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RESEARCH ARTICLE

IN-VITRO ANTI-CANCER POTENTIAL OF WHOLE PLANT OFLANTANA CAMARA EXTRACT USING HELA CELLS LINE

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Ethanolic Extract, Lantana Camara, MTT Assay, HeLa Cells

Abstract

Background: The aim of this study was to examine the anticancer property of Lantana Camara on HeLacells.
Material and Methods: Using ethnomedical datasubmission , the Indian medicinal plant(L.Camara) which are used in traditional medicine for cancer diseases were collected. The crude extracts was prepared by ethanolic extraction methods using standard protocols. The anticancer effects on alcoholic extracts of Lantana Camaraplants functioning a proliferation of cancerous cell lines, which are HeLa cell lines. Which make use of cytotoxicity assay, an cell viability, OD value and the IC_{50} of L.camarawas determined. This extracts sign influential for the treatment of cervical cancer.
Results: The ethanolic extract of L.camaraexhibit good cytotoxicity for-which concentration dependent. It repose contrasting ,incase of ethanolic extracts, the cell viability was begin to increase while the concentration of extract increases. It states that not just the concentration of extract is experience the effect on cell viability, even the methods and solvents of extraction are chief in make aneffects on cell line.
Conclusion: The ethanolic extract of L.camarareveal the cytotoxic effects on HeLa cells for which the plants used as anticancer herbal drugs, our outcome shows a cytotoxic activity on cancer cells.

Introduction:-

Cervical cancer be the fourth most common cancer among the women worldwide. Primary prevention and screening have being the most efficient for decreasing the healthcare complicationand mortalityassign to cervical cancer. In United States and other developing countries, Most screening and diagnostic efforts are mean for early identification of high-risk human papillomavirus (HPV) lesions via HPV testing and pap smears.

Cervicalcancer are become apparent to the cervix, which are due to the abnormal growth of cells that have the capacity to occupy or spread to other parts of the body.[1] Typically there is no symptoms are seen early. Later, the symptoms which may include abnormal vaginal bleeding, pelvic pain or pain during sexual intercourse. While bleeding after sex , it may not be serious, but it may also indicate the presence of cervical cancer.[2]

Human papillomavirus infection (HPV) cause more than 90% of cases.[3] Most of the women are not affected by cervical cancer, who had HPV infections.[4,5] HPV 16 and 18 strains are responsible for nearly 50% of high grade

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cervical pre-cancers.[6] Another risk factors include smoking, a weak immune system, birth controlw pills, starting sex at a young age, and having many sexual partners, but these are less important.[7]

L.Camara is ancrucial medicinal plant with tolerable medicinal uses in standard medication system. It exist a remedy in many health issues in definite parts of the World. Lantana camara (common lantana) a flowering plant species in verbena family (verbenaceae), American tropics is the native of Lantana camara[8]. It is grown from its native Central and South America to around 50 countries,it is an invasive species. L.Camarashows high morphological variation because of its extensive breeding. The biological type of L.Camara population are more. This species has diploid (n= 22), triploid (n= 33), tetraploid(n=44) and pentaploid(n= 55) varieties. Dissimilarploidy extent are biologically important in on interfering capacity on the species L.camara in the domestic range on tropical America develop while the small group as 1 diameter. L.camara also breed asexually. Vegetative breed happens by stratified horizontal stems and give rise to root system. The leaves are broadly ovate, opposite, and simple and have a strong odour when crushed. L.camara has small tubular-shaped flowers, where by four petals and are arranged in clusters in terminal areas of stems. Flowers are in many different colours, including red, yellow, white, pink and orange, which differ depending on location in inflorescences, age and maturity[9].

Materials Required:-

DMEM medium, Fetal Bovine serum(FBS) and antibiotics solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide)(5mg/ml)were from Sigma, (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plalte and wash beakers were Tarson (India).

Authentication of Herb:

Lantana Camaraplant were collected locally and identified by Prof. JOHN ROBINSON, PG ASST. IN BOTONY, Thanthai Hans RoeverHr.Sec.School,Perambalur .

Procedure

Cell Culture:

HELA (Human cervical cancer cell line) was purchased from NCCS, Pune and were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% $co_{2\ at\ 37}$℃

MTT assay:[10][11]

The LC Test sample was tested for in vitro cytotoxicity, using HELA cells by MTT assay. Briefly, the cultured HELA cells were harvested by trypsinization and pooled in a 15ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into the 96-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48 hrs at $_{37}$℃. The wells were washed with sterile PBS and treated with various concentration of the LantanaCamara.

Test sample in a serum-free DMEM medium. Each sample was replicated three timed and the cells were incubated at $_{37}$℃ in a humidified 5% co2 incubator for 24h. After incubation, MTT(20µL of 5mg/ml) was added toeach well and the cells were incubated for another 2-4h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220µL) was aspirated off the wells and washed with 1× PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100µL) was added and the plate was shaken for 5min. The absorbance for each well was measured at 570nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software(USA).

Formula Cell Viability% = Test OD/Control OD ×100

Observation of Morphological Changes

Cells plated in 96-well culture plates(1×10^5 cells per well) in DMEM containing 10% FBS for 24h were treated with or without L.Camara at various concentrations. After 24h, the cells were observed under the inverted phase contrast microscope and photographs were taken.

DNA Fragmentaion-based Apoptosis Analysis

HeLa cell lines(1×10^5cells/ml)were cultured in 25cm^2 tissue culture flasks for 24h followed by the addition of the extracts and incubated again for 24h. Cells were harvested, washed with PBS, and lysed in buffer containing 10mM

Tris-Hcl, 10 mM EDTA, 0.5% triton X-µg/ml RNase A, and 200 µg/ml proteinase K. DNA was precipitated with isopropanol and was then suspended in the Tris-EDTA solution. Samples were resolved by using 0.8% agarose gel and visualized using UV transillumination.

Results And Disscussion:-

Pharmacognostical character:

Colour:

The colour of Leaf is (Green), Flower is (Pink), Fruit is (Black when ripen), Stem is (Light stew), and Root is (Pale Yellow).

Taste:

Peppery Taste

Odour:

Somewhere between cat urine, gasoline and fermented citrus.

Shape:

The shape of the leaves are ovate, opposite and simple.

Qualitative Estimation Of Phytochemical Constituents

Preliminary phytochemical analysis of whole plant extract of lantanacamara

+indicates presence, -indicates absence

S.NO	PHYTOCHEMICAL TESTS	METHANOLIC EXTRACT
1.	Carbohydrates	+
2.	Saponins	+
3.	Tannins	+
4.	Glycosides	+
5.	Flavonoids	+
6.	Phenols	+
7.	Proteins	+
8.	Triterpenoids	+
9.	Quinolones	+

Table 1:- Phytochemical analysis of whole plant extract of lantana camara.

Pharmacological Studies:

Evaluation of Anti-Cancer Activity:

Viability of HeLa cells by MTT Assay:

When HeLa cells were treated with the ethanolic extract of L.Camaraplant , there was a concentration-dependent cytotoxic effect. As the concentration increased from 10 to 500 µg/ml, percentage of inhibition increased from 5.5% to 88.5%. At a concentration of 200 %µg/ml, there was a drastic decrease (32.6%) in cell viability(Table 2). IC_{50} value was found to be 109.9 µg/ml from the graph. But in the case of ethanol extracts, as the concentration increased, the percentage of cell viability is decreased.

S. No	Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
	Control	100	100	100	100
	500 µg/ml	3.08483	4.90654	8.70536	5.5655774
	400 µg/ml	15.6812	12.1495	9.15179	12.327517
	300 µg/ml	19.5373	22.1963	24.3304	22.021298
	200 µg/ml	40.874	32.0093	25	32.627794
	100 µg/ml	41.6452	41.1215	44.1964	42.321056
	80 µg/ml	54.4987	58.6449	54.2411	55.794882
	60 µg/ml	68.1234	65.6542	64.5089	66.095509
	40 µg/ml	79.9486	72.8972	76.1161	76.320618
	20 µg/ml	82.0051	82.243	83.9286	82.725568
	10 µg/ml	82.5193	92.9907	91.2946	88.934859

- **Cell Viability(%):**

Table 2:-

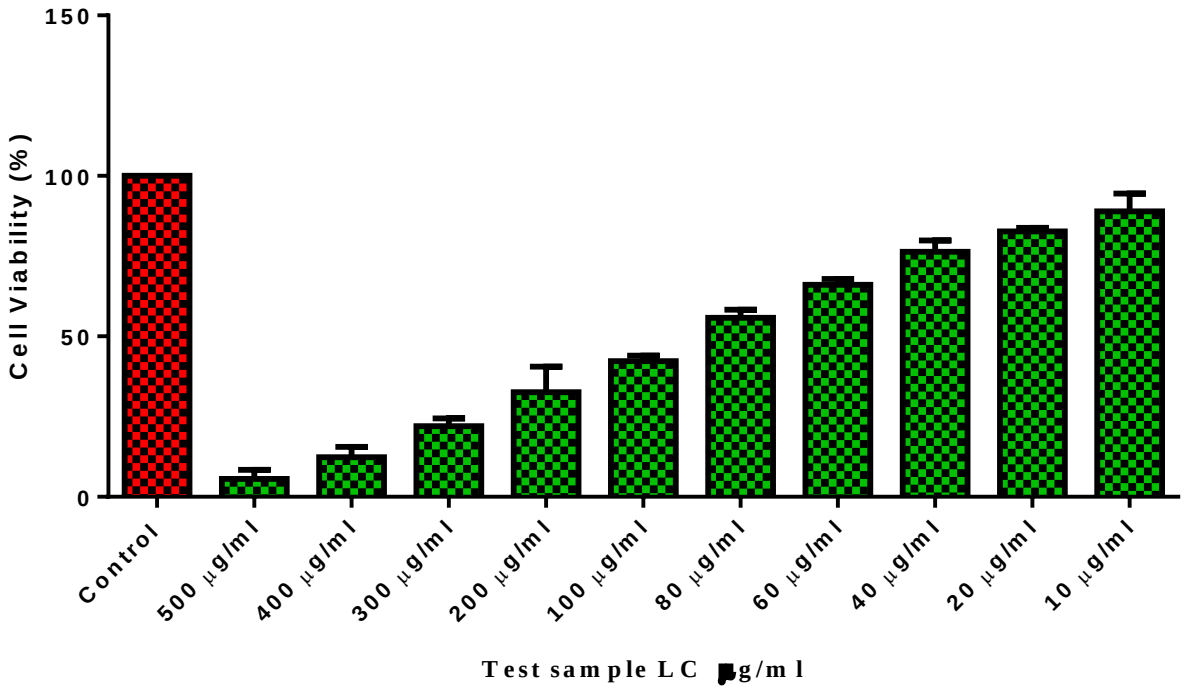

Figure 1:-

- **IC_{50} value of tested sample: 102.9 µg/ml:**

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		2.012
HillSlope		-1.623
IC50		102.9
Std. Error		
LogIC50		0.02199
HillSlope		0.1258
95% Confidence Intervals		
LogIC50		1.967 to 2.057
HillSlope		-1.880 to -1.365

IC50		92.74 to 114.1
Goodness of Fit		
Degrees of Freedom		28
R square		0.9599
Absolute Sum of Squares		1433
Sy.x		7.153
Number of points		
Analyzed	3	30

Table 3:-

- **OD Value at 570**

S. No.	**Tested sample concentration (µg/ml)**	**OD value at 570 nm (in triplicates)**		
1	Control	0.389	0.428	0.448
2	500 µg/ml	0.012	0.021	0.039
3	400 µg/ml	0.061	0.052	0.041
4	300 µg/ml	0.076	0.095	0.109
5	200 µg/ml	0.159	0.137	0.112
6	100 µg/ml	0.162	0.176	0.198
7	80 µg/ml	0.212	0.251	0.243
8	60 µg/ml	0.265	0.281	0.289
9	40 µg/ml	0.311	0.312	0.341
10	20 µg/ml	0.319	0.352	0.376
11	10 µg/ml	0.321	0.398	0.409

Table 4:-

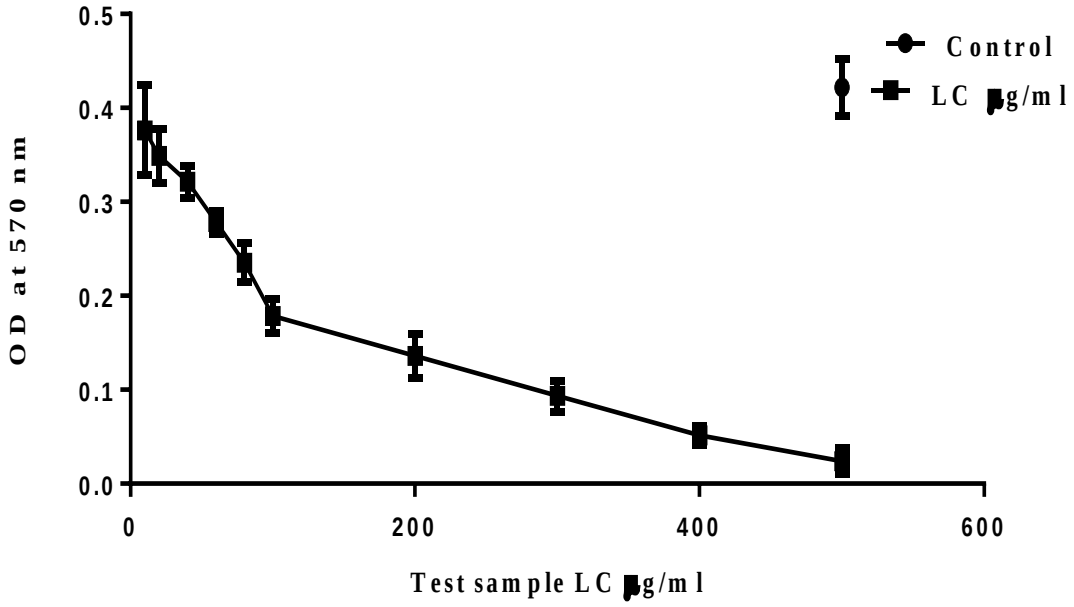

Figure 2:-

- **Images of control cells and HeLa treated cells:**

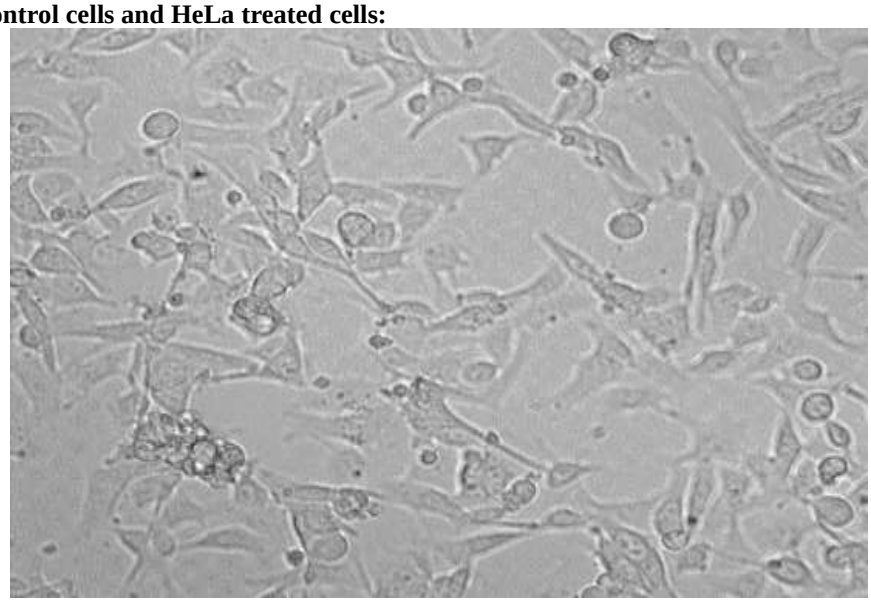

Figure 3:- Controlled cells.

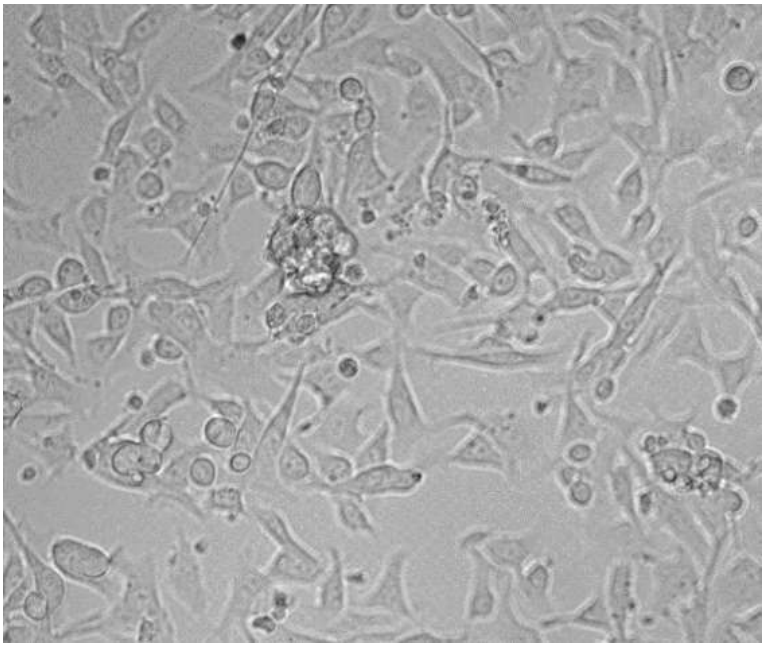

Figure 4:- LC 500 µg/ml.

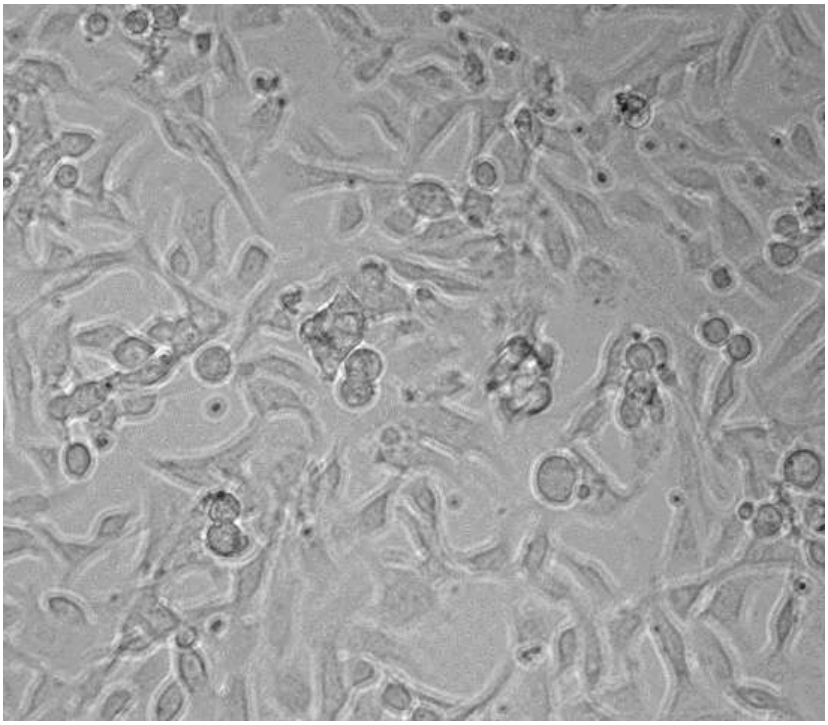

Figure 5:- LC 300 µg/ml.

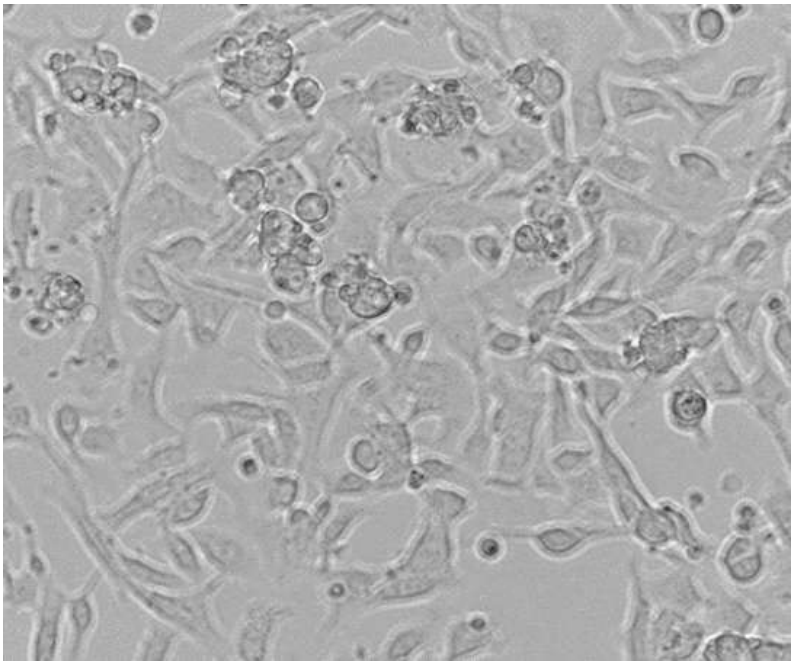

Figure 6:- LC 100 µg/ml.

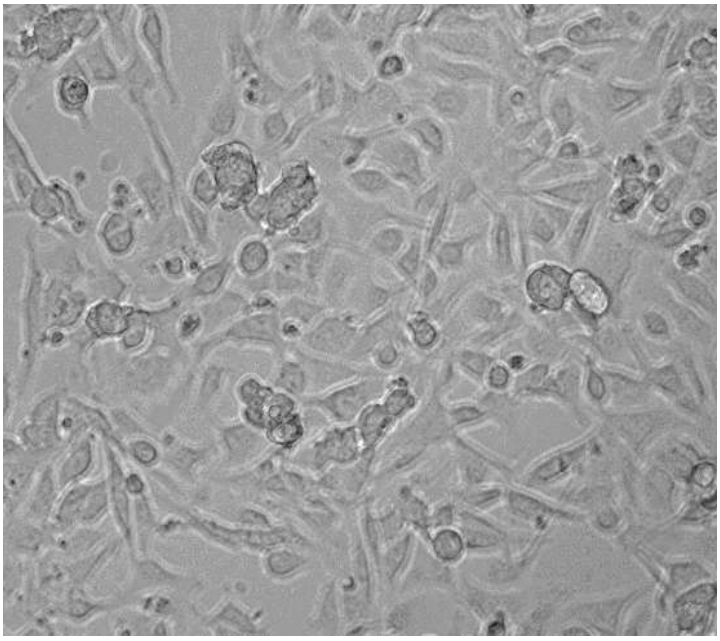

Figure 7:- LC 50 µg/ml.

Conclusion:-

In conclusion, the results of the present work shows that the ethanolic extract of Lantana Camara activated the apoptotic pathway in HeLa cells. The capability of the extract to activate and carry out apoptosis in cervical carcinoma cells is clear and the MTT assay suggests a mitochondrial involvement. The anticancer activity of whole plant ethanolic extract of L.Camarahas not been reported in the literature. The IC_{50} value was found to be 102.9 µg/ml.L

The habitual anticancer drugs used in cancer, they are toxic and have more side effects. The Lantana Camaraplant showing its good anticancer activity , potential and important is non-toxicity to normal healthy lymphocytes.

In future, we are subjected to isolation and purification of the active component and animal studies exploring their anticancer activity.

The Lantana Camaratest sample was tested for invitro cytotoxicity using HeLa cells by MTT assay. Then, the cells were plated at a density of 1×10^5 cells/well (200 µL)into the 96-well tissue culture plate in DMEM, 10% FBS and 1% antibiotics solution (24-48 hrs) at 37℃.

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