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العدد التاسع
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التقييم النسيجي والاستماتة لورم الغدي للثدي البشري المزروع (MCF-7) المعالج بالسكريات
الدهنية البكتيرية المتعددة: دراسة في الجسم الحي (*In Vivo*)

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المستخلص:

الخلاصة: هدفت هذه الدراسة إلى إجراء التقييم النسيجي والتحري الكمي عن تحفيز الاستماتة (الموت الخلوي المبرمج) في كتل أورام سرطان الغدي للثدي البشري المزروعة (MCF-7) داخل جسم الكائن الحي (*in vivo*) بعد المعالجة بالحقن الموضعي المباشر داخل الورم للسكريات الدهنية المتعددة (LPS) المشتقة من بكتيريا *Escherichia coli*، ومقارنة كفاءتها بالعلاج الكيميائي القياسي بعقار الدوكسوروبيسين (Doxorubicin). يُعد سرطان الثدي المسبب الرئيسي للوفيات السرطانية بين النساء عالمياً وفي العراق، وتستدعي معضلة المقاومة الدوائية للعلاجات التقليدية استكشاف عوامل حيوية مناعية بديلة وموجهة.

المواد وطرائق العمل: تم استحداث أورام الزرع غير المتجانس (Xenograft) بحقن 1×10^6 من خلايا MCF-7 تحت جلد الخاصرة لفئران التجارب عديمة الشعر (BALB/c Nude mice)، إناث، بأعمار ٦-٨ أسابيع وأوزان 20 ± 2 غم). وبعد التحقق من نمو الكتلة الورمية وبلوغها حجماً قابلاً للقياس والجس خلال ١٤ يوماً من الحقن، قُسمت الحيوانات عشوائياً إلى ثلاث مجاميع تجريبية متساوية ($n = 3$ فئران لكل مجموعة): مجموعة السيطرة السالبة غير المعالجة (تقتصر على حقن المحلول الملحي الفسيولوجي المعقم PBS داخل الورم)، ومجموعة عولجت بالحقن داخل الورم بالـ LPS بتركيز ١٠٠ ميكروغرام/مل ($100 \mu\text{g/mL}$ بحجم ٥٠ ميكرو لتر)، ومجموعة السيطرة الموجبة التي عولجت بالحقن داخل الورم بعقار الدوكسوروبيسين بتركيز ١٠ ملغم/مل (10 mg/mL بحجم ٥٠ ميكرو لتر). استمرت فترة المعالجة لمدة ١٤ يوماً بمعدل جرعة واحدة كل ٣ أيام (إجمالي ٥ جرعات داخل الورم). عقب استكمال بروتوكول العلاج، استؤصلت الأورام لإجراء التقييم المرضي



النسجي المصبوغ بالهيماتوكسيلين والإيوسين (H&E)، بالتوازي مع القياس الكمي لمعدل الموت الخلوي المبرمج باستعمال صبغة (Annexin V / PI) التآقية.

النتائج: كشف الفحص النسيجي لشرائح أورام مجموعة الـ LPS عن حدوث تفكك بنيوي واسع للنسيج الورمي، وتناقص ملحوظ في الكثافة الخلوية مما يعكس تثبيطاً فعالاً لتكاثر الورم، مع ظهور مساحات واسعة من التخر التخريري، وتغيرات نووية تنكسية بارزة شملت تغلط النوى (Pyknosis) وتفتتها (Karyorrhexis)، بخلاف مجموعة السيطرة غير المعالجة التي حافظت على بنيتها المتراصة ذات النوى الشاذة مفرطة الانقسام. كما أظهر القياس التآقي لصبغة Annexin V تقوفاً إحصائياً معنوياً واضحاً لمجموعة الـ LPS في رفع معدل الاستماتة الخلوية الكلية بنسبة بلغت (٦٥.٠٠ ± ٠.١٣%)، مقارنة بمجموعة الدوكسوروبيسين (٣٣.١٠ ± ٠.٣٤%) ومجموعة السيطرة غير المعالجة (٣.٤٠ ± ٠.٢٢%) بقيمة معنوية عالية ($P > ٠.٠٠٠٠١$).

الاستنتاج: أثبتت السكريات الدهنية المتعددة (LPS) قدرة فائقة وفعالية مضادة للأورام من خلال تحفيز الاستماتة الشديدة وإحداث التخر النسيجي الانتقائي داخل الكتلة الورمية، متفوقة على المعالجة بالدوكسوروبيسين بالتراكيز المختبرة، مما يبرز الأهمية العلاجية لاستعمال مشتقات السموم البكتيرية كبديل مناعي واعد في استهداف الأورام الصلبة المقاومة.

الكلمات المفتاحية: سرطان الثدي؛ خلايا MCF-7؛ السكريات الدهنية المتعددة (LPS)؛ الدوكسوروبيسين؛ الاستماتة؛ الفحص النسيجي؛ الفئران العارية؛ دراسة داخل الجسم الحي.

Histological and Apoptotic Evaluation of Transplanted Human Breast Adenocarcinoma (MCF-7) Treated with Bacterial Lipopolysaccharides: An In Vivo Xenograft Study

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ABSTRACT

Background & Aim: Breast cancer (BC) remains the most prevalent malignancy and the primary cause of cancer-related mortality among females worldwide and across Iraq. Overcoming chemotherapeutic resistance necessitates the investigation of targeted immunogenic endotoxins. The aim of this study was to evaluate the histopathological



efficacy and quantify the apoptotic induction triggered by direct intratumoral administration of bacterial lipopolysaccharides (LPS) derived from *Escherichia coli* compared to conventional doxorubicin chemotherapy in an *in vivo* human breast adenocarcinoma (MCF-7) athymic nude mouse xenograft model.

Materials and Methods: Female athymic BALB/c nude mice (6–8 weeks old, weighing 20 ± 2 g) were transplanted subcutaneously in the right flank with 1×10^6 MCF-7 cells. Upon establishment of palpable solid tumors (14 days post-inoculation), mice were assigned to three experimental groups ($n = 3$ mice per group): an untreated negative vehicle control group (receiving sterile phosphate-buffered saline, PBS), an LPS-treated group (100 $\mu\text{g/mL}$, administered in 50 μL intratumorally), and a positive control group treated with doxorubicin (10 mg/mL , administered in 50 μL intratumorally). The treatment regimen was sustained for 14 days, administered every 3 days for 5 intratumoral injections. Harvested tumor specimens were processed for histopathological examination using hematoxylin and eosin (H&E) staining, alongside quantitative evaluation of programmed cell death rates using the Annexin V / Propidium Iodide (PI) fluorescence apoptosis assay.

Results: Histopathological analysis demonstrated profound tumor architecture disruption, massive cellular depletion indicative of marked tumor proliferation arrest, confluent coagulative necrosis, pyknosis, and karyorrhexis within the LPS-treated cohort. In contrast, doxorubicin induced moderate, localized degenerative changes, while untreated control sections displayed dense sheets of pleomorphic, hyperchromatic malignant cells with high mitotic activity. Quantitative Annexin V/PI apoptotic assessment revealed a statistically significant increase in total apoptotic rates in the LPS group ($65.00 \pm 0.13\%$) compared to the doxorubicin cohort ($33.10 \pm 0.34\%$) and the untreated vehicle control ($3.40 \pm 0.22\%$) ($P < 0.0001$).

Conclusion: Direct intratumoral administration of bacterial LPS at 100 $\mu\text{g/mL}$ exhibited potent oncolytic and pro-apoptotic efficacy against solid MCF-7 xenografts, demonstrating superior tumor destruction and apoptotic induction over conventional doxorubicin under the investigated conditions. These findings support the potential application of bacterial endotoxin



derivatives as promising immunotherapeutic agents against solid refractory breast carcinomas.

Keywords: Breast Cancer; MCF-7; Lipopolysaccharides (LPS); Doxorubicin; Apoptosis; Histopathology; Nude Mice Xenograft; In Vivo Study.

1. INTRODUCTION

Breast cancer (BC) represents the most frequently diagnosed oncological disease and the leading cause of cancer mortality in women globally. In Iraq, breast cancer constitutes the foremost female malignancy, accounting for approximately 34% of all registered female cancer cases and remaining the leading contributor to female cancer deaths according to official records from the Iraqi Cancer Board and the World Health Organization (Iraqi Cancer Board, 2019; Alwan & Afshari, 2022). While conventional therapeutic approaches, including surgical intervention, endocrine therapy, external beam radiotherapy (RT), and systemic chemotherapy (CT), have extended overall survival, long-term clinical efficacy is often undermined by intrinsic or acquired chemoresistance, drug-induced organ toxicity, and secondary metastatic dissemination (García et al., 2016).

Evasion of programmed cell death (apoptosis) is an established hallmark of aggressive malignant neoplasms. Intrinsic resistance to classic chemotherapeutic agents—such as the anthracycline doxorubicin—is predominantly orchestrated by the disruption of balanced pro- and anti-apoptotic signaling cascades within the mitochondrial pathway (Askari et al., 2020). Pro-apoptotic mediators such as Bax and Bak promote mitochondrial outer membrane permeabilization, facilitating cytochrome c release and subsequent activation of initiator caspase-9 and downstream executioner caspases-3 and -7 (Yang et al., 2014; Prathyusha et al., 2022). However, aberrant upregulation of anti-apoptotic factors and multidrug efflux transporters regularly restricts the efficacy of standard chemotherapy (SCT), emphasizing the clinical imperative for novel bioactive formulations capable



of reactivating apoptotic pathways through alternative molecular mechanisms.

To circumvent chemoresistance, therapeutic interest has increasingly centered on microbial-derived immunostimulatory molecules. Inspired by early clinical observations from Coley's toxins, the antitumor utility of bacterial components exploits localized host immune activation coupled with direct cytotoxicity (Chen et al., 2015). Lipopolysaccharides (LPS), the predominant macromolecular glycolipid residing on the outer membrane of Gram-negative bacteria, act as potent pathogen-associated molecular patterns (PAMPs) specifically recognized by Toll-like receptor 4 (TLR4) (Hong et al., 2015). Although systemic dissemination of LPS triggers dangerous inflammatory endotoxemia, controlled and localized intratumoral delivery has been shown to induce vascular disruption, recruit localized tumoricidal effector cells, and stimulate endogenous production of tumor necrosis factor- α (TNF- α) and reactive oxygen species (ROS), thereby selectively precipitating tumor cell death (Huang et al., 2013; Nokhandani et al., 2020).

Despite these known properties, precise comparative in vivo investigations evaluating detailed histopathological transformations alongside quantitative apoptotic indices induced by localized intratumoral LPS delivery versus standard doxorubicin in established human breast adenocarcinoma xenografts remain scarce. Therefore, this study was designed to evaluate histopathological tumor response and quantitatively determine the apoptotic induction achieved by intratumoral administration of LPS (100 μ g/mL) compared with doxorubicin (10 mg/mL) in an in vivo MCF-7 xenograft nude mouse model.

2. METHODOLOGY

2.1. Ethics Statement and Experimental Animals

Female athymic nude mice (BALB/c nude, aged 6–8 weeks, weighing 20 ± 2 g) were obtained from the Kut Technical Institute (Wasit, Iraq). The experimental study was conducted at the College of Science, University of Wasit. Animals were housed in pathogen-free, individually ventilated microisolator cages under controlled environmental conditions (temperature: 22 ± 2 °C; relative humidity: $55 \pm 5\%$; 12-hour light/dark cycle) with



unrestricted access to autoclaved commercial rodent diet and sterilized water.

2.2. Reagents and Chemicals

Purified lipopolysaccharides (LPS) derived from *Escherichia coli* (serotype O111:B4) and analytical-grade doxorubicin hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reconstitution of working solutions was conducted under sterile conditions using pyrogen-free phosphate-buffered saline (PBS, pH 7.4). The Annexin V-FITC / Propidium Iodide (PI) Apoptosis Detection Kit was obtained from standard commercial diagnostic suppliers (BD Biosciences / Sigma-Aldrich).

2.3. Cell Culture and Tumor Inoculation

The human breast adenocarcinoma cell line MCF-7 (ER-positive, PR-positive, HER2-negative) was obtained from the Cell Bank of the Pasteur Institute (Tehran, Iran). Cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cultures were maintained in a humidified incubator at 37 °C with 5% CO₂. Confluent monolayers (80–90%) were harvested using 0.25% trypsin-0.02% EDTA solution. Cellular viability and enumeration were established using the 0.4% trypan blue exclusion assay counted via a standard Neubauer hemocytometer, confirming viability exceeding 95% (Freshney, 2015).

To establish xenograft models, recipient nude mice received a subcutaneous inoculation of 1×10^6 viable MCF-7 cells resuspended in 100 µL of sterile physiological saline into the right lateral dorsal flank. Tumor progression was monitored daily. Solid, palpable subcutaneous tumor nodules developed uniformly within 14 days post-inoculation. Tumor dimensions were measured using digital vernier calipers, and tumor volume was verified using the standard formula: $\text{Volume} = (\text{Length} \times \text{Width}^2) / 2$, confirming established tumors with volumes of 100–150 mm³ prior to treatment allocation.



2.4. In Vivo Experimental Treatment Design

Following tumor confirmation on day 14, tumor-bearing mice were randomly assigned to three experimental cohorts (n = 3 mice per group):

- Group 1 (LPS Treatment Group): Received direct intratumoral injections of purified LPS at a concentration of 100 µg/mL (administered in a volume of 50 µL per mouse).
- Group 2 (Positive Control - Doxorubicin): Received direct intratumoral injections of doxorubicin hydrochloride at a concentration of 10 mg/mL (administered in a volume of 50 µL per mouse).
- Group 3 (Negative Vehicle Control): Received volume-matched intratumoral injections of sterile vehicle (50 µL pyrogen-free PBS, pH 7.4).

The therapeutic protocol was conducted over 14 days, with intratumoral injections administered every 3 days (5 doses on days 1, 4, 7, 10, and 13). Administration was executed using fine 30-gauge micro-needles inserted into multiple planes across the tumor mass to assure homogenous intramural delivery.

2.5. Histopathological Processing

Twenty-four hours following the final treatment administration, animals were humanely euthanized via carbon dioxide inhalation in conformity with standard ethical guidelines. The intact tumor masses were surgically excised, rinsed in ice-cold PBS, and fixed immediately in 10% neutral buffered formalin (NBF) for 48 hours. Tissues were dehydrated through an ascending graded series of ethanol (70%, 80%, 95%, and 100%), cleared in xylene, and embedded in paraffin wax. Serial sections were cut at 4–5 µm using a rotary microtome (Leica Biosystems), mounted on silane-coated glass slides, deparaffinized, rehydrated, and stained with Hematoxylin and Eosin (H&E) following standardized histology protocols (Bancroft & Layton, 2018). Morphological alterations—including architectural organization, cellular density, nuclear pleomorphism, pyknosis, karyorrhexis, and areas of necrotic degeneration—were evaluated under an Olympus BX53 high-resolution brightfield light microscope fitted with a digital imaging camera.



2.6. Quantitative Apoptosis Determination (Annexin V / PI Fluorescence Assay)

Quantitative detection of apoptotic versus necrotic populations was executed using the Annexin V-FITC / PI dual staining assay (Vermes *et al.*, 1995). Freshly excised tumor specimens were enzymatically disaggregated with collagenase type IV (1 mg/mL) in serum-free RPMI medium at 37 °C for 30 minutes, followed by gentle mechanical dissociation. Cell suspensions were passed through a 70 µm nylon mesh strainer and centrifuged at 1200 rpm for 5 minutes. Pelleted cells were washed twice with cold PBS and resuspended in 1× Binding Buffer at a density of 1×10^6 cells/mL. Tumor cells were co-incubated with 5 µL Annexin V-FITC and 5 µL Propidium Iodide (PI) in the dark for 15 minutes at room temperature (25 °C). Quantitative fluorescence was evaluated immediately using fluorescence microscopy and spectrometry to determine the percentage of total apoptotic cells (Annexin V-positive cells, encompassing both early apoptotic Annexin V+/PI- and late apoptotic Annexin V+/PI+ subsets).

2.7. Statistical Analysis

Quantitative data are presented as Mean $\pm$ Standard Deviation (SD). Statistical significance among the three experimental cohorts was calculated by One-Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc multiple comparison test using GraphPad Prism software (version 9.0). A P-value of < 0.05 was considered statistically significant.

3. RESULTS

مجلة العلوم الأساسية
للعلوم التربوية والنفسية وطرائق التدريس للعلوم الأساسية

3.1. HISTOPATHOLOGICAL FINDINGS

Histological evaluation of primary MCF-7 tumor sections stained with H&E demonstrated marked morphological variations between the untreated control and the respective therapeutic groups (Figure 1):

• **Group 3 (Untreated Negative Control Group):** Tumor sections displayed characteristic, aggressive malignant architecture of untreated MCF-7 breast adenocarcinoma. The tissue exhibited densely packed, hypercellular sheets and clusters of atypical epithelial cells embedded within sparse fibrovascular stroma. Nuclei were significantly enlarged, hyperchromatic, and pleomorphic, exhibiting prominent nucleoli and

frequent abnormal mitotic figures. Cytoplasmic boundaries were intact with moderate basophilia, displaying negligible spontaneous tissue necrosis or apoptotic body formation [Figure 1c].

• **Group 2 (Positive Control - Doxorubicin, 10 mg/mL):** Tumor sections showed moderate degenerative changes characterized by localized areas of cellular shrinkage, loss of intercellular cohesion, and modest necrotic regions. Malignant nuclei exhibited early pyknosis (nuclear condensation), alongside surviving tumor cell islands that maintained viable architecture, indicating partial antineoplastic response [Figure 1b].

• **Group 1 (LPS Treatment Group, 100 µg/mL):** Histological sections exhibited extensive architectural disruption, widespread cellular depletion, and prominent confluent zones of coagulative necrosis. Neoplastic cells in this cohort displayed classic hallmarks of advanced apoptosis and cell death, including profound nuclear pyknosis, karyorrhexis (nuclear fragmentation), cell rounding, and marked cytoplasmic eosinophilia. The viable tumor cell density was considerably lower than that observed in either the doxorubicin- or vehicle-treated cohorts, demonstrating robust oncolytic and antiproliferative efficacy [Figure 1a].

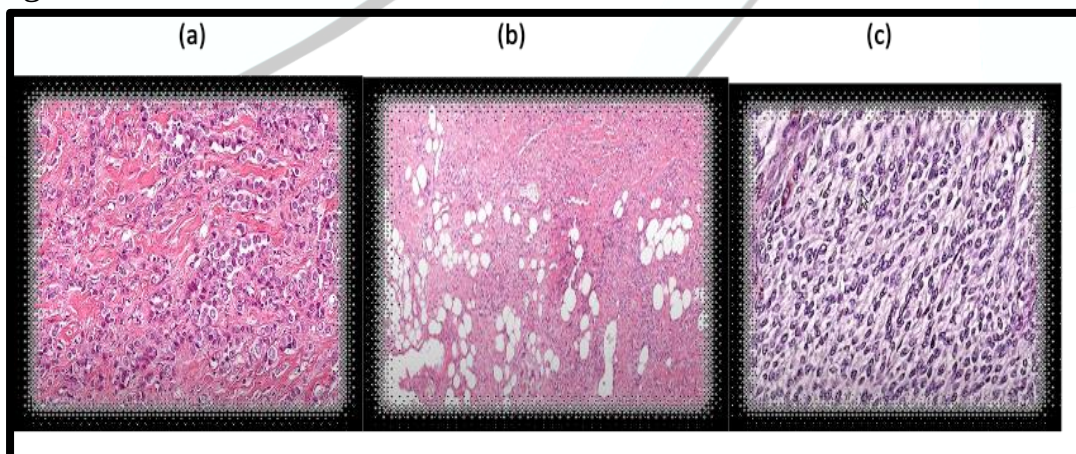


Figure 1: Representative photomicrographs of H&E-stained MCF-7 breast adenocarcinoma xenograft sections: (a) Group 1 (LPS-treated group, 100 µg/mL): displaying extensive coagulative necrosis, profound cellular loss,



pyknotic nuclei, and marked karyorrhectic fragments; (b) Group 2 (Positive control - Doxorubicin, 10 mg/mL): exhibiting moderate cell shrinkage, focal necrotic areas, and partial nuclear condensation alongside surviving malignant tumor clusters; (c) Group 3 (Untreated negative control group): showing dense hypercellular malignant morphology, atypical pleomorphic hyperchromatic nuclei, high mitotic rate, and intact cellular organization. (H&E stain; original magnification: 400×; scale bar = 50 μ m).

3.2. QUANTITATIVE APOPTOSIS ASSESSMENT (ANNEXIN V / PI FLUORESCENCE ASSAY)

Quantitative evaluation of apoptotic cell death using Annexin V/PI dual fluorescence staining demonstrated that local intratumoral LPS administration induced a pronounced cytotoxic and pro-apoptotic response that significantly exceeded both the positive control and untreated cohorts (Table 1 and Figure 2).

In the LPS-treated group (100 μ g/mL), the total apoptotic rate reached $65.00 \pm 0.13\%$, approximately double the apoptotic efficacy of the standard chemotherapeutic doxorubicin group ($33.10 \pm 0.34\%$). Conversely, the untreated vehicle control cohort exhibited only baseline spontaneous apoptosis ($3.40 \pm 0.22\%$). Fluorescence microscopic analysis confirmed that the LPS-treated cells exhibited intense green surface fluorescence corresponding to Annexin V binding to translocated phosphatidylserine, with a high proportion demonstrating dual orange-red nuclear PI staining, characteristic of advanced apoptosis and secondary cellular breakdown.



Table 1: Comparative quantitative evaluation of apoptotic index (Annexin V/PI) across experimental groups in MCF-7 xenograft-bearing nude mice.

Experimental Cohort	Treatment Regimen	Intratumoral Concentration	Apoptotic Rate (%) [Mean $\pm$ SD]	Statistical Significance (P-value)
Group 1	Lipopolysaccharide (LPS)	100 μ g/mL	65.00 $\pm$ 0.13	vs. Group 2: P = 0.0003 vs. Group 3: P < 0.00001
Group 2	Doxorubicin (Positive Control)	10 mg/mL	33.10 $\pm$ 0.34	vs. Group 3: P < 0.001
Group 3	Untreated Vehicle (Negative Control)	0 μ g/mL (PBS Vehicle)	3.40 $\pm$ 0.22	Baseline Reference

One-way ANOVA with Tukey's post-hoc pairwise comparisons showed that apoptosis induction in the LPS group was statistically higher than in the doxorubicin cohort (P = 0.0003). Both active treatment regimens induced statistically significant increases in programmed cell death relative to the untreated vehicle control group (P < 0.001 and P < 0.00001, respectively).



Figure 2: Comparative bar chart depicting the apoptotic rate (%) measured via Annexin V/PI fluorescence across experimental cohorts: Group 1 (LPS 100 μ g/mL, blue bar), Group 2 (Doxorubicin 10 mg/mL, red bar), and Group 3 (Untreated vehicle control, green bar). The Y-axis quantitatively reflects the percentage of total apoptotic cells (%). Data are plotted as Mean $\pm$ SD (**P < 0.001, ***P < 0.0001).

4. DISCUSSION

Chemotherapeutic resistance in invasive breast adenocarcinoma remains a profound clinical challenge, primarily attributable to cellular survival adaptations, restricted vascular drug delivery within dense tumor microenvironments, and acquired resistance against anthracyclines such as doxorubicin (Askari et al., 2020; García et al., 2016). In this investigation, we evaluated the therapeutic potential of direct intratumoral administration of purified bacterial lipopolysaccharide (LPS, 100 μ g/mL) against established MCF-7 xenografts in athymic nude mice, focusing on histopathological restructuring and quantitative apoptosis rates compared with standard doxorubicin chemotherapy.

Our histopathological findings demonstrated that direct localized delivery of 100 μ g/mL LPS provoked profound oncolytic destruction, characterized by extensive architectural breakdown, marked depletion of viable tumor parenchyma, confluent coagulative necrosis, pyknosis, and karyorrhexis. In contrast, tumors treated with doxorubicin at 10 mg/mL exhibited moderate degenerative changes and localized apoptotic foci, with significant islands of intact malignant epithelium surviving. These microscopic observations correlated directly with our quantitative Annexin V/PI fluorescence measurements, in which LPS induced an apoptotic rate of $65.00 \pm 0.13\%$, surpassing the $33.10 \pm 0.34\%$ achieved by doxorubicin ($P = 0.0003$). This marked reduction in cell density and widespread nuclear destruction confirms that LPS effectively arrests tumor cellular proliferation and triggers mass execution of programmed cell death.

The pronounced antitumor activity mediated by LPS in this in vivo system operates through dual complementary mechanisms. Firstly, direct exposure of MCF-7 cells to high localized concentrations of LPS induces



hyperactivation of Toll-like receptor 4 (TLR4). While baseline, chronic systemic exposure to endotoxins has been associated in some settings with inflammatory cell migration and epithelial-to-mesenchymal transition (EMT) via NF- κ B signaling (Huang et al., 2013; Hong et al., 2015), acute and high localized intratumoral delivery shifts cellular balance toward massive mitochondrial membrane destabilization, excessive reactive oxygen species (ROS) production, and cytochrome c leakage (Prathyusha et al., 2022). This intense oxidative stress triggers initiator caspase-9 and executioner caspase cascades, overcoming the anti-apoptotic thresholds conferred by endogenous Inhibitor of Apoptosis Proteins (IAPs) such as survivin and XIAP (Im et al., 2015; Askari et al., 2020).

Secondly, in vivo intratumoral delivery leverages the localized host innate immune microenvironment. Although athymic nude mice lack mature T-lymphocytes, they possess fully functional innate immune effectors, including macrophages, dendritic cells, and natural killer (NK) cells. Local engagement of TLR4 by LPS stimulates tumor-associated macrophages to undergo M1 polarization, triggering localized bursts of tumor necrosis factor-alpha (TNF- α) and nitric oxide (NO) directly inside the tumor core (Won et al., 2005; Lin & Lin, 2020). Binding of endogenous TNF- α to its receptor complex activates the extrinsic apoptotic pathway via caspase-8, which subsequently truncates Bid (tBid), converging onto the intrinsic mitochondrial death pathway and markedly amplifying apoptotic signaling (Liu et al., 2017; Nokhandani et al., 2020). Importantly, this receptor-mediated, oxidative killing cascade bypasses classical drug efflux pumps (e.g., P-glycoprotein/MDR1) that commonly reduce the intracellular concentration and therapeutic efficacy of doxorubicin (Askari et al., 2020).

Our observations are in strong agreement with Nokhandani et al. (2020), who demonstrated that combining bacterial lipopolysaccharide with chemotherapeutic agents synergistically potentiates cytotoxicity and apoptotic index in MCF-7 cells via enhanced cell cycle arrest and caspase activation. Furthermore, our findings corroborate the fundamental immunological concepts established by Coley's toxins and current attenuated bacterial immunotherapies (e.g., intravesical BCG),



demonstrating that targeted bacterial products can elicit potent oncolytic and apoptotic responses within localized tumor microenvironments (Chen et al., 2015). By using the direct intratumoral route, therapeutic concentrations concentrate within the neoplastic lesion while circumventing systemic endotoxemic toxicity, providing a compelling rationale for localized endotoxin-based therapy in refractory solid malignancies.

CONCLUSIONS

The present study demonstrates that direct intratumoral administration of bacterial lipopolysaccharides (LPS) at 100 µg/mL exhibits superior antineoplastic and pro-apoptotic efficacy in an in vivo human breast adenocarcinoma (MCF-7) athymic nude mouse xenograft model compared to standard doxorubicin chemotherapy. LPS treatment induced profound histopathological disintegration, extensive coagulative necrosis, marked cellular depletion, and nuclear karyorrhexis, alongside a significantly higher quantitative apoptotic index (65.00% vs. 33.10%, $P = 0.0003$). These findings highlight the promising therapeutic potential of locally administered bacterial endotoxins as targeted cytotoxic and apoptotic-inducing agents against solid refractory breast carcinomas.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this paper.

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