SOME NEUROPHARMACOLOGICAL AND BEHAVIOURAL EFFECTS OF AN ACTIVE FRACTION FROM HERPESTIS MONNIERA, LINN (BRAHMI).

By

D.K. Ganguly* and C.L. Malhtora,

Department of Pharmacology, Lady Harding Medical College, New Delhi.

Herpestis monniera is a small annual creeping plant, N.O. *Schrphularinae*. In India, the plant was known by the vernacular name of "Brahmi" and was extensively used in folk medicines particularly in cases of insanity, hysteria and epilepsy. A chemically pure saponin named as '*hersaponin*', m.p. 232-234°C (decomp), in addition to D-mannitol and potassium salts were isolated from *Herpestis monniera*, (13). The first alkaloid isolated from the plant showed a few pharmacological properties (2,8,9). *eial* Malhotra, studied the neuropharmacologscal effects of *hersaponin*. It was reported to have a sedative action in mice, reduced amphetamine toxicity of aggregated mice. *Hersaponin* also delayed the rate of disappearance of blood pentobarbital sodium in dogs and had a mild inhibitory action on the oxygen uptake of the rat brain homogenate studied by Warburg technique.

These experimental evidences suggested that the drug might have a potent tranquil lizing action. So it was thought worthwhile to evaluate the tranquillizing properties of the drug as far as possible, as the psychotropic action of *Herpestis monniera* (HM) has not yet been evaluated.

MATERIALS AND METHODS

1. *Spontaneous Motor Activity (SMA) in Rats :—*

(a) The apparatus consists of a light triangular aluminium cage (27X27X17 cm.) made of aluminium wire. The three flattened legs of the cage were mounted on Mary's tambours. Movements of the rat in the cage are transmitted by air-pressure changes to the writing point by means of another tambour which leaves a record on the rotating drum. A second writing point provides a time-record. The effect of drugs on SMA was studied in rats of albino variety weighing between 150 and 190 *gms*. which were of specific strain. The animals were fasted overnight and each animal was placed in the cage individually at a time and after placing the animal in the cage ten minutes were allowed to pass for the initial excitement due to the exploratory behaviour of the animals before recording. Chlorpromazine (CPZ) was taken as a control drug for comparison. Each dose was tested on a group of six animals and each group received three different doses of CPZ (1, 2 & 3 *mg/kg. i.p.*), and HM (2.5, 5 & 8 *mg.*/100 *g., i.p.*). Another saline treated group served as control. The effect was studied 15 to 20 mins. after the administration of HM and 30 mins. after CPZ.

*Present address.. Department of Pharmacy, Jadavpur University, Calcutta.

(*b*) The effect of HM and CPZ was also studied on drug induced stimulation us the same experimental device. Amphetamine was injected at a dose of 2 *mg.* *i.p.* and 15 mins. prior to testing. HM was administered simultaneously with phetamine and CPZ 15 mins. prior to amphetamine and 30 mins. prior to f recording.

All drugs were administered intraperitoneally. HM was in fine suspens in normal saline and the volume injected was between 0.5 to 1.0 *ml.* for all drugs. The activity was recorded for ten minutes.

2. *Hypothermic effects :—*

(*a*) Hypothermic effects of HM, CPZ and 5-HT were studied in albino mice weigh between 22 to 35 *gms.* of either sexes. Different doses of drugs were administe intraperitoneally. Each dose was administerd in ten mice. Each animal se ed its own control as the rectal temperature was taken before and after the dr administration. The rectal temperature was recorded by a clinical therm meter, the bulb was lubricated with glycerine. HM was administered 15 mins., C 30 mins. and 5-HT 20 mins. prior to testing. The experiments were perform in a room where constant temperature of 22 to 23°C was maintained. T temperature of each animal was taken for 60 secs. The dose for HM were 2.5, 8, and 12 *mg*/100. *gm.* CPZ-2, 3, 4, and 5 *mg./Kg.* and 5-HT 5, 10 and 20 *mg./K* administered intraperitoneally.

(*b*) In a second series of experiments the effect of iproniazid, 100 *mg./Kg.*, i.p. w studied on the hypothermic effects of the above drugs. Iproniazid was adm nistered one hour prior and a separate group treated only with iproniazid served a control.

(*c*) In a third series of experiments, the effect of lysergic acid diethylamide (LSD 2 1 *mg./Kg., i.p.*, administered one hour prior to testing was studied.

3. *Forced co-ordinated motor activity in mice :*

The apparatus consists of two main components ; an A.C. motor of 1/4 h.p. and a ge box which transmits the rotation of motor shaft at differential speed to a pulley shaft which set at right angles to the former through a conical rack and penion arrangements. The ro tion of the pulley shaft was controlable. A uniform wooden rod 43 *cm.* long and 2.5 *c.m.* diameter was fitted into the central hole of the pulley shaft and the speed of the pulley shaft wa adjusted that the r.p.m. of the rod was 16/*min.* The experiemental procedure employed w after Kinnard and Carr (1957). The mice weighed between 18 to 24 g. of either sex. The an mals were divided in different groups, each group having five animals. Four such groups we used for each dose and the results were expressed as the mean "fall-time' of the four group HM was injected in doses of 2.5, 5 and 8 *mg.*/100 *gm., i.p.* and CPZ as a standard drug in dos

of 1,2 & 3 *mg./kg*, i.p., 15 and 30 mins. prior to testing respectively. The 'fall-time' means the time each animal stayed on the rotating rod till it fell down. The average of five 'fall-time's in each group was taken as a unit result. After placing the animal on the rotating rod the time was noted for a maximum period of 120 secs. both for control as well as treated groups.

4. Effect on 'Waltzing' mice

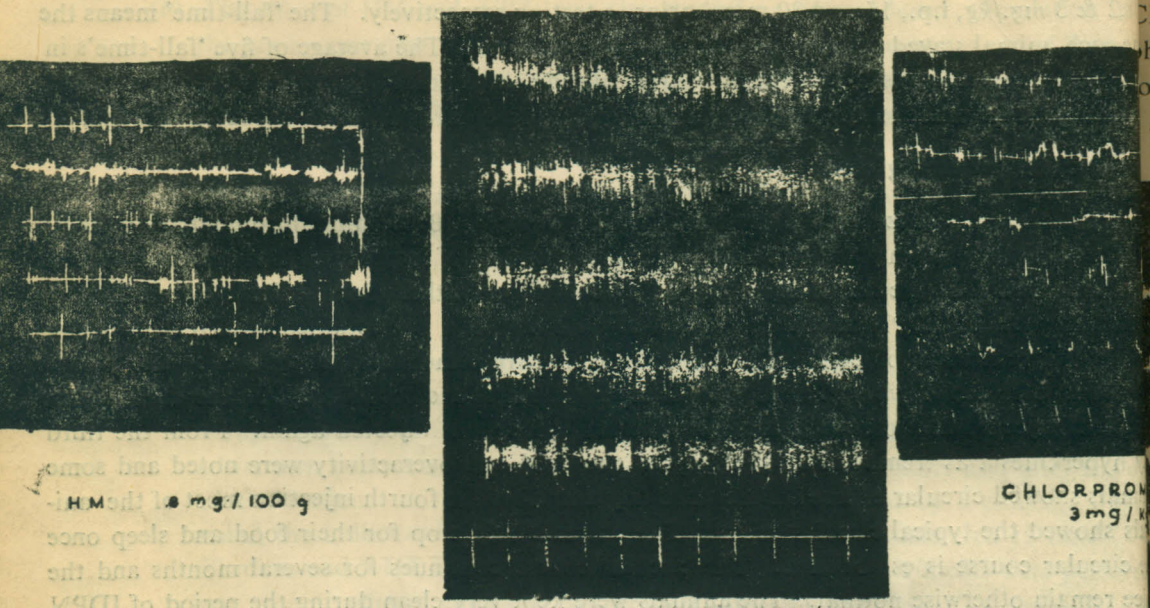
Thuillier and Nakazima (14) demonstrated the effect of B.B. iminodipropionitrile (IDPN) on mice. Chronic administration of IDPN produced a state of hyperkinesia and overactivity in mice and finally a 'circular course' which persisted for months. Such animals are termed as *'Waltzing mice' or 'IDPN mice'. The effects of CPZ and HM were studied on IDPN mice em-* ploying different doses of each. The course of IDPN treatement was as follows : IDPN was injected at a dose of 1 *gm./kg*. intraperitoneally on the first and second day, on the third day no drug was injected and on the fourth day the same dose was injected again. From the third day hyperkinesia as tremor and twtichings of the head and overactivity were noted and some animals showed circular course on the third day and after the fourth injection most of the animals showed the typical circular course. The animals only stop for their food and sleep once the circular course is established. The circular course continues for several months and the mice remain otherwise normal. The animals were kept very clean during the period of IDPN treatment, and only a few animals died during the course.

5. *Effect on 5-HT level of whole rat brain*

Adult male albino rats weighing between 130 and 175 *gms.* of the same breed were used. The animals were divided into three groups of ten animals each; one group was utilized for control value of 5-HT level, the second group was injected with 10*mg.*/100 *gm.* i.p. of HM and the brains were taken out after 30 mins. of injection. Reserpine, 2 *mg./Kg., i.p.*, was injected into the third group of animals and the removal of brain was done after 120 mins. of drug administration. The extraction of 5-HT from the brain tissue was done by the method of Amine, Crawford and Gaddum (1). The weighing of the brain and the addition of required volume of acetone were completed within two mi nutes of brain removal. The assay of 5-HT was performed on rat's uterus made oestrus by injecting stilbestrol in arachis oil, 0.1 *mg./Kg.*, s.c., on the previous day as prescribed by Gaddum, Peart and Vogt. (5). Dejalon's ringer solution containing atropine (10^6) was used as bath fluid and the bio-assay was performed on the same day of extraction. To ascertain that the extract contained no other active substance except 5-HT, they were tested again after a potent LSD blockade.

RESULTS.

1. The effect of HM and CPZ on SMA of normal rats is illustrated in Fig. 1 and the results are summarised in Table I.



EFFECT OF HM AND CHLORPROMAZINE ON SMA OF RATS.

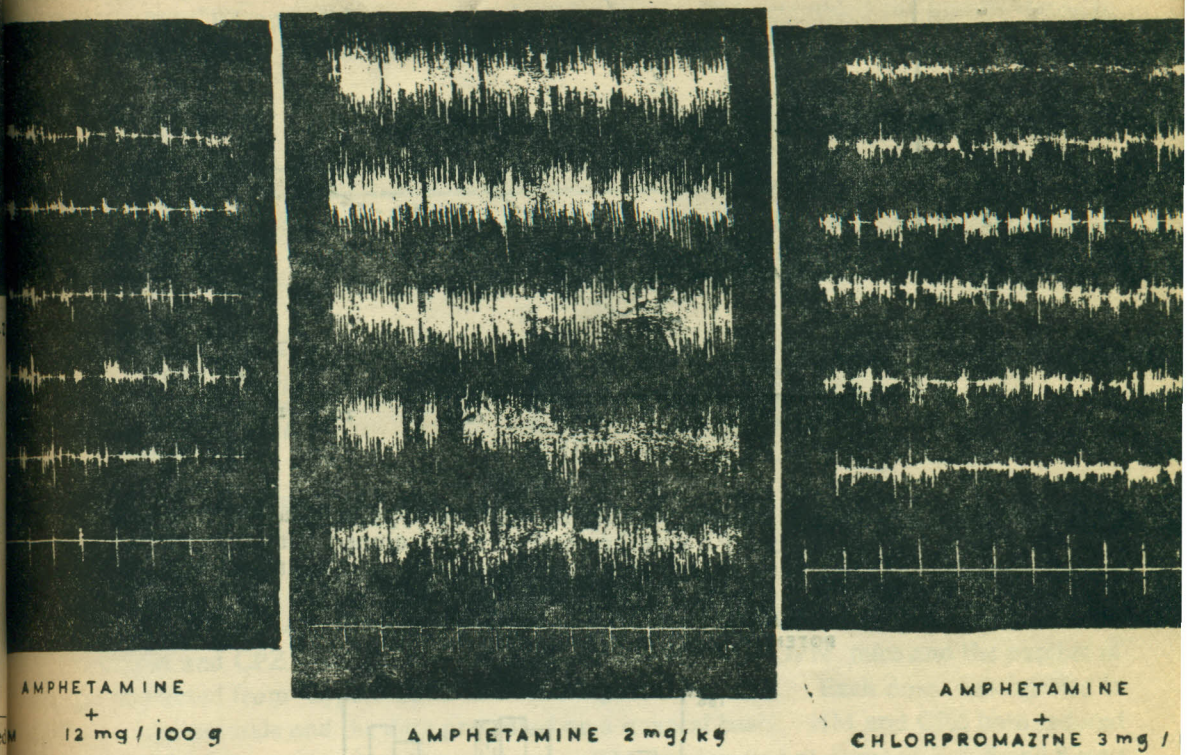
Fig. 1

TABLE I

Effect of HM (c) and chlorpromazine on normal and amphetamine stimulated rats.

Drug	Dose i.p.	Spontaneous motor activity (SMA)	
		Non-amphetamine rats (N=6)	Amphetamine stimulated rats (N=6)
Control		+++	+++++
HM (c)	2.5 *mg/100gm*	++ (+/—)	+++++
	5 *mg/100gm*	++	++++(+/
	8 mg/100 *gm*	+	+++
			++
CPZ	1 *mg/kg*	++	++++
	2 *mg/kg*	+(+/—)	+++
	3 *mg/kg*	+	++

HM like CPZ reduced the SMA of normal rats and the equipotent doses of HM and CPZ were found to be 8 *mg./100 gm.* and 3 *mg./Kg.*, *i.p.* respectively. But in cases of amphetamine stimulateds rats, HM required a higher dose (12 *mg./100 gm.*) to diminish SMA to the same extent as caused by CPZ, 3 *mg./Kg.*, i.p. (Fig. 1a). Lower doses of HM (2.5 and 8 *mg./100 gm.*, *i.p.*) did not have any appreciable effect on amphetamine stimulated rats.



EFFECT OF HM AND CHLORPROMAZINE ON SMA OF AMPHETAMINE STIMULATED RATS.

Fig. 1a

2. The degree of hypothermia produced by different doses of HM, CPZ & 5-HT is shown in Fig. 2.

The maximum fall of body temperature was produced by CPZ and minimum with 5-HT. Iproniazid, a known enzyme-inhibitor when injected alone at a dose of 100 *mg./Kg.*, ip,., did not produce any change of body temperatnre but when injected before HM, CPZ and 5-HT, potentiated their hypothermic effects and it may be assumed that the increased hypothermia was due to the increased level of 5-HT (Fig. 3). **L.S.D.** also was investigated upon the hypothermia

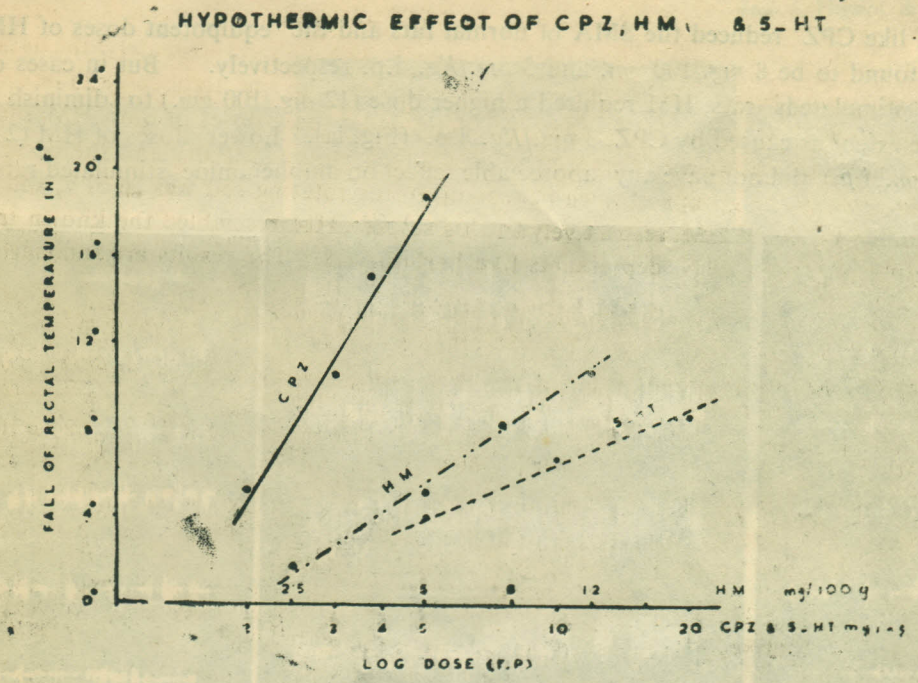


Fig. 2

effects of the three durgs-the antagonism of HM induced hypothermia was to a lesser ext than 5-HT and antagonism of CPZ was the least. LSD alone did not have any signific effect on noraml body temprature of mice. The results are represented in Fig. 3.

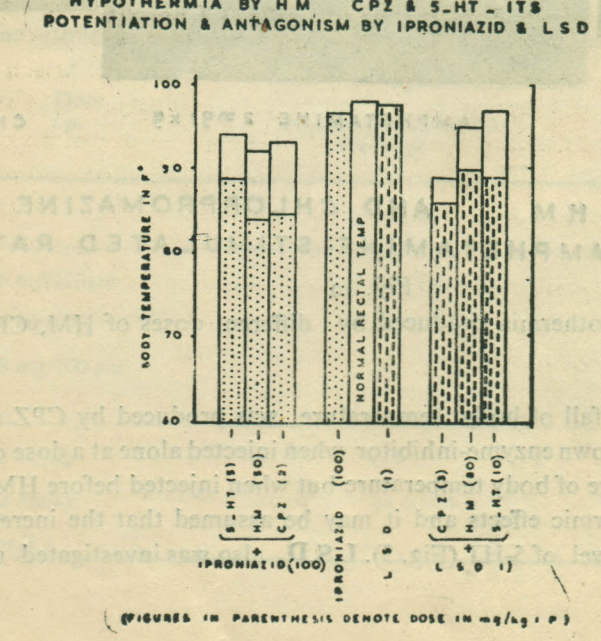


Fig. 3

(3) It is seen that CPZ and HM reduced the SMA approximately to the same extent at a dose level of 3 *mg./Kg.*, & 8 *mg.*100/*gm.* i.p. respectively, though unfortunately the data could not be expressed numerically, the reduction of SMA as shown in Fig. 1 appeared to be approximately about 80-90% as compared to the control. Under these doses of both HM and CPZ the animals were still capable of performing the forced motor activity and the decrease of length of the time of the groups of mice to remain on the rotating rod was 56 51% and of the conrrol time for CPZ and HM. respectively In this respect, HM resembled the known transqui lizers and differed from C.N.S. depressants like barbiturates. The results are summarised in-Table II.

TABLE II

Effect of HM and chlorpromazine on 'forced co-ordinated motor activity' in groups of mice (N=5/gp).

Drug	Dose i.p.	Number of; gruop (5/gp)	Average 'falltimes' S.E.	reduction%
Control	..	25	109 secs.	
Cylorpromazine	1 *mg/kg.;*	4	101±7	8%
	2 *mg/kg.;*	4	74±9	32%
	3 *mg/kg.;*	4	48±6	56%
HM	2.5 *mg*/100*gm*	4	111	Nil.
	5 ,,	4	88	20%
	8 ,,	4	54	51%

(4) HM and CPZ in different doses were administered in IDPN mice and the number of animals protected from hyperkinesia and circling were counted. Each dose was injected in a group of ten animals and the data presented on a quantal basis. HM and CPZ both reduced the number of circling mice in different doses. HM was injected at doses of 2.5, 5 and 8 *mg./* 100 *gm.*, and CPZ at 1, 2 and 3 *mg./Kg.*, i.p. The drug treated animals at such doses did not show any ataxia and neurological deficit. HM and CPZ were injected 15 and 30 mins. prior to observation respectively. Saline was injected in a different group to serve as control.

It is observed that HM and CPZ abolished agitation and circling movements of IDPN mice and the animals did not exhibit marked sedation, ataxia or any other signs of neurological deficit. The results are tabulated in Table III.

(5) To confirm the role of 5-HT in HM's mechanism of action as suggested by the evidence of antagonism of some of its effects by LSD. and potentiation by iproniazid,the 5-HT content of the rat whole brain was estimated after injection of HM, 10 *mg./*100 *gm. i.p.* and at the same time in another group of animals 5-HT level was estimated in reserpinised rats as

TABLE III

Effect of HM(cr)and chlorpromazine on IDPN induced hyperkinesia and cricling in mice

Drug.	Dose (*i.p*)	No. of mice	abolition of hyperkinesia%	Abolition of circling%
Saline	0.4 *ml.*	15	Nil	Nil
HM	2.5 *mg*/100*gm* ;	14	Nil	21.4
	5 ,,	15	26.6	46.7(P*0.05)
	8 ,,	15	66.6	80.0 (P*0.01)
Chlorpromazine	1 *mg/kg.*	12	8.3	25
	2 ,,	15	33.3	60*0(P∠0.05)
	3 ,,	13	76.9	92.3(P∠0.01)

*Probability (P) calculated by chisquare test.

a standard drug. The results showed (Table IV) that there was significant reduction of mal level of 5-HT after reserpine from 249.04± 80.32 ng. to 131.3± 130.74 *ng./gm.* of tissue (P∠O..01) On the other hand after HM, the reduction was to a much lesser ex from 249.04±80.32 to 208.78±53.48 *ng./gm.* This reduction of 5-HT level was stat cally insignificant too.

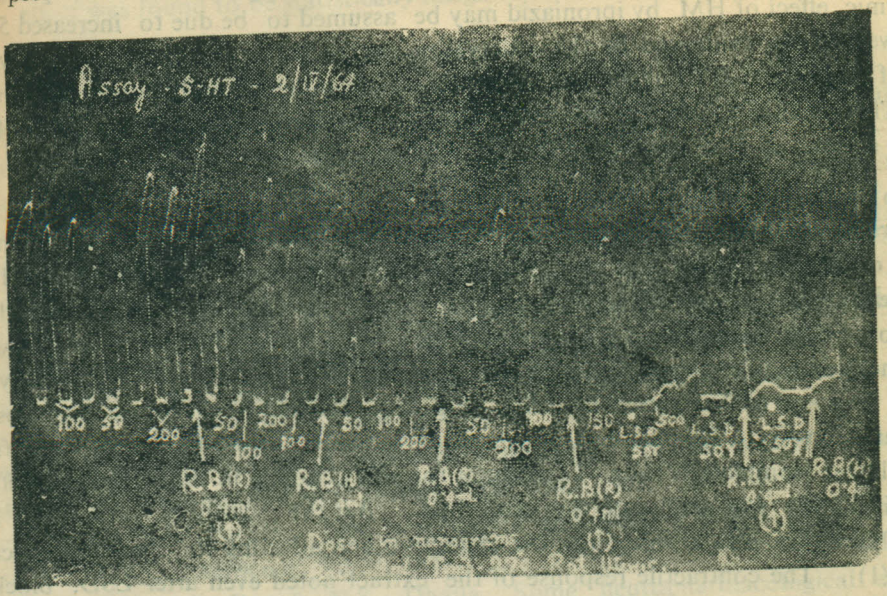
TABLE IV

Brain serotonin content before and after drugs.

No. of rats.	5-HT content in nanogram/gm. of fresh tissue					
	Control	Mean ±S.D.	Reserpine; (2 *mg/kg i.p.*)	Mean ±S.D.	HM(cr) 10 *mg*/100*gm*	Mean ±S.D.
1	236.16	..	139.83	..	276.57	..
2	139.05	..	130.5	..	257.38	..
3	258.93	249.04	103.43	131.3	146.15	208.
4	113.4	±80.32	142.05	±13.74	150.36	±53.
5	304.92	....	130.23	P∠0.01	228.87	P>0.
6	182.47	..	135.22	..	218.34	..
7	331.12	..	150.36	..	141.97	..
8	326.79	..	144.45	..	166.79	..
9	326.79	..	112.72	..	225.00	..
10	271.17	..	124.26	..	276.38	..

'P' value calculated from 't' test.

After potent LSD. blockade (5 *ng./ml.*) it was observed that a few extracts still gave a contraction. The nature of these contractions differed to that of standard 5-HT in respect that they were : (a) slow in nature (b) the latency of contraction was usually more than 40 to 60 secs. whereas the standard 5-HT responded within maximum 15 secs. of its addition to the bath and (c) a degree of either a reduction or increase of the standard 5-HT contractions were usually noted which could not be blocked by LSD. An example of such an interfering substance's presence is shown in Fig. 4. Such values were not taken into account.



Ftg. 4

DISCUSSION

One of the most obvious signs of sedation in animals is the reduction of so-called SMA. Spontaneous activity of rats and mice is depressed by most of the tranquillizers, *e.g.* chlorpromazine, meprobamate, *etc.* (12, 4, 1), reserpine (10) *etc.*, a property which is shared by HM too. HM like CPZ reduced the SMA of rats and the equipotent doses of HM and CPZ were found to be 8 *mg.*/100*gm.* and 3 *mg./Kg., i.p.* The effect of HM on SMA differed from other drugs of barbiturate groups as hexobarbital, pentothal and amobarbital produced hyperactivity in small and subataxic doses, marked reduction only in ataxic doses (quoted from Riley and Spinks. 11). From the results it was found that HM was approximately 25 times less potent then CPZ in reducing the SMA. The SMA of amphetamine stimulated rats was also reduced by HM but a higher dose was required. The equipotent doses for reduction of the SMA of amphetamine stimulated rats for CPZ and HM were found to be 3 *mg./Kg.* and 12 *mg./*100 *gm., i.p.*, respectively.

It was shown that the sedative effects of tranquillizers like CPZ and reserpine as sured by potentiation of barbiturate sleep or reduction of activity have been found to b portional to the accompanying fall of body temperature and it was consequently been gested that sedation is caused by interference with the mechanism of temperature regula (7). HM, CPZ and 5-HT all reduced the body temperature of mice. 5-HT was also stu along with as LSD. antagonised the effect of HM (unpublished data) and it was thoug be probable that 5-HT has some role in the drugs mechanism of action. The potentiati hypothermic effect of HM by iproniazid may be assumed to be due to increased 5-HT which was further evidenced by the observation that LSD. antagonised the hypothe effects of HM like 5-HT but did not have any significant effect on hypothermia produce CPZ. There is thus an indirect evidence of serotonin release as one of the probable m nism of action of HM simulating that of reserpine (3).

To confirm the role of 5-HT in HM's mechanism of action the 5-HT content of th whole brain was studied after injection of HM and as there was resemblence with rese in some of its effcts, 5-HT was also estimated after reserpine. The 5-HT level after was decreased but to a much lesser extent than after reserpine and was statistically ins ficant too. While estimating 5-HT content of rat's brain it was noted on a few occassions some other contractile substances were also extracted along with 5-HT indicated by failu block the response of the extracts by LSD. (Fig. 4). The additional contractile substanc difficult to identify. This method of extraction ensured the separation of 5-HT from s tance P almost completely, (1). To account for acetylcholine in the tissue extract, add of atropine (10^6) was made into the bath fluid. But the possiblity of K salts, ATP, pitiutary hormones, *etc.* coming into the extraction and affecting the preparation can n ruled out (1). The contractile response of the extract noted even after LSD. blockade discarded and the results were not included.

SUMMARY

A battery of pharmacological tests were performed to evaluate the drug's tranquilli properties employing many of the methods and techniques followed conventionally.

(*a*) The drug reduced the spontaneous motor activity in nonstimulated rats like o tranqullizers but was less effective in antagonising the spontaneous motor acti of amphetamine stimulated rats.

(*b*) The drug produced graded lowering of rectal temperature in mice. The effect found to be potentiated by iproniazid, an enzyme inhibitor and antagonised by L a known peripheral antagonist of serotonin.

(*c*) It was observed that the animals (mice) were capable of performing forced m activity after injection of HM at doses which reduced markedly the spontaneous m activity.

(*d*) HM inhibited the circling and hyperkinesia induced by chronic intraperitoneal administration of B.B. iminodipropinitrile in different doses without producing ataxia and loss of righting reflex.

(*e*) 5-HT content of whole rat brain was assayed before and after HM and it was observed that the drug slightly diminished the 5-HT content of rat's brain but the effect was found to be statistically insignificant.

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