Neuroprotective Effects of Pramipexole in Parkinson's Disease Induced by Rotenone in Mice

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Abstract

Background: Pramipexole (PPX), an agonist for Dopamine (DA), has been used to treat both early and advanced Parkinson's Disease (PD).

Objectives: This study was done to evaluate the neuroprotective effect of PPX in a DA neuron degeneration model of PD induced by rotenone.

Methods: Thirty six male mice were used and divided into three equal groups. The first group, the control group, received only the vehicle (sunflower oil) for a period of 7 weeks (49 days). The second group received rotenone (2mg/kg; IP) dissolved in sunflower oil daily for 49 days. The third group received a combined treatment of rotenone (2mg/kg, IP) and PPX (1mg/kg, IP) daily for 49 days. Behavioral tests were performed a day prior to drug administration and then once weekly along the duration of drugs or vehicle administration. At the end of the 49 days all animals were sacrificed and their midbrains were subjected to immunohistochemical analysis for dopaminergic neurons staining for Anti-Tyrosine Hydroxylase (TH) antibodies. Midbrain tissues were also isolated for biochemical measurements.

Results: Behavioral tests revealed that PPX counteracted the rotenone effect in mice locomotor activity and catalepsy. Immunohistochemistry results showed that PPX inhibited the rotenone-induced loss of TH-immunopositive neurons in the substantia nigra pars compacta. Biochemical measurements demonstrated that PPX treatment significantly reversed the rotenone-induced decrease in midbrain DA level. Interestingly, PPX ameliorate the rotenone-induced increase in the malondialdehyde and nitric oxide levels as well as the decrease in total antioxidant capacity.

Conclusion: The results indicate the beneficial effect of PPX against the dopaminergic neurodegeneration induced by chronic IP administration of rotenone. This neuroprotective effect seems to be mediated by inhibition of rotenone induced oxidative stress and nitric oxide overproduction and through maintenance of the cellular antioxidant status.


Introduction

PARKINSON'S Disease (PD) is one of the leading causes of neurologic disability in elderly [1]. Its pathological hallmark is the specific and progressive degeneration of dopaminergic neurons in the Substantia Nigra Pars compacta (SNpc) [2] which results in extrapyramidal motor dysfunction accompanied by progressive impairment of autonomy, mood, and cognitive functions [3-5]. Although the mechanisms of neurodegeneration in PD are not fully understood, oxidative stress, excessive free-radical formation, environmental toxins, and/or endogenous neurotoxins may be involved in the progressive loss of nigrostriatal Dopamine (DA) neurons [6,7].

Rotenone is widely used as an insecticide and fish poison. Several studies reported that chronic exposure to rotenone causes highly selective nigrostriatal dopaminergic degeneration that is associated with neurochemical, behavioral and neuropathological features of PD [8,9]. Previous studies found that rotenone causes a partial inhibition of mitochondrial complex i which in turn leads to increased oxidative stress [8,10,11]. Rotenone may induce superoxide production in cultured microglia [12]. The increased production of ROS may result in oxidative damage to the proteins and DNA of neural cells and cause the cells to undergo dysfunction and eventually lead to necrosis or apoptosis [8]. Moreover, chronic rotenone administration can lead to significant injury to the nigrostriatal system, mediated by increased generation of nitric oxide [13]. ROS, especially superoxide and hydroxyl radicals, can interact with nitric oxide to form peroxynitrite [12,14]. The potent oxidant peroxynitrite may directly oxidize DA and lead to the dopaminergic neurons damage [15,16]. Nitric oxide fosters dopamine depletion and is associated with
neurotoxic processes underlying PD [17]. Therefore, strategies that protect cells at the level of the mitochondria appear to have protective potential in PD [18].

Pramipexole (PPX) is a synthetic amino-benzothiazole derivative. It has been shown to be a selective and specific full DA receptor agonist with high affinity and selectivity for the DA D2 receptor subfamily, and particularly the D3 receptor subtype. Pramipexole is a non-ergot dopamine agonist with actions similar to those of bromocriptine. PPX is used successfully in the treatment of Parkinson's disease [19]. Previous studies reported that PPX protects against MPTP, 6-hydroxydopamine, levodopa, glutamate and metamphet-amine-induced neurodegeneration [20-25].

Various studies were performed to clarify the possible neuroprotective mechanism of PPX. Many authors reported that PPX neuroprotective effect is directed to specific sites on dopamine neurons and receptors, which is an action that it does not share with bromocriptine and pergolide suggested that PPX, like other dopamine agonists, reduces dopamine synthesis turnover in mice during repeated injections of amphetamine and, thus, reduces the free radical formation during this process [26-29]. This is considered to contribute to the neuroprotective effect of PPX. Other investigators reported that PPX attenuates intracellular processes such as the mitochondrial transition pore opening that is associated with programmed cell death [30,31]. Du and colleagues indicated that PPX stimulates a mesencephalic-derived neurotrophic activity [32]. Pan and colleagues reported that PPX has biological regulatory effects on dopaminergic neuron-associated genes, which may explain both the slower decline of imaged dopamine transporter and the neuroprotective effect [33]. Further, Izumi and colleagues concluded that PPX has a neuroprotective effect and protects dopaminergic neurons from glutamate neurotoxicity by the reduction of intracellular dopamine content, independently of dopamine D2-like receptor activation [24]. In vitro and in vivo studies of PPX in Parkinson disease models show that it decreases the phosphorylation of alpha-synuclein, which may contribute to its neuroprotective properties [34].

In view of the putative neuroprotective effects of PPX reported elsewhere, the present study was designed to evaluate the possible neuroprotective effects of pramipexole in rotenone-induced PD in mice and to clarify the possible mechanism underlying the evaluated protective effects.

Material and Methods

I- Drugs and chemicals:

- Rotenone and pramipexole were obtained from Sigma-Aldrich (China). Rotenone was dissolved in sunflower oil. Pramipexole was dissolved in saline to obtain the necessary doses. Mouse dopamine ELISA kit was obtained from (Chongqing Biospes Co., Ltd., China). Total antioxidant capacity kit was obtained from (Biodiagnostic, Egypt). Tyrosine hydroxylase antibody kit was obtained from (Geneexpression Biotechnologies, USA). 2-Thiobarbituric acid was obtained from (Novus USA). Power-StainTM 1.0 poly HRP DAB kit was obtained from (MP Biomedicals Inc., France). Malondialdehyde bis-(dimethylacetal) was obtained from (Merk, Germany).

II- Animals:

- Thirty six (36) male mice of average weight 25-35g were purchased from Assiut University Animal House from March 2014 to January 2015. Mice were housed in groups in clean capacious macrolene cages (up to 4 per cage) under standard laboratory conditions including aerated room with suitable temperature (25 ±5ºC) maintained at good light. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol of the research was approved by the Ethical Committee for Research at Assiut University. Mice were divided into 3 groups. Each group contains 12 animals as follows:

  Group 1: Control:
  - Mice received the vehicle (sunflower oil 4ml/kg, IP) daily for a period of 7 weeks (49 days).

  Group 2: Rotenone treated:
  - Mice received rotenone (2mg/kg, IP) daily for a period of 7 weeks (49 days).

  Group 3: Rotenone plus PPX:
  - Mice received rotenone (2 mg/kg, IP) and PPX (1 mg/kg, IP) daily for a period of 7 weeks (49 days).

III- Behavioral assessment:

- Behavioral tests were performed a day prior to drug administration and then once weekly along the duration of the experiments (7 weeks). All mice groups were subjected to the following behavioral tests:
  - Locomotor activity. The locomotor activity of the tested animals was quantitatively estimated...
by using an activity cage (Model 7401, Ugo basile, Comerio, Italy). The activity was monitored continuously for a period of 5 minutes. Locomotion was expressed in terms of total photo beam crossing counts per 5 minutes per animal [38].

- Exploratory behavior (rearing). When placed in a clear cylinder, animals will engage in exploratory behavior, including rearing. During rearing behavior, the forelimbs will contact the wall of the cylinder. For this test, the animals was placed in a clear plexiglass cylinder (height = 30cm, diameter = 20cm) for 5 minutes. To be classified as a rear, the animal had to raise forelimbs above shoulder level and make contact with the cylinder wall with either one or both forelimbs. Removal of both forelimbs from the cylinder wall and contact with the table surface was required before another rear was scored [36].

- Catalepsy. Each animal was hung by the paws on a vertical grid. The vertical grid apparatus is an open box of 8cm X 55cm X 5cm, set vertically. The back side of the vertically standing box is made of a wire mesh of 0.8cm X 0.8cm, the front side is open and the other sides are made of black plexiglass. For stability, the bottom of the apparatus has a 5cm extension to the front. In the experiment, each mouse was carefully placed inside the apparatus at 3cm from the top, facing upward, and was left free to turn around and climb down. The trials were videotaped. The videos were replayed for recording the total time taken for the mouse to make a turn, climb down and reach the floor by its forepaw [37].

IV- Biochemical estimations:

After performance of behavioral tests, animals were sacrificed by decapitation. Midbrain tissues were obtained from each mouse for biochemical measurements. The midbrain was rinsed in ice-cold saline, blotted carefully and weighed. Then the midbrain was placed in a glass homogenizer containing 2ml saline. After homogenization, by using motor-driven Teflon pestle (Glas-Col, USA), the homogenate was centrifuged for 10 minutes at 1000rpm and the supernatant was used for estimation of dopamine and Malondialdehyde (MDA) levels as well as total antioxidant capacity.

- Determination of midbrain dopamine level:

Dopamine was estimated by use of Enzyme-linked Immune-Sorbert Assay (ELISA) technology as described by Cavalcanti et al., [38].

- Determination of Midbrain Malondialdehyde (MDA) Level:

Malondialdehyde (MDA), an end product of lipid peroxidation, is a major indicator of oxidative stress and its level was determined in tissue homogenates spectrophotometrically by use of thiobarbituric acid reactive substances method previously described by Ohkawa et al., [39].

- Determination of the midbrain nitrite level:

Nitric oxide was measured as its stable oxidative metabolite nitrite. Serum nitrite concentration was assayed by using Griess reagents as described by Green et al., [40].

- Determination of midbrain total antioxidant capacity:

The determination of the anti-oxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H₂O₂). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H₂O₂ is determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro-2-hydroxy benzensulphonate to a colored product [41].

V- Immunohistochemistry:

Sections from mid brain were trimmed after fixation in 10% formalin for 8 hours, dehydrated, and paraffin embedded. Cutting of blocks was nearly at the same level. Four gm sections were mounted on positively-charged Super frost slides (Thermo Scientific, Menzel, Braunschweig, Germany). The sections were deparaffinized in xylene for 30 minutes, rehydrated in descending graded alcohol up to distilled water. Antigen retrieval was done by 10mm sodium citrate buffer solution, pH 6.0 for 12 minutes. The sections were incubated with monoclonal mouse anti-human antibody against tyrosine hydroxylase protein at 1:50 dilution (Code no. NB100-2051, Novus USA). The resulting immune complex was detected by use of HRP DAB kit (Cat. no. 52-0017, Genemed Biotechnologies, USA) at room temperature and following the instructions attached with the kit. Negative and positive controls were included in each staining series. Mouse substantia nigra was used as positive controls.

Evaluation of Tyrosine Hydroxylase (TH)-positive neurons: The positivity for TH was detected as brownish staining of the cytoplasm of neurons [42]. The total numbers of TH-stained neurons were counted independently by two observers (Dr. Dalia Elsers and Dr. Najla) blinded to the group under a light microscope (40X magnification). The mean
VI- Statistical analysis:

The data were collected, tabulated and statistically analyzed, using a personal computer with "Statistical Package for the Social Sciences" (SPSS), version 19, (SPSS Inc., Chicago Illinois USA) for windows and GraphPad Prism version 5.00 for Windows, (Graph Pad Software, San Diego California USA). The quantitative data were presented in the form of mean ± standard error of mean (S.E.). The significance of differences was determined by an analysis of variance (ANOVA). Further statistical analysis for post hoc comparisons was performed using the Least Significant Difference (LSD) test. The difference considered statistically significant at $p<0.05$ and highly significant at $p<0.01$.

Results

1- Behavioral assesment:

• Locomotor activity:

The locomotor activity of mice received rotenone was significantly lower than that of vehicle-treated control group with $p<0.01$. This reduction started on day 14 of our experiment and continued till the day 49 when the least activity was recorded. However, mice received PPX plus rotenone showed a significant increase in spontaneous locomotor activity in comparison to rotenone-only group with $p<0.01$. This increase was observed on the day 28 and continued till the day 49 (Table 1).

• Exploratory behavior (rearing):

Rotenone treated group showed a significant reduction in exploratory behavior in comparison to vehicle-treated control group with $p<0.01$. This reduction started at the day 28 and continued till the day 49 when least activity was recorded. On the day 28, treated group with PPX plus rotenone showed a significant increase in exploratory behavior in comparison to rotenone-only group with $p<0.01$ (Table 2).

• Catalepsy:

On performance of the grid test, rotenone treated group showed a significant increase in descending time latency as compared to vehicle-treated control group with $p<0.01$, this increase was noticed on the day 14. However, PPX treated group showed a significant decrease in descending latency time as compared to rotenone-treated group with $p<0.01$. This reduction started on the day 21 and reached its maximum on the day 49 (Table 3).

2- Midbrain dopamine level:

Chronic IP rotenone administration was associated with significant ($p<0.01$) decrease in midbrain dopamine level in comparison to vehicle-treated group. Simultaneous administration of PPX and rotenone was associated with a highly significant elevation of midbrain dopamine level as compared with rotenone only group with $p<0.01$ (Table 4).

3- Immunohistochemistry:

Tyrosine-Hydroxylase (TH) Immunohistochemical examinations demonstrated a significant ($p<0.01$) decrease in number of TH-immunopositive neurons in rotenone treated group in comparison to vehicle treated control group. However, concurrent administration of pramipexole with rotenone was associated with a significant ($p<0.01$) increase of TH-immunopositive neurons number as compared to rotenone treated group Fig. (1A-D).

4- Oxidative stress parameters:

• Midbrain Malondialdehyde Level (MDA):

Induction of PD in mice was associated with a significant increase in midbrain MDA level when compared with vehicle-treated control group with $p>0.01$. However, concomitant administration of PPX and rotenone was associated with significant decrease in midbrain MDA level as compared to rotenone only treated group with $p>0.01$ Fig. (2A).

• Midbrain nitrite level:

Chronic IP administration was associated with highly significant increase in midbrain nitrite (the major metabolite of nitric oxide) level when compared with control group with $p>0.01$. In contrast, administration of PPX in concomitant with rotenone was associated with significant decrease in midbrain nitrite level as compared to rotenone only treated group with $p>0.05$ Fig. (2B).

• Midbrain total antioxidant capacity:

Induction of Parkinsonian-like disorder by use of rotenone was associated with highly significant decrease in midbrain total antioxidant capacity when compared with vehicle-treated control group with $p>0.01$. However, concomitant administration of PPX with rotenone was associated with a significant increase in midbrain total antioxidants capacity as compared to rotenone only treated group with $p>0.05$ Fig. (2C).
Fig. (1): Expression of Tyrosine Hydroxylase (TH) in studied groups. (A) Shows TH-positive neurons in normal mice (control group). (B) Demonstrates decreased number of TH-positive neurons in the rotenone group. (C,D) Show increased number of TH-positive neurons in comparison with the rotenone group and nearly similar to control group.

Fig. (2): Effects of rotenone and Pramipexole (PPX) on: Mid brain Malondialdehyde (MDA) level (A), mid brain nitrite level (B), and mid brain total antioxidant capacity (C). Animals were treated with vehicle (control), rotenone (2mg/kg), or rotenone (2mg/kg) and PPX (1mg/kg). Data represent mean with S.E. of 6 mice analyzed using one way ANOVA followed by LSD test.

**: p > 0.01 highly significant difference as compared to vehicle control group.
#: p > 0.05 significant difference as compared to rotenone group.
##: p > 0.01 highly significant difference as compared to rotenone group.
**Table (1): Effect of pramipexole on rotenone-induced alteration in locomotor activity.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Locomotor activity (frequency)</th>
<th>Day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>269.6</td>
</tr>
<tr>
<td><strong>Rotenone</strong></td>
<td></td>
<td>218.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>211.7</td>
</tr>
<tr>
<td><strong>Pramipexole + Rotenone</strong></td>
<td></td>
<td>215.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>135.6</td>
</tr>
</tbody>
</table>

Animals were treated with vehicle (control), rotenone (2mg/kg), or rotenone (2mg/kg) and PPX (1mg/kg).

Data represent mean ± S.E. of (6-8) mice.

*p<0.05 significant as compared to control of respective week.

*p<0.05 significant as compared to rotenone treated group of respective week.

(a, b, c, d, e, f, g) *p<0.05 significant as compared to 1 st, 7 th, 14 th, 21 st, 28 th, 35 th, 42 th respectively day observations of same treatment group.

**Table (2): Effect of pramipexole on rotenone-induced alteration in exploratory behavior.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rearing (frequency)</th>
<th>Day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td>28.5±2.9</td>
</tr>
<tr>
<td><strong>Rotenone</strong></td>
<td></td>
<td>26.1±1.6</td>
</tr>
<tr>
<td><strong>Pramipexole + Rotenone</strong></td>
<td></td>
<td>31.1±2.8</td>
</tr>
</tbody>
</table>

Animals were treated with vehicle (control), rotenone (2mg/kg), or rotenone (2mg/kg) and PPX (1mg/kg).

Data represent mean ± S.E. of (6-8) mice.

*p<0.05 significant as compared to control of respective week.

*p<0.05 significant as compared to rotenone treated group of respective week.

(a, b, c, d, e, f, g) *p<0.05 significant as compared to 1 st, 7 th, 14 th, 21 st, 28 th, 35 th, 42 th respectively day observations of same treatment group.

**Table (3): Effect of pramipexole on rotenone-induced alteration in descending latency off a vertical grid (catalepsy).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Descending latency (sec)</th>
<th>Day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td>9.4±0.8</td>
</tr>
<tr>
<td><strong>Rotenone</strong></td>
<td></td>
<td>9.5±0.8</td>
</tr>
<tr>
<td><strong>Pramipexole + Rotenone</strong></td>
<td></td>
<td>9.1±0.9</td>
</tr>
<tr>
<td><strong>Rasagiline + Rotenone</strong></td>
<td></td>
<td>10.0±1.0</td>
</tr>
</tbody>
</table>

Animals were treated with vehicle (control), rotenone (2mg/kg), rotenone (2mg/kg) and PPX (1mg/kg).

Data represent mean ± S.E. of (6-8) mice.

*p<0.05 significant as compared to control of respective week.

*p<0.05 significant as compared to rotenone treated group of respective week.

(a, b, c, d, e, f, g) *p<0.05 significant as compared to 1 st, 7 th, 14 th, 21 st, 28 th, 35 th, 42 th respectively day observations of same treatment group.

**Table (4): Effect of pramipexole on rotenone-induced alteration in midbrain dopamine level.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Midbrain dopamine level (µg/mg.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.67±0.16</td>
</tr>
<tr>
<td>Rotenone</td>
<td>0.69±0.06**</td>
</tr>
<tr>
<td>Pramipexole + Rotenone</td>
<td>1.77±0.09##</td>
</tr>
</tbody>
</table>

Animals were treated with vehicle (control), rotenone (2mg/kg), or rotenone (2mg/kg) and PPX (1mg/kg).

Data represent mean ± S.E. of (6-8) mice.

**Discussion**

Parkinson’s Disease (PD) a neurodegenerative disorder for which no preventive or long-term effective treatment strategies are available. Epidemiologic studies have failed to identify specific environmental, dietary or lifestyle risk factors for PD [44]. However, oxidative stress in the SN is the most broadly accepted hypothesis for the etiopathology of PD [45].
Rotenone is well known complex inhibitor that reproduces features of Parkinson's disease [36]. In the present study we induced effective progressive PD manifestations that can be seen from the behavioral, biochemical and immunohistochemical examination results. Behavioral assessment revealed that chronic intraperitoneal administration of rotenone causes loss of spontaneous locomotor activities, decline in exploratory behavior and catalepsy. These results coincide with the observation of other investigators [9,35,37,46]. Biochemical examination showed that chronic rotenone administration caused a significant decline in the level of neurotransmitter dopamine in midbrain region. This study result was further supported by earlier and more recent study reports showing decline in the neurotransmitter DA level following rotenone exposure [35,47,48]. Immunohistochemical examination showed that systemic administration of rotenone resulted in nigrostriatal cell damage and decreased tyrosine hydroxylase immunoreactivity in the SN, this result was consistent with results of previous studies [25,49-51]. However, other researchers have found that the effect of systemic administration of rotenone in rodents is widespread [52]. The damage inflicted by rotenone on the brain is thus may not limited to the nigrostriatal pathway. Nevertheless, our results demonstrate that chronic intraperitoneal administration of rotenone causes nigrostriatal dopaminergic degeneration that is associated with biochemical and behavioral features of PD.

Pramipexole (PPX), a non-ergot dopamine receptor agonist, has been used in the management of Parkinson's disease, alone or as an adjunct to levodopa therapy in more advanced stages of the disease [53]. In the present study PPX administration produces a significant beneficial effect on locomotor impairment induced by rotenone as it prevented the loss of spontaneous locomotor activities, the decline in exploratory behavior and the increase in cataleptic behavior; this finding is consistent with the findings of Gad El-Hak et al., (2012) [37]. Moreover, PPX counteracted the decrease in the level of dopamine neurotransmitter in midbrain region; this result is consistent with that of Anderson et al., (2001) [54]. Furthermore, PPX prevented the loss of dopaminergic neurons in the SNpc of rotenone-treated mice; this result is agreed with that of Inden et al., (2009) [25] and Gad El-Hak et al., (2012) [37]. These findings are related with that of Kim et al., (2015) [55] who suggested that PPX may protect DA neurons from the damaging effect of 6-hydroxydopamine (6-OHDA).

Concerning oxidative stress, the present study revealed that chronic rotenone administration caused elevation in MDA and nitrite levels as well as decline in total antioxidant capacity in midbrain region. The significant elevation in the level of MDA (a marker of lipid peroxidation, which indicates increased free radical production with consequent attack on membrane lipids) was in accordance with several in vitro and in vivo rotenone models consistently demonstrate an increase in lipid peroxidation [48,51,56,57]. The level of nitrite which is the major nitric oxide metabolite was also increased; this increase is most likely due to elevated nitric oxide production due to induction of the inducible form of Nitric Oxide Synthase (iNOS) [58]. The finding of elevated nitrite level was consistent with findings of Bashkatova et al., [59] and Abdel-Salam et al., [51]. Moreover, the increase in oxidative stress induced by rotenone was also demonstrated by decreased total antioxidant capacity, suggesting consumption of these scavenger molecules by free radical excess. This decline in total antioxidant capacity was in line with the observations recorded by Kaur et al., [57] and Abdel-Salam et al., [51].

The present study revealed that simultaneous daily administration of PPX with rotenone inhibited the remarkable increase in the mid brain Malondialdehyde and nitrite levels as well as the decrease in mid brain total antioxidant capacity. These results suggesting decreased oxidative and nitrosative stress on administration of PPX. These findings were further supported by previous and recent findings showing that PPX possess antioxidant properties [20,23-25,60-64]. Furthermore, our results were related with that of Uberti et al., [65] who demonstrated that PPX inhibited generation of H2O2-induced reactive oxygen species in SH-SY5Y neuroblastoma cell in a DA receptor independent way. Similar findings were obtained by Ferrari-Toninelli et al., [66] reported that both S (−) and R (+) pramipexole enantiomers are mitochondria-targeted antioxidants and suggested that the antioxidant, neuroprotective activity of these drugs is independent of both the chiral 6-propyl amino group in the pramipexole molecule and the DA receptor stimulation.

In conclusion, these results suggest that PPX has neuroprotective effect against the dopaminergic neurodegeneration induced by chronic IP administration of rotenone. This neuroprotective effect may be mediated by inhibition of rotenone induced oxidative stress and nitric oxide overproduction and through maintenance of the cellular antioxidant status.
References


