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EFFECT OF DIAZEPAM ON THE CNS EXCITATION AND BEHAVIOURAL CHANGES INDUCED BY HARMALINE AND ITS NEWLY SYNTHESIZED ANALOGUES

Z.S. SAIFY, JAWAID FARHAD, NOUSHEEN MUSHTAQ, FOZIA NOOR, SHAMOONA TAKWEEN* AND SHAMIM AKHTAR
*Department of Pharmaceutical Chemistry, *Department of Pharmacology*
Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

The neuropharmacologic profile of harmala alkaloids has been studied and found to have central effects like convulsions, catalepsy, or altered startle response. In number of studies the effects of diazepam, on harmaline and other beta carboline containing compounds-induced tremors were investigated. The present study was undertaken to examine the effect of diazepam on pretreated animals with harmaline and its synthesized phenacyl and coumarine analogues. Diazepam successfully inhibited the tremor and convulsions and attenuated the other behavioural response produced by Harmaline and its derivatives.

INTRODUCTION

Peganum harmala is a plant known since the first century A.D. and still, currently used for therapeutic purposes. Harmaline (7-Methoxy-1-methyl-4,9-dihydro-3H-b-carboline) the active principle of the plant seeds, and its derivatives, cause visual troubles, loss of coordination, agitation and delirium, and, at high doses, it can produce paralysis (Lamchouri, *et al.,* 2002).

Harmaline is beta-carboline related compound, which has been proposed to be endogenous ligand for imidazoline receptors. The inferior olive and cerebellum are implicated by primary essential tremor studies. Administration of the beta-carboline alkaloid, harmaline, causes the neurons of the inferior olive to fire synchronously and to act as a pacemaker for the generation of tremor. Harmaline tremor in animals shares many features with essential tremors, and the inferior olive has been identified as the source of oscillation in the animal model. The effect of harmaline and beta-carboline related compounds on the activity of locus coeruleus (LC) neurons was studied by extracellular recordings techniques. Intracerebroventricular administration of harmaline increased the firing rate of LC neurons (Ruiz-Durantez, *et al.,* 2001; Deuschl, *et al.*, 2000 and Lutes, 1988).

Experimental and clinical studies clearly suggest the role of gamma-aminobutyric acid (GABA) in the pathogenesis of tremors (Tariq, 2001 and Mizoule, 1985). Neurophysiological and neurochemical evidence indicates that benzodiazepines facilitate inhibitory neurotransmission mediated by GABA (Rappaport, 1984).

Diazepam was investigated to change the frequency of tremors induced by different CNS active agents. Diazepam markedly depressed the power spectral density of the harmaline and other beta carboline derivatives induced tremor and reduced the tremor frequency (Shinozaki, 1985, Schweri, 1982 and Robertson, 1980)**.** Number of others studies were carried out to observe the

relationship of harmaline and other beta carboline derivatives induced tremors and other CNS activities and diazepam (Szmigielski, 1979, Mariani, 1978, Mao, 1975 and Saano, 1982).

EXPERIMENTAL

Animals:

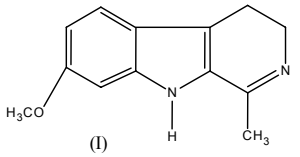
Female albino mice of +NMRI strain weighing 25-30 mg.

Drugs and Chemicals*:*

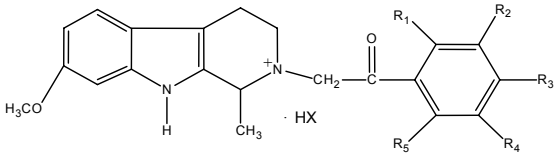
Harmaline (Merk), Test compounds, Diazepam (Roche Pharma. Ltd.), Water for injection and DMSO (Merk).

Method:

Animals were divided in to six groups (n=6). Group 1-5 were injected (i.p.) Harmaline(I) and 4 test compounds(II-V) (50mg/kg) respectively where as last group served as control (water for injection/ 2%DMSO). Diazepam in the dose of (10 mg/kg) was given (i.p.) after 10 minutes of administration of test and parent compounds (i.p.) at the dose of 50mg/kg. The effect of diazepam on the behavioural parameters showed by parent compound and synthetic derivatives was observed.

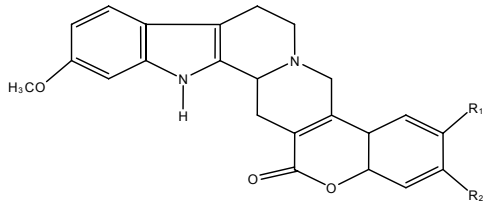


(I)



(II) R_1 = H R_2 = NO2 R_3 = H R_4 = H R_5 = H X = Br

(III) R_1 = H R_2 = OH R_3 = OH R_4 = H R_5 = H X = Cl



(IV) $R_1 = OCH_3$ $R_2 = OCH_3$

(V) R_1 = H $R_2 = OCH_3$

Table-1
Effect of Harmaline and its derivatives on mice behaviour

	Control	Harmalin (I)	Compound (II)	Compound (III)	Compound (IV)	Compound (V)
Dose	–	50mg/kg	50mg/kg	50mg/kg	50mg/kg	50mg/kg
Route of administration	i.p.	i.p.	i.p.	i.p.	i.p.	i.p.
Behaviour						
Aggressive	–	+++	+++	–	+	++
Fear full	–	–	–	–	–	–
Passive	–	–	–	–	–	–
CNS Excitation						
Motor Activity	–	++	+++	–	++	++
Tremors	–	++++	+++	–	++	++
Convulsions	–	++++	+++	–	++	++
Startle response	–	++	++	–	++	++
Fasciculation	–	–	–	–	–	–

Table-2
Effect of Diazepam on the mice treated with Harmaline and its analogues

	Control	Harmalin (I)	Compound (II)	Compound (IV)	Compound (V)
Dose of Diazepam	10mg/kg	10mg/kg	10mg/kg	10mg/kg	10mg/kg
Route of administration	i.p.	i.p.	i.p.	i.p.	i.p.
Behaviour					
Aggressive	–	++	++	+	++
Fear full	–	–	–	–	–
Passive	–	–	–	–	–
CNS Excitation					
Motor Activity	–	++	++	+	+
Tremors	–	–	–	–	–
Convulsions	–	–	–	–	–
Startle response	–	+	+	+	+
Fasciculation	–	–	–	–	–

RESULTS AND DISCUSSION

The effects of diazepam, on Harmaline (7-Methoxy-1-methyl-4,9-dihydro-3H-b-carboline) (I) and its newly synthesized analogues induced tremors and other behavioural changes were investigated in mice, given in Table-2. Synthesis of Harmaline derivatives (II-V) and their behavioural studies were reported earlier, given in Table-1 (Saify *et al*., 1997 and 2003).

The tremorgen, harmaline, causes a bursting pattern of activity in inferior olivary neurons, the effects of which are transmitted throughout the olivocerebellar circuit to other regions of the CNS (Frostholm, 2000). The pharmacological profile of harmala alkaloids on CNS was reported and showed many significant changes (Pranzatelli, 1987). Increased CNS activity in the form of electrically or chemically induced seizures is also known to alter the properties of GABA(A) receptors (Frostholm, 2000).

Diazepam markedly depressed the harmaline-induced tremor and reduced the tremor frequency (Shinozaki, 1985). Diazepam has been found to inhibit both the tremor and mechanism of cerebellar cyclic GMP caused by harmaline by a neurotransmission in the cerebellum (Rappaport,1984).

Among the four derivatives including two phenacyl analogues 2-(7-Methoxy-1-methyl-1,3,4,9-tetrahydro-β-carbolin-2-yl)-1-(3-nitro-phenyl)-ethanone (II), 1-(3,4-Dihydroxy-phenyl)-2-(7-methoxy-1-methyl-1,3,4,9-tetrahydro-β-carbolin-2-yl)-ethanone (III) and two coumarine derivatives 7-(methoxy-β-carboline),15-24,dehydro(19,20-dimethoxy)coumarine(IV), 7-(methoxy-β-carboline)15-24,dehydro(20-methoxy)coumarine (V), compound (III) was eleminated because this compound was found inactive during behavioural investigation (Table 1).

Diazepam was injected intraperitonealy (i.p.) at the dose of 10mg/kg after 10 minutes of (i.p.) administration of parent (Harmaline) and 3 derivatives and subsequent behavioural changes were observed. Diazepam successfully inhibited the tremors and convulsions of parent and compound 2-(7-Methoxy-1-methyl-1,3,4,9-tetrahydro-β-carbolin-2-yl)-1-(3-nitro-phenyl)-ethanone (II) and 7-(methoxy-β-carboline),15-24,dehydro(19,20-dimethoxy)coumarine (IV) within 30 minutes and of compound, 7-(methoxy-β-carboline)15-24,dehydro(20-methoxy)coumarine (V) within 10 minutes after administration.

Diazepam given to the group of mice injected with derivatives 7-(methoxy-β-carboline),15-24,dehydro(19,20-dimethoxy)coumarine (IV) and 7-(methoxy-β-carboline)15-24,dehydro(20-methoxy)coumarine(V), showed more or less same response but the significant difference is in the time of inhibition. Diazepam inhibited the convulsion and tremors induced by (V) earlier as compare to (IV) Both the compounds have same structure with only difference of methoxy groups at 19 and 20–carbon. Compound (V) in which diazepam effectively inhibited the convulsions and tremors earlier has only one methoxy group at 20–carbon as compare to compound IV which has two methoxy groups at 19 and 20–carbon. On the basis of structural activity relationship it can be suggested that number and position of methoxy group may be playing any role in the early inhibition of convulsions and tremors by diazepam.

In other behavioural response diazepam produce almost the same effect in both the coumarine containg analogues of harmaline given to mice. Diazepam given to animals treated with phenacyl derivatives of harmaline had not exhibited any pronounced effects.

Diazepam cannot completely inhibited the behavioural activity other than convulsions and tremors showed by the parent and its derivatives but decreases the intensity more in compounds (IV) and (V) as compare to Harmaline and compound (II).

The present study is the initial study and it can only be suggested that all the derivatives may be following the same pathway as of the harmaline to induce tremors and convulsions but in the case of other behavioural response further detailed study on receptor level is necessary to predict the mode of action of the synthesized compounds

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