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Investigation of the selective cytotoxic effect of *Lamium purpureum* extracts on MCF-7 breast cancer cells

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ABSTRACT

Objective: This study aims to evaluate the selective cytotoxicity and underlying cell death mechanisms of *Lamium purpureum* extract/fractions against MCF-7 luminal A breast cancer cells in comparison to normal mammary epithelial cells.

Materials and Methods: The cytotoxicity of five different extract/fractions of *Lamium purpureum* on MCF-7 breast cancer and HME1 mammary epithelial cells was assessed using the Cell Counting Kit-8 (CCK-8) assay. The most selective fraction was further investigated via JC-1 mitochondrial membrane potential (MMP) analysis and Annexin V/Propidium Iodide (PI) double staining to determine the precise mode of cell death.

Results: The *Lamium purpureum* n-hexane (LPH) fraction showed the highest cytotoxicity against MCF-7 cells (IC50 = 30.41 µg/mL) with a favorable selectivity index (SI) of 1.39. Mechanistic assays revealed that LPH did not induce mitochondrial depolarization in MCF-7 cells. Annexin V/PI staining confirmed a dose-dependent induction of primary necrotic cell death. This membrane collapse is consistent with the lipid-based bioactive constituents of *Lamium purpureum*.

Conclusion: The LPH fraction acts as a selective cytotoxic agent that bypasses classical mitochondrial apoptosis in MCF-7 cells, thereby executing a dominant necrotic cell death pathway.

Keywords: Breast cancer, *Lamium purpureum*, Cytotoxicity

1. INTRODUCTION

Breast cancer is the most frequently diagnosed cancer among women and ranks as the second leading cause of cancer-related mortality [1]. The biological structure of breast cancer is remarkably complex. This heterogeneity primarily arises from the presence of various molecular subtypes, each exhibiting distinct clinical behaviors, prognosis, and therapeutic responses [2]. Clinically, breast cancer is divided into four molecular subtypes: luminal A, luminal B, HER2-positive, and triple-negative. Among these, luminal A is the most common subtype found in breast cancer cases [3]. Consequently, *in vitro* models representing this phenotype (e.g., MCF-7 cell line) serve as fundamental tools for evaluating novel therapeutic strategies.

Standard therapeutic strategies for breast cancer currently integrate surgery, radiation, chemotherapy, and endocrine therapy. These traditional treatment methods can cause side effects such as fatigue, nausea, hair loss, skin irritation, and lymphedema [4]. In addition, drug resistance develops with the long-term use of chemotherapeutic drugs [5]. However, the anticancer potential of plant-derived natural products has emerged as a rapidly growing area of scientific interest [6].

The anticancer properties of the Lamiaceae family have been extensively studied. These plants have a variety of bioactive compounds, such as flavonoids, phenolic acids, terpenoids, and essential oils. These bioactive compounds are known to inhibit the proliferation of cancer cells [7]. Within this family, *Lamium purpureum* has a long history in traditional medicine because of its remarkable anti-inflammatory, antibacterial, and antioxidant properties. Despite its ethnopharmacological background,

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research on the anticancer effects of *Lamium purpureum* against breast cancer remains strictly limited [8].

To address this gap in the literature, the present study aims to evaluate the anticancer potential of *Lamium purpureum* extracts and their respective fractions. The cytotoxic effects of these fractions on MCF-7 luminal A breast cancer and HME-1 mammary epithelial cells were determined using the (CCK-8) assay. The precise cell death mechanisms of the most potent fraction were investigated through JC-1 mitochondrial membrane potential (MMP) analysis and Annexin V/Propidium Iodide (PI) double staining. This is the first study to elucidate the cytotoxic mechanisms of *Lamium purpureum* against breast cancer.

2. MATERIALS and METHODS

Preparation of plant extracts

Lamium purpureum extract/fractions—comprising ethanol (LPE), aqueous ethanol (LPAE), chloroform (LPC), ethyl acetate (LPEA), and n-hexane (LPH)—were prepared according to previously published methodology [9]. Each extract/fraction was dissolved in 100% dimethyl sulfoxide (DMSO) to yield a 60 mg/mL stock solution. Lower working concentrations were prepared via serial dilution before application to the cells.

Cell culture

The human breast cancer cell line MCF-7 (ATCC HTB-22) and the immortalized human mammary epithelial cell line hTERT-HME1 (ATCC CRL-4010) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Both cell lines were maintained at 37°C in a humidified incubator with 5% CO_2. The culture medium was refreshed three times per week for both cell lines.

Cell viability and cytotoxicity

Cell viability and cytotoxicity were assessed after 24 hours of exposure to *Lamium purpureum* extract/fractions at different concentrations (0, 10, 25, 50, 75, 100 µg/mL) using the CCK-8 colorimetric assay (n = 3). The protocol provided by the manufacturer was followed (Enhanced Cell Counting Kit 8, E-CK-A362, Elabscience, China). The selectivity index (SI) was determined by calculating the ratio of the half-maximal inhibitory concentration (IC50) value of healthy cells to that of cancer cells [10].

Mitochondrial membrane potential (MMP) depolarization

Mitochondrial membrane potential depolarization was assessed using the JC-1 dye [11]. Briefly, cells were seeded onto 96-well black opaque plates at a density of $5x10^4$ cells/well (n = 3). LPH was applied to the cells for 24 hours. The assay was performed following the manufacturer's instructions (JC-1 Mitochondrial Membrane Potential Assay Kit, 10009172, Cayman Chemical, USA).

Annexin V/Propidium Iodide (PI) double staining

Apoptotic and necrotic cell populations were determined via Annexin V/PI double-staining [12]. Cells were seeded in 6-well plates at a density of $2x10^5$ cells/well and treated with *Lamium purpureum* extracts at various concentrations for 24 hours. Following incubation, the cells were stained according to the manufacturer's protocol (E-CK-A237, Elabscience, China). Green and red fluorescence signals were subsequently captured using an Olympus CKX41 microscope equipped with a CKX-RFA fluorescence illuminator (Olympus, Japan).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 8.01). Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Differences were considered statistically significant at *P<0.05; **P<0.01; ***P<0.001.

3. RESULTS

The IC50 and SI values of *Lamium purpureum* extract/fractions (LPE, LPAE, LPC, LPEA, LPH) on MCF-7 and HME1 cell lines after 24 hours of exposure are presented in Table I.

Table I. *IC50 values (in µg/mL) of Lamium purpureum extract/fractions on MCF-7 human breast cancer and HME-1 human mammary epithelial cell lines for 24h*

Lamium purpureum extract/fractions	Cell line		Selectivity Index (SI)
	MCF-7	HME-1	
LPE	59.98	29.62	0.49
LPAE	204.7	16.61	0.08
LPC	116.5	77.89	0.67
LPEA	63.45	40.16	0.64
LPH	30.41	42.39	1.39

LPE: L. purpureum ethanol, LPAE: L. purpureum aqueous ethanol, LPC: L. purpureum chloroform, LPEA: L. purpureum ethyl acetate, LPH: L. purpureum n-hexane

The n-hexane fraction exhibited the highest cytotoxicity against MCF-7 cells with an IC50 value of 30.41 µg/mL. The IC50 of LPH against HME-1 cells was 42.39 µg/mL, resulting in an SI of 1.39. All other fractions (LPE, LPAE, LPC, LPEA) yielded SI values below 1.0 (ranging from 0.08 to 0.67). Based on the cytotoxicity and selectivity profiles, LPH was utilized for subsequent mechanistic analyses.

Mitochondrial membrane potential was evaluated using JC-1 staining to assess the involvement of the intrinsic apoptotic pathway (Figure 1). Following a 24-hour treatment with LPH, no statistically significant change in the green/red fluorescence ratio was observed in MCF-7 cells. In contrast, LPH treatment resulted in significant mitochondrial depolarization in the HME-1 cell line.

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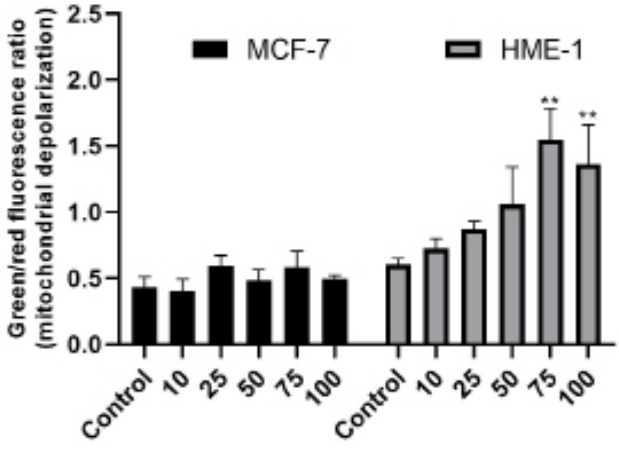


Figure 1. *Green/red fluorescence ratios for the MCF-7 human breast cancer and HME-1 human mammary epithelial cell lines for 24h **P<0.01*

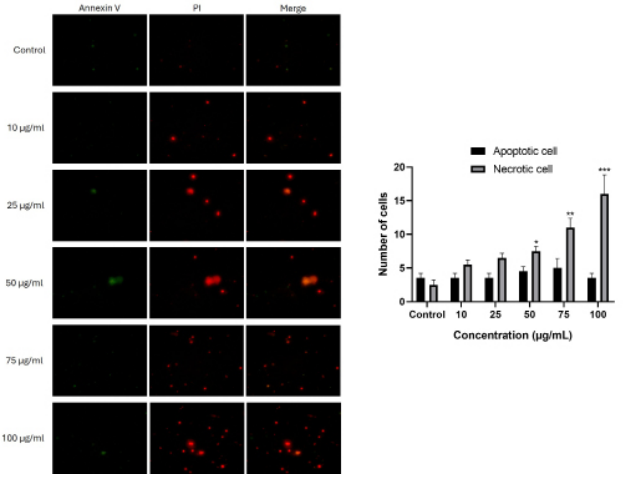


Figure 2. *Annexin V/PI staining of MCF-7 cells after 24 hours of exposure to Lamium purpureum n-hexane fraction (LPH) under fluorescence microscope *P<0.05; **P<0.01; ***P<0.001*

The cell death induced by LPH in MCF-7 cells was quantified via Annexin V/PI double staining and fluorescence microscopy (Figure 2). A 24-hour exposure to LPH (10-100 µg/mL) induced a dose-dependent increase in the number of necrotic cells. This increase was statistically significant at 50, 75, and 100 µg/mL. The population of apoptotic cells remained at basal levels, with no statistically significant increase across all tested concentrations. Fluorescence imaging demonstrated direct PI uptake, indicating a primary loss of membrane integrity.

4. DISCUSSION

One of the most important determinants of the clinical success of a chemotherapeutic candidate is its ability to selectively target tumor cells while minimizing damage to healthy cells. According to the literature, a SI greater than 1 indicates a desirable and preferential selectivity toward tumor cells over healthy cells [13]. In our study, the LPH fraction showed lower toxicity against the HME-1 mammary epithelial cell line, reaching an SI value of 1.39. In contrast, the SI values of all other fractions (LPE, LPAE, LPC, LPEA) remained below 1.0. These low SI values indicate that these fractions exert cytotoxic effects on healthy cells rather than cancer cells. Due to their non-selective toxicity, these fractions were considered inappropriate for further analysis. Therefore, among the *Lamium purpureum* fractions evaluated, only LPH demonstrated selective anticancer potential. Consequently, only the LPH fraction was subjected to MMP and Annexin V/PI staining analyses.

The JC-1 assay was employed to investigate whether LPH activates the intrinsic apoptotic pathway. No significant mitochondrial membrane depolarization was observed in MCF-7 cells following LPH treatment. These findings indicate that LPH-induced cell death is unlikely to involve the classical mitochondria-dependent apoptotic pathway. In contrast, significant mitochondrial depolarization was detected in healthy HME-1 cells at higher LPH concentrations.

Since no mitochondrial depolarization was observed in MCF-7 cells, Annexin V/PI double staining was performed under fluorescence microscopy to characterize the mode of cell death induced by LPH. Annexin V binds phosphatidylserine, which translocates to the outer membrane while preserving membrane integrity in the early stages of apoptosis. However, PI intercalates into the nuclei of necrotic cells where membrane integrity has been completely lost. As a result, PI (red fluorescence) is a marker of necrosis, and Annexin V (green fluorescence) is a marker of apoptosis. Early apoptotic cells are defined as Annexin V^+/PI^-. Necrotic cells are defined as Annexin V^-/PI^+. Cells that are Annexin V^+/PI^+ are defined as late apoptotic [12]. The quantitative results showed that LPH application triggered a clear dose-dependent trend of necrotic cell death in MCF-7 cells. In contrast, apoptotic cells remained at basal levels at all concentrations and showed no statistically significant increase.

Collectively, these findings indicate that LPH predominantly induces necrotic cell death in MCF-7 cells, while having little or no effect on the activation of the classical intrinsic apoptotic pathway.

The chemical constituents of *Lamium purpureum* have been extensively detailed in a previous study [14]. Gas chromatography-mass spectrometry (GC-MS) analyses presented in the study have identified several bioactive constituents mainly including palmitic acid, 7-tetradecenal, octadecadienoic acid, acetic acid, and stearate. The high concentration of these lipid-based compounds provides a strong mechanistic explanation for the necrotic effects observed in the MCF-7 cell line. Excess exposure to fatty acids, such as palmitic and octadecadienoic acids, is known to induce lipotoxicity. This lipotoxicity disrupts cell membrane fluidity and lipid bilayer integrity, physically lysing the cells rather than activating apoptotic pathways [15]. Since n-hexane is a highly non-polar solvent, the LPH fraction is predominantly

enriched with these lipophilic fatty acids [16]. Therefore, the targeted necrotic cell death and the rapid cellular membrane collapse in our study are highly consistent with the lipophilic bioactive profile of the LPH fraction.

Taken together, our findings demonstrate that the LPH exhibits selective cytotoxicity toward MCF-7 breast cancer cells while exhibiting minimal toxicity toward normal mammary epithelial cells. Unlike many plant-derived anticancer agents that primarily induce apoptosis, LPH appears to suppress tumor cell viability predominantly through a non-apoptotic, necrosis-associated mechanism, potentially driven by lipophilic constituents enriched in the fraction. However, this study has certain limitations, particularly the use of a single breast cancer cell line and the lack of *in vivo* tumor models. Nevertheless, comprehensive phytochemical characterization, together with analyses across diverse breast cancer cell lines and *in vivo* tumor models, will be essential for identifying the active compounds responsible for these effects and further evaluating their therapeutic potential.

Compliance with Ethical Standards

Ethical approval: Ethical approval was not required for this study, as it involves *in vitro* experiments using commercially available cell lines.

Conflict of interest: The authors declare that there is no conflict of interest.

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