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Evaluation of Anticonvulsant Potential of Alcoholic Extract of Fruits of Capsicum Annuum and Sapindus Emarginatus and Polyherbal Formulation, Formepi-4 In Albino Mice

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ABSTRACT

Epilepsy is one of the most common chronic diseases of the nervous system. In recent years many plants have been tested for their Anti-epileptic properties. This study aims in evaluating the anti-epileptic activity of the *Capsicum annuum* and *Sapindusemarginatus* and a polyherbal formulation prepared by the combination of *Capsicum annuum, Sapindus emarginatus, Withania somnifera, Hemidesmus indicus* (Formepi-4). Study was carried out using albino mice (25 – 30 grams) 8-10 weeks old, of either sex. The animals were divided into five groups containing six animals in each group. The results for maximal electroshock induced seizures, pentylenetetrazole induced seizures and strychnine induced seizures were expressed as Mean ± Standard Error of Mean. Paired Student's t-test was used to analyze the level of significance. Maximal electroshock induced seizures, Pentelenetetrazole induced seizures and strychnine induced seizures were significantly protected in mice by ethanolic extract of *C.annuum*, and Methanolic extract of *S.emarginatus*, with Formepi-4 showing a 100% protection. The alcoholic e xtract of fruits of *Capsicum annuum* and *Sapindus emarginatus* and polyherbal formulation (Formepi-4) possesses Anti-Epileptic activity against seizures induced by different mechanisms.

Keywords: *Capsicum annuum, Sapindus emarginatus, Withania somnifera, Hemidesmus indicus,* Anti- epileptic activity, Experimental animals

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INTRODUCTION

Epilepsy is one of the most common chronic diseases of the nervous system affecting about 50 million people throughout the world[1]. Around 80%of the epileptic cases are seen in the developing countries [2] and each year, for every 1, 00,000 people, 16 – 51 new cases of epilepsy are recorded [3] . The World Health Organization describes epilepsy as "a chronic disorder characterized by recurrent seizures, which may vary from a brief lapse of attention or muscle jerks, to severe and prolonged convulsions. These seizures are caused by sudden, usually brief, excessive electrical discharges in a group of neurons"[2].

Numerous allopathic medications are prescribed for the treatment of epilepsy andthe Anti-epileptic drugs (AED's) which have been licensed in the past decade for the treatment of epilepsy in children and adults are "Gabapentin, lamotrigine, Topiramate, Tiagabine, Oxcarbazepine, Levetiracetam and Zonisamide"[4]. The major disadvantages of using modern therapy AED's are chronic side effects, drug interactions, drug resistance and lack of efficacy of the drugs[5,6]. Studies have shown that despite the use of these drugs 25-30% of patients continue to have seizures[7], cognitive side effects[8],neuropsychological dysfunctioning[9], vascular occlusive diseases, and development of refractory epilepsy[10]. For many patients cognitive side effects are worse than the actual seizures, thus contributing to a poor quality of life[8]**.** So it could be said that unfortunately the AED currently used, not only fail to control seizures but also lead to unacceptable side effects. Thus, there is a need for newer and more effective AED.

Natural products obtained from plants, animals and minerals are the greatest contribution; and their advent showed a remarkable development in the medicinal treatment of various diseases[11]. These traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need[12]. In recent years plants have been tested for their Anti-epileptic properties. The phytochemical and pharmacological studies [6] carried out has revealed numerous biologically active compounds having the same mechanism of action as their synthetic counterparts and are more beneficial and safe[13], but there seems to be no literature which has reported on the effect of combination of herbal drugs for the treatment of epilepsy. This research focuses on the use of natural productsfor the control of epilepsy evaluating the anti-epileptic activity of the *Capsicum annuum* and *Sapindusemarginatus* and a polyherbal formulation prepared by the combination of *Capsicum annuum,Sapindusemarginatus, Withaniasomnifera, Hemidesmusindicus* (Formepi-4).

MATERIAL AND METHODOLOGY

Description of plants

Capsicum annum Linn is an upright, attractive shrub with a height less than 1m, belonging to the family Solanaceae. The fruits are long, cylindrical and yellow, orange or red in color with a smooth and shiny surface with a characteristic odour and pungent taste. It is the most widely cultivated species around the world[14], Africa and India producing the drug on commercial scale. Extracts are used particularly in treating Anti-inflammatory conditions[14].

*Sapindus emarginatus*Vahl commonly known as Soap nut tree belongs to the family Sapindaceae. It is most commonly found in the hilly regions of India[15]. It is a medium sized deciduous tree about 8-10m tall with many branches[16]. All parts of the plants are used traditionally as Anti-pruritic, Anti hyperlipidemic, Anti-inflammatory, Anti-microbial, emetic and also as a hair tonic[15].

Withania somnifera L Dunal belongs to the family Solanaceae. It is popularly known as Ashwagandha, Winter cherry[17] or Indian Ginseng[18] It is a green colored shrub found in Afghanistan, Pakistan, Srilanka, South Africa, Egypt and India. It is mostly grown in the drier parts of India[17]. It is widely used in Ayurvedic medicines as a nerve tonic and memory enhancer with anti-stress, anti-aging and anti-oxidant properties[18] Toxicity studies done on the plant reveals that Ashwagandha is a safe compound[19]

*Hemidesmus indicus*R.Br is popularly known as Indian Sarsaparilla. It belongs to the family Asclepiadaceae and is widely distributed in India[20]. The roots of the plant are woody, sweet in taste and have a cooling effect. Roots are well known drugs in ayurvedic medicine [21] and are used for the treatment diarrhea, respiratory disorders, skin diseases, bronchitis, asthma, epileptic fits in children, urinary diseases, loss of appetite and rheumatism [20].

Experimental animals

Study was carried out using albino mice (25 – 30 grams) 8-10 weeks old, of either sex. They were obtained from the animal house facility of Shadan Institute of Medical Sciences, India with the approval from institution ethical committee (Reg. No. 83/1999/CPCSEA). Animals were housed in polypropylene cages maintained under standard condition (12 hours light / dark cycle; 25± 3^0C) and had free access to standard rat/ mice feed (Hindustan Liver Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment.

Collection of plants

Fruits of *Capsicum annuum* were purchased from the local market, where as fruits of *Sapindus emarginatus*, roots of *Withania somnifera* and roots of *Hemidesmus indicus* were obtained from the local supplier (Totakailasham, Pinnavari Street, Warangal). The fruits and roots were authenticated by Dr. P. Veera Reddy, M.D. (AYU), Professor/Civil surgeon, Govt. Ayurvedic College, Warangal.

Preparation of extracts

The active principles of the fruits and roots of the plant were extracted into different solvents such as absolute alcohol, methanol and distilled water.

Preparation of extract of Capsicum annuum:

Fresh ripe fruits of *C. annuum* were dried and powdered using electric blender. Forty gram of chili powder was extraction with 800 ml of absolute alcohol, by continuous stirring for 24 hours. The filtrate obtained through Whatmann No. 1 filter paper was collected in a round bottom flask. The excess of alcohol was removed by distillation. The remaining alcohol was completely evaporated by pouring the extract residue into a test tube and keeping it in boiling water bath. The thick red crude residue was weighed; percentage yield of extract was calculated and the extract was suspended in appropriate volume of 1% Tween 80 for the experiment[22].

Preparation of extract of Sapindus emarginatus:

The dried powder material (500g) of the pericarp of *S. emarginatus* was extracted with methanol (2000ml) in a soxhlet apparatus. The extract was then distilled, evaporated and dried in vacuum. The resulted extract was brownish dried gum resin in nature which was then weighed; percentage yield of extract was calculated and as per demand the extract was suspended in appropriate volume of 1% Tween 80 for the experiment[23].

Preparation of extract of Withania somnifera:

The roots of *W. somnifera* were dried in an incubator for two days at 40°C, crushed in an electrical grinder and then the powder was separated. Fifty grams of powder was extracted in 250 ml of distilled water for 18 hours in a soxhlet apparatus. The extract was collected and dried at reduced pressure; percentage yield of extract was calculated, stored at 0-4°C and used for the next seven days of the experiment[24].

Preparation of extract of Hemidesmus indicus:

The roots of *H.indicus* were shade dried and powdered (100gms). The powder was extracted using 500ml sterile distilled water in Turmix electrical extractor. The juice obtained was filtered and residue was removed. The extract obtained was concentrated under vacuum to obtain a solid,

which was weighed and percentage yield was calculated. It was stored in refrigerator for the experiment[25.]

Preparation of polyherbal formulation, formepi-4:

The extracts of *C.annuum, S. emarginatus, H.indicus* and *W. somnifera* which were prepared as the procedure prescribed above were mixed in 0.25:1:1:1 proportion and then suspended in 1% Tween 80 for pharmacological experiments.

Preliminary phytochemical screening:

The preliminary phytochemical screening was carried out on the extracts of *Capsicum annuum, Sapindus emarginatus* and Formepi-4 for the detection of various phytochemicals. To carry out the test for common phytochemicals 200 mg of extracted material was dissolved in 10 ml of distilled water and filtered. This filtrate extract was used to carry out the phytochemical screening.

Drugs:

The drugs which are used in the current study are Phenytoin (Epsolin, Cadilla Healthcare Ltd, India, Diazepam (Calmpose, Ranbaxy Research Laboratories, India), Lignocaine hydrochloride (Xylocaine, AstraZeneca Pharma Limited, India), Pentylenetetrazole (Hi-media laboratories Private Limited, India), Strychnine nitrate (Shakun Enterprises Private Limited, India)

Acute toxicity studies:

A preliminary pharmacological study was conducted to assess the acute pharmacological effects and safety effects of the drug. Acute toxicity test is a test in which a single dose of the drug is used in each animal on one occasion to analyze gross behavior toxicity.

The acute toxicity study was carried on mice weighing about 20-25gm as per International Conference on Harmonization (ICH) guidelines. Overnight fasted mice received test extract at a dose of 200mg/kg intraperitoneally. Then the animal was observed continuously for one hour. LD_{50} was calculated by the Miller and Tainter method[26, 27]. In which the observed percentage mortality was converted into probits. The values thus obtained were plotted against log dose and the LD_{50} value is the dose corresponding to probit 5.

The therapeutic dose of the drug is considered as 1/10 of LD_{50} value.

For general behavioral, neurological, autonomic profiles and to find out percentage of mortality observations were tabulated according to Irwin's table. For this the following check list was employed (*Table 2*).

The extract of fruits of *Capsicum annuum*, pericarp of *Sapindus emarginatus* and formepi - 4 was found to be safe at the maximum dose of 500mg/kg, 2000mg/kg and 1000mg/kg body

weight respectively by intraperitoneal route. After 48 hours mice was found to be well tolerated. There was no mortality and no signs of toxicity. The stimulatory depressive and autonomic profile was found to be normal. No signs of mortality were observed at the doses of 500mg/kg, 2000mg/kg and 1000mg/kg body weight for extract of fruits of *Capsicum annuum*, pericarp of *Sapindus emarginatus* and Formepi-4 respectively. The extracts were found to be safe at these doses.

Table 1: Preliminary phytochemical test of C. annum, S. emarginatus and Formepi - 4

S.No.	Phytochemical test	*Capsicum annum*	*Sapindusemarginatus*	Formepi - 4
1.	Test for Alkaloids	+	+	+
2.	Test for Carbohydrates	+	+	+
3.	Test for Flavanoids	+	+	+
4.	Test for Proteins	+	-	+
5.	Test for Amino acids	+	-	+
6.	Test for Tannins	-	-	+
7.	Test for Steroids	+	-	+
8.	Test for Triterpenoids	-	+	+
9.	Test for Fats & Oils	+	-	+
10.	Test for Saponins	+	+	+
11.	Test for Glycosides	-	-	+

+→presence of compounds; -→absence of compounds

Table 2: Acute Toxicity Studies of the Extracts and Formepi-4 on Mice

S.no	Treatments	Capsicum		Sapindus	Formepi-4	
	Effects/ Dose	2000mg/kg	500mg/kg	2000mg/kg	2000mg/kg	1000mg/kg
A	**Stimulation**					
1	Hyperactivity	+	0	0	0	0
2	Piloerection	0	0	0	0	0
3	Twitching	+	0	0	0	0
4	Rigidity	0	0	0	0	0
5	Irritability	++	0	0	+	0
6	Jumping	0	0	0	0	0
7	Clonic convulsions	0	0	0	0	0
8	Tonic convulsions	0	0	0	0	0
B	**Depression**					
1	Ptosis	0	0	0	0	0
2	Sedation	+	0	0	+	0
3	Loss of reflex	++	0	0	+	0
4	Loss of traction	0	0	0	0	0
5	Loss of Pinna reflex	+	0	0	+	0
6	Catatonia	++	0	0	0	0
7	Ataxia	++	0	0	+	0
8	Loss of muscle rigidity	++	0	0	+	0
9	Analgesia	++	0	0	+	0

C	**Autonomic Reflexes**					
1	Straub's tail	+	0	0	+	0
2	Laboured respiration	++	0	0	++	0
3	Cyanosis	+	0	0	+	0
4	Reddening	+	0	0	+	0
5	Abnormal secretions	0	0	0	+	0
6	Balancing	+	0	0	+	0

0→normal; +→moderate effect; ++→marked effect

Methods employed in screening of anticonvulsant activity:

Maximal electroshock induced seizures (MESIS):

The animals were divided into five groups containing six animals each group (*Table 3*).Tonic convulsions of the hind extremities of the mice was induced by passing alternating electrical current of 50 Hz and 150 mA for 0.2 sec through corneal electrodes.

These groups were treated for the respective drugs for 15 days prior to the induction of convulsion. The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined for each dose group[28].

*Pentylenetetrazole induced seizures (PTZIS)***:**

The animals were randomly divided into five groups containing six animals each (*Table 3*). Seizures were induced in mice with standard convulsing agents, pentylenetetrazole (60 mg/kg., s.c,) after 30 min of drug treatment and the animals were observed for one hour for tonic convulsion episode. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period was noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity[28].

*Strychnine induced seizures (SIS)***:**

The animals were divided into five groups randomly, each group containing six animals each (Table 3). Seizures were induced in mice with standard convulsing agents, strychnine (2 mg/kg, i.p) after 30 min of drug treatment and the animals were observed for one hour for tonic convulsion episode. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period was noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity.

Table 3: Various methods employed in screening of anticonvulsant activity (6 animals in each group)

Groups	MESIS*	PTZIS**	SIS***
Group 1	Control	Control	Control
Group 2	*Capsicum annuum* (50mg/kg, i.p)	*Capsicum annuum* (50mg/kg, i.p)	*Capsicum annuum* (50mg/kg, i.p)
Group 3	*Sapindus emarginatus* (250 mg/kg, i.p)	*Sapindus emarginatus* (250 mg/kg, i.p)	*Sapindus emarginatus* (250 mg/kg, i.p)
Group 4	Formepi-4 (100mg/kg, i.p)	Formepi-4 (100mg/kg, i.p)	Formepi-4 (100mg/kg, i.p)
Group 5	Phenytoin (25mg/kg, i.p)	Diazepam (0.5mg/kg, i.p)	Diazepam (0.5mg/kg, i.p)

*mesis - maximal electro shock induced seizures, **ptzis - pentylenetetrazole induced seizures, ***sis - strychnine induced seizures

Statistical Analysis:

The results for maximal electroshock induced seizures, pentylenetetrazole induced seizures and strychnine induced seizures were expressed as Mean ± Standard Error of Mean. Paired Student's t-test was used to analyze the level of significance. A *p* value of <0.05 was considered as statistically significant. It was done using Graph pad 5.0 software versions.

RESULTS AND DISCUSSION:

MES produced hind limb tonic extension seizures in all the animals. The control mice showed tonic limb extension for the duration of 18.4±0.29 sec. Ethanolic extract of *C.annuum* (50mg/kg) protected 83.33 % of mice and alter the incidence of seizures to a significant extent. Methanolic extract of *S. emarginatus*(250mg/kg) protected 66.67% of mice and considerably decreased the duration of HLTE produced by MES. Formepi-4 (100 mg/kg)protected 100% of the animals and significantly reduced the duration of the seizure. The standard antiepileptic drug, phenytoin (25mg/kg) also protected all the animals and significantly reduced the duration of HLTE (Table 4).

PTZ produced tonic seizures in all the animals. Ethanolic extract of *C.annuum*(50mg/kg) protected 33.33% of animals against PTZ induced seizures and did not affect the onset of seizures to any significant extent. *S. emarginatus* extract (250 mg/kg) protected 83.33% of mice and significantly delayed the latency of seizures. Formepi-4 (100 mg/kg) significantly prolonged the latency of seizures produced by PTZ, and protected all the mice against the seizures. The standard antiepileptic drug, diazepam (0.5mg/kg) profoundly antagonized the seizures produced by PTZ (Table 5).

Table 4: Effect of extracts and formepi-4 on maximal electroshock induced seizures in mice

S.no	Treatments	Dose(mg /kg)	Duration of HLTE(sec)	Quantal protection	% protection
1	Control	-	18.43±0.29	4/6	66.67
2	*Capsicum annuum*	50	12.34 ± 0.38**	5/6	88.33
3	*Sapindus emarginatus*	250	14.89 ± 0.49**	4/6	66.67
4	Formepi-4	100	9.44±0.34***	6/6	100
5	Phenytoin	25	7.71±0.31***	6/6	100

values are mean±sem (n=6); **p<0.05 (compared with control using student's t-test), ***p< 0.0001(compared with control using student's t-test)

Table 5: Effect of extracts and formepi-4 on pentylenetetrazole induced seizures in mice

S.No	Treatments	Dose(mg/kg)	Onset of seizures(min)	Quantal protection	% protection
1	Control	-	3.04 ± 0.24	1/6	16.67
2	*Capsicum annuum*	50	3.55 ± 0.25 ns	2/6	33.33
3	*Sapindus emarginatus*	250	6.48 ± 0.33 **	5/6	83.33
4	Formepi-4	100	9.18 ± 0.40 ***	6/6	100
5	Diazepam	0.5	11.59 ± 0.42 ***	6/6	100

values are mean±sem (n=6); ns-p value not significantly different (compared with control using student's t-test), **p< 0.01(compared with control using student's t-test), ***p< 0.0001(compared with control using student's t-test)

Strychnine (2 mg/kg) elicited tonic seizures in all the animals. Ethanolic extract of *C.annuum* (50mg/kg) significantly delayed the latency but did not alter the incidence of seizures produced by strychnine to any significant extent. *S. emarginatus* (250mg/kg) significantly prolonged the latency of seizures produced by strychnine and provided 83.33% of protection to the animals. Formepi-4 (100mg/kg) totally protected the animals as that of the standard anti-epileptic drug diazepam and both significantly delayed the latency of seizures (Table 6).

Table 6: Effect of extracts and formepi-4 on strychnine induced seizures in mice

S.no	Treatments	Dose(mg/kg)	Onset of seizures(Mins)	Quantal protection	% protection
1	Control	-	4.16 ± 0.46	1/6	16.67
2	*Capsicum annuum*	50	6.18 ± 0.31 **	1/6	16.67
3	*Sapindus emarginatus*	250	7.60 ± 0.33 **	5/6	83.33
4	Formepi-4	100	9.28 ± 0.28 ***	6/6	100
5	Diazepam	0.5	10.95 ± 0.44 ***	6/6	100

values are mean±sem (n=6);,**p< 0.05 (compared with control using student's t-test),***p< 0.0001 (compared with control using student's t-test)

Maximal electroshock induced seizures:

The result of present study showed that the extract of *Capsicum annuum* and Formepi-4

decreased the duration of tonic hind leg extension. So *C.annuum* and Formepi-4 acts on the voltage dependent sodium ion channels thereby preventing the repetitive firing of action potential and thus producing their anticonvulsant effect.

Pentylenetetrazole induced seizures:

PTZ produces its anticonvulsant effect by inhibiting the activity of GABA at $GABA_A$ receptors there by inhibiting the neuronal responsiveness and activity by increasing the chloride ion conductance through opening of the chloride-ion channel. *Sapindus emarginatus* and Formepi-4 increased the latency of convulsion thereby decreasing the seizure threshold by acting on the GABAergic system.

Strychnine induced seizures:

Strychnine induces convulsions by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing the spinal reflexes. The results shows that *Sapindus emarginatus* and Formepi-4 increases the latency of convulsion more than *Capsicum annuum*, but all the three i.e., *C.annuum* and *S. emarginatus* and Formepi-4 showed protection against strychnine induced convulsions which suggests that *C. annuum* and *S. emarginatus* and Formepi-4 probably acts on glycinergic transmission.

CONCLUSION

To conclude, the alcoholic extract of fruits of *Capsicum annuum* and *Sapindus emarginatus* and polyherbal formulation (Formepi-4) possesses Anti-Epileptic activity against seizures induced by different mechanisms. New herbal anti-epileptic drugs are in demand as the synthetic drugs are not useful in all cases. Further detail investigations are required to study the exact mechanism of action.

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