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L-carnitine mitigates UVA-induced skin tissue injury in rats through downregulation of oxidative stress, p38/c-Fos signaling, and the proinflammatory cytokines

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ABSTRACT

UVA comprises more than 90% of the solar UV radiation reaching the Earth. Artificial lightening lamps have also been reported to emit significant amounts of UVA. Exposure to UVA has been associated with dermatological disorders including skin cancer. At the molecular level, UVA damages different cellular biomolecules and triggers inflammatory responses. The current study was devoted to investigate the potential protective effect of L-carnitine against UVA-induced skin tissue injury using rats as a mammalian model. Rats were distributed into normal control group (NC), L-carnitine control group (LC), UVA-Exposed group (UVA), and UVA-Exposed and L-carnitine-treated group (UVA-LC). L-carnitine significantly attenuated UVA-induced elevation of the DNA damage markers 8-oxo-2′-deoxyguanosine (8-oxo-dG) and cyclobutane pyrimidine dimers (CPDs) as well as decreased DNA fragmentation and the activity of the apoptotic marker caspase-3. In addition, L-carnitine substantially reduced the levels of lipid peroxidation marker (TBARS) and protein oxidation marker (PCC) and significantly elevated the levels of the total antioxidant capacity (TAC) and the antioxidant reduced glutathione (GSH) in the skin tissues. Interestingly, L-carnitine upregulated the level of the DNA repair protein proliferating cell nuclear antigen (PCNA). Besides it mitigated the UVA-induced activation of the oxidative stress-sensitive signaling protein p38 and its downstream target c-Fos. Moreover, L-carnitine significantly downregulated the levels of the early response proinflammatory cytokines TNF-α, IL-6, and IL-1β. Collectively, our results highlight, for the first time, the potential attenuating effects of L-carnitine on UVA-induced skin tissue injury in rats that is potentially mediated through suppression of UVA-induced oxidative stress and inflammatory responses.

1. Introduction

Solar light is the major source of UVA reaching the Earth [1]. In addition, several artificial lightening sources have been reported to emit variable amounts of UVA [2,3]. Although UVA has relatively low energy as compared to other UV components, it penetrates deeper into the skin and is considered as a major risk factor in the skin carcinogenesis [4–7]. UVA induces damage to a variety of critical biomolecules [1,8–10]. Its damaging effects are largely mediated through generation of reactive oxygen species, ROS [10–12]. It has been reported that UVA generates a variety of ROS including superoxide anion radical, hydroxyl radical, hydrogen peroxide, and singlet oxygen [10,13–16]. ROS interact with DNA, causing DNA lesions particularly 8-oxo-2′-deoxyguanosine, 8-oxo-dG [9,12,17]. Energy transfer to DNA induces cyclobutane pyrimidines dimers, CPDs [17,18]. Accumulation of DNA lesion such as 8-oxo-dG and CPDs has been linked to mutagenesis and carcinogenesis [19,20]. In the same context, ROS interact with other cellular biomolecules, causing lipid peroxidation and protein oxidative modifications as well as initiation of inflammatory responses [9,21,22]. Importantly, modification of proteins that are involved in DNA repair may boost mutagenicity of UVA [10,19].

L-carnitine is a naturally occurring quaternary amine that plays

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important role in transport of fatty acids across the mitochondrial membrane for subsequent oxidation and energy production [23]. In addition to it critical role in energy metabolism, L-carnitine exhibits antioxidant and antiapoptotic properties [24–27]. It has been shown that L-carnitine scavenges superoxide anion radical and the strong oxidant hydrogen peroxide [24]. In addition, it scavenges hydroxyl radical and prevents its generation through Fenton reaction [28,29]. L-carnitine also increases the activity and expression of the antioxidant enzymes superoxide dismutase and catalase in human hepatocytes [30].

Based on its antioxidant properties, L-carnitine may protect against UVA-induced, ROS- mediated skin tissue injury. The aim of the current study, thus, was to evaluate the potential protective effect of L-carnitine against UVA-induced skin tissue injury using rats as an experimental mammalian model.

2. Material and methods

2.1. Animals

Male Wistar rats weighing 200–230 g (50 days old) were housed in groups of four per polypropylene cage. Rats were acclimatized to Taif University animal facility for ten days before starting the experimental work. Constant conditions of temperature (23 ± 2 °C), humidity (60 ± 10%) and light/dark cycle (12 h/12 h) were maintained all over the experimental period. Standard commercial rat chow and water were allowed *ad libitum*. All procedures related to animal care, treatment, and sampling were conducted in compliance with the guidelines of National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Ethical committee approval number (2017/TU/Pharmacy/02).

2.2. Chemicals and kits

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2, 4-dinitrophenyl hydrazine (DNPH), 5, 5′- dithiobis [2-nitrobenzoic acid] (DTNB), and L-carnitine were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were of high purity. Total antioxidant capacity (TAC) kit was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). TNF-α, IL-6 and IL-1β kits were purchased from Ray Biotech (Norcross, GA, USA). Caspase-3 colorimetric assay kit and DNA fragmentation assay kit (TiterTACS In Situ Detection Kit) were purchased from R & D systems (Minneapolis, MN, USA). 8-oxo-2′-deoxyguanosine assay kit was purchased from Trevigen (HT 8-oxo-dG, Trevigen, Inc., Gaithersburg, MD, USA). Cyclobutane pyrimidine dimer assay kit was purchased from Cell Biolab (OxiSelect™, Cell Biolab, Inc, San Diego, CA, USA). DNA extraction kit was purchased from Qiagen (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany). Rat skin dissociation kit (Rat Skin PrimaCell™ I) were purchased from Chi Scientific (Chi Scientific, Inc, USA). Antibodies for PCNA and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies for total and phosphorylated forms of p38 and c-Fos were purchased from cell signaling (Cell Signaling Technology, Inc, USA).

2.3. Experimental design and treatment protocol

Thirty-two male Wistar rats were randomly distributed into four groups of eight-animal each. The dorsal area of all rats was shaved (4 cm^2) using a shaving machine. The shaved skin in different rat groups was clean and free of any injury. The treatment protocol was as the following: Group-1 (Normal control, NC): animals in this group were not exposed to any UVA or any treatment all over the experimental period. Group-2 (L-carnitine control, LC): animals in this group were treated with 300 mg/kg body weight of L-carnitine orally by gastric gavage once daily for one week. Group-3 (UVA-Exposed, UVA): rats in this group were directly illuminated with a single dose of UVA (20 J cm^{-2}) using the commercially available high intensity UVA lamp (BlakRay-B100-A, 230 W, 8900 μW/cm^2, main peak emission 365 nm at 25 cm distance, UVP, Cambridge, UK, exposure period was 37.5 min). During illumination, the animals were in their polypropylene cages and the distance between the UV light source and the animals was *ca.* 25 cm. Group-4 (UVA-Exposed and L-carnitine-treated group, UVA-LC): rats in this group were handled the same way as UVA group except that the animals were treated with 300 mg/kg body weight of L-carnitine orally by gastric gavage once daily for one week before exposure to UVA. UVA exposure was done 2 h after the last dose of L-carnitine. Doses of UVA and L-carnitine were consistence with previously published work [1,31].

2.4. Sample preparation

Twelve hours after the exposure to UVA, rats in all groups were euthanized by decapitation under pentobarbital sodium anesthesia (65 mg/kg, ip) [32] to collect skin tissue samples. The skin tissues were quickly removed, rinsed in ice cold saline, and divided into three parts for DNA extraction, tissue homogenization and skin tissue dissociation. DNA was extracted using the commercially available Qiagen kit (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Skin tissue samples designated for homogenization were weighed and homogenized (10% w/v) in phosphate-buffered saline (PBS) using T25 digital ultra-turrax homogenizer (IKA-Werke GmbH & Co. KG, Germany). Homogenates were then centrifuged for 15 min at 10000 × g and 4 °C. The supernatant was used for determination of the proposed biochemical parameters. Skin tissues designated for tissue dissociation was used to make a single cell suspension of epidermal keratenocytes using the commercially available Rat Skin PrimaCell™ I (Chi Scientific, Inc, USA). The resulted single cell suspension was used for determination of the level of DNA fragmentation in different experimental groups.

2.5. Measured parameters

2.5.1. Measurement of 8-oxo-2′–deoxyguanosine

Levels of the oxidative DNA damage biomarker 8-oxo-2′–deoxyguanosine (8-oxo-dG) in skin tissues of UVA-Exposed and control rats were evaluated using the commercially available HT 8-oxo-dG ELISA II kit (Trevigen, Inc., Gaithersburg, MD, USA). The assay employs standard 8-oxo-dG immobilized to 96-well plate. Specific antibody (anti 8-oxo-dG monoclonal antibody) is employed to bind competitively to the immobilized 8-oxo-dG and to that in test samples. Upon washing, antibodies that bind to 8-oxo-dG in the test samples are washed away while antibodies bind to the immobilized 8-oxo-dG are retained. Detection was performed with HRP-conjugated secondary antibody and a colorimetric substrate. Optical density is inversely proportional to the level of 8-oxo-dG present in the test samples [33]. The optical density was measured using SPECTRAmax PLUS^{384} microplate spectrophotometer (Molecular devices, Sunnyvale, California, USA).

2.5.2. Measurement of cyclobutane pyrimidine dimer

CPDs level was determined using the commercially available immunoassay colorimetric ELISA kit (OxiSelect, Cell Biolab, INC.) according to the manufacturer's instructions. CPD-DNA standards or test DNA samples were first heat-denatured before adsorption onto a 96-well DNA high-binding plate. The CPDs present in the standard or the test sample were probed with anti-CPD antibody followed by HRP-conjugated secondary antibody. The level of CPD in the test sample was determined using a standard curve that is prepared from a predetermined CPD-DNA standard [34].

2.5.3. Determination of caspase-3 activity

The commercially available colorimetric assay kit (R &D systems) was used for determination of the activity of the apoptotic marker caspase-3 according to the manufacturer's instructions as described

previously [35]. The method based on the ability of caspase-3 in the test sample to cleave a labeled substrate DEVD-pNA (acetyl-Asp-Glu-Val-Asp p-nitroanilide) with subsequent release of a chromophore (p-nitroanilide, pNA). The optical density was then measured spectrophotometrically at 405 nm.

2.5.4. Determination of DNA fragmentation

DNA fragmentation in a single cell suspension of the skin epidermal keratinocytes were determined in different experimental groups using the colorimetric TiterTACS In Situ Detection Kit (R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The assay based on labeling the DNA nicks with biotinylated nucleotide using terminal deoxynucleotidyl transferase (TdT). The labeled DNA nicks are then allowed to react with streptavidin-HRP conjugate followed by the HPR colorimetric substrate TACS-Sapphire. The color produced is proportional to the level of the DNA fragmentation in the sample and can be measured spectrophotometrically at 450 nm. The optical density was measured using SPECTRAmax PLUS^{384} microplate spectrophotometer (Molecular devices, Sunnyvale, California, USA) and results were presented as a relative optical density to that of the normal control group.

2.5.5. Determination of PCNA, p38 MAPK, and c-Fos protein abundance in the skin tissues

Western blotting analysis was used to determine the protein abundance of PCNA, total and phosphorylated forms of p38 MAPK, and total and phosphorylated forms of c-Fos in the skin tissue homogenates of different experimental groups in accordance with the previously described work [36,37] using the corresponding primary antibodies. Briefly, equal amounts of protein (40 μg/lane) form the supernatants of the skin tissue homogenates of different experimental groups were subjected to SDS-PAGE and Western blotting analysis. PC10 primary antibody (sc-56, Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution of 1: 2000) and goat anti-mouse secondary antibody (dilution of 1: 3000, Bio-Rad) were used to detect PCNA. Rabbit monoclonal primary antibodies (Cell Signaling Technology, Inc, USA) for p38 (D13E1), phosphorylated p38 (12F8), c-Fos (9F6), and phosphorylated c-Fos (D82C12) were used to detect the corresponding proteins in a dilution of 1:1000. Sc-1616-R primary antibody (dilution of 1: 300, Santa Cruz Biotechnology, Santa Cruz, CA) was used for the loading control β-actin. The density of different protein bands were quantitated and normalized to the loading control in intermediately exposed films using ImageJ program (ImageJ, NIH, USA).

2.5.6. Measurement of lipid peroxidation

Level of thiobarbituric acid reactive substances (TBARS) was measured to evaluate lipid peroxidation in the skin tissue samples according to the previously described method [38]. Briefly, equal volumes of the skin tissue homogenates and thiobarbituric acid (TBA) reagent (0.5% w/v TBA prepared in 20% w/v TCA) are allowed to react for 30 min at 95 °C. The reaction was then stopped by placing on ice. After centrifugation (10000 × g for 15 min at 4 °C), the optical density of the supernatant was measured at 532 nm using Optima 3000 Nano UV–Visible spectrophotometer (Optima, Itabashi-ku, Tokyo, Japan). The results were expressed as TBARS relative optical density at 532 nm.

2.5.7. Measurement of protein carbonyl content

Previously described method of Hawkins et al. [39] was employed to determine the levels of the protein oxidation marker (protein carbonyl content). Proteins in the skin tissue samples were allowed to react with 2, 4- dinitrophenyl hydrazine (DNPH). Proteins were then separated by TCA precipitation and re-dissolved in guanidine hydrochloride. The absorbance of protein solution was then measured spectrophotometrically at 370 nm.

2.5.8. Determination of total antioxidant capacity

Total antioxidant capacity (TAC) in the skin tissues of different experimental groups was evaluated using Cayman TAC kit in accordance with the manufacturer's instructions. Briefly, the procedure allows competition between antioxidants in the test samples and a standard antioxidant (2, 2-azino-di-[3-ethylbenzthiazoline sulphonate, ABTS) to react with a standard oxidant. Higher levels of oxidized TBTS indicate lower levels of TAC. The optical density of the oxidized ABTS was measured spectrophotometrically at 405 nm and the levels of TAC were determined using a standard curve [40].

2.5.9. Determination of reduced glutathione

Previously described method of Ellman [41] was used for determination of reduced glutathione (GSH) levels in the skin tissue homogenates. Briefly, deproteinated skin tissue homogenates were allowed to react with 5, 5′- dithiobis [2-nitrobenzoic acid] (DTNB). The optical density of the colored solution produced was then measured spectrophotometrically at 412 nm.

2.5.10. Determination of the proinflammatory cytokines

Ray Biotech ELISA kits were used for determination of the proinflammatory cytokines TNF-α, IL-6, and IL-1β levels in the skin tissues of different experimental groups in accordance with the previously described method [42]. Briefly, cytokines in the skin tissue samples were allowed to interact with corresponding antibodies pre-coated to a 96-well plate. Biotinylated antibodies and HRP-linked streptavidin were applied sequentially. Eventually, 3, 3′, 5, 5′-tetramethylbenzidine (TMB) reagent was added to the wells and the optical density was then measured at 450 nm. Standard curves were used to calculate the cytokines levels.

2.6. Statistical analysis

One way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test was used to analyze the statistical significance among groups. SigmaPlot 12 statistics software (Systat Software, Inc., San Jose, CA) was used to construct graphs and perform statistical analysis. Data were expressed as mean ± standard deviation. Differences were considered significant at $p < 0.05$.

3. Results

3.1. L-carnitine ameliorates UVA-induced DNA damage and reduces apoptotic cell death

To explore the possible protective effect of L-carnitine against UVA-induced DNA damage and apoptotic cell death, DNA damage markers 8-oxo-dG and CPDs along with the activity of the apoptotic marker caspase-3 and the level of DNA fragmentation were evaluated in the skin tissue of different experimental groups. The results showed that exposure to UVA resulted in 6.9-fold increase in 8-oxo-dG level (Fig. 1A), 4.4-fold increase in CPDs level (Fig. 1B) as well as 4-fold increase in the caspase-3 activity (Fig. 1C) and 3.5-fold increase in the level of DNA fragmentation (Fig. 1D) in UVA-Exposed group (UVA) compared to unexposed normal control group (NC). Administration of L-carnitine, however, effectively reduced the levels of both 8-oxo-dG and CPDs by 52% and 25% respectively (Fig. 1A&B, UVA-LC group). In addition, it diminished the activity of caspase-3 and reduced DNA fragmentation to 52% and 57% of their values in UVA group respectively (Fig. 1C&D, UVA-LC group).

3.2. L-carnitine mitigates UVA-induced oxidative stress

Illumination of shaved-skin rats with UVA resulted in obvious increase in the lipid peroxidation marker, TBARS (Fig. 2A) along with the protein oxidation marker, PCC (Fig. 2B) in UVA group as compared to

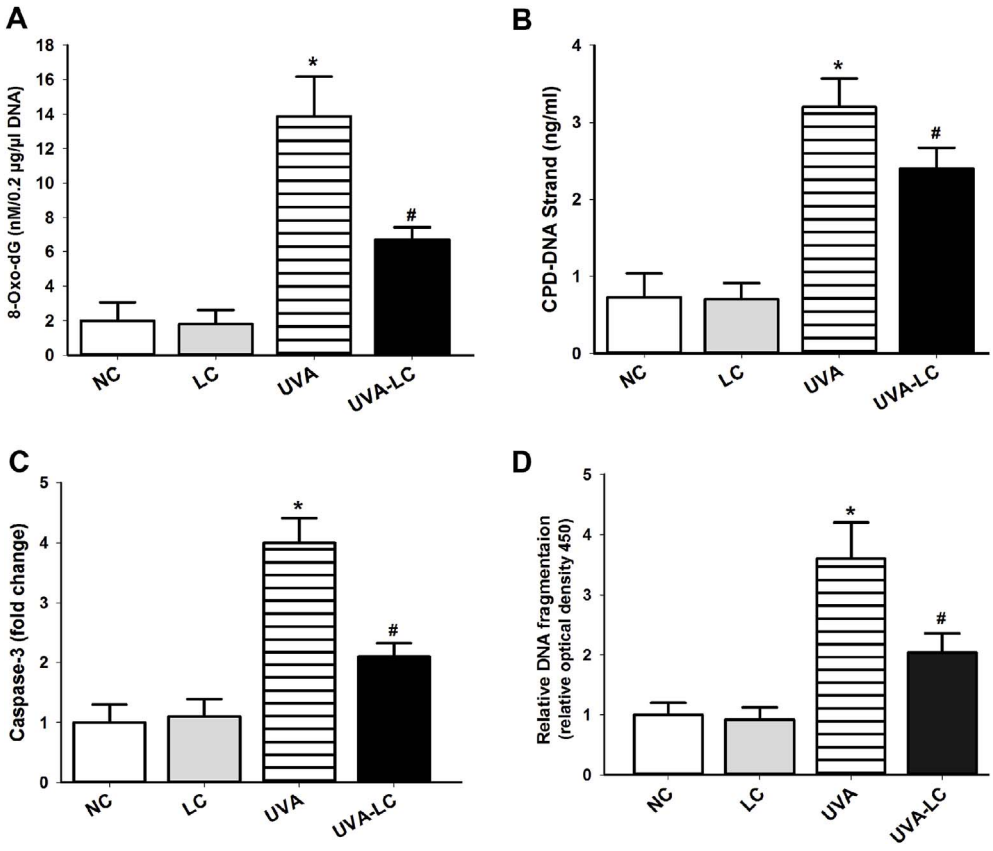

Fig. 1. Levels of the DNA damage and apoptotic markers in the skin tissues. Levels of 8-oxo-2′–deoxyguanosine, 8-oxo-dG (A); cyclobutane pyrimidine dimers, CPD-DNA (B); and the activity of the apoptotic marker caspase-3 (C); and the level of the DNA fragmentation (D) were measured in the skin tissues of the normal control (NC), L-carnitine control (LC), UVA-Exposed (UVA), and UVA-Exposed and L-carnitine-treated (UVA-LC) groups. Data are presented as mean ± standard deviation. (*) significant difference from NC, (#) significant difference from UVA group, $p < 0.05$ (n = 8). 8-oxo-dG is expressed as nM per µl of the used DNA sample (0.2 µg/µl) and CPD-DNA is expressed as ng per ml of the used DNA sample (4 µg/ml).

NC group. In the same context, it substantially diminished the antioxidant defense in the skin tissues as demonstrated by a significant reduction in the levels of the total antioxidant capacity, TAC, as well as reduced glutathione, GSH (Fig. 3). L-carnitine administration, however, significantly attenuated lipid and protein oxidative modification as revealed by significant reduction in the levels of TBARS and PCC (Fig. 2, UVA-LC group). Besides it boosted the antioxidant defense mechanisms in the skin tissues as demonstrated by significant elevation of TAC and GSH levels (Fig. 3, UVA-LC group).

3.3. L-carnitine upregulated PCNA protein abundance and attenuated p38 and c-Fos activation

Although UVA is less energetic than UVB, it has been reported that UVA-induced DNA damage is more mutagenic than that of UVB [19]. Damage to critical DNA repair proteins by UVA is believed to be the underlining cause of the higher mutagenic potential of UVA [10]. To evaluate the possible damaging effects of UVA on the critical DNA repair protein PCNA, protein abundance of PCNA was determined using Western blotting analysis. The results showed that exposure to UVA significantly lowered the PCNA protein abundance compared to the normal control group (Fig. 4). Interestingly, administration of L-carnitine significantly upregulated PCNA protein abundance compared to the UVA group (Fig. 4, UVA-LC group). The results also indicated that exposure of the shaved-skin rats to UVA induced activation (phosphorylation) of both p38 and its downstream target c-Fos. L-carnitine administration, however, significantly reduced the UVA-induced activation of both p38 and c-Fos (Fig. 6).

3.4. L-carnitine downregulates the levels of the proinflammatory cytokines

Exposure of shaved-skin rats to UVA markedly elevated the levels of the early response proinflammatory cytokines TNF-α, (296%), IL-6 (320%) and IL-1β (235%) in UVA group as compared to NC group (Fig. 5). Administration of L-carnitine efficiently downregulated the levels of TNF-α (48% reduction), IL-6 (47% reduction) as well as IL-1β (39% reduction) (Fig. 5, UVA-LC group).

4. Discussion

Solar light as well as several artificial lightening lamps represent chief sources of exposure to UVA radiation [2,3,10]. UVA has been implicated in a variety of dermatological disorders including skin aging and carcinogenesis [43–45]. The ability of UVA to induce DNA damage such as 8-oxo-dG and CPDs is linked to its mutagenic potential [19,20,46]. Consistent with the ability of UVA to induce DNA damage, the results of the current work revealed that exposure of the shaved-skin rats to UVA significantly elevated the levels of 8-oxo-dG and CPDs in UVA-Exposed group as compared to the normal control groups, NC (Fig. 1). Unlike shorter-wavelength UV radiation, UVA damages DNA indirectly through generation of ROS [9,11]. In the range of 320–400 nm, UVA produces the powerful ROS, singlet oxygen, through interaction with endogenous photosynthesizes such as porphyrins, flavins, and quinones [11,12]. UVA is also capable of generating other ROS such as hydrogen peroxide, superoxide anion, and hydroxyl radicals [47]. ROS interact directly with DNA, causing DNA oxidative damage particularly 8-oxo-dG [48,49]. Triplet energy transfer from UVA-excited chromophores to DNA resulted in formation of CPDs [18]. The current study revealed that administration of L-carnitine significantly decreased levels of both 8-oxo-dG and CPDs by 52% and 25% respectively as compared to UVA group (Fig. 1, UVA-LC group). The ability of L-carnitine to attenuate the oxidative DNA damage is potentially related to its ROS scavenging properties. Previous studies have shown that L-carnitine scavenges a variety of ROS including superoxide anion radical, hydrogen peroxide, and hydroxyl radical [28,29]. Beside it boosts the activity and expression of the antioxidant enzymes superoxide dismutase and catalase [30]. The results highlight the potential attenuating effect of L-carnitine on UVA-induced DNA damage in the

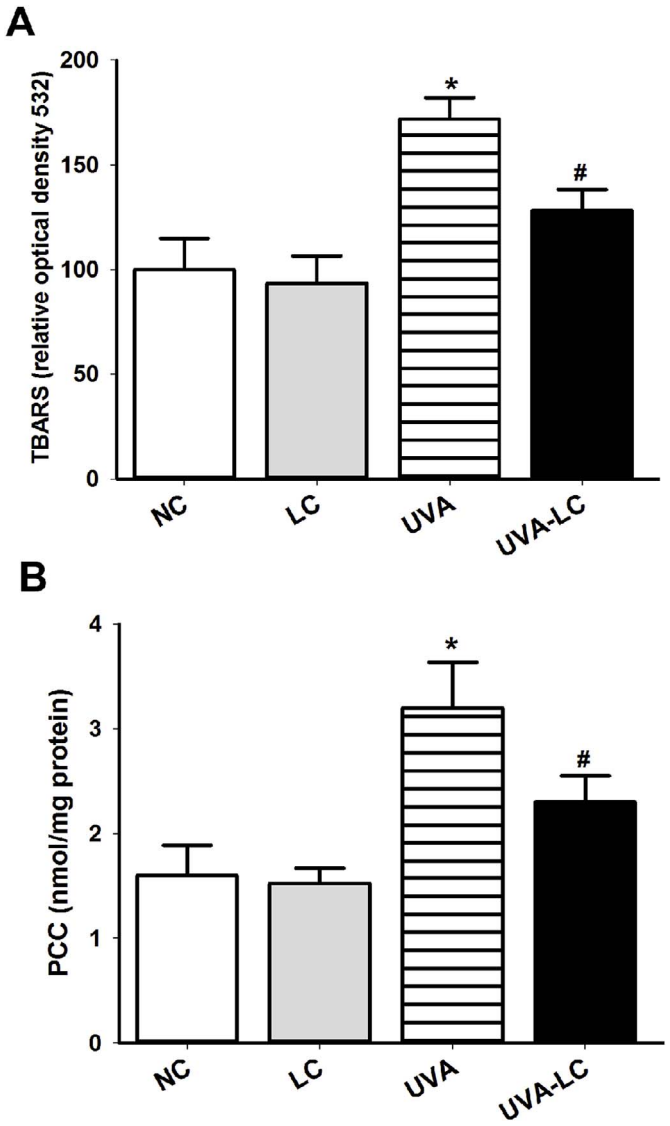

Fig. 2. Levels of oxidative stress markers in the skin tissues. Levels of the lipid peroxidation marker thiobarbituric acid reactive substance, TBARS (A); the protein oxidation marker protein carbonyl content, PCC (B) were evaluated at the end of the experiment in the skin tissues of different experimental groups; normal control group (NC), L-carnitine control group (LC), UVA-Exposed group (UVA), and UVA-Exposed and L-carnitine-treated group (UVA-LC). Data are presented as mean ± standard deviation. (*) significant difference from NC group and (#) significant difference from UVA group, $p < 0.05$ (n = 8).

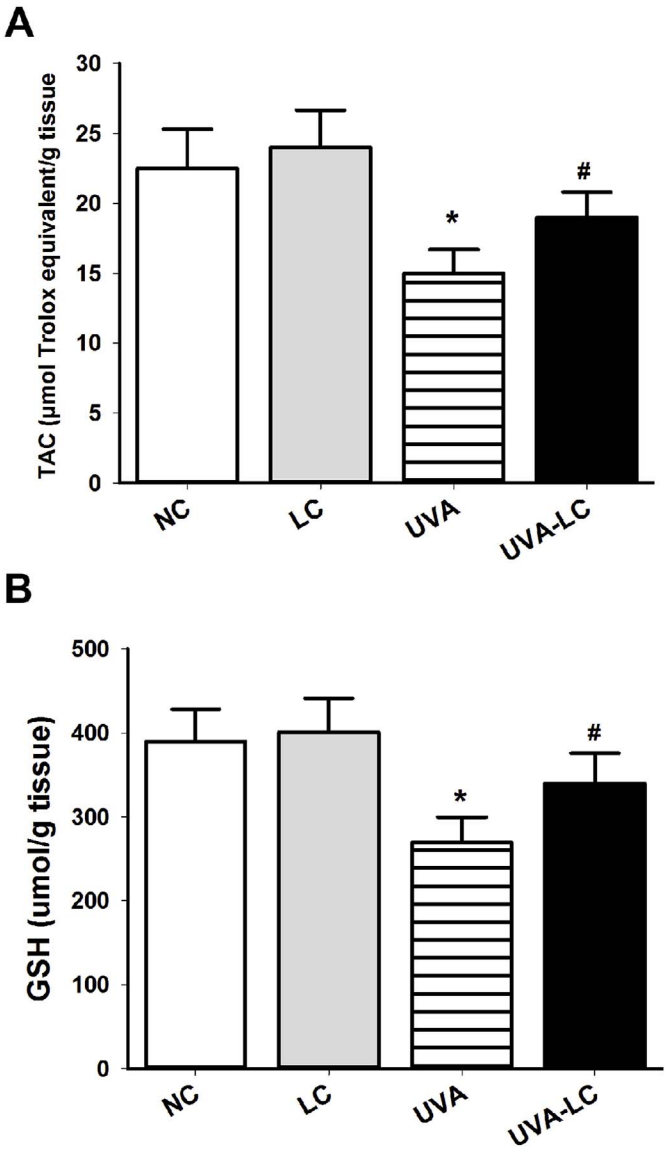

Fig. 3. Antioxidants levels in the skin tissues. Levels of total antioxidant capacity, TAC (A) and reduced glutathione, GSH (B) were evaluated in the skin tissues of the normal control group (NC), L-carnitine control group (LC), UVA-Exposed group (UVA), and UVA-Exposed and L-carnitine-treated group (UVA-LC). Data are presented as mean ± standard deviation. (*) significant difference from NC group and (#) significant difference from UVA group, $p < 0.05$ (n = 8).

skin tissues.

It has been reported that CPDs stalls replication fork and is a major inducer of apoptotic cell death [12,47]. In addition, 8-Oxo-dG has been implicated in the UV-induced apoptotic cell death [47,50]. Furthermore, UVA-generated ROS have been reported to induce apoptotic cell death independent of DNA lesions [47,51]. In consistence with these studies, the current work showed that exposure of shaved-skin rats to UVA induced 4-fold increase in the activity of the apoptotic cell death marker caspase-3 and 3.5-fold increase in the level of DNA fragmentation, an important hallmark of cellular apoptosis, compared to the normal control group (Fig. 1 C&D). L-carnitine, however, significantly diminished the activity of caspase-3 and the level of the DNA fragmentation (Fig. 1C&D, UVA-LC group) which may be due to its ability to scavenge different types of ROS with subsequent reduction in 8-Oxo-dG and CPDs levels, signifying the mitigating effect of L-carnitine on UVA-induced skin tissue injury.

In addition to induction of DNA lesions and subsequent initiation of apoptosis, UVA-generated ROS interact with other cellular macromolecules, causing lipids and proteins oxidative modifications [9,21]. In line with these studies, the current work showed a state of oxidative stress in the skin tissues of the UVA-Exposed rats. The levels of both lipid peroxidation marker, TBARS, and protein oxidation marker, PCC, were significantly elevated (Fig. 2) while the levels of both total antioxidant capacity, TAC, and the antioxidant reduced glutathione, GSH, were significantly decreased (Fig. 3). Peroxidation of membrane lipids has been reported to disrupt cellular membranes including mitochondrial membrane [52,53]. Damage of the mitochondrial membrane releases cytochrome c to the cytoplasm with subsequent initiation of the intrinsic apoptotic pathway [54] which explains the ability of ROS to induce DNA-independent apoptotic cell death. L-carnitine administration significantly decreased levels of both TBARS and PCC (Fig. 2, UVA-LC group) and boosted the levels of both TAC and GSH (Fig. 3, UVA-LC group), reinforcing its mitigating effect on UVA-induced skin tissue injury.

The homotrimeric protein PCNA is a crucial player in DNA replication and repair processes. As a sliding clamp, PCNA increases the

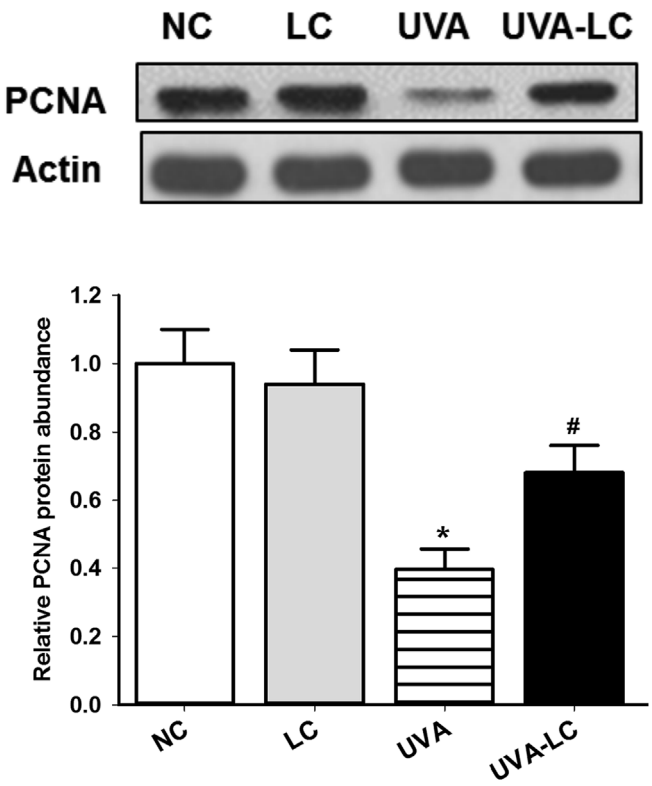

Fig. 4. PCNA protein abundance in the skin tissues. PCNA Western blotting (upper panel) and its quantification (lower panel) were evaluated at the end of the experiment in the skin tissues of different experimental groups: normal control group (NC), L-carnitine control group (LC), UVA-Exposed group (UVA), and UVA-Exposed and L-carnitine-treated group (UVA-LC). Data are presented as mean ± standard deviation. (*) significant difference from NC group and (#) significant difference from UVA group, $p < 0.05$. Western blotting was done in triplicates using pooled tissue homogenates.

processivity of DNA polymeases and plays essential roles in mismatch, and base and nucleotide excision repair [10,55]. Exposure to UVA induces different oxidative modifications to cellular proteins. UVA induces oxidation-based PCNA subunit crosslinking and oxidation-dependent degradation of other cellular proteins such as protein tyrosine phosphatases [10,56]. The results of the current work showed that exposure to UVA decreased PCNA protein abundance as detected by Western blotting analysis (Fig. 4). Decreased abundance of such critical DNA repair protein by UVA may explain, at least in part, the previous observation that UVA-generated CPDs have higher mutagenic potential than that generated by UVB [10,18,19]. While PCNA protein degradation may represent a plausible explanation for the observed decrease in PCNA abundance, the possibility of oxidative modification of PCNA epitope should not be excluded. Interestingly, L-carnitine administration upregulated PCNA protein abundance as compared to UVA-Exposed group (Fig. 4, UVA-LC group). The ability of the antioxidant L-carnitine to upregulate PCNA protein levels after UVA exposure supports the notion that oxidation is required for the observed change in the PCNA abundance.

UVA has been implicated in activation of the mitogen-activated protein kinases (MAPKs) including p38 MAPK [57]. p38, in turn, activates several downstream signaling molecules including c-Fos [58]. As a part of the AP-1 transcription factor, c-Fos plays critical roles in regulation of expression of several genes responsible for cell arrest, apoptosis, and inflammatory responses [58,59]. It has been demonstrated that UVA-induced activation of p38 is mediated through reactive oxygen species [57,60]. The current work showed that exposure of the shaved-skin rats to UVA induced activation (phosphorylation) of both p38 and its downstream target c-Fos. L-carnitine administration, however, significantly reduced the UVA-induced activation of both p38

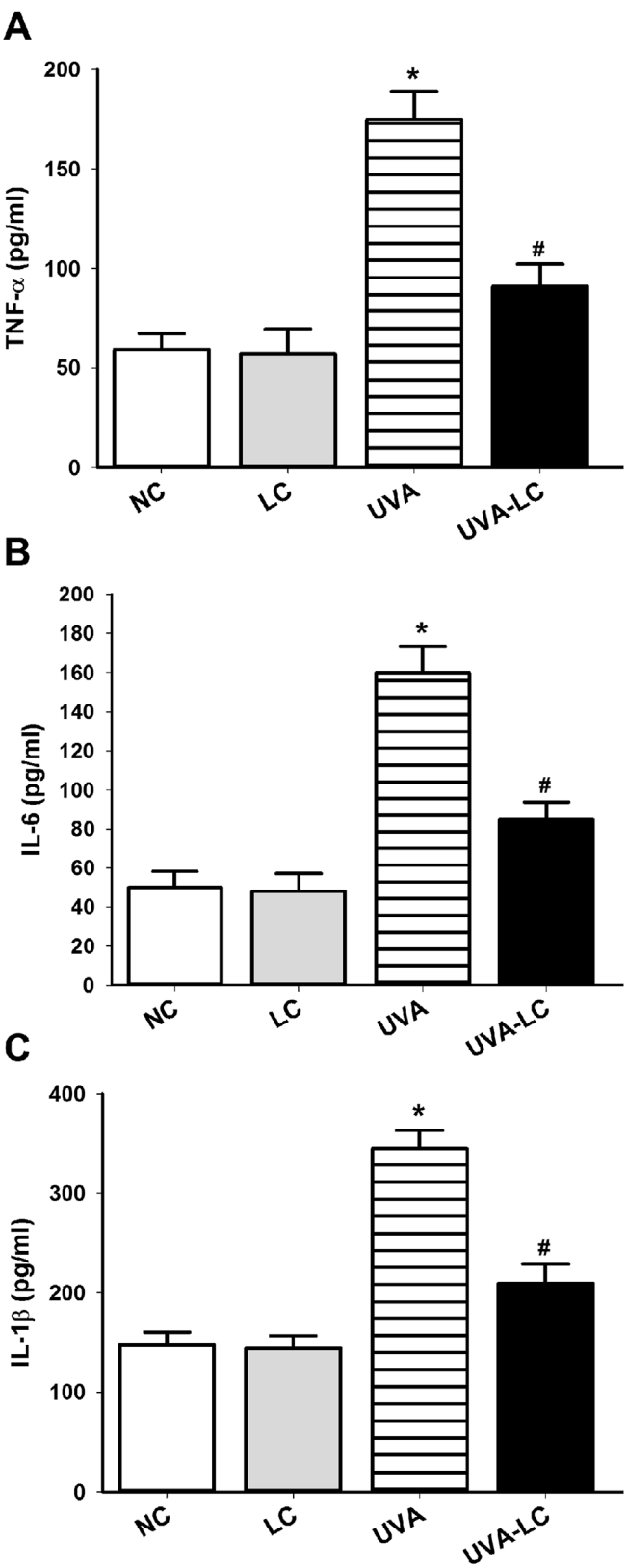

Fig. 5. Levels of the proinflammatory cytokines in skin tissues. Levels of TNF-α (A), IL-6 (B), and IL-1β (C) were determined at the end of the experiment in skin tissues of different rat groups; normal control group (NC), L-carnitine control group (LC), UVA-Exposed group (UVA), and UVA-Exposed and L-carnitine-treated group (UVA-LC). Data are presented as mean ± standard deviation. (*) significant difference from NC group and (#) significant difference from UVA group, $p < 0.05$ (n = 8).

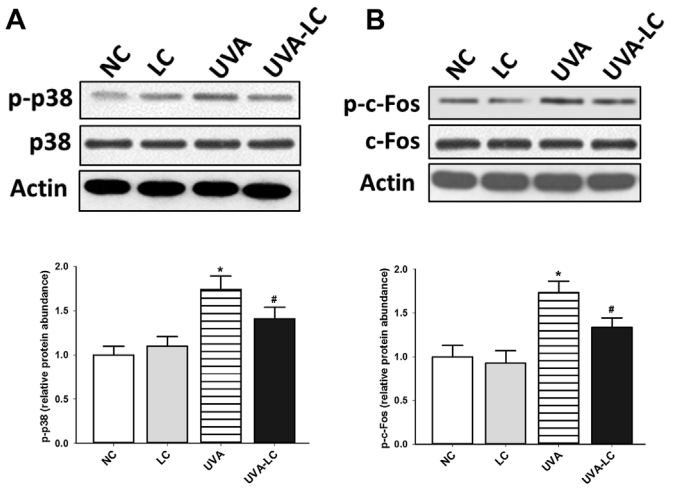

Fig. 6. p38 and c-Fos protein abundance in the skin tissues. Western blotting of total and phosphorylated forms of p38 (A, upper panel) and its quantification (A, lower panel) and Western blotting of total and phosphorylated forms of c-Fos (B, upper panel) and its quantification (B, lower panel) in the skin tissues of different experimental groups: normal control group (NC), L-carnitine control group (LC), UVA-Exposed group (UVA), and UVA-Exposed and L-carnitine-treated group (UVA-LC). Data are presented as mean ± standard deviation. (*) significant difference from NC group and (#) significant difference from UVA group, $p < 0.05$. Western blotting was done in triplicates using pooled tissue homogenates.

and c-Fos (Fig. 6), emphasizing the ameliorating effect of L-carnitine against UVA-induced skin tissue injury which may be mediated through amelioration of oxidative stress.

The current study also showed that exposure to UVA induced inflammatory response that was demonstrated by a significant elevation in the proinflammatory cytokines levels including TNF-α, IL-6, and IL-1β (Fig. 5) in the UVA-Exposed group when compared to the normal control group. Analogous to our findings, Li, M. et al. [61] has demonstrated that exposure to UVA is associated with increased levels of TNF-α, IL-6 and IL-1β as well as the oxidative stress markers in cultured skin cells. ROS have been implicated in the induction of the proinflammatory cytokines through activation of a variety of transcription factors including nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) [22]. While ROS induce inflammatory cytokines, TNF-α, in turn, has been implicated in the generation of ROS [62]. UVA, thus, generates ROS though activation of the endogenous photosensitizers as well as through induction of inflammatory responses. L-carnitine significantly downregulated the levels of TNF-α, IL-6 as well as IL-1β, supporting its mitigating activity on UVA-induced skin tissue injury. The ability of L-carnitine to downregulate the levels of the proinflammatory cytokines may be explained, at least in part, on the bases of the ability of L-carnitine to counteract the damaging effects of UVA-generated ROS.

In conclusion, our results accentuate the mitigating effect of L-carnitine on UVA-induced skin tissue injury in rats which might be mediated through downregulation of the oxidative stress, oxidative stress-sensitive signaling cascade, and the inflammatory response.

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Conflicts of interest

The authors have declared no conflict of interest.

Appendix A. Supplementary data

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Transparency document

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