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

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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

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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

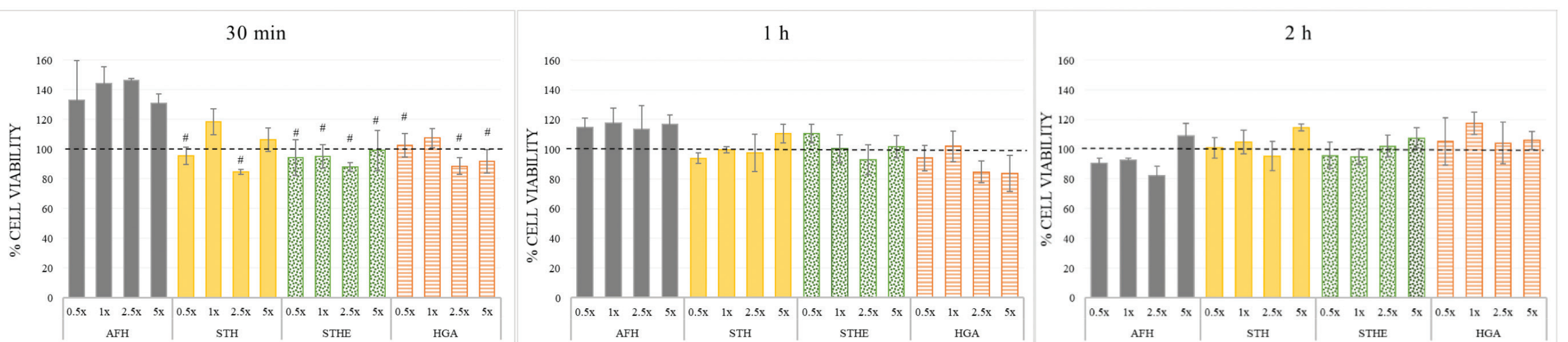

Figure 1 Cell viability of CAL 27 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). # P<0.05 vs all AFH concentrations

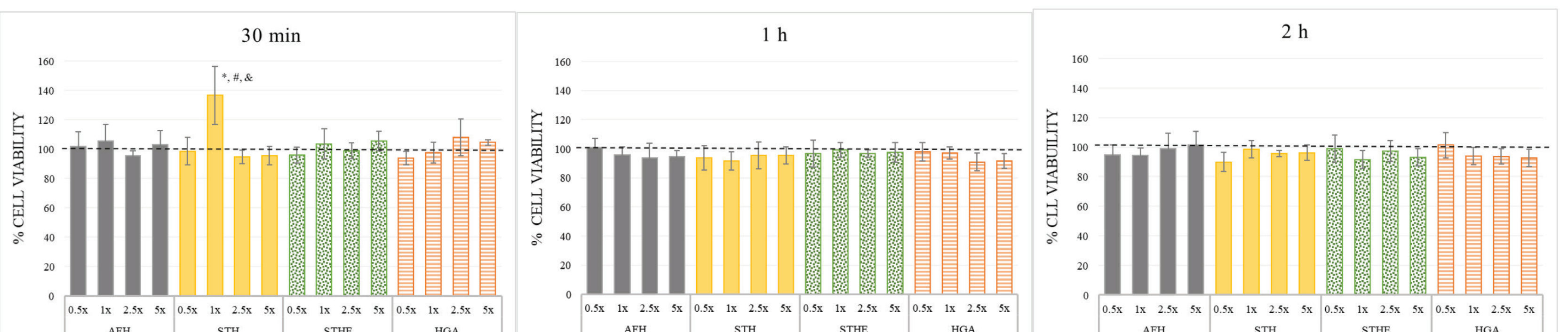

Figure 2 Cell viability of HepG2 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH)* P<0.05 vs other STH concentrations. # P<0.05 vs all AFH concentrations. & P<0.05 vs all HGA concentrations

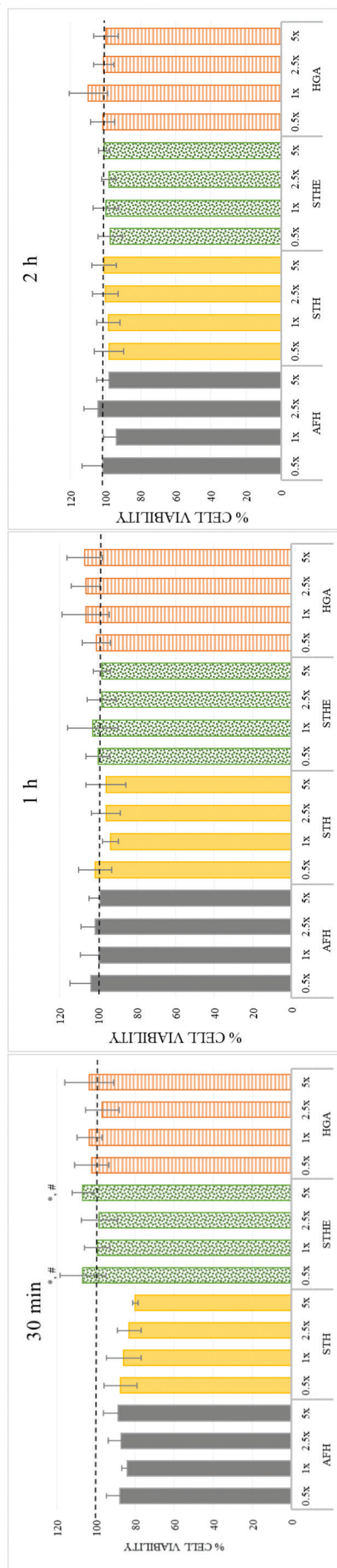

Figure 3 Cell viability of Caco-2 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). * $P<0.05$ vs all STH concentrations. # $P<0.05$ vs all AFH concentrations

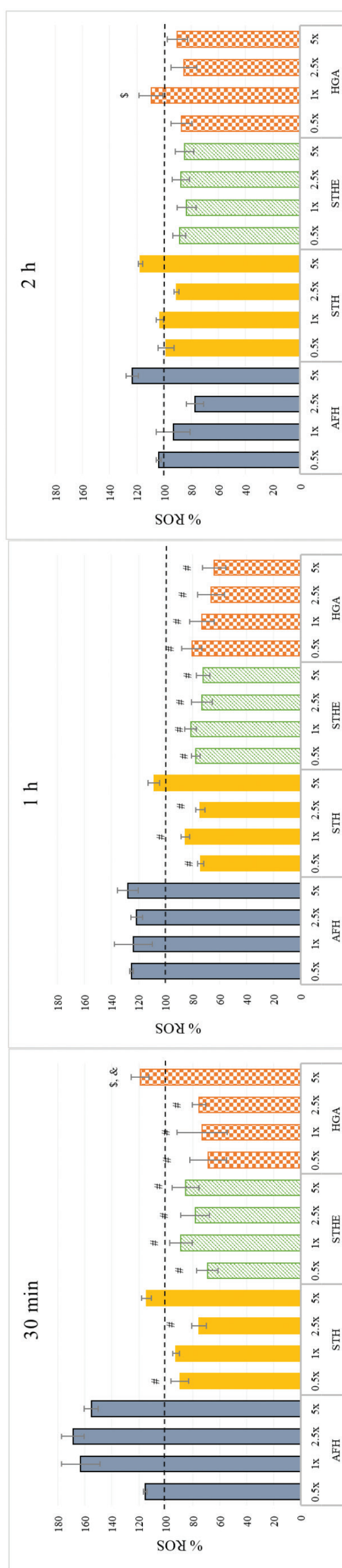

Figure 4 ROS induction in CAL 27 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). # $P<0.05$ vs all AFH concentrations. $ $P<0.05$ vs all STHE and HGA Concentrations. & $P<0.05$ vs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

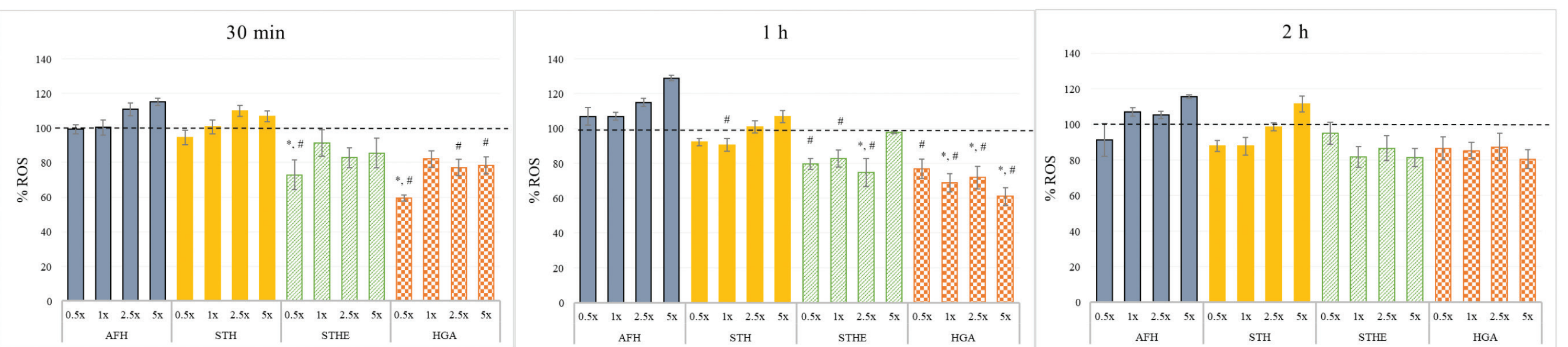

Figure 5 ROS induction in HepG2 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). * $P<0.05$ vs all STH concentrations. # $P<0.05$ vs all AFH concentrations

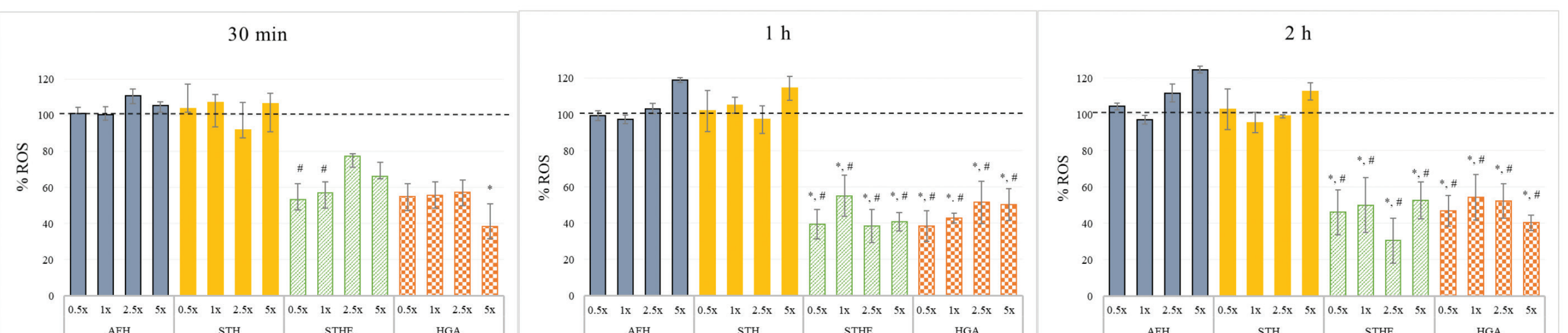

Figure 6 ROS induction in Caco-2 cells after 30 min, 1 h, and 2 h of treatment with strawberry tree honey (STH), strawberry tree honey extract (STHE), homogentisic acid (HGA), and artificial honey (AFH). * $P<0.05$ vs all STH concentrations. # $P<0.05$ vs all AFH concentrations

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.

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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