iJRASET
International Journal For Research in Applied Science and Engineering Technology

# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

**Volume:** 6 **Issue:** IV **Month of publication:** April 2018

**DOI:** http://doi.org/10.22214/ijraset.2018.4800

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
*ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887*
*Volume 6 Issue IV, April 2018- Available at www.ijraset.com*

# Effect of Fisetin on Growth of Non-Small Cell Lung Carcinoma Cells A549 and NCI-H460

Gazala Showkat[1], M. Sreepriya[2]
[1]*Department of Microbiology and Biotechnology Bangalore University, JB Campus Bangalore – 560 056, Karnataka, India*
[2]*Assistant Professor Department of Microbiology and Biotechnlology Bangalore – 560 056, Karnataka , India*

***Abstract:*** ***Lung cancer is most common cause of cancer deaths globally and is mainly classified into two major types: Small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Majority of lung cancers are NSCLCs, that account for nearly 90% of cases. Most of lung cancer cases (85%) of are due to long-term tobacco smoking. Presently tobacco smoking is the main contributor to lung cancer. Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a naturally occurring flavonoid that is abundant in various fruits and vegetables and has been reported to possesses anti-oxidative, anti-inflammatory and anti-proliferative effects in a wide variety of cancer. The aim of the present study is to investigate the growth inhibitory effect of Fisetin on non small cell pulmonary carcinoma cells A549 (adenocarcinoma) and NCI-H460 cells (large cell lung carcinoma cells) in vitro. SRB (Sulfarhodamine B) dye uptake test and NBT reduction test were performed and the results of the assays demonstrate suppressed growth of non small cell lung carcinoma cells upon treatment with Fisetin.***
***Keywords: A549, NCI-H460, Fisetin, Non small cell lung cancer***

## I. INTRODUCTION

Lung cancer is one of the commonest malignancies and is the major causes of death globally exceeding the mortality rates of colorectal, breast and prostate cancers combined [Naghma et al., 2012]. Despite improvement in diagnostics and treatment modalities, the prognosis remains poor [Makitaro et al., 2002; Barnes 2004]. The mean 5-year survival rate for NSCLC patients is approximately 15%. Even with improvements in tumor response to chemotherapy, the long-term survival rate of patients with advanced lung cancer remains remarkably low [Jemal et al., 2011]. Lung cancer can be classified into two main subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) based on the histology. Non-small cell lung cancer accounts for more than 80% of all lung cancer cases. The most common histological types are adenocarcinoma (AC), squamous cell carcinoma (SCC) and large cell lung carcinoma (LCLC] [Robinson et al., 2010]. Small cell lung cancer accounts for approximately 13% of all lung cancer cases and is highly associated with tobacco smoking [Govindan et al., 2006; Jackman and Johnson, 2005].

Natural products are found to be an important source of new drugs. Currently 50% of the anticancer drugs available in the market for therapy are derived either directly or indirectly from natural sources [Younes at al., 2007] thus natural bioactive products have gained significant interest in scientific world. Flavonoids a group of plant secondary metabolites, are the most abundant polyphenolic compounds consumed in human diet and have special ability to target several key cellular phenomenons involved in the development of cancer [Beliveau, et al., 2007; Murthy et al., 2009]. Fisetin (3,7,3′,4′-tetrahydroxyflavone) is one of the major polyphenolic flavonoids found in various fruits and vegetables such as apples, grapes, persimmons, strawberries, cucumbers, and onions. It has been reported that Fisetin can exert numerous beneficial biological activities, including antioxidant [Syed et al., 2016], anti-inflammatory [Khan et al., 2008], anti-angiogenic [Murtaza et al., 2009], hypolipidemic [Khan et al., 2015], neuroprotective [Mukhtar et al., 2015] and ant-itumor effects [Lall et al., 2016]. The aim of the current study is to investigate the effect of Fisetin on growth of non small cell lung carcinoma cell lines A549 and NCI-H460 cells.

## II. MATERIALS AND METHODS

### *A. Chemicals*

Fisetin (3,3′,4′,7-tetrahydroxyflavone) was procured from (sigma , St. Louis , MO, USA). A stock solution of 2.86mg/ ml of fisetin was prepared in DMSO and stored at -20$^0$C.

### *B. Procurement and Maintenance of cell Lines*

The NSCLC pulmonary carcinoma cell lines A549 (adenocarcinoma), NCI-H460 (large cell lung carcinoma) and HEK 293 (normal human embryonic kidney cells) were procured from National Centre for Cell Science (NCCS), Pune, India. The A-549, NCI-H460

and HEK 293 cells were cultured in Hams F12K medium (AL106A- Himedia, India) and RPMI 1640 (AL171A- HiMedia, India) and DMEM (AL007- Himedia India) respectively. The media were supplemented with 10% Fetal Bovine Serum (FBS, RM112, Himedia, India), 1X and Antibiotic and Antimycotic solution (A007, Himdia, India) for growth of cells, and cells were grown under standard growth conditions (95% humidity, 5% CO2 and temperature 37°C) in a $CO_2$ incubator.
Assays were carried out with the $IC_{50}$ concentrations of Fisetin which were fixed based on previous studies from the laboratory.

*C. SRB (Sulfarhodamine B) dye uptake test*

Cell growth was assessed by Sulfarhodamine B (Sigma-Aldrich Co., St. Louis, MO, USA) assay as previously reported (Skehan *et al.*, 1990). Briefly cell suspension containing $1X10^6$ cells /ml were plated onto 96 well plates and allowed to attach for 24h at 37°C in a 5 % $CO_2$ atmosphere. The cells were then treated with Fisetin and allowed to proliferate, cells were washed with PBS and fixed with TCA (trichloroacetic acid) at 4°C for 1h. After washing with water, cells were stained with SRB (0.4%w/v SRB in 1% v/v acetic acid in water). The protein-bound dye was solubilized with 10mM tris buffer. Intensity of the colour developed was measured at 540 nm.

*D. NBT Reduction Test*

NBT (Nitro blue tetrazolium) reduction test was performed following the method of Williams *et al.*, 1977. After proliferation of A549 and NCI-H460 cells, 10µl of nitro blue tetrazolium chloride (5mg/ml in phosphate buffered saline- pH7.4) was added to the cultured cells (1x $10^6$/ml) and incubated in a $CO_2$ incubator at 37°C for 5h. The cells were the washed three times with saline and the formazan crystals were solubilised by adding 100 µl of isopropanol. The intensity of the color developed was read at 570 nm in a micro plate.

*E. Statistical Analysis*

The experiments were carried out in triplicate on atleast three different time intervals and the mean of replicate values were taken. Values were expressed as mean ±SD. Statistical analysis of the data was determined by Student's t-test and comparisons were made between the untreated control and the treated groups.

## III. RESULTS

The results of The SRB assay and NBT assay clearly indicated that A549 and NCI-H460 cells treated with Fisetin showed marked decrease in growth which was found to be statistically significant ($p<0.001$) as compared to control cells (Fig. 1 and Fig. 2). However there was no decrease in growth upon treatment with Fisetin in normal kidney cells - HEK 293 (Fig. 3).

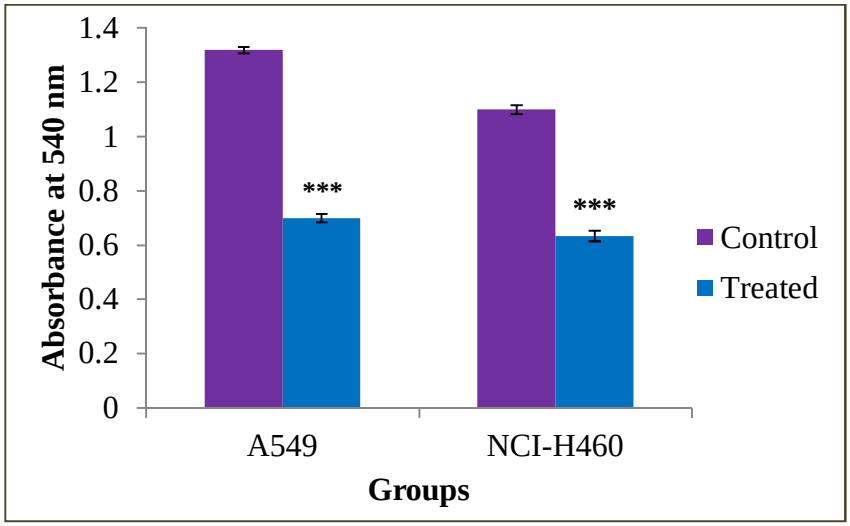



Fig. 1 Effect of Fisetin on growth of A549 and NCI-H460 cells (Sulphorhodamine B assay)

Effect of Fisetin on growth of A549 and NCI-H46 cells (SRB assay). Treatment of A549 and NCI-H46 cells with Fisetin resulted in a significant decrease in cell growth as compared with the control. Data represent mean ± SD of six replicates. Intergroup comparisons were made between the cell control and the treated group. Student's *t*-test; ***$P< 0.001$ verses control.

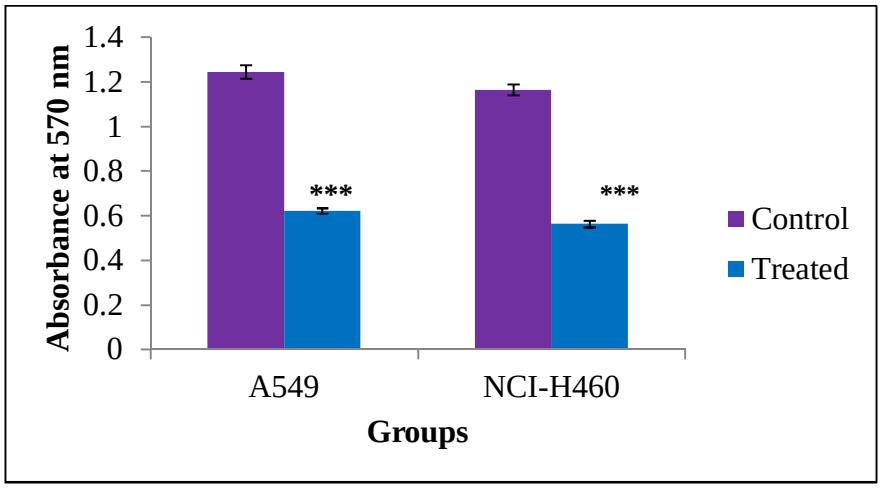



Fig. 2 Effect of Fisetin on growth of A549 and NCI-H460 cells (Nitro blue tetrazolium test)

Effect of Fisetin on growth of A549 and NCI-H46 cells (NBT assay). Treatment of A549 and NCI-H46 cells with Fisetin resulted in a significant decrease in cell growth as compared with the control. Data represent mean ± SD of six replicates. Intergroup comparisons were made between the cell control and the treated group. Student's *t*-test; ****P*< 0.001 verses control.

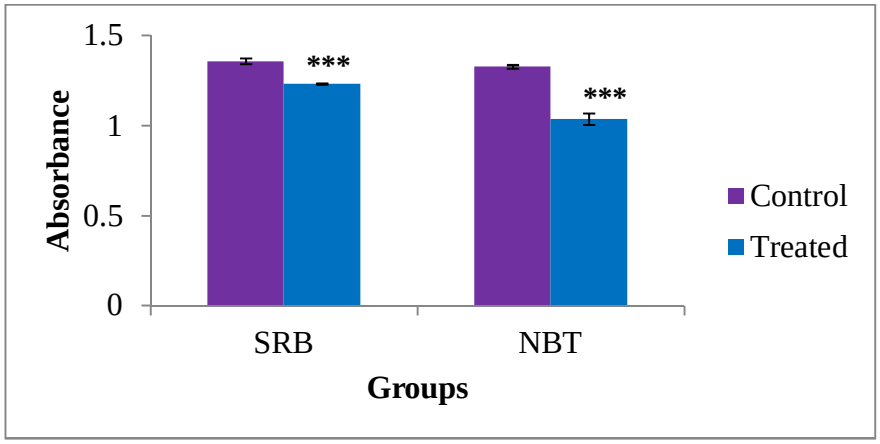



Fig. 3 Effect of Fisetin on growth of HEK-293 cells (Sulphorhodamine B assay and Nitro blue tetrazolium test)

Effect of Fisetin on growth of HEK-293 cells (SRB and NBT assay). Figure shows the effect of Fisetin on growth of HEK-293 cells. Data represent mean ± SD of six replicates. Intergroup comparisons were made between the cell control and the treated group. Student's *t*-test; ****P*< 0.001 verses control.

## IV. DISCUSSION

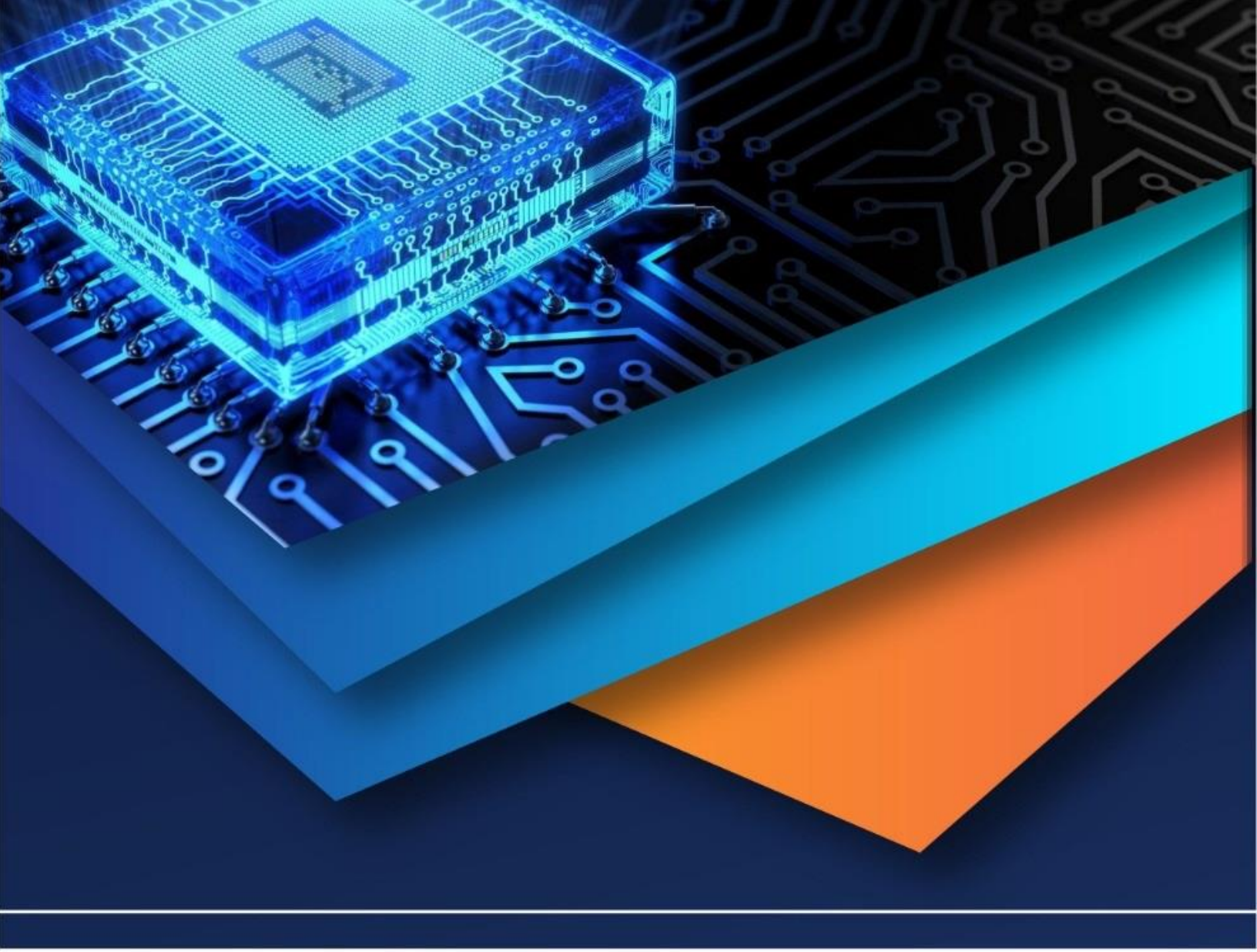
Lung cancer is the most common tumor type in the developed world and in 2012 and is the most commonly diagnosed cancer (1.82 million) and the most common cause of cancer mortality (1.6 million) worldwide [ Ferlay *et al.*, 2012; Sullivan and Planchard 2016]. Fisetin, a natural polyphenol abundantly found in several fruits and vegetables, has been reported to inhibit growth of various cancer cell lines in vitro, thus the aim of the study was to check the effect of Fisetin on growth and viability of non small cell pulmonary carcinoma cells A549 and NCI-H460. Results indicated that Fisetin was found to greatly inhibit the growth of NSCLC cells *in vitro.* As Cancer cells exhibit high proliferative potential, resistance to cell death stimuli and abnormal energy metabolism [Czarnecka *et al.*, 2007]. The sulforhodamine B (SRB) that is based on the measurement of cellular protein content is used for cell density determination, The method relies on the property of SRB, that binds stoichiometrically to proteins under acidic conditions and then can be extracted using basic conditions. Thus the amount of dye that is bound can be used as a substitute for cell mass, which was then used to measure cell proliferation. The results of SRB clearly indicated that Fisetin has significantly inhibited the growth of pulmonary carcinoma cell lines. Many tetrazolim salts, including nitro blue tetrazolium-NBT have been used to assay cell proliferation and viability. Succinate-tetrazolium reductase, is an enzyme which belongs to the respiratory chain of the

mitochondria and it is active only in viable cells [Pelicano et al., 2006]. The results of NBT found to be in agreement with the results of SRB assay. Thus the results of the present study clearly indicated the growth inhibitory effect of Fisetin in non-small cell lung cancer cells used.

After observing the growth inhibitory effects of Fisetin on human pulmonary carcinoma cells A549 and NCI-H460 by the above mentioned assays. The effect of Fisetin on human embryonic kidney cells (HEK 293) was studied and Fisetin was found to exhibit less toxic effect on human embryonic kidney cells as compared to pulmonary carcinoma cells thereby showing selective toxicity of Fisetin towards cancer cells.

## REFERENCES

[1] Naghma khan, Farrukh afaq, Fatima h. khusro, Vaqar mustafa adhami, Yewseok suh, and Hasan mukhtar. Dual inhibition of PI3k/Akt and mtor signaling in human non-small cell lung cancer cells by a dietary flavonoid fisetin. Int J Cancer. 2012; 130(7): 1695–1705.
[2] Makitaro R, Paakko P, Huhti E, Bloigu R, Kinnula VL. 1. Prospective population-based study on the survival of patients with lung cancer. Eur Respir J. 2002; 19: 1087-92.
[3] Barnes DJ. The changing face of lung cancer. 2. Chest, 2004; 126: 1718-21.
[4] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61:69–90.
[5] Robinson C.G, Bradley, J.D. The treatment of early-stage disease. Semin. Radiat. Oncol. 2010, 20: 178–185.
[6] Govindan R, Page N, Morgensztern D, Read W, Tierney R, Vlahiotis A, Spitznagel E.L, Piccirillo J. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: Analysis of the surveillance, epidemiologic, and end results database. J. Clin. Oncol. 2006; 24: 4539–44
[7] Jackman D.M, Johnson B.E. Small-cell lung cancer. *Lancet.* 2005; *366*: 1385–1396.
[8] Younes RN, Younes AD, Varella IB. Suffredini Discovery of new antitumoral and antibacterial drugs from Brazilian plant extracts using high throughput screening. Clinics. 2007; 62: 763–768.
[9] Beliveau R, Gingras, D. Role of nutrition in preventing cancer. Canadian Family Physician Medecin De Famille Canadien. 2007; 53:1905–1911.
[10] Murthy N. S, Mukherjee S, Ray G, Ray A. Dietary factors and cancer chemoprevention: an overview of obesity-related malignancies. Journal of Postgraduate Medicine. 2009; 55: 45–54.
[11] D.N. Syed, V.M. Adhami, N. Khan, M.I. Khan, H. Mukhtar. Exploring the molecular targets of dietary flavonoid fisetin in cancer, Semin. Cancer Biol. 2-016; 40–41: 130–140.
[12] N. Khan, M. Asim, F. Afaq, M.A. Zaid, H. Mukhtar. A novel dietary flavonoid fisetin inhibits androgen receptor signaling and tumor growth in athymic nude mice, Cancer Res. 2008; 68: 8555–8563.
[13] I. Murtaza, V.M. Adhami, B. Bin Hafeez, M. Saleem, H. Mukhtar. Fisetin, a natural flavonoid, targets chemoresistant human pancreatic cancer AsPC-1 cells through DR3-mediated inhibition of NF-κB. Int. J. Cancer. 2009;125: 2465–2473,
[14] N. Khan, H. Mukhtar.Dietary agents for prevention and treatment of lung cancer. Cancer Lett. 2015; 359 : 155–164,
[15] E. Mukhtar, V.M. Adhami, M. Sechi, H. Mukhtar. Dietary flavonoid fisetin binds to β-tubulin and disrupts microtubule dynamics in prostate cancer cells. Cancer Lett. 2015; 367: 173–183.
[16] R.K. Lall, V.M. Adhami, H. Mukhtar, Dietary flavonoid fisetin for cancer prevention and treatment, Mol. Nutr. Food Res. 2016; 60 : 1396–1405.
[17] Skehan P, Storeng P, Scudiero D et al. New colorimetric cytotoxicity assay for anti-cancer drug screening. J Natl Cancer Inst. 1990; 82: 1107–1112.
[18] Williams GM, Bermudez E, Scaramuzzino D. Rat hepatocyte primary cell cultures. III. Improved dissociation and attachment techniques and the enhancement of survival by culture medium. In Vitro. 1977; 13 (12):809-817.
[19] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer.2015; 136:E359–E386.
[20] Sullivan I and Planchard D. ALK inhibitors in non-small cell lung cancer: The latest evidence and developments. Ther Adv Med Oncol. 2016; 8:32–47.
[21] Pelicano H, Xu R, Du M, Feng L, Saski R, Carew J.S, Hu Y, Ramdas L, Hu L, Keating M.J, Zhano W, Plunkett W, Huang P. Mitochondrial respiration defects in cancer cells cause activation the Akt survival pathway through a redox-mediated mechanism. The Journal of Cell Biology. 2006; 175: 6, 913-923.
[22] Czarnecka A.M, Gammazza A, Felice V.D, Zummo G, Cappello F. Cancer as a Mitochondriophathy". J. Cancer Mol. 2007; 3 (3): 79-95.



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY