EFFECT OF PARNAYAVANI (COLEUS AMBOINICUS LOUR.) ON MES INDUCED EPILEPSY IN RATS

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ABSTRACT

Epilepsy is considered as the most dreadful disease & it’s prevalence has been estimated at 5–10 person per 1000. The present study was designed to investigate the antiepileptic potential of Coleus amboinicus Lour. on Maximal electroshock (MES) induced seizure model in rats. Total 50 Albino rats of wistar strain were used in the study. Animals were divided into 5 groups each having 10 rats. Fresh juice of Parnayavani i.e. Coleus amboinicus (2 ml/rat) was given in one group while the other group receives Aqueous & alcoholic extract of Parnayavani (500 mg/bw p.o.). Phenytoin (25 mg/kg bw i.p.) served as standard drug for comparision where as control group receives distil water as vehicle. In MES model delay in HLE was taken as end point. C. amboinicus leaf juice (CALJ) & alcoholic extract (CAalE) was having highest efficacy as antiepileptic drug in comparison to its aq. extract. in MES model. Thus we observed that leaf juice as well as alcoholic extracts of Coleus amboinicus possess anticonvulsant and neuroprotective activity.

KEYWORDS: Epilepsy, MES, Parnayavani, seizures.

INTRODUCTION

Humanity is suffering from various psychosomatic disorders like anxiety, depression, epilepsy, dementia, Parkinson’s (Nayak AP, 2010) and Alzheimer’s inspite of great advances in the field of medical sciences. Epilepsy is considered as the most dreadful disease amongst all, which continues to effect human beings since ancient time It’s prevalence has been estimated at 5–10 person per 1000 and it is considered as the second most common neurological disorder next to stroke (Katzung BG, 2007). The contemporary system of medicine has ample of potent antiepileptic remedies but they also have a risk of developing vast array of side effects including chronic toxicity, teratogenicity, adverse effect on cognition and behaviour etc. (Raza MF et al., 2001). Amid of all these, it is the high time to search remedies from the traditional treasure which may be proven as safe & effective antiepileptic agent.

Coleus amboinicus Lour. Synonym C. aromaticus Benth. or Plectranthus amboinicus Lour. belonging to family Lamiaceae is commonly known as Parnayavani in Sanskrit, Patta ajwain in hindi, Karpurvalli in south India & Country borage in English. It is a large, succulent, aromatic herb found throughout in India and is native of South and East Africa. The leaves of this plant are traditionally used for the treatment of severe bronchitis, asthma, diarrhoea, epilepsy, renal & vesicle calculi & fever (Bhakuni DS et al., 1969). It has been reported to exhibit antilithotic, chemopreventive & antioxidant properties (Padma, P.R. et al., 1998). Although Parnayavani have been in folklore use in Epilepsy no scientific investigation is yet to be done for establishing its antiepileptic activity. Therefore the present study aimed to study the effect of aqueous & alcoholic extract and leaf juice of Coleus amboinicus Lour. (CAaqE), (CAalE), (CALJ) which are known to have anti-epileptic property on animal model.

MATERIAL AND METHODS

The study was conducted in the P.G. deptt. of Dravyaguna, NIA, Jaipur & deptt. of Pharmacology, Pt. B.D.Sharma Post Graduate Institute of Medical Sciences, Rohtak. Total 50 Rats were taken for the study.

Collection of Plant Material:

The leaves of Coleus amboinicus were collected from the medicinal herbal garden of NIA Jaipur where it grows. The Botanical identification was carried out by BSI, Jodhpur letter no.BSI/AZC/A.19014/SE-1/Estt./162 dt.23.6.2010. Leaves were dried under shade, coarsely powdered & were packed separately in air tight containers.

Extract Preparation:

For each extraction about 200 g powdered drug was packed in Soxhlet apparatus. About 1.5 litres of solvent i.e. ethanol for alcoholic extraction & distilled water for aqueous extraction, was placed in a round bottom flask and a reflux condenser was attached above the soxhlet. The solvent was heated to boil on heating mantle & was subjected to extraction for 12 h. The filtrate was evaporated to dryness by keeping it on a water bath at 50–60˚C. This process is repeated thrice to get the required amount of extracts of Coleus amboinicus (ethanolic & aqueous extract).

Preparation of leaf juice:

About 500 g of fresh leaves of Coleus amboinicus were cut into small pieces & juice was prepared by crushing them in a mortar & pestle & by adding 30 ml distil water to it.

EXPERIMENTAL ANIMALS

Adult Albino rats of Wistar strain (weighing b/w 100–180 g) of either sex were used in the study. Rats were procured from the disease free animal house, Haryana Agricultural University (HAU), Hisar. The experimental rats were housed in poly propylene cages under laboratory conditions of 28 ± 2˚C temp with 75% relative humidity and
photoperiod of 12 h light & dark cycle. The rats were given standard pellet diet supplied by Hafed, India Limited, Rohtak) and water ad libitum, throughout the experiment.

The protocol of the study was approved by Institutional Ethical Committee (IAEC, PGIMS, Rohtak) & the experiments were carried out as per ethical guidelines for animal protection and welfare bearing the CPCSEA No: 9/IAEC/SVU/2006/dt 04.03.2006.

Chemicals:

Phenytoin (Epsolin, Cadilla, India) was used in the study. All other chemicals used were of analytical grade.

Selection of Doses:

As per OECD (2002) guideline no. 425, the LD 50 of Coleus amboinicus was estimated to be >5000 mg/kg. Hence, 1/10th of the LD 50, i.e. 500 mg/kg, dose was selected for the study. Fresh leaf juice was administered at a rate of 2 ml/rat (Baskar R. et.al., 1992).

Administration of test substance:

The test drugs were prepared by dissolving the required extracts in distilled water. The volume of administration was kept at 1 ml/kg bw. A gastric catheter was used for oral drug administration. Phenytoin was dissolved in normal saline before i.p. administration.

Induction of Epilepsy

Seizures were induced by MES methods.

Experimental design (Study Protocol)

In the present study Method of Tomar & Everett (1974) & (Cashin et al., 1962) was used. Electric shock seizures were produced by delivering a current of 150 mA through corneal electrode for a period of 0.2 sec. from an electro-convulsiometer. Animals which showed tonic extension of hind limb were selected & given overnight rest. On the next day the animals were divided into 5 groups of 10 animals each.

Group 1- Served as Control & receives normal saline (10ml/kg) as Vehicle.

Group 2- Receives Fresh leaf juice of Coleus amboinicus (CALJ) (2 ml/rat).

Group 3- Receives aqueous extract of Coleus amboinicus (CAaqE) (500 mg/kg bw p.o.)

Group 4- Receives alcoholic extract of Coleus amboinicus (CAalE) (500 mg/kg bw p.o.)

Group 5- Receives Phenytoin (25 mg/kg bw i.p.) standard drug for comparison.

Animals of Group 2 were pretreated with leaf juice of Parnayavani (Coleus amboinicus) electroshocks were given after 60 min of drug administration. Animals of Group 3 & 4 received aqueous and alcoholic extract of Coleus amboinicus respectively & again electroshocks were given after 60 min of drug administration. Group 1 and 5 were control & standard drug group respectively. Animals were considered protected if the drug prevents or delays the appearance of hind limb extension component of the Seizures. Effect of extracts on different stages of convulsions i.e. hind limb extension, flexion & clonic convulsions were also noted. Recovery & death of Rats were also recorded in each group.

Statistical Analysis

All the results were expressed as mean ± standard error of mean (SEM) and analyses of variance (ANOVA) for the data were calculated by using the SPSS (Statistical Package for Social Sciences) software.

RESULTS

Phytochemical screening

The preliminary phytochemical analysis showed presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinine, tannins, phenolic compounds and terpenoids.
Effect of CALJ, CAaqE and CAalE on MES induced epilepsy

The duration of tonic HLE in animals treated with vehicle i.e. in control group was 10.1 ± 0.53 seconds. The CAaqE treated group showed significant reduction in duration of HLE (2.3 ± 0.99) whereas CALJ, CAalE and standard drug Phenytoin treated group exhibit completely abolished HLE phase as compared to control group. The CALJ, CAaqE, CAalE and phenytoin treated group have shown 100%, 60%, 100% and 100% protection respectively (Table-1).

Table No. 1. Effect of Coleus amboinicus leaf juice (CALJ), aqueous extract (CAaqE) & alcoholic extracts (CAalE) on MES induced Seizures in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of treatment n= 10</th>
<th>Dose mg/kg</th>
<th>Duration (seconds) in various phases of convulsions</th>
<th>Mortality</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tonic flexion</td>
<td>HLE extension</td>
<td>Clonus</td>
</tr>
<tr>
<td>I.</td>
<td>Control</td>
<td>-</td>
<td>3.6 ± 0.34</td>
<td>10.1±0.53</td>
<td>5.6±0.58</td>
</tr>
<tr>
<td>II.</td>
<td>CALJ</td>
<td>2ml/rat p.o.</td>
<td>4.0±0.49</td>
<td>NIL***</td>
<td>10.5±0.84**</td>
</tr>
<tr>
<td>III.</td>
<td>CAaqE</td>
<td>500 mg/kg p.o.</td>
<td>4.1±0.433</td>
<td>2.3±0.99***</td>
<td>12.7±0.817*</td>
</tr>
<tr>
<td>IV.</td>
<td>CAalE</td>
<td>500mg/kg p.o.</td>
<td>3.2±0.388</td>
<td>NIL***</td>
<td>14.7±1.69**</td>
</tr>
<tr>
<td>V.</td>
<td>Phenytoin</td>
<td>25mg/kg i.p.</td>
<td>1.9±0.233</td>
<td>NIL***</td>
<td>2.3±0.335</td>
</tr>
</tbody>
</table>

Data represented as mean± SEM of 10 rats. Data compared with one way ANOVA
P <0.05 non significant **P <0.01 significant *** P <0.0001 highly significant

DISCUSSION

The MES induced seizures test is the most validated experimental method for assessment of antiepileptic drugs effective in generalised tonic clonic seizures (Loscher W, Schmidt D 1988) (Oliveira FA et.al., 2001). The MES model is used to identify compounds which prevents seizures spread (Kupferberg HJ 1989) (Stables JP, Kupferberg HJ 1995) and most of these compounds have the ability to inactivate voltage dependant Na+ channels in a dose dependant fashion. Such compounds suppress sustained repetitive firing in cultured neurons (Mcnamara J, 2001). In the present study CALJ, CAaqE, CAalE significantly inhibited the tonic HLE in MES test . It suggests the presence of antiepileptic compounds and their potential utility in the management of generalized tonic clonic seizures. Further CALJ and CAalE were equally potent antiepileptic agents in comparison to CAaqE. Hence CALJ, CAaqE, CAalE may be expected to have similar type of mechanism.

Carvacrol & Thymol (main active constituents of Coleus amboinicus) (Haque IU, 1988), (Pino J et al., 1989) belongs to the class of monoterpenes. Terpinoids, the oxygenated derivatives of terpenes are reported to exhibit antioxidant properties (J. Grabmann, 2005), (Johanna Grassmann et al., 2002). There is convincing evidence that in seizures, level of secondary metabolites of Lipid peroxidation (LPO) i.e. Thio barbituric acid reactive substances (TBARs) was significantly increased & antioxidant drugs facilitate the suppression of LPO enhancement (Bashkatova
V et al., 2003). *Coleus amboinicus* is reported to have antioxidant property (Rao BS et al., 2006) (Kumaran A et al., 2006). Thus it might be suggested that the suppression of LPO enhancement may be involved in the mechanism of action of antiepileptic potential.

**CONCLUSION**

Thus we conclude that leaf juice as well as alcoholic extract of *Coleus amboinicus* possess anticonvulsant and neuroprotective activity and thus can be effectively used for the treatment of epileptic seizures.

**REFERENCES**


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