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RESEARCH ARTICLE

Ultraviolet Type B-Radiation-Induced Hyperplasia and Seborrheic Keratosis is Reduced by Application of Commercial Sunscreens

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ABSTRACT

Fifty-six mice were classified into four groups; Group A (control group, n=8), Group B (exposure group, n=16), Group C (n=16) treated with sunscreen 15 minutes before UVB irradiations and group D (n=16) sunscreen treated 60 minutes before UVB exposure. Mice were irradiated 30 minutes 5days/week (12 weeks), and group C-D treated five days/week (12 weeks). Skin samples were taken in the mid and end of the experiment. The result of this study revealed that, epidermal thickness in group A was 7.155µm. At the mid-period of the experiment, severe epidermal hyperplasia was observed in group B with epidermal thickness 118.712µm, while in group C and D mild to moderate epidermal hyperplasia were noted with decreasing epidermal thickness to 64.154 and 90.042µm respectively. At the end of the experiment in Group B epidermal thickness reached to 281.35µm with seborrheic keratosis development, whereas in group C and D totally inhibited the development of seborrheic keratosis and epidermal thickness decreased again into 42.347 and 55.915µm. In conclusion, chronic UVB radiation-led to epidermal hyperplasia and seborrheic keratosis, sunscreen prevented the development of seborrheic keratosis and decreased the UVB-induced epidermal hyperplasia.

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INTRODUCTION

In recent years, skin cancer has become a threat to the population. Solar ultraviolet (UV) rays has been recognized as a potential hazard for human and animals health because of its genotoxic, mutagenic, carcinogenic and immuno-toxic properties (Tarras-Wahlberg *et al.*, 1999). UV radiation is subdivided into three distinct bands: Ultraviolet type A (UVA, 320-400nm), ultraviolet type B (UVB, 290-320nm) and ultraviolet type C (UVC, 200-290). The adverse effects of UV radiation associate with the wavelengths concerned, each has different penetration properties and potential for damage (Ouhtit *et al.*, 2000; Anitha, 2012). Prolong exposure to UV radiations has severe pathologic effects, including erythema, edema, hyperplasia, immuno-suppression, hyperpigmentation, skin aging and eventually cutaneous malignancies. UVA penetrates deeply into the dermis in opposite to, UVB that is mostly absorbed by the epidermis, with comparatively little reaching the dermis (Sheipouri *et al.*, 2012; D'Orazio *et al.*, 2013). UVB is a total carcinogen that can initiate, promote and advance the development of skin cancer (Saeed and Salmo, 2012).

The major function of skin care products is to decrease the harmful effects of UV radiation and prevent them completely (Korać and Khambholja, 2011). Sunscreen products are principally manufactured to protect the skin from the harmful effects of solar UV radiation. The components of sunscreen play a role in absorbing, reflecting, or scattering UV rays. Sunscreens are highly effective in protecting against sunburn, and they are thought to protect against the induction of skin cancer, mainly by reducing DNA damage caused by UV radiation (Ullrich *et al.*, 2002). Based on their mechanism of protective action, sunscreens are broadly divided into chemical and physical. A chemical sunscreen absorbs the UV rays, whereas the physical sunscreen reflects the harmful rays away from the skin like a temporary coat of armor (Korać and Khambholja, 2011; Anitha, 2012). There have been many reports that the application of sunscreens before UV exposure inhibits the photoaging of skin in animal models and humans (Tsukahara *et al.*, 2005). The aim of this study was to demonstrate the effect of regular use of sunscreen against the occurrence of epidermal hyperplasia and seborrheic keratosis.

MATERIALS AND METHODS

Animal model: Fifty-six albino mice of *Mus musculus* species, BALB/c strain of both sexes with the same ages (3-4 weeks) were divided into four groups: Group A (n=8); was regarded as a control group (without treatment and exposure to UVB light), Group B (Exposure group, n=16) which was exposed to UVB light only, Group C (n=16) was treated with sunscreen and left for 15 minutes then exposed to UVB radiation and Group D (n=16) also treated by sunscreen and left for 60 minutes (1 hour) then exposed to UVB radiation. Animals were housed in animal house, Department of Biology, School of Science, Sulaimani University under a controlled room temperature of about 25ºC and photo-periodicity of 12 hours light/dark system. Animals had free access to food and water. The experimental protocol was evaluated and approved by the ethics committee from the College of Veterinary Medicine/Sulaimani University.

UVB lamp: The source of the radiation was a lamp of 312nm wavelength, 15 Watts, VILBER-LOURMAT-FRANCE, with a calculated power 80mj/sec. Mice from all groups except the control group were exposed to UVB light for 30 minutes 5 d/wk (12 weeks), and this was done after shaving the mouse's dorsal skin (2X5 cm).

Sunscreen: The sunscreen, formulated by Fabriqué Par: Pella Pharmaceuticals Company/Jordan, which was a commercial waterproof formulation with labeled 50 SPF and claimed to offer broad spectrum, UVB/UVA protection. The active ingredients were included: Aqua, Glycerin, Isopropyl Myristate, Cetostearyl Alcohol & Ceteareth-20, Micronized Avobenzone, Micronized Titanium Dioxide, Glyceryl Monostearate, Sodium Phosphate Dibasic Dihydrate, Citric Acid, Methyl Paraben, Propyl Paraben, Propyl Paraben and Butylated Hydroxyanisole.

Animals from treatment groups (Group C and D) were treated with Sunscreen 5 d/wk (12 weeks) by painting the shaved area with a variable duration of pretreating such as Group C treated with sunscreen 15 minutes before exposing to UVB light while Group D was treated 1 hour before exposure.

Sample collection: Samples were taken in two different periods; first in the mid of experiment (6 weeks) included all of the control groups and a half of the other group (B-D), Second at the end of the experiment (12 weeks) the remainder half of group B-D. Mice were euthanized by cervical dislocation to take biopsies from the shaved area in dorsal skin, the samples immediately fixed at 10% neutralized formalin, processed, and embedded in paraffin blocks which were stained with Haematoxylin and Eosin stains to examine microscopically for full epidermal thickness in all groups.

Histomorphometry: Sections from the dorsal skin were examined under a light microscope; the measurement and calculation of histological sections were carried out using an image analyzer (Scope image software 9.0 "H_3D" computer system-England, digital binocular compound microscope) in the Histology Department, College of Science, Salahaddin University, Erbil. The full epidermal thickness was determined via the measuring the length of epidermis from the top stratum corneum to the bottom of stratum basale or rete-ridges in five different Medium Power Fields (X100) representing the lesion, and then the mean was calculated for each sample.

Statistical analysis: Differences between means were estimated for statistical significance using T-test (Independent and Paired) and Pearson Correlation Coefficient for determining the relationship among the groups at $P \leq 0.05$ using statistical package SPSS version 15. The results were expressed as mean±SE of the mean.

RESULTS

Group A: Gross examination of mice's skin in the control group appeared as normal, which showed a very thin skin. Microscopical examination revealed normal thin epidermis (2-3 layers) with the appearance of the dermis and its skin appendages (Fig. 1). The epidermal measurement for the cases of the control group showed in Table 1, with the group means 7.155±0.036 that was used for comparison to the other groups.

Group B: The gross pathological examination revealed variable lesions throughout the experiment period, which were started by erythema, skin swelling, increasing skin thickness that was detected through palpation, loss of hair, ulcer and friable crusts with yellow to brown color. Microscopical examination showed increasing epidermal layers in the epidermis, which was observed in the mid of the experiment (6 weeks) and regarded as severe epidermal hyperplasia and with infiltration of mononuclear inflammatory cells in the dermis such as macrophages and lymphocytes. UVB-induced epidermal benign tumor at the end of the experiment which was seborrheic keratosis acanthotic type in all cases, the characteristic of this tumor includes increasing the thickness of stratum corneum (Hyperkeratosis), thickening of epidermis due to the basaloid cell proliferation (acanthotic) with embedding multiple cysts filled with keratin called Horn cyst (Fig. 2-3).

Table 1: Epidermal thickness measurement (µm) for different cases in all groups

Cases	Group A	Groups (Experimental period in weeks)					
		B	C	D	B	C	D
		6	6	6	12	12	12
1	6.96	135.88	58.2	76.6	372.34	31.82	60.1
2	7.05	120.4	60.44	86.38	309.532	40.22	59.8
3	7.14	120.284	60.87	86.76	294.31	40.3	56.304
4	7.18	118.9	62.462	87.3	291.24	43.058	55.8
5	7.19	117.126	62.462	88.7	273.3	44.28	54.54
6	7.22	116.288	63.4	95.4	257.29	45.5	53.94
7	7.24	113.424	70.3	99.4	254.36	45.5	53.8
8	7.26	107.4	75.1	99.8	198.434	48.1	53.04
Mean±SEM*	7.155±.036	118.712±2.879	64.154±1.999	90.042±2.757	281.350±17.721	42.347±1.776	55.915±.957

*Mean±SEM.

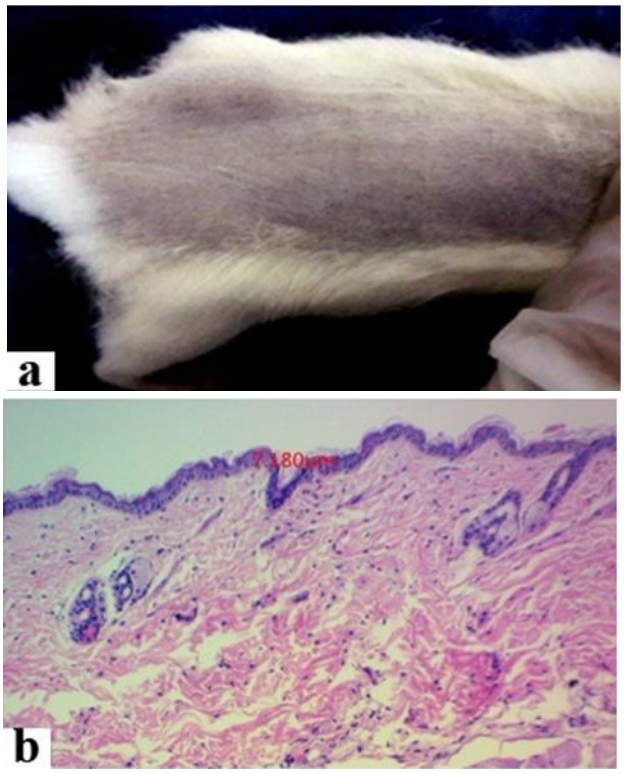

Fig. 1: Normal mouse skin appearance (a), (b) Normal mouse skin histology (H&E stains, X100).

Table 2: Increasing epidermal thickness times in two different periods of the experiment on the basis of epidermal thickness in the control group (group A).

Groups	6 weeks	12 weeks	6 weeks Vs. 12 weeks
Group A	1	1	1
Group B	16.591	39.322	2.370
Group C	8.966	5.918	1.514
Group D	12.584	7.814	1.610

The full epidermal thickness in the Group B at the mid-period of the experiment was 118.712 (Table 1-2), which was 16.591 times greater than control group (F=5.723, $r_{pearson}$=-0.923, P=≤0.031), while at the end of the experiment was equaled to 281.350 which was 39.322 times greater than normal (F=9.369, $r_{pearson}$=-0.931, P=≤0.000) and 2.370 times increased when compared with the results of 6 weeks (t=-10.893, $r_{pearson}$=0.974, P=0.000), which were statistically significant.

Group C and D: Gross lesions were also varied according to the severity of lesions, such as increasing skin thickness as observed through palpation, loss of hair in some regions, particularly in those cases that had moderate and severe lesions, while some cases showed intact skin without obvious gross lesions. Microscopical examination in group C showed an increase in epidermal thickness (Fig.4); in the mid period of the experiment was 64.154µm, indicated statistically significant. When this result compared to Group B at the same period of the experiment, 15 minutes pre-treatment with sunscreen about 7.5 times reduced the harmful effects of UVB on mouse skin ($r_{pearson}$=-0.824, P=0.012). This meant that there was a significant correlation between group B and C in opposite directions at the mid-period of the examination. At the end of the experiment, the epidermal thickness in group C became 42.347µm that was 5.918

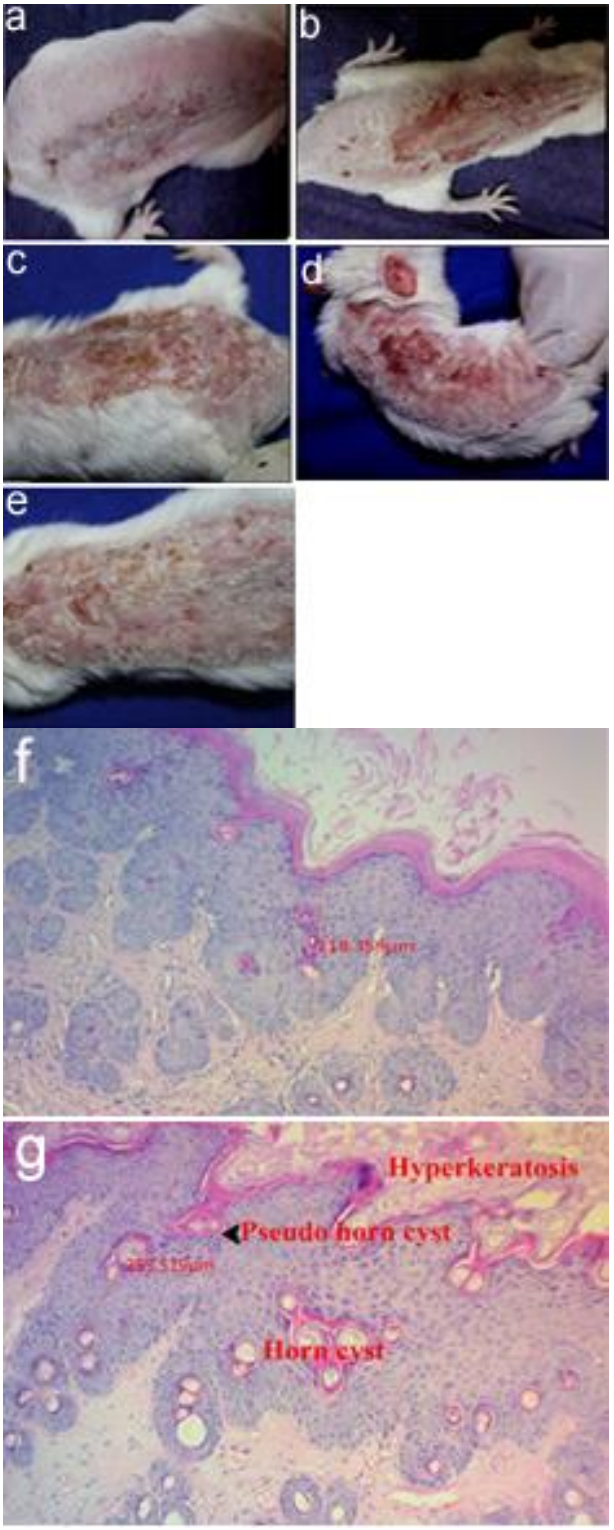

Fig. 2: (a-e) Variable gross lesions in exposed group which included (a-b) Increasing skin thickness with deep ulcers, (c-e) Friable crusts with yellow to brown coloration of the skin, (f) Increased epidermal thickness at 6 weeks of the experiment regarded as severe epidermal hyperplasia, and (g) Histological view of Seborrheic keratosis at 12 weeks of the experiment (H&E stains, X100).

times greater than the control group (F=10.544, $r_{pearson}$=0.962, P=0.006), and 1.5 times less than the epidermal thickness in the mid of the experiment (t=16.737, $r_{pearson}$=-0.977, P=0.000). This demonstrated that prolong pre-treatment (15 minutes) more effective in reducing adverse effects of UVB (Table 2), this measurement when compared to group B at the same

period there was a significant correlation between them ($r_{pearson}$=-0.959, P=0.000). The epidermal measurements in group D in the mid period of the experiment were 90.042µm, which was 12.584 times greater than control (F=16.177, $r_{pearson}$= 0.913, P=0.001) and four times less than of the exposed group with the same period ($r_{pearson}$=-0.93, 0.001). While at the end it was 55.915µm which was 7.814 times greater than control (F=14.912, $r_{pearson}$=-0.973, P=0.002), and 32 times reduced the harmful effect of UVB ($r_{pearson}$=0.893, P=0.003), also it was 1.610 times less than the epidermal thickness at the mid of experiment (t=-5.085, $r_{pearson}$=-0.896, P=0.001). The dermis in both groups (C&D) showed the decreasing number of inflammatory cells in comparison to the exposure group, especially in mild cases (Fig. 3).

DISCUSSION

Skin exposure to UV radiation is known to induce many biochemical changes including DNA damage (Mulliken *et al.*, 2012), activation or inactivation of various enzymes or proteins (El-Abaseri *et al.*, 2006), generation of reactive oxygen species (ROS) that subsequently induce skin inflammation (Guahk *et al.*, 2010), photoaging (Xu and Fisher, 2005) and skin cancer development (Soehnge *et al.*, 1997), whereas these changes depend on many variables including wavelength, dose, race, and features of the skin (Svobodova *et al.*, 2006).

The results of our study showed that exposure of mice to UVB irradiation for six weeks produced various degrees of epidermal hyperplasia along the exposed skin surface. This observation is in agreement with the previous studies which revealed that chronic UV radiation produces variable skin lesions including epidermal hyperplasia (Jin *et al.*, 2010; Saeed and Salmo, 2012; Hassan *et al.*, 2015; Hassan *et al.*, 2015). It has been reported that UV-induced activation of Epidermal Growth Factor Receptor (EGFR) increases keratinocyte proliferation and decreases apoptosis, leading to epidermal hyperplasia (El-Abaseri *et al.*, 2006; El-Abaseri and Hansen, 2007; Huang *et al.*, 2010). At the end of experiment mice in exposed group showed a benign epidermal tumor. A significant correlation was found between chronic UVB irradiation and seborrheic keratosis development, two previous studies confirmed that prolong skin exposure to UVB light induces seborrheic keratosis in mice (Haw *et al.*, 2009; Saeed and Salmo, 2012).

The regular use of sunscreens has been suggested by many public health care practitioners as a mean to decrease skin lesions produced by UV radiation. Sunscreens reduce the frequency of tumors induced experimentally in animals exposed to UV radiation.

It has been known that sunscreens postpone sunburns (Westerdahl *et al.*, 2000), decrease some UV-induced skin lesions, such as photoaging (Autier *et al.*, 2007), actinic keratosis, and nonmelanoma tumors (Darlington *et al.*, 2003; van der Pols *et al.*, 2006). While the role of sunscreen in preventing melanoma remains controversial (Mulliken *et al.*, 2012), Green *et al.*, showed that melanoma in adults may be prevented by regular use of sunscreens (Green *et al.*, 2011). Up to our knowledge till the time of this study no previous studies have mentioned the role of sunscreen in preventing seborrheic keratosis thus our study for the first time proved it.

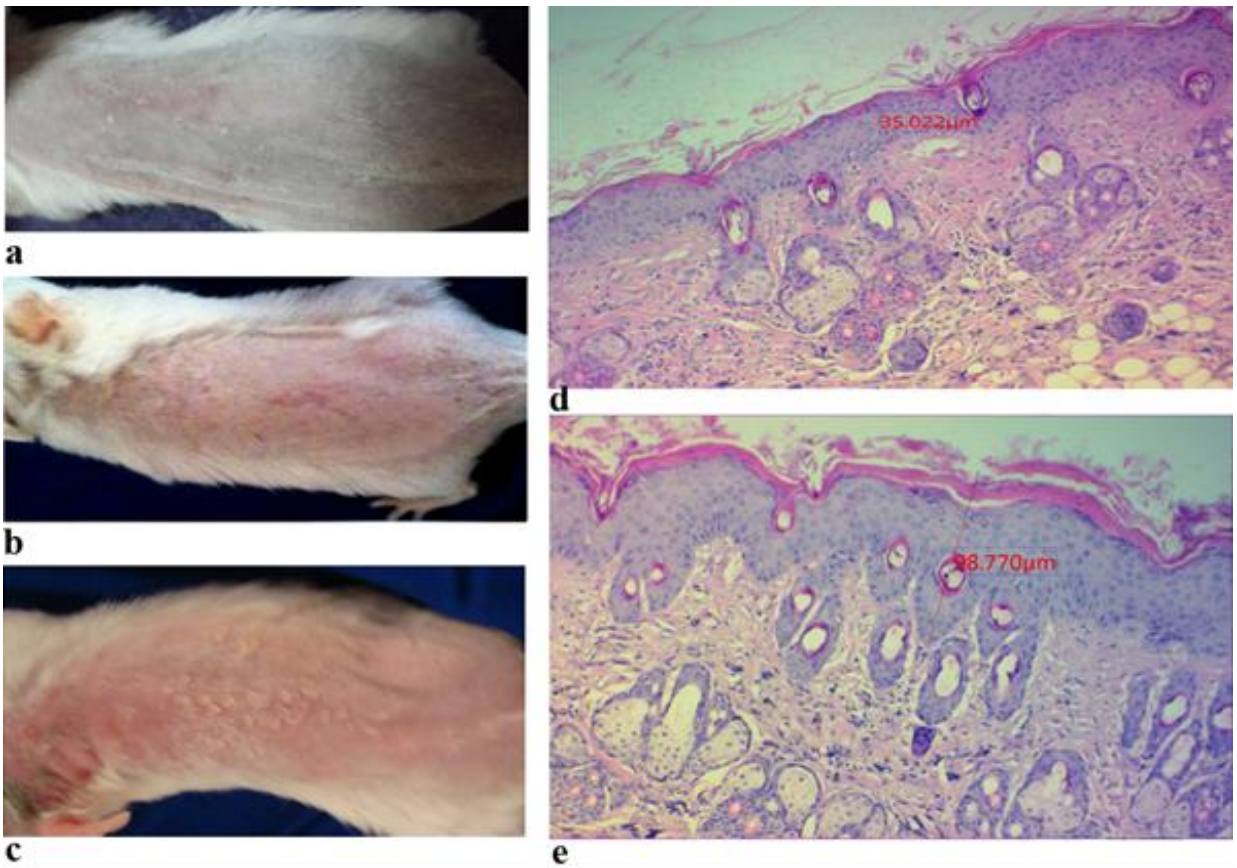

Fig. 4: Variable gross appearance of mouse skin in the treated groups (a-c), (d) and (e) showing the epidermal thickness in the treatment groups (H&E stains, X100).

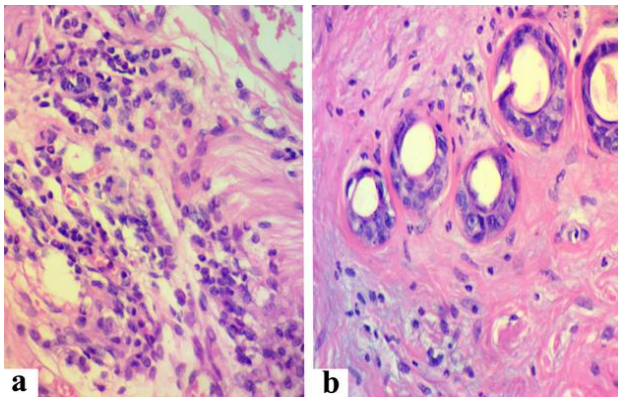

Fig. 3: (a) Severe dermal inflammation, (b) Moderate dermal inflammation (H&E stains, X400).

This study demonstrated that prolong application of sunscreen before UVB exposure significantly reduced epidermal hyperplasia but not abolishing it in both groups (C and D). This finding was in agreement with a previous research which demonstrated that application of sunscreen before UV exposure in hairless mice did not prevent the photodamage to the level of the unirradiated group (Tsukahara *et al*., 2005).

Conclusions: Application of sunscreen in the short period before UVB exposure was more protective than a long period of the application before UVB exposure. Sunscreen prevented the development of seborrheic keratosis and decreased the UVB-induced epidermal hyperplasia.

Author's contribution: AK and SMA conceived and designed the project. AK, SMA and NA executed the experiment and analyzed the tissue samples. NA Maaruf analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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