American Journal of Medical and Biological Research, 2015, Vol. 3, No. 6, 146-148
Available online at http://pubs.sciepub.com/ajmbr/3/6/2

DOI:10.12691/ajmbr-3-6-2

Soybean Protective Effect to 8-OHdG on UVB Induced-Hairless Mice

Zainuddin AS[1,*], Massi MN[2], Alam G[3], Djawad K[4], Yusuf I[2]

[1]Daya Regional Public Hospital of Makassar, South Sulawesi, Indonesia
[2]Hasanuddin University, Department of Microbiology, Faculty of Medicine, Jl. PerintisKemerdekaan Km 10 Tamalanrea, Makassar, South Sulawesi, Indonesia
[3]Hasanuddin University, Department of Pharmacognosis Phytochemistry, Faculty of Pharmacy, Jl. PerintisKemerdekaan Km 10 Tamalanrea, Makassar, South Sulawesi, Indonesia
[4]Hasanuddin University, Department of Dermatovenereology, Faculty of Medicine, Jl. PerintisKemerdekaan Km 10 Tamalanrea, Makassar, South Sulawesi, Indonesia
*Corresponding author: amirahshaleha@gmail.com

Abstract **Aims**. TheUVB light radiation inducts 8-OHdG as the photoproduct in which resulting the DNA impairment through stress oxidative pathway. Genistein is one of the basic component from soybean that the isoflavone inside conducts strong antioxidant effect. This study main purpose is to see the application of genistein's photoprotective instrument on UVB induced-hairless mice. **Method**. 24 albino mice, age 6-9 weeks, weight 15-25 grams were used as the sample of the study. These mice were divided into 6 different way of treating groups, group I as the control group which had been selected as the negative control—untreated ones, group II as the control group which had the UVB radiation, group III as UVB radiation control group with Aceton-based, group IV which had 100 ppm genistein. Group V had 200 ppm genistein and group VI had UVB radiation 3 times a week with 500 mJ dosage for 12 weeks. After 24 hours of the last UV light exposure giving, these mice were killed off to aim the skin biopsy for 8-OHdG laboratory examination. **Result**. 8-OhdG level at 100 ppm genistein control group was the lowest one rather than the UVB radiation and 400 ppm control group. And, it's not significantly showed at 200 ppm genistein control group. **Conclusion**. 100 ppm, 200 ppm and 400 ppm of genistein application showed the protective effect to 8-OHdG on UVB induced-hairless mice, which the best dose usage was 100 ppm of genistein.

Keywords: *Genistein, 8OHdG, Ultraviolet B*

Cite This Article: Zainuddin AS, Massi MN, Alam G, Djawad K, and Yusuf I, "Soybean Protective Effect to 8-OHdG on UVB Induced-Hairless Mice." *American Journal of Medical and Biological Research*, vol. 3, no. 6 (2015): 146-148. doi: 10.12691/ajmbr-3-6-2.

1. Introduction

Skin is the widest part of the body which connected directly to the outdoor environment and easily getting exposed by sunlight. Among the some well known sunlight spectrums that able to reach the skin, UVB isthe one with negative effect. UVB radiation can happenly damage the skin tissue through reactive oxygen species (ROS) mechanism. The interaction between oxidative stress with cell biomolecule such as DNA will conduct impairment in it by forming 8-OHdG photoproduct and occasionally causing serious consequence on cell [1]. This photoproduct can be used as biomarker of DNA damage through ROS formation. And, it has such main role to show chronic effect of UVB light exposure and photoaging.

There are some natural substance that experimentally proved acting as the best therapy to prevent photoaging. One of them is isoflavone which is part of the polyphenol class and has been found and isolated from soybean. It has important keyrole at preventing and treating some illness, such as cancer and chronic disease on cardiovascular system or diabetes mellitus. The main element from isoflavone is genistein that blocking tyrosin kinases, has anti oxidant effect, anti angiogenesis effect and estrogenic effect. The previous study showed that genistein has protective effect to UV light induced-photoaging. Isoflavone's extract does have strong role at preventing from photocarcinogenesis forming [10] and photoaging [2,6,11], but the study that reporting about the effect from different concentration usage of topical genistein to 8-OHdG level on UVB induced-hairless mice has never been done before. Nevertheless, this study will likely to show the photoprotective effect of genistein application on hairless mice which had been exposed by UVB radiation a few minutes earlier.

2. Material and Methods

Design. This study is a true experiment post design with control group.It was conducted at Animal laboratory of Medical faculty of Hasanuddin University (Makassar, Indonesia).

Subject. The subject of this study were 6-9 weeks old albino mice from one mother purchased from Balitbang

veterinary centre in Maros, South Sulawesi. The mice were housed in standard condition for 1 week: room temperature (28 ± 2°C), humidity 50 ± 10% and subjected to a 12 h light/ 12 h dark cycle.

Materials and Equipments. Genistein with concentration of 100, 200 and 400 PPM in lotion preparation, UVB 311nm light, calibrated radiation energy based.

Sample. The sample of this study consists of 24 mice which were divided into several perpetration groups. Inclusion criteria were healthy female albino mice spesiesswiss albino mice aged 6-9 weeks old, 15-25 grm weight. Mice which became sick or dead throughout the study were excluded.

2.1. Methods

These mice were selected randomly and divided into 6 different groups; group I as the control group which had been selected as the negative control—untreated ones, group II as the control group which had the UVB radiation, group III as UVB radiation control group with Aceton-based, group IV which had 100 ppm of genistein. Group V had 200 ppm genistein and group VI had UVB radiation 3 times a week with 500 mJ dosage for 12 weeks. After 24 hours of the last UV light exposure giving, these mice were killed off to aim the skin biopsy for 8-OHdG laboratory examination.

2.2. Ethical Clearance

This study has already gotten the ethical clearance agreement from Biomedic Ethical Comission on Hasanuddin University Faculty of Medicine's animal sample.

2.3. Statistic

The datas from this study used Kolmogorov-Smirnov' normal distribution test based, continued with Mann Whitney test based, with significant level $p<0.05$

3. Result

Sample characteristics. This study was performed on 24 albino mice eligible for inclusion criteria 15-25 g weight (18.5± 1.390) randomly divided into groups of each treatment. The average level of genistein inside the extract is 15.97%

Graphic below showed the level of 8-OhdG based on eact treatment groups. The statistic analysis between the untreated group and UVB radiation control showed meaningful result (p=0.028). There was no strong connection between the UVB radiation control and ethanol control group (p=0.689). Between the UVB radiation control group and 100 ppm soybean extract control group (p=0.02), with 200 ppm (p=0.11), and with 400 ppm (p=0.02). It showed significant higher level of 8-OhdG in 200 ppm soybean extracts control groups (Table 1).

Table 1. Level of 8-OhdG after UVB exposure for 12 weeks according to soybean extract concentration in aceton solution groups as protector

Treatment Groups	Mean ±SD
Negative control	0,085±0,013
UVB control	0,478±0.416
Aceton control	0,545±0,218
Genistein100	0,010±0,007
Genistein 200	0,189±0,324
Genistein 400	0,023±0,019

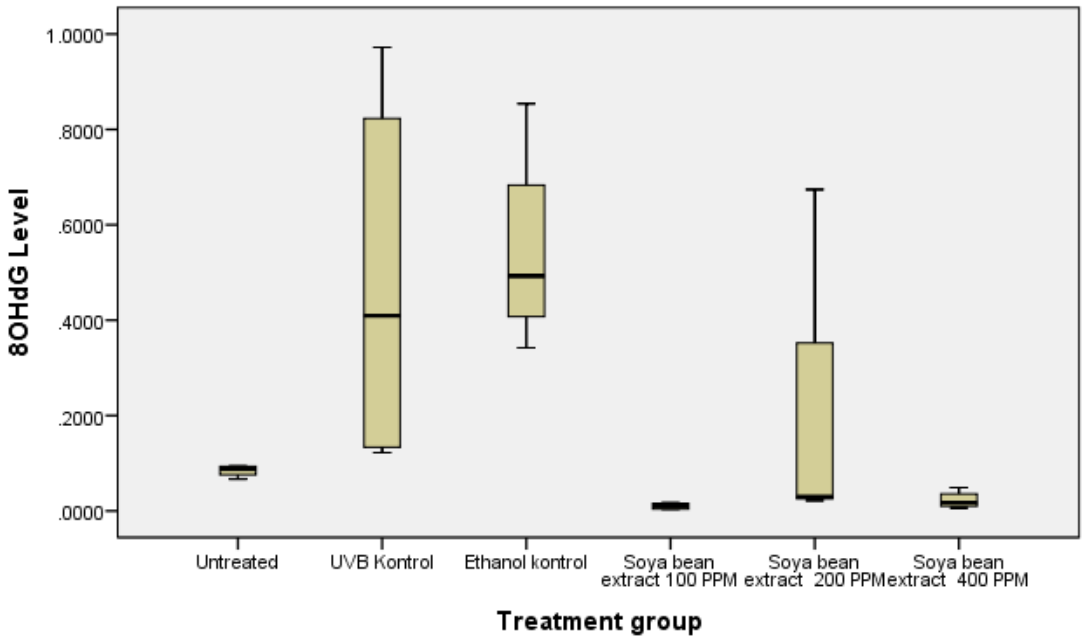

Figure 1. Graphic of 8-OHdG level according to each treatment groups of soybean extract

Statistic analysis result between the untreated control groups to 100 ppm soybean extract cocentration (p=0.02), to 200 ppm (p=0.248), to 400 ppm (p=0.02). The result between 100 ppm to 200 ppm soybean extract group (p=0.02), 100 ppm to 400 ppm (p=0.24) and 200 ppm to 400 ppm (p=0.191).

4. Discussion

UVB light radiation was absorbed by DNA keratinocyt in photon energy formulation and showed photochemistry

alteration until there'd be 64 direct photoproducts at the end of the process. The 8-OhdG was formed by reactive oxygen species (ROS) pathway undirectly. This photoproduct had induced the releasing of IL-1, IL-6 and TNF alpha on both of keratinocyt and fibroblast until the level of 8-OhdG got increased and believed to play role in photoaging process from these mediators [5].

Djawad K, 2008 reported a study about 8-OHdG expression by using 343 mJ UVB induced on hairless mice 3 times a week for 3 weeks. It showed the increasing level of 8-OhdG expression on UVB induced-hairless mice skin compared with the untreated mice.

The radiation dose that used before was responsibble to epidermal hyperplasia [3]. In our study, the UVB light radiation increased the level of 8-OhdG quiet significantly compared to untreated control groups. This information showed an evidence of DNA damage which connected with ROS formation.

ROS induction formed few pattern of DNA damage such as strand break, base modification amd DNA-protein cross links. 8-OHdG photoproduct is the DNA base modified product, a mutation-prone; G:C to T:A and becoming the biomarker of oxidative stress. Hydorxyl radical, singlet oxygen and direct photodynamic play the key role of 8-OHdG formation [4]. The previous study used hairless mice and showing the formation of 8-OHdG on mice' epidermal after 101 kJ/m2 UVB single exposure. Adriani A's research reported the 450 mJ UVB radiation 3 times a week for 12 weeks showed significant result of PCNA and 8-OHdG expression. Eventually, it did not show significant difference for the epidermal thickness. And, we decided to use 500 mJ UVB radiation for 12 weeks in our study.

The antioxidant factor such as superoxide dismutase, cathalases, glutahion reductases, ubiquinol and α-tocopherol got surpressed temporarely after the single UVB exposure frequently and it might be the reason of free radical formation that lead to 8-OHdG formation [4]. This photoproduct stimulated the transduction signal pathway which responsible to biochemistry modulation until the celullar alteration such as proliferation, apoptosis, cytokin secretion were shown as skin acute response [7].

The therapy effect of soybean extract by using 500 mJ UVB radiation showed a strong evidence to protect the skin epidermal from 8-OHdG photoproduct formation. It can be seen from the graphic where the 8-OHdg level on hairless mice that already smeared by soybean extract is lower than the UVB control group. There's been significant difference between 100 ppm soybean extract group (0.02) to 400 ppm (0.01). Compared with untreated groups, the statistic analysis result showed a significant result between 100 ppm soybean extract and 400 ppm groups but unsignificant result to 200 ppm group. Nevertheless, the graphic showed the 8-OHdG level from three groups has similar value to untreated group which the lowest level was seen from 100 ppm group. In case of this founding, 100 ppm soybean extract concentration has the better antioxidant effect among all groups. It even has the lowest level rather than the untreated group. The antioxidant effect from soybean extract was found from one of its substance called genistein in which the isoflavone inside merely being the potent element [8]. The protection effect of soybean extract came from the antioxidant enzymatic activity and oxidative stress mithocondrial modulation through down regulasi p66Shc signal pathway [9].

The protection effect of topical isoflavonoid on UVB induced-hairless mice which had been done to mice showed the antiinflammation process and immunosuppression mechanism of 20 uM of genistein. And, the genistein antioxidant effect had been shown from antioxidative enzymatic activity and free radical scavengin [9]. In our study, the increasing dose did not increase the protective effect due to the usage doses in this study too much higher. In case of this fact, we hope for the next study can use the lowest dose so we can find the proper dose as the protective effect.

References

[1] BICKERS, D.R., ATHAR, M. (2006) Oxidative Stress in the Pathogenesis of Skin Disease. *The society for investigative dermatology*, 2565-73.

[2] CHIU, T. M., HUANG, C. C., LIN, T. J., FANG, J. Y., WU, N. L. & HUNG, C. F. (2009) In vitro and in vivo anti-photoaging effects of an isoflavone extract from soybean cake. *J Ethnopharmacol*, 126, 108-13.

[3] DJAWAD, K. (2008) Protective effects of curcumin on the expression CPD, 8-OHdG, apoptosis and hyperplasia of the epidermis. Medical Faculty Makassar, Hasanuddin University.

[4] HATTORI, Y., NISHIGORI, C., TANAKA, T. (1997). 8-Hydroxy-2' Deoxyguanosine is Increased i epidermal cells of hairless mice after chronic ultraviolet B exposure. *J Invest Dermatol*, 107, 733-7.

[5] ICHIHASHI, M., ANDO, H., YOSHIDA, M., NIKI, Y. & MATSUI, M. (2009), Photoaging of the Skin. *JAAM*, 6, 46-55.

[6] KIM, S.-Y., KIM, S.-J., LEE, J.-Y., KIM, W.-G., PARK, W.-S., SIM, Y.-C. & LEE, S.-J. (2004) Protective Effects of Dietary Soy Isoflavones againts UV-Induced Skin-Aging in Hairless Mouse Model. *J Am College Nutr*, 23, 157-62.

[7] KOCHEVAR, I.E., TAYLOR, C.R., KRUTMANN, J. (2012) Disorders due to Ultraviolet Radiation. IN FITZPATRICK, T.B., EISEN, A.Z., WOLFF, K., GOLDSMITH, L. A., KATZ, S. I (Eds.) *Fitzpatrick`s Dermatology in General Medicine.* 8 ed. New York, Mc Graw Hill. 1032-43.

[8] MOORE, J.O., WANG, Y., STEBBINS, W.G., GAO, D., ZHOU, X., PHELPS, R., LEBWOHL, M. & WEI, H. (2006). Photoprotective ffect of isoflavone genistein on ultraviolet B-induced pyrimidine dimer formation and PCNA expression in human reconstitud skin and its implications in dermatology and prevention of cutaneous carcinogenesis. *Oxford Journal*, 27, 1627-34.

[9] SAEWAN, N., JIMTAISONG, A. (2013) Photoprototection of Natural Flavonoids. *Journal of Applied Pharmaceutica Science*, 129-36.

[10] WEI, H., SALADI, R., LU, Y., WANG, Y., et al. (2003), Isoflavone Genistein: Photoprotection and Clinical Implications in Dermatology. *International research conference on food, nutrition, and cancer*, 3811-8.

[11] WIDYARINI, S., ALLANSON, M., GALLAGHER, N. L., PEDLEY, J., BOYLE, G. M., PARSONS, P. G., WHITEMAN, D. C., WALKER, C. & REEVE, V. E. (2006) Isoflavonoid Photoprotection in Mouse and Human Skin is Dependent on Metallothionein. *J Invest Dermatol*, 126, 198-204.