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ORIGINAL PAPER

Biochemical and genetic alterations of oxidant/antioxidant status of the brain in rats treated with dexamethasone: protective roles of melatonin and acetyl-L-carnitine

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Abstract The current study was undertaken to investigate the protective role of melatonin (MEL) and acetyl-L-carnitine (ALC) against dexamethasone (DM)-induced neurotoxicity. Adult female rats (60) were divided into: (1) control group, (2) DM-treated group, (3) MEL-treated group, (4) ALC-treated group, (5) MEL- and DM-treated, and (6) ALC- and DM-treated group. Serum acetylcholinesterase (AchE) activity, malondialdehyde (MDA), nitric oxide (NO) level, catalase (CAT), superoxide dismutase (SOD) and glutathione-*S*-transferase (GST) activities were estimated. Gene expression of the prooxidants (NO synthases NOS-1, NOS-2 and heme oxygenases HO-1, HO-2) and antioxidant enzyme (GST-P1) as well as deoxyribonucleic acid (DNA) fragmentation analysis of brain tissue were investigated. Histological examination of the brain tissue was carried out. DM administration caused significant increase in serum AchE activity, MDA and NO levels accompanied with significant decrease in the antioxidant enzymes activity. Pretreatment with MEL or ALC prior DM has been found to reverse all the former parameters. On the genetic level, DM administration significantly increased the expression level of NOS-1, NOS-2, HO-1, and HO-2 messenger ribonucleic acids (mRNAs) and decreased that GST-P1-mRNA in brain tissue. Also, DM produced DNA fragmentation in brain tissue. Treatment with MEL or ALC prior DM administration tend to normalize the above mentioned parameters. These results were documented by the histological examination of brain tissue. The present study suggests that oxidative stress is involved in the pathogenesis of DM-induced neurotoxicity. The inhibition of oxidative stress via stimulation of the antioxidant enzymes by MEL and ALC pretreatment plays a central protective role in modulation of neurotoxicity induced by DM.

Keywords Dexamethasone (DM) · Melatonin (MEL) · Acetyl-L-carnitine (ALC) · Neurotoxicity

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Abbreviations

DM	Dexamethasone
MEL	Melatonin
ALC	Acetyl-L-carnitine
NO	Nitric oxide
MDA	Malondialdehyde

CAT Catalase
SOD Superoxide dismutase
GST Glutathione-*S*-transferase

Introduction

Glucocorticoids (GCs) are steroid hormones secreted from adrenal gland during stress. Abnormal increased levels of GCs enhanced the sensitivity of hippocampal cell for oxidative stress which is associated with atrophy in the hippocampus [14]. Thus, GCs may play a contributing role toward neuronal death and neurodegeneration [64]. Dexamethasone (DM) is the most commonly used corticosteroid because its mineralocorticoid activity is lower than that of most other steroids [62]. DM has been shown to induce apoptosis and impairs neurogenesis [24] by regulating genes involved in reactive oxygen species (ROS) generation [71]. DM induced significant decrease in hypothalamic antioxidant enzymes as catalase (CAT) and superoxide dismutase (SOD) activities [3, 16].

In mammals, the circadian system is composed of many individuals, tissue-specific, cellular clocks whose phases are synchronized by a master circadian pacemaker residing in the suprachiasmatic nuclei of the hypothalamus. The redox state has been found to be important for the molecular mechanism of the circadian clock [43]. Circadian variations of brain redox pathway enzymes have been described including nitric oxide synthase (NOS) [9], heme oxygenase (HO) [54], and glutathione-*S*-transferase P1 (GST-P1). In many cases, rhythms in enzyme activity and gene expression coincide, but in others, they are out of phase (e.g. NOS) [9].

Melatonin (MEL) is a ubiquitous biological signaling molecule that has been identified in all major taxa of organisms, different plants, invertebrates, and vertebrates. MEL has diverse physiological functions, signaling not only the time of the day, or the season of the year, but also having immunomodulatory and cytoprotective roles [51]. MEL has multiple actions as a regulator of antioxidant and prooxidant enzymes, radical scavenger and antagonist of mitochondrial radical formation. The ability of MEL and its kynuramine metabolites to interact directly with the electron transport chain (ETC) via increasing the electron flow and reducing electron leakage is unique feature by which MEL is able to increase the survival of neurons under enhanced oxidative stress [59]. Moreover, the regulation of enzymes involved in the redox pathway is one of the ways by which MEL exerts its antioxidant, cytoprotective effects in the brain. Such a regulation involves both the down-regulation of prooxidant enzymes like NOS-4 or NOS-5 and 12-lipoxygenases [66] as well as the upregulation of antioxidant enzymes like Cu/Zn-SOD and Mn-SOD [36], CAT [46], glutathione peroxidase (GPx) [10], glutathione reductase [50], or γ-glutamylcysteine synthase [69]. This action is complementary to the nonenzymatic, radical scavenger effect that MEL and some of its metabolites (*N*-acetyl-*N*-formyl-5-methoxykynuramine and *N*1-acetyl-5-methoxykynuramine) have to scavenge ROS, reactive nitrogen species, and organic radicals [63].

Acetyl-L-carnitine (ALC) is a compound acting as an intracellular carrier of acetyl groups across inner mitochondrial membranes. It also appears to have neuroprotective properties and it has been shown to reduce attention deficits in patients with Alzheimer's disease after long-term treatment [12]. ALC is effective in reducing age-related mitochondrial dysfunction and decreases oxidative damage to neurons and improves cognitive deficits [37]. Also, ALC reduces malondialdehyde and protein carbonyl levels and increases reduced GSH and SOD activity in the brains [39].

The present study was carried out to elucidate the potent role of DM in altering the oxidant/antioxidant status of the brain and to assess whether gene expression of the redox enzymes NOS-1, NOS-2, HO-1, HO-2 and antioxidant enzyme GST-P1 changes in the brain due to the treatment with DM. Also, study was extended to examine the potential damage on the deoxyribonucleic acid (DNA) as a result of DM treatment and to investigate the ameliorative effect of MEL and ALC in these issues.

Materials and methods

Experimental animals

Sixty adult female Sprague–Dawley rats (130±10 g) obtained from the Animal House Colony of the National Research Centre were enrolled in the present study. The animals were kept in plastic cages at room temperature (25±2°C) and humidity (55%) under a

12 h dark–light cycle. All animals were accommodated with laboratory conditions for at least 2 weeks before treatment and maintained under the same conditions all over the experiment. Diet and water were allowed *ad libitum*. All animals received human care in compliance with the guidelines of the Ethical Committee of Medical Research of the National Research Centre, Cairo, Egypt.

Experimental design

Animals were randomly divided into six groups (ten rats for each). The first group received saline solution orally and served as control group. The second group received DM (Amria Pharm, Egypt) in a dose (8 mg/ kg) [57] intraperitoneally (IP) daily for the first 3 weeks only. The third group was IP treated in the dark with (20 mg/kg) [2] MEL (Amoun Pharmaceutical, Egypt) three times weekly for 8 weeks. The fourth group was IP treated with (100 mg/kg) [56] ALC (MEPACO, Egypt) three times weekly for 8 weeks. The fifth group was IP treated in the dark with MEL alone three times weekly for 5 weeks and then coadministered with MEL and DM for other 3 weeks. The sixth group was IP treated with ALC alone three times weekly for 5 weeks and then coadministered with ALC and DM for other 3 weeks.

Samples collection

At the end of the experimental period, fasting blood samples were collected from retro-orbital venous plexus under diethyl ether anesthesia. Blood samples were collected in dry clean centrifuge tubes and then centrifuged at 1,800×*g* for 15 min at 4°C. Serum samples were collected and stored at −80°C in clean plastic Eppendorf tubes till analysis. The whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried, and then weighed.

Histopathological investigation

Half brain of each animal was fixed in 10% neutral buffered formalin and embedded into paraffin blocks and the histopathological examination was carried out on 5-μm-thick hematoxylin–eosin (H&E)-stained sections.

Quantitative analysis of DNA fragmentation

The second half of brain tissues was lysed in 0.5 ml of lysis buffer containing, 10 mM Tris-HCl (pH 8), 1 mM EDTA, 0.2% Triton X-100, centrifuged at 10,000 rpm (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and the supernatants (S), 0.5 ml of 25% trichloroacetic acid (TCA) was added and incubated at 4°C for 24 h. The samples were centrifuged for 20 min at 10,000 rpm (Eppendorf) at 4°C, and the pellets were suspended in 80 ml of 5% TCA, followed by incubation at 83°C for 20 min. Subsequently, to each sample 160 ml of DPA solution [150 mg DPA in 10 ml glacial acetic acid, 150 ml of sulfuric acid, and 50 ml acetaldehyde (16 mg/ml)] was added and incubated at room temperature for 24 h [15]. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

$$\%\text{Fragmented DNA} = \frac{\text{OD(S)}}{\text{OD(S)} + \text{OD(P)}} \text{ X } 100$$

Gene expression analysis

The semiquantitative RT-PCR assay was conducted to verify the gene expression of prooxidant (NOS^{-1}, NOS^{-2}, HO^{-1} and HO^{-2}) and antioxidant (GST-P1) enzymes in male rats against the exposure of different doses of DM.

RNA extraction Total ribonucleic acid (RNA) was isolated from 50 mg of brain tissue by the standard TRIzol extraction method (Invitrogen, Paisley, UK) and recovered in 100 μl molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pretreated using DNA-free™ DNase treatment and removal reagents kit (Ambion, Austin, TX, USA) following the manufacturer's protocol. The RNA concentration was determined by spectrophotometric absorption at 260 nm.

Reverse transcription The complete Poly(A) + RNA isolated from the rat brain samples was reverse transcribed into complementary DNA (cDNA) in a total volume of 20 μl using 1 μl oligo(dT) primer. The composition of the reaction mixture, termed as

master mix (MM), consisted of 50 mM $MgCl_2$, 10× reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 10 mM of each dNTP, and 50 μM of oligo (dT) [18] primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with denaturation step at 99°C for 5 min. Afterwards, the reaction tubes containing RT preparations were flash-cooled in an ice chamber until used for DNA amplification through polymerase chain reaction (PCR) [33].

Polymerase chain reaction The first strand cDNA from different rat brain samples was used as templates for RT-PCR with a pair of specific primer. The sequences of specific primer and product sizes are listed in Table 1. β-Actin was used as a housekeeping gene for normalizing messenger RNA (mRNA) levels of the target genes. The reaction mixture for RT-PCR consisted of 2.5 U of Taq Polymerase, 10 mM dNTPs, 50 mM $MgCl_2$, 10× PCR buffer (50 mM KCl; 20 mM Tris-HCl; pH 8.3; Gibco BRL, Eggenstein, Germany), and autoclaved water. Programmable temperature cycling was performed with the following cycle profile: 94°C for 1 min and then 30–40 cycles each comprising denaturation for 30 s at 94°C, annealing for 45 s at 55–60°C, and extension for 45 s at 72°C. After these cycles, the reaction tubes were kept for 5 min at 72°C and then at 4°C. The PCR products were then loaded onto 2.0% agarose gel, with PCR products derived from β-actin of the different mice samples [22].

Biochemical analysis

Serum AchE, malondialdehyde (MDA), a marker of lipid peroxidation and NO were assayed in serum according to the methods of Den Blaauwen et al. [17], Esterbauer et al. [23] and Montgomery and Dymock [45], respectively.

CAT activity in serum was estimated by the method described by Aebi [1]. SOD activity in serum was estimated using the method of Nishikimi et al. [48] and GST was estimated by the method described by Habig et al. [26].

Statistical analysis

Data were statistically analyzed according to Steel and Torrie [61] using SPSS computer program. The

Table 1 Effect of MEL and ALC treatment on serum acetylcholinesterase, MDA and NO values in DM-administered rats

	Control	DM	MEL	ALC	MEL+DM	ALC+DM
Serum acetylcholinesterase (U/l)	734.48±33.9	872.27±12.6[a] (18.76%)	724.02±28.9 (−1.42%)	735.04±33.2 (0.08%)	768.40±28.5[b] (−11.91%)	784.21±32.6[b] (−10.09%)
Serum MDA (nmol/ml)	0.83±0.02	1.24±0.04[a] (49.4%)	0.71±0.04 (−14.5%)	0.81±0.05 (−2.4%)	0.90±0.02[b] (−27.4%)	1.00±0.02[bc] (−9.3%)
Serum NO (μmol/l)	26.6±0.7	47.7±0.9[a] (79.3%)	25.7±0.5 (−3.4%)	26.2±0.7 (−1.5%)	31.9±0.7[b] (−33.1%)	34.4±0.8[bc] (−27.9%)

Each value represents the mean±SE ($n=10$)

[a] Significant difference from the control value at $P\leq 0.05$

[b] Significant difference from the DM-treated value at $P\leq 0.05$

[c] Significant difference between DM+MEL- and DM+ALC-treated groups at $P\leq 0.05$

results were presented as mean ± SE. The differences between mean values were determined by analysis of variance (ANOVA test), followed by Duncan's multiple rank test [21] using MSTAT-C computer program. Statistical significance of the relationships between variables was calculated by linear regression analysis, where $P \leq 0.05$ was considered significant.

Results

The present data showed significant increase (↑18.76%, ↑49.4% and ↑79.3%) in serum AchE, MDA, and NO values, respectively, in DM-administered group as compared with the control group. The groups treated with MEL or ALC alone showed significant changes in the serum. However, treatment with MEL or ALC prior to DM administration produced significant reduction in serum AchE (−11.91%, -10.09%), MDA (−27.4%, -9.3%), and NO (−33.1%, -27.9%) values as compared to DM-administered group (Table 1).

The results in Table 2 showed significant decrease (−21.8%, -17.3%, and −40.1%) in serum CAT, SOD, and GST activity in DM-administered group as compared to the control group. Noteworthy, the treatment with either MEL or ALC alone led to insignificant alterations in each of serum CAT, SOD, or GST activity when compared with control group. On the other hand, treatment with MEL or ALC prior to DM administration resulted in significant elevation in serum CAT, SOD, and GST activity as compared to DM-administered group.

Quantitative analysis of DNA fragmentation

The current results of the quantitative DNA fragmentation analysis for determining the potential genetic toxicity effect of DM in female rats revealed that DM was able to produce 42.4±1.9% of DNA fragmentation in brain tissues, which was highly significant ($P < 0.001$) in comparison with the control group (Fig. 1). Insignificant change was detected in DNA fragmentation rate in the brain tissue of rats administered with MEL or ALC alone as compared with the control group. However, the treatment of rats with MEL prior to DM, the rate of DNA fragmentation decreased significantly (17%; $P < 0.01$) in comparison with the DM group. The same trend was observed with ALC treatment prior to DM treatment, where the rate of

Table 2 Effect of MEL and ALC treatment on serum antioxidant enzymes activity in DM-administered rats

	Control	DM	MEL	ALC	MEL+DM	ALC+DM
Serum CAT (U/l)	2.52±0.04	1.97±0.05[a] (−21.8%)	2.59±0.05 (2.8%)	2.51±0.05 (−0.4%)	2.44±0.05[b] (23.9%)	2.35±0.07[b] (19.3%)
Serum SOD (U/ml)	0.807±0.03	0.667±0.04[a] (−17.3%)	0.880±0.03 (9.0%)	0.803±0.03 (−0.5%)	0.799±0.05[b] (19.8%)	0.769±0.06 (15.3%)
Serum GST (U/l)	370.6±1.3	222.0±0.8[a] (−40.1%)	373.9±1.1 (0.9%)	369.8±1.1 (−0.2%)	358.6±0.8[b] (61.5%)	348.8±2.1[bc] (57.1%)

Each value represents the mean±SE ($n=10$)

[a] Significant difference from the control value at $P \leq 0.05$

[b] Significant difference from the DM-treated value at $P \leq 0.05$

[c] Significant difference between DM+MEL- and DM+ALC-treated groups at $P \leq 0.05$

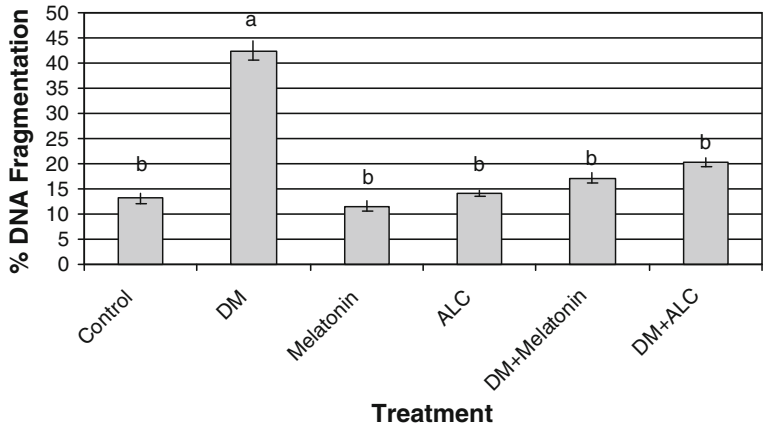

Fig. 1 Effect of melatonin and acetyl-L-carnitine against dexamethasone-induced DNA fragmentation in rat liver. [a,b]Mean values within columns with unlike superscript letters were significantly different ($P<0.05$). [b]Mean values within columns with similar superscript letters were not significantly different ($P>0.05$)

DNA fragmentation was decreased significantly (20%; $P<0.05$) compared with DM group.

Expression of the prooxidant (NOS-1, NOS-2, HO-1, and HO-2) and antioxidant (GST-P1) enzyme genes in rat brain tissues is illustrated in Figs. 2, 3, 4, 5 and 6. The results demonstrated that the expression levels of NOS-1, NOS-2, HO-1, and HO-2 mRNAs was downregulated in MEL as well as ALC treatments alone (Figs. 2, 3, 4, 5 and 6). However, MEL and ALC treatments increased the expression level of the GST-P1 in brain tissues. On the other hand, dexamethsone administration significantly ($P<0.001$) increased the expression levels of NOS-1, NOS-2, HO-1, and HO-2 mRNAs and decreased the expression level of GST-P1-mRNA in brain tissues compared with the control group (Figs. 2, 3, 4, 5 and 6). In contrary, administration MEL prior to DM significantly decreased the expression levels of NOS-1, NOS-2, HO-1, and HO-2 genes and increased the level of GST-P1-mRNA compared with DM group (Figs. 2, 3, 4, 5 and 6). Administration with ALC prior to DM significantly decreased the expression

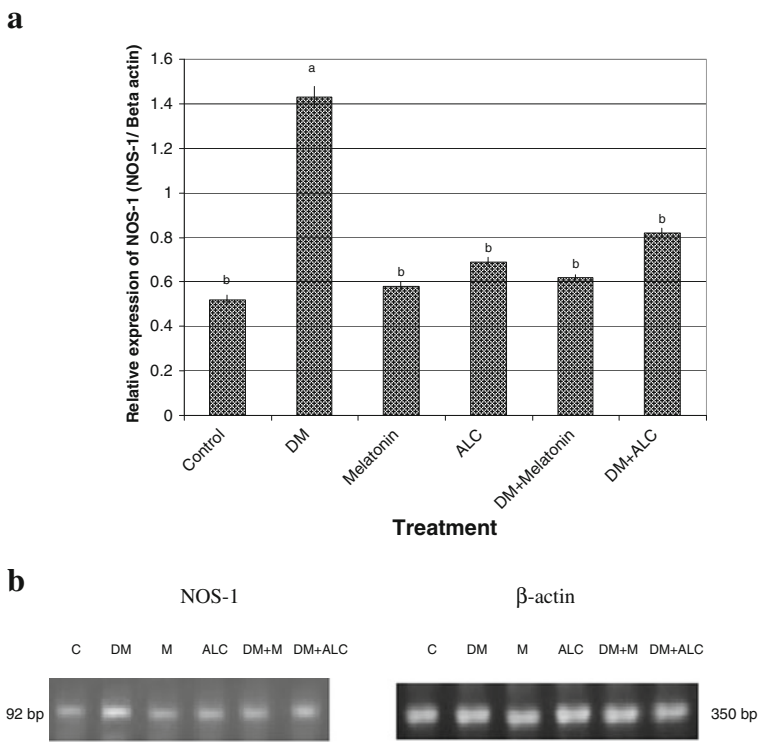

Fig. 2 Effect of melatonin and acetyl-L-carnitine against dexamethasone-induced alteration in expression level of NOS-1 gene in brain tissues of rats as determined by semiquantitative RT-PCR (**a**, **b**). The RNA recovery rate was estimated as the ratio between the intensity of NOS-1 gene and the β-actin gene. [a,b]Mean values within columns with unlike superscript letters were significantly different ($P<0.05$). [b]Mean values within columns with similar superscript letters were not significantly different ($P>0.05$). *DM* Dexamethasone, *M* melatonin, *ALC* acetyl-L-carnitine

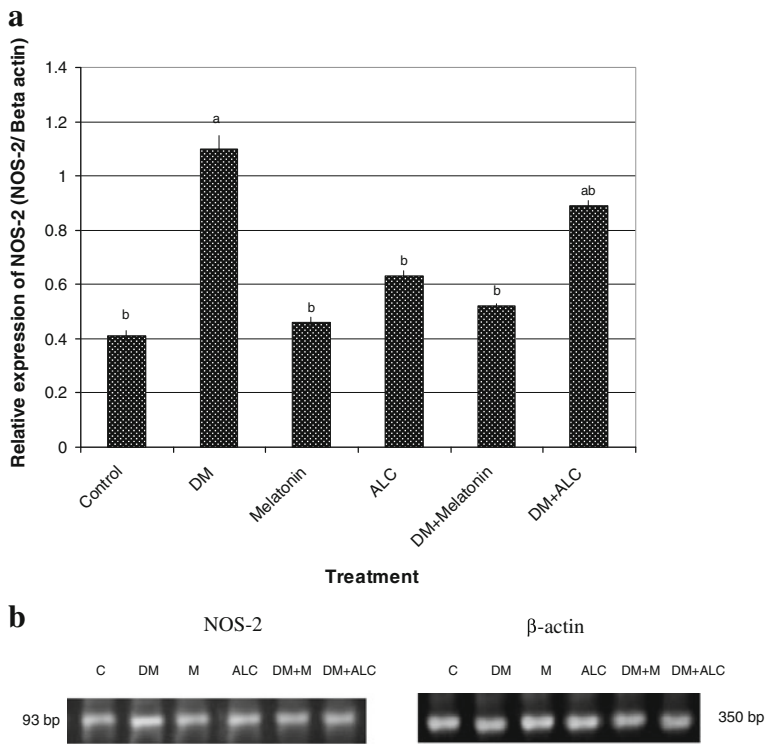

Fig. 3 Effect of melatonin and acetyl-L-carnitine against dexamethasone-induced alteration in expression level of NOS-2 gene in brain tissues of rats as determined by semiquantitative RT-PCR (**a**, **b**). The RNA recovery rate was estimated as the ratio between the intensity of NOS-2 gene and the β-actin gene. [a,b]Mean values within columns with unlike superscript letters were significantly different ($P<0.05$). [b,ab]Mean values within columns with similar superscript letters were not significantly different ($P>0.05$). *DM* Dexamethasone, *M* melatonin, *ALC* acetyl-L-carnitine

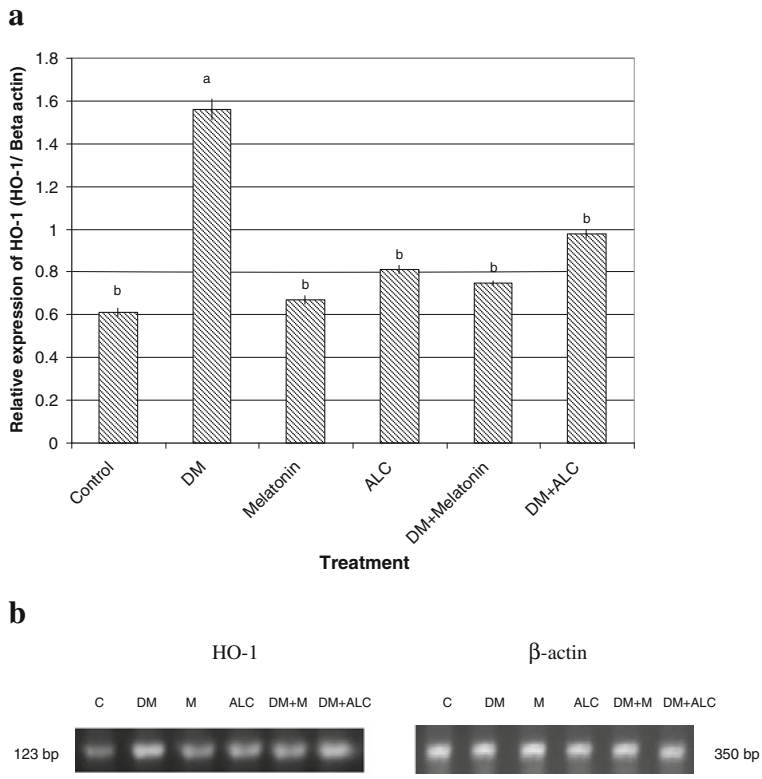

Fig. 4 Effect of melatonin and acetyl-L-carnitine against dexamethasone-induced alteration in expression level of HO-1 gene in brain tissues of rats as determined by semiquantitative RT-PCR (**a**, **b**). The RNA recovery rate was estimated as the ratio between the intensity of HO-1 gene and the β-actin gene. [a,b]Mean values within columns with unlike superscript letters were significantly different ($P<0.05$). [b]Mean values within columns with similar superscript letters were not significantly different ($P>0.05$). *DM* Dexamethasone, *M* melatonin, *ALC* acetyl-L-carnitine

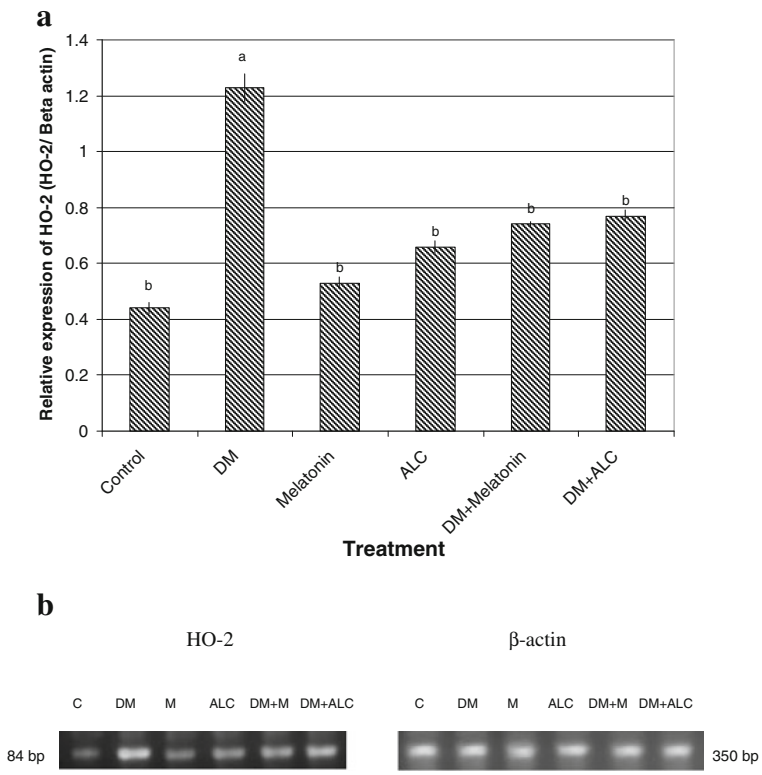

Fig. 5 Effect of melatonin and acetyl-L-carnitine against dexamethasone-induced alteration in the expression level of HO-2 gene in brain tissues of rats as determined by semiquantitative RT-PCR (**a**, **b**). The RNA recovery rate was estimated as the ratio between the intensity of HO-2 gene and the β-actin gene. [a,b]Mean values within columns with unlike superscript letters were significantly different ($P<0.05$). [b]Mean values within columns with similar superscript letters were not significantly different ($P>0.05$). *DM* Dexamethasone, *M* melatonin, *ALC* acetyl-L-carnitine

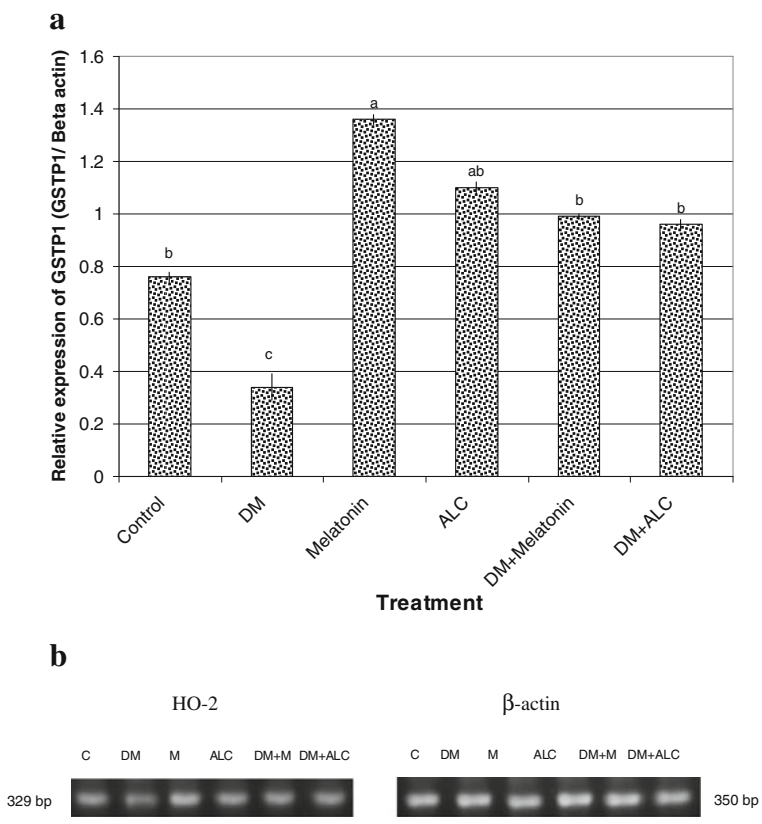

Fig. 6 Effect of melatonin and acetyl-L-carnitine against dexamethasone-induced alteration in expression level of GST-P1 gene in brain tissues of rats as determined by semiquantitative RT-PCR (**a**, **b**). The RNA recovery rate was estimated as the ratio between the intensity of GST-P1 gene and the β-actin gene. [a,b,c]Mean values within columns with unlike superscript letters were significantly different ($P<0.05$). [b,ab]Mean values within columns with similar superscript letters were not significantly different ($P>0.05$). *DM* Dexamethasone, *M* melatonin, *ALC* acetyl-L-carnitine

levels of prooxidant genes except NOS-2 gene compared with DM group (Figs. 2, 3, 4 and 5). The expression of NOS-2 gene was slightly decreased ($P > 0.05$) in the group administered with ALC prior to DM compared with DM group (Fig. 3). Also, the antioxidant GST-P1 gene was over expressed when ALC was administered prior to DM (Fig. 6).

Histological investigation

Microscopic examination of brain sections of control rats showing the highly active nerve cells that have huge nuclei relatively pale-stained. The nuclear chromatin and prominent nuclei disappeared. The surrounding relatively inactive support cells have small nuclei with densely stained condensed chromatin, and no visible nucleoli have been observed (Fig. 7). Investigation of brain sections of rat administered with DM showed the loss of the normal structure and the outlines of the cells and their nuclei. Some neurons appear like ring shape and the recently dead neurons appear dark (Fig. 8). Examination of brain sections of rat-treated MEL showed the normal structure looks like control (Fig. 9). Microscopic investigation of brain sections of rat treated with ALC showed the normal structure looks like normal sections (Fig. 10). Micrograph of brain sections of rats treated with MEL prior to DM showed neurons appeared more or less like normal (Fig. 11). Micrograph of brain sections of rats treated with ALC prior to DM showed the neurons look like normal like, but some the dark dead neurons were observed (Fig. 12).

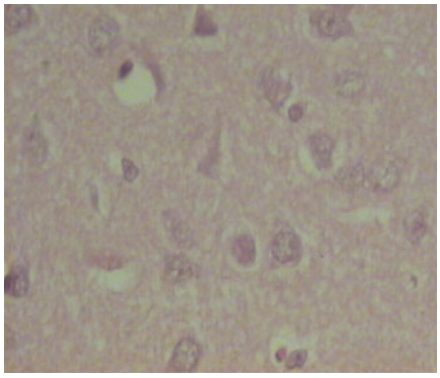

Fig. 7 Microscopic examination of brain sections of control rats showing the highly active nerve cells that have huge nuclei relatively pale-stained

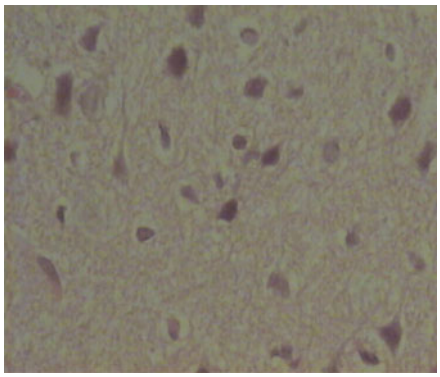

Fig. 8 Investigation of brain sections of rat administered with DM showed the loss of the normal structure and the outlines of the cells and their nuclei. Some neurons appear like ring shape and the recently dead neurons appear dark

Discussion

The current results revealed that DM administration produced significant increase in serum AchE activity, MDA, and NO levels associated with significant reduction in serum CAT, GPx, and GST activities. GCs predispose hippocampal neurons to damage during metabolic stressors. The ROS induced by DM are produced through different ways, from the mitochondrial ETC, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and xanthine oxidase in the vascular endothelium [29] or from muscle cells [44–49]. The mitochondrial ETC has been recognized as one of the major cellular generators of ROS, which include superoxide (O_2), hydrogen peroxide (H_2O_2), and the hydroxyl free radical [40]. Following this rise in H_2O_2, lipids undergo oxidation which then leads to the delayed appearance of MDA, a lipid-specific marker of oxidative stress [13–38]. It

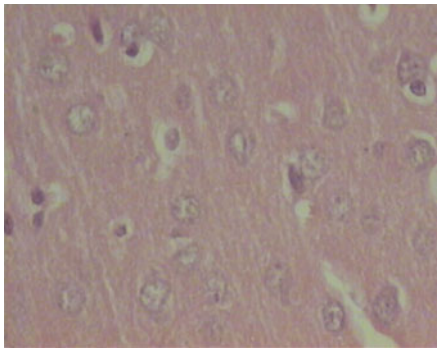

Fig. 9 Examination of brain sections of rat treated MEL showed the normal structure looks like control

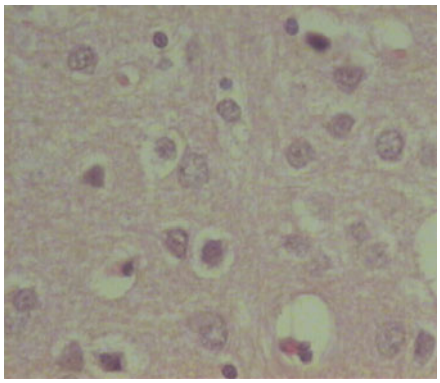

Fig. 10 Microscopic investigation of brain sections of rat treated with ALC showed the normal structure looks like normal sections

is also produced by monoamine oxidase (MAO)-A, whose expression is induced by GCs [40]. DM increased mRNA and protein expression of MAO-B in rat astrocytes [8]. GC excess enhances ROS production to cause increased production of peroxynitrite in vitro [37]. High levels of ROS deplete cellular antioxidants such as reduced GSH, which needs replenishment by energy-requiring reducing equivalents like NADPH. The reduction of GSH contents corresponds to cellular oxidative damage and death as the cellular energy is impaired [42]. GCs may alter antioxidant enzymes capacity in the brain including SOD, CAT, GPx, GST, and GSH. In the hippocampus, GCs prevents the induction of CAT and maintained the lowered GPx activity [29].

The efficacy of MEL prior to DM to inhibit the activity of serum AchE and to reduce the serum levels

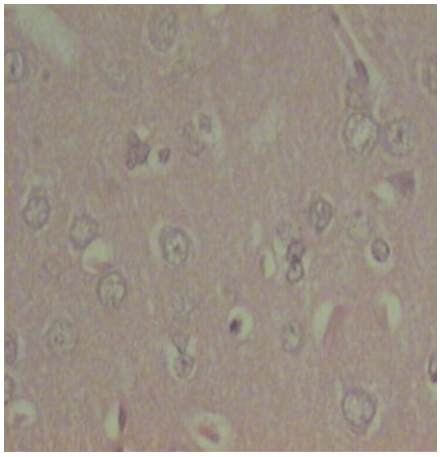

Fig. 11 Micrograph of brain sections of rats treated with MEL prior to DM showed neurons appeared more or less like normal

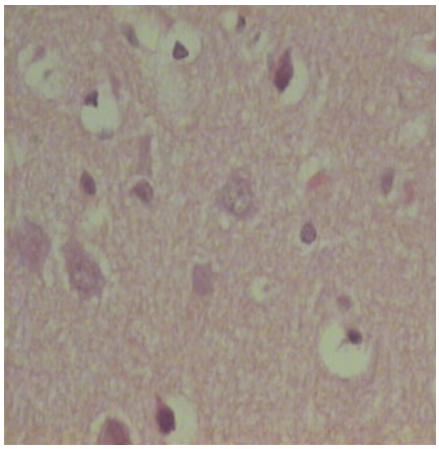

Fig. 12 Micrograph of brain sections of rats treated with ALC prior to DM showed the neurons look like normal, but some dark dead neurons were observed

of MDA and NO could be attributed to that MEL has an important role in reducing oxidative damage in the central nervous system due to the ease with which it crosses the blood–brain barrier [52]. MEL to improve cognitive functions and inhibiting AchE activity is related to its antioxidant action and anti-inflammatory activity [58]. MEL cannot only scavenge oxygen free radicals like super oxide radical (O^{-2}), hydroxyl radical (OH^{-}), peroxyl radical (LOO^{-}), and peroxynitrite anion ($ONOO^{-1}$) but can also enhance the antioxidative potential of the cell. MEL could increase the expression of mRNAs of the antioxidative enzymes [60]. Thus, it could stimulate the synthesis of antioxidative enzymes like super oxide dismutase (SOD), GPx, and also the enzymes that are involved in the synthesis of glutathione. This explains the potent role of MEL in enhancing the activity of the antioxidant enzymes in the present study.

ALC possesses unique neuroprotective, neuromodulatory, and neurotrophic properties which may play key role in counteracting various disease processes [67]. The present study showed that administration of ALC prior to DM-induced significant reduction in AchE activity, MDA, and NO levels accompanied with significant elevation in each of CAT, GPx, and GST activity. ALC has been found to increase the release of acetylcholine (ACh) in the striatum and hippocampus of rats [34–68]. L-Carnitine mediates the transfer of acetyl groups for ACh synthesis, as well as it could influence the signal transduction pathways and gene expression [25]. AChE catalyzes

the hydrolysis of Ach at cholinergic synapses, so there is a negative relationship between the AchE activity and Ach level [65].

ALC showed an antioxidant activity and antiradical capacity as it could decrease the formation of ROS [4]. L-Carnitine and its ester, ALC, facilitate the transport of long-chain free fatty acids across the mitochondrial membrane enhancing neuronal antioxidative defense [7].

L-carnitine plays a major role, as a cofactor, in the transportation of free faty acid (FFA) from cytosol to the mitochondria. FFA degrades to acyl-CoA by β-oxidation, and these substances enter the tricarboxylic acid cycle. A large amount of oxygen is consumed in this reaction and ATP is synthesized in the steps of ETC and oxidative phosphorylation. Oxygen is reduced to H_2O at the end of tricarboxylic acid cycle, thus oxygen concentration decreases resulting in the reduced ROS formation [41]. Therefore, L-carnitine attenuates oxidant injury through the inhibition of oxidative damage, mitochondria dysfunction, and ultimately inhibition of cell apoptosis [70].

L-carnitine has been found to prevent oxidative stress, regulate NO production, and enhance the activity of enzymes involved in defence against oxidative damage [25]. The antioxidant defense system is composed of mainly three enzymes: GPx, CAT, and SOD. L-Carnitine could protect these enzymes from further peroxidative damage [25].

Toxicogenomics, which uses the gene expression technologies and measures the expression of several of genes simultaneously, has the potential role in revolutionizing toxicology. Toxicogenomics have been used as tools to elucidate mechanisms and to predict toxicity [20–27]. In the present study, we have used semiquantitative RT-PCR to determine potential toxic effect of DM on the gene expression level of prooxidants (NOS-1, NOS-2, HO-1, and HO-2) and antioxidant (GST-P1) enzyme in the brain of adult female rats. Our results revealed that DM produced significant increase the gene expression of the all prooxidants accompanied with significant decrease in the antioxidant enzyme in the rat brain tissue compared with the control group. Moreover, DM induced significantly the rate of the DNA fragmentation in rat brain tissues. For our knowledge, there are no data regarding the toxic impact of DM on the mRNA or the DNA. However, Belgaumi et al. [11] reported that glucocorticosteroids are important therapeutic agents for treatment of several diseases such as lymphoblastic leukemia. Although the exact mechanism of leukemic blast cell kill is not known, GCs induces apoptosis of these cells in vitro [31]). Furthermore, Kaspers et al. [32] and Ito et al. [28] have shown that the using of DM was 5–16 times more toxic than prednisone.

The mechanism of action of DM induced toxicogenomic impacts did not publish yet. However, the present study could suggest that these toxicogenomic impacts of DM may be attributed to ROS generation in the brain of the female rats, where our present data showed more mRNA concentrations of the prooxidant enzymes (NOS-1, NOS-2, HO-1 and HO-2) and low mRNA concentrations of the antioxidant (GST-P1) enzyme in the brain tissues.

The present study revealed that administration of MEL or ALC prior to DM results in the prevention of the potential genotoxicity of DM as indicated by the significant suppression of the gene expression of prooxidant (NOS-1, NOS-2, HO-1, HO-2) and the rate of the DNA damage as well as the marked elevation of the gene expression of the antioxidant enzyme (GST-P1). These results were in great agreement with Jiménez-Ortega et al. [30]. They reported that MEL administration significantly decreased mRNAs for NOS-1, NOS-2, HO-1, and HO-2 and augmented the gene expression of the antioxidant enzymes in rat hypothalamus. Our results regarding the effect of ALC on the gene expression of the studied prooxidants and antioxidant enzyme as well as DNA fragmentation rate proved that ALC behaves as MEL in this concern.

The inhibitory effect of MEL and ALC on HO-1 and HO-2 mRNA reported in the present study can be tentatively interpreted in terms of either reduction of oxidative load by MEL and ALC (i.e., less need of HO-1 expression) and/or interference of these neuroprotective agents with the circadian signaling regulating gene expression of the prooxidant enzymes (NOS-1 and NOS-2). Since gene expression does not necessarily correlate with enzymatic activity, a more accurate evaluation of MEL and ALC effect will be given by testing their roles in the regulation of HO activity and protein levels.

The mechanisms involved in the regulation of gene expression by MEL and ALC may involve receptor-mediated and receptor-independent phenomena. Among the latter, the inhibition of ROS generation

is attractive. Since ROS plays a role in cellular signaling processes, including transcription factors activities such NF-κB and AP-1, a decrease in free radicals generation by MEL and ALC would allow the repression of redox-sensitive transcription factors, which could regulate gene transcription [35–53]. It was stated that MEL- and ALC-induced neuroprotective activity is mediated via the potentiation of other brain antioxidants (e.g., GST-P1, ascorbic acid, and other, yet unidentified, compounds) [19–47] that, by altering the cell's redox state, they could attenuate the subsequent activation of NF-κB and AP-1. Indeed, the induction of HO-1 expression is a NO-dependent process [5], and the significant inhibition of NOS-1 and NOS-2 mRNA expression given by MEL and ALC may be inhibition of the gene expression of HO-1 and HO-2 as indicated in the current study.

Further studies are needed to shed light on the mechanisms that explain MEL and ALC activity on HO-1 and HO-2 gene expression. In particular, enzyme activity assessment and Western blotting analysis of enzyme protein levels would be helpful in this respect.

Microscopic examination of brain sections of rat administered with DM showed the loss of the normal structure and the outlines of the cells and their nuclei. Some neurons appear like ring shape, and the recently dead neurons appear dark. This finding is in agreement with that of Sato et al. [55] who demonstrated that subcutaneously injecting corticosterone caused a markedly increasing in lipid hydroperoxides and protein carbonyls in the hippocampus, associated with a decreasing in the activity of antioxidative enzymes, such as SOD, CAT, and glutathione peroxidase. The ROS generated to attack the hippocampus to induce neurodegeneration, resulting in cognitive deficits in rats. On the other hand, MEL and ALC prevent oxidative stress damage in the rats' hippocampus [6–18].

In conclusion, the beneficial effects of MEL and ALC on the levels of biochemistry, molecular biology, and histology in improving the brain antioxidant status of rats administered DM are mostly mediated by their antioxidant activity and antiradical capacity. Noteworthy, MEL showed slightly more pronounced neuroprotective influence against DM-induced neurotoxicity than ALC. These encouraging results pave the way for using MEL or ALC as adjuvant therapy during long-term clinical use of DM to avoid its neurotoxic impact.

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