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Study of CNS depressant and behavioral effects of cyclopyrrolone compound zopiclone in different animal models

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ABSTRACT

Objectives: To evaluate CNS depressant and behavioral effects of zopiclone in comparison with BZD (lorazepam) by using different animal models. **Methods**: Pharmacological assays used for evaluating CNS Depressant activity are righting reflex test, pentobarbitone sleeping time potentiation, Rota rod apparatus and Incline plane performance test in albino mice. Open field apparatus (OFT) was used to study behavioral effects. Data analyzed by student's unpaired-'t' test and $p<0.05$ considered significant. **Results**: Zopiclone (7.5mg/kg p.o.) did not inhibit the Righting reflex however significant ($p<0.001$) potentiation of Pentobarbitone sleeping time and decreased fall off time in Rota rod as well as inclined plane test ($P<0.05$, $P<0.02$ respectively) were observed. In OFT increased in exploration ($P<0.001$) suggestive of anxiolysis was observed. **Conclusions**: Zopiclone (7.5mg/kg p.o.) has weak CNS Depressant activity and more selective behavioral activity compared to BZDs.

KEYWORDS

Incline plane;
Pentobarbitone sleeping time;
Rota rod apparatus;
Zopiclone.

INTRODUCTION

Advance in science and technology has contributed to an enormous improvement in the quality of life of humankind. However, modern life stress, associated trials and tribulation are responsible for the surge in incidence of variety of psychiatric disorders. Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazipam lorazepam and alprazolam etc) are the most frequently prescribed synthetic drugs for variety of condition particularly anxiety, depression, epilepsy and insomnia. But these psychoneural drugs have very serious side effects like chronic use of benzodiazepines causes deterioration of cognitive function, physical dependence and tolerance. Besides addiction liabilities, benzodiazepines adversely affect the respiratory, digestive and immune system of body and the chronic treatment with benzodiazepines often prove more harmful in the longer run[1]. In this context, a resurgence of interest in medicine is seen so as to discover newer drugs with significantly lesser side effects than that observed with conventional drugs while having comparable efficacy.

Cyclopyrrolones are a new class of psychotherapeutic agents possessing a pharmacological profile of high efficacy and low toxicity. Zopiclone is the first of the Cyclopyrrolones, chemical structure of which is completely different from BZDs. Qualitatively it possesses similar pharmacological profile as BZDs. It acts at

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GABA-BZD-Cl^--ionophore-receptor even then relatively more specific in action i.e. more potent Hypnosedative and weak anticonvulsant-muscle relaxant. This specificity is due to its distinctive binding mechanism at the site close to rather than identical to the site occupied by BZDs[2-4].

Earlier in vitro and animal studies have demonstrated that Zopiclone causes minimal impairment of psychomotor skills and mental acuity in the morning after a bedtime dose, which is thought to be due to its short half-life of about 5 hours and no long acting metabolites. Zopiclone has a low dependence liability. Thus, with its short duration of action and good tolerability profile, Zopiclone could be a good alternative to the benzodiazepine hypnotics[4,5].

Present study was therefore designed to confirm the findings of earlier studies and to evaluate CNS depressant and behavioral effects of Zopiclone in comparison with BZDs (Lorazepam) with reference to its selectivity and efficacy.

Aim and objectives

Present study is a single dose pharmacodynamic study designed to evaluate CNS depressant and behavioral effects of zopiclone by using different animal models.

To compare these actions with conventional Hypnosedative i.e. BZD (Lorazepam).

MATERIALS AND METHODS

Present study has been conducted at Government Medical College, Miraj. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC).

Experimental animals

Albino mice weighing 20-25gms of either sex, bred in Central Animal House (CAH) facility of the Government Medical College, Miraj were used for the study. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and had free access to food and water. They were acclimatized to laboratory conditions before the experiment. Pre-experiment screening for righting reflex was done 1 day prior to rule out CNS disco-ordination. The animals that show positive righting reflex were selected for study. Each animal was used once in every experiment. All experiments were carried out in daylight.

Drugs and doses

Doses were selected from earlier studies. Lorazepam (Ativan 2mg tablet obtained from Wyeth Lederle Ltd.) dissolved in DW given orally (p.o.) in the dose of 5mg/kg. Zopiclone (Zopicon 7.5mg tablet; obtained from Intas Pharmaceuticals Ltd.) suspended in 0.25% CMC, given orally (p.o.) in the dose of 7.5mg/kg, Pentobarbitone sodium given intraperitoneal (i.p.) in the dose of 40mg/kg.

Test methods

Animals were divided into various groups in such way that 6 animals were there in each group. Group-A received 0.1ml NS orally served as control for all experiments except righting reflex test where animals received Pentobarbitone (40mg/kg) IP as control, Group –B received Lorazepam (5mg/kg) served as standard & Group –C received Zopiclone (7.5mg/kg). Each animal was treated with respective drug 30 min before experimentation. Following are the details of experiments performed.

Righting reflex test

Drugs like barbiturates induce hypnosis by CNS depression easily determined by loss of righting reflex. In righting reflex test animal is are kept gently on its back over an undulated surface, normally it corrects immediately; if retained on back for 30secs or more it is recorded as loss of righting reflex. Loss of righting reflex is taken as index of CNS depression[6].

Pentobarbitone sleeping time potentiation

Pentobarbitone (barbiturate) produces quick onset of sleep as indicated by loss of righting reflex and recovery is also easily detected as the animal regain righting reflex. Animals in all three groups received the respective drugs and 30 min later treated with Pentobarbitone (40mg/kg) IP. The time interval between loss of righting reflex and reappearance of righting reflex is recorded as duration of sleep. The animal that corrects itself 3 times in 1 min is considered to have recovered from drug effect[7].

Open field apparatus behavior (OFT)

Open field apparatus (OFA) is designed as described by Gray and Lalji (1971) with little modifica-

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TABLE 1 : Observations in Righting reflex & Pentobarbitone sleeping time potentiation test

Groups (n=6)	Treatment	Righting reflex	Treatment	Duration of sleep(min) (Mean ± SEM)
A	Control (pentobarbitone 40mg/kg)	Absent	Control NS(0.1ml)	63.3±1.2
B	Lorazepam (5 mg/Kg)	Present	Lorazepam (5 mg/Kg)	85.8 ± 2.2*
C	Zopiclone (7.5 mg/Kg)	Present	Zopiclone (7.5mg/Kg)	[a]98.5 ± 4.8*

Each group consists of 6 animals. Values are Mean ± SEM, data analyzed by student's unpaired-'t' test. (*P<0.001 compared to control; [a] P<0.05 compared to Lorazepam)

TABLE 2 : Observations in Rota-rod apparatus and inclined plane performance

Groups (n=6)	Treatment	Fall off time in secs(Mean ± SEM)	
		Rota-rod apparatus	Inclined plane performance
A	Control (0.1 ml NS)	210 ± 13.4	243.3 ± 20.3
B	Lorazepam (5 mg/Kg)	156.7 ± 4.2***	133.3 ± 13.3***
C	Zopiclone (7.5 mg/Kg)	170.2 ± 6*	180.5 ± 3.1**

Each group consists of 6 animals. Values are Mean ± SEM, data analyzed by student's unpaired-'t' test. (*P<0.05, **P<0.02, ***P<0.01 compared to control)

tions. Dimensions are 100cm x 100cm x 40cm made up of themacol open from top and bottom kept on white table top; surface is divided into 25 equal squares i.e. 9 central and 16 peripheral. The animals were pretreated with the samples (Zopiclone7.5mg/kg and reference drugs) 30min before. During 5 min session of observation, each animal is placed in the corner of open field apparatus & behavior of animal as determined by ambulation (number of squares entered with both forelimbs) and, exploration (number of central squares entered) was recorded[8].

Rota rod apparatus test

The Rota rod apparatus described by Dunham and Miya (1957) was used for the test. Albino mice underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (5 rpm) for 3 min, were used for the test. The animals were treated in the same way as mentioned under test methods. During experimentation mice were placed upon the bar and the time spent upon the rotating bar (fall off time in secs) for each animal was recorded, if the animal did not fall off the fall off time is assigned to 3 min. Decrease in fall of time was taken as an index for CNS depression[9,10].

Inclined plane performance test

The plane had two rectangular plywood boards connected at one end by a hinge. One board was the base; the other was the movable inclined plane. Two plywood side panels with degrees marked on their surface were fixed on the base. Plane was kept inclined at 45^0. In preselection sessions the animals were placed at the upper part of the inclined plane and were given 5 mins to hang on or to fall off the inclined plane; the animals unable to remain on plane for 3-5mins were rejected and animals that remain on inclined plane for more than 5 min assigned to have fall off time of 5 mins. During experimentation fall off time in secs was recorded for each animal and decreased fall off time was taken as criteria for CNS depression[10,11].

Statistical analysis

Data analyzed by Student's unpaired-'t' test. All the results were expressed as mean (±SEM).

P <0.05 was considered significant.

RESULTS

Righting reflex test

Zopiclone did not inhibit righting reflex at dose of 7.5 mg/kg like Pentobarbitone (TABLE 1).

Pentobarbitone sleeping time potentiation

In the potentiation of Pentobarbitone sleep test, Zopiclone significantly increased the sleeping time in mice at dose of 7.5 mg/kg compared to controls (P<0.001) and Lorazepam 5mg/kg (P<0.05) indicating potent hypnotic activity (TABLE 1).

Rota rod apparatus test and Inclined plane performance test

Zopiclone (7.5 mg/kg) showed weak CNS depressant activity compared to Lorazepam in both the tests for assessing motor co-ordination. (TABLE 2).

Open field apparatus behavior (OFT)

In OFT Zopiclone significantly increased ambulation and exploration (P<0.001). This activity was comparable to Lorazepam (TABLE 3).

DISCUSSION

Righting reflex test and Pentobarbitone sleeping time potentiation

Barbiturate like drugs produce hypnosedation by

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TABLE 3 : Observations in OFT

Open field apparatus behavior (OFT)			
Treatment Groups (n=6)	**Dose mg/Kg**	**Number of central squares entered Mean ± SEM**	**Number of squares entered Mean ± SEM**
Control (A)	NS(0.1ml)	4.5±0.43	22.4± 1.24
Lorazepam(B)	5	13±1.5 *	43.4±1.79*
Zopiclone (C)	7.5	10.3±0.67 *	43.4±1.79*

Each group consists of 6 animals. Values are Mean ± SEM, data analyzed by student's unpaired-'t' test. (*P<0.001 compared to control)

CNS depression determined by absence of righting reflex; however hypnosedation produced by BZDs did not inhibit righting reflex suggestive of more selective actions and lack of neuronal depression. Present study clearly demonstrated that Zopiclone (7.5mg/kg) didn't cause neuronal depression. Furthermore it showed potent hypnotic activity determined by significant potentiation of Pentobarbitone sleeping time compared to Lorazepam. This potent hypnosedative activity is thought to be due to its potent agonistic activity at omega-1 receptor subtype of central BZD-receptor[12].

Rota rod apparatus test and Inclined plane performance test

Inclined plane method was originally developed for testing curare-like agents. Later on, it has been used by many authors for testing compounds for muscle relaxing activity of both centrally acting and peripheral acting muscle relaxants. Zopiclone (7.5 mg/kg p.o) made the animals unable to stay on inclined plane and decreased the fall off time. It also reduced the time spent on the revolving rod by mice in the rotarod test, a test mainly used to screen centrally acting muscle relaxants. However it was found weak myorelaxant in both tests compared to Lorazepam (5mg/kg p.o.). This study clearly demonstrated that it is a weak myorelaxant and this activity was more in inclined plane test compared to Rota rod test this represented that its muscle relaxant activity, which could be due to its peripheral actions and not due to CNS depressant activity[10].

Open field apparatus behavior (OFT)

Exploration in a new environment is an essential part of normal behavior it is determined by ambulation in OFA[13]. Animals show lower ambulation values in new environment due to anxiety and fear. Disinhibitory actions of anxiolytics increase ambulation in new environment by releasing novelty induced suppression of behavior[8]. Present study clearly demonstrated that Zopiclone (7.5mg/kg) had significant anxiolytic activity and it was comparable to Lorazepam (5 mg/kg).

CONCLUSIONS

Zopiclone (7.5mg/kg p.o.) has weak CNS depressant and potent anxiolytic activity comparable to lorazepam. Hence drug could be a good substitute for BZDs in treatment of behavioral disorders.

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