Research Article

An Evaluation of Anticonvulsant Activity of Bark of Carissa Carandas Linn. Against Phenytoin

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ARTICLE INFO

Aim: Evaluation of anticonvulsant activity of ethanolic extract of bark of Carissa carandas in mice.

Study design: In the current study the dried bark of Carissa carandas were ground to powder form then powdered bark was embedded into the Soxhlet apparatus. 250ml of 95% ethanol, which was make the temperature at 55-65°C until the dissolvable fume. The ethanolic content was removing sifted and stored in a dessicator containing dessicant (calcium chloride) until they completely dried.

Methodology: This study includes the different method for the phytochemicals screening of the ethanolic bark extract of the Carissa carandas, which show the presence of phytochemicals. The methodology of anticonvulsant study of bark of Carissa carandas by receiving isoniazid induce convulsion on the grouping of animal each groups (normal control receive normal saline, negative control receive isoniazid, positive control receive Phenytoin and two test group which contains 300mg/kg and 600mg/kg of ethanolic bark extract of Carissa carandas) contains 6 albino mice.

Results: The phytochemicals screening of ethanolic bark extract of Carissa carandas shows the presence of Flavonoids, Saponins, alkaloids, carbohydrate, phenols, tannin, protein, amino acid, Anthraquinone and cardiac glycosides. The antiepileptic potency of the plant, bark extract of Carissa carandas (BECC) had been analyzed the 300mg/kg and 600mg/kg extract protect the 50.01% and 66.67% animals respectively and the Phenytoin protect 83.33% of animals which is induce after receiving of Isoniazid induce convulsion.

Conclusion: The ethanolic extract of bark of Carissa carandas having the phytochemicals (Saponins and Flavonoids) those are responsible to treat epilepsy. The bark of Carissa carandas (600mg/kg) having the potency against isoniazid induce convulsion in comparison to the Phenytoin (25mg/kg) both having approx similar action on the treatment of convulsion.
INTRODUCTION:

EPILEPSY-
The word "epilepsy" originates from the Greek word "epilambanein," a word that means to seize or attack [1]. In epilepsy, these uncontrolled electrical discharges disrupt with the brain's regular electrical activity, severely limiting nerve cell communication [2]. Modern anticonvulsants can often control epilepsy, which is not curable but can be managed to allow for a less constrained existence by preventing or lessening the severity of the episodes [3]. Convulsions, according to Cheymol (1950), occur as a result of an abrupt, excessive, and fast discharge in the brain's grey matter [4]. Addressed are also clustering risk factors and expected clustering precipitants [5].

In the general population, learning difficulties affect about 2% of people [6]. Worldwide, epilepsy affects over 50 million individuals, and approximately two out of every three new cases are found in developing nations. Young children or persons over 65 years old are more likely than others to develop epilepsy [2]. Antiepileptic medications (AEDs) affect the brain's ion channels, metabolic enzymes, neurotransmitter receptors, and transporters. They also alter the way neurons burst, which prevents epileptic activity from spreading, and they lessen synchronization [7]. It is generally acknowledged that a shift in the ratio of synaptic excitation to inhibition, from inhibition towards excitation, can lead to the emergence of epileptiform activity. In reality, blocking synaptic inhibition is a simple method for inducing abrupt seizures in experiments [8]. The "ideal" epilepsy model, according to the 2002 National Institutes of Health (NIH), NINDS, and American Epilepsy Society (AES) Models II Workshop, mimics the biology and phenomenology of human epilepsy [9].

In animal epilepsy models, a particular loss of GABAergic neurons has been seen. GABA deficit has been seen in the brain and cerebrospinal fluid of epileptic patients. More than 40 voltage-gated potassium channels are known, and they perform a variety of tasks related to membrane hyperpolarization, such as controlling the resting potential, depolarizing the plasma membrane after action potential generation, and determining the length of an action potential. For the formation and transmission of the action potential, voltage-dependent sodium and potassium channels are the most crucial ionic pores [10, 11]. The different different markers that is responsible for epilepsy that is - sodium channel, T-type calcium channel, GABA or glutamate respectively [12]. Central nervous system effects occur due to vitamin B6 deficiency. In the present case, a deficiency of vitamin B6 was regarded to be the cause of the patient’s convulsive seizures because the serum vitamin B6 level on admission was low [13]. Primary or idiopathic generalised seizures are less well understood in terms of their pathogenic causes. Early prenatal events may result in a more diffuse or multifocal state of excitability of the neurons that is amplified with time. Cortical plasticity may be directly related to the development of subclinical neuronal explosive activity into a clinical seizure [14]. Phenytoin prevents repetitive detonation of normal brain cells [15]. PHN stabilises the rapidly inactivated state, affecting Sodium channel activities and Blockade [11, 16, 17].

HISTORICAL BACKGROUND OF ANTIEPILEPTIC TREATMENT

Beginning on May 11th, 1857, Charles Locock wrote about his treatment of potassium bromide in 15 cases of "hysterical" epilepsy in young ladies in the Lancet. Alfred Hauptmann's accidental discovery of Phenobarbital's anticonvulsant effects in 1912 was the next development. This came before Houston Merritt and Tracy Putnam's screening of prospective therapeutic drugs against feline "electrical seizures" by more than 20 years. As a result, Phenytoin was introduced in 1938 [18]. The American National Institute of Neurological Disorders and Stroke launched the Anticonvulsant Drug Development Programmed in 1975, ushering in the contemporary era of AED development. Since then, academic and pharmaceutical chemists have evaluated more than 28,000 novel chemical entities, leading to the licencing of an expanding list of AEDs [19]. Nine clinically successful medicines for the symptomatic treatment of epilepsy have been successfully developed by the Anticonvulsant Drug Development Programme since 1993 [20].
TYPES:
Abnormal neuronal discharges that are either localized or generalised define epilepsies. Following is a list of the drugs that should be chosen based on the type of seizure.

PARTIAL SEIZURES:
- SIMPLE PARTIAL - Carbamazepine, Lamotrizine, Valproic acid, Phenobarbital.
- COMPLEX PARTIAL - Phenytoin, Carbamazepine, Lamotrizine, Valproic acid.

GENERALISED SEIZURES:
- TONIC-CLONIC SEIZURE (GRAND MAL) - Phenytoin, Topiramate, other newer AEDs.
- ABSENCE SEIZURE (PETIT MAL) – Ethosuximide, Valproic acid, Clonazepam.
- ATONIC SEIZURE - Valproic acid, Lamotrizine, Ethosuximide.
- MYOCLONIC SEIZURE - Valproic acid, Lamotrizine, Other newer AEDs.

DIAGNOSIS OF EPILEPSY

- Features of seizure of diagnostic importance
  - The environment in which the incident occurred and the contributing elements
  - Any early seizure warning signs or symptoms
  - Changes in skin colour (cyanosis, pallor etc)
  - Modifications in respiratory patterns
  - Tongue biting
  - Incontinence
  - Eyes (open / closed, eye-rolling, etc)
  - Presence of limb-jerking
  - Nature of any limb-jerking
  - The length of the unconsciousness (if any)
  - Injury
  - Post-ictal features (drowsiness, headache, and confusion, limb-aching).

- The ailments most prone to be mistaken for generalised seizures are:
  - Syncope
  - Concussive seizures
  - Psychogenic non-epileptic seizures
  - Hypoglycemia
  - Breath-holding attacks
  - Narcolepsy
  - Panic attacks / hyperventilation

- Conditions that could be mistaken for partial seizures:
  - Transient ischemic attacks
  - Transient global amnesia
  - Vertigo
  - Migraine
  - Movement disorders (e.g. tics) [22].

PLAN OF WORK

Step: 1

I. Plant material was collected from the Botanical garden of R. K. Pharmacy College and sending it for authentication in BHU (Department of Botany), U.P., INDIA.

II. Extracting Bark of Plant using Soxhlet apparatus.

III. The experiment plan for the phytochemicals analysis.

Step: 2

I. Preparing animals and setting up the experiment for the anticonvulsant study.

II. Composing an assay on anticonvulsant research.

Step: 3

I. Writing a research work summary.
II. Final thesis writing.

MATERIAL AND METHODOLOGY

Material:

Plants material: Carissa Carandas Linn's bark were taken from the R. K. Pharmacy College's garden in Azamgarh, U.P., INDIA, and verified by the DEPARTMENT OF BOTANY in BHU, INDIA, using voucher specimen number Apocyna. 2023/01.

Drugs:

Phenytoin:
As a positive control test, phenytoin was purchased from Harman Finochem in Mumbai, India. Phenytoin was dispersed in distilled water and administered intraperitoneally at a dose of 25 mg/kg body weight.

Isoniazid:
As a negative control test, isoniazid was purchased from Gujarat, India's Enomark Healthcare Pvt. Ltd. Isoniazid was broken down in purified water and dosed at 300 mg/kg body weight for subcutaneous administration.

Animals:

In the experiments, Swiss mice of either sex that were 8–10 weeks old and weighed about 25–30 g were utilised. The animals were kept in polypropylene cages with a 12-hour light/dark cycle, a constant temperature of 25 to 30 degrees Celsius, and a humidity level of 45 to 65%. They were also given unlimited access to water and standard rat food (provided by Hindustan Liver Ltd., India). Before the experiment began, all of the animals spent a week becoming used to the lab environment. Prior to the start of the experiment, the Institutional Animal Ethical Committee (IAEC) evaluated and approved all experimental protocols.

METHODOLOGY:

Extraction of plants:
Whole barks were cleansed individually with refined water and water containing 0.5% chlorine. Before cutting the barks, the spikes and edges were removed, and the barks then dried at 45°C for three to five days. The dried bark of Carissa carandas was powdered and stored in a securely fastened container. For extraction, the Soxhlet device and method were used. The Soxhlet principle compartment was sealed when the powdered barks were imbedded there. 250 ml of 95% ethanol were added to the Soxhlet mechanical assembly, which was then warmed at a temperature of 55 to 65 °C until a dissolvable fume filled the fundamental chamber. At that time, the soluble fume was dense and slowly crept down into the chamber containing the powdered bark removal. The ethanolic remove was separated, maintained for 3–4 days in a dessicator with calcium chloride as a dessicant to evaporate the ethanol, and finally a buildup was obtained. The final accumulation was stored for later use in an impenetrable chamber that was kept at a deeply chilled temperature.

Grouping of animals:

<table>
<thead>
<tr>
<th>Table: 1- grouping of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
</tr>
<tr>
<td><strong>Negative control group</strong></td>
</tr>
<tr>
<td>Vehicle (normal saline) Via i.p. route</td>
</tr>
<tr>
<td>Isoniazide 300mg/kg via s. c. route.</td>
</tr>
<tr>
<td>Group of 6 mice</td>
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</tbody>
</table>
Procedure:-

- 6 Albino mice of either sex having age of 8-10 weeks old as well as their weight between 18 to 22 gm are in each group.
- Mice are treated with the test compound (BECC 300mg/kg and 600mg/kg) of the both test group of animal respectively via p.o. administration.
- The standard (Phenytoin 25mg/kg) via intraperitoneal administration to the positive control group.
- Normal controls group of animals receive the vehicle only.
- Observe 30 min after i.p. treatment and 60 min. after p.o. administration.
- Then the animals of negative control groups are injected with 300 mg/kg Isoniazide via subcutaneous route.
- Observe next 120 min
- Record the frequency of tonic and clonic seizures as well as no. of death.

RESULTS

Plant authentication:
The bark of Carissa carandas were collected in January 2023 from the territorial garden of R. K. Pharmacy College, Azamgarh and authenticated from the Prof. Nawal Kishore Dubey head of department of botany in BHU and the Voucher specimen no. Apocyna. 2023 / 01.

Subjective phytochemicals screening:

Table: 2- Analysis of phytochemicals of different extractives of C. carandas Linn.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Ethanolic Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test of Alkaloids</td>
<td>Hager’s Test</td>
</tr>
<tr>
<td></td>
<td>Tannic acid test</td>
</tr>
<tr>
<td>Test of Carbohydrates</td>
<td>Iodine test</td>
</tr>
<tr>
<td></td>
<td>Molish’s Test</td>
</tr>
<tr>
<td></td>
<td>Fehling’s Test</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Legal test</td>
</tr>
<tr>
<td>Protein</td>
<td>Biuret</td>
</tr>
<tr>
<td>Test for Phenolic</td>
<td>Ferric Chloride Test</td>
</tr>
<tr>
<td>Compounds</td>
<td>Lead Acetate</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
</tr>
<tr>
<td>Test for Saponins</td>
<td>Froth test</td>
</tr>
<tr>
<td></td>
<td>Foam test</td>
</tr>
<tr>
<td>Test for unsaturated</td>
<td>Lieberman Burchard’s Test</td>
</tr>
<tr>
<td>sterols</td>
<td></td>
</tr>
<tr>
<td>Amino acid</td>
<td>Ninhydrin test</td>
</tr>
</tbody>
</table>

(+)= Presence & (-)= Absence

The ethanolic extract of the skin of the plant Carissa carandas Linn was subjected to preliminary photochemical analysis, which revealed the existence of flavonoids, saponins, alkaloids, carbohydrates, phenols and tannins, proteins, amino acids, anthraquinone, and cardiac glycosides.
ISONIAZIDE INDUCE CONVULSION
Effect of ethanol extract of the bark of *C. carandas* (ERCC) on Isoniazide (INH) – induced seizures in mice

Table: 3 - Effect of BECC on Isoniazide (INH) – induced seizures in mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>No. of animal death due to convulsion / no. used</th>
<th>Animal was protected (%)</th>
<th>Duration of convulsion in mice (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH 300</td>
<td>Normal saline 0.25ml 6/6</td>
<td>0</td>
<td>100.0±0.45</td>
</tr>
<tr>
<td></td>
<td>BECC 25</td>
<td>83±0.33</td>
<td>32±0.50</td>
</tr>
<tr>
<td></td>
<td>BECC 300</td>
<td>50±0.01</td>
<td>64±0.83</td>
</tr>
<tr>
<td></td>
<td>BECC 600</td>
<td>66±0.67</td>
<td>43±0.50</td>
</tr>
</tbody>
</table>

Fig: 1- Animals Shows Tonic-Clonic convulsion

Doses

*Fig: 1- Animals Shows Tonic-Clonic Convulsion*
The potency of the anticonvulsant activity of standard drug of Phenytoin protects the 83.33% animals and the different-different dose range of ethanolic bark extract of *Carissa carandas* (300mg/kg and 600mg/kg) was protect the mice as 50.01% and 66.67% as respectively.

**DISCUSSION**

The neurological condition known as epilepsy is one which impacts a wide variety of people worldwide. A transitory modification in behavior caused by the disorganized, synchronized, and repetitive firing of groups of brain neurons is the hallmark of this brain illness, which occurs on a predictable and regular basis. The likelihood that a treatment would be effective in preventing generalized tonic-clonic seizures is thought to be predicted by the INH-induced seizure test. Antiepileptic medications may work by one of the following mechanisms. Normalization of seizure foci, avoidance of seizures beginning at the
focal points, prevention of post-tachycardia, and raising of the excitatory synaptic threshold lengthening of the focusing period and Potentiation of pre- or post-synaptic inhibition. Phenytoin produces prolongation of Na+ channels inactivation and inhibits the excitatory glutaminergic synapse creating a pharmacological effect. Phenytoin prevents tonic convulsions brought on by drugs, but it has no effect on chemically caused seizures of INH induce. The presence of the alkaloid components in the plant constituents that show the local anesthetic effects and its overdose also produce the convulsion but a normal dose may produce the anesthetic properties. The bark extract of Carissa carandas shows the presence anticonvulsant activities at the different-different dose range and the Phenytoin have protecting potency of anticonvulsant that is induce by the isoniazid as their convulsion inducing drug dose range. It may be the dose of the bark extract of Carissa carandas having the protecting potency from convulsion induced by the isoniazid and it is too safe to use as a natural herbs for the treatment of the convulsion. The isoniazid induce the tonic convolution and clonic convolution showing the bilateral movements arms and legs will first stiffen and limbs and head will then begin jerking as respectively as in the practical performance were shown like this types of activity that is protected approx 70% by the crude bark extract of the Carissa carandas.

CONCLUSION
In this work the evaluation of anticonvulsant activity of ethanolic bark extract of Carissa carandas against Phenytoin and I had concluded that the bark extract of Carissa carandas containing the anticonvulsant value and the Phenytoin protects the animals from the grandmal convolution induced by the isoniazid. The dose of 300 mg/kg of ethanolic bark extract show a least effect on the INH induce convulsion but the 600 mg/kg of dose of ethanolic bark extract show the better response in INH induce convulsion that induce the tonic and clonic jerking. The protective potency of BECC shows the response on tonic and clonic convulsion.

REFERENCE
21. Yvonne hart, “acute management of seizures”, © 2008 elsevier ltd. All rights reserved.