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Royal Jelly (Bee Product) Decreases Inflammatory Response in Wistar Rats Induced with Ultraviolet Radiation

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Keywords: Royal jelly; Ultraviolet radiation; Nrf 2; NF-κB; TNF alpha

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Abstract

BACKGROUND: Ultraviolet (UV) radiation damages human skin by triggering various types of cellular damage, several main factors involved are nuclear-related factor 2 (Nrf2), nuclear factor kappa-light-chain-enhancer of activated B cells (nF-kB) and pro-inflammatory cytokine, TNF alpha. Royal jelly (RJ) possesses the effect of protecting DNA and tissue against oxidative damage.

AIM: This study aimed to assess the efficacy of RJ as a protector of ultraviolet radiation, by assessing endogenous anti-oxidant expression (Nrf2), transcription factors (Nf-kB) and proinflammatory cytokines (TNF alpha).

METHODS: This study was an experimental study with post-test control group design. Thirty Wistar rats were induced by exposing 40 Watt UV-B lamps for 2 hours/day in 14 days. The rats were grouped into groups with RJ cream application with doses of 2.5%, 5%, and 10%, negative control with vaseline, and normal control. Examination of Nrf2 and NF-kB levels was carried out by ELISA. Quantitative analysis to obtain the percentage of TNF alpha expression on the tissue was entered into the ImageJ® program. Bivariate analysis was carried out by the T-test.

RESULTS: Nrf2 levels elevated following the increase of RJ dose, with the highest level was at RJ 10%. Nf-kB levels decreased following the increase of RJ dose, with the lowest level was at RJ 10%. TNF alpha expression was reduced in groups of RJ in various doses. Increased dose resulted in a more diminished level of TNF alpha.

CONCLUSION: Royal jelly cream application protected the skin from UV radiation by increasing cellular antioxidants and suppressing inflammatory cascade.

Introduction

Ultraviolet radiation (UVR) damages human skin by triggering various types of cellular damage, especially DNA damage and oxidative damage. This condition will increase the risk of skin cancer including skin melanoma [1], [2]. UVB radiation can cause loss of cellular integrity, direct damage to DNA and trigger various cellular responses including apoptosis [3] and inflammation [4] in skin cells including melanocytes (MC). However, the biological and physiological responses of normal MC to UVR are complex and are governed by various factors secreted by their neighbouring cells including keratinocytes (KC) for maintenance of MC homeostasis [5], [6], [7]. The micro-environmental conditions created by KC play a role in regulating MC responses including UVR-induced apoptosis and cell damage through paracrine factor secretions such as endothelin-1 peptides (ET-1), hypophysis and pituitary such as proopiomelanocortin (POMC), adrenocorticotropic hormones, β-endorphin and αmelanocyte-stimulating hormone (α-MSH) or corticotropin-releasing hormone (CRH) [8], [9], [10], [11], [12]. α-MSH has been recognized as an important paracrine factor that plays a protective role against UVB-induced radiation and DNA damage in MC humans. It also shows that the cytoprotective effect of α-MSH on UVR-mediated skin photodamage is associated with their ability to

suppress apoptosis, oxidative stress and inflammatory responses [13]. However, the mechanism involved in regulating the paracrine effect of KC affecting MC activity has not been investigated. Nuclear-related factor 2 (Nrf2) is the main transcription factor that regulates some phase II detoxification and antioxidant genes involved in cellular defence against oxidative stress. Nrf2 is believed to play a regulatory role in UVR-mediated oxidative stress associated with disorders in the physiology of skin cells including MC [14], [15]. Also, Nrf2 is involved in the regulation of paracrine factors such as epidermal growth factor family epigene in KC, which causes enlargement of the sebaceous gland in rats [16]. Modulation of Nrf2 can affect the function of KC associated with UVR response. Also, UVB irradiation can mediate apoptosis through oxidative stress activation from upstream mitogen-activated protein kinase (Mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38) at MC and KC. This activates nuclear factor kappa-light-chain-enhancer of activated B cells (nF-kB) and activates pro-inflammatory cytokines such as: TNF alpha [17], [18].

Royal jelly (RJ) is a product of the cephalic gland secretion of worker bees and serves as the most important part of the honeybee larva's diet, playing a major role in caste differentiation [19]. For the first 2-3 days, RJ is the only food given to all young larvae in their ripening process, while for the queen, it is a special food for the entire period of her life. This is the reason the queen of bees lives longer than other bees. RJ, one of the most effective and beneficial drugs for humans, is widely used both in traditional medicine and modern medicine and it is a controversial food supplement. RJ has the effect of protecting DNA and tissue against oxidative damage [20], [21], [22], [23], [24].

This study was the first study to assess the efficacy of royal jelly as a protector of ultraviolet radiation, by assessing endogenous anti-oxidant expression (Nrf2), transcription factors (Nf-kB) and proinflammatory cytokines (TNF alpha) in the Wistar white Ratskin tissue.

Material and Methods

Subjects

This study was an experimental study with post-test control group design. Thirty Wistar rats, aged 20 weeks, were used in this study. White Wistar rats were obtained from the Eureka Research Laboratory in Palembang. This study had received ethical approval from the Research Ethics Committee, Faculty of Medicine, Universitas Sriwijaya (No. 198/kptfkunsrirsmh/2018). White rats were maintained in a room with a temperature between 20-24°C, and a dark-light cycle for 12 hours.

UV Induction and Royal Jelly Treatment

Before induction and treatment, rats were acclimatised for seven days. UV radiation induction was carried out by exposing 40 Watt UV-B lamp for 2 hours/day; the exposure was carried out for 14 days. The rats were grouped into 5 groups: (I) 10% RJ Group: five white rats were induced with UV-B and 10% royal jelly cream was applied for 14 days. (II) 5% RJ Group: five white rats were induced with UV-B and 5% royal jelly cream was applied for 14 days. (III) RJ Group 2.5%: five white rats were induced with UV-B and were applied with 2.5% royal jelly cream for 14 days. (IV) Negative control: five white rats were induced with UV-B and vaseline cream was applied for 14 days. (V) was control: five white rats were not induced with UV-B and no cream application.

ELISA of Nrf2 and NF-kB

Examination of Nrf2 and NF-kB levels was carried out from serum samples from Wistar rats obtained from the orbital vein as much as 1 mL. Then, the serum was centrifuged at 5000 rpm for 10 minutes. The supernatant was inserted into the tube and stored at -20°C. As much as 10 µL of supernatant from each sample was put into microplate well, then incubated and continued with the addition of HRP-conjugate, Chromogen A and B and Stop Solution. The optical density value was read with a microplate reader at 450nm wavelength. The ELISA was carried out according to the manufacturer's manual (Cloud-Clone Corp®, Texas, USA).

Examination of TNF Alpha Expression

Samples of skin tissue from each experimental subject were evaporated and put into a 10% NBF solution (Leica Biosystems, Wetzlar, Germany), followed by paraffin blocks and cutting samples with a thickness of 4 um. The sample was placed on a glass object and the dehydration process was carried out by entering the sample into multilevel alcohol starting from alcohol 96%, 80%, 70%, Xylane I, II and III. Furthermore, antigen retrieval was carried out using HIER (Heat Induced Epitop Retrieval) technique. Followed by administration of anti-TNF Alpha (Cloud-Clone Corp®, Texas, USA) antibodies (1: 700) in each sample. Then proceeded with the administration of biotynilated link antibodies and streptavidine peroxidase. Then followed by DAB chromogen and counterstain with hematoxylin (Cloud-Clone Corp®, Texas, USA). Furthermore, dehydration is carried out by adding samples to alcohol starting from alcohol 70%, 80%, 96%, and Xylan I, II, III (Sigma-Aldrich®, St. Louis, Missouri, USA). Then each sample was observed under a microscope with

400 times magnification. Photographs from each subsequent sample were entered into the ImageJ® program to be carried out on quantitative analysis to obtain the percentage of TNF alpha expression on the tissue.

Statistical Analysis

Statistical analysis was performed with SPSS 24.0 (SPSS Inc., Chicago, Illinois, USA). Data were presented with mean ± SD. Bivariate analysis was carried out by the T-test between groups. P-value was considered significant at < 0.05.

Results

As exhibited in Table 1, RJ groups with various doses showed multiplied the higher level of Nrf2 compared to the negative control. Nrf2 levels elevated following the increase of RJ dose, with the highest level was at RJ 10%. Nrf2 level of RJ 10% was almost 10-fold higher compared to negative control and almost 2-fold higher compared to normal control. Nrf2 level differences in all groups were statistically significant.

Table 1: Level of Nrf2 in Skin Tissue

Group	Nrf2 Level (pg/mL)	p-Value
RJ 10%	294.18 ± 16.21	0.021*. 0.027#
RJ 5%	184.83 ± 11.55	0.001*. 0.043#
RJ 2.5%	99.11 ± 7.17	0.001*. 0.001#
Negative control	31.23 ± 1.45	0.001#
Normal	156.23 ± 10.23	0.001*

*Independent T test VS negative control; #Independent T test VS normal control.

As shown in Table 2, RJ groups with various doses showed a lower level of Nf-kB compared to the negative control. Nf-kB levels decreased following the increase of RJ dose, with the lowest level was at RJ 10%. Nf-kB level of RJ 10% was almost similar to normal control. Nf-kB level differences in all groups were statistically significant.

Table 2: Level of Nf-kB in Skin Tissue

Group	Nf-kB Level (ng/mL)	p-Value
RJ 10%	2.98 ± 0.11	0.001*. 0.011#
RJ 5%	10.83 ± 1.02	0.001*. 0.008#
RJ 2.5%	21.11 ± 7.17	0.001*. 0.001#
Negative control	26.23 ± 1.98	0.001#
Normal	2.73 ± 0.12	0.001*

*Independent T test VS negative control; #Independent T test VS normal control.

As exhibited in Figure 1, TNF alpha expression increased about 30 times in UVB-induced rats (negative control) compared to the normal group that was not UVB-induced. TNF alpha expression was reduced in groups treated with RJ in various doses. Increased dose resulted in a more diminished level of TNF alpha.

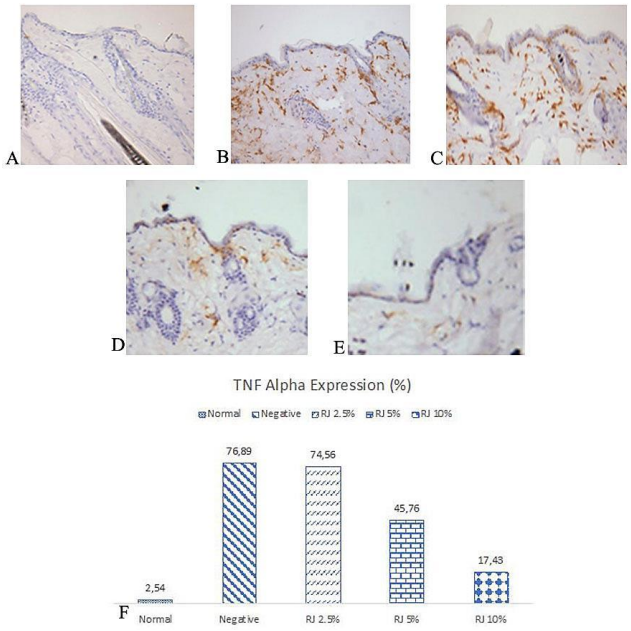

Figure 1: Expression of TNF Alpha in Skin Tissue (400x). A. Normal; B. Negative control; C. RJ 2.5%; D. RJ 5%; E. RJ 10%; F. TNF alpha expression (%)

Discussion

Ultraviolet is an electromagnetic wave with various roles in human life. Ultraviolet radiation of UV-B type causes inflammatory activation, in the form of TNF alpha expression, which increased about 30 times in UVB-induced White rats (negative control) compared to the normal group that was not UVB-induced. This showed that UVB radiation was able to trigger activation of the inflammatory cascade, in line with various studies that have previously explained [25].

Royal jelly (RJ) is a product of the cephalic gland secretion of worker bees and serves as the most important part of the honeybee larva's diet, playing a major role in caste differentiation [19]. For the first 2-3 days, RJ is the only food given to all young larvae in their ripening process, while for the queen, it is a special food for the entire period of her life. This is the reason the queen of bees lives longer than other bees. RJ, one of the most effective and beneficial drugs for humans, is widely used both in traditional medicine and in official medicine and it is a controversial food supplement. RJ has the effect of protecting DNA and tissue against oxidative damage [20], [21], [22], [23], [24]. These conditions indicate that RJ has the potential as a natural antioxidant that can suppress oxidative stress processes which are initiated by an inflammatory process induced by UVB radiation.

The group with RJ treatment showed that the higher the RJ dose, the more potential it was in

increasing the level of Nrf2. Nuclear-related factor 2 (Nrf2) is the main transcription factor that regulates some phase II detoxification and antioxidant genes involved in cellular defence against oxidative stress. The more cellular oxidants that occur will cause an increase in antioxidant production mediated by Nrf2. But, if there is a large amount of oxidant production, endogenous antioxidants are unable to compensate for the production of cellular oxidants, which will lead to a decrease in antioxidant production, which in turn will also reduce the expression of Nrf2 [26], [27], [28].

Application of RJ at a dose of 10%, 5% and 2.5% increase the level of NRF2. This showed the antioxidant potential of RJ, which suppressed cellular oxidants due to UVB radiation. In the presence of antioxidants from RJ, it would help endogenous antioxidants in overcoming cellular oxidants. The higher the level of Nrf2 shows the higher the endogenous antioxidants available in the body, so the lower the level of Nrf2 indicates the lower endogenous antioxidants available in the body. The more oxidants handled by endogenous antioxidants, the Nrf2 levels will decrease. RJ which is rich in antioxidants, will help endogenous antioxidants to suppress cellular oxidants. This caused in the groups with RJ, the level of Nrf2 were higher, which indicated the increasing number of antioxidants available in the body [29], [30], [31].

NF-kB (Nuclear factor-kappa Beta) is a transcription factor that will initiate the expression of pro-inflammatory cytokines, one of which is TNF-alpha. Application of RJ can reduce inflammation by suppressing oxidative stress [32], [33]. The mechanism of RJ in suppressing inflammation is by repressing oxidants in the body, thereby reducing the expression of NF-kB, which results in a decrease in the production of pro-inflammatory cytokines, TNF alpha.

In conclusion, royal jelly cream application protected the skin from UV radiation by increasing cellular antioxidants and suppressing inflammatory cascade.

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