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Study of Neuropharmacological Profile of Cilostazol

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ABSTRACT

Drugs with large conductance calcium activated potassium channel opening properties by virtue of their ability to promote an outflow of potassium ions, produce hyper polarization of cell membrane and decrease in the cell excitability. This modulatory role of BK channels on the glutaminergic neuro-transmission renders BK channels openers potentially useful in epilepsy. Apart from this, channels form the target for modulation for a range of neurotransmitters and have been implicated in the pathogenesis of neurological disorders. Our study was designed to investigate the neurobehavioural and anticonvulsant properties of Cilostazol. Neurobehavioural properties were evaluated using the hole board, Actophotometer and pentobarbitone-induced hypnosis. Strychnine and maximal electroshock induced convulsion tests were used to investigate the anti-convulsive actions of Cilostazol. Results show that Cilostazol significantly reduced the number of poking at both the tested doses. It also reduced the locomotor activity and showed a slight increase in sleeping time also. In addition, Cilostazol (20 & 40 mg/kg) showed protection of rats against strychnine induced convulsions but has no effect on maximal electroshock induced convulsions. The effects of cilostazol in various models were comparable with that of the standard drug. The findings from the study suggest that the Cilostazol may be neurosedative and anticonvulsant action might be by blocking the inhibitory effects of glycine.

Keywords: Cilostazol, neuroprotective, large conductance calcium activated potassium channel (BK), anticonvulsant, neurobehaviour

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INTRODUCTION

Cilostazol (CZ), has been known to be a thrombolytic and vasodilator drug that can selectively inhibit the phosphodiesterase 3 and has been approved by The Food and Drug Administration for treatment of intermittent claudication [1]. Its primary actions comprise antiplatelet aggregation, antithrombosis in feline cerebral ischemia and vasodilation due to its increased cyclic AMP level. Cilostazol treatment achieved a significant risk reduction in patients with recurrence of cerebral infarction [2]. Cilostazol has also proven its efficacy in preventing the silent brain infarction in japaneese patients with type-2 diabetes [3]. It has been also documented that cilostazol exerts neuroprotective effects on acute ischemic brain injury. Cilostazol was also reported to decrease ischemic brain infarction, inhibited apoptotic and oxidative cell death [4] and attenuated gray and whitematter damage 24 h after a focal cerebral ischemia in rats [5]. Furthermore, stroke prevention has been recently approved as a new indication of Cilostazol in Japan [6,7] However. Cilostazol has been noted to possess an additional effect that does not require the inhibition of phoshodiesterase as it has been reported to increase the outward K^+ currents by activating maxi –K channels. BK channel activation appeared to be an extra feature for the drug on account of its therapeutic plasma concentration ranging from 1 to 5 µM and lately found to stimulate BK channels with an EC_{50} of 3.5 µM[8].

Large-conductance or BK channels are one type of calcium activated potassium channel which are activated by depolarizing membrane potentials as well as by an increase in the internal calcium concentration. In nervous system, the BK channels participate in shaping action potentials and regulate neuronal excitability [9-12]. The possible anticonvulsant employment of BK openers has been demonstrated in the experimental seizure models, whereas further clinical application of these drugs can concern their potential uses for the treatment of psychoses, urinary incontinence, erectile dysfunction and gastroenteric hypermotility [13].

There are various drugs and chemicals that activate BK channels in a nonselective manner i.e., they activate BK channels along with their primary action. In our investigation we have tried to evaluate the neurobehavioural and anticonvulsant properties of Cilostazol, a non-selective BK channel opener drug.

MATERIALS AND METHOD

Animals

Adult albino Swiss mice of either sex weighing 25-35 gms and albino Wister rats of either sex weighing 150-200 gms were housed in groups of six per cage. They were maintained in well-

ventilated room temperature with relative humidity of 45-55% and natural 12h: 12h day-night cycle in propylene cages. All the experiments were carried out between 10:00 am to 2:00 pm. The animals were housed for one week, prior to the experiments to acclimatize laboratory temperature. Food was withdrawn 3 hrs before the experiment and the animals were fed with water during experiment. The experiment protocol was approved by the Institutional Animal Ethics Committee IAEC Ref.No. 290/CPCSEA/2009-PH-PCOL-01.

Drugs and chemicals:

The drugs used were Cilostazol (Cilodoc, Lupin Laboratories, India), Phenobarbitone sodium (inj) Nicholas Piramal India Ltd. Strychnine was obtained from Sas chemicals Co, India. Diazepam (inj) was obtained from Intas Laboratories, India). All chemicals and reagents used were of analytical grade. Cilostazol was made into suspension in 10% aqueous Tween 80 for oral administration and strychnine was dissolved in glacial acetic acid and made up the volume with distilled water. Animal dose was calculated according to the body mass surface ratio [14].

Experimental design:

For each of the model studied, thirty mice were randomly divided into four groups (n=6). The groups include two controls (vehicle and standard drug) and two treatment groups for doses 20 & 40 mg/kg. For anticonvulsant test, thirty rats were randomly divided into four groups (n=6) and followed the same grouping as above.

Phenobarbitone induced sleeping time

The pentobarbitone induced sleeping time that measures onset and duration of sleep was used to assess sedative activity. Mice were divided into four groups consisting of 6 per group. The first group served as control and was injected with the vehicle; while the other two groups received the CZ (20 mg/kg and 40 mg/kg respectively) and the fourth group was administered with (Diazepam 4 mg/kg, i.p). Sixty min after the administration of test drug and thirty min after the administration of the standard, all the mice were injected with pentobarbitone sodium (40 mg/kg). The sleeping time of all animals were noted by recording time interval between the loss and return of righting reflux [15].

Locomotor activity

Locomotor activity is easily measured using actophotometer which operates on photoelectric cells connected with a counter. When a beam of light falling on the photocell is cut off by the animal a count is recorded. Mice were divided into four groups consisting of 6 per group. The groups were 10ml/kg vehicle; 20, 40 mg/kg CZ; Diazepam 4 mg/kg. Mice are placed

individually in the activity cage floor 10 min. Basal activity score of animals are recorded before and after the administration of CZ and diazepam [16].

Hole Board Test

The hole board test was used to determine potential sedative effects. The hole board apparatus consisted of wooden box (40×40×25 cm) with 16 holes (diameter, 3cm) evenly distributed in the floor. The hole board was elevated to the height of 25 cm. The animals were administered the drug 30 min before placing on the apparatus and the number of head poking during 5 min period were recorded and the percentage decrease in head poking was also calculated [17]. The procedure was repeated for all the mice in different groups. There were four groups of six mice each. The groups were treated with 10ml/kg vehicle; 20, 40 mg/kg CZ; Diazepam 4 mg/kg. After each trial, the floor of the apparatus was wiped with 70% ethanol and dried thoroughly to remove traces of previous path.

Anticonvulsant tests

a) Maximum electric shock induced convulsion method: In maximum electric shock convulsions, electric stimuli was applied to the rat through the ear electrodes (apply 150 mA for 0.2 sec). The animal was held properly, the ear electrodes were placed in the ear and the prescribed current was applied. The different phases of convulsions were noted; 1) Tonic flexion 2) Tonic extensor 3) Clonic convulsions 4) Stupor 5) Recovery or Death. Rats were divided into four groups consisting of 6 per group. The groups were 10ml/kg vehicle; 20, 40 mg/kg CZ; Diazepam 4 mg/kg. The time spent by the animal in each phase of convulsions after the 60 min after the test drug administration and 30 min after the standard administration was noted. The reduction in time or abolition of tonic extensor phase of MES induced convulsions was also recorded. (18)

b) Strychnine induced convulsion method [19] Strychnine (2mg/kg; i.p) was used to induce seizures. Rats were divided into four groups consisting of 6 per group. The groups were 10ml/kg vehicle; 20, 40 mg/kg CZ; Diazepam 4 mg/kg. Sixty min after the test drug administration and thirty min after standard administration strychnine was administered at a dose of 2 mg/kg intraperitoneally to all the group of rats. The latency of tonic extensor convulsions and death is noted during a 1h period for each animal .

Statistical Analysis:

The data were analyzed by ANOVA followed by Dunnett's 't'test. Values were considered significant when $p < 0.05$. All data were expressed as mean ±S.E.M of 6 animals per group.

RESULTS AND DISCUSSION

Phenobarbitone induced sleeping time

When mice were treated with CZ (20 mg/kg & 40 mg/kg), though there is slight increase in sleeping time, there was no statistically significant potentiation in the sleeping time. Results were shown in **Table 1.**

Table 1. Effect of CZ (Cilostazol) on phenobarbitone sleeping time

S.No	Treatment	Sleeping time in(min)
1.	Phenobarbitone sodium(40 mg/kg, i.p)	49.17±4.25
2.	Phenobarbitone sodium (40 mg/kg, i.p) + CZ (20 mg/kg)	58.27±7.54^{NS}
3.	Phenobarbitone sodium (40 mg/kg, i.p) + CZ (40 mg/kg)	65.43±9.23^{NS}
4.	Phenobarbitone sodium (40 mg/kg,i.p) + Diazepam (4 mg/kg)	152.02±0.73***

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6)

Values are mean ± SEM of 6 animals per group. Comparison was made between phenobarbitone group and phenobarbitone + drug treated groups ***p<0.001 , NS – Non-significant

Locomotor activity

When mice were treated with CZ (20 mg/kg & 40 mg/kg), the locomotion was significantly reduced with increase in the dose when compared to control. The results were also comparable with that of standard. Results were shown in Figure 1.

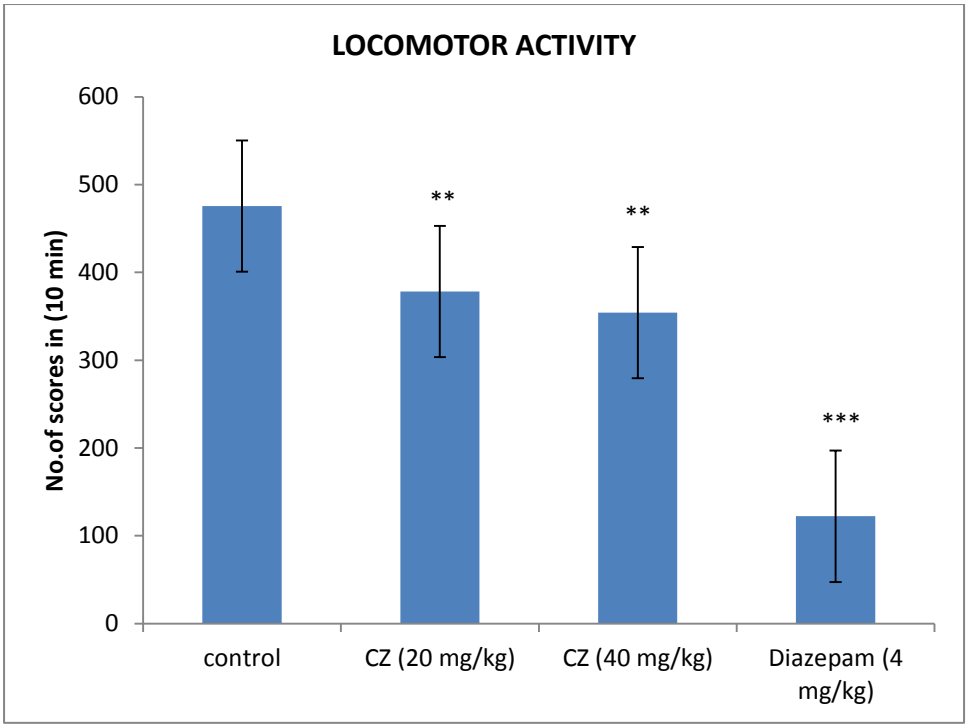

Figure 1: Effect of CZ (Cilostazol) on Locomotor activity

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6) Values are mean±SEM of 6 animals per group. Comparison was made between control group and drug treated groups ***p<0.001**p<0.01

Hole board test

Figure 2 showed the effect of CZ (20 mg/kg & 40 mg/kg) on the number of head poking in the hole board apparatus. CZ significantly reduced the number of head poking at both the tested doses. Diazepam, a well established sedative also exhibited significant decrease in the number of head poking.

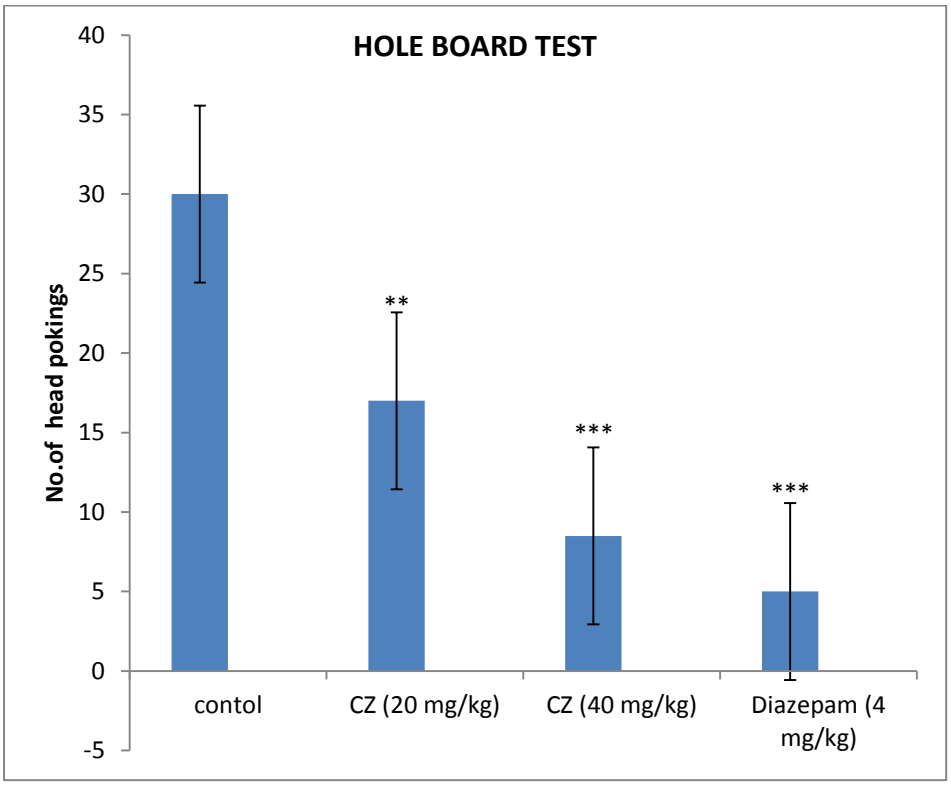

Figure 2: Effect of CZ (Cilostazol) on Hole board apparatus

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6) Values are mean±SEM of 6 animals per group Comparison was made between control group and drug treated groups**.** ***p<0.001, **p<0.01

Effect of CZ on anticonvulsant activity

Maximum electric shock induced convulsion

In this test, CZ (20 mg/kg & 40 mg/kg) did not show a significant reduction of time spent in extensor phase. Result was shown in Figure 3.

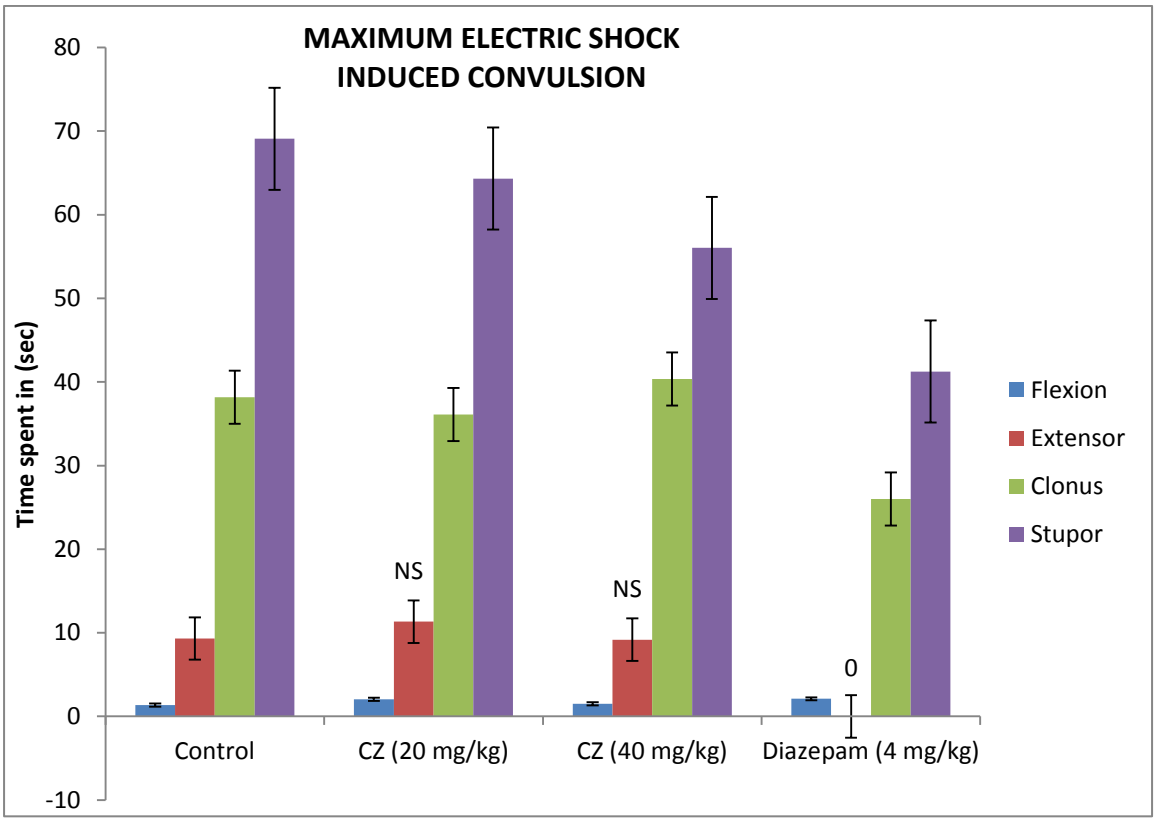

Figure 3: Effect of CZ (Cilostazol) on maximum electric shock induced convulsion

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6). Values are mean±SEM of 6 animals per group. Comparison was made between control group and drug treated groups. NS - Non significant

Strychnine induced convulsion

In this test, the onset of convulsion and death time were increased with increasing in dose CZ (20 mg/kg &40 mg/kg) when compared to control. The results were comparable with that of standard. Results were shown in Table 2.

Table 2. Effect of CZ (Cilostazol) on Strychnine induced convulsion

S.No	Treatment	Onset of convulsion (min)	Death time (min)
1.	Vehicle(10% aqueous Tween 80)	4.4±2.56	6.3±1.99
2.	CZ (20 mg/kg)	8.9±0.18*	9.23±1.78*
3.	CZ (40 mg/kg)	10.2±0.24*	12.35±4.20*
4.	Diazepam(4 mg/kg)	15.4±2.06**	16.5±1.93**

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6)

Values are mean± SEM of 6 animals per group

Comparison was made between control group and drug treated groups

*p<0.05, **p<0.01

BK channels have been involved in the etiology of neurological disorders, so the present investigation aim to study the neurobehavioural properties of non-selective BK channel opener Cilostazol along with its anticonvulsant properties . When we assessed the effect of CZ on CNS, the potentiating action of CZ on phenobarbitone induced sleeping time was not significant. But the results from the study on the locomotion and hole board test suggest that the drug may be a neurosedative [16,17]

In order to evaluate the anticonvulsant activity, we used maximum electric shock induced convulsion and strychnine induced convulsion. As strychnine induced convulsions resemble petit mal type of convulsions in man [19], we studied strychnine induced convulsions. The drug showed significant protective effect against strychnine induced convulsions at both tested doses. The convulsing action of strychnine is due to interference with postsynaptic inhibition mediated by glycine. Glycine is an important inhibitory transmitter to motoneurons and interneurons in the spinal cord and strychnine acts as a selective, competitive antagonist to block the inhibitory effects of glycine at all glycine receptors. Strychnine-sensitive postsynaptic inhibition in higher centers of the CNS is also mediated by glycine. Chances are there, that drug may act via blocking the inhibitory effect of glycine.

The drug shows no significant protection against maximum electric shock induced convulsions at both tested doses. Protection against electric shock induced seizures in rats is used as an indication for compounds which may prove effective in grand mal epilepsy. In this tonic hind limb extensions are evoked by electric stimuli. A substance is known to possess anticonvulsant activity if it reduces or abolishes the extensor phase of Maximum electric shock induced convulsions [18] In Cilostazol, there was no significant reduction in the extensor phase, so the drug may not be effective against grandma epilepsy.

CONCLUSION

In the present work emphasis was laid on preliminary neurobehavioural study of Cilostazol and its anticonvulsant effects. The findings from the study suggest that the Cilostazol may be neurosedative and might involve an interaction with glycinergic and glutaminergic system to exert its anticonvulsant effects. However, future research has to be undertaken to address the characteristic mechanism for its neuroprotective effect using other animal models, different dose level and duration.

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