Original Article

Apoptotic and Antiproliferative Effects of *Mimusops elengi* Leaf Extract in Ehrlich Ascites Carcinoma Cells

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Abstract

Context: *Mimusops elengi* Linn. (Sapotaceae) is commonly known as bakul. Traditionally, the various parts of the plants have been used in the treatment of wound healing, pain, and tumor. **Objective:** To evaluate the role of *Mimusops elengi* extract (MEE) on proliferation, apoptosis, and bcl2 gene expression in Ehrlich ascites carcinoma (EAC) cells lines and establish the possible mechanisms linked with anticancer activity. **Settings and Design:** EAC cells were treated with methanol MEE (20–400 µg/mL) in time intervals of 24, 48, and 72 h. **Materials and Methods:** The antiproliferative effect of extract was evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay; deoxyribonucleic acid (DNA) fragmentation study, cell cycle analysis, and Annexin V-fluorescein isothiocyanate (FITC) assay were performed to assess the apoptosis, and lastly, western blotting study was performed to assess the bands intensities using the ImageJ® analysis (a Java-based image processing program system, National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, Madison, WI). **Statistical Analysis Used:** The data were analyzed using one-way analysis of variance followed by the Dunnett's *post hoc* test. **Results:** MEE shows significant antiproliferative effect on EAC cell lines. In the DNA fragmentation assay, it shows significant fragmentation of DNA. In the cell cycle analysis and Annexin V-FITC assay, there was arrested in sub-G1 phase and initiation of cell apoptosis. In western blotting study, the extract shows low expression of bcl-2 and overexpression of Bax proteins. **Conclusions:** From the above result, it concludes that MEE has significant apoptosis-inducing properties.

Keywords: *Annexin V-FITC, apoptosis, cytotoxicity, Ehrlich ascites carcinoma, Mimusops elengi, western blotting*

Key Messages: *Natural products have significant properties in the management of cancer.*

Introduction

Medicinal plants have always been an essential source of remedies for many diseases and have received extensive consideration in the modern era due to their various pharmacological properties including prevention of proliferation of tumor cell, which is associated with cell cycle arrest, and initiation of apoptosis has been shown to be the common anticancer therapy.[1] It was found that activation of the cellular apoptosis is the common strategy for the treatment of cancer as it decreases severe adverse effects.[2] Currently used anticancer therapy like use of radiation and chemotherapeutic agents decimate majority of tumor cells through initiating the process of apoptosis; however, most of the cancer shows resistant to these therapies.[3] Owing to this context, plant derivatives' potential and safe anticancer agent with effective proapoptotic characteristics would be valuable.

The traditional large evergreen plant *Mimusops elengi* Linn., which belongs to the family Sapotaceae, is located in different parts of India as well in abroad such as Burma, Thailand, and Northern Australia.[4] The various parts of plants such as leaf, bark, flower, seeds, and fruits are used in the treatment of curing piles, headache, constipation, tumor, low sperm count, and mental disorders.[5,6] The bark of *M. elengi* has been attributed to many pharmacological properties including cytotoxic, protective to cardiac tissue, stomachic, and astringent.[6] Previous reports found that the leaf of this plant exhibited significant antioxidant properties,[7,8] and antiulcer,[9] antiurolithiatic,[7] anti-inflammatory,[10] and anticariogenic effects.[11] Similarly, the report also found that the extract of *M. elengi* has revealed both *in vivo* and *in vitro* antitumor properties of many cancer cell lines.[12] However, the possible mechanism of action of antitumor effect of *M. elengi* is still unclear and needs further investigation.

Phytochemical investigation of extracts of *M. elengi* displays different chemical

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constituents such as alkaloids, triterpenoids, glycosides of steroidal types, and flavonoids.[13] In addition, the bark of this plant shows constituents like different types of sterols, for example, taraxerol, V-spinosterol, alkaloid isoretronecyltiglate, and a mixture of triterpenoid saponins, whereas in the leaves, it contains hentriacontane, lupeol, and carotene.[14]

Subjects and Methods

Collection of plant material

The leaves of *Mimusops elengi* were collected and the plant was authenticated by the help of taxonomist. The leaves were shaded dried, ground to coarse powder and kept in air tight container for further use.

Chemicals

During this experiment, we have used chemicals and reagents such as trichloroacetic acid, trypan blue, 5-fluorouracil, and ethidium bromide (EB), which were procured from Merck Ltd (Vikhroli [East] Mumbai, India). Fetal calf serum was procured from Thermo Trace Ltd.; similarly, thiobarbituric acid and reduced glutathione were procured from Loba Chemie and SISCO, respectively; acridine orange (AO) was obtained from SRL lab.

Preparation of extract

The powdered material (150 g) underwent successive maceration extraction process using methanol solvent with random shaking, and then, the product was filtered and finally evaporated with the help of a rotary evaporator; the yield was 18% w/v. Then, the dried extract was kept in a freezer for the experimental purpose.[15]

Animal

Albino mice with average body weight 20 g of either sex were kept in polyacrylic cages, and all the Institute Animal Ethics Committee (IAEC) protocols were followed before the commencement of experiment.

Tumor cells

Initially, cell lines were cultured in mice model. When the cell lines reach the log phase, the ascitic fluid in the count of 2×10^6 cells per mouse form EAC cell lines was drawn, and used for our experiment.[15]

In vitro cytotoxicity study

In this study, EAC cells with count 1×10^6 cells/mL were added with phosphate-buffered saline (PBS; 0.2 M, pH 7.4). Then, various concentrations of the *Mimusops elengi* extract (MEE; 20, 50, 100, 200, 400 µg/mL) of 100 µL were mixed with cell lines. In the next step, final volume of 1 mL was maintained by adding PBS, and then incubation was carried out for 3 h at 37°C. The cell viability was measured by adding 0.4% of trypan blue in normal saline as per the standard method.[16]

Antiproliferative activity

The antiproliferative activity of MEE was performed using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye. The basic principle of this method is reduction of dye (MTT) into a blue color formazan with the help of mitochondrial dehydrogenase enzymes, suggesting mitochondrial function and cell viability.[17] In all, 100 µL of EAC cells with count 1×10^6 cells/mL was added in a 96-well plate; then, we added different concentrations of MEE such as 20, 50, 100, 200, and 400 µg/mL to the plates followed by incubation of 48 h at 37°C with 5% CO_2 atmosphere. Then, 10 µL of MTT (5 mg/mL) was added into the respective well and again incubated for 4 h followed by the addition of 100 µL of dimethyl sulfoxide (DMSO) to the solution for dissolving the formazan crystals, and absorbance was measured at 570 nm using DMSO as a negative control.

Deoxyribonucleic acid fragmentation assay

We have followed standard protocol for extraction of deoxyribonucleic acid (DNA) from both MEE-treated and control EAC cells. In this method, EAC cells were washed with PBS at 4°C, lysed with 10% sodium dodecyl sulfate (SDS), followed by the addition of potassium acetate (8 M) to the supernatant solution and incubation for 1 h at 4°C, and then, gyration was performed at 7000 rpm. Then, we have added a mixture of solvents, namely distilled phenol, chloroform, and isoamyl alcohol (25:24:1), followed by gyration at 3000 rpm for half an hour; we have repeated this process twice. In the next step, we have added RNase (20 µL/mL) to the pellet at 37°C and kept for 30 min, followed by the addition of chilled ethanol of 2 volumes, which was again kept overnight at 20°C. The solution was gyrated at 10,000 rpm for the period of 1 h at 4°C, and the formed pellet was subjected with Tris-ethylenediamine tetraacetic acid (EDTA) buffer. Finally, DNA (~2 µg) was isolated by SDS-PAGE using Tris-borate-EDTA buffer, followed by the addition of 0.5 µg/mL EB. DNA ladder was pictured under UV light.[18]

Morphological study

The objective of the morphological study was to check the cell morphology using AO and EB dye. According to Abosharaf *et al*.,[19] EAC cells with count 1×10^6 cells/mL were collected from MEE-treated and control groups and then washed properly with cold PBS solution, followed by the addition of equal proportions of AO and EB to the solution. The morphology of apoptotic cell was detected using a Leitz Diaplan florescent microscope and photographed.

Cell cycle analysis

For the determination of cell cycle phase distribution, EAC (1×10^6 cells in each case) was harvested from tumor-bearing mice after 9 days of treatment with MEE (200 and 300 mg/kg body wt.). According to Devegowda *et al*.,[20] bo th the cells of the test and control were washed twice with cold PBS. Then, cells were added with a staining agent called propidium iodide (PI; 20 µg/mL) and kept for 2 h, followed by flow cytometry analysis for measurement of cell population at G0/G1, S, and G2/M phases. Finally, the percentage of different cell distribution was determined in each phase.

Annexin V-fluorescein isothiocyanate staining

As per the cited protocol of Chirathaworn *et al*.,[21] EAC cells (1×10^6cells/mL) collected from MEE treated mice, washed

with cold PBS. After collection of cells, its added with 100 µL of binding buffer followed by the addition of 5 µL of Annexin V-FITC (Santa Cruz Biotechnology, USA) and PI in a dark environment and incubated at 37°C for 15 min. Flow cytometry was used for the examination of cells after addition of staining binding buffer (400 µL).

Western blot study

The EAC cells (1×10^6) were collected in 1X PBS and 300 mL of 1X lysis buffer was added and vortex; and followed by addition of specific amounts of protease-inhibitor cocktail (Sigma Aldrich, USA). Then, 50 µg of protein sample was boiled at 100°C for 10 min with β-mercaptoethanol, followed by cooling using ice and then separation process using 8% SDS-PAGE. After separation, the proteins present in the gels were shifted into a nitrocellulose membrane. Blocking was performed using 5% bovine serum albumin (BSA; GENEI). Western blotting was performed with the help of nitrocellulose membrane, which incubated for overnight period with rabbit anti bcl2, anti bax, and anti β-actin antibody (1:1000; Cell Signaling). In the next step, again we repeated this process using alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (1:1000 dilution) with Tris-buffered saline (TBS) buffer–5% BSA. After then the bands of alkaline phosphatase-positive were imagined in a color evolving substrate solution known as 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium (GENEI) in the standard condition like 1.5 mM Tris-HCl, pH 8.8, and water for 10 min at room temperature. The intensities of western blotting bands were checked using the ImageJ® analysis (a Java-based image processing program system, National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, Madison, WI).

Statistical analysis

Results of cytotoxicity study were presented as mean±standard error of mean (SEM). Further, the data were analyzed by one-way analysis of variance, and the Dunnett's *post hoc* test was used for *P* value calculation.

Results

Cytotoxicity study

MEE exhibited concentration-dependent cytotoxicity effect on the EAC cell line, and the half maximal inhibitory concentration (IC_{50}) value was found to be 18.61 ± 2.63 µg/mL. According to the National Cancer Institute (NCI), cytotoxicity value with IC_{50} less than 20 µg/mL of crude extract is considered to be an active anticancer agent.[22] Thus, the extract of ME could be served as a harbinger of a potential natural cytotoxic lead compound, which has immense importance for future anticancer drug discovery.

Antiproliferative activity

Treatment of EAC cells with different concentrations of MEE for a prolonged period of time leads to cell death, which suggests possible antiproliferative activity of extract.

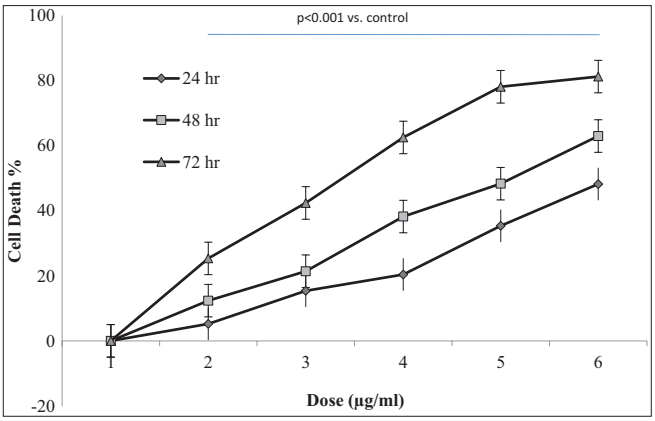

Figure 1: Effect of *Mimusops elengi* extract on the viability of Ehrlich ascites carcinoma cells. Cells were exposed to various concentrations of *M. elengi* extract at various doses for 24, 48, and 72 h, and cell viability assays were performed using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data represent mean ± standard deviation of three independent experiments with similar results. One- way analysis of variance (ANOVA), followed by Dunnett's test

The antiproliferative activity was performed using MTT reagent. The experimental result found that after 24 h of MEE administration, there was reduction of 50% of cell viability at the dose concentration of 100 µg/mL, which revealed cytotoxicity activity of MEE [Figure 1] and suggested that MEE showed potential inhibitory effect on the proliferation of EAC cell in both dose- and time-dependent manner.

Deoxyribonucleic acid fragmentation assay

A previous experimental report cited that the ladder pattern of DNA during gel electrophoresis indicates apoptosis pathway of natural compound.[23] In fact, DNA fragmentation along with chromatin condensation leads to DNA cleavage, which takes place due to stimulation of Ca^{2+} and Mg^{2+} endonucleases enzymes that helps in cutting DNA in a selective manner and producing multiple fragments.[24] During this agarose gel electrophoresis study, we have found that the MEE-treated group exhibited DNA ladder in EAC cell lines ([Figure 2 lane b and lane c], which suggests that MEE might have apoptotic-inducing properties.

Morphological study

Morphological analysis of apoptotic cells of MEE-treated EAC cell was visualized under a fluorescent microscope using AO/EB fluorescent strain; the result found was that MEE-treated cells exhibited disparate apoptotic morphological changes such as formation of apoptotic bodies, membrane blebbing, and chromatin condensation; these are indeed the characteristic features of apoptosis [Figure 3].

Cell cycle analysis

During cell cycle analysis, it was found that MEE-treated groups (200 and 300 mg/kg) show alteration in the percentage of cells at G0/G1, S, and G2/M phases when compared with the control group. MEE at the dose of 300 mg/kg body weight showed increase in the percentage of cells in the sub-G1

Figure 2: Deoxyribonucleic acid agarose gel electrophoresis of Ehrlich ascites carcinoma tumor cells. Deoxyribonucleic acid was extracted from Ehrlich ascites carcinoma tumors. Genomic deoxyribonucleic acid (~2 µg) was loaded and subjected to electrophoresis at 50 V. Induction of apoptosis (ladder formation) can be readily seen in *Mimusops elengi* extract-treated group. Lane A (Ehrlich ascites carcinoma control), lane B (*M. elengi* extract 200 mg/kg), and lane C (*M. elengi* extract 300 mg/kg)

phase; however, there was a decrease in the percentage of cells in the G0/G1 phase. There was no significant changes in the percentage of cells in the S phase and G2/M phase when compared with controls, suggesting that MEE has the ability to induce apoptosis in EAC cells.

Annexin V-fluorescein isothiocyanate staining

The reports found that flow cytometry was generally used to assess cell distribution when cancer cells were applied to a potential apoptotic agent or natural compound.[25] Owing to this, the apoptotic potential of MEE was evaluated by flow cytometry using Annexin V-FITC and PI strain. The viable, apoptotic, late apoptotic, and necrosis cell populations are indicated in the Q3, Q4, Q2, and Q1 quadrants of the density plot, respectively. After EAC cell lines were treated with MEE at 200 and 300 mg/kg for 48 h, both treated and untreated cells were stained with Annexin V-FITC and PI. The result shows that there was a reduction in the percentage of viable cell population in the Q3 quadrant in a dose-dependent manner from 92.1 to 21.3% and increase in the percentage of apoptotic cells in the Q4 quadrant from 7.9 to 32.1%, whereas the percentage of late apoptotic cell population increased to 46.2% in the Q2 quadrant in comparison to that in the untreated or control group. The result revealed that MEE has significant apoptotic effect in EAC cells [Figure 4], which suggests that plant extracts might have apoptosis-inducing properties.

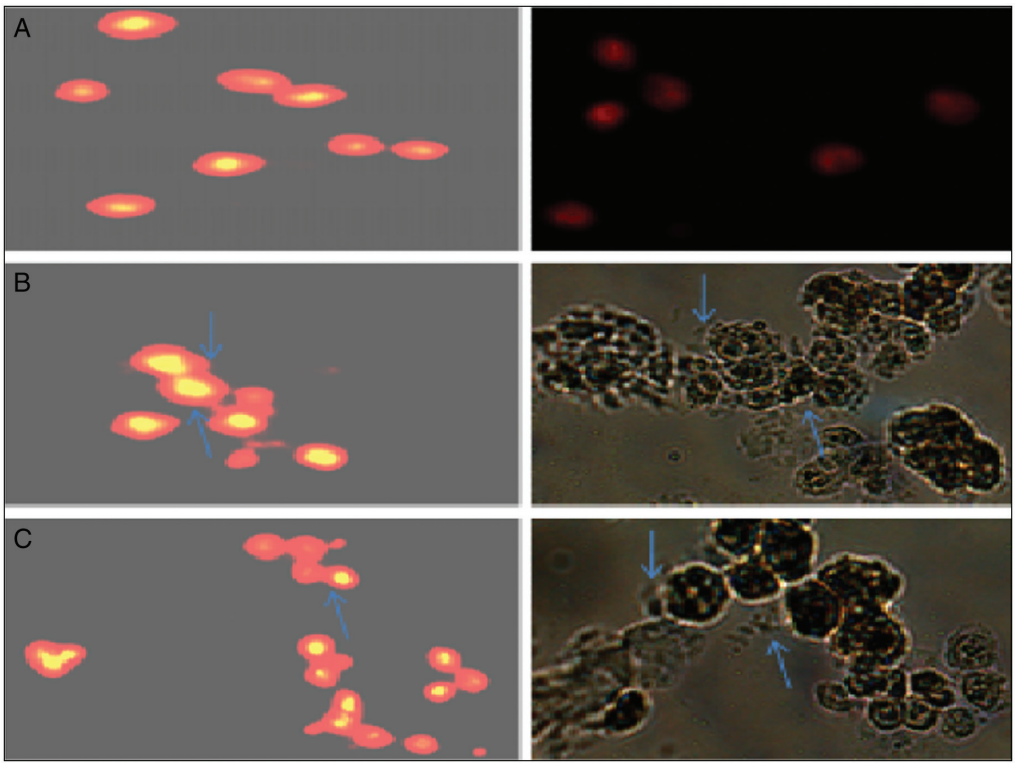

Figure 3: Effect of *Mimusops elengi* extract on Ehrlich ascites carcinoma cells showing characteristic apoptotic morphology when stained with ethidium bromide and acridine orange stain. (a) Control, (b) *M. elengi* extract 200 mg/kg treated, and (c) *M. elengi* extract 300 mg/kg treated. Arrows indicate membrane blebbing, apoptotic bodies, and condensed chromatin

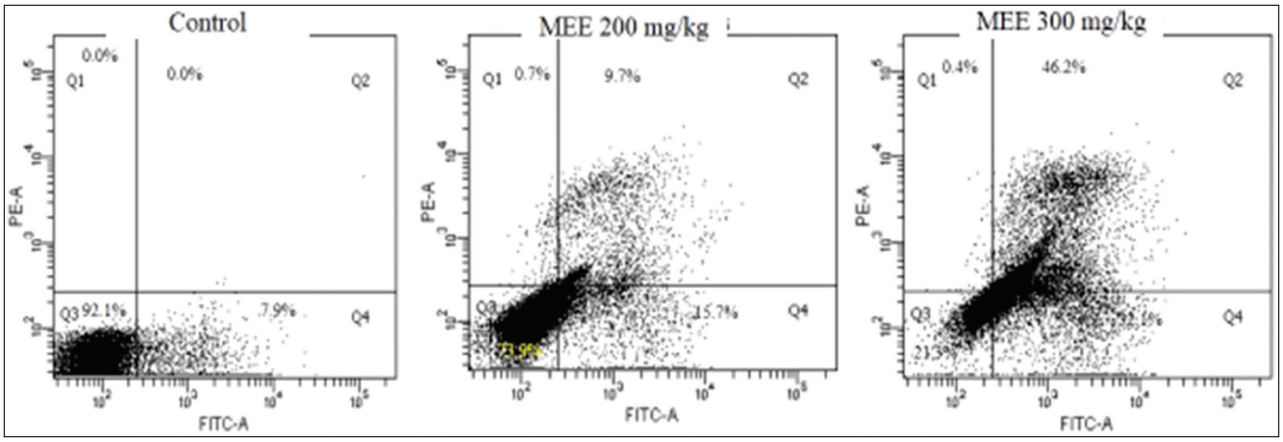

Figure 4: Apoptosis induction effect of *Mimusops elengi* extract on Ehrlich ascites carcinoma cell determined by Annexin V-fluorescein isothiocyanate/ propidium iodide flow cytometry

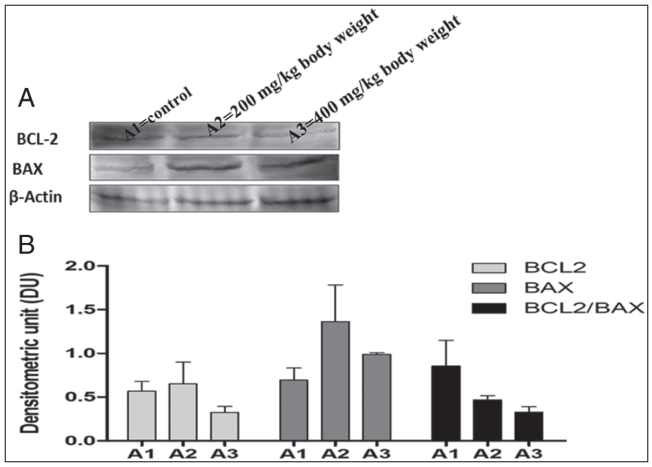

Figure 5: Effect of *Mimusops elengi* extract on Bcl-2, Bax, and Bcl 2/Bax in Ehrlich ascites carcinoma cells

Western blotting analysis

The Bcl-2 family members, for example Bcl-2 (antiapoptotic) and Bax (proapoptotic), are the key mediator for induction and implementation of apoptosis in various tumor cells.[26] A report cited that balance of proapoptotic and antiapoptotic is known to be vital for deciding whether cells will die or survive.[27] In the western blotting study, we have checked the expression of Bax and Bcl-2 genes in MEE induced in EAC cells; the result found was that upregulation of proapoptotic Bax gene and antiapoptotic Bcl-2 gene became downregulated in a dose-dependent manner as shown in Figure 5, which indicates molecular mechanism of MEE induces apoptosis.

Discussion

Apoptosis or programmed cell death is a vital pathway for proper development of cell and cancer prevention. The most important characteristic feature of apoptosis is morphological and biochemical changes during cell death in response to a cytotoxic agent.[26] Recent studies showed that development of tumor growth and neoplastic transformation were due to the program are due deregulation of apoptosis pathway i.e., imbalance of Bcl-2 family proteins.[28] The most important parameter is overexpression of protein, namely Bax, and downregulation of Bcl-2 proteins indicates the initiation of apoptosis by the plant extract by regulating apoptosis protein. Initiation of apoptosis in EAC tumor cells has been shown to be the most common anticancer model conjoint by various cancer treatments. Hence, exploring possible anticancer agents with apoptotic potential could be helpful for the future antitumor candidate. A previous report cited the anticancer role of natural compound by inducing the apoptotic pathway.[23] In a nutshell, the claim by traditional Indian therapists that MEE has significant anticancer activities has been partly corroborated by our previous experiments using the EAC cell line.[12]

Conclusion

From this study, it can be concluded that previously reported anticancer activity of the aforementioned plant extract was due to its marked apoptotic activity. This can be partially proved in this multiple experiment, which varies from antiproliferative assay to western blotting study. Hence, from the above discussion, we have concluded that methanolic leaf extract of *M. elengi* could be a promising phytotherapeutic candidate for the effective treatment of cancer. However, proper importance and further scientific investigation are required to develop the drug for use in clinical trials.

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Authors' contribution

Conceptualization: Biswakanth Kar and Pallab Kanti Haldar; literature survey: Biswakanth Kar, Pallab Kanti Haldar, Goutam Rath, and Goutam Ghosh; and data analysis, interpretation, and manuscript draft: Biswakanth Kar, Pallab Kanti Haldar, Goutam Ghosh, and Goutam Rath.

Ethical approval

The present experiment was performed after getting Institutional Animal Ethical Committee approval (AEC/PHARM/1503/04/2015), Jadavpur University.

Consent for publication

I, the undersigned, give my consent for the publication of identifiable details within the text ("Materials and Methods") to be published in the above journal and article.

Data availability

Data are available on request from the authors.

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There is no source of funding for this research work.

Conflicts of interest

The authors declare that they have no competing interests.

References

1. Ekbatan SS, Li XQ, Ghorbani M, Azadi B, Kubow S. Chlorogenic acid and its microbial metabolites exert anti-proliferative effects, S-phase cell-cycle arrest and apoptosis in human colon cancer caco-2 cells. Int J Mol Sci 2018;19:723.
2. Zhang Z, Liu Y, Wang K, Zhu K, Zheng X, Wang L, *et al*. Activation of type 4 metabotropic glutamate receptor promotes cell apoptosis and inhibits proliferation in bladder cancer. J Cell Physiol 2019;234:2741-55.
3. Zhang Z, Zheng X, Luan Y, Liu Y, Li X, Liu C, *et al*. Activity of metabotropic glutamate receptor 4 suppresses proliferation and promotes apoptosis with inhibition of Gli-1 in human glioblastoma cells. Front Neurosci 2018;12:1-16.
4. Kar B, Kumar RBS, Karmakar I, Dola N, Bala A, Mazumder UK, *et al*. Antioxidant and *in vitro* anti-inflammatory activities of *Mimusops elengi* leaves. Asian Pac J Trop Biomed 2012;2:S976-80.
5. Singh V, Pandey VN. Total phenolic content and free radical scavenging activity of *Mimusops elengi* L. leaves. Asian J Pharm Clin Res 2019;12:297-9.
6. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Allahabad, India: Lalit Mohan Basu; 1988. p. 1494-6.
7. Ashok P, Koti BC, Vishwanathswamy AH. Antiurolithiatic and antioxidant activity of *Mimusops elengi* on ethylene glycol-induced urolithiasis in rats. Indian J Pharmacol 2010;42:380-3.
8. Kar B, Nepal A, Kumar RBS, Dolai N, Bhattacharya S, Mazumder UK, *et al*. Antioxidant and anti-inflammatory properties *Hymenodictyon excelsum* bark. Orient Pharm Exp Med 2013;13:103-11.
9. Shah PJ, Gandhi MS, Shah MB, Goswami SS, Santani D. Study of *Mimusops elengi* bark in experimental gastric ulcers. J Ethnopharmacol 2003;89:305-11.
10. Rani S, Rahman K. Molsari (*Mimusops elengi* Linn.): A boon drug of traditional medicine. Int J Pharm Sci Res 2017;8:17-28.
11. Gami B, Pathak S, Parabia M. Ethnobotanical, phytochemical and pharmacological review of *Mimusops elengi* Linn. Asian Pac J Trop Biomed 2012;2:743-8.
12. Kar B, Kumar RB, Bala A, Dolai N, Mazumder UK, Haldar PK. Evaluation of antitumor activity of *Mimusops elengi* leaves on Ehrlich's ascites carcinoma-treated mice. J Diet Suppl 2012;9:166-77.
13. Sahu NP, Mandal NB, Banerjee S, Siddiqui KAI. Chemistry and biology of the triterpenes and saponins from seeds of *Mimusops elengi*. J Herbs Spices Med Plants 2001;8:29-37.
14. Khare CP. Indian Medicinal Plants. Vol. 8. New York: Springer Science, Business Media, LLC; 2007. p. 416-7.
15. Haldar PK, Bhattacharya S, Bala A, Kar B, Mazumder UK, Dewanjee S. Chemopreventive role of *Indigofera aspalathoides* against 20-methylcholanthrene-induced carcinogenesis in mouse. Toxicol Environ Chem 2010;92:1749-63.
16. Gangopadhyay A, Chakraborty M, Haldar PK, Chaulya NC, Ghosh A. Evaluation of anticancer activity of ethanolic extract of cyperus kyllingia endl. in Ehrlich ascites carcinoma-induced Swiss albino mice. Asian J Pharm Clin Res 2018;11:482-5.
17. Fernández T, Cerdá Zolezzi P, Aulicino P, Caldas Lopes E, Wagner M, Ricco R, *et al*. Immunobiological features of the galactoside lectin L-Lc isolated from the Argentine mistletoe Ligaria cuneifolia. J Ethnopharmacol 2003;85:81-92.
18. Patra S, Muthuraman MS. Gracilaria edulis extract induces apoptosis and inhibits tumor in Ehrlich ascites tumor cells *in vivo*. BMC Complement Altern Med 2013;13:331.
19. Abosharaf HA, Salah M, Diab T, Tsubaki M, Mohamed TM. Biogenic silver nanoparticles induce apoptosis in Ehrlich ascites carcinoma. Biomed Res Ther 2020;7:4100-13.
20. Devegowda PS, Srinivas Balaji K, Shivaramu Prasanna D, Ramesh Swaroop T. Pro-apoptotic activity of novel 4-anilinoquinazoline derivatives mediated by up-regulation of bax and activation of poly(ADP-ribose) phosphatase in Ehrlich ascites carcinoma cells. Med Chem (Los Angeles) 2016;11:570-8.
21. Chirathaworn C, Kongcharoensuntorn W, Dechdoungchan T, Lowanitchapat A, Sa-nguanmoo P, Poovorawan Y. *Myristica fragrans* Houtt. Methanolic extract induces apoptosis in a human leukemia cell line through SIRT1 mRNA downregulation. J Med Assoc Thai 2007;90:2422-8.
22. Mutee AF, Salizawati MS, Ghazali FC, Al Hassan FM, Lim CP, Ibrahim K, *et al*.Apoptosis induced in human breast cancer cell line by *Acanthaster planci* starfish extract compared to tamoxifen. African J Pharm Pharmacol 2012;6:129-34.
23. Kim C, Kim B. Anti-cancer natural products and their bioactive compounds inducing ER stress-mediated apoptosis: A review. Nutrients 2018;10:1021.
24. Wyllie AH, Kerr JF, Currie AR. Cell death: The significance of apoptosis. Int Rev Cytol 1980;68:251-306.
25. Vermeulen K, Strnad M, Havlícek L, Van Onckelen H, Lenjou M, Nijs G, *et al*. Plant cytokinin analogues with inhibitory activity on cyclin-dependent kinases exert their antiproliferative effect through induction of apoptosis initiated by the mitochondrial pathway: Determination by a multiparametric flow cytometric analysis. Exp Hematol 2002;30:1107-14.
26. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: The balancing act of Bcl-2 family proteins. Nat Rev Mol Cell Biol 2019;20:175-93.
27. Onyeagucha B, Subbarayalu P, Abdelfattah N, Rajamanickam S, Timilsina S, Guzman R, *et al*. Novel post-transcriptional and post-translational regulation of pro-apoptotic protein BOK and anti-apoptotic protein Mcl-1 determine the fate of breast cancer cells to survive or die. Oncotarget 2017;8:85984-96.
28. Adams JM, Cory S. The BCL-2 arbiters of apoptosis and their growing role as cancer targets. Cell Death Differ 2018;25:27-36.