STUDIES ON WITHANIA-ASHWAGANDHA, KAUL (PART-V) : THE EFFECT OF TOTAL ALKALOIDS (ASHWAGANDHOLINE) ON THE CENTRAL NERVOUS SYSTEM

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The total extract of *Withania ashwagandha*, Kaul, has been reported to have sedative effect in different species of animals, biphasic action on various smooth muscles, prolonged hypotensive, bradycardiac and respiratory stimulating actions (9, 10). Subsequent investigations showed that the actions of the total extract on the cardiovascular system and respiration were due to its alkaloidal content. The mechanism of hypotensive, bradycardiac and respiratory stimulating actions of the total alkaloids was also elucidated (12). The effects of total alkaloids on different smooth muscles have already been reported (14). The present paper deals with the neuro-pharmacological actions of the total alkaloids of *Withania ashwagandha*.

MATERIALS AND METHODS

The total alkaloids from the roots of *Withania ashwagandha* were extracted by the method described earlier (12). The total alkaloidal fraction has been provisionally named as 'Ashwagandholine'. A 2 per cent suspension of ashwagandholine was prepared in 10 per cent propylene glycol using 2 per cent gum acacia as suspending agent. Equivalent quantity of 2 per cent gum acacia suspension in 10 per cent propylene glycol was always used for control experiments. The effect of solvent control was found to be insignificant and hence omitted from text. A 2 per cent suspension of ashwagandholine was instilled in rabbit's eye several times for a period of 10 minutes. There was no sign of irritation of the conjunctival sac. All drugs were administered intraperitoneally unless otherwise stated.

1. *General effects on behaviour and acute toxicity :* Adult albino mice (20 to 30 gm), albino rats (120 to 220 gm), mongrel dogs (2.5 to 7.5 kg), rhesus monkeys (2.5 to 3.5 kg) and cats (2.0 to 3.0 kg) of both sexes were employed. Ashwagandholine was administered in graded doses and the general effects on behaviour were noted.

Acute toxicity was studied in albino rats and mice. Animals were observed for 48 hours. LD_{50} was calculated by the method of Litchfield and Wilcoxon (8).

2. *Analgesic activity* : The analgesic activity of ashwagandholine was studied in albino rats by the rat tail-hot wire technique using an analgesiometer.

3. *Anticonvulsant activity :* Effect of ashwagandholine was studied (a) on the supra-maximal electroshock seizures (extensor tonic spasm of hind leg) induced by a

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convulsometer and (b) on the maximal chemoshock seizures induced by 70 mg/kg Metrazol subcutaneously in albino rats.

4. *Metrazol and amphetamine toxicity*: The effect of ashwagandholine and/or reserpine was studied on the amphetamine and Metrazol toxicity in aggregated mice. Methyl-amphetamine sulphate 20 mg/kg and Metrazol 70 mg/kg were used. Ashwagandholine was administered one hour and reserpine four hours prior to the administration of the stimulants. The deaths were counted after 24 hours.

5. *Body temperature*: The rectal temperature in eight mice was recorded at 0, 15, 30, 60, 90 and 120 minutes after the administration of ashwagandholine (200 mg/kg).

6. *Hypnotic potentiating activity*: The effect of ashwagandholine on the sleep induced by different anaesthetics was studied. Eight mice were used for each study. Four sets of experiments were performed. In the first group the time of peak hypnotic effect was found by giving pentobarbital sodium 30 mg/kg after 0, 15, 30, 60, 90 and 120 minutes of administration of ashwagandholine. It was found to be 60 minutes. In the second set, the effect of ashwagandholine on the different doses of pentobarbital was studied in mice whilst in the third group, the effect of different doses of ashwagandholine on hypnosis induced by pentobarbital sodium 30 mg/kg was studied. In the last group, effect of ashwagandholine on sleep induced by hexobarbital 75 mg/kg, ethanol 3 gm/kg and urethane 1.5 gm/kg, was studied.

7. *Effect of ambient temperature on hypnotic potentiating activity*: Effect of ashwagandholine and reserpine was studied on pentobarbital sodium induced hypnosis in mice at ambient temperatures of 21°C, 28°C and 37°C. Experiments were conducted in the months of February/March when the room temperature was 21°C (20.5°C to 21.5°C). For studying the effect of 28°C and 37°C ambient temperatures on sleeping time, the experiments were conducted in a thermostatically controlled incubator. The mice were placed in the incubator at least an hour prior to the start of the experiment. In each group, eight mice were used.

8. *Effect of lysergic acid diethylamide and dibenzyline on hypnotic potentiating activity*: Effect of lysergic acid diethylamide (LSD) 1 ug/kg was studied on the pentobarbital hypnotic potentiating action of ashwagandholine and reserpine in mice. All the drugs were injected one hour prior to the administration of pentobarbital.

RESULTS

1. *General effects on behaviour* : (a) *Rats & Mice*—Ashwagandholine was administered in graded doses of 100-600 mg/kg. The effects were same in both the species of animals. In doses of 150 to 200 mg/kg, the animals were more docile and allowed free handling. In doses of 300 mg/kg and above, there was decrease in spontaneous movements, sluggish response to stimuli and diminished muscle tone. Power to maintain righting reflex was present. The onset of action was within 15 minutes and lasted for 2 to 10 hours depending upon the dose. There was loss of appetite for 24-48 hours after drug administration. LD_{50} in rats was 465 (332 to 651) mg/kg and in mice it was 432 (299 to 626) mg/kg. Animals died due to clonic convulsions and respiratory depression.

(b) *Dogs, Cats & Monkeys*—Ashwagandholine was given in doses of 50-200 mg/kg. Two to four animals were used for each dose. With dose of 50 mg/kg, there was taming effect and the animals preferred to sit quietly in the corner of the cage; if left undisturbed they went off to sleep. With 100-200 mg/kg, there was generalised depression of central nervous system including respiration. Animals were unable to stand up and response to stimuli was sluggish. The effect lasted varying from 2 to 10 hours in different animals. In surviving animals, there was loss of appetite for 24-48 hours. Typical effects in dogs are given in Table 1.

TABLE 1

WAA=Ashwagandholine=Total alkaloid

Effect of WAA in dogs

Dose mg/kg	No. of dogs	Heart rate	Respiration	Rectal Temp	Effect in General behaviour etc.
50	2	Tachycardia	Mild depression	Decrease by 3°C	General activity mildly depressed. Prefers to lie in a corner undisturbed. No death.
100	2	,,	Moderate/ marked depression	Decrease by 4°C	Dogs constantly and severely scratch the back of the head. Marked depression of activity. Remain lying down even on disturbing. Death of 2 dogs due to respiratory failure after 1 and 2½ hrs.
200	2	Tachycardia followed by bradycardia	Marked depression	Decrease by 4°C	Same as above but more marked. Dogs unable to stand. Do not move on disturbing. Dogs died of respiratory failure.

TABLE 2

WAA=Ashwagandholine=Total alkaloid

Effect of WAA on maximal Metrazol seizures and Supra-maximal electro-shock seizures in rats.

WAA mg/kg	Maximal Metrazol seizures			Supra-maximal electro-shock seizures				
	No. of Rats	Effect of WAA	Deaths	No. of Rats	Effect of WAA	Onset of action	Duration of action	Deaths
0	8	—	1/8	8	—	—	—	0/8
100	6	Aggravated*	2/6	6	No effect	—	—	0/6
200	6	Aggravated	5/6	6	No effect	—	—	0/6
400	6	Aggravated	5/6	6	Protection†	30 min.	3-5 hrs	1/6

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

†Extensor tonic spasm of hind leg protected.

2. *Analgesic activity* : Ashwagandholine in doses of 100 to 400 mg/kg had no analgesic activity in rats.

3. *Anticonvulsant activity* : The effects of ashwagandholine in doses of 100 to 400 mg/kg on the maximal Metrazol seizures and supra-maximal electro-shock seizures in rats have been given in Table 2. Ashwagandholine increased the total duration of convulsions induced by Metrazol. It also significantly increased the death rate of Metrazol treated rats, increase being from 12.5 per cent (1/8) to 66.7 per cent (12/18). Ashwagandholine had no effect on electro-shock seizures in doses of 100 to 200 mg/kg. But it prevented the appearance of extensor tonic spasm of hind legs in doses of 400 mg/kg.

4. *Metrazol and amphetamine toxicity* : The effects of ashwagandholine and/or reserpine on Metrazol and methylamphetamine toxicity in mice have been studied and results are given in Table 3. Ashwagandholine was found to significantly increase the

TABLE 3

WAA=Ashwagandholine=Total alkaloid.

Effect of WAA and/or reserpine on metrazol and amphetamine toxicity in mice.

Methyl amphetamine or Metrazol mg/kg	WAA mg/kg	Reserpine mg/kg	No. of mice	Death percent
Methylamphetamine				
20	—	—	12	25.0
20	150	—	12	66.7
20	—	1	8	0.0
20	150	1	8	75.0
Metrazol				
70	—	—	12	50.0
70	150	—	12	91.7
70	—	1	8	87.5
70	150	1	8	91.7

Metrazol and methylamphetamine toxicity in mice. Reserpine, however, decreased the methylamphetamine toxicity while increased the Metrazol toxicity in mice. Reserpine had no effect on the potentiating effect of ashwagandholine on methylamphetamine toxicity.

5. *Body temperature* : Ashwagandholine significantly reduced the body temperature of mice. The onset of effect was after 15 minutes of drug administration and was maximum after 1 hour (Fig. 1A).

6. *Hypnotic potentiating activity* : Ashwagandholine significantly enhances the duration of sleep induced by pentobarbital sodium in mice. The potentiation was maximum when pentobarbital sodium was given one hour after the administration of ashwagandholine (Fig. 1B). There seems to be time course relationship between the hypothermic and the hypnotic potentiating activities of ashwagandholine (Fig. 1).

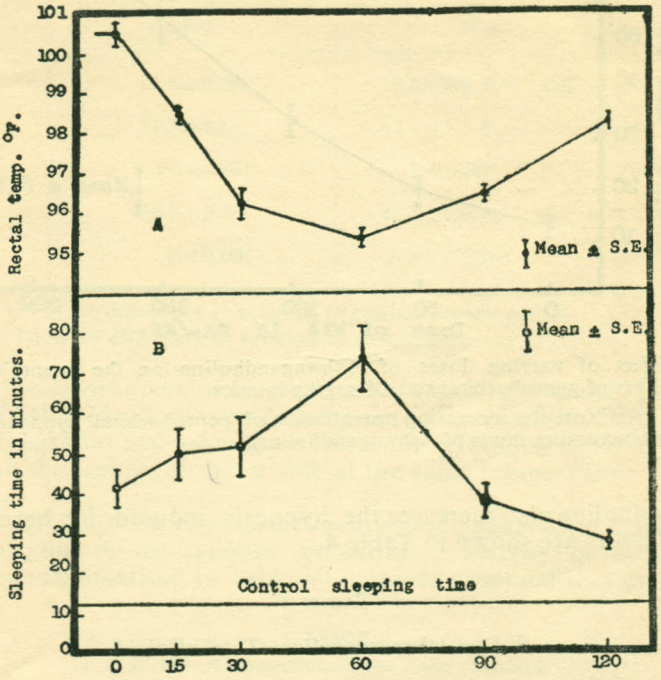

Fig. 1—*A*. Effect of Ashwagandholine 200 mg/kg on the rectal temperature in mice.

B. Effect of preadministration of Ashwagandholine 200 mg/kg at varying intervals on sleeping time of mice following pentobarbital sod. 30 mg/kg Control sleeping time is of mice treated with pentobarbital sod. alone.

Note that the hypothermic effect of ashwagandholine was maximum at 60 min. after the drug and the maximum potentiation of pentobarbital hypnosis was also when pentobarbital sod. was given 60 min. after ashwagandholine administration.

Effect of 150 mg/kg of ashwagandholine on the threshold hypnotic dose of pentobarbital sodium in mice was not significant as the percentage of mice falling asleep did not change after 15, 20, 25 and 30 mg/kg.

With different doses of ashwagandholine i.e., 50, 100, 150 and 200 mg/kg there was marked increase in the hypnotic potentiating activity of pentobarbital sodium (Fig. 2).

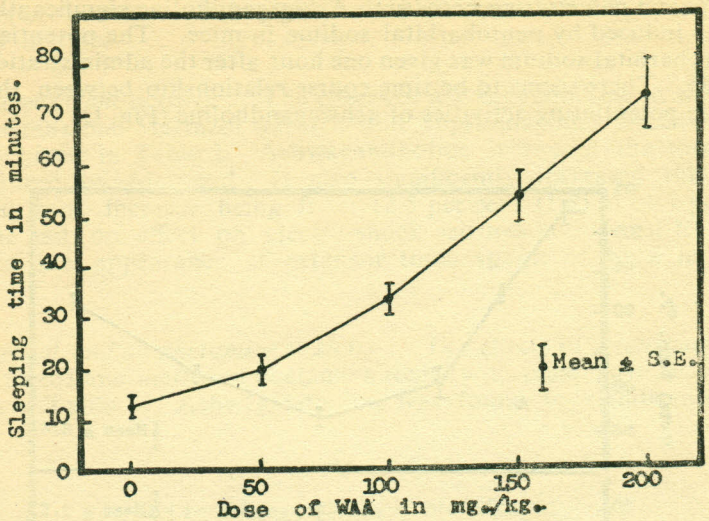

Fig. 2—Effect of varying doses of Ashwagandholine on the hypnotic effect of pentobarbital sod. 30 mg/kg in mice.

Note the increasing potentiation of pentobarbital hypnosis by increasing doses of Ashwagandholine.

Ashwagandholine also increases the hypnosis induced by hexobarbital, ethanol and urethane. Results are shown in Table 4.

TABLE 4

WAA=Ashwagandholine=Total alkaloid

Effect of WAA on the hypnosis induced by different anaesthetics in mice.

	Sleeping time in minutes (Mean ± S. E.)			
	Pentobarbital 30 mg/kg	Hexobarbital 75 mg/kg	Ethanol 3Gm/kg	Urethane 1.5 Gm/kg
Control	9.8 ± 0.9(1)*	57.4 ± 7.1(1)	10.3 ± 2.2(1)	53.1 ± 4.4(1)
W A A	43.3 ± 2.0(4.4)	98.1 ± 3.2(1.7)	21.8 ± 4.0(2.1)	251.2 ± 10.8(4.7)
150 mg/kg	P∠0.01	P∠0.01	P∠0.05	P∠0.01

*Figures within parentheses give the mean sleeping time ratio as compared to control.

7. *Effect of ambient temperature on the hypnotic potentiation activity* : The results have been summarised in Table 5. The increase in the ambient temperature from 21°C to 37°C did not significantly alter the control pentobarbital sleeping time. But the

TABLE 5

WAA=Ashwagandholine=Total alkaloid.

Effect of WAA and reserpine on pentobarbital sodium (30 mg/kg) sleeping time at 21·c, 28·c and 37·c ambient temperatures.

	Sleeping time in minutes (Mean±S. E.)		
	21.c	28·c	37·c
Control	12.5±0.06	9.8±0.9 P_1**>0.05	10.9±0.08 P_1>0.05
WAA	54.3±4.6	42.1±1.5 P_1	20.8±3.1 P_1 0.0
150 mg/kg	P*<0.001	P<0.001	P 0.0
Reserpine	76.8±9.3	43.3±4.9 P_1	26.5±3.2 P_1 0.0
1 mg/kg	P<0.001	P<0.001	P 0.0

*Probability (P) of no difference in relation to control sleeping time.
**Probability (1_1) of no difference in relation to sleeping time at 21·c.

same increase in the environmental temperature markedly decreased the hypnotic potentiating activity of ashwagandholine and reserpine. However, the sleeping time of mice treated with pentobarbital and ashwagandholine (or reserpine) at 37°C was still significantly higher than the control sleeping time at the same temperature.

8. *Effect of LSD and dibenzyline on hypnotic potentiating acitivity* : The effects of LSD and dibenzyline on the hypnotic potentiating activity of ashwagandholine and reserpine have been summarised in Table VI. It was observed that pretreatment of mice

TABLE 6

WAA=Ashwagandholine=Total alkaloid

Effect of LSD (1 microgm/kg) and dibenzyline (5 mg/kg) on the pentobarbital (30 mg/kg) hypnotic potentiating activity of WAA and reserpine in mice.

	Sleeping time in minutes (Mean ± S. E.)		
	Pentobarbital	Pentobarbital + L S D	Pentobarbital +Dibenzyline
Control	12.8±0.7	13.2±0.9 P_1**>0.05	13.4±0.8 P_1>0.05
W A A	50.5±5.4	47.8±4.8 P_1>0.05	56.9±4.2 P_1>0.05
150 mg/kg	P*<0.001	P<0.001	P<0.001
Reserpine	80.4±6.9	36.2±2.8 P_1<0.01	40.1±4.8 P_1<0.01
1 mg/kg.	P<0.001	P<0.01	P<0.01

*Probability (P) of no difference in relation to control sleeping time.
**Probability (P_1) of no difference in relation to sleeping time of mice without LSD or dibenzyline.

with LSD or dibenzyline did not significantly alter the hypnotic potentiating activity of ashwagandholine. However, dibenzyline and LSD signicantly antagonised the hypnotic potentiating activity of reserpine.

DISCUSSION

Ashwagandholine has been found to have a depressant effect on the cerebral functions in albino rats, mice, dogs, cats and monkeys. In low dosages it made the animals more docile and co-operative, and the animal could be easily handled. The depression on the whole was of a tranquillizer— sedative type because the animals never passed to hypnotic stage. However, this effect did not appear to be due to any generalised depression of central nervous system because it had no analgesic activity and did not protect rats against Metrazol induced seizures. On the other hand it aggravated the convulsions produced by Metrazol in rats and increased Metrazol toxicity in rats and mice. In these respects it resembles the tranquillizer—reserpine ; which has been shown by Dews (5) to exacerbate Metrazol seizures. But unlike reserpine, it protected the rats against electroshock seizures in toxic doses.

Burn and Hobbs (2) reported that reserpine and chlorpromazine pretreatment reduces the mortality in amphetamine treated aggregated mice. In the present investigations, ashwagandholine has been found to aggravate amphetamine toxicity in mice. It may be due to the fact that ashwagandholine is the total alkaloidal fraction i e. a mixture of many alkaloids.

Ashwagandholine has been found to potentiate the hypnotic action of anaesthetics belonging to different chemical groups i. e. barbiturates, ethanol and urethane. This potentiation was observed even with a low dose of 50 mg/kg (approximately 1/9 of LD_{50} in mice). It is of interest to note that even with 100 mg/kg of ashwagandholine alone, there was no detectable effect on the behaviour of rats and mice. It has been shown that the drug produced hypothermia, was maximum after one hour of drug administration. The hypnotic potentiating action of ashwagandholine has also been found to be maximum after one hour of administration of the drug. It was further observed that the ambient temperature had a great influence on the degree of hypnotic potentiation by ashwagandholine. An increase in ambient temperature from 21°C to 37°C, (when drug induced hypothermia is not expected to occur) markedly reduced but did not abolish the potentiating action of ashwagandholine. The results show that the hypnotic potentiating action of the drug is closely related to the hypothermic action of the drug. However, this is not the sole mechanism because even at 37°C ashwagandholine could prolong significantly the sleep induced by pentobarbital. Ashwagandholine, therefore, resembles many other drugs e. g. reserpine, chlorpromazine (7), promethazine (3), thoridazine (16) and hersaponin (11), which have all been reported to prolong barbiturate hypnosis and to produce hypothermia.

Barbiturate hypnotic potentiating action of ashwagandholine was not found to be antagonised by the serotonin antagonist—LSD and the sympatholytic agent—dibenzyline. LSD has been reported to antagonise the barbiturate hypnotic potentiating action of reserpine (1), acorus oil (4), and hersaponin (11), without affecting the potentiating action of chlorpromazine (1). Recently, it has been shown that pretreatment with dibenzyline can also antagonise the hypnotic potentiating activity of reserpine, acorus oil and hersaponin without affecting the action of chlorpromazine (12).

Ashwagandholine did not significantly affect the threshold hypnotic dose of pentobarbital sodium in mice indicating that it does not increase the brain sensitivity to pentobarbital sodium.

The earlier investigations (9), showed that the total extract of withania ashwagandha had a sedative effect in different species of animals and also pentobarbital hypnotic potentiating activity in mice. A comparison of the central actions and toxicity of the total extract and the total alkaloids shows that the latter are approximately more than twice as active as the total extract. In the case of total extracts the deaths of the animals following intraperitoneal injection were due mainly to peritonitis because of the irritant effect of the extract. The total alkaloids, however, have been found to be devoid of any irritant effect on rabbit's eye and the deaths were usually due to respiratory failure.

Withania ashwagandha roots are known to possess a number of alkaloids (6, 15). Therefore, it is possible that the variety of pharmacological actions, that the total alkaloids have on different systems of the body, might be due to the presence of different alkaloids.

SUMMARY

Effects of total alkaloids of Withania ashwagandha (Ashwagandholine) roots have been studied on the central nervous system. Ashwagandholine had a taming effect and a mild depressant effect on the central nervous system (tranquillizer—sedative type) in monkeys, cats, dogs, albino rats and mice. It had no analgesic activity in rats. It exacerbated the convulsions produced by Metrazol but in toxic doses protected against supra-maximal electroshock seizures in rats. It also increased Metrazol toxicity in rats and mice and amphetamine toxicity in mice. It produced hypothermia in mice. There was potentiation of barbiturate, ethanol and urethane induced hypnosis in mice. The potentiation of barbiturate induced hypnosis was related to the hypothermic action of ashwagandholine. The potentiating effect could not be antagonised by d-lysergic acid diethylamide and dibenzyline. Intraperitoneal LD_{50} in rats was 465 mg/kg. and LD_{50} in mice was 432 mg/kg. Death of animals were usually because of respiratory failure. Ashwagandholine appeared to be approximately more than twice as active as the 'total extract'. The total alkaloids are also devoid of any irritant effect on mucous membrane.

ACKNOWLEDGEMENT

The authors are thankful to Shri S. S. Chatwal, P. Box 522, New Delhi for supplying genuine crude drugs.

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