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Research Article

Synergistic Cytotoxicity of Natural Gallic Acid from *Vitis vinifera* and Tamoxifen in Human Osteosarcoma U2OS

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ABSTRACT

Osteosarcoma is a prevalent and aggressive bone malignancy, primarily affecting children and adolescents. While tamoxifen (TAM) is a commonly used chemotherapeutic agent, its application is often limited by adverse side effects. Gallic acid (GA), a naturally occurring polyphenol, has demonstrated promising anticancer properties and may offer a safer alternative or adjunct in cancer therapy. This study investigates the effects of GA alone and in combination with TAM on the viability and morphology of human osteosarcoma (U2OS) cells. An *in vitro* model was employed using the MTT assay to assess cell viability after 72 hr of treatment. The half maximal inhibitory concentration (IC_{50}) and combination index (CI) were calculated to evaluate cytotoxicity and drug interaction. Three combination ratios of GA:TAM (75:25, 50:50 & 25:75) were tested. GA and TAM alone exhibited IC_{50} values of 8.258 ± 1.047 µg/mL and 1.814 ± 1.205 µg/mL, respectively. The 25:75 combination yielded the lowest IC_{50} (1.475 ± 1.189 µg/mL) and a CI of 0.637, indicating a synergistic effect. Other combinations showed higher IC_{50} and CI values, suggesting reduced efficacy. Morphological analysis using phase contrast microscopy revealed apoptotic features such as cell shrinkage and rounding in treated cells, particularly in the 25:75 combination group. In conclusion, GA, especially when combined with TAM at a 25:75 ratio, enhances therapeutic efficacy while potentially reducing toxicity. This combination may represent a promising strategy for improving osteosarcoma treatment outcomes.

Key words: Cytotoxicity, gallic acid, osteosarcoma, synergistic effect, tamoxifen

INTRODUCTION

Osteosarcoma is the most common primary malignant bone tumour, particularly affecting children and adolescents during periods of rapid growth. It accounts for approximately 20–40% of all bone cancers globally and represents about 2-3% of paediatric malignancies (American Cancer Society, 2024). The disease typically arises in the metaphyseal regions of long bones, such as the femur, tibia, and humerus, and is characterised by aggressive growth and a high propensity for metastasis, especially to the lungs. According to the American Cancer Society, the five-year relative survival rate for localised osteosarcoma is approximately 77%, but this drops significantly to 25% for metastatic cases (Dean, 2016).

In Malaysia, bone cancer ranks among the top five most common cancers in children and adolescents aged 0 to 19 years. According to the National Cancer Institute, the proportion of bone cancer cases in this age group increased from 6.75% (2007–2011) to 7.20% (2012–2016). Specifically, osteosarcoma accounted for 50.4% of cases in children aged 0–14 years and 49.6% in adolescents aged 15–19 years. A male predominance was observed, with 51.2% of cases in boys aged 0–14 and 63.2% in boys aged 15–19, compared to 48.7% and 36.8% in girls, respectively (National Cancer Institute Malaysia, 2020).

Histologically, osteosarcoma can be classified into several high-grade subtypes, including osteoblastic, chondroblastic, fibroblastic, small cell, telangiectatic, and high-grade surface variants. Clinically, patients often present with localised pain, swelling, and occasionally pathological fractures without preceding trauma (Dean, 2016). Epidemiological data also indicate a higher incidence in males compared to females, with a male-to-female ratio of approximately 1.43:1 (American Cancer Society, 2024). Standard treatment modalities for osteosarcoma include surgery, chemotherapy, and, less commonly, radiotherapy. While these approaches have improved survival rates, they are often associated with significant short- and long-term side effects. Surgical complications may include infection, graft failure, and bleeding. Chemotherapy, depending on the regimen, can lead to cardiotoxicity, nephrotoxicity, and ototoxicity. Radiotherapy, though rarely used, may impair wound healing and damage adjacent healthy tissues (Dean, 2016).

Tamoxifen (TAM), a selective oestrogen receptor modulator, is widely used in breast cancer treatment due to its ability to bind oestrogen receptors and inhibit oestrogen-dependent tumour growth (Dean, 2016). Interestingly, studies have explored its potential in osteosarcoma therapy, particularly in combination with other chemotherapeutic agents such as doxorubicin (Li *et al*., 2024). However, TAM is not without risks, as it may cause serious complications, including thromboembolism, stroke, and ocular toxicity (Meier & Jick, 1998; Parkkari *et al*., 2003). Given the limitations of conventional therapies, there is growing interest in plant-derived compounds for cancer treatment. Gallic acid (GA), a naturally occurring polyphenol found in various fruits and

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plants, has demonstrated promising anticancer properties. It induces apoptosis and inhibits proliferation in several cancer cell lines, including osteosarcoma. Toxicological studies have shown that GA exhibits a favourable safety profile, with a no-observed-adverse-effect level (NOAEL) of 1000 mg/kg/day identified in mice following sub-chronic exposure (Rajalakshmi *et al*., 2001).

Recent research has also highlighted the potential of other polyphenols, such as tannic acid, in enhancing the efficacy of chemotherapeutic agents. For instance, Kasiram *et al*. (2022) demonstrated that tannic acid significantly improved the apoptotic and antiproliferative effects of cisplatin in human osteosarcoma cells, suggesting that polyphenolic compounds may serve as effective adjuncts in osteosarcoma therapy. Combination therapy, which involves using multiple agents to target cancer cells synergistically, has gained traction due to its potential to enhance efficacy and reduce drug resistance. Recent studies have shown that combining agents such as kinase inhibitors can significantly improve outcomes in osteosarcoma by mitigating compensatory oncogenic signalling pathways (Li *et al*., 2024).

In this study, we investigate the combined effects of TAM and GA on human osteosarcoma U2OS cells. While both agents have shown individual anticancer activity, their synergistic potential in osteosarcoma remains underexplored. We aim to evaluate cell viability, morphological changes, and the combination index (CI) to determine the therapeutic efficacy of this novel combination. The findings may provide preliminary insights into potential combination strategies for osteosarcoma treatment; however, further studies are required to evaluate selectivity and safety in normal cells.

MATERIALS AND METHODS

Materials

Cell revival and subculture

The human osteosarcoma cells, U2OS (HTB-96™), were used in this study. The cells were purchased from the American Type Culture Collection (ATCC®) (Manassas, VA 20110). U2OS was maintained in McCoy's 5A modified medium (Invitrogen, Massachusetts, USA) supplemented with 10% (v/v) foetal bovine serum (Invitrogen, Massachusetts, USA) and 1% (v/v) penicillin-streptomycin (Gibco, Gaithersburg, USA). The cells were incubated in a humidified atmosphere of 5% CO_2 incubator at 37°C and closely monitored every 24 hr. All cell culture procedures were conducted under sterile conditions in a Class II Biosafety Cabinet (BSC) to prevent contamination.

Preparation for cell treatment

Gallic acid (GA), a naturally extracted compound from the seeds of *Vitis vinifera* (catalogue number: CFN99624), was purchased from Chemfaces (Daejeon, South Korea), while tamoxifen (TAM) was obtained from Sigma Aldrich (St. Louis, Missouri, USA). *Vitis vinifera*, commonly known as the grapevine and locally referred to in Malaysia as "anggur," is native to temperate regions such as the Mediterranean and Europe. Both compounds were dissolved in dimethyl sulfoxide (DMSO) (Nacalai Tesque, Japan) to prepare stock solutions at a concentration of 10 mg/mL. For all treatments, the final DMSO concentration in each well did not exceed 0.1% (v/v), a level widely considered non-toxic and routinely used in cell culture studies (Hassan & Ahmad 2024).

TAM was used as the positive control, and untreated cells served as the negative control. Treatment groups consisted of cells exposed to GA alone and to combinations of GA and TAM. For combination treatments, GA and TAM were mixed at volume ratios of 75:25, 50:50, and 25:75 (GA:TAM), maintaining a final stock concentration of 10 mg/mL. These stock solutions were serially diluted to obtain working concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039, 0.02, and 0.01 mg/mL.

Cells were seeded at a density of 5×10^4 cells/mL in 96-well plates and incubated overnight at 37°C in a 5% CO_2 incubator to allow for cell adhesion. Subsequently, cells were treated with the prepared serial dilutions and incubated for 72 hr under the same conditions. All treatments were performed in triplicate across three independent experiments to ensure the reliability and reproducibility of the results.

MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Nacalai Tesque, Japan) was performed to determine the half-maximal inhibitory concentration (IC_{50}) of U2OS cells, using a method adapted from Li *et al*. (2012). The MTT working solution was prepared at a concentration of 5 mg/mL in phosphate-buffered saline (PBS). This solution was then added to each well of the control and treatment groups. The 96-well plate was wrapped in aluminium foil and incubated in a 5% CO_2 incubator at 37°C for 4 hr. Following incubation, the supernatant was aspirated from each well and replaced with dimethyl sulfoxide (DMSO) to solubilise the purple formazan crystals. The plate was then shaken for 30 min to ensure complete solubilisation. Absorbance (optical density) was measured at 570 nm using an ELISA microplate reader (Tecan, Switzerland). DMSO was used as the blank. The percentage of viable cells was calculated using the following formula:

$$\text{Percentage of viable cells } (\%) = \frac{(\text{OD value of treated cells} - \text{OD value of blank})}{(\text{OD value of untreated cells} - \text{OD value of blank})}$$

The dose-response curve of cell viability (%) against the final concentration was plotted, and the IC_{50} values were identified by using the GraphPad Prism software, version 9.

Combination analysis

GA and TAM were combined at several percentage ratios (GA:TAM) (v:v) with decreasing order of TAM dose, which were 25:75, 50:50, and 75:25. The chosen combination ratios were modified from those of Tsakalozou *et al*. (2012). GA and TAM were combined at a concentration of 10 mg/mL according to the selected ratios and serially diluted to produce concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039, 0.02, and 0.01 mg/mL. The percentages of cell viability of each serially diluted combined agent of every selected ratio were used for combination analysis. The combination analysis was conducted

according to a method described by Chou (2006) using the combination index (CI). CI allowed the quantification of multiple drug interactions based on the calculation using the CI equation. The CI value was measured automatically with the CompuSyn software. The obtained CI value specified the degree of drug interactions, in which CI < 1 indicated synergistic effects between the two drugs used, CI= 1 indicated additive effects, and CI > 1 indicated antagonistic effects (Chou, 2006).

Cell morphology observation by a phase-contrast inverted microscope

The morphological changes of U2OS cells were observed and compared after treatment with GA, TAM, and the combination of GA and TAM. Cells were seeded at a concentration of 5 × 10^4 cells/mL per well and incubated overnight at 37°C in a 5% CO_2 incubator. After that, the cells were treated with GA, TAM and a combination of GA and TAM at IC_{50} for 24, 48, and 72 hr at 37°C in 5% CO_2 incubator. The morphological changes, including adherent cells, elongated cell shapes, non-adherent cells, and irregular cell morphology (such as rounded and shrunken forms), were observed using a phase-contrast inverted microscope (Carl Zeiss, Germany). Three images were captured for each treatment and control group at 200X magnification.

Statistical analysis

Data were analysed using GraphPad Prism version 9.0. Data obtained were expressed as mean ± SD (Standard deviation) from three independent experiments (n=3). The data obtained were tested for normality and homogeneity of variance through the Shapiro-Wilk test. Then, the statistical comparison was performed using the two-way repeated measure ANOVA, followed by Tukey's honest significant difference (HSD) post hoc test. The results were considered significantly different if P<0.05.

RESULTS

Determination of half maximal Inhibitory Concentration (IC_{50}) and cell viability via MTT assay

In this study, the cytotoxic effects of gallic acid (GA), tamoxifen (TAM), and their combinations on human osteosarcoma (U2OS) cells were evaluated using the MTT assay after a 72-hr incubation period. The IC_{50} value for GA alone was 8.258 ± 1.047 µg/mL, while TAM exhibited greater potency with an IC_{50} of 1.814 ± 1.205 µg/mL. Combination treatments were tested at three different GA:TAM ratios. The 75:25 ratio yielded an IC_{50} of 6.923 ± 1.144 µg/mL, the 50:50 ratio resulted in 3.447 ± 1.119 µg/mL, and the 25:75 ratio demonstrated the most potent cytotoxic effect, with an IC_{50} of 1.475 ± 1.189 µg/mL. Among the tested combinations, the 25:75 ratio showed the highest cytotoxicity, as reflected by the lowest IC_{50} value. Table 1 summarises the IC_{50} values for all treatment groups. Figures 1 and 2 illustrate the concentration-dependent cytotoxic effects of the treatments on U2OS cells. Notably, the GA-treated group exhibited a lower percentage of cell viability compared to other groups, with viability decreasing as the concentration increased.

Table 1. Half maximal inhibitory concentration (IC_{50}) values of treated human osteosarcoma cell lines (U2OS)

Treatment	IC_{50} (µg/mL)
Gallic acid (GA)	8.258 ± 1.047
Tamoxifen (TAM)	1.814 ± 1.205
GA:TAM (75:25)	6.923 ± 1.144
GA:TAM (50:50)	3.447 ± 1.119
GA:TAM (25:75)	1.475 ± 1.189

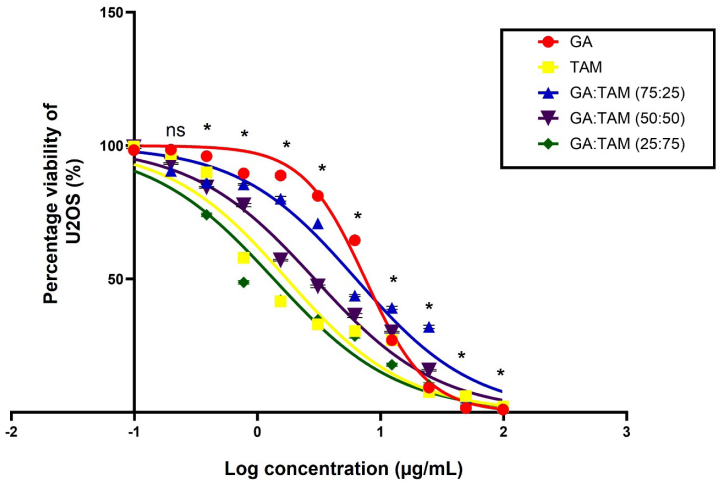

Fig. 1. Percentage viability of the human osteosarcoma cell line (U2OS) versus log concentration (µg/mL) for GA, TAM, and GA:TAM combinations. Data are presented as mean ± SD from three independent experiments (n=3). Statistical analysis was performed using two-way repeated ANOVA, followed by Tukey's honest significant differences (HSD) post-hoc test, comparing each concentration to the first (control) point within each treatment. ns indicates P≥0.05, * indicates P<0.05. Only GA and TAM at the first two concentrations were not significantly different from the control; all other concentrations showed significant reductions in viability (p<0.05).

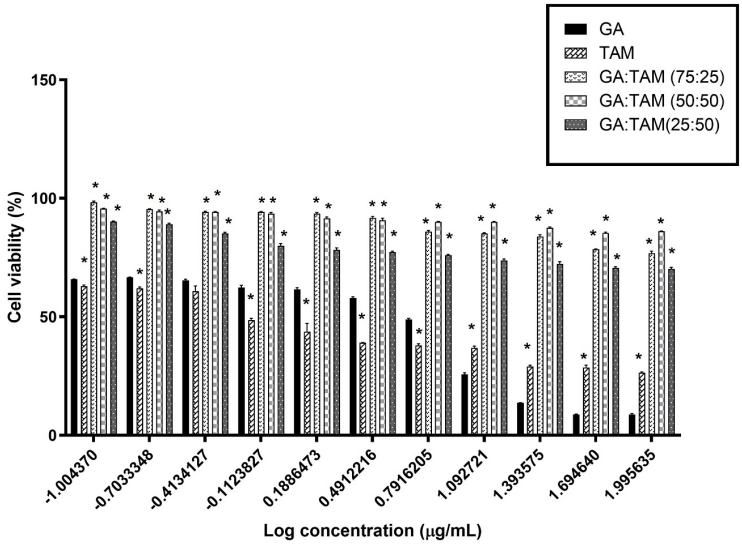

Fig. 2. Bar chart represents the percentage of cell viability versus the logarithmic final concentration of gallic acid (GA), tamoxifen (TAM), and different combination ratios of GA and TAM. Results are presented as the mean ± standard deviation (SD) from three independent experiments. The statistical analysis was performed using two-way ANOVA, and the result was considered statistically significant if $P<0.05$. Cell viability decreased significantly as concentration increased, with combination treatments showing greater reductions compared to GA or TAM alone.

Synergistic effect

To evaluate the pharmacological interaction between GA and TAM, the combination index (CI) was calculated using CompuSyn software. The CI provides a quantitative measure of drug interaction, where values greater than 1 indicate antagonism, values equal to 1 suggest an additive effect, and values less than 1 reflect synergism (Chou, 2006). The combination ratio of GA:TAM at 75:25 yielded a CI of 1.554, while the 50:50 ratio produced a CI of 1.131. Both values exceed 1, indicating antagonistic interactions in U2OS cells. In contrast, the 25:75 ratio resulted in a CI of 0.637, demonstrating a synergistic effect between the two agents. These findings suggest that the therapeutic efficacy of GA and TAM is highly dependent on their relative concentrations. A summary of CI values for all combination ratios is presented in Table 2.

Table 2. Combination index (CI) values of different combination ratios of gallic acid (GA) and tamoxifen (TAM)

GA:TAM Ratio	CI Value	Indication
25:75	0.637	Synergism
50:50	1.131	Antagonism
75:25	1.554	Antagonism

Cell morphology of U2OS cell lines

Before attachment, U2OS cells appeared rounded and were freely suspended in the culture medium. Following overnight incubation, the cells adhered to the slide surface and adopted a flat, elongated morphology. Treatments were subsequently administered to all experimental groups, while the control group remained untreated. At the initial time point (0 hr), cells across all groups exhibited a uniform flat and elongated shape (Figure 3a–d). By 36 hr, morphological changes began to emerge in the treated groups; cells exposed to GA, TAM, and their combination showed signs of shrinkage and rounding, in contrast to the untreated control group, which maintained its original morphology (Figure 3e–h). After 72 hr of treatment, pronounced morphological alterations were observed in all treated groups (Figure 3j–l), with cells appearing more rounded and shrunken, and some showing overlapping and clustering in localised regions. Additionally, treated cells exhibited reduced proliferation, and areas of cell clearance suggested cell death or detachment. These changes were absent in the control group, which continued to display healthy, elongated cells with no signs of deterioration (Figure 3i).

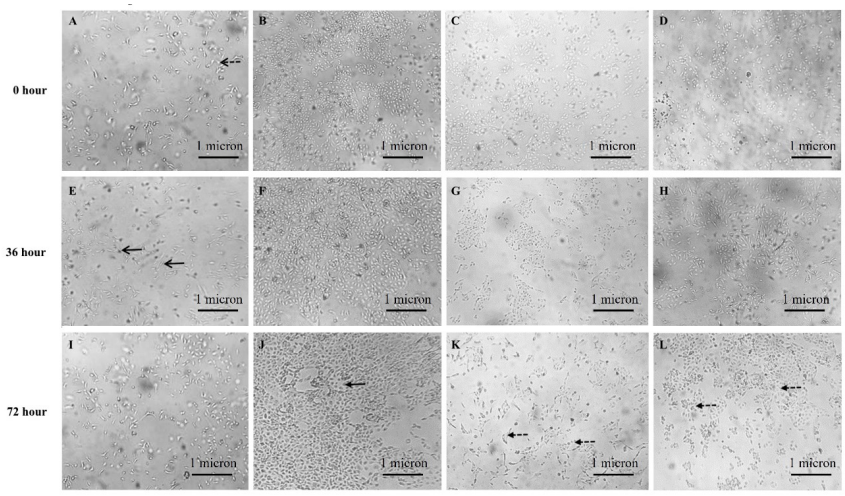

Fig. 3. Morphological changes of U2OS cells treated with gallic acid (GA), tamoxifen (TAM), and their combination were observed at different time points using a phase-contrast inverted microscope (Carl Zeiss, Germany) at 200x magnification. Panels A–D: baseline (0 hr), E–H: 36 hr, I–L: 72 hr. These observations include negative control and treated groups, highlighting the effects of GA, TAM, and their combination on U2OS cell morphology over time.[<--- represent adherent cells, ⟵ represent elongated cell shape, ⟵ represent non-adherent cells, <--- represent irregular cell shape (rounded & shrunken)].

DISCUSSION

Osteosarcoma is a highly aggressive bone malignancy predominantly affecting adolescents and young adults. Despite advances in chemotherapy, treatment resistance and toxicity remain significant challenges. This study addresses the need for alternative or adjunctive therapies by evaluating the cytotoxic effects of gallic acid (GA), a natural polyphenol, alone and in combination with tamoxifen (TAM), a well-established chemotherapeutic agent. As shown in Table 1 and Figures 1–2, both GA and TAM significantly reduced the viability of U2OS osteosarcoma cells, with the combination treatment at a 25:75 (GA:TAM) ratio yielding the lowest half maximal inhibitory concentration (IC_{50}), indicating enhanced cytotoxicity. Morphological analysis (Figure 3) further supported these findings, revealing apoptotic features such as cell shrinkage, rounding, and detachment in treated cells, particularly after 72 hr.

GA has been widely studied for its anticancer properties. Liang *et al*. (2012) showed that GA induces apoptosis in osteosarcoma cells via mitogen-activated protein kinase (MAPK) pathway modulation. Similar effects were observed in bladder cancer cells by Liao *et al*. (2018), confirming GA's broad-spectrum anticancer activity. TAM, although primarily used in hormone-responsive breast cancer, has shown efficacy in non-hormonal cancers. Ouyang and Li (2013) demonstrated that TAM enhances doxorubicin-induced apoptosis in osteosarcoma cells by inhibiting drug efflux. The current study builds on these findings by showing that GA, when combined with TAM, produces a synergistic cytotoxic effect, particularly at the 25:75 (GA:TAM) ratio. This is quantitatively supported by the combination index (CI) values presented in Table 2, where the 25:75 ratio yielded a CI of 0.637, indicating synergism. Interestingly, Ghatreh Samani *et al*. (2020) reported similar synergistic effects when combining TAM with lauryl gallate in breast cancer cells, suggesting that gallate derivatives may enhance TAM's efficacy across different cancer types. This supports the hypothesis that combining TAM with plant-derived polyphenols could be a promising strategy for improving therapeutic outcomes.

The combination index (CI) is a quantitative measure of drug interaction. According to Roell and colleagues (2017), identifying synergistic interactions is crucial for optimising combination therapies. In this study, the 25:75 GA:TAM ratio yielded a CI of 0.637, indicating a synergistic effect. In contrast, the 50:50 and 75:25 ratios showed antagonistic interactions with CI values of 1.131 and 1.554, respectively. Meletiadis *et al*. (2007) highlighted that drug interactions are concentration-dependent, with different ratios producing varying effects. Wang *et al*. (2010) demonstrated that increasing concentrations of carboplatin and gemcitabine altered their CI values, shifting from synergism to antagonism in bladder cancer cells. These findings underscore the importance of optimising drug ratios for maximal therapeutic benefit.

Further supporting this approach, Kasiram *et al*. (2022) demonstrated that tannic acid, a plant-derived polyphenol, significantly enhanced the cytotoxic effects of cisplatin in U2OS cells. The study showed increased apoptosis and reduced cell proliferation, reinforcing the therapeutic potential of polyphenolic compounds in osteosarcoma treatment. Erdogan & Usca (2025) showed that GA enhances olaparib-induced cell death and overcomes resistance in U2OS cells by modulating apoptosis-related proteins. Hohagen *et al*. (2024) developed GA-conjugated silica nanoparticles that reduced ROS levels and suppressed migration in osteosarcoma cells. Zang *et al*. (2024) highlighted hydrogel-based platforms for targeted doxorubicin delivery, emphasising the importance of combination strategies. Shagufta *et al*. (2024) reviewed tamoxifen hybrids for multi-targeted cancer therapy, supporting the rationale for combining TAM with natural compounds. Cai *et al*. (2025) emphasised the role of flavonoids in osteosarcoma treatment, including GA derivatives, through modulation of key signalling pathways. GA enhances TAM efficacy likely through increased ROS generation and activation of apoptosis pathways, including MAPK signalling and caspase activation, thereby promoting cell death and overcoming drug resistance (Hassani *et al*., 2023).

While the *in vitro* findings are promising, this study is limited by its scope. The experiments were conducted on a single cell line (U2OS), and the mechanisms underlying the observed synergy were not explored in detail. Additionally, the long-term effects and potential toxicity of the combination treatment were not assessed. Another limitation is the use of ×200 magnification for morphological observations; higher magnification (×400 or ×600) would have allowed clearer visualisation of hallmark apoptotic

features. These limitations highlight the need for further *in vivo* studies and molecular investigations. The observed synergy between GA and TAM opens avenues for developing combination therapies that are both effective and less toxic. Future research should focus on validating these findings in animal models, exploring the molecular pathways involved, and assessing the impact on drug resistance. Investigating other gallate derivatives, such as lauryl gallate and tannic acid, may also yield valuable insights into optimising combination regimens.

CONCLUSION

This study provides evidence that GA, especially when combined with TAM at a 25:75 ratio, significantly reduces osteosarcoma cell viability and induces morphological changes consistent with apoptosis. These findings support further exploration of natural compound–drug combinations, such as GA with TAM, for osteosarcoma therapy.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

American Cancer Society. 2024. Survival rates for osteosarcoma [WWW Document]. American Cancer Society. URL https://www.cancer.org/cancer/types/osteosarcoma/detection-diagnosis-staging/survival-rates.html (accessed 7.22.25).

Cai, Z., Pan, J., He, X., Wang, W. & Zhou, Z. 2025. Therapeutic potential of flavonoids in osteosarcoma: Molecular mechanisms and clinical implications. Pharmacognosy Magazine, 21(1): 1-13. https://doi.org/10.1177/09731296241305887

Chou, T.C. 2006. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacological Reviews, 58(3): 621-681. https://doi.org/10.1124/pr.58.3.10

Dean, L. 2016. Tamoxifen therapy and CYP2D6 genotype. In: Medical Genetics Summaries. V. Pratt, H. McLeod & W. Rubinstein (Eds.). National Center for Biotechnology Information (US), Bethesda. URL https://www.ncbi.nlm.nih.gov/books/NBK247013 (accessed 7.22.25).

Erdogan, M.K. & Usca, A.B. 2025. Gallic acid enhances olaparib-induced cell death and attenuates olaparib resistance in human osteosarcoma U2OS cell line. Current Issues in Molecular Biology, 47(2): 104. https://doi.org/10.3390/cimb47020104

Ghatreh Samani, K., Farrokhi, E., Tabatabaee, A., Jalilian, N. & Jafari, M. 2020. Synergistic effects of lauryl gallate and tamoxifen on human breast cancer cell. Iranian Journal of Public Health, 49(7): 1324-1329. https://doi.org/10.18502/ijph.v49i7.3586

Hassan, S.N.H. & Ahmad, F. 2024. Considering dimethyl sulfoxide solvent toxicity to mammalian cells and its biological effects. Experimental Oncology, 46(2): 174-178. https://doi.org/10.15407/exp-oncology.2024.02.174

Hassani, S., Ghanbari, F., Lotfi, M., Alam, W., Aschner, M., Popović-Djordjević, J., Shahcheraghi, S.H. & Khan, H. 2023. How gallic acid regulates molecular signaling: role in cancer drug resistance. Medical Oncology, 40: 308. https://doi.org/10.1007/s12032-023-02178-4

Hohagen, M., Saraiva, N., Kählig, H., Gerner, C., Del Favero, G. & Kleitz, F. 2024. Silica nanoparticle conjugation with gallic acid towards enhanced free radical scavenging capacity and activity on osteosarcoma cells in vitro. Journal of Materials Chemistry B, 12: 6424-6441. https://doi.org/10.1039/D4TB00151F

Kasiram, M.Z., Hapidin, H., Abdullah, H., Hashim, N.M., Azlina, A. & Sulong, S. 2022. Tannic acid enhances cisplatin effect on cell proliferation and apoptosis of human osteosarcoma cell line (U2OS). Pharmacological Reports, 74(1): 175-188. https://doi.org/10.1007/s43440-021-00330-3

Li, Y., Zhang, J., Ma, D., Zhang, L., Si, M., Yin, H. et al. 2012. Curcumin inhibits proliferation and invasion of osteosarcoma cells through inactivation of Notch-1 signaling. FEBS Journal, 279(12): 2247-2259. https://doi.org/10.1111/j.1742-4658.2012.08607.x

Li, Z., Tu, C., Zhang, W. & Qi, L. 2024. Enhanced combination treatment on osteosarcoma by mitigating drug-induced oncogenic signaling pathway activation [WWW Document]. Gene Expression Omnibus. URL https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE278302 (accessed 7.22.25).

Liang, C.Z., Zhang, X., Li, H., Tao, Y.Q., Tao, L.J., Yang, Z.R. et al. 2012. Gallic acid induces the apoptosis of human osteosarcoma cells *in vitro* and *in vivo* via the regulation of mitogen-activated protein kinase pathways. Cancer Biotherapy and Radiopharmaceuticals, 27(10): 701-710. https://doi.org/10.1089/cbr.2012.1245

Liao, C.C., Chen, S.C., Huang, H.P. & Wang, C.J. 2018. Gallic acid inhibits bladder cancer cell proliferation and migration via regulating fatty acid synthase (FAS). Journal of Food and Drug Analysis, 26: 620-627. https://doi.org/10.1016/j.jfda.2017.06.006

Meier, C.R. & Jick, H. 1998. Tamoxifen and risk of idiopathic venous thromboembolism. British Journal of Clinical Pharmacology, 45(6): 608-612. https://doi.org/10.1046/j.1365-2125.1998.00733.x

Meletiadis, J., Stergiopoulou, T., O'Shaughnessy, E.M., Peter, J. & Walsh, T.J. 2007. Concentration-dependent synergy and antagonism within a triple antifungal drug combination against Aspergillus species: analysis by a new response surface

model. Antimicrobial Agents and Chemotherapy, 51(6): 2053-2064. https://doi.org/10.1128/AAC.00873-06

National Cancer Institute. n.d. Summary of Malaysia National Cancer Registry Report 2012-2016 [WWW Document]. Institut Kanser Negara. URL https://nci.moh.gov.my/images/pdf_folder/SUMMARY-OF-MALAYSIA-NATIONAL-CANCER-REGISTRY-REPORT-2012-2016.pdf (accessed 07.22.25).

Ouyang, Z. & Li, X. 2013. Inhibitory effects of tamoxifen and doxorubicin, alone and in combination, on the proliferation of the MG63 human osteosarcoma cell line. Oncology Letters, 6: 970-976. https://doi.org/10.3892/ol.2013.1487

Parkkari, M., Puhakka, H., Laatikainen, L. & Summanen, P. 2003. Ocular side effects of tamoxifen therapy. Acta Ophthalmologica Scandinavica, 81(5): 495-499. https://doi.org/10.1034/j.1600-0420.2003.00116.x

Rajalakshmi, K., Devaraj, H. & Niranjali Devaraj, S. 2001. Assessment of the no-observed-adverse-effect level (NOAEL) of gallic acid in mice. Food and Chemical Toxicology, 39(9): 919-922. https://doi.org/10.1016/S0278-6915(01)00022-9

Roell, K.R., Reif, D.M. & Motsinger-Reif, A.A. 2017. An introduction to terminology and methodology of chemical synergy - perspectives from across disciplines. Frontiers in Pharmacology, 8: 158. https://doi.org/10.3389/fphar.2017.00158

Shagufta, Ahmad, I., Nelson, D.J., Hussain, M.I. & Nasar, N.A. 2024. Potential of covalently linked tamoxifen hybrids for cancer treatment: Recent update. RSC Medicinal Chemistry, 15(6): 1877-1898. https://doi.org/10.1039/D3MD00632H

Tsakalozou, E., Eckman, A.M. & Bae, Y. 2012. Combination effects of docetaxel and doxorubicin in hormone-refractory prostate cancer cells. Biochemistry Research International, 2012: 832059. https://doi.org/10.1155/2012/832059

Wang, S., Zhang, H., Cheng, L., Evans, C. & Pan, C.X. 2010. Analysis of the cytotoxic activity of carboplatin and gemcitabine combination. Anticancer Research, 30(11): 4573-4578.

Zang, C., Tian, Y., Tang, Y., Tang, M., Yang, D., Chen, F. et al. 2024. Hydrogel-based platforms for site-specific doxorubicin release in cancer therapy. Journal of Translational Medicine, 22: 879. https://doi.org/10.1186/s12967-024-05490-3