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Original Research Article

Comparison between the effect of *Commiphora gileadenesis* and metformin on blood glucose levels and lipid profile in diabetic mice

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Abstract

Purpose: *To investigate the effect of C. gileadensis on blood glucose levels and lipid profiles in diabetic mice.*
Methods: *A total of 60 male mice were divided randomly into 6 groups of 10 each. Type 2 diabetes was induced with streptozotocin (55 mg/kg body weight) intraperitoneally. The experiment consisted of six groups: negative control, diabetic non-treated, and diabetic groups treated with 200 mg/kg body weight per day of sap, methanolic, and acetone extracts of C. gileadensis orally for 4 months. Furthermore, the sixth group was diabetic mice treated with 55 mg/kg of metformin daily by intragastric gavage for 4 months. After 4 months of treatment, blood samples were collected from the retro-orbital venous plexus in non-heparinized tubes from all mice and random blood glucose levels, lipid profile, and hemoglobin A1c (HbA1c) were evaluated.*
Results: *The random blood glucose levels, triglyceride, total cholesterol, and low-density lipoprotein (LDL) levels were significantly ($p < 0.0001$) reduced in diabetic mice treated with C. gileadensis sap, a methanolic extract of C. gileadensis, and an acetone extract of C. gileadensis compared with non-treated diabetic group. Moreover, diabetic mice treated with C. gileadensis sap had significantly ($p < 0.01$) lower total cholesterol and LDL compared with those treated with metformin. Diabetic mice treated with C. gileadensis sap, methanol, and acetone extract had significantly ($p < 0.0001$) higher HDL levels compared to untreated diabetic group. However, C. gileadensis sap significantly increased HDL in diabetic mice compared to metformin ($p < 0.01$).*
Conclusion: *The sap, methanolic and acetone extracts of C. gileadensis normalize random blood glucose, HbA1c, and lipid profiles in diabetic mice. The sap of C. gileadensis exhibits greater efficacy compared to other extracts and metformin in reducing random blood glucose levels and improving lipid profiles.*

Keywords: *Commiphora gileadensis, Diabetes mellitus, Hemoglobin A1c (HbA1c), Lipid profile*

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INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by persistent high blood sugar levels (hyperglycemia), a condition that is caused by abnormalities in the production and/or function of insulin [1]. Scientific researchers have demonstrated that diabetes has a detrimental impact on quality of life by amplifying risk factors for stroke, amputation, renal failure, and blindness, resulting in significant morbidity and premature mortality [2]. Despite the availability of

numerous oral hypoglycemic medicines such as glucagon-like peptide 1 receptor agonists (GLP-1RA), dipeptidyl peptidase-4 inhibitors (DPP-4), sodium-glucose cotransporter 2 inhibitors (SGLT-2) and insulin, there remains no permanent cure for diabetes [3]. Diabetes mellitus has emerged as a significant healthcare concern in the 21st century. Based on the existing population of 150 million cases, it is projected that the number of cases will increase to 220 million by 2010, and further rise to 300 million by 2030 [4]. Therapeutic approaches for diabetes mellitus have advanced so far. However, some anti-diabetic medications have severe consequences, including hypoglycemic coma as well as liver and kidney diseases [5].

Herbal medicine is a fast-growing branch of medicine that requires attention. For thousands of years, plants have played a crucial role in improving the overall quality of human life. Also, they have been used by individuals as valuable medicinal components [6]. The World Health Organization (WHO) reports that 80 % of the global population depends on traditional medicine as their primary source of healthcare. The predominant therapeutic approach involves the utilization of plant extracts or their bioactive constituents. This assertion is supported by the observation that medicinal plants frequently offer cost-effectiveness and minimal adverse effects compared to regularly prescribed medications [7]. More than 1200 plant species are used to treat diabetes mellitus worldwide. Many of these plants have been tested in the laboratory and found to have effective hypoglycemic or anti-hyperglycemic activity. These medicinal herbs provide oral hypoglycemic ingredients for new pharmaceutical formulations and as dietary supplements [8].

One of those plants that have long been used to treat a variety of ailments is *Commiphora gileadensis* (*C. gileadensis*). *C. gileadensis* is a small tree under the genus *Commiphora* and the *Burseraceae family. C. gileadensis* is widespread throughout Arab, including Saudi Arabia, which is known there as Besham [9]. This shrub grows near the “Sarawat Mountains” in western Saudi Arabia. Arabic names for *C. gileadensis* include balm of Makkah, basin, and bisham. Hellenistic and Roman utilized *C. gileadensis* balsam as a scent. A previous study has investigated the analgesic, diuretic, and antihypertensive properties of *C. gileadensis* [10]. *In vivo* and *in vitro* experiments found that *C. gileadensis* sap has an antibacterial effect [11]. A prior study indicated that *C. gileadensis* methanolic extract enhances wound healing [12].

This study investigated the effect of several extracts derived from *C. gileadensis* on blood glucose and lipid profiles in diabetic mice.

EXPERIMENTAL

Plant collection

C. gileadensis was collected from the Alaab Valley, located near Al-Madinah region of western Saudi Arabia. *C. gileadensis* is the most famous plant in Saudi Arabia. Leaves and fallen branches were collected by an expert in March 2023.

Preparation of *Commiphora gileadensis* sap

After cutting *C. gileadensis* branch tips (5 mm in length), 100 mL of the sap was collected immediately. The sap was centrifuged for 10 min at 10,000 rpm, agitated for 15 min at room temperature and thereafter 50 g was diluted in ethanol. The supernatant was stored at -20 °C for further analysis [13]. Total amount of this sap used in the trial was 5 kg for 120 days.

Preparation of *Commiphora gileadensis* methanol extract

After washing, the leaves and branches of *C. gileadensis* were dried at 40 °C in a hot-air oven, sieved to remove contaminants and ground into fine powder. The powder (10 g) was macerated in 100 mL of 100 % methanol in a sterile funnel for 24 h. The funnel was forcefully shaken before filtration using sterile filter paper. The extract was thereafter concentrated in a water bath at 40 °C and refrigerated at 4 °C for 2 weeks [11]. Total amount of extract used in the study was 5 kg for 120 days.

Preparation of *Commiphora gileadensis* acetone extract

After drying at 60 °C for 6 h in a vacuum oven, *C. gileadensis* leaves and branches were crushed into powder using blades. The sample (10 g) was immersed in 200 mL of acetone at room temperature for 3 days, and replenished daily (50 mL) on a magnetic stirrer. The extract was concentrated using a rotary evaporator. The extract was stored at -20 °C for further analysis [14]. Total amount of extract used in the study was 5 kg for 120 days.

Study design

A total of 60 male BALB/c mice, two months old, weighing 20–25 g, were obtained from the Umm Al-Qura University animal house and randomly

assigned to 6 groups (n = 10 in each group). The mice were housed in a normal rodent cage to acclimatize for 2 weeks under standard conditions (well-ventilated room, room temperature, 12 h light / dark cycle, with uninterrupted access to feed and water *ad libitum*).

Group 1 was designated as negative control. Mice in this group received no treatment. Group 2 was the positive control group (untreated diabetic group). Mice in this group received streptozotocin intraperitoneally (55 mg/kg) for five consecutive days to induce diabetes mellitus [15]. Group 3 was designated as diabetic *C. gileadensis* methanolic extract-treated group. Diabetes mellitus was induced in the second group and the animals received *C. gileadensis* methanolic extract (200 mg/kg) via intragastric gavage (after 1 week of streptozotocin administration) once daily for four months. Group 4 was designated as diabetic *C. gileadensis* acetone extract-treated group.

Animals received *C. gileadensis* acetone extract (200 mg/kg) once daily via intragastric gavage following the administration of streptozotocin for four months. Group 5 received *C. gileadensis* sap (200 mg/kg per day) via intragastric gavage after one week of streptozotocin administration for four months. Group 6 received metformin (55 mg/kg) daily by intragastric gavage after one week of streptozotocin administration for four months.

The animals were fed standard diet for rodents and had unrestricted access to tap water. Random blood glucose levels were assessed every five days. At the end of treatment, blood samples were collected to assess glycalated haemoglobin (HbA_1c) and lipid profile comprising total cholesterol, triglycerides, low-density lipoprotein and high-density lipoprotein. The study was approved by the National Committee for Bioethics at Taif University (approval no. HAO-02-T-105) and complied with the internationally accepted guidelines for the Care and Use of Laboratory Animals [16].

Viscosity

The viscosity of *C. gileadensis* extracts and sap was measured using a viscometer (BDRRL digital rotatory viscometer 0.1 - 2000000 cp/mpa.s).

Body weight

The body weight was measured for 10 weeks using a digital balance (OHAUS, Model: Scout Pro SPU601, China). Each mouse in the six groups was weighed before streptozotocin injection (day 1), and 2, 4, 6, 8, and 10 weeks after treatment with different *C. gileadensis* extracts, sap and metformin.

Blood collection

Blood samples were withdrawn from the retro-orbital venous plexus into non-heparinized tubes before and 6 days after streptozotocin administration. Blood samples were centrifuged at 2500 rpm for 15 min, and the resulting sera were preserved at - 80 °C. Random blood glucose levels were assessed every 3 days following treatment with the extracts and sap of *C. gileadensis*. The HbA1c and lipid profile were assessed after 4 months following treatment with *C. gileadensis*.

Random blood glucose levels and HbA_1c

Random blood glucose levels were assessed by colorimetric assay, and HbA_1c was tested after 4 months using a glycohemoglobin kit (POINTE Scientific Inc., USA).

Lipid profile estimation

A colorimetric assay was used to determine triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

Statistical analysis

Analysis was done using Prism software version 10 (GraphPad software, San Diego, USA). Results were expressed as mean ± standard deviation (SD). Weight, HbA_1c, triglyceride, total cholesterol, HDL, and LDL comparisons among the groups were made using one-way analysis of variance (ANOVA). Comparison between blood glucose levels in the same group was done using Wilcoxon signed-rank test. Furthermore, the comparison of blood glucose levels over 21 days was done using two-way ANOVA. $P < 0.05$ was considered statistically significant using the Bonferroni / Dunn test.

RESULTS

Viscosity of *C. gileadensis* methanol extract, acetone extract, and sap

Viscosity of *C. gileadensis* methanol extract was 3201 centipoises (cps), while the acetone extract was 2147 cps. The *C. gileadensis* sap was more viscous (9121 cps).

Body weight

In the 10th week, methanol, acetone extract, and sap-treated groups showed significantly higher weights compared to untreated diabetes group ($p < 0.05$; Figure 1).

Blood glucose levels

Treatment with methanol, acetone extract and sap significantly lowers blood glucose levels ($p < 0.0001$) 12th, 15th, 18th, and 21st day following treatment compared to untreated diabetic group. Furthermore, *C. gileadensis* sap restores normal blood glucose levels on the 12th day, while methanol and acetone extracts on the 18th day. Also, *C. gileadensis* sap significantly ($p < 0.01$) reduced blood glucose levels on the 9th, 12th, and 15th day compared to metformin-treated group (Figure 2).

Level of HbA_1c

Treatment with *C. gileadensis* methanol extract, acetone extract, or sap exhibited significantly lower ($p < 0.05$) HbA_1c values compared to untreated group. HbA_1c value was non-significantly lower in the *C. gileadensis* sap-treated group compared to the diabetic metformin-treated group (Figure 3).

Lipid profile

Treatment with methanol, acetone extract, and sap of *C. gileadensis* significantly lowers triglycerides compared to untreated group ($p < 0.0001$). Diabetic mice treated with *C. gileadensis* sap exhibited the lowest total cholesterol levels (Figure 4 A).

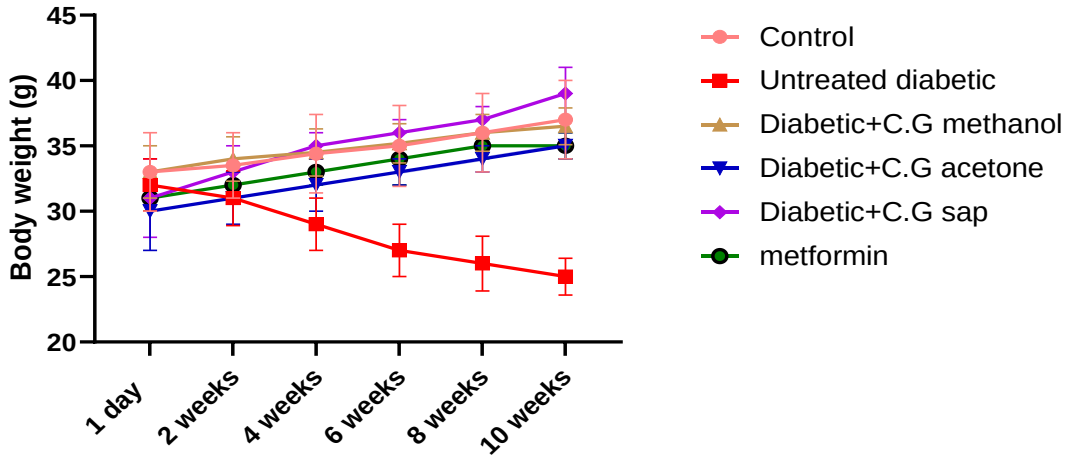

Figure 1: Effect of treatment on the weight of animals

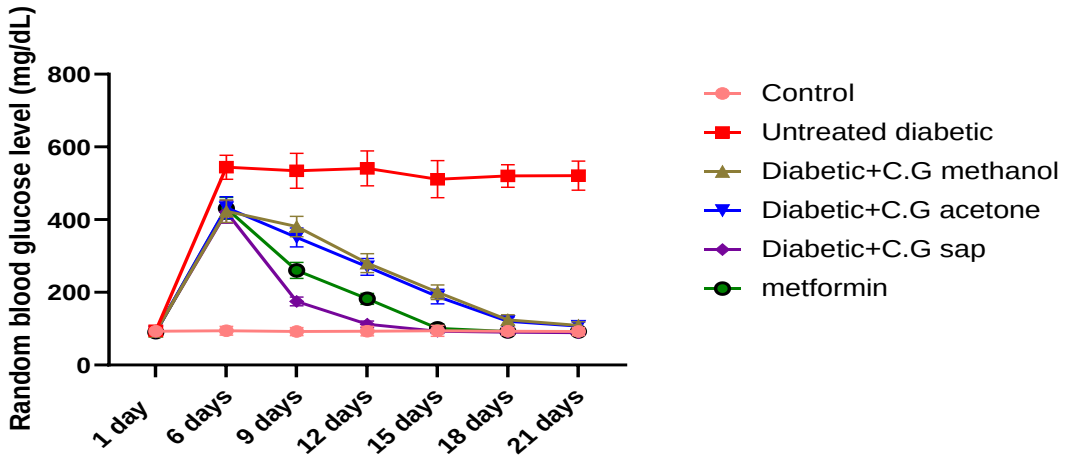

Figure 2: Effect of treatment on random blood glucose levels

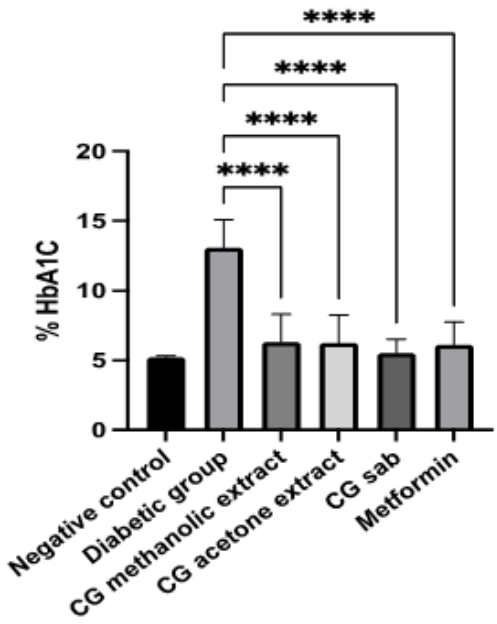

Figure 3: $HbA_{1}c$ level

Diabetic animals treated with methanolic extract, acetone extract, and sap of *C. gileadensis* exhibited significantly lower total cholesterol levels compared to untreated diabetic mice ($p < 0.0001$; Figure 4 B). Furthermore, diabetic mice treated with *C. gileadensis* sap had significantly lower total cholesterol levels compared to metformin ($p < 0.01$; Figure 5). Regarding LDL diabetic mice treated with *C. gileadensis* methanol extract, acetone extract, or sap, exhibited dramatically reduced LDL levels in comparison to the untreated diabetic cohort ($p < 0.0001$). Moreover, diabetic mice treated with *C. gileadensis* sap had significantly lower LDL than metformin-treated group ($p < 0.01$; Figure 6). The diabetic group treated with *C. gileadensis* methanol extract, acetone extract, or sap had significantly greater HDL levels than the untreated diabetic group ($p < 0.0001$). In addition, HDL was significantly increased in *C. gileadensis* sap-treated group compared with metformin-treated group ($p < 0.01$; Figure 7).

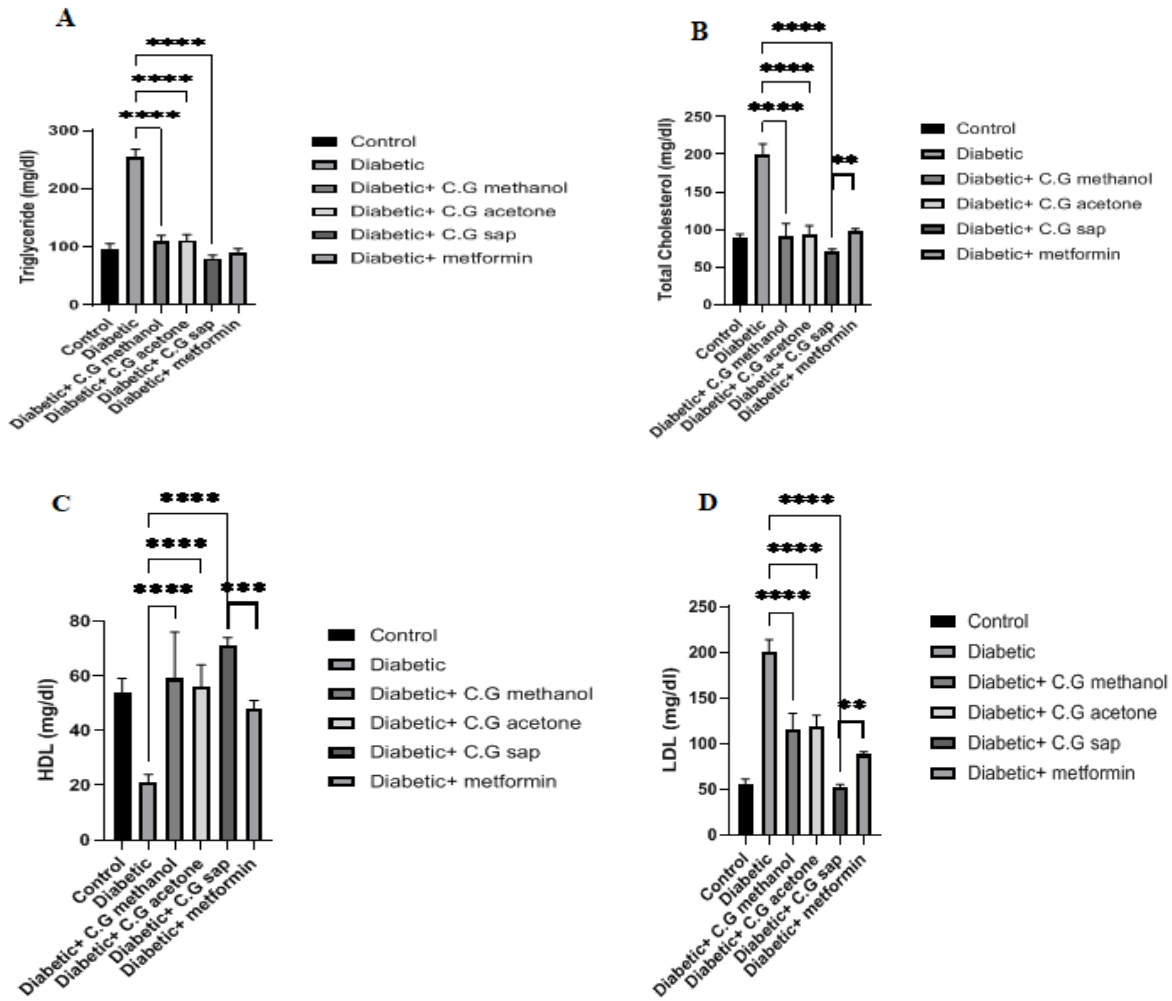

Figure 4: Lipid profile. A: triglyceride levels (mg/dL), B: total cholesterol levels (mg/dL), C: HDL levels (mg/dL), LDL levels (mg/dL)

DISCUSSION

Medicinal plants or plant-based medicines are cost-effective worldwide to prevent and/or treat diabetes. Many underdeveloped countries manage diabetes and other illnesses with plant-based therapy. Many modern medications are structurally derived from traditional medicinal plant components. Streptozotocin is specifically toxic to pancreatic β-cells due to the presence of large levels of glucose transporter 2 (GLUT2) proteins in these cells. As a result, it induces type 1 diabetes mellitus [15].

Antioxidant activity of *C. gileadensis* has been attributed to the presence of various free radical scavengers, including saponins, flavonoids, volatile oils, sterols, and triterpenes [17]. Another study observed that aqueous extract of *C. gileadensis* improved liver and kidney function and reduced blood glucose levels in diabetic rats. Findings from this present study revealed that the sap, methanol and acetone extracts of *C. gileadensis* reduced random blood glucose levels. The *C. gileadensis* sap demonstrated the most significant effect in reducing random blood glucose levels compared to methanol and acetone extracts. Also, sap of *C. gileadensis* showed more efficacy than metformin in lowering blood glucose levels. *C. gileadensis* sap, along with its methanol and acetone extracts, decreased HbA_1c. Compared to metformin, sap of *C. gileadensis* had superior activity in reducing HbA_1c. Metformin was used as an anti-diabetogenic medication in the current study because it is the first-line drug for diabetes therapy and has no hypoglycemia induction.

Previous studies demonstrated that aqueous extract of *C. gileadensis* effectively decreased levels of triglycerides, total cholesterol, and LDL in diabetic rats, restoring them to normal levels. However, aqueous extract of *C. gileadensis* increased HDL levels in rats [15]. The present study demonstrated that the sap, methanol extract, and acetone extract of *C. gileadensis* reduced triglyceride, total cholesterol, and LDL levels, while concurrently boosting HDL comparable to metformin. The current investigation demonstrated that several extracts derived from *C. gileadensis* exhibited various levels of antidiabetic activity. The sap demonstrated significantly higher antidiabetic effects compared to its methanolic and acetone extracts. These differences in antidiabetic efficacy may be attributed to the diversity in viscosity of the sap, methanol and acetone extracts. Specifically, sap of *C. gileadensis* showed higher viscosity. Observed viscosity between *C. gileadensis* sap and the methanol and acetone extracts suggests that the former exhibited a greater abundance of free radical scavengers, including saponins, flavonoids, volatile oils, sterols, and triterpenes. Consequently, the *C. gileadensis* sap demonstrated significantly higher antioxidant activity compared to methanol and acetone extracts. This antioxidant activity may improve the function of β-cells and restore their ability to produce insulin.

CONCLUSION

The sap, methanol and acetone extracts of *C. gileadensis* reduce random blood glucose levels, HbA1c, and lipid profiles in diabetic mice. However, the sap is more potent than metformin in reducing random blood glucose levels. A human-based study is recommended to evaluate the antidiabetic effect of *C. gileadensis*, as well as an *in vitro* study of the effect of *C. gileadensis* on β-cell cultures to determine the mechanism through which this occurs.

DECLARATIONS

Acknowledgement/Funding

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Ethical approval

See ‘Experimental’ section.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors.

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