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# EVALUATION OF ANTI CONVULSANT ACTIVITY OF AERIAL PARTS OF *SARCOSTIGMA KLEINII* WIGHT AND ARN

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***Abstract:*** **Current therapeutic treatment for convulsion is associated with a wild variety of side effects. The folkloric uses of plant to health care can indicate an important source of new pharmaceuticals. In the present study, the antiepileptic activity of *Sarcostigma kleinii* was evaluated against electrically chemically induced seizures. The seizures were induced in mice by maximal electric shock, picrotoxin and isoniazid models. All drugs were administered orally 1hr prior to induction of seizures. The study duration was 7 days. The antiepileptic activity of *Sarcostigma kleinii* was compared with the standard anticonvulsive agents phenytoin and diazepam. The data was analysed by one way ANOVA followed by Dunnett's test.**

***Keywords:*** **Epilepsy, Ethanolic extract, Isoniazid, Maximal electro shock, Picrotoxin, *Sarcostigma kleinii*.**

## 1. INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behaviour due to the disordered, synchronous and rhythmic firing of populations of brain neurons[1]. It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients[2,3] . The conventional antiepileptic agents like phenytoin, carbamazepine and sodium valproate carry with them several serious side effects notably neurotoxicity[4]. As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like gabapentin, vigabatrin, lamotrigine etc are used supplemental to the conventional agents[1].Thus it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in items of drug related toxicity. Numerous traditionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders.[5] *Sarcostigma klenii* Wight & Arn (Icacinaceae) a large perennial woody climber grows up on large trees. Mainly found in sacred groves. The plant is also termed as odal, vellayodal etc.The leaves are simple, alternate, oblong lanceolate and flowers are small,yellow in axillary spicate racemes. Fruits are bright orange drupes fleshy contains single embedded stony seeds. The plant parts are used for various treatments in ayurveda. Plant posess various ayurvedic medicinal properties .Plant pacifies vitiated vata, arthritis, anorexia, worm infestation, skin diseases, hysteria, epilepsy, ulcers and headache[6,7]. Based on literature search, no study has been carried out to scientifically validate the folkloric uses of *Sarcostigma kleinii* Wight &Arn in the treatment of convulsive disorders. Hence the study was carried out to investigate the anti-convulsant effects of the *Sarcostigma kleinii* Wight & Arn in mice.

## 2. MATERIALS AND METHODS

### PLANT MATERIAL:

### I. Collection and Authentication of plant materials:

Aerial parts of the plant *Sarcostigma kleinii* Wight. & Arn were collected from the place Pala, Kottayam district, Kerala during the month of February and authenticated by Asst. Prof. Rogimon P. Thomas, Department of Botany, C.M.S

College, Kottayam, and Kerala. The voucher specimens of the plant were kept in library with register number DPS/MGU/RIMSR/HERB7**,** for further reference.

**II. Processing of sample:**

The aerial parts of the plant were collected, cleaned thoroughly with distilled water and the desired plant parts were dried under shade for 30 days. The shade dried aerial parts were pulverized in a mechanical grinder to obtain coarse powder.

**III. Preparation of extracts:**

The powdered plant were subjected to extraction by Soxhlet method using ethyl alcohol as solvent. Evaporation of solvent from the extract was done by distillation method. A sticky mass were obtained after evaporation of solvent. The samples were stored at 10ºC till further use. At the time of administration a suspension was prepared by using the extract in 1% w/v of sodium carboxy methyl cellulose (sodium CMC).

**PHYTOCHEMICAL ANALYSIS:**

**I. Ethanolic extract of *Sarcostigma kleinii* Wight. & Arn:**

Ethanolic extract of *Sarcostigma kleinii* Wight. & Arn were subjected to preliminary phytochemical screening [8,9,10].

**EXPERIMENTAL ANIMALS:**

Swiss albino mice of either sex 20-30 gm of body weight obtained from Animal house, Department of Pharmacology, RIMSR, Kottayam. Animals were kept in standard animal house condition. Mice were housed in groups of 6 per cage. All the animals were maintained under standard conditions, that is room temperature 26 ±1°C, relative humidity 45-55% and 12:12 h light-dark cycle. The cages were maintained clean and all experiments were conducted between 9am to 4pm.

**I. Acute toxicity study:**

Swiss Albino Mice of either sex (20 - 25 g weight ) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 423 and animals were observed for mortality and behavioral changes [11].

**II. Ethical approval:**

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Regional Institute of Medical Sciences and Research Centre, Kottayam.(1702/po/c /06 /CPCSEA2014) and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**DRUGS AND CHEMICALS:**

Ethanol (Spectrum Chemicals), Carboxy Methyl Cellulose (India Sea Foods), Tab. Diazepam (Abbott Laboratory limited.), Tab.Phenytoin (Abbott Laboratory limited.), Picrotoxin (Hi Media), Isoniazid(Kemphasol).

## 3. PHARMACOLOGICAL SCREENING

**ANTI CONVULSANT ACTIVITY:**

**I. Effect of EESK on maximal electric shock model:**

Effect of EESK was tested against electrically induced convulsions. Swiss albino mice of either sex with a body weight between 20 -25g were divided into three groups of six animals each, Group A served as control and was administered with 1% sodium CMC, Group B with phenytoin (25 mg/kg p.o.) and served as standard. Group C with extract (400mg/kg p.o.) for seven consecutive days .On the seventh day one hour after oral administration of 1% sodium CMC/standard/extract to respective groups; MES seizures were induced by electro -convulsiometer. The electroshock was applied via ear clip electrodes separately to each mouse. The stimulus duration was 0.2sec and the current frequency 45mA (60Hz). The mice were placed in a rectangular plastic cage with an open top, permitting full view of animal's motor responses to seizure in the pilot study of various phases of convulsions. This current intensity will elicit complete tonic extension of the hind limbs in control mice[12]. The following parameters were recorded during 1hr test session:

- Onset of clonic seizures
- Duration of tonic extensor seizures
- Percentage protection from seizures

**II. Effect of EESK on PIC induced chemical model:**

Swiss albino mice of either sex with a body weight between 20 -25g were divided into three groups of six animals each, Group A served as control and was administered with 1% sodium CMC, Group B with diazepam (5mg/kg p.o.) and served as standard. Group C with extract (400mg/kg p.o.) for seven consecutive days .On the seventh day one hour after oral administration of control/standard/extract to respectively different groups; PIC (7mg/kg) was administered intraperitoneally[13]. The following parameters were recorded during test session of initial 30min and upto 24hr:

- Latency of seizures
- Percentage protection from seizures

**III. Effect of EESK on INH induced chemical model:**

Swiss albino mice of either sex with a body weight between 20 -25g were divided into three groups of six animals each, Group A served as control and was administered with 1% sodium CMC, Group B with diazepam (5mg/kg p.o.) and served as standard. Group C with extract (400mg/kg p.o.) for seven consecutive days .On the seventh day one hour after oral administration of control/standard/extract to respectively different groups; INH (250mg/kg) was administered intraperitoneally [13]. The following parameters were recorded during test session of initial 30min and upto 24hr:

- Latency of seizures
- Percentage protection from seizures

**STATISTICAL ANALYSIS:**

All data were represented as mean ±SEM values. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparison was made against the vehicle treated groups were performed using the Dunnett's - tests. The level of statistical significance adopted was $P < 0.05$.

## 4. RESULTS

**ACUTE TOXICITY:**

Acute toxicity study for EESK was performed according to OECD guidelines 423 using Female Swiss albino mice. At 2000mg/kg, the extract was neither produced mortality nor the signs of morbidity. Hence the dose 400mg/kg ($1/5^{th}$ of 2000mg/kg) was selected for further studies.

**PHYTOCHEMICAL ANALYSIS:**

The results of the chemical tests performed in the screening, revealed the presences of flavonoids, alkaloids, tannins, carbohydrates, glycosides in the ethanolic extract of aerial parts of *Sarcostigma kleinii* Wight. & Arn.

**TABLE: I : Phytochemical analysis of aerial parts of *Sarcostigma kleinii***

| Phytochemicals | Ethanolic extract |
|---|---|
| Flavonoids | + |
| Alkaloids | + |
| Tannins | + |
| Phenols | + |
| Glycosides | + |
| Triterpenoids | + |
| Amino acids | - |
| Saponins | - |

‘‘+ ’’ indicates presence and ‘‘– ’’ indicates absences of the phytochemical constituents.

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**ASSESSMENT OF ANTI CONVULSANT ACTIVITY:**

**MAXIMAL ELECTRIC SHOCK (MES) INDUCED MODEL:**

Standard drug (phenytoin 25 mg/kg, P.O.) and EESK 400 mg/kg, P.O, both,more significantly decreased onset of clonic and duration of tonic extensor seizures in MES induced convulsion,when compared to control.

**TABLE II: Effect of Ethanolic extract of *Sarcostigma kleinii* and Phenytoin in MES model**

| GROUPS | % PROTECTION FROM SEIZURES |
|---|---|
| Control (1% CMC, P.O) | 16.6 |
| Standard drug (phenytoin 25mg/kg, P.O) | 100 |
| EESK (400mg/kg, P.O) | 66.6 |

Values are expressed as Mean ± SEM, for 6 animals, *P<0.05, **P<0.01, significantly when compared with control group.

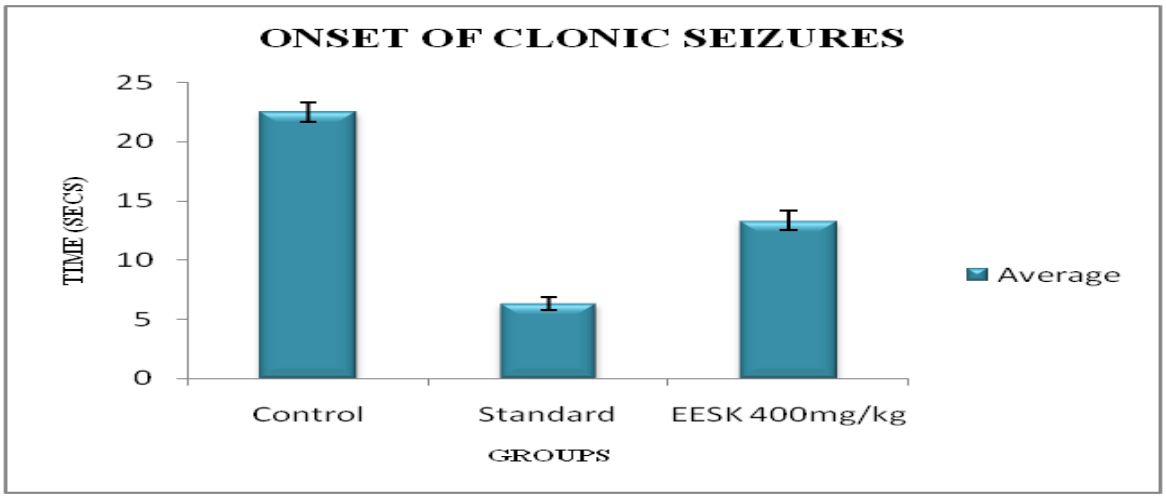


**Fig. 1: Effect of EESK (400 mg/kg) and standard (phenytoin 25 mg/kg) onset of clonic seizures in mice by MES induced model ,compared with vehicle treated control (1% CMC).**

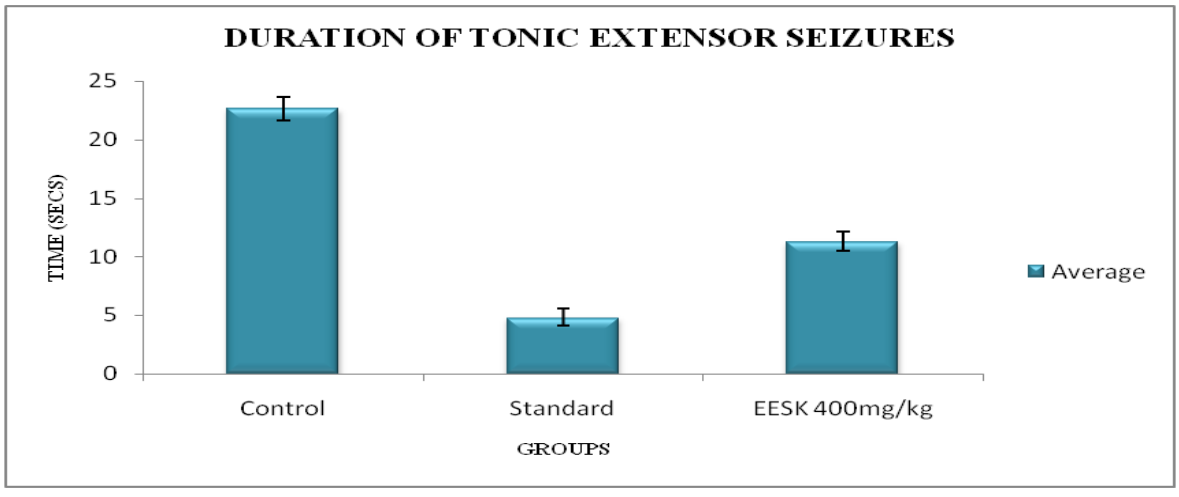


**Fig. 2: Effect of EESK (400 mg/kg) and standard (phenytoin 25mg/kg) duration of tonic extensor seizures in mice by MES model, compared with vehicle treated control (1% CMC).**

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**PICROTOXIN (PIC) INDUCED MODEL:**

Standard drug (diazepam 5 mg/kg, P.O) and EESK, 400 mg/kg, P.O, both, more significantly increased the seizure latency and percentage protection in mice, when compared to control.

**TABLE III: Effect of Ethanolic extract of *Sarcostigma kleinii* and diazepam in PIC induced model**

| GROUPS | % PROTECTION FROM SEIZURE (1HOUR) |
|---|---|
| Control (1% CMC, P.O) | 0 |
| Standard drug (Diazepam 5mg/kg, P.O) | 100 |
| EESK (400mg/kg, P.O) | 50 |

Values are expressed as Mean ± SEM for 6 animals, *P<0.05, **P<0.01, significantly when compared with control group.

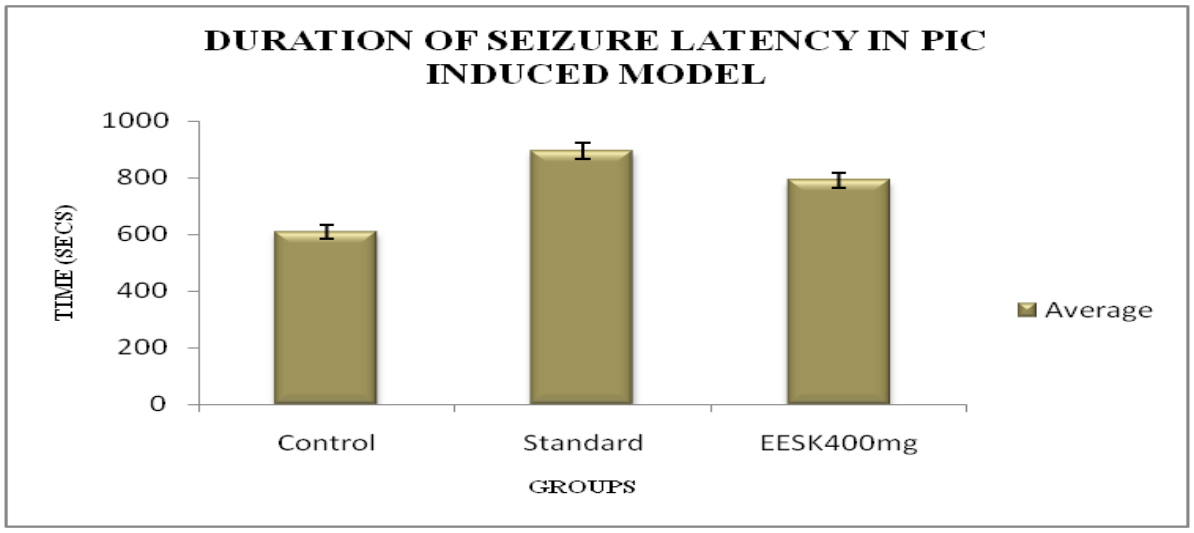


**Fig. 3: Effect of EESK (400 mg/kg) and standard (diazepam 5 mg/kg) increased duration of seizure latency in mice, compared with vehicle treated control (1% CMC).**

**ISONIAZID (INH) INDUCED MODEL:**

Standard drug (diazepam 5mg/kg, P.O) and EESK (400 mg/kg, P.O) both, more significantly increased duration of seizure latency and percentage protection in mice, when compared to control.

**TABLE IV: Effect of Ethanolic extract of *Sarcsotigma kleinii* and diazepam in INH induced model**

| GROUPS | % PROTECTION FROM SEIZURES (1 HOUR) |
|---|---|
| Control(1% CMC, P.O) | 0 |
| Standard drug (diazepam 5mg/kg, P.O) | 100 |
| EESK (400mg/kg, P.O) | 50 |

Values are expressed as Mean ± SEM for 6 animals, *P<0.05, **P<0.01, significantly when compared with control group.

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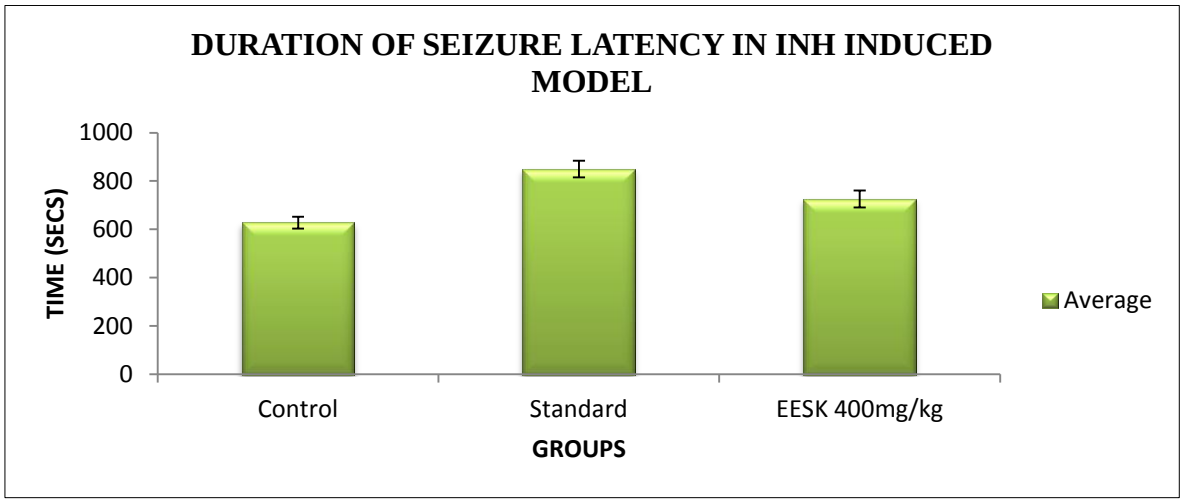


**Fig. 4: Effect of EESK (400 mg/kg) and standard (diazepam 5 mg/kg) increased duration of seizure latency in mice, Compared with vehicle treated control (1% CMC).**

## 5. CONCLUSION

Medicinal plants have served as sources of readily accessible, inexpensive, and effective medication since the earliest times known to man. Several ethnomedicinal plants have been found to possess neurobehavioral profile and serve as alternative to modern medicine. Biological evaluation and scientific validation of the ethnomedicinal plants are the need of the hour.*Sarcostigma kleinii,* (Icacinaceae) has not yet been evaluated for its activity on the CNS. Furthermore,there are no reports about anti-convulsant effects of *Sarcostigma kleinii* . The aim of the present study was to evaluate the anti-convulsant properties produced by ethanolic extract of the aerial parts of *Sarcostigma kleinii* in behavioural models. The models used are well efficient and easy to perform. The acute oral toxicity study of ethanolic extract of *Sarcostigma kleinii* was carried out in accordance to OECD guidelines 423.

The findings indicated that the ethanolic extract of *Sarcostigma kleinii* was found to be devoid of any serious toxic symptoms and no mortality was found at the dose of 2000 mg/kg. Based on these results the dose 400 mg/kg were selected for the further pharmacological evaluation.In MES-induced convulsion animals represent grandmal type of epilepsy. It has often been suggested stated that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread. Moreover, MES-induced tonic extension phase can be prevented either by drugs that inhibit voltage-dependent Na+ channels such as phenytoin, valaproate, feblamate and lamotrigine or by drugs that block glutaminergic excitation mediated by the N methyl- D-Aspartate (NMDA) receptor such as feblamate. The extract (400mg/kg) and the drug phenytoin (25mg/kg) when compared to control treated produced significant decrease in onset of clonic and duration of tonic extensor seizure (secs) in mice.

PIC exerts its convulsant effect by blocking $GABA_A$ receptor-linked chloride ion channel which, normally opens to allow increased chloride ion conductance into the brain cells following activation of $GABA_A$ receptors by GABA. Standard drug diazepam (5mg/kg) showed no convulsion after PIC treatment, it protects 100% of animals. The EESK (400mg/kg) significantly increased seizure latency (secs) and protects upto 50% of animals when compared to control group. Isoniazid exerts its convulsive effect by inhibiting GABA synthesis. It is a potent monoamine oxidase (MAO) inhibitor and a glutamic acid decarboxylase (GAD) inhibitor (enzyme involved in GABA synthesis) thus increases the brain monoamine content and inhibited GABA,synthesis respectively thereby leading to CNS excitation and convulsions. Standard drug diazepam (5mg/kg) showed no convulsion after INH treatment, it protects 100% of animals. The EESK (400mg/kg) significantly increased seizure latency (secs) and protects upto 50% of animals when compared to control group. Isoniazid induced seizures was carried out to further confirm the GABA enhancing activity of the plant extract.

The phytochemical constituents of the plant include phenols, flavonoids, alkaloids and tannins. One of these substances may be involved in the anticonvulsant activity of the extract. The extact mechanism of action of *Sarcsotigma kleinii* has to be known and biochemical evidence of neurotransmitter levels in the brain should be studied.

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