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Pharmacological validation for sarasvata arista (An ayurvedic formulation) on maximal electro shock induced epileptic model

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ABSTRACT

In the present study, an Ayurvedic poly herbal formulation, Sarasvata arista (SVA) was validated for its protective effect against seizures during epileptic attacks. A total daily dose of 1.5 ml/ 100 gram of rat was administered to the test animals. After 15 days, seizures were induced by Maximal Electro Shock (MES) and the duration of various phases of epilepsy were observed and compared with the control animals. A significant ($P < 0.01$ and $P < 0.001$) reduction in the time taken for righting reflex was noted. The levels of some of the biogenic amines were also estimated and a significant level of restoration in dopamine, serotonin and nor adrenaline was observed in the forebrain region of epilepsy induced rats treated with SVA.

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KEYWORDS

Sarasvataarista;
Seizures;
Maximum electro shock
(MES);
Dopamine;
Serotonin;
Nor adrenaline.

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 affecting human beings. According to several publications this can amount to 70% of the people with epilepsy, with a high prevalence of about 0.8% in children below the age of seven years^[2]. An alternative drug therapy for the treatment of this disease is the use of a poly herbal alcoholic formulation called Arista, a preparation of Ayurveda.

Aristas are liquid preparations which contain active constituents like alkaloids, sterols, flavanoids and other derivatives. Sarasvataarista is also one of such product which has twenty three active ingredients available in

Tamil Nadu, India. The usual method of production of aristas involves shade drying of the ingredients, grinding, fermenting with self generated alcohol and final collection by filtration.

The main active ingredients are Ashwagandha, Bramhi and Shatavari. All these plants are well known for their potential to treat nervine disorders^[3].

The verbal interviews made with patients of known epilepsy and Ayurvedic physicians in our environment led us further investigate the effect of Sarasvata arista and to validate it pharmacologically on MES induced seizures and estimation of some biogenic amines in the fore brain region of rats.

MATERIALS AND METHODS

Experimental animals

In bred adult Wistar rats weighing between 150g

and 200g of either sex were obtained from our own animal house, Department of Pharmacology and Toxicology, C.L.Baid Metha college of Pharmacy, Tamil Nadu, India. The animals were maintained in a well ventilated room with 12h light/dark cycle in polypropylene cages. Ethical committee clearance was obtained for the conduct of the study from IAEC (Ref No. IAEC/XIII/3/CLBMCP/2006-2007 dt 19-04-2007). Standard pellet feed and drinking water were provided ad libitum throughout the study period. The rats were randomly divided into three groups of six rats each such that the weight differences between the means of the groups was ± 2.0 SD. Group A animals (saline controls) were administered with 0.5ml of normal saline, Group B animals (positive controls or drug controls) received phenytoin 20mg/kg, intraperitoneally and Group C were administered with Sarasvata arista 1.5ml/100g of rat orally for 15 days. All the animals were maintained with normal feed and drink.

Poly herbal formulation

The poly herbal extract, Sarasvata arista was purchased from an Ayurvedic manufacturer [IMCOPS] in the city with the batch number SVA- BD- 002. The calculated dose for each rat was administered orally in two portions at 1h interval per day using a gastric feeding needle (CU. FNC-16-13).

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.

MES induced seizures

On the 15th day, seizures were induced to all the groups of animals by using electro convulso meter. Maximal electro Shock (MES) seizures were elicited by a 60Hz alternating current of 150 milliamps intensity for 0.2 seconds. 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^[4]. The duration of various phases of epilepsy was observed and the percentage protection was estimated by observing the number of animals showing abolition of hind limb tonic extension (HLTE) or extension not

greater than 90 degrees using the formula,

$$\% \text{ protection} = \left[\frac{\text{Mean of observations in control} - \text{Mean of observations in drug treated}}{\text{Mean of observations in drug treated}} \right] \times 100$$

Mean of observations in drug treated

Biogenic amine estimation

Biogenic amines in the fore brain of the rat were estimated Spectrofluorimetrically^[5]. The rats were sacrificed by cervical dislocation, since sacrificing by overdose of anesthesia may alter the brain monoamines^[6]. After sacrificing, the brain was rapidly removed and the fore brain was dissected on a cooled microtome at -20°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 nor adrenaline, dopamine and serotonin). The readings were limited to the neither excitation maxima 395-485 nm for nor adrenaline, 330-375 nm for dopamine and 360-470nm for serotonin using spectrofluorimeter.

Statistics

Results are presented as mean \pm S.E.M; for animal weights as SD and n represents the number of rats used for each group. Significant differences were determined by use of ANOVA followed by Dunnet's t test. $P < 0.05$ was considered significant.

RESULT

Effect on MES induced seizures

Phenytoin treated animals exhibited 100% protection against MES induced seizures whereas SVA 1.5ml/100 g exhibited 47% protection. SVA and phenytoin did not show any significant change in the duration of tonic flexion and clonic convulsions. A significant $P < 0.01$ and $P < 0.001$ reduction in the time taken for the righting reflex was noted.

Effect on biogenic amine estimation

A significant $P < 0.001$ increase in the dopamine, serotonin and nor adrenaline level was noted in the fore

Full Paper

TABLE 1: Effect of SVA on MES induced convulsions

Group	Drugs used	Flexion(seconds)	Extensor(seconds)	Clonos (seconds)	Stupor(seconds)	Recovery(seconds)
I	Control	5±0.85	13.3±0.86	13.4±1.62	5.83±1.014	184.4
II	Phenytoin	3.5±0.56 ^{a***}	0	8.5±1.67 ^{a***}	1.16±0.65 ^{a***}	176.2
III	SVA	3.33±0.33 ^{b**}	1.16±0.17 ^{b***}	5.33±0.33 ^{b***}	18±0.57 ^{b**}	113.3

Values are mean ± SEM of Six observations; Comparisons between: a-Group I and Group II, b-Group II Vs Group III, c-group III Vs Group IV; Statistical significant test for comparison was done by ANOVA, followed by Dunnet 't' test; *P<0.05, **P<0.01, ***P<0.001.

TABLE 2: Effect of SVA on Serotonin, Dopamine and Nor adrenaline in forebrain of epilepsy induced rat brain

Group	Drug used	Serotonin (ng/g of wet tissue)	Dopamine (ng/g of wet tissue)	Nor adrenaline (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	84.75±0.86 ^{b***}	253.73±2.18 ^{b***}	53.43±1.09 ^{b***}
IV	SVA	91.92±0.68 ^{c**}	302.45±0.87 ^{c**}	70.79±1.09 ^{c**}

Values are mean ± SEM of Six observations; Comparisons between: a-Group I and Group II, b-Group II Vs Group III, c-group III Vs Group IV; Statistical significant test for comparison was done by ANOVA, followed by Dunnet 't' test; *P<0.05, **P<0.01, ***P<0.001.

brain region for SVA treated animals.

DISCUSSION

The use of ayurvedic preparations in the management of epileptic seizures has increased because these preparations fit into the cultures of peoples and are not usually as expensive as the more refined orthodox drugs. Besides, these orthodox drug posses many side effects, contraindications and possible interactions with drugs used simultaneously.

SVA was found to be rich in alkaloids, reducing sugars, steroids, proteins, phenols, flavanoids, gums, glycosides, saponins as determined by a customary procedure^[7]. The outcome of our verbal interviews with epileptic patients and ayurvedic physicians impelled us to use a dose of 1.5ml/100g body weight of the tested animals.

A significant reduction in the time required for the recovery (righting reflex) was observed in this study (TABLE 1), which proves that SVA was providing a beneficial effect in controlling MES induced seizures.

The administration of SVA significantly restored the brain levels of serotonin, dopamine and nor adrenaline, which could be attributed to the significant protection offered against MES induced seizures (TABLE 2). This increase in the brain monoamine level is by inhibiting the mono amine oxidase (MAO), an enzyme responsible for destruction of biogenic amines tends to raise the seizure threshold^[8].

Serotonin, (5-Hydroxy tryptamine) is an inhibitory neurotransmitter involved in the regulation of mood,

sleep, anxiety, arousal and aggression. Serotonin agonists, precursors and neuronal uptake inhibitors are reported to enhance neuroleptic catalepsy^[9]. Increase in the serotonergic transmission raises the threshold of PTZ induced seizures in many animal test systems, thereby protecting against PTZ induced convulsions^[8].

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^[10]. These effects are mediated by dopaminergic receptors situated in several parts of brain including substantia nigra.

Nor adrenaline 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^[11]. Over activation of glutamate receptors may lead to delayed neuro degeneration as a result of increased influx of calcium ions into neurons. Well established drugs like phenytoin, carbamazepine and benzodiazepines exerts their action by inhibiting calcium calmodulin stimulated protein phosphorylation in presynaptic nerve terminal^[4]. A low concentration of dopamine in cerebellum also has an inhibitory effect on glutamate^[11]. Inhibition of prostaglandin synthesis is also reported to increase the brain levels of dopamine and nor adrenaline, which also causes an inhibition of seizure activity^[12].

In conclusion, we have found that administration of SVA 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 mono amine oxidase enzyme. One more possible mechanism involved in the antiepileptic effect of SVA may be by the decreased influx of calcium ions.

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