Effect of Melatonin on Parkinson-Like Syndrome Induced by I-Methyl-4-Phenyl Tetrahydropyridine in Mice

Minerva K Faami1, Mokhtar Taha2, Mona F El-Karan1, Omyma G Ahmed1

Departments of Physiology, Faculty of Medicine1; Pathology, Faculty of Veterinary Medicine2; Assiut University

ABSTRACT

This work was carried out on 144 adult male albino mice; 72 adult mice were divided into 3 groups (MPTP or Parkinsonian model, prophylactic, and treated). Each group consisted of 24 adult male mice. Parkinson’s model group was injected daily S.C. with 30 mg MPTP / kg for 3 successive days. Prophylactic group was injected with low dose of melatonin (0.5 mg / mice / day) I.P. for 2 weeks prior to MPTP injection. The treated group was injected firstly with MPTP and then treated with therapeutic dose of melatonin (1.0 mg / mice / day). The other 72 mice were considered as control groups. The effects of MPTP in inducing Parkinsonian like syndrome and the protective effect of melatonin were studied in each group by clinical tests, histopathological study, immunohistochemical techniques, molecular examination and biochemical analysis. The clinical studies included, observation for the appearance of muscle tremors in the experimental animals, recording of number of movement / minute by activity cage apparatus, recording gripping time on rotating rod (seconds) by Rota rod apparatus and recording descending time (seconds) on vertical pole by pole test. Blood sample was taken from each animal immediately before scarification to be used for estimation of plasma levels of free radicals (nitric oxide & lipid peroxidation products) and antioxidant enzymes (glutathione peroxidase & superoxide dismutase) by using spectrophotometer. Brain from each mouse of all studied groups was dissected and preserved for histopathological examination and immunohistochemical studies. Anatomically localized substantia nigra was freshly used for molecular studies that included detection of DNA fragmentation and Tunnel Technique. In Parkinsonian model group, as a result of MPTP administration, obvious resting tremors and significant decrease in the frequency of movement / minute, loss of ability to coordinate the limb movement, seen as significant decrease in gripping time on rotating rod and prolonged the descending time of pole test. It is concluded that MPTP administration led to significant reduction motor activity of the mice. Histopathological and immunohistochemical studies of Parkinsonian model showed severe degenerative changes in most dopaminergic neurons. In spite of the marked degeneration and necrosis of dopaminergic neurons, apoptotic cells could not be detected by molecular studies in all Parkinson’s model subgroups. Hence apoptosis had undetectable role in the pathogenesis of neuronal death in MPTP neurotoxicity. Biochemical analysis of blood samples of Parkinsonian model group revealed significant progressive increase in plasma levels of free radicals: nitric oxide (NO) and lipid peroxidation products (LP). Meanwhile, plasma levels of antioxidant enzymes, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) decreased significantly in all subgroups when compared with that of its control values. All Parkinson’s model subgroups had typical clinical features of Parkinsonian like syndrome, which were positively correlated with the measured plasma oxidant indices and antioxidant enzymes. While in prophylactic group of mice, administration of melatonin significantly decreased parkinsonian symptoms when compared with that of Parkinson’s model group. Histopathological and immunohistochemical studies, of slides representing the prophylactic group of mice, revealed an obvious increase in the number of dopaminergic neurons and the TH- positive neurons of substantia nigra and red nuclei. Also, the majority of dopaminergic neurons of SN and red nuclei preserved their normal histological architecture. Also, melatonin administration to this group of mice decreased significantly the plasma levels of free radicals and increased significantly the antioxidant enzymes in comparable to that of Parkinson’s model group. Melatonin performed its neuroprotective role through its ability to inhibit the formation of free radicals or scavenge them. Hence, application of melatonin in low protective dose succeeded in protecting the vulnerable dopaminergic neurons against the administered MPTP and restoring their neurotransmitter with consequent reestablishing the normal motor activity. Application of melatonin in high therapeutic dose to treated group succeeded to decrease parkinsonian symptoms significantly in comparable to that of Parkinson’s model group.
model group. However, the improvement in clinical test was more obvious in prophylactic than treated group. Histopathological and immunohistochemical examinations showed that, application of melatonin as a therapeutic agent failed to regenerate the MPTP lesioned neurons as shown by histopathological and immunohistochemical studies. In spite of this, melatonin may allow sprouting of dopamine terminals with subsequently restoring the released neurotransmitters from substantia nigra and improving the detectable clinical signs of MPTP induced Parkinsonism. Biochemical analysis of blood samples of this group revealed significant decrease of plasma free radicals (LP& NO) and significant increase antioxidant enzymes (GSH-Px and SOD). Hence administration of melatonin as a therapeutic agent was able to inhibit the production of oxidant indices and to enhance the antioxidant enzymes. In spite of this, the increased antioxidant enzymes may not be sufficient to treat the lesioned neurons after MPTP toxicity. In conclusions: MPTP succeed to induce typical Parkinson’s model. Melatonin has antioxidant protective role, so its application in low prophylactic dose is beneficial in protecting the neurons against the injected neurotoxin MPTP. However, application of melatonin as therapeutic agent after MPTP neurotoxicity failed to regenerate the MPTP damaged neurons, in spite of this it may allow sprouting of dopamine terminals with subsequent restoring the released neurotransmitters from SN and improving the detectable clinical signs of MPTP induced Parkinsonism. (Egypt J. Neurol. Psychiat. Neurosurg., 2007, 44(1): 363-377).

INTRODUCTION

Parkinson’s disease is a common and disabling neurodegenerative disease in which the dopaminergic neurons in the substantia nigra pars compacta undergo pathological cell death. Substantia nigra is the principle site of degeneration in Parkinson’s disease.\(^1\) Dopamine-replacement strategy formed the basis of most symptomatic treatments for Parkinson's disease. In spite of this, the long-term dopamine-replacement therapies were characterized by many side effects, as dyskinesia. Hence, the concept of application of non-dopaminergic therapy for Parkinson's disease has been attracted great interest. However, it has been proved difficult to devise a non-dopaminergic therapy with great efficacy and low side effect.\(^2\)

Parkinsonian like syndrome is an example of neurodegenerative disease resulting from oxidative stress of the brain tissue where the brain is more liable for oxidative stress and prone to free radical damage. Globus pallidus and substantia nigra) are rich in iron and vitamin C that under some conditions can greatly accelerate free radical generation.\(^3\)

Melatonin has a potent antioxidant action and it has been used prophylactically to reduce amyloid beta protein toxicity of Alzheimer’s disease.\(^4\) Also, melatonin protected against neurological injuries induced by trauma,\(^5\) stroke,\(^6\) ischemic reperfusion injury,\(^7,8,9\) and aging process.\(^10\) In addition, melatonin is used to reduce oxidative damage in several models of Parkinson’s disease induced by MPTP and 6-hydroxy dopamine.\(^11,12,13\)

Aim of The Work

The aim of the work is to investigate the ability of low melatonin dose in prophylaxis or preventing MPTP induced Parkinsonian like syndrome in mice and to find the role of high therapeutic dose of melatonin in curing it. Clinical tests, histopathological examination, Immunohistochemical studies, molecular techniques and biochemical analysis were employed to evaluate the most sensitive method for diagnosis the onset and persistence of MPTP neurotoxicity. Moreover, the molecular techniques (DNA fragmentation & Tunnel technique) were adopted to investigate the possible mechanisms of neuronal death (apoptosis versus necrosis) in MPTP induced PD.

MATERIALS AND METHODS

Materials

Chemicals used:
A. Neurotoxic agent (for induction of Parkinson
model): 1-methyl-4-phenyl 1,2,3,6
tetrahydropyridine (MPTP) M-0896 - SIGMA.
B. Protective agent: N-Acetyl-5-
methoxytryptamine (Melatonin) M-5250-
SIGMA.

Animals used:
One hundred and forty four healthy adult
male albino mice (Sprague-Dawley) were of the
same age were used. The mice were randomly
divided into four groups:

**Group 1:** Parkinson’s model Group (MPTP
group): that received a SC injection of 30 mg
MPTP /kg body weight/ day.

**Group 2:** Prophylactic Group: that received low
dose of melatonin (0.5 mg / mice/ day)
according to El-Karn, Omyma and Kim.

**Group 3:** (Treated Group): that received therapeutic
dose of melatonin (1mg/mice/day) IP at 5 PM
for 3 successive weeks.

**Control group (I):** control for Parkinson’s model
group, was injected S.C. with 1 ml. saline / day

**Control group (II):** control for prophylactic
group, was injected with low dose of
melatonin (0.5 mg / mice / day).

**Control group (III):** control for treated group,
was injected with high dose of melatonin
(1.0 mg / mice / day).

**Methods**

- **Monitoring the animal motor activity:** by using the activity cage apparatus, (Plate I)
  according to Spiraki.

- **Monitoring the animal motor power:** by using the Rota Rod apparatus according to
  Dunham and Miya (Plate II), and pole test (Plate III) which was described by Ogawa et
  al.

- **Methods used for determination of some biochemical parameters:**
  a. Lipid peroxidation product in plasma was determined according to method
     described by Aruoma et al.
  b. Nitric oxide in plasma was determined by evaluating its oxidation products
     (nitrates and nitrites) by using Griess

  c. Superoxide dismutase in plasma was determined according to the method of
     Misra and Fridovich.
  d. Glutathione peroxidase in RBCs was determined according to Hafeman et al.

- **Histopathological examination:**
  Brains were immersed in freshly prepared Bouin’s fixative (for histopathological
  examination) or in neutral buffered formalin (for Tunnel technique and
  immunohistochemical studies). Midbrain and substantia nigra is anatomically located.
  Gallocyanine chrom-alum stain was used to demonstrate Nissle’s granules, nuclei and
  nucleoli according to Drury and Walington.

- **Immunohistochemical method:**
  a. **Glial fibrillary acidic protein (GFAP):** Immuno histochemical staining of tissue
     sections with rabbit antimouse GFAP polyclonal antibody was used to
demonstrate the reactive astrocytes.
     Technique: (Avidin-Biotin peroxidase according to O’Callaghan).
  b. **Tyrosine Hydroxylase (TH):** Avidin Biotin peroxidase technique was used for
     staining of tyrosine hydroxylase positive
     neurons of paraffin midbrain sections as
described for GFAP. The primary
     antibody is rabbit antimouse polyclonal
     antibody to tyrosine hydroxylase

- **Molecular biological techniques:** For
  studying the mechanisms of neuronal death in
  the experimental groups (necrosis versus
  apoptosis), the following techniques were
carried out. Electrophoresis of genomic DNA
  from midbrain tissue as described by Brown
  et al.

**RESULTS**

The effects of MPTP in inducing
Parkinsonian like syndrome and the protective
effect of melatonin were studied in each group by
clinical tests, histopathological study,
immunohistochemical techniques, molecular examination and biochemical analysis.

The clinical studies included, observation for the appearance of muscle tremors in the experimental animals, recording of number of movement /minute by activity cage apparatus, recording gripping time on rotating rod (seconds) by Rota rod apparatus and recording descending time (seconds) on vertical pole by pole test.

In Parkinsonian model group, as a result of MPTP administration, obvious resting tremors in fore and hind limbs of injected mice were observed. Also, MPTP injection to this group led to significant decrease in the frequency of movement/ minute (Table 1), Loss of ability to coordinate the limb movement “seen as: significant decrease in gripping time on rotating rod (Table 2), and prolonged the descending time of pole test (Table 3)”. There were significant correlations between average values of movement / minute, gripping time, and descending time with the studied days as they changed progressively throughout the duration of the experiment. It is concluded that MPTP administration led to significant reduction motor activity of the mice.

Histopathological and immunohistochemical studies of Parkinsonian model showed severe degenerative changes in most dopaminergic neurons. Also, marked losses of dopaminergic neurons and TH-positive neurons were observed within 24 hours of the last injection. These degenerative effects persisted throughout the duration of the experiment. In spite of the marked degeneration and necrosis of dopaminergic neurons, apoptotic cells could not be detected by molecular studies in all Parkinson’s model subgroups. Hence apoptosis had undetectable role in the pathogenesis of neuronal death in MPTP neurotoxicity.

Biochemical analysis of blood samples of Parkinsonian model group revealed significant progressive increase in plasma levels of free radicals (nitric oxide and lipid peroxidation products). Meanwhile, plasma levels of antioxidant enzymes, glutathione peroxidase and superoxide dismutase decreased significantly in all subgroups when compared with that of its control values (Table 4). Plasma levels of NO showed positive significant correlation with plasma LP and both NO and LP revealed negative correlations with plasma GSH-Px and SOD.

All Parkinson’s model subgroups had typical clinical features of Parkinsonian like syndrome, which were positively correlated with the measured plasma oxidant indices and antioxidant enzymes. These correlations can be explained as following, MPTP injection to this group led to oxidative stress, as manifested by increasing formation of oxidant indices and reducing the m RNA of antioxidant enzymes and decreasing their plasma levels. Oxidative stress permanently damaged the nigral dopaminergic system in mice with consequent reduction of the released dopamine, which is responsible for appearance of clinical signs of PD disease.

While, in prophylactic group of mice, administration of melatonin significantly increased the average values of movement /minute and gripping time and reduced significantly the average values of descending time at the end of the experiment when compared with that of Parkinson’s model group. In addition, after melatonin administration to this group, the resting tremors became unclearly manifested even after MPTP injection.

Histopathological and immunohistochemical studies, of slides representing the prophylactic group of mice, revealed an obvious increase in the number of dopaminergic neurons and the TH-positive neurons of substantia nigra and red nuclei. Also, the majority of dopaminergic neurons of SN and red nuclei preserved their normal histological architecture.

Also, melatonin administration to this group of mice decreased significantly the plasma levels of free radicals and increased significantly the antioxidant enzymes in comparable to that of Parkinson’s model group and control group (Table 5).

Melatonin performed its neuroprotective role through its ability to inhibit the formation of free radicals or scavenge them and increase m RNA of antioxidant enzymes with increasing their plasma levels.
levels. This enhancement in plasma antioxidant enzymes and the reduction in oxidant indices constitute the major factors that rescue the dopaminergic neurons and TH positive neurons in SN and red nuclei from the neurotoxin, MPTP. Hence, application of melatonin in low protective dose succeeded in protecting the vulnerable dopaminergic neurons against the administered MPTP and restoring their neurotransmitter with consequent reestablishing the normal motor activity.

Application of melatonin in high therapeutic dose to treated group succeeded to increase significantly the average values of movement /minute and gripping time and decrease significantly the average values of descending time in comparable to that of Parkinson’s model group (Tables 6, 7). However, the improvement in clinical test was more obvious in prophylactic than treated group (Table 8).

Histopathological and immunohistochemical examinations showed that, application of melatonin as a therapeutic agent failed to regenerate the MPTP lesioned neurons as shown by histopathological and immunohistochemical studies. In spite of this, melatonin may allow sprouting of dopamine terminals with subsequently restoring the released neurotransmitters from SN and improving the detectable clinical signs of MPTP induced Parkinsonism.

Biochemical analysis of blood samples of this group revealed significant decrease of plasma free radicals (LP& NO) and significant increase antioxidant enzymes (GSH-Px and SOD) (Table 9). Hence administration of melatonin as a therapeutic agent was able to inhibit the production of oxidant indices and to enhance the antioxidant enzymes. In spite of this, the increased antioxidant enzymes may not be sufficient to treat the lesioned neurons after MPTP toxicity.

### Table 1. Average values of movement / minute (spontaneous locomotor activity test) of Parkinson’s model versus control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 11</th>
<th>Day 18</th>
<th>Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>MPTP</td>
<td>Control</td>
<td>MPTP</td>
<td>Control</td>
<td>MPTP</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>84</td>
<td>59</td>
<td>90</td>
<td>19</td>
</tr>
<tr>
<td>SE</td>
<td>1.7</td>
<td>3.3</td>
<td>1.4</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>P Value</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>%Deviation from control</td>
<td>85%</td>
<td>97%</td>
<td>70%</td>
<td>100%</td>
<td>23%</td>
</tr>
</tbody>
</table>

N.B.: All data are mean ± SE. P<0.001 = ***, P<0.01 = **, P< 0.5 = *, SE= stander error, N = number of animals.

### Table 2. Average values of gripping time (seconds) on rotating rods (Rota-Rod test) of Parkinson’s model versus control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 11</th>
<th>Day 18</th>
<th>Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>MPTP</td>
<td>Control</td>
<td>MPTP</td>
<td>Control</td>
<td>MPTP</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>65</td>
<td>30</td>
<td>69</td>
<td>25</td>
</tr>
<tr>
<td>SE</td>
<td>2.2</td>
<td>4</td>
<td>2.1</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>P Value</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>
% deviation from mean of control | 62% | 94% | 43% | 99% | 37% | 108% | 30% | 97% | 19% | 102%

N.B.: All data are mean ± SE, P < 0.001 = ***, P < 0.01 = **, P< 0.5 = *, SE = stander error, n = number of animals.

**Table 3.** Average values of descending time (seconds) (pole test) Parkinson’s model versus control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Items</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 11</th>
<th>Day 18</th>
<th>Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean MPTP</td>
<td>Control</td>
<td>MPTP</td>
<td>Control</td>
<td>MPTP</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>9.8</td>
<td>13</td>
<td>10</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.8</td>
<td>1.1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>P Value</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>% to mean of control</td>
<td>106%</td>
<td>87%</td>
<td>91%</td>
<td>144%</td>
<td>104%</td>
<td>188%</td>
</tr>
</tbody>
</table>

N.B.: All data are mean ± SE. P < 0.001 = ***, P < 0.01 = **, P< 0.5 = *, SE = stander error.

**Table 4.** Average values of plasma oxidants indices (NO & LP) and antioxidant enzymes (GSH-Px & SOD) of all MPTP subgroups (A, B, C and D) that sacrificed at day 4, 11, 18 and 25 respectively.

<table>
<thead>
<tr>
<th>Oxidant &amp; antioxidant</th>
<th>Items</th>
<th>Day 4 Subgroup (A)</th>
<th>Day 11 Subgroup (B)</th>
<th>Day 18 Subgroup (C)</th>
<th>Day 25 Subgroup (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO (µmol/L)</td>
<td>Mean±SE (n =6)</td>
<td>10.53±0.41</td>
<td>10.83±0.31</td>
<td>12.50±0.56</td>
<td>15.17±0.60</td>
</tr>
<tr>
<td>Range</td>
<td>9.2–11.5</td>
<td>10.1–11.7</td>
<td>11.1–14.25</td>
<td>13.5–16.75</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>10.5</td>
<td>10.7</td>
<td>12.75</td>
<td>15.25</td>
<td></td>
</tr>
<tr>
<td>LP (µmol/L)</td>
<td>Mean±SE (n =6)</td>
<td>4.45±0.48</td>
<td>4.68±0.37</td>
<td>13.16±0.68</td>
<td>17.61±1.82</td>
</tr>
<tr>
<td>Range</td>
<td>2.7–6.15</td>
<td>3.4–5.8</td>
<td>11.4–15.25</td>
<td>12–23.01</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4.515</td>
<td>4.8</td>
<td>13.9</td>
<td>18.775</td>
<td></td>
</tr>
<tr>
<td>GSH-Px (EU/mgHb)</td>
<td>Mean±SE (n =6)</td>
<td>33.73±3.78</td>
<td>36.53±2.9</td>
<td>22.16±1.77</td>
<td>16.46±0.88</td>
</tr>
<tr>
<td>Range</td>
<td>24.4–49.64</td>
<td>29.4–49.85</td>
<td>15.1–26.86</td>
<td>15.92–29.2</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>32.425</td>
<td>34.855</td>
<td>22.878</td>
<td>19.915</td>
<td></td>
</tr>
<tr>
<td>S.O.D (U/L)</td>
<td>Mean±SE (n =6)</td>
<td>2.77±0.93</td>
<td>2.77±0.94</td>
<td>1.11±0.56</td>
<td>0.88±0.37</td>
</tr>
<tr>
<td>Range</td>
<td>0.00–6.66</td>
<td>0.00–6.66</td>
<td>0.00–3.33</td>
<td>0.00–6.66</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.495</td>
<td>2.5</td>
<td>0.83</td>
<td>0.83</td>
<td></td>
</tr>
</tbody>
</table>

N.B.: n. = Number of animals. All data are mean SE NO = nitric oxide. LP = lipid peroxidation products. GSH-Px = glutathione peroxidase. SOD = Superoxide dismutase. All data are mean±SE. SE = Standar error. NS = Non-significant. P<0.05 = *, P<0.01 = ** P<0.001 = *** n = number of animals.

**Table 5.** Comparison between average values of plasma oxidant indices (NO& LP and antioxidant enzymes (GSH-Px &SOD) of Prophylactic group and its control (II) at the end of the experiment.

<table>
<thead>
<tr>
<th>Oxidant &amp; antioxidant</th>
<th>Items</th>
<th>Prophylactic</th>
<th>Control (II) 0.5 mg Mel.</th>
<th>P values Prophylactic vs Control</th>
<th>% deviation from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.O (µmol/L)</td>
<td>Mean±SE (n =24)</td>
<td>8.00±0.22</td>
<td>4.15±0.20</td>
<td>***</td>
<td>192.86%</td>
</tr>
<tr>
<td>Range</td>
<td>5.9–9.65</td>
<td>3.00–6.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>8.175</td>
<td>4.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>L.P (μ mol/L)</th>
<th>Mean±SE (n.=24)</th>
<th>2.41±0.17</th>
<th>1.42±0.09</th>
<th>***</th>
<th>169.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>0.90–3.74</td>
<td>0.540–2.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.66</td>
<td>1.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH-Px (EU/mgHb)</td>
<td>Mean±SE (n.=24)</td>
<td>55.42±1.56</td>
<td>63.46±1.65</td>
<td>***</td>
<td>87.34%</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>42.61–68.49</td>
<td>52.2–75.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>55.365</td>
<td>62.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.O.D (U/L)</td>
<td>Mean±SE (n.=24)</td>
<td>5.81±0.58</td>
<td>5.82±0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1.66–9.96</td>
<td>1.66–9.96</td>
<td>NS</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>6.6</td>
<td>5.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.B.: NO = nitric oxide, LP = lipid peroxidation products. GSH-Px = glutathione peroxidase. SOD = Superoxide dismutase. All data are mean ± SE. SE = Stander error. NS = Non-significant. P < 0.05 = *. P < 0.01 = ** P < 0.001 = ***, n = number of animals.

**Table 6.** Comparison between average values of movement / minute (spontaneous Locomotor activity test) of Parkinson’s model and treated subgroups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 4</th>
<th>Day 11</th>
<th>Day 18</th>
<th>Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPTP</td>
<td>Treated</td>
<td>MPTP</td>
<td>Treated</td>
</tr>
<tr>
<td>Mean</td>
<td>59</td>
<td>56</td>
<td>19</td>
<td>59</td>
</tr>
<tr>
<td>SE</td>
<td>1.4</td>
<td>1.7</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>P Value</td>
<td>N.S.</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

N.B.: All data are mean ± SE NS= non significance, P < 0.001 = ***, P < 0.01 = **, P < 0.5 = *, SE= stander error, N= number of animals.

**Table 7.** Comparison between average values of griping time (seconds) on rotating rods (Rota-Rod test) of treated and Parkinson’s model groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 4</th>
<th>Day 11</th>
<th>Day 18</th>
<th>Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPTP</td>
<td>Treated</td>
<td>MPTP</td>
<td>Treated</td>
</tr>
<tr>
<td>Mean</td>
<td>30</td>
<td>33</td>
<td>25</td>
<td>44</td>
</tr>
<tr>
<td>SE</td>
<td>2.1</td>
<td>2.7</td>
<td>1.2</td>
<td>2.8</td>
</tr>
<tr>
<td>P Value</td>
<td>NS</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

N.B.: NS = non significance, P < 0.00 =***, P < 0.01 =**, P < 0.5 =*, SE= stander error.

**Table 8.** Comparison between average values of movement / minute (spontaneous locomotor activity test) of MPTP, prophylactic and treated groups at the end of the experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MPTP Group</th>
<th>Prophylactic Group</th>
<th>Treated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.5</td>
<td>88</td>
<td>77</td>
</tr>
<tr>
<td>SE</td>
<td>1.2</td>
<td>0.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Table 9. Average values of plasma oxidant indices (NO & LP) and antioxidant enzymes (GSH-Px & SOD) of all treated subgroups.

<table>
<thead>
<tr>
<th>Oxidant &amp; antioxidant</th>
<th>Items</th>
<th>Day 11 Subgroup (A)</th>
<th>Day 18 Subgroup (B)</th>
<th>Day 25 Subgroup (C)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean±SE (n. =8)</td>
<td>Mean±SE (n. =8)</td>
<td>Mean±SE (n. =8)</td>
<td>D11 vs D18</td>
</tr>
<tr>
<td>N.O (µmol/L)</td>
<td>Mean±SE (n. =8)</td>
<td>11.18±0.49</td>
<td>9.39±0.63</td>
<td>7.98±0.38</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>9.00–13.2</td>
<td>6.48–11.85</td>
<td>6.45–9.7</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>10.925</td>
<td>9.225</td>
<td>8.05</td>
<td>NS</td>
</tr>
<tr>
<td>LP (µmol/L)</td>
<td>Mean±SE (n. =8)</td>
<td>6.31±1.32</td>
<td>3.52±0.45</td>
<td>2.42±0.32</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>3.00–12.5</td>
<td>1.73–5.38</td>
<td>1.38–3.84</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>4.7</td>
<td>3.65</td>
<td>2.15</td>
<td>NS</td>
</tr>
<tr>
<td>GSH-Px (EU/mgHb)</td>
<td>Mean±SE (n. =8)</td>
<td>40.25±2.01</td>
<td>43.62±1.38</td>
<td>50.99±2.8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>31.32–47.5</td>
<td>39.3–50.01</td>
<td>35.91–60.0</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>40.846</td>
<td>43.23</td>
<td>51.15</td>
<td>*</td>
</tr>
<tr>
<td>S.O.D (U/L)</td>
<td>Mean±SE (n. =8)</td>
<td>1.22±0.41</td>
<td>2.5±0.77</td>
<td>3.93±0.77</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.00–3.33</td>
<td>0.00–6.66</td>
<td>1.6–6.66</td>
<td>**</td>
</tr>
</tbody>
</table>

N.B.: NO = nitric oxide. LP = lipid peroxidation products. GSH-Px = glutathione peroxidase. SOD = Superoxide dismutase.
All data are mean ± SE. SE = Stander error. NS = Non-significant. P < 0.05 = *. P < 0.01 = ** P < 0.001 = ***, n = number of animals.
Fig. (1): Coronal Section of midbrain of adult male albino mice at the level of superior colliculus in subgroup A of Parkinson’s model group showing cellular swelling, diffuse chromatolysis of nuclear chromatin of dopaminergic neurons of substantia nigra (arrow), disappearance of their nuclei as well as loss of the cytoplasmic Nissle’s granules. (Gallo cyanine chrom-alum stain, X 400).

Fig. (2): Coronal Section of midbrain of melatonin treated mice at the level of superior colliculus showing obvious degeneration and necrosis of red nuclei neurons. Many neurons showed karyolysis of their nuclear chromatin and absence of nucleoli (Arrow), condensation of Nissle’s granules (Arrow head) as well as intercellular edema (Star). (Gallo cyanine chrom-alum stain X 400).
Fig. (3): Coronal section of control adult albino mice midbrain at the level of superior colliculus showing the anatomical localization and distribution of tyrosine hydroxylase positive dopaminergic neurons of SN (Arrow). (ABC technique x 40).

Fig. (4): Coronal section of control adult male albino mice midbrain at the level of superior colliculus showing normal tyrosine hydroxylase positive dopaminergic neurons of SN where peroxidase activity is indicated by brownish cytoplasmic granules (Arrow). (ABC technique x 200).
DISCUSSION

In this study we reviewed the use of different neurotoxic agents as well as variety of animal models for inducing experimental Parkinsonian like syndrome. Systemic administration of MPTP selectively destroyed nigrostriatal dopaminergic neurons in the brain of subhuman primates producing acute, irreversible Parkinson's syndrome that resemble idiopathic Parkinson's disease.27,28

In all Parkinson’s model subgroups, the average values of gripping time (seconds) on rotating rods of Rota rod test showed gradual significant decline throughout the experimental period when compared with that of its control values. These results agreed with the results of Rozas et al.29, and Ogawa, et al.19.

In the present work, histopathological examination of midbrain tissues in Parkinson’s model using Galloctanine chrom-alum stain demonstrated neuropathic alterations both in the substantia nigra and red nuclei of the affected animals. This finding proved that zona compacta of SN is considered the target site for MPTP neurotoxicity. Similar results were reported by Davis et al.30, Burns, et al.27; Marsden31, and Langston et al.32. In addition, pathological changes of red nuclei evident in our study were recorded also by few authors.33,34 This suggests that red nuclei may be involved in pathogenesis of PD.
In our Parkinson’s model group, examination of SN and red nuclei showed drastic degenerative changes of dopaminergic neurons, which were expressed by cell swelling, diffuse chromatolysis, disappearance of the cytoplasmic Nissl’s granules. Many investigators recorded similar finding as: Hallman et al.\textsuperscript{28}, C ochiolo et al.\textsuperscript{35}.

Our results demonstrated proliferation of astroglia and microglia cells, which accompanied the neurodegenerative process of SN and red nuclei in all Parkinson’s model subgroups. Many investigators also mentioned the role of gliosis in neurodegenerative diseases.\textsuperscript{13,36}

The mode of neuronal death in neurodegenerative diseases like Parkinsonism as well as in experimental neurotoxicity is still under debate. Two mechanisms of death are known and subjected to wide range of investigations: necrosis versus apoptosis. In the present study, light microscopic examination of SN and red nuclei of Parkinsonian model group revealed degenerative changes and lytic necrosis of most of dopaminergic neurons that suggested necrosis as the predominant mechanism of neuronal death. However, apoptotic –like neurons appeared sporadically distributed in some cases. To resolve this point, genomic DNA extracted from midbrain tissue samples was subjected to Agarose gel electrophoresis. In addition, in situ end labeling technique (Tunnel technique) was performed on paraffin tissue sections of this group. Typical ladder pattern of DNA fragmentation as well as tunnel positive neurons could not be demonstrated in all subgroups that ruled out apoptosis as a possible mechanism of neuronal death in our in vivo experiments. Our results are in agreement with other in vivo studies of many investigators, Jeon et al.\textsuperscript{37}, and Usha et al.\textsuperscript{38}, who failed to determine apoptotic cells in their experimental studies.

In the present work biochemical analysis of blood samples taken from experimental animals of all Parkinsonian model subgroups showed that plasma either levels of nitric oxide or lipid peroxidation increased gradually throughout the whole experimental period, which proved significant positive correlation with the studied days ($r = 0.8$, $r = 0.87$ respectively) as they increased progressively by time. The increase of plasma nitric oxide and lipid peroxidation was due to oxidative stress induced by MPTP injection and it participated in neuronal damage.

Also, strong significant correlations could be detected between the plasma levels of oxidant indices (NO & LP) and average values of clinical tests (SLMA, griping time and descending time). It may be interpreted that increase NO and LP may participate in loss of DA neurons of SN and depletion of its dopamine release. These changes in chemical indices run parallel with the histopathological, immunohistochemical and clinical results.

Our study revealed that plasma levels of antioxidant enzyme, glutathione peroxidase (GSH-Px) reduced gradually significantly throughout the experimental period. Our results are in agreement with the study of Reiter et al.\textsuperscript{39}, who detected low levels of antioxidative defense system (which included superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase), in nigrostriatal dopaminergic pathway of Parkinsonian patients, that may constitute the main factors of neuronal death in Parkinsonian patients. So, administration of antioxidant agents might be essential for prevention of cell death in Parkinson’s disease.

**Melatonin administration:**

Different antioxidant agents has been used by previous investigators to prevent MPTP induced Parkinsonian like syndrome in mice. However, in our experimental design, we preferred the use of melatonin as antioxidant agent for neuroprotection due to many advantages:

* The endogenous production of melatonin falls dramatically with age so the loss of this antioxidant may contribute to the incidence of some age associated neurodegenerative diseases.\textsuperscript{39}
Melatonin crosses blood brain barrier and after its exogenous administration it is found in high concentrations in the brain.\textsuperscript{40} In addition, it is a universally acting free radical scavenger and antioxidant.\textsuperscript{40} Besides, melatonin has undetectable side effects.\textsuperscript{40}

In the current study, the antioxidant role of melatonin was investigated through different parameters as clinical, biochemical, histopathological, immunohistochemical and molecular studies. All these previous parameters were adopted to define the mechanisms of melatonin neuroprotection against MPTP neurotoxicity. Administration of melatonin to both prophylactic and treated groups in our experiment, improved the average values of various clinical tests significantly when compared with that of Parkinsonian model group. However, the improvement was more obvious in prophylactic than treated group.

**Histopathological and immunohistochemical examinations:**

Histopathological examination of midbrain tissue sections in the therapeutic group of mice revealed persistent neurodegenerative changes of the dopaminergic neurons of SN and red nuclei that indicate the irreversible effects of MPTP on these target sites and failure of melatonin to regenerate these lesioned neurons. The fact that brain cells do not renew after its damage may explain the inability of melatonin to rescue the dopaminergic neurons after MPTP neurotoxicity in the experimental melatonin treated model.

In prophylactic group, melatonin was given as prophylactic agent in association with MPTP administration. Histopathologically, both SN and red nuclei of this group of mice manifested a remarkable neuronal recovery that proved the neuroprotective potential of melatonin coadministration with MPTP neurotoxicity. In addition, melatonin administration to this group preserved the majority of the tyrosine hydroxylase immunoreactive neurons populating the SN and red nuclei as well. Other investigators confirmed neuroprotective effects of melatonin, on nigrostriatal dopaminergic system in the in vivo experiments.\textsuperscript{16,41}

**Biochemical analysis:**

In the present study prophylactic group showed that the average values of plasma nitric oxide decreased significantly when compared with that of Parkinsonian model group at the end of the experiment. Also in treated group (received MPTP then melatonin) the plasma levels of nitric oxide began to decrease significantly after the second week of melatonin treatment when compared with that of Parkinsonian model group and continued to decrease until reached maximum reduction at the end of the experiment.

Our results showed that co-administration of melatonin with MPTP prevented the increase in lipid peroxidation and enhanced antioxidant enzymes in striatum, hippocampus and midbrain of mice. Similar findings were recorded by other investigators.\textsuperscript{12,42}

**REFERENCES**


