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In vitro Assessment of cytotoxic and apoptotic potential of Palmitic acid for Breast cancer Treatment

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Abstract: **Breast cancer is the second leading cause of cancer deaths in women worldwide. A number of strategies for the cure of breast cancer have been described such as surgery, chemotherapy and radiation; still, therapeutic effectiveness is characteristically temporary and commonly lacking with progressive period. As a result, the requisite for rational methods to breast cancer therapy is required. Palmitic acid (PA) is the most common saturated free fatty acid and plays an important role in cancer. However, the mechanism underlying the effect of PA on breast cancer is still to be elucidated.**

Experimental: In our study we have hypothesized that PA would have anticancer activity on MCF-7 Breast cancer cell line.

Results: Pamitic acid reduced cell viability in MCF-7 Breast cancer cells, apoptotic nuclear morphology of cells was observed by DAPI (4',6-diamidino-2-phenylindole) staining after 48 and 72 hrs treatment, comet assay showed the increase tail length and DNA fragmentation. Treatment with PA enhanced the expression of apoptosis-related proteins including caspase-3, 9, Bax and p53 whereas the expression of anti-apoptotic protein Bcl-2 was decreased.

Conclusion: Pamitic acid sensitizes breast cancer cells, induced apoptosis and may be considered for upcoming studies as a promising therapeutic compound for human breast cancer.

Keywords: **Breast cancer, Palmitic acid, apoptosis, inflammation, therapeutic action.**

1. INTRODUCTION

Cancer is a major health problem worldwide. One in eight deaths around the world is due to cancer [1]. In advanced countries it represents the first or second cause of death. Thus, imperative action is necessary to reduce the threat of this ailment, predominantly in emerging countries in which the occurrence and frequency of this disease are predictable to increase. Breast cancer is the subsequent leading cause of cancer death for women in the world. One of the major challenges for a better prognosis of breast cancer patients is the ineffectiveness of chemotherapy [2]. According to American cancer society it has been estimated that in 2017, 1,688,780 new cancer cases diagnosed and 600,920 cancer deaths will occur in the US [3]. It has been showed that National Cancer Institute (NCI) has documented that prevention is a critical component in minimizing the number of individuals afflicted with cancer [4]. Most of the studies in the past few years have shown that there are varieties of dietary and natural compounds with chemopreventive properties like garlic, green tea, soy and grape [5–7]. A number of recent studies also documented that natural compounds play a vital role

against cancer [8–10]. Classification of biologically active compounds which has therapeutic properties from natural products and endorse the nutritional compounds as an economical substitute to anticancer drug therapies which are toxic, immune-suppressive, mutagenic, and even carcinogenic [11-13]. Major treatment strategies include surgery, immunotherapy, and radiotherapy [14–15].

The majority of anticancer drugs aim to destroy cancer cells and decrease tumor load but are relatively ineffective against some phases of tumorigenesis [16, 17]. Thus, alternate strategies to prevent tumorigenesis are immediately required. In the past few decades, epidemiological studies have recommended that a healthy diet and way of life are significant for the prevention of breast cancer. Dietary fatty acids are one of the major intensively studied dietary factors. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and trans fatty acids (TFA) have been found to augment the risk of cancer where as specific polyunsaturated fatty acids (PUFA) are indicated to have anticancer effects [18]. There has been a growing public concern during the last years about the significant interplay between nutrition and health. Fat is an indispensable macronutrient of the human diet and vegetable oils are highly consumed fat. High-fat diet, essentially in saturated fatty acids (SFA), has been the hub of several dietary procedures that aims in the decrease of cardiovascular diseases, obesity-related diseases and, cancer prevention recently [19]. Palmitic acid, a saturated fatty acid, is the major constituent of refined palm oil. Controversial studies have reported potential unhealthy effects of palm oil due to the high palmitic acid content in recent time [20]. It has been well recognized that n-3 PUFA can restrain the progress of cancers by inhibition of cellular proliferation and induction of apoptosis. Cell culture studies investigating the effects of n-3 PUFA on murine and human breast cancer cells provide significant insights into the mechanisms underlying this inhibitory effect [21]. In the present study, we have demonstrated for the first time that PA enhances the upregulation of protein expressions of caspase3 & 9, bax, p53 and down regulation of the expression of Bcl-2, in MCF-7 breast cancer cells. Thus, our findings raise the possibility that PA could be a candidate therapy for the treatment of breast cancer.

2. MATERIAL AND METHODS

Procurement of chemicals:

Palmitic acid was purchased from Sigma–Aldrich (USA). DMEM (Dulbecco's modified Eagle's medium) powder, Neutral Red, MTT (3-(4,5-dimethylthiazole-2-yl)-2,5 diphenyl tetrazolium bromide) and 0.25% trypsin with EDTA mixture were purchased from Himedia (India). Fetal bovine serum (FBS) was from Gibco (USA). All the other chemicals and reagents were purchased from Merck and were of molecular biology grade.

Procurement Cell lines and maintenance:

Breast cancer cell line (MCF-7) was procured from NCCS Pune, India. MCF-7 was cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Bovine Serum and antibiotics in a humidified atmosphere of 5% CO2 at 37°C in culture dishes/flasks. Stock culture was maintained in the exponential growth phase by passaging as monolayer culture using in 0.02% EDTA. The dislodged cells were suspended in complete medium and reseeded routinely.

Cytotoxicity assay:

The cytotoxic effect was assessed in breast cancer cell exposed to different concentrations of palmtic acid by the MTT assay. 3-(4,5-dimethyl-2-yl)-2,5-diphynyl tetrazolium bromide (MTT) is metabolic substrate, which is reduced by the mitochondrial succinate dehydrogenase enzyme and forms formazan crystal. Cells were seeded over night at the number of 1×10^4 per well and then incubated with various concentration of PA for 24, 48 and 72 hrs respectively. At the end of the treatment, medium was removed and cells were incubated with 20µl of MTT (5mg/ml in PBS) in fresh medium (50µl) for 4 hrs in CO2 incubator. After four hours formazan crystal, formed by mitochondrial reduction of MTT were solublized in DMSO (Dimethyl sulfoxide) (150µl/well) and the absorbance was read at 570 nm after 10 min incubation on the iMark Microplate Reader (Bio-Rad, USA). Percent cytotoxicity was expressed as IC_{50} [22].

Neutral red dye uptake assay:

Neutral red (3-amino-m-dimethylamino-2-methylphenazine hydrochloride) assay determined the accumulation of the neutral red dye in the lysosomes of viable, uninjured cells. The palmitic acid was incubated with cells for 24, 48 and 72hrs respectively. Neutral red dye (100µg/ml) was dissolved in serum free medium (DMEM). The pH of the neutral red solution was adjusted in all the experiments to 6.35 with the addition of KH_2PO_4 (1 M). 10µl of neutral red was incubated

with the cells for 1 hour. After that, washed with phosphate buffer saline (PBS) and added 1ml of elution medium (EtOH/AcCOOH, 50%/1%) followed by gentle shaking for 10min so that complete dissolution was achieved. The absorbance was taken at 540nm using iMark Microplate Reader (Bio-Rad, USA). Percent cytotoxicity was expressed as IC_{50} [23].

DAPI Staining:

Nuclear morphology was evaluated by fluorescence microscopy following DAPI staining. MCF-7 cells were treated with palmtic acid for 48 and 72 hours. Then cells were washed with PBS (pH 7.4), fixed with ice cold 70% ethanol and resuspend in DAPI, and incubated for 15 min at 37^0C wrapped in aluminium foil. The cells were then washed with PBS and examined under Nikon Eclipse fluorescence microscope (Nikon Instruments Inc., NY, and USA [24].

Single cell gel electrophoresis (comet assay) for the DNA Damage:

MCF-7 (~1×10^5 cells) was exposed to IC_{50} value of palmitic acid for 48 and 72 hrs. The cell pellet was suspended in 100 µl PBS (Ca2+ and Mg2+ free) and further processed for Comet assay. Single cell alkaline gel electrophoresis was done as performed earlier [25]. Analysis of the slides and scoring was done same day using image analysis system (Komet 5.5; Kinetic Imaging, Liverpool, UK) attached to an Olympus fluorescent microscope (CX41, Olympus Optical Co, Tokyo, Japan) and a COHU 4910 integrated CC camera fitted with 510-560 nm excitation and 590 nm barrier filters (COHU, San Diego, CA, USA). Tail length i.e. migration of DNA from the nucleus was measured as the main parameter to assess cell's DNA damage.

Apoptosis by DNA Laddering assay:

Cells were harvested after treatment of Palmitic acid (IC50) for 48 and 72 hrs and centrifuged at 5000 rpm for 5 min at 4^0C. The cell pellets were washed with PBS and DNA ladder was isolated through kit (G Biosciences, USA). DNA was electrophoresed on 1.8% (w/v) agarose gel to visualize the ladder under UV light and photograph was taken under the Gel Doc system (Bio-Rad, USA).

Western Blot:

Western blot analysis was carried out using cytosolic as well as nuclear fractions [24] of breast cancer cells MCF-7 treated with IC_{50} value of palmitc acid for 48 hours. Protein concentration was determined by using Bradford reagent and lysates were resolved on 15% sodium dodecyl sulphate (SDS) polyacrylamide gels. The proteins were then electro transferred onto nitrocellulose membrane (Sigma, St. Louis, MO, USA). After blocking with 5% nonfat milk in Tris-buffered saline (TBS, 0.1 M, pH 7.4), blots were subjected to various primary antibody incubations with caspase-3, 9, Bax and Bcl-2 (R & D System, USA) at 4^0C overnight. Protein abundance of GAPDH served as a control for protein loading. Membranes were incubated with secondary antibody with respect to primary antibody and diluted at an appropriate dilution in 1% BSA, for 1 h at room temperature. After each step, blots were washed thrice with Tris-buffer saline-Tween 20 (TBST). The membranes were then rinsed with TBST and developed for 3-5 min at room temperature with the developing buffer [tablet of diamino benzidine tetrahydrochloride (DAB), 5 mL, 0.1 mol/L PBS, PH7.4, 5 µL 30 % H_2O_2].

3. RESULTS

Cytotoxicity effect of Palmitic acid:

Palmitic acid (PA) showed cytotoxicity effect on MCF-7 cell line in dose and time dependent manner (Figure 1). IC_{50} value of PA against MCF-7 cell line were 158.14 ± 0.27, 118.87± 0.22, and 94.64 ± 0.13 µM respectively for the 24, 48 and 7hrs. However, in case of NR assay, PA showed IC_{50} value 161.04, 125.93 and 96.38 µM respectively for the 24, 48 and 7hrs. Result obtained from both cytotoxicity assays were significant correlation ($r, > 0.983$; $p < 0.05$).

DAPI staining for the apoptosis:

Cell death was confirmed through the fluorescence microscopic study. Results revealed that the treated cells (IC_{50}) for 48 and 72 hrs (figure 2b, 2d) demonstrated the altered nuclear morphology. Control MCF-7 cells showed normal/regular morphology (figure 2a, 2c). However, in both 48 and 72 hrs treatment cell nuclear morphology seems to have nuclear condensation, nuclear blebbing, nuclear fragmentation and overall morphological changes as depicted in figure 2b, 2d.

Single cell gel electrophoresis (comet assay) for the DNA Damage:

DNA breakage in MCF-7 was tested in presence of IC50 value of palmitic acid of 48 and 72 hrs through the comet assay. It has been observed that treated cells showed significant DNA damage as compared to control shown in Figure 3. However, the tail length was found more in case of MCF-7 treated IC50 value for the 72 hrs as compared to 48 hrs.

Genomic DNA Fragmentation:

As shown in figure 4, the DNA fragments were clearly observed as a ladder pattern in the palmitic acid (IC_{50} value) treated all MCF-7 cell line as compared to the without treated control. DNA fragmentation is characteristically associated with apoptotic process that genomic DNA breaks into pieces through the endogenous enzyme,

Expressional analysis of Apoptotic and Anti-apoptotic protein:

For proficient apoptosis, caspases family, p53, Bax need to activate whether the signal is initiated by the intrinsic or extrinsic pathway. In the present study, we have found that treated (IC_{50} value for 48 hrs) demonstrated the high expression of p53, Bax, caspase 3 and caspase 9 as compared to the untreated control. However, the Bcl2 (anti-apoptotic) showed the decreased level of expression in case treated as compared to untreated control. GAPDH was taken as internal control.

4. DISCUSSION

Cancer is a complex disease in which intracellular signal transduction system gets deregulated leading to the occurrence and progression of cancer. One of the essential approaches for the treatment of cancer is chemotherapy. Chemotherapeutic drugs which are currently used for treating different types of cancers have hazardous side effects. As a result, recent research is mostly paying attention on herbal plants and their products that have been studied for being safe and for the treatment and prevention of cancer [26]. The results obtained from the cytotoxicity assays viz., MTT and NR indicated that breast cancer cell line (MCF-7) showed time and dose dependent viability loss by the treatment of PA. Chemotherapy drug induced apoptosis in cell through a cascade of mechanism, which includes nuclear fragmentations, laddering pattern of genomic DNA, nuclear alteration, blebbing. Our finding also supports the similar pattern after treatment of PA. Apoptosis is a program cell death elicited during a multiplicity of physiological circumstances and is highly synchronized by several types of gene products that endorse or obstruct cell death at diverse stages [27]. Bcl-2 is anti-apoptotic gene and well-known as an important apoptosis-regulator protein [28], generally blocking apoptosis and its over expression can cause breast cancer leading to delay of cell survival [29]. Bax is a pro-apoptotic protein member of the Bcl-2 family of proteins. It possesses pore-forming activity to enhance mitochondrial membrane permeability, and can also form a homodimer with Bcl-2 to increase the effects of apoptotic response [29,30]. It has been reported by Raisova *et al.* that the Bax/Bcl-2 ratio establish the susceptibility of cells to apoptosis [31]. Therefore, a low Bax/Bcl-2 ratio is associated with enhanced survival of breast cancer cells and resistance to apoptosis, and vice versa. It has been reported that diets rich in *n*-3 PUFA, such as fish and canola oil, decrease the abundance of Bcl-2 and up-regulates Bax expression to induce program ell death, thus decrease BC risk [32]. In our study we have reported for the first time that PA reduced the expression of Bcl-2 and increased the expression of Bax in breast cancer cells. Activation of caspases play a significant role in a programmed cell death generated by different stimuli. Caspase-8 activates caspase- 9-mediated apoptotic pathway through cleavage of Bid and then activate caspase-3 by cleavage of procaspase-3. In our study we found that treatment of PA enhances the upregulation of caspase-3, 9 in breast cancer cells. NF-κB is a functional regulator of genes implicated in proliferation of cells, migration, and angiogenesis [33,34]. Impaired regulation of NF-κB activation in cancer cells, will lead to deregulated expression of the anti-apoptotic genes under the control of NF-κB [100]. It has been reported that NF-κB inhibits the activity of p53, a tumor suppressor gene known to activate apoptosis in cells with damaged DNA [35]. Therefore, constitutive NF-κB expression may contribute to the development and progression of breast cancer. In our results we have reported for the first time that the expression of p53 found to be increase when treated with PA in breast cancer cells; MCF-7. Outcomes from our study thus demonstrated a novel function of PA and augmented the significance of PA as a useful chemotherapeutic compound.

5. CONCLUSION

In conclusion, our results demonstrated for the first time that treatment of PA considerably augments apoptosis in breast cancer cells via Bcl-2, caspases-dependent apoptotic in MCF-7 cells for the first time. Further investigation is however, needed to explore the association among the above mechanisms. Hence, our results for the first time propose that treatment of PA could be an effective strategy for human breast cancer therapy.

Conflict of interest:

No

Acknowledgements:

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REFERENCES

[1] S. vel Szic, A. Palagani, B. Hassannia et al., "Phytochemicals and cancer chemoprevention: epigenetics friends or foe?" in Phytochemicals- Bioactivities and Impact on Health, InTech, Rijeka, Croatia, 2011.

[2] Hailan Liu, Yang Liu, and Jian-Ting Zhang. A new mechanism of drug resistance in breast cancer cells: fatty acid synthase overexpression-mediated palmitate overproduction. Mol Cancer Ther 2008;7(2). February 2008.

[3] American cancer society. Facts and Figures 2017.

[4] Chang Hui1, Xie Qi1, Zhang Qianyong1, Peng Xiaoli2, Zhu Jundong1*, Mi Mantian. Flavonoids, Flavonoid Subclasses and Breast Cancer Risk: A Meta-Analysis of Epidemiologic Studies.

[5] A. R. M. R. Amin, O. Kucuk, F. R. Khuri, and D. M. Shin, "Perspectives for cancer prevention with natural compounds,"
Journal of Clinical Oncology, vol. 27, no. 16, pp. 2712–2725, 2009.

[6] T. M. De Kok, S. G. Van Breda, and M. M. Manson, "Mechanisms of combined action of different chemopreventive dietary compounds:areview," European Journal of Nutrition,vol.47,no. 2, pp. 51–59, 2008.

[7] S. D. Stan, S. Kar, G. D. Stoner, and S. V. Singh, "Bioactive food components and cancer risk reduction," Journal of Cellular Biochemistry, vol. 104, no. 1, pp. 339–356, 2008.

[8] T. Kuno, T. Tsukamoto, A. Hara, and T. Tanaka, "Cancer chemoprevention throughthe induction of apoptosis by natural
compounds," Journal of Biophysical Chemistry, vol. 3, no. 2, pp. 156–173, 2012.

[9] J.-A. Shin, J. S. Kim, I.-S. Hong, and S.-D. Cho, "Bak is a key molecule in apoptosis induced by methanol extracts of Codonopsis lanceolata and Tricholoma matsutake in HSC-2 human oral cancer cells," Oncology Letters, vol. 4, no. 6, pp.1379–1383, 2012.

[10] Q. J. Wu, Y. Yang, E. Vogtmann et al., "Cruciferous vegetables intake and the risk of colorectal cancer: a meta-analysis of observational studies," Annals of Oncology, vol. 24, no. 4, pp. 1079–1087, 2013.

[11] S. R. McWhinney, R. M. Goldberg, and H. L. McLeod, "Platinum neurotoxicity pharmacogenetics," Molecular Cancer Therapeutics, vol. 8, no. 1, pp. 10–16, 2009.

[12] M. Minami, S. Matsumoto, and H. Horiuchi, "Cardiovascular side-effects of modern cancer therapy," Circulation Journal, vol. 74, no. 9, pp. 1779–1786, 2010

[13] J. W. Jang, C. S. Kay, C. R. You et al., "Simultaneous multitarget irradiation using helical tomotherapy for advanced hepatocellular carcinoma with multiple extrahepatic metastases," International Journal of Radiation Oncology, Biology, Physics, vol. 74, no. 2, pp. 412–418, 2009.

[14] A. Kane and I. Yang, "Interferon-gamma in brain tumor immunotherapy," Neurosurgery Clinics of North America,vol.21,
no. 1, pp. 77–86, 2010.

[15] Y. Refaely and D. Weissberg, "Surgical management of tracheal tumors," The Annals of Thoracic Surgery, vol. 64, no. 5, pp. 1429– 1432, 1997.

[16] Menendez, J.A.; Lupu, R.; Colomer, R. Exogenous supplementation with omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA; 22:6n-3) synergistically enhances taxane cytotoxicity and downregulates Her-2/neu (c-erbB-2) oncogene expression in human breast cancer cells. Eur. J. Cancer Prev. 2005, 14, 263–270.

[17] Ravacci, G.R.; Brentani, M.M.; Tortelli, T., Jr.; Torrinhas, R.S.; Saldanha, T.; Torres, E.A.; Waitzberg, D.L. Lipid raft disruption by docosahexaenoic acid induces apoptosis in transformed human mammary luminal epithelial cells harboring HER-2 overexpression. J. Nutr. Biochem. 2013, 24, 505–515.

[18] The Role of n-3 Polyunsaturated Fatty Acids in the Prevention and Treatment of Breast Cancer Liu J, Ma DW. Nutrients. 2014 Nov 18;6(11):5184-223.

[19] Berger, N.A. Obesity and cancer pathogenesis. Ann. N. Y. Acad. Sci. 2014, 1311, 57–76.

[20] Biological and Nutritional Properties of Palm Oil and Palmitic Acid: Effects on Health Annamaria Mancini, Esther Imperlini, Ersilia Nigro, Concetta Montagnese, Aurora Daniele 2,3, Stefania Orrù 1,2 and Pasqualina Buono. Molecules 2015, 20, 17339-17361.

[21] Rose, D.P.; Connolly, J.M. Effects of fatty acids and inhibitors of eicosanoid synthesis on the growth of a human breast cancer cell line in culture. *Cancer Res.* **1990**, *50*, 7139–7144.

[22] M. A. Khan, M. Zafaryab, S. H. Mehdi, J. Quadri, M. M. A. Rizvi. Characterization and carboplatin loaded chitosan nanoparticles for the chemotherapy against breast cancer *in vitro* studies. Intl. J. Biol. Macromol. 2017, 97, 115–122.

[23] Repetto G, del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat Protoc. 2008;3(7):1125-31.

[24] M. A. Khan, M. Zafaryab, S. H. Mehdi, I. Ahmad, M. M. A. Rizvi. Characterization and anti-proliferative activity of curcumin loaded chitosan nanoparticles in cervical cancer. Intl. J. Biol. Macromol. 2016, 93, 242–253.

[25] M. A. Khan, K. Alam, S. H. Mehdi, M. M. A. Rizvi. Genotoxic effect and antigen binding characteristics of SLE auto-antibodies to peroxynitrite-modified human DNA. Arch. Biochem. Biophys. 2017, 635, 8–16

[26] Samarghandian S, Nezhad MA, Mohammadi G (2014).Role of caspases, Bax and Bcl2 in Chrysin induced Apoptosis in the A549 human lung adenocarcinoma epithelial cells, Anticancer Agents Med Chem. 14: 901–909.

[27] Elmore, S. Apoptosis: A review of programmed cell death. *Toxicol. Pathol.* **2007**, *35*, 495–516.

[28] Jacobson, M.D.; Burne, J.F.; King, M.P.; Miyashita, T.; Reed, J.C.; Raff, M.C. Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. *Nature* **1993**, *361*, 365–369.

[29] Van Slooten, H.J.; Clahsen, P.C.; van Dierendonck, J.H.; Duval, C.; Pallud, C.; Mandard, A.M.; obelle-Deroide, A.; van de Velde, C.J.; van de Vijver, M.J. Expression of Bcl-2 in node-negative breast cancer is associated with various prognostic factors, but does not predict response to one course of perioperative chemotherapy. *Br. J. Cancer* **1996**, *74*, 78–85.

[30] Oltvai, Z.N.; Milliman, C.L.; Korsmeyer, S.J. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* **1993**, *74*, 609–619. 92. Raisova, M.; Hossini, A.M.; Eberle, J.; Riebeling, C.; Wieder, T.; Sturm, I.; Daniel, P.T.; Orfanos, C.E.; Geilen, C.C. The Bax/Bcl-2 ratio determines the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis. *J. Investig. Dermatol.* **2001**, *117*, 333–340.

[31] Raisova, M.; Hossini, A.M.; Eberle, J.; Riebeling, C.; Wieder, T.; Sturm, I.; Daniel, P.T.; Orfanos, C.E.; Geilen, C.C. The Bax/Bcl-2 ratio determines the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis. *J. Investig. Dermatol.* **2001**, *117*, 333–340.

[32] Ion, G.; Akinsete, J.A.; Hardman, W.E. Maternal consumption of canola oil suppressed mammary gland tumorigenesis in C3(1) TAg mice offspring. *BMC Cancer* **2010**, *10*, 81.

[33] Dolcet, X.; Llobet, D.; Pallares, J.; Matias-Guiu, X. NF-κB in development and progression of human cancer. *Virchows Arch.* **2005**, *446*, 475–482.

[34] Wu, J.T.; Kral, J.G. The NF-κB/IκB signaling system: A molecular target in breast cancer therapy. *J. Surg. Res.* **2005**, *123*, 158–169.

[35] Mayo, M.W.; Wang, C.Y.; Cogswell, P.C.; Rogers-Graham, K.S.; Lowe, S.W.; Der, C.J.; Baldwin, A.S., Jr. Requirement of NF-κB activation to suppress p53-independent apoptosis induced by oncogenic Ras. *Science* **1997**, *278*, 1812–1815.

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APPENDICES - A

Figure Legends:

Figure 1: Cytotoxic effect of Palmitic acid was evaluated on Breast cancer cell line (MCF-7) through the MTT and NR assay in dose as well as time dependent manner.

Figure 2: Nuclear morphology capture through fluorescence microscope after staining with Dapi at 40X. (a), (c) represent the control for 48 and 72 hrs. (b), (d) represent the treated for 48 hrs and 72 hrs.

Figure 3: Palmitic acid induced DNA breakage in MCF-7 cells. Images of comet assay, Control (Without treated), PA (Palmitic acid IC_{50} concentration for 48 and 72 hrs) capture through the fluorescence microscope. Tail length was measured in both treated and untreated cells in µm.

Figure 4: Fragmentation of genomic DNA in untreated (C) and treated (1, 2) MCF-7 cell line.

Figure 5: Expression analysis of apoptotic and anti-apoptotic proteins and Gel.Net software was used to measured the band density of both control and treated sample.

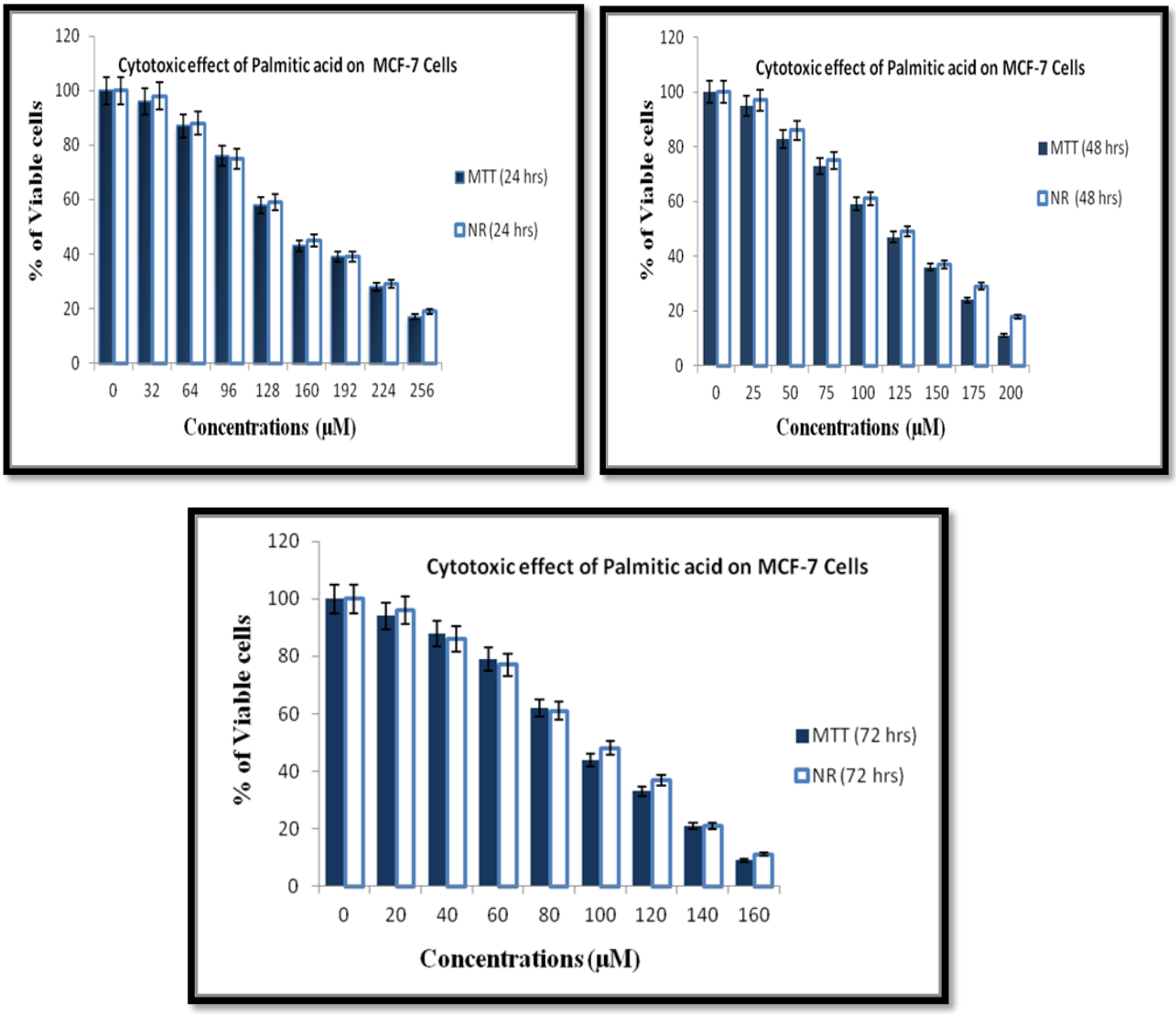

Figure 1:

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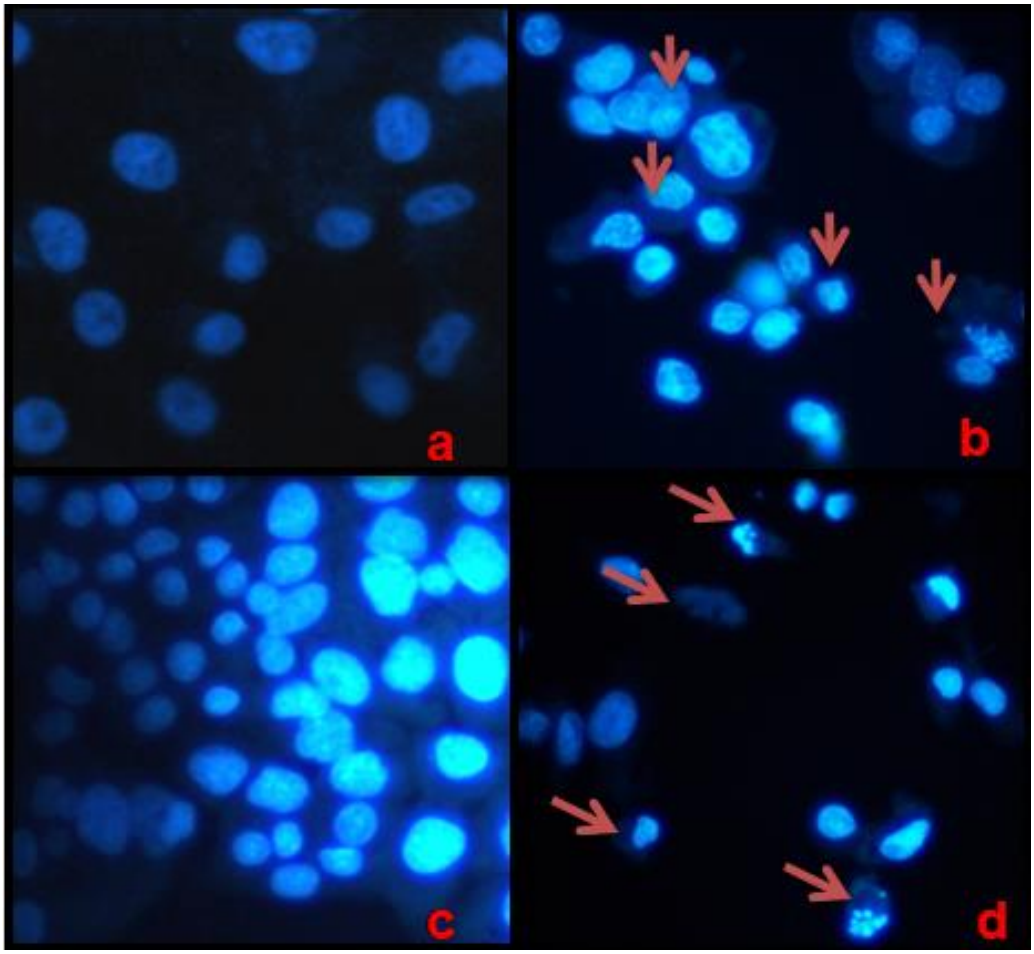

Figure 2:

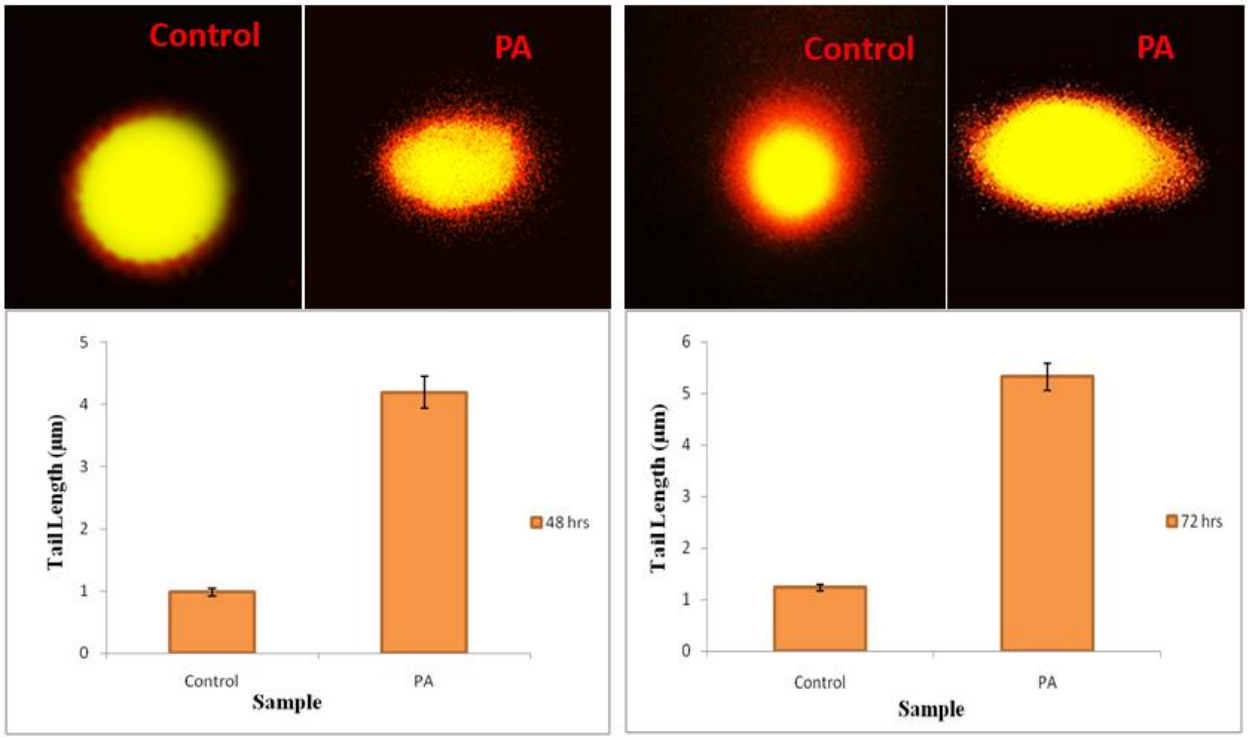

Figure 3:

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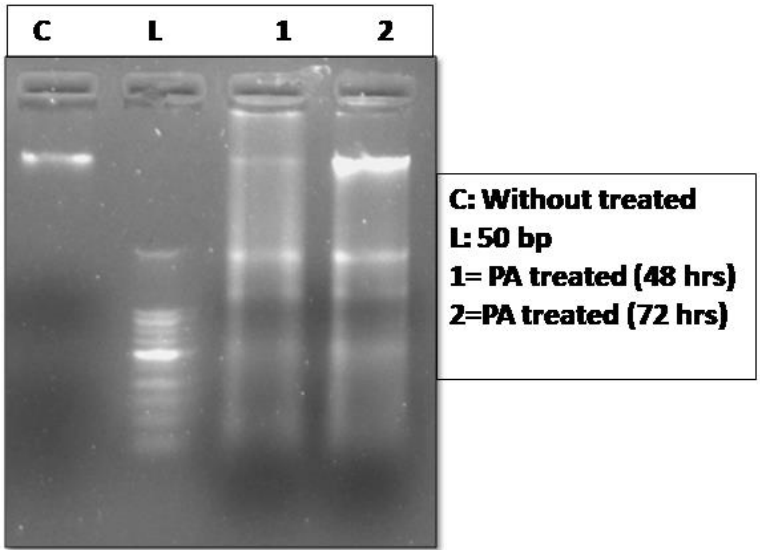

Figure 4:

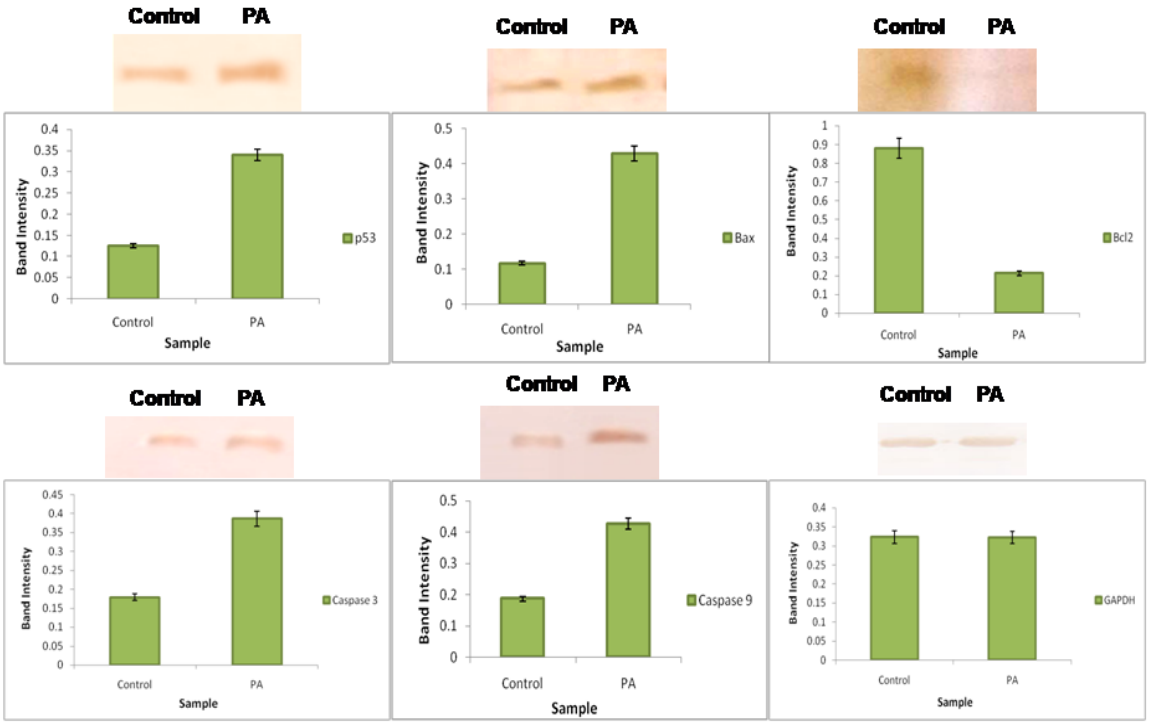

Figure 5: