Dose-dependent protective effect of selenium in rat model of Parkinson's disease: neurobehavioral and neurochemical evidences
5.1 Introduction

Parkinson’s disease (PD) is second most common progressive neurodegenerative disorder primarily affecting individuals between the age of 50 and 60, although young adults and even children can be affected by this devastating disease (Dawson, 2000). Parkinson’s disease is due to selective degeneration of dopamine containing neurons in the midbrain. In addition to the loss of dopaminergic neurons, there are indications of increased oxidative stress such as glutathione depletion, iron deposition, increased markers of lipid peroxidation, oxidative DNA damage, protein oxidation (Jenner and Olanow, 1998; Perry et al., 1982; Reiderer et al., 1989; Sian et al., 1994) and reduction in the activity of complex-1 (NADPH Co Q reductase) of the mitochondrial respiratory chain (Schapira et al., 1990). The malfunction of the basal ganglia circuits is responsible for the cardinal motor symptoms of Parkinson’s disease such as tremors at rest, muscular rigidity, bradykinesia/akinesia, stooped posture and instability (Sian et al., 1999).

6-OHDA is a selective catecholaminergic neurotoxin (Ungerstedt, 1968) widely used to investigate the pathogenesis of Parkinson's disease (Breese and Breese, 1998). The specific neurotoxicity of 6-OHDA has been associated with its uptake and accumulation by transport mechanism specific for catecholaminergic neurons (Sachs and Jonsson, 1975; Ljungdahl et al., 1991). The most popular animal model of Parkinson’s disease produced by unilateral stereotaxic injection of 6-OHDA into the substantia nigra or medial forebrain bundle of rats. However, recently it has been demonstrated that intrastriatal injection is more useful for the study of neuroprotection or neurotrophic therapies in Parkinson’s disease (Kirik et al., 2001; Shults et al., 2000). Under physiological conditions 6-OHDA is rapidly and nonenzymatically oxidised by molecular oxygen to form H2O2 and corresponding quinones.

Recently antioxidant supplementation in animal models of Parkinson’s disease has shown promising effects (Roghani and Behzadi, 2001; Moussaoi et al., 2000; Pong et
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al., 2000; Soto-Otoro et al., 2000). The neurotoxicity of methamphetamine in Se-deficient diet has potentiated the reduction of DA and its metabolites, 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) in nigrostriatal regions of the animals (Kim et al., 1999). The Se-supplementation significantly blocked dopaminergic toxicity and protect neurons in the substantia nigra from damage caused by oxidative stress (Imam et al., 1999; Cassarino et al., 1997; Castano et al., 1993; Haung et al., 1994; Boadi et al., 1991). MPTP may cause parkinson's disease in mammals and its toxicity aggravated by dietary Se-deficiency and reversed by Se-sublimated diet in mice (Kim et al., 2000). The low Se diet has increased the levels of malondialdehyde in the brains of MPTP treated mice. Selenium is present in the active site of glutathione peroxidase (GPx; Rotruck et al., 1973; Li et al., 1990). This enzyme plays a crucial role in scavenging the excess of H2O2 in the brain tissues as there is low catalase activity in brain (Halliwell, 1992). Accumulative evidences indicate a positive correlation between GPx activity and selenium intake levels and resistance to oxidative damage (Castano et al., 1993; Haung et al., 1994; Jimenez et al., 1995).

The aim of the present study was to analyze the neuroprotective role of selenium in rat model of the Parkinsonism.

5.2 Materials and Methods
5.2.1 Chemicals

Glutathione (oxidised and reduced), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), dopamine (DA), 3,4-dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA), 3,4-dihydroxybenzylamine (DHBA), 6-hydroxydopamine hydrobromide (6-OHDA) and heptane sulphonic acid were purchased from Sigma-Aldrich Foreign Holding
Chemical Company, India. Other chemicals were of analytical grade.

5.2.2 Animals

Male Wistar rats obtained from Central Animal House of Hamdard University, weighing 200-230 g at the start of the experiment were used. Rats were housed in groups of four animals per cage. They were maintained on a 12 h dark-light cycle (light on from 6:00 A.M. to 6:00 P.M.) and provided free access of rat chow and water. The experiments were in accordance with university guidelines and was approved by Animal Ethics Committee of the University.

5.2.3 Experimental groups

Experiment 1: Experiment one was carried out to evaluate the effect of sodium selenite (0.1, 0.2 and 0.3 mg/kg b. wt., i.p.) pretreatment for 7 days on open field test, muscular coordination, dopamine and its metabolites. The rats were divided into eight groups, each having 8 animals. Group 1: vehicle treated sham operated control group (S), group 2: sodium selenite 0.1 mg/kg b. wt. treated sham operated group (S+Sel), group 3: sodium selenite 0.2 mg/kg b. wt. treated sham operated group (S+Se2), group 4: sodium selenite 0.3 mg/kg b. wt. treated sham operated group (S+Se3), group 5: vehicle treated lesioned group (L), group 6: sodium selenite 0.1 mg/kg b. wt. treated lesioned group (L+Sel), group 7: sodium selenite 0.2 mg/kg b. wt. treated lesioned group (L+Se2), group 8: sodium selenite 0.3 mg/kg b. wt. treated lesioned group (L+Se3).

Experiment 2: This experiment was carried out to evaluate the effect of sodium selenite (0.1, 0.2 and 0.3 mg/kg b. wt., i.p.) pretreatment for 7 days on circling behavior, antioxidant enzymes, glutathione content (GSH) and lipid peroxidation (LPO). The rats were divided into eight groups, each having 16 animals. Tissues of
two rats in each group were pooled for the estimation of antioxidant enzymes, GSH and LPO.

On 8th day, 2 μl vehicle (0.2 % ascorbic acid in normal saline) was infused in striatum of groups 1-4 (S, S+Sel, S+Se2 and S+Se3) and 12.5 μg 6-OHDA in 2 μl vehicular solution to groups 5-8 (L, L+Sel, L+Se2 and L+Se3) as described in intrastriatal administration of 6-OHDA.

5.2.4 Intrastriatal administration of 6-OHDA

Unilateral lesions in right striatum were made in Wistar male rats. The animals were anesthetized with 400 mg/kg chloral hydrate, i.p. The rats were placed in stereotaxic frame and skull was exposed. Through a skull hole, 28-gauge Hamilton syringe of 5 μl was attached to micro-injector unit and piston of the syringe was lowered manually to the right striatum. Lesions coordinates were used as described by the Lee et al., (1996), briefly AP-0.5, L-2.5, V-5 mm relative to bragma and ventral from dura with the tooth bar set at 0 mm. (Paxinos & Watson, 1986). Rats were either infused 2 μl vehicle or 12.5 μg 6OHDA in 2 μl vehicular solution over 5 min and needle was left in place for 3 min before slowly retracting it.

5.2.5 Tissue preparation

After three weeks, animals were sacrificed and brains were taken out quickly and kept on ice. Striatum and substantia nigra were dissected out by cutting coronal section of 1.0 mm thickness using rat brain matrix using rat brain atlas Tissue preparation.

After three weeks, animals were sacrificed by overdose of pentabarbitone and brain was taken out quickly and kept in ice. Striatum and substantial nigra were dissected out on dry ice and processed as described in material and method section of chapter-2.
5.2.6 Neurochemical assay

5.2.6.1 Estimation of Catecholamines

The concentrations of dopamine and its metabolites were quantified in striatum by modified HPLC coupled with electro chemical detector as described in materials and methods section of chapter two. The amount of dopamine and its metabolites were calculated using a standard curve generated by determining ratio between three known amounts of amine or its metabolites and a constant amount of internal standard.

5.2.6.2 Glutathione peroxidase (Selenium dependent)

Selenium dependent GPx activity was measured at 37°C by coupled assay system in the striatum as described in material and method section of chapter-2. GPx activity was defined as nmol NADPH oxidized min⁻¹ mg⁻¹ protein.

5.2.6.3 Glutathione reductase

The glutathione reductase activity was measured in the striatum as described in material and method section of chapter-2 and was calculated as nmol NADPH oxidized min⁻¹ mg⁻¹ protein using molar extinction coefficient of 6.22 x 10³ M⁻¹ cm⁻¹.

5.2.6.4 Catalase

The catalase activity was measured in striatum as described in material and method section of chapter two. The catalase activity was calculated in terms of nmol H₂O₂ consumed min⁻¹ mg⁻¹ protein.

5.2.6.5 Glutathione S-transferase

The glutathione-S-transferase activity was determined in striatum as described in material and method section of chapter two and the enzymatic activity was calculated as nmol CDNB conjugate formed min⁻¹ mg⁻¹ protein using a molar extinction coefficient of 9.6 x 10³ M⁻¹ cm⁻¹.
5.2.6.6 Lipid peroxidation

LPO was measured in the substantia nigra fluorometrically as described in material and method section of chapter two. Flurometer was calibrated with quinine sulphate solution. LPO expressed as relative fluorescent unit (RFU).

5.2.6.7 Estimation of reduced glutathione

The reduced glutathione content (GSH) in the brain was determined through spectrophotometrically (lambda-20, Perkin Elmer) as described in material and method section of chapter two. The GSH concentration was calculated as nmol GSH/g tissue.

5.2.6.8 Protein

Protein concentration was determined using bovine serum albumin as a standard as described in material and method section of chapter two.

5.2.7 Behavioral Study

The behavioral tests were started after 2 weeks of lesioning. The experiment was performed between 9.00 a.m. to 4.00 p.m. in laboratory at standard optimal conditions. All tests were performed and analyzed by subject blind to the experiment.

5.2.7.1 Open field test

Open field tests were recorded automatically by Video path analyzer (Coulborn Instrument, USA) as described in material and method section of chapter two. Behavioral testing was started 10 sec after placing the rat over a black surface in the center of open field chamber.

5.2.7.2 Turning behaviour

Turning behaviour was recorded by an individual blind to the experiment as
described in the materials and methods section of the chapter two.

5.2.7.3 Rotarod (Muscular coordination)

Omni Rotor (Omnitech electronics Inc., Columbus Ohio) was used to evaluate muscular in-coordination. Detailed method is described in material and method section of chapter two. Drug naïve animals were trained on the rotarod (10 r.p.m.), so that they can stay on rotating rod for at least 180 second (cut off time). After 2 weeks of lesioning, rats were again tested for endurance performance.

5.3 Statistical Analysis

Results are expressed as mean ± SEM. ANOVA with post hoc analysis was used to analyze differences between the groups. Tukey-Kramer post hoc test was applied to serve significance between groups where needed. Multi-correlation analysis test was applied to find correlation between more than two groups.
5.4 Results
5.4.1 Effect of Se on brain dopamine metabolism

Table 1 shows the content of dopamine and its metabolites in sham operated control group (S) and lesioned group (L) and protection with selenium. The level of dopamine (78%), DOPAC (70%) and HVA (68.5%) was depleted significantly in L.

Table 1: Effect of selenium and 6-OHDA lesioning on dopamine and its metabolites in striatum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>DA (ng/mg tissue)</th>
<th>DOPAC (ng/mg tissue)</th>
<th>HVA (ng/mg tissue)</th>
<th>DOPAC/DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>9.14±0.35</td>
<td>1.33±0.16</td>
<td>1.15±0.14</td>
<td>0.142±0.016</td>
</tr>
<tr>
<td>Se1</td>
<td>9.06±0.41 (-0.9%)</td>
<td>1.32±0.09 (-0.7%)</td>
<td>1.14±0.21 (-0.8%)</td>
<td>0.140±0.021 (-1.4%)</td>
</tr>
<tr>
<td>Se2</td>
<td>9.21±0.38 (1.5%)</td>
<td>1.35±0.11 (1.5%)</td>
<td>1.13±0.08 (-1.7%)</td>
<td>0.146±0.014 (1.4%)</td>
</tr>
<tr>
<td>Se3</td>
<td>9.28±0.27 (1.5%)</td>
<td>1.36±0.17 (2.3%)</td>
<td>1.17±0.13 (1.7%)</td>
<td>0.146±0.002 (1.4%)</td>
</tr>
<tr>
<td>L</td>
<td>1.99±0.30 (-78.0%)</td>
<td>0.40±0.11 (-70.0%)</td>
<td>0.36±0.10 (68.5)</td>
<td>0.24±0.04 (-41.6%)</td>
</tr>
<tr>
<td>L+Se1</td>
<td>4.26±0.84 (113.0%)</td>
<td>0.83±0.15 (107.0%)</td>
<td>0.68±0.089 (87.5%)</td>
<td>0.19±0.016 (21.0%)</td>
</tr>
<tr>
<td>L+Se2</td>
<td>7.05±0.82 (252.0%)</td>
<td>0.99±0.19 (148.7%)</td>
<td>0.82±0.11 (127.0%)</td>
<td>0.17±0.032 (29.0%)</td>
</tr>
<tr>
<td>L+Se3</td>
<td>8.29±1.01 (310.0%)</td>
<td>1.12±0.25 (178.0%)</td>
<td>0.94±0.18 (161.0%)</td>
<td>0.14±0.019 (41.6%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, (n=16). *p<0.001 compared to S, **p<0.05, ***p<0.01, ****p<0.001 compared to L. The percentage change in parentheses of Se1-Se3 compared with S and percentage change in parentheses of L+Se1 to L+Se3 compared with L.
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Dose dependent protective effect of selenium as compared to S group. The ratio of DOPAC/DA in L group was increased by 41.6% as compared to S group. The pretreatment with Se in L+Sel to L+Se3 groups has protected the level of dopamine, DOPAC and HVA significantly and dose dependently as compared to L group. The ratio of DOPAC/DA in L+Sel to L+Se3 groups was depleted dose dependently (21, 29 and 41.6%) as compared to L group.

Table 2: Effect of selenium and 6-OHDA lesioning on antioxidant enzyme activity in striatum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GPx (nmol NADPH oxidized/min/mg protein)</th>
<th>GR (nmol NADPH oxidized/min/mg protein)</th>
<th>GST (CDNB conjugate formed/min/mg protein)</th>
<th>CAT (nmol H2O2 consumed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>26.08±2.0</td>
<td>22.74±3.5</td>
<td>10.84±1.6</td>
<td>3.12±0.45</td>
</tr>
<tr>
<td>Sel</td>
<td>26.50±1.4 (1.7%)</td>
<td>23.66±2.8 (5.0%)</td>
<td>11.52±1.5 (6.0%)</td>
<td>3.02±0.43 (3.0%)</td>
</tr>
<tr>
<td>Se2</td>
<td>28.19±2.3 (8.0%)</td>
<td>24.28±2.2 (8.0%)</td>
<td>12.3±1.1 (13.0%)</td>
<td>3.22±0.46 (3.5%)</td>
</tr>
<tr>
<td>Se3</td>
<td>32.73±3.2 (25.0%)</td>
<td>26.90±1.9 (25.0%)</td>
<td>13.48±0.5 (24.0%)</td>
<td>3.64±0.58 (15.7%)</td>
</tr>
<tr>
<td>L</td>
<td>11.16±1.5 (-51.7%)</td>
<td>6.78±0.3 (-69.8%)</td>
<td>5.92±1.2 (-45.4%)</td>
<td>1.29±0.38 (-142.0%)</td>
</tr>
<tr>
<td>L+Sel</td>
<td>17.48±1.6 (51.5%)</td>
<td>15.43±1.9 (127.5%)</td>
<td>7.75±1.2 (30.5%)</td>
<td>1.85±0.33 (43.8%)</td>
</tr>
<tr>
<td>L+Se2</td>
<td>22.34±2.6 (100.0%)</td>
<td>17.46±3.6 (157.0%)</td>
<td>8.29±0.7 (49.6%)</td>
<td>2.33±0.37 (82.0%)</td>
</tr>
<tr>
<td>L+Se3</td>
<td>24.36±3.4 (118.0%)</td>
<td>21.32±2.1 (214.8%)</td>
<td>10.06±1.9 (70.5%)</td>
<td>2.82±0.4 (118.8%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, (n=16). *p<0.001 compared to S, *p<0.05, **p<0.01. ***p<0.001 compared to L. The percentage change in parentheses of Se1-Se3 compared with S and percentage change in parentheses of L+Sel to L+Se3 compared with L.

5.4.2 Antioxidant enzymes activity in Se treated rats

Table 2 shows the effect of sodium selenite (0.1, 0.2 and 0.3 mg/kg b. wt.) on the activity of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and catalase (CAT). The activity of antioxidant enzymes in groups
significantly in L group as compared to S group. The selenium has protected the activity of these enzymes in L+Se1 to L+Se3 groups significantly and dose dependently as compared to L group.

5.4.3 Effect of Se on LPO

Fig. 1 shows the effect of various doses of sodium selenite in S groups and protection in L groups. The level of LPO was not altered in S+Se1 to S+Se3 groups but its level was significantly elevated in L group as compared to S group. Treating the rats with various doses of selenium in L+Se1 to L+Se3 groups has depleted the level of LPO significantly and dose dependently.

Fig 1: Effect of selenium and 6OHDA lesioning on lipid peroxidation in substantia nigra. Values are expressed as mean ± SEM. †p<0.001 compared to S, *p<0.05, **p<0.01, ***p<0.001 compared to L.
5.4.4 Effect of Se on GSH

Fig. 2 shows the protective effect of sodium selenite on GSH level in lesioned group. The level of GSH was not elevated significantly in S+Se1 to S+Se3 groups but its depletion in L group was significant as compared to S group. The selenium has elevated its level significantly and dose dependently in L+Se1 to L+Se3 groups as compared to L group.

Fig 2: Effect of selenium and 6-OHDA lesioning on GSH in striatum. Values are expressed as mean ± SEM. †p<0.001 compared to S, *p<0.01, **p<0.001 compared to L.
5.4.5 Behavioral Study
5.4.5.1 Rotation

The effect of sodium selenite was evaluated on amphetamine-induced rotations in lesioned rats (Fig. 3a). Rats of lesioned groups rotated towards lesioned side (ipsilateral rotations) following amphetamine administration and the rotation was decreased significantly (p<0.001) and dose dependently in L+Se1 to L+Se3 groups as compared to L group. No significant alteration was observed in S+Se1 to S+Se3 groups as compared to S group.

5.4.5.2 Rotarod (Spontaneous motor activity)

Fig. 3b shows a significant (p<0.001) depletion in muscles coordination in L group as compared to S group. Selenium (0.1, 0.2 & 0.3 mg/kg b. wt.) was found to be effective in partial recovery of muscular in-coordination in dose dependent...
manner in L+Se1 to L+Se3 groups as compared to L group. No significant alteration was observed in S+Se1 to S+Se3 groups as compared to S group.

5.4.5.3 Rearing

The rearing activity was significantly lower (p<0.001) in L group in comparison to S group (Fig. 3c). The rearing was increased with all three doses of selenium in L+Se1 to L+Se3 groups as compared to L group. Groups S+Se1 to S+Se3 has shown no significant alteration in rearing as compared to S group.

5.4.5.4 Locomotor

Distance traveled was decreased significantly in L group (p<0.01) as compared to S group and it was reversed by selenium dose dependently in L+Se1 to L+Se3 groups as compared to L group (Fig. 3d). No alteration was observed in distance traveled and locomotion in S+Se1 to
S+Se3 groups as compared to S group.

5.4.5.6 Time spent in locomotion

Time spent in locomotion was depleted significantly (p<0.01) in L group rats as compared to S group (Fig. 3e). There was significant and dose dependent recovery in L+Sel to L+Se3 groups (52%, 63% and 82%) as compared to L group.

![Fig 3e: Effect of selenium and 6-OHDA lesioning on locomotion. Values are expressed as mean ± SEM. †p<0.001 compared to S, *p<0.01, **p<0.001 compared to L.](image)

5.4.5.7 Stereo events

Stereo events were decreased (41%) significantly (p<0.01) in L group and selenium supplementation has elevated its level significantly and dose dependently in L+Sel to L+Se3 groups (19%, 33% and 50%) as compared to L group (Fig. 3f).

![Fig 3f: Effect of selenium and 6-OHDA lesioning on stereo events. Values are expressed as mean ± SEM. #p<0.001 compared to S, *p<0.01, **p<0.001 compared to L.](image)
5.5 Discussion

The experiment presented here demonstrates the ability of selenium to partially protect the toxicity of the nigrostriatal dopaminergic neurons induced by 6OHDA injection. Selenium reduces the ipsiversive rotations induced by amphetamine (Fig. 3a). These rotations can be considered as reliable indicator of nigrostriatal dopaminergic depletion (Schwarting and Huston, 1996). Schwarting and Huston (1997) have shown that ipsilateral rotation may take place when nigrostriatal lesioning is more than 74%, where the partially lesioned rats do not rotate after such treatment. Thus a marked decrease in rotation may be due to a protective effect of selenium on dopaminergic neurons against 6OHDA toxicity. Selenium reduced the increase in dopamine utilization. It is noteworthy that greater the dopamine depletion higher the increase in dopamine metabolism (Zigmond, 1990) which is probably linked to a compensatory mechanism on the part of the remaining neurons (Agid, 1973; Lavieller et al., 1978; Robinson and Whishaw, 1988, Robinson et al., 1990, 1994). Moreover, the turnover of dopamine is a better index of neuronal functioning than dopamine levels itself. It may be noted that the treatment with selenium in combination with 6OHDA allowed the remaining neurons to recover at the level of functioning closer to that of their initial activity. Such an improvement is again in good agreement with the antioxidant enzyme and behavioral data reported in the present work. The present study demonstrates that selenium supplementation prevent the striatal dopamine, also proving our earlier results (Imam et al., 1999).

The rota rod and open field experiments have indicated that vehicle injection has not caused deterioration of motor performance in the rats, while lesioned group (L)
has shown depletion in locomotion, low stereotypic events and poor coordination. The preinjection of selenium has protected these events significantly. The behavioral effects are closely linked to the degree of dopamine dysfunction (Schwarting et al., 1991).

The brain dopamine has long been thought to play a role in neurotoxicity which undergoes spontaneous oxidation to toxic quinone and other electrophilic species and causing Parkinson's disease (Halliwell, 1992; Bing, 1990). It was also found that dopamine in solution undergoes auto-oxidation, results in the production of reactive quinone derivatives and \( \text{H}_2\text{O}_2 \) (Slivka and Cohen, 1985; Graham et al., 1978; Sinet et al., 1980). The in vivo administration of antioxidant inhibits reduction in brain dopamine levels (Roghani & Behzadi, 2001). Thus, the preventive effect of selenium might be due to reduction in auto-oxidation of dopamine by enhancing antioxidant enzymes activity, particularly GPx which shows relatively high activity in striatum and substantia nigra comparatively to other brain regions (Brannan et al., 1980). \( \text{H}_2\text{O}_2 \) is detoxified to \( \text{H}_2\text{O} \) by GPx and partially by catalase (Halliwell, 1992) and GST catalyses the detoxification of oxidized metabolites of catecholamines (\( \alpha \)-quinone) and may serve as an antioxidant system preventing degenerative cellular process (Bacz et al., 1997).

The brain is thought to be vulnerable to oxidative damage due to its high oxygen consumption, presence of high levels of polyunsaturated fatty acids and non-regenerative nature of neurons (Floyd, 1991 & Floyd and Carney, 1992 and Halliwell, 1992). It is also well known that brain has poor catalase activity and moderate activity of GPx and superoxide dismutase (Coyle and Puttfarct, 1989; Halliwell, 1992). In
addition, substantia nigra is rich in iron (Youdim, 1988; Ben-Shachar et al., 1995) and selenium deficiency cause a secondary overload of iron (Chareon-Kawamoto and Yasumoto, 1995; Rafałowska et al., 1989). Thus, a selenium deficient condition may potentiate Fenton reaction in the brain, especially in substantia nigra consistently. Iron induced lipid peroxidation has been shown to increase dopamine uptake in the rat brain synaptosomal fraction and this effect could partially be attenuated by addition of selenium in the selenite form (Rafałowska et al., 1989). Moreover, Se deficiency has greater neurotoxic effect of MPTP (Johannessen et al., 1986) than vitamin E deficiency in rat striatum (Vizuate et al., 1994). Methamphetamine administration to Se deficient animals exhibited a higher Cu, Zn-SOD activity (Kim et al., 1999), elevated levels of malondialdehyde (Sutphin and Buckman, 1991) and reduction in DA and its metabolites, DOPAC (Kim et al., 2000).

6-OHDA generated oxidative stress was particularly blocked by selenium by inducing antioxidant defense mechanism. 6OHDA and auto-oxidation of dopamine produced H2O2 with subsequent Fe2+ catalyzed conversion to hydroxyl radicals (Graham, 1984; Gotz et al., 1994; Ebadi et al., 1996) which trigger the reaction and damage the DNA, membrane lipid, carbohydrate and proteins. These radicals also damage the mitochondrial electron transport system, decompartmentation of intracellular calcium homeostasis, induction of proteases, increase membrane lipid peroxidation and finally cell damage (Halliwell, 1992; Youdim et al., 1993). 6-OHDA potentiates lipid peroxidation in substantia nigra and striatum, which may be the results of diminish activities of antioxidant enzymes and low level of glutathione. Reduction of brain glutathione by L-buthionine sulfoximine potentiate the dopamine
depleting action of 6-OHDA in rat striatum (Garcia et al., 2000).

The impairment of Ca2+ homeostasis in the CNS may be the triggering event that leads to the development of neurodegeneration during oxidative stress (Orriens et al., 1992). Selenium has been shown to block Ca2+ channel with the efficacy comparable to calcium antagonist verapamil (An et al., 1992). Hence, in the light of above data, it may be suggested that protective effect of selenium against 6-OHDA neurotoxicity may be at least in part due to its calcium blocking activity. The selenium which is an essential part of our diet may be used as a best tool for the protection of neurodegeneration especially in Parkinson’s disease.