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The Role of Vitamin C in Photosensitivity Attenuation of Antimicrobial Quinolones Group

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Abstract: Photosensitivity is a common side effect of ciprofloxacin that appears after exposure to UV radiations. The aim of the present study is to investigate the damaging effect of ultraviolet-A radiation (3.96kJ/m^2; 60min, 45min, and 30min) on the antibacterial activity of ciprofloxacin (100mg/kg; p.o; daily for 7 consecutive days) in Esch. coli infected rats. Photosensitivity was induced due to exposure of the dorsal side of shaved rats to UV-A lamp radiation. The adverse effects related to skin photosensitivity and hepatic toxicity were evaluated and the protective role of Vit. C (180mg/kg; daily for 7consecutive days) against UV-A induced oxidative stress was clarified. Our results showed that UV-A has induced skin tissue injury in rats denoted by skin scoring results (redness, edema, hemorrhage, oozing and shrinkage), and induced oxidative stress and inflammatory biomarkers in the skin homogenate and altered total leukocyte count in serum, and liver function parameters such as serum albumin and total protein levels. Photosensitivity criteria induced by ciprofloxacin were bound to be proportional to UV-A exposure time. However, oral administered Vit. C exhibited antioxidant and anti-inflammatory activity by normalizing skin contents of lipid peroxide (MDA), glutathione (GSH), tumor necrosis factor-α (TNF- α) and the total leukocytic count (TLC). Moreover, by testing serum total protein and albumin levels were they increased, whereas the relative organs weights of spleen and thymus were normalized by Vit.C. These results were confirmed to histopathological alterations in skin result. The present study concludes that orally administered Vit.C has prophylactic antioxidant and anti-inflammatory effects against ciprofloxacin-induced phototoxicity on exposure to UV-A radiation in Esch. coli systematic infected rats.

Key words: Ciprofloxacin, ultraviolet A radiation, Photosensitivity, Vit.C, Rats.

1. Introduction

Drug-induced photosensitivity refers to a cutaneous adverse event occurring due to the combined interaction of a drug (topically or systemically administered) and radiation from the sun. The spectrum of cutaneous manifestations varies from trivial pricking and itching on sun-exposed sites to a severe sunburn reaction with blistering [1]. Phototoxic reactions can manifest within minutes or hours of drug administration and are non-immunological, resulting from direct tissue injury. A potential mechanism for tissue damage involves drug-potentiated oxidation of cellular lipids, proteins and DNA often mediated by reactive oxygen species (ROS) generation; free radicals, superoxide anions, hydroxyl radicals and singlet oxygen [2]. UV radiation constitutes about 10% of the total light output of the Sun [3]. Many practical

applications of UV radiation derive from its biological effects due to interactions with organic molecules [4]. The ultraviolet radiation that reaches the earth's surface, more than 95% is the longer wavelengths of UVA, with the small amount UVB [5]. UVA effects are dominated by indirect DNA damage caused by reactive oxygen species such as singlet oxygen that induce apoptosis of skin-infiltrating T cells, T-cell depletion, and induction of collagenase-1 expression in human dermal fibroblast [6]. UVA has the longest wavelength with the lowest energy and can penetrate deeply into the skin dermis and cause aging effects [7]. One of the most obvious acute effects of UV on the skin is the induction of inflammation. If the dose of UV exceeds a threshold damage response, keratinocytes activate apoptotic pathways and die [8].

A quinolone antibiotic is a member of a large group of broad-spectrum antibacterial that share a bicyclic core structure related to the compound 4-quinolone [9]. They are used in human and veterinary medicine to treat bacterial infections, nearly all quinolone antibiotics in modern use are fluoroquinolones, which contain a fluorine atom in their chemical structure and are effective against both Gram-negative and Gram-positive bacteria [10]. Quinolone antibiotics are one of the well-known photosensitizers that induce phototoxicity. Treatment with both UV radiation and quinolone showed significantly increased back skin swellings, increase sunburn cells and decreased epidermal Langerhans cells [11]. Approved fluoroquinolone drugs include levofloxacin (Levaquin), ciprofloxacin (Cipro), moxifloxacin (Avelox), norfloxacin (Noroxin), ofloxacin (Floxin), and gemifloxacin (Factive) [12]. Ciprofloxacin is one of the most widely used antibiotics. It is chemically related to nalidixic acid and induces photosensitization of human skin [13]. The most common side effects of ciprofloxacin are nausea, diarrhea, vomiting, elevating liver enzymes, kidney, skin sensitivity to sunlight and irregular heartbeat [14]. Report that some patients treated with oral ciprofloxacin developed an erythematous skin reaction when exposed to sunlight [15].

Antioxidants that are reducing agents can also act as pro-oxidants. For example, vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide, however, it will also reduce metal ions that generate free radicals through the Fenton reaction [16].

$2\ Fe^{3+}$ + Ascorbate (vit C) $\rightarrow 2\ Fe^{2+}$ + Dehydroascorbate

$2\ Fe^{2+} + 2\ H_2O_2 \rightarrow 2\ Fe^{3+} + 2\ OH^{\cdot} + 2\ OH^{-}$

Vitamin C (Vit C) is a common term used for L-ascorbic acid, dehydro-L-ascorbic acid (the oxidized form of L-ascorbic acid), and L-ascorbic acid salts (sodium, potassium, and calcium L-ascorbate). L-ascorbic acid constitutes the majority (80%-90%) of vit C in food. It is found in rose hips, blackcurrants, and citrus fruits but can also be synthesized from glucose [17]. Vit.C is an essential micronutrient required for normal metabolic functioning of the body required for the conversion of the procollagen to collagen by oxidizing proline residues to hydroxyproline. In other cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins [18].

2. Materials and Methods

Animals:

Fifty six adult male albino rats weighing 150-180g were used in the present study. They were obtained from the breeding colony maintained at the animal house of the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). Animals had free access to food, water and libitum.

They were maintained at (25 ± 2) °C and 40–60% relative humidity with 12-h light–dark cycle. Animals were subjected to an adaptation period of one week in the animal house before experimentation.

Chemicals:

Ciprofloxacin purchased from Zhrjiang Guobang, (China) was used in a dose (100mg/kg; p.o; once daily for 7 consecutive days) Yoshikawa et al. [19] and Ascorbic Acid "Vit.C" was purchased from El-Nasr Company, (Egypt) was used in a dose (180 mg/kg; p.o, once daily for 1 week) Hathcock et al. [20] were used in this study. All test substances were freshly prepared in saline and orally administrated.

Escherichia coli (E. coli) NCTC 9001 bacterial strain was obtained from Microbiology department in the NODCAR.They were grown on nutrient agar for an incubation period of 24 hours at 37º C in aerobic conditions. The colonies were verified for their viability and purity. This was followed by bacterial sub-culture on nutrient broth for an incubation period of 24 hours at 37º C in aerobic conditions prior to testing. Bacterial viable count was carried out. Turbidity was then adjusted in the Mac Farland range ($1x10^8$ CFU/mL). The test organism (Escherichia coli) activity was confirmed with a pilot work, using a UV-spectrophotometer (Schimadzu, USA) at absorbance of 546 nm. These bacteria served as test pathogens for in vitro assay and in vivo bacterial infected animal model. Each animal in the infected group was injected with 1.8×10^8 CFU/mL i.p for once of E.coli at a dose that kills 10% of rats according to Linde [21] and Virkamäki &Yki-Järvinen [22].

UVA lamp332-370nm (was calibrated at radiometry) and power density 0.11mw/cm2 at a distance of 25cm.They were divided into three groups according to doses 1.98, 2.97, 3.96kJ/m2 at exposure times 30, 45, 60 minutes respectively.

Experiment Design:

At the beginning of the experiment adult male albino rats weighing approximately150-180g were randomly allocated into seven groups. Each group consisted of 8 rats. The animals were treated according to the following scheme:

Group 1, 2 and 3 include normal control rats, UV-A exposed rats for 60min and ciprofloxacin administered bacterially infected non exposed UV-A rats respectively. Group 4, 5 and 6 rats receiving ciprofloxacin+ E.coli in vivo bacterially infected rats exposed to UV-A lamp radiation for 30, 45 and 60 min. respectively, and served as positive control. Group 7 rats receiving ciprofloxacin + E.coli in vivo bacterially infected and exposed to UV-A lamp radiation for 60 min + Vit.C.

In all experiments, dorsal side of shaved rats, using sterile surgical blade, is exposed to UV-A lamp radiation at the 5, 6, 7th days from the beginning of the experiment.

Grade of skin reaction (redness, edema, hemorrhage , shrinkage and oozing) at 24, 48 and 72 hr. after UV-A irradiation were conducted once daily. Skin was excised then, homogenized in ice cold saline using electronic homogenizer (Ezister Daihan Scientific Co., Ltd., Korea) to prepare 10 % w/v homogenate and stored at -80ºC.

For the determination of malondialdehyde (MDA), reduced glutathione (GSH), and Tumor Necrosis Factor-α (TNF-α), the skin homogenates were divided into aliquots after 3000 r.p.m. centrifugation for 15 min at 4°C using cooling centrifuge (Hermile Labortechnik, Wehingen, Germany). Determination of MDA content was carried out according to the method of **Buege and Aust [23]** with a slight modification in the incubation period according to the method of **Denizet et al. [24]**, whereas Glutathione content was determined in skin homogenate according to the method described by **Beutler et al [25]**.The skin TNF-α

was estimated according to **Brenner et al. [26]**, utilizing the commercially available Boster's rat TNF-α ELISA [enzyme-linked immune- sorbent assay] kit. In addition; total leucocytic count took place according to the methods of **Ingram and Minter [27]** using the whole blood, hem cytometer and a special pipette of WBCs.

At the end of experiments (at the 8th day) animals were anaesthetized using ether then blood samples were collected from retro-orbital plexus of each animal using a capillary tube in non-heparinized tubes and serum was separated by centrifugation (MPw-350, Warsaw, Poland) for 20 min at 4000 r.p.m. and stored at -80°C in order to measure liver function parameters such as serum albumin and total protein levels using a test Diamond reagent kit according to the method described by **Burtis and Ashwood [28]** and **Gornal et al. [29]** respectively. Another blood samples were collected from each animal using a capillary tube in heparinized tubes for UV visible spectroscopic analysis.

Histopathological examination:

Autopsy samples were taken from skin of rats in the different experimental groups then fixed in 10% formalin prepared in saline then samples were processed using a graded ethanol series and embedded in paraffin. Paraffin sections were cut into 6µm-thick slices and stained with hematoxylin and eosin (H&E) according **Bancroft et al. [30]** for light microscopic examination.

Statistical Analysis:

Results were expressed as mean ± SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey–Kramer Multiple Comparison Test. Probability values of less than 0.05 were considered statistically significant. Whereas the graphs were drawn using a prism computer program (GraphPad software Inc. V5, San Diego, CA).

3. Results and Discussion

- Skin scoring

Immediately after UVA irradiation of rats' measurement of auricular thickness and erythema score of rat skin were conducted at the same time once daily. The skin scores parameters obtained from animals is presented in **Fig. (1)** these scores are related to redness **(A)**, edema **(B)**, hemorrhage **(C)**, shrinkage **(D)** and oozing **(E)**. It is noticeable that the skin scores increased were in groups with increasing days of exposure. The obtained results indicated that in all of the tested groups all the skin scores were increased in comparison with the normal control group. However, co-administration of VIT.C and ciprofloxacin for 7 consecutive days in Esch. coli infected rats and exposed to UV-A all the skin scores showed a significant decrease and the scores approaches to control values.

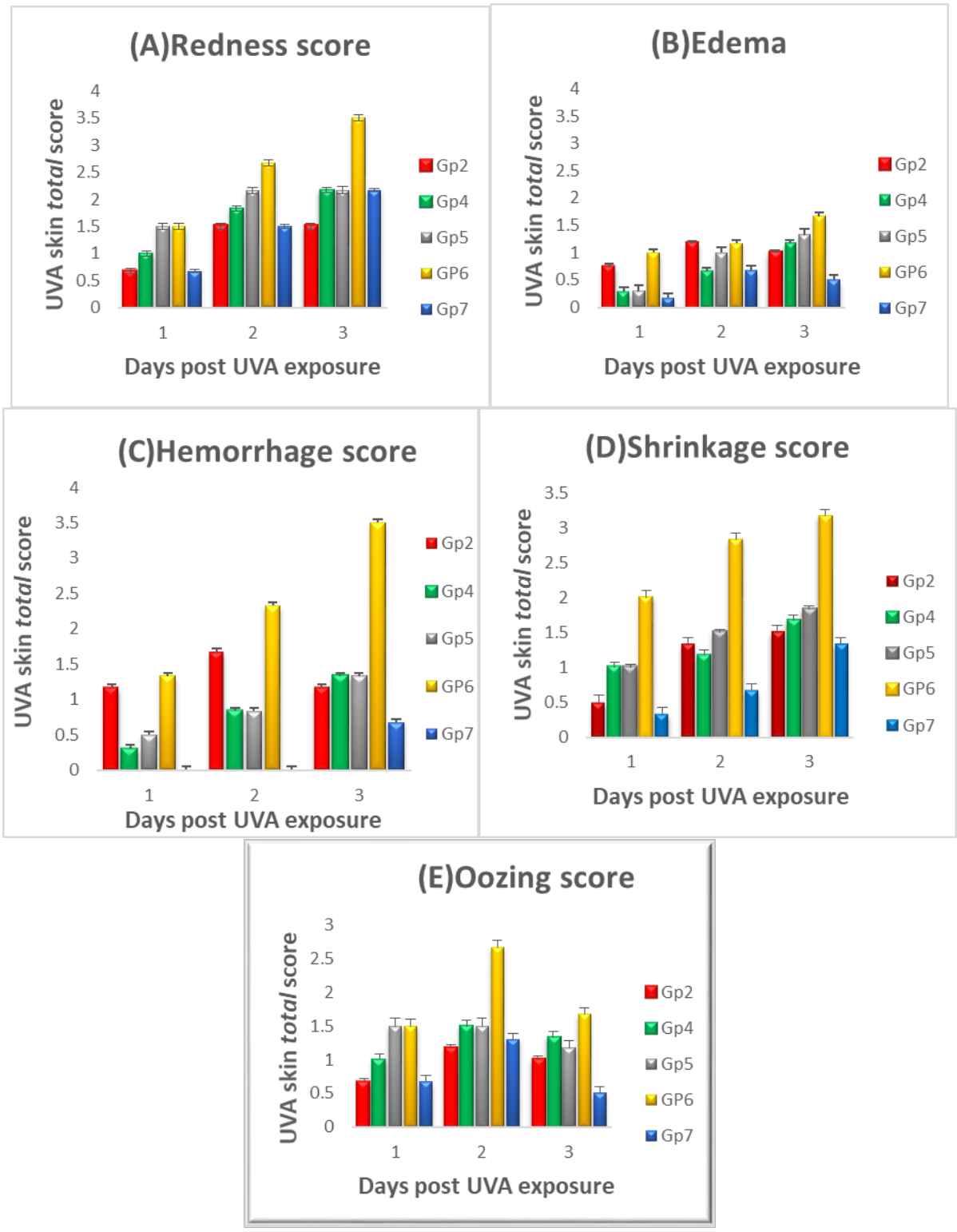


Figure 1. Skin scoring parameters {redness (A), edema (B), hemorrhage (C), shrinkage (D), oozing (E)}
Skin Score: 0 no reaction, 1 mild reaction, 2 moderate reaction, 3 intense reaction, 4 severe reaction
Skin scores increased in groups with days of exposure.

- Oxidative stress and inflammatory biomarkers in the skin homogenate.

In table (1) the obtained results of MDA, GSH and TNF-α contents indicated that in groups 2and 6 the skin MDA and TNF-α contents were increased by 128%, 200% and 88%, 380% respectively accompanied by decreased skin GSH content by 13% and 43% respectively as compared with normal rats. In group 4 and 5 the skin MDA and TNF-α contents were decreased by 55%, 62% and 26%, 30% respectively accompanied by increased skin GSH content by 3%, 26% respectively as compared with group 6. However, co-administration of group7 the skin MDA and TNF-α contents were decreased by 56%, 72% respectively accompanied by skin GSH content were increased by 42% as compared with group 6.

Table 1. skin malondialdehyde (MDA), reduced glutathione (rGSH) and tumor necrosis factor-alpha (TNF-*α*) contents for all groups:

Parameters / ***Groups***	**MDA content** (nmol/g wet tissue)	**GSH content** (nmol/g wet tissue)	**TNF-α content** (pg/g tissue)
1	6.99 ± 0.01	1.62±0.06	4.8 ± 0.01
2	15.94* ± 1.2	1.40* ±0.06	9.05* ± 1.6
3	9.86± .89	1.26*±0.02	15.4*± 2.09
4	7.91# ± 0.33	1.17*#±0.03	16.03*# ± 0.33
5	9.4# ± 0.93	0.91*± 0.02	17.10*# ± 0.93
6	20.68* ± 1.67	0.93* ±0.02	23.02* ± 1.67
7	9.04 #± 0.90	1.32 *# ±0.06	6.4#± 1.63

Data are expressed as means ± SEM of six rats per group

Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test.

*Significantly different from normal group at $p<0.05$.

#significantly different from group 6 (Cipro+E.coli+ UV-A60) at $p<0.05$.

-Total blood leucocytic count (TLC), Serum Total protein (TP) and Albumin(ALB):

In table (2) the obtained results indicated that in groups 2 and 6 the total leukocyte count (TLC) in blood was increased by 45% and 84% respectively accompanied by a decreased serum total protein (TP) and albumin (ALB) levels by 21, 31% ,12%, and21% respectively as compared with normal rats. Group 4and 5 showed decreased TLC by 26% and28% respectively accompanied by increased TP and ALB levels by 9%,28%, 15%and 19% respectively as compared with group 6. However, co-administration in group 7 decreased TLC by 31% accompanied by increased TP and ALB levels by as 27%, 20% respectively as compared group6.

Table 2. Total leucocyte count in blood and serum total protein, albumin levels for all groups.

Parameters / Groups	Total leucocyte count in blood ($x10^3$)	Total Protein Level (g/dl)	Albumin Level (g/dl)
1	4.08 ± 0.27	6.75 ± 0.12	4.31 ± 0.11
2	5.90* ± 0.21	5.30* ± 0.24	3.81* ± 0.14
3	6.87* ± 0.47	5.91*± 0.11	3.85 ± 0.13
4	5.38# ± 0.32	5.96*# ± 0.07	4.07# ± 0.01
5	5.58# ± 0.34	5.11* ± 0.16	3.91# ± 0.10
6	7.53* ± 0.51	4.67* ± 0.20	3.41* ± 0.11
7	5.2# ± 0.33	5.95*# ± 0.17	4.09# ± 0.08

Data are expressed as means ± SEM of six rats per group**.** Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test. *****Significantly different from normal group at $p<0.05$. **#** significantly different from group 6 (Cipro+E.coli+ UV-A60) at $p<0.05$.

-Relative thymus and spleen weight:

The weights of spleen and thymus relative to body weight in all groups are illustrated in fig.2. (A), the obtained results of group 6 indicated that the thymus relative weight to body weight was decreased by 44% as compared with normal rats. In groups, 4 and 5 relative thymus weight to body weight was increased by 64% and 68% respectively as compared with group 6. However, co-administration of Vit C in group7 almost normalized relative thymus weight to body weight as compared with group 6.

In fig.2 (B), the obtained results indicated in groups 2, 5and 6 relative spleen weight were increased by 48, 27and 50% respectively as compared with normal rats. In group 4 relative spleen weight was decreased by 24% was obtained as compared with group 6. However, co-administration of Vit C in group7 indicated almost normalized relative spleen weight as compared with group 6.

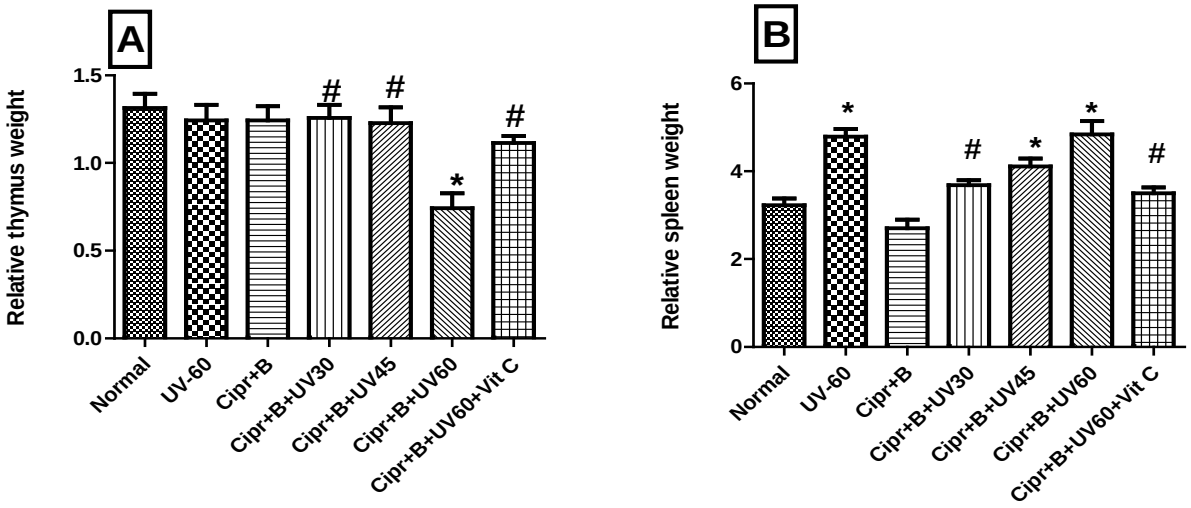
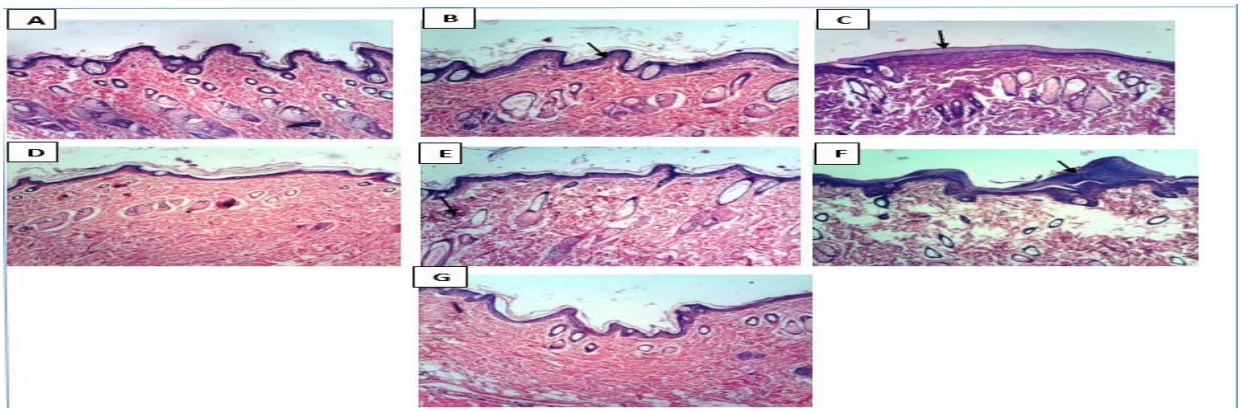


Figure 2. Relative weight of thymus (A) and spleen (B) for all groups.
Data are expressed as means ± SEM of six rats per group
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test.
*Significantly different from normal group at $p<0.05$.
#significantly different from group 6 (Cipro+E.coli+ UV-A60) at $p<0.05$.

Figure 3. Histopathological alterations in skin induced by photosensitivity of ciprofloxacin by UV-A radiation in Esch. coli infected rats.



-Histopathological examination

The histopathological alterations in skin induced due to photosentivity of ciprofloxacin by UV-A radiation in **fig.3 (A):** Skin of rat from group 1 showed no histopathological changes. **(B)**: Skin of rat from group 2 showed slight thickening and vacillations of prickle cell layer. **(C)**: Skin of rat from group 3 showed hyperkeratosis and necrosis of keratin layer. **(D):** Skin of rat from group 4 showed no histopathological changes. **(E, F):** Skin of rat from groups 5 and 6 showed few dermal inflammatory cells infiltration; necrosis of keratin layer respectively. **(G):** Skin of rat from group 7 showed no histopathological changes (H&E ×100).

Photosensitivity skin reactions are evaluated in many studies in which the intensity of all skin reactions was graded on a sensitization score [**31**] [**32**]. A total clinical severity score for skin lesions was defined as the sum of the individual score grades as 0 (none), 1 (mild), 2 (moderate), 3 (intense), 4 (severe) for each of five signs and symptoms (redness, edema, hemorrhage, shrinkage, oozing) **[33] [34] [35]** was considered by **Glucksberg et al. and Wiesmann et al. [36]** [**37**]. In this study, the groups of

animals exposed to UVR-A, showed a higher prevalence of erythema, edema, hemorrhage, oozing and shrinkage. The intensity of different inflammatory skin reactions showed positive correlation between the UVradiation absorption and time of exposure. Ciprofloxacin administration aggravated skin phototoxicity erythema, irritation and cutaneous eruption or inflammation scoring. These observations were in accordance with **Kim et al. [38]** who illustrated that the skin exposure to solar irradiation and photo reactive xenobiotic may produce abnormal skin phototoxicity reaction that are identified by skin irritation, erythema, pruritis, and edema that are similar to those of the exaggerated sunburn. Similar observations were recorded by **Yoshikawa et al.** [**39**] who studied this ciprofloxacin induced dermal phototoxic reactions in microminipigs.

The number of leukocytes in the blood is often an indicator of disease, and thus the white blood cell count is an important subset of the complete blood count **[40]**. Infection is one of the causes of leukocytosis [**41**]. In the present study, total white blood cells count leukocytosis was detected in all UVA exposed animal groups. This increase was significant in UVA60, but non-significant in UVA45 and UVA30 and UVA60 treated with vitamin C. This evidence is in accordance with **EbruÇet et al. [42]** who observed an increase of total leukocyte number in UV acute exposed mice group and mentioned that UVA and UVB exposure induce the production of immunomodulatory cytokines in keratinocytes and melanocytes of the skin that leads to changes in innate immune system. On the other hand, the groups of animals exposed to UVA with concomitant administration of ciprofloxacin, there was an increase in leucocyte count. This increase was significant in UVA60. This finding agreed with **Astrid et al. [43**] who mentioned that ciprofloxacin- treated animals changes are considered to be secondary to UV treatment and not ciprofloxacin related since the group of animals administered only ciprofloxacin showed a reduced white blood cell counts **[44].**

Moreover, exposure of animals to UVA60 significantly increased tumor necrosis factor alpha (TNF-α) content in skin homogenate. This increase was extravagated by concomitant administration ciprofloxacin in infected animals, besides, it was a dose dependent. These findings agreed with **Zhan et al. [45]** Who mentioned that UV exposure increased the contents of the inflammatory cytokines IL-1β, IL-6, IL-10, and TNF-α in the mice skin; in response to UV irradiation keratinocytes secrete cytokines and chemokines, which activate and recruit leukocytes to the skin. **Maverakis et al. [46]** studied the role of thymus T-lymphocyte in the production of TNF-α in septic rats. On the other hand, **Xue et al. [47]** verified that ciprofloxacin resulted in an even higher production of TNF-α and modified systemic and localized immune function.

Due to the ability of sunlight to penetrate the skin layer to reach blood flux, several drugs, both systemic and topic, could represent a risk factor for oxidative damage. This opinion is in line with the present study that showed photosensitivity of ciprofloxacin by UV-A lamp produced an alteration in oxidative stress biomarkers in rat's skin, which was manifested as an increase in malondialdhyde (MDA) accompanied with a decrease in reduced glutathione (rGSH) skin contents. Similar results have been reported in previous studies as **Sophia et al. [48]** that showed that MDA formation has been documented in cultured skin cells and has also been observed in murine and human skin exposed to a wide range of spectral fractions and doses of solar UVradiation. Another possibility is that after acute and chronic UVradiation exposure; the lipid peroxidation product malondialdehyde (MDA) accumulates in tissue under conditions of increased oxidative stress, and the occurrence of MDA-derived protein epitopes, including dihydropyridine-lysine (DHP), has recently been substantiated in human skin and these changes were UV radiation exposure time dependent **[48]**.

In the present study, it was showed that UVA60 and UVA45 significantly changed relative spleen and thymus weights in septic ciprofloxacin treated rats. These findings agreed with **Nasti and Timares and Xiao [49] [50]** who illustrated the role of proinflammatory cytokines in radiation injury showing highly increased IL-1β, IL-18 and IL-33 expression in mouse thymus, spleen and/or bone marrow cells enhancing these cells. **Wang et al. [51]** mentioned that inflammatory circumstances were associated with splenomegaly. **Lu and Zhang [52]** studies proved that suberythemal doses of ultraviolet light could affect the immune system in rodents as well as in humans, photo damage of collagen polypeptide, T-lymphocytes, spleen index, thymus index and lymphopoiesis levels in UV-induced damage stress rats in which enhanced thymus index and spleen index were detected.

Vitamin C acts as a co-factor for the proline and lysine hydroxylases that stabilise the collagen molecule tertiary structure, and it also promotes collagen gene expression **[53] [54] [55]**. In the present study, UVA exposed animal group that was treated with vitamin C there is an absolute predominance of improving all skin scoring criteria including redness, edema, oozing, hemorrhage and shrinkage. This evidence is in accordance with **Eberlein-Konig and Wagener et al. [56] [57]** who mentioned that vitamins C had dramatically increased photoprotective effect against UVA- induced cutaneous damage. This protective effect is obtained when vitamin C is applied either topically or orally **[58]**. Vitamin C-mediated prevention of radiation injury from acute UV exposure is relatively easily demonstrated through quench oxidants and repair the resultant UVA damage **[59] [60]**. Accordingly, this study revealed an immunoprotective effect of vitamin C that was manifested as significant reduction of reticuloendothelial indices of the organs used in the study, spleen and thymus as compared with UVA60 group. This evidence was in accordance with **Carr and Maggini [61]**.

4. Conclusion

Our study concludes that orally administration of Vit.C has prophylactic antioxidant and anti-inflammatory effects against ciprofloxacin induced photo-toxicity on exposure to UV-A radiation in Esch. coli systematic infected rats.

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Conflict of interest

The authors declare no conflict of interest.

References

[1]. Seto Y, Hosoi K, Takagi H (2012). Exploratory and regulatory assessments on photo safety of new drug entities. Curr Drug Saf; 7: 140-148.

[2]. Bracchitta G, Catalfo A, Martineau S (2013). Investigation of the phototoxicity and cytotoxicity of naproxen, a non-steroidal anti-inflammatory drug, in human fibroblasts. Photochem Photobiology Sci; 12: 911-22.

[3]. Priya TB, Divya V and Jyothi KN (2016). Effects of Environmental Parameters on Efficiency of the Solar Photovoltaic Cell academia.edu. Advances in Life Sciences.

[4]. Valeur B and Berberan-Santos (2012). Molecular fluorescence: principles and applications.

[5]. Sage E, Girard PM and Francesconi S (2012). Unravelling UVA-induced mutagenesis. Photochemical &Photobiological Sciences; 11(1): 74-80.

[6]. Juzeniene A and Moan J (2012). Beneficial effects of UV radiation other than via vitamin D production. Dermato-endocrinology; 4(2):109-117.

[7]. World Health Organization (WHO) (2016)."The known health effects of UV".

[8]. Antony R.Young, Joël Claveau, Ana Beatris Rossi (2017). Ultraviolet radiation and the skin: Photobiology and sunscreen photoprotection. J Am Acad Dermatol; 76:S100- S109.

[9]. Kyriacos C Nicolaou and Stephan Rigol (2018). A brief history of antibiotics and select advances in their synthesis-The Journal of Antibiotics; 71: 153–184.

[10]. James C. Sacchettini, Eric J. Rubin, Joel S. Freundlich (2008). Drugs versus bugs: in pursuit of the persistent predator Mycobacterium tuberculosis-Nature Reviews Microbiology; 6: 41–52.

[11]. Heeb S, Fletcher MP; Chhabra SR, Diggle SP, Williams P, Cámara M (2011). Quinolones: from antibiotics to auto inducers. FEMS Microbiology Reviews; 35(2): 247–274.

[12]. Jourdan M Cancienne, Stephen F Brockmeier, Scott A Rodeo, Chris Young and Brian C Werner (2017). Early postoperative fluoroquinolone use is associated with an increased revision rate after arthroscopic rotator cuff repair. Knee Surgery, Sports Traumatology, Arthroscopy; 25(7): 2189-2195.

[13]. Chhorvoin Om, Frances Daily, Erika Vlieghe, James C. McLaughlin and Mary-Louise McLaws (2017). Pervasive antibiotic misuse in the Cambodian community: antibiotic-seeking behaviour with unrestricted access. Antimicrobial Resistance & Infection Control; 6(1): 30.

[14]. Sun YW1, Heo EP, Cho YH, Bark KM, Yoon TJ, Kim TH (2001). Pefloxacin and ciprofloxacin increase UVA-induced edema and immune suppression; 17(4):172-177.

[15]. Filipe Monteiro, Margarida Rato and César Martins (2016). Drug-induced photosensitivity: Photoallergic and phototoxic Reactions-Clinics in dermatology; 34(5): 571-581.

[16]. Michael Lawson, Klaudia Jomova, Patrik Poprac, KamilKuča Kamil, Musílek and Marian Valko (2017). Free Radicals and Antioxidants in Human Disease.

[17]. Ameye LG and Chee WSS (2006). Osteoarthritis and nutrition. From nutraceuticals to functional foods: a systematic review of the scientific evidence. Arthritis research & therapy; 8(4), R127.

[18]. Domenico Mastrangelo, Lauretta Massai, Giuseppe Fioritoni and Francesco Lo Coco (2016). Vitamin C against Cancer. Intechopen; 68746.

[19]. Yoshikawa T, Takahashi Y, Kawaguchi H, Utsunomiya S, Miura N, Izumi H, Miyoshi N, Tanimoto A (2013). A dermal phototoxicity study following intravenous infusion administration of ciprofloxacin hydrochloride in the novel microminipigs. Toxicologic pathology; 41(1): 109-113.

[20]. Hathcock JN, Azzi A, Blumberg J, Bray T, Dickinson A, Frei B, Jialal I, Johnston CS, Kelly FJ, Kraemer K, Packer L (2005). Vitamins E and C are safe across a broad range of intakes. The American journal of clinical nutrition; 81(4): 736-745.

[21]. Linde K (1978). Use of attenuated mutants of Pasteurella multocida strain, pathogenic to calves, in experimental mouse model studies. 4. Immunogenicity of selectlrevertants of temperature-sensitive, streptomycin-dependent mutants as potential vaccine strains. Arch ExpVet; 32: 943-949.

[22]. Virkamaki A and Yki-Jarvinen H (1994). Mechanisms of insulin resistance during acute endotoxemia. Endocrinology; 134: 2072–2078.

[23]. Buege JA and Aust SD (1978). Microsomal lipid peroxidation. Methods Enzymol; 302-310.

[24]. Deniz S, Erdincler AS, Figen I, Tanju B and Gulden G (1997). Lipid peroxidation and oxidant status in experimental animals: effect of aging and hypercholestrolemic diet. Clin Acta; 265: 77-84.

[25]. Beutler E, Duron O and Kelly BM (1963). Improved method for determination of blood glutathione. J Lab Clin Med; 61: 882-888.

[26]. Brenner DA, O'Hara M, Angel P, Chojkier M and Karin M (1989). Prolonged activation of jun and collagenase genes by tumor necrosis factor-α Nature; 337:661–663.

[27]. Ingram M and Minter FM (1969). Semiautomatic preparation of cover glass blood smears using a centrifugal device. Am J Clin Pathol; 51(2): 214-221.

[28]. Burtis CA and Ashwood ER (1999). Tietz Text book of clinical chemistry.W.B. Saunders (3rd edn); 1400.

[29]. Gornal AG, Bardawill CJ, David MM (1949). Determination of serum protein by means of Biuret reaction. J Biol Chem; 177: 571-576.

[30]. Bancroft JD, Stevens A, and Turner DR (1996). Theory and practice of Histological Techniques (4th edition). pp. 99-112.

[31]. Owen K (1998). Comparative grepafloxacin phototoxicity in mouse skin. J Antimicrob Chemother; 42: 261–264.

[32]. He Q, Fang G, Wang Y, Wei Z, Wang D, Zhou S, Fan S, Yuan Z (2006). Experimental evaluation of cyadox phototoxicity to Balb/c mouse skin. Photodermatol Photoimmunol Photomed; 22(2): 100-104

[33]. Matsuda H, Watanabe N, Geba GP (1997). Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. Int Immunol; 9: 461-466.

[34]. Sumiyoshi K, Nakao A, Ushio H (2002): Transforming growth factor-β1 suppresses atopic dermatitis- like lesions in NC/Nga mice. Clin Exp Allergy; 31: 309-314

[35]. Miyauchi-Hashimoto H, Okamoto H, Sugihara A, Horio T(2005). Therapeutic and prophylactic effects of PUVA photochemotherapy on atopic dermatitis-like lesions in NC/Nga mice. Photodermatol Photoimmunol Photomed; 21(3):125-130.

[36]. Glucksberg H, Storb R, Fefer A (1974). Clinical manifestation of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. Transplantation; 18: 295-304.

[37]. Wiesmann A, Weller A, Lischka G, Klingebiel T, and Kanz L, Einsele H (1999). Treatment of acute graft-versus-host disease with PUVA (psoralen and ultraviolet irradiation): results of a pilot study. Bone Marrow Transplant; 23(2): 151-155.

[38]. Kim K, Park H, Lim KM (2015). Phototoxicity: Its Mechanism and Animal Alternative Test Methods. Toxicol Res; 31(2): 97-104.

[39]. Yoshikawa T, Takahashi Y, Kawaguchi H, Utsunomiya S, Miura N, Izumi H, Miyoshi N, Tanimoto A (2013). A dermal phototoxicity study following intravenous infusion administration of ciprofloxacin hydrochloride in the novel microminipigs. Toxicol Pathol; 41(1): 109-113.

[40]. Maton D, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, Wright JDand Kulkarni DV (1997). Human Biology and Health. Englewood Cliffs, New Jersey, US: Prentice Hall.

[41]. Goldman L and Schafer AI (2012). Goldman's Cecil medicine (24th Ed.). Philadelphia. 978.0.8089.2437.1(Interantional Edition)

[42]. Ebru Çet n and Çi dem Alt nsaat (2006). Effects of Ultraviolet Radiation on Some Immunological Parameters in Rats American-Eurasian J Agric & Environ Sci; 1(1): 31-36.

[43]. Astrid A Reus, Mustafa Usta, Julia D Kenny, Peter J Clements, Anthony M Lynch and Cyrille AM Krul (2012). The in vivo rat skin photomicronucleus assay: phototoxicity and photogenotoxicity evaluation of six fluoroquinolones. Mutagenesis; 27(6): 721-729.

[44]. Fuller CJ, May MA and Martin KJ (2000). The effect of vitamin C and vitamin E supplementation on LDL oxidizability and blood cells in young smokers. JAm Coll Nutr; 19(3): 361-369.

[45]. Zhan JYX, Wang XF, Li YH (2016). Andrographolide Sodium Bisulfate Prevents UV-Induced Skin Photoaging through Inhibiting Oxidative Stress and Inflammation. Mediators of Inflammation; 2016: 3271451.

[46]. Maverakis E, Miyamura Y, Bowen MP, Correa G, Ono Y, Goodarzi H (2010). Light, Including Ultraviolet. Journal of autoimmunity; 34(3): J247-J257.

[47]. Xue H, Field CJ, Sawyer MB, Dieleman LA, Baracos VE.(2009)- Prophylactic ciprofloxacin treatment prevented high mortality, and modified systemic and intestinal immune function in tumour-bearing rats receiving dose-intensive CPT-11 chemotherapy. British Journal of Cancer; 100(10):1581-1588.

[48]. Sophia L Park, Rebecca Justiniano, Joshua D Williams, Christopher M Cabello, ShuxiQiao and Georg T Wondrak (2015). The Tryptophan-Derived Endogenous Aryl Hydrocarbon Receptor Ligand 6-Formylindolo [3, 2-b] Carbazole Is a Nanomolar UVA Photosensitizer in Epidermal Keratinocytes. Journal of Investigative Dermatology; 135(6): 1649-1658.

[49]. Lamore SD, Azimian S, Horn D, Anglin BL, Uchida K, Cabello CM, Wondrak GT (2010). The malondialdehyde-derived fluorophore DHP-lysine is a potent sensitizer of UVA-induced photooxidative stress in human skin cells. J of Photochemistry and Photobiology Biology; 101(3): 251-264.

[50]. Nasti TH and Timares L (2012). Inflammasome activation of IL-1 family mediators in response to cutaneous photodamage. Photochem Photobiol; 88(5): 1111-1125.

[51]. Xiao M (2016). The Role of Proinflammatory Cytokine Interleukin-18 in Radiation Injury. Health Physics; 111(2): 212-217.

[52]. Wang C, Xu CX, Alippe Y (2017). Chronic inflammation triggered by the NLRP3 inflammasome in myeloid cells promotes growth plate dysplasia by mesenchymal cells. Scientific Reports; 7: 4880.

[53]. Lu YM and Zhang H (2012): Effects of electroacupuncture on T-lymphocytes, spleen index, thymus index and lymphopoiesis levels in strenuous exercise-induced stress rats. Zhen Ci Yan Jiu; 37(2): 136-139.

[54]. Kishimoto Y, Saito N, Kurita K, Shimokado K, Maruyama N, Ishigami A(2013). Ascorbic acid enhances the expression of type 1 and type 4 collagen and SVCT2 in cultured human skin fibroblasts. Biochem Biophys Res Commun; 430(2): 579-584.

[55]. Hinek A, Kim HJ, Wang Y, Wang A (2014). Mitts TF Sodium L-ascorbate enhances elastic fibers deposition by fibroblasts from normal and pathologic human skin. J Dermatol Sci; 75(3): 173-182.

[56]. Pullar JM, Carr AC, Vissers MCM (2017). The Roles of Vitamin C in Skin Health. Nutrients; 9 (8): 866.

[57]. Eberlein-Konig B and Ring J (2005). Relevance of vitamins C and E in cutaneous photoprotection. J Cosmet Dermatol; 4: 4-9

[58]. Wagener FADTG, Carels CE, Lundvig DMS (2013). Targeting the Redox Balance in Inflammatory Skin Conditions. International Journal of Molecular Sciences; 14(5): 9126-9167.

[59]. Placzek M, Gaube S, Kerkmann U, Gilbertz KP, Herzinger T, Haen E, Przybilla B (2005). Ultraviolet B-induced DNA damage in human epidermis is modified by the antioxidants ascorbic acid and D-alpha-tocopherol. J. Investig. Dermatol; 124: 304-307.

[60]. Miura K and Green AC (2015). Dietary antioxidants and melanoma: Evidence from cohort and intervention studies. Nutr Cancer; 67: 867-876.

[61]. Carr AC and Maggini S (2017). Vitamin C and Immune Function. Nutrients; 9 (11): 1211.