Journal of Neonatal Surgery
ISSN (Online): 2226 -0439
Vol. 14, Issue 22s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Phytochemical Screening and Cytotoxic Potential of *Lantana camara* Leaf Extract against MCF-7 Cells: A Cell Morphology and MTT Assay Approach

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Cite this paper as: Y. Vishwanadham, M. Omkar Balanarsimha, B. Madhavilatha, B. Parijatha, P. Raja shekar, D. Roopa, Ch. Krishna Mohan, (2025) Phytochemical Screening and Cytotoxic Potential of Lantana camara Leaf Extract against MCF-7 Cells: A Cell Morphology and MTT Assay Approach., *Journal of Neonatal Surgery*, 14 (22s), 1083-1092.

ABSTRACT

Objective: The present study aimed to evaluate the cytotoxic potential of *Lantana camara* leaf extract against MCF-7 human breast cancer cells using the MTT assay and morphological analysis.

Methods: Dried leaves of *Lantana camara* were extracted using acetone in a Soxhlet apparatus.

Phytochemical Screening: The crude extract was screened for secondary metabolites, which confirmed the presence of flavonoids, alkaloids, phenolics, and terpenoids.

Cell Culture and Treatment: MCF-7 cells were cultured in a 96-well plate and treated with various concentrations (50, 100, and 250 µg/mL) of the extract after 12 hours of incubation.

Cytotoxicity Evaluation: Cell morphology was observed microscopically, and MTT assay was performed at 24, 48, and 72 hours post-treatment to assess cytotoxicity.

Results: The extract exhibited time- and dose-dependent cytotoxic effects. Significant morphological alterations were observed at

250 µg/mL. The IC_{50} values were determined to be 153.54 ± 0.85 µg/mL (24 h), 147.16 ± 1.13 µg/mL (48 h), and 141.98 ± 1.15 µg/mL (72 h), suggesting progressive cytotoxicity with extended exposure.

Conclusion: The findings support the potential anticancer activity of *Lantana camara*, likely due to its rich phytochemical content. Further studies are recommended to isolate active compounds and validate their efficacy in vivo.

Keywords: *Lantana camara, Acetone extract, MCF-7 cells, Cytotoxicity, MTT assay, Cell morphology*

1. INTRODUCTION

Cancer was a heterogeneous collection of diseases that were defined by abnormal cell growth and the ability to metastasize to other areas of the body. It occurred as a result of genetic mutations that interfered with normal cellular control, resulting in abnormal growth. These mutations could be initiated by causes such as genetic predisposition, environmental exposures (tobacco, radiation, and carcinogens), infections, and lifestyle. Cancer could arise in nearly any tissue, developing as solid tumors or invading the blood and immune system, as in leukemia and lymphoma. The most common types were breast

cancer, and prostate cancer. Early detection by screening and improved treatments such as surgery, chemotherapy, radiation therapy, and targeted therapies had dramatically increased survival. Despite these advances, cancer remained a leading global health problem, necessitating ongoing research, education, and preventive measures.

1.1 Breast cancer

Breast cancer was defined as a malignancy that originated in the tissues of the breast. It typically presented with signs such as palpable lumps, changes in breast shape, dimpling of the skin, nipple discharge, recent nipple inversion, rejection during breastfeeding, and red or scaly patches on the skin. As one of the leading causes of cancer-related deaths worldwide, breast cancer contributed significantly to the global disease burden, with approximately 19.3 million new cases and an estimated 10 million deaths reported in 2020. Among these, breast cancer accounted for around 2.3 million new cases, representing 11.7% of all diagnosed cancers, making it the most frequently occurring cancer and the fifth leading cause of cancer mortality globally.

Most breast cancers originated from the epithelial lining of the milk ducts or the lobules that supply the ducts. Tumors arising from the ducts were termed ductal carcinomas, while those developing in the lobules were known as lobular carcinomas. A definitive diagnosis was typically established through histopathological examination of biopsy specimens.

Once diagnosed, further investigations were conducted to assess the extent of disease spread and to formulate an appropriate treatment plan.

The most common subtypes of invasive breast cancer (IBC) included invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC). In IDC, cancer cells began in the milk ducts and infiltrated surrounding breast tissues, often spreading via the lymphatic and circulatory systems. This subtype accounted for approximately 70–80% of all invasive breast cancers. ILC, on the other hand, originated in the milk-producing lobules and was generally more challenging to detect during early stages. In addition to these, other aggressive variants such as triple-negative breast cancer—characterized by the absence of estrogen, progesterone, and HER2 receptors—and inflammatory breast cancer, which involved blockage of lymphatic vessels in the skin, also posed serious clinical challenges due to their rapid progression and limited treatment options.

1.2 MCF-7:

MCF-7 cells exhibited a cobblestone-like morphology and typically grew as monolayers with strong intercellular adhesion. These cells formed microtissue structures that included luminal spaces.

Size: The diameter of MCF-7 cells ranged between approximately 19.9 µm and 33.9 µm.

Growth: After 24 hours in culture, the cells tended to aggregate into small clusters forming microtissues. By the third day, they began to develop luminal structures, which became more distinct and filled with PAS-positive secretions by the fifth day of incubation.

Additional Characteristics: MCF-7 cells demonstrated a low capacity for metastasis and were considered less aggressive than hormone-independent lines such as MDA-MB-231. They retained several characteristics of differentiated mammary epithelial cells and expressed specific genes, including the WNT7B oncogene. Due to their well-defined phenotype and reproducibility, MCF-7 cells were widely used as an in vitro model for breast cancer studies, particularly in the development and testing of chemotherapeutic agents and in research on drug resistance mechanisms.

1.3 Plant information

Lantana camara, a member of the Verbenaceae family, was an ornamental shrub that had naturalized as an invasive weed in many parts of India. The plant featured curved prickles along its branches, grew to a height of approximately 2–3 meters, and spread its branches laterally across 1–2 meters. Its mature leaves appeared dull green, had a rough texture, emitted an unpleasant odor, and caused skin irritation upon contact. These leaves typically measured between 5 and 9 cm in length. The plant bore young flowers arranged in sub-umbellate clusters, while its fruits were drupes measuring around

0.5 cm in diameter—initially green, turning dark blue upon ripening. Flowering usually occurred between April and May, followed by fruiting that continued through November to December.

Traditionally, every part of *Lantana camara* was employed in various medicinal practices across the world. The leaves were used for their reported antitumor, antibacterial, and antihypertensive properties, while the roots were applied in folk medicine for the treatment of ailments such as malaria and rheumatism.

Figure-1: *Lantana camera* leaves

2. MATERIAL AND METHODS

2.1 ***Plant Material:***

Leaves of *Lantana camara* were collected from Narapally in January 2025. The harvested leaves were thoroughly washed, then air-dried at room temperature for seven days. Once completely dried, they were ground into a fine powder using a mortar and pestle. The powdered plant material was stored in a sealed plastic bag until further use for extraction.

2.2 ***Plant extract:***

Approximately 50 grams of powdered *Lantana camara* leaves were subjected to extraction using acetone as the solvent for 72 hours through the Soxhlation method. The resulting acetone extract was then concentrated by evaporating the solvent under reduced pressure. The viscous crude extract obtained was collected and transferred into a clean, labeled vial and stored appropriately for subsequent analyses, including phytochemical screening and evaluation of cytotoxic potential.

2.3 ***Phytochemical Analysis:***

• Test for Tannins/Polyphenols:

A few drops (3–4) of 10% ferric chloride solution were added to the diluted plant extract. The appearance of a blue coloration indicated the presence of gallic tannins, while a green coloration suggested the presence of catechol tannins.

• Test for Reducing Sugars:

To 0.5 mL of plant extract, 1 mL of distilled water and 5–8 drops of Fehling's solution were added, followed by gentle heating. The formation of a brick-red precipitate confirmed the presence of reducing sugars.

• Test for Quinones:

Freshly prepared 1 mL of $FeSO_4$ solution and a few drops of ammonium thiocyanate were added to the extract, followed by the gradual addition of concentrated sulfuric acid. The development of a deep red color indicated the presence of quinones.

• Test for Glycosides (Molisch's Test):

To the extract, 5 mL of Molisch's reagent and concentrated sulfuric acid were added carefully. The formation of a violet ring at the interface confirmed the presence of glycosides.

• Test for Flavonoids (Shinoda Test):

Four milliliters of extract were mixed with 1.5 mL of 50% methanol and a small magnesium ribbon. After warming, 5–6 drops of concentrated HCl were added. A red coloration indicated the presence of flavonoids.

• Ammonia Test for Flavonoids:

Five milliliters of the extract were treated with dilute ammonia, followed by the addition of concentrated sulfuric acid. The appearance of a yellow precipitate confirmed the presence of flavonoids.

• Test for Terpenoids:

Approximately 0.2 g of the extract was mixed with 2 mL of chloroform, followed by the addition of 3 mL of concentrated H_2SO_4. A reddish-brown interface indicated the presence of terpenoids.

- Tests for Alkaloids:
- Mayer's Test: To 2 mL of the extract, 1 mL of Mayer's reagent was added. The formation of a pale yellow precipitate confirmed the presence of alkaloids.
- Dragendorff's Test: The extract was acidified with 2% H_2SO_4 and warmed. A few drops of Dragendorff's reagent were added, resulting in an orange-red precipitate, indicating the presence of alkaloids.
- Test for Saponins:

Two grams of powdered sample were boiled in 20 mL of distilled water. After filtration, 5 mL of the filtrate was vigorously shaken with distilled water. The formation of persistent froth indicated the presence of saponins.

- Test for Volatile Oils:

Two milliliters of the extract were mixed with 0.1 mL of sodium hydroxide and a few drops of dilute hydrochloric acid. The appearance of a white precipitate indicated the presence of volatile oils.

- Test for Cardiac Glycosides:

Five milliliters of plant extract were treated with 2 mL of glacial acetic acid and one drop of ferric chloride solution, followed by the careful addition of concentrated sulfuric acid. The formation of a violet or green ring suggested the presence of cardiac glycosides.

- Test for Steroids:

One gram of the plant extract was dissolved in a few drops of acetic acid, followed by the addition of concentrated sulfuric acid. The appearance of a green coloration indicated the presence of steroids.

2.4 Biological Screening

2.4.1 Cell culture:

MCF-7 cells were sub-cultured in-house at ACTREC, with the original cell line procured from ATCC. The cells were maintained in 75 cm^2 canted-neck vented flasks (Corning) containing Dulbecco's Modified Eagle's Medium (DMEM; Gibco Invitrogen, Paisley, UK), and incubated under a humidified atmosphere with 5% CO_2 at 37°C. Cell cultures (passage numbers 30–50) were supplemented with 10% fetal Jovine serum, 1% non-essential amino acids, 1% penicillin (1000 U/mL), 1% streptomycin (1000 µg/mL), and 1% amphotericin B (250 U/mL).

Cells were enzymatically dissociated using 0.25% trypsin with 1 mM EDTA and sub-cultured into 75 cm^2 plastic flasks at a seeding density of 2.2×10^4 cells/cm^2. The culture medium was refreshed every 48 hours. Cell confluence was monitored microscopically and treatment was initiated after 12 hours of seeding to minimize spontaneous differentiation.

2.4.2 Cell Morphologystudies:

Screening of test samples (LC 50, 100, 250µg / m l) against Morphological screening of MCF-7 Cells:

MCF-7 cells were treated 12 hours post-seeding with varying concentrations (50, 100, and 250 µg/mL) of the test extract (LC) for morphological analysis. Cell morphology was examined at 24, 48, and 72 hours post-treatment. Microscopic observations were performed using an Axiovert 200M phase-contrast microscope at 10× magnification, and images were captured using Axiovision Rel software.

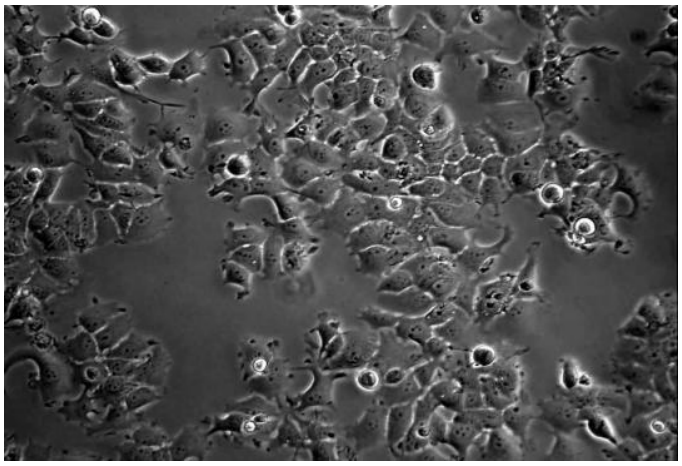


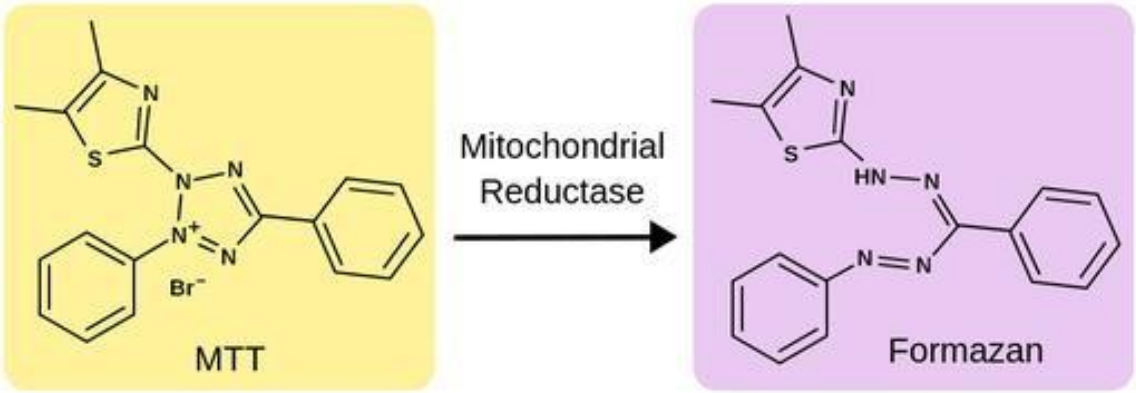
Figure 2: MCF-7 Cell lines

2.4.3 MTT assay:

MTT Assay Principle:

The MTT cell proliferation assay employed a colorimetric approach to evaluate and monitor cellular viability and growth. The assay utilized reagents sufficient to perform up to 960 tests in 96-well plates or 192 tests in 24-well plates. After seeding, cells were treated with various compounds that could potentially influence proliferation. Viable cells converted the yellow tetrazolium salt (MTT) into insoluble purple formazan crystals through the action of mitochondrial dehydrogenase enzymes.

An increase in formazan intensity reflected enhanced cell proliferation, whereas a decrease indicated cytotoxic effects or compromised growth conditions. This assay method was broadly applicable to a wide range of eukaryotic cells, including both adherent and suspension types, and could also be adapted for use with specific tissues. Additionally, the reagent had proven useful in evaluating proliferation in microorganisms such as bacteria, yeast, fungi, protozoa, and even in cultured mammalian and fish cell lines.



Assay Protocol:

- MCF-7 cells were plated at a density of 1 × 10^5 cells per well in 100 µL of culture medium in 96-well clear-bottom tissue culture plates and allowed to adhere for 24 hours.
- After incubation, cells were treated with various concentrations of the test extract (LC) ranging from 10 to 300 µg/mL (10, 50, 100, 150, 200, 250, and 300 µg/mL), each in triplicate. An additional 20 µL of fresh culture medium was added to each well to ensure uniform volume across all wells. The treated plates were incubated for 24, 48, and 72 hours.
- Following the incubation period, the media were carefully aspirated, and the cells were gently washed twice with phosphate-buffered saline (PBS) to remove any residual compounds.
- Subsequently, 15 µL of MTT reagent (prepared in PBS to a final concentration of 0.5 mg/mL) was added to each well. The plates were incubated at 37°C for 3 hours until visible intracellular purple formazan crystals developed.
- The MTT solution was then discarded, and 100 µL of DMSO was added to each well to solubilize the formazan crystals. The plates were gently agitated on an orbital shaker for one hour at room temperature.
- Finally, the absorbance of each well was measured at 570 nm using a microplate reader. The optical density values were used to calculate the percentage of viable cells in each treatment group compared to the untreated control.

3. RESULTS AND DISCUSSION

3.1 ***Phytochemical analysis:***

The acetone extract of *Lantana camara* leaves was subjected to preliminary phytochemical screening, and the results are presented in Table 1. The analysis confirmed the presence of several classes of secondary metabolites, including tannins/polyphenols, glycosides, flavonoids, terpenoids, alkaloids, cardiac glycosides, and steroids. Tests for reducing sugars, quinones, saponins, volatile oils, and quinoline-type alkaloids were found to be negative.

These findings support earlier reports that *Lantana camara* contains diverse bioactive phytochemicals, many of which are known to exhibit anticancer, antioxidant, and anti-inflammatory activities. The presence of flavonoids and terpenoids in particular may have contributed significantly to the observed cytotoxic effects.

S.NO	TESTS	Acetone Solvent Extraction
1	Test of tannins/polyphenol	+
2	Test for reducing sugar	-
3	Test for quinine	-
4	Test for glycosides	+
5	Test for flavonoids	+
6	Dil. NH3 test	+
7	Test for terpenoids	+
8	Test for alkaloids	+
9	Dragondraff's reagent test	-
10	Test for saponins	-
11	Test for volatile oils	-
12	Test for cardiac glycosides	+
13	Test for steroids	+

Table-1: Phytochemical analysis of LC extract

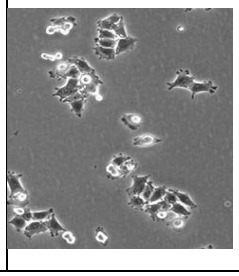
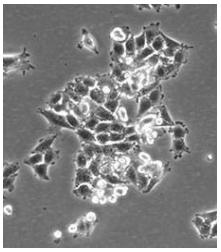
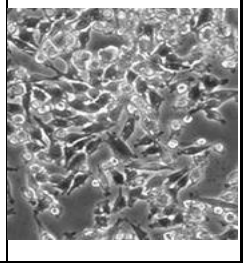
3.2 ***Cell morphology:***

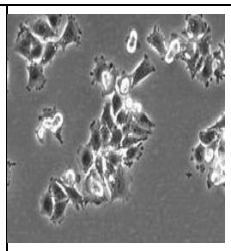
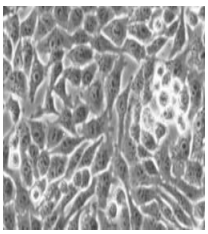
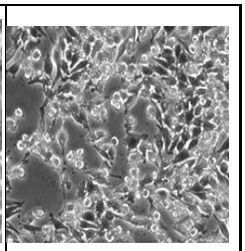
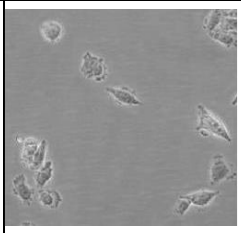
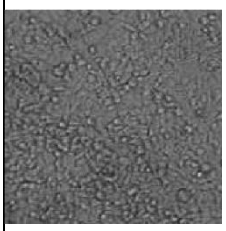
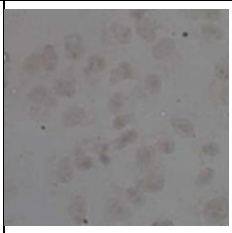
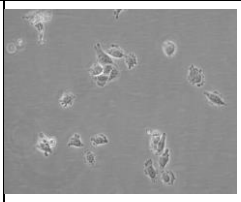
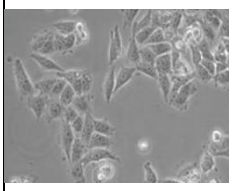
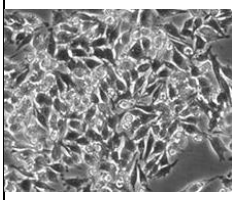
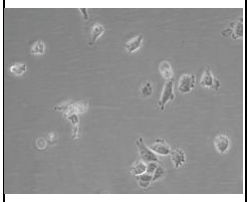
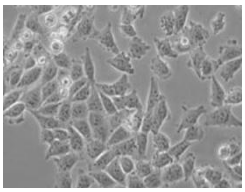
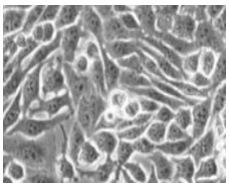
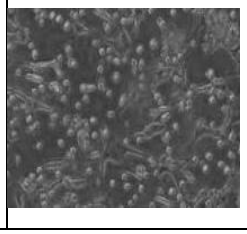
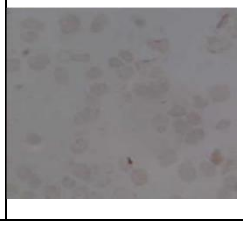
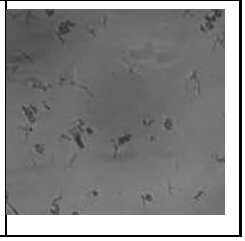
Morphological observations of MCF-7 cells treated with the LC extract (50, 100, and 250 µg/mL) were performed at

24, 48, and 72 hours post-treatment. Changes in cell morphology were compared with control and positive control (Cisplatin at 50 µM) groups. Untreated control cells retained normal morphology, while cells exposed to higher extract concentrations showed typical cytotoxic changes such as rounding, shrinkage, and detachment from the surface (Table - 2).

These morphological alterations became more prominent with increased exposure duration and concentration, particularly at 250 µg/mL, suggesting induction of apoptosis or necrosis-like cell death. The results correlate with the phytochemical profile of the extract, reinforcing the potential of *Lantana camara* constituents in disrupting cancer cell viability.

Table-2: Cell Morphological studies against MCF-7 cells

S.No	Compounds	Morphological Examination against MCF-7		
		24 hr's	48 hr's	72 hr's
1	Control			

2	**Negative control**			
3	**Positive control (Cisplatin @ 50 µM)**			
4	**LC treatment @ 50 µg/ml**			
5	**LC treatment @ 100 µg/ml**			
6	**LC treatment @ 250 µg/ml**			

3.3 ***MTT assay:***

The cytotoxic effect of the *Lantana camara* leaf extract on MCF-7 cells was quantitatively evaluated using the MTT assay at 24, 48, and 72-hour intervals. As shown in Table-3, cell viability decreased progressively with increasing extract concentrations and exposure durations, indicating a clear dose- and time-dependent cytotoxic response.

The IC_{50} values were calculated to be 153.54 ± 0.85 µg/mL at 24 hours, 147.16 ± 1.3 µg/mL at 48 hours, and 141.98 ± 1.15 µg/mL at 72 hours, respectively. In comparison, the standard chemotherapeutic agent Doxorubicin showed significantly lower IC_{50} values, highlighting its higher potency, but also supporting the moderate efficacy of the natural extract. These findings are consistent with the morphological observations and phytochemical data, indicating that the acetone extract of *Lantana camara* possesses bioactive compounds capable of inhibiting breast cancer cell growth.

Table-3: Cell cytotoxicity studies.

Conc in µg/ml	% Viability at 24 hrs	% Viability at 48 hrs	% Viability at 72 hrs
10	90.12	86.24	86.03
50	73.25	71.79	70.15
100	57.17	55.83	54.24

150	43.62	41.15	40.08
200	37.06	35.12	33.64
250	21.36	19.51	17.53
300	17.05	16.97	15.31
IC50 in µg/ml	**IC50 = 153.54 ± 0.85**	**IC50 = 147.16 ± 1.3**	**IC50 = 141.98± 1.15**
Doxorubicin	IC50 = 52.37 ± 0.7 µM	IC50 = 49.13 ± 0.5 µM	IC50 = 48.62 ± 0.4 µM

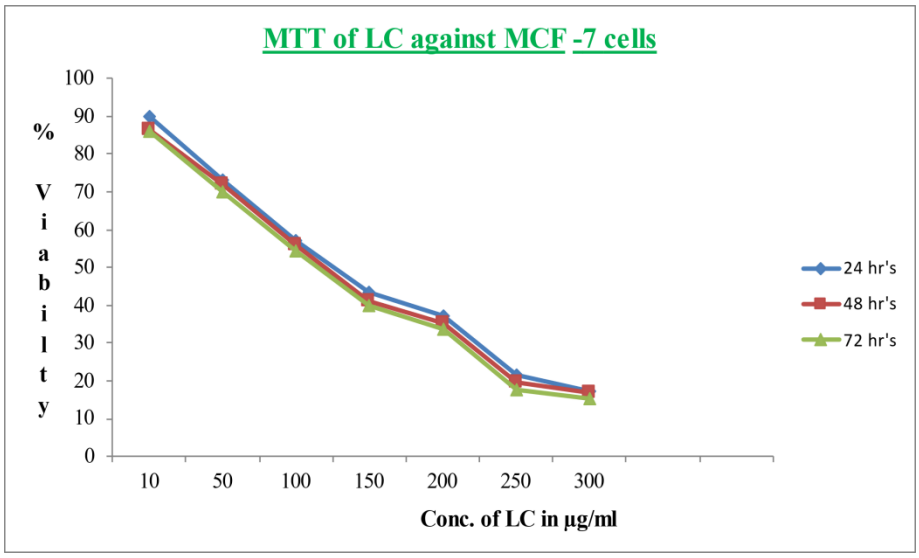


Figure-3: Cell cytotoxicity studies

4. CONCLUSIONS

This study demonstrated that the crude acetone extract of *Lantana camara* leaves exerted significant cytotoxic effects on MCF-7 breast cancer cells in a concentration- and time-dependent manner. The observed activity may be attributed to the presence of bioactive phytochemicals such as flavonoids, alkaloids, phenolics, and terpenoids. The progressive decrease in IC_{50} values over 24, 48, and 72 hours (153.54 ± 0.85, 147.16 ± 1.13, and 141.98 ± 1.15 µg/mL, respectively) indicated enhanced efficacy with prolonged exposure.

Notably, morphological changes at the highest tested concentration (250 µg/mL) further supported the extract's potential in inducing cytotoxicity. These findings suggest that *Lantana camara* could serve as a promising source of natural anticancer agents. However, further studies are warranted to isolate and characterize the active constituents and to validate these findings through in vivo models.

Conflict of Interest: The authors declare no conflict of interest.

Acknowledgements:

We are Grateful to the Management and Dean to carried out our research works and provided research facilities at school of Pharmacy, NNRESGI, Hyderabad.

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