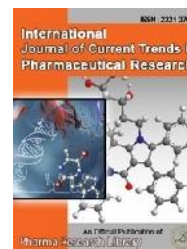




International Journal of Current Trends in Pharmaceutical Research

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RESEARCH ARTICLE

Preparation, Phytochemical Analysis and Pharmacological Evaluation of Antiepileptic and Anti-Oxidant Activity of Ethanolic Seed Extract of *Caesalpinia Crista* in Rats

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ABSTRACT

Epilepsy is a disorder of the central nervous system characterized by periodic loss of consciousness with or without convulsions associated with abnormal electrical activity in the brain. In some cases it is due to brain damage, but in most cases the cause is unknown. Epilepsy is a common, sometimes chronic, neurological condition with physical risks and psychological and socioeconomic consequences which impair quality of life. It is estimated that there are more than 10 million in India and more than 50 million people with epilepsy worldwide. Epilepsy foundation has also estimated that every 1 in 26 people in United States of America will develop epilepsy at some point in their lifetime. The prime requirements for successful management of epilepsy are a complete diagnosis and selection of an optimal treatment to benefit the patient as it is most commonly observed in paediatrics and children, who needs extreme care and counselling by an experienced doctor. The present review article focuses on providing the basic understanding on all aspects of epilepsy as a neurological disorder, considering its classification, causes, diagnosis, and various types of treatments, thus focusing on model of care to be designed in order to prevent, manage or control its occurrence as it cannot be cured.

Keywords: Epilepsy, Brain, Electrical activity, Diagnosis, Treatment

ARTICLE INFO

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PAPER QR-CODE

Article History: Received 21 October 2018, Accepted 21 November 2018, Available Online 15 January 2018

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Citation: Shaik Reshma, et al. Preparation, Phytochemical Analysis and Pharmacological Evaluation of Antiepileptic and Anti-Oxidant Activity of Ethanolic Seed Extract of *Caesalpinia Crista* in Rats. *Int. J. Curnt. Tren. Pharm. Res., Res.*, 2019, 7(1): 19-24.

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1. Introduction

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological, and social consequences of this condition. Seizures result from an electrochemical disorder in the brain. Brain cells use chemical reactions to produce electrical discharges. Each brain cell either excites or inhibits other brain cells with its discharges. When the balance of excitation and inhibition in a region of brain is moved too far in the direction of excitation, then a seizure can result^[1-3].

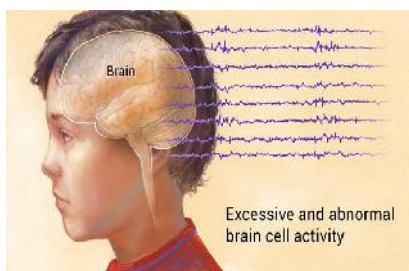


Fig 1: Epileptic Brain

Classification of Epileptic Seizures^[8-13]

This classification is based on the clinical expression of the seizure and the electroencephalographic picture during and between the seizures.

I. Partial Seizures (seizures beginning locally)

- A. Simple partial seizures (consciousness not impaired)
- B. Complex partial seizures (with impairment of consciousness)
- C. Partial seizures secondary generalized.

II. Generalized Seizures (bilaterally symmetrical and without local onset)

- A. Absence seizures (petit mal)
- B. Tonic-clonic seizures (grand mal)
- C. Myoclonic seizures
- D. Atonic seizures
- E. Tonic seizures
- F. Clonic seizures

III. Unclassified Epileptic Seizures (inadequate or incomplete data)

Causes (Etiologies) of Seizures^[17-20]

The medical word for "cause" is "etiology." Etiology of seizures varies with the type of seizure, whether it starts focally in one part of the brain or whether it is apparently generalized all over the brain at the start. However, some people with epilepsy find that certain 'triggers' make a seizure more likely. Possible triggers include: Stress or anxiety Some medicines such as anti-depressants, anti-psychotic medication, anti-malarial (by lowering the seizure threshold in the brain) Lack of sleep or tiredness Irregular meals which may cause a low blood sugar level Heavy alcohol drinking Flickering lights such as from strobe lighting or video games Menstruation cycle in woman Illnesses which cause fever such as 'flu or other infections Causes for Focal Seizures Focal seizures, more commonly

referred to as partial simple seizures, occur when there is an abnormal electrical discharge in one part of the brain. The symptoms are usually motor, but can be sensory or emotional. These seizures do not spread to the entire brain and there is no loss of consciousness.

Diagnosis of Epilepsy^[21]: The diagnosis of epilepsy may be difficult. This may be because of wrong or old ideas about epilepsy. Some parents become overprotective towards children with epilepsy. This is understandable, but may need to be resisted for the child's best interests. Like a lot of conditions, it is sometimes the attitude towards the condition that may be more disabling than the condition itself. If you find that you are over-anxious or become depressed because of epilepsy, it may be best to have counseling. Ask your doctor for advice about this. History & Physical Examination The most important diagnostic test in epilepsy is a careful history, taking detailed information on the nature of the patient's episodes. To an experienced clinician, the events should sound like seizures.

Treatment

Conventional treatment of epilepsy consists primarily of anticonvulsant medications. Although these drugs often control or reduce the frequency of seizures, some patients show little or no improvement. A number of dietary modifications, nutritional supplements, and hormones have been found to be beneficial for some patients with epilepsy.

Table 1: Recommended drugs for Epilepsy

Classification	Drugs
Sodium channel blockers	Phenytoin, Carbamazepine, Oxcarbazepine, Lamotrigine, Lacosamide
GABA Receptor agonists	Lorazepam, Diazepam, Clonazepam, Phenobarbitol, Tigabine, Vigabatrin, Gabapentin, Valproic acid

The main aim of present research work Preparation, Phytochemical analysis and Pharmacological evaluation (Antiepileptic activity and anti-oxidant activity) of ethanolic seed extract of caesalpinia crista in rats.

2. Materials and Methods

Plant profile:

Scientific name: *Caesalpinia Crista*

Telugu name: Ghacchakaya

Family: Fabaceae

Description:

This plant has profound medicinal use and is proved to have adaptogenic activity, anthelmintic activity, anti-inflammatory activity, antipyretic activity, analgesic activity, anti-amyloidogenic activity, antibacterial activity, antidiabetic activity, antifilarial activity, antioxidant activity, nootropic activity, immunomodulatory activity, hypoglycemic activity and hepatoprotective activity.

Chemical constituents: The seeds contain fatty oil which contains glycerides of some acids eg. Palmitic acid, stearic acid and phytosterols.



Fig 2: Caesalpinia Crista leaves and seeds

Collection of plant material:

The Seeds of *Caesalpinia Crista*. was collected from local market of Rajymandry, Andhra Pradesh, India. The Seeds were dried under shade, powdered and stored in an air tight container. Preparation of extract The collected Seeds was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 120g of powdered materials were extracted with ethanol (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in normal saline and used for the experiment. The percentage yield of prepared extract was Determined.

Phytochemical analysis

The ethanolic extract of *Caesalpinia Crista*. was subjected to qualitative analysis for the various phyto-constituents. Standard methods were used for preliminary qualitative phytochemical analysis of extract.

Acute toxicity study

Acute toxicity studies were performed according to organization for economic co-operation and development OECD guidelines 429 (2001). Animals were divided in groups (n=4) and fasted for 4 h with free access to water only. The MEPG extracts was administered orally in doses of 5,50,300 and 2000 mg/kg to different groups of mice and observed over 24 hr. for mortality and physical/ behavioural changes.

Experimental Animals

Wister albino rats weighing between 150-200gm each were used for this experiment. They were Mahavier Hyderabad .India. The animals were kept under standard condition in an animal house approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA). They were housed in polypropylene cages and maintained at 27±2o C; The animals were given standard diet. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref. No. /IAEC/XI/07/SPCP/2009-10.

Methods:

Determination of in vitro Antioxidant activity

Free radical scavenging (DPPH) assay:

The free radical scavenging activity of MEPG was measured in vitro by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay using the method of Blois (1958). About 0.3mM solution of DPPH in 100% ethanol was prepared

and 1ml of this solution was added to 3ml of the extract dissolved in ethanol at different concentrations (10–50 _g/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC50 value of the crude extract was compared with that of ascorbic acid, which was used as the standard.

Antiepileptic activity:

Effect on Pentylene tetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 160 to 220 gm were divided into four groups of six animals each.

The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas,

Group-II received standard drug (Diazepam, 4mg/kg) intraperitoneally,

Group-III –received EECC 250mg/kg.p.o

Group IV–received EECC 500mg/kg.p.o

For 20 days. On the 20th day, Pentylene tetrazole (PTZ) (90mg/kg body weight, s.c) was administered to all the groups to induce clonic convulsions. Animals were observed for a period of 30mins post – PTZ administration. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery/Death (% recovery or % of survival) due to PTZ [83-90].

Statistical analysis:

The data were expressed as Mean ± S.E.M. and statistically analyzed using one way ANOVA followed by Tukey-Kramer's Multiple comparison test, p.

Maximal electro-shock induced seizure model:

In this type of seizure model, the albino wister rats were divided into five groups with five animals each.

Group-1 served as solvent control, received distilled water (0.4 ml/ animal),

Group-2 received standard drug (phenytoin 20mg /kg) intraperitoneally,

Group-3 received EECG 100 mg/kg,

Group-4 received EECG 250 mg/kg

Group-5 received EECG 500 mg/kg

All the test group drugs were dissolved in distilled water and administered by orally 45min prior to the maximal electro- shock. The shock was induced in animal by passing a sinusoidal electrical current of 150 mA for 0.2 sec duration through Electro convulsimeter using ear (pinnal) electrodes. Positive control phenytoin (20mg / kg) was administered 1 hour prior to including maximal electro-shock. The animals were placed in separated cages and observed, duration of convulsions, gaining of righting reflex and postictal period for 30 minutes as a cut off period. Finally observed for behaviour and mortality of the animal upto 24 hours.

Statistical analysis: The continuous data collected are to be analysed by using AVONA and “post ANOVA t-test” p value >0.05 are accepted as significant.

Lithium pilocarpine induced seizures:

Albino wister rats of either sex weighing 160 to 220 gm were divided into four groups of five animals each. First group received vehicle control received distilled water (2ml/kg).

Group - 2 received standard drug with (diazepam 4 mg/kg),

Group -3 received EECC 250 mg/kg. ip.

Group- 4 received EECC 500 mg/kg .ip. Convulsions were induced by administration of pilocarpine (30 mg/kg/ip) 24 hr after lithium sulphate (3mEq/kg/ip) administration. EECC extract were administrated inthe increasing order of dose as mentioned above. The severity of status epilepticus was observed every 15 min till 90min and there after every 30min till 180min. All observation such as fictive scratching, tremors, head nodding and forelimb clonus were recorded in tabular form and all the data were analyzed using one- way analysis of variance(ANOVA).

Statistical analysis: The data were expressed as Mean ± S.E.M. and statistically analyzed using one way ANOVA. p<0.01,p<0.001are accepted as significant.

3. Results and Discussion

Acute Oral Toxicity Study

Acute oral toxicity was carried out by up-down regulation method. It is found that EECC were safe at limit dose 2000 mg/kg with no mortality in studied subjects.1/10th of these doses i.e. 250 mg/kg and 500 mg/kg were used in the subsequent study respectively.

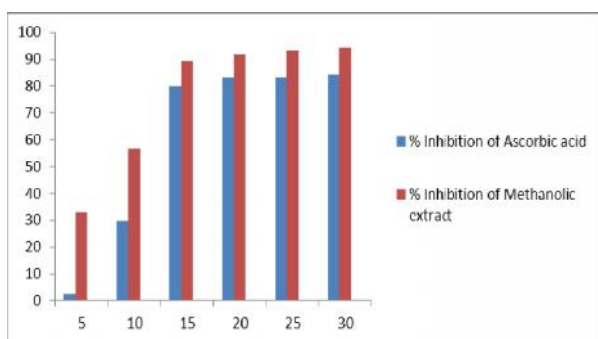


Fig 3: Free radical scavenging activity (DPPH) of EECC

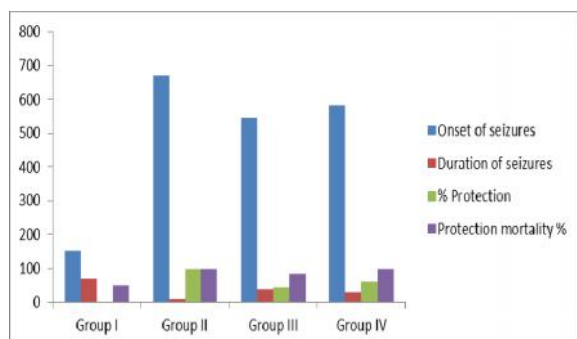


Fig 4:Effect of EECC on PTZ induced seizures in rats

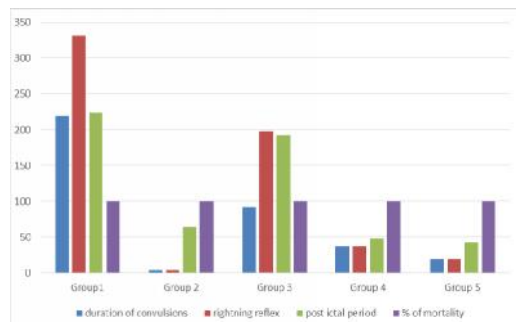


Fig 5: Effect of EECC on MES induced seizures in rats

Discussion

DPPH is stable free radical at room temperature and accepts an electron / hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity. The results indicate that the extract reduces the radicals to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principle. DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electron taken up. In the present study, lipid peroxidation and nitrite levels increased after status epileptics. In addition, in the normal physiological state, changes in neuronal activity are accompanied by alterations in the metabolic rate, which induce modifications in cerebral blood flow. There is clinical and experimental evidence of reduced oxygen availability after status epileptics.

4. Conclusion

Based on these results PTZ, lithium-pilocarpine, MES induced epileptic rat model demonstrated that the ethanol extract of EECC produces significant Anti-epileptic activity and antioxidant activity. These epileptic activity which comparable to Diazepam and phenytoin. It also produces significant anti-oxidant activity which comparable to standard ascorbic acid. Further investigation is need for isolation active compounds responsible for action.

Table 2: Phytochemical analysis

S.NO	Compound	Results
1	Flavonoids	++
2	Steroids	-
3	Saponins	+
4	Alkaloids	+++
5	Tannis	+
6	Glycoside	---
7	Aminoacids	+++

+ Present - Absent

Table 3: Acute oral toxicity studies

S.No	Dose	Behavioral changes	Mortality Rate
1	5µg/kg	Nil	Nil
2	50µg/kg	Nil	Nil
3	300µg/kg	Nil	Nil
4	2000µg/kg	Nil	Nil

Table 4: DPPH assay of EECC

S. No	Concentration (µg/ml)	Absorbance (Ascorbic acid)	Absorbance (Methanolic extract)	% inhibition of Ascorbic acid	% inhibition of methanolic extract
1	5	0.2380	0.3546	2.61±0.33	32.88±0.33
2	10	0.1719	0.3956	29.66±0.44	56.54±0.36
3	15	0.0469	0.4629	80.08±0.54	88.91±0.53
4	20	0.0415	0.5739	83.01±0.35	92.00±0.63
5	25	0.0410	0.5956	83.22±0.53	93.11±0.55
6	30	0.0390	0.6827	84.02±0.35	94.28±0.33

Table 5: Effect of EECC on PTZ induced seizures in rats

Groups	Design	Onset of seizures(Sec)	Duration of seizures(Sec)	Protection Convulsions %	Protection mortality %
I	Normal	152.52±1.25	70.42 ±0.15	0	50
II	Diazepam4mg/kg	670.5±0.65**	12.0 ±2.53**	100	100
III	EECC 250mg/kg	543.6±0.12**	40.25 ±0.12*	44.90	83.35
IV	EECC 500mg/kg	584.3 ±035**	28.25±0.35**	61.70	100

Values are expressed as mean ± SEM of six observations. Comparison between Group I Vs Group II, Group II Vs Group III & Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. *p<0.05; **p<0.001.

Table 6: Effect of EECC on MES induced seizures in rats

Groups	Design	Duration of seizures (Mean±SE) sec	Gaining of righting reflex (Mean±SE) sec	Post-ictal period (Mean±SE) sec	percentage of mortality %
I	Normal control	222±5.83	332±12.41	224±14.35	100
II	Phenytoin 25mg/kg	3.4±1.43**	3.4±1.43**	64±5.09**	100
III	EECC 100mg/kg	92±8.00**	198±5.83**	192±15.29*	100
IV	EECC 250mg/kg	37±1.37**	37±1.37**	48±5.83*	100
V	EECC500mg/kg	18.2±1.98**	18.2±1.98**	42±4.89**	100

The continuous data collected are to be analyzed by using ANOVA and post ANOVA t-test".

p value *p>0.05; **p<0.001. are accepted as significant.

Table 7: Effect of EECC on lithium-pilocarpine induced seizures in rats

Time after pilocarpine (min)	Group 1 Vehicle	Group 2 Diazepam	Group 3 (250mg/kg)	Group 4 (500mg/kg)
15	1.5±0.2	1.0±0.14	1.40± 0.45	0.00±0.00*
30	3.00±0.4	0.50±0.4**	2.00±0.30	1.00±0.2*
45	3.0±60	0.7±0.4**	2.3±0.30	1.00±0.10*
60	3.3±0.2	1.0±0.5*	3.02±0.50	2.20±0.1*
75	4.00±0.20	1.5±0.2*	3.8±0.2	2.10±0.10*
90	4.6±0.2	1.5±0.2**	3.50±0.3	2.00±0.20*
120	3.80±0.20	1.0±0.2 2*	3.00±2.0	2.00±0.10*
150	1.6±0.4	0.50±0.1*	2.00±0.4	1.30±0.10
180	0.5±0.2	0.20±0.11**	1.00±0.3	0.50±0.10

p value *p<0.01; **p<0.001. are accepted as significant.

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