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TO EVALUATE THE ANTI-EPILEPTIC ACTIVITY OF AQUEOUS ROOT EXTRACT OF *HEMIDESMUS INDICUS* IN RATS.

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Epilepsy is the most frequent neurodegenerative disease. It is a state of recurrent, spontaneous seizures. The antiepileptic efficacy of the root extract of *Hemidesmus indicus* on Maximal electroshock (MES) and Isoniazid (INH) induced convulsion was investigated in our studies. Convulsions were induced by administration of INH or by electric current. *Hemidesmus indicus* was administered to Group III, IV and V in oral doses ranging from 100, 300 and 500 mg/kg respectively. Where as Group I and group II received 2 % aqueous tragacanth and diazepam (4 mg/kg i.p.) respectively, one hour before the administration of INH or by electric current.

In MES induced convulsion method, the extract of *Hemidesmus indicus* at the different concentration (100, 300 and 500mg/kg) significantly reduced the time spent in hind limb extensor phase. In INH induced convulsion model the induction time of the convulsions measured. The *Hemidesmus indicus* extract significantly reduced the onset of convulsions. This indicates that *Hemidesmus indicus* possess antiepileptic property similar to standard drug diazepam. Thus, in conclusion, *Hemidesmus indicus* is could be regarded as a favourable anticonvulsant agent.

KEYWORDS: Diazepam, *Hemidesmus indicus*, MES.

INTRODUCTION

A mental or neurological disorder encompasses broad range of conditions that result in dysfunction of brain, spinal cord and nerves[1]. In this modern era, epilepsy is the most frequent neurodegenerative disease. Epilepsy usually begins in childhood, potentially impeding education, employment, social relationships and development of a sense of self-worth[2]. Epilepsy is among the disorders that are strongly associated with significant psychological and social consequences for everyday living[3].

Besides a number of allopathic medications available, there is considerable evidence of an increase in demand for medicinal plants, as these plants have no side effects rather it is beneficial to provide sustainability to the body. Hence, the present project has been undertaken for screening of the root extract of *Hemidesmus indicus* for anti-epileptic activity in experimental animals, as *Hemidesmus indicus* has not been studied for its anti-epileptic activity despite of its uses in treatment of epileptic fits in children[4] and chronic nervous disorders in traditional medicine.

Hemidesmus indicus is rich in ketones, saponins, tannins, sterols, ß-sitosterol, stigmasterol, and sarsapic acid[5]. The inquistiveness to determine the anti-epileptic activity of *Hemidesmus indicus* was propelled by the presence of its active constituents and to corroborate its traditional claim.

MATERIAL AND METHODS

Drugs: Isoniazid (s.d. Fine-Chem. LTD, Mumbai, India), diazepam (calmpose inj. Ranbaxy, India) were used in this study.

Plant material: The roots of *Hemidesmus indicus* were collected from local market of Tumkur and authenticated by a Botanist.

Extraction of plant material and Preparation of extract: The roots of *Hemidesmus indicus* were shade dried. The dried roots were crushed to a coarse powder (100 gm) and extracted with water under reflux for 36 hours to obtain the aqueous extract of roots of *Hemidesmus indicus* (AERHI). The extract was concentrated by evaporation and dried in air. The extract was stored in a refrigerator and reconstituted in 2% aqueous tragacanth just before use.

Animals: Albino rats of either sex, weighing between 180-220 gm were used in this study. They received standard diet and water *ad libitum*. They were divided into different groups, with each group containing 6 animals. Clearance to carry out the work was obtained from the Institutional animal ethical committee bearing no. SSCPT / IAEC. Clear /53/2007–08 dated 22/09/2007.

Safety evaluation testing: The safety of the aqueous extract of roots of *Hemidesmus indicus* was ascertained by the up and down method. Sequential dosing of animals was done with 50mg/kg, 200mg/kg, 500mg/kg, 1000mg/kg, 2000mg/kg and observing the animals for gross behavioural changes for 24hours[6].

Anti-epileptic activity: Single dose study, the parameters were assessed after oral

Single Dose Study

Table-1. Effect of aqueous extract of *Hemidesmus indicus* on maximal electroshock seizures in rats.

Groups	Treatment	Route	Duration of hind limb extensor in sec (Mean ± SEM)
I	2 % aqueous Tragacanth	Oral	11.83 ± 0.31
II	Diazepam (4mg/kg)	i.p	2.87 ± 0.17**
III	AERHI (100 mg/kg)	Oral	9.00 ± 0 .20*
IV	AERHI (300 mg/kg)	Oral	7.17 ± 0.31**
V	AERHI (500 mg/kg)	Oral	5.33 ± 0.33**

n = 6, Values are mean ± SEM, *P<0.05, ** P<0.001 compared to the Control group.

Single Dose Study

Table-2. Effect of aqueous extract of *Hemidesmus indicus* on INH induced convulsions in rats.

Groups	Treatment	Route	Onset of convulsions (min)
I	2 % aqueous Tragacanth	Oral	17.33 ± 0.61
II	Diazepam (4 mg/kg)	i.p	43.50 ± 1.06**
III	AERHI (100 mg/kg)	Oral	22.17. ± 1.16*
IV	AERHI (300 mg/kg)	Oral	34.17 ± 0.65**
V	AERHI (500 mg/kg)	Oral	39.67 ± 0.67**

n = 6, Values are mean ± SEM, *P<0.05, ** P<0.001 compared to the Control group.

Multiple Dose Study

a. Effect after 15 days administration of AERHI.

Table-3. Effect of aqueous extract of *Hemidesmus indicus* on maximal electroshock seizures in rats.

Groups	Treatment for 15 days	Route	Duration of hind limb extensor in sec (Mean ± SEM)
I	2 % aqueous Tragacanth	Oral	12 ± 0.89
II	Diazepam (4mg/kg)	i.p	1.67 ± 0.33**
III	AERHI (100 mg/kg)	Oral	7.33 ± 0 .33**
IV	AERHI (300 mg/kg)	Oral	5.17 ± 0.31**
V	AERHI (500 mg/kg)	Oral	3 ± 0.26**

n = 6, Values are mean ± SEM, *P<0.05, ** P<0.001 compared to the Control group

Table-4: Effect of chronic administration of AERHI (15 days) on INH induced convulsion in rats.

Groups	Treatment (15 days)	Route	Onset of convulsions (min)
I	2 % aqueous Tragacanth	Oral	19.33 ± 0.49
II	Diazepam (4 mg/kg)	i.p	44.67 ± 1.26**
III	AERHI (100 mg/kg)	Oral	27.17. ± 0.83**
IV	AERHI (300 mg/kg)	Oral	36.67 ± 0.67**
V	AERHI (500 mg/kg)	Oral	41.67 ± 0.56**

n = 6, Values are mean ± SEM, *P<0.05, ** P<0.001 compared to the Control group.

b) Effect after 30 days administration of AERHI.

Table-5. Effect of chronic administration of AERHI (30 days) on MES method in rats.

Groups	Treatment for 30 days	Route	Duration of hind limb extensor in sec (Mean ± SEM)
I	2%aqueous Tragacanth	Oral	11.67± 0.33
II	Diazepam (4mg/kg)	i.p	1.33 ± 0.21**
III	AERHI (100 mg/kg)	Oral	5.33 ± 0 .33**
IV	AERHI (300 mg/kg)	Oral	3.17 ± 0.31**
V	AERHI (500 mg/kg)	Oral	1.83 ± 0.31**

n = 6, Values are mean ± SEM, *P<0.05, ** P<0.001 compared to the Control group.

Table 6: Effect of chronic administration of AERHI (10days) on INH induced convulsions in rats

Groups	Treatment for 30 days	Route	Onset of convulsions (min)
I	2 % aqueous Tragacanth	Oral	20.17 ± 0.60
II	Diazepam (4 mg/kg)	i.p	51.17 ± 0.48**
III	AERHI (100 mg/kg)	Oral	30.50 ± 0.76**
IV	AERHI (300 mg/kg)	Oral	40 ± 0.58**
V	AERHI (500 mg/kg)	Oral	44.33 ± 0.42**

n = 6, Values are mean ± SEM, *P<0.05, ** P<0.001 compared to the Control group.

administration of the single dose of the AERHI. The experiments were conducted 1 hour after the oral administration. Multiple dose study, In this study the animals daily received the suitable oral dose
of the AERHI for a period of 30 days. The parameters were assessed on the 15th and 30th day after the administration of particular day dose.

1) Maximal electroshock induced seizures: One hour after the administration of aqueous extract of *Hemidesmus indicus*, MES seizures were induced by electroconvulsometer. A 150 mA current was delivered transauricularly for 0.2 sec in rats. This current intensity elicited complete tonic extension of the hind limbs in control. Various phases of convulsions, viz. tonic flexion, extension, clonus and mortality due to convulsions, were timed[7].

2) Isoniazid induced convulsions: one hour after the administration of AERHI, isoniazid at dose of 300mg was administered. The mice were placed in isolated perplex chamber, during the next 120 min the occurrence of clonic seizures, tonic seizures and death is recorded. The percentage of seizures or death occurring in the control group taken as 100%[8]. The suppression of these effects in the treated groups is calculated as percentage of controls. In both methods, The aqueous extract of root of *Hemidesmus indicus* was administered to Group III, Group IV and Group V of rats (n = 6) in oral doses ranging from 100 mg/kg, 300 mg/kg and 500 mg/kg respectively. Where as Group I and group II received 2 % aqueous tragacanth and diazepam (4 mg/kg *i.p.*) respectively, the duration of tonic hind leg extension was noted.

RESULTS

Single dose study:

a) MES test: The duration of the hind limb extension for control group was 11.83 ± 0.31 sec. AERHI produced a dose dependent decrease in the duration of hind limb extensor phase. The maximum decrease was seen with a dose of 500 mg/kg of AERHI (Table-1).

b) INH induced convulsions: Table-2 shows data obtained from experiment conducted with INH induced convulsions. In animals treated with 2% aqueous tragacanth, the onset of convulsion appeared at 17.33 ± 0.61 min. AERHI in doses of 100, 300 and 500 mg/kg significantly increased the latency of onset of convulsions.

Multiple dose study:

a) MES test: In this, the results were summarized in

Table-3 for 15 days treatment of AERHI and Table-5 for 30 days treatment. In both experiments it has been observed that, the duration of the hind limb extension AERHI produced a dose dependent decrease in the duration of hind limb extensor phase. The maximum decrease was seen with a dose of 500 mg/kg of AERHI with showed the result of 3 ± 0.26 and 1.83 ± 0.42 for 15th day and 30th day respectively.

b) INH induced convulsions*:* Table-4 (15 days) and Table-6 (30 days) showed that AERHI in all doses protected against convulsions induced by INH. The dose of 500 mg/kg of AERHI showed highest protection in both 15th day and 30th day of the experiment. Chronic administration of the AERHI considerable increased the time for the onset of convulsion after the INH administration. AERHI at 500mg/kg dose showed 41.67 ± 0.56 min on 15thday where as on 30th day the time for the onset of convulsion was 44.33 ± 0.42, signifying that chronic administration of the AERHI increases protection against convulsions induced by INH.

DISCUSSION

Epilepsy is the most common of chronic neurological disorders and it imposes the biggest burden on health care systems. Epilepsy is a symptom of a variety of conditions, and the mortality may be different for each condition. Deaths are likely to be caused by the background aetiology of the epilepsy, for example: tumors, trauma, degenerative conditions, or cerebrovascular diseases[9].

INH induced seizure bear a semblance to petitmal epilepsy[10] and MES seizures to grandmal epilepsy[11]. Evidence indicates that imbalance between excitatory and inhibitory neurotransmission in the brain is a main cause contributing to seizure development in both, experimental and clinical conditions[12]. Gamma-aminobutyric acid (GABA) is the predominant inhibitory neurotransmitter in the CNS. Impairment of GABA function is widely recognized to provoke seizures, whereas facilitation has an anticonvulsant effect. GABA is synthesized from glutamate, exclusively in GABAergic neurons, by the action of the enzyme glutamic acid decarboxylase. Upon synaptic release, GABA acts on its three specific receptors, $GABA_A$, $GABA_B$, and the newly characterized $GABA_C$. GABA is removed from the synaptic cleft into localized nerve terminals and glial cells, by specific membrane-bound transport molecules. After removal from the synapse, GABA is either recycled to the readily releasable neurotransmitter pool (GABAergic nerve terminals only) or metabolized (neurons and glial cells) to the inactive molecule succinic acid semialdehyde by the action of the mitochondrial enzyme GABA-transaminase[13]. The convulsion in MES method is due to the disturbed activity of GABA in the brain and the convulsant action of isoniazid, involves the disruption of GABAergic neurotransmission in the central nervous system. It has been reported that isoniazid inhibits glutamic acid decarboxylase (GAD), an enzyme that catalyzes the synthesis of GABA from glutamic acid. Several anticonvulsant drugs in current clinical use facilitate GABA neurotransmission by different mechanisms: barbiturates, benzodiazepines, and other antiepileptic modulate the action of GABA by enhancing chloride currents in channels linked to different receptor sites; other antiepileptics reduce the degradation of GABA by blocking GABA transaminase or by inhibiting reuptake of GABA into the presynaptic terminals[14]. Phytochemical screening of *Hemidesmus indicus* revealed the presence of ketones, saponins, tannins, sterols, ß-sitosterol, stigmasterol, and sarsapic acid[5]. One of these components might act by increasing GABA synthesis, increase release, afford allosteric receptor facilitation or reduce inactivation. Therefore we postulate that AERHI might have a definite impact on the GABAergic system.

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