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Research Article

Effect of *Syzygium cumini* (jamun) seed powder on dyslipidemia: a double blind randomized control trial

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ABSTRACT

Background: Diabetes is a metabolic syndrome characterized by disturbance in carbohydrate, fat and protein metabolism. Dyslipidemia, commonly associated in diabetes, is major risk factor for macrovascular complications leading to CAD, major contributor to mortality associated with diabetes. Managing DM without side effects is challenge that attracts researchers toward plant based new products. Many studies have found anti-diabetic and anti-hyperlipidemic properties of seeds of *Syzygium cumini*, attributed to saponins, glycosides and flavonoids. So it should be further explored for its benefits. The aim was to study the effect of jamun seed powder on dyslipidemia in type 2 DM.

Methods: Patients with type 2 DM were randomly divided in two groups- group A was supplemented with 10 gms/day jamun seed powder and group B was given placebo powder. Patients and investigators were blinded about treatment allocated. Lipid profile was noted at baseline and 30th, 60th and 90th day. All the data was collected and analyzed at the end of study.

Results: Improvement in dyslipidemia was seen after 60 days of supplementation with *S. cumini* seed powder. Statistically significant decrease in cholesterol levels by 10.55% and 15.79% in mean triglyceride levels by 8.28% and 13.66%, LDL-c levels by 10.29% and 14.50% was noticed at 60th and 90th day, respectively, reduction in VLDL-c levels by 9.38%, 12.90% and 20.69% was noted at 30th, 60th and 90th day. HDL-c increased significantly by 11.11% and 13.89% in males and 10.81% and 16.21% in females after 60 and 90 days of supplementation with *S. cumini* seed powder.

Conclusions: A significant overall effect of *S. cumini* supplementation was found in improvement of lipid profile in type 2 diabetes subjects. However, above results are seen in small number subjects, further multicenter studies with larger sample size, supplementation dose and time should be planned and its effects in detail should be explored.

Keywords: *Syzygium cumini*, Dyslipidemia, Diabetes mellitus, Seed powder

INTRODUCTION

Diabetes is a common metabolic disease characterized by disturbance in carbohydrate, fat and protein metabolism due to deficient insulin synthesis from pancreatic beta cells or defect in peripheral insulin action or both. Diabetes is associated with various complications contributing to morbidity and mortality associated with diabetes. Approximately, 80% of deaths in patients with diabetes are attributable to cardiovascular disease (CVD).[1] It is well-established that dyslipidemia is a major risk factor for macrovascular complications in patients with type-2 diabetes mellitus (T2DM) and affects 10%-73% of this population.[2-4] The characteristic features of diabetic dyslipidemia are a high plasma triglyceride

concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance. In observational studies, HDL may be the most consistent predictor of CHD in type 2 diabetes subjects, followed by triglyceride and total cholesterol.[5]

Baseline data from the United Kingdom Prospective Diabetes Study (UKPDS) showed that both decreased HDL and elevated LDL predicted CHD. All international guidelines recommend aggressive management of lipids in this population.[2,6,7] Treatment of lipid abnormalities has the potential to reduce cardiovascular events more than 50%, to rates that are seen in countries with lower cholesterol and less atherosclerotic burden. This leads to the expectation that treatment of elevated lipid levels will allow patients with diabetes to lead longer healthier lives.[8]

Various drugs are available for management of diabetes. However a drug with multimodal action leading to improvement in complications such as dyslipidemia, hypertension as well as hyperglycemia of diabetes mellitus is still to be discovered. *Syzygium cumini* has been studied and found to have favorable effect on improvement of hyperglycemia in type 2 diabetes patients.[9-12]

Some studies also support its beneficial effect in improvement of hypertension in type 2 diabetes subjects.[13,14] The aim of our study was to bring into light improvement in dyslipidemia in type 2 diabetes patients after supplementation with *Syzygium cumini* seed powder.

Aims and objectives

To study the effect of *S.cumini* supplementation on Lipid profile in patients with type 2 diabetes mellitus.

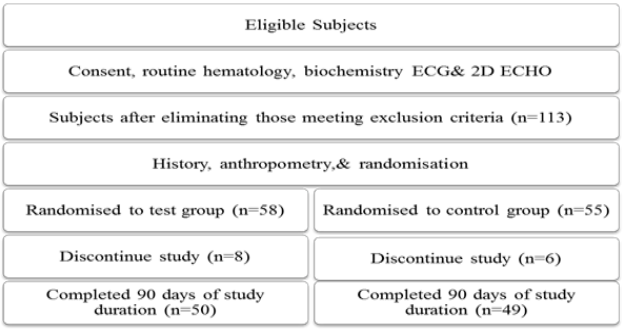
METHODS

Study was conducted over a period of one year from November 2014 to November 2015. Study design was single centred, double blind, randomized controlled parallel designed trial.

Study was conducted on a total of 99 patients with type 2 diabetes mellitus with poorly controlled blood sugar levels, FPG >126 mg/dl and post prandial blood sugar >180 mg/dl during continued treatment with oral hypoglycemic agents. Patients with cardiovascular, cerebrovascular, liver disease or renal failure were excluded from study to avoid any unknown serious side effects.

Patients with sepsis, malignancy or terminally ill patients were also excluded to avoid unforeseen adverse effects. Pregnant patients with diabetes were also excluded from study to avoid, if any, harmful effect on fetus.

Patients taking insulin for glycemic control were excluded from study as there are chances of self-monitoring and change in dose of insulin according to glycemic levels and thus hindering the study results. Before enrollment, details about nature and utility of study were explained to all patients and informed consent was taken.



Patients included in study were randomized and assigned to 2 groups:

- Group A (test group): Type 2 diabetes mellitus patients received *S.cumini* seed powder supplementation.
- Group B (control group): Type 2 diabetes mellitus patients received placebo.

Subjects in test group were given *S.cumini* seed powder was given in a dose of 5gms twice daily before meal, a total of 10gms per day and subjects in control group were given placebo powder without having any hypoglycemic effect or effect on dyslipidemia.

Anti-diabetic medications were continued as same. Baseline Total cholesterol, LDL-c, VLDL-c, HDL-c and triglyceride levels were noted and were repeated on 30^{th}, 60^{th}, 90^{th} day.

Patients and all investigators were blinded to the treatment assigned throughout the study. Patients were advised to report any adverse reactions to treating physician in charge of patients. Treatment allocated was enclosed in sealed envelope to which physician in charge of patients had access to only in case of an emergency.

RESULTS

Table 1 show that mean total cholesterol levels in test group decreased from 220±33.60 mg/dl to 199±30.85 mg/dl and 190±28.81 mg/dl after 60 and 90 days.

Percentage decrease in group A was 10.55%, an absolute value of 21 mg/dl, which was more than critical difference of 12.38 mg/dl, so was a significant improvement. In the control group mean total cholesterol

levels decreased by 0.50% after 30 days, absolute value of 1mg% which was less than required critical difference. When both groups are compared there was significant difference in baseline mean total cholesterol value between two groups p=0.001 which became non-significant p=0.119 after 30 days.

Table 1: Mean total cholesterol (mg/dl) of patients with type-2 diabetes mellitus in test group and control group during different visits.

	Day 0	Day 30	Day 60	Day 90	CD##
Group A (test) (n=50)	220±33.60	209±32.14	199±30.85	190±28.81	12.38
Group B (control) (n=49)	201±24.97	200±23.85	200±24.64	200±25.07	9.81
Percent change in Group A		↓5.26	↓10.55	↓15.79	
Percent change in Group B		↓0.50	↓0.50	↓0.50	
t-value	3.18	1.57	0.177	1.84	
p-value#	0.001	0.119	0.859	0.068	

p value is significant at p<0.05, #p value between the two groups by unpaired student t test, ##CD-critical difference value between the four groups by one way anova.

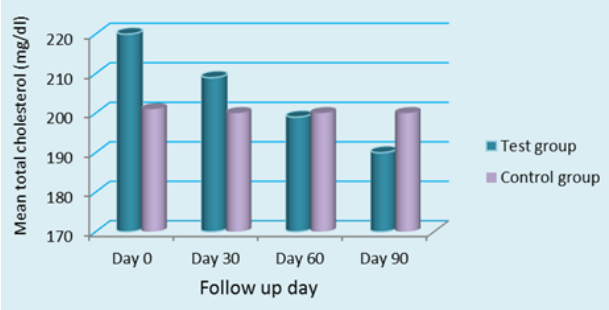


Figure 1: Change in mean total cholesterol levels in test group and control group after supplementation with jamun seed powder and placebo, respectively.

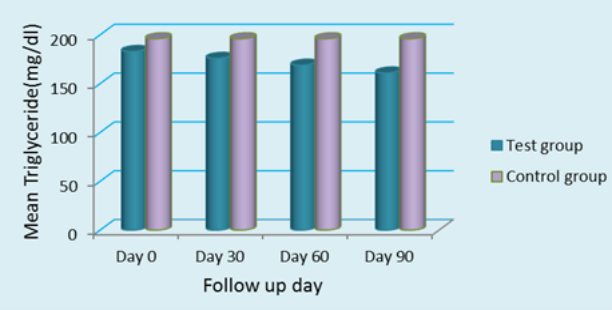


Figure 2: Change in mean triglyceride levels in test group and control group after supplementation with jamun seed powder and placebo, respectively.

Table 2: Mean triglyceride (mg/dl) of patients with type-2 diabetes mellitus in test group and control group during different visits.

	Day 0	Day 30	Day 60	Day 90	CD##
Group A (test) (n=50)	183±39.19	176±37.09	169±36.08	161±34.71	14.51
Group B (control) (n=49)	196±40.84	196±39.30	196±40.04	196±39.49	15.81
Percent change in Group A		↓3.98	↓8.28	↓13.66	
Percent change in Group B		0	0	0	
t-value	1.62	2.52	3.56	4.69	
p-value	0.108	0.015	0.0001	0.0001	

p value is significant at p<0.05, #p value between the two groups by unpaired student t test, ##CD-critical difference value between the four groups by one way anova.

It can be observed from Table 2 that on day 0 mean serum triglyceride level in group A was 183±39.19mg/dl and in group B was 196±40.84mg/dl (p=0.108).

After 30 days mean serum triglyceride level in group A was 176±37.09 mg/dl and in group B was 196±40.04 mg/dl (p=0.015). Calculated CD for test group was 14.51 mg/dl, which was achieved after 60 days in test group; it corresponds to percentage decline by 8.28%.

Table 3 shows that LDL-c levels decreased significantly from 150±26.62 mg% to 136±24.44 mg% after 60 days,

decline was more than calculated critical difference of 10.06 mg/dl, which was required for the value to become significant. In control group there was a rise in mean LDL-c levels by 0.70% after 60 days. When both groups are compared there was statistically significant difference ($p<0.05$) in mean LDL-c level at baseline, being higher in test group, which was observed to be non-significant ($p> 0.05$) after 30 days and 60 days of supplementation with jamun seed powder. However after 90 days of supplementations with *S.cumini* seed powder there was statistically significant difference ($p<0.05$) in mean LDL-c values was observed, value being lower in test group.

Table 3: Mean LDL-cholesterol (mg/dl) of patients with type-2 diabetes mellitus in test group and control group during different visits.

	Day 0	Day 30	Day 60	Day 90	CD##
Group A (test) (n=50)	150±26.62	143±25.50	136±24.44	131±25.47	10.06
Group B (control) (n=49)	142±22.46	142±22.27	143±22.75	143±23.17	9.03
Percent change in Group A		↓4.89	↓10.29	↓14.50	
Percent change in Group B		0	↑0.70	↑0.70	
t-value	2.09	0.21	1.47	2.45	
p-value#	0.039	0.834	0.144	0.016	

p value is significant at $p<0.05$, #p value between the two groups by unpaired student t test, ##CD-critical difference value between the four groups by one way anova.

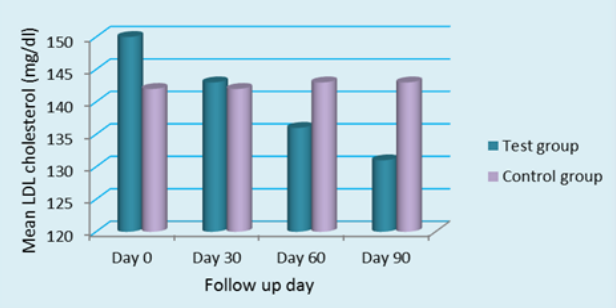


Figure 3: Change in mean LDL-c levels in test group and control group after supplementation with jamun seed powder and placebo, respectively.

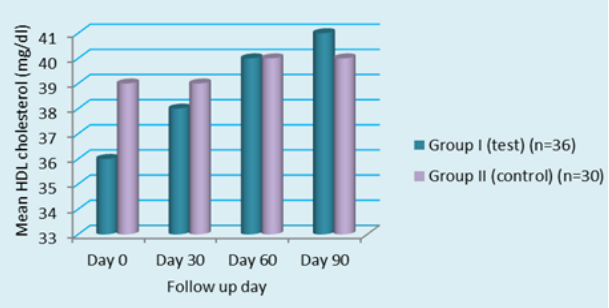


Figure 4: Change in mean HDL-c levels of male subjects in test group and control group after supplementation with jamun seed powder and placebo, respectively.

Table 4: Mean HDL-c (mg/dl) of male patients with type-2 diabetes mellitus in test group and control group during different visits.

	Day 0	Day 30	Day 60	Day 90	CD##
Group A (test) (n=36)	36±7.98	38±7.84	40±7.42	41±7.08	3.53
Group B (control) (n=30)	39±5.73	39±5.68	40±5.96	40±5.51	2.92
Percent change in Group A		↑5.56	↑11.11	↑13.89	
Percent change in Group B			↑2.56	↑2.56	
t-value	1.45	0.751	0.199	1.00	
p-value#	0.151	0.455	0.842	0.321	

p value is significant at $p<0.05$, #p value between the two groups by unpaired student t test, ##CD-critical difference value between the four groups by one way anova.

It can be observed from Table 4 that there was statistically significant rise in mean plasma HDL-c value in test group males after *S.cumini* supplementation for 60 days and 90 days by 11.11% and 13.89 % respectively. In control group there was increase in mean plasma HDL-c value by 2.56% after 60 days which was less than

required CD, so non-significant. When both groups are compared there was no statistically significant difference between two groups (p<0.05). It can be observed from Table 5 that there was statistically significant rise in mean plasma HDL-c value by 16.21% in test group after *S.cumini* supplementation for 90 days. In control group there was increase in mean plasma HDL-c value by 2.56% after 90 days which was statistically non-significant. When both groups are compared, it was found that there was no statistically significant difference between two groups (p<0.05).

Table 5: Mean HDL-c (mg/dl) of female patients with type-2 diabetes mellitus in test group and control group during different visits.

	Day 0	Day 30	Day 60	Day 90	CD##
Group A (test) (n=14)	37±8.04	39±7.95	41± 8.199	43± 8.16	6.36
Group B (control) (n=19)	39±7.29	39±6.87	39± 7.11	40± 7.03	4.57
Percent change in Group A		↑5.40	↑10.81	↑16.21	
Percent change in Group B		0	0	↑2.56	
t-value	0.656	0.058	0.647	1.116	
p-value#	0.516	0.954	0.522	0.272	

p value is significant at p<0.05, #p value between the two groups by unpaired student t test, ##CD-critical difference value between the four groups by one way anova.

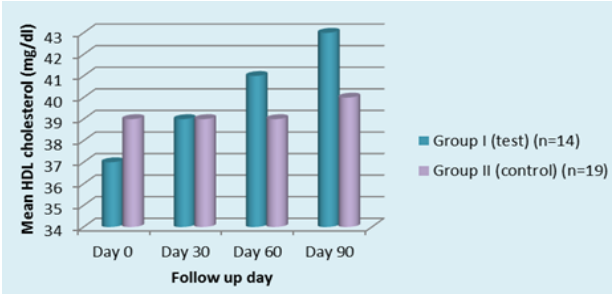


Figure 5: Change in mean HDL-c levels of male subjects in test group and control group after supplementation with jamun seed powder and placebo, respectively.

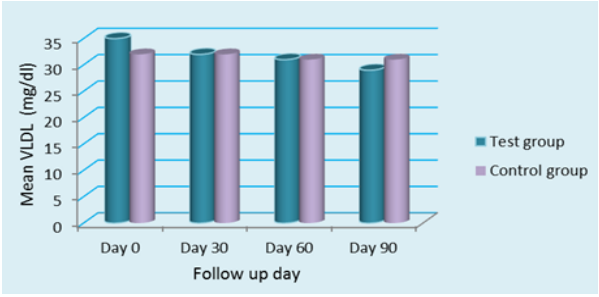


Figure 6: Change in mean VLDL-c levels in test group and control group after supplementation with jamun seed powder and placebo, respectively.

Table 6: Mean VLDL-c (mg/dl) of patients with type-2 diabetes mellitus in test group and control group during different visits.

	Day 0	Day 30	Day 60	Day 90	CD##
Group A (test) (n=50)	35±6.22	35±6.22	31±5.24	29±5.59	2.24
Group B (control) (n=49)	32±10.45	32±6.69	31±6.87	31±6.74	3.13
Percent change in Group A		↓9.38	↓12.90	↓20.69	
Percent change in Group B		0	↓3.23	↓3.23	
t-value	1.80	0	0	1.67	
p-value#	0.074	0	0	0.098	

p value is significant at p<0.05, #p value between the two groups by unpaired student t test, ##CD-critical difference value between the four groups by one way anova.

It can be observed from table 6 that VLDL-c levels decreased in test group from 35±6.22 mg/dl to 35±6.22 mg/dl, 31±5.24 mg/dl and 29±5.59 mg/dl after 30, 60 and 90 days respectively. Calculated CD for test group was 2.24 mg/dl which was achieved after 60 days. Total percentage decline after 90 days was 20.69%. In control group there was decrease in mean VLDL-c levels by 3.23% after 60 days, which was less than required CD of

3.13 mg/dl, to be considered as significant. When both groups were compared there was no statistically significant difference noticed between two groups (p>0.05).

DISCUSSION

There was statistically significant reduction in mean cholesterol levels from 220±33.60mg/dl to 199±30.85mg/dl and 190±28.81mg/dl after 60 and 90 days of supplementation with *Sygyium cumini* seed powder. Mean triglyceride levels decrease significantly from baseline of 183±39.19mg/dl to 169±36.08mg/dl and 161±34.71mg/dl after 60 and 90 days of supplementation, a percentage reduction by 8.28% and 13.66% respectively.

However reduction after 30 days of supplementation by 3.98% was not statistically significant. There was statistically significant reduction in plasma LDL-c levels from baseline of average 150±26.62mg/dl to 136±24.44 mg/dl and 131±25.47mg/dl after 60 and 90 days of supplementation with jamun seed powder, a percentage reduction by 10.29 % and 14.50% respectively. However reduction by 4.89% after 30 days of supplementation was statistically non- significant.

There was significant increment in plasma HDL-c levels after supplementation with jamun seed powder in male subjects, from baseline of 36±7.98mg/dl to 40±7.42mg/dl and 41±7.08mg/dl, percentage increment by 11.11% and 13.89%, after 60 and 90 days respectively. In female subjects HDL-c increase from 37±8.04mg/dl to 43±8.16 mg/dl, percentage increment by 16.21% after 90 days of supplementation. Mean VLDL levels also reduced significantly by 9.38%, 12.90% and 20.69% after 30,60 and 90 days, respectively.

During his study, Moses CRA observed reduction of serum cholesterol levels from 207.2 mg/dl to 172.6 mg/dl after 45 days of supplementation which rose again to 185.7mg/dl after 90 days.[13]

Virmani P et al noticed that cholesterol levels increased by 9.53% after supplementation with jamun seed powder for 4 weeks, whereas decreased by 7.68 % in group managed by diet and exercise.[11] LDL levels increased by 12.2% in patients who received jamun seed powder supplementation and reduced by 14.4% in patients managed by diet and exercise. After supplementation with jamun seed powder triglycerides levels were reduced by 10.35% whereas an increase by 6.7% was noticed in group managed by diet and exercise. There was a slight increase in HDL-c levels in both groups. Difference in results in both studies can be due to difference in sample size (99 versus 20), also it can be due to different dietary habits of both populations.

Sahana DA et al noticed highly significant rise in HDL-c value from baseline of 39.7±9.6mg/dl to 47.3±6.8mg/dl (p=0.001) after supplementation with jamun seed powder for 90 days.[12]

Improvement in lipid profile was noticed in many preclinical studies. In study by Sharma AK, Bharti S et al in department of Pharmacology, all India Institute of Medical Sciences on high-fat diet/streptozotocin–induced (HFD–STZ) diabetic rats, they found that treatment with 200mg/kg *S.cumini*, 400mg/kg S.cumini, and metformin for 21 days resulted in significant decreases in serum TC (39.87%, 44.24%, and 44.88%, respectively), TG (43.06%, 46.73%, and 48.57%, respectively) and LDL-c (28.32%, 32.89%, and 35.29%, respectively) compared with diabetic control rats (p< 0.01); additionally, there was a significant increase in HDL-c (14.60%, 20.22%, and 30.34%, respectively) (p <0.01).[16] The 100 mg/kg dose of SC did not significantly affect the lipid profile of diabetic rats. Potent hypoglycemic, insulin sensitizing, and hypo-lipidemic activity in HFD–STZ induced diabetic rats was proposed to be due to increased PPAR γ and PPAR α protein expressions.

In another study by Sharma B, Balomajumder C, Roy P on streptozotocin induced diabetic rats after giving flavonoid rich extract of *S.cumini* seed (300mg/kg/day, 15days, FPG. and other biochemical parameters like glycogen biosynthesis, glucose homeostatic enzyme (glucose-6-phosphatase, hexokinase) activities demonstrated significant (p<0.05) improvement as compared to diabetic counter parts.[17]

Further, the flavonoids also stimulated 16% increase in insulin release in vitro from pancreatic islets. The hypo-lipidemic action after this extract supplementation was also noticed by significant (p<0.05) decrease in the levels of LDL (27% MD, 29% SD), triglycerides (about 35% MD, 37% SD) and increase in HDL (21% MD, 34% SD) over untreated diabetic rats. The mentioned action of this plant extract was found to be through dual up regulation of both the peroxisome proliferators-activated receptors (PPAR alpha and PPAR gamma) up to about 3-4 folds (over control) and their capacity to differentiate 3T3-L1 preadipocytes.

In another study by Sharma SB in 2003 to find out hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *S.cumini* in alloxan-induced diabetic rabbits, they found that ethanolic extract of seeds exhibited significant hypolipidemic effect as evident from fall in total serum cholesterol (TC)/high density lipoprotein cholesterol (HDL-c) ratio, serum low density lipoprotein cholesterol (LDL-c) levels and decreased activity of HMG-CoA reductase.[18]

In their study on streptozotocin-induced diabetes in rats, Ravi K et al found that STZ-induced diabetic rats showed significant increase in the levels of cholesterol, phospholipids, triglycerides and free fatty acids and plasma lipoproteins (HDL, LDL, VLDL-cholesterol), which were considerably restored to near normal by

S.cumini seed kernel supplementation. It was proposed that this effect might be due to the presence of flavonoids, saponins, glycosides and triterpenoids in the extract.[19]

In their study on streptazocin induced diabetic rats, Patel SS et al found a significant decrease in serum LDL-c, VLDL-c, total cholesterol and triglyceride levels after treatment with Dihar (polyherbal formulation containaing *S.cumini, Momordica charantia, Emblica officinalis, Gymnema sylvestre, Enicostemma littorale, Azadirachata indica, Tinospora cardiofolia and Curcuma longa*), however rise in HDL levels was not significant.[20]

Siddiqui MS et al in 2014, during their study on alloxan induced diabetic mice, they observed that after treatment with *Syzygium cumini* orally for 21 days, there was significant reduction in TG-C, LDL-C, VLDL-C and increase in HDL-C levels. Moreover no toxic symptoms were observed.[21]

CONCLUSION

A significant overall effect of *S. cumini* supplementation was found in improvement of lipid profile in type 2 diabetes subjects. However, above results are seen in small number subjects, further multicenter studies with larger sample size, supplementation dose and time should be planned and its effects in detail should be explored.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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