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Short Communication

Anti Epileptic Activity of Poly Herbal Extract from Indian Medicinal Plants

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Abstract

With the introduction of allopathic drugs, the use of crude drugs from medicinal plants is on the decline and subsequently this traditional knowledge may be lost in the near future. In the present study, a poly herbal extract comprising of *Withania somnifera* Dunal, a medicinal plant used in many neuro protective Ayurvedic preparations along with five other medicinal plants were evaluated for its protective effect against seizures induced by Maximal Electro shock (MES) method in rats. A daily dose of 250 and 500 mg/kg of the extract was administered to the animals for 15 days, after which seizures were induced by Maximum electro shock method and the duration of various phases of epileptic attacks were recorded and compared with the control animals. A significant ($P<0.01$ and $P<0.001$) reduction in the time taken for righting reflex (recovery) was noted in the experimental animals. The levels of biogenic amines such as dopamine, serotonin and nor-adrenaline in the forebrain region were also estimated and a significant level of restoration was observed in the extract treated animals. Significant results were observed in the estimated parameters thereby justifying the use of these medicinal plants in the treatment of epilepsy.

Keywords: Anti epileptic activity; Biogenic amines; Maximal Electro Shock; Poly herbal extract; Seizures.

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1. Introduction

Traditional medicinal practices have remained as a component of health care system of many societies in spite of the availability of well-established alternatives [1]. Epilepsy is a condition, which causes seizures to occur. It is one of the most common chronic diseases

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affecting human beings. According to several publications this can amount to 70% of the people with epilepsies, with a high prevalence of about 0.8% in children below the age of seven years [2]. These observations have led to a shift in focus to the use of herbal remedies in the management of epileptic seizures, probably because these measures fit into the cultures of people and are not usually as expensive as the more refined orthodox drugs. Besides, these orthodox drugs possess many side effects, contraindications and possible interactions with drugs used simultaneously.

The alternative drug therapy for the management of this disease can be by the use of medicinal plants and their active principles. In the present study plants from India with a traditional claim of anti epileptic activity and neuro protective properties were selected and a poly herbal extract was prepared in aqueous medium.

The medicinal plants for the study were selected in such a way that their availability is maximized in many parts of the world. The plants selected were *Withania somnifera* Dunal (Solanaceae) which possess antiepileptic properties [3] and widely used as a sedative in cases of senile debility [4], *Bacopa monnieri* Linn (Scrophulariaceae) has been included for its anti epileptic property [5], *Chlorophytum borivillianum* (Liliaceae) for its valuable effects as a nervine and general tonic for strength and vigor [6], *Glycyrrhiza glabra* Linn (Legumoniaceae) and *Curcuma longa* Linn (Zingeberaceae) were included for their general tonic effects [7] and *Terminalia arjuna* Roxb (Combretaceae) for its tonic effects. The plant is said to have a potential to relieve symptomatic complaints in hypertension [8].

2. Materials and methods

2.1. Plant material

The plant materials used for the preparation of the extract such as *Withania somnifera*, *Bacopa monnieri*, *Chlorophytum borivillianum*, *Curcuma longa*, *Glycyrrhiza glabra* and *Terminalia arjuna* were purchased from an Ayurvedic store (Chennai, Tamil Nadu, India). All the plants purchased were checked for purity and authenticity in the Department of Pharmacognosy, C.L. Baid Metha College of Pharmacy and voucher specimens were deposited in the Department of Pharmacognosy, C.L. Baid Metha College of Pharmacy (Chennai, Tamil Nadu, India).

2.2. Preparation of the Polyherbal extract

Coarsely powdered, shade dried roots of *W. somnifera* (2500 g), leaves of *B. monnieri* (1500 g), *C. borivillianum* (1000 g), rhizomes of *C. longa* (500 g), *G. glabra* (500 g) and barks of *T. arjuna* (400 g) were extracted with distilled water for 72 h in a soxhlet extractor. The dark colored extract obtained was cooled, filtered and the solvent recovered by distillation *in vacuo* [9]. The residue so obtained was used for subsequent experiments. The w/w yield in terms of dry material was 19. 4%. The dried extract was suspended in 5% Carboxyl methyl cellulose (CMC) for animal administration.

2.3. Animals

Healthy adult Wistar albino rats between 2 and 3 months of age and weighing about 200-250 g were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light: 12 h dark cycle; 27±1°C; 60% humidity). They were fed with standard rat pellet diet (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. Conduct of the study was approved y the Institutional Animal Ethical Committee of CLBMCP, Chennai, India (Approval No: IAEC/XIII/20/CLBMCP/ 2006-07 dated 17-08-2006).

2.4. Drugs and chemicals

Serotonin, dopamine and nor adrenaline used in the standard readings for the estimation of bioamines were obtained from Sigma (USA) and other chemicals used were of analytical reagent grade.

2.5. Acute toxicity studies

Wistar albino rats of either sex weighing 200-250 g selected by random sampling technique was performed as per OECD-423 guidelines (acute class method) [10]. The animals were fasted overnight, provided only water, after which the poly herbal extract was administered to the respective groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups, observed for 14 days. If mortality was observed in 2 or 3 animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 h.

2.6. Estimation of duration of epileptic seizures

The animals were divided into four groups (n=6) and Group I animals served as control receiving 1 ml of 5 % CMC p.o, Group II served as drug control receiving phenytoin 20 mg/kg, p.o and Group III and IV animals were administered with the poly herbal extract at doses of 250 and 500 mg/kg, p.o for 15 days respectively. On the 15^{th} day, seizures were induced to all the groups of animals using electro convulso meter. A 60 Hz alternating current of 150 milliamps intensity elicited maximal electro shock (MES) seizures for 0.2 second. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities [11]. The observed duration of various phases of epilepsy was tabulated.

2.7. Estimation of biogenic amines

The animals were divided into five groups (*n*=6) and Group I animals served as control for reference standards, Group II animals served as negative control receiving 1 ml of 5 % CMC p.o, Group III served as drug control receiving phenytoin 20 mg/kg p.o, Group IV animals were administered with the poly herbal extract at a dose of 250mg/kg p.o and Group V animals received poly herbal extract at a dose of 500 mg/kg p.o for 15 days. On the 15th day, seizures were induced to all the groups except Group I animals using electro convulso meter and biogenic amines in the fore brain of the rat were estimated [12]. The rats were sacrificed by cervical dislocation, since sacrificing by over dose of anesthesia may alter the brain monoamine levels [13]. After sacrificing, the brain was rapidly removed and the fore brain was dissected on a cooled microtome at -20^{o} C. The fore brain region was weighed and fore brain of two rats of the same group were pooled and homogenized with 6 ml of cold acidified butanol. Each homogenate pool served as a tissue sample for the respective groups. Internal standards were prepared by the addition of known amounts of mixed standards, (500 µg each of noradrenaline, dopamine and serotonin). The readings were limited to the neither excitation maxima 395-485 nm for noradrenaline, 330-375 nm for dopamine and 360- 470 nm for serotonin. The results were expressed as ng/g of wet brain tissue [14].

3. Results and Discussions

3.1. Effect on MES induced Seizures

The poly herbal extract exhibited a dose dependent significant (P<0.01 and P<0.001) reduction in various phases of epileptic seizure on comparison with the reference standard phenytoin 20 mg/kg, p.o. There was also a significant reduction in the time required for the righting reflex (recovery) in the extract treated groups (Table 1).

Table 1. Effect of Poly herbal extract on MES induced convulsions in rats.

Group	Drug	Flexion (Sec)	Extension (Sec)	Clonus (Sec)	Stupor (Sec)	Recovery (Sec)
I	Control	5±0.85	13.3±0.86	13.4±1.62	5.83±1.014	184.4
II	Phenytoin (20 mg /kg)	3.5±0.56^{a***}	0	8.5±1.67^{a***}	1.16±0.65^{a***}	176.2
III	PHE (250 mg / kg)	3.33±0.33^{b***}	1.33±0.33^{b***}	5.33±0.33^{b***}	23±1.73^{b**}	132.67
IV	PHE (500 mg / kg)	2.33±0.33^{b***}	1.16±0.17^{b***}	4.67±0.88^{b***}	18±0.57^{b**}	113.30

Values represent mean of six observations.
Comparisons between: a – Group I vs. Group II, b – Group II vs. Group III and Group V.
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test.

$^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P < 0.001$.

3.2. Effect on biogenic amine estimation

A significant $P < 0.001$ increase in the dopamine, serotonin and noradrenaline level was noted in the fore brain region for extract treated animals (Table 2).

Table 2. Effect of Poly herbal extract on levels of biogenic amines in forebrain of epilepsy induced rat.

Group	Drug	Serotonin (ng/g of wet tissue)	Dopamine (ng/g of wet tissue)	Noradrenaline (ng/g of wet tissue)
I	Control	166.58±1.64	394.27±2.78	95.55±1.33
II	MES	63.05±0.65^{a***}	124.50±0.19^{a***}	32.89±0.47^{a***}
III	Phenytoin (20 mg/kg)	84.75±0.86^{b***}	253.73±2.18^{b***}	53.43±1.09^{b***}
IV	PHE (250 mg/kg)	91.92±0.68^{c***}	302.45±0.87^{c***}	70.79±1.09^{c***}
V	PHE (500 mg/kg)	105.12±0.66^{c***}	322.70±0.84^{c***}	66.37±1.59^{c***}

Values represent mean of six observations. MES = Maximal Electro Shock Induced Group; PHE = Poly Herbal Extract administered Group.
Comparisons between: a – Group I vs. Group II, b – Group II vs. Group III, c – Group II vs. Group IV and Group V.
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test
$^{*}P < 0.05$, $^{**}P< 0.01$, $^{***}P < 0.001$.

A significant reduction in the time required for the recovery (righting reflex) was observed in this study (Table 1), which proves that PHE was providing a beneficial effect in controlling MES induced seizures. It was not surprising that the administration of PHE significantly increased the brain levels of serotonin, dopamine and noradrenaline, which could be attributed to the significant protection offered against MES induced seizures (Table 1). The increase in the brain monoamine level by inhibiting the monoamine oxidase (MAO), an enzyme responsible for destruction of biogenic amines tends to raise the seizure threshold [15]. Serotonin (5-Hydroxy tryptamine) is an inhibitory neuro-transmitter involved in the regulation of mood, sleep, anxiety, arousal and aggression. Serotonin agonists, precursors and neuronal uptake inhibitors are reported to enhance narcoleptic catalepsy [16]. The increase in the serotonergic transmission raises the threshold of Pentylenetetrazole (PTZ) induced seizures in many animal test systems, thereby protecting against PTZ induced convulsions [15]. Dopamine activation seems to be crucial with respect to a lasting internal encoding of motor skills. Dopamine is also believed to provide a teaching signal to parts of brain responsible for acquiring new behavior. In insects, a similar effect has been demonstrated with respect to octopamine, a chemical relative of dopamine [17]. These effects are mediated by dopminergic receptors situated in several parts of brain including substantia nigra.

Noradrenaline has also a role to play in the control of seizures, but less significantly when compared with other biogenic amines, as it is mainly concerned with blood pressure regulation. It has a potential for biphasic effect of glutamate in the cerebellum and would inhibit glutamate release at low concentrations [18]. Over activation of glutamate receptors may lead to delayed neuro degeneration as a result of increased influx of calcium ions into neurons. The well-established drugs like phenytoin, carbamazepine and benzodiazepines exerts their action by inhibiting calcium calmodulin stimulated protein phosphorylation in presynaptic nerve terminal [11]. A low concentration of dopamine in cerebellum also has an inhibitory effect on glutamate [18]. Inhibition of prostaglandin synthesis is reported to increase the brain levels of dopamine and noradrenaline, which also causes an inhibition of seizure activity [19].

4. Conclusion

In conclusion, we have found that administration of PHE for 15 days increased the seizure threshold in MES induced rats and its possible mechanisms may be due to the inhibition of prostaglandin synthesis and monoamine oxidase enzyme. One more possible mechanism involved in the antiepileptic effect of PHE may be by the decreased influx of calcium ions. The exact mechanism of action of each individual principle remains to be studied in our laboratory.

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