

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

Fatmawati Fatmawati<sup>1</sup>, Erizka Erizka<sup>2</sup>, Rachmat Hidayat<sup>3\*</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia; <sup>2</sup>Department of Microbiology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia; <sup>3</sup>Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

## Abstract

**Citation:** Fatmawati F, Erizka E, Hidayat R. Royal Jelly (Bee Product) Decreases Inflammatory Response in Wistar Rats Induced with Ultraviolet Radiation. Open Access Maced J Med Sci. 2019 Sep 15; 7(17):2723-2727. <https://doi.org/10.3889/oamjms.2019.704>

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

**\*Correspondence:** Rachmat Hidayat. Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia. E-mail: dr.rachmat.hidayat@gmail.com

**Received:** 24-May-2019; **Revised:** 16-Jul-2019; **Accepted:** 17-Jul-2019; **Online first:** 29-Aug-2019

**Copyright:** © 2019 Fatmawati Fatmawati, Erizka Erizka, Rachmat Hidayat. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

**Competing Interests:** The authors have declared that no competing interests exist

**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-κB) 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-κB) 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-κB 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-κB 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), hypophysin and pituitary such as proopiomelanocortin (POMC), adrenocorticotropic hormones, β-endorphin and melanocyte-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- $\kappa$ B) 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- $\kappa$ B) 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/kptfkunsriismh/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- $\kappa$ B

Examination of Nrf2 and NF- $\kappa$ B 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  $\mu$ L 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  $\mu$ m. 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 Epitope 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 biotinylated 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.

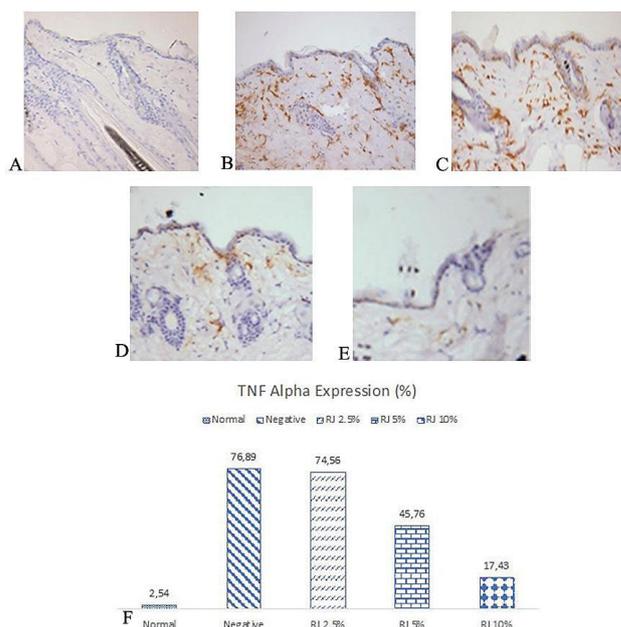
As shown in Table 2, RJ groups with various doses showed a lower level of Nf-kB compared to the negative control. Nf-kB levels decreased following the increase of RJ dose, with the lowest level was at RJ 10%. Nf-kB level of RJ 10% was almost similar to normal control. Nf-kB level differences in all groups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

## References

- Agar N, Young AR. Melanogenesis: a photoprotective response to DNA damage. *Mutat Res.* 2005; 571:121-132. <https://doi.org/10.1016/j.mrfmmm.2004.11.016> PMID:15748643
- Anna B, Blazej Z, Jacqueline G, Andrew CJ, Jeffrey R, Andrzej S. Mechanism of UV-related carcinogenesis and its contribution to nevi/melanoma. *Expert Rev Dermatol.* 2007; 2(4):451-69. <https://doi.org/10.1586/17469872.2.4.451> PMID:18846265 PMID:PMC2564815
- Premi S, Wallisch S, Mano CM, Weiner AB, Bacchiocchi A, Wakamatsu K, et al. Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science.* 2015; 347:842-7. <https://doi.org/10.1126/science.1256022> PMID:25700512 PMID:PMC4432913
- Gledhill K, Rhodes LE, Brownrigg M, Haylett AK, Masoodi M, Thody AJ, et al. Prostaglandin-E2 is produced by adult human epidermal melanocytes in response to UVB in a melanogenesis-independent manner. *Pigment Cell Melanoma Res.* 2010; 23:394-403. <https://doi.org/10.1111/j.1755-148X.2010.00696.x> PMID:20236442 PMID:PMC2881306
- Bowen AR, Hanks AN, Allen SM, Alexander A, Diedrich MJ, Grossman D. Apoptosis regulators and responses in human melanocytic and keratinocytic cells. *J Invest Dermatol.* 2003; 128:48-55. <https://doi.org/10.1046/j.1523-1747.2003.12010.x> PMID:12535197
- Coleman DJ, Chagani S, Hyter S, Sherman AM, Löhr CV, Liang X, Ganguli-Indra G, et al. Loss of keratinocytic RXR $\alpha$  combined with activated CDK4 or oncogenic NRAS generates UVB-induced melanomas via loss of p53 and PTEN in the tumor microenvironment. *Mol Cancer Res.* 2015; 13:186-98. <https://doi.org/10.1158/1541-7786.MCR-14-0164> PMID:25189354 PMID:PMC4297739
- Slominski A, Zmijewski MA, Skobowiat C, Zbytek B, Slominski RM, Steketee JD. Sensing the environment: regulation of local and global homeostasis by the skin neuroendocrine system. *Adv Anat Embryol Cell Biol.* 2012; 212:v,vii,1-115. [https://doi.org/10.1007/978-3-642-19683-6\\_1](https://doi.org/10.1007/978-3-642-19683-6_1)
- Bohm M, Wolff I, Scholzen TE, Robinson SJ, Healy E, Luger TA, et al. Alpha-Melanocyte-stimulating hormone protects from ultraviolet radiation-induced apoptosis and DNA damage. *J Biol Chem.* 2005; 280:5795-802. <https://doi.org/10.1074/jbc.M406334200> PMID:15569680
- Hyter S, Coleman DJ, Ganguli-Indra G, Merrill GF, Ma S, Yanagisawa M, et al. Endothelin-1 is a transcriptional target of p53 in epidermal keratinocytes and regulates ultraviolet-induced melanocyte homeostasis. *Pigment Cell Melanoma Res.* 2013; 26:247-58. <https://doi.org/10.1111/pcmr.12063> PMID:23279852 PMID:PMC3663331
- Kadekaro AL, Kavanagh R, Kanto H, Terzieva S, Hauser J, Kobayashi N, et al. Alpha-Melanocortin and endothelin-1 activate anti apoptotic pathways and reduce DNA damage in human melanocytes. *Cancer Res.* 2005; 65(10):4292-9. <https://doi.org/10.1158/0008-5472.CAN-04-4535> PMID:15899821
- Slominski A, Wortsman J, Luger T, Paus R, Solomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. *Physiol Rev.* 2000; 80:979-1020. <https://doi.org/10.1152/physrev.2000.80.3.979> PMID:10893429
- Slominski A, Zmijewski M, Zbytek B, Tobin DJ, Theoharides TC, Rivier J. Key role of CRF in the skin stress response system. *Endocr Rev.* 2013; 34:827-84. <https://doi.org/10.1210/er.2012-1092> PMID:23939821 PMID:PMC3857130
- Slominski A, Wortsman J, Tobin DJ. The cutaneous serotonergic/melatonergic system: securing a place under the sun. *FASEB J.* 2005; 19(2):176-94. <https://doi.org/10.1096/fj.04-2079rev> PMID:15677341
- Swope VB, Abdel-Malek ZA. Significance of the melanocortin-1 and endothelin-B receptors in melanocyte homeostasis and prevention of sun-induced genotoxicity. *Front Genet.* 2016; 7:146. <https://doi.org/10.3389/fgene.2016.00146> PMID:27582758 PMID:PMC4987328
- Böhm M, Luger TA, Tobin DJ, García-Borrón JC. Melanocortin receptor ligands: new horizons for skin biology and clinical dermatology. *J Invest Dermatol.* 2006; 126(9):1966-75. <https://doi.org/10.1038/sj.jid.5700421> PMID:16912693
- Imokawa G. Autocrine and paracrine regulation of melanocytes in human skin and in pigmentary disorders. *Pigment Cell Res.* 2004; 17(2):96-110. <https://doi.org/10.1111/j.1600-0749.2003.00126.x> PMID:15016298
- Imokawa G, Yada Y, Miyagishi M. Endothelins secreted from human keratinocytes are intrinsic mitogens for human melanocytes. *J Biol Chem.* 1992; 267(34): 24675-80.
- Song X, Mosby N, Yang J, Xu A, Abdel-Malek Z, Kadekaro AL. Alpha-MSH activates immediate defense responses to UV-induced

- oxidative stress in human melanocytes. *Pigment Cell Melanoma Res.* 2009; 22:809-18. <https://doi.org/10.1111/j.1755-148X.2009.00615.x> PMID:19659742
19. Tada A, Suzuki I, Im S, Davis MB, Cornelius J, Babcock G, et al. Endothelin-1 is a paracrine growth factor that modulates melanogenesis of human melanocytes and participates in their responses to ultraviolet radiation. *Growth Differ.* 1998; 9:575-84.
20. Waster P, Rosdahl I, Öllinger K. Cell fate regulated by nuclear factor- $\kappa$ B- and activator protein-1-dependent signalling in human melanocytes exposed to ultra- violet A and ultraviolet B. *Br J Dermatol.* 2014; 171:1336-46. <https://doi.org/10.1111/bjd.13278> PMID:25046326 PMCID:PMC4298246
21. Cagnol S, Chambard JC. ERK and cell death: mechanisms of ERK-induced cell death-apoptosis, autophagy and senescence. *FEBS J.* 2010; 277:2-21. <https://doi.org/10.1111/j.1742-4658.2009.07366.x> PMID:19843174
22. Wada T, Penninger JM. Mitogen-activated protein kinases in apoptosis regulation. *Oncogene.* 2004; 23(16):2838-49. <https://doi.org/10.1038/sj.onc.1207556> PMID:15077147
23. Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res.* 2002; 12:9-18. <https://doi.org/10.1038/sj.cr.7290105> PMID:11942415
24. Shi Q, Zhang W, Guo S, Jian Z, Li S, Li K, et al. Oxidative stress-induced overexpression of miR-25: the mechanism underlying the degeneration of melanocytes in vitiligo. *Cell Death Differ.* 2015; 23:496-508. <https://doi.org/10.1038/cdd.2015.117> PMID:26315342 PMCID:PMC5072443
25. Moritz RFA, Southwick EE. *Bees a superorganisms: An evolutionary reality.* 1st ed. Springer-Verlag, Berlin: Germany, 1992. [https://doi.org/10.1007/978-3-642-84666-3\\_1](https://doi.org/10.1007/978-3-642-84666-3_1)
26. Nagai T, Inoue R. Preparation and the functional properties of water and alkaline extract of royal jelly. *Food Chem.* 2004; 84:181-6. [https://doi.org/10.1016/S0308-8146\(03\)00198-5](https://doi.org/10.1016/S0308-8146(03)00198-5)
27. Nagai T, Inoue R, Suzuki N, Nagashima T. Antioxidant properties of enzymatic hydrolysates from royal jelly. *J Med Food.* 2006; 9:363-7. <https://doi.org/10.1089/jmf.2006.9.363> PMID:17004899
28. Liu JR, Yang YC, Shi LS, Peng CC. Antioxidant properties of royal jelly associated with larval age and time of harvest. *Altern Med Rev.* 2008; 13:330-3.
29. Jamnik P, Goranovič D, Raspor D. Antioxidative action of royal jelly in the yeast cell. *Exp Gerontology.* 2007; 42(7):594-600. <https://doi.org/10.1016/j.exger.2007.02.002> PMID:17383134
30. El-Nekeety AA, El-Kholy W, Abbas NF, Ebaid A, Amra HA, Abdel-Wahhab MA. Efficacy of royal jelly against the oxidative stress of fumonisin in rats. *Toxicol.* 2007; 50(2):256-69. <https://doi.org/10.1016/j.toxicol.2007.03.017> PMID:17490698
31. Silici S, Ekmekcioglu O, Eraslan G, Demirtas A. Antioxidative effect of royal jelly in cisplatin- induced testes damage. *Urology.* 2009; 74(3):545-51. <https://doi.org/10.1016/j.urology.2009.05.024> PMID:19616287
32. Inoue S, Koya-Miyata S, Ushio S, Iwaki K, Ikeda M, Kurimoto M. Royal jelly prolongs the life span of C3H/HeJ mice; correlation with reduced DNA damage. *Exp Gerontol.* 2003; 38(9):965-9. [https://doi.org/10.1016/S0531-5565\(03\)00165-7](https://doi.org/10.1016/S0531-5565(03)00165-7)
33. Kanbur M, Erasian G, Beyaz L, Silici S, Liman BC, Altinordulu S, et al. The effects of royal jelly on liver damage induced by paracetamol in mice. *Exp Toxicol Pathol.* 2009; 61(2):123-32. <https://doi.org/10.1016/j.etp.2008.06.003> PMID:18693095