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Research Article
Photoprotective Effects of Oral L-Glutathione Supplementation on Epidermal Hyperplasia in UVB Irradiated Balb/C Mice

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Abstract

Background and Objective: Extensive exposure of skin to ultraviolet radiation B (UVB) causes early inflammatory response and epidermal hyperplasia. L-Glutathione is an antioxidant and anti-inflammatory agent that can protect skin from photodamage. This study aimed to determine the protective effect of oral L-glutathione supplementation on UVB induced Balb/c. **Materials and Methods:** Eighteen female BALB/c mice were randomly divided into 3 groups: (1) Control group (n = 6), without UVB irradiation and L-glutathione administration; (2) UVB irradiated group (n = 6), irradiated with UVB dose of 250 mJ/cm^2 for 3 minutes and (3) treatment group (n = 6), irradiated with UVB and treated with 0.02 mL of 100 mg kg^{-1} of L-glutathione by oral gavage. The treatment was given for 14 days and UVB irradiation was given on days 9, 11 and 13. **Results:** Mice irradiated with UVB and treated with L-Glutathione showed prominent reduction of skin scaling and erythema. Oral supplementation of L-glutathione significantly reduced the skinfold thickness ($p<0.05$) as compared to UVB-irradiated group. Mice irradiated with UVB and treated with L-Glutathione showed reduced leukocyte infiltration and epidermal hyperplasia. **Conclusion:** Oral supplementation of L-glutathione can influence the cutaneous and early inflammatory response to UVB irradiation. Therefore, L-glutathione has the potential to be developed as a photoprotective agent.

Key words: Antioxidant, epidermal hyperplasia, erythema, L-glutathione, photoprotection, skin scaling, ultraviolet radiation B

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As skin is our outermost organ, it is most susceptible to such environmental stressors as solar ultraviolet radiation (UV), which is a carcinogen. The skin serves as an interface between internal homeostasis and environmental stressors[1] such as UV. The UV spectrum includes UVA (315-400 nm), UVB (280-315 nm) and UVC (200-280 nm)[2]. In the UV spectrum, UVB is considered the most damaging and genotoxic to the skin[3]. UVB is mainly absorbed in the epidermis which consists mainly of keratinocytes. UVB causes keratinocytes to undergo cell cycle arrest. When cells are damaged, they require time to repair or undergo apoptosis, if the damage is irreversible. Epidermal keratinocyte cell proliferation is then accelerated and can lead to epidermal hyperplasia[4-6]. Besides, UVB irradiation can also affect the various parts of the skin, causing edema, skin scaling and skin cancer[7,8].

There are many ways to block the exposure of skin to ultraviolet radiation. One way is to use topical commercial sunscreens formulated as cream and lotions. However, these topical sunscreens are not effective as they have short half-life and they require frequent reapplication[9,10]. Researchers have discovered that some commercial sunscreens contain such preservatives as paraben and methylparaben which aid in increasing the half-life of the sunscreens and prevent contamination by microorganisms. However, methylparaben can induce oxidative stress, cellular lipid peroxidation and cell death[11-13].

Oral antioxidant supplementation is increasingly used to maintain optimal body function. It has been shown to be an effective strategy to inhibit oxidative stress and reduce DNA damage brought about by excessive exposure to UVB[14,15]. L-Glutathione, also known as reduced glutathione, is a strong antioxidant. L-Glutathione plays a key role in multiple biological functions, including scavenging free radicals mainly hydrogen peroxides, detoxification of xenobiotics and it is also involved in the cellular metabolism process[16,17]. Several studies have shown that L-glutathione supplementation prevents carcinogenesis, improves immune function and also eliminate toxics chemicals[18-20]. In the present study, we aimed to determine the protective effect of oral glutathione supplementation on epidermal hyperplasia in UVB irradiated BALB/c mice.

MATERIALS AND METHODS

Preparation of L-Glutathione: The L-glutathione used was a gift from Dr. Nabisarr Mustan, Cambridge Herbal Sdn Bhd (Sungai Buloh, Malaysia). Mice were treated with L-glutathione at a dose of 100 mg kg^{-1} [21]. The dose was prepared fresh daily by adding the L-glutathione in corn oil and then vortexed until the powder was dissolved completely. All the mice were fasted for 4 hours before being treated with 0.2 ml of dissolved L-glutathione by oral gavage.

Animal and experimental design: Eighteen female BALB/c mice were supplied by the Faculty of Veterinary Medicine, University Putra Malaysia. The animals were kept at the animal house, Department of Biomedical Science, Centre of Health and Applied Sciences, Universiti Kebangsaan Malaysia, under 12 h light/dark cycles at controlled room temperatures . All mice were given free access to a standard pellet diet and drinking water.

The animals were randomly divided into three groups: Vehicle control group (n = 6), which were not exposed to UVB irradiation and not treated with L-glutathione; exposure group (n = 6), which were exposed to UVB irradiation only and the treatment group (n = 6), which were exposed to UVB irradiation and also treated with 100 mg kg^{-1} L-glutathione. The treatment was administered for fourteen successive days[22]. Briefly, the dorsal parts of the mice were shaved using an electric shaver (Phillips, Malaysia). Mice from both groups (exposure and treatment group) were then exposed to UVB irradiation for 3 min at dose of 250 mJ cm^{-1} on the 9th, 11th and 13th day of treatment[22]. The source of irradiation was a lamp of 312 nm of wavelength, 15 watts (UVP, USA).

The use of the mice was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (UKMAEC NO: FSK/2017/AHMAD ROHI/22-NOV./887-NOV.-2017-JULY-2018) and guidelines were strictly followed.

Skin morphology observation: The dorsal skin of the mice was observed for such morphological changes as redness, scaling and thickening of skin before the mice was euthanized by cervical dislocation on day 15.

Skin edema formation evaluation: Edema formation was evaluated by measuring skinfold thickness[23]. The midline dorsal skin of the mice was lifted up at the neck and skin-fold thickness was measured mid-way between the shoulder and hip using a Harpenden skinfold caliper (Baty, United Kingdom).

Histopathological observation: The tissue specimens were fixed using 10% neutral buffered formalin for 24 h and embedded in paraffin blocks. The specimens were then

cut into sections of 5 μm thickness for histopathology observation. Hematoxylin and Eosin (H and E) staining was performed to assess the structural alteration, epidermal hyperplasia and also leukocyte infiltration.

Statistical analysis: Data analysis was performed using the Statistical Packages for the Social Sciences (SPSS) version 23.0 and expressed as mean±standard error mean (SEM). One-way ANOVA was used to determine skinfold thickness between groups. The differences among means were inspected using post hoc Tukey test and were considered to be statistically significant at $p<0.05$.

RESULTS

Effects of L-glutathione on morphological changes in UVB irradiated skin tissue: As shown in Fig. 1, the UVB irradiated group showed obvious skin scaling as compared to the vehicle control group. Furthermore, the UVB irradiated group also presented with severe erythema and skin thickening, as compared to the vehicle control group. However, the mice which were administered with oral L-glutathione had reduced erythema and no obvious scaling on the skin, as compared to UVB irradiated group.

Effects of L-glutathione on cutaneous edema in UVB irradiated skin tissue: Based on Fig. 2, the skinfold thickness of the UVB irradiated (UVB) group (1.725±0.079 mm) increased significantly, as compared to the vehicle control (VC) group (1.0±0.035 mm) ($p<0.05$). Oral supplementation of L-glutathione (L-GSH) significantly reduced the skinfold thickness (1.087±0.047) ($p<0.05$) as compared to UVB irradiated group (1.725±0.079 mm).

Effect of L-glutathione on histopathological changes in UVB irradiated skin tissue: Figure 3 and 4 illustrated the histopathological evaluation of mice skin from the various groups. Based on Fig. 3, the vehicle control group showed the normal characteristics of mice skin, that is, epidermis, dermis and hypodermis. However, the UVB-irradiated group showed epidermal hyperplasia and hyperkeratosis, as compared to the vehicle control group. On the contrary, the group which was irradiated with UVB and administered L-glutathione showed less severe epidermal hyperplasia, as compared to the group which was only UVB-irradiated but was not administered L-glutathione.

As shown in Fig. 4, epidermis and dermis layers were observed in the vehicle control group. Furthermore, there was no sign of infiltration of leukocytes. However, the UVB-irradiated group showed leukocyte infiltration, as compared to the vehicle control group. In the oral supplementation of L-glutathione treatment group, there was marked reduction of leukocytes in the dermis layer, as compared to the UVB irradiated group.

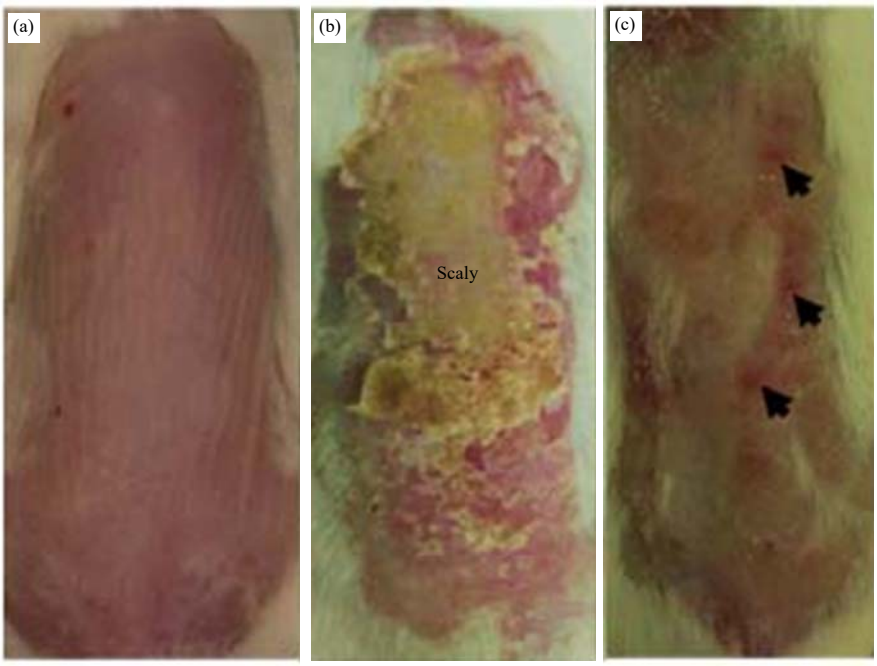

Fig. 1(a-c): The morphology of the skin observed. (a) Vehicle control group, (b) UVB irradiated group and (c) Treatment group
The black arrowhead (←) indicates slight erythema

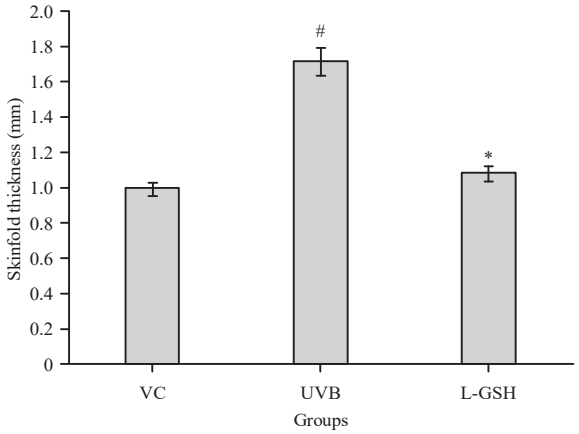

Fig. 2: Evaluation of cutaneous edema by measuring the skinfold thickness.

Bar chart showed results of skinfold thickness in different groups represented by the mean±standard error mean (S.E.M) (n = 6). #Statistically significant difference in comparison to the vehicle control group ($p<0.05$), *Statistically significant difference in comparison to the UVB exposure group ($p<0.05$)

DISCUSSION

Multiple exposures to ultraviolet radiation that generate reactive oxygen species (ROS) in the body can lead to such cutaneous malignancies as epidermal hyperplasia, erythema, hyperpigmentation and skin cancer[24]. Antioxidant supplements, such as L-glutathione, have gained considerable attention as photoprotective and anti-melanogenic agents[25]. The supplementation of L-glutathione is an effective strategy to increase intracellular glutathione as it eventually restores tissue glutathione and enhances ROS metabolism[26]. The present study showed the photoprotective effect of oral supplementation of glutathione on epidermal hyperplasia and edema formation in UVB irradiated Balb/c mice.

Acute UVB exposure can lead to skin scaling which may result in hyperkeratosis, actinic keratosis and hyperplasia in the epidermis. Besides, skin erythema is one of the early inflammatory responses to UVB irradiation[24,27]. Our study showed that UVB irradiated mice which had been administered L-glutathione showed no obvious skin scaling and less erythema (redness) than UVB irradiated mice which had not been administered L-glutathione. We suggest that such oral antioxidants as L-glutathione can prevent and protect mice skin from UVB-induced skin scaling and erythema[28]. This may be due to L-glutathione's ability to restore and maintain the level of intracellular glutathione in

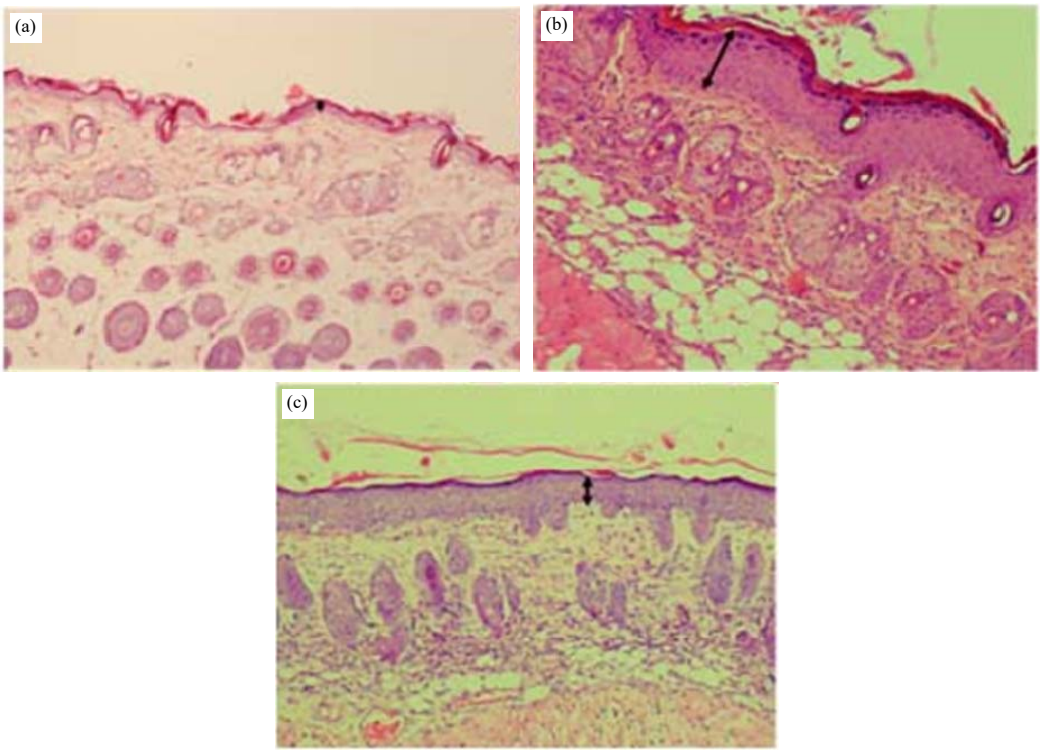

Fig. 3(a-c): Histological observation to show epidermal hyperplasia (double sided arrow). (a) vehicle control group, (b) UVB irradiated group and (c) L-glutathione treatment group

Hematoxylin-eosin staining; Pictures are shown at magnification ×10. Scale bar: 100 µm

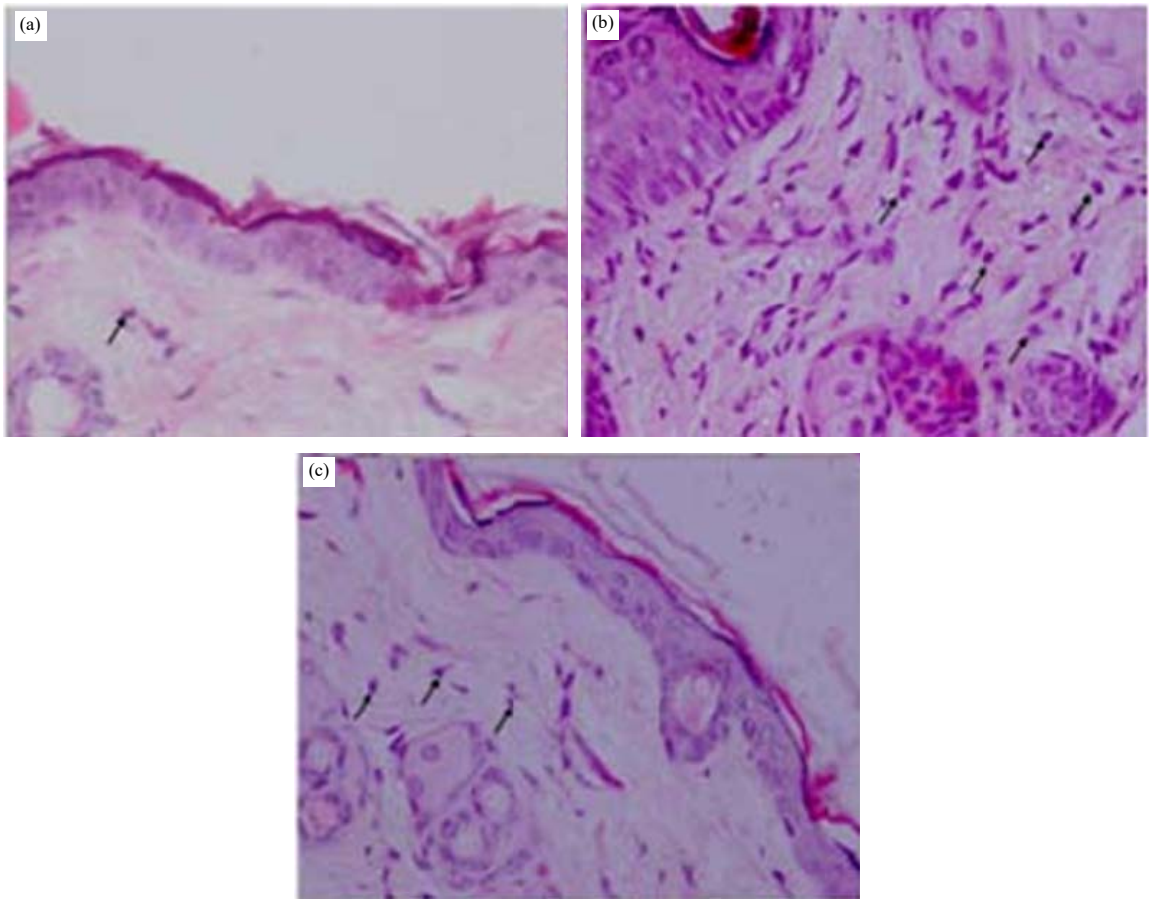

Fig. 4: Histological observation to show leukocyte infiltration (arrow) in the dermis layer. (a) Vehicle control group, (b) UVB irradiated group and (c) L-glutathione treatment group
Hematoxylin-eosin staining; Pictures are shown at magnification ×40. Scale bar: 100 μm

order to scavenge the diverse oxidants such as superoxide anion, hydroxyl radical, nitric oxides and carbon radical induced by UVB[29,30].

Acute UVB irradiation stimulates inflammatory responses associated with the formation of cutaneous edema[31]. Edema is brought about by the enlargement of lymphatic vessels with hyperpermeable function, which drains lymphatic fluids into the epidermis[32]. In our study, the formation of cutaneous edema was determined by measuring skinfold thickness[27]. Our study demonstrated that UVB irradiated mice which had been administered L-glutathione orally had significantly less skinfold thickness, compared to UVB irradiated mice which had not been administered L-glutathione. Hence, L-glutathione shows the potential of inhibiting cutaneous edema formation. In agreement with our findings, the administration of other oral antioxidant supplements such as lutein and zeaxantin has been associated with reduced cutaneous edema in the decreased swelling of mice ear following UVB irradiation[33,34].

The infiltration of leukocytes in the epidermis is one of the inflammatory responses after exposure to UVB irradiation. UVB-induced leukocyte infiltration is a potential source of ROS production in the skin. Generation of ROS triggers such host defense responses as the promoting of endothelial dysfunction and secretion of various kind of chemokines and cytokines in order to recruit the inflammatory cells especially neutrophils to infected tissue[35-37]. In the present study, we found that oral L-glutathione reduced the infiltration of leukocytes in the dermis layer of UVB-irradiated mice. The antioxidant-rich supplement may counteract the UVB-induced ROS production and may interrupt the hyper-permeability of endothelial cells and also inhibit the secretion of various chemokines. In another study, antioxidants such as alpha lipoic acid significantly reduced the leukocyte infiltration in UVB induced corneal stroma[38]. Besides, green tea polyphenol, epigallocatechin-3-gallate (EGCG), which has strong antioxidant activity, has also markedly reduced leukocyte infiltration in UVB irradiated mice skin[39].

The direct exposure of skin epidermis to UVB radiation also leads to alteration in the physiological and biochemical features of the skin. These include keratinocyte proliferation and epidermal hyperplasia[40]. These features are protective because they provide time for the damaged cells to recover. However, extensive exposure to UVB can lead to the activation of proliferating cell nuclear antigen (PCNA) that would accelerate epidermal proliferation and affect the epidermal homeostasis[41]. In the present study, hematoxylin and eosin staining were performed to obtain morphological insights into the effect of L-glutathione on the epidermis of UVB irradiated mice skin. We found that L-glutathione brought about obvious reduction of epidermal thickening and keratinocyte proliferation induced by the UVB irradiation. Hence, L-glutathione may restore the endogenous tissue glutathione level which protects the cells from oxidative damage[42].

CONCLUSION

This study demonstrated the protective effect of L-glutathione in mice skin against UVB-irradiated epidermal hyperplasia. Oral L-glutathione can act as an anti-inflammatory agent by inhibiting such early inflammatory responses as cutaneous edema formation and leukocyte infiltration. Hence, L-glutathione has the potential to be developed as a photoprotective agent.

SIGNIFICANCE STATEMENT

This study has demonstrated that L-glutathione can help prevent complications brought about by prolong exposure to solar radiation. This study will help researchers uncover critical areas of UVB-induced cutaneous responses that have not been explored. Thus, a new theory on L-glutathione as an UVB protective agent may be developed.

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REFERENCES

1. D'Orazio, J., S. Jarrett, A. Amaro-Ortiz and T. Scott, 2013. UV radiation and the skin. Int. J. Mol. Sci., 14: 12222-12248.
2. Matsumura, Y. and H.N. Ananthaswamy, 2004. Toxic effects of ultraviolet radiation on the skin. Toxicol. Applied Pharmacol., 195: 298-308.
3. Vina, J., C. Perez, T. Furukawa, M. Palacin and J.R. Vina, 1989. Effect of oral glutathione on hepatic glutathione levels in rats and mice. Br. J. Nutr., 62: 683-691.
4. Marrot, L. and J.R. Meunier, 2008. Skin DNA photodamage and its biological consequences. J. Am. Acad. Dermatol., 58: S139-S148.
5. Kang, T.H., H.M. Park, Y.B. Kim, H. Kim and N. Kim *et al*., 2009. Effects of red ginseng extract on UVB irradiation-induced skin aging in hairless mice. J. Ethnopharmacol., 123: 446-451.
6. Lee, S., C.M. Kim, J.H. Lee, K. Lee, K.S. Cho and E.S. Kim, 2017. Effect of hemp fiber on UVB-induced epidermal cell proliferation and PCNA expression. Genes Genomics, 39: 667-673.
7. Tebbe, B., 2001. Relevance of oral supplementation with antioxidants for prevention and treatment of skin disorders. Skin Pharmacol. Applied Skin Physiol., 14: 296-302.
8. Zhang, D., C. Lu, Z. Yu, X. Wang and L. Yan *et al*., 2017. *Echinacoside alleviates* UVB irradiation-mediated skin damage via inhibition of oxidative stress, DNA damage and apoptosis. Oxid. Med. Cell. Longevity, Vol. 2017. 10.1155/2017/6851464
9. Parrado, C., N. Philips, Y. Gilaberte, A. Juarranz and S. Gonzalez, 2018. Oral photoprotection: Effective agents and potential candidates. Front. Med., Vol. 5. 10.3389/fmed.2018.00188
10. Lim, H.W., M.I. Arellano-Mendoza and F. Stengel, 2017. Current challenges in photoprotection. J. Am. Acad. Dermatol., 76: S91-S99.
11. Sambandan, D.R. and D. Ratner, 2011. Sunscreens: An overview and update. J. Am. Acad. Dermatol., 64: 748-758.
12. Rodford, R., 1997. Safety evaluation of preservatives. Int. J. Cosmet. Sci., 19: 281-290.
13. Handa, O., S. Kokura, S. Adachi, T. Takagi and Y. Naito *et al*., 2006. Methylparaben potentiates UV-induced damage of skin keratinocytes. Toxicology, 227: 62-72.
14. Lee, C.W., H.H. Ko, C.Y. Chai, W.T. Chen, C.C. Lin and F.L. Yen, 2013. Effect of *Artocarpus communis* extract on UVB irradiation-induced oxidative stress and inflammation in hairless mice. Int. J. Mol. Sci., 14: 3860-3873.
15. Kurutas, E.B., 2016. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr. J., Vol. 15. 10.1186/s12937-016-0186-5
16. Suthanthiran, M., M.E. Anderson, V.K. Sharma and A. Meister, 1990. Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. Proc. Nat. Acad. Sci. USA., 87: 3343-3347.
17. Lu, S.C., 2009. Regulation of glutathione synthesis. Mol. Aspects Med., 30: 42-59.

18. Schwartz, J.L. and G. Shklar, 1996. Glutathione inhibits experimental oral carcinogenesis, p53 expression and angiogenesis. Nutr. Cancer, 26: 229-236.
19. Furukawa, T., S.N. Meydani and J.B. Blumberg, 1987. Reversal of age-associated decline in immune responsiveness by dietary glutathione supplementation in mice. Mech. Ageing Dev., 38: 107-117.
20. Kim, S.J., D. Han, B.H. Ahn and J.S. Rhee, 1997. Effect of glutathione, catechin and epicatechin on the survival of *Drosophila melanogaster* under paraquat treatment. Biosci. Biotechnol. Biochem., 61: 225-229.
21. Yabuki, Y. and K. Fukunaga, 2013. Oral administration of glutathione improves memory deficits following transient brain ischemia by reducing brain oxidative stress. Neuroscience, 250: 394-407.
22. Park, J.M., J.K. Cho, J.Y. Mok, I.H. Jeon, H.S. Kim, H.J. Kang and S.I. Jang, 2012. Protective effect of astragalin and quercetin on ultraviolet (UV)-irradiated damage in HaCaT cells and Balb/c mice. J. Korean Soc. Applied Biol. Chem., 55: 443-446.
23. Kim, H., 2016. Garlic supplementation ameliorates UV-induced photoaging in hairless mice by regulating antioxidative activity and MMPs expression. Molecules, Vol. 21, No. 1. 10.3390/molecules21010070
24. Lee, J.H., H.T. An, J.H. Chung, K.H. Kim, H.C. Eun and K.H. Cho, 2002. Acute effects of UVB radiation on the proliferation and differentiation of keratinocytes. Photodermatol. Photoimmunol. Photomed., 18: 253-261.
25. Dumoulin, M., D. Gaudout and B. Lemaire, 2016. Clinical effects of an oral supplement rich in antioxidants on skin radiance in women. Clin. Cosmet. Investig. Dermatol., 9: 315-324.
26. Uchida, H., Y. Nakajima, K. Ohtake, J. Ito, M. Morita, A. Kamimura and J. Kobayashi, 2017. Protective effects of oral glutathione on fasting-induced intestinal atrophy through oxidative stress. World J. Gastroenterol., 23: 6650-6664.
27. Afaq, F., V.M. Adhami and N. Ahmad, 2003. Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. Toxicol. Applied Pharmacol., 186: 28-37.
28. Godic, A., B. Poljsak, M. Adamic and R. Dahmane, 2014. The role of antioxidants in skin cancer prevention and treatment. Oxid. Med. Cell. Longev., Vol. 2014. 10.1155/2014/860479
29. Pizzorno, J., 2014. Glutathione!. Integr. Med. (Encinitas), 13: 8-12.
30. Bickers, D.R. and M. Athar, 2006. Oxidative stress in the pathogenesis of skin disease. J. Invest. Dermatol., 126: 2565-2575.
31. Bishop, T., D.W. Hewson, P.K. Yip, M.S. Fahey, D. Dawbarn, A.R. Young and S.B. McMahon, 2007. Characterisation of ultraviolet-B-induced inflammation as a model of hyperalgesia in the rat. Pain, 131: 70-82.
32. Sawane, M., H. Kidoya, F. Muramatsu, N. Takakura and K. Kajiya, 2011. Apelin attenuates UVB-induced edema and inflammation by promoting vessel function. Am. J. Pathol., 179: 2691-2697.
33. Lee, E.H., D. Faulhaber, K.M. Hanson, W. Ding, S. Peters, S. Kodali and R.D. Granstein, 2004. Dietary lutein reduces ultraviolet radiation-induced inflammation and immunosuppression. J. Investig. Dermatol., 122: 510-517.
34. Gonzalez, S., W. Astnersan, D. Goukassina and M.A. Pathaak, 2003. Dietary lutein/zeaxanthin decreases ultraviolet B-induced epidermal hyperproliferation and acute inflammation in hairless mice. J. Invest. Dermatol., 121: 399-405.
35. Vostalova, J., A. Zdarilova and A. Svobodova, 2010. *Prunella vulgaris* extract and rosmarinic acid prevent UVB-induced DNA damage and oxidative stress in HaCaT keratinocytes. Arch. Dermatol. Res., 302: 171-181.
36. Mittal, M., M.R. Siddiqui, K. Tran, S.P. Reddy and A.B. Malik, 2014. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signall., 20: 1126-1167.
37. Roberts, L.K. and D.G. Beasley, 1995. Commercial sunscreen lotions prevent ultraviolet-radiation-induced immune suppression of contact hypersensitivity. J. Investig. Dermatol., 105: 339-344.
38. Chen, B.Y., D.P.C. Lin, L.C. Chang, T.P. Huang and H.J. Liu *et al*., 2013. Dietary α-lipoic acid prevents UVB-induced corneal and conjunctival degeneration through multiple effects. Investig. Ophthalmol. Visual Sci., 54: 6757-6766.
39. Katiyar, S.K. and H. Mukhtar, 2001. Green tea polyphenol (-)-epigallocatechin-3-gallate treatment to mouse skin prevents UVB-induced infiltration of leukocytes, depletion of antigen-presenting cells and oxidative stress. J. Leukoc. Biol., 69: 719-726.
40. Jiang, S.J., J.Y. Chen, Z.F. Lu, J. Yao, D.F. Che and X.J. Zhou, 2006. Biophysical and morphological changes in the stratum corneum lipids induced by UVB irradiation. J. Dermatol. Sci., 44: 29-36.
41. Cho, Y.S., K.H. Lee and J.W. Park, 2010. Pyrithione-zinc prevents UVB-induced epidermal hyperplasia by inducing HIF-1α. Korean J. Physiol. Pharmacol., 14: 91-97.
42. Divya, S.P., X. Wang, P. Pratheeshkumar, Y.O. Son and R.V. Roy *et al*., 2015. Blackberry extract inhibits UVB-induced oxidative damage and inflammation through MAP kinases and NF-κB signaling pathways in SKH-1 mice skin. Toxicol. Applied Pharmacol., 284: 92-99.