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SHORT COMMUNICATION

Protective Effect of *Withania somnifera* Root Extract on Electrographic Activity in a Lithium-pilocarpine Model of Status Epilepticus

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The present study investigated the anticonvulsant profile of *Withania somnifera* (*W.s*) in a lithium-pilocarpine model of status epilepticus (SE) in rats. Acute treatment with the root extract of *W.s* prolonged the latency to forelimb clonus but failed to protect against mortality. Acute pretreatment with *W.s* root extract enhanced the antiepileptic effect of diazepam and clonazepam. Rats chronically administered *W.s* (100, 200 mg/kg, p.o. × 7 d), when subjected to lithium-pilocarpine challenge showed a reduced mortality rate. Electrophysiological data further support the behavioural findings, as the root extract brought about a parallel change in seizure activity as paroxysmal spike activity appeared only from the 60 min record. Moreover, the seizure activity seemed to subside by 4 h in comparison with the control. The protective effect of the root extract appears to involve GABAergic mediation. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Aswagandha (*Withania somnifera*) has been shown to possess anticonvulsant properties in acute models in rats and mice via a GABAergic mechanism (Kulkarni and Verma, 1993; Kulkarni *et al.*, 1993), against amygdaloid kindling in rats (Kulkarni and George, 1995), and also against pentylenetetrazol (PTZ)-induced kindling in mice (Kulkarni and George, 1996). The alcohol extract of the roots was reported to enhance PTZ-induced seizure latency following chronic administration. In the present study an attempt was made to screen *W.s* root extract for its antiepileptic potential in a lithium-pilocarpine model in rats.

MATERIALS AND METHODS

Status epilepticus was induced by the method of Honchar and colleagues (1983) by administering lithium chloride (3 meq/kg, i.p.) followed 21 h later by pilocarpine (30 mg/kg, s.c.) in adult male Wistar rats. The drugs were administered 30 min prior to pilocarpine challenge. The animals were observed for a period of 100 min by noting the onset of forelimb clonus with rearing (F.C+R) and subsequent mortality over 24 h. For EEG studies, under ketamine anaesthesia, rats were stereotaxically implanted with both cortical (bilateral), and unipolar, (unilateral) electrodes in the right hippocampus (2.8 mm posterior, 2 mm lateral and 3.5 mm ventral) according to the Paxinos and Watson atlas (1982). SE was defined as continuous convulsions for a period longer than 30 min associated with continuous spikes of high amplitude. The changes in EEG (amplitude and frequency) were recorded alternately for cortex and hippocampus before lithium (basal), 30 min after clonazepam and *W.s* treatments (20.5 h post lithium), and after administering pilocarpine (21 h post lithium) at 30 min intervals up to 90 min, and finally after 4 h of pilocarpine. Six hours after pilocarpine challenge, randomly selected brains were prepared for histological verification (haematoxylin and eosin staining) of electrode sites.

Statistics. All results are given as mean ± SD. The onset of forelimb clonus with rearing was subjected to a Kruskal–Wallis one-way analysis of variance output test followed by Student's *t*-test. For EEG studies, descriptive statistics for amplitude and frequency in the cortex and hippocampus at various time points was calculated as the mean and standard deviation for all the groups separately. Freidman's test (non-parametric test) was applied to find out at what time from the basal value, changes become significant for both the parameters (amplitude and frequency). Area under the curve was compared among the groups by Kruskal-Wallis test. In the case of overall significance, a multiple range test was applied. A value of $p < 0.5$ was considered statistically significant.

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Drugs. Lithium chloride (Merck, Germany), pilocarpine nitrate and atropine sulphate (Boehringer Ingelheim, Germany), ketamine (Themis Chemicals Ltd., Bombay, India), *Withania somnifera* root extract (Gufic India Ltd., Bombay), phenytoin (Parke-Davis, Bombay, India), sodium valproate (Reckitt and Colman, India), diazepam (Ranbaxy Labs, Delhi, India) and clonazepam (Sauter Labs, UK) were used. Doses selected for each drug were based on previous studies reported from our laboratory.

RESULTS AND DISCUSSION

Lithium followed by pilocarpine challenge induced SE as indicated by behavioural and EEG changes (George and Kulkarni, 1996; George *et al.*, 1997). Given *per se* acutely, *W.s* delayed the onset of F.C + R but could not reduce the mortality rate. Chronic pretreatment of rats with the root extract (50, 100 and 200 mg/kg, p.o.) for 7 days followed by lithium-pilocarpine challenge showed neuroprotection as it reduced the mortality to 60% but not the latency of F.C + R. However, the above results were not significant. All the anticonvulsants studied namely diazepam (1, 2.5, 5), clonazepam (0.25, 0.5, 1), sodium valproate (100, 300) and phenytoin (50, 100) produced a dose-dependent protection. *W.s* when given in combination with the above anticonvulsants was able to reduce significantly the effective dose of diazepam and clonazepam to offer full protection with no subsequent mortality (Table 1). EEG alterations closely paralleled the behavioural changes in all the three groups tested. In the control group, seizure activity was shown to occur in both the regions from 30 min and was continuous for several hours. Pretreatment with clonazepam (1 mg/kg, i.p.) completely blocked the development of paroxysmal spike activity and the lethality. Acute pretreatment with *W.s* (100 mg) resulted in the appearance of well synchronized, high voltage spiking only by 60 min which seemed to subside by 4 h. However, there was no reduction in the mortality. The increase in hippocampal amplitude with *W.s* pretreatment was markedly less compared with the lithium-pilocarpine control rats (Fig. 1B). Even the cortical amplitude showed a reduced amplitude with W.s pretreatment until 60 min compared with the control group (Fig. 1A). The hippocampal and cortical frequency did not differ much in either the control or *W.s* pretreated groups. With clonazepam (1 mg) pretreatment there was no significant change from the basal value of amplitude, and frequency from the basal value (Fig. 1). The histological study revealed that the electrode tips terminated within the hippocampus itself.

GABA is a predominant inhibitory neurotransmitter involved in SE (Wasterlain *et al.*, 1993) since benzodiazepines which modulate the $GABA_A$-benzodiazepine receptor complex with the associated Cl^- ionophore, are the main drugs used in SE. Earlier *in vitro* and *in vivo* studies from our laboratory have shown a $GABA_A$-receptor mediated anticonvulsant property of *W.s* root extract (Kulkarni and Verma, 1993; Kulkarni *et al.*, 1993). The root extract dose-dependently abolished the tonic extensor phase against PTZ-induced seizures in

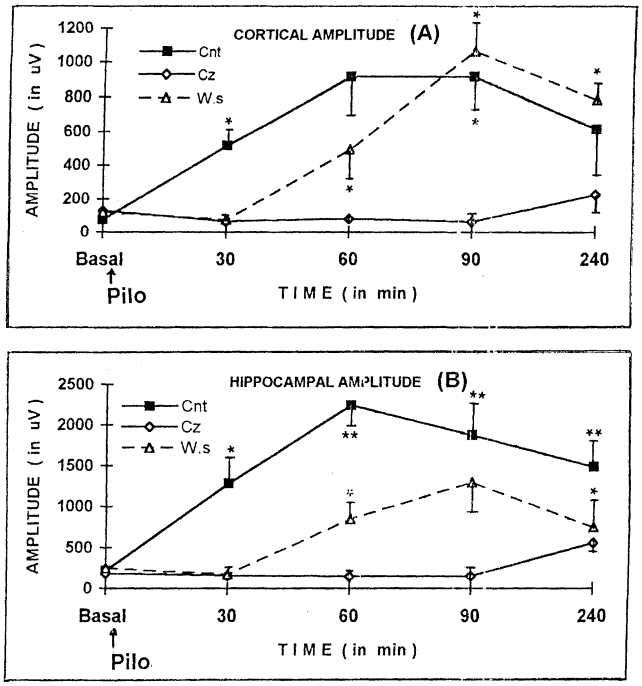

Figure 1. Time course changes in the cortical **(A)** and hippocampal **(B)** amplitude (in μV) in Li-pilocarpine model of SE following different drug pretreatment in rats. $n = 4$–6 in each group. Cnt, Cz, Ws: Cortical/hippocampal amplitude of control, clonazepam (1 mg), *W. somnifera* (100 mg) groups. Pilo: Pilocarpine, $*p < 0.05$, $**p < 0.01$ (significance with respect to the basal values, Freidmans test). Overall significant difference exists between the area under the curves of Cnt and Cz; Cz and Ws (in **A**); and between Cnt and Cz (in **B**) at $p < 0.01$ (Kruskal-Wallis test followed by multiple range test).

Table 1. Protective effect of *W. somnifera* root extract alone and in combination with known anticonvulsants in lithium pilocarpine-induced status epilepticus (SE) in rats.

Treatment (mg/kg)	Onset of F.C + R	Mortality (%)
Control [Li + Pilo]	25.4 ± 0.97	100
Acute study		
W.s (100)	56.0 ± 2.12^{NS}	100
Dz (1.0)	61.8 ± 11.0^{NS}	66.6
Dz (2.5)	60.4 ± 15.73^{NS}	50
Dz (5.0)	100 ± 0.0^{b}	0
W.s (100) + Dz (2.5)	85.0 ± 5.01^{a}	20
SV (100)	38.2 ± 5.2^{a}	80
SV (300)	74.83 ± 9.09^{a}	66
W.s (100) + SV (300)	75.8 ± 5.94^{a}	66
Cz (0.25)	72.0 ± 11.09^{a}	40
Cz (0.5)	100 ± 0.0^{b}	0
Cz (1.0)	100 ± 0.0^{b}	0
W.s (100) + Cz (0.25)	100 ± 0.0^{b}	0
Phy (50)	34.2 ± 2.13^{NS}	100
Phy (100)	79.0 ± 7.16^{a}	40
W.s (100) + Phy (100)	78.2 ± 7.45^{a}	40
Chronic study		
W.s (50) × 7d	45.0 ± 9.6^{NS}	83
W.s (100) × 7d	50.2 ± 5.87^{NS}	60
W.s (200) × 7d	57.2 ± 11.3^{NS}	66.6

F.C + R, forelimb clonus with rearing; W.s, *Withania somnifera* root extract; Dz, diazepam; SV, sodium valproate; Cz, clonazepam; Phy, phenytion; NS not significant. Each value represents mean ± SD. $n = 6$–9 in each group. $^{a}p < 0.05$, $^{b}p < 0.01$ (Kruskal-Wallis test followed by Student's *t*-test).

mice and combined administration of GABA (100 mg) and the *W.s* (30 mg) produced significant potentiation of the protective effect of GABA. Mehta *et al.* (1991) reported that the methanol extract of *W.s* inhibited the specific binding of [^{3}H]GABA and [^{35}S]TBPS; enhanced the binding of benzodiazepine agonists such as [^{3}H] flunitrazepam to their putative receptor sites, suggesting that the root extract may be acting at the level of GABA receptor site. Acute administration of the *W.s* extract (100 mg/kg) following stage III amygdaloid kindling in rats resulted in a significant reduction in the severity of motor seizures as was evident from the amplitude and frequency of EEG (Kulkarni and George, 1995). These behavioural, functional receptor studies and the present electrophysiological studies suggest that *W.s* root extract contains a component which has a GABA-mimetic activity (presumably this component is able to pass the blood–brain barrier) which could be responsible for its observed anticonvulsive effects.

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