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Pharmacology

EVALUATION OF CENTRALLY ACTING SKELETAL MUSCLE RELAXANT ACTIVITY OF ALCOHOLIC EXTRACTS OF ASHWAGANDHA (WITHANIA SOMNIFERA) ROOTS IN ALBINO MICE.

Sarita Panigrahy — Assistant professor Department of pharmacology Gayatri Vidya Parishad Institute of Health Care and Medical Technology Madhurawada Visakhapatnam Andhra Pradesh 530048.

Sangeeta Panigrahy* — Assistant professor Department of microbiology Gems Medical college road, Aditya Educational society srikalum,Ragulu, Andhra pradesh 532484. *Corresponding Author

ABSTRACT

BACKGROUND:Skeletal muscle relaxants are drugs that are used to relax and diminish tightness in muscles. Many medicinal plants have known to have skeletal muscle relaxant activity. In past studies some Polyherbal formulation containing Ashwagandha as one of the ingredients and its fat extract have shown to have skeletal muscle relaxant activity in experimental animal models. This study is intended to evaluate the skeletal muscle relaxant activity of alcoholic extracts of Withania Somnifera (Ashwagandha) roots in albino mice, as the literature regarding this extract is scarce.

METHODOLOGY: Standard drug (diazepam), different doses of Alcohol extract of ashwagandha (50,100, 150 mg/kg) were given orally to mice and 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 among the standard drug and treated mice was taken as an index of muscle relaxation.

RESULTS:The test extract at its different doses 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. On comparison with diazepam, different doses of Alcohol extract showed weak relaxant activity.

CONCLUSIONS : The three different doses of Alcoholic extract showed a dose dependent rise in muscle relaxant action. The results are promising for further investigation of efficient skeletal muscle relaxant activity.

KEYWORDS : Ashwagandha, Albino Mice, Diazepam, Rotarod

INTRODUCTION

Lance's definition of 1980 is still relevant and is widely accepted. It states "Spasticity is a motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyper-excitability of the stretch reflex, as one component of the upper motor neuron syndrome."[1] During 16th century, European explores found that natives in the Amazon Basin of south America were using curare, an Arrow poison that produced skeletal muscle paralysis, to kill animals. The active compound, d-Tubocurarine and its modern synthetic derivatives have had a major influence on the practice of anaesthesia and surgery and have proved useful in basic mechanism involved in neuromuscular transmission. By 1943, neuromuscular blocking drugs became established as muscle relaxants in the practice of anaesthesia and surgery to facilitate endotracheal intubation and optimise surgical conditions while ensuring adequate ventilation.[2] 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 drugs of choice.[3]Most 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.As an alternative to drugs, traditional medicines derived from medicinal plants are used by about 60% of the world's population. Herbal medicines have been widely recognised by physicians and patients for their better therapeutic value, few adverse effects and low cost compared to modern medicines.WithaniaSomnifera,better known as Ashwagandha or"Indian ginseng,"has been a staple of Ayurvedic medicine for over 3000 years.[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."[5]The species name *somnifera* means "sleep-inducing" in Latin. Various studies in animal models have shown that ashwagandha has anticonvulsant[6], antidepressant[7], antianxiety[8], hepatoprotective[9], anti – inflammatory[10], immune-modulatory[11]etc.In past studies some Polyherbal formulation containing Ashwagandha as one of the ingredients have shown skeletal muscle relaxant activity[12] and its fat extract have shown to have skeletal muscle relaxant activity in experimental animal models.[13]

But the literature regarding the Alcoholic extracts of ashwagandha alone is limited. Therefore, this study is designed to evaluate the skeletal muscle relaxant activity of alcoholic extracts of WithaniaSomnifera (Ashwagandha) roots in albino mice.

OBJECTIVES

1. To measure the skeletal muscle relaxant activity of alcoholic extracts of ashwagandha (withania somnifera) roots in albino mice.
2. To compare its relaxant activity with standard drug Diazepam.

MATERIALS AND METHOD

Study Centre

The present study was carried out in the postgraduate research laboratory, Department of pharmacology, Alluri sitaramaraju academy of medical sciences, Eluru, with the approval of institutional ethical committee

Materials

Rota rod, Tuberculin syringes**,** Glass beakers, Stirring rod, Animal weighing balance**,** Animal cages**,** Stop watch.

Drugs

Normal saline, Ashwagandha roots (Aqueous and Alcoholic extract) from Laila Nutraceuticals, Vijayawada. Aqueous extract contains Total Withanolides% assay by HPLC (0.15 %), and Alcoholic extract contains Total Withanolides% assay by HPLC (2.52 %), Diazepam tablets (10mg). All drugs were dissolved in distilled water and administered orally.

Animals

Albino mice of either sex weighing about 40 – 50 grams were procured from central animal house, Department of pharmacology, Alluri sitaramaraju academy of medical sciences, Eluru and kept in cages providing food pellets and water. Ethical clearance was obtained from Institutional animal ethical committee and was implemented according to the guidelines of committee of purpose of control and supervision of the experiments on animals (CPCSEA) India.

EXPERIMENTAL METHODS

Rota Rod Test

Recording the fall off time from the rotating rod by using rota rod apparatus devised by Dunham and Miya.[14]Mice with a weight between 40 and 50 grams endure a pre-test on the apparatus. Only those animals which have proved their skill to remain on the revolving rod for at least 2 minuteswere used for the test.Mice were divided into five groups, each group comprised of six mice. Group I served as control received normal saline (10ml/kg), group II received standard drug diazepam (10mg/kg), groups III, IV, V received alcoholic extract of ashwagandha orally at a dose of 50,100 and 150 mg/kg. The fall off time from the rotating rod was noted after 60 minutes. The variance in

fall off time from the rotating rod between standard and treated mice was taken as an index of muscle relaxation.

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 alcoholic extract at a dose of 50, 100, 150mg/kg formed a significant decrease in fall off time (10.3%, 13.1%, and 16.5 % respectively) after 60minutes of oral administration. Similarly, animals with diazepam (10mg/kg) orally show a significant decrease in fall off time (78.5%) after 60 minutes.

As seen in Table 2, the mean fall of time from the rotating rod after giving standard drug (diazepam - 10mg/kg) is45.3±3.3Sec and after giving test drug (alcoholic extract – 50, 100 and 150 mg/kg) are 173.3, 179 and 163.1 sec respectively. So, present study concludes that alcoholic extracts of ashwagandha at doses of 50,100 and 150mg/kg are having less skeletalmuscle relaxant activity as compared to standard drug. And the percentage of muscle relaxant effect of diazepam is78.5% when compared to alcoholic extract at dose of 50,100 and 150 mg/kg (10.3%, 13.1%, 16.5 % respectively).

As seen in Figure 1, the mean fall of free ride time before giving standard drug (diazepam) is 211 Sec and the meanfall 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 testbetween 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. (Alcoholic extracts - 50mg/kg, 100mg/kg,150mg/kg) administration. i.e. the mean falls off time from the rotating rod before giving test extract at doses of 50, 100, 150mg/kg are 193 sec, 205.6Sec, 194.3 Sec and after giving test drug are 173.3Sec, 179sec and 163 Sec respectively, thus indicates the skeletal muscle relaxant activity of aqueous extract of ashwagandha at different doses.

As seen in Figure 2, the percentage of muscle relaxant effect of diazepam (10mg/kg) is 78.5% and that of Alcoholic extract at doses of 50 mg/kg, 100 mg/kg and150mg/kg are 10.3%, 13.1% and 16.05% respectively. So, this indicates that Alcoholic extract of Ashwagandha at doses of 50mg/kg ,100mg/kg and 150 mg/kg are having less skeletalmuscle relaxant activity when compared to standard drug diazepam (10mg/kg). Fig.2 also indicates that the three differentdoses of aqueous extract showed a dose dependent increase in percentage of myorelaxation .Maximum relaxation was observed with the dose of 150mg/kg of alcoholic extract.

Table 1: Centrally Acting Muscle Relaxant Activity Of Standard And Alcoholic 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	10	194	40	78.5%
		200	55	
		220	40	
		196	52	
		226	35	
		230	50	
Alcoholic Extract	50	182	158	10.03%
		194	172	
		188	170	
		206	190	
		190	170	
		200	180	
Alcoholic Extract	100	196	176	13.01%
		194	170	
		202	172	
		206	184	
		216	180	
		220	192	
Alcoholic Extract	150	184	159	16.05%
		192 188	162 166	
		200	164	
		206	170	
		196	158	

Table 2: Effect Of Alcoholic 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	10	211	45.3	78.5
Alcoholic extract	50	193 ± 3.5	173.3±4.4	10.03
Alcoholic extract	100	205.6±4.3	179±3.4	13.01
Alcoholic extract	150	194.3±3.3	163.1±1.8	16.05

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.

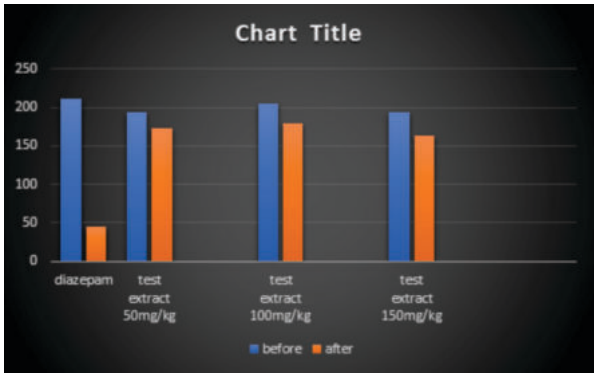

Figure 1: Fall Of Free Ride Time With Diazepam And Different Doses Of Test Drug

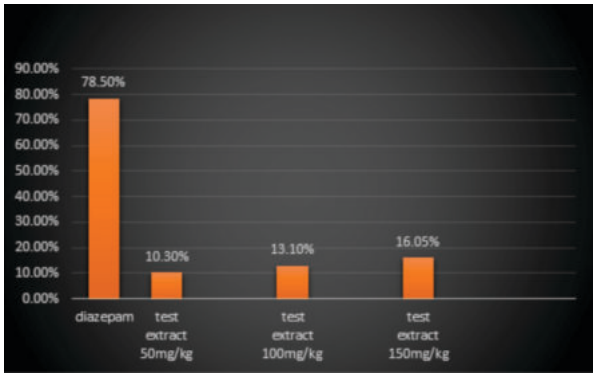

Figure 2: Percentage Of Muscle Relaxant Effect Of Alcoholic Extract (test Drug) At Doses Of 50mg/kg,100mg/kg And 150mg/kg.

DISCUSSION

In a study by Jayanthi MK et.al, (2012)[13] the fat extract of withania somnifera (Ashwagandha) root was evaluated for anxiolytic activity. A mixture of Ashwagandha root paste, ghee and water in the ratio of 1:4:16 was prepared and boiled till the water content evaporated. Ghee portion was filtered and collected in an air-tight container. The 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 rotarod test when compared to control (P value <0.01). Low dose of drug (100 mg/kg) did not show any significant effect at 30 and 45 min 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 by Mohammed Abid.et.al,(2013)[12], 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 .The dose of HAEPHF (Hydro alcoholic extracts of polyherbal formulation) used were 500, 1000 and 2000 mg/kg. The results of the study concluded that animals treated with HAEPHF (500 mg/kg) did not show significant decrease in time spent on rotating rod while those treated with HAEPHF (1000 and 2000 mg/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 (2 mg/kg).

In another study by S.Prasad and C.L Malhotra (1961)[15], the effect of the Alkaloidal fractions (Acetone, Alcohol and Water soluble) of WITHANIA SOMNIFERA on central nervous system was studied. 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 64 mg/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 Present study the alcoholic extract of Ashwagandha root at all doses showed neuropharmacological actions.

In present study, alcoholic 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 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 all three doses of alcoholic extract.

This concludes that diazepam is having better skeletal muscle relaxant activity. And the three different doses of alcoholic extract showed a dose dependent increase in muscle relaxant activity.

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 haave been isolated and studied. A sitoindoside is awithanolide containing a glucose molecule at carbon 27.

Much of Ashwagandha's pharmacological activity has been attributed to two main withanolides - withaferin A andwithanolide 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 Alcoholic 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 alcoholic extracts of Ashwagandha roots have been evaluated for its skeletal muscle relaxant activity by using rota rod method.

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