**Protective Effect of Ginkgo Biloba Extract and Pumpkin Seed Oil Against Neurotoxicity of Rotenone in Adult Male Rats**


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**Abstract:** The principal goal of the current work was to investigate the protective effect of Ginkgo biloba (Egb) extract and pumpkin seed oil in separate against rotenone induced neurotoxicity in male rats. Adult male albino rats were orally treated with Ginkgo biloba (Egb) extract (Egb) at a dose of 150 mg/kg body weight or with pumpkin seed oil (PSO) at a dose of 40 mg/kg body weight once a day for 70 days and on the 21st day, rotenone (2.5 mg/kg body weight) was given orally for 50 days. Dopamine (DA) and norepinephrine (NE) contents in striatum, cerebellum and cerebral cortex were determined on 40, 55 and 70 days of treatment. Also, the striatum Na⁺/K⁺-ATPase activity, serum and striatum lipid peroxidation, nitric oxide (NO), reduced glutathione (GSH), total antioxidant capacity (TAC), and serum testosterone level were evaluated with histological investigation of striatum. Results revealed that rotenone administration for 50 days produced significant increase in striatum and serum lipid peroxidation and NO levels, while, significant decrease in DA in striatum and cerebral cortex was detected. Also, striatum Na⁺/K⁺-ATPase activity, serum and striatum GSH, TAC levels and serum testosterone level were significantly decreased as a result of rotenone administration. On the other hand, the administration of Egb or PSO resulted in marked improvement in the all studied parameters. Noteworthy, Egb produced more pronounced protective effect against rotenone-induced neurotoxicity than PSO. In conclusion, the present study provided clear evidence that Egb possesses a promising activity against rotenone-induced neurodegeneration. Thus, it may be useful against neurotoxicity induced by environmental neurotoxins. Our study also suggested the possibility of PSO usefulness in limiting toxicant-induced oxidative stress.

**Key words:** Ginkgo biloba extract; Pumpkin seed oil; Rotenone; Neurotoxicity; Dopamine; Norepinephrine; Oxidative stress.

**INTRODUCTION**

Brains of patients suffering from neurodegenerative diseases undergo many changes, such as the degradation of neural membrane glycerophospholipids, the disruption of protein synthesis and degradation, and the generation of reactive oxygen species (ROS) and reactive nitrogen species. Among these, oxidative stress and nitrosative stress are major factors that affect the death process.¹,²,³

Parkinson’s disease (PD) is regarded as the second most common neurodegenerative disorder in humans and it affects about 2% of the population over the age of 60 years. Clinically, PD is a disorder of motor function characterized by tremor, slow and decreased movement (bradykinesia), muscular rigidity, poor balance and problems in gait.⁴ Pathologically, PD patients possess loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and frequently have Lewy bodies, eosinophilic intracellular inclusions composed of amyloid-like fibers and α-synuclein.⁵ PD may have a genetic basis for susceptibility for an early onset form but the occurrence of the more prevalent late onset form does not have an established genetic basis.⁶

A reduction of complex I activity has been demonstrated in the mitochondria of PD patients,⁶ and the complex I inhibitors such as environmental toxins are involved in some cases of PD. Factors such as drinking water from wells and exposure to agricultural chemicals have been investigated and claimed as support for an association between pesticide and increased PD.⁶ It is well known that the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MTPP) after its conversion to one of the most prominent mitochondrial complex I inhibitors 1-methyl-4-phenylpyridinium (MPP⁺) produces PD symptoms with severe motor symptoms, striatal dopamine depletion and loss of tyrosine hydroxylase immunoreactivity in humans, monkeys and various other species.⁷,⁸ Previous studies demonstrated that chronic systemic exposure to rotenone (an inhibitor of mitochondrial NADH dehydrogenase, a naturally occurring toxin and a commonly used pesticide)
reproduces many features of PD in rats, including nigrostriatal dopaminergic degeneration and formation of alpha-synuclein-positive cytoplasmic inclusions in nigral neurons.[6,9]

Rotenone, a naturally occurring compound derived from the roots of several tropical and subtropical plant species belonging to the genus *Derris* and *Lonchorcarpus*, is the active ingredient in hundreds of pesticide products widely used as a household insecticide and as a tool for eradicating nuisance fish populations in lakes and reservoirs.[9] Rotenone is extremely hydrophobic, and it crosses biological membranes easily for access to the cytoplasm of dopaminergic neurons.[9,10]

Rotenone has been shown to lead to selective dopaminergic cell death in vivo[9,10] and selective nigrostriatal dopaminergic degeneration in rats infused intravenously or subcutaneously, as well as it induced degeneration of non-dopaminergic neurons in both the basal ganglia and the brainstem.[11] In vitro studies, revealed that the dopaminergic neurons were more sensitive to rotenone-induced toxicity than other neuronal cells and glial cells. [9,12,15,16,18] The possible result of complex I inhibition by rotenone is the increased formation of ROS, creating oxidative damage within the cell. Oxidative stress has been implicated in PD.[3] Moreover, increased oxidative damage to lipids,[14,20,21] DNA,[20,21] and proteins[22] has been observed in PD substantia nigra pars compacta, along with decreased levels of reduced glutathione.[20]

Antioxidants, free radical scavengers and other such agents have the potential for therapeutic development for PD.[21] *Ginkgo biloba* is a plant with a high antioxidant power capable of scavenging various reactive oxygen species, including superoxide, peroxy radical, and hydroxyl radical,[22] and inhibiting or reducing the functional and morphologic impairments observed after lipid peroxidation.[24] *Ginkgo biloba* has been reported to enhance the activities of superoxide dismutase and catalase and to decrease lipid peroxidation in striatum, substantia nigra, and hippocampus, the major sites damaged in Parkinson’s and Alzheimer’s diseases.[22] *Ginkgo biloba* extract (EGB) exhibited protective properties against animal models of hypoxia,[24] excitotoxicity and focal as well as global cerebral ischemia.[22] EGB is a potent inhibitor of brain monoamine oxidases.[26] Some studies[33] have shown antiapoptotic properties of EGB. Some other studies reported that *Ginkgo biloba*, possibly through the antioxidant properties of its flavonoids, was able to protect hippocampal cells against toxic effects induced by amyloid beta peptides[22] and possess protective effect on the PD *in vitro*.[33]

Pumpkin seed oil (PSO) is a natural product commonly used in folk medicine for hypertension and atherosclerosis treatments.[14] It is rich in many antioxidants and beneficial nutritional supplements such as essential fatty acids, amino acids (especially tyrosine and L phenylalanine) and phytosterols (e.g. beta-sitosterol), β-carotenes, lutein and selenium.[34] The seeds also contain L-tryptophan and omega (-6 and -3) fatty acids which help to alleviate the symptoms of PD.[33] Pumpkin seeds have a very high concentration of the antioxidant vitamin E.[36,37]

The principal goal of this study was to evaluate the protective role of extract of *Ginkgo biloba* or pumpkin seed oil against rotenone induced neurotoxicity in rats. This could be fulfilled through the determination of dopamine (DA) and noradrenaline (NE) levels in different brain regions (striatum, cerebellum and cerebral cortex), and Na+/K+-ATPase activity in striatum. Lipid peroxidation, nitric oxide (NO), reduced glutathione (GSH) levels and total antioxidant capacity (TAC) in striatum and serum have been estimated. Serum testosterone level has been also assayed. The study was also extended to investigate striatum histologically.

**MATERIALS AND METHODS**

**Experimental Design:** Adult male albino rats were obtained from The Holding Company for Biological Products and Vaccines, VACSERA, Cairo, Egypt. The range of animals weight was 120-150g. The animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12-hour light-dark cycle at 25±1°C. They were provided with tap water and balanced diet *ad libitum*. Animals were divided into 6 groups each was comprised of thirty rats as follows: The first group was served as control which was received corn oil as vehicle (CON group) for 70 days. The second group was orally administrated corn oil which repeated every day for 20 days and on the 21st day the animals were daily orally administrated with rotenone (Sigma) in a dose of 2.5 mg/Kg b.wt. dissolved in corn oil[30,36] (ROT group) for 50 days. The third group was orally received *Ginkgo biloba* extract (EGB) "Arab Company for Pharmaceutical and Medicinal Plants, MEPACO" at a dose of 150 mg/kg b.wt. dissolved in water[30] for 70 days. The fourth group was orally administrated with EGB (150 mg/Kg b.wt.) for 70 days and on the 21st day they were orally administrated with rotenone (2.5 mg/Kg b.wt.) for 50 days (EGB & ROT). The fifth group was orally received pumpkin seed oil (PSO) "Arab Company for Pharmaceutical and Medicinal Plants, MEPACO" at a dose of 40 mg/kg b.wt.[14] for 70 days. The sixth group was orally administrated with PSO (40 mg/Kg b.wt.) for 70 days and on the 21st day they were orally administrated with rotenone (2.5 mg/Kg b.wt.) for 50 days (PSO & ROT). The animals were decapitated after 40, 55 and 70 days post-treatment (n=10).

After decapitation of animals (eight from each subgroup), the blood samples were collected, allowed to stand for half an hour and then centrifuged at 3000 rpm for 15 min. under cooling to separate serum. Each
Statistical analysis: The obtained data were presented in Tables as mean ± standard error. One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan’s test using a statistical package program (SPSS version 10.0).

RESULTS AND DISCUSSION

Effect of EGB and PSO on Dopamine (DA) Content of Striatum, Cerebellum and Cerebral Cortex of Adult Male Albino Rats Treated with Rotenone: The results in Table (1) showed that administration of rotenone (ROT) caused significant decline in DA level of both striatum and cerebral cortex after 55 and 70 days. These results are in agreement with those of Di Monte; Hirata and Nagatsu; and Ren et al. Hirata and Nagatsu demonstrated that rotenone inhibits tyrosine hydroxylase (TH), suggesting the inhibition of biosynthesis responsible for dopamine and norepinephrine. Another suggested mechanism for rotenone-induced reduction in dopamine content in the studied brain areas is the increased process of oxidative deamination. On the other hand, administration of Ginkgo biloba extract (EGB) or pumpkin seed oil (PSO) produced significant increase in DA level in striatum at all time intervals of the experiment and after 55 and 70 days in cerebellum of EGB-treated group. The increment in dopamine content in EGB-treated group may be due to kaempferol, major ingredient of EGB, which is considered as a potent monoamine oxidase B inhibitor (MAO-B), that prevents the degradation of DA and increases its availability. Regarding the increasing effect of PSO on dopamine content in striatum could be due to the high concentration of vitamin E in this oil. Vitamin E has been shown to increase dopamine anabolism in the brain by activating the tyrosine hydroxylase, which is a rate limiting enzyme for the biosynthesis of the neurotransmitters in the brain. Non-significant change was detected in DA content of striatum, cerebellum and cerebral cortex of EGB & ROT-treated group at all time intervals versus control group. Similarly, non-significant change was demonstrated in DA level in cerebellum and cerebral cortex of PSO & ROT-treated group as compared to control. In comparison to ROT group, treatment of EGB & ROT showed significant increase in DA level of striatum at all time intervals and after 55 and 70 days in PSO & ROT-treated group. The protective effect of Ginkgo biloba extract could be attributed to the ability of this extract to stabilize and protect the mitochondrial function by improving the mitochondrial membrane potential and reversing the decrease in ATP production as well as to its potent effect against oxidative stress. Ginkgo biloba extract and its bilobalide could increase the antioxidant enzyme activities to overcome the oxidative damage caused by ROT. In addition, flavonoid (mainly flavonol glycoside), another pharmacologically active constituent identified in EGB, has direct free radical scavenging activities that also play a role in EGB’s wide range of antioxidative effects. PSO could reduce the effect of rotenone as a neurotoxin, due to its high content of vit. E.
Adachi et al.\(^{[56]}\) demonstrated that the activity of tyrosine hydroxylase, which is a rate limiting enzyme for the biosynthesis of the neurotransmitters in the brain, was significantly lower in the vitamin E deficient rats than that of the controls. This means that monoamine anabolism in the vit. E deficient rat brain is impaired and the supplementation with vitamin E reversed this effect. In addition, PSO may have the capability to preserve neuronal resistance and probably to recover the atrophying neurons.\(^{[65]}\)

**Effect of EGB and PSO on Norepinephrine Content of Striatum, Cerebellum and Cerebral Cortex of Adult Male Albino Rats Treated with Rotenone**: As illustrated in Table (2), ROT administration induced significant decrease in NE content after 70 days only in the cerebral cortex area of the brain. This decrement in NE content is mainly due to that rotenone inhibits TH.\(^{[51]}\) Mitochondrial dysfunction may also responsible for this effect of ROT on NE content since mitochondrial disorder has a major role in the production of cellular ROS. The drawback of mitochondrial function induced by ROT is due to the ability of ROT to inhibit complex I activity in the mitochondrial respiratory chain which was similarly observed not only in SN pars compacta of PD patients\(^{[62]}\) but also in their platelets.\(^{[63]}\) This inhibition can lead to the generation of ROS, which when produced in the near vicinity and targeted the respiratory chain, may lead to further inhibition with subsequent ROS production which ultimately resulted in mitochondrial damage.\(^{[64]}\) Mitochondrial related energy failure may also disrupt the vesicular storage of neurotransmitters, leading to increased free cytosolic concentrations of the autooxidizable neurotransmitter.\(^{[65]}\) Administration of EGB or PSO caused non-significant change in NE level of all examined brain areas at all time intervals of the experiment. In addition, non-significant change in NE level in each of striatum, cerebellum and cerebral cortex was noticed at all time intervals in EGB & ROT or in PSO & ROT-treated groups as compared to control. Administration of ROT to EGB-treated (EGB & ROT) group or to PSO-treated (PSO & ROT) group showed significant increase in NE content of striatum after 70 days as compared with rotenone group. The protective effect of *Ginkgo biloba* and pumpkin seed oil may be attributed to their antioxidant activity and scavenging capability of various reactive oxygen species.

**Effect of EGB and PSO on the Lipid Peroxidation Level in Striatum and Serum of Adult Male Albino Rats Treated with Rotenone**: As shown in Table (3), the results indicated significant increase in lipid peroxidation level in striatum and serum of ROT group at all intervals of experiment with a maximum elevation after 70 days. Increased lipid peroxidation in the present study is in agreement with Dexter *et al.*\(^{[66]}\) and Good *et al.*\(^{[67]}\) ROS may attack any type of molecules, but their main target appears to be polyunsaturated fatty acids, which is the protector against lipid peroxide formation.\(^{[68]}\) Jenner *et al.*\(^{[69]}\) have also shown elevated levels of lipid hydroperoxide, an earlier component of the lipid peroxidation cascade in the parkinsonian SN, indicating the damaging impact on cell membrane structure due to elevated ROS.\(^{[70]}\) Thus the increased lipid peroxidation products suggested that ROS have an important role in the pathogenesis of neurodegenerative diseases.\(^{[71]}\) Administration of EGB or PSO to rats treated with ROT caused a significant increase in lipid peroxidation level in striatum after 40 days when compared with control. After 70 days in PSO & ROT group showed significant increase in serum lipid peroxidation level as compared to control. At the same time, the change in lipid peroxidation in both striatum and serum were significantly decreased at all experimental intervals as compared with ROT group in EGB & ROT or in PSO & ROT group. The decrement in lipid peroxidation in EGB & ROT is due to that *Ginkgo biloba* is an antioxidant capable of scavenging various reactive oxygen species, including superoxide, peroxyl

Table 2: Effect of *Ginkgo biloba* (EGb) and pumpkin seed oil (PSO) administration on norepinephrine content (ng/g tissue) in striatum, cerebellum and cerebral cortex of adult male albino rats treated with rotenone (ROT).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Norepinephrine of striatum (ng/g tissue)</th>
<th>Norepinephrine of cerebellum (ng/g tissue)</th>
<th>Norepinephrine of cerebral cortex (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 days</td>
<td>55 days</td>
<td>70 days</td>
</tr>
<tr>
<td>CON</td>
<td>2162±35.6</td>
<td>2172±57.1</td>
<td>2119±32.6</td>
</tr>
<tr>
<td>ROT</td>
<td>2140±75.38</td>
<td>2123±65.0</td>
<td>1974±9.6</td>
</tr>
<tr>
<td>EGB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGB &amp; ROT</td>
<td>2211±91.5</td>
<td>2112±71.5</td>
<td>2338±65.7</td>
</tr>
<tr>
<td>PSO</td>
<td>2205±60.9</td>
<td>2214±42.7</td>
<td>2330±43.6</td>
</tr>
<tr>
<td>PSO &amp; ROT</td>
<td>2238±66.2</td>
<td>2299±82.7</td>
<td>2175±49.8</td>
</tr>
</tbody>
</table>

The number of animals was 8 in each group. Data are expressed as mean ± SE. a: Significant change at p < 0.05 with respect to control group CON, b: Significant change at p < 0.05 with respect to ROT group.

Table 3: Effect of *Ginkgo biloba* (EGb) and pumpkin seed oil (PSO) administration on lipid peroxidation level in striatum (nmol/mg protein) and serum (nmol/ml) of adult male albino rats treated with rotenone (ROT).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxidation of striatum (nmol/mg protein)</th>
<th>Lipid peroxidation of serum (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 days</td>
<td>55 days</td>
</tr>
<tr>
<td>CON</td>
<td>6.2±0.3</td>
<td>6.5±0.2</td>
</tr>
<tr>
<td>ROT</td>
<td>8.4±0.4</td>
<td>9.1±0.4</td>
</tr>
<tr>
<td>EGB</td>
<td>5.9±0.3</td>
<td>5.8±0.3</td>
</tr>
<tr>
<td>EGB &amp; ROT</td>
<td>7.4±0.9</td>
<td>6.6±0.4</td>
</tr>
<tr>
<td>PSO</td>
<td>5.9±0.3</td>
<td>6.5±0.2</td>
</tr>
<tr>
<td>PSO &amp; ROT</td>
<td>7.6±0.2</td>
<td>6.9±0.5</td>
</tr>
</tbody>
</table>

The number of animals was 8 in each group. Data are expressed as mean ± SE. a: Significant change at p < 0.05 with respect to control group CON, b: Significant change at p < 0.05 with respect to ROT group.

radical and hydroxyl radical.**23** *Ginkgo biloba* has been reported to enhance the activities of superoxide dismutase and catalase activities and to decrease lipid peroxidation in striatum, substantia nigra, and hippocampus, the major sites damaged in Parkinson’s and Alzheimer’s diseases.**23** It is also likely that the flavonoid fraction of *Ginkgo biloba* has an important role in this respect.**23,26** While, decreased lipid peroxidation in PSO & ROT group may be due to carotinoids and vitamin E in pumpkin seeds that protect against oxidative damage of membranes, organelles and protein.**23,25,24** Tocopherols and selenium present in pumpkin-seed oil might contribute, by their combined synergistic action to constitute an important antioxidant defense mechanism against free radical mediated lipid peroxidation of cell membrane.**23**

Effect of EGB and PSO on the Nitric Oxide Level in Striatum and Serum of Adult Male Albino Rats Treated with Rotenone: Table 4 (Table 4) showed significant increase in nitric oxide level of striatum and serum in ROT group at all experimental days and the maximum elevation was occurred in striatum and in serum after 70 days. The generation of NO in striatum and serum in agreement with the studies carried by Bashkatova et al,**13** Li et al.**14** and Testa et al.**15** which demonstrated that this may be due to decrements in ATP, where it can lead to mitochondrial membrane depolarization and contribute to glutamate excitotoxicity and further free radical-mediated injury involving nitric oxide and peroxynitrite.**15** Microglial activation is associated with the degeneration of dopaminergic (DAergic) neurons in PD patients.**16** Activation of microglia in response to injury is associated with an upregulation of inducible nitric oxide synthase (iNOS) resulting in increased production of NO. Increased immunostaining for iNOS has been previously detected in the SN pars compacta of PD brains.**17,18** In addition, administration of EGB induced significant decline in striatum NO level after 70 days compared to control. Administration of EGB and ROT caused non-significant change in NO level of striatum and serum at all experimental days as compared to control, however they produced significant decrease in NO level in both striatum and serum at all time intervals compared with ROT group. EGB was already described in 1994 as an NO scavenger.**19** Beside its NO-scavenging ability, EGB has been shown to reduce NO release after ischemia in the brain**20** and heart after heat stress**21,22** as well as after lipopolysaccharide-stimulated macrophages by reducing iNOS mRNA and protein expression.**23,24** The ginkgolides A and B, the active ingredients of EGB, inhibited NO production of lipopolysaccharide-stimulated microglia**25,26** While, administration of PSO and ROT induced significant increase in NO level in striatum at all days of
experiment as compared to control. After 55 and 70 days, a significant increase in NO level was detected in PSO & ROT group as compared to control. In comparison with ROT group at all time intervals, significant decrease in serum NO level was demonstrated. The protective effect of PSO may be due to the presence of α-tocopherol and selenium in this oil which have been shown to scavenge free radicals including peroxynitrite radicals. 

**Effect of EGB and PSO on the Reduced Glutathione Level in Striatum and Serum of Adult Male Albino Rats Treated with Rotenone:** It is clear from the data in Table (5) that ROT administration caused significant decrease in striatum glutathione (GSH) level at all experimental days and in serum glutathione level after 55 and 70 days. Oxidative stress has been widely implicated to play a key role in rotenone-induced dopaminergic neuron injuries. GSH is a well-known endogenous antioxidant. From the present results and previous studies, it was shown that rotenone reduced GSH levels. GSH normally acts through a combination of various reduction and conjugation reactions to protect cells against both exogenous toxicants and the reaction of endogenous compounds. One possible explanation for the decreased level of GSH is the defective synthesis, utilization, and degradation of GSH as well as changes in the activity of glutathione peroxidase and glutathione reductase that could also lead to its reduction. EGb or PSO administration showed significant elevation in striatum and serum glutathione level at all time intervals as compared to control. In addition, compared to control, administration of EGB & ROT or PSO & ROT showed non-significant change in GSH level in striatum after 40 and 55 days however after 70 days, significant decrease in GSH of striatum was observed. While serum GSH showed non-significant change at all experimental days in the last two groups as compared to control. Significant increase in striatum and serum glutathione levels in EGB & ROT group at all experimental days as compared with ROT group. Moreover, significant increase in GSH level of striatum at all time intervals and after 70 days only in serum was noticed in PSO & ROT group as compared with ROT group in both groups. The protective effect of *Ginkgo biloba* extract has been shown to be due to increase in the protein level and activity of antioxidant enzymes such as superoxide dismutase and catalase as well as of glutathione (GSH) reductase in mouse liver. Similarly, the activity of γ-glutamylcysteiny1 synthetase, the rate limiting enzyme of GSH synthesis, was enhanced by EGB. The protective effect of PSO may be due to the tocophersols and selenium contents.

**Effect of EGB and PSO on the Total Antioxidant Capacity in Striatum and Serum of Adult Male Albino Rats Treated with Rotenone:** As illustrated in Table (6) that ROT induced significant reduction in total antioxidant capacity (TAC) of striatum and serum at all experimental days. Oral administration of *Ginkgo biloba* extract or pumpkin seed oil for 70 consecutive days showed significant increase in TAC of striatum after 55 and 70 days regarding to EGB and after 70 days with respect to PSO as compared to control. In addition, administration of rotenone to rats treated with EGB or PSO showed non-significant change in TAC in striatum of EGB & ROT and PSO & ROT groups and in serum of EGB & ROT group at all experimental days as compared to control. Significant decrease in TAC in serum of PSO & ROT group was observed after 55 and 70 days as compared to control. TAC in striatum showed significant increase after 55 and 70 days in EGB & ROT group and after 55 days in PSO & ROT group as compared to ROT. Serum TAC in EGB & ROT or PSO & ROT group as compared to ROT recorded significant increase at all corresponding time intervals. These data supported by the previous studies that showed the powerful antioxidant activity of EGB and PSO.

**Effect of EGB and PSO on the Na⁺/K⁺-ATPase Activity of Striatum of Adult Male Albino Rats Treated with Rotenone:** According to the data in Table (7), significant decrease in brain Na⁺/K⁺-ATPase activity of ROT group at all experimental days. Rotenone as a well known neurotoxin has been shown to reduce Na⁺/K⁺-ATPase activity and level which may be due to complex I inhibition of the mitochondrial electron transport chain, which in turn reduces the ATP-producing capacity of cells. Significant increase in striatum Na⁺/K⁺-ATPase activity of EGB or PSO group was occurred at all experimental days as compared to control. Also, striatum Na⁺/K⁺-ATPase activity in EGB & ROT group was significant by increased at all experimental days, while in PSO & ROT, striatum Na⁺/K⁺-ATPase activity showed significant increase at the beginning (40 days), then returned to the normal value after 55 days and finally it decreased significantly at the end (70 days) as compared to control. In comparison with ROT group, EGB & ROT or PSO & ROT showed significant elevation in striatum Na⁺/K⁺-ATPase activity at all experimental days. The protective effect of the present medicinal plants could be due to the antioxidant properties of *Ginkgo biloba* extract and pumpkin seed oil. Since Nicolson demonstrated that administration of antioxidants can prevent excess oxidative membrane damage, restore mitochondrial and other cellular membrane functions.

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**Table 4:** Effect of Ginkgo biloba extract (EGB) and pumpkin seed oil (PSO) administration on nitric oxide level in striatum (µmol/mg protein) and serum (µmol/l) of adult male albino rats treated with rotenone (ROT).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Nitric oxide of striatum (µmol/mg protein)</th>
<th>Nitric oxide of serum (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 days</td>
<td>55 days</td>
</tr>
<tr>
<td>CON</td>
<td>33.4±3.0</td>
<td>34.9±2.8</td>
</tr>
<tr>
<td>ROT</td>
<td>40.7±2.2</td>
<td>41.2±1.9</td>
</tr>
<tr>
<td>EGB</td>
<td>28.2±6.2</td>
<td>31.8±2.4</td>
</tr>
<tr>
<td>EGB &amp; ROT</td>
<td>34.7±6.5</td>
<td>34.3±2.6</td>
</tr>
<tr>
<td>PSO</td>
<td>31.4±3.1</td>
<td>32.9±1.1</td>
</tr>
<tr>
<td>PSO &amp; ROT</td>
<td>38.2±1.7</td>
<td>37.9±1.1</td>
</tr>
</tbody>
</table>

The number of animals was 8 in each group. Data are expressed as mean ± SE., a: Significant change at p > 0.05 with respect to control group (CON), b: Significant change at p < 0.05 with respect to ROT group.

**Table 5:** Effect of Ginkgo biloba extract (EGB) and pumpkin seed oil (PSO) administration on reduced glutathione level in striatum (µmol/mg protein) and serum (µmol/ml) of adult male albino rats treated with rotenone (ROT).

<table>
<thead>
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<th>Groups</th>
<th>Reduced glutathione of striatum (µmol/mg protein)</th>
<th>Reduced glutathione of serum (µmol/ml)</th>
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<tbody>
<tr>
<td></td>
<td>40 days</td>
<td>55 days</td>
</tr>
<tr>
<td>CON</td>
<td>1.16±0.05</td>
<td>1.16±0.06</td>
</tr>
<tr>
<td>ROT</td>
<td>1.08±0.04</td>
<td>0.98±0.06</td>
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<tr>
<td>EGB</td>
<td>1.22±0.01</td>
<td>1.38±0.02</td>
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<tr>
<td>EGB &amp; ROT</td>
<td>1.14±0.05</td>
<td>1.14±0.06</td>
</tr>
<tr>
<td>PSO</td>
<td>1.29±0.03</td>
<td>1.30±0.03</td>
</tr>
<tr>
<td>PSO &amp; ROT</td>
<td>1.16±0.05</td>
<td>1.13±0.04</td>
</tr>
</tbody>
</table>

The number of animals was 8 in each group. Data are expressed as mean ± SE., a: Significant change at p > 0.05 with respect to control group (CON), b: Significant change at p < 0.05 with respect to ROT group.

**Table 6:** Effect of Ginkgo biloba extract (EGB) and pumpkin seed oil (PSO) administration on total antioxidant capacity in striatum (mM/mg protein) and serum (mM/l) of adult male albino rats treated with rotenone (ROT).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total antioxidant capacity of striatum (mM/mg protein)</th>
<th>Total antioxidant capacity of serum (mM/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 days</td>
<td>55 days</td>
</tr>
<tr>
<td>CON</td>
<td>9.97±0.43</td>
<td>9.66±0.78</td>
</tr>
<tr>
<td>ROT</td>
<td>8.27±0.34</td>
<td>7.09±0.50</td>
</tr>
<tr>
<td>EGB</td>
<td>11.8±1.02</td>
<td>11.62±0.69</td>
</tr>
<tr>
<td>EGB &amp; ROT</td>
<td>8.59±0.57</td>
<td>9.88±0.43</td>
</tr>
<tr>
<td>PSO</td>
<td>10.23±0.82</td>
<td>11.19±0.79</td>
</tr>
<tr>
<td>PSO &amp; ROT</td>
<td>9.59±0.32</td>
<td>9.57±0.75</td>
</tr>
</tbody>
</table>

The number of animals was 8 in each group. Data are expressed as mean ± SE., a: Significant change at p > 0.05 with respect to control group (CON), b: Significant change at p < 0.05 with respect to ROT group.

Effect of EGB and PSO on Testosterone Level in Serum of Adult Male Albino Rats Treated with Rotenone: The data in Table (7) showed that, oral administration of rotenone to rats produced significant decrease in testosterone level after 55 and 70 days. Our findings are in accordance with those of Alam and Schmidt,[96] who hypothesized that Complex I inhibitors, which are responsible for reproducing symptoms of PD in rats, also deplete serum testosterone as well as ATP levels in the peripheral organs, such as the adrenal glands and testis. The depletion of energy levels and excess oxidative stress due to complex I inhibition could be responsible for decreasing testosterone level in the current study. Significant elevation of serum testosterone level was detected in EGB group after 55 and 70 days and at all experimental days in PSO group as compared to control. Similarly, administration of rotenone to rats...
treated with EGb (EGb & ROT) group revealed significant increase in testosterone after 70 days, while it significantly increased in all experimental days in PSO & ROT group as compared to control. Also, significant increase was found in testosterone level as compared to ROT group after 55 and 70 days in EGb & ROT group and at all corresponding time intervals in PSO & ROT group. The elevated testosterone level in the present study may be due to quercetin (an active ingredient of EGb) which could increase testosterone production through increasing intracellular cAMP levels and steroidalgenic protein expression in testis, which is responsible for facilitating testosterone synthesis in Leydig cells.\textsuperscript{[97]} Regarding the increased testosterone level due to PSO administration could be explained by the presence of fatty acids which able to inhibit 5α reductase that convert testosterone into dihydrotestosterone and consequently leading to increase testosterone level.\textsuperscript{[92]} Also, PSO contains zinc in appreciable level.\textsuperscript{[98]} Zinc may play a role in regulating testosterone secretion and its supplementation increased serum testosterone levels. Zinc enhances human chorionic gonadotropin-induced production of cAMP and consequently testosterone in rat testis. Additionally, zinc increases the conversion of androstenedione to testosterone in the periphery.\textsuperscript{[102]}

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na+/K-ATPase activity of striatum (μmol p/h/mg protein)</th>
<th>Testosterone level of serum (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>40 days</td>
<td>55 days</td>
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<td>CON</td>
<td>6.4±0.6</td>
<td>6.72±0.5</td>
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<tr>
<td>ROT</td>
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<tr>
<td>EGb</td>
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<td>EGb &amp; ROT</td>
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<tr>
<td>PSO</td>
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<td>7.2±0.6*</td>
</tr>
<tr>
<td>PSO &amp; ROT</td>
<td>7.0±0.4*</td>
<td>6.4±0.6*</td>
</tr>
</tbody>
</table>

The number of animals was 8 in each group. Data are expressed as mean ± SE. a: Significant change at p < 0.05 with respect to control group (CON), b: Significant change at p < 0.03 with respect to ROT group

**Histological Investigation in Striatum of Different Treated Groups:** Microscopic examination of striatum section of control rat showed the neuron with the surrounding supporting cells with normal nuclei which showed dispersed chromatin and prominent nucleoli. The cytoplasm of these cells was basophilic (Figure 1).

The neurons of rotenone treated animals showed extensive neuronal damage by virtue of loss of pigmented neurons and the cells appear to be smaller and shrunken as compared to the control cells, indicating that apoptosis has occurred (Fig 2a). In addition, the cells are small in number after 35 days of rotenone treatment (Figure 2b). Figure (2c; after 50 days of rotenone treatment) shows signs of damage as evident by disappearance of cells and the presence of cytoplasmic inclusions of lewy bodies. Moreover, structural alterations of the surviving neuronal cells can be also observed. The present histopathological studies showed that rotenone caused degeneration and apoptosis of the striatum characterized by the presence of lewy body. Previous studies indicated that oxidative stress may interact with other pathophysiological mechanisms, including genetic lesions, to create PD pathology.\textsuperscript{[99]} For example, oxidative stress may contribute to α-synuclein aggregation seen in PD and mitochondrial dysfunction models. α-Synuclein damaged by free radicals is more prone to aggregation.\textsuperscript{[100]} In vivo rotenone infusion generates both increased insoluble protein carbonyls and increased α-synuclein aggregation.\textsuperscript{[9,101]} Oxidative modification of α-synuclein in PD brain can lead to protein aggregation.

![Fig. 1: Micrographs of striatum of control rat showing the normal neurons after 40 days (a), 55 days (b) and 70 days (c) (H & E X 40).](image-url)
Microscopic investigation of striatum sections of rats-treated with *Ginkgo biloba* extract showed normal structure of neurons and the surrounding cells like control at all days of investigation (Figure 3).

Examination of striatum sections of rat-treated with *Ginkgo biloba* extract and rotenone showed the neurons more or less like normal (Figure 4a; after 40 days). Slight degeneration was found after 55 days (Figure 4b). Loss of the number of neurons can be seen after 70 days (Figure 4c). This mean that the neurons of striatum sections of rats-treated with *Ginkgo biloba* extract showed significant protection against rotenone neurotoxicity.

Microscopic investigation of striatum sections of rats-treated with pumpkin seed oil showed healthy neurons as well as the surrounding cells at all days of investigation (Figure 5).

Examination of striatum sections of rats-treated with pumpkin seed oil and rotenone showed the neurons more or less like normal (Figure 6a; after 40 days), while some apoptotic neurons were seen after 55 days (Figure 6b). More over degenerative neurons and more apoptotic neurons were noticed after 70 days (Figure 6c). These findings indicated that the treatment with PSO resulted in some protection against rotenone-induced neurodegeneration. Generally, the histological results revealed that the treatment with EGb or PSO protects the brain against rotenone-induced neurological apoptosis, where *Ginkgo biloba* extract or pumpkin seed oil are heralded as antioxidant agents. Noteworthy, EGb appeared to be more neuroprotective agent than PSO.

It can be concluded that Ginkgo biloba extract has a potential activity against rotenone-induced neurodegeneration. Therefore, it may be useful against neurotoxicity induced by environmental neurotoxins. Pumpkin seed oil has an effective role in limiting toxicant-induced oxidative stress.
Fig 6: Micrograph of striatum of rat treated with pumpkin seed oil (a) for 40 days and rotenone for 20 days, (b) for 55 days from treatment with pumpkin seed oil and for 35 days from rotenone treatment and (c) for 70 days from treatment with pumpkin seed oil and for 50 days from rotenone treatment (H & E X 40).

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REFERENCES


