A study on the effects of rotenone on the midbrain in the adult male albino rats

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Abstract

**Background aims.** Rotenone is a widely used insecticide has a neurodegenerative effect on the dopaminergic cells of substantia nigra (SN) of midbrain producing Parkinsonism. The aim of this study is to study the effects of rotenone when injected subcutaneously on the dopaminergic cells of the substantia nigra of the midbrain.

**Methods.** The effects of rotenone on the SN of midbrain and the were determined histopathologically, immunohistochemically, and by transmission electron microscopy.

**Results.** Subcutaneous rotenone produced Parkinsonism through producing degeneration of the dopaminergic cells of SN of the midbrain. **Conclusion.** These results indicate that, rotenone has a neurodegenerative effect on the dopaminergic cells of the substantia nigra of the midbrain producing parkinsonism.

**Key words:** Dopaminergic neurons, neurodegeneration, Parkinsonism, rotenone, Substantia nigra.

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Introduction

Rotenone is an effective natural broad spectrum pesticide and insecticide [1] that is extracted from the roots of certain tropical and subtropical legume plants and widely used all over the world since decades. Its use in agriculture can cause serious pollution of streams and reservoirs since it can easily reach the soil with heavy rains [2]. The degradation can sometimes persist for months according to a variety of factors including light, temperature, depth in the soil, dose and the presence of organic debris, for example decomposition occurs faster as the temperature of the water is higher [3]. It is often formulated as dusts, powders and sprays for use in gardens and on food crops [4]. It is whitish in color and odorless [5].

Rotenone is a potential cause of Parkinsonism because the long-term exposure to rotenone causes cellular changes in the form of injury to central dopaminergic neurons, degeneration and apoptosis of substantia nigra dopaminergic neurons and the formation of Lewy bodies in neurons indicating neurodegenerative effects [6]. Parkinsonism is the second most prevalent neurodegenerative disease after Alzheimer's disease [7] and the first most common motor neurodegenerative disease [8] affecting about 1–3% of the population over 50 years of age and 5% of the population...
over 65 [9] as its incidence and severity increases with age [10] and consistently affects males more than females and has a genetic susceptibility leading to familial and sporadic cases of Parkinsonism [11]. Also it was evidenced that Parkinsonism is more common in diabetics than non-diabetics and more in cases of uncontrolled diabetes than whose diabetes is well managed and controlled because diabetes helps the damage of nerve cells while the antidiabetic drugs suppress the progression of Parkinsonism through the inhibition of microglial activation [12]. In addition, Parkinsonism is 12% higher in urban than rural areas [13] and is heritable only in approximately 5% of familial cases [14].

Material and methods
Twenty adult male albino rats were used in this study of two months age and each weighing 200 - 250 grams. The animals were divided into two groups (One of them was the control group while the other was the experimental one in which the animals were treated by rotenone for induction of Parkinsonism) each of them consists of ten rats.

Induction of Parkinsonism
Parkinsonism was induced by rotenone (Sigma-Aldrich, St. Louis, MO, USA). Rotenone was dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) and injected subcutaneously in a dose of 1.5 mg/kg body weight every forty eight hours for six doses [15].

Histopathological evaluation of midbrain
Haematoxylin and eosin staining was used for morphological assessment and for the detection and evaluation of the dopaminergic melanin pigment producing cells in the SN of midbrain [16].

Electron microscopy
Ultrathin sections stained by uranyl acetate and lead citrate were examined using a TEM 10 transmission electron microscope (Zeiss, Jena, Germany) to evaluate the ultrastructural changes of the dopaminergic cells of the substantia nigra [17, 18].

Results
Histopathological examination:
A) Results of hematoxylin and eosin:
In the control group, the SN of midbrain appeared normal with the presence of the characteristic densely packed dopaminergic cells (Figure 1 a), while in the group exposed to rotenone, there was marked reduction in the number of dopaminergic cells in the SN with numerous vacuoles (Figure 1 b).

(Figure 1). Photomicrographs of midbrain sections of male albino rats stained with hematoxylin & eosin showing normal structure with dopaminergic cells (arrows) containing melanin pigment in the SN in the control group (A), while in the group exposed to rotenone
(B), there is decreased number of dopaminergic cells [D] and the appearance of degenerative vacuoles [V]. Scale bar 10 μm. X 100.

**B) Results of transmission electron microscopy (TEM):**

In the control group, the SN showed normal mitochondria with normal membranes and cristae and normal nuclear morphology with dispersed chromatin. Also, the myelin sheaths around the axons appeared normal i.e. thick, regular and continuous (Figure 2 a). In the rotenone group, there was degeneration of the dopaminergic cells of SN in the form of mitochondrial distension and ruptured cisterns. The cytoplasm showed vacuoles. Also there were thinned interrupted myelin sheaths around the axons (Figure 2 b).

(Figure 2). Transmission electron micrographs of the midbrain of male albino rats showing a dopaminergic cell with normal nucleus [N], nuclear homogenously distributed chromatin [C], normal mitochondria [M], Golgi apparatus [G] and normal myelin sheaths [S] i.e. thick, regular and continuous in the control group micrograph (A). Scale bar 500 μm. X 30000. While the rotenone group micrographs (B) showing degenerative changes; condensate nuclear chromatin [C] in the center of the nucleus [N], degenerated mitochondria [M] (Distended with ruptured cisterns), numerous degenerative cytoplasmic vacuoles [V] and the myelin sheaths [S] are thin, irregular and discontinuous. Scale bar 2 μm. X 10000.

**Motor evaluation (Rota rod test)**

The motor balance and motor coordination were evaluated by rota-rod test in which each rat was placed on a horizontal rotating rod and the latency (Time in seconds spent from putting the animal on the rotating rod till it falls to the ground) was recorded. The results indicated highly significant drop of the rota-rod test in group B (The rotenone group) compared to group A (The control group) indicating motor disturbances of Parkinsonism induced by rotenone. In group C (The OSCs-treated group) there was a marked motor improvement when compared to group B indicating the ability of OSCs to reach the substantia nigra of midbrain to replace the dopaminergic cells damaged by rotenone as shown in **table 1 & figure 5**.

**Table 1:** Mean ± SD of the rota-rod test of the three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
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<tbody>
<tr>
<td>Group A (Control Group)</td>
<td>63.9000</td>
<td>9.96048</td>
</tr>
<tr>
<td>Group B (Rotenone Group)</td>
<td>20.6000</td>
<td>6.96340</td>
</tr>
<tr>
<td>Group C (OSCs-treated group)</td>
<td>46.1000</td>
<td>6.33246</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>43.5333</td>
<td>19.62007</td>
</tr>
</tbody>
</table>

ANOVA = 75.665, P value <0.001 (HS)

**Discussion**

In the current study, rotenone was used to induce Parkinsonism because rotenone is a widely used broad spectrum insecticide and pesticide and easily produced by extraction from the roots and stems of several tropical and subtropical plant species, especially those belonging to the genera Lonchocarpus and Derris [19]. Rotenone can be absorbed
through the gastrointestinal mucosa if ingested orally [20].

In agreement with our study, Schuler and Casida, (2001) [21] detected that rotenone administration leads to the appearance of motor and non-motor parkinsonian manifestations as it inhibits the activity of NADH-ubiquinone reductase leading to the formation of reactive species, such as superoxide anions, and the formation of these reactive species affects the dopaminergic cells of SNpc seriously because of the high levels of metabolism in substantia nigra, the high prevalence of glial cells, and particularly the low levels of antioxidant defenses in this region. Also, Pan et al. (2009) [22] have shown that rotenone induces neuronal degeneration through the induction of apoptosis and oxidative stress and it produces Parkinsonism through highly selective neurodegeneration of dopaminergic cells.

In our study, injection of rotenone into the rats resulted in degenerative effects on the substantia nigra of the midbrain specimens. These neurodegenerative changes were in the form of marked reduction in the number of dopaminergic cells. This was in accordance with Alam and Schmidt, (2002) [23] as they detected that, rotenone destroys dopaminergic neurons of SNpc leading to depletion of the neurotransmitter dopamine with appearance of the parkinsonian symptoms in rats. Also, Kim et al. (2009) [24] explained that, rotenone crosses the blood brain barrier to enter the nerve cells then, inside the nerve cells, rotenone inhibits mitochondrial respiration, thus elicits ATP deficiency, produces reactive oxygen species, and disrupts Ca2+ homeostasis, downstream mitochondrial damage leading to release of cytochrome C and caspase-3 activation and finally causing apoptotic death.

Another finding was mitochondrial degeneration in the form of swollen vacuolated mitochondria with loss of their cisternae. This is in agreement with the findings of Dauer and Przedborski, (2003) [6] who detected that pathogenesis of Parkinsonism includes oxidative stress in the form of mitochondrial dysfunction and degeneration, and with Cabezas et al. (2012) [25]. Concerning the functional motor evaluation using the open field test and rota-rod test, the current findings were in agreement with the results of many previous studies such as those done by Fleming et al. (2013) [22].

Conclusion

The current study, rotenone that is a widely used insecticide and pesticide has a neurodegenerative effect on the dopamine producing cells of the substantia nigra of the midbrain leading to Parkinsonism.

References


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