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Targeting oxidative stress attenuates malonic acid induced Huntington like behavioral and mitochondrial alterations in rats

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ABSTRACT

Objective of the present study was to explore the possible role of oxidative stress in the malonic acid induced behavioral, biochemical and mitochondrial alterations in rats. In the present study, unilateral single injections of malonic acid at different doses (1.5, 3 and 6 µmol) were made into the ipsilateral striatum in rats. Behavioral parameters were accessed on 1st, 7th and 14th day post malonic acid administration. Oxidative stress parameters and mitochondrial enzyme functions were assessed on day 14 after behavioral observations. Ipsilateral striatal malonic acid (3 and 6 µmol) administration significantly reduced body weight, locomotor activity, motor coordination and caused oxidative damage (lipid peroxidation, nitrite, superoxide dismutase, catalase and glutathione) in the striatum as compared to sham treated animal. Mitochondrial enzyme complexes and MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolinium bromide) activity were significantly inhibited by malonic acid. Vitamin E treatment (50 and 100 mg/kg, *p.o.*) significantly reversed the various behavioral, biochemical and mitochondrial alterations in malonic acid treated animals. Our findings show that targeting oxidative stress by vitamin E in malonic acid model, results in amelioration of behavioral and mitochondrial alterations are linked to inhibition of oxidative damage. Based upon these finding present study hypothesize that protection exerted by vitamin E on behavioral, mitochondrial markers indicates the possible preservation of the functional status of the striatal neurons by targeting the deleterious actions of oxidative stress.

1. Introduction

Huntington disease is a neurodegenerative disorder, characterized by psychiatric disturbances, choreiform movements (Scattoni et al., 2007), and pathologically caused selective degeneration of medium spiny GABAergic neurons in the striatum (Davies and Ramsden, 2001; Leavitt et al., 2006). Another pathological characterization is bioenergetic defect due to activity of succinate dehydrogenase, (Calabresi et al., 2001) an enzyme that is a component of both the Krebs cycle and complex II of electron transport chain. Free radicals are now accepted as important mediators of tissue injury in several neurodegenerative states (Beal, 1996; Simonian and Coyle, 1996) and in some pathological conditions (Sun et al., 2005). In fact, free radicals attack membrane lipids, proteins and nucleic acids, which can cause cell damage or death (Halliwell, 1992). In this scenario, antioxidants play a fundamental role as scavengers (Jara-Prado et al., 2003; Perez-De La Cruz et al., 2006; Sutherland et al., 2005, 2006; Posser et al., 2006).

Earlier reports demonstrated that some Krebs cycle intermediates can act as antioxidants against a variety of in vitro and in vivo pro-oxidant situations (Yamamoto and Mohanan, 2003; Puntel et al., 2005). Conversely, literature has indicated that succinate inhibits the NADH- and NADPH-dependent lipid peroxidation (Takayanagi et al., 1980). Succinate can also prevent lipid peroxidation induced by Fe (II)/ADP (Takayanagi et al., 1980) or NADPH/Fe (III)/ADP (Cavallini et al., 1984). Malonic acid is a reversible inhibitor of enzyme succinate dehydrogenase reported to induce mitochondrial dysfunction, which in turn can trigger superoxide radicals generation, secondary excitotoxicity, and apoptosis (Dedeoglu et al., 2002). Thus, agents that restore mitochondrial function are thought to play an important role in preventing malonic acid pro-oxidant activity (Matthews et al., 1998; Fernandez-Gomez et al., 2005).

Biochemical studies of Huntington disease brain tissue demonstrated multiple defects in the caudate decreased complex II activity and decreased complex II–III activity and no alteration of complex I or IV activities) (Mann et al., 1990; Bonsi et al., 2006). Although various lines of evidence demonstrate the involvement of mitochondrial dysfunction in the pathogenesis of Huntington disease yet the mechanism by which mitochondrial inhibitors causes neuronal death is not fully understood. For all these purposes, in this study we evaluated whether the behavioral disturbances produced by malonic acid largely used as phenotypic models of Huntington disease

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are able to resemble the kinetic impairment observed in the human disorder, as well as the possible participation of oxidative damage through treatment with vitamin E in the course of these behavioral deficits elicited by malonic acid, infused intrastriatally rats. The behavioral deficits evaluated here considered markers of motor activity and motor coordination. In addition, abnormal behavior was correlated with changes in some biochemical and mitochondrial dysfunction markers of striatal damage. Vitamin E is major lipid-soluble chain-breaking antioxidant in mammals and plays an important role in normal development and physiology. Deficiency of vitamin E (whether dietary or genetic) results in primarily nervous system pathology, including cerebellar neurodegeneration, Down's syndrome and progressive ataxia (abnormal gait).

2. Materials and methods

2.1. Animal

Male Wistar rats (250–300 g) bred in the Central Animal House of Panjab University, Chandigarh were used. Animals were acclimatized to laboratory conditions prior to experimentation. The animals were kept under standard laboratory conditions of a 12 h light and dark cycle with food and water ad libitum. All the experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the Indian National Science Academy Guidelines for the use and care of experimental animals.

2.2. Drugs and treatment schedule

Malonic acid (Sigma Chemical, USA) was dissolved in the normal saline and administered unilaterally in the striatum (4 µl). Animals were divided into nine groups. Group-1 naïve (without surgery), group-2 sham (surgery without intrastriatal injection) received normal saline, groups 3 to 5 received intrastriatal malonic acid 1.5, 3 and 6 µmol single injections respectively. Naïve animals received vitamin E [(±)-α-Tocopherol (Sigma, USA)] (50 and 100 mg/kg, *p.o.*), *per se* treatment in groups 6 and 7. While vitamin E (50 and 100 mg/kg, *p.o.*) treatment against intrastriatal malonic acid 6 µmol administration was considered as groups 8 and 9 respectively.

2.3. Intrastriatal administration of malonic acid

Animals were anesthetized with thiopental sodium (45 mg/kg, *i.p.*). The surface of the skull was exposed by making an incision on the scalp. Malonic acid (1.5, 3 and 6 µmol) injections were made into the right striaturn by means of a 28-gauge stainless steel needle attached to the Hamilton syringe. Injections were made via a l–2 mm diameter hole made in the skull using a small hand drill at anterior + 1.7 mm; lateral ± 2.7 mm; ventral-4.8 mm from bregma and dura as described in Paxinos and Watson (2007). Malonic acid was injected in a volume of 4 µl delivered over a period of 2 min, and injection needle was left in the place for another 1.5 min to allow diffusion of the injected drug solution.

2.4. Behavioral assessments

2.4.1. Body weight change

The body weight was recorded before malonic acid administration (1st day) and on last day (14th) after the behavioral quantification.

2.4.2. Assessment of gross behavioral activity (locomotor activity)

The locomotor activity was monitored using an actophotometer (IMCORP, Ambala, India) on weekly intervals (1st, 7th, and 14th days). The motor activity was detected by infrared beams above the floor of the testing area. Animals were placed individually in the activity chamber for a 3-min acclimation period before starting actual activity tasks. Each animal was observed over a period of 5 min and the total activity was expressed as counts per 5 min (Kumar et al., 2007). The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room.

2.4.3. Rotarod activity

All the animals were evaluated for the motor coordination by using the rotarod on the 1st, 7th and 14th days, after malonic acid injection. The rats were given a prior training session before actual recording on rotarod apparatus (IMCORP, Ambala, India) to acclimate the environment. Rats were placed on a rotating rod with a diameter of 7 cm (speed 25 rpm). The cut off time on rotating rod was fixed as 90 s and three separate trials were given to each rat performed. The average result was expressed as described by Kumar et al. (2007).

2.5. Biochemical assessments

2.5.1. Dissection and homogenization

On the 14th day, animals were randomly divided into two groups, one for biochemical estimations and the other for mitochondrial enzyme complex estimations after the behavioral assessments. The animals were sacrificed by decapitation immediately after behavioral assessments. The brains were removed, forebrain was dissected out and cerebellum was discarded. Brains were put on ice and striatum were separated and weighted. A 10% (weight/volume) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 × *g* for 15 min and aliquots of supernatants were separated and used for the biochemical estimation.

2.5.2. Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in the brain was performed according to the method of Wills (1996). The amount of malondialdehyde (MDA), a measure of lipid peroxidation, was measured by reaction with thiobarbituric acid at 532 nm using a Shimadzu Spectrophotometer (UV-Pharmaspec 1700 Shimadzu, Japan). The values were calculated using the molar extinction coefficient of the chromophore ($1.56 \times 105\ M^{-1}\ cm^{-1}$).

2.5.3. Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al. (1982). Equal volumes of supernatant and Greiss reagent were mixed, and this mixture was incubated for 10 min at room temperature in the dark. Absorbance at 540 nm was measured with a Shimadzu Spectrophotometer (UV-Pharmaspec 1700 Shimadzu, Japan). The concentration of nitrite in the supernatant was determined from the sodium nitrite standard curve.

2.5.4. Estimation of catalase

Catalase activity was assayed by method of Luck (1971), wherein the breakdown of H_2O_2 product measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer (1.25×10^{-2} M H_2O_2), 0.05 ml of supernatant of the brain homogenate (10%), and the change in absorbance was recorded at 240 nm using Shimadzu Spectrophotometer (UV-Pharmaspec 1700 Shimadzu, Japan). Enzyme activity was calculated by using millimolar extinction coefficient of H_2O_2 (0.07).The results were expressed as mM of H_2O_2 decomposed per milligram of protein/min.

2.5.5. Reduced glutathione (GSH) estimation

Reduced glutathione (GSH) in striatum and cortex was estimated according to the method of Ellman (Ellman, 1959). 1 ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested

at 4 °C for 1 h. The sample was centrifuged at 1200×*g* for 15 min at 4 °C, 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5-dithiobis (2-nitrobenzoic acid)(DTNB). The yellow color developed was read immediately at 412 nm using Shimadzu Spectrophotometer (UV-Pharmaspec 1700 Shimadzu, Japan). Results were calculated using molar extinction coefficient of chromophore ($1.36\times10^4\ M^{-1}\ cm^{-1}$).

2.5.6. Total glutathione estimation

Total glutathione was assayed by the method of Zahler and Cleland (1968). The method is based on the reduction with dithioerythritol and determination of the resulting monothiols with DTNB in the presence of arsenite. Oxidized glutathione (GSSG) is quantified by subtracting the value of glutathione reduced from total glutathione. Redox ratio of reduced glutathione/oxidized glutathione (GSH/GSSG) was also calculated.

2.5.7. Superoxide dismutase activity

Superoxide dismutase activity was assayed according to the method of Kono (1978) wherein the reduction of nitrazobluetetrazolium (NBT) was inhibited by the superoxide dismutase, is measured at 560 nm using spectrophotometer (UV-Pharmaspec 1700 Shimadzu, Japan). Briefly, the reaction was initiated by the addition of the hydroxylamine hydrochloride to the mixture containing nitrazobluetetrazolium (NBT) and sample. The results were expressed as unit/mg protein.

2.5.8. Protein estimation

The protein was measured by the Biuret method using bovine serum albumin as a standard (Gornall et al., 1949).

2.6. Mitochondrial complex estimation

2.6.1. Isolation of rat brain mitochondria

Rat brain mitochondria were isolated by the method of Berman and Hastings (1999). The brain regions were homogenized in an isolation buffer with Ethylene Glycol Tetraacetic Acid (EGTA) (215 mM Mannitol, 75 mM sucrose, 0.1% BSA, 20 mM HEPES, 1 mM EGTA, pH-7.2). The homogenates were centrifuged at 13,000×*g* for 5 min at 4 °C. The pellet was resuspended in the isolation buffer with EGTA and spun again at 13,000×*g* for 5 min. The resulting supernatant was transferred to new tubes and topped off with the isolation buffer containing EGTA and spun again at 13,000×*g* for 10 min. The pellet containing pure mitochondria was resuspended in the isolation buffer without EGTA.

2.6.2. COMPLEX-I (NADH Dehydrogenase activity)

Complex-I was measured spectrophotometrically by the method of King and Howard (1967). The method involves the catalytic oxidation of NADH to NAD+ with subsequent reduction of cytochrome c. The reaction mixture contained 0.2 M glycyl glycine buffer pH 8.5, 6 mM NADH in 2 mM glycyl glycine buffer and 10.5 mM cytochrome c. The reaction was initiated by the addition of a requisite amount of solubilized mitochondrial sample. The absorbance change at 550 nm was followed for 2 min.

2.6.3. COMPLEX-II (Succinate Dehydrogenase)

Succinate Dehydrogenase (SDH) was measured spectrophotometrically according to the method of King (1967). The method involves the oxidation of succinate by an artificial electron acceptor, potassium ferricyanide. The reaction mixture contained 0.2 M phosphate buffer pH 7.8, 1% BSA, 0.6 M succinic acid and 0.03 M potassium ferricyanide. The reaction was initiated by addition of the mitochondrial sample and the absorbance change at 420 nm was followed for 2 min.

2.6.4. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide) assay

The MTT assay is based on the reduction of (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-H-tetrazolium bromide (MTT) by hydrogenase activity in functionally intact mitochondria. The MTT reduction rate was used to assess activity of the mitochondrial respiratory chain in isolated mitochondria by the method of Liu et al. (1997). Briefly, 100 µl mitochondrial samples were incubated with 10 µl MTT for 3 h at 37 °C. The blue formazan crystalswere solubilized with dimethylsulfoxide and measured by an ELISA readerwith a 580 nmfilter (Model 680Microplate Reader, Bio-Rad Japan).

2.6.5. Mitochondrial complex-IV (Cytochrome oxidase) assay

Cytochrome oxidase activity was assayed in brain mitochondria according to the method of Sottocasa et al. (1967). The assay mixturecontained 0.3 mM reduced cytochrome c in 75 mM phosphate buffer. The reaction was initiated by addition of the solubilized mitochondrial sample and absorbance change at 550 nm was followed for 2 min.

2.7. Statistical analysis

One specific group of twelve ($n=12$) animals was assigned to a specific drug treatment. All the values were expressed as means ± S.E.M. The data was analyzed using two way analysis of variance (ANOVA) followed by Tukey's test for behavioral alterations and one way ANOVA followed by Tukey's test for biochemical and mitochondrial alterations. In all the tests, criterion for statistical significance was $P<0.05$.

3. Results

3.1. Effect of vitamin E on malonic acid induced change in body weight

There was no significant change in the initial and final body weight of sham group as compared to naïve groups. However, malonic acid (1.5, 3 and 6 µmol) treatment significantly decreased body weight as compared to sham treated group in dose dependent manner. Vitamin E (50 and 100 mg/kg, *p.o.*) significantly improved the body weight as compared to malonic acid (6 µmol) treated group (Fig. 1). Vitamin E (50 and 100 mg/kg, *p.o.*) *per se*, did not produce any significant effect on the body weight as compared to naïve and sham operated group.

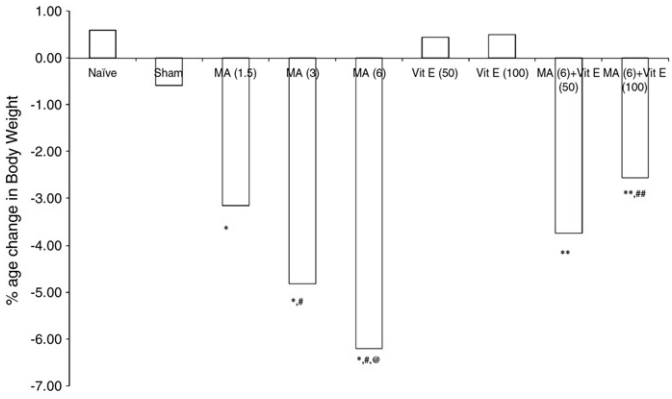

Fig. 1. Effect of vitamin E on malonic acid induced change in body weight. Values are expressed Mean + S.E.M. $^{*}P<0.05$ as compared to control, $^{\#}P<0.05$ as compared to MA (1.5), $^{@}P<0.05$ as compared to MA (3.0), $^{**}P<0.05$ as compared to MA (6.0), $^{\#\#}P<0.05$ as compared to vitamin E (50), (ANOVA followed by Tukey test).

3.2. Effect of vitamin E on malonic acid induced alterations in gross behavioral activity and rotarod performance

There was no significant change in the locomotor activity and rotarod performance of sham group as compared naïve group. Malonic acid (3 and 6 µmol) significantly impaired locomotor activity and rotarod performance as compared to naïve and sham group. Malonic acid (1.5 µmol) did not produce any significant effect on the locomotor and rotarod performance. Vitamin E (50 and 100 mg/kg, *p.o.*) significantly improved the locomotor activity and rotarod performance on 7th and 14th days in the malonic acid (6 µmol) treated group. While vitamin E (50 and 100 mg/kg, *p.o.*) *per se*, did not have any significant effect on locomotor activity and rotarod performance (Figs. 2 and 3).

3.3. Effect of vitamin E on malonic acid induced lipid peroxidation, nitrite level, superoxide dismutase and catalase levels

Intrastriatal administration of malonic acid (1.5, 3 and 6 µmol) significantly increased lipid peroxidation and nitrite concentration, while a significant decrease in superoxide dismutase and Catalase activities in rat brain striatum. Vitamin E (50 and 100 mg/kg, *p.o.*) significantly attenuated the increase in lipid peroxidation and nitrite concentration (Table 1) in malonic acid (6 µmol) treated animals. Further vitamin E (50 and 100 mg/kg, *p.o.*) treatment significantly restored the superoxide dismutase and catalase activity in rat brain striatum (Table 1). However vitamin E (50 and 100 mg/kg, *p.o.*) *per se*, did not produce any significant effect on the lipid peroxidation and nitrite concentration.

3.4. Effect of vitamin E on malonic acid induced total glutathione, reduced glutathione oxidized glutathione and redox ratio alterations

Intrastriatal administration of malonic acid significantly (1.5, 3 and 6 µmol) depleted reduced glutathione and redox ratio levels in rat brain striatum as compared to sham group. In addition intrastriatal malonic acid (1.5, 3 and 6 µmol) caused a significant rise in oxidized glutathione ($P<0.05$). However, malonic acid (1.5, 3 and 6 µmol) did not produce any significant decrease in total glutathione levels. Vitamin E (50 and 100 mg/kg, *p.o.*) significantly restored the reduced glutathione levels, redox ratio and oxidized glutathione levels (Table 2) as compared to malonic acid (6 µmol) treated animals. However vitamin E (50 and 100 mg/kg, *p.o.*) *per se*, did not produce any significant effect on the reduced glutathione levels, redox ratio, oxidized glutathione and total glutathione level.

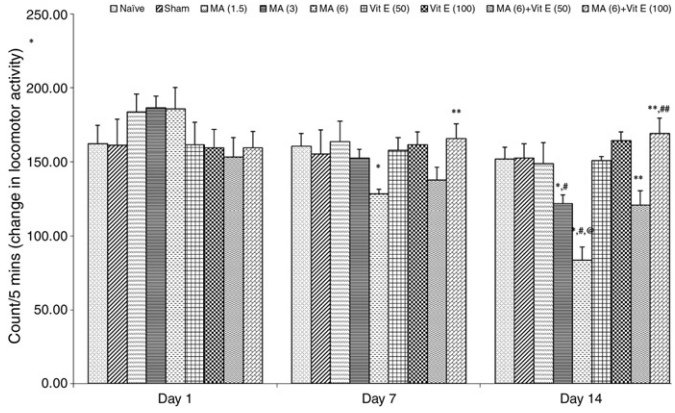

Fig. 2. Effect of vitamin E on malonic acid induced alterations in gross behavioral activity. Values are expressed Mean + S.E.M. $^{*}P<0.05$ as compared to control, $^{\#}P<0.05$ as compared to MA (1.5), $^{@}P<0.05$ as compared to MA (3.0), $^{**}P<0.05$ as compared to MA (6.0), $^{\#\#}P<0.05$ as compared to vitamin E (50), (ANOVA followed by Tukey test).

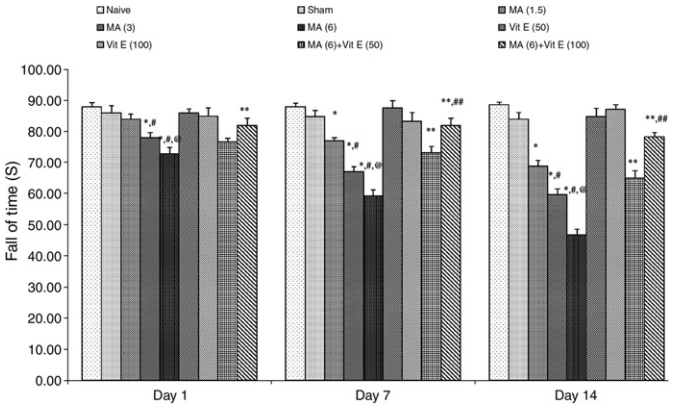

Fig. 3. Effect of vitamin E on malonic acid induced alterations in rota rod performance. Values are expressed Mean + S.E.M. $^{*}P<0.05$ as compared to control, $^{\#}P<0.05$ as compared to MA (1.5), $^{@}P<0.05$ as compared to MA (3.0), $^{**}P<0.05$ as compared to MA (6.0), $^{\#\#}P<0.05$ as compared to vitamin E (50), (ANOVA followed by Tukey test).

3.5. Effect of vitamin E on malonic acid induced mitochondrial complex-I (NADH Dehydrogenase activity) and complex-II (Succinate Dehydrogenase (SDH) activity) alterations

There was a significant decrease in the complex-II activity in striatum of the malonic acid (3 and 6 µmol) treated group, but no significant effect on the complex-I activity as compared to sham group ($P<0.05$). Further, vitamin E (50 and 100 mg/kg, *p.o.*) treatment restored the mitochondrial complex-II activity significantly ($P<0.05$) as compared to malonic acid (6 µmol). However, vitamin E (50 and 100 mg/kg, *p.o.*) *per se*, did not have any significant effect on the mitochondrial complex-I and II levels as compared to sham group (Figs. 4 and 5).

3.6. Effect of vitamin E on malonic acid induced MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and mitochondrial complex-IV (Cytochrome oxidase) activity

A significant decrease in the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) activity was observed in malonic acid (3 and 6 µmol) treated group as compared to sham treated group ($P<0.05$), however, no significant effect was observed in mitochondrial complex-IV activity in malonic acid (1.5, 3 and 6 µmol) treated group as compared to sham treated group in rat brain striatum. Vitamin E (50 and 100 mg/kg, *p.o.*) significantly restored the MTT activity as compared to malonic acid (6 µmol) treated animals ($P<0.05$). However, vitamin E (50 and 100 mg/kg, *p.o.*) *per se*, did not have significant effect on the MTT ability as compared to sham group (Figs. 6 and 7).

4. Discussion

The core findings of the present study put forward evidence for antioxidants as the adjuvant therapy for the management of the Huntington like symptoms. At behavioral levels, vitamin E significantly attenuates reduction in body weight, locomotor activity and rotarod performance in the rats. In other findings vitamin E significantly attenuate the rise in lipid peroxidation, nitrite concentrations and also restored various endogenous antioxidants like glutathione, SOD and catalase etc. these findings governs the potential of antioxidant's in attenuating the Huntington's disease like symptoms. Interestingly vitamin E treatment also significantly restored the mitochondrial functions (mitochondrial enzyme complexes) provide oversee towards the involvement of mitochondrial dysfunction and oxidative stress in Huntington disease pathogenesis.

Table 1
Effect of vitamin E on malonic acid induced lipid peroxidation, nitrite level, superoxide dismutase and catalase levels of brain striatum.

Group	Malondialdehyde levels (n mole of MDA/mg protein)	Nitrite Levels (μ mole of Nitrite/mg protein)	Catalase levels (μ mole of H_2O_2/min/mg protein)	Superoxide dismutase levels (unit/mg protein)
Naive	4.75 ± 0.07	113 ± 1.65	10.29 ± 0.17	2.19 ± 0.20
Sham	4.99 ± 0.08	121 ± 1.65	9.88 ± 0.31	2.07 ± 0.16
MA (1.5)	6.61 ± 0.09[a]	136 ± 1.47[a]	8.96 ± 0.19[a]	1.71 ± 0.12
MA (3.0)	7.77 ± 0.05[a,b]	159 ± 1.89[a,b]	7.24 ± 0.31[a,b]	1.31 ± 0.12[a,b]
MA (6.0)	9.03 ± 0.03[a,b,c]	193 ± 1.65[a,b,c]	5.48 ± 0.21[a,b,c]	0.80 ± 0.08[a,b,c]
Vit. E (50)	4.34 ± 0.02[NS]	122 ± 2.07[NS]	9.76 ± 0.32[NS]	104.34 ± 0.08[NS]
Vit. E (100)	4.43 ± 0.03[NS]	124 ± 2.89[NS]	9.98 ± 0.21[NS]	103.38 ± 0.04[NS]
MA (6.0) + Vit. E (50)	7.84 ± 0.05[d]	148 ± 2.48[d]	6.97 ± 0.31[d]	1.27 ± 0.02[d]
MA (6.0) + Vit. E (100)	6.41 ± 0.15[d,e]	118 ± 2.48[d,e]	9.60 ± 0.17[d,e]	1.99 ± 0.021[d,e]

Values are expressed Mean + S.E.M. (ANOVA followed by Tukey test). [NS] Not significant.
[a] $P<0.05$ as compared to Sham.
[b] $P<0.05$ as compared to MA (1.5).
[c] $P<0.05$ as compared to MA (3.0).
[d] $P<0.05$ as compared to MA (6.0).
[e] $P<0.05$ as compared to vitamin E (50).

Regarding the efficacy of vitamin E, there are several conflicting reports, as Flint Beal's group, who pioneered the quinolinic acid model of Huntington's disease, found that none of the antioxidants vitamin E, β-carotene, or ascorbic acid provided protection against quinolinate-induced striatal neurotoxicity when administered systemically for several days prior to toxin challenge (Beal et al., 1988). While some other findings also suggest that vitamin E plus coenzyme Q10 provide partial improvement against 3NP-induced striatal energy deficits in aged rats (Kasparova et al., 2006). Ehrnhoefer and his group report the protective effect of epigallocatechin-gallate (another antioxidant) against mHtt toxicity in a yeast model and slowed motor function decline in a transgenic mouse model (Ehrnhoefer et al., 2006). Double-blind placebo-controlled study also did not report any significant effect of α-tocopherol treatment on a cohort of 73 Huntington's disease patients (Peyser et al., 1995). These conflicts and controversy has been explained on the basis of differences in the experimental models used during the studies.

As Huntington disease is characterized by selective degeneration of striatal neuron (Ribeiro et al., 2006), choreiform movements and clinically by associated psychiatric disturbances and cognitive impairment. Malonic acid infusion into striatum caused metabolic stress on several neuronal populations (Browne et al., 1997) and results in the loss of striatal dopamine and ϒ-amino butyric acid content as well as the retrograde loss of nigral dopaminergic cell bodies (Browne et al., 2006; Moy et al., 2000). Intrastriatal injection of malonic acid has been reported to cause reactive oxygen species formation (Ferger et al., 1999) and leading to the oxidative stress. Although, the entire mechanism by malonic acid causes neuronal death is not fully understood, there is evidence linking impaired mitochondrial function and related consequences (Calabresi et al., 2001).

With this background, present study standardized the dose of the malonic acid which mimics Huntington disease like symptoms in the rodents. Three doses of malonic acid (1.5, 3 and 6 μmol) were tried based on literature (Moy et al., 2000; Maragos et al., 2004). Malonic acid with gradual increase in dose caused significant alterations in locomotor activity (hypoactivity), impaired motor coordination (rotarod performance) as seen in Huntington disease patients. However, malonic acid in lower doses did not produce any significant alterations in behavior. Reduction in body weight could be due to impaired energy metabolism and oxidative burden. However, impairment in locomotor activity, rota rod performance can be attributed to striatal neurodegeneration (particularly dopaminergic motor neurons). This striatal neurodegeneration could be due to generation of free radicals due to impaired mitochondrial functions and selective and high vulnerability of dopaminergic motor neurons. It seems that malonic acid induced energy impairment and in turn free radical generation leads to selective neurodegeneration in different areas of brain causing behavioral alterations. Further vitamin E (well known potent antioxidant) treatment significantly reverse the behavioral alteration in malonic acid treated amimals further providing support to the role of oxidative stress in pathogenesis of malonic acid induced neurotoxicity in animals.

Oxidative stress and apoptosis play pivotal roles in the pathogenesis of neurodegenerative diseases. *In vitro* and *In vivo* evidences reported that malonic acid cause damage to dopaminergic neurons

Table 2
Effect of vitamin E on malonic acid induced total glutathione, reduced glutathione oxidized glutathione and redox ratio alterations in brain striatum.

Group	Total glutathione (μ mole of Glutathione/mg protein)	Reduced glutathione (μ mole of GSH/mg protein)	Oxidized glutathione (μ mole of GSSG/mg protein)	Redox ratio (GSH/GSSG)
Naive	138.69 ± 1.49	55.94 ± 1.14	78.75 ± 2.62	0.76 ± 0.04
Sham	138.25 ± 1.05	53.64 ± 0.79	84.61 ± 3.31	0.63 ± 0.02
MA (1.5)	137.73 ± 0.53[NS]	43.49 ± 0.61[a]	94.24 ± 1.14[a]	0.46 ± 0.01[a]
MA (3.0)	136.41 ± 2.89[NS]	33.60 ± 1.05[a,b]	102.81 ± 1.84[a,b]	0.33 ± 0.02[a,b]
MA (6.0)	135.45 ± 2.63[NS]	23.01 ± 1.66[a,b,c]	112.44 ± 1.96[a,b,c]	0.20 ± 0.02[a,b,c]
Vit. E (50)	135.93 ± 2.37[NS]	54.23 ± 1.35[NS]	81.70 ± 2.23[NS]	0.66 ± 0.03[NS]
Vit. E (100)	138.20 ± 2.43[NS]	55.02 ± 1.85[NS]	83.18 ± 2.32[NS]	0.66 ± 0.04[NS]
MA (6.0) + Vit. E (50)	136.06 ± 2.36[NS]	41.74 ± 1.84[d]	94.33 ± 1.62[d]	0.44 ± 0.03[d]
MA (6.0) + Vit. E (100)	136.76 ± 1.66[NS]	51.10 ± 0.70[d,e]	85.66 ± 2.36[d,e]	0.60 ± 0.04[d,e]

Values are expressed Mean + S.E.M. [NS] Not significant.
[a] $P<0.05$ as compared to Sham.
[b] $P<0.05$ as compared to MA (1.5).
[c] $P<0.05$ as compared to MA (3.0).
[d] $P<0.05$ as compared to MA (6.0).
[e] $P<0.05$ as compared to vitamin E (50). (ANOVA followed by Tukey test).

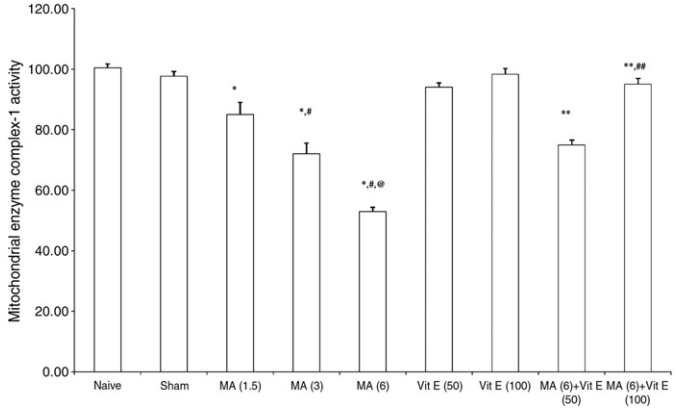

Fig. 4. Effect of vitamin E on malonic acid induced Mitochondrial Complex-I alterations. Values are expressed Mean + S.E.M. $^{*}P<0.05$ as compared to control, $^{\#}P<0.05$ as compared to MA (1.5), $^{@}P<0.05$ as compared to MA (3.0), $^{**}P<0.05$ as compared to MA (6.0), $^{\#\#}P<0.05$ as compared to vitamin E (50), (ANOVA followed by Tukey test).

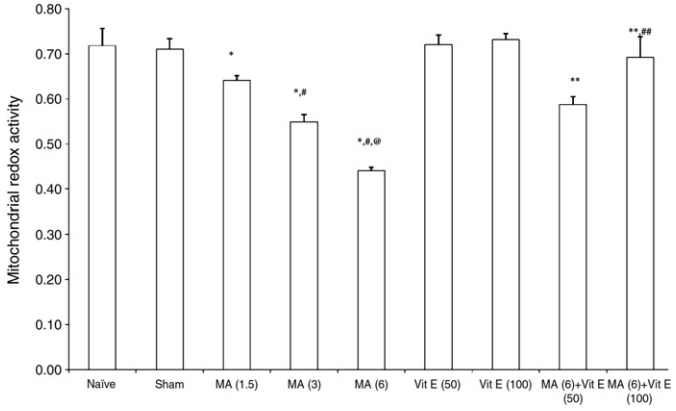

Fig. 6. Effect of vitamin E on malonic acid induced alterations MTT activity. Values are expressed Mean + S.E.M. $^{*}P<0.05$ as compared to control, $^{\#}P<0.05$ as compared to MA (1.5), $^{@}P<0.05$ as compared to MA (3.0), $^{**}P<0.05$ as compared to MA (6.0), $^{\#\#}P<0.05$ as compared to vitamin E (50), (ANOVA followed by Tukey test).

possibly by involving oxidative stress, free radical generation and secondary excitotoxicity (Ferger et al., 1999; Zeevalk et al., 2000). In the present study, intrastriatal malonic acid administration caused oxidative damage as evidenced by increased lipid peroxidation, nitrite levels and depletion of the antioxidant defense such as reduced glutathione, total glutathione, catalase and redox ratio levels. Besides, there was an increase in the oxidized glutathione levels, suggesting the involvement of oxidative damage. Further, vitamin E treatment significantly reversed the behavioral alterations as well as oxidative damage suggesting that oxidative damage could be involved in the pathogenesis of the malonic acid induced behavioral alterations. These findings are in line with earlier findings where cyclic nitrone spin trapping agents (free radicals scavenger) (Zeevalk et al., 1998; Santamaria et al., 2003; Perez-Severiano et al., 2004), monoamine oxidase inhibitors (that attenuate DA catabolism) (Thomas et al., 1996) and N-methyl-D-aspartate receptor antagonists proved their efficacy against malonic acid induced toxicity (Zeevalk et al., 2000; Thomas et al., 1996; Maragos et al., 2004).

Various lines of evidence demonstrate the involvement of mitochondrial dysfunction in the pathogenesis neurodegenerative disorders including Huntington disease (Koroshetz et al., 1997; Cicchetti et al., 2000). Biochemical studies of brain tissue of Huntington disease patients demonstrated the multiple defects in the caudate such as decreased complex II activity (Butterworth et al., 1985) and decreased complex II–III activity (Mann et al., 1990; Parker et al., 1990). Also ultra structural abnormalities in mitochondria have been described in Huntington disease cortical tissue (Gardian and Vecsei, 2004). In the present study, intra striatal injection of malonic acid decreased the activity of mitochondrial complex I, II, IV and MTT ability in striatum region. Malonic acid has been reported to cause ATP depleletion, which in further sequences leads to the impaired ability of mitochondria to sequester the Ca^{2+} which in turn activate caspase-9 and caspase-3 and caused neuronal damage due to apoptosis. However, it is still not clear the interaction of striatum and cortex in mediating gross behavioral alterations. Further, vitamin E significantly restored the mitochondrial complex enzyme functions, suggesting the involvement of oxidative stress in mitochondrial enzyme dysfunction. However, role of antioxidant in ATP regeneration cannot be neglected as one of the possible mechanisms that explain neuroprotection.

In conclusion, our results suggest that the altered behavioral patterns produced by malonic acid resembles Huntington disease and may be linked to functional changes in nerve tissue due to early reactive oxygen species formation and mitochondrial dysfunction. Further, treatment with vitamin E significantly reverses the behavioral, biochemical and mitochondrial markers of Huntington disease, proving evidence regarding the role of oxidative damage in pathogenesis of Huntington disease and thus these findings governs the potential of antioxidants in attenuating the Huntington disease like symptoms.

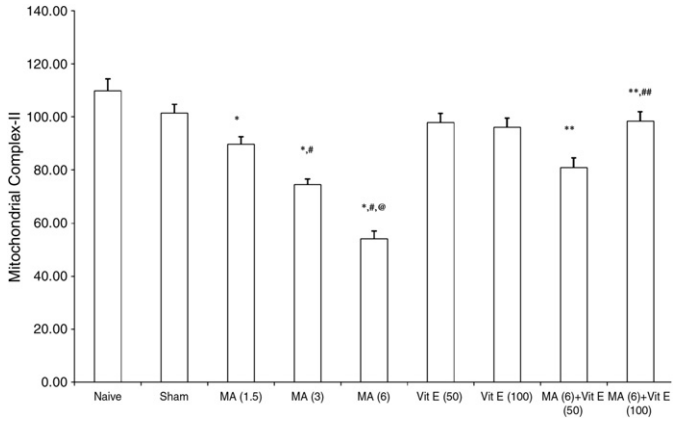

Fig. 5. Effect of vitamin E on malonic acid induced Mitochondrial Complex-II alterations. Values are expressed Mean + S.E.M. $^{*}P<0.05$ as compared to control, $^{\#}P<0.05$ as compared to MA (1.5), $^{@}P<0.05$ as compared to MA (3.0), $^{**}P<0.05$ as compared to MA (6.0), $^{\#\#}P<0.05$ as compared to vitamin E (50), (ANOVA followed by Tukey test).

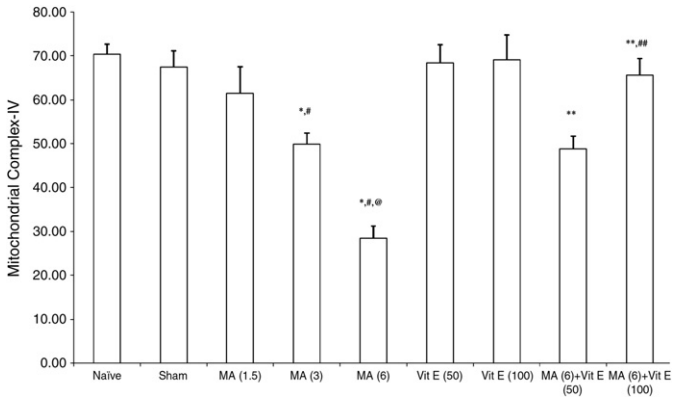

Fig. 7. Effect of vitamin E on malonic acid induced Mitochondrial Complex-IV activity. Values are expressed Mean + S.E.M. $^{*}P<0.05$ as compared to control, $^{\#}P<0.05$ as compared to MA (1.5), $^{@}P<0.05$ as compared to MA (3.0), $^{**}P<0.05$ as compared to MA (6.0), $^{\#\#}P<0.05$ as compared to vitamin E (50), (ANOVA followed by Tukey test).

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References

Beal, M.F., 1996. Mitochondria, free radicals, and neurodegeneration. Curr. Opin. Neurobiol. 6, 661–666.
Beal, M.F., Kowall, N.W., Swartz, K.J., Ferrante, R.J., Martin, J.B., 1988. Systemic approaches to modifying quinolinic acid striatal lesions in rats. J. Neurosci. 8, 3901–3908.
Berman, S.B., Hastings, T.G., 1999. Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. J. Neurochem. 73, 1127–1137.
Bonsi, P., Cuomo, D., Martella, G., Sciamanna, G., Tolu, M., Calabresi, P., Bernardi, G., Pisani, A., 2006. Mitochondrial toxins in Basal Ganglia disorders: from animal models to therapeutic strategies. Curr. Neuropharmacol. 4, 69–75.
Browne, S.E., Bowling, A.C., MacGarvey, U., Baik, M.J., Berger, S.C., Muqit, M.M., Bird, E.D., Beal, M.F., 1997. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann. Neurol. 41, 646–653.
Browne, S.E., Yang, L., DiMauro, J.P., Fuller, S.W., Licata, S.C., Beal, M.F., 2006. Bioenergetic abnormalities in discrete cerebral motor pathways presage spinal cord pathology in the G93A SOD1 mouse model of ALS. Neurobiol. Dis. 22, 599–610.
Butterworth, J., Yates, C.M., Reynolds, G.P., 1985. Distribution of phosphate-activated glutaminase, succinic dehydrogenase, pyruvate dehydrogenase and gamma-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. J. Neurol. Sci. 67, 161–171.
Calabresi, P., Gubellini, P., Picconi, B., Centonze, D., Pisani, A., Bonsi, P., Greengard, P., Hipskind, R.A., Borrelli, E., Bernardi, G., 2001. Inhibition of mitochondrial complex II induces a long-term potentiation of NMDAmediated synaptic excitation in the striatum requiring endogenous dopamine. J. Neurosci. 21, 5110–5120.
Cavallini, L., Valente, M., Bindoli, A., 1984. Comparison of cumene hydroperoxide- and NADPH/Fe3+/ADP-induced lipid peroxidation in heart and liver submitochondrial particles. Mechanisms of protection by succinate. Biochim. Biophys. Acta 795, 466–472.
Cicchetti, F., Prensa, L., Wu, Y., Parent, A., 2000. Chemical anatomy of striatal interneurons in normal individuals and in patients with Huntington's disease. Brain Res. Brain Res. Rev. 34, 80–101.
Davies, S., Ramsden, D.B., 2001. Huntington's disease. Mol. Pathol. 54, 409–413.
Dedeoglu, A., Ferrante, R.J., Andreassen, O.A., Dillmann, W.H., Beal, M.F., 2002. Mice overexpressing 70-kda heat shock protein show increased resistance to malonate and 3-nitropropionic acid. Exp. Neurol. 176, 262–265.
Ehrnhoefer, D.E., Duennwald, M., Markovic, P., Wacker, J.L., Engemann, S., Roark, M., Legleiter, J., Marsh, J.L., Thompson, L.M., Lindquist, S., Muchowski, P.J., Wanker, E.E., 2006. Green tea (−)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. Hum. Mol. Genet. 15, 2743–2751.
Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82, 70–77.
Ferger, B., Eberhardt, O., Teismann, P., de Groote, C., Schulz, J.B., 1999. Malonate-induced generation of reactive oxygen species in rat striatum depends on dopamine release but not on NMDA receptor activation. J. Neurochem. 73, 1329–1332.
Fernandez-Gomez, F.J., Galindo, M.F., Gómez-Lázaro, M., Yuste, V.J., Comella, J.X., Aguirre, N., Jordán, J., 2005. Malonate induces cell death via mitochondrial potential collapse and delayed swelling through an ROS-dependent pathway. Br. J. Pharmacol. 144, 528–537.
Gardian, G., Vecsei, L., 2004. Huntington's disease: pathomechanism and therapeutic perspectives. J. Neural Transm. 111, 1485–1494.
Gornall, A.G., Bardawill, C.J., David, M.M., 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177, 751–766.
Green, L.C., Wagner, D.A., Glgowski, J., Skipper, P.L., Wishnok, J.S., Tannebaum, S.R., 1982. Analysis of nitrate, nitrite, and [15 N] nitrate in biological fluids. Ann. Biochem. 126, 131–138.
Halliwell, B., 1992. Reactive oxygen species and the central nervous system. J. Neurochem. 59, 1609–1623.
Jara-Prado, A., Ortega-Vazquez, A., Ruano, L.M., Rios, C., Santamaria, A., 2003. Homocysteine-induced brain lipid peroxidation: effects of NMDA receptor blockade, antioxidant treatment, and nitric oxide synthase inhibition. Neurotox. Res. 5, 237–243.
Kasparova, S., Sumbalova, Z., Bystricky, P., Kucharska, J., Liptaj, T., Mlynarik, V., Gvozdjakova, A., 2006. Effect of coenzyme Q10 and vitamin E on brain energy metabolism in the animal model of Huntington's disease. Neurochem. Int. 48, 93–99.
King, T.E., 1967. Preparation of succinate dehydrogenase and reconstitution of succinate oxidase. Methods Enzymol. 10, 322.
King, T.E., Howard, R.L., 1967. Preparations and properties of soluble NADH dehydrogenases from cardiac muscle. Methods Enzymol. 10, 275.
Kono, Y., 1978. Generation of superoxide radical during auto-oxidation of hydroxylamine and an assay of superoxide dismutase. Arch. Biochem. Biophys. 186, 189–195.
Koroshetz, W.J., Jenkins, B.G., Rosen, B.R., Beal, M.F., 1997. Energy metabolism defects in Huntington's disease and effects of coenzyme Q10. Ann. Neurol. 41, 160–165.
Kumar, P., Padi, S.S., Naidu, P.S., Kumar, A., 2007. Cyclooxygenase inhibition attenuates 3-nitropropionic acid-induced neurotoxicity in rats: possible antioxidant mechanisms. Fundam. Clin. Pharmacol. 21, 297–306.
Leavitt, B.R., Van Raamsdonk, J.M., Shehadeh, J., Fernandes, H., Murphy, Z., Graham, R.K., Wellington, C.L., Raymond, L.A., Hayden, M.R., 2006. Wild-type huntingtin protects neurons from excitotoxicity. J. Neurochem. 96, 1121–1129.
Liu, Y., Peterson, D.A., Kimura, H., Schubert, D., 1997. Mechanisms of cellular 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolinium bromide (MTT) reduction. J. Neurochem. 69, 581–593.
Luck, H., 1971. Catalase. In: Bergmeyer, H.U. (Ed.), Methods of Enzyme Analysis. Academic Press, New York, pp. 885–893.
Mann, V.M., Cooper, J.M., Javoid-Agid, F., Agid, Y., Jennert, P., Schapira, A.H., 1990. Mitochondrial function and parental sex effect in Huntington's disease. Lancet 336, 749.
Maragos, W.F., Young, K.L., Altman, C.S., Pocernich, C.B., Drake, J., Butterfield, D.A., Seif, I., Holschneider, D.P., Chen, K., Shih, J.C., 2004. Striatal damage and oxidative stress induced by the mitochondrial toxin malonate are reduced in clorgyline-treated rats and MAO-A deficient mice. Neurochem. Res. 29, 741–746.
Matthews, R.T., Yang, L., Jenkins, B.G., Ferrante, R.J., Rosen, B.R., Kaddurah-Daouk, R., Beal, M.F., 1998. Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. J. Neurosci. 18, 156–163.
Moy, L.Y., Zeevalk, G.D., Sonsalla, P.K., 2000. Role for dopamine in malonate-induced damage in vivo in striatum an in vitro in mesencephalic cultures. J. Neurochem. 74, 1656–1665.
Parker, W.D., Boyson, S.J., Luder, A.S., Parks, J.K., 1990. Evidence for a defect in NADH: ubiquinone oxidoreductase (complex I) in Huntington's disease. Neurology 40, 1231–1234.
Paxinos, G., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates, 6th edition. Academic Press, San Diego.
Perez-De La Cruz, V., Gonzalez-Cortes, C., Pedraza-Chaverri, J., Maldonado, P.D., Andres-Martinez, L., Santamaria, A., 2006. Protective effect of S-allylcysteine on 3-nitropropionic acid-induced lipid peroxidation and mitochondrial dysfunction in rat brain synaptosomes. Brain Res. Bull. 68, 379–383.
Perez-Severiano, F., Rodriguez-Perez, M., Pedraza-Chaverri, J., Maldonado, P.D., Medina-Campos, O.N., Ortiz-Plata, A., Sanchez-Garcia, A., Villeda-Hernandez, J., Galvan-Arzate, S., Aguilera, P., Santamaria, A., 2004. S-allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. Neurochem. Int. 45, 1175–1183.
Peyser, C.E., Folstein, M., Chase, G.A., Starkstein, S., Brandt, J., Cockrell, J.R., Bylsma, F., Coyle, J.T., McHugh, P.R., Folstein, S.E., 1995. Trial of d-alpha-tocopherol in Huntington's disease. Am. J. Psychiatry 152, 1771–1775.
Posser, T., Moretto, M.B., Dafre, A.L., Farina, M., da Rocha, J.B.T., Nogueira, C.W., Zeni, G., Ferreira, J.D.S., Leal, R.B., Franco, J.L., 2006. Antioxidant effect of diphenyl diselenide against sodium nitroprusside (SNP) induced lipid peroxidation in human platelets and erythrocyte membranes: an in vitro evaluation. Chem. Biol. Interact. 164, 126–135.
Puntel, R.L., Nogueira, C.W., Rocha, J.B.T., 2005. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro. Neurochem. Res. 30, 225–235.
Ribeiro, C.A., Grando, V., Dutra Filho, C.S., Wannmacher, C.M., Wajner, M., 2006. Evidence that quinolinic acid severely impairs energy metabolism through activation of NMDA receptors in striatum from developing rats. J. Neurochem. 99, 1531–1542.
Santamaria, A., Salvatierra-Sanchez, R., Vazquez-Roman, B., Santiago-Lopez, D., Villeda-Hernandez, J., Galvan-Arzate, S., Jimenez-Capdeville, M.E., Ali, S.F., 2003. Protective effects of the antioxidant selenium on quinolinic acid-induced neurotoxicity in rats: in vitro and in vivo studies. J. Neurochem. 86, 479–488.
Scattoni, M.L., Valanzano, A., Pezzola, A., March, Z.D., Fusco, F.R., Popoli, P., Calamandrei, G., 2007. Adenosine A2A receptor blockade before striatal excitotoxic lesions prevents long term behavioural disturbances in the quinolinic rat model of Huntington's disease. Behav. Brain Res. 176, 216–221.
Simonian, N.A., Coyle, J.T., 1996. Oxidative stress in neurodegenerative diseases. Ann. Rev. Pharmacol. Toxicol. 36, 83–106.
Sottocasa, G.L., Kuylenstierna, B., Ernster, L., Bergstrand, A., 1967. An electron-transport system associated with the outer membrane of liver mitochondria. A biochemical and morphological study. J. Cell Biol. 32, 415–438.
Sun, J., Huang, S.H., Tan, B.K.H., Whiteman, M., Zhu, Y.C., Wu, Y.J., Ng, Y., Duan, W., Zhu, Y.Z., 2005. Effects of purified herbal extract of *Salvia miltiorrhiza* on ischemic rat myocardium after acute myocardial infarction. Life Sci. 76, 2849–2860.
Sutherland, B.A., Shaw, O.M., Clarkson, A.N., Jackson, D.M., Sammut, I.A., Appleton, I., 2005. Neuroprotective effects of (+)-epigallocatechin gallate after hypoxia–ischemia-induced brain damage: novel mechanisms of action. FASEB J. 19, 258–260.
Sutherland, B.A., Rahman, R.M.A., Appleton, I., 2006. Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. J. Nutr. Biochem. 17, 291–306.
Takayanagi, R., Takeshige, K., Minakami, S., 1980. NADH- and NADPHdependent lipid peroxidation in bovine heart submitochondrial particles. Dependence on the rate of electron flow in the respiratory chain and an antioxidant role. Biochem. J. 192, 853–860.
Thomas, C.E., Ohlweiler, D.F., Carr, A.A., Nieduzak, T.R., Hay, D.A., Adams, G., Vaz, R., Bernotas, R.C., 1996. Characterization of the radical trapping activity of a novel series of cyclic nitrone spin traps. J. Biol. Chem. 271, 3097–3104.
Wills, E.D., 1996. Mechanism of lipid peroxide formation in animal tissue. Biochem. J. 99, 667–676.
Yamamoto, H., Mohanan, P.V., 2003. Effect of α-ketoglutarate and oxaloacetate on brain mitochondrial DNA damage and seizures induced by kainic acid in mice. Toxicol. Lett. 143, 115–122.
Zahler, W.L., Cleland, W.W., 1968. A specific and sensitive assay for disulfides. J. Biol. Chem. 243, 716–719.
Zeevalk, G.D., Bernard, L.P., Nicklas, W.J., 1998. Role of oxidative stress and the glutathione system in loss of dopamine neurons due to impairment of energy metabolism. J. Neurochem. 70, 1421–1430.
Zeevalk, G.D., Manzino, L., Sonsalla, P.K., 2000. NMDA receptors modulate dopamine loss due to energy impairment in the substantia nigra but not striatum. Exp. Neurol. 161, 638–646.