STUDIES ON ALKALOIDS OF HERPESTIS MONNIERA, LINN[1]

By

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Herpestis monniera, Linn (N. O. *Scrophularineae*) has been extensively used in the Ayurvedic system of medicine for various nervous disorders such as insanity, epilepsy, hysteria etc. It has been claimed to be a 'cardiac and nervine tonic' (Chopra, 1933). Several workers (Bose and Bose, 1931; Basu and Walia, 1944; Basu and Pabrai, 1947) reported the presence of alkaloids in the plant. Sastry, Dhalla and Malhotra (1959) isolated a glycoside saponin principle, hersaponin, from the plant. Hersaponin was found to have cardiotonic effect in frogs and sedative action in rats and guineapigs (Malhotra and Das, 1959). Further neuropharmacological studies on hersaponin showed that it potentiates the hypnosis produced by barbiturates and ethanol in mice acting by more than one mechanism (Malhotra, Das and Dhalla, 1960). As no systematic studies have been conducted on the alkaloids of the plant, the present work was taken up.

METHODS

Chemical

The air dry powdered herb of *Herpestis monniera* was extracted with various solvents. The extractives gave positive tests for the presence of alkaloids with Dragendorff's and Mayer's reagents. The yields of the total alkaloidal content by gravimetric methods using the effective solvents were, prollius fluids, 0.03 per cent; cold 45 per cent alcohol, 0.02 per cent; cold 95 per cent alcohol, 0.03 per cent; hot 95 per cent alcohol 0.05 per cent; ammoniacal 95 per cent hot alcohol, 0.04 per cent, and hot 95 per cent alcohol containing 1.0 per cent tartaric acid, 0.03 per cent.

Total alkaloids.—These were isolated here from hot 95 per cent acloholic extractive. The extractive was concentrated under reduced pressure and treated with ether to remove chlorophyll. The residue was digested with 1 per cent hot hydrochloric acid. The acid solution was freed from fatty material and the bases were extracted with chloroform after making it alkaline with ammonia. The chloroform extract containing the alkaloids was washed, dried and evaporated to dryness.

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The total alkaloidal fraction was subjected to paper chromatography using *n*-butanol : acetic acid : water mixture (4: 1: 5) as the solvent. The chromatogram, when observed under ultra violet light, showed a fluorescent band having Rf value 0.97. On spraying the chromatogram with modified Dragendorff's reagent (Block, Durrum and Zweig, 1958), three bands having Rf values 0.66,0.56 and 0.97, were observed.

Presence of nicotine.—The chemical tests of the alkaloidal fraction indicated that one of the constituents may be nicotine.

On circular chromatographic paper (a) total alkaloids, (b) total alkaloids and nicotine and (c) nicotine were spotted. n-butanol : acetic acid : water mixture (4 :1 : 5) was used as solvent. The developed chromatogram on spraying with modified Dragendorff's reagent, showed that the central band (having Rf value 0.66) of the alkaloids coincided with that of nicotine. From the above tests it appears that one of the three alkaloids of *Herpestis monniera* is nicotine.

Pharmacological

The alkaloidal fraction was suspended in 10 per cent propylene glycol for all pharmacological studies. Experiments were performed on anaesthetised (with intraperitoneal pentobarbital sodium 35 mg/kg) mongrel dogs, isolated ileum of rabbits, isolated rectus muscles of frogs, and isolated frog's hearts by usual techniques. Contractions of the nictitating membrane of dogs were recorded following administration of drugs and electrical stimulation of preganglionic sympathetic fibres with a current of 5 to 10 volts of 60 per secs frequency for 15 to 30 secs each time. Drugs were either administered through cannulated femoral vein, or through the proximal end of the occluded ipsilateral lingual artery to the superior cervical ganglion.

The acute toxicity of *Herpestis monniera* alkaloids (HMA) was determined on albino mice by intraperitoneal administration. The LD_{50} was calculated by the method of Litchfield and Wilcoxon (1949). Four doses were used between LD_0 and LD_{100} using 10 mice for each dose level.

RESULTS

Effect on blood pressure, heart rate and respiration of dog —HMA in doses of 0.2 to 2.0 mg/kg intravenously, produced an initial transient fall followed by rise in blood pressure of 2 to 5 minutes duration. There was moderate degree of bradycardia and short lived respiratory stimulation. The respiratory stimulation was however, followed by slight depression. Repeated

administration of the same dose of the drug at intervals of 2 to 5 mins showed tachyphylaxis. Atropine sulphate, 1 mg/kg intravenously, blocked the negative chronotropic and the initial hypotensive responses. Intravenous administration of pentolinium tartrate 2 mg/kg blocked all the cardio-vascular and respiratory responses of the drug. Nicotine bitartrate was found to have actions similar to HMA. Nicotine bitartrate, 0.1 mg/kg however produced slightly more hypertension than that by 1 mg/kg of HMA (Fig. 1).

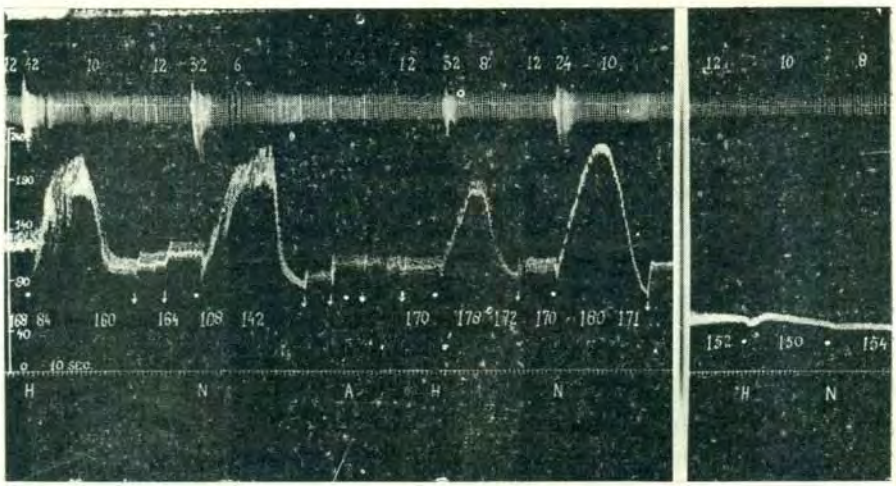

Fig. 1. Effect of *Herpestis monniera* alkaloids (H) 1 mg/kg and nicotine bitartrate (N) 0.1 mg/kg on blood pressure, heart rate and respiration of dog. From above downwards: respiratory rate, respiration, blood pressure, heart rate and time signal. Dots indicate points of drugs administration and arrows indicate stop of kymograph. A : atropine sulphate 1 mg/kg. In between two panels pentolinium tartrate 2 mg/kg was given intravenously.

Effect on superior cervical ganglion of dog.—Electrical stimulation of preganglionic cervical sympathetic chain, intravenous adrenaline hydrochloride 5 μg/kg, intra-arterial (leading to the superior cervical ganglion) HMA 0.1 mg and nicotine bitartrate 20 μg produced contractions of the nictitating membrane. Intra-arterial administration of pentolinium tartrate 100 μg either completely blocked or markedly reduced the effects of electrical stimulation of preganglionic sympathetic fibres, nicotine and HMA. The effect of adrenaline was however unaffected (Fig. 2).

Effect on isolated ileum of rabbit.—HMA was found to have a spasmodic effect on the isolated ileum of rabbit in concentrations of 10^{-6} to 2×10^{-5}.

The spasm was followed by relaxation. Repeated exposures of the ileum to the same concentration of HMA at short intervals showed tachyphylaxis. Nicotine bitartrate was found to have similar effect in concentrations of 2×10^{-7} to 2×10^{-5}. Height of the spasm produced by 10^{-5} HMA was found to be in between those of 5×10^{-6} and 10^{-5} nicotine bitartrate.

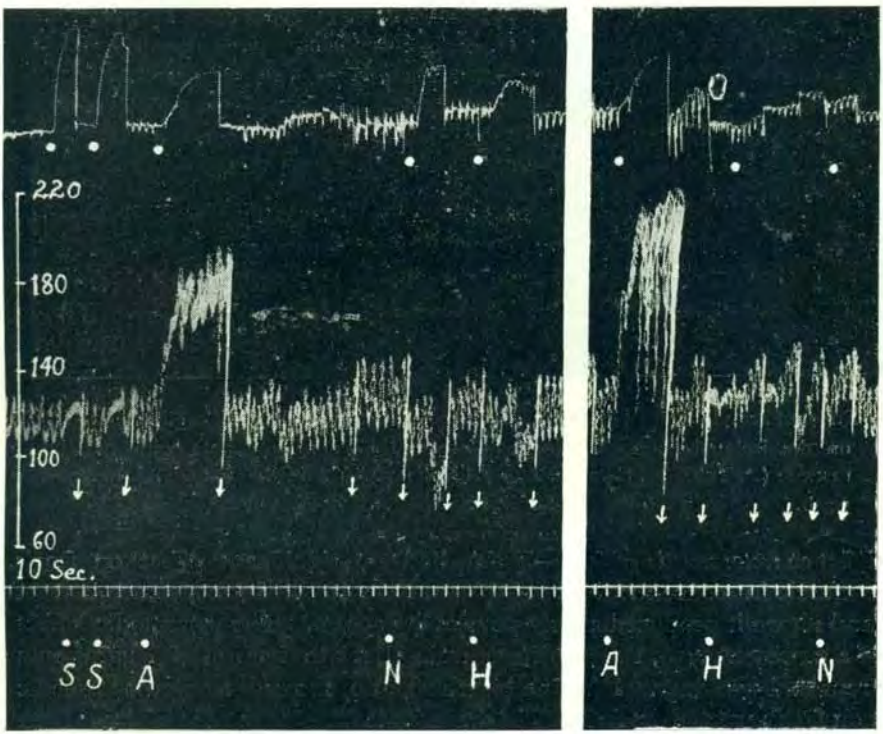

Fig. 2. Effect of intra-arterial (to the superior cervical ganglion) HMA (H) 0.1 mg, nicotine bitartrate (N) 20 μg, and intravenous adrenaline hydrochloride (A) 5 μg/kg on nictitating membrane of dog. From above downwards: nictitating memberane, blood pressure and time signal. S, electrical stimulation of preganglionic sympathetic fibres. Dots indicate points of drug administration and arrows indicate stops of kymograph. In between two panels pentolinium tartrate 100 μg was given intra-arterially.

Atropine sulphate 10^{-6} blocked the spasmodic effects of HMA 10^{-5}, nicotine bitartrate 5×10^{-6} and acetylcholine 2×10^{-6}. Pentolinium tartrate 2×10^{-5}, however, blocked the spasms produced by HMA and nicotine without affecting that of acetylcholine (Fig. 3).

Pretreatment of ileum with HMA 10^{-5} completely blocked the spasmodic effect of nicotine bitartrate 10^{-5} and *vice versa*. Both these drugs, however, did not alter the action of acetylcholine 2×10^{-6} on ileum.

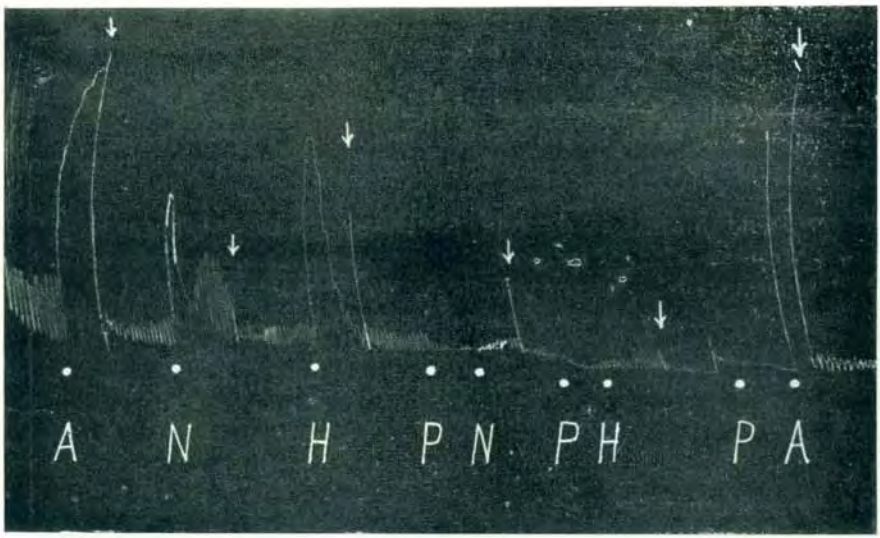

Fig. 3. Effect of acetylcholine (A)2 x 10^{-6}, nicotine bitartrate (N) $5x10^{-6}$ and HMA (H)10^{-5} on the isolated ileum of rabbit, before and after pretreatment with pentolinium tartrate (P) $2x10^{-5}$. Dots indicate addition of drug in the bath. Arrows indicate wash.

Effect on rectus muscle of frog.—HMA had no significant effect on frog's rectus muscle in concentrations of 10^{-6} to 5×10^{-6}. In concentrations of 10^{-5} and above it produced slowly developing spasm. The spasmodic effect was of a persistent type, the muscle returning back to its initial length only after repeated washings. Nicotine bitartrate also showed similar spasmodic effect on frog's rectus muscle. Height of the spasm produced by HMA 2×10^{-5} was found to be between those of 10^{-5} and 2×10^{-5} nicotine bitartrate.

HMA 10^{-6} to 10^{-5} did not significantly alter the acetylcholine 2×10^{-5} induced spasm. Spasmodic effect of HMA 2×10^{-5} and nicotine 2×10^{-5} could not be abolished by gallamine triethiodide 10^{-4}. But pretreatment of the muscle with gallamine triethiodide 10^{-4} either markedly reduced or completely prevented the HMA and nicotine induced spasms (Fig. 4).

Effect on isolated perfused frog's heart.—HMA in doses of 0.1 to 2 mg had no significant effect on frog's heart, but in doses of 4 to 8 mg it produced slight bradycardia. Nicotine also produced bradycardia in doses of 2 to 4 mg.

Acute toxicity in albino mice.—HMA had been injected intraperitoneally in albino mice in graded doses from 1 to 10 mg/100g body weight. Ten mice were used for each dose level. Respiratory stimulation and extensive spasm of the tail were observed in all the mice. Upto the dose of 2.5

mg/100g there was decrease in the spontaneous activity. In the dose of 5 mg/100 g there was ptosis, dilatation of pupil and marked decrease in spontaneous activity. Loss of righting reflex was observed in 30 per cent of mice. In doses of 7.5 to 10 mg/100 g there was marked ptosis, absence of spontaneous activity and marked decrease in response to stimuli There was rigidity of the body and loss of righting reflex in 50 to 100 per cent of the mice. Respiratory stimulation was followed by marked depression. In some of the mice there were clonic convulsions. LD_{50} (with 19/20 confidence limits) was found to be 8.5 (7.7 to 9.35) mg/100 g body weight.

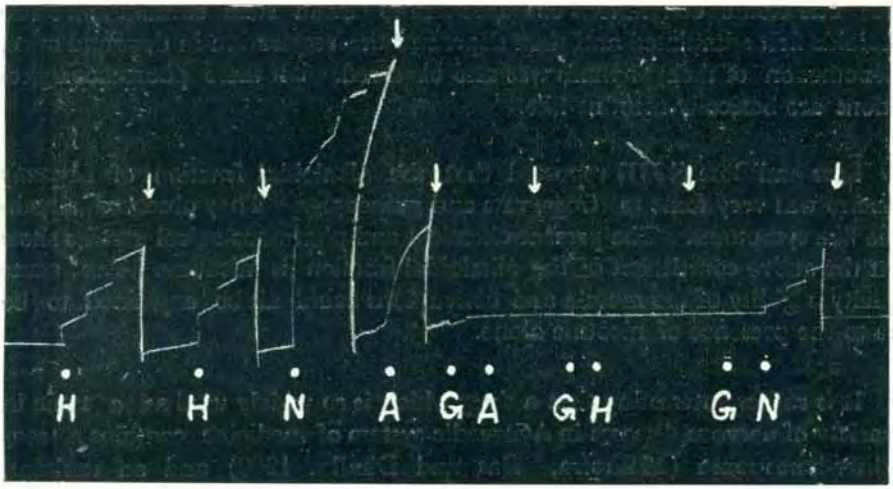

Fig. 4. Effect of HMA (H) $2x10^{-5}$, nicotine bitartrate (N) $2x10^{-5}$ and acetylcholine chloride (A) $2x10^{-6}$ on isolated frog's rectus before and after pretreatment with gallamine triethiodide (G) 10^{-4}. Dots indicate drug administration and arrows indicate wash. Contractions of nicotine and HMA have been recorded for 3 mins and of acetylcholine for 1 min.

Nicotine bitartrate 2 mg/100 g was injected intraperitoneally in 10 mice. It produced decreased spontaneous activity, diminished response to stimuli, loss of righting reflex (in 5 mice), rigidity of the body, clonic convulsions, pupillary dilatation and respiratory stimulation followed by depression. Three out of the 10 mice died.

DISCUSSION

The maximum amount of alkaloidal content of the plant material was obtained when extracted with hot 95 per cent alcohol. Chromatographic analysis of the alkaloidal fraction of the plant indicated the presence of three alkaloids which was in confirmation of the report of Basu and Walia

(1944). The specific colour reactions and the chromatographic test showed that one of the three alkaloids was nicotine, the presence of which in the plant has not been reported so far.

The alkaloidal fraction has been found to specifically stimulate the parasympathetic and the sympathetic ganglia. The stimulation was however, followed by blockade as shown on rabbit's ileum. It had spasmodic effect on frog's rectus muscle which could be prevented by a neuro-muscular blocking agent, gallamine triethiodide. This indicates that the action on the skeletal muscle, at least in part was through the neuro-muscular junctional apparatus. The stimuant action of the alkaloids on the skeletal muscle was expressed as rigidity in mice. The clonic convulsions could be due to brain stem stimulation. The alkaloids first stimulated and then depressed the respiration in dogs and mice. Phenomenon of tachyphylaxis was also observed. All these pharmacological actions are basically nicotine-like.

Bose and Bose (1931) reported that the alkaloidal fraction of *Herpestis monniera* was very toxic to frogs, rats and guineapigs. They observed strychnine like symptoms. The present chemical and pharmacological studies show that the active constituent of the alkaloidal fraction is nicotine. The acute toxicity, rigidity of the muscle and convulsions could all be explained to be due to the presence of nicotine alone.

It is rather interesting that a drug which is so widely used as a tonic in a variety of nervous diseases in Ayurvedic system of medicine contains a tranquiliser-hersaponin (Malhotra, Das and Dhalla, 1960) and an excitant, nicotine.

SUMMARY

The plant *Herpestis monniera* has been found to contain 3 alkaloids, one of them being nicotine as evidenced by chemical and pharmacological tests.

The total alkaloidal fraction stimulated autonomic ganglia followed by blockade, produced spasm of the skeletal muscle acting through neuromuscular junctional apparatus, stimulated respiration followed by depression, showed phenomena of tachyphylaxis and produced rigidity and convulsions in mice. LD_{50} was found to be 8.5 mg/100g when injected intraperitoneally in mice.

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