National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

A study on the anticonvulsant activity of *Withania somnifera* (Dunal) in albino rats

Santhosh Kumar Raju, Basavanna PL, Nagesh HN, Ajay D Shanbhag

Department of Pharmacology, Mysore Medical College and Research Institute, Mysore, Karnataka, India

Correspondence to: Santhosh Kumar Raju, E-mail: santhudoc@gmail.com

Received: June 06, 2016; **Accepted:** July 12, 2016

ABSTRACT

Background: The present study is an investigation of antiepileptic activity of *Withania somnifera* (*Ashwagandha*) is a well-known plant which is being used in tuberculosis, emaciation, sterility, and it has immunomodulatory, anabolic, anti-inflammatory, and antianxiety activities. **Aims and Objectives:** To find the efficacy of the alcoholic extract of *W. somnifera* (Dunal) in preventing experimentally-induced seizures. **Materials and Methods:** The alcoholic extract of *W. somnifera* was subjected to pilot study and then screened for anticonvulsant activity on maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced seizures models in albino Wistar rats. Animals were treated with *W. somnifera* at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg body weight and compared the results with control and standard. **Results:** Study results showed that *W. somnifera* extract at the dose of 300 mg/kg body weight when compared to control group highly significant ($P < 0.01$) reduction of hindlimb tonic extension and postictal depression in MES. PTZ-induced seizures showed significantly reduced mean duration of hindlimb tonic flexion, hindlimb tonic extension, clonus, and stupor and there was no postictal depression. **Conclusions:** The alcoholic extract of *W. somnifera* (Dunal) has shown a significant anticonvulsant effect at the dose of 300 mg/kg body weight, both in MES method and PTZ method and has given higher protection rate against pentylenetetrazol seizure than MES.

KEY WORDS: *Withania somnifera*; Pentylenetetrazole; Maximal Electroshock Model

INTRODUCTION

Epilepsy is a common neurological disorder having important medical, social, and psychological consequences. Even though it was recognized as early as 2000 B.C, new concepts about its pathogenesis, etiology, and treatment are brought out almost every year.[1] In the past, the treatment of epilepsy was based on superstitious religious beliefs and ignorance. However, the present day concept of the treatment of epilepsy is very much different from what it was earlier.[2] The investigators who have worked on epilepsy have used various chemicals for treating epilepsy, starting from bromides (which provide the first rational treatment for patients suffering from epilepsy) to recent newer antiepileptics.[3]

In spite of the vast number of drugs introduced for the treatment of epilepsy, there is still a need for an ideal antiepileptic agent, with properties such as broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability, and low cost.[4]

India has a rich treasure of medicinal plants and has contributed to the development of the well-known system of Ayurveda, the science of life.[5,6] Many medicinal plants from India have been shown to have activity by the traditional methods of psychoneuropharmacology.[7,8]

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2016.6.0615112072016	

Withania somnifera (Dunal/*Ashwagandha*) is considered to be one of the best rejuvenating agents in Ayurveda. It is indicated in tuberculosis, emaciation, sterility, and disease caused by deranged vata, its external application is indicated in tumors. Recent pharmacological researches showed *W. somnifera* have immunomodulatory, anabolic, anti-inflammatory, and antianxiety activities.[9,10]

In the light of developments cited above, an attempt has been made in this work to find out the effects of alcoholic extract of the stem and root bases of an indigenous plant *W. somnifera* on experimentally-induced seizures. Since ancient times, the stem and root bases of *W. somnifera* are being used in EPILEPSY (APASMARA) along with Vacha, Brahmi, Shankapushpi, and Jatamansi in ayurvedic system of medicine.

Hence, the work has been undertaken to find the efficacy of the alcoholic extract of *W. somnifera* (Dunal) in preventing experimentally-induced seizures.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology, JJM Medical College, Davangere.

Materials

Preparation of solutions of standard and test drug.

Sodium valproate

The standard solution of sodium valproate was prepared by dissolving 600 mg of sodium valproate in 10 ml of propylene glycol, at room temperature. Pure sodium valproate powder was obtained from Torrent Pharmaceutical Limited, Ahmedabad. The solution was freshly prepared each time. This was protected from direct sunlight. This solution has a concentration of 60 mg/ml.

Pentylenetetrazol

The solution of pentylenetetrazol was prepared by dissolving 560 mg of pentylenetetrazol in 20 ml of distilled water, at room temperature. This solution has concentration of 14 mg/ml. Pentylenetetrazol powder was obtained from Sigma-Aldrich Chemical Corporation, Bengaluru.

Test compound of W. somnifera

The test compound *W. somnifera* solution was prepared by dissolving 3 g of alcoholic extract of *W. somnifera* in 50 ml of propylene glycol. This solution has a concentration of 60 mg/ml.

Extraction of Test Compound from *W. somnifera*

About 50 g of stem and root bases of *W. somnifera* (Dunal/*Ashwagandha*) powder obtained from Ashwini Ayurvedic Medical College, Davangere. This powder was wrapped in a butter paper and put into thimble, with 500 ml of alcohol in a round bottom flask and subjected to SOXHLATION for 24 h. Dark brown solution of extract with alcohol was collected. Dark brown sticky paste like extract obtained after evaporation of alcohol.

Since the extract is not soluble in water, propylene glycol was used as a vehicle for the alcoholic extract of *W. somnifera*. Propylene glycol may produce some effects on central nervous system. Hence, propylene glycol was administered to other groups (control and standard groups) of animals so that if propylene glycol has any action, it will be similar to all animals.

Experimental Animals

Albino rats of either sex of average weight (150-200 g) which were inbred in the central animal house JJM Medical College Davangere, were used to induce convulsions by both electroshock and pentylenetetrazol.

All the test animals were allowed food and water ad libitum both being withdrawn just before experimentation. All the test animals were subjected further experiment of this study after 24 h (to avoid any possible "kindling" effect). All the preparations were administered intraperitoneally after the interval of 30 min.

The above test animals were subjected for maximal electroshock (MES) model and pentylenetetrazole (PTZ) model. Both models were consists of five groups of six animals each.

Experimental Methods

MES-induced seizure

Albino Wistar rats of either sex weighing 150-200 g were divided into four groups of six animals each. The first group received vehicle control (1 ml propylene glycol intraperitoneal), whereas Group-II received standard drug (300 mg/kg) intraperitoneally, Group-III, IV, and V received alcoholic extract of *W. somnifera* 100 mg, 200 mg, and 300 mg, respectively, in propylene glycol intraperitoneally. After the interval of 30 min, they were subjected to MES stimulation through transauricular electrodes with a current strength of 150 mA for 0.2 s by using techno electro convulsometer. The duration of various phases of epilepsy was observed. The percentage protection was estimated by observing the number of animals showing abolition of Hindleg tonic extension (or) extension not >90°.[10]

PTZ-induced seizures

Albino Wistar rats of either sex weighing 150-200 g were divided into four groups of six animals each. The first

group received vehicle control (1 ml propylene glycol intraperitoneal), whereas Group-II received standard drug (300 mg/kg) intraperitoneally, Group-III, IV, and V received alcoholic extract of *W. somnifera* 100 mg, 200 mg, and 300 mg, respectively, in propylene glycol intraperitoneally. After the interval of 30 min, pentylenetetrazol (70 mg/kg body weight) injection subcutaneously (to the scruff of neck through 27 gauge needle) was administered to all the groups to induce clonic convulsions. The duration of various phases of epilepsy was noted.

Pilot study showed that the alcoholic extract of *W. somnifera* at the dose of 400 mg/kg body weight was found to be toxic (5 out of 6 animals were died).

The parameters studied in both methods were:

1. Seizure latency (time taken for onset of seizure)
2. Hindlimb tonic flexion
3. Hindlimb tonic extension
4. Clonus
5. Stupor (unconsciousness) (from the end of clonus to regain consciousness)
6. Postictal depression (from the regain of consciousness to animals could walk away) duration of each parameter was recorded in seconds.

The results of the experiment are tabulated, and the results were analyzed statistically, and tests of significance were found out using Student's *t*-test.

RESULTS

Study results showed that W. somnifera extract at the dose of 300 mg/kg body weight when compared to control group highly significant ($P < 0.01$) reduction of hindlimb tonic extension and postictal depression in MES. PTZ-induced seizures showed significantly reduced mean duration of hindlimb tonic flexion, hindlimb tonic extension, clonus, and stupor and there was no postictal depression (Tables 1 and 2, Figures 1 and 2).

DISCUSSION

In India, studies have reported the prevalence rate of epilepsy varying from 1710 to 9780 cases/million populations. The modern conventional antiepileptic drugs (AEDs) are effective in approximately 50% of patients; many cases remain resistant to AED treatment.[11] These drugs are associated with vast array of side effects including chronic toxicity, teratogenicity, adverse effects on cognition and behavior among others.[12] Thus, due to aforementioned reasons and others, it is pertinent to look for affordable and conventional alternative medicine with view to providing a better protection and activities, particularly medicinal plants.

In this study, alcoholic extract of *W. somnifera* which was screened for anticonvulsant activity by MES and pentylenetetrazol-induced seizures. The test compound

Table 1: Effect of alcoholic extract of *W. somnifera* on MES-induced seizers in albino rats

Group	Treatment	Hindlimb tonic flexion	Hindlimb tonic extensor	Clonus	Stupor	Recovery	Percentage protection
I	1 ml of propylene glycol	3±0.58	12.16±0.48	10.3±0.66	97±6.38	132±5.34	0
II	300 mg/kg of sodium valproate in propylene glycol	-	-	3.16±0.48***	-	-	100
III	100 mg/kg of test drug*	2.66±0.33^{NS}	10.83±0.79^{NS}	10.33±0.85^{NS}	93.3±4.4^{NS}	132.5±5.38^{NS}	0
IV	200 mg/kg of test drug*	2.3±0.56^{NS}	9.3±2.09^{NS}	9.5±1.56^{NS}	76±5.38^{NS}	104.16±21.28^{NS}	16.66
V	300 mg/kg of test drug*	1.5±0.72^{NS}	4.3±2.09**	8±1.39^{NS}	48.5±21.97^{NS}	56±25.27*	50

**P<0.02 (highly significant), *P<0.05 (significant).*W. somnifera*: *Withania somnifera*, NS: Not significant, MES: Maximal electroshock

Table 2: Effect of alcoholic extract of *W. somnifera* on PTZ-induced seizers in albino rats

Group	Treatment	Seizure latency	Hindlimb tonic flexion	Hindlimb tonic extensor	Clonus	Stupor	Recovery	Percentage protection
I	1 ml of propylene glycol	443.8±23.8	3±0.25	12.16±0.79	23.6±3.09	70.66±5.65	24.66±24.67	0
II	300 mg/kg of sodium valproate in propylene glycol	64.16±64.16**	0.33±0.33**	1.5±1.5**	2.83±2.83**	5.8±5.8***	27.5±27.5^{NS}	100
III	100 mg/kg of test drug*	443.8±28.70^{NS}	2.8±0.83^{NS}	8.7±1.84^{NS}	21.6±4.14^{NS}	58.3±12.47^{NS}	22.5±22.5^{NS}	16.66
IV	200 mg/kg of test drug*	398.2±30.07^{NS}	1.33±0.88^{NS}	4±2.59*	8.83±3.88*	20.16±12.77**	-	66.66
V	300 mg/kg of test drug*	259.83±82.48^{NS}	0.66±0.67*	1.5±1.5***	5.5±3.93**	5.8±5.83***	-	83.33

**P<0.02 (highly significant), *P<0.05 (significant), *W. somnifera: Withania somnifera*, NS: Not significant, PTZ: Pentylenetetrazole

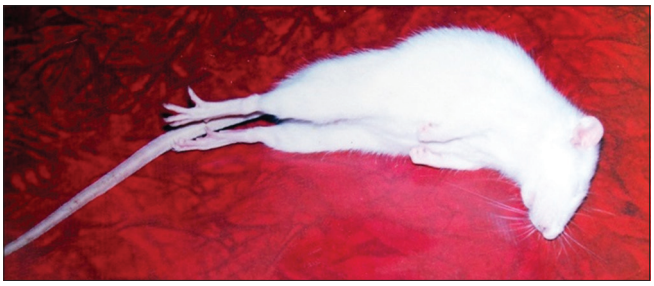

Figure 1: MEZ model showing *Withania somnifera*-induced hindlimb extension in albino rats

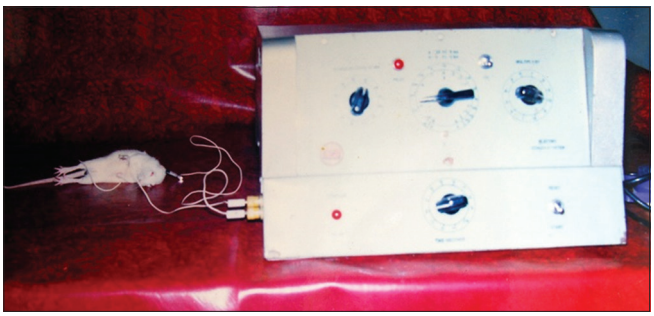

Figure 2: Pentylenetetrazole model showing *Withania somnifera*-induced hindlimb extension in albino rats

showed anticonvulsant activity by both methods in a dose-dependent manner.

Our study showed the test dose of 100 mg/kg; there was no reduction in the duration of phases of convulsions when compared to control group in both MES and PTZ models. Kulkarni and George study showed administration of Asgand root extract was found to reduce jerks and clonus in 70% and 10% animals, respectively, with dose of 100 mg/kg and reduction in the severity of PTZ-induced convulsions was evident from electroencephalogram wave pattern.[13]

Analysis of the results of the groups, those received the test compound at the dose of 100 mg/kg body weight, when compared to control groups no significant protection was observed both in MES and PTZ-induced convulsions. Although only one animal was protected against clonus, hindlimb tonic flexion, hindlimb tonic extension, stupor and postictal depression in PTZ-induced seizure, statistically not significant.

The animals received the test dose of 200 mg/kg body weight and were showed reduced mean duration of various phases of convulsions when compared to control group in MES method but was statistically not significant, and in PTZ method, mean duration of clonus, hindlimb tonic flexion, hindlimb tonic extension, and stupor was reduced significantly ($P < 0.05$) and showed absent postictal depression.

W. somnifera extract at the dose of 300 mg/kg body weight when compared to control group highly significant ($P < 0.01$) reduction of hindlimb tonic extension and postictal depression was observed in MES. PTZ-induced seizures showed significantly reduced mean duration of hindlimb tonic flexion, hindlimb tonic extension, clonus, and stupor, and there was no postictal depression.

There was no seizure latency showed in MES method, but in PTZ, there was reduction of mean duration of seizure latency in Group-IV and V but statistically not significant. The standard group (sodium valproate, II) was protected in very highly significant ($P < 0.001$) manner.

Asgand root extract showed reduction in severity of motor seizures induced by electrical stimulation in right basolateral amygdaloid nuclear complex through bipolar electrodes. The protective effect of Asgand extract in convulsions has been reported to involve GABAergic mediation.[14]

It is interesting to note that many rats in PTZ method died after exhibiting various phases of convulsions such as clonus/twitching hind tonic flexion and extension, stupor and postictal depression. The high rate of mortality is probably due to a combination of factors which include marked depression of vital medullary centers by persisting high concentration of circulating pentylenetetrazol and hypoxia and exhaustion resulting from the initial and recurrent seizures.

Swinyard et al., have considered abolition of hindlimb tonic extension as the protective and point against MES-induced seizures. According to this criterion, sodium valproate (300 mg/kg) showed 100% protection with the test compound 100 mg/kg, the percent of protection was nil, whereas at the doses of 200 mg/kg the percent of protection was 16.66% and 50%, respectively.

Bury et al., have considered the prevention of clonic seizure <5 s, as the protective end point against PTZ stimulation. According to this criterion, the percent of protection is 100% by sodium valproate (300 mg/kg). The test compound gave 83.33% of protection at the dose of 300 mg/kg, whereas 50% and 16.66% of protection is obtained at the doses of 200 mg/kg and 100 mg/kg, respectively.

Above-mentioned results showed that the test compound, i.e., alcoholic extract of *W. somnifera* has got best anticonvulsant activity at the maximum dose of 300 mg/kg body weight. When compared to sodium valproate, the test compound has anticonvulsant activity which is closer to sodium valproate; hence, it may be useful in both partial and generalized tonic-clonic (grand mal) and absence (petit mal) seizures.

Mechanism of action could be explained by the drugs that are active in the MES test often have phenytoin like effect on voltage-dependent Na+ channels, although drugs that act specifically to block NMDA-type excitatory amino acid receptors or that increase synaptic norepinephrine levels are

also effective in this test. On the other hand, pentylenetetrazol test evaluates the ability of potential AEDs to prevent clonic seizures activity in this seizure model often indicates that a drug can affect GABAergic brain system, either by enhancing brain GABA level or by altering the sensitivity of post synaptic GABA receptors. Specific drugs, such as ethosuximide and trimethadione which may act by blocking T-type voltage dependent Ca^{++} channels, are also effective in the PTZ test. Hence, alcoholic extract of *W. somnifera* may act by any of the above-said mechanisms or in combination.

It is hoped that *W. somnifera* (Dunal) (*Ashwagandha*) extract provide a promising anticonvulsant drug having higher efficacy against absence (petit mal seizures and moderate efficacy in generalized tonic-clonic (grand mal) seizures with no or acceptable adverse effect like mild sedation, more work is needed to isolate the active principle and to further evaluate it to that availability of such drug becomes a reality.

CONCLUSIONS

The alcoholic extract of *W. somnifera* (Dunal) has shown a significant anticonvulsant effect at the dose of 300 mg/kg body weight, both in MES method and PTZ method, but the anticonvulsant action is comparatively less at 200 mg/kg and 100 mg/kg doses. The test compound has given higher protection rate against pentylenetetrazol seizure than MES, which shows that the test compound is probably more useful in the absence (petit mal) seizures than generalized tonic-clonic (grand mal) seizure and may resemble sodium valproate in its action. Further study is required to confirm and to isolate the extract active principle present in the alcoholic extract of *W. somnifera*, which is actually responsible for its anticonvulsant property.

REFERENCES

1. Adams RD, Victor M. Textbook of Principles of Neurology. 4th ed. New York: Oxford University Press; 1989. p. 249-70.
2. Shorvon SD. Epilepsy - A General Practice Perspective. Philadelphia, PA: Lippincott-Raven; 1998.
3. Craig CR, Stitzel RE. Modern Pharmacology. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 1964. p. 414.
4. Satoskar RS, Bhandarkar SD. Textbook of Pharmacology and Pharmacotherapeutics. 13th ed., Vol. 1. Tripathi, MD: Essentials of Medical; 2005. p. 1-112.
5. Chadvick D. Seizures, epilepsy and other episodic disorders. Brain's Disease of the Nervous System. 10th ed. Oxford: Oxford Medical Publication; 1993. p. 697-733.
6. Vaidya AD. The status and scope of Indian medicinal plants acting on central nervous system. Indian J Pharmacol Soc. 1997:29:S340-3.
7. Shore PA, Giachetti A. Reserpine: Basic and clinical pharmacology. In: Iversen L, Iversen SD, Synder SH, editors. Handbook of Psychopharmacology. Vol. 10. New York: Plenum Press; 1958. p. 197-219.
8. Dandiya PC, Chopra YM. CNS acting drugs plants indigenous to India. Int J Pharm. 1970;2:67-90.
9. Dhawan BN. Centrally acting agents from Indian plants. In: Koslow SH, Murthy RS, Coelho GV, editors. Decades of the Brain. India - USA Research in Mental Health and Neurosciences. Rockville, MD: Rockville Institute of Mental Health; 1995. p. 197-207.
10. Prakash SK. Pharmacotherapeutic Aspects of *Ashwagandha* (*Withania somnifera,* Dunal) in Ayurveda. 1997. p. 1-10.
11. Heinemann U, Draguhn A, Ficker E, Stabel J, Zhang CL. Strategies for the development of drugs for pharmacoresistant epilepsies. Epilepsia. 1994;35 Suppl 5:S10-21.
12. Raza M, Shaheen F, Choudhary MI, Suria A, Rahman AU, Sombati S, et al. Anticonvulsant activities of the FS-1 subfraction isolated from roots of *Delphinium denudatum*. Phytother Res. 2001;15(5):426-30.
13. Kulkarni SK, George B. Anticonvulsant action of *Withania somnifera* root extract against pentylene tetrazole (PTZ) - Induced convulsions in mice. Phytother Res. 1996;95(10):447-9.
14. Kulkarni SK, Sharma A, Verma A, Ticku MK. GABA receptor mediated anticonvulsant action of *Withania somnifera* root extract. Indian Drugs. 1993:305-12.

How to cite this article: Raju SK, Basavanna PL, Nagesh HN, Shanbhag AD. A study on the anticonvulsant activity of *Withania somnifera* (Dunal) in albino rats. Natl J Physiol Pharm Pharmacol 2017;7(1):17-21.

Source of Support: Nil, **Conflict of Interest:** None declared.