

## **STUDIES ON WITHANIA ASHWAGANDHA, (PART II): EFFECT OF TOTAL EXTRACT ON CARDIO-VASCULAR SYSTEM, RESPIRATION AND SKELETAL MUSCLE.\***

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(Received on October 22, 1959.)

It has been reported by Malhotra, Das and Dhalla (1960) that the extract of the roots of *W. ashwagandha* produces sedation of varying degrees in different species of animals and has a biphasic action on smooth muscles. The present report deals with the actions of total extract of roots of *W. ashwagandha* on the cardio-vascular system, respiration and skeletal muscle.

## MATERIALS AND METHODS

Aqueous suspension of 70 percent ethyl alcohol extract of air dried roots of *W. ashwagandha* have been used for all investigations. The method of preparation of extract has been previously reported (Malhotra, Das and Dhalla, *loc. cit.*) Equivalent quantity of distilled water was used for control experiments in all cases. Doses have been expressed in terms of dried extractive.

**Intact circulation.**—Experiments were performed on mongrel dogs (4 to 11.5 kg.) of both sexes, anaesthetised with intraperitoneal pentobarbital sodium 35 mg./kg. Blood pressure was recorded from the common carotid artery and respiration by means of Brodie's tambour. In a few experiments Lead II E. C. G. was also recorded. Carotid baroreceptor reflex was tested by occluding both the common carotid arteries for 30 seconds, the femoral arterial pressure having been recorded. Contractions of nictitating membrane were recorded following adrenaline 10 to 50  $\mu$ g./kg. and electrical stimulation of pre- and post-ganglionic fibres by an electronic stimulator with a current of 5 to 10 volts of 60/second frequency for 15 to 30 seconds each time. The peripheral end of cut right vagus was also electrically stimulated with the same parameters but for 5 seconds each time, and Lead II E. C. G. was recorded. Ventricular contractions in open chest dogs were recorded on kymograph with the help of universal lever. In addition, experiments were

\*Studies carried out under "Drug Research Unit" with grant from Indian Council of Medical Research.

performed on decerebrate and spinal dogs by the techniques of Burn (1952). All injections were made through cannulated femoral vein unless otherwise stated. Other routes of administration were-(i) introduction into the stomach through a Ryle's tube, (ii) injection into common carotid artery, and (iii) injection into the lateral ventricle through a Collisson's intraventricular cannula, the fluid being allowed to drain out through a needle introduced into cisterna magna.

*Dog hind limb perfusion.*—Dogs were bled to death under ether anaesthesia, and one of the hind limbs of the same dogs were perfused through the femoral artery with oxygenated blood and mammalian Ringer's solution (in the ratio of 1 : 10) under a constant pressure head. The perfusate was collected from the femoral vein and measured. The extract was injected into the perfusion tube.

*Heart-Lung preparation of dogs.*—Experiments were performed on preparations made by the method of Krayer and Mendez (1942). The dogs weighed between 8.0 and 12.0 kg. Total blood volume at the beginning of experiment was between 500 and 800 ml. Systemic output was measured by Weese stromuhr (100 ml./stroke). Arterial pressure was recorded with a mercury manometer, right atrial pressure with a water manometer and cardiac rate was counted from Lead II E. C. G. records. The extract was added in the venous reservoir.

*Isolated rabbits' and amphibian hearts.*—Isolated rabbits' hearts were prepared by the method of Langendorff. Isolated frog's hearts were perfused by the usual method. Straub's ventricle preparations were also used in confirmatory experiments. These were prepared by the method of Straub (1912) with the use of the cannula described by Krayer, Linstead and Todd (1943).

*Frog's rectus muscle.*—Isolated rectus muscle of frog was suspended in a bath containing frog's Ringer solution and contractions produced by drugs were recorded on smoked drum.

## RESULTS

### 1. Cardio-vascular system.—

#### (a) *Intact circulation.* —

The total extract was administered in varying doses of 0.05 mg. to 1.0 gm./kg. intravenously in 15 dogs. With 0.5 mg./kg. dose there was slight transient fall in arterial pressure and slight transient bradycardia (Fig. 1). But in doses of 5 mg. to 500 mg./kg. it produced slight to moderate degree of bradycardia and moderate to marked fall in arterial pressure lasting from 2 minutes to 2 hours depending upon the dose administered (Fig. 1 and 2).

It did not produce any cardiac irregularity. 1.0 gm./kg. dose was fatal, death being due to respiratory failure. The extract did not show tachyphylaxis. It did not produce any significant E. C. G. changes.

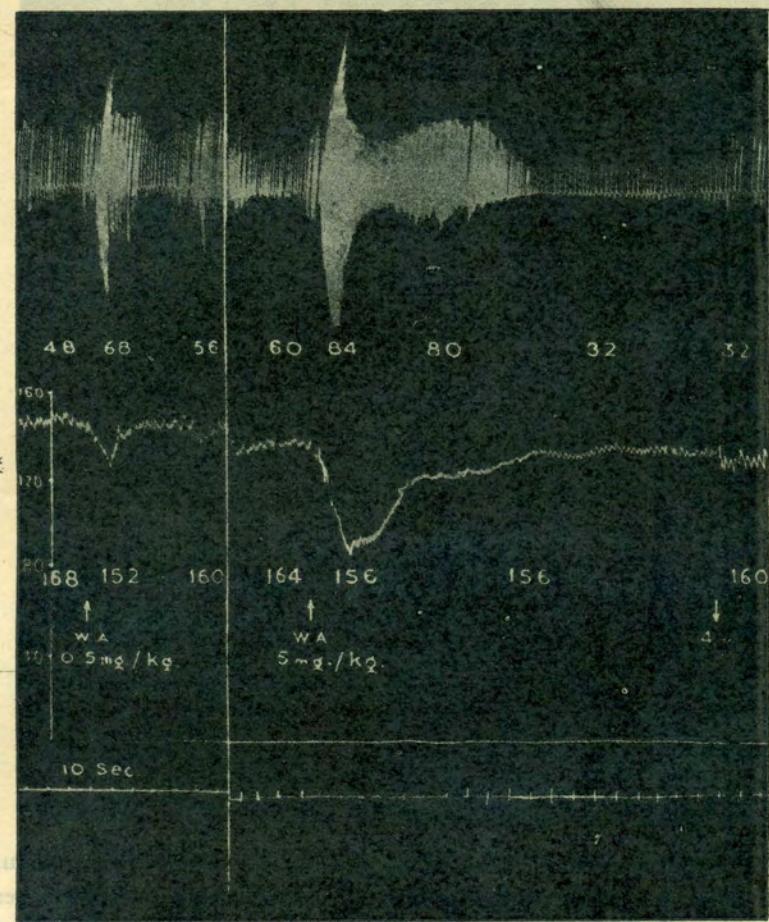
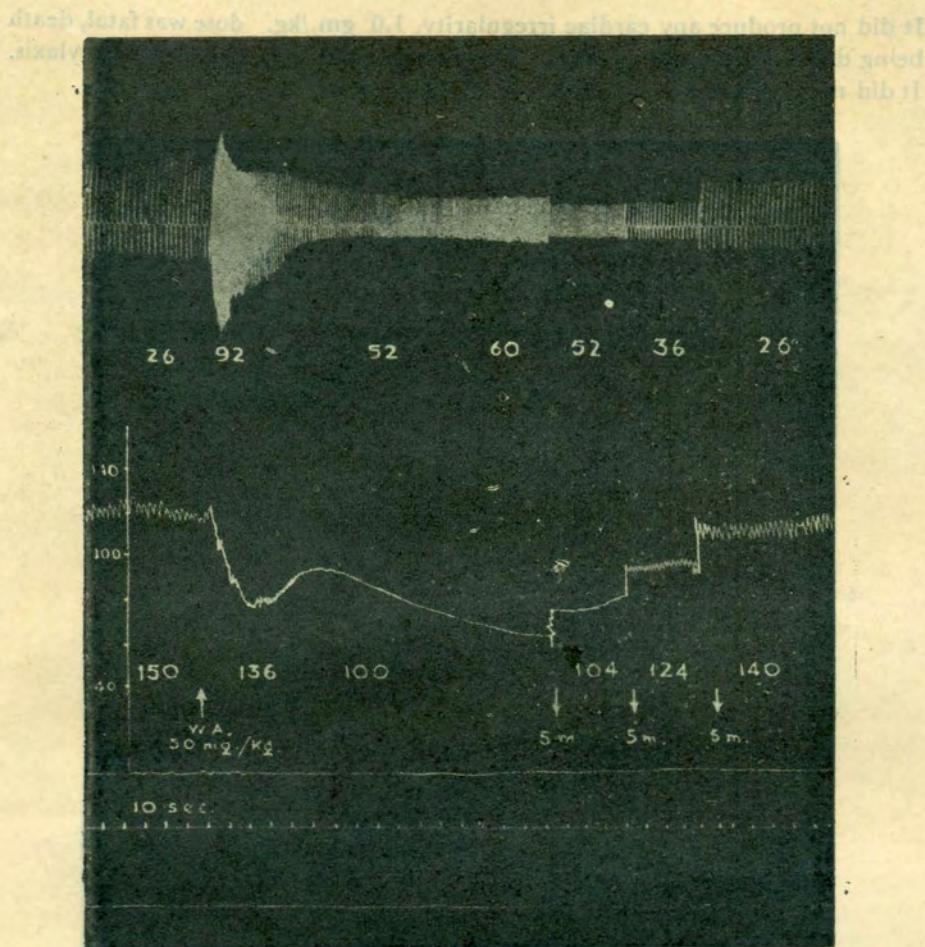


Fig. 1. Effect of intravenous *W. ashwagandha* 0.5 mg./kg. and 5 mg./kg. on respiration, arterial pressure and heart rate of dog. From above downwards: respiration; respiratory rate; arterial pressure and heart rate per minute; arrow indicating '4 minutes stop' of kymograph.  
Note the respiratory stimulation (followed by depression with the higher dose), hypotension and slight bradycardia.

Oral administrations in doses of 0.5 to 1.0 gm./kg. produced slight bradycardia and slight hypotension of very gradual onset, fall being maximum after about 2 hours and the effect lasting for 4 to 6 hours.



**Fig. 2.** Effect of intravenous W. ashwagandha 50 mg./kg. on respiration, arterial pressure and heart rate of dog. From above downwards : respiration ; respiratory rate ; arterial pressure ; heart rate per minute ; arrows indicating stops of kymograph for the duration in minutes.  
Note marked respiratory stimulation, prolonged hypotension and marked bradycardia.

**Carotid baroreceptor reflex.**—In 6 dogs, the extract in doses of 50 to 150 mg./kg. blocked the carotid baroreceptor reflex as long as the hypotension continued (Fig. 3).

Intracarotid and intraventricular injections of the extract in 4 dogs in doses of 0.5 to 5.0 mg./kg. did not produce any significant fall in blood pressure or heart rate. Sometimes it produced slight rise in blood pressure.

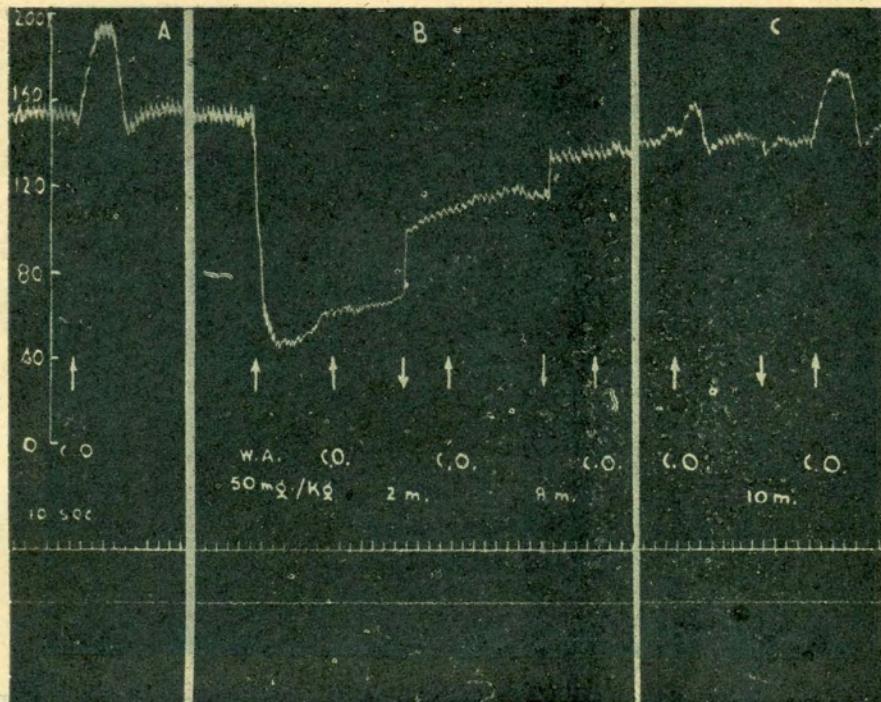


Fig. 3. Effect of *W. ashwagandha* 50 mg./kg. on the carotid baroreceptor reflex in dog. Each time both the common carotid arteries have been occluded for 30 seconds (C.O.). Kymograph has been stopped for 2, 8 and 10 minutes at the downward arrow marks. Time interval between A and B—2 minutes, and B and C—12 minutes.

Note that *W. ashwagandha* blocked the carotid baroreceptor reflex.

*Autonomic ganglionic stimulant.*—The effect of the extract was studied in 10 dogs on the pressor and respiratory stimulating actions of nicotine bitartrate given intravenously in doses of 10 to 100  $\mu$ g./kg. The extract in doses of 50 to 150 mg./kg. was found to completely abolish the action of nicotine on the arterial pressure and heart rate. But it did not affect the respiratory stimulating action of nicotine.

*Autonomic ganglionic blocking agent.*—The extract in doses of 25 to 150 mg./kg. was given in 6 dogs before and after complete blockade of autonomic ganglia by intravenous administration of hexamethonium tartrate. Following blockade of autonomic ganglia, as tested by nicotine bitartrate, the hypotensive effect of the extract was very markedly diminished. In some of the experiments there was a mild to moderate degree of pressor response and mild tachycardia (Fig. 4).

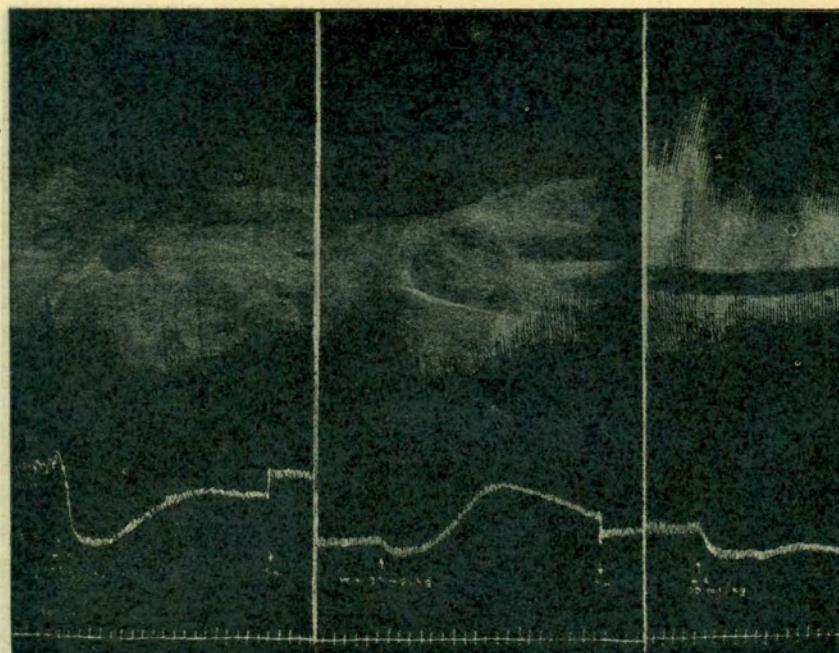


Fig. 4. Effect of *W. ashwagandha* 35 mg./kg. on blood pressure and ventricular contractions of dog. Between A and B, hexamethonium 150 mg. and between B and C, Hydergine 2 cc. have been given. Kymograph has been stopped for 5 minutes and 7 minutes at the arrow marks. Systolic contractions are downwards.

Note the increase in force of ventricular contractions in A and B. In A there is marked hypotension, in B moderate hypertension and in C mild hypotension.

*Nictitating membrane.*—In 6 dogs the extract, in doses of 50 to 150 mg./kg., markedly inhibited the contractions of nictitating membrane when stimulated through the preganglionic sympathetic chain but had insignificant effect on the contractions produced by stimulation of post-ganglionic fibres and also by intravenous adrenaline 10 to 50  $\mu$ g./kg. (Fig. 5).

*Vagal stimulation* :—The peripheral end of the cut right vagus was electrically stimulated in 5 dogs for 5 second periods before and at varying intervals after intravenous extract in doses of 25 to 150 mg./kg. (Fig. 6). In 3 dogs acetylcholine was also used as a cardiodecelerator agent. It was found that the extract completely blocked the cardiodecelerator effect of vagal stimulation while it did not alter the action of acetylcholine.

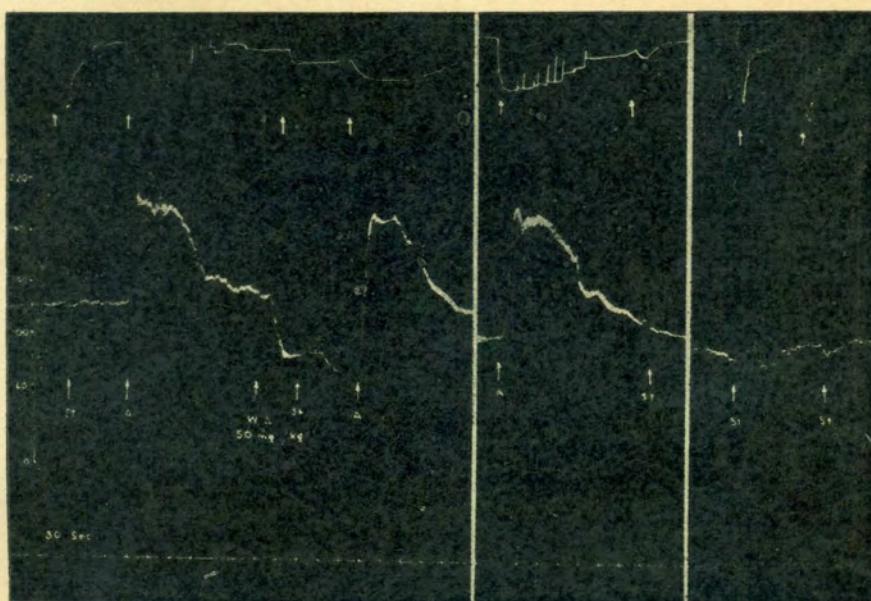
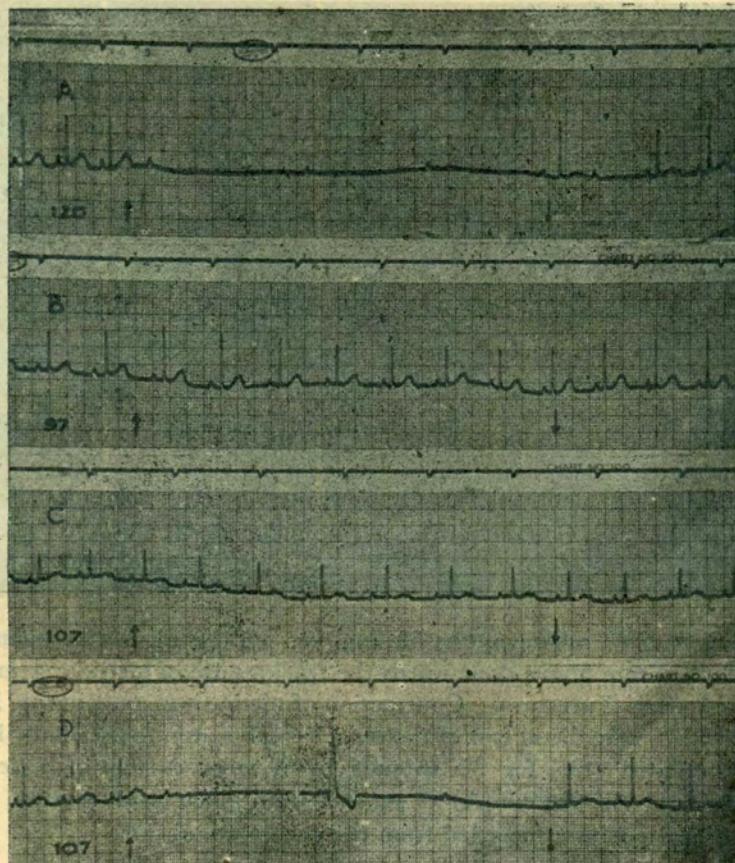


Fig. 5. Effect of *W. ashwagandha* 50 mg./kg. on the nictitating membrane of dog. From above downwards the tracings are of nictitating membrane and arterial pressure. Contraction of nictitating membrane is downwards. The distal end of the cut cervical sympathetic chain has been stimulated (St.) for 30 seconds by 6 volts current of 60/second frequency; Adrenaline (A) 50 µg. has been given intravenously. Carotid arterial pressure recorded from the contralateral side.

Note the marked diminution of contraction of nictitating membrane when cervical chain was stimulated but only insignificant effect on adrenaline induced contraction.

*Atropinisation.*—In 6 dogs the extract, in doses of 50 to 150 mg./kg. intravenously, was repeated again 15 minutes after administration of atropine sulph. 2 mg./kg. Atropinisation slightly reduced the hypotensive and bradycardiac effects of the extract.

*Sympathomimetics.*—Varying doses of adrenaline and noradrenaline were administered in 10 dogs before and at varying intervals after intravenous administration of the extract in doses of 25 to 150 mg./kg. It was found that the extract did not antagonise or inhibit the actions of adrenaline and noradrenaline on cardiovascular system. But in some dogs the extract potentiated the pressor response to these sympathomimetics.



6. E. C. G. tracing showing the effect of W. ashwagandha 50 mg./kg. on the vagal stimulation in dog. In between the arrow marks the peripheral end of cut right vagus nerve was stimulated for 5 seconds by 5 volts current of 60/second frequency. A—initial ; B, C and D—1/2, 3 and 10 minutes respectively after intravenous W. ashwagandha. Numerals indicate heart rate.

Note that W. ashwagandha blocked the bradycardic effect of vagal stimulation. It also produced diminution of R voltage and depressed bifid T wave.

**Sympatholytics.**—In 4 dogs the extract was given after complete sympathetic blockade by intravenous dihydroergotamine or Hydergine (equimolar mixture of dihydroergocornine, dihydroergokryptine and dihydroergocristine). The extract, when given after Hydergine or dihydroergotamine, produced very slight fall in blood pressure and had insignificant effect on heart rate.

In 4 dogs the extract, in doses of 50 to 150 mg./kg., was given intravenously. It was repeated after ganglionic blockade and again after sympathetic blockade. It was found that with the first dose of the extract there was depressor response, with the second dose there was mild to moderate degree of rise in blood pressure or slight fall in blood pressure and with the third dose there was only slight fall in blood pressure (Fig. 4).

*Decerebrate and spinal dogs.*—In 5 decerebrate and spinal dogs the extract in doses of 50 to 150 mg./kg. produced either slight fall or mild to moderate rise in blood pressure. There was no consistent effect on cardiac rate.

The result of the experiments in intact animal with ganglionic stimulant, sympathomimetics and sympatholytics were confirmed in spinal and decerebrated dogs (Fig. 7).

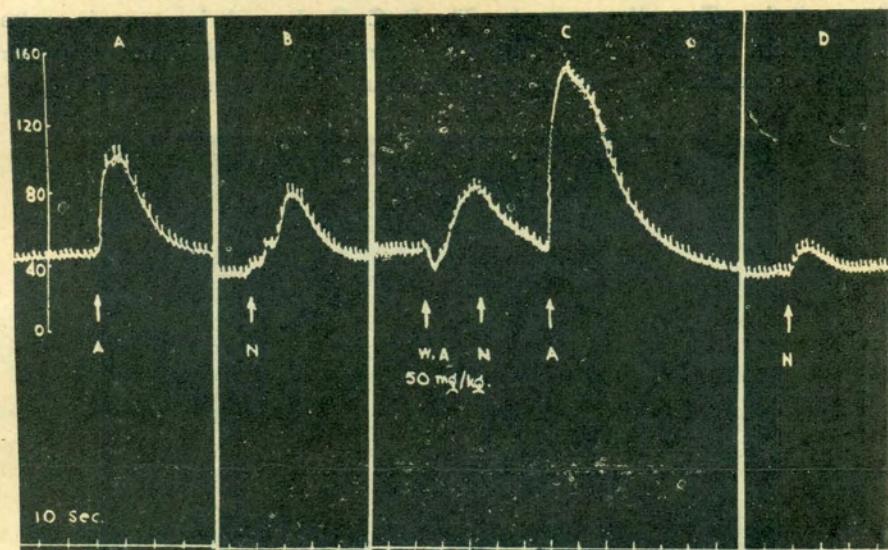


Fig. 7. Effect of *W. ashwagandha* 50 mg./kg. on the pressor responses of adrenaline (A) 100 µg. and nicotine bitartrate (N) 1 mg./kg. in decerebrated dog. Interval between A and B—2 minutes, B and C—5 minutes, C and D—5 minutes.

Note the transient and slight hypotensive effect followed by pressor response of *W. ashwagandha*. It also blocked the pressor response of nicotine but potentiated that of adrenaline.

#### (b) Dog hind limb perfusion.—

In 5 experiments on perfused dog's hind limb it was found that the extract, in doses of 25 to 300/mg., had very little and variable effect on the peripheral vascular tone.

(c) *Dog heart in situ* :—

In 5 dogs the extract, in doses of 50 to 150 mg./kg., produced either slight diminution or slight rise in the amplitude of ventricular contractions as recorded on kymograph. In these doses there was no cardiac irregularity. The extract when given after hexamethonium tartrate produced significant increase in ventricular contractions and when given after hydergine the ventricular contractions diminished in amplitude (Fig. 4).

(d) *Isolated hearts*.—

(i) *Heart-Lung preparation of dogs*.—In 5 Heart-Lung preparations the extract in 1:3,000 concentration had no significant effect while in 1:750 and 1:500 concentrations produced tachycardia, increased cardiac output, slight rise in arterial pressure and slight fall in right atrial pressure. On replacing the blood of the venous reservoir with fresh defibrinated blood without any drug, all the effects disappeared. The E. C. G. showed increase in voltage of R wave. In 1:250 concentration the extract produced ventricular extrasystole, ventricular tachycardia and ventricular fibrillation (Fig. 8).

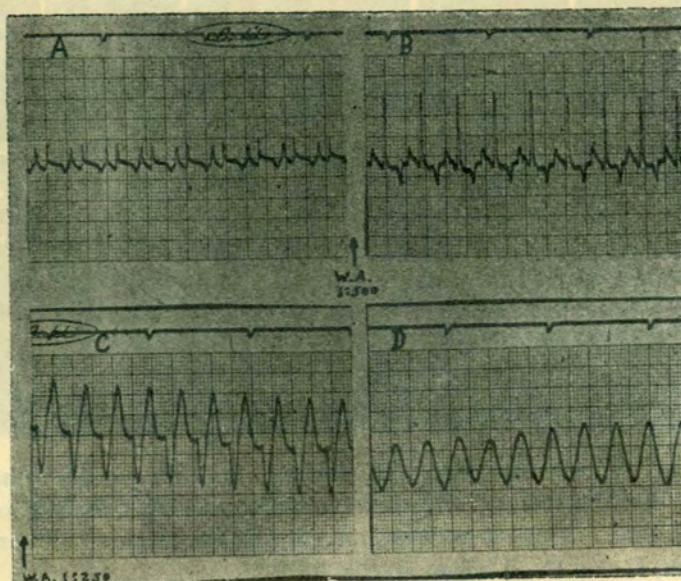


Fig. 8. Effect of extract of *W. ashwagandha* in concentrations of 1 : 500 and 1 : 250 on the E. C. G. in Heart-Lung preparation of dog. A—normal; B—with the extract 1 : 500; and C and D with the extract 1 : 250.

Note the marked increase in voltage of R in 1 : 500 concentration, and with 1 : 250 concentration ventricular tachycardia ending in ventricular fibrillation.

(ii) *Isolated rabbits' heart.*—On the Langendorff's preparation of rabbits' heart the extract was given in single doses from 1.0 to 70 mg. The results have been summarised in Table 1.

TABLE 1.

*Effect of W. ashwagandha on isolated rabbits' hearts.*

Dose in mg.	Number of expts.	Effect on cardiac :				
		Rate	Contractility	Tone	Rhythm	Coronary flow
1.0 to	6	No change	Insignifi-cant	No change	No change	No change
5.0						
10.0 to	6	No change	Increase*	No change	No change	Insignifi-cant
20.0						
25.0 to	6	Bradycardia	Decrease	Decrease	Irregular	Decrease
70.0						sometimes

Note :—\*Transient decrease followed by increase.

The total extract had no significant effect on rabbits' hearts in doses of 1 to 5 mg. while in doses of 10 to 20 mg. it produced a transient depression followed by tachycardia and increased force of contraction, there being no effect on the rhythm and coronary flow. In higher doses it had cardio-depressant effect, bradycardia, decreased cardiac contractility, irregularities and cardiac standstill in diastole (Fig. 9).

(iii) *Isolated frogs' hearts.*—The extract was given in perfused frogs' hearts in single doses of 1 to 150 mg. The results have been summarised in Table 2.

In all the experiments there was an initial depressant effect, which could be mild, producing only diminished contractility and bradycardia, or produce cardiac standstill in diastole. This effect was usually temporary and was

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followed by slight or moderate tachycardia, moderate or marked increase in force of contraction of heart, sometimes increased cardiac tone and rarely irregularities. In doses of 60 to 150 mg. the heart usually did not recover from the standstill. (Fig. 10.)

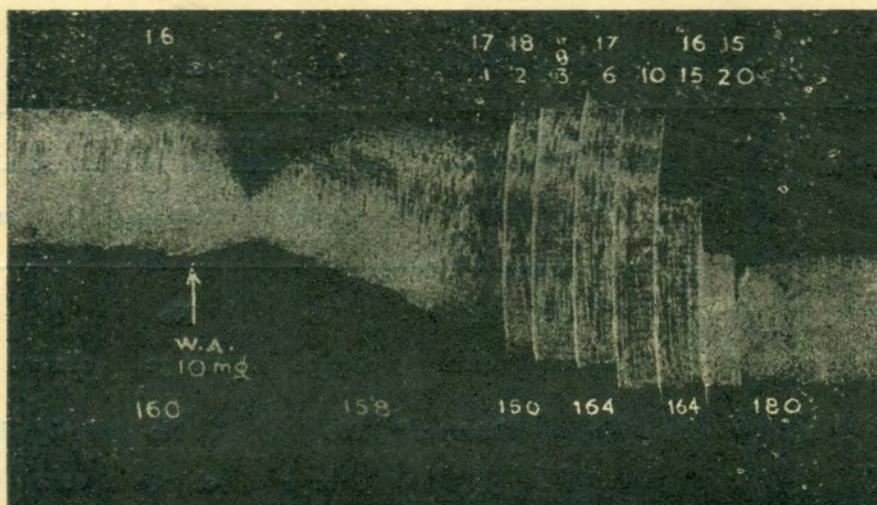


Fig. 9. Effect of W. ashwagandha 10 mg. on isolated perfused rabbit's heart. Numerals from above downwards are—coronary flow in ml. per minute; time in minutes after drug injection; and heart rate per minute. Systole is upwards.

Note the transient diminution followed by increase in force of contraction ; slight bradycardia followed by tachycardia.

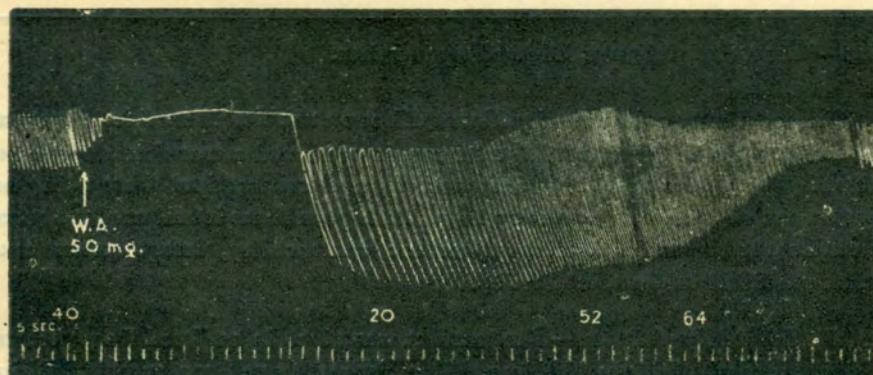


Fig. 10. Effect of W. ashwagandha 50 mg. on isolated perfused frog's heart. Numerals are heart-rate per minute. Systole is downwards.

Note the temporary cardiac standstill followed by marked increase in force of contraction ; bradycardia followed by tachycardia.

In few of the experiments with atropinised heart it was found that atropinisation could slightly reduce the initial cardiodepressant effect of the extract.

In addition, isolated frogs' hearts were continuously perfused with frog's Ringer solution containing the extract in concentrations ranging from 1 : 1,000 at 1 : 10. The results have been given in Table 3.

TABLE 2.

*Effect of single doses of *W. ashwagandha* on perfused frogs' hearts.*

Dose in mg.	No. of expts.	Effect on cardiac:			
		Rate	Contractility	Tone	Rhythm
1 to 5	10	Insignificant	Insignificant	No change	Normal
10 to 15	Decrease	Decrease	No change	Normal,	
50	or	followed by increase	change	sometimes	
	Standstill	Standstill	or increase	irregular	
	followed by increase				
60 to	12	Standstill	Standstill, increase if recovery takes place	No change or increase	Sometimes irregular on recovery
150					

The extract had variable effects when administered as a continuous perfusion. In some experiments there was bradycardia, decreased cardiac contractility and even cardiac standstill in diastole. In others there was tachycardia, increased force of contraction and tone. In the former case, when the perfusion fluid was changed to normal Ringer's solution, it was found that the heart rate and contractility increased more than the control level.

The cardiac effects of the extract were confirmed by experiments on 10 Straub's ventricles. According to him 0.05 to 0.1% of the extract produces depression of the heart muscle. At 0.05 to 0.1% concentration it causes a slight decrease in the force of contraction and a slight increase in the rate of contraction. At 0.1% depression of the heart muscle is marked.

TABLE 3.

Effect of continuous perfusion of *W. ashwagandha* on frogs' hearts.

Conc. of drug	No. of expts.	Effect on cardiac :			
		Rate	Contractility	Tone	Rhythm
0.1%	3	Insignificant	Insignificant	No change	Normal
0.2 to	8	Slight	Slight	No change	Normal
0.4%		bradycardia* or slight tachycardia	decrease* or increase	or increase	
1.0%	6	Bradycardia* or tachycardia	Decrease* or increase	No change or increase	Irregular sometimes
2 to 10%	8	Standstill	Standstill	Decrease	—

Note :--\*On changing to normal Ringer solution there were positive inotropic and positive chronotropic effects.

## 2. Respiration :—

The extract in doses of 0.1 to 1.0 mg./kg. intravenously had transient stimulant effect on respiration. The increase was both in rate and in depth of respiration. In doses of 2 to 10 mg./kg. it produced moderate degree of stimulation lasting for few minutes (Fig. 1). With 20 mg. to 70 mg./kg. there was moderate or marked stimulation of respiration lasting for 10 to 30 minutes. The stimulation was more of rate and less of depth. Sometimes respiration became rapid and shallow. (Fig. 2). The stimulation was followed by depression in some dogs. In doses of 100 mg. to 1.0 g./kg. the initial stimulation was always followed by moderate or marked respiratory depression, and deaths were due to respiratory failure. Respiratory stimulation was also seen in decerebrate dogs and after autonomic ganglionic blockade irrespective of the effect on blood pressure. Intracarotid administration of extract in doses of 0.5 to 5.0 mg./kg. had no significant effect on respiration. Intraventricular administration of 0.5 to 5.0 mg./kg. produced significant respiratory stimulation after a latent period of 2 to 4 minutes-probably the time taken by the extract to reach the fourth ventricle. Respiratory

stimulation was also seen when the extract was introduced into the stomach of anaesthetised dogs in doses of 0.5 to 1.0 g./kg. when hypotension was minimal but respiratory stimulation was appreciable and lasted for 2 to 4 hours.

### 3. Frog Rectus muscle.—

The extract in concentrations of 1 : 5,000 to 1 : 100 had slight spasmodyic effect on frogs' rectus muscle. The spasm was of gradual onset and could not be antagonised by 1 : 5,000 Flaxedil. But pretreatment of muscle with Flaxedil prevented the effect of the extract on the muscle. Acetylcholine (1 : 1 million) induced spasms, in muscles pretreated with the extract in concentrations of 1 : 250 and 1 : 100, were increased as compared to the control responses.

### DISCUSSION

The present investigations show that the extract of *W. ashwagandha* roots have a prolonged hypotensive, bradycardiac and respiratory stimulating actions in dogs. The hypotensive action appears to be chiefly due to ganglion blocking action of the extract as shown by blockade of vagal ganglion, superior cervical ganglion and pressor response to nicotine. The marked diminution or absence of depressor response of the extract in decerebrate and spinal animals, and after ganglion blocking agent, also indicate a ganglionic action. The absence of hypotension following intracarotid and intraventricular injections, exclude the possibility of hypotension due to any central action. The mild hypotension seen after ganglionic and sympathetic blockade does not appear to be due to peripheral vasodilatation. It might be of cardiac origin as evidenced by the action on frog's, rabbit's and intact dog hearts. This component is parasympathomimetic like in nature as it is affected by atropinisation. It appears, therefore, that the hypotension due to the extract is due to more than one site of action-autonomic ganglionic blockade and mild cardiac depression of parasympathomimetic nature.

Occasional pressor response of the extract in decerebrate and spinal animals and after ganglionic blockade, occasional potentiation of adrenaline, and the blockade of the pressor response by sympatholytics show that it has a sympathomimetic like action. The rise in blood pressure, however, in such cases appears to be mainly cardiac in origin. The predominant cardiac effect is supported by the positive inotropic and chronotropic actions of the extract on isolated frogs' and rabbits' hearts, and effect on heart-lung preparation of dog, and ventricular tracings of intact dog. However, this is a weak action which can only be demonstrated when the predominant hypotensive effect is blocked.

The extract has a specific respiratory stimulant action in dogs due to central stimulation of respiratory centre. This principle appears to be different from the hypotensive principle because on oral administration, respiratory stimulation is significant while hypotension is minimal. The respiratory stimulating action in albino rats on oral and intraperitoneal administration had already been reported (Malhotra, Das and Dhalla, loc. cit.).

The present investigations show that the extract of *W. ashwagandha* has multiple sites of action which might be due to the presence of different chemical fractions. This drug, with its prolonged hypotensive, bradycardiac and sedative actions and potentiation of hypnotic effect of barbiturate, might become an useful agent for the treatment of hypertension. The pharmacological studies of the total alkaloids of *W. ashwagandha* are already in progress.

#### SUMMARY

- Effect of 70 percent alcoholic extract of *W. ashwagandha* roots have been studied on the cardiovascular system and respiration in certain species, viz. dogs, frogs and rabbits.
- The extract has prolonged hypotensive, bradycardiac and respiratory stimulating actions.
- The hypotensive effect has been found to be mainly due to ganglion blocking action, but also to a minor degree due to parasympathomimetic like action.
- The extract has also got a weak sympathomimetic like action on heart.
- The respiratory stimulation is due to central stimulation.
- It produces contractions of frog's rectus muscle.
- The multiple sites of action appear to due to the presence of more than one active chemical constituent.
- The results have been discussed.

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