Anticonvulsant activity of alcoholic extract of fruits of Capsicum annuum

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ABSTRACT

The anticonvulsant activity of ethanolic extract of fruits of Capsicum annuum was evaluated in albino mice. The ethanolic extract of fruits of Capsicum annuum (50mg/kg), was studied for their anticonvulsant effect on maximal electroshock induced seizures (MES) and pentylenetetrazole (PTZ) induced seizures and strychnine (STY) induced seizures in mice. The period of hind limb tonic extension in case maximal electroshock induced seizures and beginning of convulsions in case of pentylenetetrazole induced seizures and strychnine induced seizures were noted. The percentage mortality was calculated. As well as the concentration of biogenic amines (dopamine noradrenaline and serotonin) was estimated in the brain of the mice. C.annuum significantly reduced the duration of seizures by MES. The effect of C.annuum on PTZ and STY induced convulsions was not marked. The data propose that the ethanolic extract of C.annuum will produce its anticonvulsive effect by acting on voltage dependent sodium ion channels since it reduced the duration of seizures produced by maximal electroshock.

1. INTRODUCTION

Epilepsy is a general chronic neurological disorder characterized by repeated unprovoked seizures\(^1\). These seizures are transient signs and/or symptoms of abnormal, excessive or concurrent neuronal activity in the brain\(^2\). About 50 million people worldwide have epilepsy, with approximately 90% of these people being in developing countries. Epilepsy is more probable to occur in young children or people over the age of 65 years; though it can occur at any time\(^3\).

A large number of agents called anti-epileptic drugs are available to treat various types of seizures with the objective to reduce seizure regularity and severity with in a framework of acceptable level of side effects. The ideal anticonvulsant drugs would repress all seizures without causing any unwanted effect. Unfortunately drugs used currently are unsuccessful to control seizures activity in some patients but they frequently cause side effects. In addition safety, tolerability, efficiency, expenses especially in long term therapy, serum drug monitoring etc. are other limitations with synthetic anti-epileptic drugs. Further a large number of drug interactions seen with almost all current anti-epileptic drugs make it more difficult to easily control seizures\(^4\).

Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need\(^5\). Bacopa monnieri\(^6\), Ficus platyphylla\(^7\), Viscum capense\(^8\), Clerodendrum infortunatum\(^9\), Carissa carandas\(^10\), Spondias mombin\(^11\), Boerhaavia diffusa\(^12\) and Opuntia vulgaris\(^13\) are the drugs which are extensively used in the treatment of epilepsy in ayurvedic and unani system of medicine. The saponins which are present in these drugs are mainly thought to be responsible for their anti-epileptic activity. In the present study Capsicum annuum contains the saponins as their main chemical constituents so the scope of these plants to treat epilepsy will be evaluated because of the presence of saponins as their main chemical constituent.

2. MATERIALS AND METHODS

Collection of drug: Fruits of Capsicum annuum were purchased from the local market. Fruits of capsicum annuum, were authenticated by Dr. K. Madhava chetty, Ph.D., Asst Professor/Dept of botany, S.V. University, Tirupati.

Preparation of extract:

Preparation of extract of Capsicum annuum: Fresh ripe fruits of C. annuum were dried and powdered. Forty gram of chili powder was subjected to extraction with 800 ml of absolute alcohol, by continuous stirring for 24 hour. The crude extract was then filtered through...
Whatmann No. 1 filter paper and the filtrate was collected in round bottom flask. The excess of alcohol was removed by distillation. The extract was poured into a test tube and kept in a boiling water bath till alcohol completely gets evaporated. The thick red crude residue was weighed; percentage yield of extract was calculated and as per the demand the extract was suspended in suitable volume of of 1% Tween 80 for the experiment. 

Animals used: Albino mice of either sex, weighing about 25-30 grams were used in experiments. They are obtained from the animal house facility of Shadan Institute of Medical Sciences with an approval from institution ethical committee. Animals were housed in polypropylene cages maintained under standard condition (12 hours light / dark cycle; 25± 3 °C) and had free access to standard rat's mice feed (Hindustan Liver Ltd., India) and water ad libitum. All the animals were acclimatized to laboratory condition for a week before start of experiment.

Drugs and chemicals used: Phenytoin, Diazepam, Pentylonetetrazole, Strychnine nitrate, Ophthalaldehyde, Noradrenaline Hydrochloride, Serotonin hydrochloride and Dopamine Hydrochloride all the laboratory grade reagents are used for the study.

Preliminary phytochemical screening: The preliminary phytochemical screening was approved on the extract of Capsicum annuum, for the detection of various phytochemicals. Alkaloids (Dragendorff’s test, Mayer’s test, Hager’s test, Wagner’s test) Carbohydrates (Molish’s test, Fehling’s test, Benedict’s test), Flavanoids (Shinoda test, Alkaline reagent test), Proteins (Biuret test, Xanthoproteic test, Trichloroacetic acid test), Amino acids (Millon’s test, Ninhydrin test), Tannins (Ferric chloride test, Bromine water test), Steroids and triterpenoids (Liebmann-Burchard test, Salkowski test, Sulfur test), Fixed oils and fats (Spot test, Saponification test), Saponins (Forth formation test, Hemolytic test) and Glycosides (Borntrager’s test, Baljjet’s test, Keller-Killiani test, Picric acid test.

Acute toxicity studies: A preliminary pharmacological study was conducted to assess the gross behavioral effects and safety effects of the drug. The acute toxicity study was carried on mice weighing about 20-25gm as per ICH guidelines. Overnight fasted rats received test extract at a dose of 200mg/kg intraperitoneal route. Then the animal was observed constantly for one hour. LD50 was calculated by the Miller and Tainter method. In which the observed percentage mortality was converted into probits. The values thus obtain were plotted against log dose and the LD50 value is the dose corresponding to probit 5. The therapeutic dose of the drug is considered as 1/10 of LD50 value. For general behavioral, neurological, autonomic profiles and to find out percentage of mortality observations were tabulated according to Irwin's table. For this the following check list was employed:

**Stimulation:** Hyperactivity, Piloerection, Twitching, Rigidity, Irritability, Jumping, Clonic convulsions, Tonic convulsions

**Depression:** Ptosis, Sedation, Loss of reflex (sleep), Loss of traction, Loss of Pinna reflex, Catatonia, Ataxia, Loss of muscle rigidity, Analgesia.

**Autonomic reflexes:** Straub's tail, Labored respiration, Cyanosis, Reddening, Abnormal secretions, balancing.

**Methods employed in screening of anticonvulsant activity:**

1. Electrically induced seizures: In the electrically-induced seizure experiment, the maximal electroshock (MES) method will be engaged. In brief, tonic convulsions of the hind extremities of the mice was induced by passing alternating electrical current of 50 Hz and 150 mA for 0.2 sec through corneal electrodes. All the animals were made into three groups containing six animals in each group. Group 1 was treated as control, Group 2 was treated with Capsicum annuum (50mg/kg, i.p) and Group 3 was treated with phentoin (25mg/kg, i.,) for 15 days prior to the induction of convulsion. The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined for each dose group.

2. Pentylonetetrazole induced seizures: The animals were arbitrarily divided into three groups containing six animals each. Group 1 was treated as control, Group 2 was treated with Capsicum annuum (50mg/kg, i.p) and Group 3 was treated with diazepam (5mg/kg, i.p). Seizures were induced in mice with standard convulsing agents, Pentylonetetrazole, after 30 min of drug treatment and the animals were observed for one hour for tonic convulsion episode. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period was noted. The capability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as a sign of anticonvulsant activity.

3. Strychnine induced seizures: The animals were randomly divided into three groups containing three animals each. Group 1 was treated as control, Group 2 was treated with Capsicum annuum (50mg/kg, i.p) and Group 3 was treated with diazepam (5mg/kg, i.p). Seizures were induced in mice with standard convulsing agents, strychnine (2 mg/kg, i.p) after 30 min of drug treatment and the animals were observed for one hour for tonic convulsion episode. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period was noted. The capability...
of the plant extract to prevent or postponement the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity.

Estimation of biogenic amines:22

Grouping of animals and induction of seizures: All the animals will be divided into four groups containing six animals each group. Group 1 was treated as Normal in which seizures will not be induced, Group 2 was treated as control in which seizures will be induced, Group 3 was treated with Capsicum annuum (50mg/kg, i.p), and Group 4 was treated with phenytoin (25mg/kg, i.p). On the 15th day, seizures were induced to all the groups excluding Group 1 animals using electroconvulsometer and biogenic amines in the forebrain of the mice were estimated.

Brain dissection and extraction of biogenic amines: The conscious mice were decapitated and the brain was removed within 30 sec from the skull and the forebrain region was dissected out. Weighed quantity of tissue and was homogenized in 0.1 M hydrochloric acid – butanol for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm. 0.08 mL of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 mL of heptane and 0.025 mL 0.1 M hydrochloric acid. After 10 min of forceful shaking, the tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase (0.02 mL) was used for estimation of Serotonin, Nor Adrenaline and Dopamine assay.

Estimation of Noradrenaline and dopamine: The assay represents a miniaturization of the trihydroxide method. To 0.02ml of hydrochloric acid phase, 0.05ml of 0.4M hydrochloric acid and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml 0.1 M iodine solution in ethanol for oxidation. The reaction was stopped after two minutes by addition of 0.01ml sodium sulphite in 5m sodium hydroxide. Acetic acid was added 1.5 minutes later. The solution was then heated to 100°C for 6 minutes. When the sample again reached room temperature, excitation and emission spectra were read in the microcuvette at 395-485nm for noradrenaline and 330-375nm for dopamine uncorrected instrument values.

Estimation of serotonin: For 5-HT determination, the O-pthalaldialdehyde (OPT) method was employed. From the OPT reagent 0.025ml were added to 0.02ml of the hydrochloric acid extract. The fluorophore was developed by heating at 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, excitation estimation spectra or intensity reading at 360-470 nm were taken in the micro cuvette.

Statistical analysis:23 The results for electrically induced seizures, pentylenetetrazole induced seizures and strychnine induced seizures were expressed as Mean ± Standard Error of Mean. Paired Student’s t-test was used to analyze the level of significance. A p value of <0.05 was considered as statistically significant. The results for biogenic amines were expressed as Mean ± Standard Error of Mean. The Significance of differences among the group was assessed using one way analysis of variance (ANOVA). The test followed by Dunnet’s test p values less than 0.05 were considered as statistically significant. It was done using Graph pad 5.0 software versions

3. RESULTS AND DISCUSSION

Phytochemical screening: The preliminary qualitative phytochemical analysis of ethanolic extract of Capsicum annuum showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, steroids, fats and oils, and saponins;

Acute toxicity studies: The acute toxicity studies showed that the extract of fruits of Capsicum annuum, was found to be safe at the maximum dose of 500mg/kg by intraperitoneal route. The LD 50 values of extract of fruits of Capsicum annuum, was found to be 501.18 mg/kg body weight.

duration of 18.43±0.29 sec. Ethanolic extract of C.annuum at the dose of 50 mg/kg showed tonic limb extension for the duration of 12.34 ± 0.38 and protected 83.33 % of mice and alter the incidence of seizures elicited by MES to a significant extent.. The standard antiepileptic drug, phenytoin (25mg/kg) also protected all the animals and significantly reduced the duration of HLTE.

Table 1. LD50 Values of the Extracts and Formulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LD50(mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsicum annuum</td>
<td>501.18</td>
</tr>
</tbody>
</table>

Electroshock induced seizures: MES produced hind limb tonic extension seizures in all the animals used. The control mice showed tonic limb extension for the duration of 18.43±0.29 sec. Ethanolic extract of C.annuum at the dose of 50 mg/kg showed tonic limb extension for the duration of 12.34 ± 0.38 and protected 83.33 % of mice and alter the incidence of seizures elicited by MES to a significant extent.

Table 2. Effect of Ethanolic extracts and Capsicum annuum on MES Induced Seizures in Mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose(mg/kg)</th>
<th>Duration of HLTE(sec)</th>
<th>Quantal protection</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>18.43±0.29</td>
<td>4/6</td>
<td>66.67</td>
</tr>
<tr>
<td>C.annuum</td>
<td>50</td>
<td>12.34 ± 0.38</td>
<td>5/6</td>
<td>88.33</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>25</td>
<td>7.71±0.31***</td>
<td>6/6</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n=6); **P<0.001(compared with control using student's t-test) ***P< 0.0001(compared with control using student's t-test)
Pentylentetrazole induced seizures: PTZ produced tonic seizures in all the animals used. A dose of 50mg/kg of ethanolic extract of C.annuum protected 33.33% of animals against PTZ induced seizures and did not affect the onset of seizures to any significant extent. The standard antiepileptic drug, diazepam (5mg/kg) profoundly antagonized the seizures produced by PTZ.

Strychnine induced seizures: Strychnine (2 mg/kg) elicited tonic seizures in all the animals used. Ethanolic extract of C.annuum (50mg/kg) significantly delayed the latency, but did not alter the incidence of seizures produced by strychnine to any significant extent. A standard anti-epileptic drug diazepam and both significantly delayed the latency of seizures.

Table 3. Effect of Ethanolic extracts and Capsicum annuum On PTZ Induced Seizures In Mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose, i.p</th>
<th>Onset of seizures(min)</th>
<th>Quantal protection</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5.12±1.18</td>
<td>1/6</td>
<td>16.67</td>
</tr>
<tr>
<td>C annuum</td>
<td>50 mg/kg</td>
<td>3.20 ± 0.21 ns</td>
<td>2/6</td>
<td>33.33</td>
</tr>
<tr>
<td>Diazepam</td>
<td>5 mg/kg</td>
<td>10.43 ± 0.62 ***</td>
<td>6/6</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n=6); ns-p value not significantly different **P< 0.005***P< 0.0001 (compared with control using student’s t-test)

Table 4. Effect of Extracts and Formepi-4 on Strychnine Induced Seizures in Mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose, i.p</th>
<th>Onset of seizures(Mins)</th>
<th>Quantal protection</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>6.10 ± 0.23</td>
<td>1/6</td>
<td>16.67</td>
</tr>
<tr>
<td>C annuum</td>
<td>50 mg/kg</td>
<td>7.65 ± 0.41 **</td>
<td>4/6</td>
<td>66.67</td>
</tr>
<tr>
<td>Diazepam</td>
<td>5 mg/kg</td>
<td>12.95 ± 0.90 ***</td>
<td>6/6</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n=6); *P<0.05 (compared with control using student’s t-test); ***P< 0.0001 (compared with control using student’s t-test)

Table 5. Effect of extracts and formulation on the levels of biogenic amines on seizures induced mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Noradrenaline (ng/g of wet tissue)</th>
<th>Dopamine (ng/g of wet tissue)</th>
<th>Serotonin (ng/g of wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>131.16±2.09</td>
<td>288.17±4.32</td>
<td>155.67±4.05</td>
</tr>
<tr>
<td>MES</td>
<td>59.83±2.29 a</td>
<td>130.33±3.33 a</td>
<td>87.16±2.72 a</td>
</tr>
<tr>
<td>Capsicum annuum</td>
<td>79.16±3.01 b1</td>
<td>199.5±3.45 b1</td>
<td>108.83±2.96 b1</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>114.67±4.01 b1</td>
<td>254.5±4.26 b1</td>
<td>130.17±3.61 b1</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n=6); a-***P< 0.001 (compared with control using ANOVA followed by Dunnet’s t-test)b1-**P< 0.05 (compared with MES using ANOVA followed by Dunnet’s t-test)b2-***P< 0.001 (compared with MES using ANOVA followed by Dunnet’s t-test)

A significant increase in the noradrenaline, dopamine and serotonin concentration was noted in the fore brain region of C.annuum, treated animals.

Discussion: Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain. The present study was designed to evaluate anti convulsant potential of alcoholic extract of fruits of Capsicum annuum in experimental mice. For the screening of anticonvulsant activity three methods are used namely Maximal electroshock method, Pentylentetrazole induced seizures and Strychnine induced seizures. Apart from this the concentration of biogenic amines was estimated in mice brain.

Preliminary phytochemical screening: The preliminary qualitative phytochemical analysis of ethanolic extract of Capsicum annuum showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, steroids, fats and oils, and saponins.

Acute toxicity studies: Intraperitoneal administration of ethanolic extract of Capsicum annuum at 100mg/kg did not produce any mortality, while the dose 2000mg/kg caused 100% mortality in mice. The LD₅₀ value was found to be 501.18 mg/kg by i.p route.

Electrically induced seizures: MES produces convulsions mainly by opening the voltage dependent sodium ion channels there by causing the repetitive firing of action potential [24]. On MES the result of present study showed that the extract of Capsicum annuum decreased the duration of tonic hind leg extension in maximal electroshock-induced seizures. Capsicum annuum may be acts on the voltage dependent sodium ion channels there by preventing the repetitive firing of action potential and thus produce their anticonvulsant effect. PTZ produces its anticonvulsant effect by inhibiting the activity of GABA at GABAₐ receptors there by inhibiting the neuronal responsiveness and activity by increasing the
chloride ion conductance through opening of the chloride-ion channel \[^{25}\]. Strychnine induces convulsions by directly antagonizes the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing the spinal reflexes \[^{26}\]. The results shows that \textit{Capsicum annuum} increases the latency of convulsion, showed protection against strychnine induced convulsions which suggests that \textit{Capsicum annuum} probably acts on glycinerergic transmission.

**Effect on biogenic amines:** A significant P < 0.001 increase in the dopamine, serotonin and noradrenalin level was noted in the fore brain region for extract treated animals. \textit{Capsicum annuum} administration significantly increased the brain levels of serotonin, dopamine and noradrenaline, which could be attributed to the significant protection offered against MES induced seizures. The increase in the brain monoamine level by inhibiting the monoamine oxidase, an enzyme responsible for destruction of biogenic amines tends to raise the seizure threshold\[^{27}\].

Noradrenaline in the CNS is formed by the \(\alpha\)-hydroxylation of dopamine and is considered the primary inhibitory neurotransmitter. Attenuation of synaptic noradrenaline levels have been shown to exert proconvulsant effect in models of seizures disorders whereas increasing noradrenaline neurotransmission has been shown to reduce seizure activity. Noradrenaline has a potential for biphasic effect of glutamate in the cerebellum and would inhibit glutamate release at low concentrations. Over activation of glutamate receptors may lead to delayed neurodegeneration as a result of increased influx of calcium ions into neurons.

Alterations in central dopaminergic levels are responsible in part for the onset and continuance of many seizure disorders. The inhibition of \textit{Substantia niagra} has been shown to attenuate seizure disorders. The SN projects dopaminergic neurons to the caudate putamen and in turn receives GABAergic afferents from caudate putamen via one of the two pathways. The first pathway known as direct pathway offers a direct monosynaptic GABAergic projections to the caudate putamen to \textit{Substantia niagra}. The second pathway (indirect) involves GABAergic projections from caudate putamen to the lateral \textit{Globus pallidus}. The \textit{Globus pallidus} then projects GABAergic efferent’s to the subthalamic nucleus that finally exerts the glutaminergic tone on \textit{Substantia niagra}. Both the \textit{Substantia niagra} and caudate putamen have been thought to play major roles in interruption and triggering of seizure generation respectively. Seizure control appears to be partly regulated to direct pathway and its ability to potentiate GABAergic activity within the \textit{Substantia niagra}. The antiepileptic activity of the indirect pathway is exemplified following the attenuation of seizure activity after local administration of NMDA agonists either in substantia niagra or subthalamic nucleus. It would appear that these pathways acting through the substantia niagra control seizure propagation, despite the fact that they exert opposite effects on substantia niagra neuronal activity.

Serotonin mediates its action in the mammalian CNS through 7 classes of receptors (5HT\(_{1-7}\)) within its classification. There are at least 4 subtypes (5HT\(_{1-4}\)) which are thought to modify neuronal excitability and/or neurotransmission release. In the brain, the prominent 5HT cell bodies have been shown to either inhibit or excite GABAergic interneuron’s to modify excitatory responses. The compensatory activation of serotonergic neurotransmission that exists in epilepsy generated an increase in serotonin turnover.

**4. CONCLUSION**

The present study was conducted to evaluate the anti convulsant potential of alcoholic extract of fruits of \textit{Capsicum annuum} in experimental mice by Maximal electroshock method, Pentylenetetrazole induced seizures and Strychnine induced seizures. Apart from this the concentration of biogenic amines was estimated in mice brain.

The preliminary qualitative phytochemical analysis of ethanolic extract of \textit{Capsicum annuum} showed the presence of alkaloids, sapoines, carbohydrates, flavonoids, proteins, amino acids, No signs of mortality were observed at the doses of 500mg/kg, 2000mg/kg and 1000mg/kg body weight for extract of fruits of \textit{Capsicum annuum}, pericarp of \textit{Sapindus emarginatus} and \textit{Formepi-4} respectively. The extracts were found to be safe at these doses. The LD 50 values of extract of fruits of \textit{Capsicum annuum}, was found to be 501.18, mg/kg b.w \textit{Capsicum annuum} decreased the duration of tonic hind leg extension in maximal electroshock-induced seizures probably by acting on voltage gated sodium ion channels, showed protection against strychnine induced convulsions probably acting on glycinerergic transmission.

By this study it can be concluded that \textit{Capsicum annuum} produces anticonvulsant effect by acting on sodium ion channels, inhibition of prostaglandin synthesis and monoamine oxidase enzyme as well as by the decreasing the influx of calcium ions acting on the glycinerergic transmission; \textit{Capsicum annuum}, exhibits their anti convulsant effect by acting on voltage gated sodium ion channels, inhibitory GABAergic transmission, inhibition of prostaglandin synthesis and monoamine oxidase enzyme as well as by the decreasing the influx of calcium ions acting on the glycinerergic transmission "So the present study indicates the alcoholic extract of fruits of \textit{Capsicum annuum} possess the anti-epileptic activity by different mechanism". However, the exact mechanism and the
active principle by which these extracts exert their action remain unclear. Further studies are required to study the individual mechanism of actions.

REFERENCES


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