Study of the Possible Modulatory Effect of Resveratrol and Coenzyme Q10 on MPTP-Induced Parkinsonism in Mice

Mohamed N. Abdel Rahman and Ayman A. Elsheikh

Department of Pharmacology, Faculty of Medicine, Tanta University, Egypt.
aymanahe@yahoo.com

Abstract: Background: Parkinson's disease is a motor system disease caused by factors that compromise survival of the dopaminergic neurons in the substantia nigra. The mechanisms of degeneration of these neurons include mitochondrial dysfunction, oxidative stress and neuroinflammation. CoQ10 is a fat-soluble vitamin found in the inner mitochondrial membrane and is involved in the electron transport chain that supplies energy to vital organs. Resveratrol is a natural polyphenolic compound found in grapes and red wine that had been shown to offer protective effects in cancer, cardiovascular and neurodegenerative diseases. Objective: To study the effect of the combination of L-dopa with CoQ10 or resveratrol in comparison with L-dopa alone on MPTP-induced parkinsonism in mice. Methods: Fifty albino mice were divided into 5 equal groups: control group, MPTP group, L-dopa+MPTP group, L-dopa+CoQ10+MPTP group, and L-dopa+resveratrol+MPTP group. Catalepsy score, striatal dopamine, TNF-α, NO, mitochondrial complex I activity and ATP were measured. Results: The combination between L-dopa and either CoQ10 or resveratrol induced significant increase in striatal ATP, dopamine and mitochondrial complex 1 activity with significant decrease in striatal TNF-α and NO with significant improvement in catalepsy score compared to the group that received L-dopa alone or MPTP-treated group. Conclusion: The combination of L-dopa and CoQ10 or L-dopa and resveratrol has a better effect than L-dopa alone on MPTP-induced parkinsonism in mice.
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Key words: Resveratrol, Coenzyme Q10, MPTP, Parkinsonism.
Abbreviations: CoQ10, coenzyme Q10; NO, nitric oxide; MPTP, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; TNF-α, tumour necrosis factor alpha; ATP, adenosine triphosphate.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting approximately 1% of the population older than 60 years. Classically, PD is considered to be a motor system disease and its diagnosis is based on the presence of a set of cardinal motor signs that are consequence of death of dopaminergic neurons in the substantia nigra pars compacta **(Jiao *et al.*, 2012)**.The molecular mechanisms underlying the pathogenesis of PD have not been completely elucidated. However, some progress has been made in identifying factors that compromise survival of the dopaminergic neurons in the substantia nigra. These mechanisms include mitochondrial dysfunction, oxidative stress, excitotoxicity and neuroinflammation **(Mosley *et al.*, 2006; Miller *et al.*, 2009; Barnum and Tansey, 2010; Prediger *et al.*, 2011).**

Dopamine is a neurotransmitter that can undergo metabolism either by monoamine oxidase (MAO) or by autooxidation, producing H_2O_2, superoxide anion and hydroxyl radicals. In addition, nitric oxide (NO), which is produced through inflammation-induced microglia activation or excitotoxic insults, may also play a role in the pathogenesis of PD. Formation of peroxynitrite anions through the combination of ROS with NO may confer additional toxicity to dopaminergic neurons **(Miller et al., 2009).**

MPTP (1-methyl - 4-phenyl - 1, 2, 3, 6-tetrahydropyridine) is an artificial narcotic agent that had been shown to exert selective toxicity in the SN and to produce a parkinsonian-like disease in animals as well as in humans. Consequently, MPTP is an established and popular agent for producing animal models of PD **(Schober, 2004).** The pathogenic mechanisms possibly involved in the neurodegeneration induced by MPTP include mitochondrial dysfunction, oxidative stress and activation of apoptosis **(Prediger *et al.*, 2011).** Mitochondrial complex I inhibition by MPTP is thought to underlie the neurodegenerative process in PD. Moreover, overproduction of NO due to both cytosolic and mitochondrial inducible nitric oxide synthases (iNOS) causes free radicals generation and oxidative stress, contributing to mitochondrial dysfunction and neuronal cell death caused by MPTP **(Acuna-castroviejo *et al.*, 2011)**

Coenzyme Q10 (CoQ10) is a fat-soluble vitamin-like substance found in the inner mitochondrial membrane. It is normally involved in a series of enzymatically catalyzed sequential reactions necessary to carry out oxidative phosphorylation via

the electron transport chain which is an essential process that supply energy to vital organs such as the brain, heart, muscles and kidneys **(Kim *et al.*, 2010).** CoQ10 is the electron acceptor for mitochondrial complexes I and II and a powerful antioxidant. A correlation was reported between mitochondrial CoQ10 levels and activities of complexes I and II/III in PD patients **(Del Hoyo *et al.*, 2010).**

Resveratrol is a natural polyphenol found in grapes and red wine. It had been shown to offer protective effects against many cardiovascular and neurodegenerative diseases and cancer**.** Although the mechanisms of action of resveratrol have not yet been clearly elucidated, many studies have attributed this to its antioxidant, anti-inflammatory and anti-apoptotic effects **(Bi *et al.*, 2005; Blanchet *et al.*, 2008; Sun *et al.*, 2010).** Resveratrol protects dopaminergic neurons through inhibition of activation and release of proinflammatory factors and decreased production of reactive oxygen species **(Zhang *et al.*, 2010).**

2. Materials and Methods:

Drugs and chemicals:

Resveratrol, MPTP and other chemicals were obtained from Sigma–Aldrich Chemical Co. CoQ10 (Coenzyme Q10 capsule, 30 mg) was obtained from MEPACO, Egypt and L-dopa (levocar® tablet, levodopa® 250 mg plus carbidopa 25 mg) was obtained from ACAPI Co., Egypt.

Mice and treatment protocol:

The present study was carried out on 50 Albino mice weighing 20-25 grams collected from local source with free access to food and tap water ad libitum through the whole period of the work. All the experiments were conducted according to the National Research Council's guidelines. Animal handling was followed according to Helsenki declaration of animal ethics. Mice were divided into five equal groups each of 10 mice as follows: **(I)** control group received intraperitoneal injection of normal saline for 28 consecutive days; **(II)** received intraperitoneal injection of MPTP dissolved in normal saline in a dose of 30 mg/kg body weight at 24 hrs intervals for 28 consecutive days **(Lee and Lee, 2011)**; **(III)** received L-dopa in a dose of 10 mg/kg/day orally **(Alam and Schmidt, 2004)**; **(IV)** received L-dopa in a dose of 10 mg/kg/day orally concomitantly with CoQ10 in a dose of 200 mg/kg/day orally **(Faust et al., 2009)**; **(V)** received L-dopa 10 mg/kg/day orally concomitantly with resveratrol in a dose of 50 mg/kg/day orally **(Blanchet et al., 2008).** The treatment with L-dopa, CoQ10 or resveratrol was started 2 weeks before and continued during administration of MPTP.

Catalepsy score:

The development of parkinsonism was detected at 28 days from starting induction with MPTP, by occurrence of tremors and observation of bradykinesia and rigidity in mice that further quantified by catalepsy score. The first part was the grid test where the mouse was hung by its paws on a vertical grid (25.5 cm wide and 44 cm high with a space of 1 cm between each wire), and the time for the mouse to move its paws or any sort of first movement was recorded. The second part was the bar test where the mouse was placed with both forepaws on a bar (9 cm above and parallel from the base), and the time of removal of the paw was recorded **(Alam and Schmidt, 2004).**

Isolation of striata:

At the end of the work, all mice were decapitated. Brain of each mouse was immediately excised, washed with ice-cold saline and freezed at −70 °C. Then, striata of the two hemispheres were isolated and weighed. One striatum was processed for assay of striatal dopamine levels and striatal mitochondria were isolated for estimation of tumour necrosis factor alpha (TNF-α), nitric oxide (NO), mitochondrial complex I activity and mitochondrial levels of ATP.

Preparation of brain mitochondria:

The striatum of each mouse was collected in the following medium (10 mM Tris-HCl, 1 mM EGTA, 0.32 M sucrose) obtained by dissolving 10.94 g sucrose, 1.21 g Tris-HCl, and 0.38 g EGTA in 100 ml distilled water and adjusted to pH 7.8. Homogenization was done in 9 volumes of this cold medium with three or four strokes using Teflon pestle homogenizer. Then the homogenate was centrifuged at 700 ×g for 10 min at 4 °C, the supernatant was centrifuged for 20 min at 1000 ×g to obtain mitochondria pellets that were washed once with the previous collecting buffer to remove microsomal and cellular contamination. Finally the mitochondria were resuspended in 9 volumes of the collecting buffer, pH 7.8 **(Turpeenoja et al., 1988)**. Mitochondrial protein was determined using Lowry method **(Lowry et al., 1951)**.

Assay of striatal dopamine levels:

The striatum part of each mouse was homogenized in ice-cold n-butanol as 10% (W/V) using Teflon pestle homogenizer, centrifuged at 1000 × g and supernatant stored at −70 °C for Fluorometric assay of striatal dopamine levels according to method described by **Ciarolone (1978)**.

Assay of mitochondrial complex I activity (NADH: CoQ oxido-reductase activity)

It was measured spectrophotometrically according to the method of **Birch-Machin et al. (1994)**, by following the decrease in the absorbance due the oxidation of NADH at 340 nm with the use of extinction coefficient = 6.81 l/mmol/cm.

Assay of mitochondrial ATP level

It was measured spectrophotometrically using Adenosine 5′-Triphosphate (ATP) determination Kit supplied by Sigma-Aldrich Chemical Co., according to the principal based on the reaction between 3-phosphoglycerate and ATP catalyzed by phosphoglycerate kinase. The reaction was coupled with a dephosphorylation reaction using the enzyme glyceraldehyde phosphate dehydrogenase (GAPD) that involved the oxidation of NADH. Formation of NAD was then quantitated by measuring the decrease in the absorbance at 340 nm a measure of the amount of ATP originally present is obtained **(Adams, 1963)**.

Estimation of striatal TNF-α and NO

TNF-α was estimated by ELISA using mouse TNF-α kits supplied by RayBiotech, Inc. Tissue nitrite and nitrate were estimated as an index of NO production **(Cortas and Wakid, 1990)**.

Statistical analysis:

Values of the measured parameters were expressed as mean ± SD. One way-ANOVA (F value) was used to test significance of the difference among more than two arithmetic means followed by Scheffe test to test the difference between each two means. Pearson's correlation coefficient (r) was applied to correlate between the parameters. The significance was considered at p values <0.05.

3. Results:

Effect of different treatments on catalepsy score (Table 1):

Administration of MPTP to mice induced significant increase in catalepsy score of either grid test or bar test compared to the normal control group. Administration of L-dopa induced significant decrease in catalepsy score of either grid test or bar test compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in catalepsy score compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in catalepsy score compared to the group that received L-dopa alone.

Effect of different treatments on striatal dopamine (Table 2, Fig.1):

Administration of MPTP to mice induced significant decrease in striatal dopamine level compared to the normal control group. Administration of L-dopa induced significant increase in striatal dopamine compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in striatal dopamine compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in striatal dopamine compared to the group that received L-dopa alone.

Effect of different treatments on striatal TNF-α and NO levels (Table 2, Fig.2,3):

Administration of MPTP to mice induced significant increase in striatal TNF-α and NO levels compared to the normal control group. Administration of L-dopa produced non-significant effect on striatal TNF-α and NO levels compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in striatal TNF-α and NO levels compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant decrease in striatal TNF-α and NO levels compared to the group that received L-dopa alone.

Effect of different treatments on mitochondrial complex I activity (Table 2, Fig.4):

Administration of MPTP to mice induced significant decrease in mitochondrial complex I activity compared to the normal control group. Administration of L-dopa produced non-significant effect on mitochondrial complex I activity compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in mitochondrial complex I activity compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in mitochondrial complex I activity compared to the group that received L-dopa alone.

Effect of different treatments on mitochondrial ATP level (Table 2, Fig.5):

Administration of MPTP to mice induced significant decrease in mitochondrial ATP level compared to the normal control group. Administration of L-dopa produced non-significant effect on mitochondrial ATP level compared to MPTP-treated group. Concomitant administration of

either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in mitochondrial ATP level compared to MPTP-treated group. Concomitant administration of either L-dopa and CoQ10 or L-dopa and resveratrol induced significant increase in mitochondrial ATP level compared to the group that received L-dopa alone.

Correlation between catalepsy score and biochemical parameters (Table 3):

In the group II (MPTP-induced parkinsonism group), there was significant negative correlation of catalepsy score with striatal dopamine, mitochondrial complex I activity and mitochondrial ATP while it showed significant positive correlation with striatal TNF-α and striatal NO level.

Table (1): Comparison between the different studied groups for catalepsy score

Catalepsy score	Groups				
	I	II	III	IV	V
	Control	MPTP	MPTP + L-dopa	MPTP + L-dopa + Q10	MPTP + L-dopa + Resveratrol
Grid test	7.42 ± 1.23	60.5 ± 3.3	46.1 ± 4.13	33.5 ± 4.29	29.5 ± 3.48
		P1 <0.05	*P2 <0.05*	*P2 <0.05* *P3 <0.05*	*P2<0.05* *P3 <0.05*
Bar test	8.2 ± 1.02	53.59± 3.39	39.5 ± 3.69	29.5 ± 3.83	25.5 ± 3.29
		P1 < 0.05	*P2 <0.05*	*P2 <0.05* *P3 <0.05*	*P2 <0.05* *P3 <0.05*

Number of mice in each group = 10
Values expressed as mean ± SD.

Scheffe test:
P1: group I vs group II
P2: group II vs group III, group IV & group V
P3: group III vs group IV and group V

Table (2): Comparison between the different studied groups for the measured biochemical parameters (Striatal ATP, striatal dopamine, mitochondrial complex I activity, striatal TNF-α and striatal NO).

Group / Parameter	Group I Control	Group II MPTP	Group III MPTP+ L-dopa	Group IV MPTP + L-dopa + Q10	Group V MPTP + L-dopa + Resveratrol
Striatal ATP nmol/mg	12.19±0.98	4.33±0.19*	4.51±0.82$^{@}$	8.68±0.94$^{\#\$}$	9.96±1.11$^{\#\$}$
Striatal dopamine ng/mg	95.32±3.12	36.2±3.09*	64.6±2.8$^{\#}$	81.07±4.21$^{\#\$}$	85.24±3.3$^{\#\$}$
Mitochondrial complex I activity nmol/min/mg	30.36±1.14	13.29±0.72*	13.48±1.12$^{@}$	23.88±1.1$^{\#\$}$	24.25±1.09$^{\#\$}$
Striatal TNF-α pg/mg	82.33±15.55	215.63±20.6*	210.63±12.41$^{@}$	121.34±15.4$^{\#\$}$	108.12±17.12$^{\#\$}$
Striatal NO µmol/gm	0.08±0.01	0.35±0.02*	0.32±0.06$^{@}$	0.16±0.02$^{\#\$}$	0.13±0.01$^{\#\$}$

Number of mice in each group = 10
Values expressed as mean ± SD.
* Significant compared to control group
# Significant compared to MPTP group
$ Significant compared to MPTP + L-dopa group
$^{@}$ Non significant compared to MPTP group

Table (3): Correlation of catalepsy score (Grid test and Bar test) with the biochemical parameters in MPTP-induced parkinsonism (Group II).

Parameter	Grid test, r_1	Bar test, r_2
Striatal ATP	−0.616*	−0.623*
Striatal dopamine	−0.506*	−0.613*
Mitochondrial complex I activity	−0.832*	−0.697*
Striatal TNF-α	0.624*	0.616*
Striatal NO	0.716*	0.685*

*Significant at P < 0.05

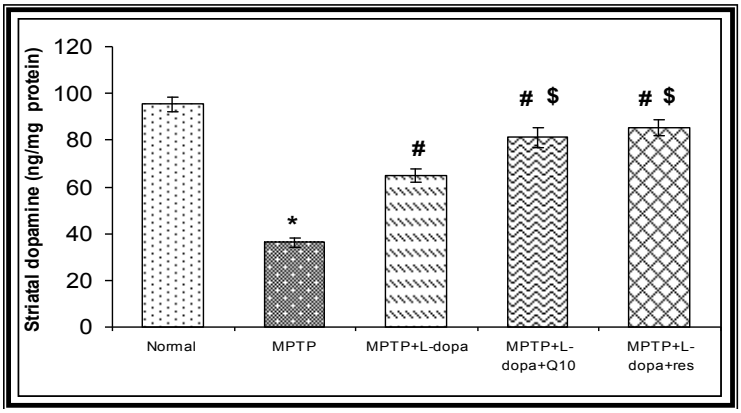

Figure (1): Striatal dopamine (ng/mg protein) mean ± SD in all studied groups

* Significant compared to control group
# Significant compared to MPTP group
$ Significant compared to MPTP + L-dopa group

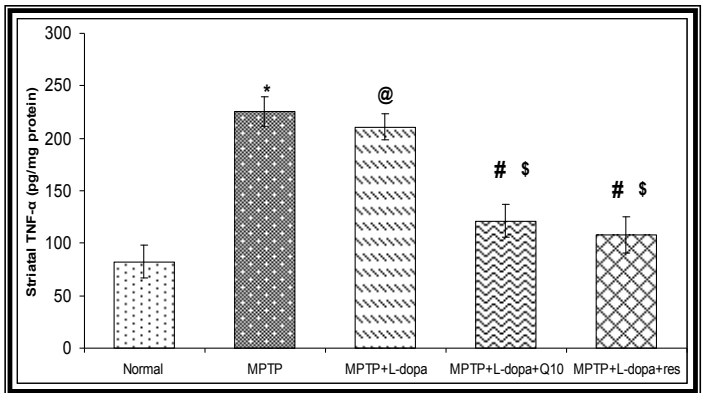

Figure (2): Striatal TNF-α (pg/mg protein) mean ± SD in all studied groups

* Significant compared to control group
# Significant compared to MPTP group
$ Significant compared to MPTP + L-dopa group
@ Non significant compared to MPTP group

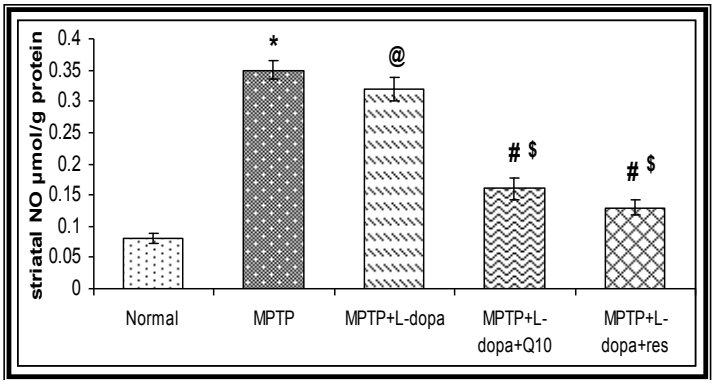

Figure (3): Striatal NO (µmol/g protein) mean ± SD in all studied groups.

* Significant compared to control group # Significant compared to MPTP group
$ Significant compared to MPTP + L-dopa group @ Non significant compared to MPTP group

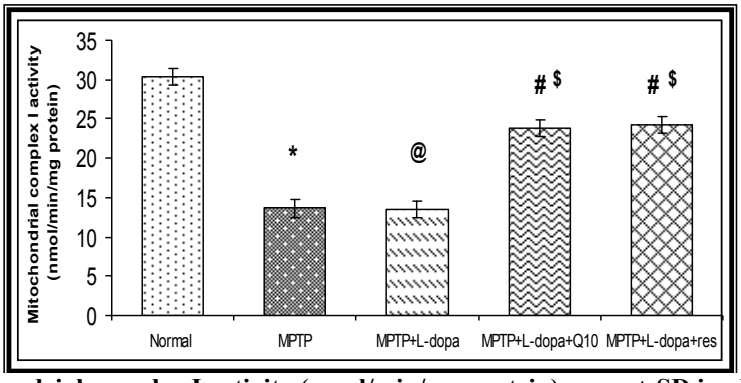

Figure (4): Mitochondrial complex I activity (nmol/min/mg protein) mean ± SD in all studied groups
* Significant compared to control group # Significant compared to MPTP group
$ Significant compared to MPTP + L-dopa group @ Non significant compared to MPTP group

4. Discussion:

Parkinson's disease (PD) is a movement disorder characterized by progressive degeneration of nigrostriatal dopaminergic (DA) neurons **(Dauer and Przedborski, 2003).** Lines of treatment of PD are limited and mainly affect the symptoms without significant disease-modifying effect. For this reason, understanding the molecular pathology and the cause of dopaminergic cell loss will lead to new lines of treatment that may prevent and cure the disease **(Savitt *et al.*, 2006)**.

MPTP is an artificial narcotic agent that had been shown to produce a parkinsonian-like disease in animals as well as in humans. In the present study, the administration of MPTP to mice induced a model of parkinsonism resembling the basic findings in human where bradykinesia and rigidity were manifested as an increase in catalepsy score and striatal TNF-α and NO level with significant decrease in striatal dopamine, mitochondrial ATP and complex I activity compared to the normal control group. These results were in agreement with other studies that indicated that exposure to MPTP caused nigrostriatal dopaminergic degeneration associated with neurochemical and behavioural features of PD **(Schober, 2004; Blanchet *et al.*, 2008**; **Soliman *et al.*, 2010, Prediger *et al.*, 2011).**

L'Episcopo *et al.* (2010) reported that selective degeneration of DA neurons in the subtantia nigra (SN) is a pathological hallmark of both PD and MPTP animal model of PD. The decline of dopamine in the striatum is associated clinically with progressive bradykinesia, tremors, rigidity and postural instability. **Soliman *et al.*(2010)** reported that MPTP mediates dopaminergic degeneration by inhibiting electron transport chain activity at complex I where decreased production of ATP through the electron transport chain can bring about rapid neuronal depolarization and a calcium mediated cascade of cell death. Also, MPTP induces key enzymes involved in the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and contributes to DA neuronal death. Additionally, **Kao *et al.* (2010)** found that MPTP increases production of proinflammatory cytokines, such as TNF-α and IL-1β that contribute to DA neuronal death. Other studies demonstrated that MPTP activates the brain-resident immune cells which lead to increased production of inflammatory cytokines. Moreover, it was found that if TNF-α receptor expression is genetically suppressed, microglial activation is absent and MPTP-induced neurotoxicity is significantly blunted **(Blanchet *et al.*, 2008)**. **Gao and Hong (2008)** reported that MPTP directly led to dopaminergic neuronal damage initially and then the damaged neurons release toxic soluble factors such as α-synuclein, which in turn induced microglial activation and production of proinflammatory factors and contributed to additional neuronal damage. This proposed pathogenesis was illustrated in the current study by the significant correlation between the catalepsy score and the neurochemical parameters obtained.

L-dopa is the precursor of dopamine that is used clinically in treatment of PD and dopamine-responsive dystonia in combination with a peripheral decarboxylase inhibitor (e.g. carbidopa) to reduce nausea and vomiting associated with L-dopa therapy and allow a greater proportion of L-dopa to enter the brain **(Savitt *et al.*, 2006)**. Although its administration in the present work caused symptomatic improvement in the form of reduction of catalepsy score with restoration of striatal dopamine levels that was in concordance with **Alam and Schmidt (2004)**, but it did not show any significant effects on striatal complex I activity, ATP levels, TNF-α or NO level. These results were in accordance with **Mogi *et al.* (1999)** who reported that L-dopa didn't increase the level of TNF-α in the brain in PD. In addition, there is a concern that L-dopa might be

toxic to dopamine neurons as it undergoes oxidative metabolism and has the potential to generate cytotoxic free radicals by their two free hydroxyl residues on their benzene ring **(Schapira and Olanow, 2004)**. The effects of L-dopa on NO production in the DA neurons and striatal DA terminals, in which degenerated DA neurons have been observed in PD, remain unclear. However, few reports have suggested that dopamine modulates NO release and/or production. **Kashihara *et al.* (1998)** reported that systemic treatment with L-dopa did not enhance NO production in the striatum of rats. In contrast, **Itokawa *et al.* (2006)** reported that L-dopa may increase NO production in the striatum of mice. **Melis *et al.* (1996)** showed that dopamine agonists increased NO production by activating D_2 receptors in the paraventricular nucleus of the hypothalamus. **Ben-Shachar et al. (2004)** reported that treatment with L-dopa was associated with elevated dopamine concentrations in the brain with significant reduction in the activity of complex I of the mitochondrial respiratory chain. Additionally, a significant decrease in striatal ATP concentrations was detected which was associated with dopamine-derived neurotoxic effects. This was attributed to the inhibitory effect of dopamine on pyruvate- and succinate-dependent electron transport.

CoQ10 is an essential biological cofactor of the electron transport chain that serves as an important antioxidant in mitochondrial and lipid membranes **(Littarru and Tiano, 2007)**. CoQ10 is able to penetrate blood brain barrier and can modulate the mitochondrial electron transport chain, modulate mitochondrial apoptosis and generally reduce oxidative stress in mitochondria **(Muqit *et al.*, 2006)**. Because of these functions, CoQ10 has attracted attention as a neuroprotective agent in neurodegenerative disorders linked to mitochondrial defects or oxidative stress, such as PD **(Storch *et al.*, 2007)**.

The results of the present study demonstrated the role of administration of CoQ10 in combination with L-dopa in amelioration of PD where it caused restoration of the striatal dopamine, complex I activity and ATP levels as well as decrease in the catalepsy score, striatal TNF-α and NO level compared to MPTP treated group. These results were in agreement with **Yang *et al.* (2009)** who reported that treatment with CoQ10 significantly blocked MPTP-induced oxidative stress and reduced pathological changes which contribute to a cascade of pathological changes in striatal neurons. Also, it was suggested that CoQ10 reduced α-synuclein aggregates and decreased brain oxidative stress. CoQ10 is a particularly important antioxidant in the inner mitochondrial membrane, where it transfers electrons from complexes I/II to complex III with final synthesis of ATP and can directly scavenge free radicals through interactions with α-tocopherol **(Littarru and Tiano, 2007)**. It also prevents apoptotic cell death by blocking Bax binding to mitochondria, and by inhibiting activation of the mitochondrial permeability transition. **Beal (2003)** suggested that CoQ10 may activate mitochondrial uncoupling proteins (UCP) leading to reduction in mitochondrial-free radical generation which offers marked neuroprotection against the MPTP toxicity. **Bessler *et al.* (2010)** reported that CoQ10 can modulate the immune function and decreases TNF-α production. **Schmelzer *et al.* (2007)** showed that Co Q10 decreases TNF-α secretion in human and murine monocytic cell lines by its anti-inflammatory effects. Moreover, **Abd El-Gawad and Khalifa (2001)** reported that CoQ10 protects against lipid peroxidation and NO generation in rat brain by its antioxidant and radical scavengering effect. The present study showed that combined administration of CoQ10 and L-dopa induced significant improvement in the biochemical parameters with significant decrease in catalepsy score compared to L-dopa treated group. This can be explained by the antioxidant and anti-inflammatory effects of CoQ10 with its ability to prevent depletion and regenerate endogenous antioxidants, decrease production of inflammatory cyokines and restore brain level of dopamine with increased ATP production through electron transport chain.

Resveratrol is a type of natural phenols produced by several plants under the attack of pathogens such as bacteria or fungi. The effects of resveratrol are currently a topic of numerous animal and human studies. In mouse and rat experiments, anticancer, anti-inflammatory, blood sugar-lowering and other beneficial cardiovascular effects of resveratrol have been reported (**Elliott and Jirousek, 2008)**.

In the present study, the combination of resveratrol with L-dopa induced significant increase in striatal ATP, dopamine and mitochondrial complex I activity with significant decrease in catalepsy score, striatal TNF-α level and striatal NO level compared to MPTP-treated group. These results were in accordance with recent studies reporting the protective effects of resveratrol against MPTP-induced motor coordination impairment, hydroxyl radical overloading and neuronal loss **(Blancet *et al.*, 2008; Anandhan *et al.*, 2010; Sun *et al.*, 2010)**.

Anandhan *et al.* (2010) showed that pretreatment of resveratrol significantly reversed the toxic effects of MPTP by increasing the levels of striatal dopamine, GSH and activities of the antioxidant enzymes along with enhanced behavior

performance. **Bi *et al.* (2005) and Zhang *et al.* (2010)** reported that resveratrol inhibits NO and TNF-α production by lipopolysaccharide-activated microglia and protects dopamine neurons against lipopolysaccharide-induced neurotoxicity through its anti-inflammatory and antioxidant properties.

Ryan *et al.* (2010) reported that resveratrol suppresses oxidative stress, restores mitochondrial function and increases complex I activity. **Zini et al. (2002)** suggested that resveratrol-induced limitation of dysfunction of mitochondria isolated from rat brain in an anoxia-reoxygenation model with at least three mechanisms: antioxidant properties, action on complex III and a membrane stabilizing effect. **Zhang *et al.* (2010)** reported that microglia were the targets of resveratrol action and resveratrol exerted neuroprotection through the inhibition of microglial activation. Microglial activation leads to reaction of superoxide anion with NO to form highly reactive intermediates which have an important role in neurodegeneration. Other studies suggested that NO serve as secondary messengers to enhance the gene expression, encoding a variety of proinflammatory factors. In addition, increased levels of cytokines such as TNF-α, IL-1β and interferon-γ have been demonstrated in the substantia nigra of patients with PD. Resveratrol inhibits both direct microglial activation induced by lipopolysaccharides or reactive microgliosis induced by MPTP. Also, the inhibition of accumulation of inflammatory cytokines conferred resveratrol-mediated significant neuroprotection on DA neurons **(Bi *et al.,* 2005)**. Recently, resveratrol was shown to prevent neurotoxicity triggered by 6-hydroxydopamine (6-OHDA). This model involves chronic inflammation, mitochondrial dysfunction, oxidative stress and loss of dopaminergic neurons in the substantia nigra. Several studies demonstrated that resveratrol significantly decreased the levels of COX-2, TNF-α, mRNA and COX-2 protein expression in the substantia nigra and hence its neuroprotective effect **(Kim *et al.,* 2006; Gong *et al.,* 2010; Sun *et al.,* 2010)**.

The present study showed that the combination of resveratrol and L-dopa induced significant improvement in the biochemical parameters with significant decrease in catalepsy score compared to L-dopa treated group. This can be attributed to the antioxidant and anti-inflammatory properties of resveratrol with its ability to inhibit NO and TNF-α production by microglia and improve the functions of electron transport chain in the striatal mitochondria with increased production of ATP. *In conclusion,* the combination of L-dopa and CoQ10 or L-dopa and resveratrol has a better effect than L-dopa alone on MPTP-induced parkinsonism in mice.

Corresponding author
Ayman A. Elsheikh
Department of Pharmacology, Faculty of Medicine, Tanta University, Egypt.
aymanahe@yahoo.com

References:

Abd El-Gawad HM, Khalifa AE. (2001). Quercetin, coenzyme Q10, and L-canavanine as protective agents against lipid peroxidation and nitric oxide generation in endotoxin-induced shock in rat brain. Pharmacol Res. 43(3):257-63.

Acuña-Castroviejo D, Tapias V, López LC, Doerrier C, Camacho E, Carrión MD, Mora F, Espinosa A, Escames G. (2011). Protective effects of synthetic kynurenines on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in mice. Brain Res Bull. 85(3-4):133-140.

Adams H. (1963). Adenosine 5'-triphosphate, determination with phosphoglycerate kinase. Methods of Enzymatic Analysis. 539-543.

Alam M, Schmidt WJ. (2004). L-DOPA reverses the hypokinetic behaviour and rigidity in rotenone-treated rats. Behav. Brain Res. 153 (2): 439–446

Anandhan A, Tamilselvam K, Vijayraja D, Ashokkumar N, Rajasankar S, Manivasagam T. (2010). Resveratrol attenuates oxidative stress and improves behaviour in MPTP challenged mice. Annals of Neurosciences. 17 (3): 113-119.

Barnum CJ, Tansey MG. (2010). Modeling neuroinflammatory pathogenesis of Parkinson's disease. Prog Brain Res. 184:113-132.

Beal MF. (2003). Bioenergetic approaches for neuroprotection in Parkinson's disease. Ann Neurol. 53 (3): 39–47.

Ben-Shachar D, Zuk R, Gazawi H, Ljubuncic P. (2004). Dopamine toxicity involves mitochondrial complex I inhibition: implications to dopamine related neuropsychiatric disorders. Biochemical Pharmacology. 67(10):1965-74

Bessler H, Bergman M, Blumberger N, Djaldetti M, Salman H. (2010). Coenzyme Q10 decreases TNF-alpha and IL-2 secretion by human peripheral blood mononuclear cells. J Nutr Sci Vitaminol (Tokyo). 56(1):77-81.

Bi XL, Yang JY, Dong YX, Wang JM, Cui YH, Ikeshima T, Zhao YQ, Wu CF. (2005). Resveratrol inhibits nitric oxide and TNF-alpha production by lipopolysaccharide-activated microglia. Int Immunopharmacol. 5(1):185-93.

Birch-Machin MA, Briggs HL, Saborido AA, Bindoff LA, Turnbull DM. (1994). An evaluation of the measurement of the activities of complexes I–IV in the respiratory chain of human

skeletal muscle mitochondria. Biochem Med Metab Biol. 51:35–42.

Blanchet J, Longpré F, Bureau G, Morissette M, DiPaolo T, Bronchti G, Martinoli MG. (2008). Resveratrol, a red wine polyphenol, protects dopaminergic neurons in MPTP-treated mice. Prog Neuropsychopharmacol Biol Psychiatry. 32(5):1243-1250.

Ciarlone AE. (1978). Further modification of a fluorometric method for analyzing brain amines. Microchemical Journal. 23 (1): 9–12

Cortas N, Wakid NW. (1990). Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin. Chem. 36: 1440-1443

Dauer W, Przedborski S. (2003). Parkinson's disease: mechanisms and models. Neuron. 39 (6):889-909.

Del Hoyo P, García-Redondo A, de Bustos F, Molina JA, Sayed Y, Alonso-Navarro H, et al. (2010). Oxidative stress in skin fibroblasts cultures from patients with Parkinson's disease. BMC Neurol. 10: 95.

Elliott PJ, Jirousek M. (2008). Sirtuins: novel targets for metabolic disease. Curr Opin Investig Drugs. 9 (4): 371–8.

Faust K, Gehrke S, Yang Y, Yang L, Beal M, Lu B. (2009). Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a Drosophila model of Parkinson's disease. BMC Neuroscience. 10:109.

Gao HM, Hong JS. (2008). Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends Immunol. 29: 357–365.

Gong QH, Li F, Jin F, Shi JS. (2010). Resveratrol attenuates neuroinflammation-mediated cognitive deficits in rats. J Health Science. 56 (6): 655-663.

Itokawa K, Ohkuma A, Araki N, Tamura N, Shimazu K. (2006). Effect of l-DOPA on nitric oxide production in striatum of freely mobile mice Neuroscience Letters. 402: 142–144

Jiao Y , Lu L, Williams R, Smeyne1 R. (2012). Genetic Dissection of Strain Dependent Paraquat-induced Neurodegeneration in the Substantia Nigra Pars Compacta PLoS One. 7(1): e29447.

Kao CL, Chen LK, Chang YL, Hsu MC, Chen YC, Chen YL, Ku HH, Hwang J. (2010). Resveratrol protect human endothelium from H2O2- induced oxidative stress and senescence via sir t1activation .journal of Atherosccl erosis and Th rombosis. 17: 970-979.

Kashihara K, Sakai K, Marui K, Shohmori T. (1998). l-DOPA does not facilitate nitric oxide production in the rat striatum and substantia nigra: in vivo microdialysis study. Life Sci. 63: 59–64.

Kim YA, Lim SY, Rhee SH. (2006). Resveratrol inhibits inducible nitric oxide synthase and cyclooxy- genase-2 expression in beta-amyloid-treated C6 glioma cells. Int J Mol Med. 17:1069–75.

Kim YH, Kim YS, Kang SS, Cho GJ, Choi WS. (2010). Resveratrol Inhibits Neuronal Apoptosis and Elevated Ca2+/Calmodulin-Dependent Protein Kinase II Activity in Diabetic Mouse Retina. Diabetes. 59 (7): 1825–1835.

L'Episcopo F, Tirolo C, Caniglia S, Testa N, Serra PA, Impagnatiello F, Morale MC, Marchetti B. (2010). Combining nitric oxide release with anti-inflammatory activity preserves nigrostriatal dopaminergic innervation and prevents motor impairment in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. Journal of Neuroinflammation. 7:83

Lee DH, Lee YJ. (2011). Astaxanthin protects against MPTP/MPP+-induced mitochondrial dysfunction and ROS production in vivo and in vitro. Food Chem Toxicol. 49(1): 271–280.

Littarru GP, Tiano L. (2007). Bioenergetic and antioxidant properties of coenzyme Q10: recent: developments. Mol Biotechnol. 37:31–7.

Lowry OH, Rosebrough NJ, Farr AI, Randall RJ. (1951). Protein measurement with the Folin-Phenol reagent. J Biol Chem. 193: 265–275

Melis MR, Succu S, Argiolas A. (1996). Dopamine agonists increase nitric oxide production in the paraventricular nucleus of the hypothalamus: correlation with penile erection and yawning, Eur J Neurosc. 8: 2056–2063.

Miller R, James-Kracke M, Sun G, Sun A. (2009). Oxidative and Inflammatory Pathways in Parkinson's Disease. Neurochemical Research. 55-65.

Mogi M, Togari A, Tanaka K, Ogawa N, Ichinose H, Nagatsu T. (1999). Increase in level of tumor necrosis factor (TNF)-alpha in 6-hydroxydopamine-lesioned striatum in rats without influence of systemic L-DOPA on the TNF-alpha induction. Neurosci Lett. 268(2):101-104.

Mosley R, Benner E, Kadiu I, Thomas M, Boska M, Hasan K, Laurie C, Gendelman H. (2006). Neuroinflammation, Oxidative Stress and the Pathogenesis of Parkinson's Disease. Clin Neurosci Res. 6(5): 261–281.

Muqit MM, Gandhi S, Wood NW. (2006). Mitochondria in Parkinson disease: back in fashion with a little help from genetics. Arch. Neurol. 63: 649–654.

Prediger RD, Aguiar AS Jr, Moreira EL, Matheus FC, Castro AA, Walz R, De Bem AF, Latini A, Tasca CI, Farina M, Raisman-Vozari R. (2011).

The intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a new rodent model to test palliative and neuroprotective agents for Parkinson's disease. Curr Pharm Des. 17(5):489-507.

Ryan MJ, Jackson JR, Hao Y, Williamson CL, Dabkowski ER, Hollander JM, Always SE. (2010). Suppression of Oxidative Stress by Resveratrol After Isometric Contractions in Gastrocnemius Muscles of Aged Mice. J Gerontol A Biol Sci Med Sci. 65(8): 815–831.

Savitt JM, Dawson VL, Dawson TM. (2006). Diagnosis and treatment of Parkinson disease: molecules to medicine. J Clin Invest. 116 (7):1744–1754.

Schapira AHV, Olanow CW. (2004). Neuroprotection in parkinson disease. Mysteries, myths, and misconceptions. JAMA. 291: 358–364.

Schmelzer C, Lorenz G, Lindner I, Rimbach G, Niklowitz P, Menke T, Döring F. (2007). Effects of Coenzyme Q10 on TNF-alpha secretion in human and murine monocytic cell lines. Biofactors. 31(1):35-41.

Schober A. (2004). Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. Cell and Tissue Research. 318 (1): 215-224.

Soliman Y, Jackson T, Mazzio E, Soliman KF. (2009). The Effects of Piroxicam in the Attenuation of MPP+/MPTP Toxicity In Vitro and In Vivo. Neurochem Res. 34(2): 304–310.

Storch A, Jost WH, Vieregge P, Spiegel J, Greulich W, et al. (2007). Randomized, double-blind, placebo-controlled trial on symptomatic effects of coenzyme Q10 in parkinson disease Arch. Neurol. 64 (7): 938–944.

Sun AY, Wang Q, Simonyi A, Sun GY. (2010). Resveratrol as a Therapeutic Agent for Neurodegenerative Diseases. Molecular Neurobiology. 41 (2-3): 375-383.

Turpeenoja L, Villa R, Magri G, Stella G. (1988). Changes of mitochondrial membrane proteins in rat cerebellum during aging. Neurochem Res. 13: 859–865.

Yang L, Calingasan NY, Wille EJ, Cormier K, Smith K, Ferrante RJ, Beal MF (2009). Combination therapy with coenzyme Q10 and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's diseases. J Neurochem. Jun;109(5):1427-39.

Zhang F, Shi JS, Zhou H, Wilson B, Hong JS, Gao HM. (2010). Resveratrol protects dopamine neurons against lipopolysaccharide-induced neurotoxicity through its anti-inflammatory actions. Mol Pharmacol. 78(3): 466-77.

Zini R, Morina C, Bertellib A, Bertellic A, Tillementa JP. (2002). Resveratrol-induced limitation of dysfunction of mitochondria isolated from rat brain in an anoxia-reoxygenation model. Life Sciences. 71(26): 3091–3108.

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