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# Studies of CNS Activities of Some Mannich bases of 1,3,4-Oxadiazole

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**Abstract:** The present study demonstrated the various Central Nervous System effects like depressant, sedative-hypnotic, anticonvulsant and anxiolytic activities of some Mannich bases of 2,5-disubstituted-1,3,4-oxadiazole on albino mice. Before their evaluation for Central Nervous System activities, these derivatives were subjected to preliminary screening for anticonvulsant activity and toxicity studies concluding that the compounds are non toxic and Central Nervous System depressant in nature. Various animal models were utilized, such as, for depressant activity: ambulatory activity, Hole-board and Rotarod; for sedative-hypnotic activity: Ketamine-induced sleeping time; for anticonvulsant activity: Maximum Electroshock, Pentylene tetrazole induced convulsions and Strychnine induced convulsions; for anxiolytic activity: Staircase test, Elevated Plus Maze model. It was found that the test compounds of doses 50, 100 and 150 mg $kg^{-1}$, showed significant dose dependent increase in the above Central Nervous System activities when administered intra peritoneally.

**Key words:** Anticonvulsant, sedative, hypnotic, anxiolytic, CNS depressant

## INTRODUCTION

Brain function and nervous system are the most important aspects of physiology that define the difference between humans and other species. Disorders of brain function and nervous system (central and peripheral) due to improper balance in neurotransmitter levels, whether primary or secondary, to malfunction of other systems, are a major concern of human society and a field in which pharmacological intervention plays a key role (Katzung, 2004).

Epilepsy, psychosis, insomnia, Parkinsonism, depression, Alzheimer's disease and anxiety are some clinically important CNS disorders. Among these, epilepsy, anxiety and insomnia are the areas of interest in this study. There are various classes of drugs of the type of anticonvulsants, anti-depressants, sedative-hypnotics, anti-Parkinsons and anti-Alzheimers available in the market. But the major limitations include addiction, withdrawal symptoms and increase in the dosage during prolonged intake due to lesser effectiveness in the prescribed dosage.

Although, a variety of agents for different Central Nervous System (CNS) activities are available and symptomatic, but neither are effective, prophylactic nor possess proper cure. Compliance with medication is a major problem because of the need for long term therapy together with the unwanted effects of many drugs. Hence, a lot of effort is being focused to investigate a new drug which overcomes the limitations of currently used CNS active drugs.

Therefore, nowadays, the need for novel drugs has been the recent lookout for effective CNS therapy, as a result of which, heterocyclic moieties are being explored. Amongst many heterocyclic rings, 1,3,4-Oxadiazole moiety is of the prime focus in this research work.

1,3,4-Oxadiazole moiety and their Mannich bases constitute one of the most active classes of compounds possessing diverse pharmacological and microbiological activities like antibacterial (Goswami *et al*., 1984; Kulkarni and Rowhani, 1989), antifungal (Khan and Bahel, 1976; Ram *et al*., 1977), anti-inflammatory (Satyanarayana *et al*., 2000) and anticonvulsant (Ram and Pandey, 1974; Jaiswal *et al*., 1978). These test

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compounds were synthesized using ester of benzimidazole moiety which was then converted to its hydrazide, then cyclized to its oxadiazole and then coupled with certain aromatic and aliphatic amines to yield Mannich bases of 2,5-disubstituted-1,3,4-oxadiazole-2-thione (Vijayaraghavan *et al*., 2009). The final derivatives were synthesized using conventional method as well as using microwave synthesizer. Using microwave synthesizer proved to be less time consuming and more product yielding than the conventional method. These compounds were then purified and subjected to spectral analysis including Infra Red (IR), Nuclear Magnetic Resonance spectroscopy (NMR). Elemental analysis such as Carbon, Hydrogen, Nitrogen (CHN) was also performed. These analysis have proved the proper synthesis of the test compounds. These derivatives were previously screened for antibacterial activity, thus displaying effective therapeutic efficacy.

Four compounds were taken for the preliminary anticonvulsant and toxicity studies. Amongst them, only two compounds showed good anticonvulsant activity and were very less toxic, therefore, these two derivatives were shortlisted and were evaluated for their CNS activities using animal models like ambulatory activity, Hole-board, Rotarod, Ketamine induced sleeping time, Maximum Electroshock, PTZ induced convulsions, Strychnine induced convulsions, Staircase test, Elevated Plus Maze.

## MATERIALS AND METHODS

**Animals:** The project was performed in one year period from July 2007 to April 2009 in Bharati Vidyapeeth College of Pharmacy, C.B.D., Navi Mumbai. The animals used for the experiments were BALB/c albino mice weighing between 18-24 g and age between 8-12 weeks purchased from Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai, India. They were housed in groups of 5 in a polypropylene cage of size 25×18.5×12.5 cm under good hygienic conditions in the registered Animal House of Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai. Bedding of rice husk was replaced every 3 days so as to maintain good hygienic conditions. Ambient temperature of 25±1°C, relative humidity of 45-55% and 12:12 h light: dark cycles were maintained in the animal house. Animals had free access to water and standard pelleted diet obtained from Amrut Laboratories, Sangli. All the experiments were conducted according to guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India with their procedures and protocols reviewed and approved by the Institutional Animal Ethical Committee (IAEC), constituted under CPCSEA. All the animals were routinely examined for infections, disorders and injuries and they were treated ethically and humanly.

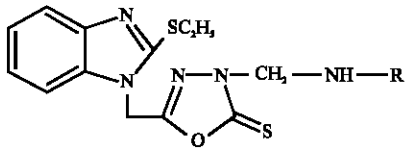


Fig. 1: The structure of test compounds. Where, TC I: R= 4-Nitrophenyl, TC II: R = 2-Chloro-4-fluorophenyl, TC III: R = 3-Chlorophenyl, TC IV: R = Phenyl

**Chemicals and instruments:** Phenytoin sodium I.P. was procured from Alcon Biosciences Pvt. Ltd. Wadhwan, whereas Diazepam (calmpose) Inj. I.P. was obtained from Ranbaxy, Mumbai. Pentylene tetrazole (PTZ) was procured from Himedia Laboratories Pvt. Ltd. Mumbai, Ketamine Hydrochloride Inj. I.P. from Neon Laboratories Ltd. Palghar, Thane. The various instruments used were Rotarod from the manufacturer Dolphin, Electroconvulsometer and activity cage from Shreeji.

**Synthetic test compounds:** The synthetic test compounds (TC, Fig. 1) were procured from Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai. Chemically these compounds are Mannich bases of 5-benzimidazole-3-substituted-1,3,4-oxadiazole-2-thione (Vijayaraghavan *et al*., 2009). All the compounds were pure and characterized by spectroscopic techniques like IR, $^1$H NMR by the providers.

**Preparation of doses:** All the synthetic test compounds were relatively insoluble in water hence 5% gum acacia suspension was used. For doses greater than 100 mg kg$^{-1}$, a 2% acacia suspension was employed. All other dilutions were made with 0.9% saline solution. Injection volume was less than 1-2% of animal body weight.

**Preliminary screening of test compounds for anticonvulsant activity:** Since, Mannich Bases of 1,3,4-oxadiazole-2-thione have been reported for their anticonvulsant activity in Pentylene Tetrazole (PTZ) induced convulsions model of epilepsy (Chaudhary *et al*., 1978), the four test compounds TC I, TC II, TC III and TC IV at a dose of 100 mg kg$^{-1}$ i.p., were preliminarily tested for anticonvulsant activity against PTZ (80 mg kg$^{-1}$ i.p.)

induced convulsions in albino mice. The compounds having higher activities were short listed for further evaluation of CNS activity.

**Acute oral toxicity test:** Malathi and Gomaz (2008) an acute oral toxicity study in mice was carried out for determining median Lethal Dose ($LD_{50}$). According to OECD guidelines 425, Up and Down or Staircase method was used for carrying out toxicity tests. Animals were dosed two at a time at a minimum of 48 h intervals. Dose progression factor was 3.2. Doses were selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550, 1750/2000, 5000 mg $kg^{-1}$ with six animals per group. Each animal was observed carefully for the signs of toxicity as well as for mortality in the first 30 min after dosing and then occasionally for further 4 h and daily thereafter for a period of 14 days. The number of mice dying during 48 h period was recorded.

**Acute i.p. toxicity test:** Doses were selected from sequence of 10, 30, 100, 300, 1000 30 mg $kg^{-1}$ dose was used as starting dose.

**Ambulatory activity:** Aguorre-Hernandez *et al.* (2007) the test was performed using activity cage consisting of a metallic box 36×36×12.5 cm and small stainless steel rods over which the mice are placed one at a time for the test period. The activity cage was covered upwards with transparent plexiglass so as to avoid the disturbances from external environment. The floor of the box was divided into 20 squares of equal size. The cage was placed in sound attenuated room. 30 min after i.p. administration of test drug or the standard, the mice were placed in the centre of the activity cage. The number of squares explored by the animals during a 5 min test period was recorded. The number of squares crossed by the mouse at each dose level was counted for a 5 min period and was compared with the control group.

**Hole-board test:** Vogel (2002) and Woode *et al.* (2009a) the hole-board was made up of wooden ply material and had size of 40×40 cm with 16 holes of 3 cm diameter each, distributed evenly on the floor. The board was elevated at a height of 30 cm so that the mouse poking its nose into the hole does not see the bottom. Thirty minutes after i.p. administration of test drug or the standard, the mouse was placed on the hole-board and the number of nose poking was counted for a period of 5 min. The number of counts for nose-poking of treated animals were recorded and compared with that of the control group.

**Rotarod test:** Vogel (2002) and Woode *et al.* (2009b) the apparatus consisted of a horizontal metallic rod of 2.5 cm diameter and 45 cm in length and was divided into 3 sections by 2 plexiglass partitions, thereby allowing simultaneous testing of 3 mice. It was attached to a motor with the variable adjusted speed of 20 rpm. The rod was positioned at a height of 20 cm above the table top in order to discourage the animals from jumping off the roller. Thirty minutes after i.p. administration of the test compound or the standard, the mice were placed on the rotating rod and observed for their falling from the rotarod as well as the time required falling from the rod in a 3 min test session. The number of animals from each group and the time required for falling from the rotarod during a 3 min test period were recorded for every drug concentration.

**Ketamine induced sleeping time:** Mimura and Namiki (1990) 30 min after i.p. administration of test drug or the standard, the mice were injected (i.p.) with 100 mg $kg^{-1}$ dose of ketamine. At this dose level, all vehicle treated animals produce loss of righting reflex or sleep. The animals were placed on their backs and observed for their wakefulness and regaining of righting reflex. This period was recorded as the duration of sleep. The values of onset as well as duration of sleep were recorded at each dose level in the test drug treated animals and compared with that of the control group.

**Maximum electroshock (MES) test:** Vogel (2002) and Ambawade *et al.* (2002) test was started 30 min after i.p. injection with the vehicle/test compounds/standard drug. Electroconvulsometer with two ear clips was used to deliver the stimuli. The intensity of stimuli was 45 mA for 0.2 sec. Under these conditions, all vehicle treated mice showed the characteristic tonic extensor convulsions. The animals were observed closely for 5 min. Disappearance of the Tonic Clonic Hind Limb Extension (TCHLE) convulsions was used as positive criteria. Inhibition of the seizures by the test compounds relative to control was calculated.

**Pentylene tetrazole (PTZ) induced convulsions:** Vogel (2002) and Ambawade *et al.* (2002) test was started 30 min after i.p. injection with the vehicle/test compounds/standard drugs. 80 mg $kg^{-1}$ of PTZ i.p. induced convulsions and produced 100% mortality in all the vehicle treated animals. Each animal was placed into an individual cage for observations lasting 1 h. Protected animals were observed for next 24 h for mortality. The number of protected animals in the treated groups was calculated as a percentage of affected animals in the control group. The time interval between PTZ injection and occurrence of seizures was measured and compared with the control group. The delay of onset was calculated in comparison with the control group.

**Strychnine induced convulsions:** Vogel (2002) 2 mg $kg^{-1}$ strychnine nitrate i.p. induced convulsions and mortality in all vehicle treated animals. The time until the occurrence of tonic extensor convulsions and death was noted during 1 h period. The number of protected animals in the treated groups was calculated as percentage of affected animals in the control group. The time interval between the strychnine injection and the occurrence of seizures was measured and compared with the control group. The delay of onset was calculated in comparison with the control group.

**Staircase test:** Vogel (2002) and Simiand *et al.* (1984) Staircase was made up of wooden ply material and composed of 5 identical steps of 2.5 cm height, 10 cm width and 7.5 cm depth. The internal height of the walls was constant along the whole length of the staircase. The staircase model was placed inside a light and sound attenuated room. The mice were placed on the floor of the box with their back to the staircase. The number of steps climbed and the number of rears were counted over a period of 5 min. After each test, the box was cleaned in order to eliminate any olfactory cues which might modify the behavior of the next animal. The average number of steps and rearings of the control group were recorded. The values for treated animals were compared to those of the control group.

**Elevated Plus Maze (EPM) test:** Vogel (2002) Rabbani *et al.* (2003, 2004) the elevated plus maze apparatus was made up of wooden ply material and consisted of two open arms (16×5 cm) and two closed arms (16×5×12 cm) with an open roof. They were arranged so that the open arms are opposite to each other and all the four arms are connected together with a central square of 5×5 cm. The entire maze was elevated at a height of 25 cm and was placed inside a light and sound attenuated room (Kumar and Kalonia, 2007). The number of entries into the open arm and closed arm and the time spent in the open arms was measured during a 5 min period. Thirty minutes after i.p. administration of test drug or the standard, the mice was placed in the centre of the maze facing towards one of the closed arms. Number of entries as well the time spent in the open arm was recorded. The values for the test compound treated groups were compared with that of the vehicle treated and the standard drug treated groups.

## RESULTS

**Preliminary screening for anticonvulsant activity:** The anticonvulsant activity of the synthesized compounds was determined by evaluation of the ability of the compounds to protect mice against convulsions induced by a PTZ. Test compounds I and II (Table 1) showed greater anticonvulsant activity in mice and were short listed for further evaluation of CNS activity.

Table 1: Preliminary screening of test compounds (TC) at 100 mg $kg^{-1}$ (i.p.) for its anticonvulsant activity against PTZ (80 mg $kg^{-1}$ i.p.) induced convulsions in albino mice

| Test compounds | No. of animals tested | No. of animals protected | No. of deaths | % protection |
|---|---|---|---|---|
| TC I | 10 | 8 | 2 | 80 |
| TC II | 10 | 8 | 2 | 80 |
| TC III | 10 | 5 | 5 | 50 |
| TC IV | 10 | 3 | 7 | 30 |

Data are presented in the form of number of mice protected and killed using PTZ induced convulsions test

Table 2: Results of acute (i.p.) toxicity

| Dose (m $kg^{-1}$) | No. of deaths |
|---|---|
| 250 | 0 |
| 260 | 1 |
| 275 | 3 |
| 285 | 4 |
| 300 | 6 |

Data are presented in the form of number of mice killed using the test compounds

**Acute oral toxicity:** Test compounds when tested at 175, 550 and 1750 mg $kg^{-1}$ doses did not produce mortality. Test compound at 2000 mg $kg^{-1}$ dose showed death of one animal out of two. The study was continued at the same dose level by taking three more animals. Two out of five animals showed death. Thus oral $LD_{50}$ value for test compound was >2000 mg $kg^{-1}$. Since, $LD_{50}$ value was more than 2000 mg $kg^{-1}$, the main study was eliminated. Maximum safe dose found was 1500 mg $kg^{-1}$.

**Acute i.p. toxicity:** The test compounds at 30, 100 mg $kg^{-1}$ dose did not produce mortality. But at 300 mg $kg^{-1}$ dose, they showed mortality in both the animals. Thus, the dose was decreased by 0.7 and for tested animals at 200 mg $kg^{-1}$ dose level and no mortality was found. The study was continued at higher doses (225, 250, 275 mg $kg^{-1}$) to find out the maximum safe dose 225 and 250 mg $kg^{-1}$ doses did not show any mortality hence they were considered safe. At 275 mg $kg^{-1}$ dose, one out of two animals showed death. The main test was conducted with each group of six animals by taking three intermediate dose levels in between the 250 and 300 mg $kg^{-1}$ dose levels. From Table 2, maximum safe dose (i.p.) was found to be 250 mg $kg^{-1}$, minimum toxic dose (i.p.) was 300 mg $kg^{-1}$ and the $LD_{50}$ value (i.p.) was found to be 275 mg $kg^{-1}$.

**Ambulatory activity:** In the vehicle treated control group, the mean number of squares explored by the mice was

Table 3: Effect of diazepam and different doses of test compounds (TC) on the number of squares explored and percentage decrease in squares explored of an activity cage apparatus during a 5 min test session in mice

| Treatments | Dose (mg kg$^{-1}$) | No. of squares explored in 5 min (Mean±SEM) | Percentage decrease in squares explored |
|---|---|---|---|
| Control | 10 mL kg$^{-1}$ | 58.83±3.987 | - |
| Diazepam (Std) | 0.3 | 20.00±1.789* | 66 |
| TC I | 50 | 38.50±2.849* | 34.55 |
| TC I | 100 | 26.50±1.360* | 54.95 |
| TC I | 150 | 15.33±1.022* | 73.94 |
| TC II | 50 | 42.83±2.301* | 27.2 |
| TC II | 100 | 28.33±2.076* | 51.84 |
| TC II | 150 | 19.50±1.893* | 66.85 |

Results are expressed as Mean±SEM, (n = 6). *p<0.05 as compared to vehicle treated control group was considered significant. Statistics used is one way ANOVA followed by Dunnett's test

Table 4: Effect of diazepam and different doses of test compounds (TC) on number of nose poking and percentage decrease in nose poking in the hole-board test apparatus during a 5 min test session in mice

| Treatments | Dose (mg kg$^{-1}$) | No. of nose poking in 5 min (Mean±SEM) | Percentage decrease in nose poking1 |
|---|---|---|---|
| Control | 10 mL kg$^{-1}$ | 29.33±4.24 | - |
| Diazepam (Std) | 0.3 | 16.50±1.996* | 43.74 |
| TC I | 50 | 27.17±1.797 | 7.36 |
| TC I | 100 | 22.33±1.238 | 23.87 |
| TC I | 150 | 14.17±1.701* | 51.69 |
| TC II | 50 | 28.17±1.302 | 3.95 |
| TC II | 100 | 24.38±2.257 | 16.88 |
| TC II | 150 | 14.00±1.238* | 52.26 |

Results are expressed as Mean±SEM, (n = 6). *p<0.05 as compared to vehicle treated control group was considered significant. Statistics used is one way ANOVA followed by Dunnett's test

58.83. Diazepam (0.3 mg kg$^{-1}$) as a reference standard reduced the squares explored by 66%. Injection of test compounds at 50, 100, 150 mg kg$^{-1}$ showed significantly and dose dependently decrease in the number of squares explored (Table 3).

**Hole-board test:** In the vehicle treated control group, the mean number of nose poking by the mice was 29.33. Diazepam (0.3 mg kg$^{-1}$) as a reference standard showed decrease in number of nose poking by 43.74%. Injection of test compounds at 50, 100, 150 mg kg$^{-1}$ showed not significantly but dose dependently decrease in the number of nose poking. Test compound at a dose of 50 mg kg$^{-1}$ did not affect the counts of nose poking indicating absence of activity at lower dose levels (Table 4).

**Rotarod test:** The vehicle treated control group animals did not fall from the rotarod within the 3 min test session which indicates normal motor activity. The test compound treated animals at 50 and 100 mg kg$^{-1}$ doses produced

Table 5: Effect of diazepam and different doses of test compounds (TC) on the number of animals' falls from the rotarod (20 rpm) during a 3 min test session in mice

| Treatments | Dose (mg kg$^{-1}$) | No. of animals falling within 3 min | Average time required for falling (sec) |
|---|---|---|---|
| Control | 10 mL kg$^{-1}$ | 0 | - |
| Diazepam (Std) | 2 | 6 | 58.66 |
| TC I | 50 | 0 | - |
| TC I | 100 | 0 | - |
| TC I | 150 | 3 | 160 |
| TC II | 50 | 0 | - |
| TC II | 100 | 0 | - |
| TC II | 150 | 2 | 149.5 |

Results are expressed as no. of animals falling and average time of their fall

similar effect as that of control group. But the test compounds at 150 mg kg$^{-1}$ doses occasionally produced falls from the rotarod. At higher doses (>150 mg kg$^{-1}$), animals significantly fell from the rotarod (Table 5).

**Ketamine induced sleeping time:** In the vehicle treated control animals, the righting reflex was lost after 139.5 sec. Diazepam as a reference standard at dose of 0.5 mg kg$^{-1}$ reduced the latency to sleep by 48.5%. Injection of test compounds (30 min prior to ketamine) dose dependently suppressed the latency to sleep. In the vehicle treated control group, the duration of sleep was 29.52 min. Diazepam as a reference standard at the dose of 0.5 mg kg$^{-1}$ increased the duration of sleep by 86.44%. Injection of test compounds at 50, 100, 150 mg kg$^{-1}$ showed significant and dose dependant increase in duration of sleep (Table 6).

**Maximum Electroshock Test (MES):** In the vehicle treated control group, 100% of the mice exhibited convulsions indicated by Tonic-Clonic Hind Limb Extensions (TCHLE). In the control group, the TCHLE time of convulsions was 22.2 sec. Phenytoin as a reference standard reduced both TCHLE as well as stupor by 66.22 and 78.50%, respectively. TC I at the dose of 50 mg kg$^{-1}$ showed similar effects as that of control on TCHLE phase of convulsions. TC I at the dose of 100 and 150 mg kg$^{-1}$ showed significantly and dose dependently decreases in TCHLE as well as stupor phase of convulsions. TC II at doses of 50, 100 and 150 mg kg$^{-1}$ significantly and dose dependently produced a decrease in TCHLE and stupor phase of convulsions (Table 7).

**Pentylene tetrazole (PTZ) induced convulsions:** The vehicle treated control group showed onset of seizure after 2.305 min and also showed 100 % mortality against 80 mg kg$^{-1}$ dose of PTZ. Diazepam (4 mg kg$^{-1}$ i.p.) as a reference standard significantly increased the onset of seizure and showed 100% protection against PTZ convulsions. Test compounds at 50, 100 and 150 mg kg$^{-1}$

Table 6: Effect of diazepam and different doses of test compounds (TC) on latency to sleep, percentage decrease in latency to sleep, duration of sleep and percentage increase in duration of sleep in ketamine-induced sleeping time test in mice

| Treatments | Dose (mg kg$^{-1}$) | Latency to sleep (sec) (Mean±SEM) | Percentage decrease in latency to sleep | Duration of sleep (min) (Mean±SEM) | Percentage increase in duration of sleep |
|---|---|---|---|---|---|
| Control | 10 mLkg$^{-1}$ | 139.50±5.59 | - | 29.52±3.765 | - |
| Diazepam (Std) | 0.5 | 71.83±2.4* | 48.5 | 55.04±0.534* | 86.44 |
| TC I | 50 | 128.70±4.088 | 7.75 | 40.60±0.72* | 37.53 |
| TC I | 100 | 114.67±3.403* | 17.8 | 45.09±0.328* | 52.74 |
| TC I | 150 | 94.00±2.236* | 32.61 | 52.58±0.65* | 78.12 |
| TC II | 50 | 131.83±2.227 | 5.5 | 38.11±1.77* | 29.1 |
| TC II | 100 | 121.20±1.642* | 13.12 | 43.00±1.05* | 45.66 |
| TC II | 150 | 99.50±2.217* | 28.67 | 50.02±1.04* | 69.44 |

Results are expressed as Mean±SEM, (n = 6). *p<0.05 as compared to vehicle treated control group was considered significant. Statistics used is one way ANOVA followed by Duunett's test

Table 7: Effect of Phenytoin and different doses of test compounds on tonic-clonic hind limb extensions (TCHLE), percentage decrease in TCHLE, stupor and percentage decrease in stupor in the Maximal Electroshock (MES) induced convulsions test in mice

| Treatments | Dose (mg kg$^{-1}$) | TCHLE (sec) (Mean±SEM) | % decrease in TCHLE | Stupor (Mean±SEM) | Percentage decrease in stupor |
|---|---|---|---|---|---|
| Control | 10 mL kg$^{-1}$ | 22.20±1.02 | - | 143.00±14.57 | - |
| Phenytoin (Std) | 25 | 7.50±1.041* | 66.22 | 30.75±4.347* | 78.5 |
| TC I | 50 | 22.00±1.0 | 0.9 | 120.80±7.228* | 15.52 |
| TC I | 100 | 14.17±0.946* | 36.17 | 86.25±12.81* | 39.7 |
| TC I | 150 | 11.75±1.25* | 47.07 | 47.60±2.249 | 66.7 |
| TC II | 50 | 19.17±0.872* | 13.65 | 122.20±5.616 | 14.55 |
| TC II | 100 | 16.33±0.667* | 26.44 | 89.17±13.32* | 37.64 |
| TC II | 150 | 13.00±1.155* | 41.44 | 58.67±4.006* | 58.97 |

Results are expressed as Mean±SEM, (n = 6). *p<0.05 as compared to vehicle treated control group was considered significant. Statistics used is one way ANOVA followed by Duunett's test

Table 8: Effect of diazepam and different doses of test compounds on onset of seizure and percentage of protected animals in the PTZ-induced convulsions test in mice

| Treatment | Dose (mg kg$^{-1}$) | Onset of seizure (min) (Mean±SEM) | % protection in 1 h | Percentage recovery in 24 h |
|---|---|---|---|---|
| Control | 10 mL kg$^{-1}$ | 2.305±0.145 | 0 | - |
| Diazepam (Std) | 4 | 13.77±0.700* | 100 | 100 |
| TC I | 50 | 8.225±0.720* | 83.33 | 66.66 |
| TC I | 100 | 9.378±0.691* | 83.33 | 83.33 |
| TC I | 150 | 10.220±.560* | 83.33 | 83.33 |
| TC II | 50 | 8.427±0.709* | 50 | 50 |
| TC II | 100 | 9.975±0.759* | 83.33 | 83.33 |
| TC II | 150 | 10.517±0.230* | 83.33 | 83.33 |

Results are expressed as Mean±SEM, (n = 6). *p<0.05 as compared to vehicle treated control group was considered significant. Statistics used is one way ANOVA followed by Duunett's test

significantly and dose dependently extended the time required to produce first seizures. But in the case of unprotected animals, the time required to produce the seizure was not extended. Protection against PTZ convulsions was not dose dependent and it produced 83.33% protection at almost all dose levels (Table 8).

**Strychnine induced convulsions:** Strychnine at 2 mg kg$^{-1}$ i.p. dose produced 100% mortality in the vehicle treated control group. Both the test compounds treated animals at any of the three dose levels tested did not show any protection against the strychnine induced convulsions.

**Staircase test:** In the vehicle treated control group, number of steps climbed and rearing was 23.33 and 22.17, respectively. Diazepam (2 mg kg$^{-1}$) as a reference standard showed significant increase in number of steps climbed by 60% and decrease in rearing by 37.75%. Test compounds at 50 mg kg$^{-1}$ dose did not show any prominent effect on the activity. Test compounds at 100 mg kg$^{-1}$ showed a slight increase in steps climbing and a significant decrease in rearings. Test compounds at 150 mg kg$^{-1}$ showed a decrease in steps climbing and also showed a prominent and significant decrease in rearings (Table 9).

**Elevated plus maze (EPM) test:** Average number of entries in open arm was 2.66 and time spent on open arm was 11.5 sec in the vehicle treated control group. Diazepam (1 mg kg$^{-1}$) treated the mice which showed a significant increase in the number of open arm entries and also the time spent on the open arms. Test compounds showed increase in number of entries in open arm and also dose dependent increase in the time spent on open arm. Test compound at 150 mg kg$^{-1}$ showed a decrease in the number of entries in open arm as compared to 100 mg kg$^{-1}$ dose (Table 10).

Table 9: Effect of diazepam and different doses of test compounds (TC) on the number of steps climbed, rearings and percentage decrease in rearings in the staircase model during 5 min test session in mice

| Treatments | Dose (mg kg$^{-1}$) | No. of steps climbed in 5 min (Mean±SEM) | No. of rearingsin 5 min (Mean±SEM) | Percentage decrease in rearing |
|---|---|---|---|---|
| Control | 10 mL kg$^{-1}$ | 23.33±2.565 | 22.17±2.007 | - |
| Diazepam (Std) | 2 | 37.33±1.453* | 13.80±1.281* | 37.75 |
| TC I | 50 | 24.67±1.667 | 18.00±1.155* | 18.81 |
| TC I | 100 | 26.33±1.116 | 14.67±1.054* | 33.83 |
| TC I | 150 | 12.00±1.211* | 8.66±0.882* | 60.94 |
| TC II | 50 | 26.33±1.406 | 16.83±2.136 | 24.09 |
| TC II | 100 | 29.17±1.701 | 12.83±1.249* | 42.13 |
| TC II | 150 | 15.83±1.515* | 7.33±1.145* | 66.94 |

Results are expressed as Mean±SEM, (n = 6). *p<0.05 as compared to vehicle treated control group was considered significant. Statistics used is one way ANOVA followed by Duunett's test

Table 10: Effect of diazepam and different doses of test compounds (TC) on the number of entries in the open and closed arms and time spent in the open arm of an elevated plus maze model during a 5 min test session in mice

| Treatments | Dose (mg kg$^{-1}$) | No. of entries in open arms during 5 min (Mean±SEM) | No. of entries in closed arms during 5 min (Mean±SEM) | Time spent on open arms (sec) during 5 min (Mean±SEM) |
|---|---|---|---|---|
| Control | 10 mL kg$^{-1}$ | 2.667±0.558 | 13.000±1.693 | 11.50±1.258 |
| Diazepam (Std) | 1 | 14.000±1.065* | 15.000±1.390 | 63.67±1.801* |
| TC I | 50 | 4.667±0.558 | 14.500±1.893 | 18.17±1.167* |
| TC I | 100 | 11.000±0.856* | 17.830±2.509 | 29.50±1.708* |
| TC I | 150 | 6.330±0.715* | 8.330±1.174 | 47.00±2.490* |
| TC II | 50 | 4.667±0.558 | 15.000±1.390 | 16.00±1.414 |
| TC II | 100 | 9.500±1.258* | 14.330±1.229 | 36.00±2.160* |
| TC II | 150 | 7.167±1.195* | 4.833±0.601* | 43.83±2.136* |

Results are expressed as Mean±SEM, (n = 6). *p<0.05 as compared to vehicle treated control group was considered significant. Statistics used is one way ANOVA followed by Duunett's test

## DISCUSSION

The toxicity tests results showed that the oral $LD_{50}$ value was 7 times greater than the i.p. $LD_{50}$ value which indicated a lower bioavailability of the test compounds by the oral route, but a wide margin of safety, which indicates that they may be safe in overdose.

In the ambulatory activity test, the number of squares explored by the mouse indicates its ambulatory behavior which is related to general motor activity and locomotion of the treated animals. This test allows the differentiation between various types of sedative and stimulant drugs. Both the test compounds showed significantly and in a dose dependent manner, a decrease in the number of squares explored by mice. Reduction of ambulatory activity observed after treatment with both the test compounds at a dose of 150 mg kg$^{-1}$ was more pronounced than that produced by diazepam. Thus, the observations made and the results obtained from the present study indicate that both the test compounds have a sedative action and possess CNS depressant activity.

In the Hole-board test, the test compounds showed dose dependent decrease in the number of nose pokings. The decrease in the nose poking activity at the higher dose levels was even more pronounced than that shown by the reference standard diazepam. This indicates a decrease in the curiosity or exploratory behavior of test animals and also provides evidence in favor of a CNS depressant action (Sonavane *et al*., 2001; Suba *et al*., 2002).

A decrease in the ambulatory activity of the test compounds does show the sedative effect, but it can also be due to a variety of causes other than sedation, such as motor impairment or muscle relaxation for which the Rotarod test is the most widely used and accepted method. The animals did not fall from the rod at lower doses, but at a higher dose, they fell, which indicates a possible skeletal muscle relaxant or motor in-coordination inducing effect of the test compounds only at higher doses (Achliya *et al*., 2004; Manna *et al*., 2005).

In the ketamine induced sleeping time, administration of both the test compounds dose dependently shortened the latency to sleep which is observed as faster loss of righting reflex and prolonged the duration of sleep which indicates that the test compounds possess sedative-hypnotic activity. However, the time required for latency to sleep was higher and the duration of sleep was shorter than that produced by the reference standard diazepam at all the three dose levels of the test compounds.

In the Maximum Electroshock test, from the results, there was dose dependant decrease in TCHLE as well as stupor phase of convulsions. Thus the test compounds have likely therapeutic efficacy against generalized tonic-clonic seizures (Sayyah *et al*., 2005). TC II produced better protection in MES test as compared to TC I. Both the test compounds at all dose levels were not found to be as effective as the reference standard phenytoin (25 mg kg$^{-1}$) in our study.

In the PTZ test, single acute administration of diazepam (4 mg $kg^{-1}$) significantly prolonged the onset of seizure and showed 100% protection against PTZ induced convulsions. Both the test compounds significantly and dose dependently prolonged the time required to produce first seizures and also offered significant protection against PTZ seizures. As the test compounds protected against PTZ convulsions, they could be explored further as potential agents for the treatment of Petit Mal epilepsy. These test compounds are not as effective as the reference standard diazepam which showed 100% protection against PTZ induced convulsions (Khosla and Pandhi, 2001).

In the strychnine induced convulsions, both the test compound treated animals produced mortality at all three dose levels tested, which indicates that the compounds do not have a possible effect on strychnine induced convulsions. This leads us to the probability that the anticonvulsant action of the test compounds involves mechanisms other than the potentiation of glycine action.

In the staircase test, diazepam (2 mg $kg^{-1}$) as a reference standard showed a significant increase (60%) in the number of steps climbed and a decrease (37.75%) in rearings, thus confirming its anxiolytic activity. The test compounds showed significant and dose dependent decrease in rearings indicating their anxiolytic activity. (Abid *et al*., 2006; Bhatacharya and Satyan, 1997; Soulimani *et al*., 1997).

In the elevated plus-maze, as compared with the control, diazepam increased the number of entries in the open arm and the time spent in the open arm, thus confirming its anxiolytic effect. At higher dose, the decrease in open arm entries may be due to sedative effect. Both the test compounds produced a dose dependent increase in the time spent in the open arm, which proves their anxiolytic activity.

## CONCLUSIONS

In conclusion, this study provides the evidence that both the test compounds possess sedative and antidepressant properties in mice. These effects are not accompanied by motor in-coordination. The results indicate that the test compounds have anticonvulsant effects on MES and PTZ induced convulsions. Though they decreased the latency to sleep and increased the duration of sleep when given with other hypnotics like ketamine, they do not show hypnotic action of their own when used alone. Also, results from sedative-hypnotic test and rotarod test may be attributed to the sedative and central depressant action of the compounds and not due to muscle relaxation. Most of the results obtained with the test compounds correlate with the action of benzodiazepines, thus, the mechanism of action for anticonvulsant and anxiolytic activity may involve GABA–benzodiazepine-chloride channel receptor opening. The exact mechanism of action of these compounds remains to be elucidated.

In summary, the present study demonstrates that the test compounds have central depressant, sedative-hypnotic, anticonvulsant and anxiolytic properties. Further studies are necessary for the biochemical estimation of various bioamines/neurotransmitter levels in the brain and also to identify the exact mechanisms responsible for their activities.

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