Ameliorative Effect of Aqueous Extract of Tetracarpidium Conophorum (African Walnut on Salt Induced Hypertensive Wistar Rats

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Abstract
This work was designed to investigate the antihypertensive properties of aqueous extract of Tetracarpidium conophorum (TC) in salt-induced hypertensive rats. A total of thirty (30) male wistar rats were used for this study. The rats were randomly divided into six groups (A-F) of five rats each. Hypertension was induced in the rats except group A which served as the normotensive control group. The rats in groups (B-F) were placed on 8% NaCl in the diet/drinking water for 21 days and then treated with 70 mg/kg, 140 mg/kg, 210 mg/kg body weight of aqueous extract of Tetracarpidium conophorum (AETC) and lisinopril 5 mg/kg respectively for additional 21 days. Acute toxicity studies using LD₅₀ assays showed AETC to be virtually non-toxic (LD₅₀ >700 mg/kg body weight). Phytochemical analysis of crude extract indicates the presence of flavonoids, saponins, tannins, alkaloids and Phenols. Salt loading significantly increased the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and heart rate. Treatment showed significant (p < 0.05) decrease in SBP, DBP, MAP, heart rate, total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein and increased high density lipoprotein and body weight as compared with the salt -loaded untreated group. There was no significant difference (p>0.05) in SBP, DBP, MAP, heart rate and body weights of salt -loaded groups treated with AETC and the salt loaded group treated with lisinopril. These results indicate that AETC (African walnut) possesses antihypertensive effect in hypertensive rats.

Keywords: hypertension, African walnut, lipid profile, blood pressure


1. Introduction

Tetracarpidium conophorum (T C) (African walnut), a tropical rambling perennial woody plant of the family Euphorbiaceae is widely distributed and consumed by the inhabitants of Africa [1]. TC is often found growing wild as a climber in the forest regions of Africa and India [2]. It is commonly referred to as African walnut because of its origin in the West Africa rainforest and as Nigerian walnut by Nigerians. It is known as Ekporo by Efik and Ibibios of Cross River and Akwa-ibom states, Ukpa by Igbos, Awusa by Yoruba, Okwe by Edo, Gawudi bairi by Hausas. [3,4]. It is found in Akwa Ibom, Cross River, Lagos, Kogi, Osun and Oyo States. The plants cultivated principally for the nuts which are cooked and consumed as snacks. [5]. TC, like many plants in Africa and other parts of the world has been proven to have decorative, nutritive, medicinal, agricultural and industrial values over the years. Several studies on TC indicates that it contains bioactive compounds such as oxalates, phylates, saponins and alkaloids which partly shows the use of the seeds, leaves and roots in herbal medicine [3,6]. TC is considered to be a herb in Traditional Chinese Medicine. They are said to tonify kidneys, strengthen the back and knees, moisten the intestines and move stool. TC is known to be rich in protein, fat, carbohydrate but low in fiber and ash content [7]. The nut have also been found to be very good source of Vitamin A, B₁, B₂, B₆, E, folate, potassium, sodium, manganese, copper, chloride, iron and ascorbic acid [2]. TC contains plant based polyunsaturated fatty acids such as alpha-linolenic acid. Furthermore, the nut of TC contain Omega-3 essential fatty acids and since it cannot be manufactured within our bodies and must therefore be ingested [8].

Tetracarpidium conophorum has been reported to have anti hyperglycaemic activity [9,10]. It has been revealed by [10] to be useful in ulcer treatment. Parameters such as gastric volume, pH, total and free acidity and ulcer index were used as indicators for antilulcerogenic activity of TC.
Results of studies have shown that TC has a high potential as an antimicrobial plant [11]. A study by [12] revealed that the nuts are effective in tackling male fertility by boosting sperm productions in testicles. [13] reported that TC possesses antioxidant activities. A chemical evaluation of TC nuts revealed it can be useful to manage cardiovascular disease due to the presence of tocopherol [6]. However, despite the various medicinal values of TC there is paucity of information on its antihypertensive properties. Hypertension is sustained elevation of arterial blood pressure above the normal range expected in a particular age group. Hypertension is seen when the blood pressure of an individual is ≥140mmHg systolic, and/or ≥90mmHg diastolic [13]. Hypertension is regarded as the most common cardiovascular diseases and is a major public health issue in developed as well as developing countries. Although it is common and readily detectable, it often leads to lethal complications if untreated [15]. In Nigeria, not less than 46.6% of individuals over 15 years of age are Hypertensive [16]. Furthermore, hypertension may be of unknown cause (essential hypertension) it may also result from kidney disease, including stenosis of the renal artery, endocrine disease (such as Cushing’s syndrome) or disease of the arteries (such as coarctation of the aorta) when it is known as secondary hypertension. Complications that may arise from hypertension include atherosclerosis, heart failure cerebral hemorrhage and kidney failure. Some cases of hypertension can be managed by eradicating the cause. The increasing rate of hypertension and concomitant high cost of antihypertensive drugs with its associated side effects which many indigent Nigerians cannot afford, there is need to explore non pharmacological antihypertensive agents that may be available in Nigeria flora, that are affordable with little or no side effects. TC has been reported to contain some chemical constituents that may exert antihypertensive effect. However, there is paucity of information on the antihypertensive effect of TC, this work is designed to investigate the potential of TC nuts as an antihypertensive agent on blood pressure (SBP, DBP, MABP), Heart rate, Lipid profile (TC, TG, HDL, LDL and VLDL), Body weight.

2. Materials and Methods

2.1. Plant Collection and Identification

Mature nuts of TC were purchased from a local market in Enugu. The plant was identified and authenticated by Mr. Onyekachukwu, C J of the department of Plant Science and Biotechnology, University of Nigeria, Nsukka where voucher specimen number (UNH NO. 98a) was deposited.

2.2. Preparation of tetracapidium conophorum Extract

The method of [17] and [18] was used to prepare the nuts. The nuts were boiled at 100°C for 2 hours and allowed to cool. The shells were removed and the white colored nuts washed thoroughly. The nuts were grounded with an electric blender. A weight of 1173 g of blended walnut was obtained. 500 g was macerated with 1000 ml of distilled water and allowed for 24 hr. After 24 hr it was filtered using Whatman filter paper (No 1) and the residue was discarded and filtrate collected into a plastic container, covered and stored in the refrigerator at a temperature of 4°C.

2.3. Phytochemical Analysis

The standard method of [19,20] was used in the analysis of some of the phytochemical of Tetracarpidium conophorum nuts.

2.4. Acute Toxicity Studies

Acute toxicity was determined using the method of [21]. The LD₅₀ was carried out in two phases. The animals (mice) were fasted for 24 hours before administration of aqueous extract of Tetracarpidium conophorum (AETC) intraperitoneally.

Phase one: at total of 21 mice of 3 mice in each group was used. The following dosages were used, 5000mg/kg, 4500mg/kg, 4000mg/kg, 3500mg/kg, 3000mg/kg, 2000mg/kg and 1000mg/kg depending on their body weight. They were observed for 24 hours for signs of toxicity, after 24 hours all 21 mice were dead.

Phase two: 10 mice of 2 per group were administered with a dosage of 100mg/kg, 200mg/kg, 300mg/kg, 400mg/kg and 500mg/kg. They were allowed for 24 hours for toxicity signs, at the end of 24 hours all 10 mice were alive.

Dose administered to each mouse was calculated thus:

\[
\text{Weight of animal (kg) x Dose (mg/kg)} \times \frac{\text{Stock Conc. (mg/ml)}}{} = \text{Dose (mg/kg)}
\]

2.5. Experimental Animals

30 male Wistar rats weighing 120 -150g were used for this study. The animals were obtained from the Animal House Unit, College of Health Sciences, Benue State University, Makurdi. The rats were allowed one week acclimatization before the commencement of the experiment. The rats were fed with pelletinized growers feed (Grand Cereals Ltd, Jos) and were allowed access to water ad libitum before and during experiment.

2.6. Induction of Hypertension in Rats

The rats were induced to hypertension according to the method of Ani et al., 2017. They were randomly divided into 6 groups (A-F) of 5 rats each. Group A was normal control while hypertension was induced in group (B-F). The baseline body weights and blood pressure were measured before induction of hypertension. Rats in group (B-F) were fed 8% NaCl in their feed and drinking water ad libitum for 21 days and their blood pressure and weights recorded.

They were all confirmed hypertensive and treated with 70 mg/kg AETC, 140 mg/kg AETC, 210 mg/kg AETC and Lisinopril 5 mg/kg respectively. Measurement of blood pressure and body weights were taken weekly during treatment while lipid profile was analyzed at the end of treatment.
2.7. Dosage Design

Using approximated value of the LD<sub>50</sub> (700mg/kg). Tainter and Miller’s method of 10% low dose, 20% medium dose and 30% high dose, the dosage for the experiment was designed as follows:

Low dose: \( \frac{10 \times 700 \text{mg/kg}}{100} = 70 \text{mg/kg (AETC)} \)

Medium dose: \( \frac{20 \times 700 \text{mg/kg}}{100} = 140 \text{mg/kg (AETC)} \)

High dose: \( \frac{30 \times 700 \text{mg/kg}}{100} = 210 \text{mg/kg (AETC)} \)

2.8. Experimental Design

Group A: Normotensive
Group B: Hypertensive, no treatment
Group C: Hypertensive + 70 mg/kg AETC
Group D: Hypertensive + 140 mg/kg AET
Group E: Hypertensive + 210 mg/kg AETC
Group F: Hypertensive + Lisinopril (5 mg/kg).

3. Measurement of Blood Pressure

The blood pressure was measured according to the method of Ani et al, 2017 using Non-invasive Blood Pressure Meter (NIBP) (LE 5001) by Pan Lab technology, Barcelona, Spain was used for the determination of the SBP, DBP, Heart rate and Mean Arterial Pressure (MAP).

The rats were restrained from movement with a transparent glass restrainer. The rat’s tail was immersed in water at temperature of 45°C with a thermostat and allowed for about 30 seconds for easy blood flow. The sensitive BP meter was switched on and allowed to acclimatize for 2 minutes. The animals were covered with a piece of dark clothes to reduce anxiety. The tail cuff/transducer was introduced to the base of the tail and selector switch turned on immediately; the readings were displayed on the LCD screen. An average of 3 readings was taken for each rat.

3.1. Measurement of Body Weights

A digital weighing balance (Max 1500g d 0.1g) by Adam equipment was used. The equipment was switched on and a transparent glass rat restrainer was placed on it and tarred, each rat was placed on the weighing balance and the weight displayed on the screen was recorded.

3.2. Measurement of Lipid Profile

At the end of the experiment the serum total cholesterol [22], triglyceride [23] and high density lipoprotein cholesterol [24] were determined by enzymatic method. Serum low density lipoprotein cholesterol was calculated using the Friedewald equation [25] using analyzed values of total cholesterol, HDL and Triglycerides.

\[
\text{LDL} = \frac{\text{Total Cholesterol} - \text{HDL} - \text{Triglyceride}}{2.17}
\]

3.3. Collection of Experimental Samples

At the end of 21 days, blood was collected from the rats into plain sample bottles by Cardiac puncture under light chloroform anesthesia after an overnight fast. The blood was collected into plain sample bottles, the sample was left to coagulate a little and placed in a 12 bucket centrifuge and spun for 15 minutes at 4000 revolution per minute. The serum was collected into another plain sample bottle stored in the refrigerator and was later taken to the laboratory for lipid profile analysis.

3.4. Statistical Analysis

The data were presented as mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA). Further analysis was done using Bonferroni post-hoc test using GraphPad prism. p<0.05 were considered to be statistically significant.

4. Results

4.1. Phytochemical Screening

The qualitative and quantitative phytochemical screening of extract revealed the presence of alkaloids, saponins, flavonoids, phenols and tannins as shown in Table 1.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Unit</th>
<th>Qualitative</th>
<th>Quantitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>%</td>
<td>++</td>
<td>0.42</td>
</tr>
<tr>
<td>Saponins</td>
<td>%</td>
<td>++</td>
<td>8.26</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>%</td>
<td>+</td>
<td>3.22</td>
</tr>
<tr>
<td>Phenols</td>
<td>%</td>
<td>++</td>
<td>1.87</td>
</tr>
<tr>
<td>Tannins</td>
<td>%</td>
<td>++</td>
<td>0.49</td>
</tr>
</tbody>
</table>

+ Trace, ++ Moderate.

Table 2 is the systemic blood pressure changes in various groups. The result indicated significant difference (p < 0.05, p < 0.01 and p < 0.001) in the SBP as compared with the Normotensive group. There was significant decrease (p< 0.001 and p < 0.01) in SBP when compared with the salt-loaded untreated group. There was no significant (p > 0.05) difference between the salt-loaded treated groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DAY 0</th>
<th>DAY 21</th>
<th>DAY 28</th>
<th>DAY 35</th>
<th>DAY 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>123 ± 1.60</td>
<td>128.0 ± 3.80</td>
<td>127.6 ± 1.63</td>
<td>127.6 ± 1.08</td>
<td>137.2 ± 3.73</td>
</tr>
<tr>
<td>B</td>
<td>121.6 ± 2.77</td>
<td>167.6 ± 0.75</td>
<td>166.6 ± 4.87</td>
<td>161.2 ± 4.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>168.4 ± 1.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>121.2 ± 3.80</td>
<td>167.4 ± 0.51&lt;sup&gt;s&lt;/sup&gt;</td>
<td>132.6 ± 7.39</td>
<td>151.0 ± 11.34</td>
<td>137.2 ± 7.44&lt;sup&gt;s&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>124.0 ± 2.12</td>
<td>168.6 ± 1.12&lt;sup&gt;s&lt;/sup&gt;</td>
<td>137.8 ± 0.80</td>
<td>135.4 ± 4.83&lt;sup&gt;s&lt;/sup&gt;</td>
<td>130.8 ± 1.91&lt;sup&gt;s&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>123.2 ± 2.35</td>
<td>173.2 ± 0.97&lt;sup&gt;s&lt;/sup&gt;</td>
<td>141.2 ± 3.47</td>
<td>137.2 ± 2.63&lt;sup&gt;s&lt;/sup&gt;</td>
<td>132.4 ± 3.14&lt;sup&gt;s&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>124.2 ± 0.66</td>
<td>172.8 ± 1.48&lt;sup&gt;s&lt;/sup&gt;</td>
<td>138.0 ± 1.98</td>
<td>135.0 ± 1.82&lt;sup&gt;s&lt;/sup&gt;</td>
<td>129.2 ± 1.46&lt;sup&gt;s&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
The treated groups was observed. Significant difference (p > 0.05) among the salt-loaded groups when compared with the salt-loaded untreated group. No significant difference (p < 0.05, p < 0.01 and p < 0.001) when compared with group B.

Table 3 shows the diastolic blood pressure changes in the various groups. The salt loaded groups showed significant difference (p < 0.01 and p < 0.001) when compared with the normotensive group. Significant decrease (p < 0.01 and p < 0.001) in MABP of the normotensive group. Significant decrease (p < 0.01 and p < 0.001) was observed in DBP of the salt-loaded treated groups when compared with the salt-loaded untreated group. There was no significant difference (p > 0.05) between the salt-loaded treated groups.

Table 4 shows the changes in heart rate. A significant difference (p < 0.05) was observed between salt-loaded treated with 70 mg/kg AETC and salt-loaded untreated group on day 42. There was significant difference (p < 0.05) in the salt loaded groups treated with 70 mg/kg AETC and 140 mg/kg AETC when compared with the salt-loaded treated group with 210 mg/kg AETC.

While Table 5 shows the result of the changes in body weights. Significant difference (p > 0.01 and p > 0.001) was observed in salt-loaded treated groups when compared with Normotensive group. Significant difference (p < 0.05, p < 0.01 and p < 0.001) was observed in salt-loaded treated groups when compared to the salt-loaded untreated group.
Table 7. Lipid Profile of all experimental groups after treatment

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>VLDL (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.450 ± 0.083</td>
<td>1.050 ± 0.040</td>
<td>1.307 ± 0.008</td>
<td>0.667 ± 0.058</td>
<td>0.477 ± 0.109</td>
</tr>
<tr>
<td>B</td>
<td>2.783 ± 0.026*</td>
<td>1.193 ± 0.015B</td>
<td>0.720 ± 0.017B</td>
<td>0.523 ± 0.023B</td>
<td>0.540 ± 0.006A*</td>
</tr>
<tr>
<td>C</td>
<td>1.757 ± 0.043Acse</td>
<td>0.837 ± 0.015Esse</td>
<td>0.920 ± 0.021Ese</td>
<td>0.450 ± 0.012Esse</td>
<td>0.417 ± 0.012E**</td>
</tr>
<tr>
<td>D</td>
<td>1.783 ± 0.047Acse</td>
<td>0.837 ± 0.013se</td>
<td>0.970 ± 0.032se</td>
<td>0.417 ± 0.003se</td>
<td>0.383 ± 0.004se</td>
</tr>
<tr>
<td>E</td>
<td>1.800 ± 0.040Bse</td>
<td>0.827 ± 0.009Bse</td>
<td>1.033 ± 0.011Bse</td>
<td>0.387 ± 0.024Bse</td>
<td>0.373 ± 0.003Bse</td>
</tr>
<tr>
<td>F</td>
<td>2.250 ± 0.136C</td>
<td>1.003 ± 0.038C</td>
<td>1.090 ± 0.066C</td>
<td>0.673 ± 0.164C</td>
<td>0.453 ± 0.017C</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM. *p < 0.05, *p < 0.01 and *p < 0.001 when compared with group A; †p < 0.01 and †p < 0.001 when compared with group B; ‡p < 0.05 and ‡p < 0.01 when compared with group F.

Table 7 indicates the results of the lipid profile. The result indicate significant decrease in the levels of TC, TG, LDL and VLDL and increase in HDL of the salt-loaded treated groups as compared with the salt-loaded untreated group. Significant difference (p<0.05, p<0.01 and p<0.001) in the lipid profile of the salt-loaded groups when compared with the normotensive group. There was significant difference (p<0.01 and p<0.001) in the salt loaded treated groups when compared with the salt-loaded untreated group. Also, there was significant difference (p<0.05 and p<0.01) in the salt- loaded groups treated with 70 mg/kg, 140 mg/kg and 210 mg/kg AETC when compared with the salt-loaded group treated with losinipril 5 mg/kg.

5. Discussion

The use of traditional medicine and medicinal plants in Africa and Nigeria specifically as a normal approach to the maintenance of health is an age long approach and is gaining more awareness due to its efficiency and recent advances in research in this area [26]. This study was carried out to investigate the antihypertensive effect of aqueous extract of TC nut, a well known fruit with antioxidant properties, on salt - loaded hypertensive rats. In this present study, the rats were induced by placing them on 8% NaCl diet. Chronic salt - loading has been reported to increase blood pressure in rats Models [29,30,31]. There was a significant increase in the blood pressure of the rats at the end of 3 weeks induction. This may be as a result of sympathetic over activity which may contribute to salt - induced blood pressure elevations through impaired excretory function [32]. Dietary flavonoids and saponins have been reported to have antihypertensive activities in experimental animals. Significant decrease in SBP, DBP and MABP was observed after weeks of treatment with AETC. The decrease in blood pressure may be due to action of flavonoids and saponins contained in the extract. The result of this present study agrees with those from earlier studies where saponin constituents were used in treating hypertensive rats [33,34] have been found to decrease blood pressure. Flavonoids have also been reported to decrease blood pressure in hypertensive rats [35,36]. Flavonoids have been described as a vasodilator [37,38,39]. Flavonoids and saponins in Tetracarpidium conophorum can be attributed to have enhanced the lowering of the cardiovascular parameters (SBP, DBP, MAP and Heart rate) in the hypertensive rats. The main vasodilatory mechanism of flavonoids seems to be the inhibition of protein kinase C or decrease Ca2+ uptake may also contribute to their vasodilatory effects [37]. The L - arginine content of TC nuts have been attributed the role of an anti hypertensive drug by producing nitric oxide in vivo, which act as a vasodilator of the blood vessels and smooth muscles [38]. TC nuts have been proven effective for keeping the arteries flexible and reducing the damage caused by fatty food to the arteries; this is attributed to the high content of Omega-3 fatty acids, phytosterols and antioxidants in the nuts. It is possible these were responsible for lowering the SBP, DBP, MAP and Heart rate in the groups treated with AETC. Adequate intake of dietary fiber lowers cholesterol level risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer [41]. The study also showed a decrease in the total cholesterol (TC), Triglyceride (TG), low density Lipoprotein (LDL) and very low density lipoprotein (VLDL) with an increase in the high density lipoprotein (HDL) in the hypertensive treated groups as compared to the hypertensive control.HDL enables cholesterol and triglycerides to be transported within the bloodstream. In healthy individuals, about thirty percent blood cholesterol is carried by HDL. HDL are able to remove cholesterol from within the artery atheroma and transport it back to the liver for excretion or re-utilization. Those with higher levels of HDL seem to have fewer problems with cardiovascular diseases, while those with low HDL levels (Less than 40mg/dl or about 1 mmol/L) have increase rates of heart disease. [42] LDL particles appear harmless until they are within the blood vessel walls and oxidized by free radicals [43]. Studies have shown that Vitamin B5 (Pantothine) inhibits several enzymes and co-enzymes that make cholesterol. It blocks the activity of one co-enzyme involved in cholesterol synthesis, HMG-COA by 50%. It lowers cholesterol and heals arteries in rabbits on experimental diet, may modify lipid deposition in major arteries by affecting lipoprotein composition and/or exerting arterial protective effect [44]. It is possible Pantothine in Tetracarpidium conophorum nuts contributed to the cholesterol lowering effect. Pantothine lowers cholesterol acting as a statin which locks an enzyme that the body uses to manufacture cholesterol [45]. The possible mechanism underlying the role of AETC against lipid peroxidation could be by scavenging free radicals. A significant decrease in body weight was observed at the end of the 3 weeks of salt- loading, its weight loss appears to have a stronger blood pressure lowering effect [46,47,48] and suppression of the plasma renin activity to enhance fluid loss in hypertensive rat. In this study, significant increase in the body weights was observed after first week of treatment with reduction in the blood pressure. This is not in agreement with other reports in which reduction in weight gain of hypertensive treated rats was observed [49,50]. However, other studies...
with *Tetracarpidium conophorum* nuts have shown an increase in the body weights of experimental rats.

6. Conclusion

This study has shown that AETC have the potential to ameliorate salt-induced hypertension and reduce lipid peroxidation in Wistar rats. Thus, the consumption of *Tetracarpidium conophorum* nuts should be encouraged. There is need to research on its possible preservation as the plant is seasonal. Further studies, should be carried out to ascertain the mechanism of action through which it reduces blood pressure.

Acknowledgements

The authors sincerely acknowledge all the staff of Department of Pharmacology & Therapeutics and Animal House Unit, College of Health Sciences, Benue State University Makurdi for their permission to use their NIBP equipment through the Technical efforts of the Senior Technologist (Mr. Ani Celestine Okafor) who devoted much of his time to ensure the success of this research work and most importantly Prof Nwachukwu Daniel Chukwu who initiated and ensured thorough supervision of this research.

Conflict of Interest

None to declare.

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