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Peptide pACC1-Nano Complex Abrogates ACC1-Mediated Lipogenesis in Breast Cancer Cells Via Modulating p53/NF-κB Signaling Cascades

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Abstract

Objective

Cancer cells encompass comprehensively altered metabolic circuits for the growth and survival that underlie the malignant phenotype. pACC1 also referred to as peptide protein produced from chitosan, has been applied recently to develop targeted treatment that has numerous pharmacological activities towards DMBA-induced mammary cancer cells. Materials Methods: Bio Concept Labs located at Delhi (India) supplied the peptide pACC1 and meanwhile Sigma Aldrich Company (America) provided us sodium tripolyphosphate and chitosan (MW-210 kDa). Antibodies were obtained from Cell Signaling Technology, USA and finally 5-tetradecyl-oxy-2-furoic acid (TOFA) offered by Santa Cruz Biotechnology from India respectively. The following methods were carried out like Cell line and culture, pACC1–Chitosan Nanoparticles, MTT assays, Flowcytometry assays, DNA fragmentation assays, detection of western blot and Gene expression analysis. A malignant tumour alters the way that cells produce energy and execute biosynthesis process. Apoptosis, or programmed cell death, could be originated and improved their ability to create new fats (de novo lipogenesis) at the time of interrupted. Signalling proteins linked to apoptosis are triggered by rising oxidative stress, which results in cell arrest in the G2/M phase. Notably, expression of IKK and NF-kB protein expression were remarkably altered when Acetyl-CoA Carboxylase alpha (ACC1) and fatty acid synthase (FASN), suppression taken place simultaneously by PCN influence. Duel staining and DCFH-DA Photomicrograph were further confirming the utmost oxidative stress status of cells which was influenced by PCN. ACC1 and FSN gene expression were found to be down regulated. Conclusion: All the experiential evidence supported by positive control 5-(Tetradecyloxy)-2-furoic acid (TOFA) inhibitor of Fatty acid synthesis. The current study focused that PCN may potentially induce oxidative stress mediated apoptosis and down regulation of inflammatory signals in breast cancer cell.

Keywords: Lipogenesis, Nanoparticles, Oxidative Stress, Peptide, Pacc1, Tofa

Introduction

Cancer cells encompass comprehensively altered metabolic circuits for the growth and survival that underlie the malignant phenotype. Thus, exploiting metabolic functional nodes as therapeutic targets perhaps results in successful killing of cancer cells while leaving normal cells. This kind of approach pharmacologically opens a wide therapeutic window. Hence, effectual therapy would be achieved with extensive safety. Development and progression of cancer are frequently associated with increased *de* novo production of fatty acids in the tumor cell [1]. Many experimental results confirm that lipid biosynthesis of tumor tissue is comparable to that of liver tissue [2]. Certain metabolic alterations serve as a major functional process for advancement of cancer, including metastasis and the epithelial-to-mesenchymal transitions are also assembled to strengthened lipogenesis. Through, mutually transcriptional and post-transcriptional pathways, this activities necessitates the coordinated control of many lipogenesis-enabling enzymes [3,4]. Acetyl-CoA Carboxylase (ACC) is encoded by the human chromosome and exist in the form of two different nature namely ACC-α

(ACC1) and ACC-β (ACC2). The biological functional attributes and tissue distribution followed by these two enzymes are quite different. Since, it is the initial and rate-limiting enzyme in fatty acid synthesis mostly exits in the cytosol. The stated that the process of catalyses the ATP-dependent conversion of acetyl-CoA into malonyl-CoA, a potential molecule that inhibits carnitine palmitoyl transferase I (CPT-I) and functions as a substrate for fatty acid synthase (FASN to supress fatty acid beta-oxidation and de novo fatty acid synthesis [5].

Excessive levels of ACC1 were found in several malignancies, such as endometrial, colorectal, and breast cancer cell lines [6-8]. A tumour suppressing promotor gene and the BRCA1 protein is essential to perform several physiological functions, including transcription, ubiquitination, cell cycle control and repairing of DNA. The Znf C3HC4-RING domain, the BRCA1 serine domain and two BRCA1 C-terminal (BRCT) domains were the four basic domains constitutes a protein referred as BRCA1. An elongated structure with numerous repeats of 90–100 amino acid residues is the dual repeat BRCT domain of BRCA1 [9,10].

Much evidence suggested that BRCA1 affects lipogenesis through binding to p-ACC1 at BRCT domain, providing a new mechanism by which BRCA1 may exert a tumor suppressor function. Peptide p-ACC1 with the sequence of 1258-1271AA provides molecular evidence for direct interactions betweenBRCA1 and ACC1 [11]. PACC1 also referred to as peptide protein produced from chitosan, has been applied recently to develop targeted treatment that has numerous pharmacological activities towards DMBA-induced mammary cancer as reported [12].

In current investigation, we report PCN-J04 to alleviate the load of ACC1 in MCF-7 cells and signifies the contribution of pACC1 peptide on apoptotic, inflammatory and lipogenic markers and compared first attempt with ACC1 inhibitor 5-tetradecyloxy-2-furoyl-CoA (TOFA).

Materials and methods

Numerous suppliers provided the research materials. The supplier of peptide pACC1 was procured from Bio Concept Labs in Delhi, India. Sigma Aldrich in the USA provided the sodium tripolyphosphate and chitosan, which has a molecular weight range of 210 kDa. Furthermore, the USA-based company Cell Signalling Technology delivered us antibodies. Moreover, Santa Cruz Biotechnology in India supplied 5-tetradecyl-oxy-2-furoic acid, or TOFA. Each and every other substance that was executed in this research was based on analytical grade.

Cell Line followed by Culture

MCF-7 patient breast carcinoma cell line was acquired from the National Centre for Cell Sciences (NCCS) in Pune, India, and cultured in Dulbecco's modified Eagle's media with a 10% volume of fetal bovine serum (heat inactivated FBS) and one percent antibiotic-antimycotic mixture. Cells have been propagated to fullness at 37°C in a 5% CO2 environment (Shell lab, USA).

PACC1– Chitosan Nanoparticles

Following the technique outlined, peptide pACC1–Chitosan Nanoparticles (PCN) were created due to ionic cross-linking chitosan along with sodium tripolyphosphate (TPP) anions. For twelve subsequent hours, chitosan (2 mg per millilitre) was immersed in 0.25% (v/v) acetic acid and stirred for 10 °Celsius [13]. The chitosan-peptide combination (100 µg/mL pACC1 peptide dissolved in HPLC-grade water) was blended with an aqueous TPP solution (0.75% w/v) according to ratio of 2:1. The mixture was continuously stirred for duration about six hours at 4 °C. For 20 minutes at 4 °C, the resultant dispersion was transformed to centrifugation at 13,000 ×g (Remi, India). The PCN were collected, freeze-dried, and remained at -55 °C for later exposure after the supernatant ready to disposed.

MTT Assay

Adaptation of conventional MTT test (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) to measure mitochondrial dehydrogenase functional activities within healthier cells, cell viable possibility was followed in reference to the methodology [14]. In order to achieve 90% confluence, MCF-7 cells were cultivated in 96-well plates, with estimate around 3×10^6 cells individually for every well and incubated for 24 hrs. Following incubation, new media containing different doses of PCN-J04 and TOFA both of which had preceding been dissolved in DMSO were incorporated into the culture medium.

MTT solution was then incused to each well followed at endpoint concentration of 5 mg/mL for 24-hours incubation period and the plate was then incubated for 4 hrs at 37 °C temperature in dark environs. After incubation completed process, the cells were resuspended in 200 µL of DMSO, and the media was disposed away from the setup. An ELISA reader (Bio-Rad, Hercules, CA, USA) was assisted to measure the absorbance of the resultant formazan product at 570 nm with a reference wavelength displayed as 650 nm. For additional research, the IC50 values were considered.

Dual Staining Assays

Apoptosis changes were analyzed by fluorescence microscopic techniques using probes coated with fluorescent such as acridine orange (AO) and ethidium bromide (EB) stains [15]. After treatments, discard the medium, rinsed with PBS 2 times and stained with 100µg/mL AO and EB. Followed by incubation for 20 min at ambient condition under absence of sun light and washed with warm PBS to remove excess dye. The shape of the cell was observed using a

fluorescent microscope (λEx/λEm=490/530 nm) and photographed. Fluorescent intensity was measured at 535 nm using a spectrofluorometer.

Flowcytometry Assays

The cell cycle distribution was determined using flow cytometry analysis [16]. MCF-7 cells (2X105 cells/ml) were plated in 60-mm dishes and shortly after 24 h incubation completed, fresh media containing IC50 level effective dose of drugs was treated at various time intervals such as for 3, 6 as well as 12 hrs. Floating and adherent cells were collected, washed with PBS and fixed in 70% ethanol overnight. Fixed cells wiped and underwent incubation with a propidium iodide (PI) staining solution (50 mg/ml PI, 100 mg/ml RNase A and PBS) in the state of sunlight absence for 30 min at 37 o C. At least 10,000 cells were monitored using a BD FACSCantoTMflowcytometer (BD Bioscience, San Jose, CA, USA).

DNA Fragmentation Assays

To assess DNA fragmentation, agarose gel electrophoresis has to be initiated. The procedure began by seeding 1 million MCF-7 cells placed in 6-well culture dishes. Once the cells attained 70% confluency, they were exposed to increasing doses of the drug at IC50 concentration and incubated for about 48 hours. Following incubation, cells were collected, centrifuged, and cleansed around 2 times with ice-cold PBS. The cell pellet was then disrupted from the buffer solutions containing Tris-HCl, EDTA, and Triton X-100. After 10 minutes kept at ice condition, the lysate was centrifuged and the resulting supernatant comprises of RNA and fragmented DNA was extracted with phenol and phenol-chloroform-isoamyl alcohol. This process effectively isolated the fragmented DNA, discarding intact chromatin, as previously described [17]. Afterwards increasing the aqueous phase to 300 mM NaCl, two volumes of ethanol were incorporated to precipitate the nucleic acids. After attaining cleaning process with 70% ethanol was finished and allowed to dried in moist condition and the pellet was immersed in 20 µL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5). After RNA digestion with an enzyme RNase A (0.6 mg/mL) for thirty mins at 37 °Celcius, DNA samples were collected and administered to 1.5% agarose gel electrophoresis analysis. The gels were remained to electrophoresed, stained using ethidium bromide and examined with the aid of an instrument UV transilluminator.

Western Blot Analysis

Extracted proteins (30 µg) from every single group underwent heated process in sample buffer (0.2 M Tris-HCl buffer pH 6.8, 10% (v/v) glycerol, 2.0 % (w/v) SDS, 0.02 % (v/v) β-mercaptoethanol) for 5 min. Then, equal concentrations of each protein samples were resolved by 10% (v/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electro-transferred on to polyvinylidene difluoride (PVDF) membrane using semi dry method at 60mA for90 min (Medox, India). The cell membrane was barred with 1xTBS containing 5% BSA at room temperature for 1 h and followed by incubation full night with major antibody against Bcl-2, Bax, caspase-3, p53, PRAP, IKKα, IKKβ, NF-κB, FASN, and ACC1 above diluted value of 1:5000 and β-actin at 1:2000 1hat room temperatures. After washing, the membrane was then re-incubated with horseradish peroxidase (HRP)-connected secondary antibody (Genei, India) 1:10000 for 1hat room temperature. The inclusion of Diaminobenzidine (DAB) using as a substrate enables to visualize protein-antibody molecular combinations. Finally, Image J software was implemented with respect to quantify the relative intensity (NIH, Bethesda, MD).

Gene Expression Analysis

MCF-7 cells with the assistance of Trizol reagent (Genei, India) enable to extract total nucleic RNA. Using instrument spectrophotometer, the RNAs' concentration and purity were guaranteed. The mRNA synthesis was examined using RT-PCR. Following the manufacturer's instructions, one microgram of total RNA from each sample underwent reverse transcription (RT) to convert cDNA (AuPreP TM Gold cDNA Synthesis Kit, Life Technologies Pvt. Ltd., INDIA). As accompanied by the manufacturer (Thermocycler, BenchTop, USA), a polymerase chain reaction (PCR) was implied and amplified using PCR ready mix (Bioserve Biotechnologies (India) Pvt. Ltd). The primer designer 3 apps were used to frame the pattern sequences of corresponding sense and anti-sense primers in order for ACC1 (Forward 5'-CCGGCCAAGTTTGGTTTCCG-3' Reverse 5'-ACTTCCACAAACCAGCGTCT-3'), FASN (Forward 5'-TCTACACCACCATCCTGAACAAA-3' Reverse 5'- AGCCAAAGGAGTTGATGC CC-3'), and β-Actin (Forward 5'-TGTTACCAACTGGGACGACA-3' Reverse 5'-ACATCTGCTGGAAGGTGGAC-3'). Reaction substances have been fixed on 1.2 percent loaded with agarose gels labeled with ethidium bromide and observed using a UV transilluminator. Gene expression was assessed by standardizing with β-Actin PCR product (J-Image Software APPS).

Results

Effect of PCN-J04 on Cell Viability

The cytotoxic impact of PCN-J04 and TOFA on MCF-7 cell lines was performed according to the MTT test. Living cells minimize MTT, and the resulting formazan product modifies related to cell viability. PCN-J04 and TOFA treatment (6, 12, 25, 50, and 100 µg/mL for 24 h) of MCF-7 cells enhanced dependent on dosage toxicity to cells, with approximate IC_{50} values of 4.78 and 2.89 µg/mL for TOFA and PCN-J04, correspondingly (Figure 1A-B). These values were applied for future prospects. The survive cell proportion treated with PCN-J04 and TOFA reduced dramatically when compared to the untreated group (Figure 1C).

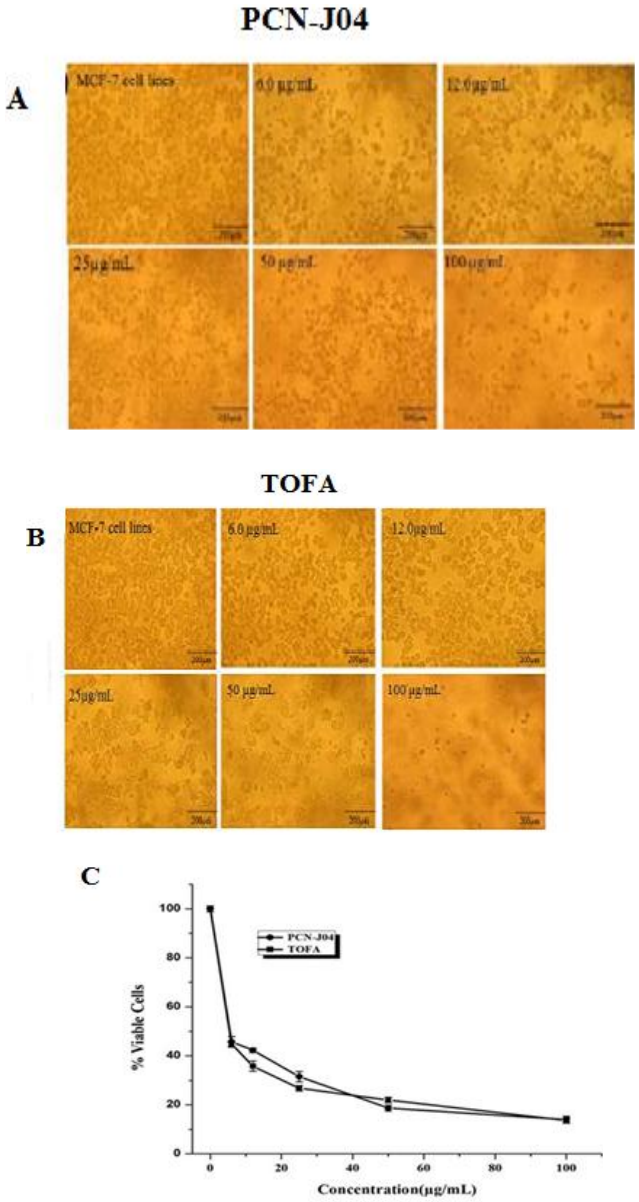
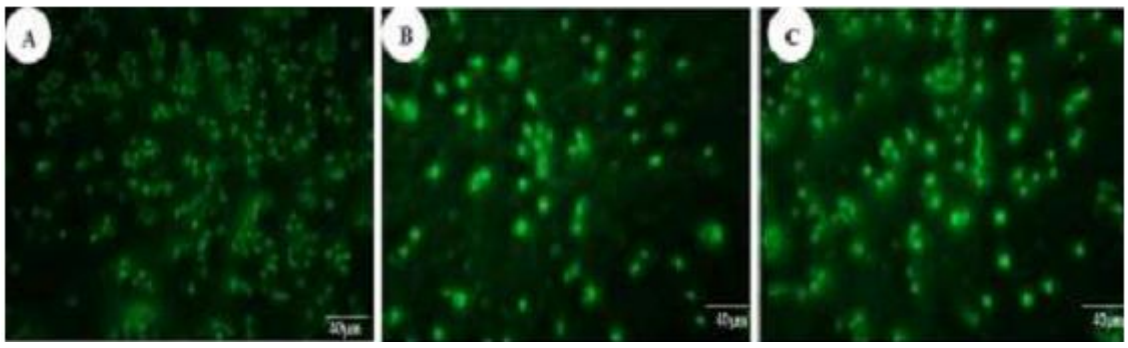


Figure 1: Dose Dependent Effect of PCN-J04 and TOFA Induced Cell Viability after 24 h. A) MCF-7 Cells Treated with Different Concentrations of PCN-J04 (6, 12, 25, 50 and 100 µg/ml of Final Volume Of Peptide pACC1 Containing PCN-J04).B) MCF-7 Cells Treated with Different Concentration of TOFA (6, 12, 25, 50 and 100µg/ml. C) Dose Dependent Effects of PCN-J04 and TOFA. Values are Presented As Mean ±SE of three Experiments in each Group.

Implications of PCN-J04 on Intracellular ROS Production

For the purpose to induce oxidative stress, cells were treated with IC50 doses of PCN-J04 and TOFA. DCFH-DA fluorescence measured ROS levels within cells and showed a direct correlation with ROS materials. Figure 2 shows that PCN-J04 and TOFA treatment significantly increased ROS levels in MCF-7 cell line as a result of 285 ± 12% and 264 ± 17%, respectively, as seen by increased green fluorescence relative to the control group.



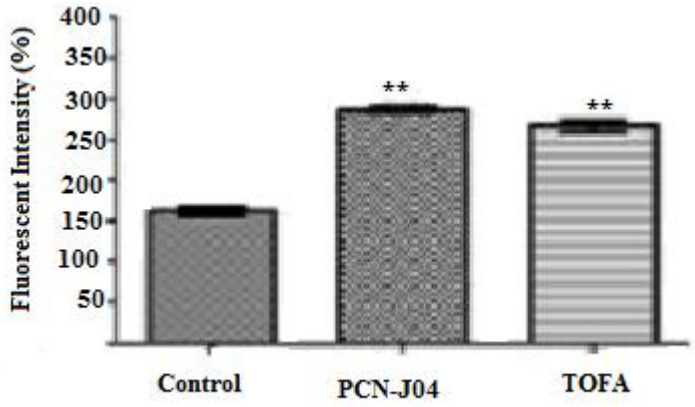
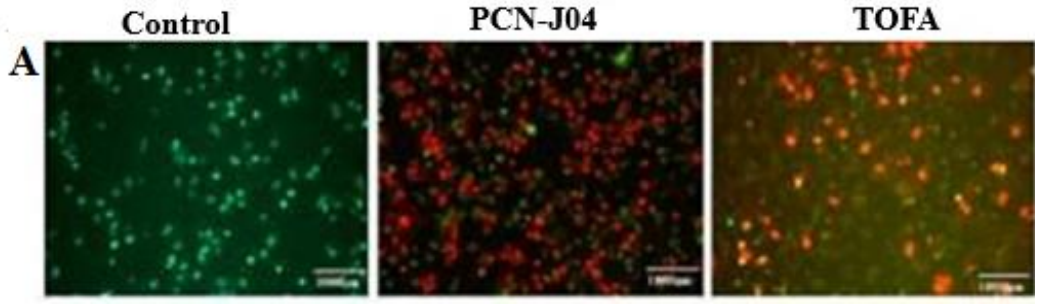


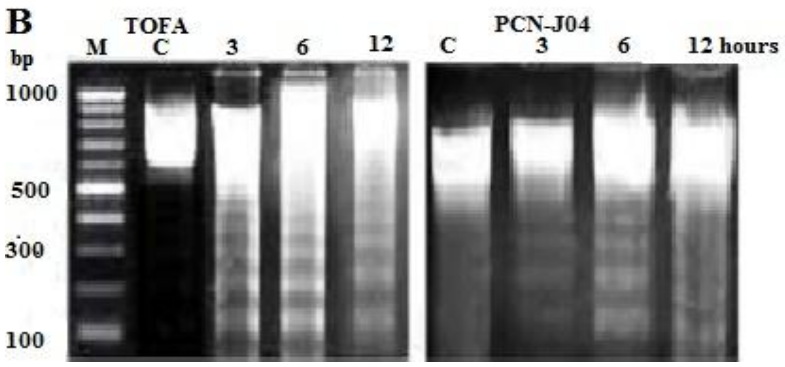
Figure 2: Effect of PCN-J04 on intracellular ROS Formation as Stained by DCFH-DA Photomicrograph Showing the oxidative Stress Effect of upon Treatment of PCN-J04 and TOFA.A) Control B) PCN-J04 C) TOFA and D) Fluorescent Intensity of Treated Cells Showing Significant Increase in the Levels of ROS as Compared to Control. Values are Presented as Mean±SE of three Experiments in each group ** P< 0.05 vs control.

Effect Of Pcn-J04 On Apoptsis

Dual AO/EB labelling was implied to assess the nuclear appearance of apoptotic cells, including chromatin condensation surrounding the membrane of the nuclear envelope. The results distinguish between the beginning of apoptosis (yellow) and delayed apoptosis (orange/red) in PCN-J04/TOFA-treated cells. In contrast, the control group showed homogenous brilliant green living cells (Figure 3A). A DNA fragmentation test was applied to determine the process involved due to cell demise caused by PCN-J04 and TOFA. The cells underwent therapy at different time intervals (3, 6, and 12 hours); a characteristic ladder arrangement of inter nucleosomal fragmentation was detected in cells after 3, 6 and 12 hours of PCN-J04 and TOFA treatment respectively (Figure 3B).

These findings indicate that PCN-J04 and TOFA constitute resilient catalysts of apoptosis in MCF-7 cell lines. The flowcytometry analysis determined the level towards in which reduction of viable cell population was caused due to cell cycle arrestor-induced apoptosis or either of the two (Figure 3C). The results suggest that treatment to PCN-J04 and TOFA exhibited a distinct sub-G1 peak, indicating an apoptotic cell population, which was clearly visible in cells subjected to the IC_{50} concentrations of PCN-J04 and TOFA. The gradual increase of Sub-G1 levels from the 3 h to the 12 h treatment of PCN-J04 suggests that a brief exposure to PCN-J04 may be sufficient for initiating apoptosis. Figure 3C illustrates that shortly after 12 hours of therapy with PCN-J04 and TOFA, over sixty percent of cells became stuck in the G2/M phase. However, the persistent in lipogenic block without any significant stoppage occurred in G2/M-phase. This shows that certain apoptotic occurrences due to lipogenic block have not been associated to mitotic arrest.





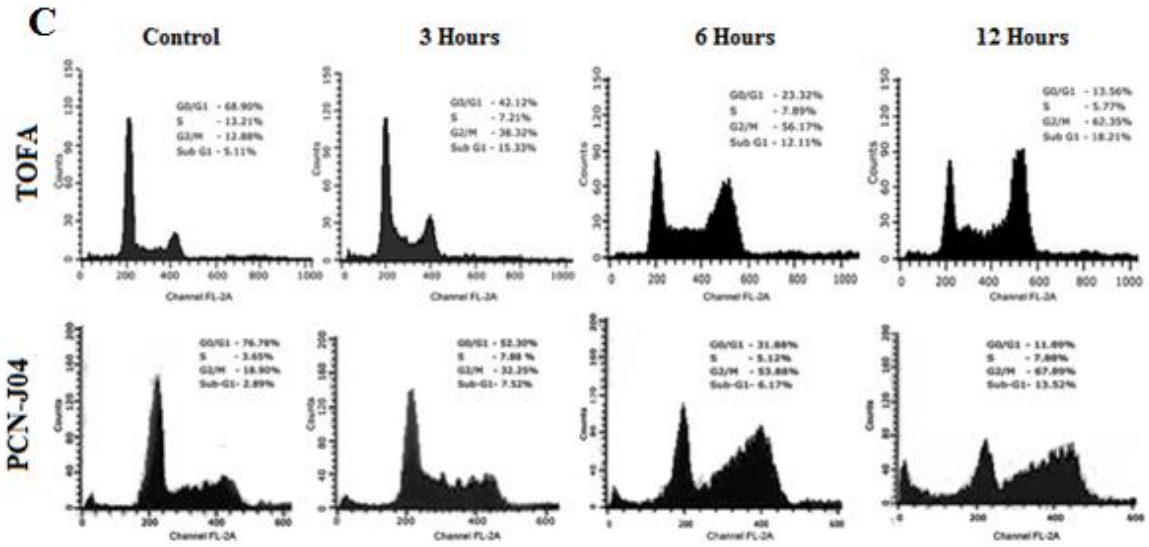


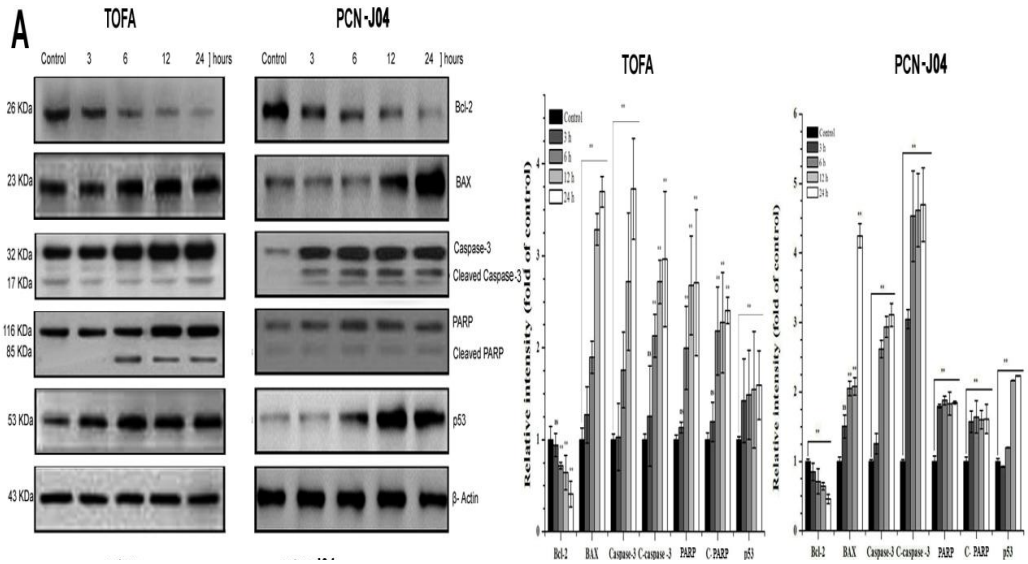
Figure 3: Apoptotic Effect of PCN-J04. A) Photomicrograph Showing the Effect of PCN-J04 (2.89 µg/ml) and TOFA (4.78 µg/ml) Induced Apoptotic Morphological Changes in MCF-7 cells. B) DNA Fragmentation of PCN-J04 and TOFA exposed MCF-7 cells for 3, 6 and 12 h. C) Flowcytometric Analyses of MCF-7 Cells Treated with IC50 Concentrations of PCN-J04 and TOFA at 3, 6 and 12 h cultures.

Influence of PCN-J04 on the Levels of Bcl-2, Bax, PRAP, Caspase-3, and p53

The stable between anti-apoptotic and pro-apoptotic proteins in the Bcl-2 family, specifically signifies the Bcl-2/Bax ratio, and serves as a regulatory process to determine cellular exposure to apoptosis via the intrinsic signaling way. Consequently, the expression levels of Bax and Bcl-2 were assessed through immunoblotting (see Figure 4). The findings stated that statistically significant increase ($P<0.05$) in Bax levels alongside a reduction in Bcl-2 following treatment with PCN-J04 and TOFA. Densitometric analysis indicated that Bax levels rose to be an approximate 2.8-fold, while Bcl-2 levels decreased by 0.8-fold compared to the control group after 24 hours of treatment with PCN-J04 and TOFA. Additionally, it was confirmed that the influence of PCN-J04 on pro-apoptotic and anti-apoptotic markers initiated as early as 3 hours' post-treatment. In contrast, TOFA did not exhibit a significant effect on the Bcl-2/Bax ratio after 3 hours of treatment.

The traditional signs of apoptosis were investigated in order to ascertain the biological process of cell death triggered by PCN-J04 and TOFA. In numerous forms of controlled cell death, caspase-3 cleavage occurs early and promotes PARP cleavage. The findings showed that the activity of both the initiator caspase-3 and the executor PRAP accompanied the further disruption of Bax/Bcl-2 in MCF-7 cells exposed by PCN-J04 and TOFA (Figure 4).

These findings enhance preceding research indicating that PCN-J04 appears functioning within three hours after therapy. However, during 3-hour treatments, TOFA showed without any significant ($P<0.05$) changes in PRAP and Caspase-3 levels. Many intrinsic responses to stress, including as genotoxic harm, oncogene stimulation, impairment of normal cell connections and oxygen or food deprivation engage the p53. Moreover, one of the metabolic stressors that triggers p53 that result in a restricted food supply to a cell or dysregulated nutrient-sensing strategies is lipogenic injury. Therefore, the following PCN-J04 and TOFA therapy, raise in p53 status could be observed (Figure4).



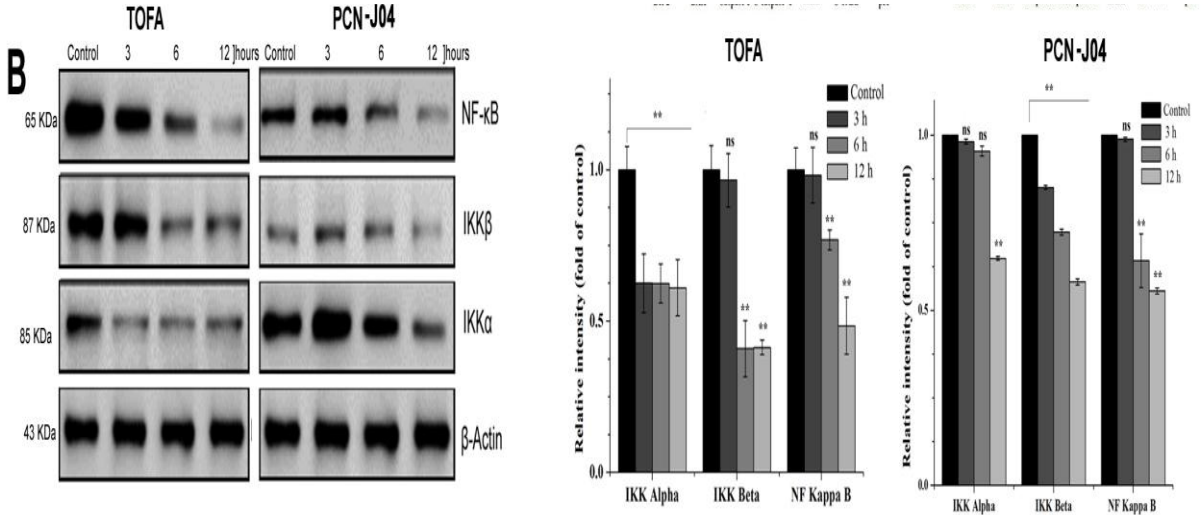


Figure 4: Immunoblot Analysis of Apoptotic Marker Proteins. Values are presented as mean ±SE of three Experiments in each Group, aP<0.05 vs Control, nnon-Significant vs Control.

PCN-J04 Alters the Inflammatory Protein Synthesis

In tumours' inflammatory processes, NF-κB is able to transfer into the nucleus and favour transcription of desired genes because the inhibitors of nuclear factor kappa-B kinase subunit alpha and beta (IKKs) phosphorylate and boost their cleavage. Acetylation of targets including PGC-a, STAT3, NF-κB, and NF-κB target genes may change in response to alteration in acetyl-CoA availability. IKKα, IKKβ, and NF-κB proteins were immunoblotted to determine the manner in which PCN-J04 regulates inflammatory proteins. The findings showed that PCN-J04 and TOFA both reduce the amount of expression of inflammatory markers (Figure 5). Nevertheless, TOFA disrupted the apoptotic pathway's early initiation of action by producing efficient NF-κB blockage within 3 hours.

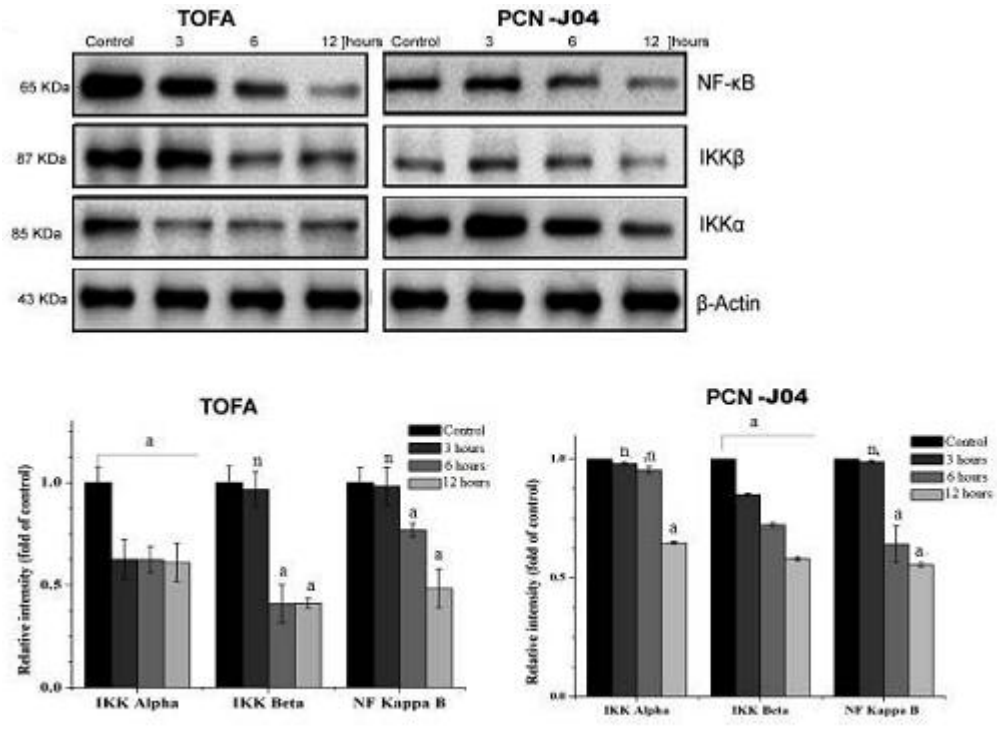


Figure 5: Immunoblotting of Inflammatory Marker Proteins. Values are Presented as mean ±SE of three Experiments in each group, aP<0.05 vs control, nnon-Significant vs Control.

Suppressions of Fatty Acid Production Process by PCN-J04

The potential cytotoxicity of PCN-J04 has been researched and target level protein molecules, such as ACC1 and FASN, in MCF-7 cells was evaluated. The lipid production of these cells in response to TOFA and PCN-J04 was illustrated in Figure 6. ACC1 is abundantly expressed in MCF-7 and is responsive to TOFA and PCN-J04, resulting in substantial suppression of fatty acid production ($p < 0.05$). As a result, TOFA and PCN-J04 demonstrate that blocking fatty acid production and reduces the likelihood of survival of MCF-7 cells.

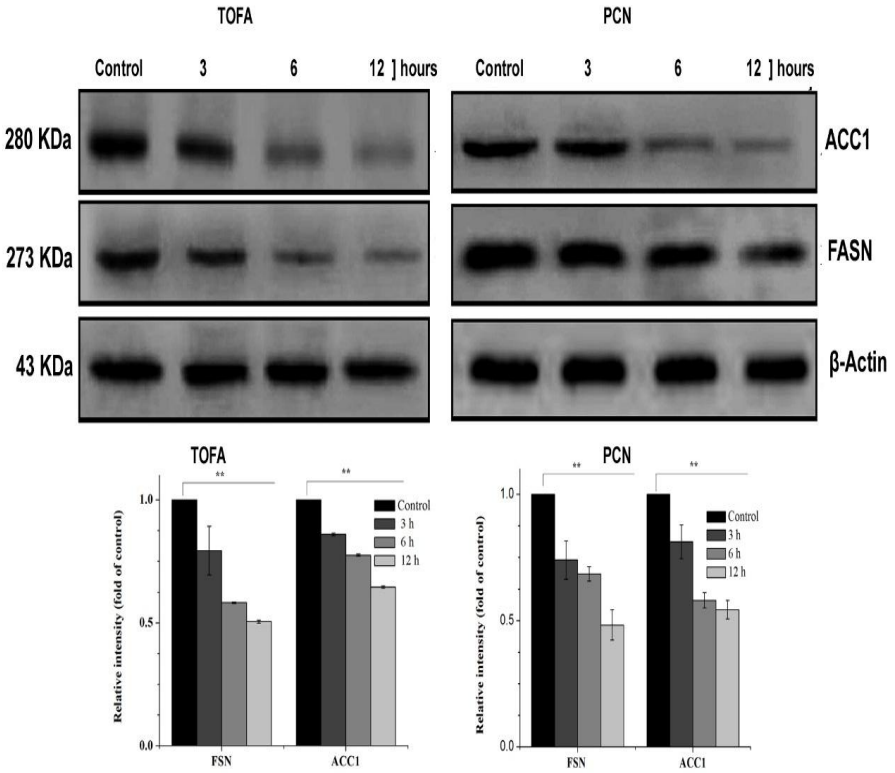


Figure 6: Inhibition of Fatty Acid Synthesis by PCN-J04.Immunoblot Expression of ACC1 and FASN. Values are Presented as mean ±SE of three Experiments in each group P<0.05 vs Control.**

Impact of PCN-J04 on Gene Expression of ACC1 and FASN

Alterations in transcriptional levels happened are frequently related with modifications to the manifestation of proteins. To corroborate the down regulation of ACC1 transcripts, an RT-PCR investigation has to be conducted. (Figure7A). The data revealed that the drop in ACC1 expression, followed by FASN, endured for up to 12 hours. Nonetheless, the PCN-J04 treated cells displayed neither substantial change in the expression of ACC1 and FASN genes after 3 hours of treatment (Figure 7B). However, TOFA-treated cells revealed sustained reductions in ACC1 and FASN gene expression initial at 3 hours. After 12 hours of PCN-J04 therapy, the expression of ACC1 (0.65) and FASN (0.54) genes decreased dramatically.

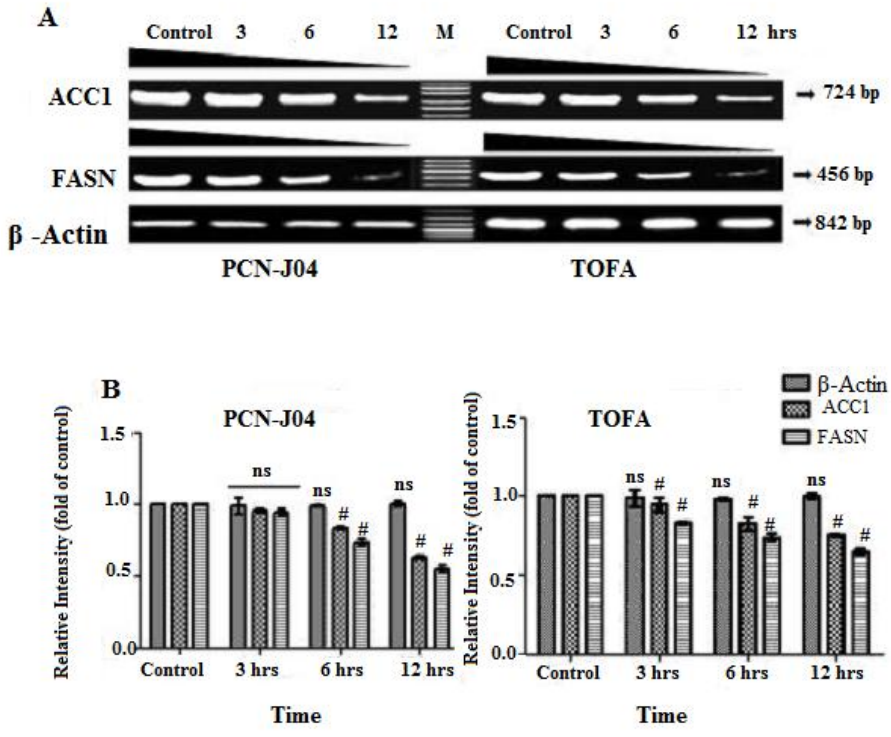


Figure 7: RT-PCR Analysis of ACC1 and FASN Genes. A) Agarose gel Electrophoresis of RT-PCR for ACC1 and FASN Genes Expression .B) Densitometry values of RT-PCR, nsnon –Significant vs Control, #P< 0.05 vs Control.

Discussion

BRCA1's ability to influence lipogenesis by lowering the amount of cytosolic ACC1 in cells of the mammary gland is closely linked to the phosphorylation-dependent pACC1 binding ability in the BRCT domain. Ser79, Ser1200, Ser327, and Ser1263 are the main phosphorylation sites of ACC1, and suggested to be a crucial in system operation. Additionally, BRCT domain repeats of BRCA1 bind specifically to the phosphorylated Ser1263 location of ACC1 as illustrated by glutathione S-transferase pull-down tests [18]. On the MCF-7 cell line, the pACC1 peptide-loaded chitosan nanoparticles displayed an apoptotic cascade triggered due to the oxidative stress enhance. PCN-J04's apoptotic impact was contrasted with that of TOFA, an ACC1 allosteric regulator. Tandem repetitions of BRCT domains can be noticed in BRCA1, and there is the possibility of not much aggregation, which contributes to rapid unwinding in the Free State, as seen in the pACC1 peptide compounds BRCT domain. TOFA promotes dose-dependent apoptotic in cells with human lung cancer [19]. These report were similar to our findings and both PCN-J04 and TOFA have a concentration-dependent apoptotic impact on MCF-7 cells.

A number of steps involved to convert carbons from citrate to reactive fatty acids. Essential fatty acid synthesis is mostly carried out by ATP citrate lyase, Acetyl-CoA carboxylase-1, which is the key pace-limiting enzyme and fatty acid synthase. Preventing these enzymes might impede cancer cells from surviving and multiplying. Furthermore, new findings clearly revealed that fatty acids required component for cancer cell persistence. Many enzyme inhibitors have limited effects on non-malignant cells for the clinical success of these therapies.

Phospholipids that include choline, such as phosphatidylcholine and sphingomyelin, are mostly found in the outer layer of the plasma membrane [20]. For the stability of membranes, this chemical distribution and compositions are required. Cells with damaged plasma membranes can absorb ethidium bromide (EB). This occurrence may thus lead to EB staining of cells, which might be caused by a decrease in phospholipids in the plasma membrane.

One of the features of apoptosis involves the fragmentation of genomic DNA into high molecular weight was suggested to be the final stage of the anticancer activity pathway [21]. It has been determined that Bax creates holes in the outer mitochondrial membrane, enabling cytochrome c to be released from the mitochondria into the cytoplasm. Furthermore, according to the Bax overexpression may led to inhibiting the action of anti-apoptotic proteins like Bcl-2 [22].

De novo lipogenesis limitation in B16-F10 murine melanoma cells elevates ROS levels and generates the intrinsic apoptotic process [23]. Lipogenesis regulation reduces tumour ingenuity by activating a caspase /PARP-mediated apoptotic mechanism in prostate cancer cells as well as animals harbouring xenograft human prostate cancers [24].

There is a possible approach in which p53 transforms the cellular stress response by functioning as a transcription factor to regulate the expression of genes for apoptosis including Bax. Augmented evidence describes p53 regulating non-genotoxic metabolic stresses, including hypoxia, metabolic stress like reduced lipogenesis and acidosis. The decision between apoptosis and growth arrest following lipogenesis inhibition is greatly influenced by p53 status [25]. Hence, the current investigation demonstrates that the continuous lipogenic block leads to activation of Bax followed by caspase / PARP-mediated apoptotic pathway and continuous elevation of p53 in the cell.

Lipogenesis inhibitors produce quick and persuasive inhibition of DNA replication by limiting phospholipid synthesis, resulting in a block in the cell cycle before G1 through the accumulation of p53 and of its main downstream effector. The present study also demonstrates that MCF-7 cells led to G2/M continuous cell cycle arrest and induced apoptosis, which was associated with the accumulation of p53.

Nuclear factor- kappa B (NF-κB) also counters programmed cell death. In vitro studies have demonstrated that reduction lipids as the result of biguanides may inhibit tumor progress by preventing p53-induced autophagy , and its treatment has an inhibitory effect on NF-κB [26]. With the liberation of NF-κB dimers following activation of IKKs, their steady state localization is normally shifted to the nucleus [27].The activation of NF-κB through over expression of IKK-β is a phenomenon due to unregulated lipogenesis [28]. These amendments were signified in our results; the continuous reduction in IKK-α, IKK-β and NF-κB levels were reported upon treatment with PCN-J04 as well as TOFA. The tumor oxidative stress may cause degradation of phospholipids by oxidation [29]. Polyunsaturated acyl chains are more susceptible to peroxidation; inhibition of lipogenesis generates a huge variety of lipid peroxidation products and renders cells more susceptible to oxidative stress–induced cell death. Modulation of *de novo* lipogenesis affects the sensitivity of cancer cells to oxidative stress and leads to apoptosis [30].

The ACC1 acts as a substrate for AMPK (Adenosine Monophosphate-activated Protein Kinase). AMPK is activated in response to lipid metabolic stresses and hypoxia naturally. An enhanced level of AMPK stimulates fatty acid oxidation and inhibition of ACC1 [31]. Hence, once the cell has undergone starvation of lipids following serial biochemical events make it worsen further to lead apoptosis which is much warranted in the effective clinical management of cancer prognosis.

Conclusions

Augmented confirmation achieved by the present investigation evidently demonstrates that of inhibition ACC1 by pACC1peptide loaded chitosan nanoparticles and TOFA as well leads to continuous activation of apoptotic cascade and

down regulation of inflammatory signals, which further supports its novelty for anticancer activity.

Author Contribution

Dharmar Manimaran and Vasan Palanisamy: conceptualization, methodology, carried out experiment, writing the original manuscript. Dharmar Manimaran: Data acquisition, writing review and editing. Dharmar Manimaran and Vasan Palanisamy: contributed to data curation, formal analysis, software. All the authors read and approved the final manuscript.

Conflict of Interest

The authors have declared having no conflict of interest concerning this article.

Consent to Participate

All authors have approved the final version of the manuscript.

Consent for Publication

All authors have approved the final version of the manuscript publication.

Ethical Approval

No ethics approval is required.

Sample Availability

Samples of the compounds are available from the authors.

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