

# STUDIES ON WITHANIA ASHWAGANDHA, KAUL, (PART IV) THE EFFECT OF TOTAL ALKALOIDS ON THE SMOOTH MUSCLES\*

By

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The pharmacological actions of the total extract of the roots of *Withania ashwagandha* were reported by Malhotra, Das and Dhalla (3, 4). It was found to have sedative effect in different species of animals, biphasic action on various smooth muscles, prolonged hypotensive, bradycardiac and respiratory stimulating actions. Sastry, Dhalla and Malhotra (7) have isolated the total alkaloids, dulcitol, glucose, hentriacontane and inorganic salts from the roots of the plant. The effects of the total alkaloids of *Withania ashwagandha* on cardio-vascular system and respiration have been analysed in detail and reported in an earlier communication (5). The present paper deals with the effect of the total alkaloids on various smooth muscles.

## MATERIALS AND METHODS

Four gm. of the total alkaloids were dissolved in 10 c.c. alcohol and diluted with distilled water to give a final concentration of 4 percent suspension of total alkaloids. Equivalent quantity of 10 per cent alcohol was always used for control experiments. The effect of solvent control was found to be insignificant and hence omitted from the text. In all experiments papaverine was used for comparison.

1. *Isolated smooth muscles* :—Effects of the total alkaloids of *Withania ashwagandha* (Ashwagandholine) were studied on isolated ileum of rabbit, guinea pig and albino rat, uterus of albino rat and tracheal chain of dog by the routine methods. The antispasmodic effect of Ashwagandholine was tested against spasms induced by acetylcholine chloride, histamine diphosphate, barium chloride and serotonin creatine phosphate. Approximate  $ED_{50}$  was calculated by plotting log-dose percentage inhibition curve. Different doses of the drugs were used against each spasmogen and at least five experiments were conducted for each dose.

2. *Rat hind leg perfusion* :—The hind legs of albino rats were perfused by the technique of Burn (1). The perfusion rate was recorded on a smoked drum using a Condon's drop recorder. The antispasmodic effect of the drugs was tested against barium chloride induced vasoconstriction.

3. *Experimental asthma in guinea pig* :—Experimental asthma was produced in guinea pigs by exposing them to aerosols of 5 per cent acetylcholine chloride and 1.5 per cent histamine diphosphate at a pressure of 300 mm. of mercury. The animals were pretreated with either Ashwagandholine or papaverine intraperitoneally and after an interval of 45 to 60 minutes were exposed to the appropriate aerosol challenge. The control animals were pretreated with the same volume of blank solvent. The period of

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exposure before the animals developed asthma was noted. If no asthma developed within 600 seconds, the animal was removed from the aerosol chamber, and the animal was considered to be completely protected.

4. *Ileum in situ of dog* :—Six mongrel dogs of either sex were anaesthetised with pentobarbital sodium 35 mg./kg. given intraperitoneally. Contractions of the ileum *in situ* were recorded with the help of Cushney's myocardiograph. Carotid arterial pressure was also recorded. All drugs were administered intravenously.

#### RESULTS

1. *Isolated ileum and uterus* :—Ashwagandholine had relaxant effect on the isolated ileum of albino rat, guinea pig and rabbit (Fig. 1). The effect on amplitude of contractions was variable. It also decreased the amplitude of contractions of rat uterus (Fig. 2).

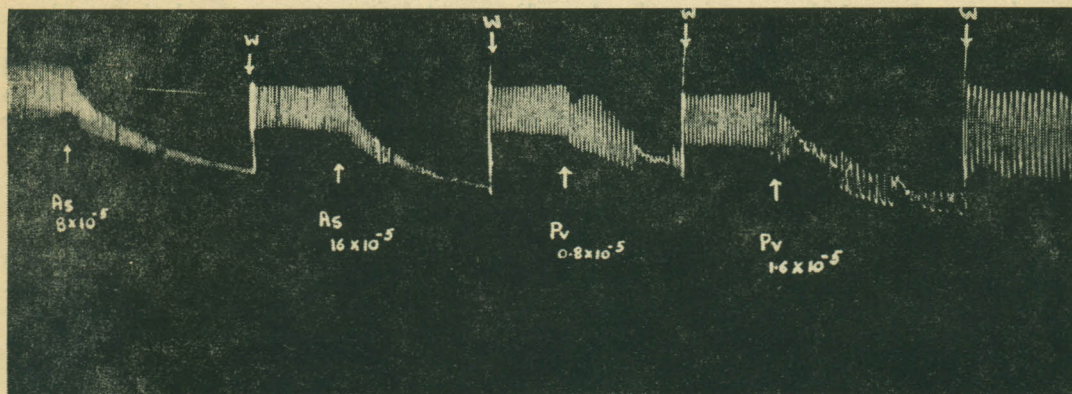


Fig. 1. Effect of Ashwagandholine (As) and Papaverine (Pv) on isolated rabbit's ileum. W—washed. Note the intestinal relaxation.

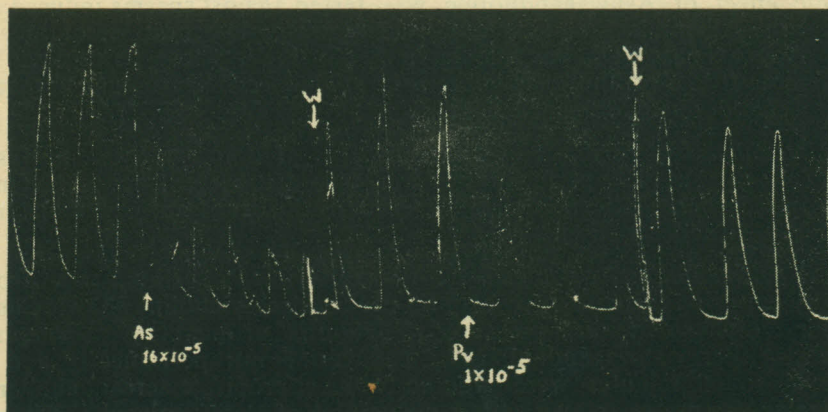


Fig. 2. Effect of Ashwagandholine (As) and Papaverine (Pv) in isolated rat's uterus, W—washed. Note the reduction in the amplitude of uterine contractions.



In some experiments it was found that Ashwagandholine in very low concentrations increased the tone and the amplitude of contractions of isolated ileum of rabbit, guinea pig and rat, but more so in rabbit. But with the increase in the concentration there was decrease in the spasmodic effect. Such spasmodic effect was not seen in isolated rat uterus. Pretreatment of the ileum with pentolinium tartrate ( $20 \mu\text{g/c.c.}$ ) completely blocked while atropine sulphate ( $10 \mu\text{g/c.c.}$ ) markedly reduced the spasmodic effect of Ashwagandholine (Fig. 3). The effects have been summarised in Table I. Rat ileum was found to be the most sensitive whilst rat uterus the least sensitive to both the

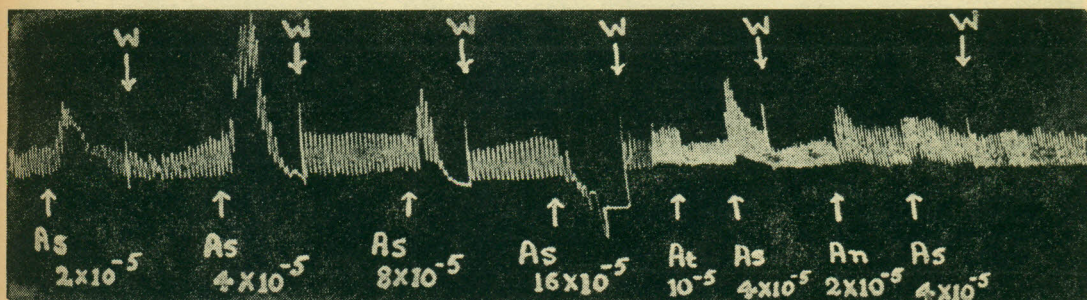


Fig. 3. Effect of Ashwagandholine on isolated rabbit's ileum before and after pretreatment with atropine sulphate (At)  $10^{-5}$  and pentolinium tartrate (An)  $2 \times 10^{-5}$  W—washed. Note that Ashwagandholine in concentrations of  $2 \times 10^{-5}$ ,  $4 \times 10^{-5}$  and  $8 \times 10^{-5}$  had spasmodic effect while in higher concentration ( $16 \times 10^{-5}$ ) had relaxant action. Also note that atropine sulphate markedly reduced and pentolinium tartrate completely abolished the spasmodic effect of Ashwagandholine.

TABLE I

*Effect of Ashwagandholine and papaverine on isolated mammalian tissues*

Decrease in tone and/ or decrease in motility.	Rabbit ileum		Rat ileum		Rat uterus	
	As.	Pap.	As.	Pap.	As.	Pap.
No action ...	20*	3	8	1	40	2
Slight ...	40	6	20	3	100	8
Moderate ...	80	8	60	5	160	12
Marked ...	160	16	100	10	240	20

As. : Ashwagandholine.

Pap. : Papaverine hydrochloride

\*Concentration in  $\mu\text{g/c.c.}$ —Average of 6 experiments at each dose level.



drugs—Ashwagandholine as well as papaverine. Papaverine was, however, approximately 7 to 12 times stronger relaxant than Ashwagandholine in this respect.

The antispasmodic activity of Ashwagandholine against acetylcholine chloride (0.2  $\mu\text{g./c.c.}$ ), histamine diphosphate (0.2  $\mu\text{g./c.c.}$ ), barium chloride (0.1 mg./c.c.) and serotonin creatinine phosphate (0.4  $\mu\text{g./c.c.}$ ) induced spasms in isolated ileum of rabbit, guinea pig and rat, and uterus of rat have been summarised in Table II. The antispasmodic effect of Ashwagandholine against acetylcholine chloride and barium chloride was

TABLE II

*The comparative antispasmodic activity (approximate  $ED_{50}$ ) of Ashwagandholine and papaverine against various spasmogens*

	Acetylcholine chloride spasm	Histamine diphosphate spasm	Barium chloride spasm	Serotonin creatine phosphate spasm
1. <i>Rabbit ileum</i> Ashwagandholine Papaverine	35* 11	— —	27 8	— —
2. <i>Rat ileum</i> Ashwagandholine Papaverine	40 18	— —	35 16	— —
3. <i>Guinea pig ileum</i> Ashwagandholine Papaverine	43 20	52 18	30 20	— —
4. <i>Rat Uterus</i> Ashwagandholine Papaverine	25 18	— —	36 13	981 812
5. <i>Dog tracheal chain</i> Ashwagandholine Papaverine	380 156	— —	311 142	— —

\*Concentration in microgm/c.c. showing 50% inhibitions of spasm. Derived from Log—dose percentage inhibition curve.

nearly of the same degree in these tissues. It antagonised the histamine spasms in guinea pig ileum also to nearly the same extent. But its antispasmodic action against serotonin was about 20 to 40 times weaker. Spasmolytic activity of papaverine was also of a similar pattern but it was about one and a half to three times stronger in activity than Ashwagandholine in this respect.

2. *Tracheal chain* :—Ashwagandholine as such had usually no relaxant effect on dog tracheal chain preparations. Pretreatment of tracheal chain for 5 minutes with Ashwagandholine in concentration of 0.2 to 2.0 mg./c.c. reduced the acetylcholine (1  $\mu\text{g./c.c.}$ ) induced spasms by varying degrees. In lower concentrations the drug had no significant action. The antispasmodic effect of Ashwagandholine was about two to three times weaker than that of papaverine.



3. *Blood vessels* :—Ashwagandholine in doses of 1 mg. to 20 mg. was given in six experiments of rat hind quarter perfusion. The drug alone had no significant effect on perfused blood vessels but it was found to have antispasmodic effect against barium chloride (5 mg.) induced vasoconstriction. The spasmolytic activity of Ashwagandholine was found to be much weaker than that of papaverine (Fig. 4).

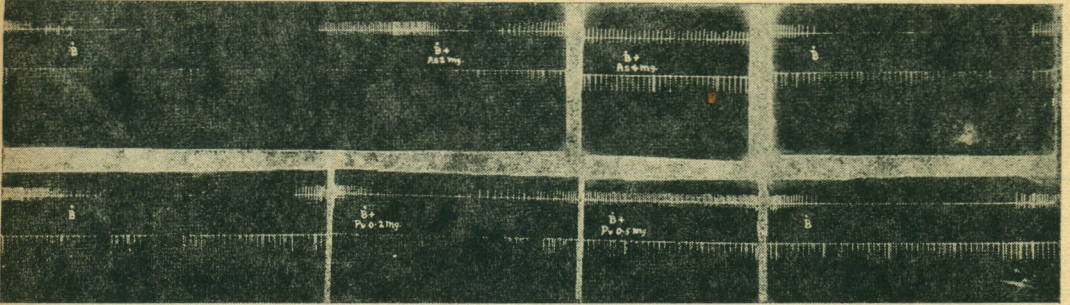


Fig. 4. Effect of Ashwagandholine (As) and Papaverine (Pv) on barium chloride (B) 5 mg. induced vasoconstriction in rat hind quarter perfusion experiment.

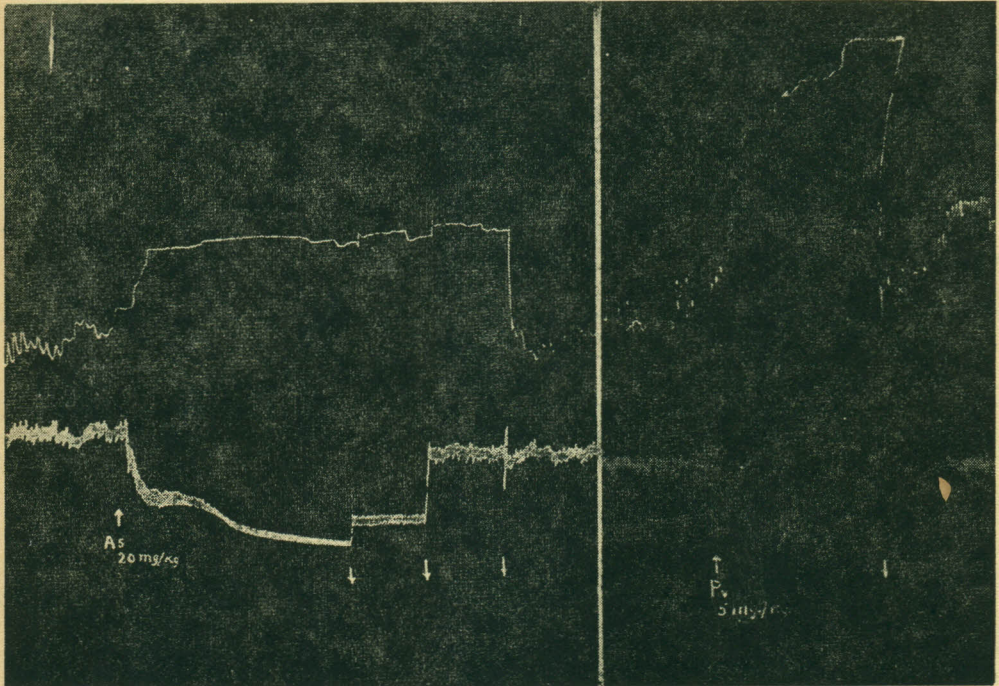


Fig. 5. Effect of intravenous Ashwagandholine (As) and Papaverine (Pv) on dog's ileum *in situ*. Tracings from above downwards are ileal contraction and arterial pressure. Arrows pointing downwards indicate stop of kymograph for 20 minutes each time. Contractions of ileum are downwards. Note the intestinal relaxation.



4. *Ileum in situ of dog*:—Ashwagandholine was given in doses of 4 mg. to 40 mg./kg. in 6 dogs. In doses of 10 mg. to 40 mg./kg., it produced prolonged moderate to marked relaxation of dog's intestine. The relaxant effect was, however, found to be much weaker than that of papaverine (Fig. 5) Ashwagandholine had prolonged slight to marked degree of hypotensive effect in doses of 4 mg. to 40 mg./kg.

5. *Experimental asthma in guinea pig*:—The effect of Ashwagandholine on experimental asthma in guinea pigs has been summarised in Table III. Papaverine in doses of 50 mg. and 100 mg./kg. was used for comparison. The results showed that

TABLE III

*Effect of Ashwagandholine and papaverine on experimental asthma in guinea pigs*

Drug	Dose mg./Kg.	No. of animals used	Acetylcholine aerosol 5 per cent	No. of animals used	Histamine aerosol 1.5 per cent
Control	...	18	148±22*	20	140±22
Ashwagandholine	40 mg.	6	158±20 (P>0.05)†	6	172±32 (P>0.05)
Ashwagandholine	80 mg.	8	198±28 (P<0.05)	8	255±48 (P<0.05)
Papaverine	50 mg.	4	229±26 (P<0.05)	4	320±110 (P<0.05)
Papaverine	100 mg.	6	398±47 (P<0.01)	6	485±43 (P<0.01)

\*Mean (±S.D.) time in seconds before asthma occurred.

†Probability of no significance as compared to relative controls.

Ashwagandholine in the dose of 40 mg./kg. had no significant protective action against acetylcholine and histamine induced asthma. But in dose of 80 mg./kg. Ashwagandholine significantly delayed the onset of asthma in guinea pig, but the effect was much weaker than that of papaverine.

#### DISCUSSION

Ashwagandholine has been found to have varying degrees of relaxant and spasmolytic effects on the various smooth muscles in different species of animals. The drug produced much better relaxation of isolated rat's ileum as compared to that of rabbit and guinea pig. The uterus of rat was also relaxed but the effect was weaker. It had no direct detectable relaxant action on dog tracheal chain and perfused blood vessels of rat. Though it antagonised the spasms induced by acetylcholine chloride, histamine diphosphate and barium chloride on the various tissues to nearly the same degree but the anti-serotonin action was only seen in relatively very high concentrations. In general, the



pattern of relaxant and spasmolytic activity of Ashwagandholine was similar to that of papaverine with the following differences. The former was a weaker relaxant and spasmolytic as compared to the latter. Papaverine was 7 to 12 times stronger relaxant but only one and a half to three times stronger spasmolytic than Ashwagandholine in most of the tissues studied. It, therefore, appears that the mechanism of action of Ashwagandholine on smooth muscles is a papaverine like direct non-specific musculotropic action. However, the spasmolytic activity of the Ashwagandholine is relatively more than its direct relaxant action. In addition, Ashwagandholine in lower concentration sometimes showed a weak spasmolytic effect in isolated tissues. This action is probably mediated through autonomic ganglia, as the effect could be blocked by ganglion blocking agent. While studying the cardio-vascular effects of the total alkaloids the autonomic ganglion blocking action has been well established (5). Probably in very low concentrations it might also be stimulating the ganglia, which could be demonstrated in some isolated smooth muscle preparations.

The present investigations, therefore, provide the pharmacological basis for the use of Ashwagandha (Sanskrit) in asthma and as a uterine sedative in Ayurvedic system of medicine (2) ; (6).

#### SUMMARY

##### I

1. The actions of total alkaloids of *Withania ashwagandha* (Ashwagandholine) have been studied on various smooth muscles in different species of animals. Papaverine has been used for comparison.

2. Ashwagandholine had relaxant effect, and antispasmodic effect against various spasmogens on intestinal, uterine, bronchial, tracheal and blood vascular muscles.

3. The pattern of smooth muscle activity of Ashwagandholine was similar to that of papaverine which suggested a direct musculotropic action.

4. The activity of Ashwagandholine\* was several times weaker than that of papaverine.

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