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Research Article

ANTI-CONVULSANT PROPERTY OF SYNTHESIZED COMPOUND DIHYDROPYRIMIDINONE-5 IN LABORATORY ANIMALS

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ABSTRACT

Objective: The present study was undertaken to investigate the anti-convulsant effects of dihydropyrimidinone-5 (DHPM-5) in animal models.

Materials and Methods: Swiss albino mice were used for the study. The test drug and standard drugs were administered once daily for a period of 14 days. Maximal electroshock (MES) induced convulsion and strychnine induced convulsion models were used for the study. Phenytoin (PHT) and Diazepam were used as standard agents throughout the study.

Results and Discussion: Anti-convulsant studies with test drug showed a significant protection in MES induced convulsion models in a dose-dependent manner. There was a significant ($p<0.05$) decrease in the duration of tonic hind limb extension at both the doses of test drug (200 mg/kg) in MES model. Compared with the control group, treatment with test drug had no significant effect on onset and duration of convulsions in the strychnine induced seizure model. As expected, the animals treated with diazepam 4 mg/kg increased onset, duration of convulsions and latency to death as compared with control group. In all groups, all animals had seizures and all died. DHPM-5 has an influence on the excitatory and inhibitory neurotransmission, of special interest being the increase in the gamma amino butyric acid levels.

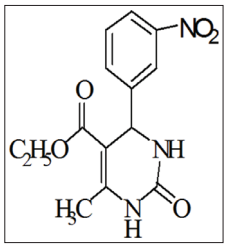
Conclusion: The presence of the chemical constituents gave strength to its anti-convulsant action. However, further research is warranted to determine the specific mode of its anti-convulsant activity.

Keywords: Dihydropyrimidinone-5, Strychnine, Seizure, Neurotoxicity.

INTRODUCTION

Epilepsy is defined as a chronic disorder of the brain that is characterized by spontaneous and recurrent seizure activity, which is triggered by the abnormal discharge of neurons [1]. Worldwide, this disease directly affects more than 50 million people and is roughly in the range of 5-10/1000 people and 100-190/100,000 people in industrialized and developing countries respectively [2]. Although several anti-convulsant drugs are used to treat seizure attacks, about 30% of patients are medicated incompletely. Furthermore, current anti-epileptic drugs have toxicity and teratogenic effects [3]. This consideration implicates search for the new anti-epileptic agents having lesser side-effects and quick onset of action.

Dihydropyrimidinones (DHPMs) are heterocyclic compounds which have gained a great importance in organic and medicinal chemistry as they have displayed a fascinating array of pharmacological properties ever since they were reported. Until now they have been explored for a plethora of biological activities *viz*. calcium channel modulator, adrenergic receptor antagonist, anticancer, antiviral, etc. and have been proved as promising [4].



From the study of clinically established drugs like phenobarbitone, it has been concluded that the structural requirements for a compound to possess anti-convulsant activity are presence of at least one aryl ring, one electron donor atom and a second donor atom in close proximity to the N-H group, forming a hydrogen bond acceptor or donor. Since DHPMs possess structural similarity to phenobarbitone, and also confirms to the structural requirements for anti-convulsant activity to some extent it was hypothesized that these compounds may possess anti-convulsant activity. This hypothesis was further supported by results of *in vitro* anti-convulsant activities of these compounds in which they have been proved to be promising. Hence, this dissertation work presents a detailed study report on synthesis, characterization and anti-convulsant activities of DHPMs [5].

METHODS

Collection of test drug (DHPM-5)

The drug DHPM-5 provided by Department of Pharmaceutical Chemistry, School of Pharmacy, BBDU, Lucknow, Uttar Pradesh, India.

Animals

Swiss albino mice (weighing between 18 and 25 g) obtained from the animal house of Babu Banarasi Das National Institute of Technology and Management, Lucknow were used in the study. They were maintained at a temperature of 22°C±5°C, relative humidity 55°C±5°C with free access to food and water ad libitum, under 12:12 light/dark cycle (light on at 8:00 hrs). All manipulations were carried out only once between 9:00 and 15:00 hrs with each animal used. The experimental protocol was approved by the Institutional Animal Ethics Committee as per the direction of the Committee for the Purpose of Control and Supervision of Experimental on Animals (UIP/CPCSEA/J-2013/05). The animals were acclimatized for a period of 7 days before the study. All efforts were made to reduce the number of animals used and treated humanely to minimize their pain and discomfort.

Drugs and chemicals

PHT (Orgamine Chemicals Pvt. Ltd., Thane), Diazepam (ALPA Laboratories Limited, Pigdamber), Strychnine (Sigma-Aldrich, USA)

were used as standard drugs. All other chemicals and reagents used for the study were of analytical grade.

Acute toxicity study

Mice were kept on overnight fasting and water was withdrawn 3-4 before administration of test compound. DHPM-5 was administered orally in increasing doses of 100, 500, 1000, 2000, and 4000 mg/kg body weight. Immediately after dosing, the mice were observed continuously for 4 hrs for symptoms of toxicity such as motor activity, tremors, convulsions, tonic extension, muscle spasm, loss of frighting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhea, salivation, and writhing. Mice were then kept under observation up to 72 hrs for any mortality. Locomotor activity was monitored using actophotometer (IMCORP, India), animals were individually placed in activity meter after 60 minutes of treatment, and total activity count was registered for 5 minutes. The locomotor activity was expressed in terms of total photobeam interruption counts/5 minutes [6].

Neurotoxicological studies

Neurotoxicity was determined using rotarod test. Mice, which were able to remain on the rotating rod, with a speed of 10 rpm for 5 minutes or more were selected and divided into two groups (n=6). The experimental group received dose 200 mg/kg (p.o.). One group received only vehicle and served as control. All animals were placed on the rotarod after 60 minutes of the treatment and average retention time on the rod was calculated. Neurotoxicity was assessed as the inability of the animal to maintain equilibrium on the rotating rod for at least 3 minutes [7,8].

Maximal electroshock (MES) induced convulsion

The anti-convulsant property of the drug in this model was assessed by its ability to protect against MES induced convulsions. The animals were first weighed and were selected for the experiment depending on the weight. Mice of either sex were used. The mice were then divided into three groups of six mice each. Group 1 received vehicle; Group 2 received 25 mg/kg b.wt. of PHT 60 minutes prior to the convulsion induction. Group 3 received 200 mg/kg b.wt. of test drug (DHPM-5), 60 minutes before to the convulsion induction. The MES (Inco Electroconvulsiometer model # 100-3) of 50 mA current for 0.2 seconds was administered through corneal electrodes to induce convulsions in the control and drug-treated animals. All the animals were treated for 14 days [9,10]. The severity of convulsions was assessed by the duration of tonic flexion, tonic extensor, clonus, stupor, and recovery phase for each animal. The ability of the drugs to prevent or delay the onset of hind limb extension was taken as an indication of anti-convulsant activity.

Strychnine induced seizure

The anti-convulsant property of the drug in this model was assessed by its ability to protect against strychnine induced convulsions. The method used was as described by Bum *et al*., 2008 [11]. The animals were first weighed and were selected for the experiment depending on the weight. Mice of either sex were used. The mice were then divided into three groups of six mice each.

Group 1 received vehicle; Group 2 received 4 mg/kg b.wt. of standard 1 (diazepam); Group 3 received 200 mg/kg b.wt. of test (DHPM-5); 60 minutes prior to the strychnine (2.5 mg/kg b.wt.) administration. Strychnine produced powerful tonic convulsions of the body and limbs. All the animals were treated for 14 days. The latency of convulsions and the % mortality was assessed for each animal for 30 minutes [12].

Statistical analysis

The different results are expressed as a mean ± standard error of the mean. The comparisons between the averages of the series of values were performed using ANOVA test, followed by a post-Tukey test.

RESULTS

Acute toxicity

In acute toxicity study, DHPM-5 did not show any mortality in mice. Even at this higher dose, i.e., 4000 mg/kg, there were no gross behavioral changes were observed, and 200 mg/kg dose were used for evaluation of scopolamine-induced memory impairment activity.

Neurotoxicological studies

In the rotarod test, the vehicle-treated mice did not demonstrate any signs of impaired motor co-ordination. Each control mouse was capable of performing the test, i.e., the mean time spent on the rotarod apparatus was 180 seconds. Similarly, DHPM-5 did not impair motor coordination OF Mice in the rotarod test at any dose. Thus, the test drug was found to have no neurotoxic effects (Table 1).

MES induced model

According to the results shown in Table 2, PHT (25 mg/kg b.wt.) and DHPM-5 ($p<0.001$) shows complete abolition of extensor phase in acute and chronic studies of MES induced convulsions.

Strychnine induced seizure

The results as shown in Table 3, diazepam and DHPM-5 significantly prolong the onset of a jerk when compared with control in both acute and chronic studies. DHPM-5 showed a significant reduction in the duration of the jerk when compared to the control in acute ($p<0.001$) and chronic ($p<0.01$) studies. The onset of seizure also prolonged by the DHPM-5 in acute and chronic studies significantly ($p<0.05$).

DISCUSSION

The present study was designed to investigate the anti-convulsant activity of DHPM-5 in MES and strychnine induced convulsions. The results of the study showed that DHPM-5 possessed significant anti-convulsant effect against MES induced convulsions, but had a mild effect on strychnine induced convulsions.

There are a number of synthetic anti-convulsant drugs currently available for use in the management, control and/or treatment of individuals with epilepsy. The mechanisms of action of anti-seizure (anti-convulsant) drugs have been broadly divided into three major categories [13]. According to McNamara [14], anti-convulsant drugs that are effective against the most common forms of epileptic seizures, namely partial and secondarily generalized tonic-clonic seizures, appear to act either by (i) reducing or limiting the sustained repetitive firing of neurons, an effect mediated by promoting and/or prolonging the inactivated state of voltage-activated Na+-channels, thereby reducing the ability of neurons to fire at high frequencies, or (ii) enhancing and facilitating gamma amino butyric acid (GABA)-mediated synaptic transmission and inhibition, an effect mediated either by a pre- or post-synaptic action. In the presence of GABA, the GABA-A (GABAA) receptor is opened, thus allowing an influx of Cl^- ions, which in turn increases membrane polarization. Some anti-seizure drugs also act by reducing the metabolism of GABA. Other anti-convulsants act at the GABAA receptors, enhancing Cl- ion influx in response to GABA, or by promoting GABA release [15]. Anti-convulsant drugs that are effective against absence seizure, a less common form of epileptic seizure, act by reducing or limiting the flow of Ca^{2+} through T-type voltage-activated Ca^{2+}-channels, thus reducing the pacemaker Ca^{2+} current that underlines the thalamic rhythm in spikes and waves seen in generalized absence seizures.

In the central nervous system (CNS), the disruption of the naturally existing balance between the concentrations of inhibitory and excitatory neurotransmitters is thought to be the main cause of

Table 1: Acute toxicity and neurotoxicity screening of DHPM-5

Treatments	Acute toxicity test	% mortality	Neurotoxicity screening
	Locomotor activity count/5 minutes		Retention time (seconds)
Vehicle	356±4.56	0	310.12±1.52
Test drug	344.20±4.11	0	302.21±1.26

DHPM-5: Dihydropyrimidinone-5

Table 2: Effect of DHPM-5 on MES induce model in mice

Serial number	Groups (n=6)	Extensor phase			
		Acute study	Chronic study	% Protection	
				Acute study	Chronic study
1	Control	8.88±0.27	33.93±0.77	0	0
2	Standard (PHT)	0.00***	0.00**	100	100
5	DHPM-5	0.92±0.12***	1.30±0.18***	100	100

MES: Maximal electroshock, DHPM-5: Dihydropyrimidinone-5, SEM: Standard error of the mean, PHT: Phenytoin. All value are given in mean±SEM, **p<0.01, ***p<0.001 as compare with the control group (one-way ANOVA followed by Tukey post-test)

Table 3: Effect of DHPM-5 on strychnine induce seizure in mice

Serial number	Groups	Onset of jerk		Duration of jerk		Onset of seizure	
		Acute	Chronic	Acute	Chronic	Acute	Chronic
1	Control	294.40±12.58	362±23.17	143.33±8.48	295.83±31.69	430.40±18.53	681±11.55
2	Standard (diazepam)	540±19.29**	754±29.48***	60.00±3.65**	101.67±14.47*	592±20.49**	884±33.78
5	DHPM-5	478±15.56*	642±19.31	49.17±6.11***	71.67±6.70**	529±20.06*	708±22.22

DHPM-5: Dihydropyrimidinone-5, SEM: Standard error of the mean. All value are given in mean±SEM, *p<0.05,**p<0.01, ***p<0.001 as compare with the control group (one-way ANOVA followed by Tukey post-test)

convulsive episodes. Electrical stimulation of certain areas of the brain results in a permanent lowering of after discharge (AD) threshold and the development of potentiality to trigger motor seizures in those areas. The lowering of AD threshold appears to be a local phenomenon, whereas the development of motor seizures involves changes that take place outside the stimulated structure [16,17]. Tissue damage or metallic ion deposits, for example, have been eliminated as a possible mechanism underlying the development of motor seizure by electrical stimulation [18]. Another possibility is that neuronal cells are being sensitized so that more and more cells near the electrode are being fired by stimulation as the treatment continues. The other hypothetical mechanism proposes an increase in strength of inter-limbic connections, so that a discharge in one structure more readily activates an independent discharge in several other structures with a consequent convergence into the "motor" structure. When enough secondary foci are triggered, the motor structure is driven which in turn drives the skeletal response [19].

The MES test is the most widely used animal model in the evaluation of anti-epileptic drugs the MES test identifies agents with activity against generalized tonic clonic-seizures using clinically established anti-epileptic drugs. MES causes several changes at the cellular level, which can disrupt the signal transduction in the neurons. One of the most important mechanism by which it causes cellular damage is facilitation of Ca^{2+} entry into the cells in large amounts and thus prolonging the duration of convulsions. Apart from Ca^{2+} ions, MES may also facilitate the entry of other positive ions like Na^{+}, blockade of which, can prevent the MES induced tonic convulsions [20]. MES induced seizures are abolished by the drugs that blocks voltage gated sodium channels like PHT and carbamazepine or by the drugs that block N-methyl-D-aspartate (NMDA) receptors like felbamate. Whereas the drugs that block T-type Ca^{2+} current in thalamus like sodium valproate [21]. PHT, a widely utilized anti-convulsant drug, predominantly exhibits a significant anti-convulsant activity in MES test and is utilized in the control of convulsive-seizures in epileptic patients.

Chemoconvulsant models for primary generalized seizures include by bicuculine (GABAA receptor antagonized), strychnine (glycine receptor antagonist) and aminophylline (adenosinereceptor antagonist). These substances block the physiological inhibitory action of glycine by a non-competitive action. This effect might explain their epileptogenic nature. Strychnine-induced seizures are different from those produced by primary GABA antagonists since they are mainly extensor tonic. These seizures are not fully relieved by acceptable doses of any of the classical anti-convulsants including benzodiazepines [22].

Strychnine, competitive antagonist of glycine receptors in the spinal cord. Although glycine is thought to act as an inhibitory neurotransmitter, a strychnine-insensitive glytine (Gly), receptor has been recently described in cultured mouse neurons that are thought to be allosterically linked to the excitatory amino acid NMDA receptor. The seizure potentiating effects of glycine are blocked by aminophosphonovaleric acid, an NMDA antagonist. In addition, in animals pre-treated with a subconvulsive dose of strychnine to block strychnine-sensitive glycine receptors (Gly), glycine enhances, rather than inhibits, NMDA-induced convulsions. Together, these results indicate that the seizure-potentiating [23] induced convulsions.

Almost all anti-epileptic drugs show the signs of sedation, hypo or (less often) hyper-location, ataxia, abnormal gait, reduced or inhibited righting reflexes and muscle relaxation in laboratory animals. These effects are commonly termed as neurotoxicity. In laboratory neurotoxicity can be determined using retard test. In a study used rotarod test to determine neurotoxic effect of DHPM-5. The drug showed no neurotoxicity as there was no sedation, normal gait, no change on righting reflexes, and all animal were able to maintain equilibrium on rotating the rod for more than 3 minutes.

DHPM was found to positive modulation GABA receptor binding. GABA is the major inhibitory neurotransmitter in the brain whereas glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmission and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy [24]. The GABAA receptor is a member of the ligand-gated ion channel superfamily, GABA being the major inhibitory transmitter in the CNS. Binding of GABA to the GABAA receptor activates a chloride ion flux through the channel, and ligands for the benzodiazepine binding site modulate the inhibitory effects of GABA. Such ligands of the benzodiazepine binding site are classified as positive allosteric modulators, antagonists, or negative allosteric modulators according to their spectrum of intrinsic efficacy toward the GABAA receptor. Positive allosteric modulators increase the frequency of chloride channel openings without altering channel conductance or duration of opening. Therapeutically, they are used as anxiolytics, anti-convulsants, sedative hypnotics, and muscle relaxants [25].

Alterations in GABAA receptor subunit expression and composition in epilepsy are well documented in human and in animal models; The changes in GABAA receptor subunit contribute to the changes in inhibitory function that might underlie epileptogenesis and occurrence of chronic recurrent seizures, Our study suggests that differential

expression of GABAA receptor subunits in the brain regions of epileptic animals plays an impotent role in the excitation/inhibition balance and the affinity to the GABA [5,26].

Although there are various reports of the DHPM-5 have been explored for a plethora of biological activities *viz*. calcium channel modulator, adrenergic receptor antagonist, anti-cancer, antiviral, etc., little effort has been extended to understand their pharmacological action on the glutamate receptors in hippocampus, the site most afflicted in epilepsy. Glutamate is the most important excitatory neurotransmitter significantly contributing to fast excitatory neurotransmission via an activation of inotropic glutamate receptors. Ligand-mediated activation of these receptors enhances cation fluxes into post-synaptic cells thereby resulting in depolarization of the post-synaptic membrane and enhanced neuronal excitability.

DHPM-5 has calcium channel modulator properties. L-type calcium channel shows the effect on NMDA receptor and they reduced the increase in glutamate dehydrogenase activity to near-controls levels. Hence, it is suggested that DHPM-5 has a definite role in decreasing glutamate excitotoxicity through L-type of calcium channel.

The present study demonstrates a dose-dependent increase in the seizure threshold (decrease in the extension/flexion ratio), suggesting that the drug (DHPM-5) might possess anti-convulsant property against the MES and strychnine model. Keeping this in view, the anti-convulsant efficacy of DHPM-5 was examined following daily administration for 14 days. The results of the chronic administration also clearly demonstrated that this drug increased the seizure threshold. However, 14 days treatment had no advantage over single dose treatment.

From our study clearly shows that DHPM-5 has an influence on the excitatory and inhibitory neurotransmission, of special interest being the increase in the GABA levels. Moreover, many investigators have suggested that the experimental protective index could express better the clinical utility between the relationship of undesired and the desired drug effect.

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