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EVALUATION OF ANTICONVULSANT EFFECT OF DIBENZO-α-PYRONE DERIVATIVES IN MICE

Sawhney Kapoor Shalini [a*], Agarwal Bharal Nidhi [b], Kumar Yatendra [a], Shrivastava Birendra [c], Sharma Pankaj [c]

[a]ITS paramedical (Pharmacy) College, Muradnagar, Ghaziabad-201206 ,U.P. India
[b]Department of Clinical Research, Faculty of Allied Health Sciences, Jamia Hamdard (Hamdard University), New Delhi-110062, India
[c]School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India

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Corresponding author:
Shalini Sawhney Kapoor,
ITS paramedical (Pharmacy) College, Muradnagar, Ghaziabad-201206 ,U.P. India

ABSTRACT

Aim: The present study was designed to explore the effect of synthesized derivatives of Dibenzo-α-pyrone an active ingredient of Shilajit on electroshock, pentylenetetrazole (PTZ)-induced seizures.

Materials and Methods: Butylamine, Diethylamine and Pyrrolidine derivatives of Dibenzo-α-pyrone were administered intraperitoneally (i.p.) at doses 10, 20 and 40mg/Kg(single dose) to observe its effect on the increasing current electroshock seizure(ICES) test and PTZ-induced seizure test. In addition, a chronic study (21 days) was also performed to assess the long term effects on electroshock and PTZ- induced convulsions. Phenytoin (25mg/kg, i.p.) was used as a standard anticonvulsant drug.

Results: The results were compared with an acute study, wherein it was found that the administration significantly raised the seizure-threshold current as compared to control group in the ICES test. Similar results were observed after chronic administration. In PTZ test, all the 3 tested doses failed to show protective against PTZ –induced seizure test.

Conclusion: These results suggested that derivatives of Dibenzo-α-pyrone appear to possess anticonvulsant activity in mice.

INTRODUCTION

Several experimental studies have reported neuroprotective and antioxidant activity of certain natural products like Dibenzo-α-pyrone(DBP). DBP have been found to be the active constituent of a multifaceted herbo-mineral drug called SHILAJIT. Shilajit having many biological activities has always provoked the researchers to inquire about its active constituents. Over sixty years of clinical research have shown that Shilajit has positive effects on humans. It increases longevity, improves memory and cognitive ability, reduces allergies and respiratory problems, nervous disorders, reduces stress and relieves

digestive troubles. It is anti-inflammatory, antioxidant, antidiabetic, antiepileptic, antimicrobial, aphrodisiac and eliminates free radicals. The research also proves that Shilajit increases immunity, strength, and endurance. It improves the quality and quantity of life and it seemed to cure all diseases. ***[1-3]***. The present study was designed to explore the effect of synthesized derivatives of Dibenzo-α-pyrones on electroshock, pentylenetetrazole (PTZ)-induced seizures. For the synthesis of the oxygenated DBP moiety, the mostly adopted method has been developed by Hurtley W.R.H et al (1870, 1929). The Hurtley condensation involved copper catalyzed condensation of 2-halobenzoic acid with various β-dicarbonyls (1, 3 - diketone) in water, alcohol/ β-carbonyl itself in the presence of a strong base.***[4]*** The mannich bases were synthesized using various primary and secondary amines.***[5-7]***

Epilepsy is one of the most common serious disorders of the brain. It is a neurological condition characterized by recurrent seizures occurring due to abnormal electrical activity in the brain. Various conventional antiepileptic drugs (AEDs) are available to treat epileptic patients. However despite the availability of these AEDs in about 20-30% patients, the seizures are not adequately controlled by these established drugs.*[8,9]*The epileptic discharges and the subsequent epileptic seizures are associated with an imbalance between the excitatory and inhibitory neurotransmitters in specific brain areas. Based on this hypothesis, various agonists or antagonists of specific receptor subtypes are found to interfere with the occurrence of epileptic event in different experimental studies.*[10-12]*

In the present study, we investigated the effects of acute administration (single dose) of Butylamine, Diethylamine and Pyrrolidine derivatives of Dibenzo-α-pyrones on electrically and pentylenetetrazole (PTZ) induced convulsions in mice. In addition, a chronic study was also performed to assess the long-term effects of drug administration on electroshock and PTZ induced convulsions in mice.

MATERIALS AND METHODS

ANIMALS

Healthy Swiss albino mice of either sex weighing 24-30 g (n=6/group) were used in the study. Animals were housed in groups of six mice per cage (43x28.6x15.5cm) with a natural light/dark cycle and provided with free access to pellet diet and water. Procedures adopted during experiments on animals and their care were conducted in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals, India, and the study was approved by Institutional Animal Ethics Committee.

DRUGS AND DOSING SCHEDULES

Butylamine, Diethylamine and Pyrrolidine Derivatives of Dibenzo-α-pyrone were used in the present study. They were administered intraperitoneally (i.p.)once (acute study) and for 21days (chronic study) at doses of 10,20 and40mg/Kg. Phenytoin was used as a standard anticonvulsant drug and injected at a dose of 25mg/kg i.p. Control animals received normal saline.*[13-18]*

METHODOLOGY

INCREASING CURRENT ELECTROSHOCK SEIZURE TEST

Increasing current electroshock seizure (ICES) test.*[19-20]* was used to determine seizure-threshold current(STC) for each animal. Starting with a current of 2mA, electroshock was delivered to each mouse via ear electrodes as a single train of pulses (20 Hz for 0.2s) with linearly increasing of 2mA/2s using an electro-convulsiometer (Techno, India). The current at which tonic hind limb extension (HLE) occurred was recorded as the STC. If no tonic HLE was observed up to a current of 30mA, electroshock was terminated and this cut off current was used in the analysis.

PTZ INDUCED SEIZURE TEST

PTZ was administered i.p. at a dose of 45mg/kg. Immediately after PTZ administration, mice were observed for latency of first myoclonic jerk and generalized clonic seizures. The observation period was limited to 30min.*[21]*

STATISTICAL ANALYSIS

The results were expressed as mean ± standard error of mean. Statistical analysis of the data was performed using one –way analysis of variance followed by post hoc Tukeys test. The p values less than 0.05 were considered significance.

RESULTS AND DISCUSSIONS

ACUTE STUDY

Effect on ICES test in mice-In single dose administration, dose of 10, 20 and 40mg /Kg significantly raised the STC as compared to the control group(p<0.001;Figure 1, 2and 3).

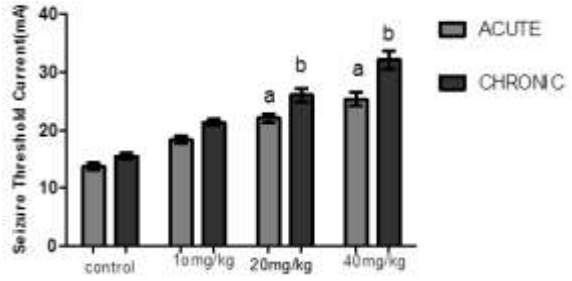

Figure1.The effect of acute and chronic administration of Butylamine derivative of Dibenzo-α-pyrone on increasing current electroshock seizure (ICES) test in mice. Values are expressed as mean ± SEM (analysis of variance (ANOVA) followed by Tukeys test). a p<0.001versus control (acute); b p<0.001versus control (chronic).

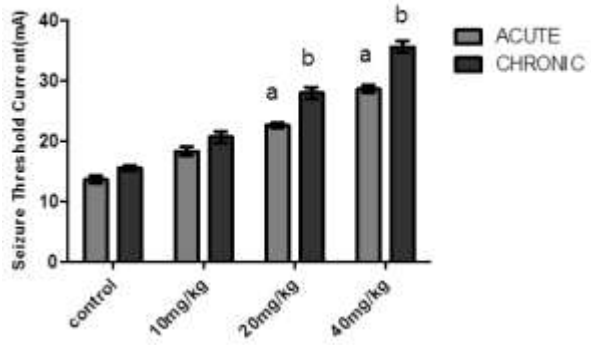

Figure2.The effect of acute and chronic administration of Diethylamine derivative of Dibenzo-α-pyroneon increasing current electroshock seizure (ICES) test in mice. Values are expressed as mean ± SEM (analysis of variance (ANOVA) followed by Tukeys test). a p<0.001 versus control (acute); b p<0.001versus control (chronic).

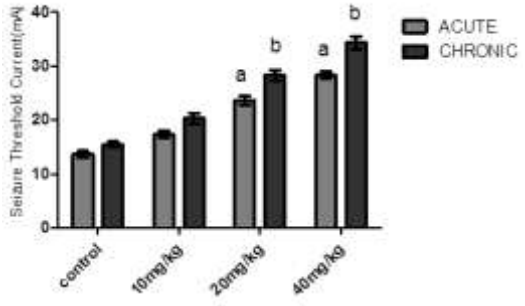

Figure3.The effect of acute and chronic administration of Pyrrolidine Derivative of Dibenzo-α-pyrone on increasing current electroshock seizure (ICES) test in mice. Values are expressed as mean ± SEM analysis of variance (ANOVA) followed by Tukeys test). a p<0.001versus control (acute); b p <0.001versus control (chronic).

Effect on PTZ-induced seizure test in mice-Single dose administration at all doses did not

show protective effect against PTZ-induced seizures in mice (Figure 4, 5and 6)

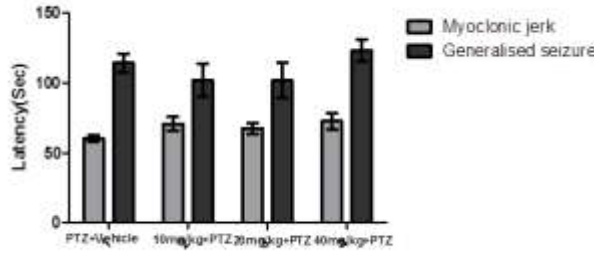

Figure 4.The effect of Butylamine derivative of Dibenzo-α-pyrone on pentylenetetrazole (PTZ)-induced seizure in mice. Values are expressed as mean ± SEM

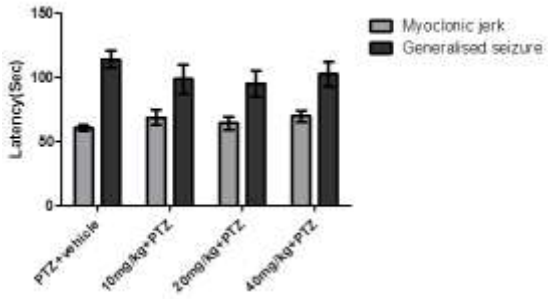

Figure 5 The effect of Diethylamine derivative of Dibenzo-α-pyrone on pentylenetetrazole (PTZ)-induced seizure in mice. Values are expressed as mean ± SEM

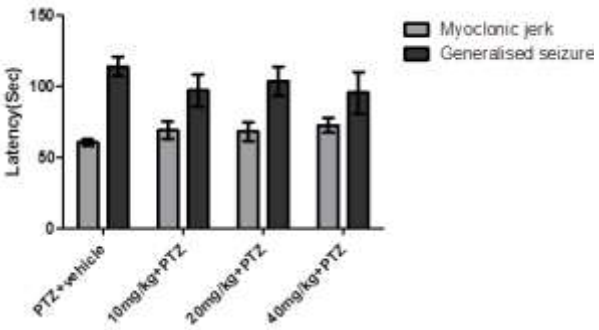

Figure 6.The effect of Pyrrolidine Derivative of Dibenzo-α-pyrone on pentylenetetrazole (PTZ)-induced seizure in mice. Values are expressed as mean ± SEM

CHRONIC STUDY

Effect on ICES test in mice-After chronic administration of derivatives for 21days, the changes in STC were similar to the results of acute study.

DISCUSSION

In the present study, we have examined the effect of synthesized Butylamine, Diethylamine and Pyrrolidine derivatives of Dibenzo-α-pyrones on ICES test in mice., which is a simple and sensitive test to identify the pro-and anticonvulsant nature of the test drug. Epilepsy is a heterogenous group of disorder caused by abnormal ion transport across cell membrane and/or an imbalance between excitatory (glutamic acid) and inhibitory (GABA) neurotransmitter systems. The results indicate that the drug in 20 and 40mg/kg was more effective in blocking the electroshock induced convulsions as significant increase in STC in the drug treated groups was observed as compared to the control group. The results were compared with the standard AED, phenytoin. The protective action offered in electroshock test might be due to alteration in the influx of cations Na+, Ca+2 or K+, leading to the inhibition of neuronal depolarization.***[22]***

In PTZ-induced seizures test, the derivatives in all the three doses did not inhibit clonic seizures caused by the administration of PTZ. Neurochemical evidences suggested that PTZ blocks GABA-mediated inhibitory effect. Activation of N-methyl-D-aspartate receptors and/or inhibition of GABA were the main factors involved in the initiation and propagation of PTZ-induced seizures. 5-HT3 receptor being a ligand gated ion channel also modulate the activity of other neurotransmitter such as norepinephrine, GABA, glysine, dopamine and acetylcholine.***[23-26]*** In the present study we tested the synthesised derivatives of Dibenzo-α-pyrones in low doses as it might be possible that in these doses they did not cause CNS inhibition lead to convulsions and thus unable to show pro or anticonvulsant effect in PTZ test.

Review of literature hints at several putative mechanisms of anticonvulsant activity. Oxidative stress has already been demonstrated to play a role in epileptogenesis and free radicals such as oxygen, superoxide and and nitrite are generated during epileptogenesis. ***[27,28]*** Secondly, the role of Brain Derived Neurotropic Factor(BDNF) in epileptogenesis has come to the forefront in recent times. It exerts a modulatory effect on neuronal excitability in hippocampus. BDNF administration has already been demonstrated to protect against hippocampal kindling with possible carry-over effect. ***[29-31]*** However, further studies are required to look at the exact mechanisms involved in the observed effect as complex changes in neuronal excitability may occur at low doses of derivatives.

CONCLUSION

The present data support that synthesized derivatives of Dibenzo-α-pyrones indicate anticonvulsant effect against electrically induced seizures and is devoid of effect on PTZ-induced seizures in mice. These findings suggested that these derivatives could be promising in the treatment of seizure disorders like epilepsy.

However, there is also scope for further experimental, biochemical and clinical studies to ascertain their anticonvulsant potential.

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