PHARMACOLOGICAL STUDIES IN THE APOCYNACEOUS GENUS ASPIDOSPERMA MART. AND ZUCC. *ASPIDOSPERMA ALBUM* (VAHL) R. BEN. AND *ASPIDOSPERMA MEGALOCARPON* MUELL. ARG.

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IN previous communications[1,2] the pharmacological properties of the alkaloids of *Aspidosperma oblongum* A.DC. have been discussed. The results obtained from studies of these alkaloids lead us to an investigation of the pharmacological properties of alkaloids found in the barks of other Aspidosperma species.

This paper deals with the alkaloids of *Aspidosperma album* (Vahl) R.Ben. and *Aspidosperma megalocarpon* Muell. Arg.

MATERIALS

The dried bark was reduced to a coarse powder and the total alkaloids extracted by classical methods. The pharmacological tests were made with the dried total alkaloid fractions, which were dissolved in 0·1 N hydrochloric acid and adjusted to *p*H 6·6 to 6·8 by addition of 0·05 N sodium hydroxide solution. Precipitation occurred if the *p*H was higher. Sodium chloride was added to the adjusted solution to give a final concentration of 0·9 per cent. for mammalian work, and 0·6 per cent. for work on frog tissues. The adjusted solution contained 10 mg. of total alkaloid per ml. and dilutions were made from this.

METHODS AND RESULTS

The pharmacological properties of the alkaloids from both barks are described together and differences indicated where they exist.

When applied to the muscle of the frog sciatic nerve–gastrocnemius muscle preparation, there was a contractural response which was reversible on washing. There was a progressive decline in the response of the muscle to indirect stimulation, and also a depression of response when the drug was applied to the sciatic nerve. Inhibition in both cases progressed to completion and was irreversible, although the muscle still responded to direct stimulation. In the frog rectus abdominis preparation the response obtained on addition to a 10-ml. bath of 10 μg. of acetylcholine chloride was inhibited by previous exposure to both alkaloids. The inhibition was proportional to the dose given (Figs. 1 and 2).

There was depression of smooth muscle tone, with inhibition of peristalsis in rat and guinea-pig ileum, and in rabbit duodenum. The tone and normal rhythmic movements of rat, guinea-pig and rabbit uterus were inhibited. The spasmogenic actions of 1-μg. doses of acetylcholine chloride on rat and guinea-pig ileum, of 10-μg. doses on

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the rat and guinea-pig uterus, and of 2-μg. doses on the rabbit duodenum were inhibited. Inhibition of the spasmogenic actions of 1 μg. of histamine acid phosphate on guinea-pig ileum and uterus was shown. A similar effect was shown for barium chloride at a dose level of 2 mg. using rat and guinea-pig uterus, and rat, rabbit and guinea-pig ileum. On the rabbit uterus antagonism to the spasmogenic action of 10 μg. of

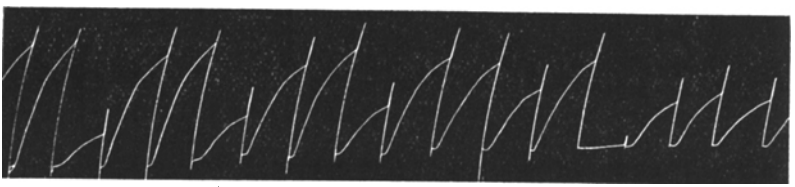

FIG. 1. Inhibition of the action of 10 μg. of acetylcholine chloride by:—A 0·2 mg., B 0·1 mg., C 0·05 mg., D 0·02 mg. and E 0·4 mg. of *A. album* alkaloids. Each dose given 3 minutes previous to the dose of acetylcholine.

adrenaline hydrochloride was shown. Higher doses of alkaloids were needed to produce on the uterus effects equivalent to those seen on the gut. In all cases a 50-ml. bath was used, containing for rat tissues oxygenated de Jalon's solution[3], and for the others oxygenated Tyrode's solution. The bath temperature was 36·5° to 37° C.

The rate, amplitude and tone of the frog heart *in situ* were gradually decreased by application of solutions of both drugs. After partial

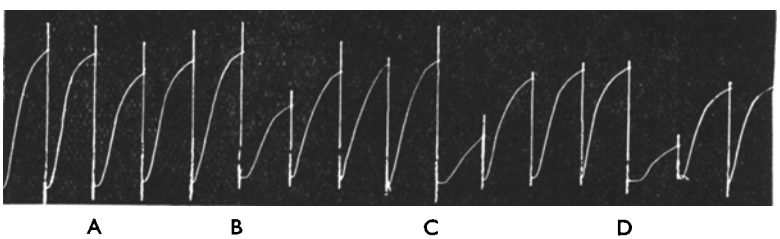

FIG. 2. Inhibition of the action of 10 μg. of acetylcholine chloride by:—A 0·1 mg., B 0·2 mg., C 0·32 mg. and D 0·4 mg. of *A. megalocarpon* alkaloids. Each dose given 3 minutes previous to the dose of acetylcholine.

cardiac depression vagal stimulation did not produce its characteristic effects. Similar effects were noted with the isolated frog's heart perfused through the aorta or sinus venosus with normal Ringer's solution or with "half-calcium" Ringer's solution, containing 1 part in 200,000 of the alkaloids. No auricular-ventricular block could be demonstrated.

The amplitude of the beat of Langendorff preparations of rabbit and kitten heart was reduced by 1 mg. of the alkaloids given as an injection into the cannula. 2 mg. administered similarly also depressed the rate. With *A. megalocarpon* alkaloids no auricular-ventricular blocking action could be seen. During the phase of recovery after a 4-mg. dose of *A. album* alkaloids a well marked but short-lived block was shown.

Similar effects were seen when the fluid perfusing the heart contained 1 in 200,000 of alkaloid. The effects were reversible. After administration of *A. megalocarpon* alkaloids (1 mg.) a well marked increase in outflow was seen. This rose from 3·3 ml. to 6·0 ml. per minute. In the isolated rabbit auricles suspended in oxygenated Ringer-Locke solution at 29° C., 1·5 mg. of alkaloids reversibly inhibited the depressant effects of 5 μg. of acetylcholine chloride.

When perfused into the abdominal aorta of the rat hind limbs preparation, solutions of *A. megalocarpon* alkaloids produced marked vasoconstriction. There was no reversal of the vasoconstrictor action of 5 μg. of adrenaline. Inconsistent results were obtained with *A. album* alkaloids which produced either marked vasoconstriction or transient vaso-dilatation followed by a return to normal tone. There was antagonism by the *A. album* alkaloids of the vasoconstrictor effects of 1 μg. of acetylcholine chloride and of the vasoconstrictor effects of 5 μg. of adrenaline hydrochloride. No vasodilator effect to administration of acetylcholine chloride was noted in this series of experiments, which numbered 10 preparations—the perfusion being carried out at room temperature. 0·5 mg. of both *A. album* and *A. megalocarpon* alkaloids dilated the blood vessels of the perfused rabbit ear. No adrenaline reversal or antagonism could be shown.

In the cat anæsthetised with ether and chloralose, administration of small doses of the drug (2 to 10 mg.) into the jugular vein caused no noticeable effects upon depth or frequency of respiration. The blood pressure was depressed after each administration, the magnitude of depression being proportional to the dose of alkaloid given. No reversal of the pressor response to doses of 5 μg. of adrenaline hydrochloride could be shown. 4-mg. doses of the alkaloids showed no protecting power in mice weighing 20 to 21 g. against the effects of lethal doses of adrenaline hydrochloride[4]. The depressor response to 5 mg. of alkaloids in the spinal cat was less marked than in the anæsthetised animal. No modification in this preparation of the pressor effects of adrenaline (1 to 2 μg.) could be shown by *A. megalocarpon* alkaloids but there was very marked potentiation of the pressor effect when *A. album* alkaloids (0·5 mg./kg.) were added..

Injection of 6 mg. of the alkaloids into the ventral lymph sac of frogs caused paralysis, loss of reflexes and death in about 50 per cent. of frogs. No convulsions were seen. With the *A. megalocarpon* alkaloids, paralysis was preceded by restlessness. In mice weighing 20 to 21 g. intraperitoneal injection of 8 mg. of the total alkaloids caused the following symptoms. 15 minutes after the injection the animals became quiet and sleepy with closed eyelids; frequent washing movements were noted; soon after there were convulsions; these alternated with periods of quiet; 6 out of 10 mice died; the others recovered within 3 to 4 hours. Similar symptoms were observed in rats at a dose level of alkaloid of 400 mg./kg.

When tested by the method of Sollmann[5] as modified by Bülbring and Wajda[6] a graded local anæsthetic activity was shown. To rule out the possibility of a generalised toxic effect upon nervous tissues or of a

neuromuscular block, immediately after sensory paralysis to the highest concentration of acid the sciatic nerve was exposed and stimulated, when the gastrocnemius muscle was found to respond normally.

No antimalarial activity was shown when the alkaloids (at 3 mg. dose level) were tested by the method of Rollo[7] against *Plasmodium berghei* in mice. When tested *in vitro* against *Entamœba histolytica* 1 part in 20,000 of the alkaloids showed some anti-amœbic activity (Table I).

TABLE I

In vitro ANTI-AMŒBIC ACTIVITY OF *A. album* AND *A. megalocarpon* ALKALOIDS

Compound and concentration	Viable amoebæ per 100	
	Incubation with drug for 24 hours	Incubation with drug for 48 hours
Emetine—		
(*a*) 1 : 200,000	4/100	3/100
(*b*) 1 : 400,000	12/100	30/100
(*c*) 1 : 800,000	40/100	many
A. megalocarpon alkaloids—		
(*a*) 1 : 20,000	24/100	3/100
(*b*) 1 : 200,000	—	many
A. album alkaloids—		
(*a*) 1 : 20,000	18/100	10/100
(*b*) 1 : 200,000	—	many

There was depression of up to 3° C. of the rectal temperature in mice weighing 23 to 25 g. when 2·0 mg. of alkaloids was given intraperitoneally.

DISCUSSION

Unlike the alkaloids of *Aspidosperma oblongum* the alkaloids of the barks of *Aspidosperma album* and *Aspidosperma megalocarpon* do not reverse the response to adrenaline on the blood pressure of the chloralosed cat although an inconsistent effect was seen on the blood vessels of the rat hind limbs preparation using *A. album* alkaloids. There was, however, antagonism to the stimulant action of adrenaline on the virgin rabbit uterus, and potentiation of the pressor response by album alkaloids to adrenaline on the spinal cat. There was no protection in mice against lethal doses of adrenaline. Antagonism to acetylcholine, histamine and barium were shown but there was no evidence that these were specific effects. A local anæsthetic action was shown using the frog lumbar plexus method of Sollman[5] as modified by Bülbring and Wajda[6]. An *in vitro* anti-amœbic action has been shown.

Preliminary qualitative chemical tests have indicated that the alkaloids contained in the above barks differ from those of the bark of *Aspidosperma oblongum* which appears to contain indole derivatives.

SUMMARY

1. The alkaloids of the barks of *Aspidosperma album* and *Aspidosperma megalocarpon* non-specifically antagonise the actions of acetylcholine, histamine and barium.

2. An *in vitro* anti-amœbic action has been shown.

3. There is no evidence of reversal of the pressor effects of adrenaline.
4. The *A. album* alkaloids potentiate the pressor response to adrenaline in the spinal cat.

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