STUDIES ON WITHANIA ASHWAGANDHA (PART I): EFFECT OF TOTAL EXTRACT ON CENTRAL NERVOUS SYSTEM AND SMOOTH MUSCLES*

By

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Uptill now two species of Withania, belonging to the family Solanaceae, had been described viz. W. coagulans, Dunal (Hindi : Akri; Sanskrit: Ashvagandha) and W. somnifera, Dunal (Hindi : Asgand; Sanskrit: Ashvagandha). Recently Kaul (1957) has identified another species- W. ashwagandha which is closely similar to W. somnifera and both till lately have been confused as the same plant. W. somnifera has been used in Ayurvedic and Yunani systems of medicine as a tonic for debilitating diseases, nervine sedative, anthelmintic, diuretic, deobstruent and aphrodisiac. It has also been used for asthma, rheumatism, uterine disorders and lumbar pains, and locally for carbuncles, ulcers and painful swellings (Kirtikar and Basu, 1933; Nadkarni, 1954). The scientific investigations on this plant date back to 1886 when Trebut reported the sedative effect of the plant due to an alkaloid. Power and Salway (1911) reported the presence of an alkaloidal principle but could not confirm the sedative effect in dogs. Majumdar (1955) claims to have isolated eight alkaloids. As no systematic pharmacological investigations of any of the species of Withania have yet been conducted, detailed pharmacological studies were taken up. The present report deals with the actions of roots of W. ashwagandha on central nervous system and smooth muscles.

MATERIALS AND METHODS

The air dried roots of W. ashwagandha were pulverized and extracted with 70 percent ethyl alcohol on a water bath. The residue was re-extracted twice and the hot filtrates were mixed and concentrated under reduced pressure till it was alcohol free. A few samples were completly dried and the average percentage extractive was found to be 11.9 percent of the roots. Aqueous suspensions were taken for all pharmacological studies. Equivalent quantity of water was used for control experiments in all cases. Doses have been expressed in terms of dried extractive.

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For central nervous system and toxicity studies adult albino mice (20 to 35 gm.), albino rats (150 to 230 gm.), rhesus monkeys (3 to 4.5 kg.), mongrel dogs (7 to 12 kg.) and rabbits (1.5 to 2.3 kg.) of both sexes were employed. The extract was administered intraperitoneally in mice, rats, monkeys and rabbits, intravenously into tail vein of mice, and orally through a tube in rats, monkeys and dogs in the fasting state. In four dogs extract was also administered intraduodenally. Duodenum was exposed under ether anaesthesia and through a small nick a small rubber tube was introduced into the duodenum and secured to the duodenal wall with a few stitches. The other end of the tube was brought out of the adbomen and the adbominal wall was closed. The tube was closed with a screw clip. No solid food was allowed for 24 hours, after which the extract was introduced into the duodenum through the tube. Control dogs were only given distilled water.

Potentiation of pentobarbital sleeping time was studied in mice. Sleep was induced in mice by intraperitoneal pentobarbital sodium 1.5 to 5.0 mg./100 gm. After the mice had fallen asleep they were laid on their backs. Time of onset of hypnosis was recorded as the time required for loss of righting reflex after injection of drug. The waking time was taken as that at which the mice righted themselves and began to move away. About half the animals were pretreated with intraperitoneal extract of W. ashwagandha and the other half with distilled water 15 minutes prior to pentobarbital administration.

Analgesic activity of the extract following intraperitoneal injection in albino rats, was tested by the rat tail-hot wire technique with the help of an analgesiometer. Control responses had been between 5 and 10 seconds, and if the rats did not respond till 30 seconds, it was recorded as analgesia. Anticonvulsant activity was tested against convulsions induced by subcutaneous metrazol 8.0 mg./100 gm. in albino rats.

ED_{50} and LD_{50} were calculated by the method of Litchfield and Wilcoxon (1949). Six to eight dosage levels were taken between 0 and 100 percent effects and an average of 13 (S. E. 4.7) animals were used for each dose.

For effects on smooth muscles, contractions of isolated rabbit's and rat's ileum, and rat's uterus suspended in a bath containing Ringer's solution were recorded. Tracheal chains were prepared by tying 2 rings of isolated trachea of dog together with cotton thread. Rings were later opened by cutting through the cartilages and suspended in an organ bath containing Kreb's solution. Spasms produced by acetylcholine 4×10^{-7} to 2×10^{-6} for 60 seconds were recorded on smoked drum.

In addition, four dogs were anaesthetised with pentobarbital sodium and the contractions of ileum in situ were recorded on smoked drum with the help of Cushney's myocardiograph. Carotid arterial pressure was also recorded.

RESULTS

1. Central nervous system :—

(a) *Albino rats and mice.*—Table 1 summarizes the sedative and analgesic activities of the extract and its acute toxicity in rats and mice.

TABLE 1.

The sedative and analgesic activities of the extract and its acute toxicity in rats and mice.

Animal species	Route of administration.	Range of doses tested mg./100gm.	No. of animals used.	Action	ED_{50} (19/20 Confidence limits) mg./100 gm.	LD_{50} (19/20 Confidence limits) mg./100 gm.	T.I. *
Mice	I. P.	20 to 140	107	Sedation	32.0 (24.6-41.6)	112.2 (97.6-129.0)	3.5
Mice	I. V.	10 to 70	113	Sedation	20.9 (16.1-27.2)	58.2 (55.4-61.1)	2.8
Rats	I. P.	75 to 200	84	Sedation	94.8 (88.6-101.5)	119.4 (111.6-127.8)	1.3
				Analgesia	101.2 (94.6-108.3)		1.2
				Sedation	398.1		
Rats	Oral	150 to 450	60		(318.4-497.5)	**	**

*T. I.—Therapeutic index, LD_{50}/ED_{50}.

** —There was no death upto the dose of 450 mg./100 gm.

Rats and mice showed depression of central nervous system following administration of the extract. There was absence of spontaneous movements, sluggish or no response to stimuli, diminution of muscle tone, inability to maintain equilibrium and delayed righting reflex. Mice were about three times more sensitive to the sedative effect of the extract as compared to rats, LD_{50} being nearly the same in both species. Onset of effect was immediate

on intravenous administration, within 15 minutes on intraperitoneal injection and within one hour on oral administration, and lasted from 30 minutes to more than 8 hours depending upon the dose. The deaths on intravenous administration were immediate or within few minutes due to respiratory failure. Following intraperitoneal administration the animals usually died 12 to 36 hours after injection but in few, deaths were within 2 hours due to respiratory failure. A few animals were given dosages nearing LD_{50}, and out of those who survived some were sacrificed after 24 hours while others after 48 hours of drug administration. It was found that those who had been injected intraperitoneally suffered from peritoneal inflammation. These animals also generally abstained from food. The extract could not be given in doses higher than 45 mg./100 gm. orally due to increased bulk and the extract used to be expelled out.

The extract had respiratory stimulant action in lower dosages in albino rats on oral and intraperitoneal administration. But in doses of 150 to 200 mg /100 gm. intraperitoneally the initial stimulation was followed by depression and few of the animals died of respiratory failure.

The extract in doses of 100 to 150 mg./100 gm. did not protect the rats against metrazol induced convulsions.

Potentiation of pentobarbital induced sleeping time.—The effect of the extract on the mean sleeping time of mice with pentobarbital sodium is given in Table 2. The extract, though in itself had no hypnotic effect in white mice, potentiated the sleep induced by pentobarbital sodium. The number of mice falling asleep was higher, onset of sleep was earlier and duration of sleep was much prolonged in the group pretreated with the extract, as compared to control group.

(b) *Monkeys.*—The extract had a sedative effect in monkeys both when given orally and intraperitoneally.

It induced vomiting and diarrhoea on oral administration. Post mortem examination of dead monkeys who were given the extract intraperitoneally showed that the abdominal cavity was full of foul smelling pus with stomach distended. The results have been summarised in Table 3.

TABLE 2.

Hypnotic effect of pentobarbital sodium, alone and in mice pretreated with W. ashwagandha.

Pentobarbital sod. mg./100 gm. I. P.	W. ashwagandha mg./100gm. I. P.	No. of mice used	Hypnotic effect % mice **	Latent period in min. (Mean±S. E) ***	Sleeping time in min. (Mean±S. E.) ***
1.5	—	35	22.9	12.1±1.0 *	1.8±1.7
1.5	50	12	25.0 (P>0.05)	11.6±1.7 * (P>0.05)	2.2±2.1 (P>0.05)
1.5	60	12	41.7 (P>0.05)	12.6±1.4 * (P>0.05)	2.9±2.4 (P>0.05)
1.5	70	12	50.0 (P<0.05)	12.2±1.6 * (P>0.05)	3.9±4.9 (P<0.05)
1.5	80	24	100.0 (P<0.01)	8.2±2.2 (P<0.01)	29.4±15.0 (P<0.01)
2.0	—	11	63.6	11.6±2.5 *	14.5±12.2
2.0	70	12	100.0 (P>0.05)	7.8±0.6 (P<0.01)	30.2± 8.4 (P<0.01)
2.5	—	18	88.9	9.5±2.8 *	29.7±17.5
2.5	70	18	100.0 (P>0.05)	5.4±0.7 (P<0.01)	59.0±30.8 (P<0.01)
5.0	—	6	100.0	4.5±2.5	81.7±26·7
5.0	70	12	100.0	2.2±0.3 (P<0.01	135.0±30.6 (<0.01)

*Latent period of those mice which slept.

**Probability (P) calculated by the X^2 test, in relation to sleep induced by pentobarbital sod. alone.

***Probability (P) calculated by the 't' test, in relation to sleep induced a by pentobarbital sod. alone.

TABLE 3

Effect of W. ashwagandha extract on monkeys.

Route of administration.	Dose in gm /kgm.	No. of Monkeys Used.	Effect.
Intraperitoneal.	0.14	2	Monkeys were quiet; lying down if undisturbed; response to stimuli quick but not aggressive; did not take food; effect for 24 hrs. No death.
„	0.35	2	Monkeys were quiet; sleeping if undisturbed; otherwise dosing; response to stimuli slow; not aggressive; effect for 24 hrs. Did not take food and died after 10 to 15 days.
„	0.7	2	Same effect as with 0.35 g./kg. but continued for 5 to 7 days when the animals died.
Oral	0.7	2	Monkeys quiet; ptosis present; sometimes dosing; less aggressive; took food but vomited; diarrhoea. Effect for 24 hours. No death.
„	1.4	2	Same as above but more in degree. Effect lasted for 48 hours. No death.

(c) *Dogs.*—The extract produced vomiting, diarrhoea and increased salivation. The sedative effect was mild in nature. The results have been summarised in Table 4.

TABLE 4

Effect of W. ashwagandha extract in dogs.

Route of administration.	Dose in gm /kgm.	No. of dogs used.	Effect.
Oral	0.28	2	Dogs avoided interference by hiding somewhere.
,,	0.56	4	Salivation and vomiting. Dogs were drowsy when undisturbed.
,,	0.70	5	Marked salivation and vomiting. Dogs were drowsy and mildly sedated. Diarrhoea.
,,	1.40	2	Marked salivation and vomiting. Dogs drowsy and sedated. Diarrhoea.
Intraduodenal.	0.70	4	Salivation and vomiting.* Dogs were drowsy and sedated. Diarrhoea.

Note :* Vomitus did not contain the drug.

(d) *Rabbits.*—The extract in doses of 0.28 gm./kg. I.P. did not produce any significant response but with 0.56 gm./kg. there was sedative effect. The rabbits were sitting quietly, allowed handling freely and lost aggressiveness towards their own species.

In 4 rabbits W. ashwagandha extract was instilled in one eye in concentrations of 5 percent and 10 percent, in the other, the control eye, distilled water was instilled. The extract produced congestion of the conjunctiva, slight constriction of pupil and the eye got covered with nictitating membrane in 10 percent concentration. The effects passed off within 24 hours. It had no local anæsthetic effect.

2. Smooth muscles :—

(a) *Ileum.*— (i) *Isolated rabbit's ileum.*— The effects of the extract on the isolated rabbit's ileum have been given in Table 5 and Fig. 1.

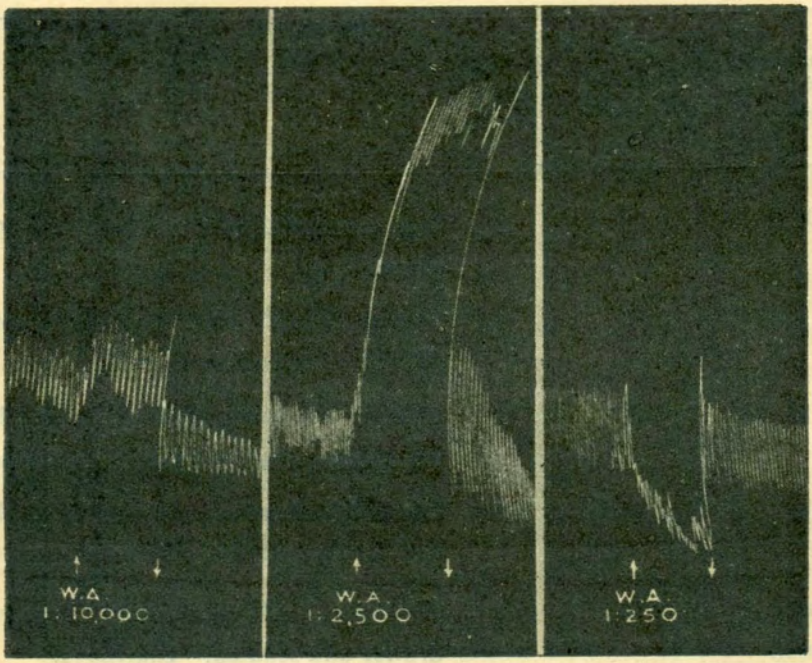

Fig. 1

Effeet of W. ashwagandha on isolated rabbit's ileum. Contraction is upwards.

Note the increase in intestional tone in low concentrations and relaxation with diminished rhythmic movements in higher concentrations.

Intestinal spasms were produced by acetylcholine and barium chloride to test for antispasmodic actions. Pretreatment of an ileal segment with the extract in concentrations of 2×10^{-3} to 10^{-2} markedly reduced or prevented acetylcholine 2×10^{-7} to 10^{-6} and barium chloride (10^{-5} to 4×10^{-5}) induced spasms. Similarly, the extract added to the bath at the height of acetylcholine and barium chloride induced spasms was an effective antispasmodic (Fig. 2.).

(ii) *Isolated rat's iteum.*— The extract decreased the tone and rhythmic movements of isolated rat's ileum in concentrations of 2×10^{-4} to 10^{-2}. In lower concentrations it had no significant action. It effectively prevented and also antagonised acetylcholine (10^{-7} to 10^{-6}) induced spasms, in concentrations of 2×10^{-4} to 10^{-2}.

TABLE 5

The action of W. ashwagandha on rabbit's ileum.

Conc. of the extract.	Action on intestinal tone	Action on rhythmic movements	
10^{-5} to 4×10^{-5}	No effect	No effect	
10^{-4}	Slight increase	No effect	
2×10^{-4} to 4×10^{-4}	Slight to marked increase	Slight increase or slight diminution	Increased rhythmic movements on washing
10^{-3}	Slight to moderate increase or slight decrease	Slight increase or slight diminution	Increased rhythmic movements on washing
2×10^{-3} to 10^{-2}	Moderate to marked relaxation	Slight or marked diminution	Increased rhythmic movements on repeated washing

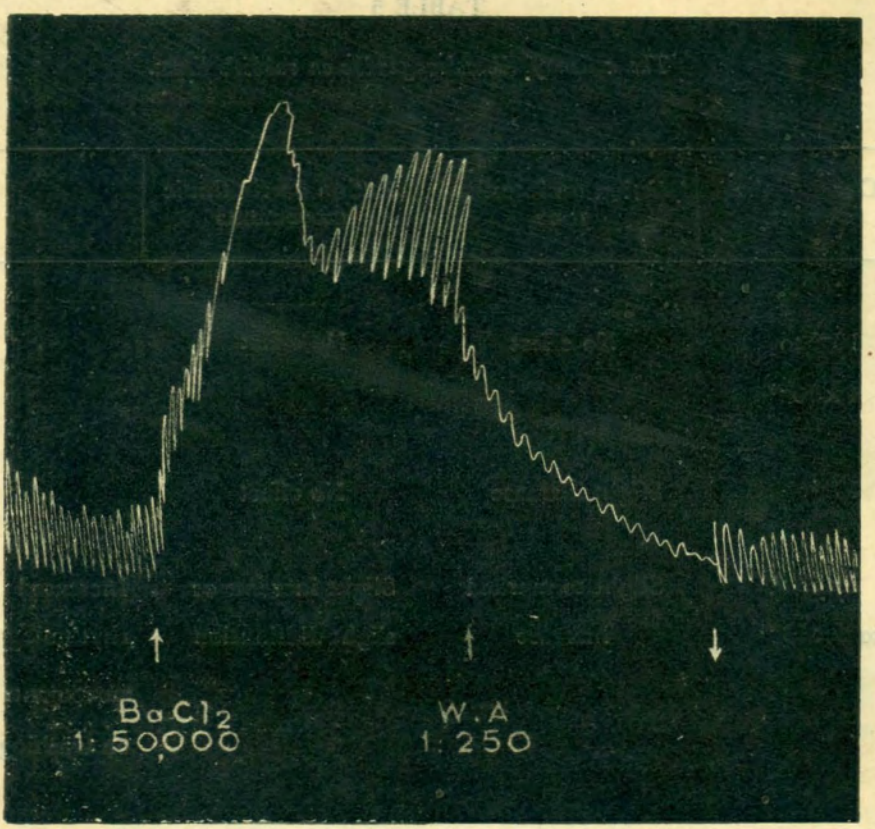

Fig. 2

Effect of W. ashwagandha 1:250 on Barium chloride (1:50,000) induced spasm in isolated rabbit's ileum.

Note the antispasmodic action.

(iii) *Intact dog's ileum.*—The extract in doses of 10 to 70 mg./kg. I.V. produced a temporary increase in intestinal tone followed by relaxation and diminution of rhythmic movements. The effect lasted for 30 minutes to 2 hours depending upon the dose administered. It also produced hypotension and bradycardia.

(b) *Trachea.*—The results have been given in Table 6 and Fig. 3.

TABLE 6

The action of W. ashwagandha on trachea of dog.

Conc. of W. ashwagandha	Action on tone of trachea	Ach. spasm in pretreated muscle	
2×10^{-4} to 4×10^{-4}	No, silght or moderate spasm	Slight increase	Normal on washing.
10^{-3} to 2×10^{-3}	No, slight or moderate spasm	Moderate Increase	Control ach. responses restored on repeated washing.
4×10^{-3} to 10^{-2}	No, or slight spasm	Slight increase or decrease	Control ach. responses restored on repeated washing.
2×10^{-2}	No, or slight spasm	Decrease	Depressed even on repeated washing

The extract had either no effect or increased the tone of tracheal muscle.

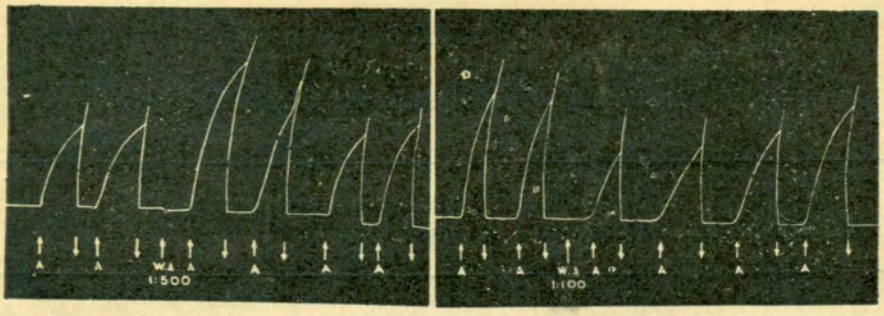

Fig. 3

Effect of W. ashwagandha on the tracheal chain of dog against acetylcholine (1:500,000) induced spasm. The trachcal chain was pretreated with W. ashwagandha for 5 minutes at the point marked W. A.

(Acteylcholine).—(A) was allowed to act for 1 minute each time.

Note that in low concentrations it potentiated but in higher concentrations reduced the acetylcholine spasm.

The spasm could not be antagonised significantly with atropine sulph. (10^{-4}). But the latter could prevent the spasmodic effect of W. ashwagandha to an appreciable degree. Pretreatment of tracheal muscle with comparatively low concentrations of W. ashwagandha for 2 to 5 minutes increased the subsequent acetylcholine induced spasm but in high concentrations the effect was opposite.

(c) *Uterus.*—The extract in concentrations of 10^{-3} and 2×10^{-3} reduced the rhythmicity and amplitude of contractions of isolated uterus of white rat. In concentrations of 4×10^{-3} and 10^{-2} it completely inhibited uterine contractions, (Fig. 4).

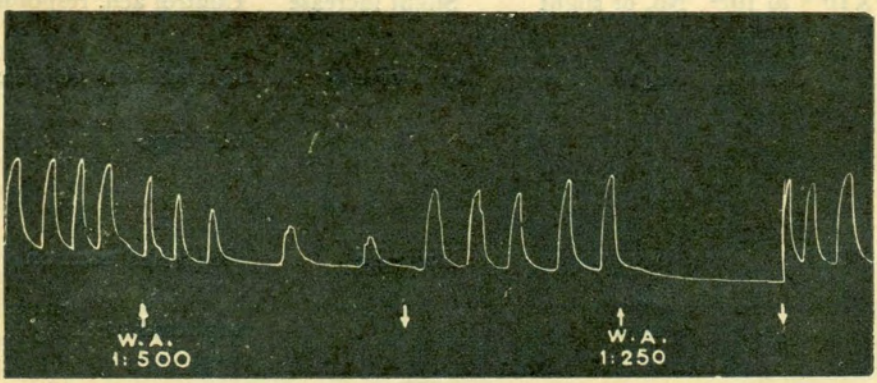

Fig. 4

Effect of W. ashwagandha 1:500 on isolated rat's uterus.

Note the depression of uterine contractions.

DISCUSSION

The investigations show that W. ashwagandha has a sedative effect on the central nervous system, specially so in white mice and to a lesser extent in monkeys, dogs, rabbits and albino rats. It also potentiates the hypnotic activity of pentobarbital in mice. It has no anticonvulsant action. The analgesic action seen in rats appears to be due to a generalised depression of the nervous system and not due to any specific analgesic action.

The extract appears to have a spasmodic effect on ileum and tracheal smooth muscles in low concentrations but in higher concentrations it relaxes the intestines and has antispasmodic action against acetylcholine and barium chloride induced spasms. Atropine could only partially antagonise the stimulant effect of the extract on smooth muscles. The uterus of rat is, however, always relaxed. Unlike other Solanaceous plants it does not have a mydriatic effect.

The extract shows irritant properties on mucous and serous membrane, conjunctiva and peritoneum. Diarrhoea and vomiting on oral administration are most probably due to the direct irritant effect. The deaths on intraperitoneal administration were mostly due to inflammation of the peritoneum. The same factor might be responsible for the spasmodic effect on smooth muscles seen in low concentrations.

The investigations justify the existing use of the drug as a nervous sedative and uterine sedative. The anthelmintic action might be due to the increased intestinal peristalsis and irritant effect on worms. The detailed study of the hypotensive effect and its other cardio-vascular actions will be the subject of next communication.

SUMMARY

1. Effects of 70 percent alcoholic extract of W. ashwagandha roots have been studied on the central nervous system and smooth muscles.

2. The extract has sedative effect in white mice, dogs, monkeys, rabbits and white rats. It significantly potentiates the hypnotic effect of pentobarbital sodium in mice.

3. It has no anticonvulsant action.

4. It has spasmodic action on ileal and tracheal muscles in small doses but in large doses it has relaxant and antispasmodic actions. It relaxes uterus.

5. LD_{50} in rats is 119.4 mg./100 gm. I. P., and in mice 112.2 mg./100 gm. I. P. and 58.2 mg./100 gm. I. V.

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