

Effects of Vitamin E and Q10 Supplementation against Doxorubicin-Induced Neurotoxicity in Rats

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Abstract

Doxorubicin (DOX) is a chemotherapeutic agent; it is widely used in human malignancies. Its long-term use can cause neurobiological side-effects. Vitamin E and Coenzyme Q10 may possess neuroprotective effects. This work was designed to investigate the effect of vitamin E and the coenzyme Q10 (CoQ10) supplementation on neurotoxicity induced by doxorubicin (DOX) in rats. Forty nine adult rats of both sexes were used in this study; the animals were randomly enrolled into seven groups of 7 rats each. Group I: negative control (rats administered corn oil); Group II: Vitamin E at a dose of 100mg/kg/d for 3 weeks ; Group III: CoQ10 at a dose of 50 mg/kg/d for 3 weeks; Group IV: positive control (Doxorubicin 2.5 mg/kg) every other day for 2 weeks; Group V: vitamin E at a dose of 100mg/kg/d for 3 weeks administered prior to Doxorubicin at dose 2.5 mg/kg every other day for 2 weeks; Group VI: CoQ10 at a dose of 50 mg/kg/d for 3 weeks administered prior to Doxorubicin at dose 2.5 mg/kg every other day for 2 weeks. Group VII: CoQ10 (50mg/kg/day), Vitamin E (100mg/kg) for 3 weeks administered prior to Doxorubicin at dose 2.5 mg/kg every other day for 2 weeks. On day twenty two of the study, brain of each animal was excised and part of it to be utilized to prepare homogenate for estimation interleukin-1 beta (IL-1 β), and interleukin-10 (IL-10); the other part of brain was used for histological examination. Vitamin E and CoQ10 significantly ($P<0.05$) decreased IL-1 β , and only combination vitamin E and CoQ10 significantly ($P<0.05$) increased IL-10 and there was an improvement in the histopathological lesions of the brain in group V, group VI and group VII compared to group IV. In conclusion both Vitamin E and CoQ10 may have protective effect against DOX-induced neurotoxicity in rats.

Keywords: Vitamin E, CoQ10, Doxorubicin, Neurotoxicity, Rats.

تأثير فيتامين (هـ) ومكمل Q10 على سمية الدماغ المستحثة بواسطة الدوكسوروبيسين في الجرذان

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الخلاصة

كان الهدف من هذه الدراسة هو تقييم تأثير فيتامين (هـ) ومكمل Q10 على السمية العصبية الناجم عن الدوكسوروبيسين في الجرذان. تم استخدام 49 جرذاً بالغاً من كلا الجنسين حيث قسمت الحيوانات بصورة عشوائية الى سبع مجموعات وفي كل مجموعة 7 حيوانات. المجموعة الاولى: مجموعة المراقبة السلبية اعطيت زيت الذرة؛ المجموعة الثانية: 100 اعطيت ملغم/كغم من فيتامين (هـ) عن طريق الفم مرة واحدة يومياً لمدة واحد وعشرون يوماً متتالياً؛ المجموعة الثالثة: اعطيت 50 ملغم/كغم من مكمل Q10 عن طريق الفم مرة واحد وعشرون يوماً لمدة أحد عشر يوماً متتالياً؛ المجموعة الرابعة: السيطرة الايجابية (2,5 ملغم/كغم من عقار الدوكسوروبيسين داخل الصفاق) سبع جرعات كل يومين لمدة أسبوعين المجموعة الخامسة: اعطيت 100 ملغم/كغم من فيتامين(هـ) عن طريق الفم مرة واحدة يومياً لمدة واحد وعشرون يوماً متتالياً قبل 2,5 ملغم/كغم من عقار الدوكسوروبيسين داخل الصفاق كل يومين لمدة أسبوعين؛ المجموعة السادسة: اعطيت 50 ملغم/كغم من مكمل Q10 عن طريق الفم مرة واحدة يومياً لمدة واحد وعشرون يوماً متتالياً قبل 2,5 ملغم/كغم من عقار الدوكسوروبيسين داخل الصفاق كل يومين لمدة أسبوعين، المجموعة السابعة: اعطيت 100 ملغم/كغم من فيتامين(هـ) و 50 ملغم/كغم من مكمل Q10 عن طريق الفم مرة واحدة يومياً لمدة واحد وعشرون يوماً متتالياً قبل 2,5 ملغم/كغم من عقار الدوكسوروبيسين داخل الصفاق كل يومين لمدة أسبوعين. في اليوم الثاني والعشرين من الدراسة. بعد التشريح واستخراج الدماغ. جزء من الدماغ استخدم لإعداد جناسه النسيج لتقدير مستويات الانترلوكين بيتا والانترلوكين-10 والجزء الاخر من الدماغ كل حيوان استخدم للفحص النسيجي. بينت النتائج فيتامين(هـ) ومكمل Q10 خفضا بشكل ملحوظ الانترلوكين بيتا ($P<0.05$) وزيادة الانترلوكين-10 ($P<0.05$) وكان هناك تحسن في النقطيع النسيجي للدماغ في المجموعة الخامسة، المجموعة السادسة والمجموعة السابعة مقارنة بالمجموعة الرابعة. في الختام، قد يكون لكل من فيتامين هـ ومكمل Q10 تأثير وقائي ضد السمية العصبية التي يسببها عقار الدوكسوروبيسين في الجرذان.

الكلمات المفتاحية: فيتامين هـ، مكمل Q10، دوكسوروبيسين، السمية العصبية، جرذان.

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Introduction

Doxorubicin (DOX) is a quinine containing anticancer and antibiotic that is used to treat various solid malignancies and lymphomas ⁽¹⁾. Anthracyclines, including doxorubicin (DOX), are the most efficacious anti-cancer drugs available; their use has extended less than ten decades despite numerous adverse effects ⁽²⁾; chemotherapeutic agents, including DOX, are responsible for brain tissue injury.

The neurobehavioral changes-induced by DOX have been indicated by authors via the utilization of rodent models ⁽³⁾.

Nowadays, it is well-documented that the central nervous system (CNS) and the immune system (IS) are intimately linked through bidirectional chemical messengers ⁽⁴⁾. Importantly, it has been implicated in neurodegenerative diseases that accompany CNS injuries ⁽⁵⁾. Authors reported that patients undergoing chemotherapy for breast cancer have consistently shown their depressed mood and decreased interest in surroundings ⁽⁶⁾.

Vitamin E (α -tocopherol and its derivatives) is a predominant chain breaking lipid-soluble antioxidant and is believed to be the primary free radical scavenger and prevent lipid peroxidation ⁽⁷⁾. Hadi N, Yousif N, Al-amran F, et al 2012 approved that MDA contents was significantly reduced after vitamin E treatment this can be explained by that, vitamin E allows free radical to abstract hydrogen atom from antioxidant molecule rather than from poly unsaturated fatty acid thus breaking chain of free radical ⁽⁸⁾.

Authors reported that cell death and many neurological deficits can be reduced by antioxidants. Coenzyme Q10 is considered as a neuroprotective agent that can prevent the cascade of cell death events in order to maintain cellular integration and restore neuronal function. CoQ10 plays a critical role as an intrinsic free radical-scavenging and antioxidant enzyme that acts against the H₂O₂-induced oxidative damage in cells ⁽⁹⁾. It directly, inhibits biomolecule oxidation and affects antioxidants in vivo although its structural characteristic allows it to diffuse into the membrane phospholipids bilayer ⁽¹⁰⁾. Furthermore, CoQ10 is a key component of the mitochondrial respiratory chain, and it has a fundamental role in oxidative phosphorylation ⁽¹¹⁾.

The aim of this study is to evaluate the effect of vitamin E and CoQ10 on DOX-induced neurotoxicity in rats.

Materials and Methods

Experimental animals

Forty nine adult albino rats of both sexes, three months old, weighing 160-250gm were used in this study; they were obtained from and maintained in the Animal House of the College of Pharmacy, Baghdad University under conditions of controlled temperature. The animals were fed commercial pellets and tap water ad libitum throughout the experiment period. The study was approved by the Scientific- and the Ethical- Committees of the College of Pharmacy/ University of Baghdad.

Drugs

Doxorubicin as hydrochloride (50 mg vial) was purchased from EBEWE Pharma, AUSTRIA. Vitamin E (soft gelatin capsule 400mg) was purchased from Geltec Private Limited, India, and CoQ10 (soft gelatin capsule 30mg) was purchased from Basic nutrition, United Kingdom.

Experimental protocol

The healthy rats were randomly divided into seven groups (7animals/group) as follows: Group I- Rats orally administration corn oil (1 ml/kg BW/day) alone for 3 weeks. This group served as control ⁽¹²⁾.

Group II- Rats orally administered vitamin E alone at a dose of 100mg/kg/day for 3 weeks ⁽¹³⁾.

Group III- Rats orally administered Q10 at dose of 50mg/kg/day alone for 3 weeks ⁽¹⁰⁾.

Group IV- Rats IP injected with doxorubicin (DOX) every other day at a dose of 2.5 mg/kg for 2 weeks ⁽¹⁴⁾.

Group V- Rats orally administered 100mg/kg/day vitamin E for 7 days before starting DOX injection and continued for 2 weeks; where, it administered 1hr prior to doxorubicin IP injected every other day at a dose of 2.5 mg/kg ^(13, 14).

Group VI- Rats orally administered 50mg/kg/day CoQ10 for 7 days before starting DOX injection and continued for 2 weeks; where, it administered 1hr prior to doxorubicin IP injected every other day at a dose of 2.5 mg/kg ^(10, 14).

Group VII- Rats orally administered 50mg/kg/day CoQ10 and 100mg/kg/day vitamin E for 7 days before starting DOX injection and continued for 2 weeks; where, they orally administered 1hr prior to

doxorubicin IP injected every other day at a dose of 2.5 mg/kg^(10,13,14).

Twenty-four hour after the end of the treatment duration, each animal was euthanized by diethyl ether. After that, the skull of each animal was broke by surgical scissor then the brain was excised for homogenate preparation and histological examination.

Preparation and Estimation of homogenate biochemical parameters:

The preparation of the brain tissue homogenate involved removal of excess blood by rinsing in ice-cold phosphate buffer saline (PBS) (pH=7.4), followed by desiccation using filter paper and then measurement the weight of each brain tissue before homogenization was performed. Then each of rats' brain tissue minced to small pieces and put in 15ml plastic test tube containing chilled PBS solution (pH=7.4); where, (tissue weight (g): PBS volume (mL) = 1:9). Homogenization was performed by means of cell lab homogenizer in icy condition. After that, the homogenate was centrifuged for approximately 20 minutes at 3000×g. The supernatant was carefully collected and stored at -20 °C until the time for the determination of interleukin-1beta (IL1β), and interleukin-10 (IL10)].

Homogenate of brain tissue samples were used for the estimation of cytokines [interlukin-1 beta (IL-1β), and interlukin-10 (IL-10)] levels by automated biochemistry analyzer (Elabsience, USA).

Histological examination

After necropsy, brain of each rat was removed and part of it was used for histopathological examination according to the routine method⁽¹⁵⁾ utilizing paraffin sections technique; the fragments were fixed in 10% formaldehyde solution, embedded in paraffin, segmented, and then stained with haematoxyline/eosin. Morphological examination of the samples was studied using light microscopy.

Statistical Analysis

Data were expressed as the mean values, mean± standard error of the mean (SEM). Unpaired Student t-test was used for testing the significant difference between two groups. The statistical significance of the differences among various groups was determined by one-way analysis of variance (ANOVA). Differences were considered statistically significant for P-value less than 0.05.

Results

Effect of Vitamin E and CoQ10 against DOX on interleukin-1beta (IL-1β) in rats' brain tissue homogenate. Table 1 showed that, there were non-significant differences (P>0.05) in levels of IL-1β in brain tissue homogenate in group of rats orally administered vitamin E (100mg/kg/day alone for 3 weeks) (Group II) compared to control group (Group I). Mean ± SEM of IL-1β levels in brain tissue homogenate were respectively, 54.2±5.8 vs. 49.5±4.2. Similarly, there were non-significant differences (P>0.05) in IL-1β levels in brain tissue homogenate in group of rats orally administered CoQ10 (50mg/kg/day alone for 3 weeks) (Group III) compared to control group (Group I). Mean± SEM of IL-1β level in brain tissue homogenate were respectively, 51.8±4.4 vs. 49.5±4.2.

Furthermore, rats IP injected with DOX every other day at a dose of 2.5 mg/kg for 2 weeks (Group IV) produced significant elevation (P<0.05) in levels of IL-1β in brain tissue homogenate compared to control group (Group I). Mean ± SEM of IL-1β level in brain tissue homogenate were respectively, 175.1±15.2 vs. 49.5±4.2. Table 1.

Moreover, there were significant reduction (P<0.05) in IL-1β levels in brain tissue homogenate in groups of rats treated with either -100mg/kg vit E prior to 2.5mg/kg of DOX (Group V), -50mg/kg CoQ10 prior to 2.5 mg/kg of DOX (Group VI), or -100mg/kg of vit E and 50mg/kg of Q10 prior to 2.5 mg/kg of DOX (Group VII) compared to group of rats IP injected with DOX every other day at a dose of 2.5 mg/kg for 2 weeks (Group IV). Mean±SEM of IL-1β level in brain tissue homogenate were respectively, 100.2±4.3 vs. 175.1±15.2, 110.2±7.9 vs. 175.1±15.2, and 108.5±10.8 vs. 175.1±15.2. Table 1.

Furthermore, table 1, showed that there were non-significant differences (P>0.05) in levels of IL-1β in brain tissue homogenate among groups of rats treated with 100mg/kg/d vit E prior to 2.5mg/kg of DOX (Group V), (50mg/kg CoQ10 prior to 2.5 mg/kg of DOX (Group VI), and (100mg/kg of vit E and 50mg/kg of CoQ10 prior to 2.5 mg/kg of DOX) (Group VII) compared among each other's.

Effect of Vitamin E and CoQ10 against DOX on interleukin-10 (IL-10) in rats' brain tissue homogenate.

Table 1 showed that there were non-significant differences (P>0.05) in IL-10 levels

in brain tissue homogenate in group of rats orally administered vitamin E (100mg/kg/day alone for 3 weeks) (Group II) compared to control group (Group I). Mean \pm SEM of IL-10 levels in brain tissue homogenate were respectively, 325 \pm 11.4 vs. 311.7 \pm 11.9. Likewise, there were non-significant differences ($P>0.05$) in levels of IL-10 in brain tissue homogenate in group of rats orally administered CoQ10 (50mg/kg/day alone for 3 weeks) (Group III) compared to control group (Group I). Mean \pm SEM of IL-10 levels in brain tissue homogenate were respectively, 334.2 \pm 13.2 vs. 311.7 \pm 11.9. Table 1.

Moreover, rats IP injected with DOX every other day at a dose of 2.5 mg/kg for 2 weeks (Group IV) produced significant reduction ($P<0.05$) in the IL-10 level in brain tissue homogenate compared to control group (Group I). Mean \pm SEM of IL-10 levels in brain tissue homogenate were respectively, 53.5 \pm 4.4 vs. 311.7 \pm 11.9 Table 1.

Furthermore, there were non-significant differences ($P>0.05$) in IL-10 levels in brain

tissue homogenate in groups of rats treated with either -100mg/kg vit E prior to 2.5mg/kg of DOX (Group V), or -50mg/kg Q10 prior to 2.5 mg/kg of DOX (Group VI), compared to group of rats IP injected with DOX every other day at a dose of 2.5 mg/kg for 2 weeks (Group IV). But, there were significant elevation ($P<0.05$) in IL-10 levels in brain tissue homogenate in groups of rats treated with-100mg/kg of vit E and 50mg/kg of CoQ10 prior to 2.5 mg/kg of DOX (Group VII) compared to group of rats IP injected with DOX every other day at a dose of 2.5 mg/kg for 2 weeks (Group IV).

Table 1 Moreover, table 1 showed there were non-significant differences ($P>0.05$) in levels of IL-10 in brain tissue homogenate among groups of rats treated with 100mg/kg vit E prior to 2.5mg/kg of DOX (Group V), (50mg/kg Q10 prior to 2.5 mg/kg of DOX (Group VI), and (100mg/kg of vit E and 50mg/kg of CoQ10 prior to 2.5 mg/kg of DOX) (Group VII) compared among each other's.

Table 1. Effect of Coenzyme Q10 and Vitamin E each alone and in combination on interleukine-1Beta (IL-1 β) and interleukine-10 (IL-10) levels in brain tissue homogenate after intraperitoneal injection of doxorubicin (DOX) in rats.

Treatment group n=7	Type of treatment	IL-1Beta (pg/ml) for homogenate of rat brain (Mean \pm SEM)	IL-10 (pg/ml) for homogenate of rat brain (Mean \pm SEM)
I	negative control/corn oil	49.5 \pm 4.2	311.7 \pm 11.9
II	vit E 100mg/kg	54.2 \pm 5.8	325 \pm 11.4
III	Q10 50mg/kg	51.8 \pm 4.4	334.2 \pm 13.2
IV	positive Control / Dox 2.5 mg/kg)	175.1 \pm 15.2 ^{*a}	53.5 \pm 4.4 ^{*a}
V	100mg/kg vit E prior to 2.5mg/kg of Dox	100.2 \pm 4.3 ^{ba}	74.5 \pm 4.3 ^{aa}
VI	50mg/kg Q10 prior to 2.5 mg/kg of Dox	110.2 \pm 7.9 ^{ca}	84.2 \pm 8.7 ^{aa}
VII	100mg/kg of vit E and 50mg/kg of Q10 prior to 2.5 mg/kg of Dox	108.5 \pm 10.8 ^{da}	104.2 \pm 15.3 ^{ba}

Each value represents mean \pm standard error of means (SEM).

Group I: control (corn oil); **Group II:** Vitamin E (100mg/kg/day); **Group III:** Coenzyme Q10 (50mg/kg/day); **Group IV:** Doxorubicin (2.5mg/kg); **Group V:** Vitamin E (100mg/kg/day) prior to Doxorubicin (2.5mg/kg); **Group VI:** Coenzyme Q10 (50mg/kg) prior to Doxorubicin (2.5mg/kg); **Group VII:** Vitamin E (100mg/kg) and coenzyme Q10 (50mg/kg) prior to Doxorubicin (2.5mg/kg).

- ^{*} = Significantly different ($P<0.05$) with respect to the control group.

- Values with non-identical small letters superscripts (a, b, c and d) are significantly different ($P<0.05$) using unpaired Student t-test.

- Values with non-identical capital letter superscripts (A) are non-significantly different ($P<0.05$) among (V, VI and VII) groups using ANOVA.

Histopathological examination of rats' brain tissue

Rats orally administered corn oil at a dose of 1ml/kg (group I), 100mg/kg of vit E (group II) and 50mg/kg CoQ10 (group III) each for 3 weeks showed normal brain section, pyramidal cell showed

open face nuclei and basophilic cytoplasm, smaller neurological cell, and blood capillary were scattered between neuron. (Figure 1,a,b,c respectively).

Histopathological changes in animals brain injected with IP dose (2.5mg/kg) of DOX characterized by neuronal degeneration and with frequent nuclear pyknosis ,Irregular darkly stained cells with pyknotic nuclei and surrounded with halos arrows were prominent (figure 1-d) compared with brain section of rats administered corn oil (group I, negative controls), group II (100mg/Kg of vit E) and group III 50mg/kg CoQ10; where normal appearance were observed in rats' brain section .

Sections of rats' brain orally administered 100mg/kg BW of vit E for 3 weeks prior to IP DOX every other day for 2 weeks at a dose of (2.5 mg/kg BW) (group V) showed congestion of blood vessels, and darkly stained nucleus surrounded with halo while other section of rats' brain of the intended group showed normal brain architecture, there weren't neurodegenerative, and hyper cellularity (figure 1-e).

Additionally, brain section of animals orally administered CoQ10 at a dose of 50mg/kg BW for 3 weeks prior IP injection of DOX every other day for 2 weeks at dose (2.5 mg/kg BW) (group VI) revealed congestion of blood vessels and, darkly stained nucleus surrounded with halo while other section of rats' brain of the intended group showed normal brain architecture, there weren't neurodegenerative, and hyper cellularity (figure1-f).

As well as, brain section of animals orally administered combination of CoQ10 and vit E at a dose of 50mg/kg and 100mg/kg BW, respectively for 3 weeks prior IP injection of DOX every other day for 2 weeks at a dose (2.5 mg/kg BW) (group VII) showed congestion of blood vessels, and darkly stained nucleus surrounded with halo; while other section of rats' brain of the intended group showed normal brain architecture; there weren't neurodegenerative, and hyper cellularity (figure 1-g).

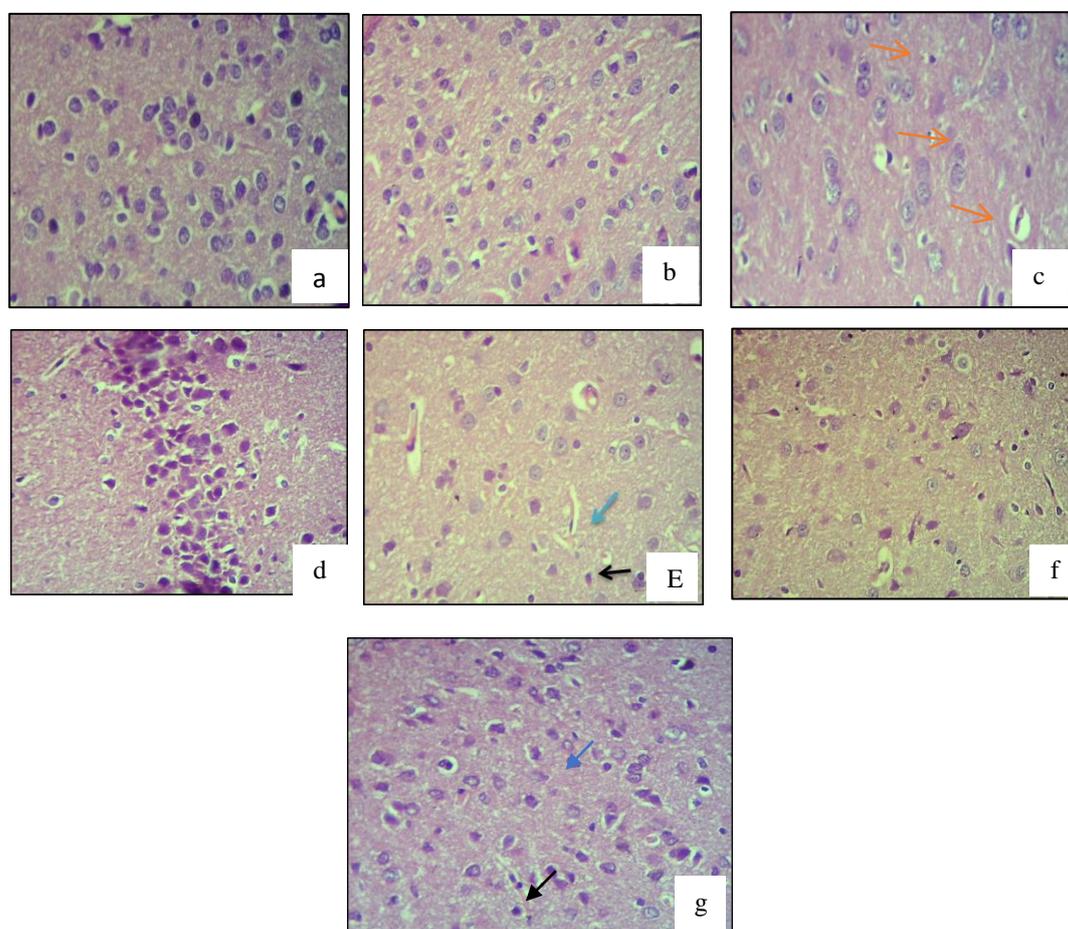


Figure 1. Histopathological section of brain in various experimental rats' groups; (haematoxyline and eosin; X40). a: group I (negative control (corn oil));b: group II (vit E 100mg/kg);c: group III (Q10 50 mg/kg);d: group IV (positive control (Dox 2.5 mg/kg));e: group V (vit E100mg/kg prior to a IP dose of Dox 2.5 mg/kg);f: group VI (Q10 50mg/kg prior to IP dose of Dox 2.5 mg/kg);g: group VII(vit E 100mg/kg, and Q10 50mg/kg prior to IP dose of DOX 2.5mg/kg)Negative control group, group II, and group III showed normal brain section. Positive control group characterized by shows marked neuronal degeneration with frequent nuclear pyknosis , and Irregular darkly stained cells with pyknotic nuclei and surrounded with halos arrows were prominent (red arrow) The lesion in groups V,VI,VII revealed mild Congestion of blood vessels (black arrow) with some appear with darkly stained nucleus surrounded with halo(blue arrow)

Discussion

Toxicity is the major factor hindering DOX treatment. The impairment of neurogenesis and increased neural apoptosis in the limbic brain regions, including the prefrontal cortex and hippocampus, is considered as one of the leading causes of depression. It was reported that DOX-mediated generation of free radicals in the brain tissues increases lipid peroxidation and protein oxidation, and alters the antioxidant defense system, eventually leading to neuropsychological changes^(16, 17). Moreover, increased generation of superoxide anions induced by DOX may elevate the level of circulating tumor necrosis factor-alpha (TNF- α) which can directly pass blood brain barrier (BBB), and activate glial cells to initiate the local production of pro-inflammatory cytokines which exacerbate the oxidative stress and neural apoptosis⁽¹⁸⁾. In addition, many inflammatory mediators such as TNF- α and nuclear factor-kappa B (NF- κ B) have been shown to be critically involved in neuroinflammation both in animal models and in patients undergoing chemotherapy⁽¹⁹⁾.

The current study revealed that DOX produced significant ($P < 0.05$) elevation in IL-1 β level in brain tissue homogenate compared to negative control group; the results are in agreement with the study of others⁽²⁰⁾. Pro-inflammatory cytokines such as IL-1 β , IL-6, TNF- α , NF- κ B, and iNOS have been demonstrated to induce abnormal behaviors, such as decreased locomotor activity, exploration, and depression⁽²¹⁾. Furthermore, DOX provoked generation of TNF- α and subsequently caused the activation of NF- κ B and iNOS and increased the expression of genes required to control infection and injury, such as IL-1 β and IL-6, indicating severe inflammatory conditions in the brain⁽²²⁾.

It has been reported that IL-10, a pleiotropic cytokine, is endogenously produced by activated immune cells including T cells, B cells and macrophages⁽²³⁾. It mainly drives a regulation of a variety of anti-inflammatory processes⁽²⁴⁾. In the brain, IL-10 is expressed by monocytes, astrocytes and microglia⁽²⁵⁾ as well as by neurons. Authors found that IL-10 expression was down regulated in the substantia nigra of patients with Parkinson's disease (PD)⁽²⁶⁾.

Moreover, researchers reported that osmotic pump infusion of IL-10 into the substantia nigra can protect against lipopolysaccharide (LPS)-induced cell death of dopaminergic neurons, with a corresponding reduction in the number of activated microglia, suggesting that the reduction in microglia-

mediated release of inflammatory mediators may contribute to the anti-inflammatory effect of IL-10. However, other researchers established that IL-10 reduced LPS-induced neuronal loss in either the presence or the absence of glial cells; and demonstrated that IL-10 inhibited LPS-induced glial activation by down regulation of pro-inflammatory mediators and up regulation of neurotrophic factors. A potential therapeutic strategy for PD is to limit development to inflammatory response⁽²⁷⁾; this is in agreement with this study; where, DOX significantly ($P < 0.05$) reduced level IL-10 (group IV) compared to group control (group I). In the current study, brain sections of DOX-treated animals under light microscope showed neuronal degeneration with frequent nuclear pyknosis, irregular darkly-stained cells with pyknotic nuclei that are surrounded with halos arrows were prominent in figure 3-18d; these findings are coinciding with the works of Mohamed, Karam and Amer, 2011⁽¹⁴⁾.

Furthermore, in the present study, vitamin E and CoQ10 administered at a dose of 100mg/kg and 50mg/kg, respectively prior to IP dose of DOX 2.5mg/kg (groups V, and VI), significantly ($P < 0.05$) lowered level of IL-1 β compared to positive control group (DOX-treated rats). Also combination of vit E and CoQ10 (group VII) significantly reduced IL-1 β . The results of this study are in line with those of others that demonstrated the dual effects of vitamin E on oxidative damage and proinflammatory cytokine (IL-1 β , IL-6 and TNF- α) production, resulting in an effective anti-inflammatory response⁽²⁸⁾. It further provided evidence of the anti-inflammatory role of vitamin E⁽²⁹⁾.

This study is also in parallel with other research that indicated that CoQ10 at low concentrations can block IL-1 production that in turn can affect the inflammatory mediators, PGE-2 and IL-6. PGE-2 is one of the most significant inflammatory mediators produced in the body. It is made in response to a variety of stimuli in a wide variety of cell types. Elevated PGE-2 has been associated with a wide number of inflammatory diseases including Alzheimer, stroke, and cancer,⁽³⁰⁾

At the same time, vitamin E and CoQ10 cause elevation in the level of IL-10 ($P > 0.05$) but non-statistically significant. However combination of vit E and CoQ10 significantly ($P < 0.05$) elevate level of protective IL-10 (group VII) compared to group IV; this may be due to

insufficient dose (50mg/kg) of CoQ10 to cause significant elevation in level of IL-10 according to other study that indicated that CoQ10 assisted in boosting of anti-inflammatory cytokine, IL10, which happened to be consistent with greater dose of CoQ10⁽³¹⁾.

Moreover, there were improvement of the histopathological lesions of the brain in groups V, VI, and group VII rats compared to positive control group IV. In conclusion, vit E and CoQ10 may have protective effect against DOX-induced neurotoxicity in rats as demonstrated by the evaluation of neurotoxicity biomarkers and histological examination. To the best of our knowledge, this is the first study that examines the effects of vit E and CoQ10 at doses 100mg/kg, and 50mg/kg respectively each alone and combination each prior to DOX on the brain.

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