

# STUDIES ON *WITHANIA ASHWAGANDHA KAUL* (PART—VI)—THE EFFECT OF THE ALKALOIDAL FRACTIONS (ACETONE, ALCOHOL AND WATER SOLUBLE) ON THE CENTRAL NERVOUS SYSTEM

By

S. PRASAD AND C.L. MALHOTRA

Department of Pharmacology, Lady Hardinge Medical College, New Delhi

The pharmacological actions of the total extract of *Withania ashwagandha Kaul*, were reported by Malhotra, Das, and Dhalla (7,8). It was found to have sedative effect in different species of animals, biphasic actions on various smooth muscles, prolonged hypotensive, bradycardiac and respiratory stimulating actions. Subsequent investigations showed that these actions of the total extract were due to its alkaloidal content. *Withania ashwagandha* roots are known to possess a number of alkaloids (5, 13). It is possible that the variety of pharmacological actions of the total alkaloids on different systems of the body might be due to the presence of different alkaloids. The mechanism of hypotensive, bradycardiac and respiratory stimulating actions of the total alkaloids was elucidated (10). The effects of total alkaloids on different smooth muscles and central nervous system have also been reported. (11, 12). The present paper deals with the neuropharmacological actions of the three alkaloidal fractions *i.e.* acetone soluble, alcohol soluble and water soluble of total alkaloids of *Withania ashwagandha*.

## MATERIALS AND METHODS

The total alkaloids from the roots of *Withania ashwagandha* were extracted by the method described earlier (10). The total alkaloidal fraction was separated into three main fractions, *i.e.* acetone soluble (75%), alcohol soluble (16%) and water soluble (9%).

(i) Acetone soluble alkaloidal fraction was used as 2 percent suspension in 10 percent Propylene glycol using 2 percent gum acacia as suspending agent. Equivalent quantity of 2 percent gum acacia suspension in 10 percent propylene glycol was always used for control experiments. The effect of solvent control was found to be insignificant and hence omitted from text.

(ii) Alcohol soluble alkaloidal fraction was used in 10 percent alcohol.

(iii) Water soluble alkaloidal fraction was used as aqueous solution. These fractions were instilled in rabbits eye several times for period of ten minutes. There was no sign of irritation of the conjunctival sac. All drugs were administered intraperitoneally unless otherwise stated.

### 1. General effects on behaviour :

Adult albino mice (20-30 g), Albino rats (100-200 g) and mongrel dogs (3.4 to 8.5 kg) of both sexes were employed. The alkaloidal fractions were used in graded doses. General effects on behaviour were noted.

## 2. Analgesic activity :

The analgesic activity of the alkaloidal fractions was studied in albino rats by the rat tail-hot wire technique using an analgesiometer.

## 3. Anticonvulsant activity :

Effect of alkaloidal fractions was studied (a) on the supramaximal electroshock seizures (extensor tonic spasm of hind leg) induced by a convulsimeter and (b) on the maximal chemoshock seizures induced by 70 mg/kg metrazol subcutaneously in albino rats.

## 4. Hypnotic potentiating activity :

The effect of alkaloidal fractions on the sleep induced by different anaesthetics was studied. As soon as the mice had fallen asleep they were gently put on their backs. The time interval was noted when they spontaneously corrected their posture. This interval was taken as sleeping time. Nine mice were used for each study. Since the peak hypnotic effect of the alkaloid was found to be 60 minutes (12), the fractions were also administered after 60 minutes. Since water soluble and alcohol soluble fractions did not show any neuropharmacological actions further work was carried on acetone soluble fraction. Three sets of experiments were performed. In the first set, the effect of acetone soluble alkaloidal fraction on the different doses of pentobarbital was studied in mice, whilst in the second group, the effect of different doses of acetone soluble alkaloidal fraction on hypnosis induced by pentobarbital sodium 30 mg/kg was studied.

In the last group, effect of acetone soluble alkaloidal fraction on the sleep induced by hexobarbital 75 mg/kg, ethanol 3 gm/kg and urethane 1.5 gm/kg was studied.

## 5. Body temperature :

The effect of acetone soluble alkaloidal fraction was studied on the rectal temperature of mice, recorded 0, 15, 30, 60, 90 and 120 minutes after the administration of alkaloids.

## 6. Effect of lysergic acid diethylamide and dibenzylene on hypnotic potentiating activity :

Effect of lysergic acid diethylamide (LSD) 1  $\mu$ g/kg, and dibenzylene (DBZ) 5 mg/kg was studied on hypnotic potentiating action of acetone soluble alkaloidal fraction and reserpine in mice. All the drugs were injected one hour prior to the administration of pentobarbital.

## RESULTS

### A. ACETONE SOLUBLE ALKALOIDAL FRACTION :

#### 1. General effects on behaviour :

(a) *Rats and Mice*—Acetone soluble alkaloidal fraction was injected in graded doses of 75 to 300 mg/kg. In doses of 110 to 150 mg/kg and above, the animals were more docile, allowed free handling, preferred to sit quietly in corner, showed sluggish response to stimuli, diminished tone and decrease in spontaneous activity. Animals maintained power of equilibrium and righting reflex. The onset of action was within 15 minutes and lasted for 8 to 12 hours depending upon the dose. Animals abstained from food for 24 to 48 hours.

(b) *Dogs*—In doses of 40-75 mg/kg, there was tachycardia, respiratory depression, animal preferred to sit or lie down quietly in one corner. There was about 2°C decrease in rectal temperature. In dose of 150 mg/kg, there was marked depression of respiration, tachycardia followed by bradycardia, animal was lying quietly, and there was marked decrease of muscle tone and loss of equilibrium. Decrease of rectal temperature was 3°C. Duration of effect was about 2 to 10 hours depending upon the dose. There was loss of appetite for 24 to 48 hours. The results are given in Table I.

TABLE I

*Effect of Acetone Soluble Alkaloidal fraction in dogs*

Dose mg/kg	No. of dogs	Heart rate	Respiration	Rectal Temperature	Effect in General Behaviour etc.
40	2	Tachycardia	Mild depression	Decrease by 1°C	Mild depression of general activity. Prefers to sit quietly. Very co-operative. No death.
75	3	Slight tachycardia	Moderate/marked depression	Decrease by 2°C	General activity markedly depressed. Prefers to lie in one corner undisturbed. No death.
150	2	Tachycardia followed by bradycardia	Marked depression	Decrease by 3°C	Dogs constantly and severely scratch the back of the head. Marked depression of activity. Remain lying down even on disturbing. Dogs unable to stand. No death.

2. *Analgesic activity* :

In doses of 75 to 300 mg/kg, acetone soluble alkaloidal fraction had no analgesic activity in rats.

3. *Anticonvulsant activity* :

In doses of 75 to 150 mg/kg, acetone soluble alkaloidal fraction increased the total duration of convulsions induced by metrazol. It also significantly increased the death rate of metrazol treated rats. Acetone soluble alkaloidal fraction had no effect on electroshock seizures in doses of 75 mg/kg. But in doses of 100 to 150 mg/kg, it prevented the extensor tonic response of hind legs of rats. The results are given in Table II.

TABLE II

*Effect of Acetone soluble alkaloidal fraction in Maximal Metrazol Seizures and Supra-maximal electroshock seizures in rats*

Acetone soluble alkaloidal fraction mg/kg	Maximal Metrazol Seizures			Supra-maximal electro-shock seizures				
	No of Rats	Effect of acetone soluble alkaloidal fraction	Deaths	No. of Rats	Effect of acetone soluble alkaloidal fraction	Onset of action	Duration of action	Deaths
0	6		0/6	7	—	—	—	0/7
75	6	Aggravated*	0/6	6	No effect	—	—	0/6
100	6	Aggravated	2/6	9	3/9 protected**	36 min.	3 hrs	0/9
125	6	Aggravated	3/6	9	5/9 protected	30 min.	3-4 hrs	0/9
150	6	Aggravated	5/6	9	9/9 protected	30 min.	3-4 hrs	0/9

\*The total duration of convulsions increased significantly as compared to control.

\*\*Extensor tonic spasm of hind leg protected.

4. *Hypnotic potentiating activity :*

Acetone soluble alkaloidal fraction in doses of 150 mg/kg significantly enhances the duration of sleep induced by pentobarbital in mice (Table III).

TABLE III

*Effect of total alkaloids and its three alkaloidal fractions (Acetone, Alcohol and water soluble) on the hypnosis induced by pentobarbital sodium 30 mg/kg in mice*

Control Pentobarbital 30 mg/kg	Sleeping time in minutes (Mean $\pm$ S.D.)			
	Total alkaloid 20 mg/kg	Acetone soluble 150 mg/kg	Alcohol soluble 64 mg/kg	Water soluble 18 mg/kg
27.4 $\pm$ 2.7	44.72 $\pm$ 2.4	51.7 $\pm$ 1.4	24.72 $\pm$ 5.28	26.17 $\pm$ 4.7
	P < 0.001	P < 0.001	P > 0.1	P > 0.5

The effect of acetone soluble alkaloidal fraction 150 mg/kg on varying doses of pentobarbital sodium was not significant. The percentage of mice falling asleep did not change after 15, 20, 25 and 30 mg/kg of pentobarbital sodium.

With different doses of acetone soluble alkaloidal fraction *i.e.* 40, 75, 110 and 150 mg/kg, there was marked increase in hypnotic potentiating activity of pentobarbital sodium.

Acetone soluble alkaloidal fraction significantly prolongs the hypnosis induced by hexobarbital, ethanol and urethane (Table IV).

TABLE IV

*Effect of Acetone soluble alkaloidal fraction on the hypnosis induced by different anaesthetics in mice*

Control	Sleeping time in minutes (Mean $\pm$ S.D.)			
	Pentobarbital 30 mg/kg	Hexobarbital 75 mg/kg	Ethanol 3 mg/kg	Urethane 1.5 gm/kg
	21.1 $\pm$ 4.8	13.0 $\pm$ 2.4	11.3 $\pm$ 2.8	14.1 $\pm$ 1.7
Acetone soluble alkaloidal fraction 150 mg/kg	42.6 $\pm$ 4.8 P < 0.02	20.6 $\pm$ 2.5 P < 0.05	24.2 $\pm$ 2.6 P < 0.01	23.7 $\pm$ 4.3 P < 0.05

5. *Body temperature :*

Acetone soluble alkaloidal fraction significantly reduced the body temperature of mice. The onset of effect was after 15 minutes of drug administration and was maximum after 1 hour.

6. *Effect of LSD and DBZ on hypnotic potentiating activity :*

The results are given in Table V. Pretreatment of mice with LSD or DBZ did not significantly alter the hypnotic potentiating activity of acetone soluble alkaloidal fraction. On the other hand, LSD and DBZ significantly antagonised the hypnotic potentiating activity of reserpine.

TABLE V

*Effect of LSD (1 micro gm/kg) and dibenzylidine (5 mg/kg) on the Pentobarbital (30 mg/kg) hypnotic potentiating activity of acetone soluble alkaloidal fraction and reserpine in mice*

	<i>Sleeping time in minutes (Mean±S.D.)</i>		
	<i>Pentobarbital</i>	<i>Pentobarbital+LSD</i>	<i>Pentobarbital+DBZ</i>
Control	27.4±2.7	25.0±3.6 P <sub>1</sub> ** > 0.05	24.6±4.7 P <sub>1</sub> > 0.5
Acetone soluble alkaloidal fraction 150 mg/kg	51.7±1.4 P* < 0.001	54.8±7.0 P <sub>1</sub> > 0.05 P < 0.01	57.9±3.1 P <sub>1</sub> > 0.05 P < 0.01
Reserpine 1 mg/kg	47.5±2.1 P < 0.001	29.1±6.3 P <sub>1</sub> < 0.02 P < 0.02	34.2±11.1 P <sub>1</sub> < 0.5 P < 0.05

\*Probability (P) of no difference in relation to control sleeping time.

\*\*Probability (P<sub>1</sub>) of no difference in relation to sleeping time of mice without LSD or DBZ.

#### B. ALCOHOL SOLUBLE ALKALOIDAL FRACTION :

In dose of 32 to 64 mg/kg, there was no sedative effect on central nervous system and general behaviour of rats and mice.

It did not exhibit any hypnotic potentiating (Table III), anticonvulsant and analgesic activity.

#### C. WATER SOLUBLE ALKALOIDAL FRACTION :

In doses higher than 5 mg/kg, it produced a weak generalised sedative and depressant effect on central nervous system, the effect was more significant with doses of 20 mg/kg. It did not exhibit any hypnotic potentiating (Table III), anticonvulsant or analgesic activity.

#### DISCUSSION

In the present study, it has been found that most of the neuropharmacological actions of the total alkaloids (Ashwagandholine) (12) 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.

The nature of action of acetone soluble alkaloidal fraction is tranquillizer-sedative type because the animals never passed to hypnotic stage. The effects do not seem to be due to any generalised depression of central nervous system because the fraction had no analgesic activity and did not protect rats against metrazol induced seizures but on the other hand, it had aggravated the metrazol convulsions produced in rats. It resembles reserpine which has been shown to exacerbate metrazol seizures (4), but unlike reserpine, it protected the rats against electroshock seizures.

Acetone soluble alkaloidal fraction has potentiated the hypnotic action of anaesthetics belonging to different chemical groups, *i.e.* barbiturates, ethanol and urethane. The hypothermic effect of acetone soluble alkaloidal fraction was maximum after one hour of administration of the drug. Malhotra *et al.* (12) have found out that ambient temperature has a great influence on the degree of hypnotic potentiation by ashwagandholine. The same may be true for acetone soluble alkaloidal fraction. This fraction like ashwagandholine (12) resembles many other drugs *i.e.* reserpine, chlorpromazine (6), promethazine (2), thoridazine (14) and hersaponin (9). All these drugs have been reported to produce hypothermia and prolong barbiturate hypnosis.

Like ashwagandholine (12), barbiturate hypnotic potentiating action of acetone soluble alkaloidal fraction was not found to be antagonised by the serotonin-antagonist-LSD and the sympatholytic agent—DBZ. LSD and DBZ have been reported to antagonise the barbiturate hypnotic potentiating action of reserpine (1), acorus oil (3) and hersaponin (9) without affecting the potentiating action of chlorpromazine (1). Acetone soluble alkaloidal fraction did not significantly affect the threshold hypnotic dose of pentobarbital sodium in mice. Like ashwagandholine (12), it does not increase the brain sensitivity to pentobarbital sodium.

The present study indicates that the acetone soluble alkaloidal fraction is responsible for the neuropharmacological actions of the total alkaloids of *Withania ashwagandha*.

#### SUMMARY

Effect of the three alkaloidal fractions, acetone soluble, alcohol soluble and water soluble of total alkaloids of *Withania ashwagandha* has been studied on the central nervous system. Acetone soluble alkaloidal fraction had a mild depressant effect on the central nervous system (tranquillizer-sedative type) in dogs, albino rats and mice. It exacerbated the convulsions produced by metrazol but protected against supra-maximal electroshock seizures in rats. It had no analgesic activity in rats. It produced hypothermia in mice. There was potentiation of barbiturate, ethanol and urethane induced hypnosis in mice. The potentiation effect could not be antagonised by lysergic acid diethylamide and dibenzylamine. The alcohol soluble and water soluble alkaloidal fractions did not exhibit any significant neuropharmacological actions. These alkaloidal fractions are also devoid of any irritant effect on mucous membrane.

#### REFERENCES

1. Brodie, B.B., P.A. Shore and S.L. Silver. Potentiating action of chlorpromazine and reserpine, *Nature*. 175:1133, 1955.
2. Courvoisier, S., J. Fournel, R. Duerot, M. Kolsky and P. Koetschet. *Arch. Int. Pharmacodyn.* 19:305, 1953. Quoted from Malhotra *et al.*, 1962.
3. Dandiya, P.C., H. Collumbine and E.A. Sellers. Studies on *Acorus calamus* (IV)—Investigation on Mechanism of action in mice. *Jour. Pharm. & Exp. Therap.* 126:334, 1959.
4. Dews, P.B. 'Pharmacology in Medicine' Edited by V.A. Drill, 2nd Edn., McGraw Hill Book Company, Inc., 325, 1958. Quoted from Malhotra *et al.*, 1962.

5. Dhalla, N.S., K.C. Gupta, M.S. Sastry and C.L. Malhotra. Comparative studies of *Withania somnifera*. *Ind. Jour. Pharm.* **23**: 126, 1961.
6. Lessin, A.W. and M.W. Parkes. *Brit J. Pharmacol.* **12**:245, 1957. Quoted from Malhotra *et al.* 1962.
7. Malhotra, C.L., P.K. Das and N.S. Dhalla. Studies on *Withania ashwagandha* Kaul (Part I). Effect of total extract on central nervous system and smooth muscles. *Ind. Jour. Physiol. & Pharmacol.* **4**:35, 1960.
8. Malhotra, C.L., P.K. Das and N.S. Dhalla. Studies on *Withania ashwagandha*, Kaul (Part II): Effect of total extract on cardiovascular system, respiration and skeletal muscles. *Ind. Jour. Physiol. & Pharmacol.* **4**:49, 1960.
9. Malhotra, C.L., P.K. Das and N.S. Dhalla. Some Neuropharmacological actions of Hersaponin—an active principle from *Herpestis monniera*, Linn., *Arch. Int. Pharmacodyn.* **129**:1960b.
10. Malhotra C.L., P.K. Das, N.S. Dhalla and K. Prasad. The effect of total alkaloids on cardiovascular system and respiration. *Ind. Jour. Med. Res.*, **49**:448, 1961.
11. Malhotra C.L., V.L. Mehta, P.K. Das and K. Prasad. Studies on *Withania ashwagandha* Kaul (Part IV) : The effect of total alkaloids on smooth muscles. *Ind. Jour. Physiol. & Pharmacol.* **9**: 9, 1965.
12. Malhotra, C.L., V.L. Mehta, P.K. Das and N.S. Dhalla. Studies on *Withania ashwagandha*, Kaul (Part V) : The effect of total alkaloids (Ashwagandholine) on the central nervous system. *Ind. J. Physiol. & Pharmacol.* **9**:127, 1965.
13. Sastry, M.S., N.S. Dhalla and C.L. Malhotra. Chemical examination of *Withania ashwagandha* Kaul. *Ind. Jour. Pharm.* **22**:205, 1960.
14. Swinyard, E.A., H.H. Wolf, G.B. Fink and L.S. Goodman. Some neuropharmacological properties of Thoridiazine hydrochloride (Mell-aril). *Jour. Pharm. & Exp. Therap.* **126**:312, 1959.