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Research Article

Anti-Epileptic Potential of hydroalcoholic extract of *Naringi crenulata* (Roxb.) o n MES induced epilepsy in rats

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ABSTRACT

Naringi crenulata (NC) leaves have demonstrated different properties of nervous system. However, the leaves have not still studied to treat epilepsy. The aim of the present study was to determine the effect of NC hydroalcoholic extract on MES induced epilepsy in rats. Maximal Electroshock (MES) produces generalized electrical stimulation of the large portions of the brain; it provokes the neurons to fire repetitively, which is the characteristic of epileptic neurons. 30 female Wistar rats were divided into 5 groups. Group I was treated with water (2ml/kg), Group II with water (2ml/kg), Group III with phenytoin and diazepam, Group IV and V NC (200 and 400mg/kg) for 14 days. On 15th day MES (150mA for 0.2sec). The present research demonstrated that NC extract possesses a potential effect to prevent MES induced seizures in addition to increase GABA levels.

Keywords: Epilepsy, *Naringi crenulata,* Maximal electroshock (MES), seizures

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CONTENTS

1. Introduction

Epilepsy is a central nervous system (CNS) disorder marked by the occurrence of recurring seizures as well as a persistent complex of somatic, vegetative, and mental symptoms. When a patient has epilepsy, he or she has at least one of the following: (a) two or more unprovoked (orreflex) seizures that are more than 24 hours apart, (b) oneunprovoked (or reflex) seizure with a recurrence risk of at least the general recurrence risk (60 percent) after two unprovoked seizures over the next 10 years, or (c) a diagnosis of an epilepsy syndrome[1].

Epilepsy patients are more likely to have epileptic seizures, which can lead to social, psychological, cognitive, and neurological problems [2.] Epilepsy affects 1–2% of the world's population, according to estimates. [3,4.] It can affect people at all ages and is linked to a slew of socioeconomic, behavioral, mental, and other medical problems for both the patient and their dear once [2,5.]

Current epilepsy treatments can inhibit the immune system and relieve symptoms; nevertheless, all standard pharmacological drugs have the potential to cause unpleasant side effects. In individuals who are in the chronic or severe phases of the disease, treatment failure is unfortunately common. Patients with epilepsy are increasingly resorting to complementary and alternative treatment as a result. For generations, *Narinigi crenulata* (Rutaceae) has been used to cure a range of diseases, including sleeplessness, anxiety, and memory enhancement. *Narinigi crenulata* has a complex of phytoconstituents that are useful in the treatment of mental deficiency, disease, and the control of a number of other problems, according to pharmacology.

Narinigi crenulata, a plant, has therapeutic potential in modulating PTZ and MES- induced seizures and oxidative response in diverse regions of the brain in Albino Wistar rats. Phytochemical screening and chromatographic profile of leaves of *Naringi crenulata* revealed the presence of alkaloids, coumarins, flavonoids, phenols, quinones, saponins, steroids, tanins, terpenoids, sugar, glycosides, xanthoprotein[6].

2. Materials and Methods

Animals:

Three months old female albino Wistar rats each weighing 100-150g was procured from Biogen lab animal supply, Bengaluru (with health certificate of the animals) having CPCSEA Registration No: 600/PO/Ere/S/02/CPCSEA and was maintained under the controlled condition of temperature (23±2^{0}C), humidity (50±5%) and 12-hour light and dark cycles. The animals were randomized into five groups of six animals each and housed in large sanitized polypropylene cageson clean special husk bedding during the experimental period. They were provided with free access to specialized rodent chow and water. Assignment of animals to each group was made in such a way that each group had mean and total body weights like the other groups. The study protocol and form B were prepared as per CPCSEA norms and the same was presented in the IAEC meeting held on 12-12-2021 at PES College of Pharmacy (IAEC approval No:PESCP/IAEC/120/2021). The animal experiments was conducted at CPCSEA registered animal house at PES College of Pharmacy, Bangalore (CPCSEA registration No-600/PO/Ere/S/02/CPCSEA validity 26/05/2022) according to the guidelines of CPCSEA, New Delhi.

Collection and Authentication of plant

Naringi crenulata plant was collected in the months of April from an authentic supplier from Bangalore, India. Dr. N.M. Ganesh Babu, Associate Professor, taxonomically recognized and validated the plant. Ethno Medicinal Garden, FRLHT, Botany, Bengaluru is a city in India.

Preparation of Extract:

The entire plant material was shade dried at ambient temperature and maintained in a 40°C oven. A mechanical grinder was used to finely grind the dried plant. After that, the powder was sieved and stored in airtight containers to continue the extraction process. *Naringi crenulata* extract was made using the soxhlet apparatus. The dried plant powder was dissolved in 70% hydroalcohol and left at room temperature for 12 hours with intermittent shaking. The supernatant was decanted and the mixture was filtered when the extraction was finished. The extract was concentrated to dryness by allowing the solvent to evaporate completely. After evaporation of the solvent, the extract was weighed and kept in air tight glass container for the determination of extractive value and preliminary phytochemical screening[7.]

The extractive value in percentage was calculated by using the following formula:

Extractive value (%) = Wt of dried extract/Wt of plant material*100

Phytochemical Screening:

The extract of *N. crenulata* was analyzed for the presence of phytochemical constituents, such as alkaloids, terpenoids, quinones, flavonoids, saponins, steroids and phenolic compounds, with the standard qualitative phytochemical methods described[8].

Anti-Epileptic Activity:

Effect of ***Naringi crenulata*** leaves against Maximal electroshock (MES) induced convulsion.

Each group comprised 6 Rats.

Group I: Control (Vehicle 2ml/kg).

Group II: Disease control (Electro-convulsive shock 150milliamps, 0.2 sec, using ear electrode).

Group III: Standard control (Phenytoin 20mg/kg bw p.o + Electro-convulsive shock 150milliamps, 0.2 sec, using ear electrode).

Group IV: Hydroalcoholic extract (Medium dose +Electro-convulsive shock 150milliamps, 0.2sec, using ear electrode).

Group V: Hydroalcoholic extract (High dose + Electro-convulsive shock 150milli amps, 0.2 sec, using ear electrode)

Induction of MES Model

Epilepsy was induced on the 15th day in all groups except group I by using the electro convulso meter. A 60 Hz alternating current of 150 milliamps intensity elicited maximal electroshock (MES) seizures for 0.2 seconds. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes before application to the rats. This increases the contact and reduces the

incidence of fatalities. The duration of various phases of epilepsy was observed.

Biochemical Parameters: One hour after the last day of treatment, the following parameters were evaluated:

- General parameters- Body weight
- Behavioral parameters- Passive avoidance test, elevated plus maze
- Euthanasia
- Brain isolation and homogenate preparation
- Tissue parameters- GABA level
- Oxidative stress parameters- Catalase, SOD, glutathione, lipid peroxidation
- Histopathological analysis

Preparation of aqueous brain homogenate

The isolated brain of each rat was washed with ice-cold phosphate-buffered saline (PBS), dried by gently blotting between the folds of a filter paper, and weighed in an analytical balance. 20% (w/v) aqueous brain homogenate was prepared in ice-cold PBS using a tissue homogenizer. The aqueous brain homogenate was centrifuged at 10,000 rpm for 20min and the clear supernatant was used for the estimation of AChE, GABAand oxidative stress parameters- Catalase, SOD, Glutathione, and lipid peroxidation.

Estimation of GABA by spectrophotometry

GABA (gamma amino butyric acid) was determined from whole brain and was isolated immediately to be transferred to homogenization tube containing 5mL of 0.01 M hydrochloric acid. Brain homogenate was transferred to bottle containing 8mL of ice cold absolute alcohol and kept for 1 h at 0 °C. The content was centrifuged for 10 min at 16,000 rpm, supernatant was collected in petridish. Precipitate was washed with 5mL of 75% alcohol for three times and washes were combined with supernatant. Next, samples were evaporated to dryness at 70 °C on water bath. To the dry mass 1mL water and 2mL chloroform were added and centrifuged at 2000 rpm. Upper phase containing GABA (2.0mL) was separated and 10mL of it was applied as spot on Whatman paper (Nº 41). The mobile phase consisted of n-butanol (50mL) acetic acid (12mL) and water (60mL). The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% hydroalcohol. The paper was dried for 1h at 90°C. Blue color spot developed on paper was cut and heated with 2mL of ninhydrin solution on water bath for 5 min. Water (5.0mL) was added to solution and kept for 1 h. Supernatant (2.0mL) was decanted and absorbance was measured at 570 nm by using spectrophotometry[10]. GABA standard was used to extrapolate absorbances of the samples.

Histopathological analysis

Animals was euthanized by cervical dislocation and the brain was perfused with phosphate-buffered saline (PBS, pH=7.4) and the prefrontal cortex and hippocampus regions were traced from the isolated brain and fixed in 10% neutral buffered formalin (NBF) solution. The cortex and hippocampus slices were used to evaluate the neuronal damage.

Statistical methods

All data was expressed as mean ± standard deviation (SD). All study groups was compared between disease treated groups by using a one-way analysis of variance (ANOVA) followed by a Dunnett's Multiple Comparison Test of Statistics using the Graph pad prism statistical program. The results was considered statistically significant if the 'p' value was <0.05 or less.

3. Results & Discussion

Extraction of *Naringi crenulata*

The extraction procedure was carried out using the dried coarse powdered sample of the *Naringi crenulata* plant by soxhlet with 70% hydroalcohol for 12 hours.

Extractive value of the Product:

The extractive value in percentage was calculated by using thefollowing formula:

Extractive value (%) = Wt of dried extract/Wt of plant material*100

Wt of dried extract= 85g

Wt of plant material= 800g

% extractive value= 85/800*100=10.62%

Table 1: Preliminary phytochemical investigation

Phytochemical Constituents	Result
Steroids	**Present**
Alkaloids	**Present**
Flavonoids	**Present**
Quinines	**Absent**
Triterpenoids	**Present**
Tannins	**Present**
Saponins	**Present**
Coumarin	**Present**
Protein	**Present**
Sugars	**Present**

The preliminary phytochemical tests was performed to verify the authenticity of the *Naringi crenulata* sample. Based on the results, it was confirmed that the procured plant sample was indeed *Naringi crenulata.*

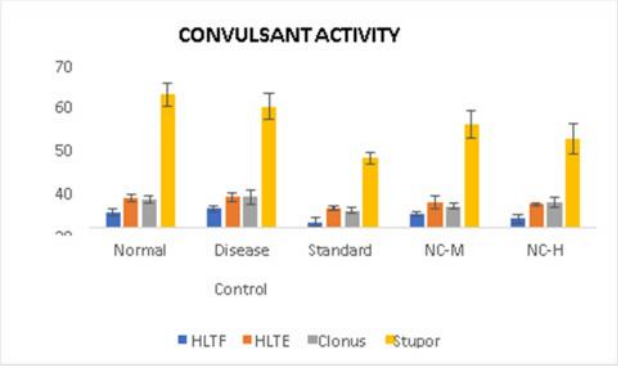

Fig 1- MES Model - Convulsant activity

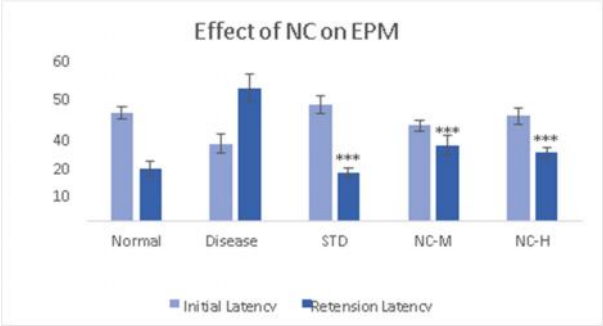

Fig 2- Effect of NC on EPM

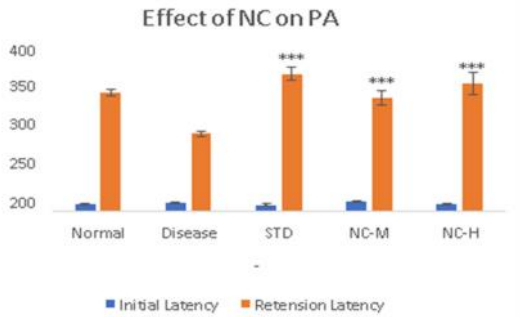

Fig 3- Effect of NC on PA

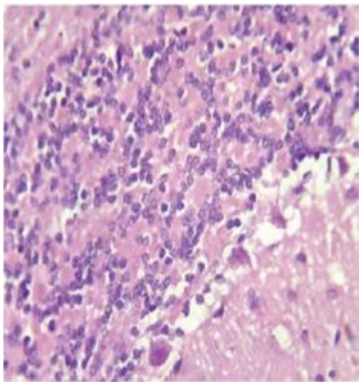

Fig: 4: Histopathology of the cortex of group I rats at 400x magnifications. The sections was stained with hematoxylin and eosin to determine the neuronal damage. The cortex of group I rats showed the presence of surrounding support cells (glial cells) having small nuclei with densely stained, condensed chromatin with no visible nucleoli.

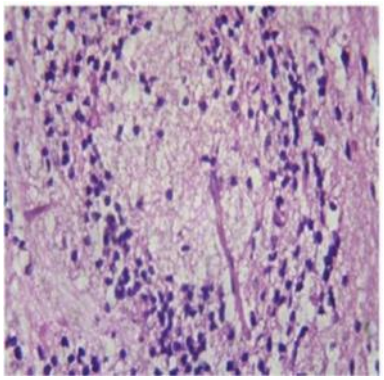

Fig 5- Histopathology of the cortex of group II rats at 400x magnifications. The sections was stained with hematoxylin and eosin to determine the neuronal damage. The cortex of group II rats showed a cluster of injured neurons revealing variation in sizes, vacuolization, shrinking, apoptosis, and lysis.

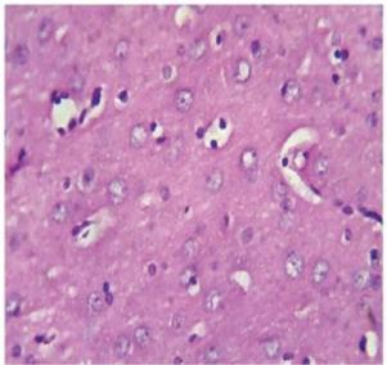

Fig 6- Histopathology of the cortex of group III rats at 400x magnifications. The sections was stained with hematoxylin and eosin to determine the neuronal damage. The cortex of group III rats showed few normal structure of the neurons. Cortex showed gliosis.

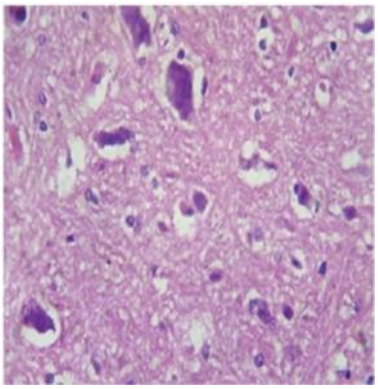

Fig 7- Histopathology of the cortex of group IV rats at 400x magnifications. The sections was stained with hematoxylin and eosin to determine the neuronal damage. The cortex of group IV rats showed normal structure of the neurons and ganglionic cells. Notice few cell damage and shrinkage of neurons.

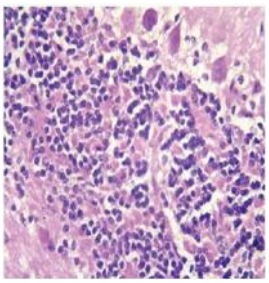

Fig 8- Histopathology of the cortex of group V rats at 400x magnifications. The sections was stained with hematoxylin and eosin to determine the neuronal damage. The cortex of group V rats showed normal structure of the neurons and basal ganglion. Notice few cell damage and shrinkage of neurons.

Discussion:

In the present study, the protective effect of hydroalcoholic extract of *N. Crenulata* against seizures was

evaluated. Seizures occur when there is abnormal synchronous neuronal firing in a section of the brain, or throughout the entirety of the brain, when networks are irregularly formed or are perturbed by a structural, infectious, or metabolic disturbance. The prevalence of epilepsy is likely to increase because more people are surviving serious head trauma, strokes, and intracranial infections, as well as living longer with primary and secondary braintumors than ever before [11]. The different animal models can be classified as those induced by chemical convulsing agents, such as penicillin and cobalt, among others; models by electrical stimulation, such as kindling and electroshock, and genetic models, such as audiogenic seizures or the Papio papio baboon model[12]. Administration of *NC* prolongs the latency to myoclonic jerks dose-dependently and also showed protection against MES-induced GTCS. The administration of NC for 15 days, showed further increase in myoclonic jerk latency, but no enhanced protection against MES-induced GTCS was observed. Thus, the results showed the anticonvulsant activity of *NC* in MES-induced seizures.

Anti convulsant activity studies

MES Model

Table 2: Effect of hydroalcoholic leaf extract of *Naringi crenulata* onMES-induced convulsions in rats

S.No	Group	HLTF	HLTE	Clonus	Stupor
1	Normal	6.33+1.79	12.50+1.53	11.83+1.77	57.38+4.9
2	Disease Control	7.83+1.46	13.00+1.82	13.04+3.05	52.02+5.8
3	Standard	2.16+1.86***	8.16+1.06***	7.00+1.29***	29.75+2.7***
4	NC-M	5.55+1.06**	10.73+2.85*	9.00+1.29**	48.13+5.9*
5	NC-H	3.59+2.04***	9.83+0.57**	10.58+2.35*	36.25+6.4***

Data represent Mean ± SD (n=6), *- P=<0.05, **-P=<0.01, ***-P=<0.001.

In MES -induced seizures, a significant difference was found in the flexion, extension, clonus and stupor as compared to the disease control group. Pre- treatment with *NC* significantly decreased (p<0.001) the time of onset of seizure at all dose level tested as compared to disease group.

Table No – 3 Elevated plus maze

Group No	Group Name	MES Induced seizures	
		Elevated plus maze	
		Initial latency (s)	Retention latency (s)
I	Normal	40.00±2.28	19.33±2.80
II	Disease Control	28.50±3.72	48.83±5.34
III	Standard	42.83±3.31	17.83±1.72***
IV	NC-M	35.17±2.04	28.00±3.46***
V	NC-H	38.83±2.99	25.17±2.04***

In MES-induced seizures, no significant difference was found in the initial transfer latency among the groups, whereas a significant difference was observed in the retention transfer latency. It was found that retention transfer latency in MES group was significantly increased (P<0.001) in comparison to vehicle control group. Pre-treatment with *NC* significantly decreased (P<0.001) the retention transfer latency at all the dose levels tested as compared to MES group, which was comparable to that of the phenytoin group.

Passive avoidance test

Table No -4

Group No	Group Name	MES Induced seizures	
		Passive avoidance test	
		Initial latency (s)	Retention latency (s)
I	Normal	17.67±2.06	293.5±6.97
II	Disease Control	28.50±2.42	192.2±6.85
III	Standard	16.00±2.28	339.5±16.23***
IV	NC-M	20.00±1.41	281.0±18.13***
V	NC-H	21.00±1.78	316.0±27.77***

There was no significant difference in initial latency amongst the groups in MES model, whereas retention latency between the groups shows significant difference (P<0.001). A significant decrease (P<0.001) in the retention latency in MES group as compared to the vehicle control group was observed. However, as compared to the MES group, pre-treatment with 200 and 400 mg/kg doses of *NC* caused a significant increase in the retention latency, which was comparable to that of phenytoin group.

4. Conclusion

The study was carried out to evaluate the antiepileptic activity of *Naringi crenulata* in experimentally induced epilepsy in rats. The hydroalcoholic extract of NC was prepared using the soxhlet technique using 70% hydroalcohol as solvent and the extract was subjected to preliminary phytochemical investigations to validate the authenticity of the sample. The rats were divided into 5 groups- Normal, Disease control, STD, NC-(Mid), NC-(High). The first group of rats were administered with vehicle (water- 2ml/kg bw po), the second group with (water- 2ml/kg bw po), the third group with phenytoin (20mg/kg bw po), fourth and fifth group with hydroalcoholic extract of NC (200mg/kg and 400 mg/kg bw po) respectively for 14 days. On the 15^{th} day all the groups except the first group will receive a electro convulsive shock (150mAfor 0.2sec). The administration of MES was done on the 15^{th} day. It was associated with a significant decline in the retention time in MES. The administration of MES also caused a reduction in the tissue levels of GABA.

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