J Appl Biol Chem (2023) 66:41, 299–304
https://doi.org/10.3839/jabc.2023.041

Online ISSN 2234-7941
Print ISSN 1976-0442

Article: Biochemistry/Molecular Biology

Crataegus pinnatifida Bunge root extract induces apoptosis of murine lung carcinoma cells *in vitro*

Minjeong Kwon[1] · Jongbeom Chae[1] · Ju-Ock Nam[1,2]

Received: 13 June 2023 / Accepted: 10 July 2023 / Published Online: 18 July 2023

Abstract This study sought to evaluate the anticancer effects of *Crataegus pinnatifida* Bunge root extract (CPE) on murine Lewis lung carcinoma cells (LLC1) *in vitro*. CPE treatment (2.5, 5, 10 μg/mL, 24 h) of LLC cells led to a dose-dependent decrease in cell viability, while CPE treatment did not have a cytotoxic effect on non-cancer cells (NIH/3T3). CPE affects LLC by flipping the plasma membrane and making the membrane more permeable; by flow cytometry, CPE-induced annexin V and propidium iodide positivity, indicating induction of apoptosis in LLC cells. In addition, CPE enhanced the expression of apoptotic proteins caspase-3 and poly (ADP-ribose) polymerase 1 (PARP-1). CPE upregulated the proapoptotic protein BCL-2-associated X while downregulating the anti-apoptotic protein B-cell lymphoma 2 (BCL-2), suggesting that CPE induces apoptosis via the mitochondrial pathway. Furthermore, CPE upregulated the phosphorylation of the mitogen activated protein kinase p38. In conclusion, the results suggest that CPE has an anticancer effect in LLC cells by inducing apoptosis via p38.

Keywords Apoptosis · Caspase-3 · *Crataegus pinnatifida* · Lung cancer · p38

Ju Ock Nam (✉)
E-mail: namjo@knu.ac.kr

[1]Department of Food Science and Engineering, Kyungpook National University, Daegu 41566, Republic of Korea

[2]Research institute of Tailored Food Technology, Kyungpook National University, Daegu 41566, Republic of Korea

Introduction

Cancer is one of the major causes of death worldwide [1]. Lung cancer has the highest overall mortality among the different types of cancers [2]. In Korea, the rate of new lung cancer diagnosis was 58.4 people per 100,000 in 2019, with a mortality rate of 36.2 people per 100,000 [3]. There are two different types of lung cancer: non-small cell lung cancer (NSCLC) and small cell lung cancer. Their incidence in Korea is 79 and 21%, respectively [4]. Although NSCLC has a higher incidence, it currently has fewer available treatment options [7].

The main treatments for NSCLC are surgery, radiation therapy, and chemotherapy [5-9]. The main purpose of the initial surgical procedure is to eliminate the solid tumor. Radiation then kills remaining cancer cells by applying radiation beams to a specific part of the patient's body [7,10]. Radiation creates free radicals, which are highly reactive and cause DNA damage to cancer cells [7,10]. Although radiation therapy is one of the most common cancer treatments, NSCLC is less sensitive to radiation than other cancers [7]. Finally, chemotherapy drugs can be used independently or mixed together in various proportions [11,12], all typically with the intended effect of inducing apoptotic cell death specifically in cancer cells [13].

Apoptosis is also known as programmed cell death; when it occurs, cells shrink, the nuclear envelope and DNA strands fragment, and the plasma membrane blebs [14]. Apoptosis signaling can occur via either the intrinsic or extrinsic pathway. The intrinsic pathway is initiated by intracellular stress, such as DNA damage and growth factor defection [15]. The extrinsic pathway is initiated by a signal from outside the cell [15]. These stimuli induce signaling cascades, resulting in the release of cytochrome C from the mitochondria. Cytochrome C combines with apoptotic protease-activating factor 1 to form an apoptosome, which activates caspase-9 [15]. Subsequently, apoptosis initiates when the effector caspases are activated [15]. The extrinsic pathway is initiated by the binding of a ligand to one of the death receptors [16]. This results in the recruitment of adapter proteins

containing death domains, such as tumor necrosis factor receptor type 1-associated via death domain or Fas-associated via death domain [17]. The adapter-protein complex binds to and activates pro-caspase-8 [18]. Active caspase-8 induces apoptosis by mediating the cleavage of proapoptotic proteins such as BH3-interacting domain death agonist [18].

When chemotherapeutic drugs induce apoptosis in cancer cells, however, patients can suffer from side effects like myelo-suppression, nausea, and vomiting [19]. To find substitutes for these harsh drugs, significant research has been done on the therapeutic potential of natural products. For example, Honokiol from Magnolia officinalis has an anti-cancer effect in colon cancer [35]. And Morusin from Morus alba L. has an anti-cancer effect in cervical cancer [35].

Crataegus species, including the fruit of the hawthorn *Crataegus pinnatifida* Bunge var. typica Schneider, have long been used for traditional medicine in Korea [20,22]. Traditionally, the fruit of *C. pinnatifida* was used to treat hypertension and cardiovascular disease [21]. Other parts of the *C. pinnatifida* plant also have therapeutic potential. For example, the leaves, wood, bark, and fruit have all been shown to have an antioxidant activity [24-26]. The leaves of *C. pinnatifida* have anti-allergic effect [23], and the wood and bark have anti-inflammatory effects [25]. Furthermore, it has been reported that *C. pinnatifida* fruit has antitumor effects in gastrointestinal cancer [27]. Plant-derived secondary products abundantly present in leaves, fruits, and roots of C. pinnatifida are flavonoids, for example, quercetin, hyperoside, and rutin [36]. The flavonoids of the genus Crataegus have recently received great attention [36].

However, the biological effects of the *C. pinnatifida* root have rarely been studied. Therefore, based on the anticancer effect of the hawthorn fruit [26], the current study was designed to investigate the anticancer effect of the hawthorn root using an *in vitro* cell model of murine lung carcinoma.

Materials and Methods

Materials

The Lewis lung carcinoma (LLC1) murine cell line and the NIH/3T3 murine fibroblast cell line were purchased from the American Type Culture Collection (Rockville, MD, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), newborn calf serum (NBCS) and trypsin were obtained from Gibco Laboratories (Grand Island, NY, USA). Cell counting kit-8 (CCK-8) was purchased from Dojindo (Tokyo, Japan). Alexa Fluor 647-conjugated annexin V and propidium iodide (PI) dye for double staining assay were purchased from Invitrogen. Primary antibodies for b-actin and B-cell lymphoma 2 (BCL-2) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibodies for BCL-2-associated X (BAX), poly (ADP-ribose) polymerase (PARP), cleaved caspase-3, p38, phosphorylated-p38 (p-p38), extracellular signal-regulated kinase (ERK), p-ERK, c-Jun N-terminal kinase (JNK), and p-JNK were obtained from Cell Signaling Technology (Beverly, MA, USA). Secondary antibodies for b-actin, BCL-2, BAX, PARP, cleaved caspase-3, p38, p-p38, ERK, p-ERK, JNK, p-JNK were obtained from Cell Signaling Technology.

Sample extract preparation and treatment

C. pinnatifida Bunge root extract (CPE) was purchased from the Korea Plant Extract Bank (Korea). CPE was prepared via methanol extraction. Cells were treated with low (2.5 μg/mL), middle (5 μg/mL), or high (10 μg/mL) concentrations of CPE, denoted as CPE-L, CPE-M, CPE-H, respectively. Control cells were treated with dimethyl sulfoxide (DMSO) alone. DMSO was added in the same concentration as the highest CPE treatment dose (10 μg/mL). These concentrations were used for all of the following assays.

Cell culture conditions

LLC1 cells were cultured in DMEM supplemented with 10% FBS and maintained in a humidified incubator with 5% CO_2 at 37 ºC. NIH/3T3 cells were cultured in DMEM supplemented with 10% NBCS and maintained in a humidified incubator with 5% CO_2 at 37 ºC.

Cytotoxicity assay

Cell proliferation was determined by using a CCK-8 kit. Cells were detached from the plate using trypsin. 5×10^3 cells were seeded per well in a 96-well plate, then placed in a humidified incubator for 24 h. Cells were treated with low, middle, or high concentrations of CPE and incubated for 24 h. After the 24-h treatment, 10 μL of CCK-8 reagent was added to each well, and plates were placed in a dark incubator. Cell viability was determined after 3 h of incubation by measuring optical density values at 450 nm using a microplate reader.

Annexin V and PI staining

1×10^6 cells were seeded in a 60 mm dish and incubated for 24 h. Cells were then treated with low, middle, or high concentrations of CPE and placed in a humidified incubator for 24 h. Cells were then detached from the dish using trypsin and centrifuged at 3000 rpm for 5 minutes. After discarding the supernatant, cells were washed with 500 μL of phosphate-buffered saline containing 2% FBS, then centrifuged again at 3000 rpm for 5 minutes. The supernatant was discarded and 200 μL of binding buffer and 10 μL of Alexa Fluor 647-conjugated annexin V antibody were added to each sample. After 15 minutes, cells were centrifuged and the supernatant was discarded. 200 μL of binding buffer and 10 μL of PI fluorescent dye were added to each sample. Samples were analyzed using flow cytometry through FL-1 (BL2) and FL-2 (RL1) filters.

Western blot

LLC1 cells were collected and lysed in lysis buffer with protease inhibitors (Xpert Protease Inhibitor Cocktail Solution, GenDEPOT) and phosphatase inhibitors (Xpert Phosphatase Inhibitor Cocktail Solution, GenDEPOT). Proteins were separated in 7.5, 10, or 15% SDS-PAGE gels, depending on target protein size, then transferred to polyvinylidene fluoride membranes. Blots were blocked in 5% skim milk for 1 h at room temperature, then incubated overnight at 4 ºC with primary antibodies: caspase3, PARP, BAX, BCL-2, ERK, p-ERK, JNK, p-JNK, p38, or p-p38 at 1000-fold dilutions and β-actin at a 5000-fold dilution. The membranes were washed with tris-buffered saline containing 0.1% TWEEN 20 and were then incubated at room temperature for an h with horseradish peroxidase (HRP)-conjugated anti-mouse antibody at a 2000-fold dilution and HRP-conjugated anti-rabbit antibody at a 3000-fold dilution. Proteins were detected using enhanced chemiluminescence reagent (GE Healthcare, Buckinghamshire, UK). Protein bands were quantified and analyzed using ImageJ software. All experiments were performed in triplicate.

Statistical analysis

All of the data were presented as mean ± standard deviation (SD). According to one-way ANOVA, analyses were considered to be significant at p <0.05*, p <0.01**, p <0.001***, and p <0.0001**** compared to the control.

Results and Discussion

CPE treatment decreases viability of LLC1 cells

Murine lung cancer LLC1 cells were treated with different concentrations of CPE (2.5, 5, or 10 μg/mL), or with DMSO as a negative control. After 24 h of treatment, cell viability was analyzed by CCK-8 assay. Higher treatment concentration yielded lower viability of LLC1 cells compared to the control (Fig. 1A). In previous studies, Mouse embryo fibroblast NIH/3T3 cell line was used as a comparison target with tumor cell line for cytotoxicity evaluation [37-39]. Thereafter, in this study, NIH/3T3 cell line is used as non-cancer cells for cytotoxicity evaluation. We performed the same experiment using the murine fibroblast NIH/3T3 cell line. No decrease in cell viability with CPE treatment was observed in the non-cancerous cells (Fig. 1B), demonstrating a cancer cell-specific cytotoxic effect of CPE.

CPE induces apoptosis of LLC1 cells

To investigate if CPE decreased cell viability by inducing apoptosis, an annexin V/PI double staining assay and a western blot assay were conducted. Annexin V staining indicates early and late apoptotic cells, while PI stains dead cells in stages of late apoptosis or necrosis. Therefore, live cells are negative for both annexin V and PI, necrotic cells are negative for annexin V and positive for PI, early apoptotic cells are positive for annexin V and

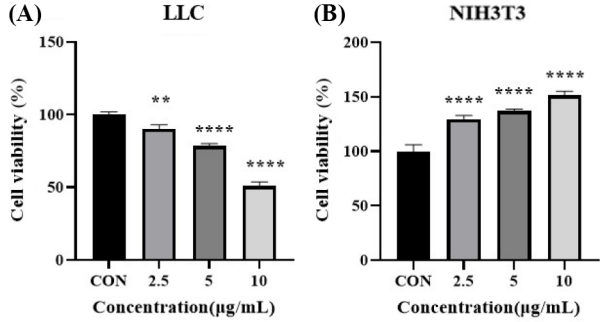

Fig. 1 CPE treatment decreases viability specifically in LLC1 cells. (A) LLC1 cells were treated with 2.5, 5, or 10 μg/mL of *Crataegus pinnatifida* Bunge root extract (CPE) for 24 h. (B) NIH/3T3 cells were treated with 2.5, 5, or 10 μg/mL of CPE for 24 h. Viability was measured by CCK-8 assay. All data are presented as mean value ± standard deviation (SD), and n =3 in each group. **p <0.01 and ****p <0.0001 compared to the control

negative for PI, and late apoptotic cells are positive for both annexin V and PI. As shown in Fig. 2A-D, the overall apoptosis rates were 19%, 33%, and 81% for CPE-L, CPE-M, and CPE-H, respectively, showing a tendency of increased apoptosis with increasing concentration of CPE (compared to a 3% rate of apoptosis in the control group). The breakdown of early and late apoptosis was 5.03 and 14.10%, respectively, for CPE-L, 13.08 and 19.50% for CPE-M, and 42.39 and 38.40% for CPE-H.

To confirm the induction of apoptosis by CPE, western blot analysis was performed. Caspase-3 acts as the apoptotic "executioner" caspase and is activated by cleavage [28]. Cleavage of PARP-1 is also considered as a hallmark of cell death during apoptosis [29], and occurs when the final protein in the apoptosis process is activated [30]. The expression of cleaved caspase-3 and cleaved PARP both significantly increased with H-CPE treatment (Fig. 2E-G). These results indicate that CPE-H induces apoptosis by activating caspase-3 and cleavage of PARP.

CPE induces apoptosis via the mitochondria-mediated intrinsic pathway

To determine whether the apoptosis induced by CPE progressed via the intrinsic or extrinsic pathway, western blot analysis was performed. In general, the intrinsic apoptosis pathway is regulated by the relative ratios of BAX and BCL-2 [31]. BCL-2 is involved in cell survival and inhibits apoptosis by various stimuli, making it a negative regulator of apoptosis [32]. BAX, on the other hand, plays an important role in the early stage of apoptosis as a proapoptotic protein [33]. BAX expression was upregulated by increasing concentrations of CPE (Fig. 3A). Overall, the ratio of BAX to BCL-2 increased significantly with CPE-H compared to the control (Fig. 3A, B). These results suggest that the apoptosis induced by CPE treatment is related to a mitochondrial pathway dependent on BCL-2 family proteins.

We confirmed that CPE triggers intrinsic, mitochondria-mediated

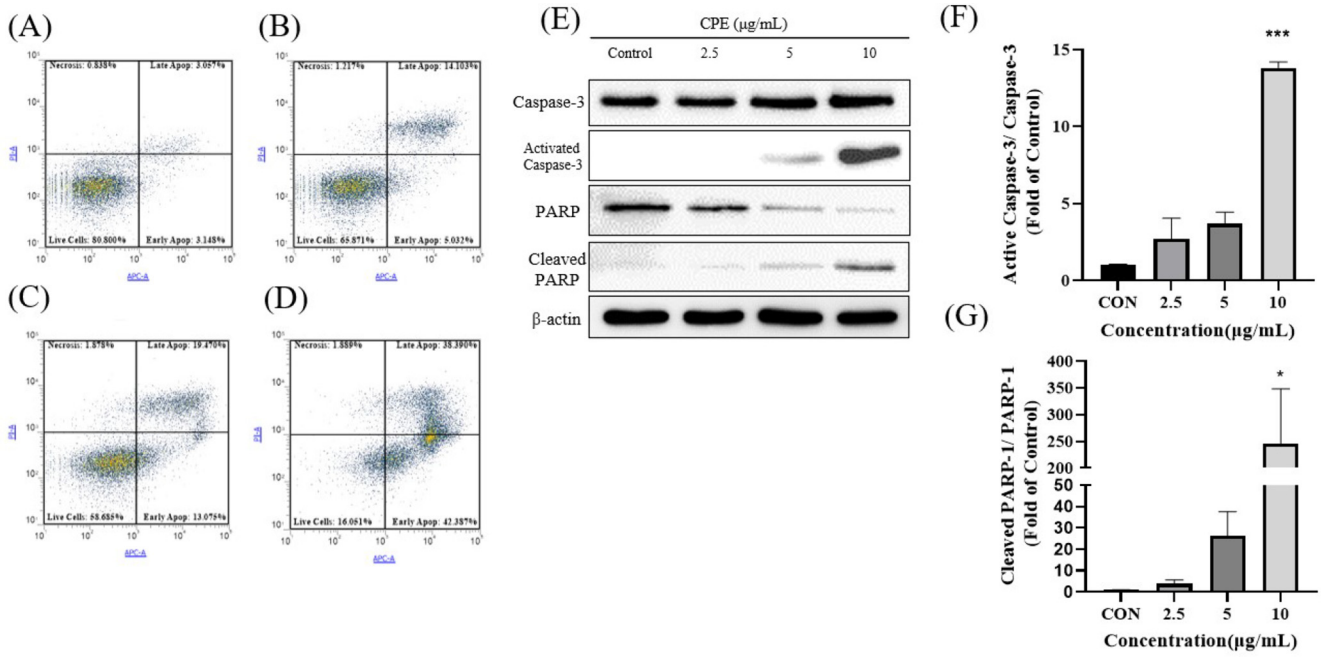

Fig. 2 CPE treatment increases apoptosis in LLC1 cells. LLC1 cells were treated with (A) DMEM medium alone or CPE (B) 2.5 μg/mL, (C) 5 μg/mL, and (D)10 μg/mL for 24 h. Cells were labeled with Alexa Fluor 647-conjugated annexin V and stained with propidium iodide, and flow cytometry was used to detect the intracellular fluorescence intensity in cells. (E) Protein expression levels of caspase-3, cleaved caspase-3, PARP, and cleaved PARP were assessed by western blot analysis following treatment with 2.5, 5, or 10 μg/mL of CPE for 24 h. β-Actin served as a loading control. (F), (G) Relative ratio of protein levels in (E). All data are presented as the mean ± SD of the percentage of the control, and n =3 in each group. *p <0.05 and ***p <0.001 compared to the control

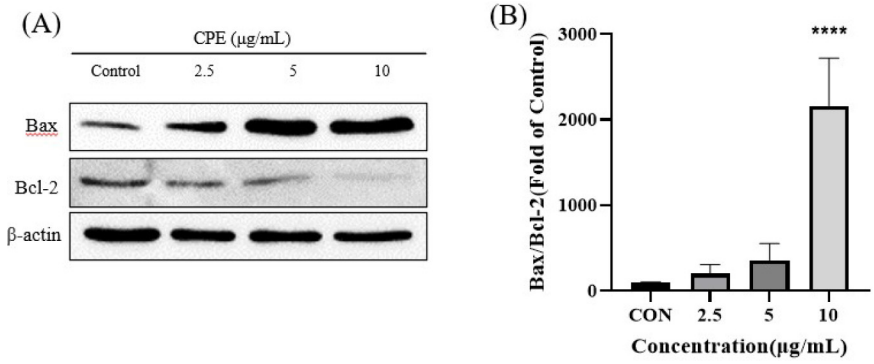

Fig. 3 CPE treatment increases BAX/BCL-2 ratio in LLC1 cells. (A) Protein expression levels of BAX and BCL-2 were assessed by western blot analysis following treatment of LLC1 cells with 2.5, 5, or 10 μg/mL of CPE for 24 h. β-Actin served as a loading control. (B) Relative ratio of protein levels in (A). All data are presented as the mean ± SD of percentage of the control, and n =3 in each group. ****p <0.0001 compared to the control

apoptosis. Our results show that CPE treatment upregulates proapoptotic BAX expression and downregulates antiapoptotic BCL-2 expression, elevating the overall BAX/BCL-2 ratio in LLC1 cells. These results suggest that the apoptosis induced by CPE treatment is related to a mitochondrial pathway dependent on BCL-2 family proteins. In conclusion, we demonstrated that CPE induces apoptosis in LLC1 cells, and that it occurs through the intrinsic mitochondrial pathway.

CPE-mediated apoptosis is induced via p38

To determine the underlying signaling pathway of CPE-induced apoptosis, we performed western blot analysis of the mitogen activated protein kinase (MAPK) p38. The MAPK signaling pathway is involved in cell proliferation and cell survival [34]. The MAPK p38 is known to be induced by cellular stress and involved in apoptosis [34]. Our results showed that both CPE-M and CPE-H treatment led to significantly increased phosphorylation

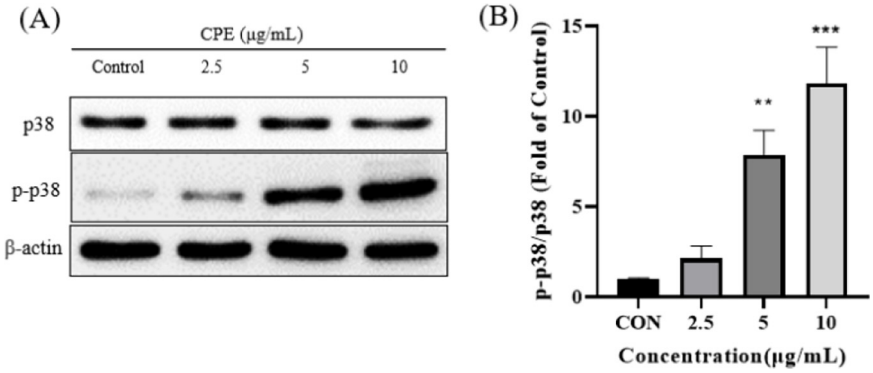

Fig. 4 CPE-induced apoptosis occurs via p38 activation. Protein expression levels of ERK, p-ERK, p38, and p-p38 were assessed by western blot analysis following treatment with 2.5, 5, or 10 μg/mL of CPB for 24 h. β-Actin served as a loading control. All data are represented as the mean ± SD of the percentage of the control, and n =3 in each group. $^{**}p$ <0.01 and $^{***}p$ <0.001, compared to the control

of p38 (7.85- and 11.82-fold over control, respectively) (Fig. 4A, B). Therefore, our results indicated that CPE induces apoptosis by activating the p38 MAPK pathway.

The results of this study showed that the natural product CPE indicated cytotoxic activity in one murine lung carcinoma cell line, without demonstrating toxicity to non-cancerous murine lung fibroblasts. We demonstrated that this cytotoxicity occurs via the intrinsic apoptotic pathway, mediated by mitochondria. Therefore, CPE merits further exploration for potential use and pharmaceutical development as a lung cancer treatment.

References

1. Bray F, Laversanne M, Weiderpass E, Soerjomataram I (2021) The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer 127: 3029–3030. doi: 10.1002/cncr.33587
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca-cancer J Clin 71: 209–249. doi: 10.3322/caac.21660
3. Kang MJ, Won Y-J, Lee JJ, Jung K-W, Kim H-J, Kong H-J, Im J-S, Seo HG (2022) Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2019. Cancer Res Treat 54: 330–344. doi: 10.4143/crt.2022.128
4. Jeon DS, Kim JW, Kim SG, Kim HR, Song SY, Lee JC, Ji W, Choi CM, Kim HC, Korean Association for Lung Cancer SKCCR (2022) Sex differences in the characteristics and survival of patients with non-small-cell lung cancer: A retrospective analytical study based on real-world clinical data of the Korean population. Thorac Cancer 13: 2584–2591. doi: doi.org/10.1111/1759-7714.14594
5. Park S, Park IK, Kim ER, Hwang Y, Lee HJ, Kang CH, Kim YT (2017) Current trends of lung cancer surgery and demographic and social factors related to changes in the trends of lung cancer surgery: an analysis of the national database from 2010 to 2014. Cancer Res Treat 49: 330–337. doi: 10.4143/crt.2016.196
6. Vinod SK, Hau E (2020) Radiotherapy treatment for lung cancer: Current status and future directions. Respirology 25: 61–71. doi: 10.1111/resp.13870
7. Huang C-Y, Ju D-T, Chang C-F, Reddy PM, Velmurugan BK (2017) A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer. Biomedicine 7. doi: 10.1051/bmdcn/2017070423
8. Lee JG, Kim HC, Choi C-M (2021) Recent trends of lung cancer in Korea. Tuberc Respir Dis 84: 89. doi: 10.4046/trd.2020.0134
9. Mithoowani H, Febbraro M (2022) Non-Small-Cell Lung Cancer in 2022: A Review for General Practitioners in Oncology. Curr Oncol 29: 1828–1839. doi: 10.3390/curroncol29030150
10. Yoshimura M, Itasaka S, Harada H, Hiraoka M (2013) Microenvironment and radiation therapy. Biomed Res Int 2013. doi: 10.1155/2013/685308
11. Gadgeel SM, Ramalingam SS, Kalemkerian GP (2012) Treatment of lung cancer. Radiol Clin N Am 50: 961–974. doi: 10.1016/j.rcl.2012.06.003
12. Sweeney CJ, Sandler AB (1998) Treatment of advanced (Stages III and IV) non—small-cell lung cancer. Curr Prob Cancer 22: 87–132. doi: 10.1016/S0147-0272(98)90010-1
13. Ricci MS, Zong W-X (2006) Chemotherapeutic approaches for targeting cell death pathways. Oncologist 11: 342–357. doi: 10.1634/theoncologist.11-4-342
14. Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke E, Blagosklonny M, El-Deiry W, Golstein P, Green D (2009) Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 16: 3–11. doi: 10.1038/cdd.2008.150
15. Lawen A (2003) Apoptosis—an introduction. Bioessays 25: 888–896. doi: 10.1002/bies.10329
16. Sayers TJ (2011) Targeting the extrinsic apoptosis signaling pathway for cancer therapy. Cancer Immunol Immun 60: 1173–1180. doi: 10.1007/s00262-011-1008-4
17. Sartorius U, Schmitz I, Krammer PH (2001) Molecular mechanisms of death-receptor-mediated apoptosis. Chembiocam *2*(1): 20–29. doi: 10.1002/1439-7633(20010105)2:1<20::AID-CBIC20>3.0.CO;2-X
18. Kantari C, Walczak H (2011) Caspase-8 and bid: caught in the act between death receptors and mitochondria. Biochim Biophys Acta 1813: 558–563. doi: 10.1016/j.bbamcr.2011.01.026
19. Sridhar T, Symonds RP (2009) Principles of chemotherapy and radiotherapy. Obstet Gynaecol Reprod Med 19: 61–67. doi: 10.1016/j.ogrm.2008.11.011
20. Li C, Son HJ, Huang C, Lee SK, Lohakare J, Wang M-H (2010) Comparison of Crataegus pinnatifida Bunge var. typica Schneider and C. pinnatifida Bunge fruits for antioxidant, anti-α-glucosidase, and anti-inflammatory activities. Food Sci Biotechnol 19: 769–775. doi: 10.1007/s10068-010-0108-9
21. Li C, Wang M-H (2011) Anti-inflammatory effect of the water fraction from hawthorn fruit on LPS-stimulated RAW 264.7 cells. Nutr Res Pract 5: 101–106. doi: 10.4162/nrp.2011.5.2.101

22. Li F, Yuan Q, Rashid F (2009) Isolation, purification and immunobiological activity of a new water-soluble bee pollen polysaccharide from Crataegus pinnatifida Bge. Carbohyd Polym 78: 80–88. doi: 10.1016/j.carbpol.2009.04.005
23. Zhang S-Y, Sun X-L, Yang X-L, Shi P-L, Xu L-C, Guo Q-M (2022) Botany, traditional uses, phytochemistry and pharmacological activity of Crataegus pinnatifida (Chinese hawthorn): a review. J Pharm Pharmacol 74: 1507–1545. doi: 10.1093/jpp/rgac050
24. Luo M, Yang X, Hu JY, Jiao J, Mu FS, Song ZY, Gai QY, Qiao Q, Ruan X, Fu YJ (2016) Antioxidant properties of phenolic compounds in renewable parts of Crataegus pinnatifida inferred from seasonal variations. J Food Sci 81: C1102–C1109. doi: 10.1111/1750-3841.13291
25. Min H-J, Kim Y-K, Bae Y-S (2017) Evaluation of biological activity on hawthorn tree (Crataegus pinnatifida) extracts. J Korean Wood Sci Technol 45: 317–326. doi: 10.5658/WOOD.2017.45.3.317
26. Duan Y, Kim M-A, Seong J-H, Chung H-S, Kim H-S (2014) Antioxidative activities of various solvent extracts from haw (Crataegus pinnatifida Bunge). Korean J Food Preserv 21: 246–253. doi: 10.11002/kjfp.2014.21.2.246
27. Yang J, Gu J, Shen Y, Cao L, Zhou H, Zhu W (2023) Effect of Shan Zha (Hawthorn or Crataegus) on gastrointestinal cancer: A network pharmacology and molecular docking study. Cancer Pathogenesis and Therapy 1: E012–E012. doi: 10.1016/j.cpt.2023.02.001
28. Ponder KG, Boise LH (2019) The prodomain of caspase-3 regulates its own removal and caspase activation. Cell Death Discov 5: 56. doi: 10.1038/s41420-019-0142-1
29. Gobeil S, Boucher C, Nadeau D, Poirier G (2001) Characterization of the necrotic cleavage of poly (ADP-ribose) polymerase (PARP-1): implication of lysosomal proteases. Cell Death Differ 8: 588–594. doi: 10.1038/sj.cdd.4400851
30. Chaitanya GV, Alexander JS, Babu PP (2010) PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. Cell Commun Signal 8: 1–11. doi: 10.1186/1478-811X-8-31
31. Raisova M, Hossini AM, Eberle J, Riebeling C, Orfanos CE, Geilen CC, Wieder T, Sturm I, Daniel PT (2001) The Bax/Bcl-2 ratio determines the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis. J Invest Dermatol 117: 333–340. doi: 10.1046/j.0022-202x.2001.01409.x
32. Gaumer S, Guenal I, Brun S, Theodore L, Mignotte B (2000) Bcl-2 and Bax mammalian regulators of apoptosis are functional in Drosophila. Cell Death Differ7: 804–814. doi: 10.1038/sj.cdd.4400714
33. Zhu L, Han MB, Gao Y, Wang H, Dai L, Wen Y, Na LX (2015) Curcumin triggers apoptosis via upregulation of Bax/Bcl-2 ratio and caspase activation in SW872 human adipocytes. Mol Med Rep 12: 1151–1156. doi: 10.3892/mmr.2015.3450
34. Gräb J, Rybniker J (2019) The expanding role of p38 mitogen-activated protein kinase in programmed host cell death. Microbiol Insights 12: 1178636119864594. doi: 10.1177/1178636119864594
35. Hashem S, Ali TA, Akhtar S, Nisar S, Sageena G, Ali S, Al-Mannai S, Therachiyil L, Mir R, Elfaki I, Muzaffar Mir M, Jamal F, Masoodi T, Uddin S, Singh M, Haris M, Macha M, Bhat AA (2022) Targeting cancer signaling pathways by natural products: Exploring promising anti-cancer agents. Biomed Pharmacother. 150: 113054. doi: 10.1016/j.biopha.2022.113054
36. Yu C, Lv D-G, Hu X-M, Deng W (2015) Changes in flavonoids concentration of Hawthorn (Crataegus pinnatifida) in response to exogenous amino acids. J Hortic For 7(9): 193–199. doi: 10.5897/JHF2015.0405
37. Danihelová M, Veverka M, Šturdík E, Jantová S (2013) Antioxidant action and cytotoxicity on HeLa and NIH-3T3 cells of new quercetin derivatives. Interdiscip Toxicol. 6: 209–216. doi: 10.2478/intox-2013-0031
38. Ahmad S, Ullah F, Zeb A, Ayaz M, Ullah F, Sadiq A (2016) Evaluation of Rumex hastatus D. Don for cytotoxic potential against HeLa and NIH/3T3 cell lines: chemical characterization of chloroform fraction and identification of bioactive compounds. BMC Complement Altern Med 16: 1–10. doi: 10.1186/s12906-016-1302-y
39. Khanavi M, Nabavi M, Sadati N, Shams Ardekani M, Sohrabipour J, Nabavi S Nabavi (2010) Cytotoxic activity of some marine brown algae against cancer cell lines. Biol Res. 43: 31–37. doi: 10.4067/S0716-97602010000100005