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Preliminary evaluation of melatonin in the kindling model of epilepsy

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ABSTRACT

During the past decades, epilepsy syndrome has been depicted across India as well as worldwide and this leads to increasing mortality and morbidity rate. Researchers are trying to investigate the responsible causes and risk factors for seizure occurrence. Epilepsy is a chronic disorder which is derived from a Latin word 'sacire' meaning 'convulsive attack' and is expressed as a paroxysmal experience appointed to atypical, unnecessary or concurrent neuronal bustle in the brain. The treatment of epilepsy involves the use of anti-epileptic drugs i.e. Sodium valproate, phenytoin, carbamazepine. Despite being treated with the available anti-convulsant drugs, this disease is still prevalent worldwide. So, as an adjuvant treatment melatonin exhibit an anti-epileptic activity in several animal models of epilepsy. However, its anti-epileptic potential has yet to be evaluated in Pentylenetetrazole (PTZ) induced model of epilepsy through kindling phenomenon. Rats were injected with a dose of (35-55 mg/kg) of pentylenetetrazole (PTZ) up to twenty days in alternate days. Observed the convulsive behavior of rats for thirty minutes immediately after PTZ injection. The entire treatment schedule includes the administration of melatonin (75 mg/kg) one hour prior to the PTZ administration. Sodium valproate was used as standard drug for this kindling model of epilepsy.

Keywords: Pentylenetetrazole, Melatonin, Sodium valproate, Gamma-amino butyric acid

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1. INTRODUCTION

Epilepsy is considered as one of the most prevailing pandemic of the twenty-first century[1] (Ben et al. 2009). According to the World Health Organization report, around fifty million people worldwide are suffering from epilepsy syndrome[2,3]. It has been suggested that the epilepsy leads to several complications[4] including central nervous system disorders[5,6] result from the complex interaction between epileptogenic, precipitating and endogenous factors.

Various clinical and animal studies revealed that epilepsy can cause multidimensional cognitive impairment. The treatment of epilepsy involves the use of anti-epileptic drugs i.e. Sodium valproate, phenytoin, carbamazepine[7]. It has been documented that sodium valproate can ameliorate γ-amino butyric acid level in epileptic animals[8]. However, along with their efficacy and potency, several adverse effects of sodium valproate have also been reported including abdominal cramps, abnormal liver function, and tremor[9,10]. Hence, it is imperative to discover novel strategies in the management of epilepsy syndrome.

Melatonin is considered as hormone of darkness as well as human time keeping machine. It accomplishes clock and calendar functions to make the human body alter to diurnal variation which impact the 24-h rhythms in physiology and behaviors of human body[11,12]. Lerner and his colleagues worked on melatonin by isolating and chemically characterizing them from mammalian pinealocytes in 1958[13]. Melatonin (also called pleiotropic hormone) exhibit chronobiotic effects linked with hormonal secretion, sleep-wake cycle, thermoregulation and other physiological events[14-16]. Melatonin have also been observed to maintain the immunity, energy metabolism, feeding behavior, free radical scavenging, cancer proliferation, maintenance of vasculature, inflammation, reproduction growth and development[17].

Melatonin also exhibits anti-epileptic activity against experimentally induced epilepsy in several animal models. Moreover, it has been reported that melatonin can protect neuronal damage in the brain of chemical-induced epileptic animals.

Several epidemiological studies have postulated that neuronal hyperexcitability is the main reason in the pathogenesis of epileptic seizure. Underlying molecular mechanism of rapid neuronal firing, it depends on three steps which include decreased inhibitory neurotransmission,

increased excitatory neurotransmission, and variation in sodium or potassium ion channels[18-19] Glutamate[20-22], GABA, Dopamine, Serotonin, Acetylcholine are some major neurotransmitters found in central nervous system[23] (Nicoll et al. 1990). Among them, GABA and glutamate (inhibitory and excitatory neurotransmitter respectively) are the important neurotransmitters for the mechanism of epilepsy[24,25]. Experimental result suggests that melatonin increases the seizure threshold by enhancing the GABA level in the hippocampus region of the central nervous system. Inhibitory neurotransmitter (GABA) precedes their effects through $GABA_A$ and $GABA_B$ receptors, which are found postsynaptically and presynaptically respectively[26-28]. Melatonin exerts their effect by opening the chloride channels through the activation of GABA-A receptors which leads to inhibition of action potential and thus effective in epilepsy syndrome[29-31].

2. MATERIAL AND METHODS

2.1. Animals

Male Wistar albino rats (180 ± 20 g) were obtained from the central animal house, Institute of Pharmaceutical Research, GLA University, Mathura and were used in this study. The animals were grouped and housed in poly-acrylic cages lined with husk under standard conditions (24 ± 2 °C temperature, 45–55 % relative humidity and 12 h light: 12 h dark cycle). Animals were fed standard pellets diet and had free access to water. The experimental protocol was carried out in accordance with the approval of the Institutional Animal Ethics Committee (1260/Po/ac/09/CPCSEA/IAEC/2018/ P.Col./R01) under strict compliance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for experimental studies (National Research Council US Committee for the update of the guide for the care and use of laboratory animals 2011)[32].

2.2. Chemicals

Melatonin was purchased from Central Drug House, India. Curcumin (Yarrow chemicals), Sodium valproate (Yarrow chemicals), Pentylenetetrazole (Star chemicals) and GABA (yarrow chemicals). All other chemicals and reagents were available commercially from local suppliers and were of analytical grade.

2.3. Animal Kindling Model of Pentylenetetrazole induced epilepsy

Rats were injected intra-peritoneally with sub convulsive doses of Pentylenetetrazole at 48 h intervals during 20 days (From 35 mg/kg to 55 mg/kg) [additional 5mg after each 2 injections][33].

2.4. Experimental Design

The acclimatized animals were divided into three groups of six rats in each. Each groups namely control, Test drug and standard. All the animals received Pentyleneterazole (PTZ) (except control) in an alternative manner for up to 20 days. Assessment of convulsion behavior in rats immediately after the PTZ injection for 30 minutes and scored according to modified Racine's scale[34].

2.5. Assessment of Behavioral parameters

According to researchers, seizure scores are evaluated under the following headings:

Table 1: Seizure scores in accordance with modified Racine's scale

Behavioral Responses	Scores
No seizure	0
Sudden immobilization	1
Ear and facial twitching	2
Facial, vibrissal and forelimb clonus/or wet dog shake behavior	3
Sporadic forelimb clonus with kangaroo posture	4
Clonic and/or tonic seizures with posture maintenance	5
Generalized tonic-clonic seizures with loss of posture tone, and turning onto the side and seizures characterized by rearing and falling	6
Death within half an hour	7

PTZ administration was withdrawn after establishment of kindling status and subsided by saline injections until the end of the protocol. After the induction of epilepsy, the treatment schedule involves the administration of test drug (melatonin 50 mg/kg) and standard drug (sodium valproate, 200 mg/kg) through oral route one hour prior to the administration of Pentylenetetrazole.

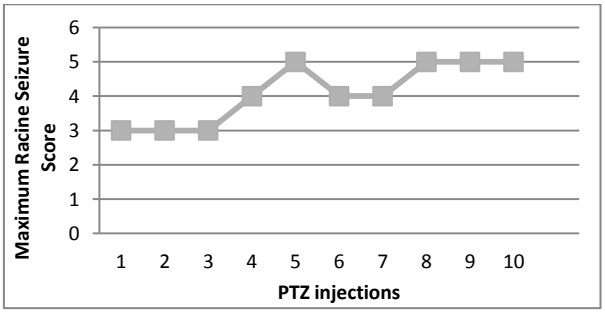

Figure 1: Graphical representation of induction of seizure during kindling progression according to Racine's seizure scale

2.6. Data Analysis

All the data were mean ± standard error of the mean (SEM). All statistical analyses of data were done using one-way analysis of variance (ANOVA) with Dunnett multiple comparison test. P< 0.05 was considered as *significant; P< 0.01 was considered as **more significant.

3. RESULTS

Table depicts the effect of melatonin (75 mg/kg) on PTZ induced changes in the level of γ-amino butyric acid (GABA). Stastical analysis revealed that melatonin treatment significantly attenuated PTZ induced decrease in the levels of GABA in wistar albino rats. However, the anti-epileptic activity of melatonin (75 mg/kg) was significantly lower compared to standard drug sodium valproate in epileptic rats Table-2.

Table 2: Drug concentration

Groups	Concentration
Control	76 ± 2.531
Test drug (Melatonin)	112 ± 2.312**
Standard drug (Sodium Valproate)	120 ± 2.112**

All values are mean ± SEM (n = 6)

(One way ANOVA followed by Dunnett multiple comparison test). ** More Significant difference at P< 0.01 vs. Control group.

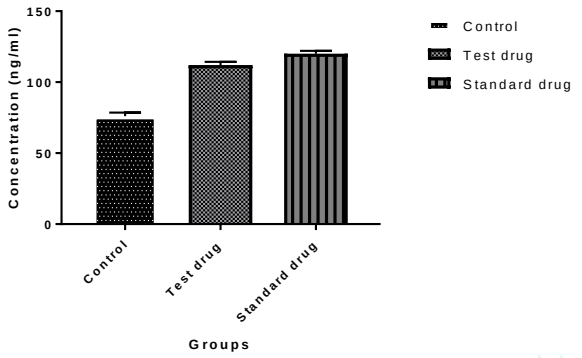

Figure 2: Effect of melatonin (75.0 mg/kg) on PTZ induced changes in to convulsion behavior of rats. All values are mean ± SEM (n = 6). ** More Significant difference at P< 0.01 vs. Control group (one-way ANOVA followed by Dunnett multiple comparison test)

4. DISCUSSION

The present study demonstrated for the first time the fact that melatonin (75 mg/kg) exhibit anti-epileptic activity in Pentyleneterazole (PTZ) induced epileptic rats. Wistar albino rats of either sex were employed in the present study because of their small size, low cost and easily availability. Hence, melatonin can perhaps be considered as an adjuvant to sodium valproate in the management of epilepsy syndrome during PTZ-induced seizure.

The relation between ion channels and neurotransmitters with epilepsy was proved in many epidemiological studies (Babb TL et al. 1987). Currently, it is known that reduction of GABA level, increase in glutamate level, and alteration in sodium or potassium ion channels are observed in the population affected from epilepsy syndrome. In our study, chemical kindling model of epilepsy was conducted. Kindling process refers to a process in which there is a repeated administration of a subconvulsant stimulus, electrical or chemical produces a gradually increasing electroencephalographic and behavioral response which culminates in a behavioral seizure. Administration of pentylenetetrazole used as an inducing agent at a dose of 25-55 mg/kg, intraperitoneal in an alternate manner for 20 days produced a seizure.

In the present study, Melatonin (an indoleamine derivative of serotonin) at a dose of 75 mg/kg, i.p. was noted to reduce hyperexcitability of neurons and thus suppress seizure by increasing the level of inhibitory neurotransmitter (GABA). Sodium valproate was used as a standard to compare the test drugs such as melatonin.

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