Original article DOI: 10.2478/aiht-2022-73-3653

Cytotoxic activity of strawberry tree (*Arbutus unedo* L.) honey, its extract, and homogentisic acid on CAL 27, HepG2, and Caco-2 cell lines

Andreja Jurič[1], Ana Huđek Turković[2], Irena Brčić Karačonji[1,3], Saša Prđun[4], Dragan Bubalo[4], and Ksenija Durgo[2]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Zagreb Faculty of Food Technology and Biotechnology, Zagreb, Croatia
[3] University of Rijeka Faculty of Health Studies, Rijeka, Croatia
[4] University of Zagreb Faculty of Agriculture, Zagreb, Croatia

[Received in April 2022; Similarity Check in April 2022; Accepted in May 2022]

Strawberry tree (*Arbutus unedo* L.) honey (STH), also known as "bitter honey", is a traditional medicine widely used in the Mediterranean area. Regardless of geographical origin, it usually has a very high content of phenolic compounds and strong antioxidant capacity. Yet, little is still known about the effects of STH, its phenolic extract (STHE), and its main bioactive compound – homogentisic acid (HGA) – at the cell level. The aim of this study was to estimate total phenolic content, DPPH radical scavenging activity, and ferric reducing antioxidant power of STH made in Croatia and investigate cytotoxic and pro-oxidative effects of STH, STHE and HGA on three human cell lines: tongue squamous cell carcinoma (CAL 27), hepatocellular carcinoma (HepG2), and epithelial colorectal adenocarcinoma cells (Caco-2) cells. These substances were tested at four concentrations (0.5–5× average human daily intake of STH) and over 30 min and 1 and 2 h. Croatian STH had a total phenolic content of 1.67 g gallic acid equivalents (GAE) per kg of honey, DPPH radical scavenging activity of 2.96 mmol Trolox equivalents (TE) per kg of honey, and ferric reducing antioxidant power (FRAP) of 13.5 mmol Fe^{2+} per kg of honey. Our results show no clear and consistent time- or concentration-dependent cytotoxicity in any of the cell lines. ROS levels in all the three cell types at almost all exposure times were not significantly higher than control. The most important observation is that the tested substances have low cytotoxicity and high biocompatibility, regardless of concentration, which is a good starting point for further research of their biological effects in other models.

KEY WORDS: colon adenocarcinoma; hepatocellular carcinoma; phenols; human cell lines; cell viability; reactive oxygen species; tongue adenosquamous carcinoma

As the incidence of cancer cases grows, so does intensify research for natural alternatives to conventional anticancer drugs with fewer side effects, greater efficiency, and lower cost of therapy. Beneficial effects of honey on human health have been known for centuries (1) and are mostly owed to the antibacterial and antioxidant properties of its bioactive constituents, such as phenolic compounds (2).

Strawberry tree *(Arbutus unedo* L.) honey (STH), also known as a "bitter honey", is a typical product of the Mediterranean (3). Several studies have shown that STH has a very high content of phenolic compounds and strong antioxidant capacity. Compared to other types, this unifloral honey has greater ability to defend the body from harmful reactive oxygen species (ROS) (4, 5). It also exerts antibacterial and anti-inflammatory activity with high anti-mutagenic and anti-proliferative properties important for tumour prevention and treatment (6, 7).

The main phenolic constituent of STH is homogentisic acid (2,5-dihydroxyphenylacetic acid, HGA), which has remarkable antioxidant, antiradical, and protective properties against thermal cholesterol degradation, comparable to those of other, better known antioxidants (8).

As STH, its phenolic extract (STHE), and HGA effects at the cell level have not been studied extensively so far, the aim of our study was to investigate their effects on ROS production and viability of human cell models of tongue squamous cell carcinoma (CAL 27), hepatocellular carcinoma (HepG2), and epithelial colorectal adenocarcinoma cells (Caco-2). To establish possible concentration-dependent effects, the cells were exposed to these substances at four concentrations for 30 min, 1 h, and 2 h. STH was also characterised for total phenolic content, radical scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP). We hoped that our findings would

Corresponding author: Andreja Jurič, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10000 Zagreb, Croatia
E-mail: *ajuric@imi.hr*, ORCID: 0000-0002-9279-5159

provide new knowledge about the beneficial effects of the tested compounds and help establish STH as a novel nutraceutical product.

MATERIALS AND METHODS

Sample preparation

The STH sample was collected in Vrgorac (43.20 °N 17.37 °E), Croatia in 2016. Its botanical origin was confirmed earlier by melissopalynological and sensory analyses (9) and identification of its specific chemical marker HGA. The mass fraction of HGA in the honey sample, also determined earlier by gas chromatography-mass spectrometry (GC-MS) (10), was 306.8 mg/kg. Its phenolic profile was determined with an ultra-high performance liquid chromatograph (UHPLC) coupled to a linear ion trap-Orbitrap hybrid mass spectrometer (LTQ Orbitrap MS) (11).

The STHE sample was prepared using a solid phase extraction (SPE) column (Oasis HLB; 200 mg; 3 mL, Waters, Etten-Leur, The Netherlands). Honey (1 g) was mixed with 15 mL of demineralised water, adjusted to pH 2 with 0.1 % hydrochloric acid, then mixed for 30 s, and passed through a 0.2 μm pore PTFE filter (LLG Labware, Meckenheim, Germany). The SPE cartridge was conditioned with 3 mL of methanol and 3 mL of acidified demineralised water. The mixture of honey and water (15 mL) was passed through the cartridge and washed with 6 mL of acidified demineralised water to remove all sugars and other polar constituents of honey. The adsorbed phenolic compounds were eluted with methanol (3 mL). The eluate was evaporated in a rotary evaporator at reduced pressure and 50 °C. From 1 g of STH we obtained 5 mg of STHE.

To control for sugar interferences, we also used a sample of artificial honey (AFH) prepared by mixing fructose (40 mg), glucose (30 mg), maltose (8 mg), and sucrose (2 mg) with 10 mL of demineralised water based on average sugar content in honey (4).

HGA was purchased from Sigma-Aldrich (Steinheim, Germany). Other chemicals and reagents were purchased from Merck (Darmstadt, Germany), unless specified otherwise.

Before we started treating the cells, all tested substances were dissolved in an RPMI-1640 cell culture medium (BioWhittaker®, Lonza, Walkersville, MD, USA).

The applied concentrations of STH, STHE, and AFH were based on estimated average daily honey intake of 50 g (two spoons; as self-reported by consumers) for a person weighing 70 kg (9). HGA concentrations were calculated based on its mass ratio in an average daily intake of honey, i.e. 15.34 mg/50 g.

To establish potential cytotoxic and pro-oxidative effects we tested the samples at concentrations ranging from 0.5× to 5× the concentration of estimated average daily honey intake. Experimental schedule is given in Table 1.

Determination of total phenolic content

Total phenolic content (TPC) was quantified using the Folin-Ciocalteau method (5). An aliquot (50 μL) of 10 % (w/v) aqueous honey solution was mixed with 1.4 mL of demineralised water and 100 μL of 2 mol/L Folin-Ciocalteu reagent, incubated at room temperature for 5 min, and then mixed with 1.5 mL of sodium carbonate solution (6 % w/v). Followed another incubation at 40 °C for 30 min. Absorbance was measured with a Cary 50 UV-Vis spectrophotometer (Varian, Mulgrave, Australia) at 765 nm. The obtained results are expressed as g of gallic acid equivalents (GAE) per kg of honey. The artificial honey was used as the sugar analogue to control for interferences, and gallic acid (Sigma-Aldrich) solutions (0–2.5 g/L) to construe the calibration curve and quantify phenols.

Determination of DPPH radical scavenging activity

To determine the radical scavenging activity (RSA) of the tested compounds we relied on a slightly modified method described by Tariba Lovaković et al. (4). An aliquot (200 μL) of honey diluted with demineralised water (1:10, w/v) was mixed with 1.8 mL of methanol and 1.5 mL of DPPH methanolic solution (0.18 mmol

Table 1 Experimental design

Experimental group	Tested concentrations*#			
	0.5 ×	1 ×	2.5 ×	5 ×
AHF – Artificial honey (g/L)	0.35	0.71	1.77	3.5
STH – Strawberry tree honey (g/L)	0.35	0.71	1.77	3.5
STHE – Strawberry tree honey extract (mg/L)	1.25	2.5	6.25	12.5
HGA – Homogentisic acid (mg/L)	0.107	0.214	0.535	1.07
Exposure				
30 min	1 h		2 h	
Cell cultures				
HepG2	Caco-2		CAL 27	

* 1× concentration represents the estimate of average daily honey intake of 50 g. # Tested concentrations were prepared using the RPMI-1640 medium

/L), vortexed vigorously, and the mixture incubated in the dark at 25 °C for 30 min. The absorbance was measured at 517 nm with a Cary 50 UV-Vis spectrophotometer. The calibration curve was construed with Trolox (Sigma-Aldrich) in the range of 0.01–0.30 mmol/L, and results are expressed as mmol of Trolox equivalent antioxidant capacity (TE) per kg of honey (mmol/kg).

Determination of ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of honey samples was measured as described elsewhere (12). An aliquot (200 μL) of 5 % (w/v) aqueous honey solution was mixed with 1.8 mL of FRAP reagent, vortexed, and incubated at 37 °C for 10 min. The FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tris(2-pyridyl)-s-triazine solution (Sigma-Aldrich), and 20 mmol/L $FeCl_3 \times 6H_2O$ solution (Kemika, Zagreb, Croatia) in a 10:1:1 ratio and incubated at 37 °C. The absorbance was measured at 593 nm against AFH. To construe the calibration curve, we used aqueous standard solutions of $FeSO_4 \times 7H_2O$ (Sigma-Aldrich) in the range of 0.01–2 mmol/L. Results are expressed as mmol of Fe^{2+} per kg of honey.

Cell lines

We used CAL 27(ATCC CRL-2095), HepG2 (ATCC HB-8065), and Caco-2 (ATCC HTB-37) cells cultured in monolayer in an RPMI-1640 medium (Lonza) supplemented with 10 % of foetal bovine serum (Gibco, Carlsbad, CA, USA) and 1 % of streptomycin (13) and passaged with a trypsin–EDTA solution (Gibco). The cultures were kept at 37 °C in a humid atmosphere with 5 % CO_2 (Forma Scientific, Austin, TX, USA).

We selected these three cell models because they originate from the most important tissues that interact with the studied compounds. Biological activity of phenolic compounds depends on their absorption in the intestine (Caco-2 model) and metabolism in the liver (HepG2 model). Tongue cells (CAL 27 model), on the other hand, are in the first contact with the compounds taken via oral route.

As HepG2 cells preserve the activities of many enzymes important for xenobiotic metabolism, they are widely used to investigate possible toxic effects of unknown substances with potential antitumor activity (14, 15). The choice of this cell line is very important when testing complex matrices such as honey, as it contains a number of biologically active compounds whose effectiveness may change due to metabolic transformation. The Caco-2 cells, in turn, are used in toxicity studies of compounds ingested with food, mostly in drug research and for the detection of substrates, inhibitors, and inducers of intestinal transporters, P-glycoproteins in particular. They retain the activities of various transporters, enzymes, and nuclear receptors, such as cytochrome P450 1A (CYP1A), sulphotransferase, glutathione S-transferase, and UDP-glucuronosyltransferase (16, 17). The CAL 27 cell line is less metabolically active than HepG2 and Caco-2, but it also possesses the activity of some CYP enzymes (18).

Cytotoxicity assay

We ran this assay, as this type of honey is quite rare and its safety profile poorly known. *In vitro* cytotoxicity was determined as described by Babich and Borenfreund (19). Briefly, CAL 27, HepG2, and Caco-2 cells were seeded in 96-well plates (10^5 cells per mL) and treated with STH, STHE, HGA, and AFH samples in four concentrations (Table 1) for 30 min, 1 h, and 2 h. After treatment, we added Neutral Red (Sigma-Aldrich) solution, incubated the cells at 37 °C for 1 h, removed Neutral Red solution, and washed the cells with phosphate buffered saline (PBS) (100 μL). Finally, we added 100 μL of solution combining acetic acid (Sigma-Aldrich), ethanol (Kemika), and demineralised water in the 0.1:5:4.9 ratio to extract the dye from the lysosomes of living cells. Cell viability was measured as colour intensity and absorption at 540 nm in a FLUOstar OPTIMA plate reader (BMG Labtech, Durham, NC, USA).

Data are expressed as percentage of viability of untreated cells (considered to be 100 %). Each concentration was tested in quadruplicate, and each experiment was repeated three times.

Induction of reactive oxygen species

In the tested cells, ROS were measured using the 2',7'-dichlorofluorescein-diacetate (DCFH-DA) fluorometric assay (20, 21). CAL 27, HepG2, and Caco-2 cells were seeded into 96-well black plates at 10^5 cells per mL and treated with STH, STHE, HGA, and AFH at concentrations given in Table 1 for 30 min, 1 h, and 2 h. After treatment, cells were washed with PBS (pH 7.4) and treated with 100 μL of DCFH-DA (50 μmol/L; Sigma-Aldrich, Steinheim, Germany). After half an hour, the intensity of fluorescence (λ_{Ex} 485 nm, λ_{Em} 520 nm) was measured, and results are expressed as percentage of ROS production by untreated cells (negative control). Each concentration was tested in quadruplicate, and each experiment repeated three times.

Statistical analysis

Statistical analyses were run on Statistica 13 for Windows (StatSoft Inc., Tulsa, OK, USA). Normality of data distribution was tested and confirmed with the Kolmogorov-Smirnov test. We assessed the effects of tested samples with the factorial ANOVA test (type II). The significance was established with Tukey's HSD *post-hoc* test, and the level of statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION

In this study, the analysed STH had a TPC of 1.67 g GAE per kg of honey. Its DPPH activity was 2.96 mmol TE per kg of honey and FRAP activity of 13.5 mmol Fe^{2+} per kg of honey. These

findings point to high antioxidant activity of STH. An earlier report on STH from Croatia (5) showed high mean phenolic content of 1.04 g GAE per kg of honey and strong DPPH activity (mean 3.32 mmol TE per kg of honey). A study of STH from Italy (6) reported the TPC of 0.972 g GAE per kg of honey, scavenging ability of DPPH radicals of 4.8±0.8 mmol TE per kg of honey, and FRAP activity of 11.7 mmol Fe^{2+} per kg of honey.

As reported earlier (11), STH used in this study contains flavonoids, phenolic acids, and phenolic acid derivatives, but the most abundant compound is HGA. It usually accounts for 50–60 % of the total phenolic content in STH samples collected in the Mediterranean area (4, 6, 22). Considering its high content in the tested STH, we assume that HGA largely contributed to the measured radical scavenging activity and ferric reducing antioxidant power. STH shows much better antioxidant features than other unifloral honeys (6, 22, 23) and is a highly effective cytoprotector against irinotecan-induced chromosome damage in human peripheral blood lymphocytes *in vitro* (24).

The cytotoxicity of all tested compounds was low (Figures 1–3). The lowest cell viability was found in Caco-2 cells after treatment with STH at fivefold daily average concentration for 30 min (Figure 3). There were no time or concentration-dependent effects of tested compounds on cell viability. This is not surprising, considering the complex phytochemical profiles of tested STH and STHE and possible synergistic and antagonistic effects of their constituents.

The phenomenon of cytotoxic effects of different types of honey has only recently been recognised and described in the literature (25, 26). Recently, Imtara et al. (27) reported cytotoxic effects of different honeys collected in Morocco and Palestine on human colon adenocarcinoma (HCT-116) and breast cancer (MCF-7) cell lines. They found a strong correlation between antioxidant content (phenols, favonoids, and favonol) and cytostatic effect in MCF-7 cells and a strong negative correlation between syringic and tannic acid and cytostatic activity in HCT-116 cells. The only study, besides ours, of STH from Sardinia (28) and a study of manuka honey from New Zealand (29) have shown cytotoxic effects against HCT-116 and metastatic colon epithelial adenocarcinoma cells (LoVo) and reduced cytotoxicity to non-tumour cells. The same authors also found that STH from Sardinia induced a higher percentage of ROS in LoVo cells, which points to a better anticancer potential compared to manuka honey. Chinese jujube honey was reported to have a cytotoxic effect on HepG2 cells (30), and Chilean ulmo honey on Caco-2 cells (31).

Also, the cytotoxic potential of HGA on human cells lines is almost unknown. Our recent *in vitro* study (24) suggests that HGA does not pose a significant threat to human lymphocytes, even at concentrations corresponding to a 10-fold daily average STH intake. This study confirms its negligible cytotoxic potential in the three cancer cell types.

Besides HGA, STH and its extract contain many other polyphenols, such as acacetin, quercetin, pinocembrin, apigenin, chrysin, *p*-hydroxybenzoic acid, *p*-hydroxyphenyl acetic acid, *p*-coumaric acid, gallic acid, ferulic acid, caffeic acid, and protocatechuic acid (5, 9). Existing literature provides ample evidence of their cytotoxic effects. For example, acacetin has been reported to induce cytotoxic effects in HepG2 cells (32), human non-small cell lung cancer A549 cells (33), human gastric carcinoma AGS cells (34), B-cells of chronic lymphocytic leukaemia (35), and MCF-7 and MDA-MB-468 human breast cancer cell lines (36). *p*-coumaric acid induced apoptosis in HCT-15 colon cancer cells (25). Gallic acid caused cytotoxicity through apoptosis and necrosis in HeLa cervical cancer cells (37), while its derivatives showed apoptotic potential in several carcinoma cell lines (38). Wang et al. (39) found that ferulic acid induced cell death in osteosarcoma lines 143B and MG63. Chang et al. (40) reported that caffeic acid induced apoptosis in HeLa cells. Kabala-Dzik et al. (41) also discovered the cytotoxic effects of caffeic acid on human breast cancer cells MDA-MB-231, although its phenethyl ester had stronger cytotoxicity. Yin et al. (42) reported apoptotic effects of protocatechuic acid on human breast cancer MCF7, lung cancer A549, HepG2 cells, HeLa, and prostate carcinoma cells LNCaP. High content of protocatechuic and *p*-hydroxybenzoic acid stood out as an important contributor to their antitumor effects (43). Choi et al. (44) reported that quercetin induced apoptosis in human breast cancer cells. Hashemzaei et al. (45) reported that quercetin significantly induced cell death in the colon carcinoma CT-26, prostate adenocarcinoma LNCaP, acute lymphoblastic leukaemia MOLT-4, and human lymphoid Raji cell lines. Pinocembrin induced apoptosis in the HCT-116 colon cancer cell line (46) and in the B16F10 and A375 melanoma cell lines (47). Budhraja et al. (48) reported that apigenin induced cytotoxicity in human leukaemia cells. Yang et al. (49) found that apigenin induced apoptosis and autophagy in hepatocellular carcinoma cells. Woo et al. (50) documented the cytotoxicity of crises in U937 leukaemia cells, while Khoo et al. (51) determined the cytotoxic effects of chrysin on different human cancer cell lines. Samarghandian et al. (52) confirmed the cytotoxicity of chrysin in human adenocarcinoma cells. From all these studies, we can conclude that the tested STH and its extract can initiate cell death through almost all known mechanisms of apoptosis and necrosis due to its complex phenolic composition.

Also, our results show that the three types of cells are differently sensitive to the same treatment, which may be related to the general stability or instability of cancer cells, as they may lose control over their division, growth, and mechanisms responsible for inducing cell death (53–57).

It should be noted that some of the tested samples stimulated the growth of treated cells. For example, STH at a daily intake concentration stimulated the growth of HepG2 cells after 30 min of treatment. HGA had a similar effect on Caco-2 cells at the same concentration after 2 h of treatment. In CAL 27 cells, cell viability was the highest with all tested substances. These effects for the tested honey and its extract can be related to their specific complex composition and interactions between compounds (additive or synergistic effects). Some cells probably grow better *in vitro* thanks

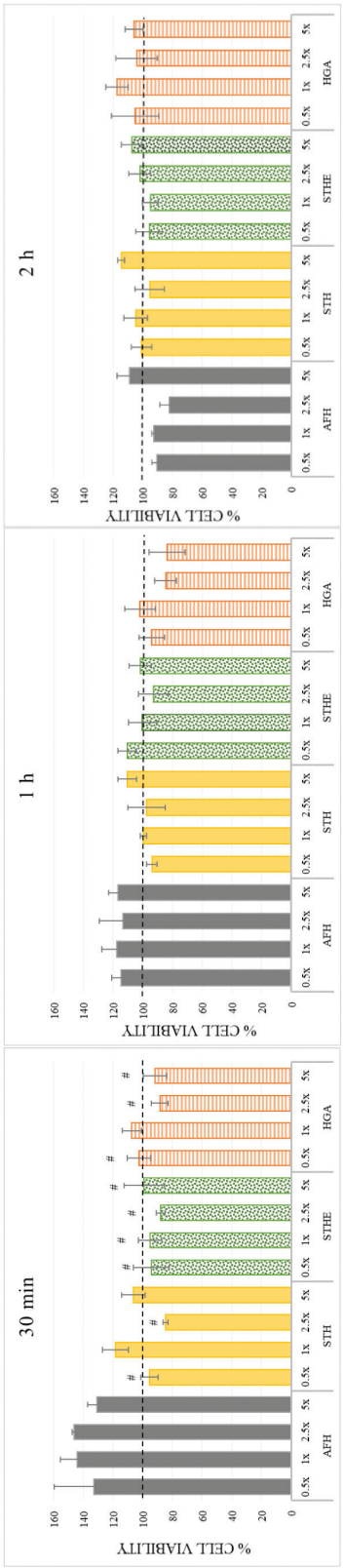

Figure 1 Cell viability of CAL 27 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). # P<0.05 vs all AFH concentrations

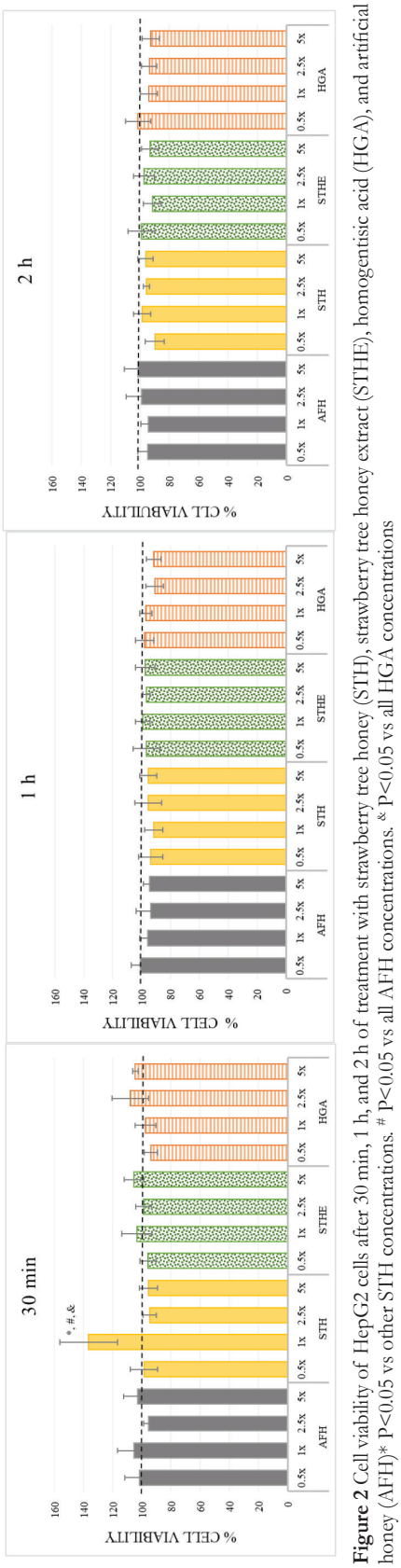

Figure 2 Cell viability of HepG2 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH)* P<0.05 vs other STH concentrations. # P<0.05 vs all AFH concentrations. & P<0.05 vs all HGA concentrations

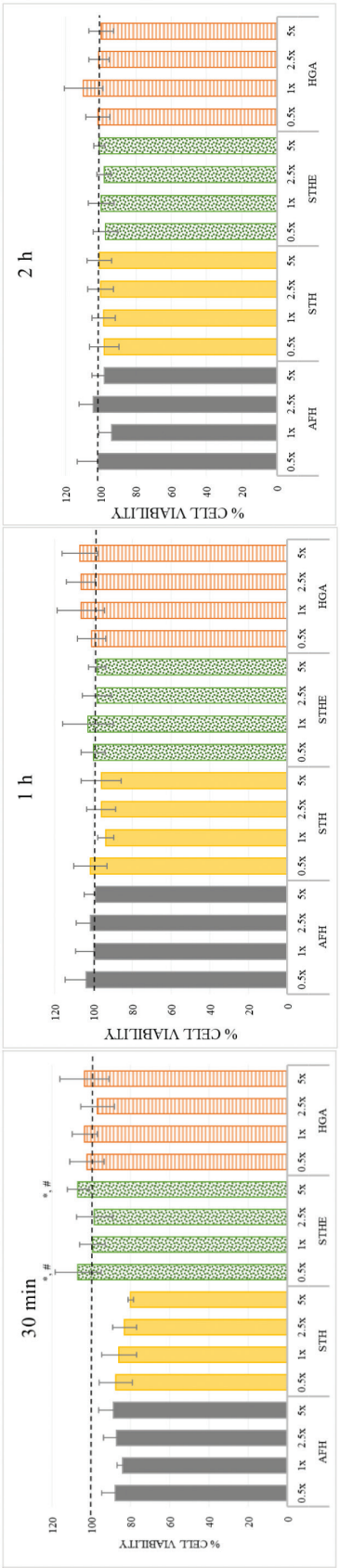

Figure 3 Cell viability of Caco-2 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). * P<0.05 vs all STH concentrations. # P<0.05 vs all AFH concentrations

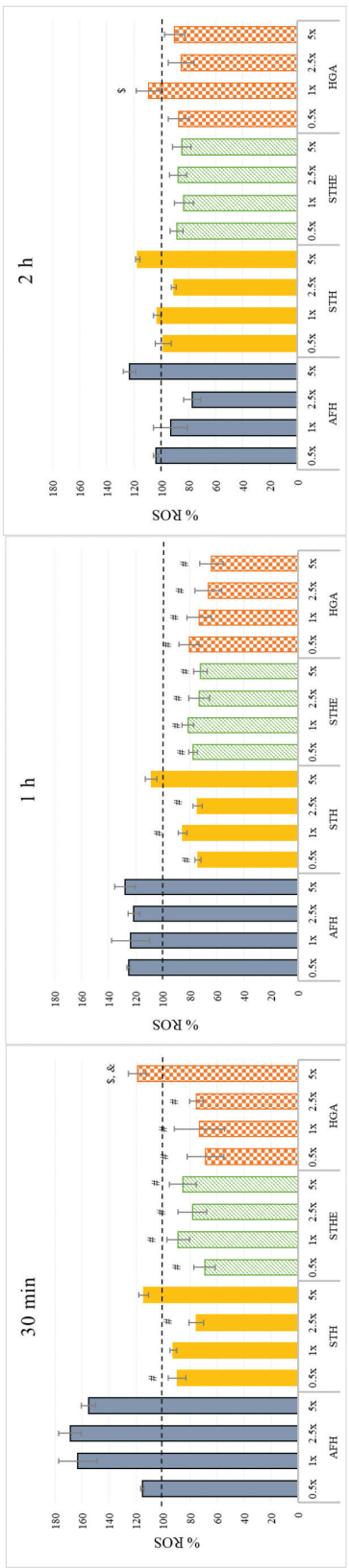

Figure 4 ROS induction in CAL 27 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). # P<0.05 vs all AFH concentrations. $ P<0.05 vs all STHE and HGA Concentrations. & P<0.05 vs other HGA concentrations

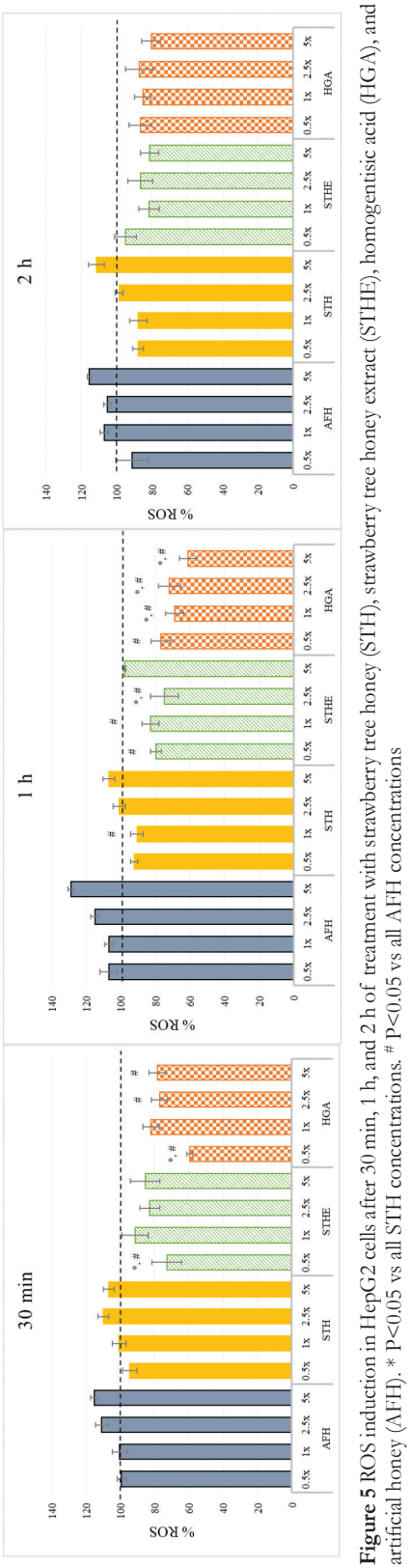

Figure 5 ROS induction in HepG2 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). * P<0.05 vs all STH concentrations. # P<0.05 vs all AFH concentrations

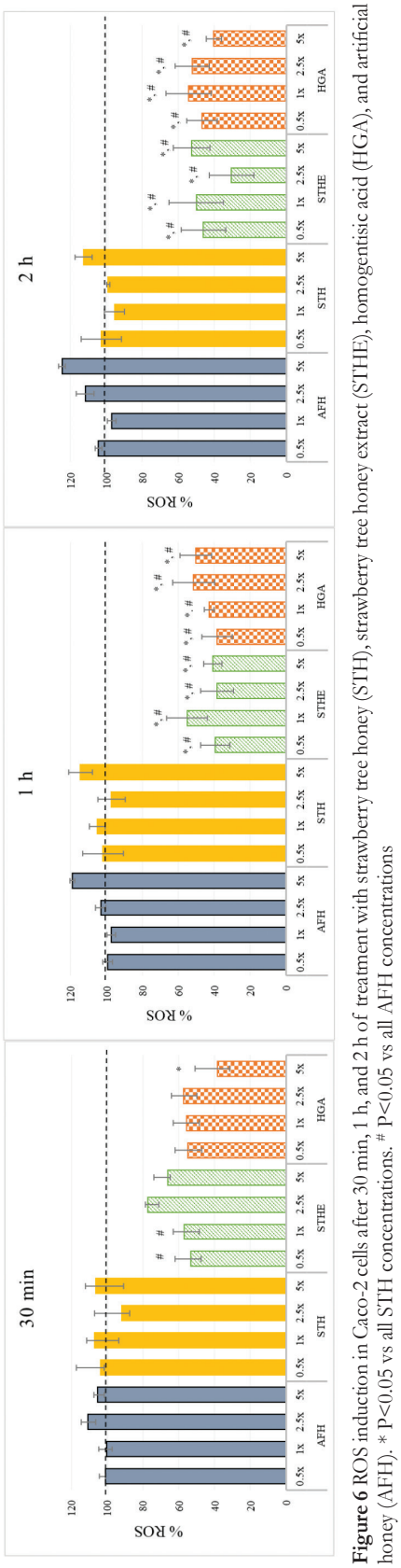

Figure 6 ROS induction in Caco-2 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). * P<0.05 vs all STH concentrations. # P<0.05 vs all AFH concentrations

to the presence of sugar in the tested samples. Furthermore, some compounds can cause cytotoxic effects at low concentrations *in vitro*, while at higher concentrations the same compound can promote the proliferation of cancer cells (58). This biphasic behaviour has also been reported for antioxidative and pro-oxidative potential of many phenolic compounds, including those in the tested sample of honey (59–61). It is believed that many polyphenolic compounds can stimulate lipid peroxidation, DNA damage, and apoptosis in healthy and carcinoma cells alike (62).

Figures 4–6 show our findings about ROS induction by the tested compounds in all three cancer cell types. AFH generally induced ROS more efficiently than STH, STHE, and HGA, but the differences were not statistically significant. STH, in turn, did not increase ROS levels significantly compared to control even at highest concentrations, regardless of cell type and time of exposure. What is more, STHE and HGA even lowered ROS production significantly compared to STH.

Similar findings have been reported for HGA at a concentration of 60 μmol/L in human fibroblast cell line cells (WI 38) exposed to hydrogen peroxide, possibly due to higher catalase activity and extracellular signal-regulated kinases (ERK), which play an important role in increasing antioxidant defences in cells (63). In contrast, Martin and Batkoff (64) have reported that HGA is capable of prooxidative activity, and in Hiraku et al. (65) observed oxidative damage caused by HGA in human DNA fragments, but at much higher concentrations than those investigated in our study. Afrin et al. (28) reported that Sardinian STH increased intracellular ROS production in HCT-116 and LoVo cells at concentration ranges of 3–60 mg/mL.

Considering the total cytotoxic effect of honey, we should take into account individual effects of its components, their concentrations, and the time of cell treatment. Pro-oxidative effects have already been reported for some of the previously mentioned phenolic components of the honey. For example, Pan et al. (34) found that acacetin stimulated ROS production in the AGS human gastric cancer cell line. Lodovici et al. (66) reported that 3-hydroxybenzoic acid enhanced oxidative DNA damage *in vitro.* The pro-oxidative properties of gallic acid have also been previously reported (67, 68). Bhat et al. (69) observed that caffeic acid caused DNA damage in human peripheral blood lymphocytes through pro-oxidative action. Maistro et al. (70) reported pro-oxidative effects of caffeic and ferulic acid in rat hepatoma tissue cells, while Chedea et al. (71) described pro-oxidative effects of quercetin and caffeic acid. Evidence of pro-oxidative activity of quercetin has also been provided by other authors (72–74). Truong et al. (75) reported the antioxidant activity of ferulic acid under some specific conditions. Miyoshi et al. (76) demonstrated the pro-oxidative potential of apigenin on human HL-60 leukaemia cells, while no similar effects were found for chrysin. However, detailed analysis of photochemical profile of honey showed a very complex matrix of bioactive compounds that can enhance individual anti- or pro-oxidative properties.

CONCLUSION

To conclude, our findings confirm low cytotoxicity and high biocompatibility of strawberry tree honey, its extract, and homogentisic acid as its major component, which is a good starting point for further research into their biological effects on other models.

REFERENCES

1. Bogdanov S, Jurendić T, Sieber R, Gallmann P. Honey for nutrition and health: A review. J Am Coll Nutr 2008;27:677–89. doi: 10.1080/07315724.2008.10719745
2. Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valéro JR. Extraction and analysis of polyphenols: Recent trends. Crit Rev Biotechnol 2011;31:227–49. doi: 10.3109/07388551.2010.513677
3. Spano N, Piras I, Ciulu M, Floris I, Panzanelli A, Pilo MI, Piu PC, Sanna G. Reversed-phase liquid chromatographic profile of free amino acids in strawberry-tree (*Arbutus unedo* L.) honey. J AOAC Int 2009;92:S73–84. doi: 10.1093/jaoac/92.4.S73
4. Tariba Lovaković B, Lazarus M, Brčić Karačonji I, Jurica K, Živković Semren T, Lušić D, Brajenović N, Pelaić Z, Pizent A. A multi-elemental composition and antioxidant properties of strawberry tree (*Arbutus unedo* L.) honey from the coastal region of Croatia: Risk-benefit analysis. J Trace Elem Med Biol 2018;45:85–92. doi: 10.1016/j.jtemb.2017.09.022
5. Jurič A, Gašić U, Brčić Karačonji, I, Jurica K, Milojković-Opsenica D. The phenolic profile of strawberry tree (*Arbutus unedo* L.) honey. J Serb Chem Soc 2020;85:1011–9. doi: 10.2298/JSC191217018J
6. Rosa A, Tuberoso CIG, Atzeri A, Melis MP, Bifulco E, Dessì MA. Antioxidant profile of strawberry tree honey and its marker homogentisic acid in several models of oxidative stress. Food Chem 2011;129:1045–53. doi: 10.1016/j.foodchem.2011.05.072
7. Tuberoso CIG, Boban M, Bifulco E, Budimir D, Pirisi FM. Antioxidant capacity and vasodilatory properties of Mediterranean food: The case of Cannonau wine, myrtle berries liqueur and strawberry-tree honey. Food Chem 2013;140:686–91. doi: 10.1016/j.foodchem.2012.09.071
8. Floris I, Pusceddu M, Satta A. The Sardinian bitter honey: from ancient healing use to recent findings. Antioxidants 2021;10(4):506. doi: 10.3390/antiox10040506
9. Jurič A. Phenolic profile and *in vitro* effects of strawberry tree honey (*Arbutus unedo* L.) on tumour cells and lymphocytes [PhD thesis]. Zagreb: University of Zagreb, Faculty of Food Technology and Biotechnology; 2021.
10. Brčić Karačonji I, Jurica K. Development and validation of gas chromatographic-mass spectrometric method for the analysis of homogentisic acid in strawberry tree (*Arbutus unedo* L.) honey. J AOAC Int 2017;100:889–92. doi: 10.5740/jaoacint.17-0148
11. Jurič A, Brčić Karačonji I, Žunec S, Katić A, Gašić U, Milojković Opsenica D, Kopjar N. Protective role of strawberry tree (*Arbutus unedo* L.) honey against cyto/genotoxic effects induced by ultraviolet B radiation *in vitro*. J Apic Res 2022. doi: 10.1080/00218839.2022.2047421
12. Lazarus M, Tariba Lovaković, B, Sekovanić A, Orct T, Jurič A, Prđun S, Denžić Lugomer M, Bubalo D. Combined approach to studying authenticity markers following spatial, temporal and production

practice trends in honey from Croatia. Eur Food Res Technol 2021;247:1511–23. doi: 10.1007/s00217-021-03728-8
13. Osmak M, Eljuga D. The characterization of two human cervical carcinoma HeLa sublines resistant to cisplatin. Res Exp Med 1993;193:389–96. doi: 10.1007/bf02576247
14. Batra P, Sharma AK. Anti-cancer potential of flavonoids: recent trends and future perspectives. 3 Biotech 2013;3:439–59. doi: 10.1007/s13205-013-0117-5
15. Mersch-Sundermann V, Knasmüller S, Wu XJ, Darroudi F, Kassie F. Use of a human-derived liver cell line for the detection of cytoprotective, antigenotoxic and cogenotoxic agents. Toxicology 2004;198:329–40. doi: 10.1016/j.tox.2004.02.009
16. Sun H, Chow EC, Liu S, Du Y, Pang KS. The Caco-2 cell monolayer: usefulness and limitations. Expert Opin Drug Metab Toxicol 2008;4:395–411. doi: 10.1517/17425255.4.4.395
17. Chang CF, Chang YC, Lin JT, Yu CW, Kao YT. Evaluation of inhibitors of intestinal UDP-glucuronosyltransferases 1A8 and 1A10 using raloxifene as a substrate in Caco-2 cells: Studies with four flavonoids of *Scutellaria baicalensis*. Toxicol In Vitro 2021;72:105087. doi: 10.1016/j.tiv.2021.105087
18. Yang SP, Raner GM. Cytochrome P450 expression and activities in human tongue cells and their modulation by green tea extract. Toxicol Appl Pharmacol 2005;202:140–50. doi: 10.1016/j.taap.2004.06.014
19. Babich H, Borenfreund E. Cytotoxicity of T-2 toxin and its metabolites determined with the neutral red cell viability assay. Appl Environ Microbiol 1991;57:2101–3. doi: 10.1128/aem.57.7.2101-2103.1991
20. Wang H, Joseph JA. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic Biol Med 1999;27:612–6. doi: 10.1016/s0891-5849(99)00107-0
21. Hempel SL, Buettner GR, O'Malley YQ, Wessels DA, Flaherty DM. Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2',7'-dichlorodihydrofluorescein diacetate, 5(and 6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate and dihydrorhodamine 123. Free Radic Biol Med 1999;27:146–59. doi: 10.1016/s0891-5849(99)00061-1
22. Tuberoso CIG, Bifulco E, Caboni P, Cottiglia F, Cabras P, Floris I. Floral markers of strawberry tree (*Arbutus unedo* L.) honey. J Agric Food Chem 2010;58:384–9. doi: 10.1021/jf9024147
23. Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by combination of spectrophotometric/fluorometric assays and chemometrics. Anal Chim Acta 2005;533:185–91. doi: 10.1016/j.aca.2004.11.010
24. Jurič A, Brčić Karačonji I, Kopjar N. Homogentisic acid, a main phenolic constituent of strawberry tree honey, protects human peripheral blood lymphocytes against irinotecan-induced cytogenetic damage *in vitro*. Chem Biol Interact 2021;349:109672. doi: 10.1016/j.cbi.2021.109672
25. Jaganathan KS, Balaji A, Vellayappan M, Asokan MK, Subramanian AP, John AA, Supriyanto E, Razak SA, Marvibaigi M. A review on antiproliferative and apoptotic activities of natural honey. Anticancer Agents Med Chem 2015;15:48–56. doi: 10.2174/1871520614666140722084747
26. Mumtaz PT, Bashir SM, Rather MA, Dar KB, Taban Q. Antiproliferative and apoptotic activities of natural honey. In: Rehman MU, Majid S, editors. Therapeutic applications of honey and its phytochemicals. Singapore: Springer; 2020. p. 345–60.
27. Imtara H, Kmail A, Touzani S, Khader M, Hamarshi H, Saad B, Lyoussi B. Chemical analysis and cytotoxic and cytostatic effects of twelve honey samples collected from different regions in Morocco and Palestine. Evid Based Complement Alternat Med 2019;2019:8768210. doi: 10.1155/2019/8768210
28. Afrin S, Forbes-Hernandez TY, Gasparrini M, Bompadre S, Quiles JL, Sanna G, Spano N, Giampieri F, Battino M. Strawberry-tree honey induces growth inhibition of human colon cancer cells and increases ROS generation: A comparison with manuka honey. Int J Mol Sci 2017;18:613. doi: 10.3390/ijms18030613
29. Afrin S, Giampieri F, Cianciosi D, Pistollato F, Ansary J, Pacetti M, Amici A, Reboredo-Rodríguez P, Simal-Gandara J, Quiles JL, Forbes-Hernández TY, Battino M. Strawberry tree honey as a new potential functional food. Part 1: Strawberry tree honey reduces colon cancer cell proliferation and colony formation ability, inhibits cell cycle and promotes apoptosis by regulating EGFR and MAPKs signaling pathways. J Funct Foods 2019;57:439–52. doi: 10.1016/j.jff.2019.04.035
30. Cheng N, Zhao H, Chen S, He Q, Cao W. Jujube honey induces apoptosis in human hepatocellular carcinoma HepG2 cell via DNA damage, p53 expression, and caspase activation. J Food Biochem 2019;43:e12998. doi: 10.1111/jfbc.12998
31. Acevedo F, Torres P, Oomah BD, de Alencar SM, Prado Massarioli A, Martín-Venegas R, Albarral-Ávila V, Burgos-Díaz C, Ferrer R, Rubilar M. Volatile and non-volatile/semi-volatile compounds and *in vitro* bioactive properties of Chilean Ulmo (*Eucryphia cordifolia* Cav.) honey. Food Res Int 2017;94:20–8. doi: 10.1016/j.foodres.2017.01.021
32. Hsu Y-L, Kuo P-L, Lin C-C. Acacetin inhibits the proliferation of Hep G2 by blocking cell cycle progression and inducing apoptosis. Biochem Pharmacol 2004;67:823–9. doi: 10.1016/j.bcp.2003.09.042
33. Hsu Y-L, Kuo P-L, Liu C-F, Lin C-C. Acacetin-induced cell cycle arrest and apoptosis in human non-small cell lung cancer A549 cells. Cancer Lett 2004;212:53–60. doi: 10.1016/j.canlet.2004.02.019
34. Pan M-H, Lai C-S, Hsu P-C, Wang Y-J. Acacetin induces apoptosis in human gastric carcinoma cells accompanied by activation of caspase cascades and production of reactive oxygen species. Agric Food Chem 2005;53:620–30. doi: 10.1021/jf048430m
35. Salimi A, Roudkenar MH, Sadeghi L, Mohseni A, Seydi E, Pirahmadi N, Pourahmad J. Selective anticancer activity of acacetin against chronic lymphocytic leukemia using both *in vivo* and *in vitro* methods: key role of oxidative stress and cancerous mitochondria. Nutr Cancer 2016;68:1404–16. doi: 10.1080/01635581.2016.1235717
36. Kandhari K, Mishra JPN, Singh RP. Acacetin inhibits cell proliferation, survival, and migration in human breast cancer cells. Int J Pharma Biol Sci 2019;9:443–52. doi: 10.21276/ijpbs.2019.9.1.58
37. You BR, Moon HJ, Han YH, Park WH. Gallic acid inhibits the growth of HeLa cervical cancer cells via apoptosis and/or necrosis. Food Chem Toxicol 2010;48:1334–40. doi: 10.1016/j.fct.2010.02.034
38. Serrano A, Palacios C, Roy G, Cespón C, Villar ML, Nocito M, González-Porqué P. Derivatives of gallic acid induce apoptosis in tumoral cell lines and inhibit lymphocyte proliferation. Arch Biochem Biophys 1998;350:49–54. doi: 10.1006/abbi.1997.0474
39. Wang T, Gong X, Jiang R, Li H, Du W, Kuang G. Ferulic acid inhibits proliferation and promotes apoptosis via blockage of PI3K/Akt pathway in osteosarcoma cell. Am J Transl Res 2016;8:968–80. PMCID: PMC4846940
40. Chang W-C, Hsieh C-H, Hsiao M-W, Lin W-C, Hung Y-C, Ye J-C. Caffeic acid induces apoptosis in human cervical cancer cells through the mitochondrial pathway. Taiwan Obstet Gynecol 2010;49:419–24. doi: 10.1016/S1028-4559(10)60092-7

41. Kabała-Dzik A, Rzepecka-Stojko A, Kubina R, Jastrzębska-Stojko Ż, Stojko R, Wojtyczka RD, Stojko J. Comparison of two components of propolis: caffeic acid (CA) and caffeic acid phenethyl ester (CAPE) to induce apoptosis and cell cycle arrest of breast cancer cells MDA-MB-231. Molecules 2017;22(9):1554. doi: 10.3390/molecules22091554
42. Yin M-C, Lin C-C, Wu H-C, Tsao S-M, Hsu C-K. Apoptotic effects of protocatechuic acid in human breast, lung, liver, cervix, and prostate cancer cells: potential mechanisms of action. J Agric Food Chem 2009;57:6468–73. doi: 10.1021/jf9004466
43. Spilioti E, Jaakkola M, Tolonen T, Lipponen M, Virtanen V, Chinou I, Kassi E, Karabournioti S, Moutsatsou P. Phenolic acid composition, antiatherogenic and anticancer potential of honeys derived from various regions in Greece. PLoS One 2014;9(4):e94860. doi: 10.1371/journal.pone.0094860
44. Choi J-A, Kim J-Y, Lee J-Y, Kang C-M, Kwon H-J, Yoo Y-D, Kim T-W, Lee Y-S, Lee S-J. Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. Int J Oncol 2001;19:837–44. doi: 10.3892/ijo.19.4.837
45. Hashemzaei M, Delarami Far A, Yari A, Heravi RE, Tabrizian K, Taghdisi SM, Sadegh SE, Tsarouhas K, Kouretas D, Tzanakakis G, Nikitovic D, Anisimov NY, Spandidos DA, Tsatsakis AM, Rezaee R. Anticancer and apoptosis inducing effects of quercetin *in vitro* and *in vivo*. Oncol Rep 2017;38:819–28. doi: 10.3892/or.2017.5766
46. Kumar MAS, Nair M, Hema PS, Mohan J, Santhoshkumar TR. Pinocembrin triggers Bax-dependent mitochondrial apoptosis in colon cancer cells. Mol Carcinog 2007;46:231–41. doi: 10.1002/mc.20272
47. Zheng Y, Wang K, Wu Y, Chen Y, Chen X, Hu CW, Hu F. Pinocembrin induces ER stress mediated apoptosis and suppresses autophagy in melanoma cells. Cancer Lett 2018;431:31–42. doi: 10.1016/j.canlet.2018.05.026
48. Budhraja A, Gao N, Zhang Z, Son YO, Cheng S, Wang X, Ding S, Hitron A, Chen G, Luo J, Shi X. Apigenin induces apoptosis in human leukemia cells and exhibits anti-leukemic activity *in vivo*. Mol Cancer Ther 2012;11:132–42. doi: 10.1158/1535-7163.MCT-11-0343
49. Yang J, Pi C, Wang G. Inhibition of PI3K/Akt/mTOR pathway by apigenin induces apoptosis and autophagy in hepatocellular carcinoma cells. Biomed Pharmacother 2018;103:699–707. doi: 10.1016/j.biopha.2018.04.072
50. Woo KJ, Jeong Y-J, Park J-W, Kwon TK. Chrysin-induced apoptosis is mediated through caspase activation and Akt inactivation in U937 leukemia cells. Biochem Biophys Res Commun 2004;325:1215–22. doi: 10.1016/j.bbrc.2004.09.225
51. Khoo BY, Chua SL, Balaram P. Apoptotic effects of chrysin in human cancer cell lines. Int J Mol Sci 2010;11:2188–99. doi: 10.3390/ijms11052188
52. Samarghandian S, Azimi-Nezhad M, Borji A, Hasanzadeh M, Jabbari F, Farkhondeh T, Samini M. Inhibitory and cytotoxic activities of chrysin on human breast adenocarcinoma cells by induction of apoptosis. Pharmacogn Mag 2016;12(Suppl 4):S436–40. doi: 10.4103/0973-1296.191453
53. Ruiz de Almodóvar JM, Núñez MI, McMillan TJ, Olea N, Mort C, Villalobos M, Pedraza V, Steel GG. Initial radiation-induced DNA damage in human tumour cell lines: a correlation with intrinsic cellular radiosensitivity. Br J Cancer 1994;69:457–62. doi: 10.1038/bjc.1994.83
54. Dunne AL, Price ME, Mothersill C, McKeown SR, Robson T, Hirst DG. Relationship between clonogenic radiosensitivity, radiation-induced apoptosis and DNA damage/repair in human colon cancer cells. Br J Cancer 2003;89:2277–83. doi: 10.1038/sj.bjc.6601427
55. Jariwalla RJ, Gangapurkar B, Nakamura D. Differential sensitivity of various human tumour-derived cell types to apoptosis by organic derivatives of selenium. Br J Nutr 2008;101:182–9. doi: 10.1017/S0007114508998305
56. Szumiel I. Intrinsic radiation sensitivity: cellular signaling is the key. Radiat Res 2008;169:249–58. doi: 10.1667/RR1239.1
57. Kustiawan PM, Puthong S, Arung ET, Chanchao C. *In vitro* cytotoxicity of Indonesian stingless bee products against human cancer cell lines. Asian Pac J Trop Biomed 2014;4:549–56. doi: 10.12980/APJTB.4.2014APJTB-2013-0039
58. Lawal AO, Ellis E. Differential sensitivity and responsiveness of three human cell lines HepG2, 1321N1 and HEK 293 to cadmium. J Toxicol Sci 2010;35:465–78. doi: 10.2131/jts.35.465
59. Martin-Cordero C, Leon-Gonzalez AJ, Calderon-Montano JM, Burgos-Moron E, Lopez-Lazaro M. Pro-oxidant natural products as anticancer agents. Curr Drug Targets 2012;13:1006–28. doi: 10.2174/138945012802009044
60. Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol 2013;51:15–25. doi: 10.1016/j.fct.2012.09.021
61. Khan ZS, Chatterjee NS, Shabeer TPA, Shaikh S, Banerjee K. Profile of triacylglycerols, phenols, and vitamin E of Manjari Medika grape seed oil and cake: Introducing a novel Indian variety. Eur J Lipid Sci Technol 2020;122(4):1900356. doi: 10.1002/ejlt.201900356
62. Eghbaliferiz S, Iranshahi M. Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: Updated review of mechanisms and catalyzing metals. Phytother Res 2016;30:1379–91. doi: 10.1002/ptr.5643
63. Kang KA, Chae S, Lee KH, Zhang R, Jung MS, You HJ, Kim JS, Hyun JW. Antioxidant effect of homogentisic acid on hydrogen peroxide induced oxidative stress in human lung fibroblast cells. Biotechnol Bioprocess Eng 2005;10:556–63. doi: 10.1007/BF02932294
64. Martin JP Jr, Batkoff B. Homogentisic acid autoxidation and oxygen radical generation: implications for the etiology of alkaptonuric arthritis. Free Radic Biol Med 1987;3:241–50. doi: 10.1016/s0891-5849(87)80031-x
65. Hiraku Y, Yamasaki M, Kawanishi S. Oxidative DNA damage induced by homogentisic acid, a tyrosine metabolite. FEBS Lett 1998;432:13–6. doi: 10.1016/s0014-5793(98)00823-0
66. Lodovici M, Guglielmi F, Meoni M, Dolara P. Effect of natural phenolic acids on DNA oxidation *in vitro*. Food Chem Toxicol 2001;39:1205–10. doi: 10.1016/s0278-6915(01)00067-9
67. Yen G-C, Duh P-D, Tsai H-L, Huang S-L. Pro-oxidative properties of flavonoids in human lymphocytes. Biosci Biotechnol Biochem 2003;67:1215–22. doi: 10.1271/bbb.67.1215
68. Sohi KK, Mittal N, Hundal MK, Khanduja KL. Gallic acid, an antioxidant, exhibits antiapoptotic potential in normal human lymphocytes: A Bcl-2 independent mechanism. J Nutr Sci Vitaminol (Tokyo) 2003;49:221–7. doi: 10.3177/jnsv.49.221
69. Bhat SH, Azmi AS, Hadi SM. Prooxidant DNA breakage induced by caffeic acid in human peripheral lymphocytes: involvement of endogenous copper and a putative mechanism for anticancer properties. Toxicol Appl Pharmacol 2007;218:249–55. doi: 10.1016/j.taap.2006.11.022
70. Maistro EL, Angeli JPF, Andrade SF, Mantovani MS. *In vitro* genotoxicity assessment of caffeic, cinnamic and ferulic acids. Genet Mol Res 2011;10:1130–40. doi: 10.4238/vol10-2gmr1278

71. Chedea VS, Choueiri L, Jisaka M, Kefalas P. *o*-Quinone involvement in the prooxidant tendency of a mixture of quercetin and caffeic acid. Food Chem 2012;135:1999–2004. doi: 10.1016/j.foodchem.2012.06.094
72. Metodiewa D, Jaiswal AK, Cenas N, Dickancaité E, Segura-Aguilar J. Quercetin may act as a cytotoxic prooxidant after its metabolic activation to semiquinone and quinoidal product. Free Radic Biol Med 1999;26:107–16. doi: 10.1016/s0891-5849(98)00167-1
73. Lee PY, Costumbrado J, Hsu C-Y, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. J Vis Exp 2012;20(62):3923. doi: 10.3791/3923
74. Yang B, Chen F, Hua Y, Huang S-S, Lin S, Wen L, Jiang Y. Prooxidant activities of quercetin, *p*-courmaric acid and their derivatives analysed by quantitative structure-activity relationship. Food Chem 2012;131:508–12. doi: 10.1016/j.foodchem.2011.09.014
75. Truong DH, Nhung NTA, Dao DQ. Iron ions chelation-based antioxidant potential *vs*. pro-oxidant risk of ferulic acid: A DFT study in aqueous phase. Comput Theor Chem 2020;1185:112905. doi: 10.1016/j.comptc.2020.112905
76. Miyoshi N, Naniwa K, Yamada T, Osawa T, Nakamura Y. Dietary flavonoid apigenin is a potential inducer of intracellular oxidative stress: the role in the interruptive apoptotic signal. Arch Biochem Biophys 2007;466:274–82. doi: 10.1016/j.abb.2007.07.026

Citotoksično djelovanje meda obične planike (*Arbutus unedo* L.), ekstrakta i homogentizinske kiseline na stanične linije CAL 27, HepG2 i Caco-2

Med obične planike (*Arbutus unedo* L.) (STH), poznat kao "gorki med", tradicionalno se koristi u narodnoj medicini na sredozemnom području. Bez obzira na zemljopisno podrijetlo, obično ima vrlo visok udio fenolnih spojeva i snažan antioksidacijski kapacitet. Ipak, još uvijek se malo zna o učincima STH-a, njegova ekstrakta (STHE), kao i dominantnoga fenolnog spoja – homogentizinske kiseline (HGA) – na staničnoj razini. Cilj ovoga istraživanja bio je utvrditi ukupni sadržaj fenola, antioksidacijski kapacitet metodom DPPH i FRAP u STH-u, proizvedenome u Hrvatskoj, te ispitati citotoksične i prooksidacijske učinke STH-a, STHE-a i HGA-e na tri ljudske stanične linije: karcinoma pločastih stanica jezika (CAL 27), hepatocelularnoga karcinoma jetre (HepG2) i adenokarcinoma epitela debelog crijeva (Caco-2). STH, STHE i HGA ispitani su u četirima koncentracijama (0,5–5× prosječni dnevni unos STH-a u ljudi) i tijekom 30 minuta te tijekom jednog i dva sata. Hrvatski STH imao je visok ukupan sadržaj fenola (1,67 g ekvivalenata galne kiseline po kg meda i snažan antioksidacijski kapacitet (2,96 mmol Trolox ekvivalenata po kg meda i 13,5 mmol Fe^{2+} po kg meda). Dobiveni rezultati ne pokazuju jasnu i dosljednu citotoksičnost, ovisno o vremenu ili koncentraciji, ni u jednoj staničnoj liniji. Razine reaktivnih kisikovih vrsta u svim trima tipovima stanica u gotovo svim vremenima izlaganja nisu bile značajno veće od kontrole. Najvažnije je zapažanje da ispitivane tvari imaju nisku citotoksičnost i visoku biokompatibilnost, bez obzira na koncentraciju, što je dobra polazna točka za daljnja istraživanja njihovih bioloških učinaka na drugim modelima.

KLJUČNE RIJEČI: adenokarcinom debelog crijeva; fenoli; hepatocelularni karcinom jetre; karcinom pločastih stanica jezika; ljudske stanične linije; preživljenje; reaktivne kisikove vrste