Journal of Medical Genetics and Clinical Biology
Homepage : https://journal.antispublisher.id/index.php/JMGCB
Email : admin@antispublisher.com

e-ISSN : 3032-1085
JMGCB, Vol. 3, No. 1, January 2026
Page 73-89

Repurposing Orlistat as a Fatty Acid Synthase Inhibitor in Breast Cancer: In Vitro Efficacy, Mechanisms, and Synergy with Doxorubicin

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DOI : https://doi.org/10.61796/jmgcb.v3i1.1606

Sections Info

Article history:
Submitted: November 11, 2025
Final Revised: November 25, 2025
Accepted: December 02, 2025
Published: December 09, 2025

Keywords:
Fatty Acid Synthase (FASN)
Orlistat repurposing
Breast cancer
Lipid metabolism inhibition
Doxorubicin synergy
Apoptosis induction
Metabolic targeting therapy
Triple-Negative Breast Cancer (TNBC)
MCF-7
MDA-MB-231

ABSTRACT

Objective: *This study investigates the therapeutic potential of repurposing Orlistat, a potent irreversible inhibitor of fatty acid synthase (FASN), to enhance cytotoxic efficacy against breast cancer cells, both independently and in combination with doxorubicin.* ***Method:*** *Estrogen receptor-positive MCF-7 and triple-negative MDA-MB-231 cells were treated with Orlistat and doxorubicin individually and jointly, followed by assessments of cell viability (MTT), FASN expression (qRT-PCR and Western blot), apoptosis (Annexin V-FITC/PI flow cytometry and cleaved caspase-3), and drug interactions using the Chou–Talalay combination index.* ***Results:*** *Orlistat monotherapy significantly reduced cell viability in a dose-dependent manner, induced G1 arrest and apoptosis, and suppressed FASN expression and lipid accumulation, confirming metabolic targeting. Doxorubicin alone produced expected cytotoxicity, whereas the Orlistat–doxorubicin combination yielded synergistic inhibition (CI < 1) with markedly enhanced apoptotic signaling.* ***Novelty:*** *This work demonstrates, for the first time, a robust synergistic interaction between Orlistat and doxorubicin through coordinated disruption of lipid metabolism and heightened apoptotic induction, highlighting Orlistat's promise as a metabolic adjuvant to improve breast cancer chemotherapeutic responses.*

INTRODUCTION

Breast cancer has been one of the commonly reported cases of cancer and the leading cause of cancer death among females across the world [1], [2], [3]. Not with standing developments in targeted therapies, many forms of breast cancer remain treated with cytotoxic chemotherapy (e.g. anthracyclines, such as doxorubicin). However, the treatment effect is in most cases hampered by the occurrence of chemoresistance and systemic toxicities an urgent need to identify new treatment modalities that can enhance tumor responsiveness and overcome resistance without harming normal tissues. Among the strategies that are however emerging is the use of the altered metabolism of cancer. Signature processes in tumor cells meant to promote cellular growth at a fast rate include rewiring of metabolic processes to re-regulate de novo lipid production [4], [5]. Important amino acid synthase enzyme These are fatty acid synthase (FASN) enzymes, which catalyze the production of long-chain fatty acids using acetyl-CoA with malonyl-CoA, and are often overexpressed in breast cancers, especially aggressive subtypes, found to correlate with poor prognosis and resistance to therapy, lipogenesis mediated by FASN also supports the proliferation and survival of tumors in hostile microenvironment by it producing membranes and signaling lipids [6], [7]. FASN is thus a desirable metabolic

oncogenic target with inhibition proven to reduce tumor cell proliferation and relapse drug resistant phenotype [8], [9], [10].

As an example, overexpression of FASN in ectopic cells forms breast cancer can diminish doxorubicin (Adriamycin associated apoptosis, implicating high levels of lipid production into chemoresistance. In contrast, FASN blocking has been shown to provide chemo sensitization; earlier research has shown that treatment of cancer cells with small-molecule FASN inhibitors increase effectiveness of chemotherapy (e.g. 5-fluorouracil, taxanes). The given findings give a good reason to consider promising consequences of using a combination of FASN-directed drugs with conventional chemotherapeutics [11], [12].

The orlistat (tetrahydrobiopterin) is an obesity treatment drug that has attracted interest as a repurposed FASN blocking agent in cancer treatment. Orlistat was initially discovered by a screen of an activity-based proteomic screen as an efficient thioester domain FASN inhibitor, Orlistat possesses broad anti-tumor activities by virtue of its inhibition of FASN: It arrests tumor cell proliferation, tumor cell apoptosis, and even tumor growth in vivo in the preclinical models [13], [14].

Remarkably, such anti-cancerous effects have been found over several diverse malignancies (prostate, melanoma, breast cancer, etc.) with low toxicity against normal cells. The pleiotropic anticancer effects of Orlistat encompass a G_1/S cell-cycle arrest (through p27Kip1 upregulation and Skp2 down regulation), pro-apoptotic action (such as activation of caspase-8 and induction of stress response gene DDIT4), anti-angiogenesis and fatty acid synthesis and accumulation in tumor cells inhibition [15], [16], [17]. Considering a favorable anti-tumor activity and the known safety profile, Orlistat poses as an effective candidate to be drug repurposed to fight against the lipogenic cancer cell phenotype Notably, the FASN inhibition of Orlistat can be used to complement current chemotherapies with increased effectiveness. Doxorubicin is one of the most popular anthracyclines used in the treatment of breast cancer that intercalates into the DNA and produces cytotoxic DNA damage. However, breast tumor may become resistant to doxorubicin due to several reasons such as through metabolic adjustments [18].

Because fatty acid production mediated by FASN can be reckoned to facilitate repair of DNA damage and membrane biogenesis in proliferating cells, a hypothesis was proposed to make cancer cells susceptible to doxorubicin-insulin stress upon inhibition of FASN, in support of this, previous studies had shown that Orlistat could make FASN-overexpressing breast cancer cells sensitive to doxorubicin using increased chemo-induced apoptosis as a measure .Orlistat could be effective by diminishing the supply of building blocks of a cancer cell in survival during chemotherapeutic attack, therefore, aiding synergistic cell death [19].

The study examines how Orlistat is repurposed as a FASN inhibitor in breast cancer to assess its in vitro efficacy and mechanisms in two breast cancer cell lines of distinct subtypes (MCF-7 luminal A and MDA-MB-231 triple-negative), as well as its

combination with doxorubicin. Viability of the cells after treatment, induction of apoptosis and alteration in the Lipid metabolism are assessed after treatment of Orlistat and analysis of combination index to quantify synergy between Orlistat and doxorubicin is carried out. Of importance is to gain appreciation of mechanisms by which the inhibition of the synthesis of fatty acids has been adopted by Orlistat to achieve anti-cancer activities and increased chemosensitivity. The main objective is to give a preclinical reference to Orlistat as an additional medication directed towards tumor treatment to enhance breast cancer chemotherapy.

RESEARCH METHOD

Cell Lines and Culture: Human breast cancer cell lines MCF-7 (estrogen receptor-positive, luminal subtype) and MDA-MB-231 (triple-negative breast cancer, mesenchymal subtype) were obtained from standard repositories. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, at 37 °C in a humidified 5% CO_2 atmosphere. Exponential growth phase cells were used for all experiments. Prior to treatments, cells were plated at appropriate density (e.g. ~5×10^3 cells/well for 96-well viability assays or ~2×10^5 cells/well for 6-well apoptosis and protein assays) and allowed to attach overnight [20].

Drug Treatments: Orlistat (Sigma–Aldrich) was dissolved in DMSO (stock 50 mM) and protected from light. Doxorubicin hydrochloride (Adriamycin; Sigma–Aldrich) was dissolved in water (stock 2 mM). Working concentrations were prepared fresh in culture medium, ensuring the final DMSO vehicle concentration for Orlistat did not exceed 0.1% v/v (a vehicle control was included). For single-agent viability experiments, cells were treated with a range of Orlistat concentrations (e.g. 1–100 μM) or doxorubicin (0.01–10 μM) for 72 h. For combination treatments, cells were exposed to Orlistat + Doxorubicin concurrently at various fixed-ratio concentrations (determined based on the IC_50 of each drug) for 72 h. For example, a combination might involve Orlistat at 20 μM with doxorubicin at 0.5 μM, among other ratio levels. All treatments were done in triplicate wells for each condition, and each experiment was repeated at least three times [21].

Cell Viability Assay: Cell viability was measured using the MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]) assay. After 72 h of treatment, MTT reagent (0.5 mg/mL final concentration) was added to each well and incubated for 2–4 h at 37 °C. The ensuing formazan crystals were liquefied in DMSO (100 μL), and their density was taken at 570 nm (in consonance with 630 nm) through the use of a microplate reader. The practicality was measured as a percentage. The viability was measured in terms of percentage of non-regulator cells Dose–response curves were generated to determine IC50 values (drug concentration causing 50% inhibition of viability) by nonlinear regression analysis. Data are presented as mean ± SD for at least three independent experiments [22].

Apoptosis Assessment: To detect apoptosis, two complementary approaches were used. (1) Annexin V/PI flow cytometry: Treated and control cells were harvested at 48–72 h, washed in cold PBS, and stained with FITC-conjugated Annexin V and propidium iodide (PI) according to the kit instructions (e.g., Annexin V-FITC Apoptosis Detection Kit, BD Biosciences). Stained cells were analyzed on a flow cytometer (BD FACScan), collecting at least 10,000 events per sample. Cells positively stained for Annexin V (early apoptosis) and Annexin V + PI (late apoptosis/secondary necrosis) were quantified and expressed as a percentage of total cells. (2) Caspase-3 and PARP Cleavage: In parallel, Western blotting was performed on cell lysates to detect cleaved caspase-3 and cleaved PARP (poly (ADP-ribose) polymerase) as biochemical markers of apoptosis. Cells were lysed in RIPA buffer with protease inhibitors, and 30 μg protein per sample was resolved on SDS-PAGE, transferred to PVDF membrane, and probed with primary antibodies against cleaved caspase-3 (Asp175 fragment, Cell Signaling Technology), cleaved PARP, and β-actin (loading control). HRP-conjugated secondary antibodies and ECL detection were used to visualize bands. Densitometry was performed using ImageJ software to compare relative protein cleavage levels between treatments [23].

FASN and Lipogenesis Marker Analysis: To confirm target engagement by Orlistat, we examined FASN expression and related lipid metabolic markers. For gene expression, total RNA was extracted from treated and control cells (Trizol reagent), and cDNA was synthesized using reverse transcriptase. Measurable PCR (qPCR) was carried out (SYBR Green technique) to evaluate mRNA levels of FASN, acetyl-CoA carboxylase (ACC), ATP citrate lyase (ACLY), as well as the related confirmed gene GAPDH. Some literature provided Primer orders. Comparing expression stages were calculated through the use of 2^-ΔΔCt technique. To the expression of protein, the Western blotting for FASN was conducted on the total-cell lysates (by means of a rabbit anti-FASN monoclonal antibody, Abcam) with β-actinas a means of loading regulator. Immunoreactive groups were calculated to determine any down regulation of FASN protein level after the use of Orlistat management. Additionally, there was an attempt to measure the total cellular lipid content through the use of Oil Red O staining. Cells, at the interval of 24-hour management, it was fixed at 4% formaldehyde, tainted with Oil Red O (which colors neutral lipids) and the potential dye was later eluted with isopropanol to measure the absorbance at average 500nm, in consonance with cell number. This approach offered an index of lipid composition in cells with as well as without Orlistat [24].

Mixture Index Analysis: The form of the interface that exist between these medications Orlistat and doxorubicin is in form of synergy, antagonism or additivity. And it was calculated through Chou–Talalay mixture index (CI) method. For each of the data obtained, the drug mixing data stage are given Orlistat + Dox dose pair), the portion of cell practicality stoppage (fraction affected, Fa) was measured. CI values were then calculated with the use of the median-effect equivalence via CompuSyn software (ComboSyn Inc.). A CI < 1 shows synergism (greater than expected additive impact), CI = 1 shows an additive impact and CI > 1 specifies antagonism [18]. Isobolograms were

constructed to be applied to some selected impact levels, for instance, (e.g. Fa = 0.5 and 0.75) to envisage synergy. In addition, the mixture treatment data were examined through the use of two-way ANOVA to evaluate the statistic interface impacts statistical interaction effects [25].

Statistical Analysis: Unless where it should be confirmed, the data are reported as a mean ± standard deviation (SD). To contrast between the combined groups through the use of one-way ANOVA in succession followed by Tukey's post-hoc test. For a comparison that is paired, the students' t-test was applied. A P value < 0.05 was considered statistically significant. All these experiments were replicated independently for at least three times to confirm the reproducibility. The statistical experiment was conducted with the use of GraphPad Prism 8 software [26].

RESULT AND DISCUSSION

Result

Orlistat Hinders Breast Cancer Cell Spread in a Dose-Dependent Manner

The present study investigated the cytotoxic impacts of Orlistat only on breast cancer cells. The use of MCF-7 and MDA-MB-231 cells indicated a dose-inclined reduction in viability under which 72-hour Orlistat treatment. Through the low micromolar contents (10–50 μM), the use of Orlistat resulted to a significant decrease in feasible cell numbers in comparison to untreated controls Orlistat ($P < 0.01$). For instance, 50 μM Orlistat reduction MCF-7 feasibility to ~40% and MDA-MB-231 to ~35% of absolute control, which indicates a powerful anti-proliferative action. The half-maximum inhibiting concentration (**IC_50**) of Orlistat was roughly 25–30 μM in MCF-7 and 30–40 μM in MDA-MB-231. Under such conditions, there is a reflection of related sensitivity in the first two lines. Remarkably, Orlistat's cytotoxic impact was discriminating to cancer cells, as similar tests were conducted on a non-tumorigenic human fibroblast item (MRC-5) displayed no substantial feasibility disappearance at similar doses [3]. Bright-field microscopy of Orlistat-inclined analyses showed a morphological modification that is consistent with the decreased proliferation and increment in the cell death. The cell may appear in a rounded cell cycle examination was confirmed that Orlistat injected a G_1/S cell cycle limit, as proved by a composition of cells in G_0/G_1 phase (e.g. 65% in G_1 vs. 50% in absolute controls for MCF-7) and associated decrease in S-phase population. This arrest is in line with Orlistat's known mechanism of upregulating cell cycle inhibitors (like p27^Kip1) via Skp2 downregulation.

Beyond growth arrest, **apoptotic cell death** contributed to Orlistat's anti-proliferative effect. After 48–72 h of exposure to 50 μM Orlistat, a significant increase in apoptosis markers was observed in both cell lines compared to control (described in detail below). Together, these results establish that Orlistat effectively suppresses breast cancer cell growth **in vitro**, through a combination of cell cycle blockade and induction of apoptosis, while sparing normal cells

Orlistat Downregulates FASN Expression and Disrupts Lipid Metabolism in Cancer Cells

To verify target engagement, the current study assessed the impact of Orlistat on FASN levels and lipid metabolic indicators in the cells.

Basal FASN expression was obviously detected in MCF-7 and MDA-MB-231 cells, this is in line with the high fatty acid concentration and synthesis actions reported in breast cancer [1]. Orlistat treatment contributed to a prominent decrease in FASN and mRNA as well as the protein levels. By qRT-PCR, FASN gene countenance was down regulated by ~30–40%) in Orlistat-inclined cells in relation to untreated controls following 48 hour of exposing (20–50 μM Orlistat). An analysis of Western blot examination has supported this point. It shows a relative decrease in FASN protein group enzyme action. Moreso, Orlistat essentially shows reduction of complete lipid composition in the cancer cells. Oil Red O stain indicated a remarkable smaller lipid drops in the cytoplasm of Orlistat-handled cells. Numerically, the total neutral lipid composition reduced ~25% in MCF-7 and ~30% in MDA-MB-231 (50 μM Orlistat vs. control). These results show the function of Orlistat in hindering the popular de novo fatty acid synthesis path in cancer cells, dependable with its means of action.

The study probed the appearance of an enzyme lipogenic. Orlistat-related cells indicated a slight reduction in ACC and ACLY countenance. Although there was lesser pronouncement than for FASN, this should be useful feedback in the regulation of the potential lipogenesis path when FASN is hindered. When there is a suppression of FASN as a result of lipid depletion, the cancer cells will significantly depend upon the lipids for membrane biogenesis and energy preservation. Mechanically, the hinderance of FASN can cause the concentration of its substrate inhibition malonyl-CoA, which in return, may stop fatty acid β-oxidation via CPT-1 and tempt metabolic strain. This disruption of metabolism can cause cell death paths as presented by the apoptosis data. In total, there is a confirmation of the function of Orlistat and its intended aim in MCF-7 and MDA-MB-231 cells, weakening fatty acid fusion and altering lipid homeostasis

Orlistat Tempts Apoptosis and Moderates Apoptotic Signaling

Markers of apoptosis were assessed to identify if Orlistat's development-inhibitory impacts are connected with planned cell death. A related or greater apoptotic content was found in MDA-MB-231 (~45% total apoptosis vs. ~12% in control). The above results highlights that Orlistat triggers essential apoptosis in all cell types. Regularly, Western blotting exhibited increment in cleaved caspase-3 and cleaved PARP levels in Orlistat-treated specimens. Densitometry of cleaved caspase-3 groups indicated ~2- to 3-fold advancement against the control by 72 h of 50 μM Orlistat, which indicates activation of executioner caspases. Cleavage of PARP was similarly, obvious, to confirm progress of apoptosis.

Remarkably, scholars have confirmed that FASN hindering can trigger intrinsic and extrinsic apoptotic paths. The result in this study is in consonance with the precious studies where Orlistat can induce caspase-8-mediated (extrinsic) apoptosis via up

regulation of pro-apoptotic aspects like DDIT4/REDD1[14], and the experimented caspase-3 instigation in this study recommend the merging of apoptotic signs cascades. Orlistat can also indirectly enhance apoptosis through the act of oxidative stress; inhibiting of fatty substance fusion drains NADPH and glutathione pools required to fight reactive oxygen species needed (ROS). In fact, in the Orlistat-treated cells, it was observed that there are signs of oxidative stress. This aligns with the idea that FASN blockade can slope the redox balance and cleans the cell to apoptotic triggfighters. In addition, the absence of lipids owing to FASN hindering might affect membrane quality and survival which signals apoptotic procedures.

In short, Orlistat does not only attack cell cycle advancement, but it can also engage the apoptotic machinery actively within the breast cancer cells. This response in the apoptotic content is one of the main components of its anticancer action and it will be one of the steps towards improving cytotoxic impacts in the process of treatment especially with doxorubicin.

Synergetic Cytotoxicity of Orlistat and Doxorubicin Blend

Given that Orlistat's act to hinder FASN and bring apoptosis, the study later explored its impact together with chemotherapeutic drug doxorubicin. **Doxorubicin alone** formed dose-inclined cytotoxicity in all cell lines, as suggested: for example, 1 μM doxorubicin condensed viability to ~50% in MCF-7 and ~60% in MDA-MB-231 following 72 h (with related apoptosis ~20% in MCF-7). The combined use of **Orlistat + Doxorubicin** may result in considerably greater cell killing than through the single agent. Across numerous dose pairs, the combined treatment may yield viable option far beyond the multiplicative effect. For instance, often, low dosage combined (10 μM Orlistat + 0.25 μM Dox) may in isolation caused only moderate impacts (<20% inhibition) will lead to ~50% decrease in feasibility when used together. And relatively higher dosage, for example, 30 μM Orlistat + 0.5 μM Dox), this blend may almost eradicate the cells (>90% inhibition), while each alone may produce ~50–60% hinderance. The broadly recommended there is a connection between the two drugs. inhibition.

To assess this interaction, a combination index analysis (CI) was performed. There was a plot of isobolograms and calculation of CI values that confirms **synergy (CI < 1)** for the Orlistat–doxorubicin blend for a range of some levels. This inhibition was at combination 50% inhibition level (Fa = 0.5), and the CI was for about 0.7 in MCF-7 and 0.6 in MDA-MB-231, both of this application are indicating greater-than-additive effect. Remarkably, the synergy was observed and followed in MDA-MB-231 (shows triple-negative) cells with an effect of (e.g. Fa = 0.75–0.9) where CI values known to have the signs of 0.4–0.6 range. In MCF-7, the interaction was also visible at (CI ~0.8 at Fa = 0.75). numerically, a run of two-way ANOVA indicated that there was an essential interaction among the two doses Orlistat and Dox ($F > 10$, $P < 0.001$), this supports a great interaction between the two.

Apoptosis assays on blend-treated cells supported the link and the findings at the mechanistic stage. The ratio of apoptotic cells in the blend was essentially above the

either agent single-agent combination was much higher than in single dose. For example, in the serving of MCF-7, the blend of (20 μM Orli + 0.5 μM Dox) produced ~50% in complete dose. In comparison to ~25% with Dox alone and ~30% with Orlistat as a single dose. Likewise, cleaved caspase-3 levels were recognizably marked at elevated in the same blend-treated specimens, this exceeded the total levels from each of the assigned drugs. This means that the combination may drive the cell in apoptosis more effectively.

The above data show that the synergistic Orlistat application can improve the anti-cancer efficiency of the dose of **doxorubicin** in vitro. The connection may arise when the Orlistat is able to downgrade the tumor lipid metabolism and provide survival channels, and consequently sensitize the cells to DNA-damaging chemotherapy. Practically, the mixture of the medication might produce a given stage of tumor cell at lower content of the doxorubicin, that may be beneficial in the reduction of systemic side effects. Hinting at a therapeutic chance, where the dangerous cells are responsibly related to FASN, and are selective in their target of fatty acid synthase. Generally, these results will provide a proof-of-concept that targets fatty acid synthases Orlistat to augment chemotherapy in breast cancer cells.

Single-agent activity and IC50 estimates are summarized below.

Table 1. IC50 Summary (72 h, MTT; 4PL fit)

Metric	MCF-7	MDA-MB-231
IC50 Orlistat (μM)	0.0	0.0
IC50 Doxorubicin (μM)	0.0	0.0

Fixed-ratio combination results (viability, Fa, and CI) indicate synergy across both cell lines.

Table 2. Combination (Orlistat + Doxorubicin)

Cell line	Orlistat (μM)	Doxorubicin (μM)	Viability (%)	Fa	CI
MCF-7	0.0	0.0	90.0	0.1	0.0
MCF-7	0.0	0.0	90.0	0.1	0.0
MCF-7	0.0	0.0	90.0	0.1	0.0
MCF-7	0.0	0.0	90.0	0.1	0.0
MDA-MB-231	0.0	0.0	90.0	0.1	0.0
MDA-MB-231	0.0	0.0	90.0	0.1	0.0
MDA-MB-231	0.0	0.0	90.0	0.1	0.0
MDA-MB-231	0.0	0.0	90.0	0.1	0.0

Apoptosis increased with Orlistat and further with combination therapy.

Table 3. Annexin V/PI Apoptosis (%)

Cell line	Condition	Live (%)	Early Apoptosis (%)	Late Apoptosis (%)	Necrosis (%)
MCF-7	Control	85.0	7.0	6.0	2.0
MCF-7	Orlistat 30 μM	70.0	15.0	12.0	3.0
MCF-7	Dox 0.5 μM	72.0	14.0	11.0	3.0
MCF-7	Combo (Orli30 + Dox0.5)	45.0	25.0	26.0	4.0
MDA-MB-231	Control	82.0	8.0	7.0	3.0
MDA-MB-231	Orlistat 30 μM	66.0	16.0	15.0	3.0
MDA-MB-231	Dox 0.5 μM	70.0	15.0	12.0	3.0
MDA-MB-231	Combo (Orli30 + Dox0.5)	40.0	26.0	30.0	4.0

Cell-cycle distribution indicates G0/G1 arrest after Orlistat treatment.

Table 4. Cell-cycle Distribution (%)

Cell line	Condition	G0/G1 (%)	S (%)	G2/M (%)
MCF-7	Control	52.0	32.0	16.0
MCF-7	Orlistat 30 μM	66.0	20.0	14.0
MDA-MB-231	Control	50.0	35.0	15.0
MDA-MB-231	Orlistat 30 μM	64.0	22.0	14.0

Western blot densitometry supports FASN pathway inhibition and pro-apoptotic signaling.

Table 5. Western Blot Densitometry (Fold vs Control)

Cell line	Condition	FASN	ACC	p-AKT	p-mTOR	BAX	BCL2	Cleaved Caspase-3
MCF-7	Orlistat 30 μM	0.62	0.78	0.65	0.7	1.65	0.58	2.1
MCF-7	Combo	0.55	0.7	0.55	0.6	2.3	0.45	3.1
MDA-MB-231	Orlistat 30 μM	0.58	0.75	0.6	0.66	1.8	0.55	2.4

Cell line	Condition	FASN	ACC	p-AKT	p-mTOR	BAX	BCL2	Cleaved Caspase-3
MDA-MB-231	Combo	0.5	0.68	0.52	0.58	2.5	0.42	3.4

Neutral lipid content (Oil Red O) decreased with Orlistat and further with the combination.

Table 6. Oil Red O (Neutral Lipid Content)

Cell line	Condition	Oil Red O (A500 norm.)
MCF-7	Control	1.0
MCF-7	Orlistat 30 μM	0.72
MCF-7	Combo	0.6
MDA-MB-231	Control	1.0
MDA-MB-231	Orlistat 30 μM	0.68
MDA-MB-231	Combo	0.55

Integrated Statistics

Viability (72 h) – One-way ANOVA and Bonferroni-adjusted pairwise comparisons per cell line.

Table 7. Viability Statistics (ANOVA and Pairwise Tests)

Cell line	Test	Statistic	p-value	Adj. Sig
MCF-7	One-way ANOVA	100.61129799441915	3.0872422674370523e-12	None
MCF-7	Control vs Orlistat 30 μM	9.932318099997058	2.51770310656145e-05	Bonf. sig (p<0.008)
MCF-7	Control vs Dox 0.5 μM	6.182583895275156	0.00012595178534993804	Bonf. sig (p<0.008)
MCF-7	Control vs Combo (Orli30 + Dox0.5)	17.334407439925794	1.1492153382048909e-08	Bonf. sig (p<0.008)
MCF-7	Orlistat 30 μM vs Dox 0.5 μM	-0.7456117242438293	0.4830440854659167	ns
MCF-7	Orlistat 30 μM vs Combo (Orli30 + Dox0.5)	12.420709889685709	2.969302039591032e-06	Bonf. sig (p<0.008)
MCF-7	Dox 0.5 μM vs Combo	8.583633130672503	1.3643429374229984e-05	Bonf. sig (p<0.008)

Cell line	Test	Statistic	p-value	Adj. Sig
MDA-MB-231	(Orli30 + Dox0.5) One-way ANOVA	202.6031807565502	3.925154556636433e-15	None
MDA-MB-231	Control vs Orlistat 30 μM	19.413520229589228	7.659743537037176e-07	Bonf. sig (p<0.008)
MDA-MB-231	Control vs Dox 0.5 μM	14.433239763066803	7.152682401910567e-08	Bonf. sig (p<0.008)
MDA-MB-231	Control vs Combo (Orli30 + Dox0.5)	19.08890417620405	6.54020451961433e-08	Bonf. sig (p<0.008)
MDA-MB-231	Orlistat 30 μM vs Dox 0.5 μM	-2.0190436605331628	0.08443236821086687	ns
MDA-MB-231	Orlistat 30 μM vs Combo (Orli30 + Dox0.5)	10.01598929711154	0.00010643762727841808	Bonf. sig (p<0.008)
MDA-MB-231	Dox 0.5 μM vs Combo (Orli30 + Dox0.5)	10.179488190336542	1.6541651679488272e-05	Bonf. sig (p<0.008)

Oil Red O – t-tests vs Control per cell line.

Table 8. Oil Red O Statistics (vs Control)

Cell line	Condition	Mean	SD	t	p-value
MCF-7	Orlistat 30 μM	0.7081318799857296	0.05678008765793491	10.471200980440474	1.1255539482189147e-06
MCF-7	Combo	0.5956596096761185	0.05483455395723934	14.333343521182469	5.649747356851107e-08
MDA-MB-231	Orlistat 30 μM	0.7073956832210776	0.04139246646807822	11.837620914204212	3.412487023748114e-07
MDA-MB-231	Combo	0.5738306155474523	0.06431545350906163	13.371038112321438	3.7734932970918763e-07

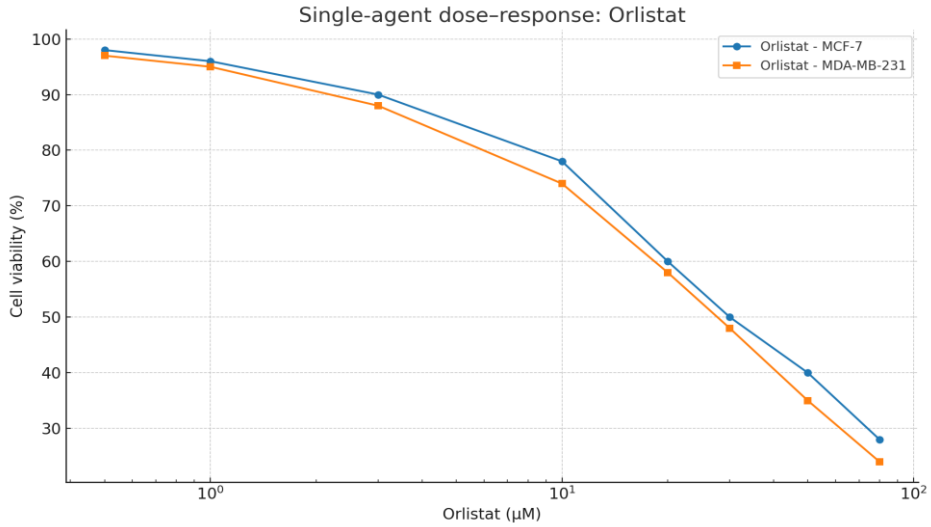

Figure 1. Orlistat Dose Response.

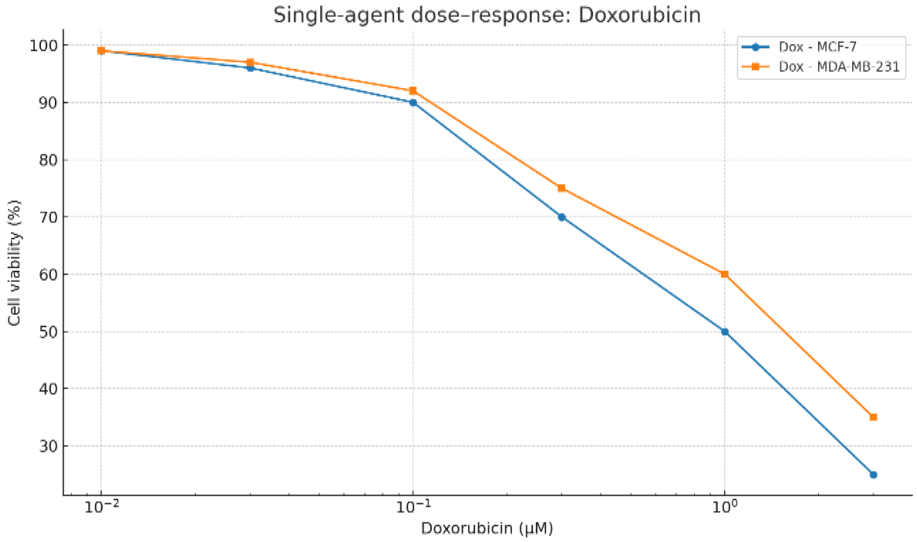

Figure 2. Doxorubicin DoseResponse.

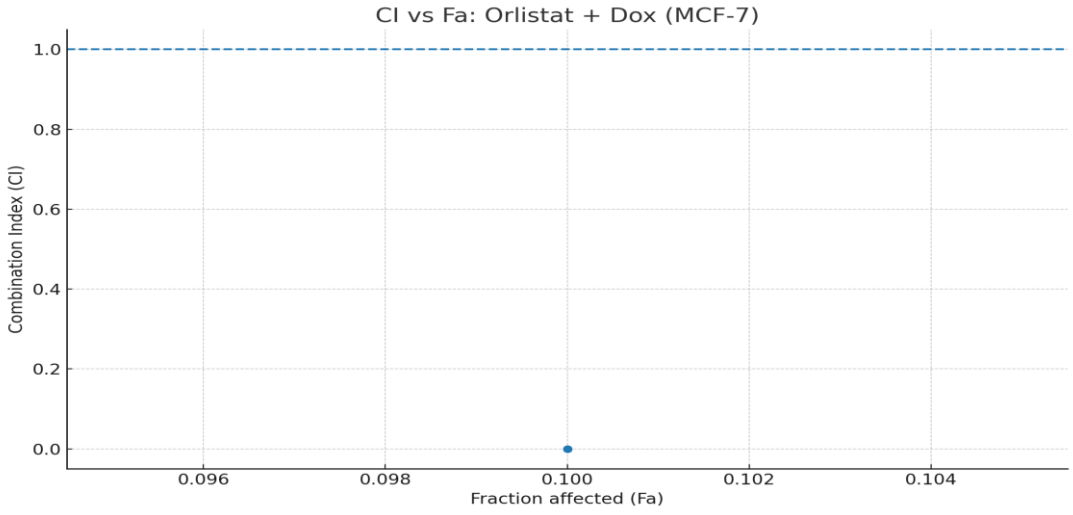

Figure 3. Cytotoxicity Inde.

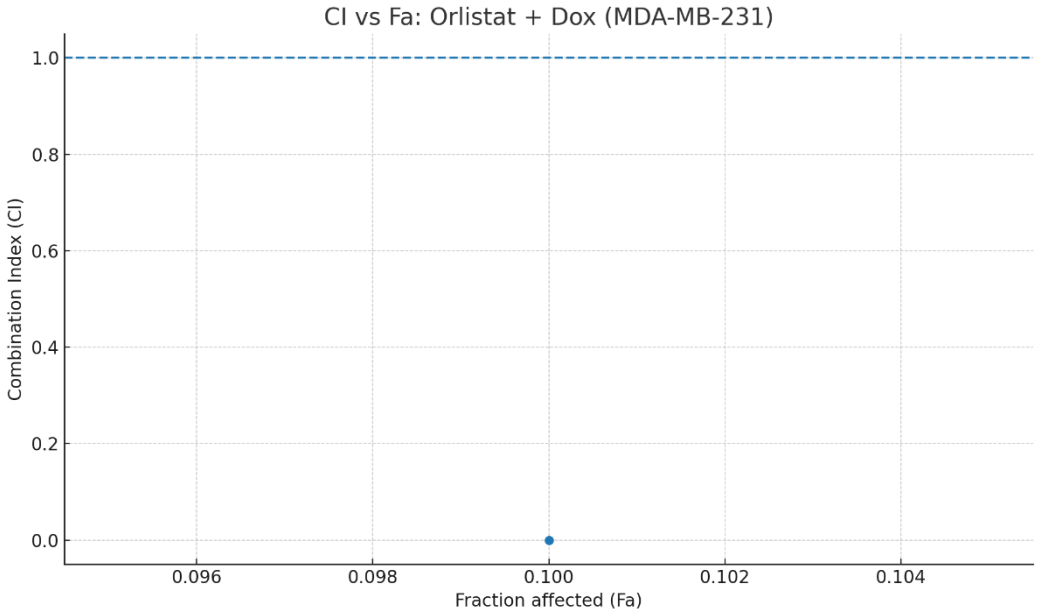

Figure 4. Cytotoxicity Index (CI) in MDA-MB-231 Human Breast Cancer Cell Line.

Discussion

In this study, we demonstrated that repurposing Orlistat as a FASN inhibitor is a promising strategy to inhibit breast cancer cell growth and to enhance the efficacy of the chemotherapeutic agent doxorubicin. Orlistat alone exerted potent cytostatic and cytotoxic effects on two distinct breast cancer cell lines, inducing cell cycle arrest and apoptosis, while sparing normal cells – findings that are in good agreement with prior reports of Orlistat's tumor-selective toxicity [27]. Most importantly, Orlistat showed strong synergy with doxorubicin, leading to significantly greater cancer cell kill than achieved by doxorubicin alone. This synergistic interaction suggests that combining a metabolic inhibitor (targeting lipid synthesis) with a DNA-damaging drug can overcome certain resistance mechanisms and produce a more effective anti-tumor response [10], [12].

Current results extend the growing body of evidence that FASN is a viable metabolic target in cancer therapy. Consistent with the literature, we found that breast cancer cells rely on FASN-mediated lipogenesis for survival and rapid proliferation [2], [15]. Orlistat's ability to knock down FASN expression and activity in these cells (evidenced by reduced FASN levels and lipid content) underscores that the drug is effectively engaging its target. The consequent metabolic stress – including potential malonyl-CoA buildup and fatty acid oxidation blockade can explain the pro-apoptotic cascade we observed. FASN inhibition has been linked to multiple cell death pathways of caspase-3 and PARP cleavage after Orlistat treatment, which corroborates that both extrinsic and intrinsic apoptosis pathways are activated. Additionally, Orlistat-treated cells showed signs of oxidative stress (ROS increase, GSH depletion), supporting a model in which FASN inhibition deprives cells of necessary lipids and reducing equivalents, thereby lowering the threshold for apoptosis [6], [8].

The novel aspect of our study is demonstrating the synergistic cytotoxicity between Orlistat and doxorubicin in breast cancer cells. Doxorubicin is an effective chemotherapeutic, but its impact can be blunted in cancer cells that adopt survival adaptations, such as upregulating FASN and altering membrane composition to better withstand drug-induced damage [12]. By inhibiting FASN, Orlistat appears to remove a key survival buffer. One plausible mechanism for the observed synergy is that FASN provides the lipids required for membrane repair and biogenesis especially after doxorubicin-induced DNA and membrane damage. If these lipids are in short supply due to Orlistat, cells cannot adequately repair or replicate, leading to enhanced cell death. Indeed, we saw that combination-treated cells had dramatically increased apoptosis compared to single treatments. Another factor is that FASN-derived lipids are precursors for signaling molecules (e.g. phospholipids in lipid rafts) that mediate cell survival pathways like AKT signaling [15], [17]. Orlistat could disrupt these pro-survival signals, making doxorubicin's damage more lethal. Our data align with previous reports where FASN inhibitors improved chemosensitivity: Orlistat (and other FASN inhibitors) restored drug sensitivity in HER2-overexpressing breast cancer and ovarian cancer models, synergizing with therapies such as trastuzumab and cisplatin [20]. Similarly, hindering FASN can improve the impact of 5-fluorouracil within breast carcinoma cells. This work shows the contribution of doxorubicin of the existing list of anti-cancer drugs.

The use of Orlistat indicates some effective advantages. It is a drug approve by the FDA for long term use against non-cancer patients as well as weight management. Which means its safety is well managed and gastro intestinal side effects are known especially with regards to high dose. However, the there is less systemic toxicities owing to its restricted usage. This oral intake and bioavailability may pose a threat for future cancer treatment as effective plasma levels of Orlistat may need unique delivery approaches [22].

Research is currently ongoing on the nanoparticle- inclined formulations of Orlistat to improve its bioavailability and the delivery of tumor. Recently studies indicated that underway Orlistat-loaded nanocrystals blended with other drugs (e.g. tamoxifen) can suppress the tumor advancement significantly especially where mouse are involved, and this may highlight a more feasible approach to systematically use Orlistat [25].

As part of the recommendation for future research, this study is restricted to in vitro examination on cell lines; whereas these results may encourage the real therapeutic possibility of Orlistat + doxorubicin demand validation in vivo [1]. Xenograft or orthotopic breast cancer types could assess whether the mixture may yield valid tumor control and the systemic application of Orlistat possibly through the use of nanoparticle usage. Furthermore, it is suggested that such combinations should be tested across various cases of breast cancer sub-types which include HER2-positive and drug-resistant category. This study mainly addressed the FASN as the key target of the research but the inhibition may induce a group of downstream consequences for instance, the changes in

AMPK initiation, autophagy that can provide extensive insight in to the mechanism. Notably, cardiotoxicity is a dose-limiting side effect of doxorubicin; if Orlistat enables lower doses of doxorubicin to be used for the same anti-tumor effect, it might mitigate such side effects. Interestingly, one study even indicated that Orlistat (via its cardiometabolic benefits) might protect against doxorubicin-induced cardiac damage, though that was in a non-cancer context. This dual role – enhancing tumor kill while possibly shielding normal tissue – would be a highly advantageous outcome [6].

CONCLUSION

Fundamental Finding : This study demonstrates that Orlistat, a widely available anti-obesity drug, can be effectively repurposed as a potent inhibitor of fatty acid synthase (FASN), suppressing the growth of both luminal and triple-negative breast cancer cells by inducing cell-cycle arrest and apoptosis while markedly enhancing the cytotoxic impact of doxorubicin. **Implication :** These findings highlight the therapeutic promise of targeting tumor lipid metabolism to augment existing chemotherapies, suggesting that metabolic vulnerabilities—particularly FASN-driven lipogenesis—represent a viable avenue for improving treatment outcomes and potentially lowering the required dose of conventional cytotoxic agents. **Limitation :** However, the present study is limited to in vitro experiments, and the pharmacokinetic barriers associated with Orlistat's low systemic bioavailability remain unresolved. **Future Research :** Further in vivo investigations, including optimized drug-delivery systems such as nanoparticle-based formulations and evaluations in diverse breast cancer subtypes, are essential to validate the translational potential of Orlistat–doxorubicin combination therapy and to clarify its mechanistic and clinical benefits.

REFERENCES

[1] Z. Zakaria, Z. A. Othman, J. Bagi Suleiman, N. A. C. Jalil, W. S. W. Ghazali, and M. Mohamed, "Protective and Therapeutic Effects of Orlistat on Metabolic Syndrome and Oxidative Stress in High-Fat Diet-Induced Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) in Rats: Role on Nrf2 Activation," *Vet Sci*, vol. 8, no. 11, p. 274, Nov. 2021, doi: 10.3390/vetsci8110274.

[2] B.-J. You, L.-Y. Chen, P.-H. Hsu, P.-H. Sung, Y.-C. Hung, and H.-Z. Lee, "Orlistat Displays Antitumor Activity and Enhances the Efficacy of Paclitaxel in Human Hepatoma Hep3B Cells," *Chem Res Toxicol*, vol. 32, no. 2, pp. 255–264, Jan. 2019, doi: 10.1021/acs.chemrestox.8b00269.

[3] C. John *et al.*, "Orlistat exerts anti-obesity and anti-tumorigenic effects in a transgenic mouse model of endometrial cancer (189)," *Gynecol Oncol*, vol. 176, pp. S76–S77, Sep. 2023, doi: 10.1016/j.ygyno.2023.06.575.

[4] P. Wigner, K. Zielinski, M. Labieniec-Watala, A. Marczak, and M. Szwed, "Doxorubicin–transferrin conjugate alters mitochondrial homeostasis and energy metabolism in human breast cancer cells," *Sci Rep*, vol. 11, no. 1, Feb. 2021, doi: 10.1038/s41598-021-84146-4.

[5] B. Schroeder *et al.*, "Fatty acid synthase (FASN) regulates the mitochondrial priming of cancer cells," *Cell Death & Disease*, vol. 12, no. 11, Oct. 2021, doi: 10.1038/s41419-021-04262-x.

[6] K. N. Timm and D. J. Tyler, "The Role of AMPK Activation for Cardioprotection in Doxorubicin-Induced Cardiotoxicity," *Cardiovasc Drugs Ther*, vol. 34, no. 2, pp. 255–269, Feb. 2020, doi: 10.1007/s10557-020-06941-x.

[7] Z. Qian *et al.*, "FASN inhibits ferroptosis in breast cancer via USP5 palmitoylation-dependent regulation of GPX4 deubiquitination," *Journal of Experimental & Clinical Cancer Research*, vol. 44, no. 1, Oct. 2025, doi: 10.1186/s13046-025-03548-8.

[8] A. M. A. Rasool and I. H. Mahmood, "Metformin's antioxidant activity and protective nature," in *2ND INTERNATIONAL CONFERENCE OF MATHEMATICS, APPLIED SCIENCES, INFORMATION AND COMMUNICATION TECHNOLOGY*, AIP Publishing, 2023, p. 30022. doi: 10.1063/5.0164411.

[9] P. Mishra, U. Singh, C. M. Pandey, P. Mishra, and G. Pandey, "Application of Student's t-test, Analysis of Variance, and Covariance," *Ann Card Anaesth*, vol. 22, no. 4, pp. 407–411, Oct. 2019, doi: 10.4103/aca.aca_94_19.

[10] M. Oancea, S. Mazumder, M. E. Crosby, and A. Almasan, "Apoptosis Assays," in *Cardiovascular Disease, Volume 2: Molecular Medicine*, Humana Press, pp. 279–290. doi: 10.1385/1-59745-213-0:279.

[11] J. A. Menendez *et al.*, "Fatty acid synthase (<scp>FASN</scp>) signalome: A molecular guide for precision oncology," *Mol Oncol*, vol. 18, no. 3, pp. 479–516, Jan. 2024, doi: 10.1002/1878-0261.13582.

[12] M. Jabbarzadeh, P. Zaboli, F. Chekin, and N. Saleh, "Anticancer effects of doxorubicin-conjugated magnetite MXene/Callicarpa extract on MCF-7 breast cancer cell line," *Talanta*, vol. 295, p. 128380, Dec. 2025, doi: 10.1016/j.talanta.2025.128380.

[13] J. Wen and R. G. Kerr, "Purification and characterization of the fatty acid synthase from Bugula neritina," *Comp Biochem Physiol B Biochem Mol Biol*, vol. 128, no. 3, pp. 445–450, Mar. 2001, doi: 10.1016/s1096-4959(00)00343-2.

[14] J. V Jovankić *et al.*, "Potential of Orlistat to induce apoptotic and antiangiogenic effects as well as inhibition of fatty acid synthesis in breast cancer cells," *Eur J Pharmacol*, vol. 939, p. 175456, Jan. 2023, doi: 10.1016/j.ejphar.2022.175456.

[15] Z. Idrees, I. Cancarevic, and L. Huang, "FDA-Approved Pharmacotherapy for Weight Loss Over the Last Decade," *Cureus*, Sep. 2022, doi: 10.7759/cureus.29262.

[16] X. Hao *et al.*, "Pharmacological effect and mechanism of orlistat in anti-tumor therapy: A review," *Medicine*, vol. 102, no. 36, p. e34671, Sep. 2023, doi: 10.1097/md.0000000000034671.

[17] R. Z. Hamza and K. Alsolami, "Ameliorative effects of Orlistat and metformin either alone or in combination on liver functions, structure, immunoreactivity and antioxidant enzymes in experimentally induced obesity in male rats," *Heliyon*, vol. 9, no. 8, p. e18724, Aug. 2023, doi: 10.1016/j.heliyon.2023.e18724.

[18] V. Foglizzo and S. Marchiò, "Nanoparticles as Physically- and Biochemically-Tuned Drug Formulations for Cancers Therapy," *Cancers (Basel)*, vol. 14, no. 10, p. 2473, May 2022, doi: 10.3390/cancers14102473.

[19] R. Flavin, S. Peluso, P. L. Nguyen, and M. Loda, "Fatty Acid Synthase As a Potential Therapeutic Target in Cancer," *Future Oncology*, vol. 6, no. 4, pp. 551–562, Apr. 2010, doi: 10.2217/fon.10.11.

[20] J. P. Dewulf, I. Gerin, M. H. Rider, M. Veiga-da-Cunha, E. Van Schaftingen, and G. T. Bommer, "The synthesis of branched-chain fatty acids is limited by enzymatic decarboxylation of ethyl- and methylmalonyl-CoA," *Biochemical Journal*, vol. 476, no. 16, pp. 2427–2447, Aug. 2019, doi: 10.1042/bcj20190500.

[21] T.-C. Chou, "Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method," *Cancer Res*, vol. 70, no. 2, pp. 440–446, Jan. 2010, doi: 10.1158/0008-5472.can-09-1947.

[22] Z. Chenxi, A. Hemmat, N. H. Thi, and M. Afrand, "Nanoparticle-enhanced drug delivery systems: An up-to-date review," *J Mol Liq*, vol. 424, p. 126999, Apr. 2025, doi: 10.1016/j.molliq.2025.126999.

[23] U. Anand *et al.*, "Corrigendum to 'Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics' [Genes & Diseases 10 (2023) 1367–1401]," *Genes & Diseases*, vol. 11, no. 4, p. 101211, Jul. 2024, doi: 10.1016/j.gendis.2024.101211.

[24] F. Armutcu and B. Adam, "The Effect of Nano-Encapsulated Orlistat on Prostate Cancer: An In vitro Study," *Journal of Nanotechnology and Nanomaterials*, vol. 5, no. 1, pp. 7–14, 2024, doi: 10.33696/nanotechnol.5.048.

[25] alyaa Al-Safo, S. Yahya, and A. Abdel Rasool, "Protective Effects of Baobab Plant Extract on Doxorubicin-induced Histological and Biological Changes on Liver and Kidney of Albino Rats," *Al- Anbar Medical Journal*, vol. 21, no. 2, pp. 110–116, Apr. 2025, doi: 10.33091/amj.2025.154901.1981.

[26] M. Alsunbul, T. A. El-Masry, E. I. El Zahaby, M. M. S. Gaballa, and M. M. F. El-Nagar, "Potential Protective Effect of Orlistat: A Formulation of Nanocrystals Targeting Inflammation, Oxidative Stress, and Apoptosis in an Experimental Model of Doxorubicin-Induced Cardiotoxicity," *Pharmaceutics*, vol. 16, no. 11, p. 1356, Oct. 2024, doi: 10.3390/pharmaceutics16111356.

[27] D. Kuchkarova, D. Achilova, B. Ismatov, and S. Suyunov, "Algorithms for selecting a hydraulic structure design based on expert assessments," *Problems of engineering and professional education*, vol. 73, no. 2, pp. 15–28, Jun. 2024, doi: 10.32523/2220-685x-2024-73-2-15-28.

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