Original article DOI: 10.2478/aiht-2019-70-3322

Toxic activity of *Prunus spinosa* L. flower extract in hepatocarcinoma cells

Teuta Murati[1], Marina Miletić[1], Josipa Kolarić[1], Vanja Lovrić[2], Danijela Bursać Kovačević[2], Predrag Putnik[2], Irena Landeka Jurčević[3], Domagoj Đikić[4], Verica Dragović-Uzelac[2], and Ivana Kmetič[1]

[1] *Laboratory for Toxicology, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*
[2] *Laboratory for Technology of Fruits and Vegetables Preservation and Processing, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*
[3] *Laboratory for Food Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*
[4] *Division of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia*

[Received in July 2019; Similarity Check in July 2019; Accepted in November 2019]

Prunus spinosa L. (blackthorn) is used in traditional medicine as a remedy for various diseases. To establish its anticancer properties, we exposed human liver cancer cells (Hep G2) to a range of blackthorn flower extract concentrations (10-200 μg/mL) and determined cytotoxic activity with the neutral red and kenacid blue methods after 24, 48, and 72 h of incubation. Statistically significant inhibitory effects on Hep G2 cellular proliferation were observed at concentrations above 50 μg/mL ($p<0.001–0.05$). Cell viability was lower when determined with neutral red than kenacid blue method. In addition, we evaluated antioxidant/prooxidant effects of the blackthorn flower extract by measuring reactive oxygen species (ROS), and the results confirmed its prooxidant behaviour within the applied concentration range. Flow cytometry determined primarily necrotic and apoptotic cell death, which provides additional evidence of its cytotoxic effect on liver carcinoma.

KEY WORDS: blackthorn; cytotoxicity; Hep G2; kenacid blue; neutral red; ROS

Croatian folk and official medicine and pharmacy lists 735 plant species used as a remedy for various diseases (1), including *Prunus spinosa* L. (blackthorn). Its petals are used medicinally as tea, syrup, fresh juice, or tincture to treat diarrhoea, anaemia, and other ailments (2). Its flower is a valuable source of phenolic compounds, such as kaempferol, quercetin, kaempferol 3-*O*-α-L-arabinofuranoside, quercetin 3-*O*-α-L-arabinofuranoside, kaempferol 3-*O*-α-L-ramnopyranoside, kaempferol 7-*O*-α-L-ramnopyranoside, kaempferol 3-*O*-β-D-xylopyranoside, and kaempferol 3-*O*-(2''-E-*p*-coumaroyl)-α-L-arabinofuranoside (3, 4). These polyphenols have potent antioxidant/prooxidant properties and a potential to treat cancer either as prophylaxis (antioxidants) or cancer cell killers (prooxidants) (5, 6).

Liver cancer is the third most common cause of death from cancer worldwide, with overall mortality-to-incidence rate of 0.93 (7). The incidence of primary liver malignancy has increased over the past 20 years, with hepatocellular carcinoma being the most common primary liver tumour (8). This is one of the reasons why current research has been focused on identifying chemotherapeutic agents that can prevent, delay, or stop tumour initiation and growth and reduce mortality.

Cytotoxicity screening models provide important preliminary data to help identify plant extracts with potential anticancer activity for future study (9, 10). The aim of this *in vitro* study was to assess the anticancer properties of *Prunus spinosa* L. flower ethanolic extract (PSBE) in human hepatoblastoma cells (Hep G2) and is an update to our recently published report on blackthorn flower extract activity at the cellular level (11).

MATERIALS AND METHODS

Reagents, solvents, and standards

Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 Ham (DMEM/F12), trypsin/EDTA solution (0.25 % trypsin with EDTA 4Na), trypan blue dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and gallic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Heat-inactivated foetal bovine serum (FBS) was obtained from GIBCO (Paisley, UK). Coomassie brilliant blue R-250 was obtained from LKB (Bromma, Sweden), and neutral red dye from Merck (Darmstadt, Germany).

Corresponding author: Professor Ivana Kmetič, PhD, Laboratory for Toxicology, Faculty of Food Technology and Biotechnology, University of Zagreb, 10000 Zagreb, Pierottijeva 6, Croatia, E-mail: *ikmetic@pbf.hr*

2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) assay kit (cat #ab113851) was obtained from Abcam (Cambridge, UK) and Muse Annexin V & Dead Cell Kit (cat #MCH100105) from Merck Millipore (Billerica, MA, USA). Ethanol was purchased from Gram-Mol (Zagreb, Croatia). Folin-Ciocalteu reagent was obtained from Kemika (Zagreb, Croatia), and anhydrous sodium carbonate from T.T.T. (Sveta Nedjelja, Croatia).

Plant material

Dried flowers of wild blackthorn (*Prunus spinosa* L.) were obtained from a local producer (Suban Ltd., Strmec, Croatia). Before analyses, the flowers were ground to powder with an electrical grinder (150 W, Imetec Dolcevita CG1, Azzano San Paolo, Italy) and the process was stopped every 15 s for 10 s to prevent sample from heating. Particle size and the powder distribution were d(0.9)≤405.24 μm; d(0.5)≤180.90 μm; d(0.1)≤16.26 μm, as determined with a laser particle size analyser (MASTERSIZER 2000, Malvern Instruments, Worcestershire, UK).

Extraction procedure

Phenolic compounds were extracted following a procedure described in detail in our previous paper (11), which was a modification of a procedure described by Bursać Kovačević et al. (12).

Determination of total polyphenols

Total phenols in the extract were determined following previously reported methods (13, 14). Briefly, 100 μL of (300 times) diluted wild blackthorn extract was mixed with 200 μL of Folin-Ciocalteu reagent and 2 mL of distilled water, and 1 mL of saturated (20 %, w/v) sodium carbonate solution was added after 3 min. The mixture was incubated at 50 °C for 30 min, and the absorbance was measured with a UV-1600 PC spectrophotometer (Geldenaaksebaan, Leuven) at 765 nm. Total phenolic content was calculated according to the gallic acid standard calibration curve (y=0.0035x, R^2=0.9995) and was 21.5 mg of gallic acid equivalents (GAE) per gram of wild blackthorn flowers.

Cell viability analysis

Human Caucasian hepatocyte carcinoma cells Hep G2 (HB-8065, American Type Culture Collection, Manassas, VA, USA) were grown in DMEM/F12 supplemented with 10 % FBS at 37 °C in a 5 % CO_2 humidified environment. Antibiotics were not used. To evaluate the cytotoxicity of PSBE, Hep G2 cells were trypsinised and seeded into a 6-well plates at a density of $5x10^4$ cells/mL (2 mL per well) and allowed to attach for 24 h. The cells were then treated in culture medium with 10 μL of PSBE to obtain the desired total phenol concentration of 10–200 μg/mL expressed as GAE. The final ethanol concentration in the experiments was never greater than 0.5 %. Cytotoxic effects were evaluated after 24, 48, and 72 h of treatment. Cells treated with ethanol (5 μL/mL) were used as controls. Cytotoxicity was assessed using two different well-established *in vitro* test methods – kenacid blue assay and neutral red (15, 16) – with minor modifications as described earlier (17). The MTT test is often used to evaluate the anticancer activity of natural or synthesised compounds, but considering literature data (and ours as well), we have found it inappropriate for plant polyphenolic compounds (18). Polyphenols α-β-γ-δ-tocotrienol and α-tocopherol that are extracted from natural sources directly reduce MTT in the absence of living cells (19, 20). Bioassays that we have selected are based on the following principles: neutral red evaluates the ability of viable uninjured cells to incorporate and bind supravital dye in the lysosomes, and kenacid blue measures changes in total cell protein content. For each method at least three experiments were performed and each applied PSBE concentration was tested in triplicate within single experiment.

Reactive oxygen species determination

Cellular ROS was measured with a DCFDA assay kit according to manufacturer's protocol. After diffusion in a cell, a stable, fluorogenic dye DCFDA is deacetylated by cellular esterases to a non-fluorescent compound that is later oxidised by ROS into fluorescent 2',7'–dichlorofluorescein (21). Hep G2 cells were seeded into a 96-well black plates at a density of 2.5×10^5 cells/mL (100 μL per well) and allowed to attach for 24 h. The cells were then washed with PBS and incubated with 25 μmol/L DCFDA (100 μL) at 37 °C for 45 min. After washing with PBS, they were exposed to PSBE (10, 50, 100, 150, and 200 μg/mL) for 3 h. Fluorescence was measured with Cary Eclipse fluorescence spectrophotometer (Varian, Palo Alto, CA, USA) at 485 nm excitation and 535 nm emission wavelengths. All concentrations were tested in quadruplicate.

Apoptosis assay

After 48 h of treatment with 10, 50, and 100 μg/mL PSBE, floating and adherent Hep G2 cells (cultured in 6-well plates at a density of $5x10^4$ cells/mL; 2 mL per well) were collected, centrifuged at 600 *g* for 1 min, and suspended in cell culture medium. Then, 100 μL aliquots of cell suspension were added to 100 μL of Muse Annexin V & Dead Cell reagent, incubated in the dark at room temperature for 20 min, and analysed with a Muse Cell Analyzer (Merck Millipore). Each PSBE concentration was tested in quadruplicate. Cytofluorimetric separation, based on externalisation of phosphatidylserine (positivity for annexin V) in apoptotic cells and simultaneous staining of dead cells with fluorescent nuclear dye 7-aminoactinomycin D (7-AAD) distinguished four cell populations in each sample: non-apoptotic live (Annexin V-negative and 7-AAD-negative), early apoptotic (Annexin V-positive and 7-AAD-negative), late-stage apoptotic and dead cells

(Annexin V-positive and 7-AAD-positive), and dead cells, mostly nuclear debris (Annexin V-negative and 7-AAD-positive) (22).

Statistical analysis

All data are presented as means ± SEM. The results were analysed with a two-tailed Student's *t*-test, and $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Cytotoxic effects of PSBE

The first significant cytotoxic events were detected with both neutral red and kenacid blue ($p<0.001$ and $p<0.05$, respectively) after 24 h of exposure to 50 µg/mL of PSBE and both methods confirmed that cytotoxicity was dose-dependent (Figure 1 and 2). However, the comparison between IC_{50} revealed differences between the methods (Table 1), as those obtained with kenacid blue were higher than those obtained with neutral red, which points to higher sensitivity of the latter. After 72 h of exposure to 200 µg/mL of PSBE, neutral red showed that almost none of the cells survived. These findings indicate that blackthorn flower polyphenols might affect lysosomes/endosomes in Hep G2 cells. However, at its lowest concentration PSBE had a positive effect on cell proliferation (although it was not statistically significant), which points future research in the direction of possible PSBE protective effects in normal cells and tissues at doses below 10 µg/mL.

Prooxidative effects of PSBE

PSBE significantly increased ROS production (Figure 3) at all tested concentrations in a dose-dependent manner. This effect is welcome in liver cancer cells. Targeting cancer cells via ROS-based mechanism has been proposed as a relatively novel, radical therapeutic approach. Cancer cells exhibit higher endogenous oxidative stress than normal cells, and pharmacological ROS insults through either greater ROS production or inhibition of ROS-scavenging activity can lead to increased (lethal) ROS levels in them (23, 24). Other studies also reported that polyphenol-rich plant extracts and pure polyphenol compounds could behave as prooxidants under certain conditions (5, 6, 25). Prooxidant molecules can act as cytotoxic agents against cancer cells by achieving toxic ROS levels associated with their apoptotic effect in various types of tumour cells (26).

Cell death induced by PSBE

Cytofluorimetric analysis of apoptotic events showed that 48 h of treatment with PSBE significantly increased ($p<0.001$) the percentage of total apoptotic cells compared to control (Figure 4). Interestingly, the lowest applied dose of PSBE (10 µg/mL) resulted in the highest percentage of early apoptotic cells, even though neutral red and kenacid blue did not detect cytotoxic effects at this dose. At higher PSBE doses (50 and 100 µg/mL) the percentage of early apoptotic cells decreased, and the majority of cells were late apoptotic or dead. Flavonoids are known to interact with the pathways signalling cell growth and apoptosis such as PI3-kinase (phosphoinositide 3-kinase), Akt/Pkb (protein-kinase B), tyrosine-kinase, P1KC (protein-1 kinase

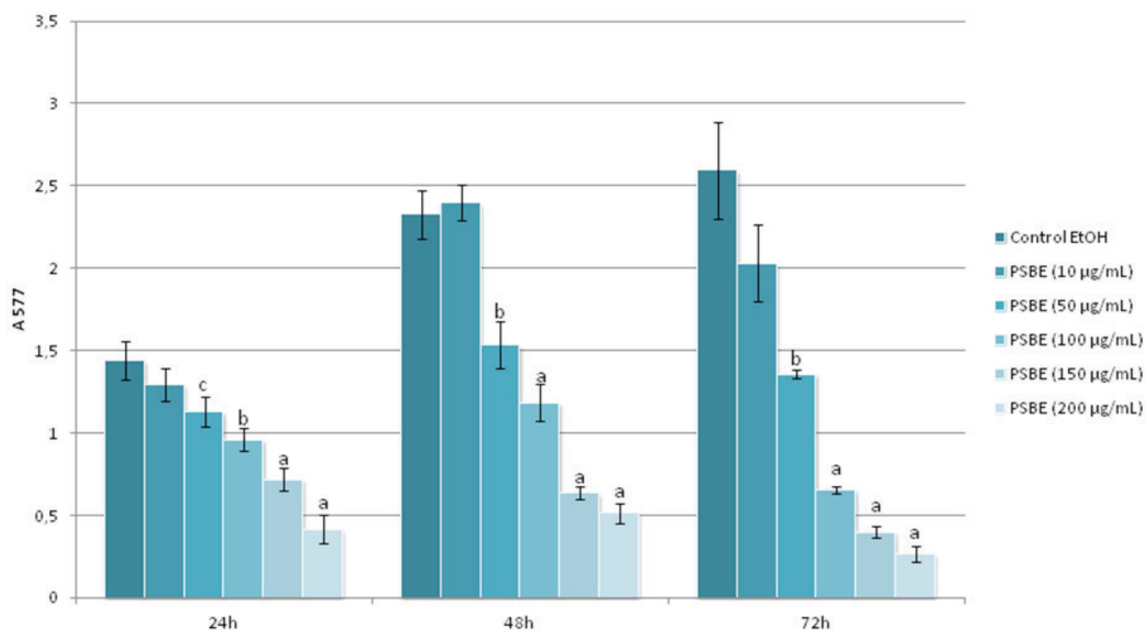

Figure 1 Effects of blackthorn flower extract on Hep G2 cell viability (mean±SEM) determined with kenacid blue after 24, 48, and 72 h; [a] $p<0.001$; [b] $p<0.005$; [c] $p<0.05$ (vs control). Control EtOH – cells treated with 5 µL/mL ethanol; PSBE 10, 50, 100, 150, and 200 µg/mL – concentrations of PSBE in growth medium

Table 1 Inhibition concentrations of *Prunus spinosa* L. flower extract in Hep G2 cells after 24, 48, and 72 h

IC µg/mL	Time					
	24 h		48 h		72 h	
Method	**KB**	**NR**	**KB**	**NR**	**KB**	**NR**
IC_{20}	64.59	37.81	36.30	19.49	20.49	24.76
IC_{50}	148.11	92.50	94.49	63.28	59.87	54.93
IC_{80}	212.90	163.42	/	127.17	121.07	95.98

KB – kenacid blue; NR – neutral red

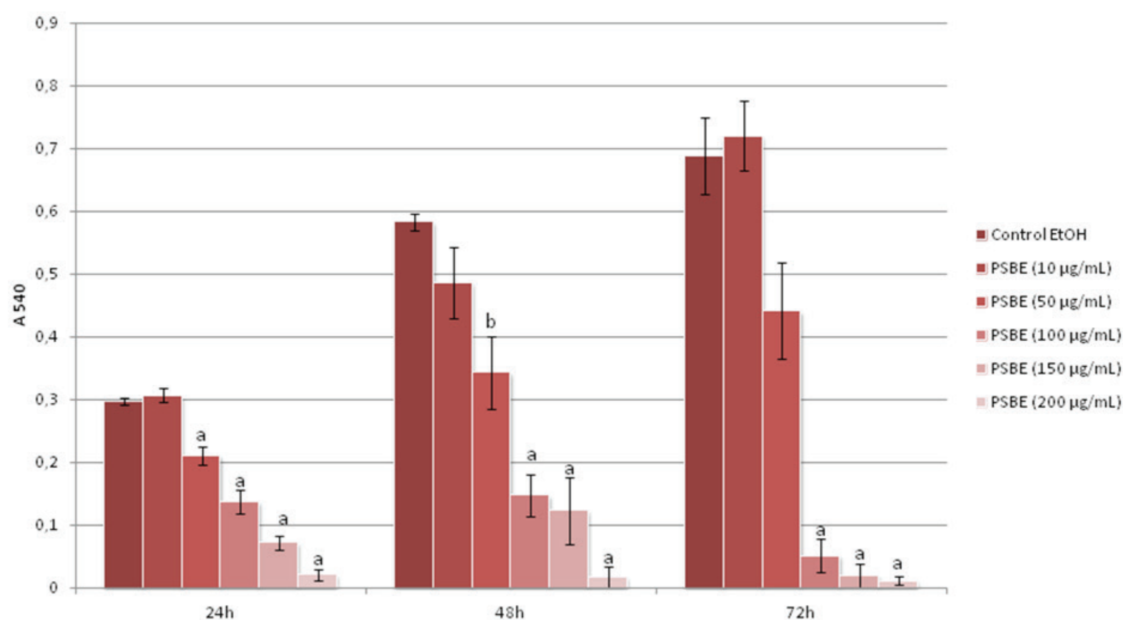

Figure 2 Effects of blackthorn flower extract on Hep G2 cell viability (mean±SEM) determined with neutral red after 24, 48, and 72 h; [a] $p<0.001$; [b] $p<0.01$ (vs control). Control EtOH – cells treated with 5 µL/mL ethanol; PSBE 10, 50, 100, 150, and 200 µg/mL – concentrations of PSBE in growth medium

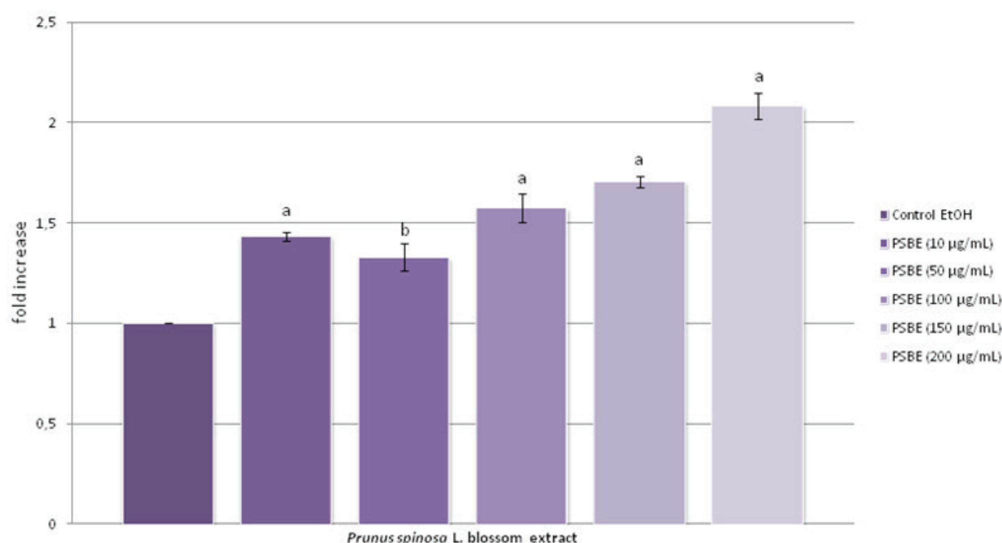

Figure 3 Mean (±SEM) increase in ROS generation in Hep G2 cells by blackthorn flower extract; [a] $p<0.001$; [b] $p<0.01$ (vs control). Control EtOH – cells treated with 5 µL/mL ethanol; PSBE 10, 50, 100, 150, and 200 µg/mL – concentrations of PSBE in growth medium

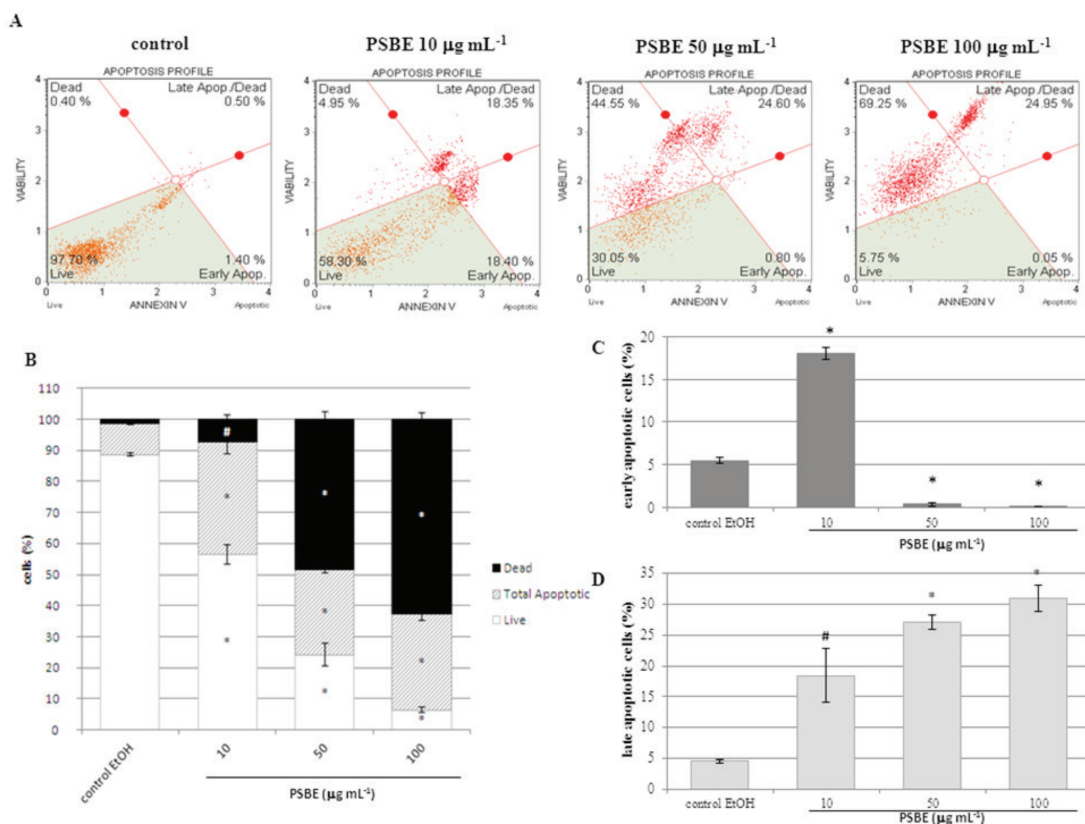

Figure 4 Cell death by apoptosis in Hep G2 cells treated with blackthorn flower extract (PSBE) at concentrations of 10, 50, and 100 μg/mL for 48 h. (A) Representative dot plot profiles of untreated and PSBE-treated cells; (B) Distribution of live, total apoptotic, and dead cells (mean±SEM); (C) Distribution of early apoptotic cells relative to the total number of cells (mean±SEM); (D) Distribution of late apoptotic/dead cells relative to the total number of cells (mean±SEM); *p<0.001, #p<0.025 vs. the respective controls

C), and MAP (mitogen-activated protein)-kinase. They can also alter growth signalling by inhibiting receptor phosphorylation or by arresting growth factors binding to receptors. Quercetin, for example, can induce thymidylate synthase-mediated apoptosis at high concentrations (27). Quercetin and kaempferol have already been detected and quantified in *Prunus spinosa* L. flower extract (3, 4), and our results seem to implicate their apoptotic signalling role in addition to prooxidative cytotoxic action. Most of the necrotic cells in our study can be explained with high cyanogenic glucoside content in the genus *Prunus*, as reported earlier (11).

CONCLUSION

Our study confirms that blackthorn flower extract in the concentration range of 50–200 μg/mL is toxic to human liver cancer cells and induces apoptotic/necrotic cell death, most likely through increased oxidative stress (ROS production). Previously, we have demonstrated similar effects in mouse hepatocarcinoma cells (Hepa 1–6) but also pointed out toxic effects in non-neoplastic, normal hepatocytes (AML-12) (11). Further studies are required in order to evaluate the *in vivo* anticancer efficacy of PSBE and selectivity regarding normal cells and tissues. Doses of PSBE below 10 μg/mL should be investigated for antioxidative and protective effects at the cellular level. Additionally, a promising area of research is combining phytomedicine or natural products with synthetic drugs or introducing these into conventional treatment regimens. Combination of natural and synthetic drugs proved to be an alternative strategy to generate synergistic anticancer effects, reduce toxicity associated with individual drug, suppress resistance associated with multiple drugs, and enhance the treatment effect (28, 29). Research of PSBE should also go in this direction.

Acknowledgments

This study was supported by the Croatian Science Foundation project no. 3035 "Application of innovative technologies for production of plant extracts as ingredients for functional food (IT-PE-FF)".

Conflicts of interest

None to declare.

REFERENCES

1. Nikolić T, Rešetnik I. Plant uses in Croatia. Phytol Balcan 2007;13:229-38.
2. Popescu I, Caudullo G. *Prunus spinosa* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz J, de Rigo D, Caudullo G, Durrant TH, Mauri A, editors. European atlas of forest tree species. Luxembourg: Publication Office of the European Union; 2016, p. 145.
3. Pinacho R, Rita Cavero Y, Astiasarán I, Ansorena D, Calvo MI. Phenolic compounds of blackthorn (*Prunus spinosa* L.) and influence of *in vitro* digestion on their antioxidant capacity. J Funct Foods 2015;19:49-62. doi: 10.1016/j.jff.2015.09.015
4. Olszewska M, Wolbiś M. Flavonoids from the flowers of *Prunus spinosa* L. Acta Pol Pharm 2001;58:367-72. PMID:11876444
5. Šamec D, Durgo K, Grúz J, Kremer D, Kosalec I, Piljac-Žegarac J, Salopek-Sondi B. Genetic and phytochemical variability of six *Teucrium arduini* L. populations and their antioxidant/prooxidant behaviour examined by biochemical, macromolecule- and cell-based approaches. Food Chem 2015;186:298-305. doi: 10.1016/j.foodchem.2014.07
6. Dorman HJD, Hiltunen R. Antioxidant and pro-oxidant *in vitro* evaluation of water-soluble food-related botanical extracts. Food Chem 2011;129:1612-8. doi: 10.1016/j.foodchem.2011.06.017
7. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917. doi: 10.1002/ijc.25516
8. Lizcano LJ, Siles M, Trepiana J, Hernández ML, Navarro R, Ruiz-Larrea MB, Ruiz-Sanz JI. *Piper* and *Vismia* species from Colombian Amazonia differentially affect cell proliferation of hepatocarcinoma cells. Nutrients 2014;7:179-95. doi: 10.3390/nu7010179
9. Cardellina II JH, Fuller, RW, Gamble WR, Westergaard C, Boswell J, Munro MHG, Currens M, Boyd MR. Evolving strategies for the selection, dereplication and prioritization of antitumor and HIV-inhibitory natural products extracts. In: Bohlin L, Bruhn JG, editors. Bioassaay methods in natural product research and development. Dordrecht: Kluwer Academic Publishers; 1999, p. 25-36.
10. Itharat A, Houghton PJ, Eno-Amooquaye E, Burke PJ, Sampson JH, Raman A. *In vitro* cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J Ethnopharmacol 2004;90:33-8. doi: 10.1016/j.jep.2003.09.014
11. Murati T, Miletić M, Štefanko A, Landeka Jurčević I, Elez Garofulić I, Dragović-Uzelac V, Kmetič I. Comparative assessment of *Prunus spinosa* L. flower extract in non-neoplastic hepatocytes and hepatoblastoma cells.‌S Afr J Bot 2019;123:36-42. doi: 10.1016/j.sajb.2019.02.006
12. Bursać Kovačević D, Putnik P, Dragović-Uzelac V, Pedisić S, Režek Jambrak A, Herceg Z. Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. Food Chem 2016;190:317-23. doi: 10.1016/j.foodchem.2015.05.099
13. Singleton VL, Rossi JAJ. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.
14. Shortle E, O'Grady MN, Gilroy D, Furey A, Quinn N, Kerry JP. Influence of extraction technique on the anti-oxidative potential of hawthorn (*Crataegus monogyna*) extracts in bovine muscle homogenates. Meat Sci 2014;98:828-34. doi: 10.1016/j.meatsci.2014.07.001
15. Clothier RH. The FRAME cytotoxicity test (Kenacid Blue). In: O'Hare S, Atterwill CK, editors. *In vitro* toxicity testing protocols. Totowa (NJ): Humana Press Inc.; 1995. p. 109-18.
16. Liebsch HM, Spielmann H. Balb/c 3T3 cytotoxicity test. In: O'Hare S, Atterwill CK, editors. *In vitro* toxicity testing protocols. Totowa (NJ): Humana Press Inc.; 1995. p. 177-87.
17. Kmetič I, Gaurina Srček V, Slivac I, Šimić B, Kniewald Z, Kniewald J. Atrazine exposure decreases cell proliferation in *Chinese Hamster Ovary* (CHO-K1) cell line. Bull Environ Contam Toxicol 2008;81:205-9. doi: 10.1007/s00128-008-9425-6
18. Craciunescu O, Constantin D, Gaspar A, Toma L, Utoiu E, Moldovan L. Evaluation of antioxidant and cytoprotective activities of *Arnica montana* L. and *Artemisia absinthium* L. ethanolic extracts. Chem Cent J 2012;6:97. doi: 10.1186/1752-153X-6-97
19. Murati T, Šimić B, Pleadin J, Vukmirović M, Miletić M, Durgo K, Kniewald J, Kmetič I. Reduced cytotoxicity in PCB-exposed *Chinese Hamster Ovary* (CHO) cells pretreated with vitamin E. Food Chem Toxicol 2017;99:17-23. doi: 10.1016/j.fct.2016.11.014
20. Han M, Li JF, Tan Q, Sun YY, Wang YY. Limitations of the use of MTT assay for screening in drug discovery. J Chinese Pharm Sci 2010;19:195-200.
21. Deng R, Hua X, Li J, Chi W, Zhang Z, Lu F, Zhang L, Pflugfelder SC, Li D-Q. Oxidative stress markers induced by hyperosmolarity in primary human corneal epithelial cells. PLoS One 2015;10:e0126561. doi: 10.1371/journal.pone.0126561
22. Acikgoz E, Guven U, Duzagac F, Uslu R, Kara M, Soner BC, Oktem G. Enhanced G2/M arrest, caspase related apoptosis and reduced e-cadherin dependent intercellular adhesion by trabectedin in prostate cancer stem cells. PloS One 2015;10:e0141090. doi: 10.1371/journal.pone.0141090
23. Zhu C, Hu W, Wu H, Hu X. No evident dose-response relationship between cellular ROS level and its cytotoxicity-a paradoxical issue in ROS-based cancer therapy. Sci Rep 2014;4:5029. doi: 10.1038/srep05029
24. Kong Q, Beel JA, Lillehei KO. A threshold concept for cancer therapy. Med Hypotheses 2000;55:29-35. doi: 10.1054/mehy.1999.0982
25. Maurya DK, Devasagayam TP. Antioxidant and prooxidant nature of hydroxycinnamic acid derivatives ferulic and caffeic acids. Food Chem Toxicol 2010;48:3369-73. doi: 10.1016/j.fct.2010.09.006
26. León-González AJ, Auger C, Schini-Kerth VB. Pro-oxidant activity of polyphenols and its implication on cancer chemoprevention and chemotherapy. Biochem Pharmacol 2015;98:371-80. doi: 10.1016/j.bcp.2015.07.017
27. Hertzog DI, Tica, OS. Molecular mechanisms underlying the anticancerous action of flavonoids. Curr Health Sci J 2012;38:145-9.
28. Mangla B, Kohli K. Combination of natural agent with synthetic drug for the breast cancer therapy. Int J Drug Dev Res 2018;10:22-6.
29. Demain AL, Vaishnav P. Natural products for cancer chemotherapy. Microb Biotechnol 2011;4:687-99. doi: 10.1111/j.1751-7915.2010.00221.x

Toksično djelovanje ekstrakta cvijeta *Prunus spinosa* L. na stanice hepatokarcinoma

Prunus spinosa L. (trnina) biljka je koja se koristi u tradicionalnoj medicini kao lijek za različite bolesti. Da bi se utvrdila njezina antikancerogena svojstva, kao model korištene su stanice humanoga hepatocelularnoga karcinoma (Hep G2) koje su izložene ekstraktu cvijeta trnine u koncentracijama 10–200 μg/mL te je određena citotoksična aktivnost s *kenacid blue* i *neutral red* metodama nakon 24, 48 i 72 sata inkubacije. Statistički značajni inhibitorni učinci na staničnu proliferaciju uočeni su pri koncentracijama iznad 50 μg/mL ($p<0,001–0,05$). Vijabilnost stanica određena metodom *neutral red* bila je niža u odnosu na onu koja je određena *kenacid blue* metodom. Dodatno su ispitani antioksidacijski/prooksidacijski učinci ekstrakta cvijeta trnine mjerenjem reaktivnih kisikovih vrsta (ROS), a rezultati su potvrdili prooksidacijsko djelovanje u primijenjenom rasponu koncentracija. Protočnom citometrijom detektiran je visok udio stanica u apoptozi i nekrozi, što dodatno upućuje na citotoksičnost u stanicama karcinoma jetre.

KLJUČNE RIJEČI: citotoksičnost; Hep G2; *kenacid blue*; *neutral red*; *ROS*; trnina