ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of The Anti-Convulsant Effect of Melatonin Hormone in Comparison to Gabapentin in Pentylentetrazol - Induced Epileptogenesis In Rats.

Raghda N El-Sayed[1], Magda M Hagras[1], Reham E Masoud[2]*, and Amira S Mohammed[1]

[1]department Of Clinical Pharmacology, Suez Canal University, Egypt.
[2]department Of Clinical Pharmacology, Portsaid University, Egypt.

ABSTRACT

Gabapentin, which is an analogue of GABA, is effective in partial seizures. In spite of its close structural relationship to GABA, gabapentin appears not to act on GABA receptors. Melatonin levels decreased in uncontrolled epileptic patients, therefore melatonin could be a potential candidate for treatment of epilepsy. To evaluate the therapeutic effect of melatonin on seizure's score in comparison with a conventional antiepileptic drug , rats were injected with sub-convulsive doses of PTZ (30 mg/kg i.p.) three times a week, or the total of 13 injections then evaluation of seizures and motor coordination by different methods were done and after sacrifice , immune cy to chemical identification of GABA-ergic neurons was done to determine mechanism of action of melatonin. We concluded that melatonin is effective neuroprotective agent which enhance GABA neurotransmission.

Keywords: epilepsy, melatonin, GABA, Gabapentin

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Traditional epilepsy treatments have side effects as dizziness, fatigue, GIT upset and may experience drug interactions. In addition, some traditional seizure medications may become less effective over time. Gabapentin, which is an analogue of GABA, is effective in partial seizures. In spite of its close structural relationship to GABA, gabapentin appears not to act on GABA receptors. It may however, alter GABA metabolism, its non-synaptic release, or its reuptake by GABA transporters(1). Melatonin is a hormone secreted by the pineal gland, which is located in the epithalamus at the center of the brain. It is also available in a synthetic form as a supplement. As the melatonin levels decreased in uncontrolled epileptic patients, therefore melatonin could be a potential candidate for treatment of epilepsy (2). Some clinical data suggested that melatonin could have a neuroprotective effect through modulating the electrical activity of neurons by reducing glutamate and enhance GABA neurotransmission. It may enhance nitric oxide synthase expression in PTZ-seizure paradigm in rats and some people may use melatonin supplements to treat the symptoms of epilepsy, it is not proven to be an effective treatment (3). In addition, melatonin has an antioxidant effect that could scavenge the free radicals generated by epilepsy(4). Therefore, the present study was conducted to elucidate the therapeutic effect of melatonin on seizure's score in comparison with a conventional antiepileptic drug, and to find its mechanism of action related to preservation of GABA neurons.

METHODS

Animals:

Thirty-two male albino rats of body weight ranging from 170-200 g were included in the study. Rats were purchased from the Ophthalmic Research Institute animal house in Giza. Rats were kept under controlled laboratory setting, normal day/night cycle, temperature 23±2 ^{o}C and humidity ranging 45-55%. Rats were housed in polypropylene cages (8 per cage) with food and water given ad libitum. Rats were acclimatized for one week before the start of the study.

All experiments were performed at the time between 10:00 AM and 2:00 PM to decrease as much as possible the circadian effects of susceptibility to seizure.

Procedures involving animals were conducted according to ethical committee guidelines that comply with national and international laws and policies.

Drugs:

- PTZ: was obtained from (Sigma Aldrich Company, USA ®), as white powder and was dissolved in normal saline.
- Gabapentin: was obtained from Delta Pharm Company ®, Cairo, Egypt as white powder and was dissolved in normal saline.
- Melatonin: was obtained from (Sigma Aldrich Company, USA ®), as white powder and was dissolved in normal saline.

Study design:

It is an experimental study that was carried out in the Pharmacology department, Faculty of Medicine, Suez Canal University.

Rats were randomly assigned into four groups 8 rats each

Group (1): saline control group, rats were injected with 0.5 ml 0.9% saline intraperitoneally (i.p.) corresponding to PTZ injection.
Group (2): disease control group, subconvulsive doses of PTZ (30 mg/kg i.p.) was injected 3 times per week (day after day), for the total of 13 injections .(This dose produces clonic seizure without mortality as detected by pilot study).
Group (3): gabapentin treated group, rats were subjected to injection of gabapentin (200mg/kg) i.p. 60 minutes after each injection with PTZ.

Group (4): melatonin treated group, rats were injected with melatonin 40mg/kg) i.p. 60 minutes after each injection with PTZ .

PTZ induced kindling

Rats were injected with sub-convulsive doses of PTZ (30 mg/kg i.p.) three times a week, or the total of 13 injections (5)

After each injection of PTZ, the following were done:

Evaluation of the seizures:

After each injection of PTZ, the convulsive behavior was observed for 30 minutes and the resultant seizures were classified according to Racine rating scale (6). The average score was calculated for each group at the end of the experiment.

The rating scale for the evaluation of seizures

Symptoms Score

No seizure response 0
Immobility, eye closure, ear twitching, facial clonus 1
Head nodding associated with more severe facial clonus 2
Clonus of one forelimb 3
Bilateral forelimb clonus without rearing 3.5
Bilateral forelimb clonus with rearing 4
Falling on a side (without rearing), loss of righting reflex accompanied by generalized tonic clonic seizures 4.5
Rearing and falling on back accompanied by generalized tonic clonic seizures 5
N.B. loss of righting reflex is defined as the inability of the animal to right itself within 30 seconds from a supine position.

Assessment of motor coordination: To evaluate the motor coordination, the rotarod and open field tests was used.

Rotarod test:

It measures balance, coordination, and motor control. The rotarod apparatus consists of a suspended rod able to run at constant or at accelerating speed.

Each rat was placed on a rod (10 cm long and 4 cm in diameter). Rats were left for at least one min on the rod for habituation. The rod rotated 6 round per minute (rpm.) over the course of 5 min. normally the animals must continuously walk forward to avoid falling of the rotarod. After the injection of PTZ, the behavior of animals were evaluated again. Falling time (time starting from putting the animal on the shaft of the rotarod, till it falls) was measured. The latency to fall in this test represents a measure for motor coordination (7).

Open field test:

The open-field test is based on the conflict between exploration of a new environment and the aversion to open spaces from which escape is prevented by a surrounding wall.

Apparatus

Assessment took place after each PTZ injection in the open field arena with the measurements 115 X 115 X 44 cm. The arena is made of dark glass and its floor is painted with white lines that form a 5 X 5 cm pattern (8).

Procedure

Each rat was placed separately in the open field space and behavioral changes were recorded for 10 minutes as recommended by (Conceicao and Frussa-Filho 1993) (9)

Hand-operated counters and stopwatches were employed to score ambulation frequency (the number of squares crossed) and number of stops (10)

Ambulation frequency was recorded each time an animal crossed a single line from one grid square into an adjacent grid with all four paws (8).

Further, activity factor (A) was calculated according to the formula:

A = Ambulation frequency (No. of squares crossed by the animal)
No. of stops

Processing of the brains

After the 13th injection, rats in all groups were anesthetized with thiopental sodium (50 mg/kg) and killed by decapitation (11). Brains were dissected on ice and divided into two lobes. One lobe was quickly frozen before cryo-sectioning. From each animal five 20 µm-thick coronal sections 100 µm apart from each other were prepared from hippocampus on positively charged slides using a Leica cryostat (CM3000, Leica Instruments GmbH, Nussloch, Germany).The sections were thaw mounted onto Super Frost/Plus object glasses (Menzel-Gläzer, Braunschweig,Germany) and stored at-20°C prior to use to perform immunohistochemistry assay for GABA-antibodies (12).

Immuno his to chemistry:

For immune cyto chemical identification of GABA-ergic neurons, slices were fixed in 4% paraformaldehyde for 24 h and then washed extensively with 0.1 ml phosphate buffer saline (PBS). Slices were incubated initially with PBS containing 5% normal goat serum and 1% Triton X-100 for 2 h followed by incubation with rabbit anti-GABA antibody (sigma, CA) at a dilution of 1:500 for 48 h at 4°C. Each incubation was followed by thorough washing with PBS (12)

Primary antibodies to S100 (rabbit polyclonal, diluted 1:800, labvision) was applied and incubated for 60 min at room temperature according to the manufacturer's protocol. DAB was used as a chromogen substrate and it was incubated for 1-3 minutes at room temperature. Mayer's Hematoxylin is used as a counterstain. Then slides were examined by light microscope (12).

Statistical analysis: We used statistical package for social sciences (SPSS) program (windows version number 16). Data derived from three groups or more was evaluated by One-way analysis of variance (ANOVA). Differences between groups were assessed by post hoc test. Other values were assessed by using chi square test. Data was considered statistically significant with a P value < 0.05

RESULTS

Disease control group that received subconvulsive doses of PTZ (30mg/kg i.p.) produced kindling starting from the seventh injection. As shown in figure (1), rats showed a significant increase in seizure score starting from the seventh injection ($p = 0.048$) and progressing with repeated PTZ injections until the 13th injection ($p = 0.00$).Gabapentin produced significant reduction in epilepsy score in comparison with disease control group that received (PTZ 30mg/kg) ($p < 0.05$) as shown in figure (1).

ISSN: 0975-8585

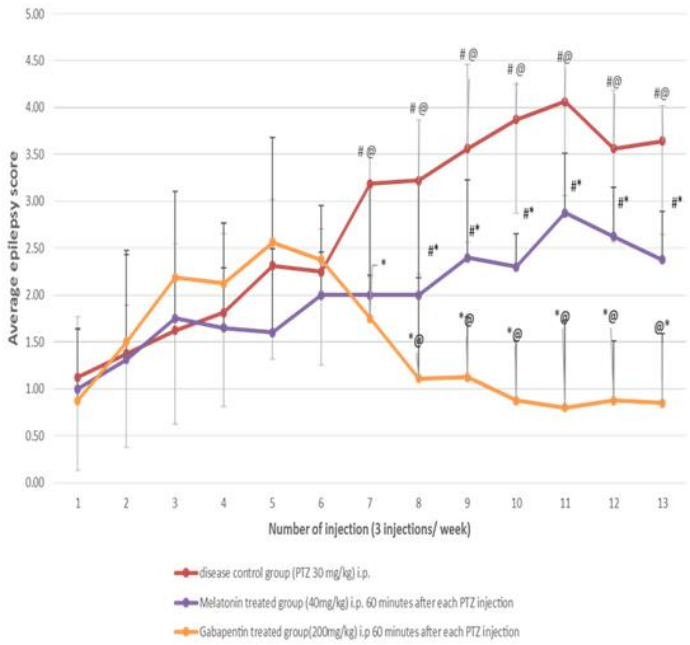

Fig (1): Mean seizure score exhibited by the rats after each injection of PTZ and the effect of treatment with gabapentin and Melatonin.

* Significantly different from PTZ control group at corresponding time at p≤ 0.05.
# Significantly different from gabapentin treated group at corresponding time at p≤ 0.05.
@ Significantly different from melatonin treated group at corresponding time at p≤ 0.05.

Melatonin (40mg/kg) i.p. which is given 60 minutes after each PTZ injection showed significant effect starting from the seventh injection and continued throughout the study, manifested as a significant decrease in epilepsy score in comparison with the corresponding disease control group that received PTZ (p = 0.012).

Additionally there was significant difference between melatonin treated group and Gabapentin treated group from the eighth injection (p = 0.034).

Behavioral assessment:

Rotarod test results

As shown in figure (2) there was a significant decrease in the latency time of falling from rotarod apparatus in the diseased- control group that received (PTZ 30mg/kg) in comparison to the control saline group starting from the fourth injection (p = 0.038) .

Melatonin-treated group did not show any significant effect on latency time compared to the PTZ-treated group in the first nine injections. However, a significant increase in the latency time starting from the 10th injection was evident in the melatonin-treated group compared to the PTZ-group (p =0.01) and progress throughout the study until the 13th injection as shown in figure (2).

By comparing the latency time of melatonin treated group and the control saline group, it was evident that the latency time of the melatonin group did not reach the normal control values, as was evident by the significant decrease in the latency time in melatonin treated group (40mg/kg) i.p. in comparison to saline group starting from the fourth injection (p = 0.04).

ISSN: 0975-8585

Treatment with gabapentin (200mg/kg), which enhanced motor coordination in the treated rats manifested as an significant increase in the latency period of falling from rotarod apparatus starting from the 5th injection as compared to PTZ- control group ($p = 0.027$).

Similarly, administration of gabapentin did not significantly reach the control saline values, however, a significant decrease in the latency time was evident in the gabapentin treated group (200mg/kg) in comparison to saline group starting from the seventh injection ($p = 0.041$).

Treatment with gabapentin (200mg/kg), enhanced motor coordination in the treated rats manifested as significant increase in the latency period of falling from rotarod apparatus starting from the 5th injection as compared to melatonin-treated group (40mg/kg), ($p = 0.027$)

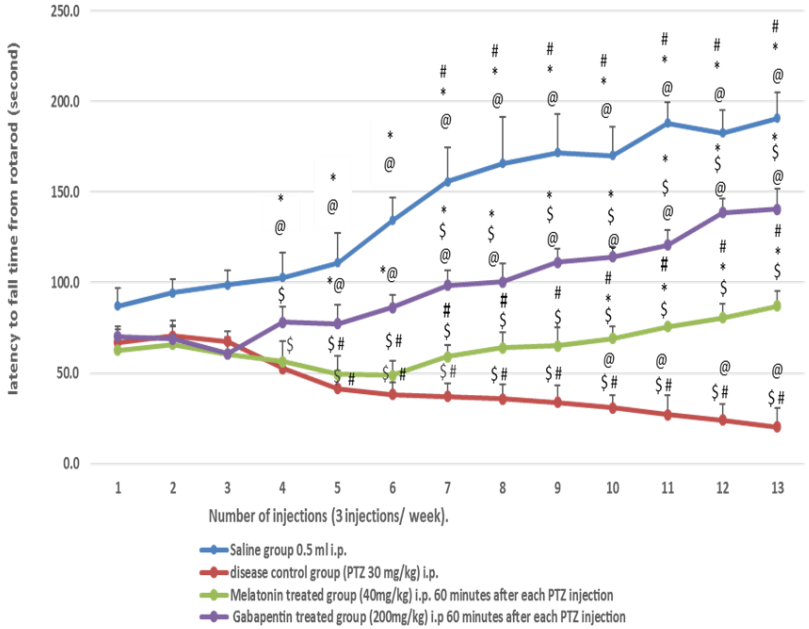

Fig (2): Mean Rotarod test score in the saline injected group, disease control group and the effect of treatment with either gabapentin or Melatonin.

$ Significantly different from saline normal group at corresponding time at $p \leq 0.05$.
* Significantly different from PTZ control group at corresponding time at $p \leq 0.05$.
# Significantly different from gabapentin treated group at corresponding time at $p \leq 0.05$.
@ Significantly different from melatonin treated group at corresponding time at $p \leq 0.05$.

Open field test results

As shown in figure (3), there was a significant decrease in the motor coordination in the PTZ group, (30mg/kg i.p.), manifested as a significant decrease in the number of squares crossed in the open field as compared to normal saline group (0.5 ml i.p.). The decrease in motor coordination was evident from the forth injection ($p = 0.044$).

In addition, there was a significant decrease in the motor coordination in melatonin treated group (40mg/kg), manifested as a significant decrease in the number of squares crossed in the open field as compared to normal saline group (0.5 ml i.p.), The decrease in motor coordination was evident from the third injection ($p = 0.046$).

ISSN: 0975-8585

Also there was a significant decrease in the motor coordination in gabapentin treated group (200mg/kg), manifested as decrease in the number of squares crossed in the open field to a significant value as compared to normal saline group (0.5 ml i.p.), it was evident from the third injection ($p = 0.043$).

On the other hand, there was a significant increase in the motor coordination in melatonin treated group (40mg/kg), as manifested by the increase in the number of squares crossed in the open field compared to PTZ control group starting from the 11th injection ($p = 0.032$).

Also there was a significant increase in the motor coordination in gabapentin treated group (200mg/kg i.p.), manifested as an increase in the number of squares crossed in the open field compared to PTZ control group (PTZ 30mg/kg i.p.) starting from the 4th injection ($p = 0.021$).

There was a significant increase in the motor coordination in gabapentin treated group (200 mg/kg i.p.), manifested as an increase in the number of squares crossed in the open field compared to melatonin treated group (40mg/kg i.p.), starting from the 5th injection till the 11th injection ($p = 0.03$).

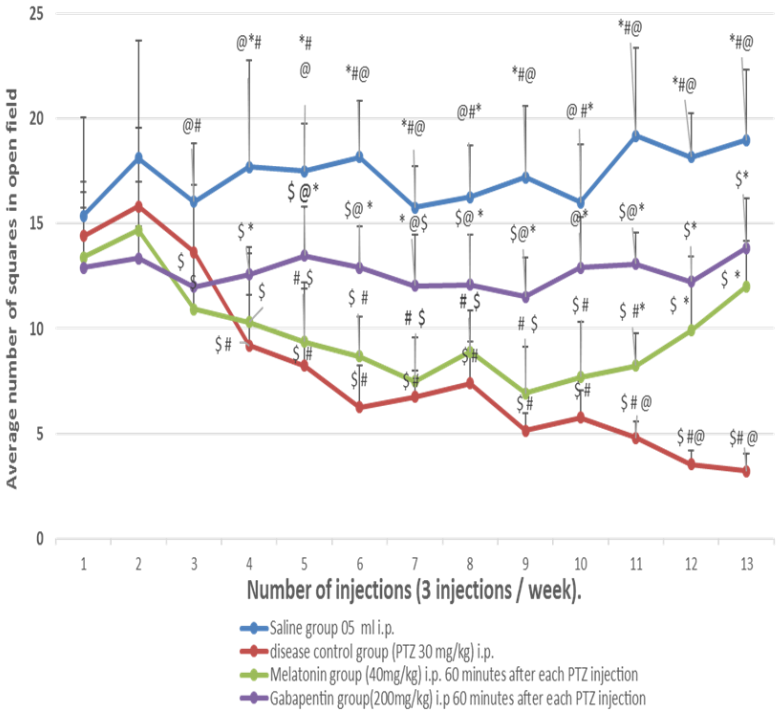

Fig (3): Mean open field test score in the saline injected group, PTZ injected group and the effect of treatment with either gabapentin or Melatonin

$ Significantly different from saline normal group at corresponding time at $p \leq 0.05$.
* Significantly different from PTZ control group at corresponding time at $p \leq 0.05$.
# Significantly different from gabapentin treated group at corresponding time at $p \leq 0.05$.
@ Significantly different from melatonin treated group at corresponding time at $p \leq 0.05$.

Anti GABA antibodies immunohistochemical staining results:

In the rats that received PTZ brain showed no cells positive for GABA staining fig. (5) normal rats showed cytoplasmic staining positive for cells active for GABA staining fig (4) while in the rats that received either gabapentin or Melatonin, brain showed more cells positive for GABA staining fig. (6) and fig. (7) for gabapentin and melatonin respectively.

ISSN: 0975-8585

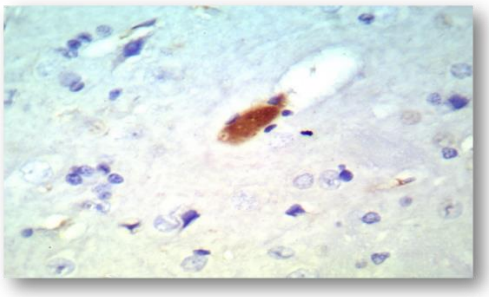

Fig (4): Hippocampal sections of the normal rat brain showing cytoplasmic staining positive for cells active for GABA staining.magnification power 400

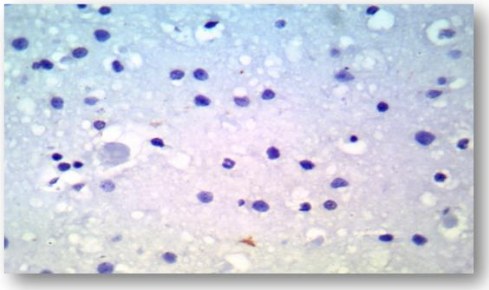

Fig (5): Hippocampal sections of the brain of rats which received PTZ showing no cytoplasmic staining positive for cells active for GABA staining.magnification power 400

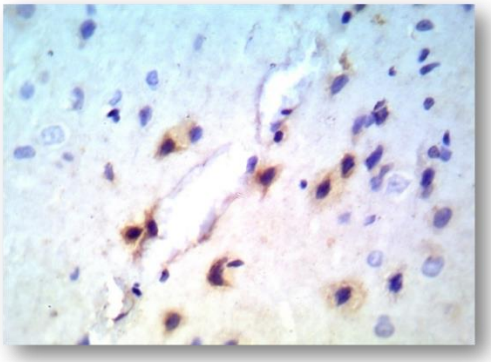

Fig (6): Hippocampal sections of the rats that receieved gabapentin showing cytoplasmic staining positive for cells active for GABA staining (arrow) magnification power 400.

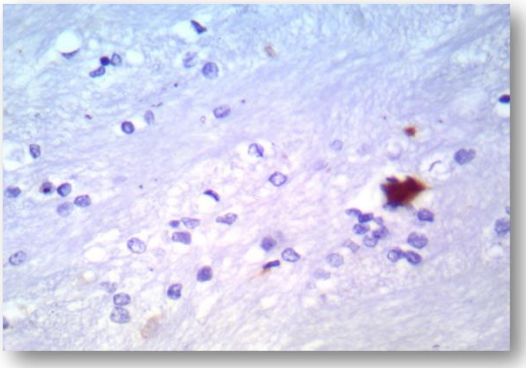

Fig (7): Hippocampal sections of the rats that receievedmelatnin showing cytoplasmic staining positive for cells active for GABA staining .magnification power 400.

DISCUSSION

In the present study, disease control group that received (PTZ 30 mg/kg i.p.) showed a significant increase in seizure score starting from the seventh injection and progressing until after the 13th injection. In addition to significant decrease in motor coordination in the diseased rats manifested as decrease in the latency period of falling from rotarod apparatus & decrease in the number of squares crossed in the open field to a significant value as compared to normal saline group.

Our results demonstrated that melatonin treated group, showed significant enhancement in the motor activity as tested by Rotarod& open field tests. In addition to significant improvement in epilepsy score, as compared by the diseased control group. However, this significant improvement was less than that showed with Gabapentin treated group.

These results is in consistent with the study of Bikjdaouene et al, 2003, which examined the effect of melatonin on brain levels of amino acids and nitric oxide after (PTZ)-induced seizures in rats. Animals were treated with melatonin (10-160 mg/kg, i.p.) 30 min before PTZ administration (100 mg/kg, s.c.), and were killed 3 hours later. At the dose of 80 mg/kg, melatonin significantly increased the latency (5.7-12.7 min) and decreased the duration (31.2-18.4 s) of the first seizure(13) .

Bikjdaouene et al. have shown that administration of melatonin 30 minutes before intraperitoneal injection of PTZ increases the latency and decreases the duration of the first seizure and reduces PTZ-induced mortality from 87.5% to 25% in this model of epilepsy in rats (13).

It has been reported that chronic but not acute melatonin treatment, reduces the incidence and mortality of PTZ-induced seizures in male gerbils. However, there is a discrepancy in the findings probably because of the differences in animal species (gerbil vs mouse), the approach used to measure seizures (seizure threshold vs seizure incidence), and dose of melatonin (0.1–10 mg/kg vs 20–80 mg/kg (13).

Also Yahyavi et al, 2006 studied the effect of melatonin in (PTZ)-induced clonic seizures in mice. Acute intraperitoneal administration of melatonin (40 and 80 mg/kg) significantly increased the clonic seizure threshold induced by intravenous administration of PTZ (14).

Solmaz et al, 2009, which investigated the antiepileptic effects of low-dose melatonin (10 mg/kg) on (PTZ)-induced experimental epilepsy on twelve male albino guinea pigs weighing 500-800 g. Latent period, seizure intensity and mortality parameters were evaluated during the epileptic seizure induced by PTZ(15).

After a recovery period of 7 days, effects of the neuroprotective agent, melatonin (which is dissolved in 2.5% ethanol-saline solution), on epileptic seizures induced by PTZ were evaluated. Solmaz et al., 2009, concluded that melatonin does not have a primary anticonvulsant effect at low doses (10 mg/kg), but it lowers the mortality rates and attenuates seizure severity while increasing the latent period (15).

Another study tested the anticonvulsant efficacy of melatonin but in an experimental model of hyperthermic febrile seizures. It included 72 male Sprague-Dawley rat pups divided into eight groups. The seizures were induced by keeping the rats in 45 °C water and the experiments were performed in two steps. In the first step, the control group was given a vehicle injection and the study groups were given a MT injection (150 mg/kg, intraperitoneal [i.p.]) at either 5, 10, or 15 min prior to the induction of the seizure to determine the anticonvulsant effects of MT and its optimum time of administration(16).

Mosińska et al, 2016 tested the anticonvulsant activity of melatonin evaluated in (PTZ) and electrically-induced convulsions. Only melatonin (50 and 100mg/kg, i.p.) significantly increased the seizure threshold. The i.p. administration of melatonin (100mg/kg) resulted in a significantly elevated PTZ seizure threshold for forelimbs tonus. The compounds did not affect muscle strength. No alteration in motor coordination was noted(16).

In the present study, Gabapentin treated group showed significant enhancement in the motor activity as tested by Rotarod& open field tests. In addition to significant improvement in epilepsy score, as compared by the diseased control group Moreover, this significant improvement was more than that showed with melatonin treated group.

These results is in consistent with the study of Rizwan et al., 2005. Effects of gabapentin and antidepressant drug combinations on convulsions and memory in mice. Administration of gabapentin led to marked reduction in seizure activity. This may be attributed to the fact that GBP increases GABA levels in the brain within 30–60 min after its administration(17)

We concluded that melatonin caused enhancement in the motor activity and improvement in epilepsy score ,may be attributed to increasing GABA levels in the brain.

REFERENCES

[1] Roger J.P. and Brian S.M. (2004): Antiseizure drugs in basic and clinical pharmacology Bertram G. Katzung 9th ed. 24: 388-389.

[2] Jain S, Besag FM. (2013): Does melatonin affect epileptic seizures? Drug Saf. ; 36(4): 207-15.

[3] Rogers S.J. and Cavazos J.E. (2011): Epilepsy. In: Talbert RL, DiPiro JT, Matzke GR, et al, eds. Pharmacotherapy: Chapter 65. A Pathophysiologic Approach. 8th Ed. New York, NY: The McGraw Hill Companies. 57-81.

[4] Aversa S., Pellegrino S., Barberi I., Reiter R. and Gitto E. (2012): Potential utility of melatonin as an antioxidant during pregnancy and in the perinatal period. J Maternal Fetal Neonatal Med 25: 207–221

[5] Atack G.R., Cook S.M., Hutson P.H., and File S.E. (2000): Kindling induced by pentylenetetrazole in rats is not directly associated with changes in the expression of NMDA or benzodiazepine receptors. Pharmacology Biochemistry Behavior. 65(4): 743-750.

[6] Racine R.J. (1972): Modification of seizure activity by electrical stimulation: motor seizure. Electro enceph Clin Neurophysiol; 32: 281-94.

[7] Mahyar J., Iran G., Mohammad R.K., Gila B., Yaqub F. and Sohrab H. (2009): Co-treatment with riluzole, a neuroprotective drug, ameliorates the 3-acetylpyridine-induced neurotoxicity in cerebellar Purkinje neurones of rats: Behavioural and electrophysiological evidence. Neurotoxicology; 30(3): 393-402

[8] Correa M., Wisniecki A. and Betz A. (2004): The adenosine A2A antagonist KF17837 reverses the locomotor suppression and tremulous jaw movements induced by haloperidol in rats: possible relevance to Parkinsonism. Behav Brain Res, 148: 47-54.

[9] Conceição I.M. and Frussa-Filho R. (1993): Effects of a single administration of buspirone on catalepsy, yawning and stereotypy in rats. Braz J Med Biology Res. 26(1): 71-4.

[10] Queiroz C.M. and Frussa-Filho R. (2000): Effects of buspirone on an animal model of tardive dyskinesia. ProgNeuropsycho pharmacol Biolpsychiaty. 23(8): 1405-1418.

[11] Vogler G.A. (2006): Anesthesia and analgesia. In: Suckow MA, Weisbroth S, Franklin CL (eds). The Laboratory Rat. Elsevier Academic Press, New York; 627–95.

[12] Deng P.Y. and Lei S. (2008): Serotonin Increases GABA Release in Rat Entorhinal Cortex by Inhibiting Interneuron TASK-3 K+ Channels. Molecular Cell Neuroscience. 39(2): 273–284.

[13] Bikjdaouene L., Escames G., León J., Ferrer J.M., Khaldy H., Vives F. and Acuña-Castroviejo D. (2003): Changes in brain amino acids and nitric oxide after melatonin administration in rats with pentylenetetrazole-induced seizures. J Pineal Res.; 35(1): 54-60.

[14] Yahyavi-Firouz-Abadi N., Tahsili-Fahadan P., Riazi K., Ghahremani M.H. and Dehpour A.R. (2006): Involvement of nitric oxide pathway in the acute anticonvulsant effect of melatonin in mice.Epilepsy Res.;68(2): 103-13.

[15] Solmaz I., Gürkanlar D., Gökçil Z., Göksoy C., Ozkan M. and Erdoğan E. (2009): Antiepileptic activity of melatonin in guinea pigs with pentylenetetrazol-induced seizures. Neurol Res. 31(9): 989-95.

[16] Mosińska P., Socała K., Nieoczym D., Laudon M., Storr M., Fichna J. and Wlaź P. (2016): Anticonvulsant activity of melatonin, but not melatonin receptor agonists Neu-P11 and Neu-P67, in mice. Behavioral Brain Res. S0166-4328(16)30176-0.

[17] Rizwan A.N., Ali A., Dua Y., Pal S.N. and Pillai K.K. (2003): Effects of gabapentin and antidepressant drug combinations on convulsions and memory in mice.Pol J Pharmacology; 55(6): 965-71.