Neuroprotective Effect of Melatonin in the Hippocampus of Rat’s Brain in Normal Aging Model

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Abstract

**Background:** Aging can be defined as decrease or loss of adaptation with increasing age. In aging nervous system goes through natural changes. Slowing of memory and thinking is a normal part of aging. It may be due to neurodegeneration in the brain cells specially hippocampus. Melatonin is a hormone produced inside the human body. It is a strong antioxidant protecting the body against noxious agents. It is hypothesized that decrease melatonin level during aging may be responsible for aging process.

**Aim:** The present study aimed to evaluate the effect of melatonin on age associated memory deficit and neurodegeneration.

**Methods:** Twenty seven male Wister albino rats, weighing 150-400gm were used in the present study. They were divided into 3 equal groups (each of 9 rats) as follow: I) Control (adult) group: Rats in this group aged from 3-4 months, II) Aged group: Rats in this group aged 22-24 months, III) Melatonin-treated group: In this group aged rats were given melatonin orally 10mg/kg once daily in 1% ethanol for 4 weeks. The following parameters were measured in all groups: Memory test by Barnes maze, histological examination and quantitative assessments of the hippocampi, measurements of Malondialdehyde (MDA), Superoxide Dismutase activity (SOD) and Advanced Glycation End product (AGEs) in the brain homogenate.

**Results:** The present study revealed that melatonin administration to aging rats significantly improved age associated hippocampal neurodegeneration, the oxidative stress in the hippocampus by decreasing MDA level and elevation of SOD level and significantly decreased AGEs in the hippocampus.

**Key Words:** Age – Neurodegeneration – Hippocampus – Melatonin.

Introduction

AGING is a process of becoming older. It is among the largest known risk factors for most human diseases and most common neurodegenerative disorders. The multisystem decline is associated with increasing pathology. Aging process is associated with several structural, chemical, and functional changes in the brain as well as neurocognitive changes. Brain aging may occur in several forms, such as progressive loss of specific neuronal populations and connections, behavioral and memory deficits, increased oxidative stress [1]. In the brain, the most sensitive regions to oxidative stress include the cerebral cortex, the hippocampus, and the striatum. Brain aging has many theories; oxidative stress hypothesis is one of these theories [2]. It is evidenced by strong correlation between increasing age and the accumulation of oxidative damage [3].

The hippocampus is one of the most vulnerable parts of age-related changes in the brain. It plays a major role in spatial learning and memory, and its aging is closely related to decrease in cognition. Alterations in the hippocampus during aging are paralleled by behavioral and functional deficits [4]. It has been proposed that the age-related changes in the hippocampus leads to alterations in brain function which may be associated with an increase in apoptosis. Also these changes may be due to decline in melatonin level in aging brain [5,6].

Advanced Glycation End products (AGEs) are substances that can be considered as a factor for development of diseases. These harmful substances affect every type of cell in the body and thought to be a factor of aging and age related diseases. During the process of aging, structural proteins are damaged by a process called glycation where it is combined with sugar. AGEs are now implicated in the development of age related diseases [7]. AGEs accumulate in the human brain with increasing age and they were found in neurofibrillary tangles and senile plaques in patients with Alzheimer’s disease. Their level was related to the severity of cognitive impairment [8].
Melatonin (N-acetyl-5-methoxytryptamine) is a highly lipophilic molecule that serves as a free radical scavenger, and it has antioxidant properties that act as endogenous buffers against oxidative stress [9,10]. Melatonin is known to modulate many physiological functions [11]. With advancing age and certain age-related diseases, the endogenous secretion of melatonin drops markedly with age, and its supplementation can ameliorate sleep disorders in the aged [12]. As melatonin is known as a strong antioxidant, we aimed to evaluate the potential neuroprotective role of melatonin to improve age related changes in memory and rat brain hippocampus.

**Material and Methods**

*Animals and experimental design:*

Twenty seven male Wister albino rats, weighing 150-400gm were used in the present experiment. All rats were handled gently, housed in groups of five per cage in standard rat cages under controlled temperature (22-24°C), humidity (30-40%) and lighting conditions (12:12h light:Dark cycle) with food and water available ad libitum animals were acclimatized to these conditions for at least 1 week prior to the experiment.

All animals received appropriate care in compliance with the Public Health Service Policy on use of laboratory animals published by the National Institutes of Health and was approved by the Ethical Committee of the College of Medicine, Menoufia University, Egypt. Work was held from February 2015 to July 2015.

Twenty seven animals were divided into 3 equal groups (n=9 each): I) Control (adult) group: Rats in this group aged from 3-4 months, weighting 150:200gm, they were not subjected to any procedure, II) Aged group: Rats in this group aged 22-24 months, weighing 300-400gm, III) Melatonin-treated group: In this group aged rats (22-24 months, weighing 300-400gm) were given melatonin orally 1.0mg/kg once daily in 1% ethanol for 4 weeks [13]. The drinking water of untreated groups contained 0.01% ethanol. Drinking water bottles were changed every other day.

At the end of the experiment (4 weeks) the spatial memory of all groups was assessed by Barnes maze test for 5 days. After which they were scarified by cervical decapitation. Their brains were divided into 2 equal halves; one half was fixed in 10% formalin for histopathological study using Hematoxylin and Eosin staining (H & E) to detect histological changes, and do histological quantitative assessments (morphometric analysis) of the hippocampus. The second halves were utilized for preparation of brain homogenate from the hippocampus. The homogenate were used for biochemical analysis of Malondialdehyde (MDA), Superoxide Dismutase activity (SOD) and Advanced Glycation End product (AGEs).

*Preparation of melatonin solution:*

Melatonin powder (BioBasic. Canada. INc): 100mg of the powder was dissolved in 1ml of absolute ethanol (Sigma-Aldrich Co., St. Louis, MO) and mixed up with tap water to concentration of 0.1 mg/ml. The solution of melatonin was freshly prepared three times a week. The bottles with melatonin solution were covered with aluminum foil [13].

*Fig. (1): Barnes maze for testing spatial memory.*

*Assessment of spatial memory:*

For the memory testing we used the methods of the Barnes maze (especially designed at Physiology Department, Faculty of Medicine, Menoufia University according to Barnes, [1979] [14,15]. The Barnes maze consists of an experimental platform with circular holes around its circumference. Below the surface is an "escape box" which can be reached by the rodent through the corresponding hole on the top of the table Fig. (1). The model is based on rodents' aversion to open spaces, which motivates the tested rat to seek a quiet place in the escape box. At first, rats were trained. During the training phase rats were placed on the circular platform for an adaptation period, after which the training period followed (4 trials/day for consecutive 4 days) this is called acquisition phase. On the next day (5th day) they were tested, this is called probe phase. All steps of the Barnes maze test were recorded using Sony video digital camera for analysis and calculation of latency (time necessary to find the escape box) and errors (total number of head deflections into incorrect holes)
during training days and probe phase in every experimental group and compared the results.

*Tissue homogenate preparation and analysis:*  
The dissected brains were rinsed with ice cold saline and placed in a petri dish, kept on ice then hippocampi were identified, dissected and weighed. The tissue was homogenized by hand in PBS PH 7.2-7.4 (Bio diagnostic Company, Egypt), then centrifuged for 20min at 4C° at speed of 2000-3000rpm (Centrifuge; Narco-Bio system, U.K.). Supernatant was taken and if any precipitation found centrifuged again. The samples were kept at 80 till the time of biochemical analysis of MDA, SOD and AGEs.

*Measurements of MDA and SOD and AGEs levels in the hippocampus:*  
MDA and SOD are measured using commercial kits (Bio diagnostic Company, Egypt), colorimetric method using spectrophotometer (Shimadzu/Double beam spectro-photometer U.V.150, Germany). Estimation of MDA was done by using the previously mentioned protocol [16] by using thiobarbituric acid reactive substance for measuring the peroxidation of fatty acids as oxidative stress marker. Colorimetric method for estimation of Superoxide Dismutase (SOD) as previously described [17] depending on the ability of SOD to inhibit the initial rate of photoactivated phenazine methosulfate mediated reduction of O2-to O2 which then reduce nitroblue tetrazolium dye. Estimation of advanced glycation end product using Eliza kits which, was ordered from Glory Science Co., Ltd (USA). It was measured according to manufacturer’s instructions [18].

*Histopathological examination and quantitative assessment (morphometric analysis) of the hippocampus:*  
The dissected hippocampi from different groups were sent to the Pathology Department, Faculty of Medicine Menoufia University, where they were submitted to routine tissue processing, including fixation in 10% neutral buffered formalin then dehydration in ascending grades of ethanol followed by immersion in xylene and finally impregnation in paraffin. 4 micron thick sections were cut from paraffin embedded blocks by microtome to be stained with haematoxylin and eosin stains for histopathological examination by (Microscope with digital camera; Olympus). For histological quantitative assessments, non-overlapping fields (400X) per section were randomly captured by a digital camera (Olympus) in the hippocampus where the entire area was analyzed. The number of different cells in the fields taken from each section was counted using (image J software; Maryland, USA). The numbers calculated for each animal in the experimental groups were considered for comparison and statistical analyses.

*Statistical analysis:*  
The data were tabulated and analyzed by SPSS (statistical package for the social science software) using statistical package version 11 on IBM compatible computer. Quantitative data were expressed as mean ± standard error of mean (X ± S.E.M). The data from control and test groups were compared using one way analysis of variance (1-ANOVA). Probability value of less than 0.05 was considered as statistically significant (* p<0.05). “n” indicates the number of tested rats.

**Results**

*Memory tests:*  
Regarding the acquisition phase of Barnes maze, the present study revealed that melatonin treatment significantly decreased the mean number of errors in the first day as compared to the corresponding value in aged group (*p*<0.001). There was significant increase in the mean number of errors in the first day in aged group when compared to normal control group (p<0.001). There was no significant difference in the mean numbers of errors between all groups in the remaining days of Barnes maze Fig. (2).

**Fig. (2): The effect of melatonin administration on aged rats on the mean number of errors/day in acquisition phase of Barnes maze. Data are expressed as mean ± S.E.M. (n=9).**

* : Sign when compared to control group.  
**: Sign when compared to aged group.

Also, the acquisition phase of Barnes maze shows, that melatonin treatment significantly decreased the mean time of escape latency in the first
day as compared to the corresponding value in aged group ($p<0.001$). There was significant increase in the mean time of escape latency in the first day in aged group when compared to normal control group ($p<0.001$). There was no significant difference in the mean time of escape latency between all groups in the remaining days of Barnes maze Fig. (3).

Regarding the probe phase of Barnes maze Fig. (4), the present study revealed that melatonin treatment significantly decreased the mean number of errors and the mean time of escape latency in the probe phase as compared to the corresponding value in aged group ($p<0.001$). There was significant increase in the mean number of errors and the mean time of escape latency in aged group when compared to normal control group ($p<0.001$).

**Fig. (4):** The effect of melatonin administration on aged rats in the mean number of errors and the mean time of escape latency (seconds) in probe phase of Barnes maze. Data are expressed as mean ± S.E.M. (n=9).

* : Sign when compared to control group. **: Sign when compared to aged group.

The level of MDA SOD and AGEs in brain homogenate of the hippocampus:

The present study showed that melatonin administration to aged group significantly decreased MDA and AGEs levels in the brain homogenate of the hippocampus ($p<0.001$). In the aged rats there was significant ($p<0.001$) increase in MDA and AGEs levels when compared to corresponding value in normal control group (Table 1). The same table showed that melatonin treatment significantly increased the SOD level in the brain homogenate of the hippocampus when compared to corresponding value in aged group ($p<0.001$). In aging rats the SOD level in the brain homogenate of the hippocampus was significantly ($p<0.001$) decreased when compared to normal control group.

Histopathological examination and quantitative assessment:

Fig. (5) shows section of rat hippocampus (CA 1 region) of all groups. In control group, it shows the three layers of CA1region: Outer polymorphic (O), middle Pyramidal (P) and inner Molecular layer (M). The pyramidal cells are closely packed together. In aged group it shows hypo cellularity of pyramidal cell layer with many apoptotic cells with darkly stained nuclei (↑). The outer polymorphic layer shows many astrocytes (curved arrow) (gliosis). In melatonin-treated group it shows that most of pyramidal cells appear on the same line with vesicular nuclei; however other pyramidal cells show pyknotic nuclei (↑). Excessive number of microglia and astrocytes (curved arrow) are also seen.
Fig. (6) shows section of rat hippocampus (CA3 region) of all groups. In control group, it shows the Outer polymorphic (O), middle Pyramidal (P) and inner Molecular (M) layers. The pyramidal cells are not densely packed as CA1 cells. In aged group it shows disarrangement of pyramidal cell layer with many pyramidal cells in the polymorphic cell layer (T). Other pyramidal cells show apoptosis with pyknotic nuclei and short axons (curved arrow). In melatonin-treated group there are many pyramidal cells that appear normal with vesicular nuclei, however other cells show apoptosis with darkly stained nuclei (T). Excess number of astrocytes and microglia (Gliosis) is still evident (curved arrow).

Fig. (7) shows section of rat hippocampus (Dentate gyrus region) of all groups. In control group, it shows the apex and parts of the 2 blades. It consists of 3 layers from outward inward: Molecular layer (M) formed of small neurons, Granular layer (G) formed of outer mature granular cells (T) and inner immature granular cells and polymorphic layer called Hilus (H) formed of different shaped neurons. In aged group there is marked disarrangement of the granular cells layer with many apoptotic cells with darkly stained nuclei (T) and apparent increased number of astrocytes and microglia (gliosis) (curved arrow). In melatonin-treated group the hilum appears with well-organized hilar cells (T) having euchromatic vesicular nuclei. Some astrocytes appear among hilar cells (curved arrow).

Histopathological quantitative assessment of CA1, CA3 and dentate gyrus regions of the hippocampus showed that melatonin administration to aged group significantly increased the percentage of pyramidal cell count ($p<0.001$) in CA1 and CA3 regions and significantly ($p<0.001$) increased granular cell count in the dentate gyrus of the hippocampus. Also, it significantly ($p<0.001$) decreased the apoptotic cell count as a percentage in the same areas (Table 2).

The same table shows that aging significantly decreased the percentage of pyramidal cell count ($p<0.001$) in CA1 and CA3 regions and significantly ($p<0.001$) decreased granular cell count in the dentate gyrus of the hippocampus. It significantly ($p<0.001$) increased the apoptotic cell count as a percentage in the same areas.
Fig. (6): Effect of melatonin administration on the CA3 region of the hippocampus in aged rats.
CA3 region of the rat hippocampus.
H & E X400
C: Control group.
A: Aged group.
M: Melatonin-treated group.

Fig. (7): Effect of melatonin administration on the dentate gyrus of the hippocampus in aged rats.
Dentate gyrus of the rat hippocampus.
H & E X400
C: Control group.
A: Aged group.
M: Melatonin-treated group.
probe phases of Barnes maze in melatonin treated phases of Barnes maze. Also, the hippocampi time of escape latency in both the acquisition and probe phases of animals. Which was indicated by hand, melatonin attenuated the memory deficits in the number of errors/day and the mean time of escape latency in both the acquisition and probe phases of Barnes maze tests of animal models of aging [22,23]. In Alzheimer patients, melatonin has been able to stabilize cognitive function [24].

Given that neuronal cell death underlies many neurological disorders, an obvious possibility would be that loss of neurons may contribute to age-related deficits in memory function [25]. In the present work, massive apoptosis were detected in different regions of aged hippocampi. Studies of autopsy material indeed suggested that humans had lost neurons during the aging process. A study of hippocampal neuron numbers in the human brain described a loss of cells in the dentate gyrus. Aging in the dentate gyrus is associated with increased expression of pro-apoptotic genes and proteins and decreased anti-apoptotic ones [6]. In our study, melatonin has not only improved the memory function of aging rats, but also ameliorated the histopathological changes detected in aging hippocampi. This result is supported by previous studies which showed that chronic melatonin administration to rats improved dendritic stability [26,27], indicating a protective effect of melatonin against hippocampal neurodegeneration. In addition, neurons died in the hippocampus of pimelecomized rats, and melatonin treatment prevented such effects [28].

Several mechanisms have been proposed to explain the neuroprotective actions of melatonin. In the present study, melatonin significantly reduced MDA level, supporting previous evidence demonstrating the inhibitory effect of melatonin on lipid peroxidation. Melatonin treatment reduced lipid peroxidation level in the brain of rats after cerebral injury [29]. It is a very efficient free radical scavenger of the highly toxic (OH) both in vitro and in vivo [30,31]. In addition to its inhibitory effect

Table (1): Effect of melatonin administration on the MDA, SOD and AGEs in brain homogenate of the hippocampus in aged rats.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Aged group</th>
<th>Melatonin group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nm/gm tissue)</td>
<td>2.67±0.57</td>
<td>24.07±2.88*</td>
<td>4.66±0.88**</td>
</tr>
<tr>
<td>SOD (U/gm tissue)</td>
<td>8.55±0.34</td>
<td>2.16±0.23*</td>
<td>6.28±0.17**</td>
</tr>
<tr>
<td>AGEs ng/L</td>
<td>74.63±2.91</td>
<td>421.62±14.21*</td>
<td>210.07±4.63**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. (n=9).
* : Sign when compared to control group.
**: Sign when compared to aged group.

Table (2): Effect of melatonin administration on the morphometric studies of the hippocampus in aged rats.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Aged group</th>
<th>Melatonin group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyramidal cell count in in CA1 as %</td>
<td>77.16±2.74</td>
<td>36.66±3.31*</td>
<td>64.83±3.54**</td>
</tr>
<tr>
<td>Apoptotic cell count in in CA1 as %</td>
<td>0.66±0.49</td>
<td>13.33±1.52*</td>
<td>0.5±0.50**</td>
</tr>
<tr>
<td>Pyramidal cell count in in CA3 as %</td>
<td>54.0±3.65</td>
<td>25.50±3.06*</td>
<td>58.33±4.77**</td>
</tr>
<tr>
<td>Apoptotic cell count in in CA3 as %</td>
<td>0.83±0.83</td>
<td>7.83±1.49*</td>
<td>0.66±0.49**</td>
</tr>
<tr>
<td>Granular cell count in in dentate gyrus as %</td>
<td>148.00±1.65</td>
<td>75.00±8.55*</td>
<td>141.00±3.83**</td>
</tr>
<tr>
<td>Apoptotic cell count in in dentate gyrus as %</td>
<td>1.00±0.51</td>
<td>34.00±7.57*</td>
<td>1.00±0.30**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. (n=9).
* : Sign when compared to control group.
**: Sign when compared to aged group.

Discussion

With the rapid increase in the elderly population, aging has become a social problem affecting the development of society and economy. In the present study, aging is associated with learning and memory impairments which, was indicated by increase in the number of errors/day and the mean time of escape latency in both the acquisition and probe phases of Barnes maze. Also, the hippocampi showed increase in apoptosis, decrease in pyramidal and granular cells and histopathological alterations in the hippocampus of aged group. On the other hand, melatonin attenuated the memory deficits in the aged brain of rats. Which was indicated by decrease in the number of errors/day and the mean time of escape latency in both the acquisition and probe phases of Barnes maze in melatonin treated group. In addition, it reduced the apoptosis and improved the histopathological alterations in the aged hippocampi.

The current study demonstrates that melatonin induced its beneficial effects via reducing lipid peroxidation and elevating antioxidant enzymes level in the aged hippocampi. Also, it decreased the level of AGEs in the brain of aged group.

Aging has been associated with deterioration in memory, which is critically supported by the hippocampus. Age-related impairments in memory have been attributed to deterioration in hippocampal function; older adults show structural and/or synaptic alterations in the hippocampus [19-21]. In the present work, memory performance was impaired compared to control, this decline was dramatically attenuated after melatonin intake. Our results are in agreement with several previous studies in animals and patients. Melatonin shortened the latencies and reduced number of errors of in water maze tests of animal models of aging [22,23]. In Alzheimer patients, melatonin has been able to stabilize cognitive function [24].

Given that neuronal cell death underlies many neurological disorders, an obvious possibility would be that loss of neurons may contribute to age-related deficits in memory function [25]. In the present work, massive apoptosis were detected in different regions of aged hippocampi. Studies of autopsy material indeed suggested that humans had lost neurons during the aging process. A study of hippocampal neuron numbers in the human brain described a loss of cells in the dentate gyrus. Aging in the dentate gyrus is associated with increased expression of pro-apoptotic genes and proteins and decreased anti-apoptotic ones [6]. In our study, melatonin has not only improved the memory function of aging rats, but also ameliorated the histopathological changes detected in aging hippocampi. This result is supported by previous studies which showed that chronic melatonin administration to rats improved dendritic stability [26,27], indicating a protective effect of melatonin against hippocampal neurodegeneration. In addition, neurons died in the hippocampus of pinelecomized rats, and melatonin treatment prevented such effects [28].
on lipid peroxidation, melatonin is a well-known antioxidant, an ability which was supported in the present study and was illustrated in significant elevation of antioxidants enzymes.

AGEs are thought to be endogenously generated by lipid peroxidation. Its accumulation inside the body accelerates aging. Thus, AGEs found in damaged tissues could be markers for oxidative stress and inflammation or may be causative factor for oxidative stress [32]. Melatonin significantly improved the level of AGEs which may be responsible for the improvement in hippocampal functions.

Beside the antioxidant ability, melatonin has been shown to play an anti-apoptotic effect. Melatonin reduced the expression of pro-apoptotic genes such as Bax and Bad in the dentate gyrus of old male rats and inhibited H$_2$O$_2$-induced apoptosis in cultured rat astrocytes via Bax and caspase-3 expression regulation [33]. In addition, early melatonin supplementation prevented the abnormal upregulation of Bax, caspase-3 and Par-4 (prostate apoptosis response-4) in cortex neurons of Tg mice (a model of Alzheimer's disease) [34]. Therefore, it is plausible to conclude that the observed amelioration of the memory deficits and histopathological alteration in the current work was at least partially attributed to melatonin ability to remedy oxidative stress and apoptosis via its antioxidant and anti-apoptotic actions. Also, its effect may be attributed to decrease in AGEs.

In conclusion, the current study demonstrated the protective effect of melatonin on the memory impairments and cell death via reducing oxidative stress and AGEs. This work further supported melatonin as a potential therapeutic candidate, which is of low cost and low toxic hazards, in slowing normal brain ageing and in the treatment of neurodegenerative diseases.

References


يمكن تعرف الشيخوخة على أنها نقصان أو فقدان تكيف وظائف الجسم مع الزيادة في العمر. يعرض الجهاز العصبي في الشيخوخة بعدة تغييرات طبيعية مثل تباطؤ الذاكرة والتفكير ويعتبر ذلك طريقة بسيطة يحدث عند الشيخوخة. قد يكون ذلك بسبب تتلك عصبى في خلايا الخصاصة العصبية لانه الجزء المسول من الذاكرة في المخ، يعتبر الميلاتونين من أحد مضادات الأكسدة القوية التي تحمي الجسم ضد الجزيئات الضارة، والميلاتونين هرمون ينتج داخل الجسم البشري ولكن تقل نسبة مع الشيخوخة. ويفترض أن انخفاض مستوي الميلاتونين أثناء الشيخوخة قد يكون هو المسؤول عن التغيرات المصاحبة لعملية الشيخوخة.

الهدف من البحث: تهدف هذه الدراسة إلى تقييم تأثير الميلاتونين على تباطؤ الذاكرة والتتكس العصبي المصاحب للشيخوخة.

طرق ومواد البحث: استخدمت سبعة ومثعونا، والتي يتراوح وزنها من 150 إلى 200 جرام، وقد تم تقسيم الفئران إلى 3 مجموعات متساوية (تتكون كل مجموعة من 9 فئران) على النحو التالي:

1- المجموعة الضابطة (الفئران الناضجة): وتتراوح أعمار الفئران في هذه المجموعة من ثلاثة إلى أربعة شهور.
2- مجموعة المسنين: وتتراوح أعمار الفئران في هذه المجموعة من اثني عشر رأئ إلى أربعة ومثعونا شهراً.
3- مجموعة المسنين المتعالجة بالميلاتونين: وتتراوح أعمار الفئران في هذه المجموعة من اثني عشر رأئ إلى أربعة ومثعونا شهراً وقد أعطيت هذه المجموعة الميلاتونين عن طريق الفم بجرعه مقدارها 10 ألف شواشر/كيرام مرة واحدة يومياً منذ ما قبل مولع الإبلول بتركز 1/4 لبادل.

الانتاج: وبعد انتهاء مدة الدراسة تم اختبار الذاكرة عن طريق مثعية بارزين لكل المجموعات، وأيضاً تم اخذ عينات من مخ الفئران وتم تحديد الحصين وتشريح، وقد استخدم العصبين لفحص النسيج ولاعير متجانس، وقد استخدم المتجانس لقياس عامل الأكسده ومصالح الأكسدة وبايضاً النواتج النهائية لعملية الغليفيكين.

النتائج: كشفت هذه الدراسة أن تناول الميلاتونين في الفئران المسن لدى اتى تحسن ملحوظ في تباطؤ الذاكرة والتتكس العصبي بشكل ملحوظ أيضاً لدى اتى خفض مستوى الأكسدة في الحصين عن طريق خفض مستوى عامل الأكسدة ورفع مستوى مضادات الأكسدة بشكل ملحوظ كما اتى أيضاً إلى خفض النواتج النهائية لعملية الغليفيكين.