RESEARCH ARTICLE

Elephantopus scaber Linn. Leaf Extract Sensitizes Doxorubicin in Inducing Apoptosis in HSC-3 Tongue Cancer Cells through Inhibiting Survivin Activity at Thr34

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Abstract

BACKGROUND: Previous research has demonstrated the effect of *Elephantopus scaber* Linn. leaf extract (ESLE) on various cancer cell lines. However, research on the effects of ESLE on oral squamous cell carcinoma (OSCC), especially tongue cancer, is still lacking. Moreover, the apoptotic mechanisms induced by ESLE are not well understood and require further exploration. Therefore, this study was conducted to investigate the effects of ESLE on cell viability and apoptosis in human squamous cell carcinoma (HSC)-3 tongue cancer cells.

METHODS: HSC-3 cells were treated with varying concentrations of ESLE, doxorubicin, and a combination of both. Cell viability and apoptosis were assessed using MTT and Sub-G1 assays. The expression levels of survivin and its phosphorylated form at threonine (Thr)34 were evaluated using Western blot analysis.

RESULTS: ESLE exhibited a concentration-dependent cytotoxic effect on HSC-3 cells in decreasing cell viability (Kruskal Wallis, p=0.001) and increasing apoptotic cells (ANOVA, p=0.001) significantly. When combined with doxorubicin, ESLE further enhanced the induction of apoptosis compared with doxorubicin alone. The combined treatment resulted in a decrease in the levels of phosphorylated survivin (p-Surv) Thr34, indicating the inhibition of survivin's anti-apoptotic function.

CONCLUSION: ESLE significantly enhances the efficacy of doxorubicin, thereby sensitizing its ability to induce apoptosis in HSC-3 tongue cancer cells. This sensitization occurs through the inhibition of survivin activity, particularly at the Thr34 phosphorylation site. These findings suggest that ESLE could serve as a potential adjuvant to improve the effectiveness of doxorubicin in inducing apoptosis in tongue cancer cells.

KEYWORDS: *Elephantopus scaber*, doxorubicin, tongue cancer, HSC-3 cells, apoptosis, Survivin, Thr34 phosphorylation

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Introduction

The field of natural product research is growing rapidly, especially in the search for effective anticancer agents from plants.(1) Natural products are particularly well-suited for this purpose due to their minimal side effects, ability to target multiple cancer processes, and potential for synergistic effects.(2) Their complex chemical diversity and capacity make them valuable candidates for drug discovery

and development.(3,4) Among many studied plants, *Elephantopus scaber* Linn., which is known as Elephant's Foot and belongs to the Asteraceae family, stands out due to its traditional use in folk medicine and its promising results in modern research.(5)

Previous research has shown that some parts of *E. scaber*, such as the leaves and the roots, possess pharmacological activities due to their rich chemical composition.(6) This plant has gained attention for its potential therapeutic benefits. *E. scaber* contains a range of bioactive metabolites, including flavonoids, triterpenoids and sesquiterpene lactones.(7) The flavonoids in the plant are well-known for their strong antioxidant and anti-inflammatory properties.(8) Triterpenoids further enhance the plant's therapeutic value with their diverse effects, including anti-inflammatory and antimicrobial activities.(9) Additionally, sesquiterpene lactones, due to their complex structures, are noted for their potent biological effects, such as inducing cell death and inhibiting cell growth.(10)

A regulated process of cell death, apoptosis, plays a vital role in removing cancerous cells and inhibiting tumor progression.(11,12) This process can be triggered by either the mitochondrial (intrinsic) pathway, which is mediated by caspase-9, or the death-receptor (extrinsic) pathway, which is mediated by caspase-8. Ultimately, both pathways converge to activate the effector caspases-3 and -7, which execute the cell death program.(13) One of the critical regulators of apoptosis is Survivin, a key member of the inhibitor of apoptosis (IAP) protein family.(14) Survivin is essential in regulating apoptosis by inhibiting caspase activity and promoting cell survival.(15) The phosphorylated variant of Survivin, known as p-Survivin (p-Surv) threonine 34 (Thr34), further modulates this function by altering its interactions with apoptotic machinery.(16) Phosphorylation at Thr34 affects Survivin's stability and its ability to bind to second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO), a mitochondrial protein that promotes apoptosis by antagonizing IAPs.(17)

Previous studies have reported the effects of *E. scaber* leaf extract (ESLE) on breast cancer cell lines (18,19) and colorectal cancer cell lines (20). However, research on the effects of ESLE on oral squamous cell carcinoma (OSCC), especially tongue cancer, is lacking. Furthermore, the apoptotic mechanisms triggered by ESLE are not well understood and require further investigation. Consequently, this study aims to examine the effects of ESLE on cell viability and apoptosis in human squamous cell carcinoma (HSC)-3 tongue cancer cells.

Methods

Preparation of ESLE

The leaves of *E. scaber* L. were acquired from Indonesian Institute for Testing Instrument Standard for Spices, Medicinal, and Aromatic Plant, Ministry of Agriculture. The ESLE was obtained using maceration technique. *E. scaber* leaves were finely minced and dried. The dehydrated material was extracted with 70% ethanol solution, followed by filtration and evaporation using rotary evaporator. The crude ESLE obtained was thereafter kept at a temperature of 4°C.

HSC-3 Cell Culture

The HSC-3 cell culture was conducted using a previously reported method (21), with specific modifications. The HSC-3 cell line was acquired from Sigma-Aldrich (St. Louis, MO, USA). HSC-3 cells were cultured in Dulbecco's modified eagle's medium (DMEM) (Sigma-Aldrich) complete medium contained 50 U/mL penicillin 50 µg/mL, streptomycin (Sigma-Aldrich) and 10% fetal bovine serum (FBS) (PAN-Biotech, Aidenbach, Germany). The cells were cultured in a humidified incubator at 37°C, 5% CO_2. The HSC-3 cells were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) solution (Sigma-Aldrich) once they reached 80% confluence.

Cell Viability Assay

The measurement of viable cells was conducted with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, following a previously reported method.(13) In 96-well plates, HSC-3 cells were placed (5×10^3/well) and treated with/without 1, 10, or 100 µg/mL ESLE or 1 µM Doxorubicin (Dankos Farma, Jakarta, Indonesia) for 24 hours. MTT was added in the treated well (100 µL/well) and incubated for 4 hours. Then, the suspension in each well was removed and dissolved in 100 µL dimethylsulfoxide (DMSO). The formazan crystal that was formed was measured using a microplate reader (Bio-Rad, Hercules, CA, USA) at OD570. The measurements for each experimental group were conducted in sextuplicate.

Sub-G1 Assay

Apoptotic HSC-3 cells were measured using the sub-G1 assay in order to evaluate the cytotoxic effects of ESLE, based on previously reported method.(13) Treated-HSC-3 cells were collected and incubated in a hypotonic fluorochrome solution (50 µg/mL of propidium iodide (Sigma-Aldrich),

0.1% Triton X-100 (Sigma-Aldrich), and 0.1% sodium citrate (Wako, Osaka, Japan)). Subsequently, the cell suspensions were incubated in darkness for 30 minutes. The fluorescence of individual nuclei was quantified using a FACSCanto II flowcytometer (Becton Dickinson, Franklin Lakes, NJ, USA), and a total of 400 events were recorded.

Western Blotting Assay

HSC-3 cells that were treated with/without various concentrations of ESLE or 25 nM YM155 (Tocris, Bristol, UK) were then harvested and incubated with radioimmunoprecipitation assay (RIPA) lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA). YM155, a survivin suppressant, was used as a positive control in this study to demonstrate its ability to reduce or inhibit p-Surv. Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) sheet. After blocking with 5% skim milk in Tris-buffered saline (TBS, 150 nM NaCl and 50 nM Tris-HCl, pH 7.4), the sheets were incubated with rabbit polyclonal anti-β-Actin (Cat. No. 4967; Cell Signaling, Danvers, MA, USA) and rabbit polyclonal anti-phospho-survivin (Thr34) (Cat. No. 8888; Cell Signaling) antibody. The secondary antibody was horseradish peroxidase-conjugated donkey anti-rabbit (Cell Signaling) IgG antibody, diluted 1:1000. The bound antibodies were visualized using Clarity Western ECL (Bio-Rad) and captured using Alliance 4.7 (UVItech, Cambridge, UK).

Statistical Analysis

The Shapiro-Wilk normality test was utilized for statistical analysis. Then, one-way ANOVA test was used to analyze the findings of a normally distributed dataset, followed by Tukey's post-hoc test. Subsequently, the results of abnormal data distribution were tested using Kruskal-Wallis test, followed by Mann-Whitney's post-hoc test.

Results

ESLE Decreased HSC-3 Viable Cells

The results in Figure 1 showed that the number of HSC-3 viable cells in 1 µM doxorubicin group (56±11.12) was significantly lower (Mann-Whitney's post-hoc test, p=0.004) than the ones in the sham group (9,212±65.58). The number of HSC-3 viable cells in ESLE-treated groups decreased significantly (Kruskal Wallis, p=0.001) in concentration-dependent manner. The number of HSC-3 viable cells in 1 µg/mL ESLE-treated group (9,268±424.76) did not significantly differ (Mann-Whitney's post-hoc test, p=0.423) than the ones in the sham group, meanwhile the number of HSC-3 viable cells in 10 µg/mL ESLE-treated group (8,173±316.61) and 100 µg/mL ESLE-treated group (6,952±602.94) differed significantly (Mann-Whitney's post-hoc test, p=0.004) than the ones in the sham group. In this MTT assay, IC_{50} concentration of ESLE in inducing apoptosis of HSC-3 cells was 222.34 µg/mL.

ESLE Increased HSC-3 Apoptotic Cells

The results in Figure 2 showed that the percentage of HSC-3 apoptotic cells in 1 µM doxorubicin group (95.73±0.48%) was significantly higher (Tukey's post-hoc test, p=0.001) than the ones in the sham group (4.62±0.48%). The percentage of HSC-3 apoptotic cells in ESLE-treated groups increased significantly (ANOVA, p=0.001) in concentration-dependent manner. The percentage of HSC-3 apoptotic cells in 1 µg/mL ESLE-treated group (6.08±0.34%) did not significantly differ (Tukey's post-hoc test, p=0.120) than the ones in the sham group, meanwhile percentage of HSC-3 apoptotic cells in 10 µg/mL ESLE-treated group (18.88±0.75%) and 100 µg/mL ESLE-treated group (33.45±2.09%) differed significantly (Tukey's post-hoc test, p=0.001) than the ones in the sham group.

Combination of 100 µg/mL ESLE with 0.25 µM Doxorubicin Increased HSC-3 Apoptotic Cells

The results in Figure 3 showed that the percentage of HSC-3 apoptotic cells in 1 µM doxorubicin group (95.71±0.47%) was significantly higher (Mann-Whitney's post-hoc test, p=0.004) than the ones in the 100 µg/mL ESLE + 0.25 µM

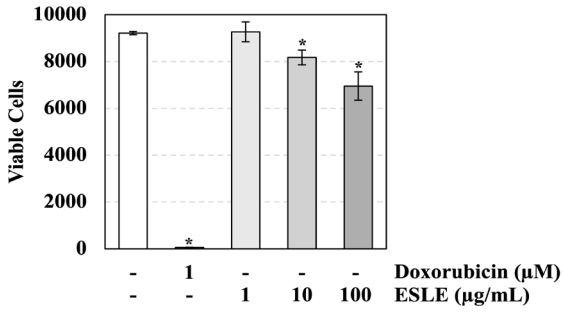

Figure 1. ESLE decreased HSC-3 viable cells in concentration-dependent manner. HSC-3 cells were starved for 12 h and subsequently treated with/without 1 µM Doxorubicin or ESLE in different concentrations for 24 h. Viable cells were measured using MTT assay as outlined in methods. The results are presented as mean±standard deviation (n=6). *Statistical significance (p<0.05) was determined using Mann-Whitney's post-hoc test when compared to the sham group.

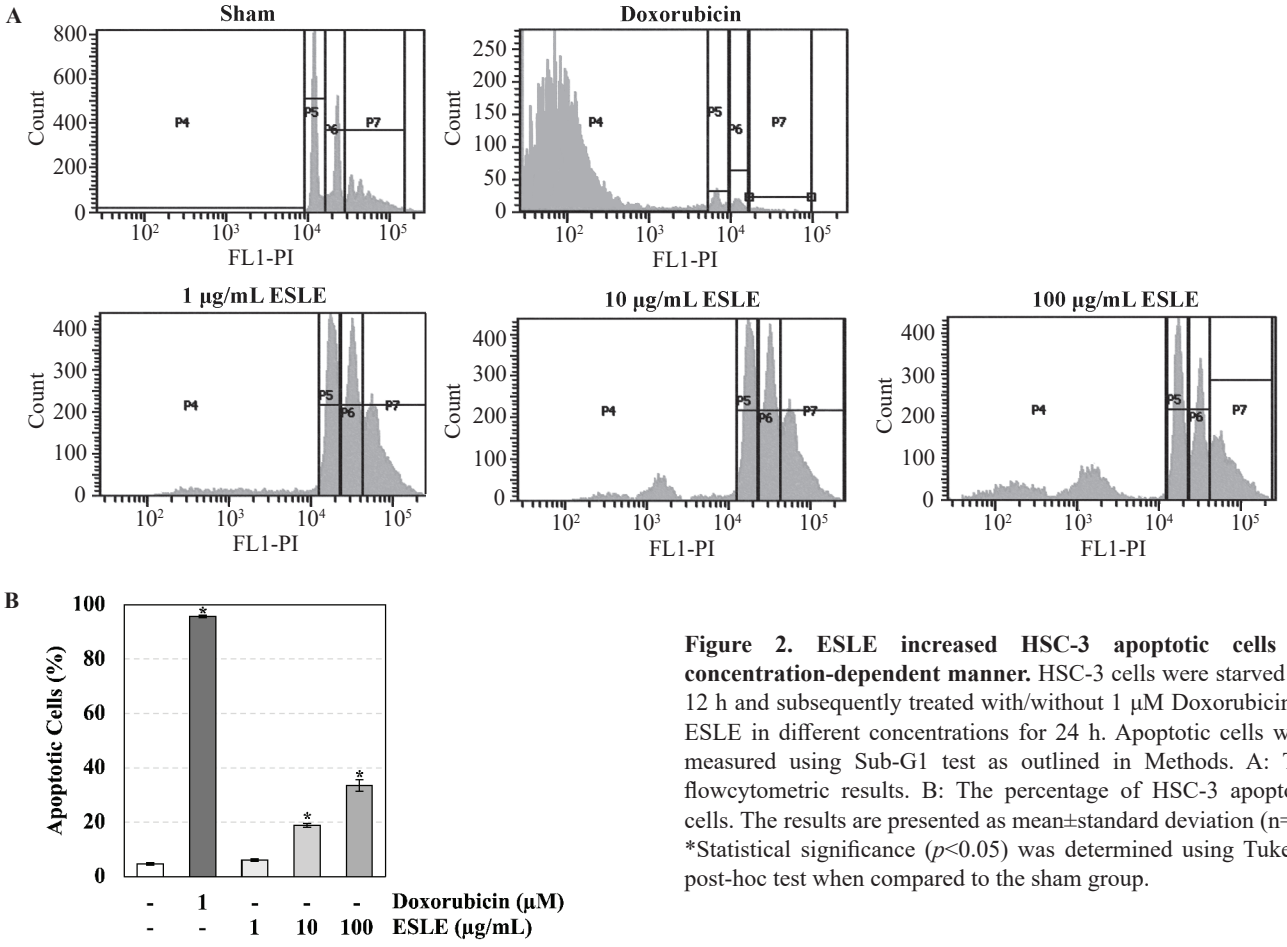

Figure 2. ESLE increased HSC-3 apoptotic cells in concentration-dependent manner. HSC-3 cells were starved for 12 h and subsequently treated with/without 1 µM Doxorubicin or ESLE in different concentrations for 24 h. Apoptotic cells were measured using Sub-G1 test as outlined in Methods. A: The flowcytometric results. B: The percentage of HSC-3 apoptotic cells. The results are presented as mean±standard deviation (n=6). *Statistical significance (p<0.05) was determined using Tukey's post-hoc test when compared to the sham group.

doxorubicin-treated group (65.00±10.05%). The percentage of HSC-3 apoptotic cells in the 0.25 µM Doxorubicin group (22.98±5.98%) and the 100 µg/mL ESLE-treated group (33.45±2.09%) were low. However, the percentage of HSC-3 apoptotic cells in the 100 µg/mL ESLE + 0.25 µM doxorubicin-treated group was significantly higher (Mann-Whitney's post-hoc test, p=0.004) compared to the 0.25 µM Doxorubicin group and the 100 µg/mL ESLE-treated group.

ESLE Decreased p-Surv (Thr34) of HSC-3 Cells

The results in Figure 4 showed that the p-Surv (Thr34) amount of HSC-3 cells in YM155 group (28±7) was significantly lower (Tukey's post-hoc test, p=0.000) than the ones in the sham group (933±49.14) (Figure 5). The β-Actin was used as a loading control. The p-Surv (Thr34) amount in ESLE-treated group decreased significantly (ANOVA, p=0.000) in concentration-dependent manner. The p-Surv (Thr34) amount in the 1 µg/mL ESLE-treated group (659±125.74), 10 µg/mL ESLE-treated group (408±36.47) and 100 µg/mL ESLE-treated group (179±62.4) differed significantly (Tukey's post-hoc test, p=0.000) than the ones in the sham group.

Discussion

In the current study, ESLE exhibited a concentration-dependent cytotoxic effect on HSC-3 cells. Results from the MTT (Figure 1) and sub-G1 assays (Figure 2) showed a reduction in viable HSC-3 cells, which was attributed to apoptosis induction. These findings align with previous studies that reported the ability of ESLE to induce apoptosis in T47D (breast cancer), MCF-7 (breast cancer), and HCT116 (colorectal cancer) cell lines.(18,19) IC_{50} of ESLE in inducing apoptosis of HSC-3 cells (222.34 µg/mL) was categorized as weak cytotoxicity (201-500 µg/mL).(22) This IC_{50} value was higher than those in inducing apoptosis of T47D cells (132.17±9.69 µg/mL) (18), MCF-7 cells (14.69±0.29 µg/mL) (19) and HCT116 cells (14.69±0.29 µg/mL) (20). However, although having weak cytotoxicity, ESLE could enhance the potency of doxorubicin in inducing apoptosis of HSC-3 cells. Specifically, the combination of 100 µg/mL ESLE with 0.25 µM doxorubicin increased the percentage of HSC-3 apoptotic cells more than treatment with either agent alone (Figure 3). These results suggest a

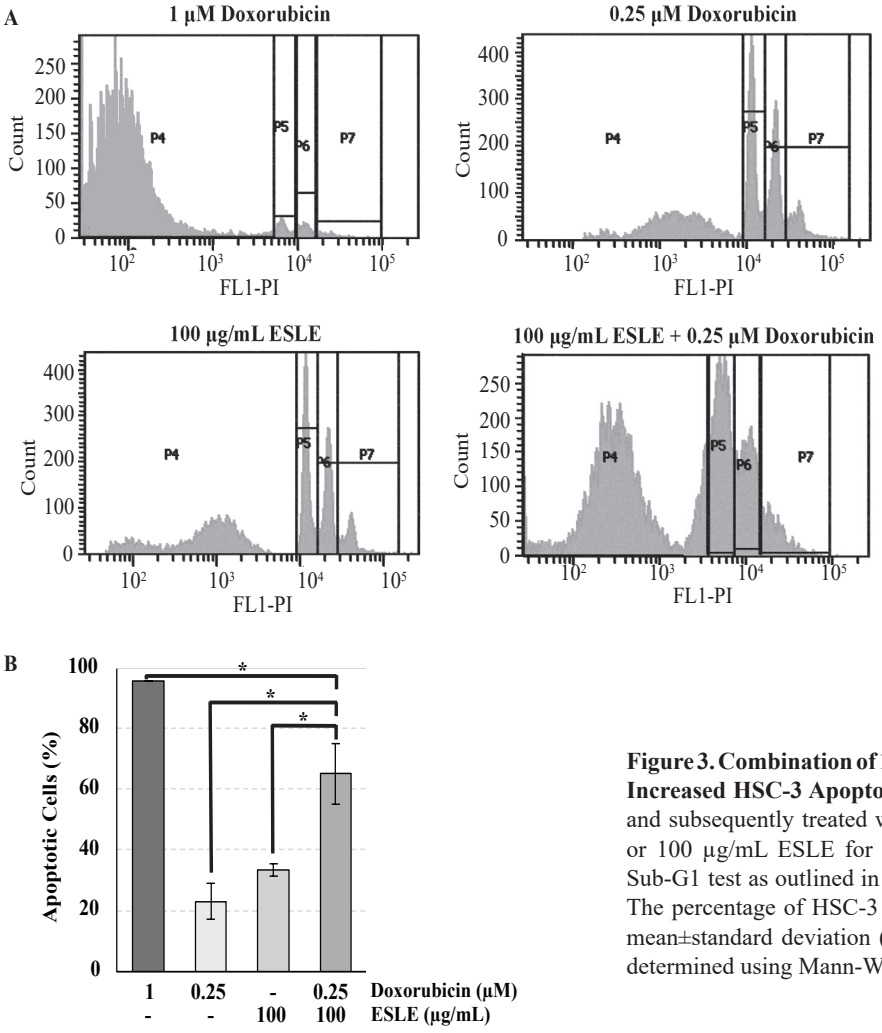

Figure 3. Combination of 100 µg/mL ESLE with 0.25 µM Doxorubicin Increased HSC-3 Apoptotic Cells. HSC-3 cells were starved for 12 h and subsequently treated with/without 1 µM or 0.25 µM Doxorubicin, or 100 µg/mL ESLE for 24 h. Apoptotic cells were measured using Sub-G1 test as outlined in Methods. A: The flow cytometric results. B: The percentage of HSC-3 apoptotic cells. The results are presented as mean±standard deviation (n=6). *Statistical significance (p<0.05) was determined using Mann-Whitney's post-hoc test.

synergistic effect of combining 100 µg/mL ESLE and 0.25 µM doxorubicin in inducing apoptosis in HSC-3 cells. This finding indicates that ESLE can sensitize the apoptotic effect of doxorubicin, potentially allowing for lower doses of doxorubicin to be used in cancer therapy, thereby reducing its associated side effects.

The observed synergy between ESLE and doxorubicin is likely due to several interacting mechanisms. This combination seems to enhance doxorubicin-induced DNA damage and inhibit survival pathways in cancer cells, making them more susceptible to doxorubicin. This result aligns with previous studies that examined the effects of ESLE in combination with tamoxifen in MCF-7 breast cancer cells.(23) These studies demonstrated that ESLE enhances the effectiveness of tamoxifen in targeting breast cancer cells. The combination of ESLE and tamoxifen led to cell cycle arrest at the S phase, downregulation of pro-survival genes heat shock protein (HSP)-105, upregulation of the pro-apoptotic genes, implicating both intrinsic and extrinsic apoptotic pathways.(23)

The present study showed that the ESLE-treated group exhibited a decrease in the p-Surv (Thr34) levels in HSC-3 cells (Figure 4) in a concentration-dependent manner. This reduction in p-Surv (Thr34) is comparable to the effect of YM155, a known survivin suppressant. YM155 has been shown to inhibit survivin expression and induce apoptosis in various cancer cell lines.(24,25) In this study, YM155 was used as a positive control to validate the effect of ESLE on survivin phosphorylation. The significant reduction in p-survivin levels in the ESLE-treated cells, similar to that observed with YM155, underscores the potential of ESLE as an effective anti-cancer agent targeting survivin. This result aligns with previous studies that showed ESLE decreased survivin expression at the transcript level.(26) Survivin, which is phosphorylated at Thr34 by the cyclin-dependent kinase (CDK)1 during the G2/M phase of the cell cycle, is crucial for its anti-apoptotic function. A reduction in p-survivin levels could disrupt the function, thereby promoting apoptosis in cancer cells.(27).

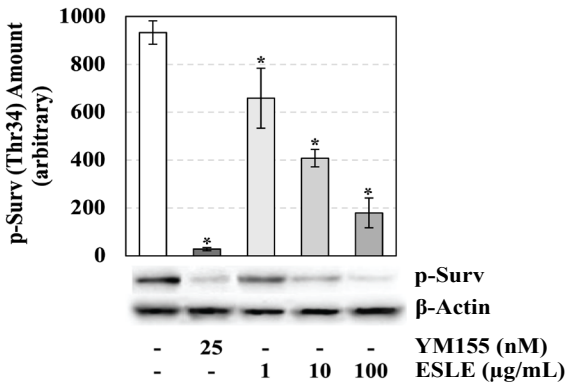

Figure 4. ESLE decreased the amount of p-Surv (Thr34) in HSC-3 cells in concentration-dependent manner. HSC-3 cells were treated with/without 25 nM YM155 or ESLE in various concentrations. The amount of p-Surv (Thr34) was measured using Western Blot, as outlined in Methods. *Statistical significance (p<0.05) was determined using Tukey's post-hoc test when compared to the sham group.

Further studies are needed to fully elucidate the mechanism of action of ESLE. Specifically, future research should examine other potential phosphorylation sites on survivin, such as Ser 70 and Ser81, may be crucial to its anti-apoptotic function. Moreover, exploring the effects of ESLE on other signaling pathways involved in cancer cell survival and proliferation could provide a more comprehensive understanding of its therapeutic potential.

Conclusion

Taken together, ESLE significantly enhances the efficacy of doxorubicin, thereby sensitizing its ability to induce apoptosis in HSC-3 tongue cancer cells. This sensitization occurs through the inhibition of survivin activity, particularly at the Thr34 phosphorylation site. These findings suggest that ESLE could serve as a potential adjuvant to improve the effectiveness of doxorubicin in inducing apoptosis in tongue cancer cells.

Authors Contribution

FS and TP were involved in conceptualizing and planning the research, performing data acquisition and collection, as well as conducting data analysis. FS and AES drafted the manuscript and designed the figures. FS, RAH, DR, and KHL aided in interpreting the results and provided critical discussion. FS, RAH, DR, TP, AES, and KHL participated in the critical revision of the manuscript.

References

1. Naeem A, Hu P, Yang M, Zhang J, Liu Y, Zhu W, Zheng Q. Natural products as anticancer agents: Current status and future perspectives. Molecules. 2022; 27(23): 8367. doi: 10.3390/molecules27238367.
2. Castañeda AM, Meléndez CM, Uribe D, Pedroza-Díaz J. Synergistic effects of natural compounds and conventional chemotherapeutic agents: recent insights for the development of cancer treatment strategies. Heliyon. 2022; 8(6): e09519. doi: 10.1016/j.heliyon.2022.e09519.
3. Eziefule OM, Arozal W, Wanandi SI, Louisa M, Wuyung PE, Dewi S, *et al.* Andrographis paniculata ethanolic extract improved doxorubicin-induced cardiac inflammation, alterations in liver function parameters and anemia. Mol Cell Biomed Sci. 2024; 8(2): 117-26.
4. Callixte C, Baptiste NJ, Arwati H. Phytochemical screening and antimicrobial activities of methanolic and aqueous leaf extracts of Carica papaya grown in Rwanda. Mol Cell Biomed Sci. 2020; 4(1): 39-44.
5. Nguyen PAT, Khang DT, Nguyen PTT, Do HDK. The complete chloroplast genome of Elephantopus scaber L. (Vernonioideae, asteraceae), a useful ethnomedicinal plant in Asia. Mitochondrial DNA B Resour. 2023; 8(9): 936-41.
6. Hiradeve SM, Rangari VD. Elephantopus scaber Linn.: A review on its ethnomedical, phytochemical and pharmacological profile. J Appl Biomed. 2014; 12(2): 49-61.
7. Hiradeve SM, Rangari VD. A review on pharmacology and toxicology of Elephantopus scaber Linn. Nat Prod Res. 2014; 28(11): 819-30.
8. Andari D, Khan FI, Jakfar SI. Methanol extract of katuk (Sauropus androgynus) leaves as an anti-inflammatory agent: Animal study in carrageenan-induced rat models of inflammation. Mol Cell Biomed Sci. 2022; 6(3): 129-34.
9. Girsang E, Lister INE, Ginting CN, Khu A, Samin B, Widowati W, *et al.* Chemical constituents of snake fruit (Salacca zalacca (Gaert.) Voss) peel and in silico anti-aging analysis. Mol Cell Biomed Sci. 2019; 3(2): 122-8.
10. Hjazi A, Alissa M, Alqasem AA, Alghamdi A, Alghamdi SA. Cynaropicrin, a sesquiterpene lactone, triggers apoptotic cell death in triple negative breast cancer cells. Mol Biol Rep. 2024; 51(1): 856. doi: 10.1007/s11033-024-09723-y. PMID: 39066893.
11. Haifa R, Sartika CR, Faried A, Hadisaputri YE, Chouw A, Wijaya A, *et al.* Potency of peripheral blood-and umbilical cord blood-derived dendritic cells and their secretomes as vaccines for cancer. Mol Cell Biomed Sci. 2024; 8(1): 31-6.
12. Sandra F, Hendarmin L, Nakao Y, Nakamura N, Nakamura S. TRAIL cleaves caspase-8,-9 and-3 of AM-1 cells: A possible pathway for TRAIL to induce apoptosis in ameloblastoma. Tumor Biol. 2005; 26(5): 258-64.
13. Sandra F, Rizal MI, Dhaniar AY, Scania AE, Lee KH. Cosmos caudatus leaf extract triggers apoptosis of HSC-3 cancer cells by decreasing bcl-2 and increasing bax. Indones Biomed J. 2024; 16(3): 285-91.
14. Wanandi SI, Syahrani RA, Suraduhita A, Yunita E, Louisa M. Andrographolide reverses doxorubicin resistance in human breast cancer stem cells by regulating apoptotic gene expressions. Indones Biomed J. 2023; 15(5): 288-96.
15. Rahman MN, Wijaya CR, Novalentina M. Survivin clinical features in cervical cancer. Mol Cell Biomed Sci. 2017; 1(1): 6-16.
16. Liao J, Qing X, Deng G, Xiao Y, Fu Y, Han S, *et al.* Gastrodin destabilizes survivin and overcomes pemetrexed resistance. Cell

Signal. 2023; 110: 110851. doi: 10.1016/j.cellsig.2023.110851.
17. Pandey SK, Paul A, Shteinfer-Kuzmine A, Zalk R, Bunz U, Shoshan-Barmatz V. SMAC/diablo controls proliferation of cancer cells by regulating phosphatidylethanolamine synthesis. Mol Oncol. 202; 15(11): 3037-61.
18. Sulistyani N, Nurkhasanah. The cytotoxic effect of Elephantopus scaber Linn extract against breast cancer (T47D) cells. IOP Conf Ser Mater Sci Eng. 2017; 259: 012006. doi: 10.1088/1757-899X/259/1/012006
19. Ho WY, Yeap SK, Ho CL, Raha AR, Suraini AA, Alitheen NB. Elephantopus scaber induces cytotoxicity in MCF-7 human breast cancer cells via p53-induced apoptosis. J Med Plants Res. 2011; 5(24): 5741-9.
20. Chan CK, Supriady H, Goh BH, Kadir HA. Elephantopus scaber induces apoptosis through ROS-dependent mitochondrial signaling pathway in HCT116 human colorectal carcinoma cells. J Ethnopharmacol. 2015; 168: 291-304.
21. Wicaksono BD, Tangkearung E, Sandra F. Brucea javanica leaf extract induced apoptosis in human oral squamous cell carcinoma (HSC-2) cells by attenuation of mitochondrial membrane permeability. Indones Biomed J. 2015; 7(2): 107-10.
22. Sajjadi SE, Ghanadian M, Haghighi M, Mouhebat L. Cytotoxic effect of Cousinia verbascifolia Bunge against OVCAR-3 and HT-29 cancer cells. J Herbmed Pharmacol. 2015; 4(1): 15-19.
23. Ho WY, Liew SS, Yeap SK, Alitheen, NB. Synergistic cytotoxicity between Elephantopus scaber and tamoxifen on MCF-7-derived multicellular tumor spheroid. Evid Based Complement Alternat Med. 2021; 2021: 6355236. doi: 10.1155/2021/6355236.
24. Mackay RP, Weinberger PM, Copland JA, Mahdavian E, Xu Q. YM155 induces DNA damage and cell death in anaplastic thyroid cancer cells by inhibiting DNA topoisomerase iiα at the ATP-binding site. Mol Cancer Ther. 2022; 21(6): 925-35.
25. Premkumar DR, Jane EP, Foster KA, Pollack IF. Survivin inhibitor YM-155 sensitizes tumor necrosis factor- related apoptosis-inducing ligand-resistant glioma cells to apoptosis through Mcl-1 downregulation and by engaging the mitochondrial death pathway. J Pharmacol Exp Ther. 2013; 346(2): 201-10.
26. Kabeer FA, Rajalekshmi DS, Nair MS, Prathapan R. Molecular mechanisms of anticancer activity of deoxyelephantopin in cancer cells. Integr Med Res. 2017; 6(2): 190-206.
27. Hu F, Pan D, Zheng W, Yan T, He X, Ren F, *et al.* Elucidating respective functions of two domains BIR and C-helix of human IAP survivin for precise targeted regulating mitotic cycle, apoptosis and autophagy of cancer cells. Oncotarget. 2017; 8(69): 113687-700.