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Therapeutic Impact of *Spirulina platensis* on Protein Alterations in Alloxan-Induced Diabetic Mice

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Abstract
Diabetes mellitus is a prevalent chronic metabolic condition largely defined by hyperglycemia and related protein modifications that lead to systemic consequences. This study sought to examine the therapeutic potential of *Spirulina platensis* (SP), a cyanobacterium recognised for its antioxidant and hypoglycemic attributes, in alleviating protein modifications caused by hyperglycemia in alloxan-induced diabetic Swiss albino mice. The experimental mice were categorised into four experimental groups: non-diabetic control, diabetic control, diabetic mice administered SP at a dosage of 15 mg/kg body weight, and non-diabetic mice administered SP. The treatment of SP resulted in a substantial drop in fasting blood glucose levels, declining from 292.33 ± 3.50 mg/dL to 121.17 ± 2.13 mg/dL over a 21 days duration. Moreover, SP supplementation markedly elevated plasma protein levels, rising from 4.72 ± 0.79 g/dL to 6.15 ± 0.53 g/dL. Electrophoretic examination of blood serum proteins demonstrated the restoration of protein band patterns, specifically the anodic proteins with molecular masses of 30.55 ± 0.31 kDa and 57.15 ± 0.26 kDa, which were markedly diminished in diabetic animals. The findings indicate that SP has advantageous effects by reducing protein changes caused by hyperglycemia, normalising glucose metabolism, and mitigating diabetic complications, thus underscoring its potential as an adjunctive therapeutic agent in diabetes mellitus management.

Keywords: Diabetes mellitus, *Spirulina platensis*, Alloxan-induced hyperglycemia, Protein alterations, Fasting blood glucose, Plasma protein, Electrophoresis.

Introduction

Diabetes mellitus (DM) is a chronic, multifactorial metabolic condition marked by persistent hyperglycemia, resulting from either impaired insulin secretion, or an insulin action, or a combination of both. This disorder presents a global health burden, affecting millions and resulting in severe complications that compromise the functionality of essential organ systems, such as the renal, neurological, and cardiovascular systems (Ahmed *et al*., 2010). Extended exposure to elevated glucose levels disturbs cellular equilibrium, resulting in the production of advanced glycation end-products (AGEs), compromised protein synthesis, and increased protease activity. These metabolic changes aggravate diabetic problems by disrupting essential cellular functions (Brownlee, 2001).

Animal models have been widely utilised to investigate the pathophysiological underpinnings of DM and assess prospective treatment approaches. The alloxan-induced diabetic mouse model is considered a dependable representation of human diabetes, offering significant insights into protein malfunctions and systemic metabolic disturbances linked to the condition (Szkudelski, 2001). This model indicates that diabetes causes protein modifications marked by heightened proteolysis, diminished anabolic processes, and impaired protein synthesis, all of which disrupt systemic homeostasis and lead to problems (Singla *et al*., 2010). Oxidative stress exacerbates these dysfunctions, resulting in extensive protein degradation and metabolic abnormalities (Giacco & Brownlee, 2010).

Spirulina platensis (SP), a unicellular cyanobacterium, has attracted considerable interest for its medicinal potential in metabolic disorders, particularly diabetes. It contains abundant bioactive components like phycocyanin, β-carotene, essential amino acids, and essential fatty acids, which confer antioxidant, anti-inflammatory, and immunomodulatory effects (Belay, 2002). Research has shown that SP effectively manages lipid profiles, regulates glycaemic levels, and diminishes oxidative stress indicators in diabetic animals (Layam & Reddy, 2006). Its elevated protein content and antioxidative characteristics indicate, it may alleviate oxidative damage and rejuvenate protein metabolism in diabetes circumstances.
Although impact of SP on glycaemic regulation and lipid metabolism is well-established, its precise effects on protein modifications and oxidative stress are still inadequately investigated. Proteins are essential for physiological function and are profoundly impacted by diabetes circumstances, experiencing glycation, aggregation, and oxidative damage. These alterations undermine protein homeostasis, facilitating disease advancement and systemic impairment. Its capacity to restore protein homeostasis *via* its antioxidative and anabolic properties presents a viable opportunity for

therapeutic intervention.
This study sought to assess the therapeutic effects of SP on protein modifications in alloxan-induced diabetic hyperglycaemic mice. Critical indicators, such as fasting blood glucose levels, electrophoretic protein profiles, and oxidative stress markers, were evaluated to clarify effectiveness of SP in re-establishing protein homeostasis and alleviating diabetes-related dysfunctions. The results indicated that SP supplementation markedly enhanced glycaemic control, normalised protein band patterns, and diminished oxidative stress, hence endorsing its potential as a supplementary therapeutic agent in the management of DM and its related problems.

Materials and Methods

Experimental Animals

Female Swiss albino mice (*Mus musculus*), weighing 22 to 27 gm, were obtained from the Central Drug Research Institute (CDRI) in Lucknow, India. The animals were housed in the animal facility of Department of Zoology at T.M. Bhagalpur University under regulated environmental conditions, specifically a constant room temperature of 23 ± 1°C, relative humidity of 50 ± 15%, and a 12-hour light-dark cycle. The mice were acclimated to their new environment for one week before the initiation of tests to guarantee adaption to the controlled setting. Throughout the trial, the animals were given a regular meal (Aashirwad Ltd., Chandigarh) and water *ad libitum*. The bedding material, composed of rice husk, was replaced every day to ensure hygiene and cleanliness. The experimental protocol conformed to the standards established by the Institutional Animal Ethics Committee (Reg. No. 5873/10) and adhered to the principles delineated in the Declaration of Helsinki.

Chemicals and Plant Material

Alloxan monohydrate, used for inducing diabetes, was obtained from Spectrochem, India. All chemicals and reagents employed in the study were of analytical grade. The *Spirulina platensis* powder, a spray-dried product of standardized quality, was sourced from Sunova Spirulina Ltd., Delhi, India.

Induction of Diabetes

Prior to the induction of diabetes, the animals had a fasting period of 16 to 18 hours. Diabetes was induced by delivering alloxan monohydrate intraperitoneally at a cumulative dose of 450 mg/kg body weight, divided into three injections of 150 mg/kg body weight each, administered at 48-hour intervals. Fasting blood glucose levels were assessed utilising a glucometer (Accu-Chek Active, Roche Diagnostics) to verify diabetes. Mice exhibiting fasting blood glucose levels over 200 mg/dL were categorised as diabetic and incorporated into the study (Szkudelski, 2001).

Experimental Design

The diabetic and control mice were randomly assigned to one of four experimental groups (n = 6 per group):
Group I (Control): Non-diabetic mice (no treatment).
Group II (Diabetic Control): Diabetic mice without any treatment.
Group III (Diabetic + *Spirulina*): Diabetic mice treated with *Spirulina platensis*.
Group IV (Control + *Spirulina*): Non-diabetic mice treated with *Spirulina platensis*.

Biochemical Analyses

Fasting Blood Glucose Levels (FBGL): Blood glucose levels were assessed at baseline (Day 0) and on days 7, 14, and 21 utilising a portable glucometer (Accu-Chek Active, Roche Diagnostics).
Protein Estimation: Plasma protein concentrations were measured utilising the Biuret method. Blood samples were obtained using tail vein puncture and centrifuged at 3000 rpm for 15 minutes to isolate the plasma. The supernatant was spectrophotometrically analysed at 540 nm to ascertain protein concentration (Gornall *et al*., 1949).
Electrophoretic Protein Banding: Protein profiles were examined *via* sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in accordance with Davis' technique (1964). Gels were stained with Coomassie Brilliant Blue to see protein bands and subsequently destained for enhanced clarity. Relative mobility (Rm) and molecular weights of protein bands were assessed utilising conventional molecular weight markers.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using two-way analysis of variance (ANOVA) to evaluate group differences. Post hoc comparisons were conducted using Student's *t*-test, with a significance threshold of $p < 0.05$. All analyses were performed using Microsoft Excel software.

Results

Effect of *Spirulina platensis* on Fasting Blood Glucose Levels

Administration of alloxan resulted in substantial hyperglycemia in mice, with FBGL escalating ($p < 0.01$) from 78.33 ± 3.88 mg/dL in the control group (Group I) to 292.33 ± 3.50 mg/dL in the diabetic control group (Group II) by day 21 (Fig 1). Treatment with SP (Group III) markedly diminished FBGL, decreasing from 255.83 ± 2.93 mg/dL on day 1 to

121.17 ± 2.14 mg/dL by day 21 ($p < 0.01$). Conversely, non-diabetic control mice administered SP (Group IV) exhibited no notable alteration in FBGL during the research duration (Fig. 1). These findings underscore the hypoglycemic potential of SP, presumably attributable to its bioactive components that regulate glucose metabolism.

Effect of *Spirulina platensis* on Plasma Protein Levels

In diabetic mice (Group II), plasma protein levels dramatically declined ($p < 0.01$) from 6.54 ± 0.23 g/dL on day 1 to 4.73 ± 0.80 g/dL on day 21 (Fig. 2). Treatment with *Spirulina platensis* (Group III) resulted in a notable enhancement of plasma protein levels, rising from 6.59 ± 0.47 g/dL on day 1 to 6.15 ± 0.54 g/dL by day 21 ($p < 0.05$). No notable alterations in plasma protein levels were detected in the control group (Group I) or in non-diabetic control mice administered SP (Group IV) during the trial (Fig 2). The findings indicate that SP exerts protective and restorative effects on protein metabolism in diabetes circumstances.

Electrophoretic Analysis of Serum Proteins

Electrophoretic analysis demonstrated notable alterations in protein banding patterns among experimental groups. The control group (Group I) exhibited seven unique protein bands with molecular weights spanning from 30.55 ± 0.31 kDa to 184.08 ± 0.78 kDa. Rm1 (anodic protein): 184.08 ± 0.78 kDa; Rm2: 163.59 ± 1.29 kDa; Rm3: 103.31 ± 0.24 kDa; Rm4: 67.12 ± 0.55 kDa; Rm5: 42.23 ± 0.91 kDa; Rm6: 36.44 ± 0.57 kDa; Rm7: 30.55 ± 0.31 kDa. In contrast, the diabetic control group (Group II) demonstrated a significant decrease in both the quantity and intensity of protein bands. By day 21, anodic proteins Rm6 and Rm7 were entirely missing. Treatment with SP (Group III) largely reinstated these protein bands, with Rm6 and Rm7 reemerging at diminished nevertheless quantifiable amounts by day 21 (Table 1 & Fig 3). The data suggest that SP plays a therapeutic function in restoring serum protein integrity, particularly anodic proteins associated with glucose and lipid metabolism, and alleviating protein changes induced by hyperglycemia in diabetic mice.

Discussion

This study examines the therapeutic efficacy of SP in alleviating metabolic abnormalities caused by hyperglycemia in alloxan-induced diabetic mice. DM is a multifaceted metabolic condition marked by persistent hyperglycemia, impaired protein metabolism, and elevated oxidative stress, leading to consequences including nephropathy, hepatopathy, neuropathy, and reproductive dysfunction (Tessari *et al*., 2011). The results highlight capacity of SP to restore protein homeostasis, diminish oxidative stress, and enhance glycaemic control, indicating its promise as an adjuvant treatment for diabetes.

The notable decrease in FBGL in diabetic mice treated with SP underscores its hypoglycemic properties. Bioactive components in SP, including phycocyanin and peptides, are recognised for their ability to augment insulin secretion and boost glucose absorption, hence facilitating glycaemic management (Layam & Reddy, 2006; Muley *et al*., 2007). Moreover, its fibre content may diminish glucose absorption in the gastrointestinal tract, hence enhancing its hypoglycemic effects. These methods highlight its potential in regulating hyperglycemia *via* insulin-mimetic effects and control of glucose metabolism.

Diabetes impairs protein metabolism by enhancing proteolysis and diminishing anabolic activity, resulting in hypoalbuminemia and compromised protein synthesis. This study found that untreated diabetic mice displayed markedly reduced plasma protein levels, corroborating these metabolic disturbances. Treatment with SP markedly reinstated plasma protein levels, presumably owing to its antioxidative capabilities, which alleviate oxidative damage to ribosomes and the endoplasmic reticulum, hence enhancing protein synthesis and diminishing proteolytic activity (Laurman, 1980). These findings underscore its contribution to enhancing protein metabolism and maintaining systemic homeostasis in diabetes circumstances.

Serum electrophoresis demonstrated notable changes in protein profiles in diabetic mice, characterised by a reduced quantity and intensity of protein bands, especially those linked to glucose and lipid metabolism. This decline aligns with diabetes-induced protein glycation and oxidative stress, which impair protein production and functionality. Nonetheless, treatment with SP reinstated the protein banding patterns, particularly the anodic proteins Rm6 and Rm7, which are essential for metabolic functions. The resurgence of these bands, albeit at diminished levels, indicates that *Spirulina* enhances protein integrity and reinstates the metabolic profile in diabetes states. Research by Cechowska-Pasko and Pałka (2000) reveals that SP enhances insulin-like growth factor (IGF), hence promoting protein anabolism and collagen synthesis.

Conclusion

This research presents strong evidence for the therapeutic efficacy of SP in alleviating protein modifications and metabolic disruptions linked to hyperglycemia in diabetic mice. The bioactive constituents of SP, such as phycocyanin, β-carotene, and essential amino acids, enhance its diverse therapeutic effects, acting as powerful antioxidants and regulators of glucose metabolism. SP has potential as a supplementary therapeutic agent for controlling diabetes and its consequences by restoring protein production, boosting glycaemic control, and improving serum protein integrity. The

restoration of plasma protein levels and the decrease in fasting blood glucose levels in *Spirulina*-treated diabetic mice highlight its function in rectifying protein metabolic disturbances and alleviating oxidative stress. The restoration of anodic proteins associated with glucose and lipid metabolism underscores its protective effects on cellular structures, facilitating the normalisation of metabolic profiles altered by DM. This study, building on previous research, underscores the efficacy of SP as a safe and natural addition to traditional antidiabetic treatments. In addition to glycaemic control, *Spirulina* promotes protein production, boosts lipid profiles, and mitigates oxidative stress, establishing it as a significant option for holistic diabetes care.

Further investigations are necessary to clarify the specific molecular processes of its actions, establish ideal dosages, and evaluate long-term safety and efficacy. Clinical research involving human patients will be crucial to corroborate these findings and ascertain the therapeutic potential of SP in practical applications. In conclusion, SP presents a promising natural remedy for the management of diabetes and its various complications. Its capacity to modulate blood glucose levels, rejuvenate protein metabolism, and mitigate oxidative stress establishes it as a formidable therapeutic agent in comprehensive diabetes management. The incorporation of SP into diabetes care strategies may signify a substantial progress in the prevention and treatment of diabetic complications.

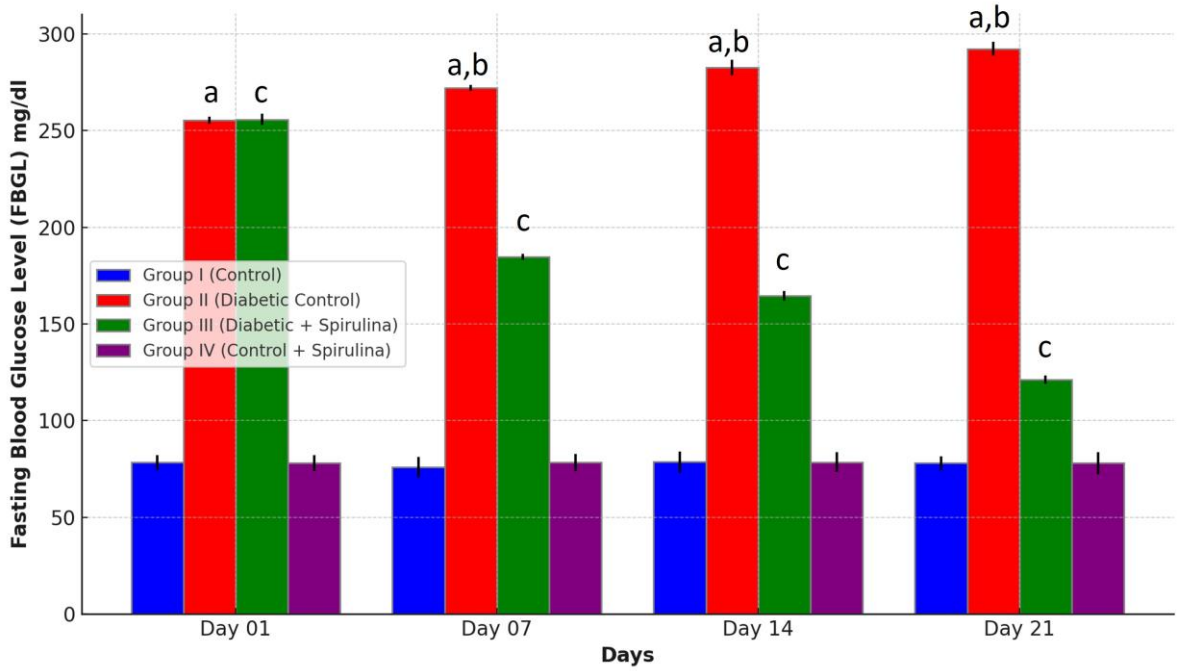

Fig. 1: Effect of *Spirulina platensis* treatment on Fasting Blood Glucose Levels (FBGL) in diabetic and non-diabetic mice. N = 6, values are expressed as mean ± SEM. Similar superscript letters indicate no statistically significant difference ($p > 0.05$). Different superscript capital letters indicate significant difference ($p < 0.05$).

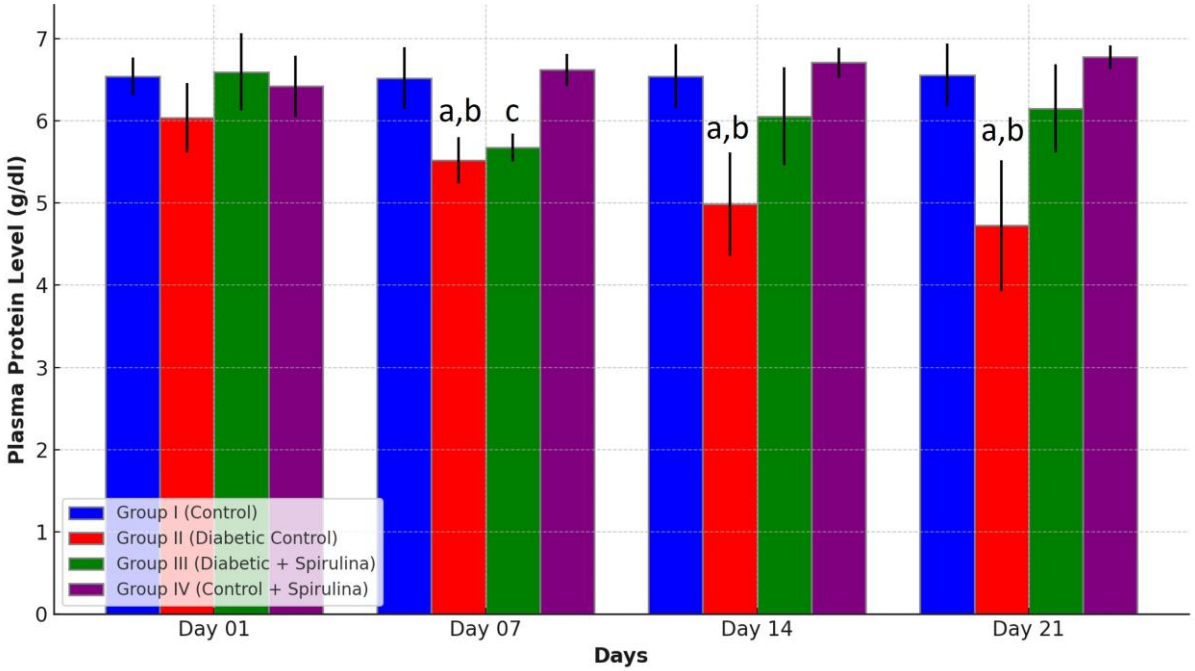

Fig. 2: Effect of *Spirulina platensis* on plasma protein levels in diabetic and non-diabetic mice. N = 6, values are expressed as mean ± SEM. Similar superscript letters indicate no statistically significant difference ($p > 0.05$). Different superscript capital letters indicate significant difference ($p < 0.05$).

Table 1: Electrophoretic Analysis of Protein Banding Patterns, Relative Mobility (Rm), and Molecular Mass (Mm) (kDa) in Serum from All Experimental Groups

Band	Relative mobility (Rm)	Molecular mass (Mm) (KD)	Nature of Protein	Group-I	Group-II	Group-III	Group-IV
1 (Rm1)	0.0694±0.02	184.08±0.78	CP	+	-	-	+
2 (Rm2)	0.208±0.01	141.25±0.67	CP	+	+	+	+
3 (Rm3)	0.354±0.03	77.62±0.92	CP	+	+	+	+
4 (Rm4)	0.444±0.02	68.39±0.21	CP	+	+	+	+
5 (Rm5)	0.708±0.01	43.85±0.16	AP	+	+	+	+
6 (Rm6)	0.792±0.02	37.15±0.26	AP	+	*6	**6	***6
7 (Rm7)	0.889±0.03	30.55±0.31	AP	+	*7	**7	+

Note: + (Present); - (Absent); CP (Cathodic Protein); AP (Anodic Protein). *6 and *7 *8 are present in day 1 in very less quantity but absent in day 07, 14 and day 21. **6 and **7 are present in day 1 in very less quantity but absent in day 07, 14 and reappeared in day 21. Rm for **6 and **7 was found to be 0.778±0.02 and 0.882±0.04 respectively and their molecular mass (kDa) were found to be 38.46±0.19 and 30.55±0.31 kDa respectively. ***6 present in all day of treatment but the concentration increases slightly in day 21. Rm for ***6 was found to be 0.792±0.03 and their molecular mass (kDa) was found to be 37.15±0.08 kDa.

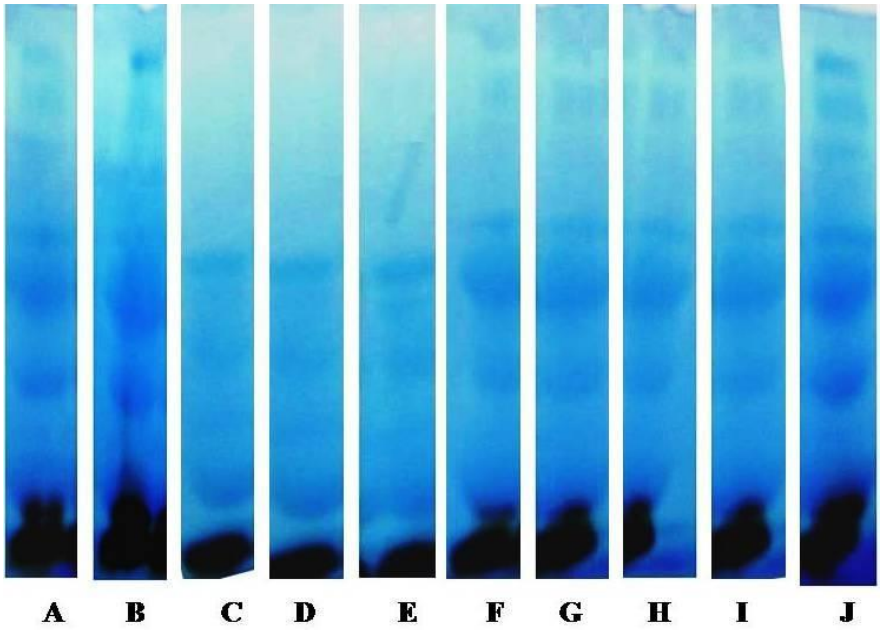

Figure 3: Blood Serum Protein Banding of Experimental Mice. (A): Group-I (Control); (B): Group-II (Diabetic Control; Day 01); (C): Group-II (Diabetic Control; Day 07); (D): Group-II (Diabetic Control; Day 14); (E): Group-II (Diabetic Control; Day 21); (F): Group-III (Diabetic Control Mice Fed with *Spirulina platensis*; Day 01); (G): Group-III (Diabetic Control Mice Fed with *Spirulina platensis*; Day 07); (H): Group-III (Diabetic Control Mice Fed with *Spirulina platensis*; Day 14); (I): Group-III (Diabetic Control Mice Fed with *Spirulina platensis*; Day 21); (J): Group-IV (Control Mice Fed with *Spirulina platensis*)

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