Evaluation of Antiepileptic Activity of Flowers of Cocos nucifera L. Against Experimentally Induced Convulsions in Rats

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The report used to be planned to analyze the antiepileptic activity of Cocos nucifera flowers against special experimentally induced convulsions in rats. In the present study, antiepileptic activity was assessed by following experimental models. Anti-convulsant in vivo models: Maximal electroshocks (MES) induced models in rats, Pentylenetetrazole (PTZ) induced in rats. Pretreatment of animals with Cocos nucifera flowers extract has reduced by half the general continuance of tonic hind leg extension, the most commonly used endpoint in assessing clonic convulsions. MES provokes repetitive neuronal firing indicates epileptic neurons. MES is the widely accepted model to demonstrate the antiepileptic property of a drug. This property is antagonistic of the plant extract could flow from to blockade of voltage-gated sodium channel or due to effect on NMDA receptors.

INTRODUCTION

Epilepsy is defined as abnormal electrical activity in the brain. Epilepsy is a common neurological characterized by paroxysmal cerebral dyshrythmia, visualizing for the reason that aphoristic trailers going from sum or disturbance of consciousness, without or with distinctive body movement, sensorial or psychiatric consequence. Epilepsy has a central origin in the brain; manifestations depend on the site of focus, regions into which the discharges spread, and postictal depression of this region. Epilepsy has been known at the time of antiquity. The coconut Cocos nucifera Linn has been defined as the  "tree about life operating room tree going from heaven in addition to nature's life” supreme gift to human beings. To each one component of the overall cocoa, the palm can be utilized to supply perfume invaluable for the overall boat club. Cocos nucifera L. is a leading type tree belonging to the family Areecaceae (palm). The common name of Cocos nucifera is the coconut plant. From the earliest time, the different parts of Cocos nucifera have been used for the treatment of various disease states in the Indian system of medicine. The purpose of the study is to evaluate the epileptic activity of flowers of Cocos nucifera against experimentally induced convulsions in rats.
MATERIALS AND METHODS

Drugs and Chemicals Phenytoin was purchased from Medico Remedies Ltd., Maharashtra, India, diazepam was procured from Prem Pharmaceuticals Ltd., Indore, Madhya Pradesh, India and pentylentetrazole (PTZ) and GABA was purchased from Yerrow chem Mumbai, India. All other solvents and chemicals were of analytical grade purchased from Hi-Media Laboratories Pvt., Ltd., Bengaluru, India.

METHODOLOGY

Preparation of extract

Fresh flowers of Cocos nucifera have always been specter dried at room temperature and coarse powdered and extracted with methanol by Soxhlet’s Apparatus. Then the extract was concentrated by using a rotary flash evaporator to obtain semisolid crude extract. The % yield of the Cocos nucifera flowers methanolic extract was found to be 22.79%. The methanolic extract was preserved and kept in an air tight bottle in a refrigerator below 10°C. The suitable concentration of stock solutions of the extract was prepared using distilled water and it is used following studies 1.

1. Detection of Preliminary phytochemical investigation.
2. Detection of Acute toxicity study in mice.
3. Evaluation of the crude extracts of Cocos nucifera flowers for the anticonvulsant property against

a. Maximal electroshock (MES) triggered convulsion models in rat
b. Pentylentetrazole (PTZ) triggered convulsion.

1. Preliminary phytochemical screening

The preliminary phytochemical screening conveys out for methanol extract of Cocos nucifera flowers for the presence of phytoconstituents. The tests for common phytochemicals were carried out by the following methods as described in the journal of preliminary phytochemical screening of Cocos nucifera flowers.

2. Determination of acute toxicity (LD50)

Acute toxicity of different extracts of Cocos nucifera flowers extract was executed on female albino mice (20-30 g). The animals have fasted overnight before the experiment. Fixed-dose (OECD Guideline No. 423) method was personalized for toxicity studies. 1/5th LD50 cut-off value of the extract was selected given that screening dose for the anticonvulsant activity of extract of Cocos nucifera flowers.

Experimental animals

The Wistar rats 150-200 g of either sex were used in the experimentation. Animal Ethics committee approval no. 1688/P0/E/2020/CPSEA, Sanzme Ltd Healthcare Business, Hyderabad. After randomization into various teams of animals were acclimatized for 10 days under standard husbandry conditions, and room temperature at 27 ± 3°, relative humidity 65 ± 10%, 12 hr. light or dark cycle. All the animals were fed with a rodent food diet (Pavan Agro’s Industries, Sangli, India) and water was allowed ad libitum under severe hygienic conditions.

3. Evaluation of the crude extract of Cocos nucifera flowers for anticonvulsant Property against

A) Maximal electroshock (MES) induced models in rats
B) Pentylentetrazole (PTZ) induced.

A. Maximal Electroshock (MES) seizure

Electrical stimulation was applied using ear electrodes. The electrodes have been moistened with saline before application. All animals were stimulated with 150mA for 0.2 seconds, with consisting voltage stimulator of 250 V. The animals are divided into five groups. Each group consists of 6 animals both sexual activities.

Group I: Normal Saline (0.5ml P.O for 15 days) + MES on 15th day
Group II: Phenytoin sodium (25mg/kg p.o for 15 days) + MES on 15th day
Group III: Cocos nucifera flower extract (CNFE) (125mg/kg p.o for 15 days) + MES on 15th day
Group IV: CNFE (250mg/kg p.o for 15 days) + MES on 15th day
Group V: CNFE (500mg/kg p.o for 15 days) + MES on 15th day

On the 15th day, the test samples were given that 1 hour before generalization of convulsions. Stiffing of tonic hind limb extension die for a measure of efficacy in this test.

B. Pentylentetrazole (PTZ) triggered convulsion

PTZ 60mg/kg LP was administered to rats. The parameter noted was the time scale(duration) of convulsions. The animals were divided into five groups. Each group consisted of 6 males and 6 females (n=12).

Group I: Normal Saline (0.5ml p.o for 15 days) + PTZ on 15th day
Group II: Diazepam (2mg/kg p.o for 15 days) + PTZ on 15th day
Group III: CNFE (125mg/kg p.o for 15 days) + PTZ on 15th day
Group IV: CNFE (250mg/kg p.o for 15 days) + PTZ on 15th day
Group V: CNFE (500mg/kg p.o for 15 days) + PTZ on 15th day

On the 15th day, the test samples were given 1 hour before induction of convulsions. Termination of the leg pain gives up for as valuate of affectivity in this test.

GABA Estimation

For the estimation of GABA levels in PTZ induced animals, animals were divided into six groups, and each group consisted of six rats. The rats were sacrificed 45 min after Cocos nucifera extract or vehicle and 30 min after diazepam. All Group of animal was sacrificed after the onset of convulsions occurs or 65 sec after PTZ injection. The rat brain was immediately isolated and transferred into a homogenisation tube and the tube containing 5 ml 0.01 M hydrochloric acid and homogenized the content. Afterward, the homogenized brain was transferred into 8 ml of the ice cold absolute alcohol containing bottle and at 0°C kept it for 1 hr. And then the content was centrifuged at 16,000 rpm for 10 min, and then the upper part or supernatant was collected in a Petri dish. Then the precipitate was washed three times with 5 ml of 75% alcohol and washes were mixed with supernatant. And the Petri dish contents were disappeared at 70 °C for dryness on a water bath under stream air. And to the dry mass 2 ml chloroform and 1 ml water were added and for 10 min centrifuged at 2,000 rpm.
And upper containing phase is GABA, in that 2.0 ml was separated and 0.1 ml of it was applied on whatman paper No.41 as spot. The mobile phase was prepared and it consists of 12 ml acetic acid, 60 ml water, and 50ml n-butanol. The chamber was kept for half an hour for saturation with the mobile phase. The chromatography paper was developed with ascending technique. The paper was dried in a hot air oven and then spread with 0.5% ninhydrin solution in 95% ethanol on chromatography paper. And then the paper was dried at 90 c for 1 hr. Blue color spot developed on paper and the blue color spot was cut and heated with 2 ml ninhydrin solution for 5 min on a water bath. Then 5.0 ml of water was added to the solution and kept for 1h. Then supernatant 2.0 ml was decanted and absorbance was measured at 570 nm.

Statistical Significance
The data obtained from the above findings were subjected to statistical analysis using one-way ANOVA followed by Tukey’s Kramer Multiple Comparison Test to assess the statistical significance of the results. The p<0.01 implies significance.

RESULTS
Practical yield
The practical yield of Cocos nucifera flowers extract from Soxhlet extraction was evaporated to dryness by using a rotary flash evaporator. The yield of Cocos nucifera flowers finds to be 22.79% w/w.

Preliminary phytochemical screening
Results of the preliminary phytochemical investigation on methanolic extract of Cocos nucifera flowers [Table 1].

Determination of acute toxicity LD₅₀
The methanolic extract of Cocos nucifera flowers turned into calculated given that acute toxicity at a dose of 2000 mg/kg p.o. in albino mice. The extracts find devoid containing mortality of the animals. Hence 2500 mg/kg were regarded as LD₅₀ cut-off value.

So the screening doses selected for the evaluation of anticonvulsant activity as per OECD guidelines No. 423 and fixed dose method are mentioned under
1. 125 mg/kg methanolic extract (1/20th of 2500 mg/kg).
2. 250 mg/kg methanolic extract (1/10th of 2500 mg/kg).
3. 500 mg/kg methanolic extract (1/5th of 2500 mg/kg).

Anticonvulsant study
Effect of Cocos nucifera flowers extract on MES induced seizures
The effect of Cocos nucifera flowers extract on MES triggered involuntary movements in rats will be summarized successfully the general in Table 2. The CNFE at 250 mg/kg and 500 mg/kg p.o. have significantly delayed the attack in addition to reducing the duration containing hind limb extension seizure (HLES) compared to control. There were been no significant alterations in the latency consisting of convulsions compared to regulating at the dose of 125mg/kg. However, the extract as well exhibited 22%, 43%, and 46% protection in hind limb extension at the doses of 125mg/kg, 250mg/kg, 500mg/kg respectively, though it all become 72% in hydantoin, standard drug-treated rats [Figure 1, 2, 3 & 4].

Effect of Cocos nucifera flowers extract on Pentylenetetrazole (PTZ) induced seizures
The effect of Cocos nucifera flowers extract on PTZ triggered seizures in rats determined in Table 3. The plant extract CNFE at 500 mg/kg p.o indicates important onset containing PTZ caused seizures in addition to less significant donic convulsions. At 125mg/kg and 250mg/kg p.o. that it also indicates onset containing involuntary movements and duration of clonic convulsions in PTZ taste-maker other than outcome find planned mathematically non-significant. CNFE at 500 mg/kg p.o exhibited 66% consisting of inoculating towards the duration containing donus convulsion, though it used to 74% in diazepam dosed grouping [Figure 5, 6, 7 & 8].

GABA Estimation
The effect of Cocos nucifera flowers extract on GABA level in PTZ triggered seizures in rats indicated in Table 4. In present research work, we found that GABA level was decreased in controlled rats were as, the title plant extract at 500mg/kg p.o has significantly increased GABA level. The extract at 125mg/kg, 250mg/kg as well multiplied GABA level but mathematically it finds to be non-significant. The standard drug has also greatly magnified the GABA level [Figure 9 & 10].

Table 1: Results of the preliminary phytochemicals investigation in the methanolic extract of Cocos nucifera flowers

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>Phenol</td>
<td>Present</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Absent</td>
</tr>
<tr>
<td>Saponins</td>
<td>Absent</td>
</tr>
<tr>
<td>Tannins</td>
<td>Absent</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Present</td>
</tr>
<tr>
<td>Aminoacids</td>
<td>Present</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Absent</td>
</tr>
</tbody>
</table>
Table 2: Effect of *C. Nucifera* flowers extract on MES model in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Flexion in (sec)</th>
<th>Extension in (sec)</th>
<th>Clonus in (sec)</th>
<th>Stupor in (sec)</th>
<th>Protection against the extension (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>0.5ml</td>
<td>12.00± 1.183</td>
<td>16.50± 0.56</td>
<td>16.50± 1.23</td>
<td>12.00± 10.87</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>Phenytoin sodium</td>
<td>25 mg/kg</td>
<td>2.50± 0.428***</td>
<td>4.60± 0.33***</td>
<td>4.80± 0.30***</td>
<td>32.83± 1.55***</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>CNFE</td>
<td>125mg/kg</td>
<td>10.33± 0.61**ns</td>
<td>12.80± 1.01**</td>
<td>13.00± 0.61*</td>
<td>90.00± 7.14*</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>CNFE</td>
<td>250mg/kg</td>
<td>8.66± 0.21*</td>
<td>9.30± 0.42***</td>
<td>12.00± 0.68**</td>
<td>77.00± 7.34**</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>CNFE</td>
<td>500mg/kg</td>
<td>4.33± 0.42***</td>
<td>8.83± 0.60***</td>
<td>7.60± 0.49***</td>
<td>40.50± 3.04***</td>
<td>46</td>
</tr>
</tbody>
</table>

Consequences have been verbalised given that Mean ± SEM, n=6. Significance at *p<0.05, **p<0.01, ***p<0.001, and non-significant v/s control.
Figure 4: Effect of C. Nucifera flowers extract on MES induced stupor in rats

Table 3: Effect of C. Nucifera flowers extract on PTZ induced model in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Onset of jerks (sec)</th>
<th>Onset of Clonus (sec)</th>
<th>Duration of Clonus (sec)</th>
<th>Onset of Extension (sec)</th>
<th>Protection against clonus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>0.5ml</td>
<td>65.00± 6.19</td>
<td>322.66± 10.08</td>
<td>22.16± 4.75</td>
<td>136.66± 20.44</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>Diazepam</td>
<td>2mg/kg</td>
<td>122.60± 6.79***</td>
<td>468.33± 16.4***</td>
<td>5.66± 0.55**</td>
<td>43.00± 1.82***</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>CNFE 125mg/kg</td>
<td>125mg/kg</td>
<td>65.16± 6.68ns</td>
<td>333.50± 17.42ns</td>
<td>19.33± 3.85ns</td>
<td>121.66± 20.60 ns</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>CNFE 250mg/kg</td>
<td>250mg/kg</td>
<td>69.83± 6.24ns</td>
<td>361.83± 21.27ns</td>
<td>13.16± 2.14ns</td>
<td>107.50± 11.50 ns</td>
<td>38</td>
</tr>
<tr>
<td>5</td>
<td>CNFE 500mg/kg</td>
<td>500mg/kg</td>
<td>101.66± 5.20**</td>
<td>434.33± 9.98***</td>
<td>7.33± 0.76*</td>
<td>45.00± 1.50***</td>
<td>66</td>
</tr>
</tbody>
</table>

Consequences have been verbalised given that Mean ± SEM, n=6. Significance at *p<0.05, **p<0.01, ***p<0.001, and non-significant v/s control.

Figure 5: Effect of C. Nucifera flowers extract on PTZ induced onset of jerks in rats

Figure 6: Effect of C. Nucifera flowers extract on PTZ induced onset of clonus in rats
Figure 7: Effect of *C. Nucifera* flowers extract on PTZ induced duration of clonus in rats

![Graph showing duration of clonus](image1)

**Treatment**

Vehicle, Diazepam, CNFE 125mg/kg, CNFE 250mg/kg, CNFE 500mg/kg

Figure 8: Effect of *C. Nucifera* flowers extract on PTZ induced onset of Extension in rats

![Graph showing onset of extension](image2)

**Treatment**

Vehicle, Diazepam, CNFE 125mg/kg, CNFE 250mg/kg, CNFE 500mg/kg

Table 4: Effect of *C. Nucifera* flowers extract on GABA level in PTZ induced model in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>GABA Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>---</td>
<td>269.85 ± 3.46</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>Vehicle</td>
<td>189.47 ± 2.88</td>
</tr>
<tr>
<td>3</td>
<td>Diazepam</td>
<td>2mg/kg</td>
<td>258.62 ± 4.03***</td>
</tr>
<tr>
<td>4</td>
<td>CNFE</td>
<td>125mg/kg</td>
<td>192.37 ± 3.62ns</td>
</tr>
<tr>
<td>5</td>
<td>CNFE</td>
<td>250mg/kg</td>
<td>204.35 ± 4.06***</td>
</tr>
<tr>
<td>6</td>
<td>CNFE</td>
<td>500mg/kg</td>
<td>246.89 ± 3.61***</td>
</tr>
</tbody>
</table>

Figure 9: Effect of *C. Nucifera* flowers extract on GABA level in PTZ induced model in rats

![Graph showing GABA level](image3)

**Treatment**

Normal control, Control, Diazepam 2mg/kg, CNFE 125mg/kg, CNFE 250mg/kg, CNFE 500mg/kg
DISCUSSION

Epilepsy is defined as paroxysmal cerebral dysrhythmia. Epilepsy (Encephalopathy) is a common neurological disease that has effects on a variety of people inside the world. It can be a disorder of the brain characterized by undeterminable and irregular (periodic) happening consisting of a transitory of behaviour due to the disordered, synchronous, and rhythmic firing of populations of brain neurons.

Currently, many synthetic anticonvulsant drugs are available for the management, control, and treatment of epileptic patients. However, most of the drugs are high cost and inaccessible and also produce adverse effects. Hence, there is a need for the potential anticonvulsant drug which is low cost and safe.

Since ancient times medicinal plants are useful drugs known to humans. Literature survey indicates that, many plants are possessing anticonvulsant activity and which can serve as an alternative to allopathic drugs. The present analysis was once well-intentioned to investigate the antiepileptic activity of methanolic extract of Cocos nucifera flowers.

In the present study, it is evident that the Cocos nucifera flower ameliorates the convulsions induced by MES and PTZ animals. The commonly used epileptic models to study antiepileptic drugs are MES and PTZ induced convulsions. The general biography was written document famous that GABAergic neurotransmission can be prevalent with the overall stimulus consisting of epilepsy.

Pretreatment of animals with Cocos nucifera flowers extract has significantly reduced the duration of tonic hind limb extension, the most commonly used endpoint in assessing clonic convulsions.

Electroshock, Electrical stimulation at the brain, provokes repetitive neuronal firing indicates epileptic neurons. MES is the widely accepted model to demonstrate the antiepileptic property of a drug. Our study exhibits that Cocos nucifera flowers have shown potential anticonvulsant effect in this model by effectively reducing hind limb extension.

MES caused involuntary movements to affect oxidative damage, in addition, to having been abolished by the drugs that block voltage-gated sodium channels like phenytoin sodium or drugs that block NMDA receptors like felbamate.

In the PTZ model convulsions are abolished by drugs that possess GABA agonists like diazepam or block T-type Ca^{2+} current in the thalamus like valproate.

In MES and PTZ models, convulsions are produced by inhibiting the activity of GABA receptors. GABA is the major inhibiting neurotransmitter implicated in epilepsy. Inhibition of neurotransmitters of GABA will cause convulsions whereas enhancement attenuates it.

Diazepam, a standard antiepileptic drug, displays glamour consequence by way of battering GABAergic neurotransmission within the unconscious mind.

The abolishing of MES-induced seizures by the Cocos nucifera flowers extract suggests anticonvulsant activity in generalized tonic-clonic seizures. The present belongings the
plant life find time for may well be imputable stymie epitelithal voltage-gated sodium channel or referable its antagonistic upshot in the NMDA receptors.

The Cocos nucifera flowers extract was also demonstrated potential anticonvulsant activity in PTZ induced convulsions and this may be due to its agonistic activity on the GABA_A receptor. This is further supported by an elevated level of GABA by the plant extract in the PTZ model.

CONCLUSION

Methanolic make time for containing Cocos nucifera flowers gave an important anticonvulsant activity against MES and PTZ caused epileptic seizure fashions. That cited utilization can be because of ubiquity containing flavonoids along with other phytochemical constituents found in the sensational slot in.

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Conflict of Interest

The authors attest that they have no conflict of interest in this study.

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BIBLIOGRAPHY


