

Effect of Co Enzyme Q10 Alone or in Combination with Vitamin C on Lipopolysaccharide-Induced Brain Injury in Rats

Nabila A. El-Laithy¹, Elsayed M.E. Mahdy², Eman R. Youness^{1*},
Nermeen Shafee³, Mohamed S.S. Mowafy⁴ and Mahmoud M. Masoud¹

¹Department of Medical Biochemistry, ³Department of Pathology,
National Research Centre, Cairo, Egypt.

²Department of Chemistry, Faculty of Science, Helwan University, Helwan, Egypt.

⁴Critical care unit, Faculty of Medicine, Cairo University, Cairo, Egypt.

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Our was to determine the impact of Coenzyme Q10 (Co Q10) and vitamin C alone or in combination on oxidative stress in brain tissue of rats during endotoxemia induced by single intraperitoneal dose of Lipopolysaccharide (LPS), 500µg/kg. Both CoQ10 & vitamin C were given orally to rats with doses (200 & 100 mg/kg) respectively for 7 successive days prior induction of endotoxemia .LPS injected, with Co Q10 with doses (100 & 200 mg/kg) & vit. C (50 & 100 mg/kg). In addition CoQ10 and vitamin C together in doses (100 & 50 mg/kg) & (200 & 100 mg/kg) respectively were added to LPS-treated rats. Then euthanized 4 hours later. Histopathological assessment of brain tissue was done. Results: LPS injection induced oxidative stress in brain tissue, resulting in marked increase in malondialdehyde (MDA), nitrite (NO) and Amyloid beta (Aβ), while decreasing reduced glutathione (GSH), paraoxonase-1 (PON1) and brain derived neurotrophic factor (BDNF). CoQ10 and vit.C administration with doses (200 & 100 mg/kg) before endotoxemia result in reduction of brain MDA, NO and Aβ, while increasing levels of GSH, PON1 and BDNF compared to controls. The addition of both Co Q10 & vit.C to LPS- treated rats lead to decrease of brain NO, MDA and Aβ, also increase of GSH, PON1 and BDNF. This effect was more obvious with high doses, this due to the ameliorating effect of both CoQ10 and vit.C on oxidative stress of brain tissue during endotoxemia. This consisted with the histopathological results. Conclusion: this work focuses on the possible role of CoQ10 & vit.C as antioxidants in protecting brain tissue.

Keywords: CoQ10 - vitamin C - LPS - oxidative stress - Aβ - BDNF.

The imbalance between the reactive oxygen species generation (ROS) and the mechanism responsible for elimination of the ROS causes oxidative stress in tissues and cells¹. Common ROS has many kinds like, hydroxyl radical, hydrogen peroxide and superoxide anion radical. Also, superoxide and high levels of nitric oxide give increase in peroxynitrite, which can perform both oxidation and nitrative stress together with similar reactive nitrogen species (RNS).

Oxidative stress has the ability to damage all cellular macromolecules, including protein side chains, sugars, nucleic acids & polyunsaturated lipids, thus plays a vital role in the pathogenesis of many neurological diseases.

Coenzyme Q10 (CoQ10) or Ubiquinone is fat-soluble, vitamin-like substance present in nearly all eukaryotic cells, mainly in mitochondria. It is a component of the electron transport chain that contributes to aerobic cellular respiration,



which produces energy in the form of ATP.² In the last few years, there has been many researches which focused on its antioxidant properties in several cellular compartments.³ CoQ10 has shown defending effect against neuronal loss in several animal models of neurodegeneration and the capacity of CoQ10 to limit disease progression in clinical trials put into consideration to explore neuroprotective activity in many of the neurodegenerative diseases.⁴

Ascorbic acid (vit.C) is a water-soluble micronutrient. It is a robust antioxidant which perform directly via scavenging of ROS and indirectly through reproducing of other antioxidant systems.⁵ Yaman *et al.*, (2010) had proved that oxidative damage to macromolecules in vivo can be minimized by supplementation with large doses of this vitamin. This is important in the synthesis and stabilization of neurotransmitters.⁶

Brain derived neurotrophic factor (BDNF), plays a vital role in supporting neuronal survival & function during development in adulthood.⁷ Mitochondrial respiratory coupling at complex I is motivated by BDNF. It plays an essential role in brain metabolism and oxygen utilization.⁸ Both glial cell line-derived growth factor and BDNF protect against H₂O₂ induced neuronal death in patients with idiopathic Parkinson's disease.⁹ Accordingly, antioxidant effects in lesioned spinal cords of rats can be produced by giving of BDNF.¹⁰

Amyloid β (A β) has been shown play a viable role in Alzheimer's disease (AD) and, thus, may play a main role in the pathogenesis of the disease. Butterfield, *et al* (2013) has shown that A β induces protein oxidation and lipid peroxidation both in vitro and in vivo, The excessive production and accumulation of A β are responsible for the progression and pathogenesis of AD.¹¹ Hence, the use of treatments to decrease A β formation or the downstream oxidative stress connected with A β could be useful in preventing, curing, or delaying the progression of AD.¹²

Lipopolysaccharide (LPS) is the main component of the outer membrane of Gram-negative bacteria and is responsible for the initiation of the host response to the microorganisms. In response to LPS, macrophages secrete pro-inflammatory cytokines as the interleukins IL-6, IL-12, IL-1 β & tumor necrosis factor TNF- α and reactive oxygen species (ROS) like superoxide anion¹³.

The current work was therefore designed to inspect the impact of oral supplementation of Co Q10 and ascorbic acid alone or in combination on brain oxidative stress in a model of endotoxemia. For this purpose, rats were injected intraperitoneally with a subseptic dose of LPS. MDA, NO, GSH, PON1 as well as BDNF and A β were determined in brain homogenate.¹⁴

MATERIALS AND METHODS

The current study was done on sixty six adult albino Wistar rats weighing from 100 to 120 gm, 3 months old that are obtained from the animal house colony of the National Research Centre (NRC), Egypt. The animals were maintained on standard diet and water, housed in stainless steel cages in controlled temperature (23 \pm 1°C). All animals were housed in animal house for a week prior to experiments. Also they received human care according to the guidelines for animal experiments which were approved by the Ethical Committee of Medical Research, National Research Centre (NRC).

Chemicals and drugs

A purified and lyophilized *E. coli* endotoxin from (Serotype 055: B5, Sigma, USA) was utilized and dissolved in sterile saline, aliquoted, and frozen at -80°C. CoQ10 were purchased from (MEPACO-MEDIFOOD, Egypt). CoQ10 was prepared by dissolving in 1% tween 80 as vehicle. Vitamin C (Sigma, MO, USA) was prepared by dissolving in distilled water.

Study design

66 Female albino rats weighing 100-120 g will be divided into 11 groups (six rats in each). Rats were treated with tween 80 (group 1). Animals treated with saline (group 2), LPS (500 μ g/kg, i.p.) (group 3), Co Q10 (200 mg/kg) (group 4), Co Q10 at 100 mg/kg + LPS (group 5), Co Q10 at 200 mg/kg + LPS (group 6), Vitamin C (100 mg/kg) (group 7), Vitamin C at 50 mg/kg + LPS (group 8), Vitamin C at 100 mg/kg + LPS (group 9), Co Q10 at 100 mg/kg + Vitamin C at 50 mg/kg + LPS (group 10) and Co Q10 at 200 mg/kg + Vitamin C at 100 mg/kg + LPS (group 11). After four hours, rats were euthanized after LPS injection. Brains were removed & washed with ice-cold saline solution (0.9 % NaCl) and stored at -80 °C for the biochemical analysis.

Lipid peroxidation Determination

The level of lipid peroxidation in the brain tissue homogenates was determined by evaluating malondialdehyde level (MDA) according to Ruiz-Larrea *et al.*¹⁵. In this assay, MDA (along with its equivalents) reacts with thiobarbiturate producing a red colored complex that read at 532 nm.

Determination of nitric oxide

Nitric oxide level in the supernatants of the brain tissue homogenate was determined indirectly by measuring the level of nitrite using the Griess reagent, according to Moshage *et al.*¹⁶.

Determination of reduced glutathione

Reduced glutathione was evaluated in supernatant of brain tissue rendering to Ellman (1959)¹⁷. This was based on the reduction of Ellman's reagent by -SH groups in GSH forming nitro mercaptobenzoic acid. The color of this acid can be determined spectrophotometrically.

Paraoxonase 1 activity determination

The activity of paraoxonase 1 (PON1) in brain tissue homogenates was measured using substrate phenyl acetate as described previously¹⁸. Herein, PON1 catalyzes phenyl acetate cleavage, forming phenol. The rate of the formation of phenol which is measured spectrophotometrically at 270 nm.

Determination of Brain derived neurotrophic factor

BDNF was measured using ELISA kit (Glory Science Co., Ltd, Del Rio, TX, USA).

Determination of Brain Amyloid β

Amyloid β was measured ELISA kit (Glory Science Co., Ltd, Del Rio, TX, USA).

Histopathological studies

Specimens of brain were dissected right away after death and fixed in 10% neutral-buffered formal saline, then dehydrated in ascending grades of alcohol (70% - 80% - 90% and finally absolute alcohol), cleared in xylene, soaked in soft paraffin wax at 55°C and entrenched in hard paraffin. Sections of 6 μ m thick were cut and stained with Haematoxylin and eosin¹⁹ for histopathological investigation.

Statistical Test

Data were given as the mean \pm SE from six rats in each group. Significance of the statistics was assessed using ANOVA test followed by post hoc LSD test. Value of less than 0.05 was considered statistically significant.

RESULTS**Brain Biochemical results****Impact of Coenzyme Q10 on oxidative stress****Malondialdehyde (MDA)**

Co Q10 at a dose 200 mg/kg b/w revealed decrease in MDA level in brain by 20.9 % ($p < 0.05$) in comparison with the control group. Following LPS, MDA in the brain tissue was significantly increased by 108.47 % ($p < 0.001$) when compared with the tween80 control group. MDA showed decrease by 27.69 % ($p < 0.05$) & 39.35 % ($p < 0.05$) in rats given Co Q10 (100 mg/kg & 200 mg/kg) and LPS compared with the corresponding LPS control value (table 1).

Nitrate (NO)

Nitrate decreased by 14.96 % ($p > 0.05$) after Coenzyme Q10 injection at a dose 200 mg/kg in comparison with the tween80 control group. In disparity, marked and significant elevation of nitric oxide by 59.09% ($p < 0.05$) following injection of LPS. Rats treated with Co Q10 and LPS, nitrates decreased in brain in dose 100mg/kg body weight by 28.73% ($p < 0.05$) and in dose 200 mg/kg b/w by 35.28% ($p < 0.05$) if compared with LPS group (table 1).

Reduced glutathione (GSH)

Coenzyme Q10 resulted in increase in GSH by 31.15% ($p < 0.05$) if compared with the tween80 control group. Following LPS, GSH in brain was significantly decrease by 23.96% ($p < 0.05$). When Coenzyme Q10 given to LPS - treated rats result in increasing by 62.92% ($p < 0.05$) and 65.73% ($p < 0.05$) in rats taken Co Q10 (100 mg/kg & 200 mg/kg b/w) and LPS compared with the corresponding LPS control value (table 1).

Impact of Coenzyme Q10 on paraoxonase activity (PON1)

The administration of only Co Q10 result in increase in PON1 activity by 19.09 % ($p < 0.05$) in comparison with the corresponding tween80 control value. Following LPS, PON1 in the brain was significantly decreased by 46.27 % ($p < 0.05$) compared with the tween80 control group. PON1 showed increase by 55.42 % ($p < 0.05$) & 74.69 % ($p < 0.05$) in rats given Co Q10 (100 mg/kg & 200 mg/kg b/w) and LPS when compared with the corresponding LPS control value (table 1).

Table 1. Effect of Coenzyme Q10 (Co Q10), LPS or both on malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), paraoxonase I activity (PON1), Brain derived neurotrophic factor (BDNF) and Amyloid β (A β) in rat brain

Parameters Groups	MDA (nmol /g. tissue)	NO (μ mol/g. tissue)	GSH (μ mol/g. tissue)	PON1 (kU/l)	BDNF (pg/ml)	A β (pg/ml)
Tween80	32.58 \pm 1.04	29.75 \pm 1.28	3.98 \pm 0.06	3.09 \pm 0.06	50.81 \pm 0.71	217.85 \pm 1.32
CoQ10(200 mg/kg)	25.77 \pm 0.92*	25.30 \pm 0.80	5.22 \pm 0.04*	3.68 \pm 0.08*	70.35 \pm 0.82*	179.72 \pm 1.87*
LPS	67.92 \pm 2.49*	47.33 \pm 0.83*	3.03 \pm 0.05*	1.66 \pm 0.05*	35.17 \pm 4.69*	543.01 \pm 3.49*
CoQ10(100 mg/kg)+LPS	49.11 \pm 1.86#	33.73 \pm 1.00#	4.93 \pm 0.03#	2.58 \pm 0.05#	41.11 \pm 0.62#	340.49 \pm 1.75#
CoQ10(200 mg/kg)+LPS	41.19 \pm 1.41#	30.63 \pm 0.03#	5.02 \pm 0.06#	2.90 \pm 0.06#	44.50 \pm 0.96#	281.41 \pm 1.16#

Results are presented as mean \pm SE, P < 0.05 = statistically significant.

*P < 0.05 versus Tween80 control

#P < 0.05 versus LPS group

Table 2. Impact of LPS, vitamin C or together on (MDA), (NO), (GSH), (PON1), (BDNF) and (A β) in rat brain

Parameters Groups	MDA (nmol /g. tissue)	NO (μ mol/g. tissue)	GSH (μ mol/g. tissue)	PON1 (kU/l)	BDNF (pg/ml)	A β (pg/ml)
Saline	30.86 \pm 1.82	28.80 \pm 1.19	4.08 \pm 0.06	3.17 \pm 0.07	54.09 \pm 0.85	214.78 \pm 1.52
Vit.C (100 mg/kg)	27.13 \pm 0.75	27.11 \pm 1.04	4.76 \pm 0.03*	3.35 \pm 0.07	60.33 \pm 1.16	195.84 \pm 1.38*
LPS	67.92 \pm 2.49*	47.33 \pm 0.83*	3.03 \pm 0.05*	1.66 \pm 0.05*	35.17 \pm 4.69*	543.01 \pm 3.49*
Vit.C (50 mg/kg)+LPS	54.62 \pm 2.35#	40.21 \pm 0.04#	3.83 \pm 0.05#	2.06 \pm 0.07#	37.66 \pm 0.78	356.44 \pm 2.39#
Vit.C (100 mg/kg)+LPS	47.32 \pm 1.49#	36.61 \pm 1.35#	4.57 \pm 0.06#	2.29 \pm 0.06#	39.6 \pm 0.57	344.72 \pm 1.82#

Results are presented as mean \pm SE, P < 0.05 considered statistically significant.

*P < 0.05 versus saline control

#P < 0.05 versus LPS control group

Table 3. Impact of both Coenzyme Q10 and Vitamin C with different concentrations in addition to LPS on (MDA), nitric oxide (NO), (GSH), paraoxonase 1 activity (PON1), Brain derived neurotrophic factor (BDNF) and Amyloid β (A β) in rat brain.

Parameters Groups	MDA (nmol /g. tissue)	NO (μ mol/g. tissue)	GSH (μ mol/g. tissue)	PON1 (kU/l)	BDNF (pg/ml)	A β (pg/ml)
Saline	30.86 \pm 1.82	28.80 \pm 1.19	4.08 \pm 0.06	3.17 \pm 0.07	54.09 \pm 0.85	214.78 \pm 1.52
Tween80	32.58 \pm 1.04	29.75 \pm 1.28	3.98 \pm 0.06	3.09 \pm 0.06	50.81 \pm 0.71	217.85 \pm 1.32
LPS	67.92 \pm 2.49*	47.33 \pm 0.83*	3.03 \pm 0.05*	1.66 \pm 0.05*	35.17 \pm 4.69*	543.01 \pm 3.49*
CoQ10(100mg/kg)						
+Vit.C(50mg/kg)+LPS	38.47 \pm 1.49#	31.07 \pm 0.33#	5.46 \pm 0.05#	4.10 \pm 0.07#	48.09 \pm 0.9#	227.85 \pm 1.59#
CoQ10(200mg/kg)						
+Vit.C(100mg/kg)+LPS	35.02 \pm 1.15#	30.46 \pm 0.07#	5.65 \pm 0.06#	4.70 \pm 0.06#	51.06 \pm 0.61#	221.09 \pm 0.87#

Results are presented as mean \pm SE, P < 0 .05 considered statistically significant.

*P < 0 .05 versus saline control

#P < 0.05 versus LPS control group

Impact of Coenzyme Q10 on derived neurotrophic factor (BDNF)

Coenzyme Q10 resulted in increase in BDNF by 38.45% (p<0.05) when compared with the tween80 group. Following LPS, BDNF decrease by 30.78% (p<0.05), Coenzyme Q10 when given to rats treated with LPS, resulted in an increase by 16.89% (p<0.05) and 26.53% (p<0.05) in rats given Co Q10 (100 mg/kg & 200 mg/kg b/w) and LPS when compared with the corresponding LPS control value(table 1).

Effect of Coenzyme Q10 on Amyloid β (A β)

The administration of only Co Q10 at a dose 200 mg/kg body weight resulted in decrease in the level of A β in brain by 17.50 % (p<0.05) if compared with the tween80 control group. Following LPS, A β in the brain was significantly increased by 149.25 % (p<0.05) when compared with the tween80 group. A β showed decrease by 37.29 % (p<0.05) & 48.17 % (p<0.05) in rats given Co Q10 (100 mg/kg & 200 mg/kg b/w) and LPS when compared with the corresponding control value of LPS (table 1).

Effect of Vitamin C on brain oxidative stress Malondialdehyde (MDA)

Vitamin C only at a dose 100 mg/kg b/w cause decrease in MDA level in brain by 12.08 % (p>0.05) in comparison with the control group. Following lipopolysaccharid, MDA level in brain was significantly augmented by 120.09 % (p<0.05) as compared with the control group. MDA showed decrease near 19.58 % (p<0.05) and 30.33 % (p<0.05) in rats given the vitamin (50 mg/kg & 100 mg/kg b/w) and LPS compared with the corresponding LPS value (table 2).

Nitric oxide (NO)

In brain, nitric oxide decreased by 5.86 % (p>0.05) after vitamin C injection at a dose 100 mg/kg b/w in comparing with the tween80 group. In contrast, marked & significant elevation of nitric oxide by 64.34% ((p<0.05) following LPS injection. In rats taken vitamin C and LPS , nitric oxide decrease in dose 50mg/kg b/w by 15.04% (p<0.05) and in dose 100 mg/kg b/w by 22.65% (p<0.05) as compared with LPS group(table 2).

Reduced glutathione (GSH)

Vitamin C resulted in increase in GSH by 16.66% (p<0.05) in comparison with the control group. Following LPS, GSH showed obvious and significant decrease by 25.83% (p<0.05). Vitamin C

when added to LPS treated rats result in increasing by 26.56% ($p < 0.05$) and 49.70% ($p < 0.05$) in rats given vitamin C (50 mg/kg & 100 mg/kg b/w) and LPS as compared with the corresponding LPS value (table 2).

Impact of Vitamin C paraoxonase activity (PON1) on brain

Administration of vitamin C only result in increase in paraoxonase activity by 5.67% ($p > 0.05$) as compared with the corresponding control value. Following LPS, PON1 in the brain was significantly diminished by 47.63% ($p < 0.05$) in comparison with the control group. PON1 showed increase by 24.09% ($p < 0.05$) and 37.95% ($p < 0.05$) in rats given Vitamin C (50 mg/kg & 100 mg/kg b/w) and LPS as compared with the corresponding LPS control value (table 2).

Impact of Vitamin C on Brain derived neurotrophic factor (BDNF)

Vitamin C resulted in increase in BDNF by 11.54% ($p > 0.05$) as compared with the control group. Following LPS, BDNF showed obvious and significant decrease by 34.97% ($p < 0.05$). Vitamin C when given to LPS-treated rats result in increasing by 7.10% ($p > 0.05$) and 12.59% ($p > 0.05$) in rats taken vitamin C (50 mg/kg & 100 mg/kg b/w) and LPS compared with the corresponding LPS control value (table 2).

Effect of Vitamin C on Amyloid β ($A\beta$)

The administration of vitamin C alone in a dose 100 mg/kg b/w cause decrease of the level of $A\beta$ in brain by 8.81% ($p < 0.05$) as compared with saline control group. Following LPS, $A\beta$ in the brain was significantly increased by 152.82% ($p < 0.001$) as compared with the control group. $A\beta$ showed decrease by 34.35% ($p < 0.05$) and 36.51% ($p < 0.05$) in rats given Vitamin C (50 mg/kg & 100 mg/kg b/w) and LPS compared with the corresponding LPS control value (table 2).

Impact of both Coenzyme Q10 & Vitamin C with different concentrations in addition to LPS on malondialdehyde (MDA)

The group treated with Coenzyme Q10 (100 mg/kg b/w), vitamin C (50 mg/kg b/w) and LPS showed decrease in MDA by 43.36% ($p < 0.05$) and the group treated with Coenzyme Q10 (200 mg/kg b/w), vitamin C (100 mg/kg b/w) and LPS showed decrease in MDA by 48.44% ($p < 0.05$) compared with the corresponding LPS control value (table 3).

Effect of both Coenzyme Q10 and Vitamin C with different concentrations in addition to LPS on NO

In brain, nitric oxide lowered by 34.35% ($p < 0.05$) in the group treated with Coenzyme Q10 (100 mg/kg b/w), vitamin C (50 mg/kg b/w) and LPS, decreased by 34.35% ($p < 0.05$) in the group treated with Coenzyme Q10 (100 mg/kg b/w), vitamin C (50 mg/kg b/w) and LPS, as compared with LPS group (table 3).

Impact of both Coenzyme Q10 and Vitamin C with different concentrations in addition to LPS on Reduced glutathione (GSH)

The group treated with Coenzyme Q10 (100 mg/kg body weight), vitamin C (50 mg/kg b/w) and LPS showed noticeable and significant increase of GSH by 80.43% ($p < 0.001$) and the group treated with Coenzyme Q10 (200 mg/kg b/w), vitamin C (100 mg/kg b/w) and LPS showed marked and significant increase in GSH by 86.71% ($p < 0.001$) as compared with the corresponding LPS control value (table 3).

Impact of both Coenzyme Q10 and Vitamin C with different concentrations in addition to LPS on brain paraoxonase activity (PON1)

The group treated with Coenzyme Q10 (100 mg/kg b/w), vitamin C (50 mg/kg b/w) and LPS showed marked & significant increase in paraoxonase by 146.98% ($p < 0.001$) and the group treated with Coenzyme Q10 (200 mg/kg b/w), vitamin C (100 mg/kg b/w) and LPS showed noticeable and significant increase in PON1 by 183.13% ($p < 0.001$) as compared with the corresponding LPS control value (table 3).

Effect of both Coenzyme Q10 and Vitamin C with different concentrations in addition to LPS on Brain derived neurotrophic factor (BDNF)

The group treated with vitamin C (50 mg/kg b/w), Coenzyme Q10 (100 mg/kg b/w) and LPS showed marked and significant increase in BDNF by 36.73% ($p < 0.05$) and the group treated with Coenzyme Q10 (200 mg/kg b/w), vitamin C (100 mg/kg b/w) and LPS showed marked and significant increase in BDNF by 45.18% ($p < 0.05$) as compared with the corresponding LPS control value (table 3).

Impact of both Coenzyme Q10 and Vitamin C with different concentrations in addition to LPS on Amyloid β ($A\beta$)

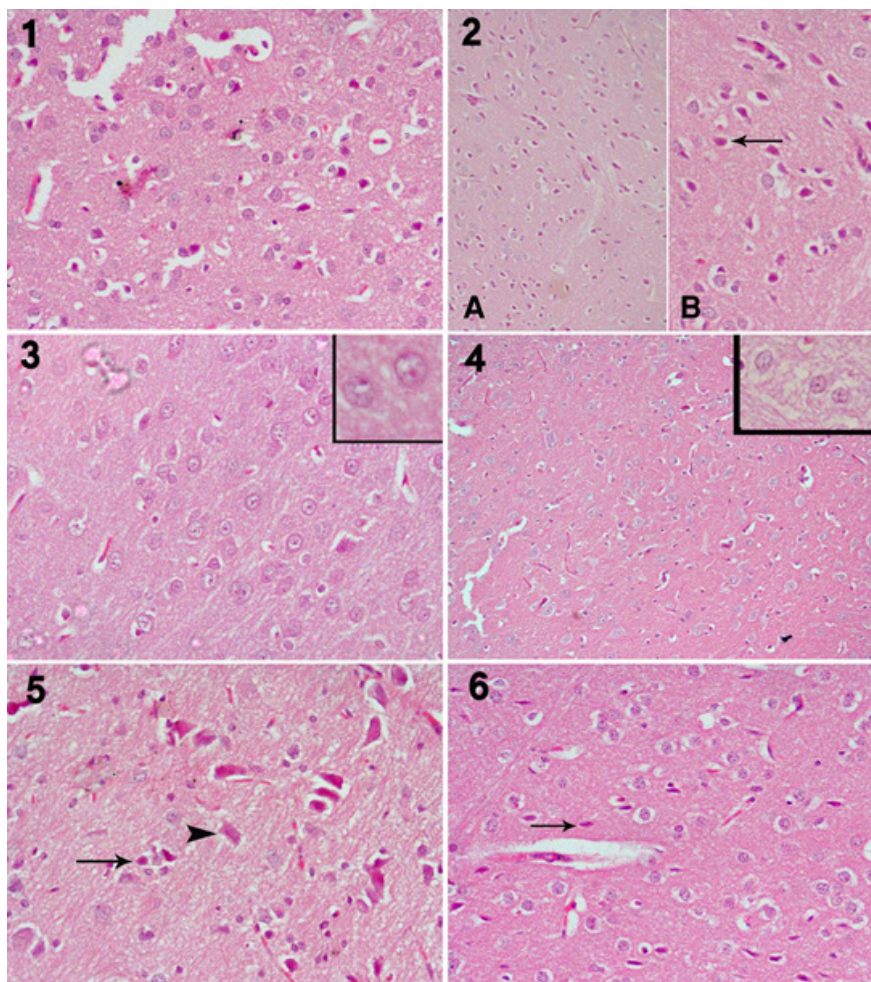
In brain tissue, $A\beta$ decreased by 58.04%

($p < 0.05$) in the group treated by Coenzyme Q10 (100 mg/kg b/w), vitamin C (50 mg/kg b/w) and LPS, and $A\beta$ decreased by 59.3 % ($p < 0.05$) in the group treated by Co Q10 (100 mg/kg b/w vitamin C (50 mg/kg b/w) and LPS, and compared with LPS group (table3).

DISCUSSION

Herein in the present study, we investigated the impact of oral administration of Coenzyme Q10 (CoQ10), vitamin C and both together on brain oxidative stress due to intraperitoneal

administration of lipopolysaccharide (LPS) in rat. Following injection of LPS at sub-septic dose of 500ig/kg, levels of reduced glutathione (GSH) in brain homogenate decreased along with the increased values of the lipid peroxidation (MDA). Glutathione tripeptide in brain (glycyl-glutamyl-cysteine) is the most plentiful cytosolic thiol and is the ultimate important antioxidant. Also it acts as a co-factor for glutathione transferase, glutathione peroxidase. Glutathione ca directly scavenge reactive oxygen species as singlet oxygen and hydroxyl radical. The ratio of oxidized and reduced types of glutathione determines the redox

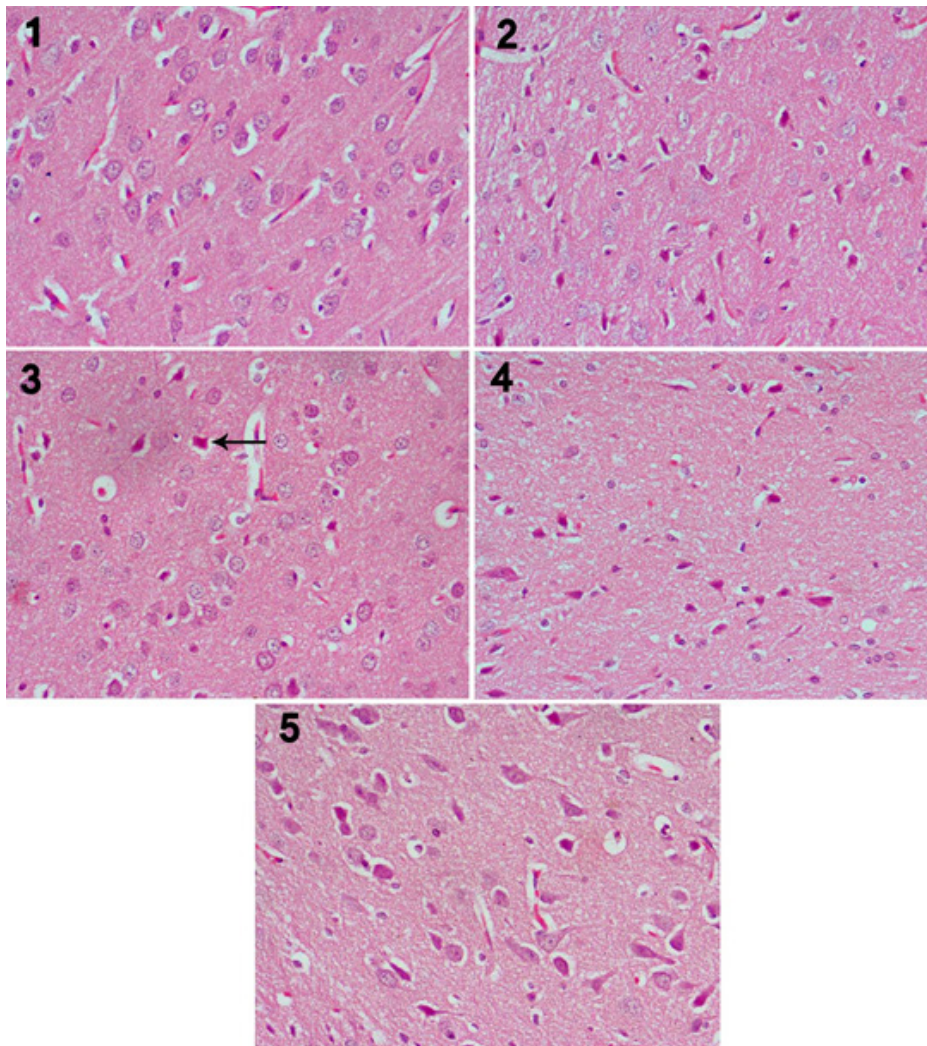


1) control vehicle rat shows normal shaped neurons. 2) control +ve (LPS) shows a large number of small dark neurons that appear in the higher magnification part with acidophilic cytoplasm and deeply stained nuclei (arrow). 3) a control -ve rat, 4) control co Q10 - 200 mg rat shows quite normal structure of cerebral cortex. 5) rat (received LPS + 100 mg co Q10) shows a noticeable reduction in dark neurons number, some are still small in size (arrow), while others began to regain their normal size (arrowhead). 6) rat (received LPS + 200 mg co Q10) shows normalization of almost all the cells

state of the cell.^{20,21} The depletion of GSH in brain reflects the consumption of the antioxidant as the augmented free radical generation by endotoxin. It was also found that elevated lipid peroxidation, depleted glutathione reductase & GSH activity occur after the peripheral injection of LPS^{22,23}.

Lipopolysaccharide also elevated nitric oxide (NO) values in brain. Diminished concentrations of nitric oxide generated by endothelial (eNOS) or neuronal (nNOS) isoforms of nitric oxide synthase (NOS) are important in

maintenance of vascularity and in neurotransmission²⁴. In contrast, a high levels of nitric oxide for long period may cause death of nerve cell. This is established in cases of inflammation, infection, ischemia and trauma as microglia is activated by reactive oxygen intermediates and inflammatory mediators. This stimulate the release of exaggerated amounts of nitric oxide through nitric oxide synthase (iNOS)²⁵. Studies indicated that not only induction of eNOS mRNA but also iNOS in brain by LPS treatment²⁶. NO motivate neurotoxicity



1) a rat (control Vit. C) shows normal cerebral cortex tissue, 2) rat (received LPS + 50 mg Vit. C) shows slight decrease in small dark neurons, 3) rat (received LPS + 100 mg Vit. C) shows only a few cells with signs of degeneration (arrow). 4) rat (received LPS + 100 mg co δ10 + 50 mg Vit. C) shows some neurons with signs of degeneration are still observed. 5) rat (received LPS + 200 mg co δ10 + 100 mg Vit. C) shows normalization of cerebral cortex tissue.

via suppression of neuronal respiration leading to glutamate release and then excitotoxicity²⁷. It inreacts with the superoxide anion producing more reactive peroxy nitrite anion (ONOO⁻), that is able to oxidize proteins, lipids, and DNA²⁸.

In our work, the bustle of paraoxonase was reduced in brain of LPS-treated rat. The (PON1) hydrolyzes the lethal metabolites of many organophosphorus pesticides. paraoxonase enzyme is formed mainly by liver cells and secreted to the plasma. It prevents oxidation of high density lipoproteins^{29,30,31}. Also PON1 conserves cellular membranes against lipid peroxidation³². The decrease in its activity has been recommended to inhance the neurodegenerative diseases, such as, Parkinsonism³³.

Our results demonstrated an inhibition of Brain-derived neurotrophic factor (BDNF) level, that acts on specific neurons of the peripheral and central nervous system³⁴. It was confirmed that the administration of LPS lowered BDNF mRNA expression levels in the hippocampus of rats³⁵. Also previous researches showed that BDNF can protect the cells against oxidative-mediated damage indicating that this factor may have antioxidant properties^{36,37}. Although Zhu *et al.*, (2014) found no change in levels of BDNF expression following LPS administration³⁸.

In our model, LPS results in an increase of brain Amyloid- β (A β), this in agreement with Zhu *et al.*, (2014). Increased expression of A β in the hippocampus was spotted following the chronic administration of LPS. This indicating that the enhanced expression of A β may be a major factor in the pathogenesis of cognitive dysfunction³⁸.

Oral pretreatment with CoQ10 for 7 days before endotoxemia produced significant brain protection as evidenced by reducing MDA content to the basal level, decreasing NO production, and A β .while there was significant increase of GSH, PON-1 and BDNF compared to control group .The neuroprotection observed in our model by CoQ10 may be refer to the fact that CoQ10 is found to be an essential component of the electron transport chain where it acts as an electron donor and acceptor. It has also been suggested that it could bridge a defect in the electron transport chain, improve membrane fluidity, and act as an antioxidant.³⁹ Matthews *et al.*, (1998) showed that oral administration of CoQ10 increases its

mitochondrial concentrations in the cerebral cortex and provided further evidence that CoQ10 can produce neuroprotective effects that might be valuable in the treatment of neurodegenerative diseases.⁴⁰

In this study the effect of both CoQ10 & vit.C administration to the LPS treated rats can alleviate the effect against LPS-induced oxidative stress in brain tissue .Concerning CoQ10 this in agreement with Song *et al.* (2017), they suggest the beneficial effect of CoQ10 on antioxidant defense system in laboratory animals under severe oxidative stress conditions⁴¹. Also Kantar *et al.*, (2005) reported the importance of vit.C as antioxidant for protection against degenerative process caused by oxidative stress⁴².

In this study, the impact of oral pretreatment of vitamin C, with dose of 100mg/kg for 7 days on brain tissue result in inhibition of MAD & NO, at the same time increased the level of GSH, this agree with Halliwell *et al.*, (1987)⁴³. D'Uscio *et al.*, (2003) said that vitamin C scavenges the ROS via very rapid electron transfer that inhibit lipid peroxidation⁴⁴. There was increase of PON-1 level which was agree with Zargari and Saeedy (2017)⁴⁵. Also there was a decrease in A β that was in agreement with Kook *et al.*, (2014). Vitamin C supplementation results in reduction of amyloid plaque deposition, BBB (blood brain barrier) disruptions and mitochondrial dysfunction in the brains of 5XFAD mice, an animal model for Alzheimer's disease (AD).⁴⁶ There was increase of BDNF concentration, although in study of Rai *et al.*, (2013) vitamin C with dose 200mg/kg resulted in diminished BDNF expression in rats that have not received stress suggesting that vitamin C acted as pro-oxidant linking the connectivity between the oxidative damage and BDNF expression⁴⁷.

The potential effect of CoQ10 and vitamin C when adding together with doses of 200 and 100 mg/kg respectively result in modulating oxidative stress in endotoxemic brain tissue generated by restrained stress may determine their clinical usefulness as supplemental nutritional therapeutic agent in disorders affecting the brain free radical metabolism .This was consistent with the result of histopathology, who found that these concentrations more effective than other (100 and 50mg/kg). As vitamin C and CoQ10 are reported to act as an effective antioxidants of major importance

for protection against diseases and degenerative processes caused by oxidative stress.

The histopathological results proved that LPS caused damage to brain tissue. Such results are emphasized by pang *et al.*, 2003 who reported that intracerebral delivery of lipopolysaccharide (LPS) preferentially induces white matter damage, loss of immunoreactivity of immature OL markers, hypomyelination and increased size of lateral ventricles⁴⁸.

In conclusion, CoQ10 treatment alone or in combination with vitamin C may be beneficial in preventing endotoxin-induced oxidative damage in brain, and therefore show potential for clinical use.

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