The Effect of *Thaumatococcus danielli* Leaf Extracts on Immunological and Oxidative Stress Markers in Rat

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**Authors’ contributions**

This work was carried out in collaboration among all authors. Author OBA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors OBA, OOF, GMS, BOE and GAA managed the analyses of the study. Author AGA managed the literature searches. All authors read and approved the final manuscript.

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**ABSTRACT**

This work demonstrated the immunological and antioxidant activities of *Thaumatococcus danielli* leaf extracts and their potential as the pharmaceutical factor for the treatment of some pathological disorders such as cancer, leucopenia, cells inflammation, dietary, atherosclerosis, neurodegenerative disease and other immunological and oxidative stress disorders. 36 albino rats of average weight 140 ± 5 g were randomly grouped into 6 with six rats per group. The rats in group A (positive control) received only 0.1% DMSO. The rats in the negative control (group B) received pyrogallol (immunosuppressor) + 0.1% DMSO for 7 days and challenged with 0.1ml Sheep Red Blood Cell (SRBC). The test groups (C to F) also received pyrogallol + 0.1% DMSO for 7 days and were also challenged with SRBC for a period of 72hours after which they were treated with High and Low doses of either petroleum ether or water extracts of *Thaumatococcus danielli* for 21days. The assessment of immunomodulatory activity was carried out using Carbon Clearance Test.
Haematological/ immunological parameters, Spleen and Plasma antioxidant parameters like Catalase (CAT), Superoxide dismutase (SOD), Glutathione Peroxidase (GPX) Activities, Lipid Peroxidation (LPO) and Malonaldehyde (MDA) concentrations were also determined. Result unveiled a significant (p<0.05) increase in the phagocytic index of both high doses of water and petroleum ether extracts compared to the negative control group. There were no significant (p<0.05) changes in the Heamatological parameters and plasma catalase, but there was a significant change in the white blood cell (WBC) count and also an improvement in the spleen and plasma antioxidant parameters. Further study is required to understand the mechanism of action of both extracts in order to exploit it in immunomodulation.

Keywords: Immunomodulation; antioxidants; oxidative stress; thaumatococcus danielli.

1. INTRODUCTION

*Thaumatococcus danielli* (Benn.) Benth. (syn: Phrynium danielli) is a multipurpose rhizomatous, perennial and monocotyledonous plant [1] which belongs to the family Marantaceae and native to West Africa, particularly Ghana, Cote d’Ivoire and Nigeria. It also exists in the Princes Islands, Angola, the Central African Republic, Uganda and Indonesia [2]. The leaves (ca45 cm x 30 cm) are ovate-elliptic rounded, truncate at the base and shortly acuminate at the apex, and of different sizes depending on the maturity of the plant [3] [4]. The flowers most prolific from July until late October, and ripening from January until mid-April are purple pinkish with short spikes and long bracts at the base of the swollen petiole [1]. The fruit furnishes the protein sweetener which is widely used in beverage, confectionery and pharmaceuticals industries. In addition, the stalks in some cases are used to line utensils in which food is prepared particularly in Southwestern, Nigeria [5]. Furthermore, *Thaumatococcus danielli* has contributed to the rural economy of some West African natives, who have been using several parts of the plant for centuries in wrapping food materials, making thatching roots, weaving baskets, mats and as taste modifier [5].

Immunomodulators may be synthetic drugs or of herbal origin. Due to the severe side effects related to synthetic drugs, immunomodulation using herbal drugs can offer an option to conventional chemotherapy for a diversity of diseases, especially when the host defense mechanism has to be activated under the conditions of an impaired immune response [6]. While testing the immunomodulatory activity, most of the studies utilize agents like cisplatin, cyclophosphamide, or corticosteroids in order to stimulate the immunosuppression in experiments [7]. These agents are also known to yield free radicals in the biological system and thereby cause oxidative stress [8] [7][9]. Whether the damage in immune responses is posterior to their power to yield oxidative stress is not clear. However, several workers have established that prooxidants inhibit the immune responses in experimental animals [9]. In addition, it has been established that the immunomodulators isolated from plant sources have antioxidant activity [10].

It is recognized that pyrogallol is a strong source of free radicals [11] and it is proved that it can inhibit the proliferation of mouse lymphocytes *in vitro* [12]. In view of this proof, it was proposed to enquire whether a potent prooxidant like pyrogallol can induce immunosuppression in rats and to test the utility of such a method to screen the immunomodulatory activity of a known agent like the alcoholic extract of *Rubia cordifolia* (RC) [13]. In order to investigate the engagement of free radicals in the influence of pyrogallol on the immune system, the markers of oxidative stress such as the lipid peroxidation (LPO) levels, reduced glutathione (GSH) concentration, superoxide dismutase (SOD) and catalase (CAT) activities were measured in the blood. In the present study, the immunomodulatory activities of water and petroleum ether extracts of *Thaumatococcus danielli* were determined in pyrogallol-induced immunosuppressed Rats.

2. MATERIALS AND METHODS

All reagents and chemicals used are of analytical grade.

2.1 Collection and Authentication of Plant Materials and Processes

The leaves of *Thaumatococcus danielli* plant were bought from Agbara local market in Ogun state of Nigeria and air-dried for 18 days to
maintain its constituents after the leaves had been authenticated by the Botanical Department, Lagos State University, Ojo. The leaves were grounded to achieve efficient extraction of the leaf extract. 400g each of the grounded leaves were soaked into 800ml of petroleum ether and water in beakers of labelled ‘P’ and ‘W’ respectively for three days. After the third day, the mixture was filtered into different beakers and lyophilized at -40°C. The lyophilized samples were stored in the refrigerator at 4°C for further use.

2.2 Experimental Animals

Fifty adult albino rats (Rattus norvegicus) were bought from Olu Research Animal, Ibadan, Oyo state. The rats were domiciled in neat metabolic cages of dimensions 33.0×20.5×19.0cm held in well aired standard housing conditions (temperature: 28–31°C; photoperiod: 12 h natural light and 12hour dark; humidity: 50–55%).

The cleanup of the cages was done twice daily. The animals were given rat pelleted food (Lagos State Agric, Ojo, Lagos Nigeria) and water. The animals whose weights ranged from 80-150g were acclimatized for two weeks before the beginning of the experiment.

2.3 Acute Toxicity Studies

The acute toxicity (LD50) of leaf extracts of T. daniellii was determined using the modified [14] method in rat using the oral route. Forty-eight rats were assigned equally into twelve well ventilated plastic cages. Ten grammes of each lyophilized extracts were suspended in 50ml of 1% DMSO. The first 6 groups were administered with petroleum ether extract while the remaining 6 groups were given water extract of Thaumatococcus daniellii orally at dosage levels of 50mg/kg, 100mg/kg, 200mg/kg, 400mg/kg, 800mg/kg and 1600mg/kg body weight in concentrated DMSO. From the petroleum ether group, 1 rat died each from 400mg/kg and 1600mg/kg groups within 72hours after the administration. On the other hand, 1 rat died from 400mg/kg group of water extract within 72hours after the administration.

2.4 Pyrogallol Administration

36 rats were willy-nilly distributed into six groups (A, B, C, D, E and F). Group A was not given pyrogallol solution because it was chosen to be a positive control while others were administered with 50mg/kg body weight of 1% pyrogallol for 7 days.

2.5 Antigen and Extract Administration

Fresh sheep red blood cell (SRBC) was hoarded from live matured sheep at Alaba Rago market in Lagos state between 07.00hrs and 08.00hrs and its preparation was done according to [15] where 0.1ml each from the fresh preparation of SRBC was challenged into the involved rats (groups B, C, D, E and F) through retro-orbital plexus followed by the administration of the Thaumatococcus daniellii extracts after 72 hours to groups C, D, E and F.

2.6 Study Design

Thirty-six rats were disservered into 6 groups according to their weight range groups. Positive Control (Group A) received only distilled water and 0.1% DMSO while the remaining groups received pyrogallol (immunosuppressor) for 7 days with their weight being determined and were challenged with 0.1ml sheep red blood cell which acted as antigen. Group B (Negative control) without treatment while the remaining groups C to F received (petroleum ether and water extracts of Thaumatococcus danielii) 72hours after SRBC (sheep red blood cell) challenged. This lasted for 21 successive days. After the 21 successive days of extracts administration, the rats were starved overnight for clearance test or phagocytic index. After the clearance test for the groups (A to F), the animal was fed and starved overnight again in preparation for the next day when the animals would be sacrificed for the evaluation of the rat oxidative stress markers, immunological and as well as its haematological parameters.

2.7 Modified Preparation of Serum/ Tissue Homogenates/ Plasma/ Erytrocytes

The procedure described by [16] was used. Under ether anaesthesia, the rats were dissected using a sterile scalpel in such a way to prevent blood taint by interstitial fluid, 5ml of the medical syringe was used to collect 4ml of blood from the cardiac system (heart) of each rat into EDTA, heparin and plain bottles depends on the type of analysis intending to perform. Each preparation was done according to Yakubu et al. [16].
2.8 Modified Clearance Test or Phagocytic Index

This method was described by [17-18]. *Thaumatococcus daniellii* extracts were used to treat the rats orally for 21 days. After 48 hrs when the last dose of the extracts was administered, animals were shot with 0.1 ml of black printer paper ink through the tail vein. Blood samples were taken at 0 and 15 mins after injection. A 50μl blood sample was mixed with 4 ml of 0.1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm. The phagocytic index K was calculated using the following equation:

\[ K = \frac{\text{Loge OD1} - \text{Loge OD2}}{15} \]

Where OD1 and OD2 are the optical densities at 0 and 15 mins respectively.

2.9 Effect on Serum Immunoglobulins

A day after the phagocytic index, all the grouped rats were sacrificed. During the sacrifice of the animals, blood was hoarded and the serum was used for the calculation of Immunoglobulin levels using method devised by Mullen [19].

2.10 Haematological/Immunological Parameters

Rats were phlebotomized from the retro-orbital plexus (under methoxyflurane anaesthesia) into EDTA tubes. White blood cells and red blood cells counts were determined using an electronic cell counter (model Zf; Coulter, Hialeah, FL) [20].

2.11 Catalase Activity (Plasma and Spleen)

Catalase (CAT) enzyme activity was estimated by the method of [21-22]

2.12 Glutathione Peroxidase Assay Method (GPx) In Plasma

GPx activity was estimated by the method described by [23] and modified by [24].

2.13 Superoxide Dismutase (Sod) Assay in Plasma

Superoxide dismutase activity was estimated by a modified method of [25].

2.14 Lipid Peroxidation in Plasma

Thiobarbituric acid reactive substances (TBARS) was assayed for lipid peroxidation in erythrocyte was carried out by a modified method of [26].

2.15 Statistical Analysis

This was done using one-way analysis of variance (ANOVA) followed by multiple comparing tests using Turkey’s HSD Post Hoc tests. Values are expressed as mean ± SEM and P < 0.05 were conceived significant.

3. RESULTS

There was a significant increase in the phagocytic index of rats administered with high doses of both petroleum ether and water extracts of *Thaumatococcus daniellii* (Group D and F) compared to untreated rats (Group B). There was also a significant increase in serum immunoglobulin of rats administered with low dose of petroleum ether extract of *Thaumatococcus daniellii* rats (Group C). There was a substantial decrease in white blood cell of rats administered low dose of water extract of *Thaumatococcus daniellii* extracts rats’ (Group E) compared to untreated (Group B) and also normalise the adverse effect of pyrogallol caused injury compared to positive control rats’ (Group A). Lastly, no significant change in neutrophil level of all the groups after rats were given *Thaumatococcus daniellii* extracts compared to positive control rats (Group A) and negative control (untreated) rats’ (Group B).

There was a significant increase in the spleen catalase activity of all the groups treated with *Thaumatococcus daniellii* extracts compared to positive control rats (Group A) and untreated rats (Group B). There was a significant increase in plasma catalase activity of the rats (Group C) treated with low dose of petroleum ether extract of *Thaumatococcus daniellii* compared to untreated rats (Group B). There was a significant decrease in superoxide dismutase of all the groups (C, D, E and F) of *Thaumatococcus daniellii* extracts compared to untreated rats (Group B) while having an increase of the rats’ groups (E and F) of *Thaumatococcus daniellii* extracts of water compared to positive control (Group A).

Result shows a significant decrease in lipid peroxidation of the rats’ groups (C, D, E and F) treated with *Thaumatococcus daniellii* extracts.
compared to untreated rats (Group B) and also, all doses of petroleum ether rats’ groups (C and D) of *Thaumatococcus daniellii* extracts can be used as corrective measure compared to positive control rats (Group A).

For Glutathione peroxidase activity, it shows a significant decrease of the rats treated with all the doses of *Thaumatococcus daniellii* compared to untreated rats’ group (B) extracts while a low dose of water extract of *Thaumatococcus daniellii* of the rats’ (group E) corrected the abnormal situation caused by pyrogallol administration compared to positive control rats (Group A).

In Table 3 There was no significant change in haemoglobin, red blood cell, percentage blood cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelets of all the rats treated with *Thaumatococcus daniellii* extracts of (Groups C, D, E and F) compared to both positive and negative control rats’ groups (A and B respectively).

4. DISCUSSION

The carbon clearance screening was done to determine the effect of drugs on the reticuloendothelial system (RES). RES is an imbue system consisting of phagocytic cells, Cells of the RES play an important role in the clearance of molecules from the bloodstream. When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation [18]. Since both High doses of water and petroleum ether extracts of T.danielli revealed a remarkable augmentation in the phagocytic index, it is hypothesized that it might be due to an increment in the activity of the reticuloendothelial system by prior treatment of the rats with both extracts. this is in line with [27].

Serum immunoglobulin contains majorly immunoglobulin M (IgM) which is an antibody that is produced by B cells. It is the largest antibody in the human circulatory. It is the first antibody that responds to initial exposure to antigen. The spleen is the major site of specific IgM. It activates complement C3b to bind to the antigen [28]. Result reveals a significant increase in serum immunoglobulin of rats administered with a low dose of petroleum ether extract of *Thaumatococcus daniellii* rats (Group C). The study also shows a significant decrease in white blood cell of rats administered low dose of water extract of *Thaumatococcus daniellii* (Group E) compared to untreated (Group B) and also normalise the adverse effect of pyrogallol caused injury compared to positive control rats (Group A). No significant change in the neutrophil level of all the groups after the administration of *Thaumatococcus daniellii* extracts compared to positive control rats’ (Group A) and negative control (untreated) rat’s (Group B).

This result agreed with [29] on comparison of Brazilian plants used to treat gastritis on the oxidativeburstof *Helicobacter pylori*-stimulated neutrophil using *Qualea parviflora* and *Qualea multiflora*. The study showed that there was a significant increase in spleen catalase activity in all the extracts administered groups compared to both positive and negative control groups. Thus, indicating that the *Thaumatococcus daniellii* extracts may be effective in correcting the depression and damages caused by pyrogallol in the rat’s system by activating the spleen to filter and remove foreign bodies from the rat red blood cell [30].

Conditions such as chronic inflammatory diseases, vulnerability to toxic chemicals, environmental pollutants, alcohol, and high-fat diet, which are known to cause impairment to the immune system, are also known to yield free radicals [8][10]. Impairment in these conditions may thus be later to over-utilization of endogenous antioxidants [8] Knight [9-10]. In view of this, Pyrogallol, which is a potent generator of superoxide radicals [31] might cause impairment to the immune response through oxidative stress. Such possible action is affirmed by the increase in lipid peroxidation and a decrease in antioxidant defense, after pyrogallol treatment. This indicates a strong relationship between pyrogallol-induced oxidative stress and immunosuppression. This has been further substantiated by the fact that the Pearson correlation analysis shows a significant correlation between the changes in LPO concentrations, a known marker of oxidative stress, and the changes in the immunological parameters.

Administration of *Thaumatococcus daniellii* extracts had no effect on plasma catalase activity except in rats treated with a low dose of petroleum ether extract which had a significant
Table 1. Effect of *Thaumatococcus daniellii* on some immunological parameter of pyrogallol–treated rats

<table>
<thead>
<tr>
<th>Treatment groups (n)</th>
<th>Phagocytic index</th>
<th>Serum immunoglobulin (ZST units)</th>
<th>White blood cell (10^6/ml)</th>
<th>Neutrophil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0223±0.004177 ab</td>
<td>0.0248±0.00605bc</td>
<td>2.123±0.59926 a</td>
<td>43.33±7.60117 a</td>
</tr>
<tr>
<td>B</td>
<td>0.0092±0.002746 a</td>
<td>0.00525±0.00239ab</td>
<td>11.80±2.91805 a</td>
<td>46.25±14.34326 a</td>
</tr>
<tr>
<td>C</td>
<td>0.0225±0.002500 ab</td>
<td>0.02925±0.00165c</td>
<td>8.80±0.77567 ab</td>
<td>60.00±14.14214 a</td>
</tr>
<tr>
<td>D</td>
<td>0.0333±0.006667 b</td>
<td>0.0030±0.00110 a</td>
<td>11.20±5.80000b</td>
<td>42.50±17.50000 a</td>
</tr>
<tr>
<td>E</td>
<td>0.02225±0.001109 ab</td>
<td>0.0165±0.00247abc</td>
<td>2.17±0.78462 a</td>
<td>42.50±16.52019 a</td>
</tr>
<tr>
<td>F</td>
<td>0.02725±0.004270 b</td>
<td>0.0207±0.00325abc</td>
<td>4.47±1.64589 ab</td>
<td>62.50±10.30776 a</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, at abc P< 0.05 values with different superscript are significantly different within column.

Table 2. The effect of *Thaumatococcus daniellii* extracts on some oxidative stress markers of pyrogallol–treated rats

<table>
<thead>
<tr>
<th>Treatment groups (n)</th>
<th>Catalase activity: spleen (U/mg)</th>
<th>Catalase activity: plasma (U/mg)</th>
<th>Superoxide dismutase (U/ml)</th>
<th>Lipid peroxidation (nmol/ml)</th>
<th>Glutathione peroxidase activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0157±0.00048b</td>
<td>0.0182±0.00017ab</td>
<td>0.0125±0.00034c</td>
<td>44.29±0.63b</td>
<td>0.756±0.014c</td>
</tr>
<tr>
<td>B</td>
<td>0.0137±0.00021a</td>
<td>0.0180±0.00000a</td>
<td>0.0130±0.00000 c</td>
<td>50.94±0.81c</td>
<td>1.704±0.005 e</td>
</tr>
<tr>
<td>C</td>
<td>0.0183±0.00025c</td>
<td>0.0188±0.00025 b</td>
<td>0.0048±0.00025a</td>
<td>43.29±0.41b</td>
<td>1.491±0.019 d</td>
</tr>
<tr>
<td>D</td>
<td>0.0180±0.00000c</td>
<td>0.0180±0.00000 a</td>
<td>0.0035±0.0005 a</td>
<td>40.69±0.3ab</td>
<td>0.560±0.001 b</td>
</tr>
<tr>
<td>E</td>
<td>0.0178±0.00025c</td>
<td>0.0180±0.00000 a</td>
<td>0.0108±0.00025b</td>
<td>37.55±1.12 a</td>
<td>0.764±0.002 c</td>
</tr>
<tr>
<td>F</td>
<td>0.0173 ±0.00025c</td>
<td>0.0180±0.00000 a</td>
<td>0.0108±0.00025b</td>
<td>36.89±1.43 a</td>
<td>0.530±0.004 a</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, at abcde P< 0.05 values with different superscript are significantly different within column.

Table 3. The effect of *Thaumatococcus daniellii* extracts on haematological parameter such as haemoglobin, red blood cell, percentage blood volume cell, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration and platelets

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Haemoglobin (g/d)</th>
<th>Red blood cell (10^6/ml)</th>
<th>Percentage Blood volume cell (PVC)</th>
<th>Mean Corpuscular Haemoglobin (MCH)</th>
<th>Mean Corpuscular Haemoglobin Concentration (MCHC)</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.78±0.36 a</td>
<td>7.81±0.24 a</td>
<td>36.08±1.10 a</td>
<td>46.28±0.13 a</td>
<td>32.68±0.07 a</td>
<td>434.00±30.37 a</td>
</tr>
<tr>
<td>B</td>
<td>10.10±1.03 a</td>
<td>6.68±0.65 a</td>
<td>30.90±3.12 a</td>
<td>46.38±0.49 a</td>
<td>32.65±0.07 a</td>
<td>616.25±88.67 a</td>
</tr>
<tr>
<td>C</td>
<td>10.30±0.43 a</td>
<td>6.82±0.29 a</td>
<td>31.50±1.29 a</td>
<td>46.30±0.33 a</td>
<td>32.78±0.06 a</td>
<td>687.50±129.31 a</td>
</tr>
<tr>
<td>D</td>
<td>10.35±0.15 a</td>
<td>6.91±0.06 a</td>
<td>31.55±0.45 a</td>
<td>45.75±0.10 a</td>
<td>32.90±0.10 a</td>
<td>685.00±34.00 a</td>
</tr>
<tr>
<td>E</td>
<td>10.63±0.95 a</td>
<td>7.10±0.63 a</td>
<td>32.38±2.88 a</td>
<td>45.73±0.06 a</td>
<td>32.80±0.04 a</td>
<td>491.25±38.80 a</td>
</tr>
<tr>
<td>F</td>
<td>11.88±0.63 a</td>
<td>7.51±0.28 a</td>
<td>36.35±1.93 a</td>
<td>48.43±0.56 a</td>
<td>32.60±0.06 a</td>
<td>451.75±55.54 a</td>
</tr>
</tbody>
</table>

All values are mean±SEM, n=6, at ap< 0.05 values with different superscript are significantly different within.
increase compared to the untreated group. Result shows a significant reduction in superoxide dismutase (SOD) of all doses of petroleum ether groups of *Thaumatococcus daniellii* compared to the positive control while all doses of water extract resulted to a significant increase in SOD activity equated to positive control.

Pyrogallol administration to rat causes oxidative stress which causes tissue damage and associated with the production of oxygen free radical leading to increased lipid peroxidation. Lipid is from the inflammatory site and can be measured in the blood. The Plasma membrane is more vulnerable to lipid peroxidation due to constant vulnerability to high oxygen tension and polyunsaturated fatty acid [32]. The increase in lipid peroxidation of pyrogallol induced rats group without treatment in this research showed that the pyrogallol had caused oxidative damage to the cell membrane as observed by [33] and that extracts of *Thaumatococcus daniellii* can act as a corrective measure though subjected to further proof.

The results show no significant change in haemoglobin, percentage blood volume cell (PVC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin or Mean Cell Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and platelets as indicated compared to both the positive and negative control groups, this can be due to the fact that the extracts have no effect on the haematological parameters.

5. CONCLUSION

The present study demonstrates that both high doses of water and petroleum ether extracts of *Thaumatococcus daniellii* shows significant Immunomodulatory effect by increasing the phagocytic activity in the immunosuppressed rats and also lowers the lipid peroxidation caused by free radicals generated by oxidants. The low dose water extract also reduces the white blood count thereby reducing stress in the rats.

Further Research would look at the neutrophil adhesion activity of the immunosuppressed rats and also understand the role of either water extracts or petroleum ether extracts of *Thaumatococcus daniellii* molecularly.

ETHICAL APPROVAL

This survey was followed through by approval from the Departmental Ethical Committee on the Care and Use of Experimental Animals for Research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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