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Original Research Article

Evaluation of centrally acting skeletal muscle relaxant activity of aqueous extract of *Withania somnifera* (ashwagandha) roots in albino mice

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ABSTRACT

Background: Skeletal muscle relaxants are used to treat both muscle spasm and spasticity, acting both as antispasmodic and antispasticity agents. In past studies some polyherbal formulations containing ashwagandha have shown skeletal muscle relaxant activity and fat extract of ashwagandha showed skeletal muscle relaxant activity in experimental animal models. This study is designed to evaluate the skeletal muscle relaxant activity of aqueous extract of *Withania somnifera* (ashwagandha) roots in albino mice, as the literature regarding them is limited.
Methods: Standard drug (diazepam) and different doses of Aqueous extract of ashwagandha (50, 100,150mg/kg) were given orally to albino mice. Skeletal muscle relaxant activity was assessed by Rota-rod apparatus. The fall off time from the rotating rod was noted for each group after 1 hour of drug administration. The difference in fall off time from the rotating rod between the standard and treated mice was taken as an index of muscle relaxation.
Results: The test extract at doses (50mg/kg, 100mg/kg and 150mg/kg) showed highly significant reduction in the time spent by the animals on revolving rod in rota rod test when compared to baseline ($p < 0.0001$). As compared with diazepam, aqueous extract (150mg/kg) showed almost equal reduction in the time spent by the animals on revolving rod in rota rod test.
Conclusions: This study indicates that the aqueous extract of ashwagandha possess central skeletal muscle relaxant activity. The results are promising for further investigation of efficient skeletal muscle relaxant activity.

Keywords: Ashwagandha, Albino mice, Diazepam, Rota rod test

INTRODUCTION

Drugs that affect skeletal muscle function include two different therapeutic groups 1) those used during surgical procedures and in intensive coronary unit (ICU) to produce muscle paralysis (that is - neuromuscular blockers) and 2) those to reduce spasticity in a variety of painful conditions (that is - spasmolytics - traditionally called as skeletal muscle relaxants).[1] When the central nervous system neuromuscular junction has interruptions in normal transmission of nerve stimuli, skeletal muscle spasms and muscle spasticity occur.[2] It is characterized by muscle over activity which, if left untreated, may lead to muscle and soft tissue contracture.

Centrally acting and directly acting muscle relaxants accompanied by physical therapy are the drugs of choice for relief of muscle spasticity. Dantrolene and diazepam are mostly the drugs of choice. Studies have shown that the skeletal muscle relaxants to be more effective than placebo in the treatment of acute painful musculoskeletal disorders and muscle spasm, while efficacy was less consistent when treating chronic disorders. When the skeletal muscle relaxants were used in combination with analgesics, pain

relief is superior to either agent used alone. Centrally acting skeletal muscle relaxants are generally prescribed either as single agents or as components of combination products. The Food and Drug Administration has approved these medications as adjuncts to rest and physical therapy for relief of acute, painful musculoskeletal problems. The side effects of antispasmodic agents and antispasticity agents cause them to be used with caution. Previous reports have shown that 10-20% of adults suffer from insomnia. *Withania somnifera*, better known as Ashwagandha or "Indian ginseng," has been a staple of Ayurvedic medicine for over 3000 years.[3] The botanical name of Ashwagandha is *Withania somnifera* belonging to solanaceae.[4] The name Ashwagandha is from the Sanskrit language and is a combination of the word ashva, meaning horse, and gandha, meaning smell. The root has a strong aroma that is described as "horse-like." In Ayurveda it is classified as rasayana (Rejuvenation) and expected to promote physical and mental health, rejuvenate the body in debilitated conditions and increases longevity.[5] Various studies in animal models have shown that ashwagandha has anticonvulsant, antidepressant, antianxiety, hepatoprotective, anti - inflammatory, immune-modulatory activity.[6-11] As there are limited studies regarding the skeletal muscle relaxant activity of Ashwagandha. Therefore, this study is designed to evaluate the skeletal muscle relaxant activity of aqueous extract of *Withania somnifera* (Ashwagandha) roots in albino mice.

METHODS

The present study was carried out in the postgraduate research laboratory, Department of pharmacology, Alluri Sita Ramaraju Academy of Medical Sciences, Eluru, with the approval of institutional ethical committee.

Drugs

Normal saline, ashwagandha roots (aqueous extract) from Laila Nutraceuticals, Vijayawada. Aqueous extract contains total Withanolides% assay by High Performance Liquid Chromatography, HPLC (0.15 %), and diazepam tablets (10mg).

Animal

Swiss albino mice of the either sex weighing 40-50gms were used. The animals were procured from central animal house, Department of pharmacology, Alluri Sita Ramaraju Academy of Medical Sciences, Eluru and maintained in standard cages with free access to food and water. Permission from Institutional Animal Ethics Committee constituted for the purpose of CPCSEA Government of India was taken.

Experimental design

Animals of each group that remain on the revolving rod for 2 or more minutes in four trials carried out in two days during morning and afternoon sessions were selected and divided into different groups. Mice were divided into five groups consisting of 6 animals each. Group I served as control which received normal saline 10ml/kg, group II received standard drug diazepam at a dose of 5mg/kg, group III, IV and V received the aqueous extract of Ashwagandha orally at a dose of 50, 100 and 150mg/kg. After the administration of control, standard and test material the fall off time from the rotating rod was noted after 60minutes. The difference in the fall off time from the rotating rod between the standard and the treated mice was taken as an index of muscle relaxation. Recording the fall off time from the rotating rod by using rota rod apparatus devised by Dunham MW et al.[12]

Statistical analysis

The data was entered through Microsoft Excel - 2007 software and analysed by the Descriptive statistics like Mean, standard deviation and standard error and the quantitative data was analysed by using the statistical test like t - test, paired test and ANOVA one - way classification. P value <0.05 considered significant.

RESULTS

All obtained data were noted and processed for Statistical analysis. As seen from Table 1, The effect of muscle relaxant activity of aqueous extract have been shown in Table 1, at a dose of 50, 100, 150mg/kg body weight produced a significant decrease in fall off time (30.4%, 61.7%, 74.9% respectively) after 60minutes of oral administration of test drug. Similarly, animals with diazepam (5mg/kg) i.p shows a significant decrease in fall off time (78.5%) after 60 minutes of standard drug administration.

As seen in Table 2, the mean falls of time from the rotating rod after giving standard drug (diazepam - 10mg/kg) is 45.3±3.3Sec and after giving test drug (aqueous extract - 50 and 100mg/kg) are 145 and 80 sec respectively. So, authors conclude that aqueous extracts of ashwagandha at a dose of 50mg/kg, 100mg/kg are having less skeletal muscle relaxant activity as compared to standard drug. And the percentage of muscle relaxant effect of diazepam is 78.5% when compared to aqueous extract at dose of 50 and 100mg/kg (30.4% and 61.7% respectively).

But at a dose of test drug (150mg/kg) the mean falls off time from rotating rod is 53.16sec. when compared to diazepam, there is less significant difference between their mean responses. This result concludes that aqueous extract (150mg/kg) was found to be effective and it exhibited activity similar to that of diazepam i.e the percentage of muscle relaxant effect of diazepam is 78.5% and that of aqueous extract at dose of 150mg/kg is 74.9%.

As seen in Figure 1, the mean falls of free ride time before giving standard drug (diazepam) is 211 Sec and the mean fall of free ride time after giving diazepam is 45.3 Sec. This

shows there is a significant difference in the mean fall of free ride time on the revolving rod in the rota rod test between the before and after standard drug (diazepam) administration, indicating the skeletal muscle relaxant activity of diazepam. Similarly, there are significant differences in the mean fall of free ride time on the revolving rod in the rotarod test between before and after test drug i.e. (Aqueous extract - 50mg/kg, 100mg/kg, 150mg/kg) administration. i.e. the mean falls off time from the rotating rod before giving aqueous extract at doses of 50, 100, 150mg/kg are 208.3 sec, 209 Sec, 212.3 Sec and after giving test drug are 145 Sec, 80 sec and 53.16 Sec respectively, thus indicates the skeletal muscle relaxant activity of aqueous extract of ashwagandha at different doses.

Table 1: Centrally acting muscle relaxant activity of standard and aqueous extract of test drug on mice (n=6; in each group).

Groups	Dose (mg/kg)	Time (sec) of animals remained without falling from rotating rod		% of myorelaxation
		Before	After	
Diazepam (standard)	10	194	40	78.5%
		200	55	
		220	40	
		196	52	
		226	35	
		230	50	
Aqueous extract	50	190	145	30.4%
		220	130	
		180	120	
		200	140	
		220	165	
		240	170	
Aqueous extract	100	192	100	61.7%
		222	90	
		184	70	
		202	65	
		222	80	
		232	75	
Aqueous extract	150	200	45	74.9%
		230	40	
		226	81	
		196	55	
		202	50	
		220	48	

Table 2: Effect of aqueous extract of ashwagandha on muscle coordination using rotarod apparatus.

Groups	Dose (mg/kg)	Mean±SE fall off time from rotating rod (sec)		Percentage of myorelaxation (%)
		Before	After	
Diazepam (standard)	10	211	45.3	78.5
Aqueous extract	50	208.3	145	30.4
Aqueous extract	100	209	80	61.7
Aqueous extract	150	212.3	53.16	74.9

The results are expressed as means±SEM; Differences in mean values between groups was analysed by a one way analysis of variance (ANOVA). Statistical significance was assessed as $p<0.05$.

As seen in Figure 2, the percentage of muscle relaxant effect of diazepam (10mg/kg) is 78.5% and that of Aqueous extract at doses of 50 mg/kg, 100 mg/kg and 150mg/kg are 30.38%, 61.7% and 74.9% respectively. So, this indicates that Aqueous extract of Ashwagandha at doses of 50mg/kg and 100mg/kg are having less skeletal

muscle relaxant activity when compared to standard drug diazepam (10mg/kg). But the higher dose of the Aqueous extract (150mg/kg) showed a greater inhibition comparable with standard drug (diazepam). Thus, the three different doses of aqueous extract showed a dose dependent increase in percentage of myorelaxation i.e. 30.38%, 61.7% and 74.9% respectively. Maximum relaxation was observed with the dose of 150mg/kg of aqueous extract.

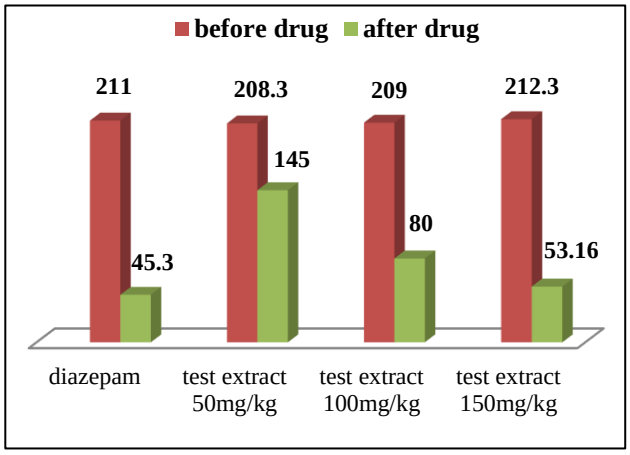

Figure 1: Fall of free ride time with diazepam and different doses of test drug.

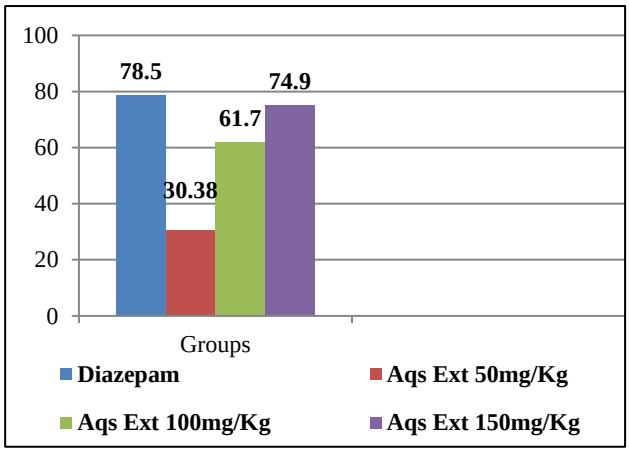

Figure 2: Percentage of muscle relaxant effect of aqeuous extract (test drug) at doses of 50mg/kg, 100mg/kg and 150mg/kg.

DISCUSSION

In a study the fat extract of *Withania somnifera* (ashwagandha) root was evaluated for anxiolytic activity.[13] Two different doses (100 and 200mg/kg), a fat extract of *Withania somnifera* was suspended in 1% gum acacia solution. The results of the study concluded that in the rota rod test, *Withania somnifera* (100mg/kg and 200mg/kg) significantly reduced the time spent by the animals on revolving rod in rota rod test when compared to control (P value <0.01). Low dose of drug (100mg/kg) did not show any significant effect at 30 and 45min time intervals. But in this present study low dose of *Withania somnifera* (50 mg/kg) also showed significant reduction in the time spent by the animals on revolving rod in the rota rod test after 60 minutes of test drug administration when compared to baseline.

In another study the polyherbal formulation consisting of hydro-alcoholic extract of leaves of *Butea frondosa*, roots of *Withania somnifera* (ashwagandha), aerial parts of *Convolvulus Pruricalis*, seeds of *Nigella sativa*, rhizomes of *Curcuma longa*, and leaves of *Azadirachta indica*, all of these drugs were screened for anti - depressant, analgesic, muscle relaxant activities.[14] The dose of HAEPHF (Hydro alcoholic extracts of polyherbal formulation) used were 500, 1000 and 2000mg/kg. The results of the study concluded that animals treated with HAEPHF (500mg/kg) did not show significant decrease in time spent on rotating rod while those treated with HAEPHF (1000 and 2000mg/kg) showed more significant decrease in time spent on revolving rod indicating the muscle relaxant activity. The test drug was found to be comparable to standard drug, diazepam (2mg/kg).

In another study, the effect of the alkaloidal fractions (acetone, alcohol and water soluble) of *Withania somnifera* on central nervous system was studied.[15] The results of this study concluded that water soluble alkaloid fraction in doses higher than 5 mg/kg dose, produced a weak generalised sedative and depressant effect on central nervous system, the effect was more significant with dose of 20mg/kg. And the alcohol soluble alkaloid fraction in dose of 32 to 64mg/kg, did not exhibit any significant neurophamacological actions. So, this study concluded that most of the neurophamacological actions of the total Alkaloids (ashwagandholine) are due to the acetone soluble alkaloidal fraction. Other two fractions, i.e. Alcohol soluble and water-soluble alkaloidal fractions are devoid of any neuropharmacological actions. In equivalent doses, acetone soluble alkaloidal fraction is 1.15 times more active than the total alkaloids. In this present study low dose of aqueous extract of *Withania somnifera* (50 mg/kg) showed significant reduction in the time spent by the animals on revolving rod in the rota rod test after 60 minutes of test drug administration when compared to baseline.

In present study, aqueous extracts at the doses of 50mg/kg, 100mg/kg and 150mg/kg showed highly significant reduction in the time spent by the animals on revolving rod in rotarod test when compared to baseline ($p < 0.0001$) which is highly significant. But when compared with standard drug diazepam, diazepam showed highly significant reduction in the time spent by the animals on revolving rod in rota rod test as compared with the two doses of aqueous extract i.e. (50mg/kg and 100mg/kg). This concludes that diazepam is having better skeletal muscle relaxant activity. But aqueous extract at a dose of 150mg/kg have showed almost equal reduction in the time spent by the animals on revolving rod in rota rod test as with standard drug diazepam. And the three different doses of aqueous extract showed a dose dependent increase in

muscle relaxant activity. Maximum muscle relaxation was observed with dose of 150mg/kg of aqueous extract.

The major biochemical constituents of ashwagandha root are steroidal alkaloids and steroidal lactones in a class of constituents called withanolides. About 12 alkaloids, 35 withanolides, and several sitoindosides from this plant have been isolated and studied. A sitoindoside is a withanolide containing a glucose molecule at carbon 27.

Much of Ashwagandha's pharmacological activity has been attributed to two main withanolides - withaferin A and withanolide D.

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing withaferin A and withanolide D possess activity against many CNS disorders.

Further biochemical and pharmacological studies are necessary to establish the exact chemical constituents and their mechanisms of action.

Further human studies are needed to prove the safety and efficacy of long-term administration of Aqueous extract of *Withania somnifera* roots.

CONCLUSION

Previous studies with polyherbral formulations containing Ashwagandha as one of the ingredients and fat extract of Ashwagandha have shown skeletal muscle relaxant activity. In the present study aqueous extracts of Ashwagandha roots have evaluated for its skeletal muscle relaxant activity by using rota rod method.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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