Hepatoprotective, Cardioprotective and Nephroprotective Actions of Essential Oil Extract of Artemisia Sieberi in Alloxan-Induced Diabetic Rats

Fawzi Irshaid, Kamal Mansi, Ahmad Bani-Khaled and Talal Aburjia

Abstract—The aim of current study is to evaluate the potential mechanism of antidiabetic action of the essential oil of Artemisia sieberi and its effects on some hematological and biochemical parameters in alloxan induced diabetic rats. Extraction of essential oil from aerial parts of A. sieberi was preformed by hydrodistillation. Fifty rats were divided into five groups. Groups I and II normal rats given 1 ml/day of dimethyl sulfoxide and 80 mg/kg bw of this oil extract, respectively. Groups III, IV and V diabetic rats given 1 ml/day of dimethyl sulfoxide, oil extract (80 mg/kg bw) and metformin (14.2 mg/kg bw), respectively. Several hematological and biochemical parameters were assessed. Oral administration of the extract resulted in a significant reduction in the mean values of blood glucose, glucagon, cholesterol, triglyceride, LDL-C, ESR, urea, uric acid, creatinine accompanied by an increase in the mean values of total protein, albumin, insulin, HDL-C, neutrophile count and PCV in diabetic rats. No significant changes in these parameters were found in the control group. The effects produced by this extract were closely similar to a standard antidiabetic drug, metformin. In conclusion, the present study indicates that the essential oil extract of A. sieberi appears to exhibit cardioprotective, nephroprotective and hepatoprotective activities in alloxan induced diabetic rats.

Keywords—Cholesterol, metformin, plant extract, uric acid.

I. INTRODUCTION

Diabetes mellitus increases risk of several serious health problems or complications including hyperlipidemia, poor metabolic control, nephropathy, hepatopathy, and cardiomyopathy [1]-[3]. These complications are considered the leading causes for death among these patients. Thus, an early control of DM is recommended as one of main strategy to prevent these complications and increase the life span of these patients. Several synthetic compounds have been used as therapeutic drugs for control of DM, including metformin [4], [5]. This drug is widely used in Jordan to regulate blood glucose level. However, most of synthetic drugs just regulate the blood glucose level and does not completely cure DM and prevent or delay the onset of its complications. Therefore, the insufficient protection by this drug necessitates the need for new treatment to prevent or delay these complications.

The use of natural products such as plant extracts is a common practice in Jordan in relieving and treating several diseases including DM [6]-[8]. The most widely used plant species in treating DM in Jordan is Artemisia sieberi (A. sieberi). This perennial shrub belongs to family Asteraceae and has a strong aromatic smell and a bitter taste. It grows abundantly in arid areas of Middle East countries and other part of the world [9]-[11]. This valuable plant can also be used to treat other ailments in the various traditional systems of medicine. Recently, we have demonstrated that essential oil extract derived from A. sieberi given as a repeated daily dose of 80 mg/Kg bw for six weeks exhibits antidiabetic activity in a diabetic animal model produced by the injection of alloxan in rats [12]. However, the mechanism of antidiabetic action of this essential oil has not yet been determined or investigated. Thus, we recommend that more studies should be conducted to explore more about the role of this extract in prevention of DM complications. Based on these data, the current investigation is aimid to determine the potential mechanism of antidiabetic action of this extract. This investigation is also aimid to explore more about other potential biological and pharmacological activities of this extract, including cardioprotective, nephroprotective and hepatoprotective activities.

II. MATERIALS AND METHODS

A. Plant material and essential oil extraction

Collection, drying, processing and extraction of essential oil from aerial parts of A. sieberi were preformed as described previously [12].
B. Experimental animals and induction of diabetes in rats

Wister rats at weights ranging from 155 to 183 g were used for the present experiment. All rats were purchased from the animal house of the Jordan University of Science and Technology, Irbid, Jordan. They were housed under standard laboratory conditions and treated as described previously [12]. Alloxan monohydrate (BOH Chemical, LTD, Poole, England) was used to induce diabetes in experimental rats as described previously [12]. After two weeks, rats with blood glucose of 200 mg/dl or more were classified as diabetic rats and were used for the subsequent experiments.

C. Experimental Design

Fifty rats were divided into five experimental groups of ten rats each. Group I consisted of normal rats that given only DMSO (0.5 ml/kg bw) and served as control group. Group II consisted of normal rats that given 80 mg/kg bw of oil extract. Group III, IV and V consisted of alloxan induced diabetic rats that given daily DMSO (0.5 ml/kg bw), oil extract (80 mg/kg bw) and metformin (14.2 mg/kg bw), respectively, for 6 weeks by an intragastric tube with free access to food and water. The experimental protocol complied with the guidelines of our animal ethics committee which was established in accordance with the internationally accepted principles for laboratory animal use and care. At the end of the experimental period, blood samples were taken from each rat by cardiac puncture protocol. Rats were sacrificed by cervical dislocation under light ether anesthesia.

D. Hematological analysis

The CBC is usually performed on an automated hematology analyzer using whole well mixed blood to which EDTA is added to prevent clotting. ESR was determined using Westengren method. Differential leucocyte count was conducted on Geimsa stained blood smears. Blood glucose level was measured immediately by Haemo-Glukotest (20-800R) glucose strips supplied by M/S Boehringer Mannheim India Ltd.

E. Biochemical analysis

Lipid profile (total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)), total protein, albumin, blood urea, uric acid and creatinine levels were measured by using Bio-Merieux Kit (Bio- Meraux Lab. reagent and product, France).

F. Insulin and glucagon determination

Serum insulin and glucagon were measured by radioimmunoassay methods (CEA-JRE-SORIN Firm, France).

G. Statistical analysis

Differences between control and experimental groups were estimated using student's t-test analysis. Within group comparisons were performed by analysis of variance using ANOVA test. Differences were considered significant if P-value is less than 0.05.

III. RESULTS

The results for the effect of the oil extract in the mean values of some hematological parameters on normal and diabetic rats are presented in Table I. Our data indicated that there were significant decreases (P < 0.05) in the mean values of blood glucose levels in alloxan-diabetic rats treated with the oil extract (Group IV) when compared to untreated diabetic rats (Group III) during the entire period of the study. There were also significant decrease (P < 0.05) in the mean values of neutrophile count and PCV accompanied by a significant increase (P < 0.05) in the mean values of ESR in untreated diabetic rats compared to control rats (Group I). While an oral injection of 80 mg/Kg bw of the oil extract and 14.2 mg/kg bw of metformin resulted in significantly elevated (P < 0.05) in the mean values of neutrophile count and PCV accompanied by a significant reduction in the mean values of ESR in diabetic rats (Groups IV and V). However, no significant alterations or fluctuations were found in the mean values of other blood parameters including hemoglobin, RBC, basophile, eosinophile and lymphocyte counts in untreated diabetic rats relative to the control rats. The effect of this essential oil dose was comparable to that of metformin administered group.

The average value of insulin was significantly lower (P < 0.05) in untreated diabetic rats compared to respective control rats, as shown in Table II. On other hand, there were significant increases (P < 0.05) in mean value of insulin in diabetic group treated with the oil extract as compared to untreated diabetic rats during the entire period of the study. The insulin mean value in diabetic rats treated with metformin (Group V) was found to be closely similar to that in untreated diabetic rats given only DMSO. Of note, the insulin mean value was significantly reduced in the diabetic rats treated with metformin (Group V) relative to the control rats. Furthermore, the diabetic rats experienced significant increased (P < 0.05) in the mean value of glucagon when compared with the control rats; whereas the mean value of glucagon in diabetic rats treated with the oil extract (Group IV) or metformin was significantly (P < 0.05) lower than that of untreated diabetic rats. The mean value of insulin was not significantly altered in the normal rats treated with the essential oil extract (Group II) relative to the control rats.

According to the data in Table III, significant increase in mean values of the serum total cholesterol, triglyceride and LDL-C were recorded in untreated diabetic rats relative to the control groups (P < 0.05). Compared with the untreated diabetic rats, oral administration of oil extract (80 mg/kg) or metformin (14.2 mg/Kg) for six weeks in diabetic rats (Group IV) resulted in significant decreased (P < 0.05) in the mean values of serum total cholesterol, triglyceride and LDL-C. Along the same line, in untreated diabetic rats, the mean values of serum HDL-C were significantly less than in the control groups (P < 0.05). While the mean values of serum HDL-C were significant higher in diabetic rats treated with the oil extract (Groups IV) when compared with the untreated
diabetic rats (P < 0.05). The effect of the oil extract dose in lipid serum profile in diabetic rats was comparable to that of metformin administered group. Serum lipid profile was not changed in normal rats treated with the oil extract (Group II) relative to the control rats.

As can be seen in Table IV, the mean values of urea, uric acid and creatinine were significantly higher in untreated diabetes rats as compared to the control rats (P < 0.05). Treatment of the diabetic rats with the oil extract and metformin for six weeks (Groups IV and V) caused a significant decrease in urea, uric acid and creatinine as compared to the untreated diabetic group (P < 0.05).

In addition, the average values of total protein and albumin were significantly lower (P < 0.05) in untreated diabetic rats compared to the control rats. On other hand, there were significant (P < 0.05) increases in total protein and albumin in diabetic groups treated with the oil extract or metformin as compared to untreated diabetic group during the entire period of the experiment.

### TABLE I

**Effect of Essential Oil of Artemisia sieberi on Levels of Some Hematological Parameters in Normal and Alloxan Induced Diabetic Rats After Six Weeks of Treatment.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>95 ± 9.6</td>
<td>101 ± 11.5</td>
<td>369 ± 26.8*</td>
<td>204 ± 29.3**</td>
<td>188 ± 19.5**</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.0 ± 1.3</td>
<td>12.3 ± 1.0</td>
<td>10.8 ± 1.0</td>
<td>11.4 ± 0.8</td>
<td>12.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>RBCs x 10⁶ µl</td>
<td>5.8 ± 0.7</td>
<td>5.9 ± 0.9</td>
<td>4.6 ± 0.5</td>
<td>5.0 ± 0.6</td>
<td>5.2 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>WBCs x 10⁶ µl</td>
<td>7.4 ± 1.0</td>
<td>6.5 ± 0.8</td>
<td>4.0 ± 1.4</td>
<td>3.9 ± 0.8</td>
<td>3.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>32.8 ± 3.7</td>
<td>36.9 ± 4.0</td>
<td>24.6 ± 3.2*</td>
<td>36.7 ± 3.4**</td>
<td>39.7 ± 3.6**</td>
<td></td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>2.7 ± 0.2</td>
<td>2.6 ± 0.9</td>
<td>3.5 ± 0.7</td>
<td>3.9 ± 0.3</td>
<td>3.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Basophiles %</td>
<td>3.5 ± 0.2</td>
<td>4.7 ± 0.6</td>
<td>4.4 ± 0.6</td>
<td>4.9 ± 0.4</td>
<td>4.5 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>63.7 ± 3.6</td>
<td>64.4 ± 4.2</td>
<td>63.5 ± 4.2</td>
<td>63.5 ± 4.2</td>
<td>60.3 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>PCV</td>
<td>37.7 ± 2.7</td>
<td>38.5 ± 1.9</td>
<td>30.7 ± 2.3*</td>
<td>35.6 ± 2.7**</td>
<td>35.4 ± 2.6**</td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>14.6 ± 2.4</td>
<td>12.6 ± 1.8</td>
<td>20.6 ± 2.4*</td>
<td>16.5 ± 1.6**</td>
<td>14.4 ± 2.9**</td>
<td></td>
</tr>
</tbody>
</table>

Values (mg/dl) are the mean values ± standard deviation of 10 rats; *: Statistically significant when compared to control group (I) at P < 0.05; **: Statistically significant when compared to untreated diabetic group (III) at P < 0.05.

### TABLE II

**Effect of Essential Oil of Artemisia sieberi on Insulin and Glucagon Levels in Normal and Alloxan Induced Diabetic Rats After Six Weeks of Treatment.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I</th>
<th>II</th>
<th>Groups</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µg/ml)</td>
<td>5.8 ± 0.3</td>
<td>6.1 ± 0.4</td>
<td>3.4 ± 0.1*</td>
<td>4.8 ± 0.1**</td>
<td>3.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Glucagon (pg/mol)</td>
<td>34.8 ± 4.6</td>
<td>38.7 ± 3.9</td>
<td>54.7 ± 8.8**</td>
<td>44.7 ± 3.7**</td>
<td>47.9 ± 6.8**</td>
<td></td>
</tr>
</tbody>
</table>

Values (mg/dl) are the mean values ± standard deviation of 10 rats; *: Statistically significant when compared to control group (I) at P < 0.05; **: Statistically significant when compared to untreated diabetic group (III) at P < 0.05.

### TABLE III

**Effect of Essential Oil of Artemisia sieberi on Plasma Lipid Profiles in Normal and Alloxan Induced Diabetic Rats After Six Weeks of Treatment.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I</th>
<th>II</th>
<th>Groups</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>114 ± 8.3</td>
<td>108 ± 10.5</td>
<td>179 ± 21.45*</td>
<td>152 ± 18.8**</td>
<td>142 ± 16.7**</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>75 ± 11.6</td>
<td>82 ± 9.3</td>
<td>128 ± 19.4*</td>
<td>97 ± 19.5**</td>
<td>95 ± 17.8 **</td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>32 ± 5.6</td>
<td>31 ± 3.2</td>
<td>22 ± 4.3*</td>
<td>30 ± 2.6**</td>
<td>28 ± 3.4**</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>30 ± 4.7</td>
<td>27 ± 3.6</td>
<td>52 ± 11.5*</td>
<td>37 ± 9.4**</td>
<td>41 ± 11.8**</td>
<td></td>
</tr>
</tbody>
</table>

Values (mg/dl) are the mean values ± standard deviation of 10 rats; *: Statistically significant when compared to control group (I) at P < 0.05; **: Statistically significant when compared to untreated diabetic group (III) at P < 0.05.
TABLE IV

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dL)</td>
<td>8.0 ± 1.0</td>
<td>7.7 ± 0.8</td>
<td>5.2 ± 0.7*</td>
<td>6.4 ± 0.8**</td>
<td>7.4 ± 1.0**</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.4 ± 0.7</td>
<td>4.3 ± 0.9*</td>
<td>1.8 ± 0.5*</td>
<td>2.9 ± 0.2**</td>
<td>3.0 ± 0.2**</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>27.8 ± 3.6</td>
<td>29.8 ± 2.5</td>
<td>37.4 ± 6.5*</td>
<td>31.6 ± 4.4**</td>
<td>29.7 ± 5.6**</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.7</td>
<td>2.6 ± 0.8*</td>
<td>1.7 ± 0.2**</td>
<td>1.6 ± 0.2**</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>2.7 ± 0.7*</td>
<td>1.4 ± 0.2**</td>
<td>1.3 ± 0.3**</td>
</tr>
</tbody>
</table>

Values (mg/dl) are the mean values ± standard deviation of 10 rats; *: Statistically significant when compared to control group (I) at P < 0.05; **: Statistically significant when compared to untreated diabetic group (III) at P < 0.05.

IV. DISCUSSION

Recently, our laboratory clearly demonstrated antihyperglycemic action of essential oil derived from A. sieberi [12]. Thus, an attempt has been made in this current work to investigate the potential mechanism of antidiabetic action of this extract in alloxan induced diabetic rats. Our current study demonstrated that continuous oral administration of the essential oil extract of A. sieberi for six weeks significantly improved hyperglycemia in diabetic rats by 45% compared with decreases of 49% with the metformin, a well known antidiabetic drug, after six weeks of oral administration. Also our data revealed for first time that this extract produced a significant increased in insulin level accompanied by significant reduction of glucagon and blood glucose levels in diabetic rats. By contrast, in the normal rats, this extract did not have any effect on blood glucose levels as well as on some hematomical and biochemical parameters investigated. The difference in these observations can be explained by the normal homeostasis mechanisms, such as the secretion of regulatory hormones. These regulatory hormones usually work in the normal animals to maintain the normal homeostasis environment.

Moreover, the destruction of cells in our study appears to be partially and not completely, because plasma insulin levels in the diabetic rats are about 58% of that in normal rats. In addition, our data also revealed that metformin did not change insulin level. The hypoglycemic action of the metformin in diabetic patients has been proposed via decreased glucose production, increased fatty acid oxidation in hepatocytes, and/or increased glucose uptake in skeletal muscle [4], [5], [13]. Thus, the mechanism of antidiabetic activity of essential oil extract appears to be not similar for that of metformin. Interestingly, our data also suggest that antidiabetic action of A. sieberi essential oil has been attributed to stimulation of insulin release from the pancreatic cells and inhibition of glucagon secretion from pancreatic cells, since chronic administration of this extract has positive effect on plasma insulin level and negative effect on plasma glucagon level in diabetic rats. This is also in agreement with some previous studies [14], [15]. These studies noted that there was selected destruction of pancreatic islet cells in alloxan induced diabetic model, since some cells do survive and plasma insulin levels in the diabetic rats are about 22% of that in normal rats, and that insulin secretion can be stimulated in the residual β-cells of these diabetic animals.

In addition to marked hyperglycemia, our result revealed that the alloxan diabetic rats developed notable hyperlipidaemia. Diabetes induced hyperlipidaemia was observed in diabetic experimental animal models, and it is associated with increase of mobilization of fat from fat cells and lipid metabolism due to the inability to utilize glucose properly [15]-[19]. This is very important, since elevated concentrations of cholesterol, triglyceride and LDL-C are important risk factors in the development of artheriosclerosis in diabetes mellitus. Our study also revealed for the first time that this extract normalized serum lipids (cholesterol, triglyceride, HDL-C, LDL-C) closely to the level of normal rats. Our findings are in consistent with a recent study by Bavarva and Narasimhacharya (2010) which reported that leaves of Leucas cephalotes lowered both plasma and hepatic lipid profiles (total lipid, triglycerides and cholesterol) and LDL-C while elevating the HDL-C levels [17]. They suggest that these improvements in lipid profiles are most likely due to its insulin-like actions of the leaves extract. Similarly, a previous study done by Lopes-Virella et al. (1983) reported that DM patients taken insulin injection showed not only elevates lipoprotein lipase activity but also lowers the plasma triglyceride concentrations [18]. Thus, it can be concluded that enhancement of insulin secretion or level is accompanied by enhancement of glucose utilization as well as a reduction of lipid level in diabetic rats. It is possible to suggest that the mechanism(s) of antihyperlipidemic effect of the A. sieberi might be similar to some of those suggested for anti-diabetic plants exhibiting antihyperlipidemic activity, such as activation of lipoprotein lipase, insulin-mediated lipolytic activity or inhibition of lipogenic enzymes or hormone-sensitive lipase [14]-[19].

Our study also showed a significant decrease in serum total protein and albumin in untreated diabetic rats, whereas total protein and albumin significantly increased after the administration of this extract. Total protein and albumin levels in blood can also be used as an indicator of liver function. Similar results were obtained when the metformin were administered orally in alloxan diabetic. These results suggest that this extract can improve some biochemical parameters that are related to liver functions.

Hyperglycemia has also been recently implicated in
initiation and development of various types of diabetic complications. Nephropathy is one of these serious microvascular complications that has been observed in diabetic individuals [2]. In addition, blood urea and creatinine concentrations were increased among uncontrolled diabetic individuals and this increase could be a result of impaired renal function due to an increased blood glucose level. Our results revealed for the first time that the mean values of these end products in the serum increased in untreated diabetic rats, while they significantly decreased after the administration of oil extracts. Thus, this extract might improve renal function which, in turn, leads to reduction in these end products. It was reported that diabetic individuals had lower serum albumin concentrations as well as higher serum uric acid and urea levels than nondiabetic individuals [20, 21]. Thus, the reduction in urea and creatinine levels probably can be explained by a reduction in blood glucose level.

Further, DM is also considered as a risk factor for cardiovascular disease, and elevated serum uric acid has been linked to cardiovascular disease, especially if accompanied with high triglyceride and low HDL. Moreover, high levels of serum uric acid, urea and creatine may act as a marker of kidney problems. Thus, it is possible to suggest that this extract might play an important role in reducing risk of kidney problems as well as cardiovascular diseases by lowering serum urea, uric acid, creatinine as well as improving lipid profile. The beneficial effects that have been seen for the first time in our study are indications of safety of essential oil extract of A. sieberi, and hence it is worth trying to study the effects of this extract on some voluntary diabetic patients.

The study of the literature indicated that free radicals are one of the main contributors to development of DM as well as its complications [1], [17], [22]-[26]. It is also worth mentioning that alloxan can induce rapid death of cells of pancreas, resulting in partial or complete loss of insulin production and leading to the development of hyperglycemia and its complications in experimental animals; and this action of alloxan was mediated by formation of free radicals [22]. Moreover, a wide range of studies have strongly supported the notion that antioxidant compounds derived from plant extracts might play a vital role in the treatment of DM and prevent or delay its complications [1], [22]-[27]. Thus, it possible to suggest that these therapeutic values of the essential oil extract could be attributed to the main antioxidant constituents of this essential oil extract of A. sieberi. Direct support for this notion was also confirmed by identification and characterization the components of essential oil derived from Artemisia species by our lab and others [11], [22], [26]-[28]. According to these analyses, essential oil is mixture of a variety of lipid soluble and volatile compounds such as terpenes and terpenoids, phenol-derived alcohol, ketone and monotepenes compounds and aliphatic components, and most of them were characterized as antioxidants.

In conclusion, this is the first study to reveal that oral administration of essential oil extract of A. sieberi exhibit cardioprotective, nephroprotective and hepatoprotective activities via enhancing insulin production and decreasing glucagon production in alloxan induced diabetic rats. Thus, oral use of this extract might positively affect the functional capacities of various rat tissues, particularly blood, heart, kidney and liver against toxic action of alloxan compound (dose of 150 mg/Kg bw). These findings clearly support the traditional use of this medicinal plant in treatment of diabetes mellitus and shed more light in the efficacy of this plant. Thus, A. sieberi appears to a valuable plant and ideally suited to be used in treatment of DM and prevent or delay the onset of its complications in humans, since this is a non-toxic plant.

ACKNOWLEDGMENT

This study was partially supported by the Deanship of Scientific Research at Al al-Bayt University. The authors would, therefore, like to appreciate the financial help and express our gratitude to the University.

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