

Faculty of Science University of Kragujevac

## BEE PRODUCT ROYAL JELLY REDUCE OXIDATIVE STRESS IN HEALTHY MRC-5 CELLS AND UPREGULATE GSTP1 EXPRESSION



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# S- INTRODUCTION

Oxidative stress, as an imbalance between oxidants and antioxidants in favour of the oxidants, primarily reactive oxidative species (ROS), causes irreparable cell damage. Among the most common ROS, besides nitrites are superoxide anion radicals  $O_2^{-}$ . This deregulation in redox homeostasis deeply impacts human health and causes various pathological conditions. Redox homeostasis in human body is strictly regulated by

the "redox-buffering" intracellular thiols, reducing molecules, such as glutathione (GSH), as well as various antioxidant enzymes. Glutathione S-transferase proteins (GSTPs) are antioxidative enzymes that control drug metabolism and protects the cell from oxidative stress by conjugating GSH with a wide variety of highly electrophilic/lipophilic substrates and reactive species. Also, the commercial use of many natural antioxidants, functional foods and nutraceuticals showed to possess the ability to reduce ROS and oxidative stress, resulting in an increase of the life span. As well-known bee product, royal jelly (RJ) has been traditionally used since ancient times due to its numerous biological properties and is considered nowadays as effective dietary supplement. It is a very potent reducer of oxidative stress *via* suppression of free radicals and increase of enzymatic and non-enzymatic components of the antioxidative system. Therefore, the subject of current investigation is evaluation of the RJ's ability to scavenge  $O_2^{--}$  and modulate *GSTP1* gene expression in healthy cells, which has not been analyzed so far, in order to offer a scientific basis for further development and use of this natural bee product in maintaining of human wellbeing.



**Figure 1.** Commercially available royal jelly supplement

# METHODS

Healthy lung fibroblast cell line MRC-5 was treated with RJ in selected concentration - 100  $\mu$ g/mL. After 24 h, the level of O<sub>2</sub><sup>--</sup> was determined using NBT test, and the obtained results are presented as nmol O<sub>2</sub><sup>--</sup> per mL and calculated in relation to the number of viable cells. The expression of *GSTP1* marker was assessed by qPCR method and results are presented as relative mRNA expression.

# RESULTS

The antioxidative potential of RJ was investigated in control and treated MRC-5 cells within 24 h and according to the results of present study, this natural bee product reduced the level of  $O_2$ .<sup>-</sup> in tested cells when compared to control values.



RJ treatment significantly upregulated *GSTP1* mRNA levels in MRC-5 cells after 24 h, when compared to the control values (untreated cells).



#### ) 3 6 9 12 15

### nmol $O_2^{-}/mL/nr.$ of viable cells

**Figure 2.** The concentration of superoxide anion radicals  $O_2^{+-}$  in control (untreated) and MRC-5 cells treated with RJ. The results are obtained after 24 h and presented as mean ± standard error from two in-dependent experiments performed in triplicates and expressed in relation to the number of viable cells. \*p < 0.05 designated statistically significant difference between treatment and control.



**Figure 3.** Relative mRNA expression of GSTP1 gene detected in MRC-5 cells 24 h after treatment with RJ. Results are presented as mean  $\pm$  standard error from two independent experiments performed in duplicates and expressed in relation to the untreated (control) cells and housekeeping gene  $\beta$ -actin. \*p < 0.05 designated statistically significant difference between treatment and control values.



RJ has an important protective effect against oxidative damage of healthy human lung fibroblasts. Due to its notable antioxidant activity, it becomes an ideal option for application in order to maintain human wellbeing.



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