positive correlation between antioxidant and anti-inflammatory properties in SPM. In summary, an overdose of TMs may have negative effects on animals' heath; however, we did not observe toxicity effects of SPM at these levels on animals.

Conclusion

Our findings showed that diabetes significantly decreased the plasma concentration of TMs and antioxidant enzymes and also increased the plasma concentration of glucose, lipid parameters, MDA, ALT, AST, TNF-α and IL-6. Our results showed that oral supplementing with SPM, at high levels (20 and 30 mg/kg body weight), increased the plasma concentration of zinc, iron, selenium and copper. The higher levels of SPM (20 and 30 mg/kg body weight) also increased the plasma antioxidant enzymes and also lowered the plasma concentration of glucose, lipid parameters, MDA, ALT, AST, TNF-α and IL-6. It seems that oral supplementing of SPM (20 and 30 mg/kg body weight) may supply essential TMs for synthesis of antioxidant enzymes; on the other hand, increased levels of antioxidants enzymes can decrease MDA and the plasma concentration of lipids, glucose, MDA, ALT, AST, TNF-α and IL-6. It can be suggested to use SPM at higher levels as a supplement for treatment of diabetes. Since levels of 20 and 30 mg/kg had similar effects on mentioned parameters, we recommend using 20 mg/kg, because of

the lower cost. In addition, TMs have higher costs and may have toxicity effects in comparison to SPM. Thus, it can be recommended to use SPM instead of TMs.

Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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