ORIGINAL ARTICLE

ANTIHYPERGLYCEMIC AND ANTIDYSLIPIDEMIC EFFECT OF CALOTROPIS FLOWERS IN EXPERIMENTAL DIABETIC RATS

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ABSTRACT

Background: Diabetes mellitus is a growing menace and the cost of treatment and debilitation is high especially in poor societies. Hyperglycemia in diabetes is associated with deranged lipid profile. Various parts of plants and herbs have been indigenously used around the world for treatment of diabetes mellitus. Root, bark, leaves and latex of Calotropis procera- have been experimentally studied for their antidiabetic effect. This study was undertaken to explore the antidiabetic and antidyslipidemic effect of the flowers of Calotropis procera.

Methods: Diabetes was induced by streptozotocin. Calotropis procera flowers aqueous extract (CFEX) was given orally in doses of 10,20,30,40 and 50 mg/kg body weight. Serum levels of total cholesterol, triglycerides, HDL and LDL were measured at the beginning of experiment and at intervals of 1 day, 1 week, 2 weeks and 3 weeks and compared with those in positive controls treated with glibenclamide and untreated negative control animals.

Results: Weight loss in CFEX-treated animals was not significantly different from positive control group. Decrease in serum glucose levels, comparable to positive controls, was observed in animals treated with CFEX 20,30,40 and 50 mg/kg body weight. The TC, TG and LDL significantly decreased while a significant increase in HDL was seen.

Conclusion: Calotropis procera flowers extract has shown antihyperglycemic and antidyslipidemic effects in experimental diabetic rats and may have the potential of a potent antidiabetic agent.

KEY WORDS: Calotropis procera flowers, diabetes mellitus, antihyperglycemic, dyslipidemia, cholesterol, triglycerides, high density lipoproteins, low density lipoproteins

INTRODUCTION

With ever-increasing incidence of diabetes mellitus around the world and in consideration of the cost of treatment[1]especially in poor societies, attention has turned to explore plant elements that are locally and cheaply or freely available and have been traditionally used by indigenous population for centuries[2].

Calotropis procera is one such plant (Fig.1), widely distributed and easily available in the tropics and subtropics, various parts of which have been studied for their effect on the hyperglycemia associated with experimental diabetes mellitus. In this regard studies have been carried out with favorable results producing hypoglycemia in experimental animalsby extracts of the root, bark, leaves and latex extracted from the plant[3,4]. Scantyliteraturehowever is available regarding the effect of flower extracts of the Calotropis procera in diabetes[5]. The leaves and latex of the plant are reported to be hepatotoxic and cardiotoxic in high doses in experimental animal studies. There is no exclusive report on ill effects of the Calotropis flowers[6,7].

This study was planned to observethe effect of aqueous extract of the flowers of Calotropis procera in experimentally induced diabetes in rats with regard to hyperglycemia and dyslipidemia associated with it.

Figure 1: Calotropis procera (family: Asclepiadaceae, genus:Calotropis, species: procera) is a tropical and subtropical drought-resistant, salt-tolerant shrub wildly growing in arid land and roadsides in Pakistan, Saudi Arabia, middle eastern, far eastern and some African countries. Inset: Calotropis procera flowers.

METHODS

Departmental approval was obtained to carry out the study in accordance with the institutional principles for biomedical and animal experimental research.

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ANIMALS
Forty-twoWistar albino male rats with an average weight of 210+23 grams were randomly divided into groups of six each as follows:
I. Negative Control:non-diabetic, no treatment; water and laboratory chew available ad libitum
II. Experimental Group: all animals treated with streptozotocin and confirmed to be diabetic; water and laboratory chew available ad libitum
a. Positive Control:5 mg/kg body weight of glibenclamideorally daily
b. CFEX 10:10 mg/kg body weight of Calotropis flower extract orally daily
c. CFEX 20: 20 mg/kg body weight ofCalotropis flower extractorally daily
d. CFEX 30: 30 mg/kg body weight of Calotropis flower extract orally daily
e. CFEX 40: 40 mg/kg body weight of Calotropis flower extractorally daily
f. CFEX 50: 50 mg/kg body weight of Calotropis flower extract orally daily

INDUCTION OF DIABETES:
Streptozotocin (STZ) purchased from Sigma Chemical Co St Louis, USAwas injected intraperitoneally at 0.05 g/kg body weight in 0.1M Citrate buffer (pH 4.5)to each experimental animal in a single dose. Blood serum glucose level was measured after seventy-two hours and animals with a glucose level of 200 mg/dl or above were considered diabetic.

PREPARATION OF CALOTROPIS FLOWER EXTRACT:
Calotropis procera flowers (Fig. 1)were freshly collected from wild growing plants in the Umm al Qura University campus at Makkah. Flowers were washed in cool running tap water to remove any dust or grit and then dried in shade at room temperature.

Flower extract was prepared as follows: dried flowers were ground to a fine powder and sifted through a kitchen strainer, 100 grams of powder was then mixed with distilled water in a flask and shaken for 36 hours in a shaker. The suspension was filtered through muslin cloth and then Whatman#1 filter. The filtrate was vacuum dried at 50 C. The yield was 4.8% (w/w).

TREATMENT:
The diabetic animals were treated for twenty-two days. The experimental animals received 10, 20, 30,40, and 50 mg/ kg body weight of Calotropis flower extract orally dailywhilepositive control group received glibenclamide 5mg/kg body weight orally daily. Water and laboratory chow were available at libitum.

PARAMETERS OF OBSERVATIONS:
Weight of the animals was recordedand blood samples were drawn at the beginning of experiment followed by intervals of 24 hours, one week, two weeks and three weeks. Blood serum was analyzed for glucose to assess the antidiabetic effect. Assessment of lipid profile included total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TAG).

DATA ANALYSIS:
Data weresubjected to statistical analysis andexpressed as mean ± SEM. Comparisons were made between the experimental and control groups using unpaired t-test. Values were considered statistically significant at p 0.05.

RESULTS

Weight of the experimental group of animals remained steady during the first two weeks of treatment with all doses of CFEX;however, after the third week there was aninsignificant decrease in weight in all treated groups as compared to the negative control group. A similar and insignificant decrease in weight was observed in the positive control group that was diabetic and treated with glibenclamide. The weight loss in the CFEX treated animals was not significant when compared with the positive control group (Table 1).

The serum glucose level significantly increased in all diabetic rats as compared to the negative control animals and remained so until the end of the study (Table 2). Whereas higher levels of glucose were observed in all treated groups at the end of first week CFEX-treated animals had aserum level comparable andinsignificantly lower than the positive controls. Similar observations were recorded for the later weeks of treatment. After the third week of treatment however,the serum glucose values although still higher than the negative control animals were not significantly different in CFEX-treated animals as compared to the positive control group (Table 2).

There was not much decrease in total cholesterol serum levels (TC) after 24 hours; afterwards the TC levels significantly and steadily decreased in all groups treated with CFEX after the first week and continued to decline during the second and third week as compared to the negative control group and the initial values in the treated group ($p = 0.0001$).The effect appeared to be more pronounced with CFEX doses of 30, 40 and 50 mg (Table 3).

Serum triglyceride (TG) levels started declining significantly after 24 hours and continued the trend so that the decrease was very highly significantwhen compared to the negative and positive control animals ($p = 0.0001$ for both) during the later period of the experiment (Table 4). The effect was more pronounced with CFEX doses of 20, 30, 40 and 50 mg. In all treated groups the TC levels were highly significantly lower ($p = 0.0001$) at the end of 3rd week than the initial levels at the beginning of the experiment.

The serum levels of high-density lipoproteins (HDL-C) indicated aninsignificant increase after one week in all theanimals treated withCFEX as compared to the negative control group; a similar and insignificant increase was noted after the first and second week when the positive and negative groups were compared (Table 5).

The low-density lipoproteins (LDL-C) serum levels slightly but significantly increased in the positive control group as compared to the negative controls at the end of first week; the difference wassustained throughout the period of experiment. In the CFEX treated groups there was no significant change after one day but afterwards a significant and steady decline was observed which continued throughout the next weeks. The decreasing levels were found to be extremely highly significant ($p = 0.0001$)when compared to both the positive and negative control groups (Table 6).

Table1: Showing weight of the animals in grams (Mean ± SEM)

The differences were not statistically significant at 95% confidence interval when compared with the negative and positive control groups and with the initial value (day 0) of the same group.

Groups	Day 0	1 Day	1 Week	2 Weeks	3 Weeks
Negative control	208 ± 18	206 ± 23	211 ± 16	218 ± 12	232 ± 21
Positive control	212 ± 22	213 ± 18	209 ± 16	198 ± 19	186 ± 14
CFEX 10 mg	216 ± 16	196 ± 12	200 ± 14	198 ± 13	202 ± 18
CFEX 20 mg	210 ± 20	199 ± 13	201 ± 16	196 ± 18	194 ± 14
CFEX 30 mg	212 ± 17	194 ± 15	199 ± 11	189 ± 19	192 ± 15
CFEX 40 mg	216 ± 19	195 ± 17	193 ± 12	185 ± 14	181 ± 12
CFEX 50 mg	209 ± 14	193 ± 13	196 ± 18	188 ± 12	192 ± 15

Table 2: Showing serum levels of glucose (mg/dl) incontrol and diabetics rats (Mean ± SEM)

[a]Significantat 95% confidence interval when compared with the negative control group
[b]Significantat 95% confidence interval when compared with the positive control group
[c]Significantat 95% confidence interval when compared with the initial value (day 0) of the same group

Groups	Day 0	Day 1	1 Week	2 Weeks	3 Weeks
Negative Control	116 ± 7.43	112 ± 6.86	108 ± 5.92	114 ± 8.74	102 ± 3.63
Positive Control	253 ± 8.97	168 ± 10.43 p-values a-0.0001 b-0.0002	144 ± 9.33 p-values a-0.0001 b-0.0002	152 ± 6.95 p-values a-0.0001 b-0.0002	149 ± 7.44 p-values a-0.0002 b-0.0002
CFEX 10 mg	285 ± 10.65	277 ± 2.36 p-values a-0.0001 b-0.0001	188 ± 7.61 p-values a-0.0001 b-0.0001	172 ± 5.83 p-values a-0.0001 b-0.0002	163 ± 7.66 p-values a-0.0001 b-0.0001
CFEX 20 mg	266 ± 9.67	254 ± 8.65 p-values a-0.0001 b-0.0001	176 ± 8.43 p-values a-0.0001 b-0.0001 c-0.0002	163 ± 7.91 p-values a-0.0001 b-0.0001 c-0.0001	147 ± 7.66 p-values a-0.0001 b-0.0001
CFEX 30 mg	275 ± 8.33	261 ± 6.76 p-values a-0.0001 b-0.0001	166 ± 5.89 p-values a-0.0001 b-0.0001 c-0.0001	159 ± 9.32 p-values a-0.0001 b-0.0001	152 ± 8.43 p-values a-0.0001 b-0.0001
CFEX 40 mg	282 ± 11.83	283 ± 8.77 p-values a-0.0001 b-0.0001	154 ± 6.65 p-values a-0.0001 b-0.0001	143 ± 8.33 p-values a-0.0001 b-0.0001	145 ± 7.88 p-values a-0.0001 b-0.0001

CFEX 50 mg	273 ± 9.45	256 ± 7.21 p-values a-0.0001 b-0.0001	163 ± 5.78 p-values a-0.0001 b-0.0001	148 ± 8.83 p-values a-0.0001 b-0.0001	134 ± 8.21 p-values a-0.0001 b-0.0001

Table 3: Showing total cholestrol serum levels (TC) mmol/l in control and diabetics rats (Mean ± SEM)
[a]Significantat 95% confidence interval when compared with the negative control group
[b]Significantat 95% confidence interval when compared with the positive control group
[c]Significantat 95% confidence interval when compared with the initial value (day 0) of the same group

Groups	Day 0	Day 1	1 Week	2 Weeks	3 Weeks
Negative Control	2.311± 0.107	2.263 ± 0.131	2.487 ± 0.131	2.337 ± 0.181	2.363 ± 0.147
Positive Control	2.443 ± 0.132	2.341 ± 0.143	2.648 ± 0.186	2.527 ± 0.193	2.853 ± 0.187
CFEX 10 mg	2.247 ± 0.137	2.231 ± 0.121	2.261 ± 0.132	1.974 ± 0.098[b] p-values b-0.0286	2.103 ± 0.137[b] p-values b-0.0089
CFEX 20 mg	2.351 ± 0.152	2.184 ± 0.153	1.964 ± 0.125[a,b] p-values a-0.332 b-0.0122	1.879 ± 0.131[b,c] p-values b-0.0195 c-0.0411	1.881 ± 0.166[b] p-values b-0.0030
CFEX 30 mg	2.148 ± 0.127	2.173 ± 0.147	1.876 ± 0.165[a,b] p-values a-0.0278 b-0.0112	1.764 ± 0.154[a,b] p-values a-0.0366 b-0.0114	1.748 ± 0.191[a,b] p-values a-0.0288 b-0.0020
CFEX 40 mg	2.336 ± 0.136	2.443 ± 0.132	2.165 ± 0.131	1.853 ± 0.132[b,c] p-values b-0.0163 c-0.0289	1.678 ± 0.183[a,b,c] p-values a-0.0153 b-0.0012 c-0.0162
CFEX 50 mg	2.241 ± 0.129	2.311 ± 0.115	2.112 ± 0.120[b] p-values b-0.0360	1.783 ± 0.144[a,b,c] p-values a-0.0376 b-0.0114 c-0.0393	1.782 ± 0.164[a,b] p-values a-0.0248 b-0.0015

Table 4: Showing triglyceride (TG) mmol/Iserum levels in control and diabetic rats; (Mean ± SEM)
[a]Significantat 95% confidence interval when compared with the negative control group
[b]Significantat 95% confidence interval when compared with the positive control group
[c]Significantat 95% confidence interval when compared with the initial value (day 0) of the same group

Groups	Day 0	Day 1	1 Week	2 Weeks	3 Weeks
Negative Control	0.521± 0.036	0.513 ± 0.031	0.531 ± 0.023	0.497 + 0.033	0.541 ± 0.028
Positive Control	0.516 ± 0.017	0.486 ± 0.021	0.479 ± 0.013	0.510 + 0.023	0.531 ± 0.017

CFEX 10 mg	2.522 ± 0.026	0.456 ± 0.017	1.411 ± 0.014[a,b,c] p-values b-0.0286	0.392 ± 0.018[a,b,c] p-values a-0.0190 b-0.0024 c-0.0021	0.376 ± 0.040[a,c] p-values a-0.0070 c-0.0120
CFEX 20 mg	0.537 ± 0.018	0.422 ± 0.022	0.413 ± 0.019[a,b,c] p-values b-0.0195 c-0.0411	0.364 ± 0.021[a,b,c] p-values a-0.00068 b-0.0009 c-0.0001	0.284 ± 0.024[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001
CFEX 30 mg	0.508 ± 0.021	0.410 ± 0.0265[a,b,c]	0.387 ± 0.222[a,b,c] p-values a-0.0366 b-0.0114	0.311 ± 0.026[a,b,c] p-values a-0.013 b-0.0002 c-0.0002	0.215 ± 0.030[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001
CFEX 40 mg	0.518 ± 0.017	0.428 ± 0.018[a,c]	0.401 ± 0.012[a,b,c] p-values b-0.0163 c-0.0289	0.316 ± 0.023[a,b,c] p-values a-0.0011 b-0.0001 c-0.0001	0.241 ± 0.029[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001
CFEX 50 mg	0.526 ± 0.019	0.412 ± 0.020[a,b,c]	0.385 ± 0.024[a,b,c] p-values a-0.0376 b-0.0114 c-0.0393	0.306 ± 0.018[a,b,c] p-values a-0.0005 b-0.0001 c-0.0001	0.236 ± 0.016[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001

Table5: Showing high-density lippoprotein cholestrol (HDL-C)mmol/l serum levels in control and diaberic rats (Mean ± SEM)

The differences were not statistically significant at 95% confidence interval when compared with the negative and positive control groups and with the initial value (day 0) of the same group.

Groups	Day 0	1 Day	1 Week	2 Weeks	3 Weeks
Negative control	4.462 ± 0.107	1.441 ± 0.102	1.732 ± 0.132	1.689 ± 0.106	1.652 ± 0.141
Positive control	1.471 ± 0.115	1.443 ± 0.121	1.484 ± 0.130	1.381 ± 0.133	1.491 ±0.143
CFEX 10 mg	1.496 ± 0.121	1.471 ± 0.111	1.583 ± 0.127	1.610 ± 0.142	1.632 ± 0.132
CFEX 20 mg	1.484 ± 20.109	1.317 ± 0.162	1.432 ± 0.153	1.597 ± 0.123	1.710 ± 0.137
CFEX 30 mg	1.498 ± 0.117	1.466 ± 0.133	1.495 ± 0.144	1.564 ± 0.141	1.686 ± 0.119
CFEX 40 mg	1.476 ± 0.133	1.323 ± 0.127	1.542 ± 0.122	1.652 ± 0.114	1.735 ± 0.142
CFEX 50 mg	1.486 ± 0.147	1.432 ± 0.126	1.574 ± 0.134	1.733 ± 0.126	1.818 ± 0.138

Table 6: Showing low-density lipoprotein cholestrol (LDL-C) mmol/lserum levels in control and diabetic rats; (Mean ± SEM)

[a]Significantat 95% confidence interval when compared with the negative control group
[b]Significantat 95% confidence interval when compared with the positive control group
[c]Significantat 95% confidence interval when compared with the initial value (day 0) of the same group

Groups	Day 0	Day 1	1 Week	2 Weeks	3 Weeks
Negative Control	0.828± 0.031	0.828 ± 0.028	0.831 ± 0.019	0.814 ± 0.0021	0.796 ± 0.031
Positive Control	0.894 ± 0.043	0.875 ± 0.030	0.910 ± 0.022[a] p-values a-0.0216	0.889 ± 0.026[a] p-values a-0.0487	0.916 ± 0.027[a] p-values a-0.0153
CFEX 10 mg	2.913 ± 0.037	0.844 ± 0.042	0.784 ± 0.037[b,c] p-values b-0.0151 c-0.0334	0.693 + 0.022[a,b,c] p-values a-0.0026 b-0.0002 c-0.0005	0.642 ± 0.031[a,b,c] p-values a-0.0056 b-0.0001 c-0.0002
CFEX 20 mg	0.921 ± 0.041	0.863 ± 0.051	0.774 ± 0.033[b,c] p-values b-0.0064 c-0.0190	0.618 ± 0.031[a,b,c] p-values a-0.00068 b-0.0009 c-0.0001	0.583 ± 0.033[a,b,c] p-values a-0.0008 b-0.0001 c-0.0001
CFEX 30 mg	0.964 ± 0.057	0.831 ± 0.023	0.685 ± 0.041[a,b,c] p-values a-0.0090 b-0.0007 c-0.0026	0.542 ± 0.023[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001	0.377 ± 0.042[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001
CFEX 40 mg	0.972 ± 0.067	0.846 ± 0.037	0.621 ± 0.038[a,b,c] p-values a-0.0006 b-0.0001 c-0.0010	0.472 ± 0.027[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001	0.292 ± 0.029[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001
CFEX 50 mg	0.941 ± 0.072	0.851 ± 0.028	0.645 ± 0.027[a,b,c] p-values a-0.0002 b-0.0001 c-0.0032	0.420 ± 0.038[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001	0.262 ± 0.033[a,b,c] p-values a-0.0001 b-0.0001 c-0.0001

DISCUSSION

Out of the several forms type II diabetes is the main category affecting major populations across the globe; ninety percent diabetics suffer from this type [1,8]. Carbohydrate metabolism is the targeted victim in diabetes mellitus which when disrupted in turn leads to derangements of lipids and proteins. The laboratory hallmarks of diabetes mellitus remain hyperglycemia and dyslipidemia associated with it; therefore, any attempt at bringing the elevated glucose and deranged lipid levels to normal or near normal is desirable and worthwhile in its management.

Streptozotocin selectively kills the beta cells in the pancreas and produces a model that effectively produces hyperglycemia and associated dyslipidemia in rats; the condition closelyresembles type II diabetes in humans. This model is reliable and has widely been used in diabetic research; it was adopted in this study. The parameters of observations i.e., serum levels of glucose, total cholesterol, triglycerides and high and low density lipoproteins were appropriate and reflected the aim of the study. Glibenclamide has been used previouslyas an antidiabetic agent in STZ-induced diabetic rats to compare the effects of other hypoglycemic compounds[9,10].

Induction of diabetes by streptozotocin leads to severe loss of weight. The weight loss observed in all the treated groups was significant when compared with the initial weight at the start of experiment; notwithstanding the fact that it was not significantly different from the positive control group treated with glibenclamide, isstill indicative that the results of treatment with CFEX were at least comparable to the test drug.

Serum glucose levels drastically dropped as compared to the negative control group after first week and continued the trend until the end of third week. The effect was more pronounced in CFEX 20, 30, 40 and 50 where the serum glucose levels were not significantly different from the positive control group. A similar observation was recorded in the group CFEX 40 where the serum levels did not indicate any difference from animals treated with glibenclamide. It seems that the effective dose of the flower extract to achieve hypoglycemic effect may be between 30 and 50 mg/kg body weight.

Antihyperglycemic effects of various other parts of Calotropis plant such as leaves, bark and root, have been

recorded by other workers [10-14]. Rathod et al[15] have shown a significant reduction in serum glucose level along with an improvement in oral glucose tolerance after short-term administration of leaf and flower extract Calotropis gigentia (a different species of the same plant) in streptozotocin-induced diabetic rats.Choudhary et al [16] in their study on alloxan-induced diabetic rats and using chloroform extract at a dose of 200 mg/kg of Calotropis gigentia recorded a moderately significant antidiabetic effect. They also observed that the extract significantly influenced maintaining the serum markers of antioxidant enzymes.Thepresent work supports their observations with respect to flowers of the plant.

The serum total cholesterol levels decreased in all groups treated with CFEX as did the triglyceride levels. The difference was very highly significant in comparison with the normal control and positive control groups of animals. The serum values for High density and low- density lipoproteins indicated a desirable change: the HDL levels increased whereas the LDL levels decreased. The difference was statistically highly significant when compared with the control groups of animals. The antidyslipidemic effect of Calotropis flower extract is not reported in literature.

The flowers of Calotropis plant contain several chemicals including flavonoids, sterol, calotropin, calotoxin, calotropagenin, glucose, glucosamine and L-rhamnose along with enzymes such as proteinase and protease (calotropain)[17].

Association of oxidative stress with several inflammatory and other pathological conditions including diabetes is well known. Whole plant methanol extract of Calotropis procera has been reported to have a very high free radical scavenging ability. This antioxidant potential of the plant is attributed to its high content of flavonoids[17,18].

The presence of glycosides, flavonoids, phenols and alkaloids reported in various parts of the Calotropis plant[17-19]; andalso present in the flowers may possibly be responsible for the antihyperglycemic and antidyslipidemiceffects of the flower extract used in this study. However, the exact pharmacological action of these compounds remains to be elucidated. The results also indicate that Calotropis flower extract is an equally good if not better (because of its antidyslipidemic effect) antidiabetic agent as glibenclamide.

Although several antidiabetic synthetic drugs are available, their cost especially for the poor societies is high which underscores the need for research to explore cheaper herbal alternatives. Calotropis procera flowers seem to have the potential of a cheap and easily accessible antidiabetic agent.

Further work, however is warranted to find out the active principals and their dosage and to elucidate the pharmacological properties and mechanism of antihyperglycemic and antidyslipidemic effects of the Calotropis procera flower extract viz a viz any toxic effects.

CONCLUSION

Calotropis procera flower extract has shown antihyperglycemic and antidyslipidemic effects in experimental animals in this study. The plant seems to have the potential of a cheap and easily accessible antidiabetic; however further research is required to explore the safety and pharmacological active principals and their mode of action.

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