Chapter 5

Evaluation of rebamipide in sub-acute doses for its action against 6-OHDA-induced toxicity in rats

5.1. Introduction

In the present scenario, repurposing of an already approved drug can be counted as major asset to address the disease protection and disease-modification at early PD stages (Bourque et al., 2018). Rebamipide, an FDA approved widely used gastrointestinal protective drug, is recently reported to reduce amyloid-β 1-42 (Aβ42) production and attenuated Aβ43-lowered cell viability in cultured SH-SY5Y human neuroblastoma cells (Fukui et al., 2017). Moreover, rebamipide suppressed diclofenac-induced intestinal permeability via mitochondrial protection in mice for the reason that it improved mitochondrial complex-I, II and V activities, mitochondrial membrane potential (MMP) and mitochondrial function (Diao et al., 2012) and act against lipid peroxidation to increase adenosine triphosphate (ATP) in hepatic ischemia/ reperfusion injury in rats (Gendy et al., 2017). Rebamipide also attenuated celecoxib-induced mitochondrial dysfunction *in vitro* (Ishihara et al., 2010) and indomethacin-induced mitochondrial damage, lipid peroxidation and apoptosis in gastric epithelial RGM-1 cells (Nagano et al., 2005). Additionally, rebamipide is shown to act against oxidative stress by decreasing inducible nitric oxide synthase (iNOS) in bone tissue in osteoarthritis rat model (Moon et al., 2012), thiobarbituric acid reactive substances (TBARS) in acetic acid-induced colitis (Sakurai et al., 1998) and anthral ulcers in rats (Ohashi et al., 2009). Rebamipide also increased antioxidant enzymes such as superoxide dismutase (SOD) in ethanol-induced gastric mucosal damage (Choi et al., 2013), acetic acid-induced colitis (Sakurai et al., 1998), anthral ulcers (Ohashi et al., 2009) and ischemia-reperfusion (Kim and Hong, 1995) in rats.

Rebamipide is reported to cross the blood brain barrier and after a single oral administration in rats the radioactivity of ^{14}C-labeled rebamipide in brain was found as 41% and 71% of plasma and blood levels respectively (Fukui et al., 2017; Shioya et al., 1989). Therefore, there is a scope to believe that rebamipide may also act against oxidative stress, mitochondrial dysfunction and related GCase deficiency as well as α-synuclein pathology involved in PD pathogenesis. The effect of rebamipide in any of the CNS-related disorders *in vivo* and PD-like neurodegenerative disorders is yet to be evaluated. Hence, the role of rebamipide is investigated for the first time against impairments in mitochondrial function and bioenergetics with α-synuclein pathology in 6-OHDA-induced model of PD *in vivo* as shown in **Figure 5.1**. Various behavioral parameters like apopmorphine-induced head rotations, grip strength, rotarod, bar catalepsy and open field tests were performed to characterize PD-like motor deficits. Striatal DA deficiency, α-synuclein concentration and GCase activity in PD model was estimated. Mitochondrial complex enzymes activities and mitochondrial bioenergetics was performed in order to assess mitochondrial function. Mitochondrial lipid peroxidation was estimated as a function of oxidative stress. Intrinsic pathway of apoptosis was expressed by caspase-9, caspase-3 and cytochrome-C proteins.

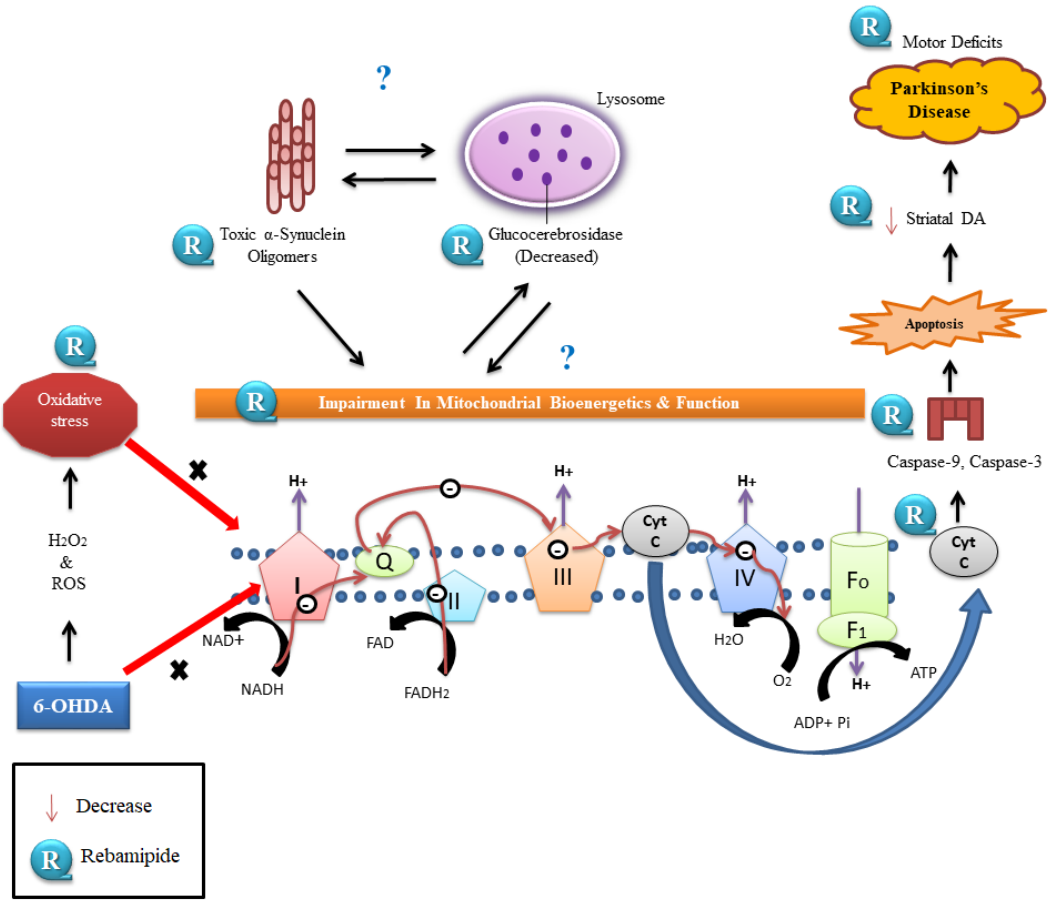

Figure 5.1 The schematic diagram of hypothesis for the evaluation of rebamipide in sub-acute doses for its action against 6-OHDA-induced toxicity in rats. The drug may increase mitochondrial complex enzyme activities, mitochondrial bioenergetics, followed by GCase enzymatic activities. It may decrease oxidative stress with α-synuclein pathology. Collectively, this may attenuate 6-OHDA-induced DA striatal degeneration and intrinsic pathway of apoptosis.

5.2. Materials and Methods

5.2.1. Animals

Charles-Foster strain of adult albino rats male (260 ± 20 g) was procured from Central Animal House; Institute of Medical Sciences, Banaras Hindu University

(IMS-BHU) and acclimatized at a temperature of 25 ± 1^0C and 45-55% relative humidity with light/dark cycle of 12:12h by keeping them in polypropylene cages. Commercial food pellets (Doodhdhara Pashu Ahar, India) and water was made available *ad libitum.* No experiments were performed for one week in order to let the animals adapt to the laboratory conditions. All the experimental procedures were carried out in compliance with the principles of laboratory animal care [National Institutes of Health guide for the care and use of Laboratory animals (NIH Publication No. 8023, revised 1978)] guidelines and approved by the Institutional animal ethical committee, BHU (Dean/2016/CAEC/33). The experiments were performed between 9:00h and 16:00h.

5.2.2. Materials

Rebamipide was received as a gift sample from Akums Drugs & Pharmaceuticals Ltd., New Delhi, India. Magnesium chloride ($MgCl_2$), KH_2PO_4, malate, pyruvate, adenosine diphosphate (ADP), succinate, oligomycin, FCCP [carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone] and rotenone were procured from Sigma-Aldrich (St. Louis, MO, USA). For the source of remaining materials, please refer Chapter 4 (page 29).

5.2.3. Surgery and Microinjection

Please refer Chapter 4 (page 30).

5.2.4. Experimental Design

The detailed experimental design is depicted in **Figure 5.2.** Animals were randomly distributed into seven groups of fifteen animals each, namely, control, sham, 6-

OHDA, 6-OHDA+R-20 (Rebamipide 20 mg/kg), 6-OHDA+R-40 (Rebamipide 40 mg/kg), 6-OHDA+R-80 (Rebamipide 80 mg/kg) and 6-OHDA+Selegiline (positive control). The day animals received 6-OHDA intrastriatal unilateral injection was regarded as day-1 (D-1). The drugs were administered to their respective groups from D-4 after the onset of behavioral deficits. 6-OHDA solution was prepared and administered into left striatum to all groups except control and sham groups as described in Chapter 4 (page 31). Ascorbic acid-normal saline solution (0.2 mg/mL) was given to sham group (Kumar et al., 2012). Selegiline is used to decrease early stage symptoms of PD (Murray and Callahan, 2003; Zhao, Q. et al., 2013) and was given as 10 mg/kg *p.o.* daily. This dose is reported to attenuate 6-OHDA induced motor deficits and striatal DA depletion in rats in Chapter 4 (Mishra et al., 2018). Rebamipide can cross the blood brain barrier after oral administration (Shioya et al., 1989). Rebamipide in the doses of 30 and 100 mg/kg was found to be effective against ethanol-induced gastric mucosal damage (Choi et al., 2013), gastric lesion induced by ischemia-reperfusion (Kim and Hong, 1995) and also in anthral ulcers in rats (Ohashi et al., 2009). The reported oral bioavailability of drug is 4.8 ± 1.4% as shown by biopharmacokinetic data (Shin et al., 2004). The radioactivity of ^{14}C-labeled rebamipide in the brain after single oral dose in rats is reported as 41% and 71% of plasma and blood levels respectively (Shioya et al., 1989). The integrity of blood brain barrier is compromised in PD patients and same is observed by 6-OHDA neurotoxin in rats also (Carvey et al., 2005). In the pilot study, it was observed that 1 mg/kg intravenous (i.v.) dose of rebamipide is effective against motor deficits when given daily once a day from D-4 to D-27 after 6-OHDA injection in rats. 10 mg/kg rebamipide was not reported to be effective against anthral ulcers in rats (Ohashi et

al., 2009). The same dose when administered once orally to rats, brain concentrations of rebamipide were not detectable at 8h (Shioya and Shimizu, 1988). On the basis of above, 20, 40 and 80 mg/kg doses of rebamipide were selected and administered *p.o.* twice a day at every 12h. Rebamipide was suspended in 0.5% carboxymethylcellulose (CMC) (Kim and Hong, 1995; Ohashi et al., 2009) and control rats were administered with 0.5% CMC *p.o.* only. The treatment schedule was carried on for twenty-four consecutive days that is from D-4 to D-27 of the experimental design. Behavioral parameters were conducted on D-0, 7, 14, 21 and 28 except for apomorphine-induced rotational behavior which was also performed on D-4. Training sessions for behavioral parameters were performed as discussed in Chapter 4 (page 32-33). The observations for open field test were recorded by ANY-MAZE behavioral tracker version 4.72 (USA) and rest of the behavioral parameters were recorded with a video camera by observers blind to the study protocol. All the animals were killed by decapitation on D-28 at 24h after the last drug dosing and SNc and striatal tissues were micro dissected from ipsilateral hemispheres. Tissues were stored immediately at -80^0C till further studies. SNc tissues were used for the estimation of GCase activity (n = 6), α-synuclein concentration (n = 6) and proteins expression of cytochrome-C, caspase-9 and caspase-3 by western blots (n = 3). Striatal monoamines were estimated by HPLC (n = 6). Mitochondrial complex enzymes activities, lipid peroxidation (n = 6) and bioenergetics (n = 3) was performed on striatal tissues.

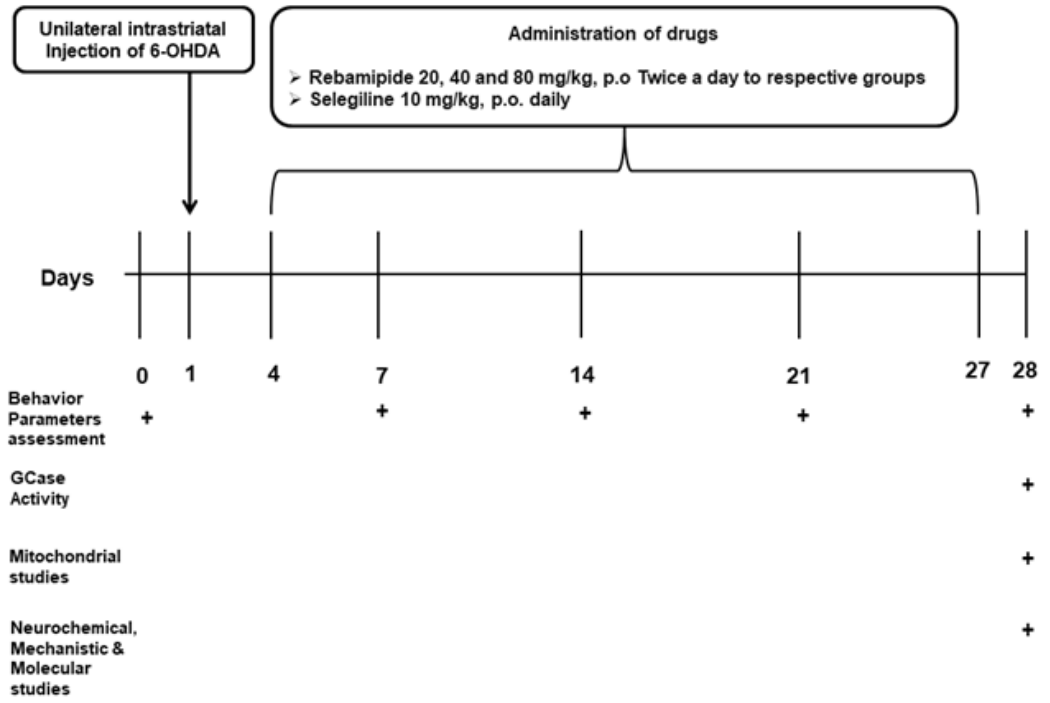

Figure 5.2 The experimental design of study for the evaluation of rebamipide in sub-acute doses for its action against 6-OHDA-induced toxicity in rats. "+" indicates the days at which the tests were performed.

5.2.5. Behavioral Parameters

5.2.5.1. Apomorphine-induced head rotation

Please refer Chapter 4 (page 34).

5.2.5.2. Open field test

Please refer Chapter 4 (page 34).

5.2.5.3. Rotarod Test

Please refer Chapter 4 (page 34).

5.2.5.4. Grip Strength test

Please refer Chapter 4 (page 35).

5.2.5.5. Bar Catalepsy Test

Please refer Chapter 4 (page 35).

5.2.6. Estimation of striatal DA and its metabolites

Please refer Chapter 4 (page 36).

5.2.7. Estimation of mitochondrial function, oxidative stress and bioenergetics

5.2.7.1. Isolation of mitochondria

Please refer Chapter 4 (page 37) for the isolation of striatal mitochondria.

5.2.7.2. Measurement of Mitochondrial respiratory complex-I, II, IV and V activity

Complex-I (NADH dehydrogenase) activity was estimated in the presence of potassium ferricyanide as an artificial electron acceptor (Shapiro et al., 1979). Reaction mixture consists of 200 µL of 10 mM potassium ferricyanide, 60 µL of 1 mM NADH in 2 mM potassium phosphate buffer and 2.64 mL of 120 mM potassium buffer. pH was maintained at 8.5 and reaction mixture was incubated for 5 min. Mitochondrial sample (100 µL) was added and was assayed fluorimetrically at Ex 350 / Em 470 at room temperature. The activity was indicated as nmol NADH oxidized/ min/ mg protein. The estimation of complex-II (succinate dehydrogenase) activity was performed by previously described method (Old and Johnson, 1989). Progressive reduction of nitrobluetetrazolium (NBT) to diformazan was measured at

570 nm and the activity was denoted as µmol formazan produced/ min/ mg protein. The measurement of complex-IV (cytochrome-C Oxidase) activity was done by reducing cytochrome-C with few crystals of sodium borohydride and neutralized to pH 7.0 with 100 mM hydrochloric acid (HCl) (Old and Johnson, 1989). Reduced cytochrome C (0.3 mM) was mixed with 75 mM phosphate buffer (pH 7.4) and reaction was initiated by adding mitochondrial sample. Decrease in absorbance was recorded for 3 min at 550 nm and results were shown as nmol cytochrome C oxidized/min/mg protein (Molar extinction coefficient = 19.6 $mmol^{-1}$ cm^{-1}). Complex V (F1F0-ATP synthase) activity was evaluated by incubating mitochondrial samples in ATPase buffer (Griffiths and Houghton, 1974). Phosphate content was measured (Fiske and Subbarow, 1925) and the values were denoted as nmol ATP hydrolyzed/ min/ mg protein.

5.2.7.3. Mitochondrial lipid peroxidation (LPO) measurement

Mitochondrial malondialdehyde (MDA) was estimated as marker of membrane lipid peroxidation as previously reported (Uchiyama and Mihara, 1978) with slight modifications (Sunderman et al., 1985). The end product of reaction was obtained as chromophore and measured at 532 nm. The results are shown as nmol MDA/mg of protein.

5.2.7.4. Evaluation of mitochondrial bioenergetics

Mitochondrial function was evaluated by using Oxytherm Clark – type oxygen electrode (OXYT1/ED, Hansatech Instruments, Norfolk UK). Mitochondria (180-200 µg) were placed in the sealed oxytherm chamber containing respiration buffer (125 mM potassium chloride, 0.1% BSA, 20 mM HEPES, 2 mM $MgCl_2$, 2.5 mM KH_2PO_4,

pH 7.2). The stirring was continued throughout the procedure and temperature was set at 37^0C. Various states of mitochondrial respiration, namely state 2, state 3, state 4 and state 5 complex-I and state 5 complex-II were measured. Respiratory control ratio (RCR) was estimated by measuring oxygen consumption during state 3 (presence of ADP) to state 4 (presence of oligomycin) (Gilmer et al., 2009; Samaiya and Krishnamurthy, 2015).

5.2.8. GCase activity measurement

Please refer Chapter 4 (page 36) for estimation of GCase activity in rat nigral tissues.

5.2.9. α-synuclein measurement

Please refer Chapter 4 (page 37) for the measurement of α-synuclein concentration in rat nigral tissues.

5.2.10. Western blot analysis for cytochrome-C, caspase-9, and caspase-3 protein expressions

Please refer Chapter 4 (page 39) for the estimation of proteins in rat nigral tissues.

5.2.11. Statistical Analysis

All the experimental datasets were expressed as mean ± SD. The statistical significance for time-dependent effects on behavioral parameters was analyzed by repeated measures of two-way ANOVA followed by post hoc Bonferroni test. All the other datasets were analyzed by one-way ANOVA followed by post hoc Student Newman-Keuls test. $p < 0.05$ was considered to be statistically significant throughout the experimental data analysis.

5.3. Results

5.3.1. Behavior Parameters

5.3.1.1. Rebamipide attenuated 6-OHDA-induced changes in apomorphine-induced rotation and cataleptic behavior in rats

Repeated measures of two-way ANOVA revealed significant differences in rotational and cataleptic behavior in rats among groups ([F (6, 490) = 533.6; $p < 0.05$], [F (6, 490) = 314.7; $p < 0.05$] respectively), time ([F (4, 490) = 409.7; $p < 0.05$], [F (4, 490) = 461.9; $p < 0.05$] respectively) and an interaction ([F (24, 490) = 83.33; $p < 0.05$], [F (24, 490) = 73.19; $p < 0.05$] respectively) between group and time **(Table 5.1).** No significant differences were found between control and sham groups. 6-OHDA caused a significant increase in head rotation induced by apomorphine in rats from D-4 (data not shown) which was increased up to 40% compared to sham groups on D-7. Progressive increase was observed in 6-OHDA toxic effects up to D-28. No effects were observed for R-20, but R-40 and R-80 significantly decreased the rotational behavior up to 16% and 20% with onset of action at D-21 and D-14 respectively in 6-OHDA-administered rats. Dose-dependent effect of rebamipide was noticed on D-14, 21 and 28. The onset of catalepsy behavior of 6-OHDA was D-14 with 62% increase than sham group. Higher doses of rebamipide caused significant decline in 6-OHDA-induced cataleptic behavior with onset of action of R-40 at D-28 (26%) and R-80 at D-21 (36%). Both R-40 and R-80 induced progressive decrease in rotational and cataleptic behavior in 6-OHDA-administered rats.

5.3.1.2. Rebamipide decreased 6-OHDA-induced changes in rotarod retention time and grip strength score

6-OHDA caused 46% and 71% reduction in both the rotarod retention time and grip strength scores respectively from D-7 compared to sham. Post-hoc analysis did not reveal any significant difference between control and sham groups. Statistical analysis by repeated measures of two-way ANOVA indicated that there were significant differences in rotarod retention time and grip strength scores among groups ([$F(6, 490) = 190.6$; $p < 0.05$], [$F(6, 490) = 803.6$; $p < 0.05$] respectively), time ([$F(4, 490) = 225.5$; $p < 0.05$], [$F(4, 490) = 944.0$; $p < 0.05$] respectively) and an interaction between group and time ([$F(24, 490) = 19.94$; $p < 0.05$], [$F(24, 490) = 102.7$; $p < 0.05$] respectively) as shown in **Table 5.1**. No change in motor parameters was observed by R-20. Onset of action for both R-40 (29% increase) and R-80 (51% increase) against 6-OHDA administration was D-21 in grip strength score. Rotarod retention time was found to be increased by 31% for both R-40 and R-80. However, the onset of action was D-28 for R-40 and D-21 for R-80. Progressive effects of R-40 and R-80 were noticed.

5.3.1.3. Rebamipide decreased 6-OHDA-induced changes in number of central squares crossed, ambulation, grooming and rearing in open field test

Open field parameters were adversely affected by 6-OHDA unilateral injection. There was no significant difference between control and sham groups. 6-OHDA significantly decreased all the parameters of open field test from D-7 (82%, 53% and 58% in ambulation, grooming and rearing respectively compared to sham) except for number of central squares crossed for which the onset of action was D-14 (57%

decrease). This delay is probably due to the further exploration of the open field by animal as indicated by number of central squares crossed. Repeated measures of two-way ANOVA showed that there were significant differences in number of central squares crossed, ambulation, grooming and rearing behavior among groups ([F (6, 490) = 536.8; $p < 0.05$], [F (6, 490) = 1941; $p < 0.05$], [F (6, 490) = 876.1; $p < 0.05$], [F (6, 490) = 1222; $p < 0.05$] respectively), time ([F (4, 490) = 893.0; $p < 0.05$], [F (4, 490) = 2131; $p < 0.05$], [F (4, 490) = 831.1; $p < 0.05$], [F (4, 490) = 1061; $p < 0.05$] respectively) and an interaction ([F (24, 490) = 121.8; $p < 0.05$], [F (24, 490) = 286.7; $p < 0.05$], [F (24, 490) = 105.7; $p < 0.05$], [F (24, 490) = 136.7; $p < 0.05$] respectively) between group and time in open field test **(Table 5.2).** Higher doses of rebamipide reduced 6-OHDA-induced impairment in open field behavior with onset of action of R-40 at D-28 and R-80 at D-21 (39% increase) for central squares crossed. R-40 and R-80 increased rearing D-28 (45% increase) and D-14 (15% increase) respectively. These doses also increased ambulation (66% and 76% respectively) and grooming (35% and 47% respectively) decreased by 6-OHDA from D-21. This proves more significant involvement of rebamipide in exploratory and displacement behavior (Coronel-Oliveros and Pacheco-Calderón, 2018; Lever et al., 2006; Smolinsky et al., 2009).

Table 5.1 Effects of rebamipide on 6-OHDA-induced alterations in motor functions as assessed by apomorphine-induced rotations, cataleptic behavior, grip strength score and rotarod retention time in rats

Groups	Apomorphine-induced rotations (Counts/5 min)	Cataleptic Behavior (sec)	Grip Strength Score	Retention Time in Rotarod Test (sec)
DAY 0				
Control	5.68 ± 0.54	1.87 ± 0.56	4.45 ± 0.37	180.30 ± 13.97
Sham	5.82 ± 0.54	1.90 ± 0.58	4.43 ± 0.31	181.30 ± 13.87
6-OHDA	5.79 ± 0.67	1.66 ± 0.51	4.43 ± 0.44	181.50 ± 20.70
6-OHDA+R-20	5.33 ± 0.58	1.72 ± 0.54	4.53 ± 0.41	171.60 ± 19.89
6-OHDA+R-40	5.28 ± 0.57	1.74 ± 0.50	4.41 ± 0.32	180.00 ± 18.27
6-OHDA+R-80	5.74 ± 0.60	1.91 ± 0.54	4.40 ± 0.28	175.40 ± 15.84
6-OHDA+Selegiline	5.88 ± 0.68	1.84 ± 0.49	4.53 ± 0.24	180.70 ± 16.32
DAY 7				
Control	6.17 ± 0.64	1.75 ± 0.41	4.34 ± 0.39	180.70 ± 10.06
Sham	5.49 ± 0.53	1.98 ± 0.44	4.23 ± 0.33	164.20 ± 8.33
6-OHDA	9.23 ± 0.94[a]	2.01 ± 0.20	1.22 ± 0.24[a]	88.17 ± 29.41[a]
6-OHDA+R-20	9.63 ± 1.12[a]	2.03 ± 0.42	1.10 ± 0.18[a]	80.11 ± 26.14[a]
6-OHDA+R-40	9.69 ± 0.85[a]	1.84 ± 0.21	1.27 ± 0.23[a]	83.36 ± 26.77[a]
6-OHDA+R-80	8.99 ± 0.64[a]	1.97 ± 0.24	1.30 ± 0.20[a]	81.50 ± 25.18[a]
6-OHDA+Selegiline	8.92± 0.62[a]	1.88 ± 0.23	1.34 ± 0.26[a]	89.67 ± 29.06[a]
DAY 14				
Control	5.76 ± 0.62	1.51 ± 0.30	4.19 ± 0.31	181.80 ± 11.75
Sham	5.91 ± 0.48	1.57 ± 0.31	4.16 ± 0.29	165.70 ± 10.43
6-OHDA	12.12 ± 1.10[a]	4.11 ± 0.40[a]	1.20 ± 0.26[a]	79.33 ± 19.15[a]
6-OHDA+R-20	11.93 ± 1.18[a]	4.21 ± 0.96[a]	0.99 ± 0.20[a]	72.49 ± 17.26[a]
6-OHDA+R-40	11.79 ± 1.07[a]	3.92 ± 0.29[a]	1.09 ± 0.21[a]	78.12 ± 15.60[a]
6-OHDA+R-80	9.69 ± 1.16[a,b,c,d]	3.80 ± 0.22[a]	1.20 ± 0.20[a]	90.34 ± 12.73[a]
6-OHDA+Selegiline	9.99 ± 1.13[b]	3.09 ± 0.17[b]	2.90 ± 0.38[b]	121.80 ± 15.02[b]
DAY 21				
Control	5.81 ± 0.64	1.95 ± 0.39	4.21 ± 0.29	180.20 ± 15.03
Sham	5.97 ± 0.55	1.96 ± 0.41	4.16 ± 0.25	166.30 ± 13.46
6-OHDA	14.22 ± 1.18[a]	6.09 ± 0.64[a]	1.45 ± 0.32[a]	93.83 ± 25.78[a]
6-OHDA+R-20	13.82 ± 1.17[a]	5.78 ± 0.60[a]	1.65 ± 0.36[a]	103.50 ± 28.33[a]
6-OHDA+R-40	11.92 ± 0.88[a,b,c]	5.87 ± 0.64[a]	2.04 ± 0.33[a,b,c]	111.30 ± 19.92[a]
6-OHDA+R-80	8.34 ± 0.53[a,b,c,d]	3.87 ± 0.44[a,b,c,d]	2.93±0.30[a,b,c,d]	135.90 ± 11.19[a,b,c,d]
6-OHDA+Selegiline	6.52 ± 0.49[b]	2.08 ± 0.28[b]	4.23 ± 0.41[b]	163.30 ± 12.02[b]
DAY 28				
Control	6.15 ± 0.67	1.91 ± 0.36	4.18 ± 0.28	181.50 ± 15.55
Sham	6.22 ± 0.42	1.89 ± 0.40	4.23 ± 0.28	171.30 ± 14.37
6-OHDA	14.97± 1.27[a]	5.78 ± 0.56[a]	1.23 ± 0.20[a]	87.53 ± 23.50[a]
6-OHDA+R-20	14.41 ± 1.14[a]	5.49 ± 0.45[a]	1.43 ± 0.27[a]	99.64 ± 27.45[a]
6-OHDA+R-40	7.89 ± 0.73[a,b,c]	4.28 ± 0.44[a,b,c]	2.66 ± 0.39[a,b,c]	127.40 ± 22.22[a,b,c]
6-OHDA+R-80	6.89 ± 0.87[b,c,d]	2.32 ± 0.33[b,c,d]	4.18 ± 0.43[b,c,d]	164.20 ± 12.19[b,c,d]
6-OHDA+Selegiline	6.82 ± 0.76[b]	2.25 ± 0.24[b]	4.25 ± 0.43[b]	167.20 ± 12.85[b]

All values are mean ± SD; n = 15; [a]$p < 0.05$ compared to sham, [b]$p < 0.05$ compared to 6-OHDA, [c]$p < 0.05$ compared to 6-OHDA+R-20 and [d]$p < 0.05$ compared to 6-OHDA+R-40 [Repeated measures of two-way ANOVA followed by Bonferroni test].

Table 5.2 Effects of rebamipide on 6-OHDA-induced alterations in motor functions as assessed by number of central squares crossed, ambulation, rearing and grooming in open field test in rats

Groups	Central Squares crossed (numbers)	Ambulation (numbers)	Rearing (numbers)	Grooming (numbers)
DAY 0				
Control	4.52 ± 0.16	45.35 ± 1.61	15.05 ± 0.49	6.71 ± 0.18
Sham	4.59 ± 0.15	46.40 ± 1.71	15.12 ± 0.47	6.65± 0.21
6-OHDA	4.59 ± 0.13	45.99 ± 2.02	15.11 ± 0.45	6.67 ± 0.20
6-OHDA+R-20	4.69 ± 0.13	46.99 ± 2.18	14.91 ± 0.41	6.72 ± 0.19
6-OHDA+R-40	4.47 ± 0.14	47.16 ± 1.83	14.62 ± 0.41	6.55 ± 0.21
6-OHDA+R-80	4.54 ± 0.13	45.23 ± 1.38	14.55 ± 0.42	6.46 ± 0.20
6-OHDA+Selegiline	4.52 ± 0.13	46.03 ± 1.33	14.52 ± 0.40	6.42 ± 0.22
DAY 7				
Control	4.47± 0.18	45.89 ± 1.91	14.42 ± 0.39	6.43 ± 0.42
Sham	4.50 ± 0.17	46.19 ± 3.59	14.39 ± 0.39	6.36 ± 0.46
6-OHDA	4.55± 0.21	8.27± 1.89[a]	6.03 ± 0.38[a]	3.02± 0.36[a]
6-OHDA+R-20	4.54 ± 0.26	7.97 ± 1.70[a]	6.09 ± 0.37[a]	3.01 ± 0.40[a]
6-OHDA+R-40	4.61 ± 0.23	8.34 ± 1.77[a]	6.00± 0.77[a]	3.18 ± 0.29[a]
6-OHDA+R-80	4.68 ± 0.24	9.11 ± 1.83[a]	6.42 ± 0.32[a]	3.22 ± 0.31[a]
6-OHDA+Selegiline	4.51± 0.23	10.04 ± 2.20[a]	6.53 ± 0.37[a]	3.32 ± 0.28[a]
DAY 14				
Control	4.53 ± 0.26	46.21 ± 2.67	15.06 ± 0.89	6.36 ± 0.39
Sham	4.64 ± 0.28	45.43 ± 2.80	14.91 ± 0.86	6.42 ± 0.43
6-OHDA	1.99 ± 0.21[a]	5.94 ± 1.57[a]	5.99 ± 0.50[a]	2.82 ± 0.23[a]
6-OHDA+R-20	2.11 ± 0.20[a]	6.39 ± 1.24[a]	5.79 ± 0.47[a]	2.75 ± 0.21[a]
6-OHDA+R-40	2.16 ± 0.17[a]	6.78 ± 1.56[a]	6.39 ± 0.72[a]	2.95 ± 0.19[a]
6-OHDA+R-80	2.21 ± 0.16[a]	7.33 ± 1.62[a]	7.05 ± 0.45[a,b,c]	3.05 ± 0.21[a]
6-OHDA+Selegiline	3.97 ± 0.22[b]	12.43 ± 2.31[b]	10.35± 0.65[b]	5.46± 0.31[b]
DAY 21				
Control	4.39± 0.23	46.03 ± 2.33	14.91 ± 0.76	6.38 ± 0.30
Sham	4.49 ± 0.22	45.05 ± 1.90	14.89 ± 0.82	6.41 ± 0.34
6-OHDA	2.20 ± 0.20[a]	8.22 ± 2.02[a]	5.66 ± 0.66[a]	2.84 ± 0.24[a]
6-OHDA+R-20	2.36 ± 0.19[a]	8.76 ± 2.23[a]	6.26 ± 0.75[a]	3.05 ± 0.28[a]
6-OHDA+R-40	2.54 ± 0.23[a,b]	23.92 ± 3.81[a,b,c]	6.95 ± 1.03[a,b]	4.37 ±0.39[a,b,c]
6-OHDA+R-80	3.59 ± 0.36[a,b,c,d]	33.69 ± 2.44[a,b,c,d]	10.59 ± 1.12[a,b,c,d]	5.32±0.51[a,b,c,d]
6-OHDA+Selegiline	4.38± 0.50[b]	43.89 ± 3.56[b]	14.20 ± 1.33[b]	6.27 ± 0.56[b]
DAY 28				
Control	4.32± 0.23	44.18 ± 2.27	14.55 ± 0.75	6.36± 0.32
Sham	4.41± 0.21	44.22 ± 1.97	14.47 ± 0.81	6.40 ± 0.33
6-OHDA	1.87 ± 0.17[a]	5.94 ± 1.50[a]	5.28± 0.73[a]	2.71 ± 0.26[a]
6-OHDA+R-20	2.08 ± 0.15[a]	6.58 ± 2.04[a]	5.53± 0.81[a]	2.88 ± 0.33[a]
6-OHDA+R-40	3.05 ± 0.28[a,b,c]	34.21 ± 6.03[a,b,c]	9.58± 1.46[a,b,c]	4.19 ± 0.45[a,b,c]
6-OHDA+R-80	4.19± 0.41[b,c,d]	43.26 ± 3.78[b,c,d]	13.86 ± 1.31[b,c,d]	6.15 ± 0.66[b,c,d]
6-OHDA+Selegiline	4.29± 0.40[b]	44.28 ± 3.80[b]	14.16 ± 1.38[b]	6.25 ± 0.67[b]

All values are mean ± SD; n = 15; [a]$p < 0.05$ compared to sham, [b]$p < 0.05$ compared to 6-OHDA, [c]$p < 0.05$ compared to 6-OHDA+R-20 and [d]$p < 0.05$ compared to 6-OHDA+R-40 [Repeated measures of two-way ANOVA followed by Bonferroni test].

5.3.2. Rebamipide ameliorated 6-OHDA-induced changes in striatal dopaminergic system

PD occurs due to death of neurons in nigrostriatal DA pathway (Dauer and Przedborski, 2003) and 6-OHDA mimics the same by decreasing striatal DA concentration in rats. One way ANOVA showed significant differences among groups in the levels of DA [$F(6, 35) = 122.0$; $p < 0.05$], DOPAC [$F(6, 35) = 75.60$; $p < 0.05$], HVA [$F(6, 35) = 25.80$; $p < 0.05$], DOPAC/DA [$F(6, 35) = 14.68$; $p < 0.05$] and HVA/DA [$F(6, 35) = 8.589$; $p < 0.05$] as shown in **Figure 5.3.** No significant differences were found between control and sham groups. 6-OHDA-administration decreased DA (67%), DOPAC (55%) and HVA (51%) levels and therefore there was increased in DOPAC/DA (28%) and HVA/DA (33%) ratios compared to sham group. Lower doses R-20 and R-40 were found to be ineffective against 6-OHDA-administered rats. However, R-80 significantly increased DA (54%), DOPAC (44%) and HVA (31%) and decreased DOPAC/DA (17%) and HVA/DA (33%) in 6-OHDA-infused rats.

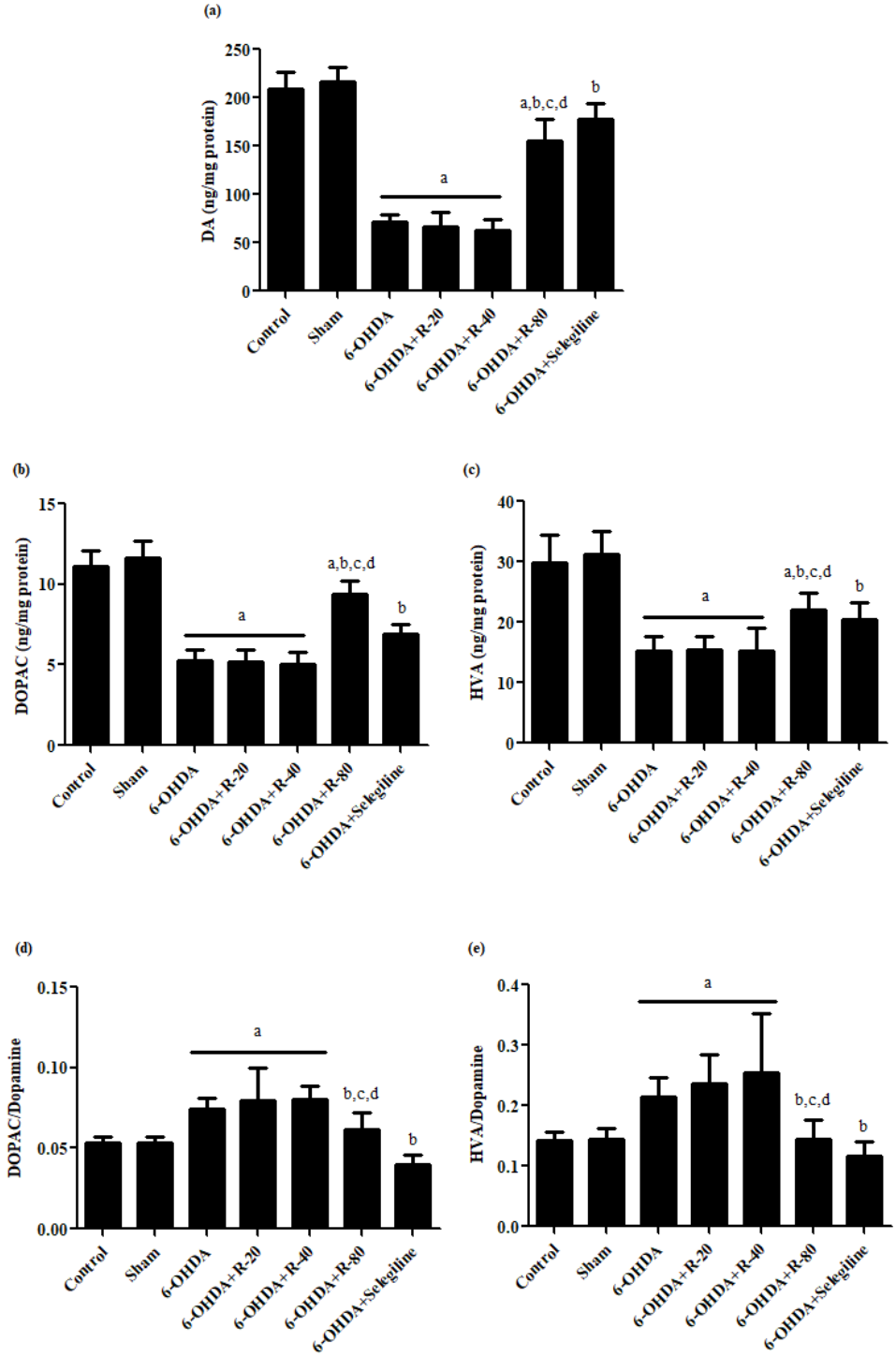

Figure 5.3 Effect of rebamipide on 6-OHDA-induced changes in the levels of DA (a), DOPAC (b), HVA (c), DOPAC/DA (d), and HVA/DA (e) in ipsilateral striatal tissues of rats. All values are mean ± SD; n = 6; $^{a}p < 0.05$ compared to sham, $^{b}p < 0.05$ compared to 6-OHDA, $^{c}p < 0.05$ compared to 6-OHDA+R-20 and $^{d}p < 0.05$ compared to 6-OHDA+R-40 [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

5.3.3. Rebamipide attenuated 6-OHDA-induced decrease in mitochondrial respiratory complex activities in rat striatal tissues

6-OHDA being a direct inhibitor of mitochondrial respiratory chain results into mitochondrial dysfunction (Blum et al., 2001). 6-OHDA administration significantly reduced the complex enzyme I, II, IV and V activities up to 74%, 50%, 55% and 61% respectively. Control and sham groups were not found to be significantly different. One-way ANOVA showed that there were significant differences in complex-I [$F(6, 35) = 48.43$; $p < 0.05$], complex-II [$F(6, 35) = 13.46$; $p < 0.05$], complex-IV [$F(6, 35) = 17.39$; $p < 0.05$] and complex-V activities [$F(6, 35) = 27.32$; $p < 0.05$] among groups **[Figure 5.4].** Higher doses increased the complex enzyme – I, II, IV and V activities dose-dependently (50%, 34%, 46% and 33% increase by R-40; and 69%, 49%, 50% and 50% increase by R-80) against 6-OHDA-infused rats.

5.3.4. Rebamipide decreased 6-OHDA-induced increase in Mitochondrial LPO activity in rat striatal tissues

Mitochondrial respiratory chain inhibition leads to increase in oxidative stress. Moreover, auto-oxidation of 6-OHDA also generates ROS which is toxic to neuron (Blum et al., 2001). Mitochondrial MDA, a marker of lipid peroxidation was estimated and 6-OHDA caused a severe increase (59%) in mitochondrial MDA activity. One-way ANOVA revealed significant differences in MDA levels [$F(6, 35) = 29.62$; $p < 0.05$] among groups **[Figure 5.5].** There was not any significant difference found between control and sham groups. MDA was decreased by R-40 and R-80 whereas R-20 was found to be ineffective in 6-OHDA-infused rats.

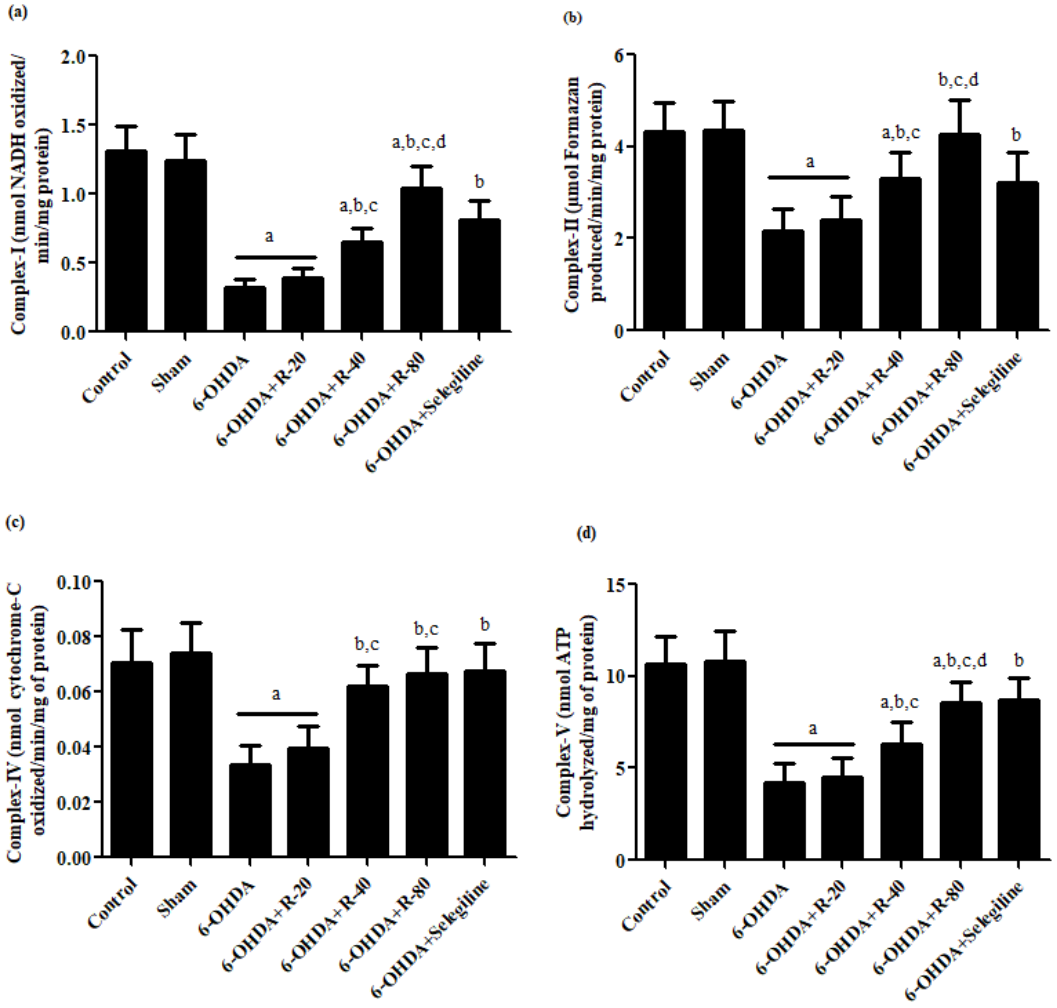

Figure 5.4 Effect of rebamipide on 6-OHDA-induced alterations in mitochondrial complex-I (a), complex-II (b), complex-IV (c) and complex-V (d) activities in ipsilateral striatal tissues of rats. All values are mean ± SD; n = 6; $^{a}p < 0.05$ compared to sham, $^{b}p < 0.05$ compared to 6-OHDA, $^{c}p < 0.05$ compared to 6-OHDA+R-20 and $^{d}p < 0.05$ compared to 6-OHDA+R-40 [One-way ANOVA followed by Student Newman–Keuls Post-hoc test].

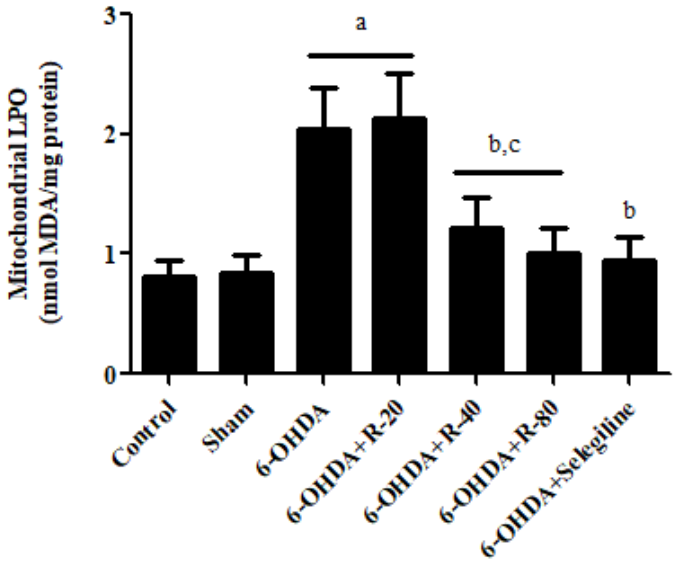

Figure 5.5 Effect of rebamipide on 6-OHDA-induced increase in mitochondrial LPO activity in ipsilateral striatal tissues of rats. All values are mean ± SD; n = 6; $^{a}p < 0.05$ compared to sham, $^{b}p < 0.05$ compared to 6-OHDA and $^{c}p < 0.05$ compared to 6-OHDA+R-20 [One-way ANOVA followed by Student Newman–Keuls Post-hoc test].

5.3.5. Rebamipide attenuated 6-OHDA-induced changes in different states of mitochondrial respiration and mitochondrial RCR in rat striatal tissues

Impairment in cellular respiration results into reduced ATP production making cells prone to oxidative stress and causes apoptosis or necrosis (Zamzami et al., 1997). The effect of various doses of rebamipide on 6-OHDA-induced changes on oxygen consumption in different states of mitochondrial respiration and mitochondrial RCR (State 3/State 4 respiration) is shown in **Figure 5.6 (a) and (b)** respectively. One-way ANOVA revealed that there were significant differences in different states of mitochondrial respiration, namely, state 2 [$F(6, 14) = 6.456$; $p < 0.05$], state 3 [$F(6, 14) = 30.57$; $p < 0.05$], state 4 [$F(6, 14) = 16.98$; $p < 0.05$], state 5 complex-I [F (6,

14) = 26.63; $p < 0.05$] and state 5 complex-II respiration [F (6, 14) = 5.581; $p < 0.05$] among groups. No significant differences were observed between control and sham groups. Mitochondrial bioenergetics was hampered in case of 6-OHDA administration because significant reduction of 49%, 43%, 50% and 27% in states 2, 3, 5 (complex I & II) of mitochondrial respiration was observed except for state 4 which was 44% increased by 6-OHDA compared to sham groups. No significant modulatory effect was elicited by R-20, but R-40 and R-80 significantly attenuated the changes induced by 6-OHDA. Both R-40 and R-80 were found to be effective to similar extent in increasing state 3 and decreasing state 4 respiration in 6-OHDA-infused rats. However, in upregulating the state-V (complex I) respiration R-80 was most effective.

A significant difference in RCR [F (6, 14) = 18.41; $p < 0.05$] was observed among groups as shown by one-way ANOVA. Control and sham groups were not found to be significantly different. Post-hoc analysis revealed that in 6-OHDA-administered group there was 68% reduction in RCR compared to sham group. RCR was restored by R-40 and R-80 up to 47% and 64% respectively in 6-OHDA-administered group.

5.3.6. Rebamipide decreased 6-OHDA-induced changes in GCase enzymatic activity and soluble α-synuclein concentration in rat nigral tissues

Figure 5.7 depicts the effects of various rebamipide doses on 6-OHDA-induced alterations in GCase activity **(a)** and soluble α-synuclein concentration **(b)** in nigral tissues of rats. One-way ANOVA showed significant differences in GCase enzymatic activity [F (6, 35) = 29.12; $p < 0.05$] and soluble α-synuclein concentration [F (6, 35)

= 13.59; $p < 0.05$] among groups. Post-hoc analysis indicated that in 6-OHDA-infused rats there was severe decrease in GCase enzymatic activity (76%) and soluble α-synuclein concentration (73%) compared to sham groups. Higher doses R-40 and R-80 dose-dependently caused marked elevations in GCase activity and soluble α-synuclein concentration against 6-OHDA-infused rats. No significant differences were observed between control and sham groups.

5.3.7. Rebamipide decreased 6-OHDA-induced increase in protein expressions of cytochrome-C, caspase-9 and caspase-3 in rat nigral tissues

Cellular death is the final outcome of 6-OHDA-induced toxicity as observed by decrease in expression of proteins like cytochrome-C, cleaved caspase-9 and caspae-3 **[Figure 5.8].** Significant differences were noticed among groups [$F (6, 14) = 50.2$; $p < 0.05$], [$F (6, 14) = 78.7$; $p < 0.05$] and [$F (6, 14) = 60.5$; $p < 0.05$] respectively as given by one-way ANOVA. Control and sham groups were not found to be significantly different. 6-OHDA-induced expression of these proteins was decreased by R-80 administration. However, R-20 and R-40 did not ameliorate the same.

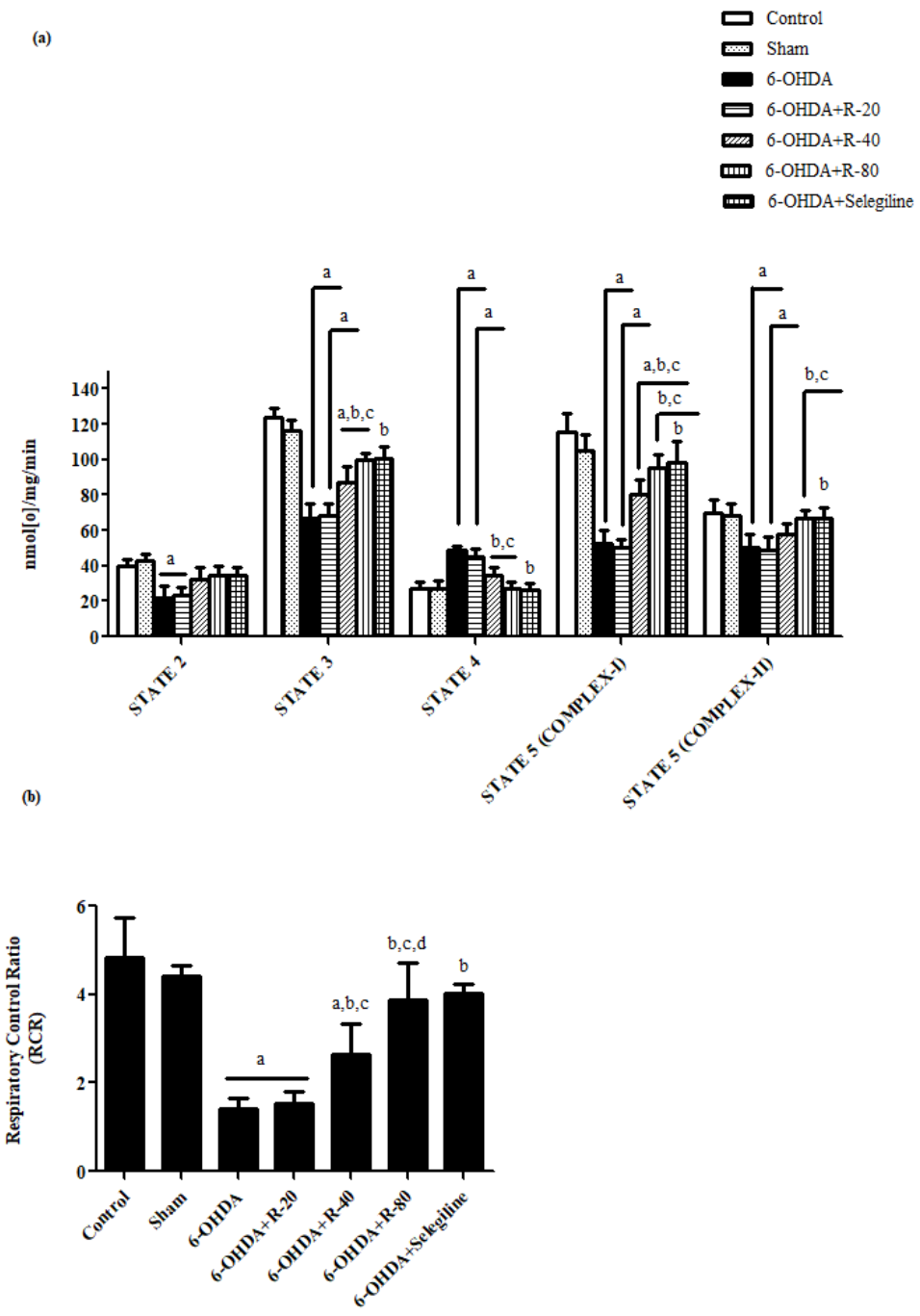

Figure 5.6 Effect of rebamipide on 6-OHDA-induced changes on oxygen consumption in different states of mitochondrial respiration (State 2, State 3, State 4, State 5 via complex-I and state 5 via complex-II) (a) and RCR (b) in rat striatal tissues. All values are mean ± SD; n = 3; $^{a}p < 0.05$ compared to sham, $^{b}p < 0.05$ compared to 6-OHDA, $^{c}p < 0.05$ compared to 6-OHDA+R-20 and $^{d}p < 0.05$ compared to 6-OHDA+R-40 [One-way ANOVA followed by Student Newman–Keuls Post-hoc test].

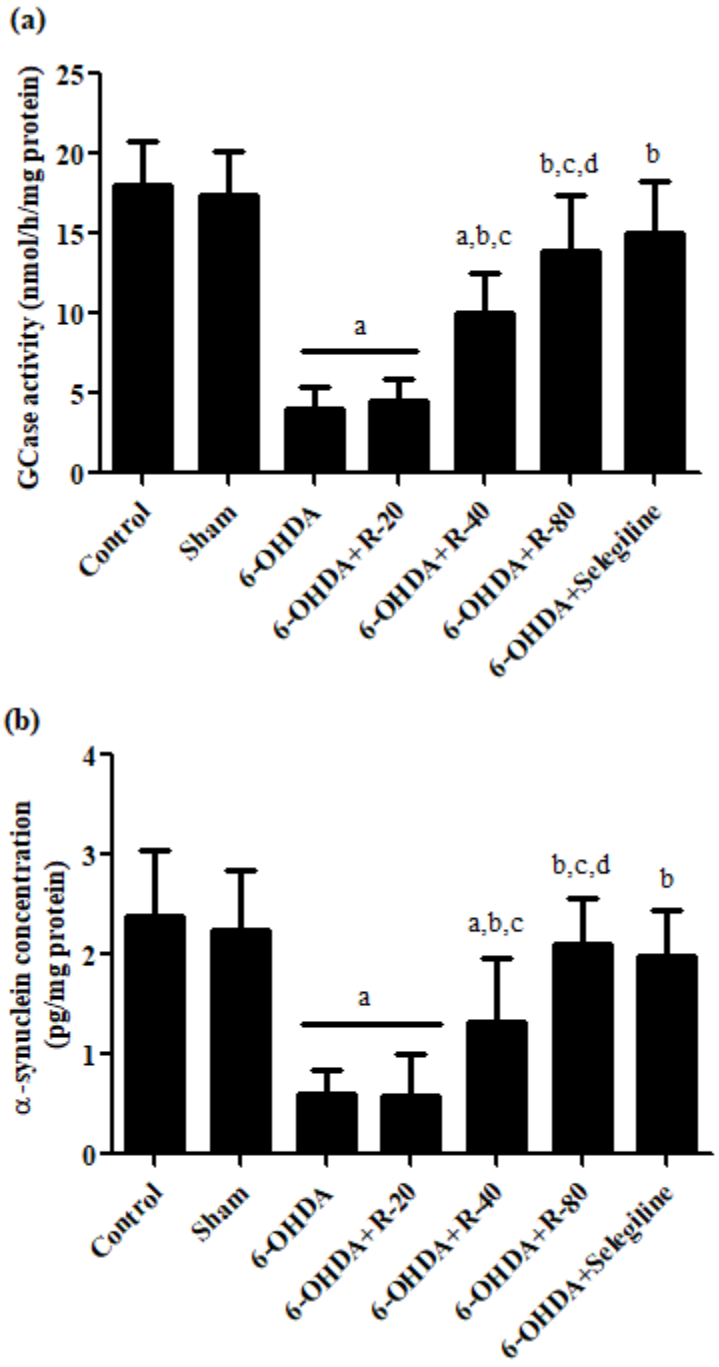

Figure 5.7 Effect of rebamipide on 6-OHDA-induced alterations in GCase enzymatic activity (a) and α-synuclein protein concentration (b) in ipsilateral nigral tissues of rats. All values are mean ± SD; n = 6; $^{a}p < 0.05$ compared to sham, $^{b}p < 0.05$ compared to 6-OHDA, $^{c}p < 0.05$ compared to 6-OHDA+R-20 and $^{d}p < 0.05$ compared to 6-OHDA+R-40 [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

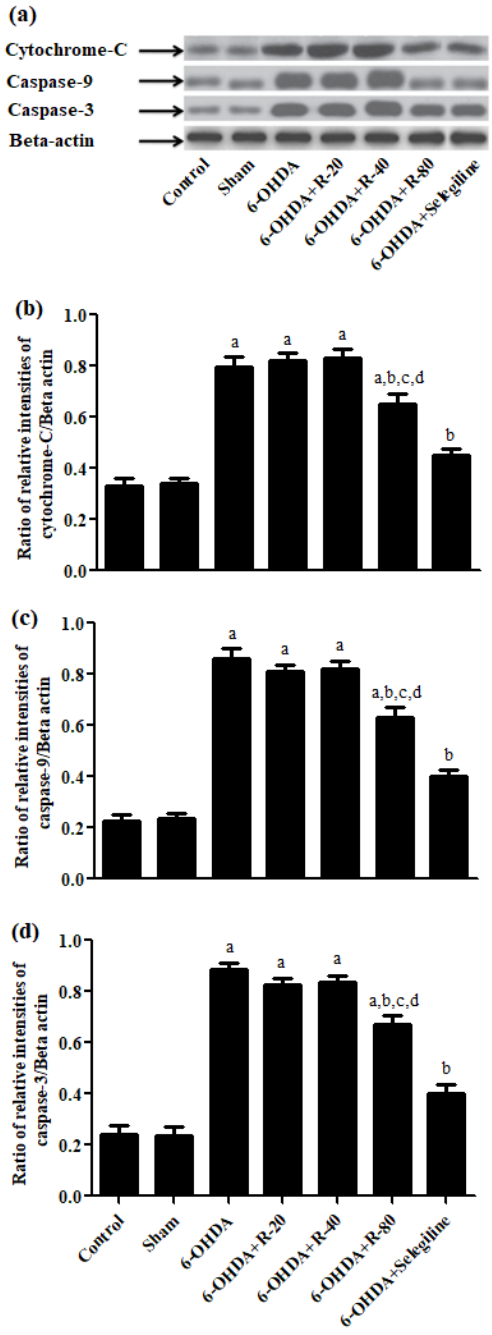

Figure 5.8 Effect of rebamipide on 6-OHDA-induced alterations in the protein expression of cytochrome-C, caspase-9 and caspase-3 in rat nigral tissues. Proteins are represented in blots (a) and histograms express the ratio of relative intensity of protein levels of cytochrome-C (b), cleaved caspase-9 (c) and cleaved caspase-3 (d) to β-actin. All values are mean ± SD; n = 3; $^{a}p < 0.05$ compared to sham, $^{b}p < 0.05$ compared to 6-OHDA, $^{c}p < 0.05$ compared to 6-OHDA+R-20 and $^{d}p < 0.05$ compared to 6-OHDA+R-40 [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

5.4. Discussion

The most important finding of the present study is the pharmacological effect of rebamipide, a popularly known anti-ulcer drug in alleviating the symptoms of 6-OHDA-induced unilateral experimental PD model in rats. Additionally, the efficacy of rebamipide against the factors responsible for pathophysiology of PD such as oxidative stress, mitochondrial dysfunction, α-synuclein pathology and GCase enzymatic depletion is also discussed for the first time.

The pathophysiology of PD involves the death of DA neurons in nigrostriatal pathway causing decreased DA release in the striatum (Dauer and Przedborski, 2003). In the present study, intrastriatal injection of 6-OHDA caused significant reduction in striatal contents of DA (67%), DOPAC (55%) and HVA (51%). These changes increased DA turnover in the ipsilateral tissues of rats as reported (Kumar et al., 2017). Highest dose of rebamipide (R-80) was required to ameliorate the effects of 6-OHDA by increasing DA and its metabolites and decreasing DA turnover in 6-OHDA-administered rats.

Dopaminergic neuronal death starts long before appearance of symptoms of PD which appear only after 60-70% DA neuronal death. PD mainly affects the motor movements of patients (Cheng et al., 2010). In the present study, 6-OHDA caused motor deficits in rats. Rotarod retention time (Rozas et al., 1997), grip strength scores (Kumar et al., 2017) and open field parameters (Van Den Buuse et al., 1986) were decreased, while apomorphine-induced contralateral rotations (Ungerstedt, 1971) and cataleptic behavior (Kumar et al., 2017) was increased in rats by 6-OHDA. Unilateral 6-OHDA intrastriatal injection gives rise to unilateral DA depletion which can be accurately measured by apomorphine-induced head rotation (Ungerstedt, 1971). 6-

OHDA-induced motor impairments were observed from D-7; however, 6-OHDA could impair cataleptic behavior and some open field test parameters like number of central squares crossed only from D-14. Catalepsy test evaluates fine motor control including acceptance and retention of abnormal posture (Batool and Haleem, 2008; Walther and Strik, 2012; Whishaw et al., 1990) and number of central squares crossed represents wider exploration of open field and locomotor activity by rats (Denenberg, 1969). This indicates that 6-OHDA probably required more time to impair fine motor movements seen from D-14 in the present study. However, once initiated, 6-OHDA effects were progressive. Increase in rotational behavior and intensity of cataleptic behavior was found to be more severe as the study progressed, thereby proving more significant role of 6-OHDA in unilateral DA depletion and fine motor control. Rebamipide, a popularly known gastro protective drug is reported to decrease Aβ42 production and increased cell viability in cultured SH-SY5Y human neuroblastoma cells (Fukui et al., 2017). In the present study, rebamipide ameliorated motor deficits. Lower dose of rebamipide was found ineffective, but both R-40 and R-80 significantly attenuated motor deficits in 6-OHDA-infused rats. Bar cataleptic behavior (Walther and Strik, 2012; Whishaw et al., 1990), open field parameters (Qian et al., 2010) and grip strength test (Pradhan et al., 2010) signify fine motor skills. Gross motor coordination is characterized by rotarod test (Qian et al., 2010; Rozas et al., 1997). Dose-dependent effects of rebamipide were observed to ameliorate both the gross and fine motor deficits. Moreover, the onset of action for R-80 was earlier than R-40, indicating high efficiency of highest dose (R-80) against 6-OHDA-induced behavioral deficits.

Decreased DA striatal release and occurrence of DA neuronal death in SNc in PD subjects is reported to be a direct consequence of mitochondrial dysfunction (Guo et al., 2013) because mitochondrial complex I deficiency is observed in SNc of PD subjects (Schapira et al., 1990). In the present study also, 6-OHDA decreased mitochondrial complex enzyme activities in rat striatal tissues. Both R-40 and R-80 increased the same in 6-OHDA-infused rats. Rebamipide is reported to suppress diclofenac-induced intestinal permeability via mitochondrial protection in mice and improved complex-I, II and V activities (Diao et al., 2012). In the present study, rebamipide increased complex-V activity which can increase ATP abundance for DA biosynthesis. Impaired mitochondrial complex enzyme activities also generate toxic radicals to induce oxidative stress (Moore et al., 2005). 6-OHDA increased mitochondrial oxidative stress in rat striatal tissues measured in terms of LPO levels as reported earlier (Kumar et al., 2017). Both R-40 and R-80 protected against oxidative stress by decreasing mitochondrial LPO. Rebamipide is reported to inhibit indomethacin-induced mitochondrial lipid oxidation in gastric epithelial RGM-1 cells and inhibit ROS production *in vitro* (Nagano et al., 2005). Rebamipide also decreased MDA induced by hepatic ischemia/reperfusion injury in rats (Gendy et al., 2017). Antioxidant effects of rebamipide may interfere with 6-OHDA pro-oxidant mechanism.

Lipid peroxidation is associated with impaired mitochondrial bioenergetics (Jun et al., 2007; Krügel et al., 2003). The effects of rebamipide were also studied on different states of striatal mitochondrial respiration and it was measured in terms of oxygen consumption at D-28 after 6-OHDA toxicity. State 2 respiration was initiated by adding its substrate pyruvate/malate which enhanced the electron transfer via

complex-I (NADH dehydrogenase) and 6-OHDA significantly decreased state 2 respiration. State 3 respiration was fueled by addition of ADP which straight away got converted to ATP due to the proton motive force induced by ETC (Samaiya et al., 2016). In healthy mitochondria these protons returned to the matrix via ATPase pump. In present study, 6-OHDA decreased state 3 respiration indicating dysfunction of mitochondrial complex enzyme V activation which leads to inadequate proton gradient and scarcity of ATP after 6-OHDA administration. 6-OHDA is reported to inhibit ATP content in SH-SY5Y cells (Hu et al., 2010) and chronic mouse model of PD also decreased striatal ATP (Patki and Lau, 2011). State 3 respiration was followed by addition of oligomycin which blocked ATPase in its open state thereby inhibiting proton gradient to travel across the channel (Nicholls and Ward, 2000). This is the reason why healthy isolated mitochondria failed to consume high oxygen during this state. 6-OHDA is reported to decrease state 3 respiration and increased state 4 respiration in rat nigral tissues (Kupsch et al., 2014). In present study, 6-OHDA caused significant increase in state 4 respiration probably due to partial damage of mitochondrial membrane (Gilmer et al., 2009; Kumar et al., 2017). It was reported that striatal mitochondria on incubation with 1 mM DA at 30^0C during 5 min caused significant decrease in state 3 and increase in state 4 respiration (Czerniczyniec et al., 2010). The ratio of state 3 to state 4 respiration gives information about RCR, the extent of coupling of ETC to oxidative phosphorylation for ATP production (Samaiya et al., 2018). In current study, 6-OHDA caused significant decrease in RCR indicating detrimental mitochondrial bioenergetics at D-28. Chronic mouse model of PD is reported to decrease RCR in striatal mitochondria (Patki and Lau, 2011) and 6-OHDA is also reported to cause a significant reduction

in RCR in nigral rat tissues (Kupsch et al., 2014). This is followed by addition of mitochondrial uncoupler (FCCP) which makes protons bypass the ATP synthase and enable them to return to matrix freely at a very fast rate. The role of FCCP is to evaluate optimum efficiency of ETC to re-establish the dissipated proton gradient (Singh et al., 2006). State 5 respiration via complex-I was decreased by 6-OHDA in the present study. This was followed by adding complex-I inhibitor (rotenone) to cease the oxygen consumption via complex-I and 6-OHDA administration decreased the same in the present study. Complex-II substrate succinate was then added to begin the respiration through complex-II (FADH dehydrogenase) which was also reduced by 6-OHDA, suggesting decreased activity of mitochondrial complex-II. 6-OHDA is also reported to decrease complex-I and –II activities in nigral brain tissues of rats (Dabbeni-Sala et al., 2001). State 3 and state 5 of mitochondrial respiration were increased and state 4 was decreased by R-40 and R-80 in 6-OHDA-administered rats indicating restoration of mitochondrial complex enzyme system. R-40 and R-80 also increased mitochondrial RCR in striatal tissues of 6-OHDA rats. Therefore, rebamipide restored impaired mitochondrial bioenergetics in 6-OHDA-infused rats.

Mitochondrial dysfunction also occurs as negative downstream effects of abnormal accumulation of α-synuclein which causes reduction in mitochondrial protein import. α-synuclein aggregates are reported to depress both basal and FCCP-stimulated respiration and caused protein thiol oxidation leading to oxidative damage (Di Maio et al., 2016). In the present study, water-soluble α-synuclein protein concentration was measured in rat nigral tissues. α-synuclein is found with monomeric structure under normal physiology and turn into water-insoluble aggregates during toxic conditions. In aged individuals, the water solubility of α-

synuclein is reduced, suggesting the formation of aggregated proteins (Budi et al., 2012). In present study, neurotoxin 6-OHDA reduced soluble α-synuclein concentration on D-28 in rat nigral tissues, indicating aggregates formation of α-synuclein as reported earlier (Coulombe et al., 2016). Both R-40 and R-80 dose-dependently increased the concentration of water-soluble α-synuclein in 6-OHDA-infused rats indicating decreased α-synuclein aggregates.

α-synuclein also impairs GCase trafficking to lysosome and makes α-synuclein-GCase complex, thus inhibiting GCase enzyme function (Mazzulli et al., 2011; Yap et al., 2011). GCase enzymatic activity is decreased by 6-OHDA in rat nigral tissues in the present study as reported earlier in Chapter 4 (Mishra et al., 2018). Rebamipide increased GCase enzymatic activity in 6-OHDA-infused rats in dose-dependent manner. Mitochondrial dysfunction was also observed due to GCase inhibition in cellular models (Cleeter et al., 2013) and brains of GCase-deficient animals (Osellame et al., 2013). The relationship is bidirectional because mitochondrial dysfunction also leads to GCase deficiency (Gegg et al., 2012). So, there is a scope to believe that in the present study rebamipide attenuated 6-OHDA-induced mitochondrial impairment and oxidative stress due to reduction in insoluble α-synuclein aggregates followed by increase in GCase activity. Bidirectional relationships are also reported between α-synuclein and GCase. Pharmacological GCase inhibition caused formation of α-synuclein aggregates (Cleeter et al., 2013; Mazzulli et al., 2011) due to accumulation of GCase substrate GC in SNc which act as scaffold for oligomeric intermediates to form oligomeric α-synuclein toxic aggregates in lysosome (Mazzulli et al., 2011). So, it is also possible that rebamipide

increased GCase activity in 6-OHDA-infused rats to reduce insoluble α-synuclein aggregates and mitochondrial impairment.

Mitochondrial dysfunction is followed by the release of cytochrome-C from the mitochondria and activation of caspase-9 and caspase-3 causing intrinsic pathway of apoptosis (Elmore, 2007). In present study, the expression of these proteins was found to be increased in nigral tissues of rats by 6-OHDA intrastriatal injection (Kumar et al., 2017). Highest dose R-80 significantly decreased the expression of these proteins in 6-OHDA-administered rats. Rebamipide is reported to inhibit indomethacin-induced caspase-3 dependent apoptosis in gastric epithelial RGM-1 cells (Nagano et al., 2005) and decreased caspase-3 in hepatic ischemia in rat (Gendy et al., 2017). Hence, R-80 was found to ameliorate 6-OHDA-induced intrinsic pathway of apoptosis. Therefore, rebamipide mitigates impairments in mitochondrial function and bioenergetics with α-synuclein pathology in 6-OHDA-induced hemiparkinson's model in rats.

5.5. Conclusions

The study showed dose-dependent effects of rebamipide in ameliorating characteristic motor deficits of PD induced by 6-OHDA in rats. The drug increased mitochondrial complex enzyme activities, mitochondrial bioenergetics and GCase enzymatic activities as well as decreased oxidative stress with α-synuclein pathology in 6-OHDA-induced hemiparkinson's rat model. Highest dose (R-80) was also found to attenuate 6-OHDA-induced DA striatal degeneration and intrinsic pathway of apoptosis. **Figure 5.9** illustrates the anti-PD action of rebamipide in 6-OHDA-

induced hemiparkinson's rat model. Hence, the results suggest therapeutic potential of rebamipide in management of PD.

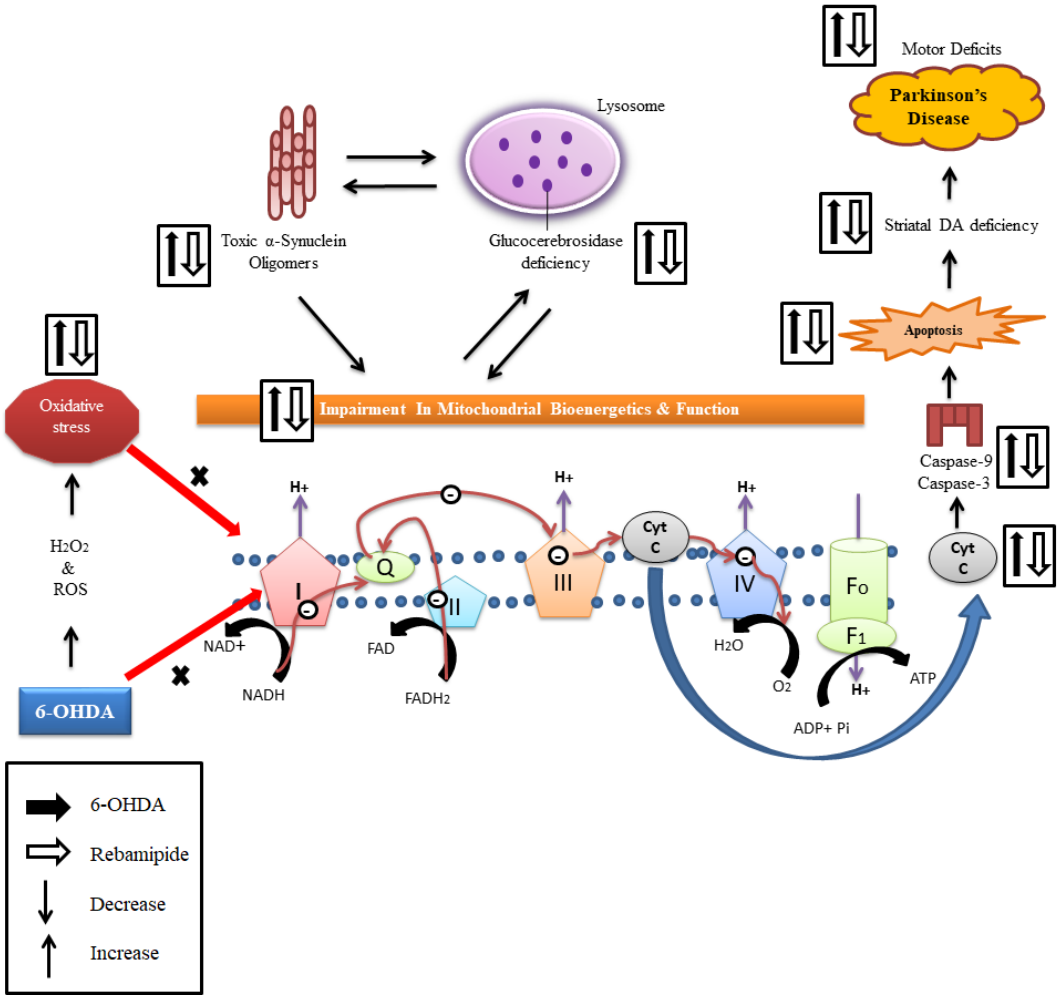

Figure 5.9 The outcome of specific objective for the evaluation of rebamipide in sub-acute doses for its action against 6-OHDA-induced toxicity in rats. Rebamipide mitigates impairment in mitochondrial bioenergetics, followed by GCase deficiency. It decreases oxidative stress with α-synuclein pathology and thereby attenuates 6-OHDA-induced DA striatal degeneration, intrinsic pathway of apoptosis and motor deficits in hemiparkinson's model in rats.