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# SOME PHARMACOLOGICAL STUDIES OF HARMIDINE HYDROCHLORIDE

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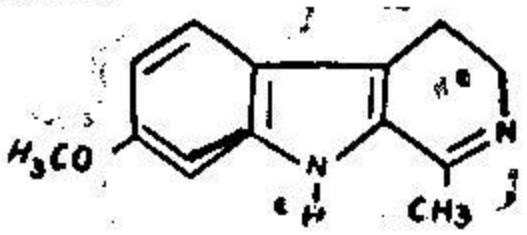
These studies were conducted to investigate some pharmacological actions of Harmidine, a newly isolated alkaloid from an indigenous drug popularly know as 'Harmal' in the Indo-Pak subcontinent. Median lethal and effective doses ($LD_{50}$ and $ED_{50}$) of Harmidine were determined in mice and rats by various routes of drug administration. The new alkaloid was found to be a safer drug and its therapeutic index was about 4 times more than that of Harmine, another harmala alkaloid. Record of behavioural pattern of Harmidine showed that it produced shivering, tremors, stiffening of legs and ataxia in rats. Harmidine (24 mg/kg, intraperitoneally) was 100% effective within 15 minutes in depressing the voluntary motor activity in mice and its effect lasted for more than 24 hours. Harmidine, when tested on sciatic nerve—gastrocnemius preparation of dogs, depressed the contraction of indirectly stimulated muscle but the depressed muscle continued to respond to direct stimulation. Harmidine completely antagonized the oxytocic effect of 5-hydroxytryptamine on the isolated rat uterus preparation. The possible mechanisms of the determined actions of Harmidine are discussed.

## INTRODUCTION

Harmidine ($C_{13}H_{14}ON_2$), whose structural formula appears below, is an indole alkaloid contained in the seeds of an indigenous plant, *Peganum harmala* Linn., which is popularly known as Harmal in the Indo-Pak subcontinent (Siddiqui, 1962).

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This plant is a bushy herb that grows wildly all over Pakistan, Northern India, Persia and some parts of Russia. In ancient and indigenous medicines, many varied medicinal properties have been attributed to the seeds of this plant. They are said to produce hallucinations, euphoria and sexual stimulation. The powdered seeds are still used by the practitioners of indigenous medicine to cure asthma, billiary colic, jaundice and tape worm infection. Watery infusions of the plant are also used to increase the flow of milk and to induce abortion. The smoke of the harmal seeds is used as a repellent of mosquitoes and other insects (Nadkarni, 1954). At present, the harmal seeds are known to contain several alkaloids including Harmine, Harmaline, Harmalol and a number of derivatives with similar pharmacological properties (Robson and Stacey, 1962). Recently, Siddiqui (1962) reinvestigated the alkaloidal constituents of this plant and isolated a new base which was assigned the name of Harmidine. This article deals with some pharmacological studies of the Harmidine hydrochloride.

## MATERIALS AND METHODS

Adult healthy albino Sprague-Dawley rats and Swiss mice were used in all the experiments. They were fed a commercial feed (Lever Brothers). Fourteen mongrel dogs (10–14 kg) were also used for the sciatic nerve-gastrocnemius preparations. Pentobarbitone (Nembutal) and d-tubocurarine were supplied in solution form. The solutions of Harmidine Hydrochloride* and 5-Hydroxytryptamine were prepared in distilled water. Concentrations of all the drugs were expressed in terms of their bases. Further dilutions were made in normal saline just before use.

### Acute Toxicity

Acute toxicity studies were carried out in rats and mice. Total number of mortalities within 24 hours were observed and the median lethal dose ($LD_{50}$) was calculated by the graphic method of De Bear (1945).

### Behavioural Pattern Record

Harmidine (6, 12 and 24 mg/kg intraperitoneally) was injected in 3 groups of 20 rats each. A 4th group of 20 animals was kept as a control which received 0.5 ml of physiological (0.9%) saline. The signs of C.N.S.

* Harmidine HCl was very kindly provided by Dr. Salimmuzzaman Siddiqui, Director, Institute of Chemistry, University of Karachi, Karachi.

depression or excitement etc., were observed in all the groups by the method of Chandhoke and Ghatak (1969).

**Voluntary Motor Activity**

Fifty adult male mice weighing 18-30 G. were divided into five groups with 10 animals each. Group I was treated with normal saline while group II, III and IV were injected intraperitoneally with 6, 12 and 24 mg/kg body weight of Harmidine. Group V was treated with Chlorpromazine 5 mg/kg. The voluntary motor activity was recorded actographically on a Grass Polygraph. The number of movements were also counted with the help of an electric telecounter. The activity was observed before and after injecting the drug and percentage decrease in activity was calculated. The median affective dose ($ED_{50}$) for this effect was calculated graphically by the method of De Bear (1945). The therapeutic index was also calculated.

**Myoneural Blocking Effect**

Sciatic nerve-gastrocnemius preparations were made in 14 dogs (10-15kg) under pentobarbitone (35 mg/kg, I/p) anaesthesia. The nerve or muscle was electrically stimulated by the method of Dutta and Pradhan (1965) at the rate of 1 pulse/sec. (duration 2 milliseconds and 4 to 12 volts). In most cases minimal voltage producing adequate contraction was used in order to prevent early fatigue of the muscle. Harmidine (25, 50 and 100 mg) was injected in the femoral artery of the same side through arterial cannula.

**5-Hydroxytryptamine (5-HT) Antagonism**

Female virgin rats (200-250 G) showing normal and regular oestrus cycle were used. The vaginal smears were obtained with a pipette and examined daily throughout the experiment. The rats received stilboestrol (100 mg/kg, I/p) on the first day of dioestrous and were killed 18 hours later. A 2 5 Cm. length of the uterine horn was suspended in a 5 ml organ bath containing De-Jalon's solution at 30°C. The drugs were added to the bath and the contractions were recorded isotonically on a kymograph with a frontal point writing lever with magnification of 5. The dose response curve of 5-HT was recorded as described by Khan and Ahmad (1969). Then, the dose of 5-HT producing response equal to the dose producing 50% of the maximum response in the presence of Harmidine was determined by gradually increasing the dose of the agonist (5-HT) and the dose ratio calculated.

## RESULTS

The acute toxicity of Harmidine determined as median lethal dose ($LD_{50}$) is shown in Table 1. The behavioural pattern observed after the administration of different doses of this alkaloid is recorded in Table 2. This compound produced a decrease in the voluntary motor activity as is clear from the Figure 1. The percentage decrease in activity, the onset and duration of this action is given in Table 3. The median effective doses ($ED_{50}$) producing depression of motor activity are presented in Table 4.

Table 1 : *$LD_{50}$ of Harmidine HCl in Albino Rats and Mice.*

| Route of Administration | Mice | Rats |
|---|---|---|
| Orally | 125 mg/kg | 148 mg/kg |
| Intraperitoneally | 89.1 mg/kg | 110.6 mg/kg |
| Subcutaneously | 100 mg/kg | 111.2 mg/kg |
| Intravenously | 44.7 mg/kg | 70.8 mg/kg |

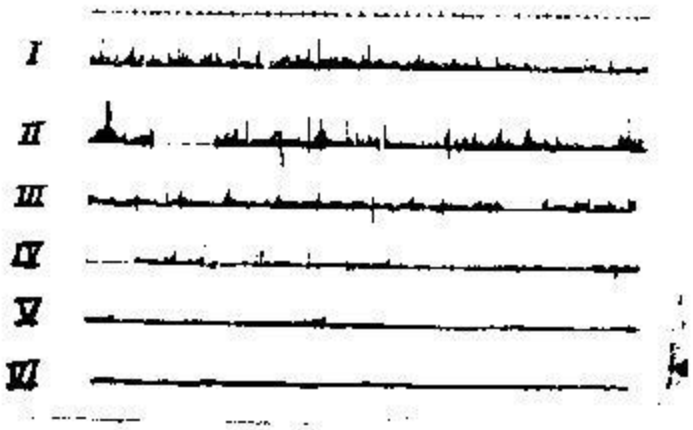
Table 2 : *Record of Behavioral Pattern of Harmidine in rats.*

| Behavioural Pattern | Normal | Harmidine (mg/kg) I/P Injection 6 | 12 | 24 |
|---|---|---|---|---|
| Awareness | 4+ | 4+ | + | ± |
| Alertness | 4+ | 4+ | + | ± |
| Spontaneous activity | 4+ | 4+ | 2+ | 0 |
| Abnormal body position | - | Shivering | Shivering | Shivering |
| Sedation | 0 | 2+ | 2+ | 4+ |
| Gait | Normal | Normal | Ataxia | Ataxia |
| Sound response | 4+ | 4+ | 2+ | ± |
| Touch response | 4+ | 4+ | 3+ | + |
| Muscular tone | Normal | Normal | Flacid | Flacid |
| Tremor twitching and convulsions | 0 | ± | Tremor (+) | Tremor (4+) |
| Rightening reflex | 4+ | 3+ | 3+ | 2+ |
| Mydriasis | — | + | 2+ | 3+ |
| Piloerection | 0 | 2+ | 3+ | 4+ |

0 = No action
+ = Slight action
3+ = Strong action
± = Doubtful action
2+ = Moderate action
4+ = Very strong action

FIGURE NO. 1

*Actographic Record of Voluntary Motor Activity.*




Speed : 2.5 mm/Sec.

I. Before Drug Administration
II. 1 hour after Normal Saline 0.5 ml I/P
III. 1 hour after Harmidine HCl 6 mg/kg I/P
IV. 1 hour after Harmidine HCl 12 mg/kg I/P
V. 1 hour after Harmidine HCl 24 mg/kg I/P
VI. 1 hour after Chlorpromazine 5 mg/kg I/P

Table 3 : *Effect of Harmidine HCl on Voluntary Motor Activity in Mice*

| Drug | Dose | %age Decrease in activity after one hour | Onset of action | Duration of action |
|---|---|---|---|---|
| Untreated Control | Saline (0.5 ml) | 0 | - | - |
| Harmidine HCl | 6 mg/kg | 10 | 30 Minutes | 2 Hours |
| Harmidine HCl | 12 mg/kg | 50 | 15 ,, | 24 ,, |
| Harmidine HCl | 24 mg/kg | 100 | 15 ,, | 24 ,, |
| Chlorpromazine | 5 mg/kg | 100 | 20 ,, | 5 ,, |

Table 4. *$ED_{50}$ and Therapeutic Index (T.I) of Harmidine HCl in Rats and Mice by Various Routes.*

| Route of Administration | Mice | | Rats | |
|---|---|---|---|---|
| | $ED_{50}$ | T. I. | $ED_{50}$ | T. I. |
| Orally | 13 mg/kg | 9.61 | 15.6 mg/kg | 9.48 |
| Intraperitoneally | 10.8 ,, | 8.20 | 12.4 ,, | 8.92 |
| Subcutaneously | 8 3 ,, | 12.04 | 9.4 ,, | 11.82 |
| Intravenously | 8.9 ,, | 5.03 | 14.16 ,, | 5.00 |

The margin of safety of Harmidine was calculated in terms of Therapeutic index ($LD_{50}/ED_{50}$). The therapeutic index values in mice by oral, intraperitoneal, subcutaneous and intravenous routes were found to be 9.61, 8.20, 12.04 and 5.03 respectively. These values in rats were respectively 9.48, 8.92, 11.82 and 5.00 (Table 4). Following administration of 25, 50 and 100 mg of Harmidine HCl, the contractions of indirectly stimulated muscle were depressed (Table 5) but the depressed muscle responded to direct stimulation (Figure 2). In the five isolated rat uterus preparations $0.48 \pm 0.25$ microgram of the 5-HT produced a response 50% of the maximum response (Dose A). In the presence of I microgram of Harmidine, $5.12 \pm 0.7$ microgram of 5-HT produced the response equal to dose A (Figure 3). The calculated dose ratio was found to be $11.2 \pm 1.9$ (Table 6).

Table 5. *Effect of Harmidine on Dog Sciatic Nerve-Gastrocnemius preparation in Situ.*

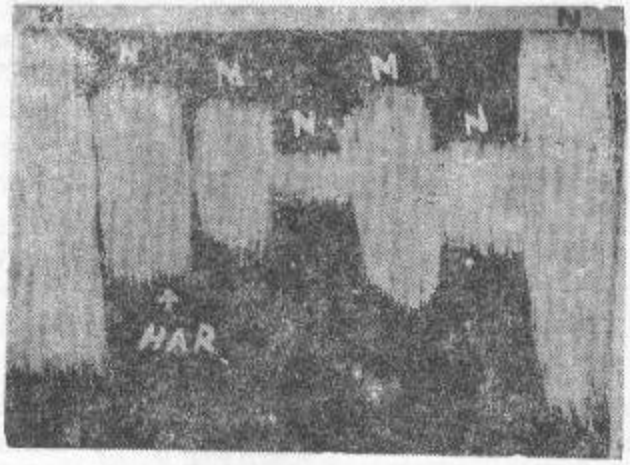
| Drug | Dose* (mg) | No. of Tests | %age reduction of Muscle Contraction (Mean ± S. E.M.) |
|---|---|---|---|
| Harmidine HCl | 25 | 3 | $30 \pm 10.5$ |
| Harmidine HCl | 50 | 3 | $65 \pm 13\ 5$ |
| Harmidine HCl | 100 | 3 | $100 \pm 0.0$ |
| d-Tubocurarine | 400 | 3 | $100 \pm 0.0$ |

* Since the effect of intra-arterial injection are more of local than of systemic nature, the doses mentioned for such experiments are given as the total amount administered rather than mg/kg.

FIGURE NO. 2

*Sciatic - Gastrocnemius Preparation*

DOG, 12 KG

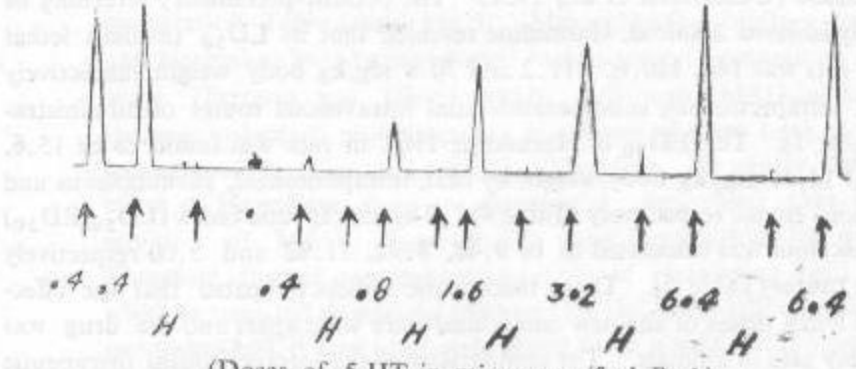



HAR = Harmidine HCl 100 mg was injected in Femoral Artery.
M = Muscle Stimulation
N = Nerve Stimulation

FIGURE NO. 3

*5-Hydroxytryptamine Antagonism by Harmidine*

(Isolated Rat Uterus Preparation)




(Doses of 5-HT in microgram/5ml Bath)
H = Harmidine HCl (1 mg/ Bath)

Table 6: *5-HT Antagonism of Harmidine HCl on Rat Uterus Preparation*

| Test No. | Ug of 5-HT producing a response 50% of the maximum response in absence of Harmidine | Ug of 5-HT producing a response 50% of the maximum response in presence of Harmidine | Dose Ratio |
|---|---|---|---|
| 1 | 0.4 | 3.2 | 8 |
| 2 | 0.4 | 6.4 | 16 |
| 3 | 0.8 | 6.4 | 8 |
| 4 | 0.4 | 6.3 | 16 |
| 5 | 0.4 | 3.2 | 8 |
| Mean±S. E. | 0.48±0.25 | 5.12±0.70 | 11.2±1.9 |

## DISCUSSION

Since antiquity the Harmal seeds have been used to produce hallucinations and euphoria but the constituents responsible for its activity were not known. As early as 1851, Goebal isolated an alkaloid Harmaline from the seeds of this plant. Another alkaloid Harmine was isolated by Fritsche in 1847 from the seeds of the same plant (Siddiqui, 1962). Gunn (1935) investigated the pharmacological actions of these alkaloids and reported that Harmine produces tremors and clonic convulsions in rats, mice, etc. Chen and Chen (1939) observed that the monkeys treated with harmine showed unsteady gait, arching of back, tendency to stand in one corner of the cage, shivering and clonic convulsion with larger doses (cited by Gershon and Lang, 1962). Harmine and Harmaline were later on shown to be reversible inhibitors of monoamine oxidase (Udenfriend *et al.*, 1958). The present preliminary screening of the newly isolated alkaloid, Harmidine revealed that its $LD_{50}$ (median lethal dose) in rats was 148, 110.6, 111.2 and 70.8 mg/kg body weight respectively by oral, intraperitoneal, subcutaneous and intravenous routes of administration (Table 1). The $ED_{50}$ of Harmidine HCl in rats was found to be 15.6, 12.4 and 14.16 mg/kg body weight by oral, intraperitoneal, subcutaneous and intravenous routes respectively (Table 4). The therapeutic index ($LD_{50}/ED_{50}$) of this alkaloid was calculated to be 9.48, 8.92, 11.82 and 5.00 respectively by these routes (Table 5). These therapeutic indices indicated that the effective and lethal doses of the new compound were wide apart and the drug was reasonably safe in animals. The comparison of the intraperitoneal therapeutic index of Harmidine with that of Harmine as reported by Gunn, (1912) was

found to be four times higher than that of Harmine showing that the former alkaloid was less toxic than the latter one.

The record of the behavioural pattern of Harmidine (Table 2) showed that the new alkaloid produced shivering, tremors stiffening of legs and ataxia in rats. These actions resembled somewhat to those already reported for Harmine and Harmaline (Gunn, 1935). As Harmidine was found to be a potent and safer compound, the present studies were extended and the effects of Harmidine HCl were also studied on the voluntary motor activity and myoneural junction. Harmidine HCl (24 mg/kg. intraperitoneally) was 100 per cent effective within 15 minutes in depressing the motor activity of mice and this effect lasted for more than 24 hours (Table 3). The exact mechanism of action of Harmidine is needed to be investigated but it can be assumed that the decrease in motor activity may be due to myoneural blocking action as 100 mg of Harmidine HCl when injected intra-arterially depressed the muscle contraction induced by the nerve stimulation (Table 5 and Fig. 2). These findings are in agreement with Dutta and Pradhan (1965) who reported that Harmine too similarly blocked the myoneural transmission in dog and rabbit nerve-muscle preparations. They also reported that some other MAO inhibitors like J.B. 516, J.B. 835 and M.O. 911 produce a similar block. In addition, Poirier *et al.* (1966) reported that the Harmine and Harmaline produced shivering and tremors in rats due to 'amine protection' action of these drugs through inhibition of monoamine oxidase (MAO). Thus it can be assumed that perhaps Harmidine too is an inhibitor of monoamine oxidase as it has also produced shivering and tremors in animals. This belief was strengthened when 1 mg of Harmidine HCl was found to completely antagonize the effect of $5.12 \pm 0.7$ of 5–HT on isolated rat uterus preparation (Table 6 and Fig 3). Many MAO inhibitors including Harmine and Harmaline have been reported to antagonize serotonin on isolated preparations (Perrault and Clevel, 1957). Although MAO-inhibitors as a class increase voluntary motor activity but many of them have also been reported to decrease it (Pletscher, 1965). Therefore, the motor activity decreasing effect of Harmidine does not disprove the hypothesis that Harmidine is an inhibitor of MAO. However, all these explanation are just speculations. Therefore, further comprehensive study of Harmidine HCl was undertaken which is reported separately (Akhtar, 1971). This alkaloid seems to be very promising and it may ultimately prove to be a safe and potent antidepressant drug. Behavioural studies in monkeys, human volunteers and clinical trials might establish its therapeutic efficacy.

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