PHARMACOLOGICAL STUDIES OF NITROHARMIDINE NITRATE ON CENTRAL NERVOUS SYSTEM

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Nitroharmidine nitrate, a modified new alkaloid of Peganum harmala has been studied for its pharmacological actions on the central nervous system. Albino mice, rats and the rabbits have been employed. The drug was tried in 250 mcg, 500 mcg, 1 mg, 2 mg and 4 mg/kg body weight doses. The drug in 250 mcg/kg B.W. doses produced slight increase in voluntary movements in mice whereas larger doses markedly reduced the activity. The drug did not induce specific block of conditioned avoidance response in rats with any dose tried. The larger doses blocked both the conditioned and unconditioned avoidance responses. In rabbits general body temperature was raised with all the doses of the drug. Variable effects on pentobarbitone induced sleeping time in rabbits was observed depending upon the time of administration of barbiturate. A mixed pattern of EEG in rabbits was revealed with smaller doses of the drug whereas larger doses produced a modified activation of EEG.

Red Indians used to take the seeds of Peganum Harmala Linnean on the occasions of feast and festivities to produce hallucinations. Two alkaloids, harmaline and harmine have been isolated and studied by Gunn (1913) and by Chen and Chen in 1939 (Gershan and Lang, 1962) and many other investigators.

Peganum harmala grows in abundance in the forests of the North Western regions of Pakistan. The seeds of Peganum harmala, in this country, are burnt and the fumes used as deoderant and antiseptic.

Siddiqui (1962) improved the methods of isolation of active principles of Peganum harmala and successfully isolated a new substance named as harmidine. This alkaloid, harmidine has been studied in our laboratories and found to be a monoamine oxidase inhibitor (Akhtar 1971; Babar 1971). Siddiqui (1972) modified the structure of harmidine with an idea of enhancing its antipsychotic and antihypertensive activities and produced mono-nitroharmidine by introducing the nitroso group in the benezene ring of harmidine.

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Chemistry

Mono-nitroharmidine nitrate, a modified alkaloid of Peganum harmala has a melting point 245.—252°C. It is fairly soluble in water and the solubility of the salt increases manifold by heating. The molecular formula of the base is $C_{13} H_{13} O_3 N_3$.

Experimental

Material

Adult albino mice weighing between 25-35G and 3-4 months old reared at 27°C temperature at our own colony were used. They were fed ad libitum with mouse feed and water.

Adult Sprague Dawly rats of either sex weighing between 250-300G and 3-4 months old reared locally and fed ad libitum with mouse feed and water, were employed in these studies.

Rabbits of either sex weighing between 1.5-2.5 kg and fed on lucerne, carrots and water were used.

Nitroharmidine nitrate supplied by the Postgraduate Institute of Chemistry, Karachi University, was used. A stock solution of the drug was made by dissolving it in distilled water heated to 60°C. Further dilutions were made in normal saline.

Actophotometer and Skinner's box were used for studying the activity of the animals. The Grass model 5D polygraph provided with additional pre-amplifier unit for EEG was employed for recording EEG tracings.

Methods

1. *Effect on Voluntary Motor Activity*

This study was made on mice. The animals were divided into seven groups and each group had 25 mice. The total volume of drug injected did not exceed 0.01 ml/G body weight.

Group 1 served as untreated control where the animals were not given any treatment.

Group 2 comprised animals treated with normal saline injected intraperitoneally.

Group 3 to 7 animals received nitroharmidine nitrate intraperitoneally in doses of 250 mcg,

500 mcg, 1 mg 2 mg and 4 mg per kilogram body weight respectively.

Each group was further divided into sub-groups each comprising of 5 animals. The activity was studied by leaving the animals of sub-group in the chamber of actophotometer for 4 hours. Readings were taken at 0, 15, 30, 60, 120 and 240 minutes after the administration of the drug.

2. *Effects on the Conditioned and Unconditioned Avoidance Response in Rats:*

The effect of nitroharmidine nitrate on conditioned avoidance response was studied by adopting the technique of Cook and Weidly (1957). The animals were divided in 7 groups and each group comprised of 10 rats. The total volume of drug solution injected was 0.5 ml intraperitoneally.

Group 1 consisted of untreated control subjected to experimental procedures without any prior treatment with drug or normal saline.

Group 2 was given normal saline intraperitoneally before experimentation.

Group 3 to 7 were treated with nitroharmidine nitrate injected intraperitoneally in 250 mcg, 500 mcg, 1 mg, 2 mg and 4 mg per kilogram body weight respectively.

Before starting each experiment the rats were tested for conditioned response and were reinformed with unconditioned stimulus to ensure 100 per cent stability of the developed conditioned response.

The rat was placed in the chamber and left undisturbed for 30 seconds. The conditioned stimulus (buzzer) was then delivered until the rat climbed over the pole for a period of 30 seconds. After the animal came back on the floor of chamber same procedure was repeated using the unconditioned stimulus (shock). Each animal was tested at 0, 15, 30, 60 120 and 240 minutes after the drug treatment.

Animals climbing over the pole within 30 seconds of the application of the conditioned or unconditioned stimulus were taken as positive while those not climbing over the pole within 30 seconds were treated as negative (Cook and Weidly, 1957).

In all the groups of animals subjected to experiment the size of pupil for moisis or mydriasis and condition of eye for ptosis or proptosis were also noted.

3. *Effect on Normal Body Temperature in Rabbits*

The room temperature kept at 26-27°C, the rectal temperature of animals was recorded with a clinical thermometer. The thermometer was kept in the rectum for 1 minute and mean of 3 consecutive readings taken at 1 minute intervals was taken. The animals showing larger departure from the mean were discarded. The temperature was recorded at 0, 15, 30, 60, 120 and 240 minutes after administration of drug.

The animals were again divided into 7 groups and each group contained 6 rabbits. The total volume of drug solution injected intraperitoneally was 1.0 ml.

The grouping of animals and treatment of groups was as per previous experiments.

4. *Effects of Barbiturate Induced Hypnosis in Mice:*

Mice weighing 25-30G, were included in this study. The animals were divided into 3 groups. The total volume of injected material was 0.01 ml/G, body weight.

Group 1 comprising of 12 animals served as control. Each animal in this group was injected pentobarbitone 50 mg/kg B.W. as 5 per cent solution (Everette et al., 1963).

Each of the 2nd and 3rd groups were further divided into 6 sub-groups and each sub-group comprised of 12 animals (Fouts and Brodie, 1956). In group 2, the animals were injected with 5 per cent solution of 50 mg/kg B.W. of pentobarbitone immediately after treatment with saline or test drug. This group was divided into 6 sub-groups as follows:-

Sub-group (i) was injected intraperitoneally 0.01 ml/G B.W. of normal saline to serve as saline treated control group.

Sub-group (ii) to (vi) were treated with 250 mcg, 500 mcg, 1 mg, 2 mg and 4 mg per kilogram B.W. nitroharmidine nitrate injected intraperitoneally.

Group 3 was also divided into 6 sub-groups. In this group the pentobarbitone was administered 15 minutes after the saline or drug treatment, otherwise the sub-groups were similar to group 2.

The sleeping time in mice was presumed to be the interval that lapsed between the loss of the righting reflex and its recovery.

5. *Effect on Electroencephalogram:*

The experiments were performed on uncurarized, conscious and physically restrained rabbits weighing between 2-2.5 kg. All the experiments were performed in noiseless surroundings in a semi dark room. A Grass model 5D polygraph was used to record the cortical

electrical activity. The stainless steel needles of resistance less than 10,000 ohms were used as electrodes inserted into the scalp of the animal (Dawson and Walter, 1944; Engel, 1947; Wada and Gibson, 1959). Cortical bipolar leads were used for EEG and the record was made through two right frontal leads inserted 6 mm in front of coronal suture; two right parietal leads inserted 6 mm behind the coronal suture and two occipital leads inserted 6 mm behind the posterior parietal lead. All the leads stopped 2 mm short of the sagital suture and the leads in each set were 2 mm, apart from each other (Gangloff and Monnier, 1957). The animals were screened and left undisturbed for 30 minutes before taking the record. The readings were taken at 0, 15, 30, 60, 120 and 240 minutes after the administration of drug.

Both the qualitative and quantitative analysis of the EEG were carried out by visual inspection (Dawson and Walter, 1944; Engel, 1947; Goldstein and Aldunata, 1960).

The animals were divided into 6 groups and each group comprised of 6 rabbits. The total volume of the drug solution injected intraperitoneally was 1.0 ml.

Group 1 and 2 served as untreated and saline treated controls whereas groups 3 to 6 received 250 mg, 500 mcg, 1 mg and 2 mg per kilogram B.W. nitroharmidine nitrate.

Results

A. *Effect on Voluntary Motor Activity in Mice*

A dose of 250 mcg/kg B.W. nitroharmidine caused an increase in the number of movements recorded at all time intervals but this change was not statistically significant. A dose of 500 mcg/kg B.W. on the other hand produced a decrease in voluntary motor activity but it was also insignificant statistically. However, higher doses of 1 mg and above brought about statistically significant decrease in voluntary motor activity at all time intervals.

A maximum decrease was observed with all these doses at 15 minutes interval. The results have been presented in Table I. Tremors, clonic convulsions and traub tail was observed with all doses.

B. *Effect of Conditioned Avoidance Response in Rats*

Nitroharmidine nitrate did not specifically block the conditioned avoidance response in rat with any dose at any time interval. A dose that brought about a block of conditioned response in an animal also produced the simultaneous block of the unconditioned response in the same animal. Doses ranging from 1 mg to 4 mg/kg B.W. brought about effect as is shown in Figure 1. Nitroharmidine nitrate in 1 mg/kg B.W. doses produced 20 per cent and 10 per cent block of conditioned and unconditioned responses at 15 and 30 minutes intervals respectively, whereas 4 mg/kg B.W. dose resulted in 100 per cent block of both conditioned and unconditioned responses at 15 minutes.

Table I: Effect on Voluntary Motor Activity of Mice at Various Intervals Treated with Different Doses of Nitroharmidine Nitrate

Group No.	*Drug Treatment*	*MEAN NUMBER OF MOVEMENTS ± S.E.**				
		After 15 *min.*	*After* 30 *min.*	*After* 60 *min.* (1 *hour*)	*After* 120 *min.* (2 *hours*)	*After* 240 *min.* (4 *hours*)
1.	Untreated control	88.4 ± 5.20	144.4 ± 11.09	236.88± 14.5	356.88± 14.4	488.6 ± 17.56
II.	Normal saline 0.01 ml/G B.W. I/P	74.32± 12.02	155.0 ± 11.7	250.08± 18.06	396.76± 23.22	494.44± 30.98
III.	Nitroharmidine 250 ug/kg B.W. I/P	84.4 ± 4.96	155.0 ± 8.59	278.76± 10.09	406.98± 11.07	544.84± 9.1
IV.	Nitroharmidine 500 ug/kg B.W. I/P	52.92± 8.06	107.40± 14.9	215.48± 15.05	322.52± 17.21	450.92± 22.59
V.	Nitroharmidine 1 mg/kg B.W. I/P	28.2 ± 2.6	46.64± 1.94	85.8 ± 11.9	186.0 ± 11.6	276.82± 30.69
VI.	Nitroharmindine 2 mg/kg B.W. I/P	13.16± 0.91	31.72± 2.21	82.52± 3.82	204.0 ± 13.91	315.56± 15.76
VII.	Nitroharmidine 4 mg/kg B.W. I/P	12.5 ± 1.21	26.8 ± 2.62	65.7± 13.9	182.5± 14.5	298.0 ± 28.35

*Standard error.

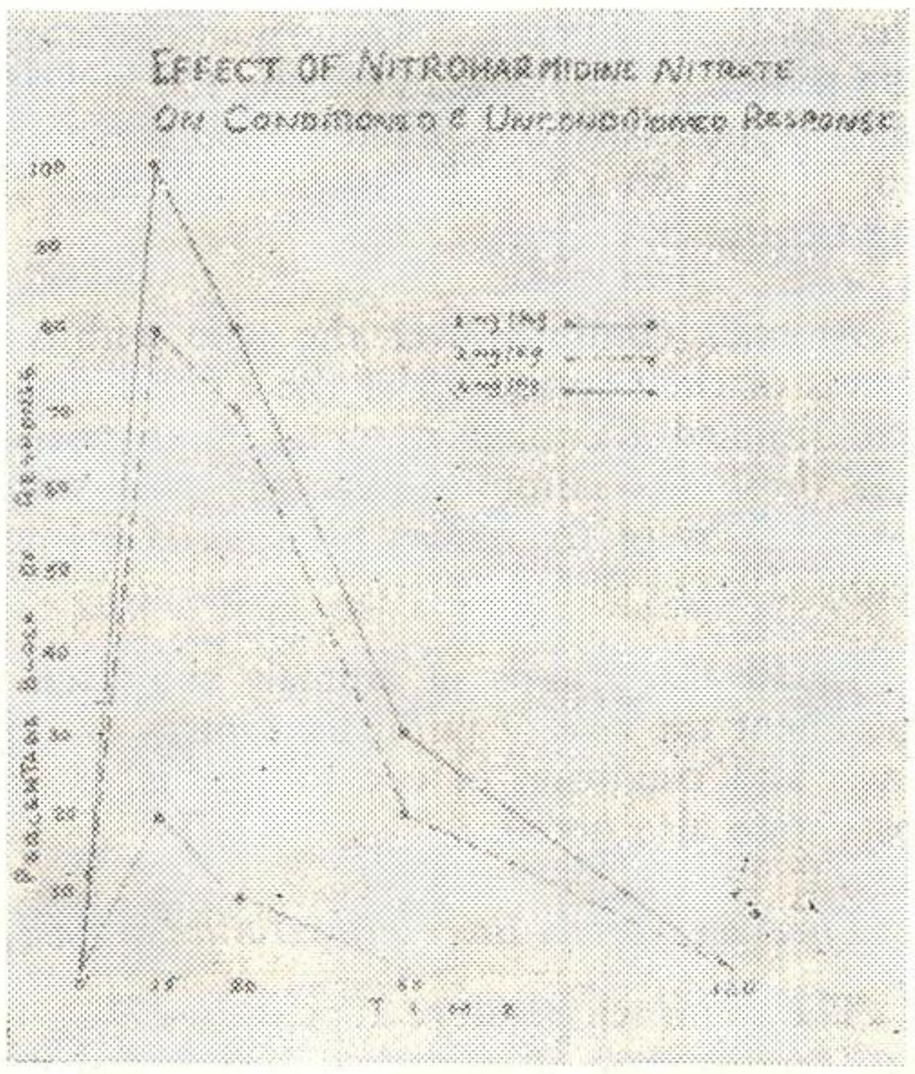

Fig. 1 : Graph showing the effect of different doses of nitroharmidine nitrate on the conditioned avoidance and unconditioned responses of rats.

The block produced was reversible and animal recovered after sometime. The time of recovery to positive response was directly proportional to the dose of the drug.

No change in the size of the pupil or condition of eye was observed in any animal with any dose at all time intervals. The animals suffered tremors, swaying gait and clonic convulsions with all the doses tried.

C. *Effect on General Body Temperature*

Nitroharmidine nitrate caused a significant rise in general body temperature with all the doses tried. The rise was observed at 15 minute and 30 minute intervals. The maximum rise was observed at 30 minutes after 4 mg/kg B.W. dose. The results have been shown in Figure 2. The temperature started subsiding after 60 minutes and was normal after 2 hours.

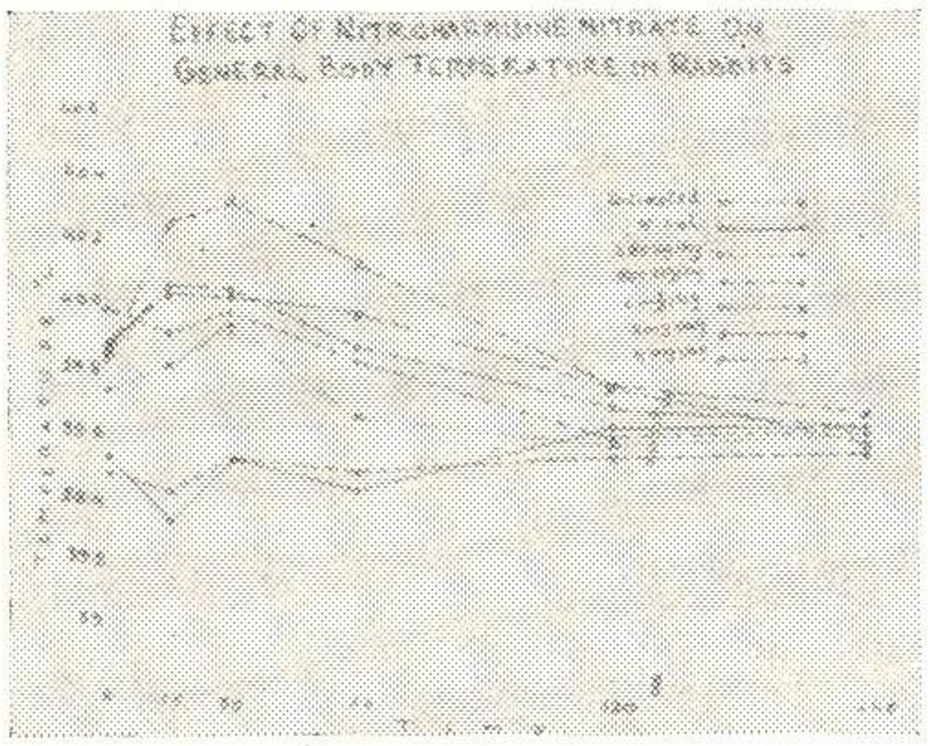

Fig. 2: Graph showing hyperthermic effect of different doses of nitroharmidine nitrate on the general body temperature of rabbits in comparison with that of normal saline.

D. *Effect on Pentobarbitone Induced Sleeping Time*

The effect of nitroharmidine nitrate on pentobarbitone induced sleeping time was variable. It was dependent upon the interval between the administration of the drug and in subsequent injection of pentobarbitone.

(*a*) When pentobarbitone was injected immediately after the administration of nitroharmidine, a remarkable decrease in the sleeping time was observed. The effect was statistically significant with 1 to 4 mg per kilogram B.W. doses. The results are shown in Table II.

(*b*) However, there was a marked increase in pentobarbitone induced sleeping time when the drug was injected 15 minutes after administration of nitroharmidine. The effect was again statistically signifiant with higher doses ranging 1-4 mg/kg B.W. The results are recorded in Table III.

E. *Effect on Electroencephalogram in Rabbits*

The normal EEG of a restrained uncurarized and conscious rabbit presented a mixed pattern of different frequencies (Figure 3). The polyrhythmic picture was equally contributed by a slow alpha rhythm of 8-10 cycles per second and the theta rhythm of 5-6 cycles/second with an intermittent 13 cycles/second resting spindles. The resting spindles or waves were more frequent when the animal was settled and calm. The saline treated group and those receiving 250 and 500 mcg/kg B.W. doses of nitroharmidine nitrate did not show any statistically significant departure from the untreated group. The groups of animals receiving 1 and 2 mg/kg B.W. doses of the drug recorded a diffuse rhythmic alert pattern of EEG at 15 minutes interval. Theta activity contributed a major portion of the EEG which was interspersed with a modified alert pattern of 3-4 cycles/second.

Table II: Effect of Nitroharmidine Nitrate on Sleeping Time in Mice with Pentobarbitone Sodium injected Immediately After Nitroharmidine.

Group No	*Sub group No*	*Name and doses of the drug*	*Mean Sleeping time in minutes± S.E.**	*Relative Sleeping time*	*P. Value*
I.		Nembutal 50 mg/kg B.W. I/P	77.5± 3.61		
II.	1	Normal saline (0.9%) sodium chloride 0.01 ml/G. B.W. 50 mg/kg B.W. I/P.	80.2± 4.54	1.0	P ⩾0.6
II.	2	Nitroharmidine nitrate 250 ug/kg B.W. I/P.+ Nembutal 50 mg/kg. B.W. I/P.	79.0± 6.8	0.99	P ⩾0.8
II.	3	Nitroharmidine nitrate 500 ug/kg B.W. I/P.+ Nembutal 50 mg/kg/ B.W. I/P.	72.1± 4.71	0.89	P ⩾0.1
II.	4	Nitroharmidine nitrate 1 mg/kg B.W. I/P.+ Nembutal 50 mg/kg B.W. I/P.	66.9± 5.17	0.83	P ⩽0.05
II.	5	Nitroharmidine nitrate 2 mg/kg B.W. I/P.+ Nembutal 50 mg/kg. B.W. I/P.	61.0± 6.81	0.76	P ⩽0.05
II.	6	Nitroharmidine nitrate 4 mg/kg B.W. I/P.+ Nembutal 50 mg/kg. B.W. I./P.	53.0± 3.07	0.66	P⩽0.001

*Standard error.

Table III: Effect of Nitroharminine Nitrate on Sleeping Time in Mice Produced with Pentobarbitone Sodium Injected 15 Minutes After Nitroharmidine.

Group No	*Sub group*	*Name and doses of the drug*	*Mean sleeping time in minutes ± S.E.**	*Relative Sleeping time*	*P Value*
I.		Sodium pentobarbitone 50 mg/kg B.W. I/P.	77.5 ± 3.61		
III.	1	Normal saline (0.9%) sodium chloride 0.01 ml/G B.W. Nembutal 50 mg/kg B.W. I/P.	79.6 ± 4.25	1.0	P < 0.6
III.	2	Nitroharmidine nitrate 250 ug/kg B.W. I/P. + Nembutal 50 mg/kg B.W. I/P.	82.25 ± 3.67	1.03	P > 0.4
III.	3	Nitroharmidine nitrate 500 ug/kg B.W. I/P. + Nembutal 50 mg/kg B.W. I/P.	85.54 ± 6.5	1.07	P > 0.2
III.	4	Nitroharmidine nitrate 1 mg/kg B.W. I/P. + Nembutal 50 mg/kg B.W. I/P.	90.16 ± 3.7	1.13	P < 0.05
III.	5	Nitroharmidine nitrate 2 mg/kg B.W. I/P. + Nembutal 50 mg/kg B.W. I/P.	97.5 ± 7.25	1.22	P < 0.05
III.	6	Nitroharmidine nitrate 4 mg/kg B.W. I/P. + Nembutal 50 mg/kg B.W. I/P.	106.0 ± 7.09	1.33	P < 0.01

*Standard error.

Table IV: Effect of Graded Doses of Nitroharmidine Nitrate on the Frequency of E.E.G. of Rabbits Recorded at Various Intervals

Sl. No.	*Treatment*	*MEAN FREQUENCY C/SECOND AT VARIOUS INTERVALS ± S.E.**					
		0 *minutes*	15 *minutes*	30 *minutes*	60 *minutes*	2 *hours*	4 *hours*
I.	Untreated control group	7.99 ± 0.32	8.37 ± 0.29	7.8 ± 0.23	7.96 ± 0.37	7.85 ± 0.29	7.80 ± 0.31
II.	Saline treated control group	8.21 ± 0.31	7.97 ± 0.15	7.87 ± 0.3	8.49 ± 0.14	8.52 ± 0.28	8.69 ± 0.5
III.	Nitroharmidine nitrate 250 ug/kg B.W. I/P	8.89 ± 0.87	7.43 ± 0.62	8.3 ± 0.61	8.9 ± 0.24	9.3 ± 0.32	9.3 ± 0.35
IV.	Nitroharmidine nitrate 500 ug/kg B.W. I/P	9.35 ± 0.61	7.49 ± 0.67	8.17 ± 0.75	8.5 ± 0.48	9.57 ± 0.86	9.36 ± 0.67
V.	Nitroharmidine nitrate 1 mg/kg B.W. I/P	7.83 ± 0.4	5.7 ± 0.22	6.45 ± 0.25	7.96 ± 0.24	7.83 ± 0.48	7.72 ± 0.28
VI.	Nitroharmidine nitrate 2 mg/kg B.W. I/P	7.94 ± 0.51	5.5 ± 0.21	6.11 ± 0.3	7.61 ± 0.44	8.17 ± 0.46	8.04 ± 0.31

*Standard error.

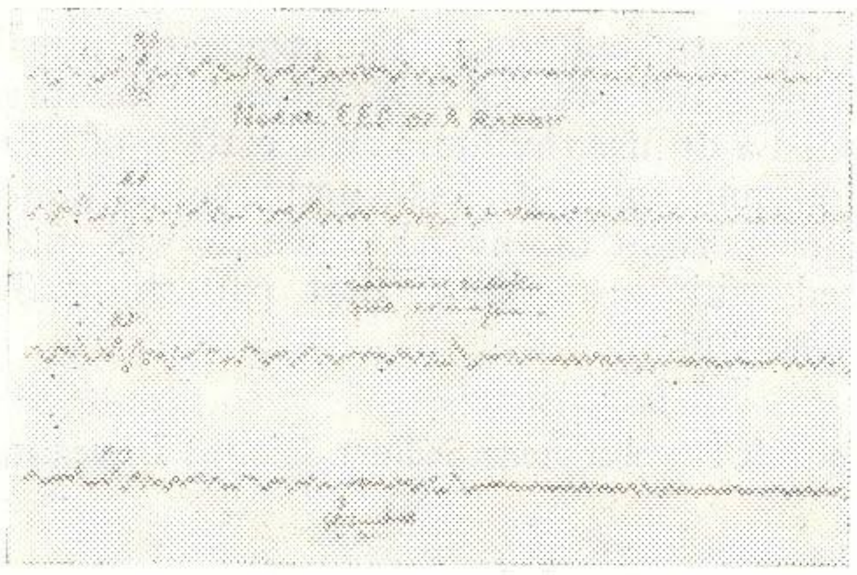

Fig. 3 : Showing the normal pattern of E.E.G. of restrained, uncurarized and conscious rabbit.

The qualitative analysis revealed a fall in mean frequency to 5.78±0.22 and 5.50±0.21 per second with 1 and 2 mg doses respectively.

These results were statistically significant. The voltage did not show undulations and it fluctuated between 30-50 uv per wave. The animal was unresponsive to alarming stimuli as the human appearance brought no significant change in the pattern of EEG. The results are presented in Table IV and Figure 4.

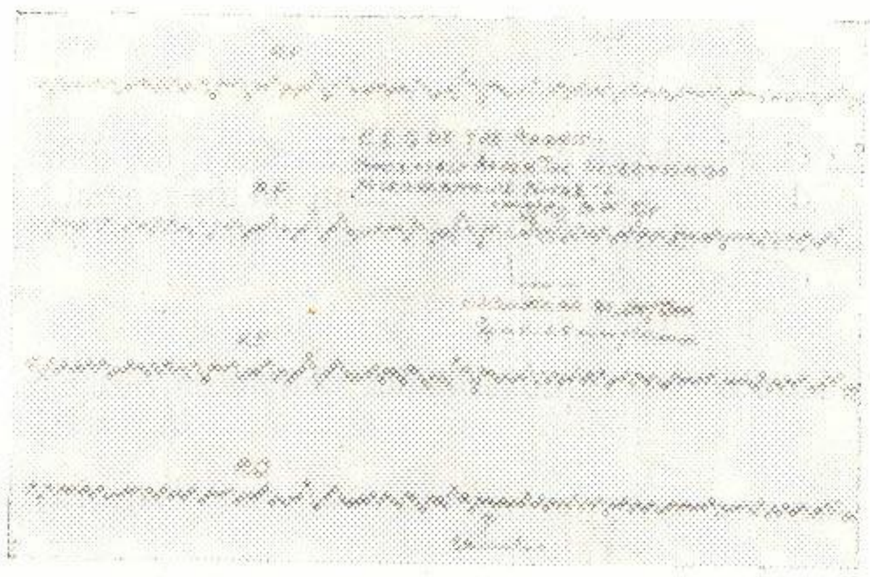

Fig. 4 : Showing E.E.G. of restrained, uncurarized and conscious rabbit immediately after an injection of nitroharmidine nitrate (1 mg/kg B.W. I/P)

This picture persisted upto 30 minutes and the normal resting pattern got restored in the subsequent readings. The mean frequency of EEG with 1 and 2 mg/kg B.W. doses of nitroharmidine nitrate at 30 minutes interval was 6.45 cycle±0.25 per second and 6.11 cycles ±0.30 per second respectively. There was a fall of 1.41 cycles per second and 1.76 cycles/second respectively and these results were statistically significant.

Discussion

Nitroharmidine nitrate in the present studies has produced tremors and clonic convulsions and traub tail in mice and swaying gait, tremors and clonic convulsions in rats. These symptoms were observed with all the doses tried. However, the intensity and onset of these effects depended upon the dose. The larger doses resulted in stiffening of the hind limbs in the rats. Gunn (1913) observed tremors and clonic convulsions in mice, rats and guinea pigs with harmine treatment.

Ghen and Ghen in 1939, Gershon and Lang (1962) reported that the monkeys treated with harmine suffered an unsteady gait and showed a tendency to stand in one corner of the cage; larger doses produced arching of the back, stifening of the leg, shivering and clonic convulsions. Nitroharmidine an alkaloid from Peganum harmala, very close in structure to harmine and harmaline has pharmacological properties very similar to these substances.

Effect on Volurtary Motor Activity

A smaller dose, 250 mcg/kg B.W. of nitroharmidine produced a slight statistically insignificant increase in voluntary motor activity in mice. Similar effects have been reported by Irwin (1961) with MAO inhibitors like iproniazid, pheniprazine and nialamide. Harmine and harmaline have been reported to be MAO inhibitors (Undenfriend et al., 1958). Similarly harmidine has also been shown to possess this property (Akhtar 1971). Nitroharmidine, a nitrose compound of harmidine appears to retain this property of the parent substance.

The larger doses of nitroharmidine produced statistically significant decrease in voluntary movements in mice. Iproniazid has been found to reduce activity in cats (Funderburk and Finger, 1962).

In Parkinson's disease, characterized by an increased muscle rigidity and involuntary tremors, the dopamine contents of the caudate nucleus and putamen are markedly decreased (Ganong 1965). There is evidence that tryptamingergic nerves participate in the control of motor functions by extra pyramidal system in the brain and 5-HT serves as a neurotransmitter (Douglas 1971). It is probable that larger doses of nitroharmidine nitrate cause selective depletion of dopamine in basal ganglia resulting in increased rigidity, increased muscle tone, tremors ataxia and decreased transitional locomotion. There is also possibility that the drug might have raised the brain 5-HT levels due to its presumed MAO inhibiting properties and this may be responsible for change in locomotor activity.

Both dopamine and acetylcholine have been considered to be involved in conduction of impulses through neuronal pathways and it has been suggested that a possible balance between two neurotransmitters may be responsible for the normal conditions of impulses (Hornykiwicz 1966; Barbeau 1969). Nitroharmidine perhaps brings about symptoms resembling parkinsonism by disrupting the balance between the two neurotransmitters.

The tremors and rigidity produced by nitroharmidine nitrate closely resembles those produced by tremorine. The tremors produced by tremorine can be antagonized with some anticholinergic drugs effective in human parkinsonism (Everett et al., 1956). It is suggestive of exploring the possibility of nitroharmidine acting through acetylcholine.

Effect on Conditioned Avoidance Response:

The conditioned avoidance response could not be specifically blocked by nitroharmidine nitrate with any dose tried. Larger doses blocked both the conditioned and unconditioned responses in rats simultaneously whereas smaller doses did not show any effect. The animals could feel the stimulus as they jumped about in the box but did not climb the pole. This inability to climb over the pole was perhaps due to the increased muscle rigidity also observed in mice. This again was probably the result of transient depletion of dopamine from the basal ganglia.

Effect on General Body Temperature

Nitroharmidine nitrate caused a significant rise in general body temperature with all the doses employed at 15 minutes interval. The duration and degree of hyperthermia increased with an increase in the dose. Gunn (1913) also observed a transient rise followed by a fall in general body temperature in rabbits when harmine and harmaline were administered. Iproniazid, a MAO inhibitor, has been found to potentiate the pyretogenic effect of injected 5-HTP a precursor of 5-HT (Horita and Gogerty, 1958).

An increased secretion of catecholamines is an important response to cold (Ganong 1965). It is also well known that stimulation of anterior hypothalamus causes hyperthermia and that of posterior hypothalamus a fall in general body temperature. There is some evidence that serotonin is a synaptic mediator in the centre controlling the mechanism activated by cold and gives rise to heat production whereas norepinephrine may play a role in the control of the centre activated by heat and lowers the general body temperature (Ganong 1965).

Nitroharmidine nitrate probably caused a direct release of catecholamines from their binding

sites in the brain like harmine. However, 5-HT appears to be released in comparatively far greater amounts than those of norepinephrine with nitroharmidine whereas harmine and harmaline are unable to produce such a selective action. The amounts of 5-HT released in response to harmine and harmaline could mask the hypothermic action of norepinephrine for a short time as was observed by Gunn (1913).

Effect on Pentobarbitone Induced Sleeping Time

Nitroharmidine nitrate produced variable effects on pentobarbitone induced sleeping time. The relative sleeping time decreased when sodium pentobarbitone was administered immediately after the nitroharmidine pretreatment. On the other hand the sleeping time increased when pentobarbitone was injected 15 minutes after the nitroharmidine administration.

Monoamine oxidase inhibitors have been reported to increase the barbiturate induced sleeping time (Fouts and Brodie, 1956; Horita, 1958). Everett et al. (1963) also noted variable effect of MAO inhibitor on the duration of the barbiturate induced sleep. These variations were related to the time interval between administration of MAO inhibitor and that of pentobarbitone.

The barbiturates induce sleep by a direct depressant effect on reticular activating system and 5-HT has been reported to potentiate the barbiturate and ethanol induced sleeping time (Lewis 1970). Harmine has been shown to increase the biogenic amine contents in the brain, not only through its MAO inhibiting properties but also by causing direct release from their binding sites in the brain. It is presumed that nitroharmidine nitrate may also be acting like harmine and prolong barbiturate induced sleeping time through an increase in the level of free 5-HT concentrations in the brain.

The reduction in the sleeping time observed after immediate administration of pentobarbitone following injection of nitroharmidine may be the result of excessive amount of impulses originating in the peripheral muscles due to shivering and increased rigidity. The impulses might be exerting a stimulant action on reticular activating system and contribute in prevention of sleep induced by the barbiturates.

The reduction in sleeping time may also be explained by the fact that nitroharmidine may alter the rate of absorption, renal excretion and metabolism of barbiturates in the body when the two drugs are given simultaneously.

Effects on EEG in Rabbits:

Himwitch (1959) observed that harmine 400 mcg/kg B.W. produced an activated pattern of EEG in rabbits. However, they failed to obtain that pattern upto 100 per cent of the recording time. Harmine, nevertheless, revealed EEG of modified activation with larger doses (5 mg/kg B.W.). It differed from the tracing with small doses that the alert pattern appeared to a lesser degree with a frequency of 3-4 cycles/second as compared to 14 cycles/second. The control alert pattern was of the order of 5-6 cycles/second.

The results obtained in present studies are in agreement with above noted findings. The smaller doses revealed a more activated pattern of EEG as compared to that in control, though the changes were not significant statistically. The polyrhythmic picture of EEG recorded in control group was equally contributed by theta activity of 4-7 cycles/second and slow alpha rhythm of 8-10 cycles/second, alongwith 14 cycles/second sleep spindles. The per cent time of theta activity, a characteristic of alert pattern, increased in EEG of rabbits treated with 250 and 500 mcg/kg B.W. of nitroharmidine nitrate. However, the larger doses (1 and 2 mg/kg B.W.) of the drug produced a modified activation of EEG like harmine.

In the present study nitroharmidine nitrate produce slowing of EEG i.e., the frequency count was decreased. This slowing may be the result of a direct action of the drug on the higher centres or it may be due to increased concentration of free 5-HT as a result of MAO inhibition. Funderburk et al. (1962) found that nialamide, a MAO inhibitor showed slow wave pattern in the EEG of the cats. They speculated that this pattern was a combined result of fatigue, 5-HT and direct action of the drug. According to Gangloff and Monnier (1957) reserpine and 5-HT may be considered as tropotrophic tranquilizers, but reticular formation is not depressed at midbrain level whereas thalamocortical levels are depressed. These drugs thereby, give a mixed arousal and relaxation pattern. Probably the mixed pattern observed with smaller doses of nitroharmidine nitrate was due to raised brain 5-HT concentration.

Costa and Rinaldi (1958) after the administration of 5-HT in rabbits found a significant increase in 5-HT levels in telencephalon, medulla, pons and midbrain with concomittent monorhythmic diffuse high voltage activity in the EEG of the animal. The cortical fast rhythm disappears and hippocampal theta waves were followed by a generalized depression of the voltage; on the basis of these observations the effects on EEG produced by nitroharmidine nitrate may be explained by increased level of free 5-HT in the rabbit brain due to its MAO inhibiting properties.

Acknowledgements

We are grateful to Dr. Saleemuzzaman Siddiqui, Director Postgraduate Institute of Chemistry, Karachi University, for a generous supply of nitroharmidine nitrate.

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