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Anticancer Activity of Sinapic Acid by Inducing Apoptosis in HT-29 Human Colon Cancer Cell Line

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Abstract

Background: Colorectal cancer is the third most lethal and fourth most commonly diagnosed cancer worldwide. Sinapic acid, a derivative of hydroxycinnamic acid, is a promising phytochemical exhibiting numerous pharmacological activities in various systems. It is a substantial chain-breaking antioxidant that operates as a radical scavenger. The aim of this research was to investigate the antiproliferative effect of sinapic acid on the HT-29 cell line, besides the mechanisms underlying this activity.

Materials and Methods: The effect of sinapic acid on the viability of HT-29 cell line was investigated using XTT assay. the levels of BCL-2, cleaved caspase 3, BAX, cleaved PARP and

8-oxo-dG were measured using ELISA. Gamma-H2AX and cytochrome C expression was assessed semi-quantitatively using immunofluorescence staining.

Results: Sinapic acid at 200 μM and higher doses produced a significant antiproliferative effect on HT-29 cells. The IC50 value was found to be 317.5 μM for 24 hours. Sinapic acid (317.5 μM) significantly elevated cleaved caspase 3, BAX, cleaved PARP and 8-oxo-dG levels. the levels of γ-H2AX foci are significantly higher while the levels of cytochrome-C are lower in sinapic acid treated HT-29 cells.

Conclusion: These results indicate that sinapic acid has an antiproliferative, apoptotic and genotoxic effect on colon cancer cells.

Keywords: Sinapic acid, Colorectal Cancer, HT-29 Cell Line, Antiproliferative Effect.

Introduction

GLOBOCAN 2018 data indicates that colorectal cancer is the third most lethal and fourth most commonly diagnosed cancer worldwide (1). Colon cancer affects nearly 150,000 Americans yearly, almost a third of whom die, (2), about 250,000 in Europe (3) and almost 1 million people all around the world (4). The interactions between genes and environment together with the excessive dietary fat intake play a crucial role in the etiology of colon cancer (5). Activated oncogenes and inactivated tumor suppressor genes can lead to hyperplasia and provide protection to cancer cells against apoptosis, giving rise to a dysfunction in cell processes (DNA replication, cell cycles, dysplasia, and immune-cell interactions) (6,7). knockout trials in mice have demonstrated that low dose (<10 Gy) gamma radiation-induced apoptosis, limited to epithelial cells, is mediated by BAX and p53 and is antagonized by bcl-2 and bcl-w (8-11). It has been demonstrated that food components known to inhibit the development of colon cancer enhance

apoptosis subsequent to DNA damage and this might represent a considerable mechanism of cancer prevention (12). Colon cancer is linked to pathological outcomes of constant oxidative stress and lipid peroxidation. Unsaturated lipids are vulnerable to oxidation and the metabolites of lipid peroxidation produce the mutagenesis promoting exocyclic DNA adducts (13).

HT-29 is a human colon cancer cell line used widely in biological and cancer research (14). HT-29 cells were initially obtained from a 44-year-old Caucasian female with colorectal adenocarcinoma in 1964, HT-29 cells form a tight monolayer while showing resemblance to enterocytes from the small intestines. HT-29 cells overexpress a mutated p53 tumor antigen (having a histidine replacing an arginine due to a mutation at position 273 in p53 gene) (15).

The conventional treatments for colon cancer include surgery, radiotherapy, chemotherapy. Furthermore, current approaches also involve targeted therapies mainly for advanced stages of colon cancer such as immunotherapy, gene therapy, cancer vaccines and cell therapy (16).

Chemotherapy-related toxicities and severe side effects such as diarrhea, provide a motivation for persistent research aimed at finding effective phytochemicals that can reduce these risks and also increase survival rates in metastatic colon cancer.

The uses of medicinal herbs and nutraceuticals have been steadily risen worldwide as their effectiveness has been proven besides being safer compared to synthetic drugs (17). Phenolic acids are renowned as reactive chemicals that have an effect on biological systems. They have got a lot of attention in the fields of pharmaceutical and medical research because they appear to play a role in the prevention of many diseases and involved in bioactivities applicable to human health. Phenolic acids exist in almost all edible plants, and the daily dietary consumption by humans is informed to be about 200mg (18, 19). Hydroxycinnamic acids form a main class of phenolic acids

found in the plant kingdom. Caffeic acid, ferulic acid, sinapic acid and p-coumaric acid are the most common hydroxycinnamic acids (20). Sinapic acid (SA) is 3,5-dimethoxy-4-hydroxycinnamic acid (Figure 1) (21). Some of the sinapic acid-rich sources are rice, wheat, oil, spices, vegetables, seeds, cereals, citrus fruits, and vinegar. Furthermore, SA has been informed to be a main active constituent of Chinese traditional medicines (22). Sinapic acid exhibits numerous pharmacological activities in various systems. Several in-vivo and in-vitro researches have been carried out to demonstrate the pharmacological features such as antioxidant, anticancer, analgesic, anti-inflammatory, and antimicrobial of SA and to clarify the mechanism of action of this compound (23). SA is considered to be a substantial chain-breaking antioxidant that fruitfully operates as a radical scavenger (24). This antioxidant activity is associated with its ability to donate hydrogen atoms and the feature to stabilize the resulting phenoxyl radicals via the conjugated system (25).

The aim of this research was to investigate the antiproliferative effect of sinapic acid on the HT-29 cell line, besides the mechanisms underlying this activity.

Materials and methods

Cell culture and cell lines

Colon adenocarcinoma cell line HT-29 was purchased from American Type Culture Collection. The cells were grown in Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 1% antibiotic mixtures of penicillin and streptomycin at 37°C in a 5% CO2 atmosphere in a humidified incubator. Before the procedure, sinapic acid (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) and diluted in the culture medium to a final DMSO concentration of less than 0.1%.

Cell viability test

Using XTT test assay (Roche Diagnostic, MA, USA), the effect of SA on the viability of HT-29 cell line was investigated. These cells were cultured at a concentration of 1×10^4 cells per well and incubated overnight before the addition of SA. After that the different concentrations (800, 400, 200 and 100 μM) of SA were applied to cells for 24 h. Untreated cells were used as a control. After incubation, 50 μL of XTT mixture was added to each well. After 4-hour incubation, the cells were shaken and the absorbance was measured using a microplate reader (Thermo Fisher Scientifi c, Altrincham, United Kingdom) at 450 nm. Cell viability was evaluated as a percentage of live cells versus control cells after each experiment was performed three times [26,27].

The measurement of BCL-2, cleaved caspase 3, BAX, cleaved PARP and 8-oxo-dG levels

The human ELISA kits of BCL-2 (BT Lab, catalog #E1832HU), cleaved caspase 3 (BT Lab, catalog # E6970HU), Bax (BT Lab, catalog #E1825HU), cleaved PARP (BT Lab, catalog #E6971HU) and 8-hydroxy-desoxyguanosine (8-oxo-dG) (BT Lab, catalog #E1436HU) were used to assess the levels of BCL-2, cleaved caspase 3, BAX, cleaved PARP and 8-oxo-dG in sinapic acid-treated and untreated HT-29 cells. HT-29 cells were cultivated into a 6-well plate and treated with 317.5 μM sinapic acid for 24 hours. HT-29 cells that had been treated with sinapic acid and those that had not were gathered and diluted in PBS. Then they were frozen and thawed three times. Following that, the quantities of BCL-2, cleaved caspase 3, BAX, cleaved PARP and 8-oxo-dG in cell lysates were assessed following the manufacturer's instructions. Bradford protein assay kit (Merck Millipore, Darmstadt, Germany) was used to calculate the total protein quantities in both experimental and control HT-29 cells (28).

Immunofluorescence staining

Cells were fixed with methanol for 5 minutes at -20°C and washed with PBS. They were then incubated with PBS containing 0.1% Triton X-100 for 15 minutes at room temperature. After washing, they were incubated with PBS containing 2% BSA for 60 minutes at room temperature. After rewashing, they were incubated overnight with monoclonal anti-gamma H2AX (Abcam, Catalog no. ab26350) and monoclonal anti-Cytochrome C (Abcam, Catalog no. ab110325) primary antibodies at a dilution ratio of 1/300 at +4ºC. Cells were washed with PBS then incubated with goat anti-mouse FITC secondary antibody at a dilution ratio of 1/50 for 45 minutes at room temperature in the dark. Finally, 4′,6-diamidino-2-phenylindole (DAPI) was applied on the washed cells and examined under fluorescence microscope. During the evaluation, positivity in the cells in the whole field was evaluated semi-quantitatively as follows; absent (-), mild (+), moderate (++) and severe (+++).

Statistical analysis

The results were stated as a mean ± standard error of the mean (SEM). Statistical evaluation of the data was done with SPSS Version 23.0 for Windows using One Way ANOVA and a postdoc Tukey test. The results obtained from BCL-2, cleaved caspase 3, BAX, cleaved PARP and 8-oxo-dG levels tests were examined using Independent Samples t Test. For anti-gamma H2AX and anti-Cytochrome C staining statistical evaluation of the data was performed by One Way ANOVA. Differences were evaluated statistically significant at *: $P < 0.05$, **: $P < 0.01$ and ***: $P < 0.001$. GraphPad Prism 8.0 software (USA) was used for data analysis and graphical presentations.

Results

Cytotoxic effect of sinapic acid on HT-29 cells

The cytotoxic effect of sinapic acid was assessed in HT-29 cells. At 200 μM and higher doses, sinapic acid significantly inhibited the growth of HT-29 cells as compared to control ($P < 0.05$). The IC50 value of sinapic acid in HT-29 cells was found to be 317.5 μM for 24 hours (Figure 2).

The effect of sinapic acid on BCL-2, cleaved caspase 3, BAX, and cleaved PARP levels in HT-29 cells

ELISA was used to evaluate the expression of apoptosis-related proteins in HT-29 cells, such as BCL-2, cleaved caspase 3, BAX, and cleaved PARP. The treatment with sinapic acid (317.5 μM) for 24 hours significantly increased cleaved caspase 3, BAX, and cleaved PARP levels ($p<0.05$). In contrast, sinapic acid had no effect on BCL-2 level ($p>0.05$) (Fig. 3).

Effect of sinapic acid on 8-oxo-dG level in HT-29 cells

ELISA was used to assess 8-oxo-dG expression in HT-29 cells in order to determine the DNA-damaging effects of sinapic acid. Treatment with sinapic acid (317.5 μM) for 24 hours significantly elevated the quantity of 8-oxo-dG ($p<0.01$) (Fig. 4)

Effect of sinapic acid on γ-H2AX and cytochrome-C levels in HT-29 cells

We stained 6 samples from both the experimental and control groups. The differences in gamma-H2AX foci and cytochrome-C quantities were statistically significant ($p <0.05$) (Table 1). It is obviously that the levels of cytochrome-C in microscope field of view 40x are lower and the levels of γ-H2AX foci are higher in the experimental samples compared with the control samples (Figure 5).

Discussion

Despite considerable progress achieved in the areas of cancer diagnosis and treatment, cancer is still one of the leading causes of death worldwide. In 2022, it is estimated that 52,580 of the total 609,360 cancer deaths in the United States will be caused by colorectal cancer, becoming the second cause of cancer death after lung cancer (29). Novel treatments for cancer often fail owing to frequent genetic changes and mutations in cancer genes. Because of the frequent side effects caused by chemotherapy, we still in need to new, more effective and safer treatments for metastatic cancers. There is increasing interest in developing drugs from various natural sources including plants, animals, and microorganisms to overcome these problems (30). Natural products have been in use as traditional medicines to treat different sorts of diseases including cancer all over the world for thousands of years. Several studies have detected a wide spectrum of biological activities of natural products such as, stimulation of the immune system, antimicrobial, anti-hepatotoxic, anti-ulcer, antioxidant, anti-inflammatory, anti-mutagenic, and anti-cancer effects (31). Sinapic acid and its derivatives have been suggested for potential use in food processing, cosmetics, and the pharmaceutical industries due to their antioxidative activity (23). Several studies have demonstrated its anti-cancer activity, Abdel Naser et al. (2020) indicated the cytotoxic effect on Human squamous cell carcinoma cell line (HEp-2) and found that the intracellular reactive oxygen species (ROS) levels were increased in sinapic acid and nano-sinapic acid treated cells as compared to the untreated cells (32). The antiproliferative, apoptotic and anti-invasive effects of SA on PC-3 (Androgen Independent Phenotype) and LNCaP (Androgen Dependent Phenotype) prostate cell lines were determined by Eroğlu M et al. (2018) (33). Hudson EA et al. (2000) demonstrated the inhibiting effect of SA on the clonogenicity of human-derived colon carcinoma cell lines (SW 480) (34). Sinapic acid inhibited the growth of cervical cancer (HeLa) and colon cancer (HT29) cell lines more effectively than the well-known histone deacetylase inhibitor, sodium butyrate. These

antiproliferative effects were mediated by induction of apoptosis (35). The cytotoxic effect of sinapic acid on Human laryngeal carcinoma cell line (HEp-2) was confirmed by Janakiraman K et al. (2015). This effect was the result of promoting apoptosis accompanied by loss of cell viability, increasing ROS levels and cell cycle arrest by the induction of G0/G1 phase arrest (36). Xinglong Hu et al. (2021) determined the anti-cancer activity of SA against lung cancer both in-vitro and in-vivo. The administration of SA was showed to ameliorate the exposure of B[a]P mediated lung cancer in swiss albino mice by a decrease in IgG and IgM level, leukocyte count, neutrophil function tests, soluble immune complex, lipid peroxidation, pro-inflammatory cytokines, tumor markers and increased phagocytic index, activity index and antioxidant defense enzymes. Besides, in-vitro studies exhibited a potential cytotoxic and apoptotic activity by increasing ROS production and caspase activity (caspase-3 and caspase-9) in human lung cancer cell lines (A549) (37). Both free SA and Leucaena leucocephala galactomannan (LLG) conjugated SA were found to have similar antiproliferative activity against human colon cancer cell lines (HCT-116 cells) according to a study conducted by Jasleen Kaur et al. (2021) (38). Antiproliferative and histone deacetylase inhibitory activity of peanut phenolics including ferulic acid, p-Coumaric acid and sinapic acid were assessed in MCF-7 breast cancer and HeLa cervical cancer cells. All the compounds were found to have antiproliferative, apoptotic and histone deacetylase inhibitory effects in both cell lines. Furthermore, all of the compounds led to cell cycle arrest at G0/G1 phase in MCF-7 cells and S-phase arrest in HeLa cells induced by p-Coumaric acid and ferulic acid (39). A study conducted by Huang Z et al (2021) demonstrated that SA inhibited growth, migration, and invasion of pancreatic cancer cells. Both the in vitro (PC cell lines PANC-1 and SW1990) and in vivo (mice injected with SW1990 cells) models indicated the anticancer activities of SA without inducing apoptosis. These results were associated with downregulation of the AKT/Gsk-3β signal pathway

198 which has been shown to play a pivotal role in inhibiting apoptosis and cancer development (40).
199 The anti-cancer activity of free and encapsulated sinapic acid was also determined against lung
200 (A549), and colon (CaCo2) cancer cell lines along with up regulation of P53 and BAX and a down
201 regulation of BCL-2 genes in both cell lines (41).

202 The present study aimed to evaluate the cytotoxic and apoptotic effect of sinapic acid on colon
203 cancer HT-29 cell lines. Our findings showed that sinapic acid had a concentration-dependent
204 cytotoxic effect on HT-29 cells. It significantly inhibited HT-29 cell proliferation in a
205 concentration-dependent manner, with an IC50 value of 317.5 μM after 24 hours.

206 One approach for treating cancer is to obtain control or stop the uncontrolled growth of cancer
207 cells. Recruiting the cell's own mechanism for death is a very effective anti-cancer method. Since
208 apoptosis evasion is a hallmark regardless of the cause or type of the cancer, inducing apoptosis is
209 one of the most successful non-surgical treatments. This induction can be achieved by either
210 stimulation of pro-apoptotic proteins or inhibition of anti-apoptotic proteins (42).

211 The levels of BCL-2, cleaved caspase 3, BAX, and cleaved PARP were measured using ELISA
212 technique to investigate the apoptotic effect of sinapic acid on HT-29 cells. The intrinsic pathway
213 of apoptosis is regulated by the BCL-2 protein family (43). Different apoptotic stimulants lead to
214 the upregulation of BH3-only proteins, which activate both BAX and BAK (44). BAX and BAK
215 then oligomerize and lead to the permeabilization of mitochondrial outer membrane which
216 represents the decisive event of intrinsic pathway (45). The permeabilization results in the release
217 of intermembrane proteins like cytochrome c which contributes with apoptotic protease-activating
218 factor-1 (APAF-1), dATP and procaspase-9 to the formation of apoptosome (46). This leads to the
219 conversion of procaspase-9 into caspase-9 within the apoptosome (43) The caspase-9 in turn
220 activates the executioner (caspase-3 and -7) (47). Executioner caspases cleave target proteins

resulting in cell apoptotic breakdown. Caspase-3 and -7 are responsible for the proteolytic cleavage of poly (ADP-ribose) polymerase-1 (PARP-1), an ADP-ribosylating enzyme essential for initiating various cellular processes, including DNA repair, regulation of chromatin structure, transcription, replication and recombination. The cleavage of PARP-1 thus prevents the recruitment of the enzyme to DNA damage sites (48). In the present study, 317.5 μM sinapic acid treatment significantly elevated pro-apoptotic Bax, cleaved caspase 3, and cleaved PARP protein expressions ($p<0.05$), while not altering anti-apoptotic BCL-2 expression ($p>0.05$). These results confirm the apoptotic effect of sinapic acid on HT-29 cells. Besides, the immunofluorescence staining of cytochrome-c showed that the number of cells with intact mitochondria is significantly lower in the experimental group compared to the control group as they released more cytochrome-c during apoptosis induced by sinapic acid.

In nuclear and mitochondrial DNA, 8-oxo-dG is a prevalent free-radical-induced oxidative lesion. Thus 8-oxo-dG has been commonly used in many studies as a biomarker to measure endogenous oxidative DNA damage, and as a risk factor for many diseases including cancer, because urinary 8-oxo-dG is a good biomarker to estimate the risk of different cancers and other degenerative diseases (49). Increasing the ROS level over the cytotoxic threshold can selectively kill cancer cells. The increased ROS level disrupts the cellular reduction-oxidation (redox) homeostasis and as a result leads to the death of cancer cells. If exogenous ROS-generating agents are triggered, the redox-imbalanced cancer cells become more susceptible than normal cells, thereby resulting in cell death (50). The 8-oxo-dG ELISA kit was used in our study to investigate DNA fragmentation in HT-29 cells after 24 hours of sinapic acid treatment. The results indicate the cytotoxic activity of sinapic acid is associated with oxidative DNA damage. γ-H2AX is a phosphorylated form of the histone, H2AX. This phosphorylation follows the formation of DNA

double strand breaks as a result of DNA damage (51). According to the results of our immunofluorescence staining of γ-H2AX, there was a significant increase in γ-H2AX levels which assures the occurrence of DNA damage as a possible mechanism of the anticancer activity of sinapic acid.

Conclusion

Sinapic acid significantly suppressed HT-29 cell growth in a concentration-dependent way. Sinapic acid treatment significantly elevated pro-apoptotic cleaved caspase 3, BAX, and cleaved PARP levels as well as mitochondrial cytochrome-c release. Sinapic acid treatment also significantly elevated 8- oxo-dG quantities in HT-29 cells and thus the cytotoxic effect of sinapic acid may be linked to oxidative DNA damage. Therefore, the present study suggests that sinapic acid has the potential to be a promising therapeutic agent for colon cancer. However, these findings need to be supported by in vivo and clinical studies.

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References

1. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019;14(2):89-103. doi: 10.5114/pg.2018.81072. Epub 2019 Jan 6. PMID: 31616522; PMCID: PMC6791134.

2. Jamal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA Cancer J Clin 2007;57: 43–66.
3. Hassan C, Zullo A, Laghi A, et al. Colon cancer prevention in Italy: cost-effectiveness analysis with CT colonography and endoscopy. Dig Liver Dis 2007;39:242–50.
4. Shibuya K, Mathers CD, Boschi-Pinto C, et al. Global and regional estimates of cancer mortality and incidence by site: II. Results for the global burden of disease 2000. BMC Cancer 2002;2:37.
5. Endo H, Hosono K, Fujisawa T, et al. Involvement of JNK pathway in the promotion of the early stage of colorectal carcinogenesis under high-fat dietary conditions. Gut 2009; 58(12): 1637–1643
6. Nakaji S, Umeda T, Shimoyama T, et al. Environmental factors affect colon carcinoma and rectal carcinoma in men and women differently. International Journal of Colorectal Disease 2003;18:481–6.
7. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. The Journal of Nutrition 2001;131:3109S–20S.
8. Merritt AJ, Potten CS, Kemp CJ, et al. The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53- deficient mice. Cancer Res 1994;54:614–17.
9. Merritt AJ, Potten CS, Watson AJM, et al. Differential expression of Bcl-2 in intestinal epithelia—correlation with attenuation of apoptosis in colonic crypts and the incidence of colonic neoplasia. J Cell Sci 1995;108(Pt 6):2261–71.

10. Pritchard DM, Potten CS, Korsmeyer SJ, et al. Damageinduced apotosis in intestinal epithelia from bcl-2-null and bax-null mice: Investigations of the mechanistic determinants of epithelial apoptosis in vivo. Oncogene 1999;18:7287–93.
11. Pritchard DM, Print C, O'Reilly L, et al. Bcl-w is an important determinant of damage-induced apoptosis in epithelia of small and large intestine. Oncogene 2000;19(34):3955–9.
12. Johnson IT. Anticarcinogenic effects of diet-related apoptosis in the colorectal mucosa. Food Chem Toxicol 2002;40:1171–8.
13. Bartsch H, Nair J and Owen RW. Exocyclic DNA adducts as oxidative stress markers in colon carcinogenesis: potential role of lipid peroxidation, dietary fat and antioxidants. Biol Chem 2002; 383(6): 915–921.
14. Martínez-Maqueda, D; et al. (2015). "HT29 Cell Line". The Impact of Food Bioactives on Health. Springer. pp. 113–124. doi:10.1007/978-3-319-16104-4_11. ISBN 978-3-319-15791 7. PMID 29787047.
15. Coudray, Anne-Marie; et al. (1 December 1989). "Proliferation of the Human Colon Carcinoma Cell Line HT29: Autocrine Growth and Deregulated Expression of the c-myc Oncogene" (PDF). Cancer Research. 49 (23): 6566–6571. PMID 2684395.
16. Mishra J, Drummond J, Quazi SH, Karanki SS, Shaw JJ, Chen B, Kumar N. Prospective of colon cancer treatments and scope for combinatorial approach to enhanced cancer cell apoptosis. Crit Rev Oncol Hematol. 2013 Jun;86(3):232-50. doi: 10.1016/j.critrevonc.2012.09.014. Epub 2012 Oct 23. PMID: 23098684; PMCID: PMC3561496.

17. Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 4:177.

18. S.A. Heleno, A. Martins, M.J.R.P. Queiroz, I.C.F.R. Ferreira, Food Chem. 173 (2015) 501–513.

19. K.L. Tuck, P.J. Hayball, J. Nutr. Biochem. 13 (2002) 636–644.

20. H.R. El-Seedi, A.M. El-Said, S.A. Khalifa, U. Goransson, L. Bohlin, A.K. Borg-Karlson, R. Verpoorte, Biosynthesis, natural sources, dietary intake, pharmacokinetic properties, and biological activities of hydroxycinnamic acids, J. Agric. Food Chem. 60 (2012) 10877–10895.

21. Hosny H, El Gohary N, Saad E, Handoussa H, El Nashar RM (2018) Isolation of sinapic acid from broccoli using molecularly imprinted polymers. J Sep Sci 41(5):1164–1172.

22. Menezes JC, Kamat SP, Cavaleiro JA, Gaspar A, Garrido J, Borges F (2011) Synthesis and antioxidant activity of long chain alkyl hydroxycinnamates. Eur J Med Chem 46(2):773–777.

23. Nićiforović N, Abramovič H, Sinapic acid and its derivatives: Natural sources and bioactivity, Compr Rev Food Sci Food Safety 13(1), 34–51, 2014. http://dx.doi.org/10.1111/1541-4337.12041.

24. Lee-Manion AM, Price RK, Strain JJ, Dimberg LH, Sunnerheim K, Welch RW (2009) In vitro antioxidant activity and antigenotoxic effects of avenanthramides and related compounds. J Agric Food Chem 57(22):10619–106224.

25. Cos P, Rajan P, Vedernikova I, Calomme M, Pieters L, Vlietinck AJ, Augustyns K, Haemers A, Vanden Berghe D (2002) In vitro antioxidant profle of phenolic acid derivatives. Free Radic Res 36(6):711–716.

26. Cartea M.E., Francisco M., Soengas P., Velasco P., Phenolic compounds in Brassica vegetables, Molecules, 2010, 16(1), 251-280.

27. Chen C. Sinapic acid and ıts derivatives as medicine in oxidative stress-ınduced diseases and aging, Oxıd Med Cell Longev, 2016, 2016, 3571614.

28. Ergul M, Bakar-Ates F. Investigation of molecular mechanisms underlying the antiproliferative effects of colchicine against PC3 prostate cancer cells. Toxicol Vitr 2021; 73. https://doi.org/10.1016/j.tiv.2021.105138.

29. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022 Jan;72(1):7-33. doi: 10.3322/caac.21708. Epub 2022 Jan 12. PMID: 35020204.

30. Thazin Nwe Aung, Zhipeng Qu, R. Daniel Kortschak and David L. Adelson. Understanding the Effectiveness of Natural Compound Mixtures in Cancer through Their Molecular Mode of Action; Int. J. Mol. Sci. 2017; 18 (3): 656. Doi: 10.3390/ijms18030656.

31. Rajamanickam S, Agarwal R. Natural products and colon cancer: current status and future prospects. Drug Dev Res. 2008 Nov 1;69(7):460-471. doi: 10.1002/ddr.20276. PMID: 19884979; PMCID: PMC2659299.

32. Abdel Naser, Gehan & Elazab, Samia & Elbolok, Amr & Elgayar, Sherif & Mohamed, Aly. (2020). Antioxidant and Apoptotic Activity of Free and Nano-Sinapic Acid on HEp-2 Cell Line. Indian Journal of Public Health Research and Development. 11. 953-958. 10.37506/ijphrd.v11i4.7790.

33. Eroğlu C, Avcı E, Vural H, Kurar E. Anticancer mechanism of Sinapic acid in PC-3 and LNCaP human prostate cancer cell lines. Gene. 2018 Sep 10;671:127-134. doi: 10.1016/j.gene.2018.05.049. Epub 2018 May 22. PMID: 29792952.

34. Hudson EA, Dinh PA, Kokubun T, Simmonds MS, Gescher A. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiol Biomarkers Prev. 2000 Nov;9(11):1163-70. PMID: 11097223.

35. Senawong T, Misuna S, Khaopha S, Nuchadomrong S, Sawatsitang P, Phaosiri C, Surapaitoon A, Sripa B. Histone deacetylase (HDAC) inhibitory and antiproliferative activities of phenolic-rich extracts derived from the rhizome of Hydnophytum formicarum Jack.: sinapinic acid acts as HDAC inhibitor. BMC Complement Altern Med. 2013 Sep 22;13:232. doi: 10.1186/1472-6882-13-232. PMID: 24053181; PMCID: PMC3848914.

36. Janakiraman, K., Kathiresan, S., & Mariadoss, A.V. (2015). INFLUENCE OF SINAPIC ACID ON INDUCTION OF APOPTOSIS IN HUMAN LARYNGEAL CARCINOMA CELL LINE.

37. Xinglong Hu, Royapuram Veeraragavan Geetha, Krishna Mohan Surapaneni, Vishnu Priya Veeraraghavan, Arunachalam Chinnathambi, Tahani Awad Alahmadi, Velu Manikandan, Kalaivani Manokaran. Lung cancer induced by Benzo(A)Pyrene: ChemoProtective effect of sinapic acid in swiss albino mice, Saudi Journal of Biological Sciences,Volume 28, Issue 12,2021, 7125-7133,ISSN 1319-562X. https://doi.org/10.1016/j.sjbs.2021.08.001

38. Jasleen Kaur, Vikrant Mehta, Gurpreet Kaur, Preparation, development and characterization of Leucaena leucocephala galactomannan (LLG) conjugated sinapic acid: A potential colon targeted prodrug, International Journal of Biological Macromolecules, Volume 178, 2021, 29-40, ISSN 0141-8130, https://doi.org/10.1016/j.ijbiomac.2021.02.132.

39. Saenglee, S.; Jogloy, S.; Patanothai, A.; Leid, M.; Senawong, T. Cytotoxic effects of peanut phenolics possessing histone deacetylase inhibitory activity in breast and cervical cancer cell lines. Pharmacol. Rep. 2016, 68, 1102–1110.

40. Huang Z, Chen H, Tan P, Huang M, Shi H, Sun B, Cheng Y, Li T, Mou Z, Li Q, Fu W. Sinapic acid inhibits pancreatic cancer proliferation, migration, and invasion via downregulation of the AKT/Gsk-3β signal pathway. Drug Dev Res. 2022 May;83(3):721-734. doi: 10.1002/ddr.21904. Epub 2021 Dec 3. PMID: 34859906.

41. Badr, D.A., Amer, M.E., Abd-Elhay, W.M. et al. Histopathological and genetic changes proved the anti-cancer potential of free and nano-capsulated sinapic acid. Appl Biol Chem 62, 59 (2019). https://doi.org/10.1186/s13765-019-0462-0.

42. Pfeffer CM, Singh ATK. Apoptosis: A Target for Anticancer Therapy. Int J Mol Sci. 2018 Feb 2;19(2):448. doi: 10.3390/ijms19020448. PMID: 29393886; PMCID: PMC5855670.

43. Zaman S., Wang R., Gandhi V. Targeting the apoptosis pathway in hematologic malignancies. Leuk. Lymphoma. 2014;55:1980–1992. doi: 10.3109/10428194.2013.855307.

44. Lomonosova E., Chinnadurai G. BH3-only proteins in apoptosis and beyond: An overview. Oncogene. 2008;27:S2–S19. doi: 10.1038/onc.2009.39.

45. Lopez J., Tait S.W.G. Mitochondrial apoptosis: Killing cancer using the enemy within. Br. J. Cancer. 2015;112:957–962. doi: 10.1038/bjc.2015.85.

46. Hassan M., Watari H., AbuAlmaaty A., Ohba Y., Sakuragi N. Apoptosis and molecular targeting therapy in cancer. BioMed Res. Int. 2014;2014 doi: 10.1155/2014/150845.

47. Green D.R., Llambi F. Cell death signaling. Cold Spring Harb. Perspect. Biol. 2015;7:a006080. doi: 10.1101/cshperspect.a006080.

48. Los M, Mozoluk M, Ferrari D, Stepczynska A, Stroh C, Renz A, Herceg Z, Wang ZQ, Schulze-Osthoff K. Activation and caspase-mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. Mol Biol Cell. 2002 Mar;13(3):978-88. doi: 10.1091/mbc.01-05-0272. PMID: 11907276; PMCID: PMC99613.

49. Ock CY, Kim EH, Choi DJ, Lee HJ, Hahm KB, Chung MH. 8-Hydroxydeoxyguanosine: not mere biomarker for oxidative stress, but remedy for oxidative stress-implicated gastrointestinal diseases. World J Gastroenterol. 2012 Jan 28;18(4):302-8. doi: 10.3748/wjg.v18.i4.302. PMID: 22294836; PMCID: PMC3261525.

50. Kim SJ, Kim HS, Seo YR. Understanding of ROS-Inducing Strategy in Anticancer Therapy. Oxid Med Cell Longev. 2019 Dec 18;2019:5381692. doi: 10.1155/2019/5381692. PMID: 31929855; PMCID: PMC6939418.

51. Kuo LJ, Yang LX. Gamma-H2AX - a novel biomarker for DNA double-strand breaks. In Vivo. 2008 May-Jun;22(3):305-9. PMID: 18610740.

Table 1

Samples	Gamma-H2AX	Cytochrome-C
Control sample-1	+	+++
Control sample-2	++	+++
Control sample-3	+	++
Control sample-4	+	+++
Control sample-5	+	++
Control sample-6	+	+++
Experimental sample-1	+++	+
Experimental sample-2	++	+
Experimental sample-3	+++	+
Experimental sample-4	+++	+
Experimental sample-5	+++	-
Experimental sample-6	+++	+
Mean values ± standard deviation		
Control group	1.16±0.40	2.66±0.51
Experimental group	2.83±0.40	0.83±0.40

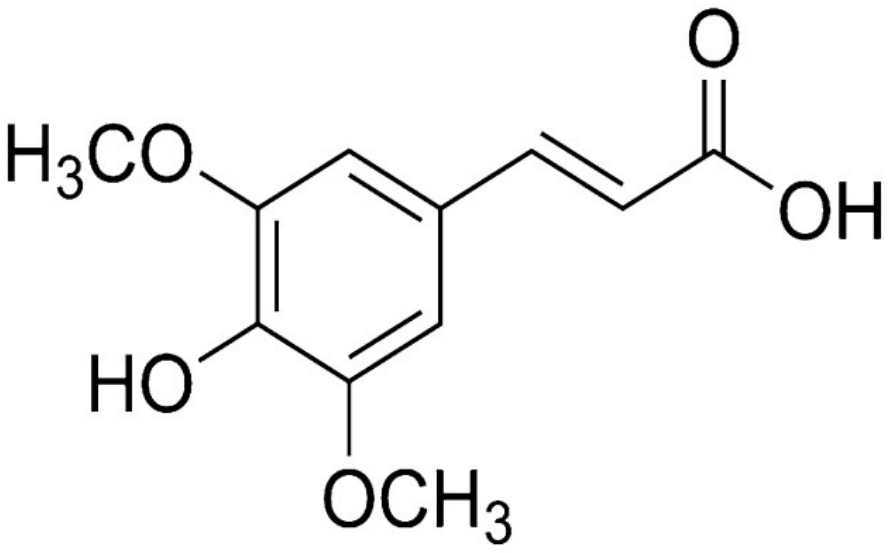
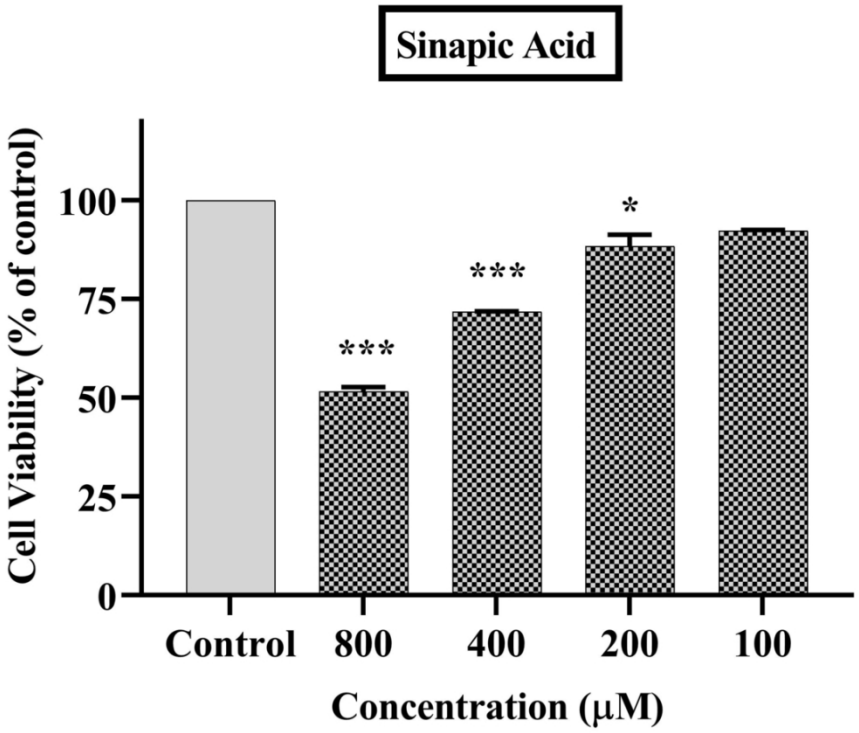


Fig. 1 Structure of Sinapic acid

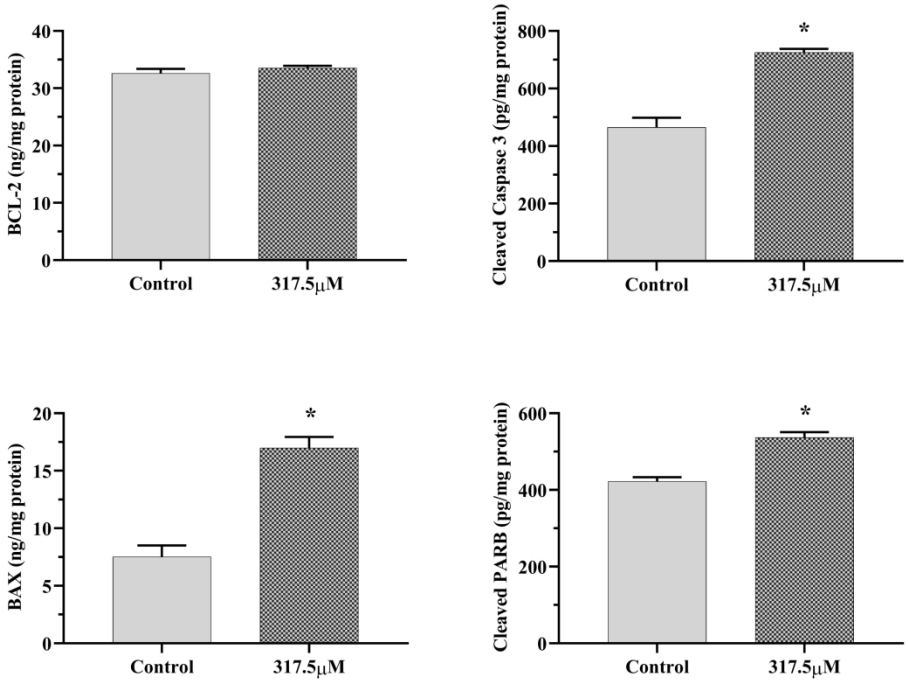
Structure of Sinapic Acid

187x136mm (96 x 96 DPI)



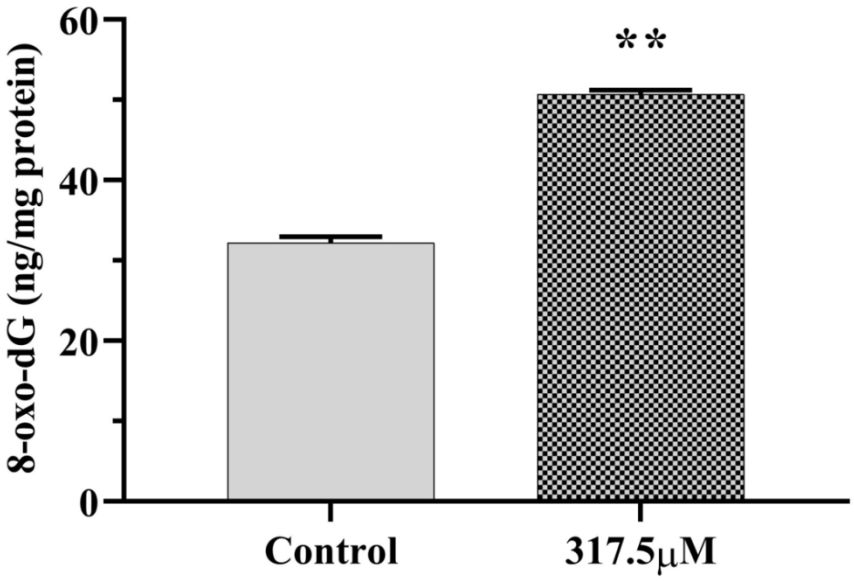
The antiproliferative effects of sinapic acid on HT-29 cells. The findings are evaluated as a percentage of viable cells versus control. The results are presented as the mean ± SEM of three samples. *: P < 0.05 and ***: P < 0.001as compared to the control group.

107x83mm (300 x 300 DPI)



Sinapic acid (317.5 μM) enhanced apoptosis of HT-29 cells. The BCL-2, cleaved caspase 3, BAX and cleaved PARP levels were assessed using the ELISA kits. Results are represented as mean ± SEM of three samples. *: P < 0.05 as compared to the control group

261x190mm (300 x 300 DPI)



Sinapic acid (317.5 μM) enhanced DNA damage of HT-29 cells. The 8-oxo-dG level was assessed using the ELISA kit. Results are represented as mean ± SEM of three samples. **: P < 0.01 as compared to the control group

105x68mm (300 x 300 DPI)

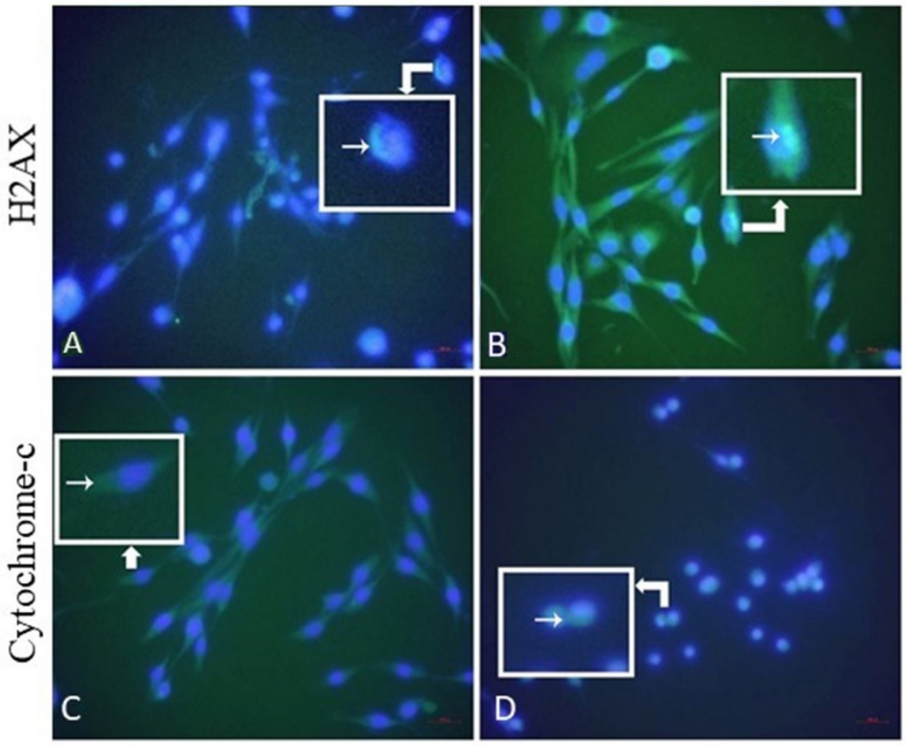


Fig 5: Gamma-H2AX expression was significantly higher in the experimental group B compared to the control group A ($P < 0.05$), while cytochrome-C expression was significantly lower in the experimental group D compared to the control group C ($P < 0.05$) x40.

515x413mm (96 x 96 DPI)