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Research Article

Effect of Ishige Okamurae Algae Extract on Omentin-1 Gene, IL-6 Gene, Glucose, Insulin, and Lipid Profile in Diabetic Rats

Samia Mahdi Ahmed[1*], Maha Ibrahim[2], Ammar A.Bayoumi[2]

[1] Taibah University, Saudi Arabia
[2] MSF. Medical Scientific Foundation for Research& Development

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***Corresponding author:**
Samia Mahdi Ahmed
E: smmohammed@taibahu.edu.sa

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ABSTRACT

Diabetes mellitus is one of the most common diseases worldwide. Synthetic medicines usually have side effects; thus seeking alternatives is preferable. Ishige Okamurae Algae might act as a hypoglycemic agent beside its other bioactive effects. In the present study, the effect of IO on diabetic rats was studied concerning Omentin-1 gene, IL-6 gene, glucose, insulin, triglycerides, total cholesterol, LDL, HDL. Ishige Okamurae was extracted and fractionated. Twenty-four male Wistar rats were divided into 4 groups. Group 1 served as control, GRP 2 served as untreated diabetic group. GRP 3 non Diabatic were administered with ethanol extract of IO at a dose of 250 mg/kg B.W , GRP 4 were diabetic and received an oral dose of 250 mg/kg Body wight (BW) of ethanol extract of Ishige Okamurae.

At the end of the treatment period, animals were euthanized under anesthesia. Blood was withdrawn from the abdominal aorta and the adipose tissues were removed and stored at −80°C until use.

RNA was extracted from the frozen adipose tissues to study the effect of IO on both Omentin-1, and IL-6 gene. Relative expression in omentin and lL-6 mRNA levels were calculated. The current study revealed that IO Algae plays a role in increasing blood insulin level, decreasing blood glucose, lowering cholesterol, decreasing triglycerides, decreasing LDL, increasing HDL, suppressing the expression of IL-6 gene, thus, reducing the harmful prolonged inflammatory effect of IL-6, whereas increasing the expression of omentin-1 gene increases sensitivity to insulin. Therefore, it may be recommended to use this algae to treat diabetes and reduce its complications.

1. INTRODUCTION

Diabetes mellitus (DM) is a medical condition that is characterized by hyperglycemia (high blood sugar) arising from the lack of insulin release or its action and has been referred to as a major public health concern of the 21st century (Atkins & Zimmet, 2010). A number of pathological processes are associated with DM, and this includes the autoimmune pancreatic β-cells destruction, leading to insufficient insulin production and the body's resistance to insulin action (Association, 2014). The excessive increase in the blood sugar levels seen in diabetic patients is linked with a long-term negative effect such as failure or damage to key organs like the kidney, eye, and heart (Association, 2014).

With the growing concern in the rise of the cases of DM, an estimated 380 million individuals have been projected to be diabetic by the year 2025(Atkins & Zimmet, 2010).The increased prevalence in the number of DM cases has a negative impact not only on the life of DM patients but also on the cost of health care (Wang, Wang, & Chan, 2013).

The currently available treatment options for DM include regular exercise, a balanced diet, hypoglycemic drugs, and insulin injection (Asante, 2013). Despite the advances in medical and pharmaceutical science and technologies, there is still a setback in the treatment of DM. For example, most diabetic drugs' ability to maintain an optimal glycemic level is less than 45% (Agarwal, Jadhav, & Deshmukh, 2014) and cannot be sustained for a long duration (Valerón & de Pablos-Velasco, 2013). Furthermore, most pharmacological diabetic drugs are associated with weight gain, heart problems, bone loss and are not safe for pregnant women (Valerón & de Pablos-Velasco, 2013).

However, natural medicines have shown great potentials in their usage as Hypoglycemic drugs (El-Kaissi & Sherbeeni, 2011). They are less toxic, cheaper with considerably lesser side effects when compared to pharmacological drugs. They can thus be considered as an alternative to normal pharmacological drugs. The long-term utilization of these natural medicines could be useful in the treatment of DM and the amelioration of its associated medical complications (Prabhakar & Doble, 2011; Wang et al., 2013).

Marine Algae are considered a prolific source of important bioactive compounds that aid in maintaining normal health and mitigating disease risks. Among the marine Algae, brown Algae and its constituent phlorotannins are widely studied globally for various biological effects by several research groups. Ishige Okamurae is an edible brown algae found abundantly in the coastal areas of Jeju Island in Korea,. It has been reported that IO exerts several biological activities, such as anti-α-glycosidase, free-radical scavenging, cytoprotective, anti-obesity, and anti-inflammatory activities Diphlorethohydroxycarmalol (DPHC). Ishige Okamurae classified into brown Algae was effective in inhibiting the production of inflammatory mediators such as IL-6 and enhance omentin gene. (Heo, S.-J.,2008 , Thomas, N.V.et al.,2011, Costa, P.Z. et al ., 2013 and Giribabu, N. et al.,2015).

Omentin is an adipokine secreted and preferentially expressed in the visceral adipose tissue (Yang et al., 2006). Several previous reports have revealed the importance of omentin in controlling metabolism and the body's response to insulin. It has also been reported to play a critical role in inflammation and could help in alleviating diabetes and the risk of developing cardiovascular disease in an AMP- and mitogen-activated protein kinase, Akt, and NF-κB pathways (Zhou, Chan, & Zhou, 2014). Omentin is found in circulation as omentin-1. Previous studies have shown the association between the pathogenesis of DM and insulin resistance with plasma omentin level (Yoo et al., 2011). In addition, its reduced expression has been found in several metabolic syndromes, including DM, insulin resistance, and obesity (Zhou et al., 2014). Thus, the insulin resistance-reducing potentials of omentin coupled with its anti-inflammatory effects offer a promising prospect for its use as a therapeutic agent for treating metabolic syndrome (Zhou et al., 2014). Therefore, in this study, the potentials of Ishige Okamurae to increase the expression of the omentin-1 gene in adipose tissue of streptozotocin-induced diabetic rats was investigated.

2. MATERIALS AND METHODS

2.1 Extraction of Ishige Okamurae

Briefly, 50% ethanolic extract of IO was fractionated using centrifugal partition chromatography (CPC 240, Tokyo, Japan) and further purified using semi preparative HPLC column (YMC-Pack ODS-A; 10 mm × 250 mm, 5μm) to obtain Isopropyl alcohol The identity of Isopropyl alcohol (99% of purity) was verified using MS fragmentation of *m*/*z* 1986.26 using ultrahigh resolution Q-TOF LC-MS/MS coupled with an electrospray ionization (ESI) resource (maXis-HD; Bruker Daltonics, Breman, Germany) According to a previously validated method (Ryu, B.; Jiang, Y., 2018).the IO extract used in this study had 1.81% ± 0.362 Isopropyl alcohol.

2.2 Experimental Animals

Twenty-four (24) male Wistar rats weighing between 180 and 200 g were purchased from the animal house of King Fahd Medical Centre, King Abdulaziz University, Jeddah, Saudi Arabia. The rats were housed at the Preclinical unit, King Fahd Medical Centre, King Abdulaziz University, and acclimatized for one week at an ambient temperature of 22°C and a 12 hr dark/12 hr light cycle. During this period, the animals were maintained on a normal diet with free access to water. This study was conducted following the guidelines of the United States National Institute of Health's revised Guide for the Care and Use of Laboratory Animals and was approved by the Ethics Committee, College of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

2.3 Induction of Diabetes

Following an overnight fast, animals were administered with an intraperitoneal injection of streptozotocin (STZ) (dissolved in 0.1 M citrate buffer (pH 4.5)), at a dose of 50 mg/kg bw. After one week, the fasting blood sugar levels of the animals were checked using a glucometer, and animals found to have fasting blood glucose level greater than 250 mg/dl were taken as being diabetic used subsequently in the study. Following this, animals were administered orally with the ethanolic extract of IO at a dose of 250 mg/kg/day for 30days.

2.4 Doses and samples collections

The twenty-four male Wistar rats were divided into four groups with six animals in each group. Group (GRP) 1 served as the control group, while GRP 2 served as the untreated diabetic group. GRP 3 non Diabatic animals were administered with the ethanolic extract of IO at a dose of 250 mg/kg B.W. Finally, animals in GRP 4 were diabetic and received an oral dose of 250 mg/kg bw of ethanolic extract of IO.

At the end of the treatment period, the animals were placed on overnight fast and euthanized under diethyl ether anesthesia, after which blood was withdrawn from the abdominal aorta and the adipose tissues were removed and then stored at −80°C until use.

2.5 Measurement of Insulin, blood glucose, level, and biochemical parameters

To obtain serum, whole blood was centrifuged at 3000 rpm for 10 min at 4ºC. The serum level of insulin was quantified using an ELISA kit (Abcam, UK). Serum glucose level was measured using a glucometer. Furthermore, the serum levels of low-density lipoprotein

(LDL), high-density lipoprotein (HDL), total cholesterol (TC), and triglycerides (TG) were measured using commercially available kits (BioVision, USA) following the manufacturer's protocol.

2.6 RNA extraction and real-time quantitative polymerase chain reaction

Following homogenization, the total RNA was extracted from the frozen adipose tissues by column method (Bio Basic, Canada). Tissue from three samples was pooled for each biological replicate, and the concentration was determined by an ultraviolet spectrophotometer (PCR max Lambada, Japan). Complementary DNA synthesis was performed with 500 ng RNA from each sample using Prime Script Reverse Transcript Reagent Kit (TaKaRa Inc., Japan). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was carried out to quantify the expression of omentin (as a target gene), compared with Beta 2 Micro globulin (B2M) (as a reference gene) using SYBR Premix Ex Taq II (TaKaRa Inc., Japan) by Rotor-Gene 6000 qPCR machine (Qiagen, Germany). The specific primers for IL-6 & omentin & B2M were 5′-GGTACATCCTCGACGGCATCT-3′; IL-6 reverse primer: 5′-GT GCCTCTTTGCTGCTTTCAC-3′, IL-6 probe: 5′-FAM-TGTTAC TCTT-GTTACATGTCTCCTTTCTCAGGGCT-TAMRA-3 & F-5′-GCTGAAGAGAACCTGGAC-3′ and R-5′ AA-TAGAGACCATCTTGTGC-3′, F-5′-CTTCAG-CAAGGACTGGTC-3′ and F-5′-TCTCGATCCCAG-TAGACG-3′, respectively. The stages of three steps qPCR were pre-denaturation at 95 °C for 5 min, 40 cycles at 95 °C for 10 s, 55 °C for omentin and IL-6 57 °C (for B2M) for 20 s, as well as 62 °C for 30 s. Melting curve analysis was performed by increasing the temperature (1 °C) from 52 to 95 °C with continues fluorescence acquisition. Relative expression in omentin & lL-6 mRNA levels was calculated using the $2^{-\Delta\Delta CT}$ method and normalized based on B2M mRNA levels.

2.7 Statistical analysis

Data are reported as mean ± SEM. Statistical analysis using one-way ANOVA with Turkey's multiple comparison test and graphs fittings were performed using Graph Pad Prism 6 software (Graph Pad Software, La Jolla, CA, USA) Differences were considered significant at $p < 0.05$.

3. RESULTS

3.1 Effects of Ishige Okamurae (IO) on serum insulin level

First, we determine the effects of Ishige okamurae (IO) on serum insulin level in diabetic rats. As shown in Fig. 1, the insulin level decreased significantly ($p < 0.0001$) in diabetic rats compared to the control animals in group 1. There was no significant difference in animals treated with V. vinifera seed and the untreated animals in group 1. Furthermore, there was a significant increase ($p < 0.0001$) in the level of serum insulin in diabetic rats treated with Ishige Okamurae (IO) extract when compared to untreated diabetic rats in group 2 (Fig. 1).

GRP 1, untreated normal rats; GRP 2, diabetic not treated rats; GRP 3, normal rats treated with IO extract; GRP 4, diabetic rats treated with IO extract. Data are mean ± SE (n = 6). Mean values are significantly different at $p<0.05$.

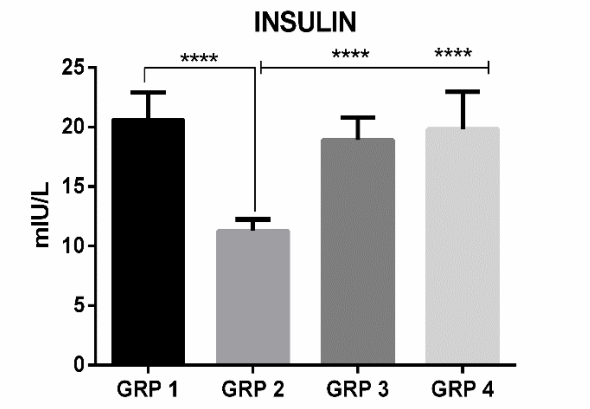


Figure 1:Effects Ishige Okamurae on serum insulin level

3.2 Effects of Ishige Okamurae on serum glucose level

The effects of IO on serum glucose levels concerning diabetic rats, was determined. As expected, diabetic rats showed a significant increase ($p < 0.0001$) in the serum level of glucose when compared with control animals in group 1 (Fig. 2). Similarly, animals in groups three and four revealed a significant decrease ($p < 0.0001$) in serum glucose levels when compared to untreated diabetic rats in group 2 (Fig. 2).

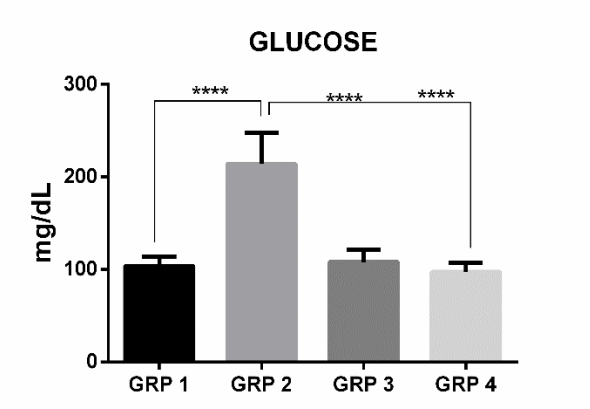


Figure 2:Effects of Ishige Okamurae on the serum glucose level

GRP 1, untreated normal rats; GRP 2, not treated diabetic rats; GRP 3, normal rats treated with IO extract; GRP 4, diabetic rats treated with IO. Data are mean ±

SE (n = 6). Mean values are significantly different at p<0.05.

3.3 Effects of Ishige Okamurae on serum biochemical parameters

Investigating the effects of **Ishige Okamurae** on the biochemical parameters of diabetic rats revealed that, serum level of cholesterol was significantly high ($p < 0.0001$) in untreated diabetic rats when compared to the control rats in group 1. However, administration of IOextract to diabetic rats in group 4 significantly reduced the serum level of cholesterol compared to untreated rats in group 2 (Fig. 3a). There was no significant difference in the serum levels of animals treated with IOextract and the untreated control animals in group 1. Furthermore, the **Ishige Okamurae** extract treated diabetic rats showed a significant decrease ($p < 0.01$) in serum TG levels when compared to untreated diabetic rats in group 2 (Fig. 3b). There was a significant elevation ($p < 0.0001$) in the serum LDL in the untreated diabetic rats when compared with the serum levels of the control animals in group 1 (Fig 3c). However, the administration of **Ishige Okamurae** extract to diabetic rats significantly decreased the serum LDL levels compared to the untreated diabetic rats in group 2. The serum level of **Ishige Okamurae** extract-treated normal rats and the untreated control rats in group 1 remained the same (Fig 3c). In addition, the HDL was significantly reduced ($p < 0.05$) in the untreated diabetic rats when compared with control rats in group 1 (Fig 4d). The administration of **Ishige Okamurae** extract to normal non-diabetic rats in group 3 did not affect the serum HDL levels when compared with the untreated rats in group 1. However **Ishige Okamurae** extract treated diabetic rats significantly increased the level of HDL when compared with untreated diabetic rats in group 3 (Fig 3d).

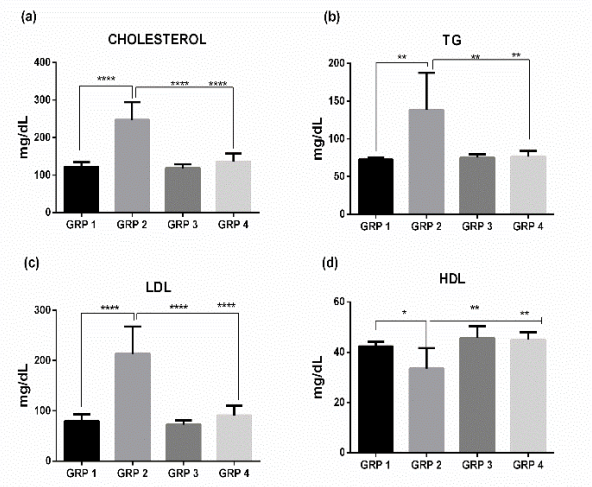
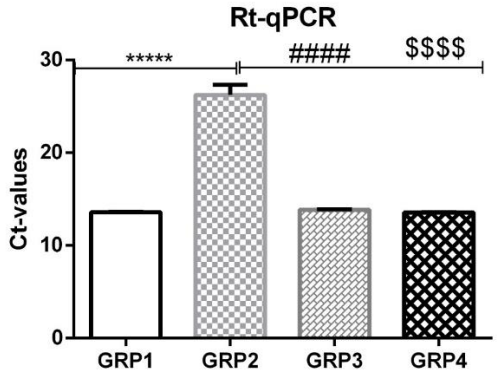


Figure 3:Effects of Ishige Okamurae on serum biochemical parameters

GRP 1, untreated normal rats; GRP 2, diabetic rats; GRP 3, rats treated with **Ishige Okamurae** extract; GRP 4, diabetic rats treated with IO extract. Data are mean ± SE (n = 6). Mean values are significantly different at p<0.05.



3 .1:Effects of Ishige Okamurae on the expression of IL-6 gene

GRP 1, untreated normal rats; GRP 2, untreated diabetic rats; GRP 3, normal rats treated with IO extract; GRP 4, diabetic rats treated with IO extract. Data are mean ± SE (n = 6). Mean values are significantly different at p<0.05.

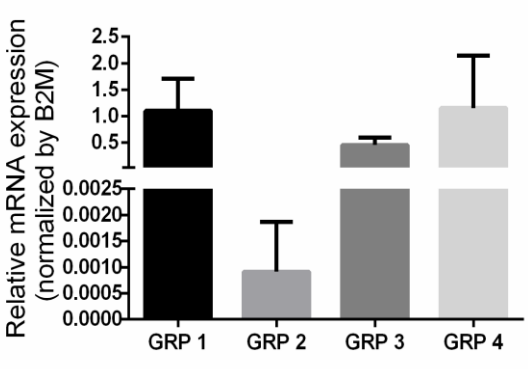


Figure 4: Effects of Ishige Okamurae on the expression of omentin gene. GRP

untreated normal rats; GRP 2, diabetic rats; GRP 3, rats treated with IO extract; GRP 4, diabetic rats treated with IO seed extract. Data are mean ± SE (n = 6). Mean values are significantly different at p<0.05.

4. DISCUSSION

Marine algae is known to have a major role as a source of food, it contains proteins, polysaccharides, dietary fibers, polyunsaturated fatty acids, minerals and vitamins (Wijesekara et al. 2011). Moreover, they are considered to have therapeutic effects, such as anti-inflammatory effect, anti-cancer, anti-viral, anti-hypertension, anti-bacterial, anti-obesity, and anti-diabetic effects (Wijesekara et al. 2011).

One of the most important marine algae is **Ishige Okamurae** alga. This brown algae is extensively studied to evaluate its nutritive and therapeutic effects.

The present study was conducted to evaluate the effect of **Ishige Okamurae** extract on

IL-6 gene expression, omentin-1 gene expression, as well as its effect on insulin level, glucose level, total cholesterol, triglyceride, LDL, and HDL. Results of this study revealed that the **Ishige Okamurae** extract increases the insulin level significantly in diabetic rats treated with **Ishige Okamurae** xtract comparing to those diabetics who were not treated with the extract (fig. 1).

Regarding blood glucose level, the **Ishige Okamurae** extract found to lower blood glucose levels significantly, in both normal and diabetic rats (fig 2).

Regarding the effect of **Ishige Okamurae** on lipids, the present study revealed that Ishige okamurae has the ability to improve and keep lipids at the optimal levels, since it decreases blood cholesterol, triglycerides, LDL whereas HDL was increased (figures 3a, 3b, 3d, 3e); thus improving lipid profile which helps in reducing the possibility of developing obesity and its pathological consequences.

Obesity is considered as a life-threatening risk factor, since it increases the risk of some diseases, such as type 2 diabetes mellitus, hypertension, coronary heart disease, stroke, and certain types of cancer. Adipocytes play a key role in obesity (storing triacylglycerol). Differentiation of pre adipocytes to adipocytes is regulated by glycerol-3- phosphate dehydrogenase (Furuyashiki et al. 2004; Cornelius et al. 1994) and other proteins such as glucose transporter 4 (GLUT4). Defect or abnormality of one of those regulating proteins will reduce the formation of adipocytes. Therefore, compounds inhibiting pre adipocyte differentiation such as Ishige okamurae may be used as anti-obesity factor (Park et al, 2013).

Thus, **Ishige Okamurae** may help to diminish the development of dyslipidemia in diabetic patients, and hence decrease coronary heart disease.

Moreover, Ishige okamurae is known to have an anti-oxidant activity. As it is known that:

reactive oxygen species (ROS), whether produced from metabolism or from external sources, it must be sustained at a harmless level. Imbalances between ROS production and the antioxidant system will result in an oxidative stress (Martindale and Holbrook 2002). Prolonged oxidative stress may lead to various pathological disorders, including cancer, inflammation, cardiovascular diseases, and diabetes (Fernando et al. 2016).

Extreme exposure of pancreatic-β cells to the high level of ROS might lead to malfunction of β cells or even death due to oxidative stress (Karunakaran and Park 2013; LeRoith et al. 2004). Thus, compounds with anti-oxidant properties such as Ishige okamurae might have a potential to be developed as anti-diabetic agents.

Therefore, **Ishige Okamurae** might decrease different pathological disorders.

Regarding Interleukin-6 (IL-6), it is a cytokine characterized by its pleiotropic action.

It modulates different functions, e.g. apoptosis, cell proliferation and differentiation. IL-6 also has an effect on the neural and endocrine systems, bone metabolism and skeletal muscles.

Usually continuous and prolonged inflammatory response might be harmful; it may induce various disorders such as Alzheimer's disease, rheumatoid arthritis, inflammatory bowel disease, cystic fibrosis, graft-versus-host disease, and cancer (Herath et al. 2016).

In the present study, IL-6 gene expression was evaluated in rats treated with Ishige Okamurae. IL-6 gene expression was found to be reduced. This is agreed with some studies such as that conducted by Kim et al. (2010); who found that a specific substance (Fucoxanthin, a major carotenoid in **Ishige Okamurae** Algae) is found to diminish the expression levels of various pro-inflammatory mediators, including IL-6.

Concerning Omentin-1, it is a 34-kDa, anti-inflammatory circulating adipocytokine (9).

Omentin-1 is secreted from the visceral fat adipose tissue [9].
Usually omentin-1 increases sensitivity to insulin; if its level is reduced it will lead to impaired glucose homeostasis. (5, 6).
In the present study, **Ishige Okamurae** was found to improve the expression of Omentin-1gene and hence increases the insulin sensitivity this might diminish the possibility of developing diabetes mellitus or improve the health of diabetic patients.

5. CONCLUSION

Diabetes is a global health problem; it might be associated with serious complications. Medicines used as anti-diabetes may have an adverse side effects. Therefore, it will be more valuable to seek a natural-based anti-diabetic drugs such as **Ishige Okamurae** algae which may play a role as anti-diabetic drug. The present study revealed that this Algae plays a key role in increasing insulin level, decreasing blood glucose, lowering cholesterol, decreasing triglycerides, decreasing LDL, increasing HDL, suppressing the expression of IL-6 gene whereas increasing the expression of omentin-1 gene.

Therefore, it may be recommended to use this algae as an anti- diabetic agent.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ETHICAL APPROVAL

MSF Medical Research Center, King Abdul Aziz University, Saudi Arabia

Reference No.634-20

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