PHARMACOLOGICAL SCREENING OF HARMIDINE HYDROCHLORIDE

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This study was conducted to screen some of pharmacological actions of Harmidine, an alkaloid isolated from a local plant popularly known as 'Harmal' or 'Asband' in Pakistan. The effects were studied in albino rats treated with Harmidine hydrochloride at the rate of 12 mg/kg of body weight alone and pre-treated with Pyridoxine. Harmidine produced shivering, ataxia, diarrhoea, reduction in motor activity, loss of postural reflex and hypothermia. Weight of animals, respiratory rate, heart rate, urinary output, estrous cycle, leucocyte and red blood cell count, bleeding time, clotting time, haemoglobin in G/100 cc of blood and differential leucocyte count remained unaffected. Harmidine probably acts on the basal ganglia and brain stem reticular formation.

The animals pretreated with Pyridoxine did not show shivering, ataxia, reduction in motor activity, loss of postural reflex and hypothermia, indicating that Harmidine probably acts by inhibiting the activity of Pyridoxine in the brain and thereby protects 5–Hydroxytryptamine and Norepinephrine of the brain from destruction by M.A.O. which needs Pyridoxine as coenzyme for its activity.

INTRODUCTION

Peganum harmala Linn. known as Harmal locally grows abundantly in Sind and the Punjab. Powdered seeds and their extracts have been used in indigenous medicine for various purposes (Nandkarni, 1945). Native American Indians used to take the seeds and extracts of various parts of the plant during feasts to produce hallucination and euphoria (Robson and Stacey, 1962).

As early as 1841, Goebal (cited by Siddique, 1962) in Germany isolated an alkaloid Harmaline from the seeds of *Peganum harmala*. Another alkaloid named Harmine was isolated by Fritsche in 1847 (cited by Gunn, 1912) from the seeds of the same plant. Gunn in 1912 investigated the pharmacological actions of these alkaloids and reported that the Harmaline and Harmine produced tremors and colonic convulsions in rats, mice, guinea-pigs and monkeys.

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Chen and Chen, 1939 (cited by Gershon and Lang, 1962) observed that monkeys treated with Harmine showed unsteady gait, arching of back and stiffening of legs. Plestcher and Gey (1959) studied the effects of Harmine and Harmaline in-vitro on liver and in-vivo on rat's brain. They reported that these alkaloids were responsible for inhibition of monoamine-oxidase (M.A.O). Turner *et al.* (1955) used Harmine in non-psychotic men and observed anxiety, tremors, restlessness and aggressive acts without hallucinations in these subjects. Poirier *et al.* (1966) reported that Harmine and Harmaline produced shivering and tremors in rats due to protective actions of these alkaloids through inhibition of M.A.O.

Siddique (1962) isolated a new alkaloid Harmidine from the seeds of *Peganum harmala*. He reported that Harmaline is actually a mixture of the two alkaloids (Harmidine 85% and Harmine 15%). Therapeutic index of Harmidine was found to be four times higher than that of Harmine (Qureshi, 1959 cited by Akhtar, 1971). These observations created an interest to investigate the pharmacological effects of Harmidine in rats.

MATERIALS AND METHODS

Two to three months old male and female Albino rats weighing 250—300 gm. reared in the animal house at 27 C were divided into four groups of 10 rats each. The first group was treated with Harmidine 12 mg/kg of body weight. The second group (control) was administered normal saline (0.9%) 2 cc/kg of body weight intraperitoneally. The third group was treated with pyridoxine 1 mg/kg of body weight intraperitoneally. The fourth group was treated with Harmidine hydrochloride 12 mg/kg of body weight one hour after the administration of Pyridoxine. Pyridoxine group was included because it acts as a co-enzyme of M.A.O. The animals were observed for the pharmacological effects of the drugs at 15–minute, 30–minute, one–hour, two–hour and 24–hour intervals after the administration of the drugs.

RESULTS

The results are represented in Tables 1 and 2. The group treated with saline and Pyridoxine did not produce such visible and noticeable effects as shivering, ataxia, reduction in motor activity and loss of postural reflex. Such effects of Harmidine were significantly antagonised by Pyridoxine.

Estrous cycle in female virgin rats remained unaffected during and after the administration of Harmidine hydrochloride 12 mg/kg of the body weight for about six weeks. The weight of the animals remained stable. Skin and hair were also unaffected.

DISCUSSION

Harmidine hydrochloride 12 mg/kg of body weight in rats has produced shivering, ataxia, diarrhoea, reduction in motor activity and loss of postural reflex. These effects are probably due to increased concentration of 5-hydroxytryptamine (5-HT) and NE (Norepinephrine) in certain parts of the brain. Qureshi and Akhtar (1972) have demonstrated a significant increase in concentration of 5-HT and NE in the hypothalamus of rats treated with Harmidine. The above mentioned effects except diarrhoea were found to be absent in the animals pre-treated with Pyridoxine (4th group). Pyridoxine a co-enzyme is essential for the activity of monoamine-oxidase (M.A.O). Harmidine probably inhibited the activity of Pyridoxine in the brain, thus decreasing or abolishing the distructive action of mono-amino-oxidase on 5-HT and NE of the brain. Pletscher and Bey (1959) reported that Harmaline and Harmine were responsible for inhibition of M.A.O. in-vitro on liver and in-vivo on rat's brain respectively. Siddique (1962) reported that Harmidine is a mixture of Harmaline (85%) and Harmine (15%).

The presence of diarrhoea was probably due to an increase in the concentration of free 5-HT in the alimentary canal, again due to the local inhibition of M.A.O. activity in the animals.

The decrease in the motor activity was not due to tranquilization but presence of spastic paralysis of the hind limbs observed in the animals treated with Harmidine (Group 1). The reduction in motor activity was nil in the animals pretreated with Pyridoxine (group 4) and the animals did not show any sign of tranquillity.

Stability of weight after the administration of the alkaloid over a period of about six weeks show that it does not interfere with the nutrition or metabolism of the animals. Absence of changes in the estrous cycle indicates that the hormonal effects on the vagina at least is not effected. Nothing can be said about the effect of the alkaloid on the uterine muscle of such animals at present. In in-vitro experiments, Harmidine has inhibited the effects of 5-HT on the isolated rat uterus preparation (Qureshi *et al.* 1970, cited by Akhtar, 1971).

In rabbits treated with Harmidine hydrochloride 12 ug/kg of the body weight, desynchronization of Electroencephalogram (EEG) was observed upto 24 hours (Babar, 1972). It is evident from the results of the present studies that Harmidine has its effects on multiple sites in the brain with multiple actions. The main site of action is most probably the brain stem reticular formation and basal ganglion. Keeping in view the effects of the alkaloid produced in

the above mentioned studies it can be said that Harmidine may prove an effective antidepressant drug. Absence of its effects on respiration, heart rate, weight, total leucocytes and erythrocytes count, haemoglobine, bleeding time, clotting time, differential leucocyte count and urine output reflect that the alkaloid may also be quite harmless. But further studies on cardiovascular system and genital tract and central nervous system are required before the final suggestion.

TABLE 1. *Latent period, peak and duration of various effects produced after administration of harmidine hydrochloride 12 mg/kg of body weight in rats.*

Effect Noted	Latent Period	Peak	Duration
Shivering and Tremors	15 minutes	1 hour	2 hours
Ataxia	30 minutes	1 hours	2 hours
Diarrhoea	30minutes	2 hours	6 hours
Reduction in Motor activtiy	15 minutes	1 hour	4 hours
Loss of postural reflex	15 minutes	—	2 hours
Respiratory rate	—	—	—
Heart rate	—	—	—
Eye reflexes	—	—	—
Diuresis	—	—	—
Rectal temperature	15 minutes	—	4 hours

TABLE 2. *Effects of Harmidine Hydrochloride 12 mg/kg of Body Weight (Subcutaneous) on Blood in Rats*

Time interval	Weight	Total leuco-cyte count	Total erythro-cyte count	Haemo-globin in G% cc of blood	Bleeding time (Minutes)	Clotting time (Minutes)	*Differential leucocyte count*				
							Lympho-cyte	Neutro-phil	Mono-cyte	Eosino-phil	Baso-phil
Zero minute	253.6 (10)	11705 (10)	8.21 (10)	89.3% (10)	3.25 (10)	4.15 (10)	31.2 (10)	64.6 (10)	1.1 (10)	1.1 (10)	0.93 (10)
15 minutes	253.6 (10)	12080 (10)	8.21 (10)	90.2% (10)	3.25 (10)	4.35 (10)	31.9 (10)	64.6 (10)	1.4 (10)	1.9 (10)	0.6 (10)
30 minutes	253.6 (9)	11815 (9)	8.16 (9)	89.2% (9)	3.45 (9)	4.4 (9)	31.6 (9)	63.3 (9)	1.4 (9)	2.8 (9)	1.3 (9)
60 minutes	253.6 (10)	11960 (10)	8.21 (10)	89.8% (10)	3.45 (10)	4.2 (10)	32.5 (10)	64.6 (10)	1.3 (10)	2.0 (10)	0.9 (10)
2 hours	253.6 (10)	9910 (10)	8.19 (10)	90.8% (10)	4.25 (10)	4.32 (10)	31.6 (10)	64.1 (10)	1.1 (10)	2.3 (10)	1.2 (10)

Note: The figures in parenthesis show the number of animals used.

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