

THOUGH A LOT OF ANTIOXIDANT ACTIVE COMPOUNDS FROM PLANT ORIGIN HAVE ANTI-INFLAMMATORY PROPERTIES, THE MECHANISMS OF THEIR ACTION ARE NOT FULLY UNDERSTOOD.

ELLAGIC ACID/ELLAGITANNINS ARE PREDOMINANT PHENOLIC COMPOUNDS OF RASPBERRIES AND THEY SIGNIFICANTLY CONTRIBUTE TO THE ANTIOXIDANT ACTIVITY OF THESE BERRIES [1]. THEREFORE, ELLAGIC ACID, RASPBERRY FRUITS AND THEIR MARC (WHERE ELLAGIC ACID/ELLAGITANNINS ARE BY FAR MORE ABUNDANT) ARE OF PARTICULAR INTEREST FROM PHARMACOLOGICAL POINT OF VIEW.

THUS, THE AIM OF THIS INVESTIGATION WAS TO EVALUATE AND TO COMPARE THE ANTI-INFLAMMATORY EFFECT OF DRY RASPBERRY FRUIT AND MARC EXTRACTS (CVS. 'Beglianka' AND 'NOVOKITAEVSKAJA'), AND ALSO ELLAGIC ACID BY ASSESSMENT OF THE PRODUCTION OF THE HYDROGEN PEROXIDE BY MURINE MACROPHAGE J774 CELLS. HYDROGEN PEROXIDE PRODUCTION BY MACROPHAGES WAS STIMULATED WITH ARACHIDONIC ACID (ARA) AND PHORBOL-12-MYRISTATE-13-ACETATE (PMA). ADDITIONALLY, THE EFFECT OF RASPBERRY EXTRACTS ON THE VIABILITY OF MACROPHAGES WAS INVESTIGATED.

HOMOGENIZED RASPBERRY FRUITS AND MARC WERE EXTRACTED WITH 80% METHANOL, FILTERED AND DRIED IN A ROTARY VACUUM EVAPORATOR AND LATER ON IN A FREEZE DRYER.

THE TOTAL PHENOLICS CONTENT (TPC) OF THE EXTRACT WAS DETERMINED WITH FOLIN-CIOCALTEU REAGENT, USING GALLIC ACID AS A STANDARD. THE ANTIRADICAL ACTIVITY WAS DETERMINED BY DPPH RADICAL SCAVENGING ASSAY [2] AND EXPRESSED AS TROLOX EQUIVALENT ANTIOXIDANT CAPACITY (TEAC). THE pH DIFFERENTIAL METHOD WAS USED FOR ANTHOCYANIN DETERMINATION. ELLAGITANNINS WERE QUANTIFIED AS ELLAGIC ACID EQUIVALENTS AFTER ACID HYDROLYSIS OF THE EXTRACTS. FREE ELLAGIC ACID WAS ANALYZED IN NON-HYDROLYZED SAMPLES. ELLAGIC ACID AND ITS DERIVATIVES WERE DETERMINED BY RP-HPLC [3]. MEASUREMENT OF HYDROGEN PEROXIDE WAS PERFORMED USING MACROPHAGE CULTURE ( $3 \times 10^5$  cell/mL) STIMULATED BY ARA AND PMA, AMPLIX RED, HORSE RADISH PEROXIDASE AND WITH OR WITHOUT ADDED RASPBERRY FRUIT/MARC EXTRACTS AND ELLAGIC ACID. MACROPHAGE VIABILITY WAS EVALUATED USING TRYPAN BLUE TEST.

THE TPC OF 'NOVOKITAEVSKAJA' FRUIT EXTRACT WAS APPROXIMATELY 2.9-FOLD LOWER THAN THAT OF 'NOVOKITAEVSKAJA' MARC EXTRACT AND 2-FOLD LOWER THAN THAT OF 'Beglianka' MARC EXTRACT (TAB. 1.). SIMILARLY, ELLAGITANNINS CONCENTRATION OF 'NOVOKITAEVSKAJA' FRUIT EXTRACT WAS APPROXIMATELY 2.6-FOLD LOWER THAN THAT OF 'NOVOKITAEVSKAJA' MARC EXTRACT AND 1.7-FOLD LOWER THAN THAT OF 'Beglianka' MARC EXTRACT. ANTHOCYANINS WERE NOT DETECTED IN 'Beglianka' MARC EXTRACTS. THE EXTRACT MADE OF 'NOVOKITAEVSKAJA' FRUITS ALSO HAD THE LOWEST TEAC VALUE (TAB. 1.).

THE EFFECT OF RASPBERRY FRUIT AND MARC EXTRACTS ON  $H_2O_2$  PRODUCTION OF PMA STIMULATED MACROPHAGES WAS CONCENTRATION DEPENDENT (FIG. 1.). ALL TESTED EXTRACT CONCENTRATIONS SIGNIFICANTLY INHIBITED  $H_2O_2$  PRODUCTION WHEN COMPARED WITH CONTROL. AT EXTRACT CONCENTRATION OF 10  $\mu\text{g/mL}$   $H_2O_2$  GENERATION INHIBITORY EFFECT OF 'Beglianka' MARC WAS 53%, OF 'NOVOKITAEVSKAJA' MARC – 51% AND OF 'NOVOKITAEVSKAJA' FRUIT EXTRACT – 32%.

RASPBERRY EXTRACTS AT A 10 – 1200  $\mu\text{g/mL}$  CONCENTRATIONS INHIBITED  $H_2O_2$  PRODUCTION IN ARA STIMULATED MACROPHAGES, ON AVERAGE, FROM 30% (AT A CONCENTRATION OF 10  $\mu\text{g/mL}$ ) UP TO 80% (AT A CONCENTRATION OF 1200  $\mu\text{g/mL}$ ) (FIG. 2.). THERE WERE NO STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN THE EFFECTS OF THE MARC EXTRACTS OF 'NOVOKITAEVSKAJA' AND 'Beglianka'. MARC EXTRACT OF 'NOVOKITAEVSKAJA' INHIBITED  $H_2O_2$  GENERATION AT A GREATER EXTENT THAN FRUIT EXTRACT AT A CONCENTRATIONS OF 10 AND 100  $\mu\text{g/mL}$ .

COMPARING THE EFFECT OF ELLAGIC ACID TO PMA AND ARA STIMULATED MACROPHAGES, SIGNIFICANT DIFFERENCE BETWEEN THESE TWO STIMULATIONS WAS DETERMINED WHEN CONCENTRATION OF ELLAGIC ACID WAS 8.75  $\mu\text{g/mL}$ . ELLAGIC ACID INHIBITED RADICAL GENERATION UP TO 75%, WHEN MACROPHAGES WERE STIMULATED WITH PMA, AND ONLY UP TO 37%, WHEN MACROPHAGES WERE STIMULATED WITH ARA (FIG. 3.).

CELL VIABILITY WAS SIGNIFICANTLY INHIBITED BY 'Beglianka' AND 'NOVOKITAEVSKAJA' MARC EXTRACTS IN A DOSE DEPENDENT MANNER (FOR 'NOVOKITAEVSKAJA' EXTRACT  $R=0.82$  AND FOR 'Beglianka' EXTRACT  $R=0.86$ ) (FIG. 4.). MARC EXTRACT OF 'NOVOKITAEVSKAJA', COMPRISING HIGHER CONTENT OF PHENOLIC COMPOUNDS, INHIBITED CELL VIABILITY MORE EFFECTIVELY THAN MARC EXTRACT OF 'Beglianka' ( $IC_{50}=130$  AND 200  $\mu\text{g/mL}$ , RESPECTIVELY). EFFECT OF ELLAGIC ACID ON MACROPHAGE VIABILITY WAS SIGNIFICANTLY HIGHER THAN THAT OF RASPBERRY MARC EXTRACTS ( $IC_{50}=23$   $\mu\text{g/mL}$ ) (FIG. 5.).

## THE EFFECTS OF ELLAGITANNIN-RICH EXTRACTS AND ELLAGIC ACID ON HYDROGEN PEROXIDE PRODUCTION BY MACROPHAGES AND ON THEIR VIABILITY

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TAB. 1. PHENOLIC COMPOSITION AND DPPH-TEAC OF RASPBERRY FRUIT AND MARC EXTRACTS.

Extract	Anthocyanins	Phenolics	Ellagitannins	Free ellagic acid	$\mu\text{mol TE/g}$ of extract
'Beglianka' marc	n.d.	101.24±3.23	32.13±1.01	1.73±0.04	503.0±31.73
'Novokitaevskaja' marc	5.25±0.14	149.34±4.01	50.12±1.62	2.74±0.07	590.2±41.06
'Novokitaevskaja' fruits	6.10±0.18	51.62±2.10	19.23±0.22	0.82±0.02	334.2±20.66

FIG. 1. THE EFFECT OF RASPBERRY FRUIT AND MARC EXTRACTS ON HYDROGEN PEROXIDE PRODUCTION (nmol), WHEN J774 MACROPHAGES WERE STIMULATED WITH PMA. EXPRESSED AS MEANS ± SE (n = 3). STATISTICAL SIGNIFICANCE IS BASED ON THE DIFFERENCE WHEN COMPARED WITH THE CONTROL CELLS (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001).

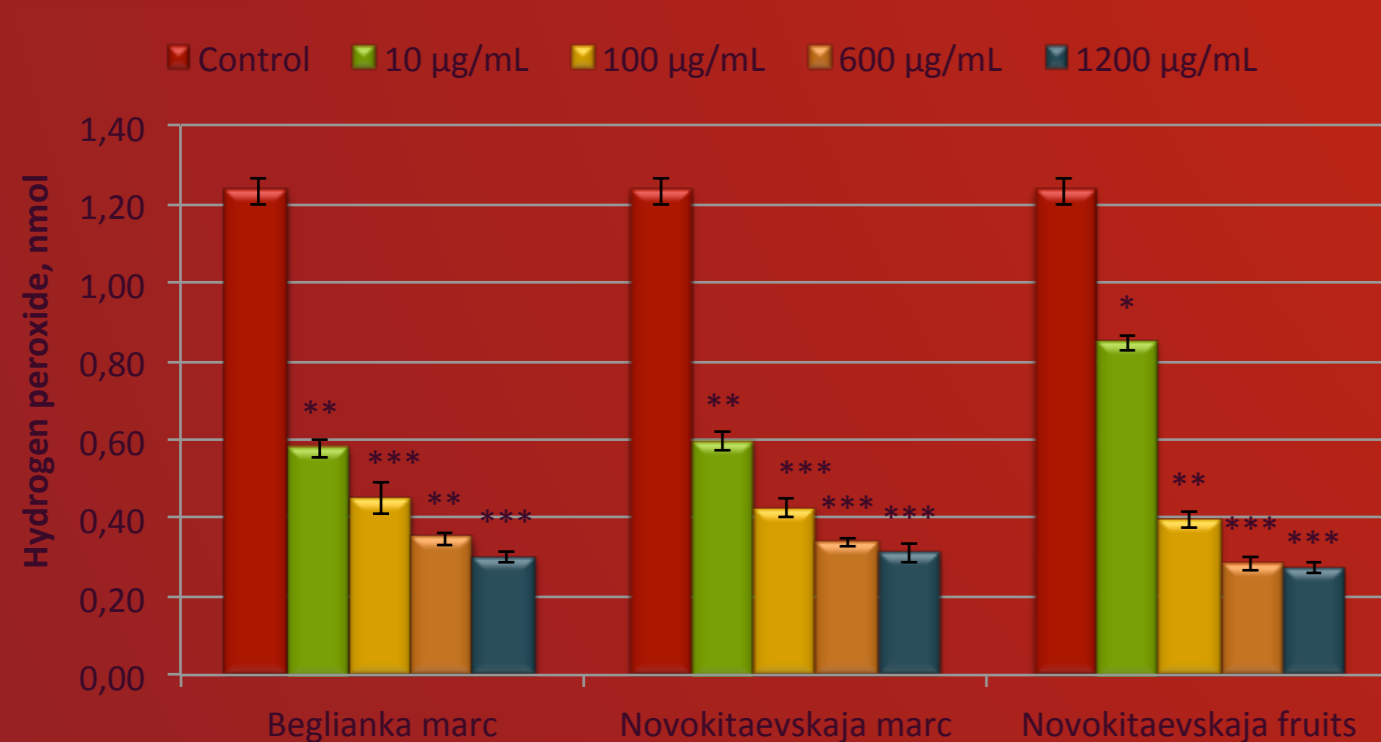


FIG. 2. THE EFFECT OF RASPBERRY FRUIT AND MARC EXTRACTS ON HYDROGEN PEROXIDE PRODUCTION (nmol), WHEN J774 MACROPHAGES WERE STIMULATED WITH ARA. EXPRESSED AS MEANS ± SE (n = 3). STATISTICAL SIGNIFICANCE IS BASED ON THE DIFFERENCE WHEN COMPARED WITH THE CONTROL CELLS (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001).

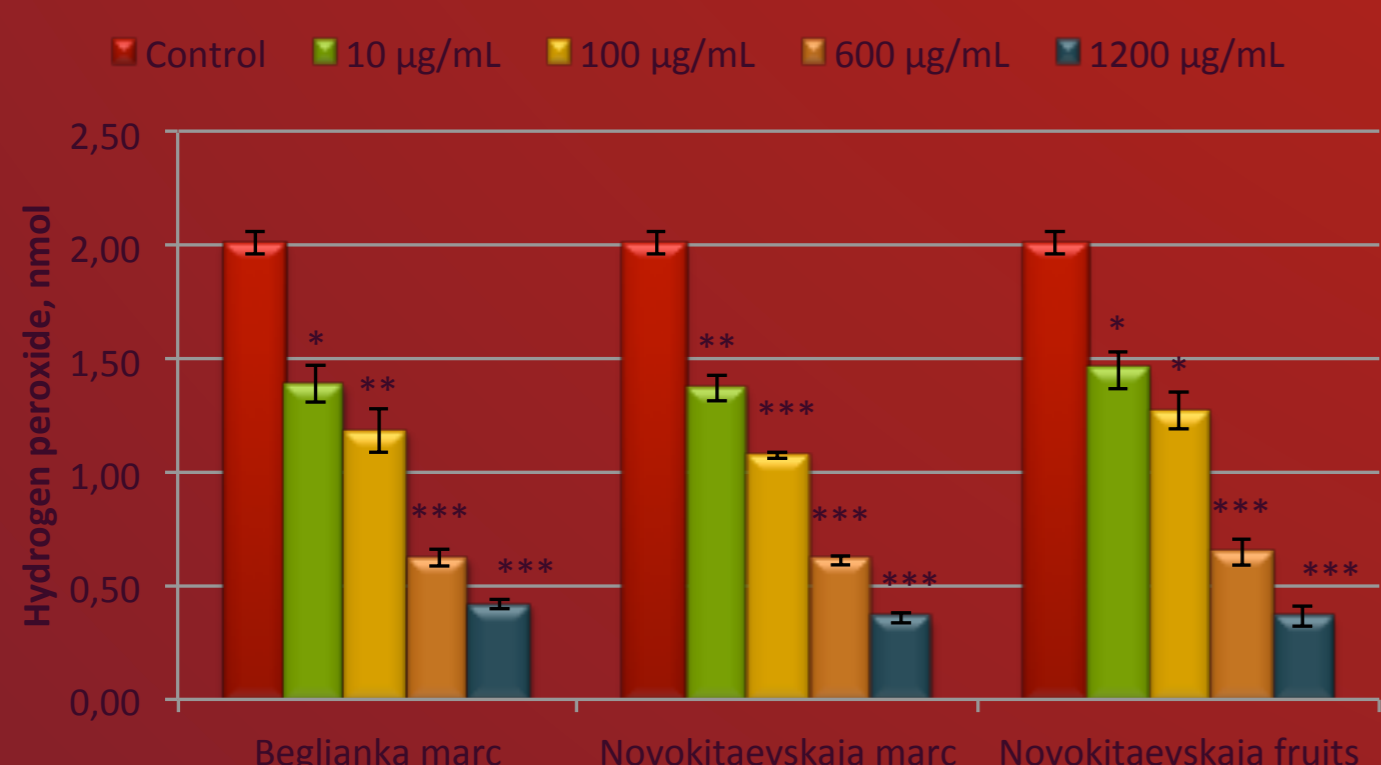


FIG. 3. THE EFFECT OF ELLAGIC ACID ON HYDROGEN PEROXIDE PRODUCTION (nmol), WHEN J774 MACROPHAGES WERE STIMULATED WITH ARA AND PMA. EXPRESSED AS MEANS ± SE (n = 3). STATISTICAL SIGNIFICANCE IS BASED ON THE DIFFERENCE WHEN COMPARED WITH THE CONTROL CELLS (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001).

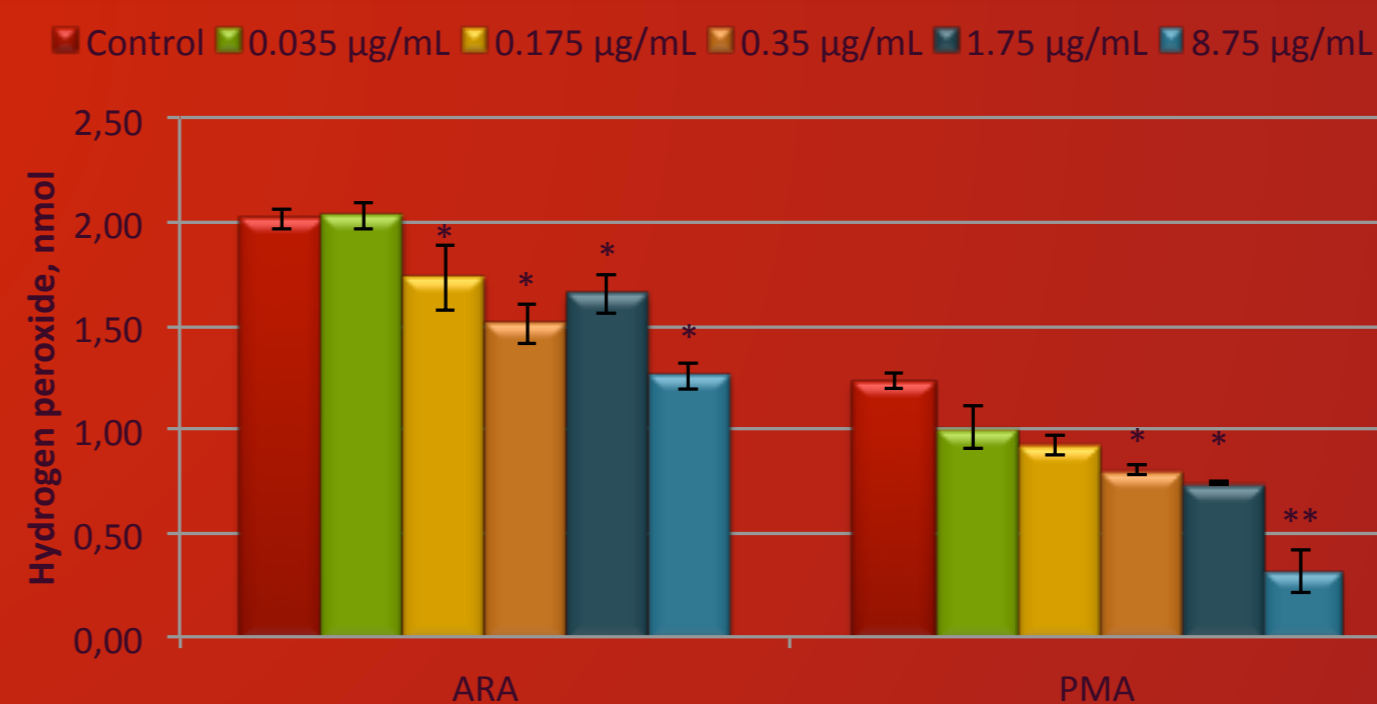


FIG. 4. THE EFFECT OF RASPBERRY MARC EXTRACTS ON J774 MACROPHAGE CELL CULTURE VIABILITY. EXPRESSED AS MEANS ± SE (n = 3). STATISTICAL SIGNIFICANCE IS BASED ON THE DIFFERENCE WHEN COMPARED WITH THE CONTROL CELLS (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001).

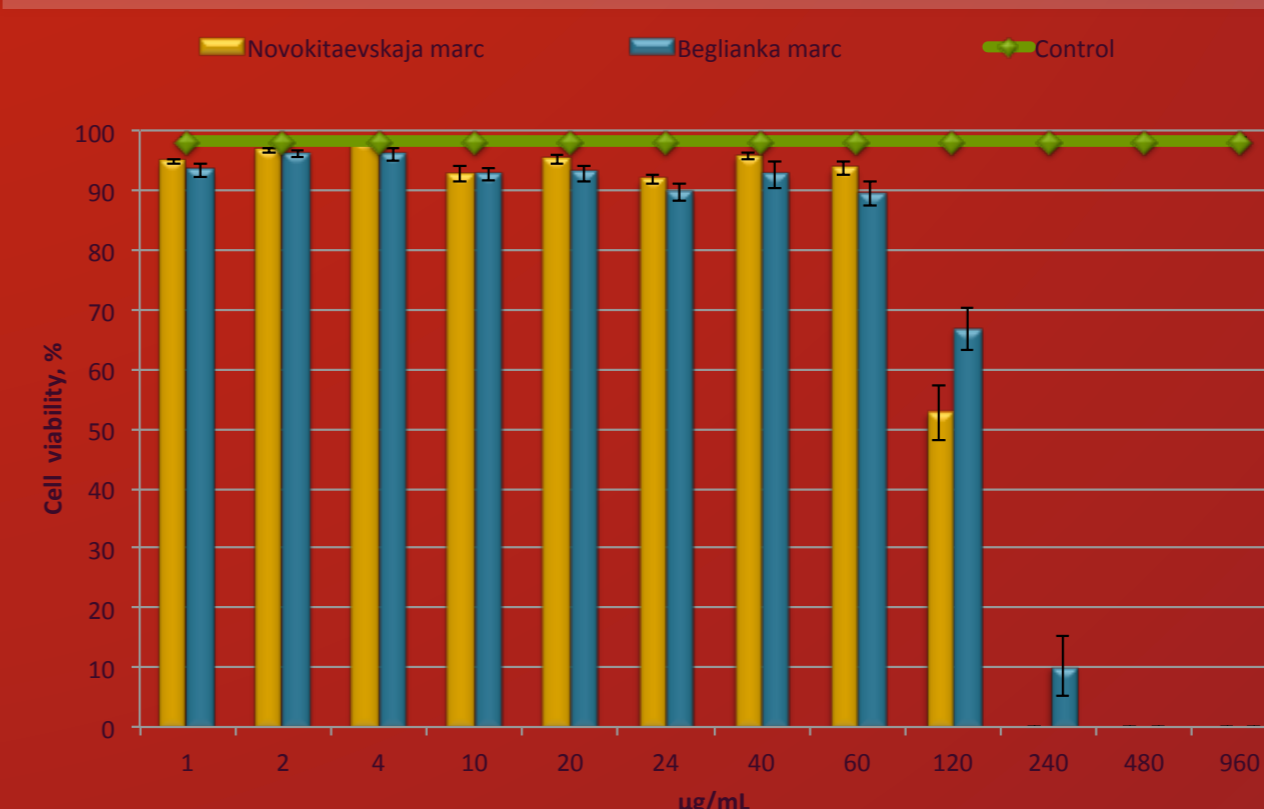
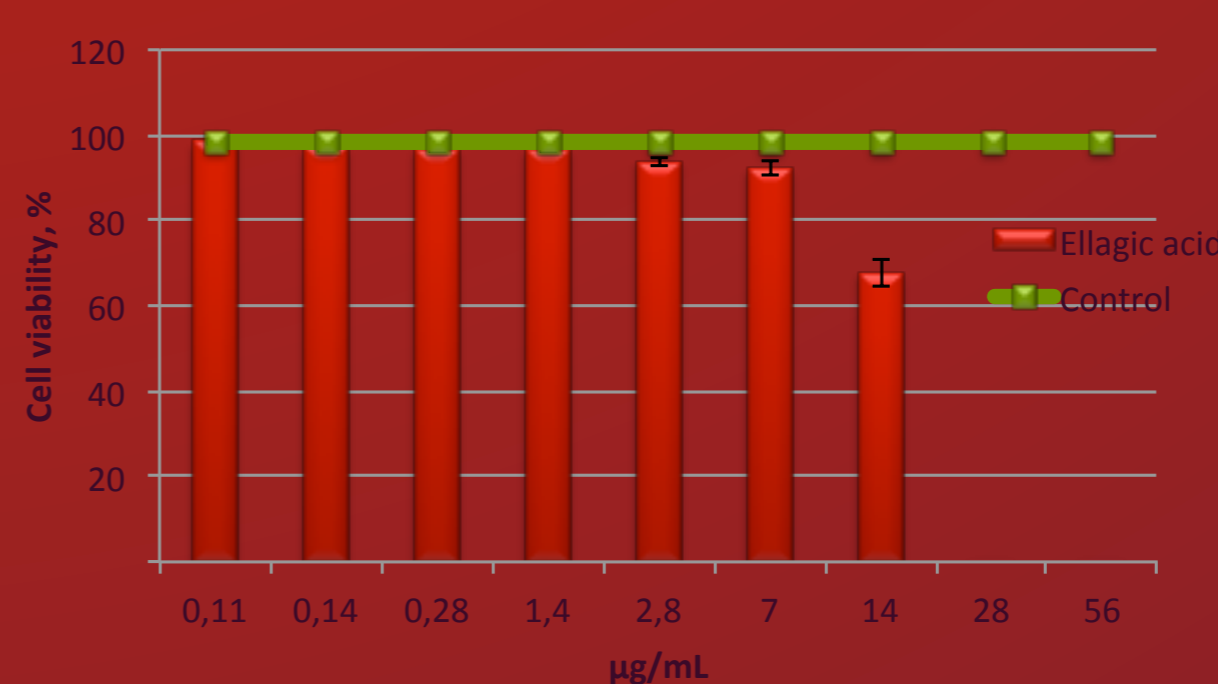


FIG. 5. THE EFFECT OF ELLAGIC ACID ON J774 MACROPHAGE CELL CULTURE VIABILITY. EXPRESSED AS MEANS ± SE (n = 3)  $\mu\text{g/mL}$ . STATISTICAL SIGNIFICANCE IS BASED ON THE DIFFERENCE WHEN COMPARED WITH THE CONTROL CELLS (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001).



## CONCLUSIONS

THE MODE OF ACTION OF RASPBERRY EXTRACT DEPENDS NOT ONLY ON CONCENTRATION BUT ALSO ON THE MECHANISM THAT TRIGGERS THE ACTIVATION OF NADPH OXIDASE. RASPBERRY EXTRACTS MAY ACT DIFFERENTLY ON PATHWAYS IMPLICATED INTO INFLAMMATION.

[1] BEEKWILDER J., JONKER H., MEESTERS P., HALL R. D., VAN DER MEER I. M., DE VOS C. H. R. 2005. ANTIOXIDANTS IN RASPBERRY: ON-LINE ANALYSIS LINKS ANTIOXIDANT ACTIVITY TO A DIVERSITY OF INDIVIDUAL METABOLITES. J. AGRIC. FOOD CHEM. [2] BRAND-WILLIAMS W., CUVELIER M. E., BERSET C. 1995. USE OF A FREE RADICAL METHOD TO EVALUATE ANTIOXIDANT ACTIVITY. LEBENS-M. WISS. UND-TECHNOL. [3] KOPONEN J. M., HAPPONEN A. M., MATTILA, P. H. TORRONEN, A. R. 2007. CONTENTS OF ANTHOCYANINS AND ELLAGITANNINS IN SELECTED FOODS CONSUMED IN FINLAND. J. AGRIC. FOOD CHEM.

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