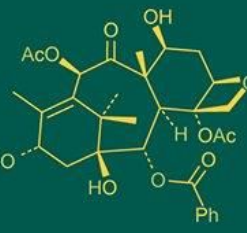
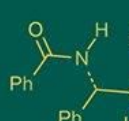


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Significance of Oleanolic Acid (OA) to counter the lung cancer

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Abstract

Chemically, Oleanolic acid (OA) is a natural pentacyclic triterpenoid abundant in the olive family. The therapeutic potential of OA has been evaluated in obesity, dyslipidemia, hypertension, and hyperglycemia, as well as, in anticancer activity. Moreover, OA is used to fabricate semi-synthetic triterpenoids in terms of high solubility, bioavailability, and efficacy for the therapeutic application of chronic disorders. Numerous derivatives of OA have been synthesized by researchers through significant alteration in the OA, less cytotoxic chemotherapeutic agents in lung cancer. CDDO (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid) has been approved in clinical trials and many more are under the pipeline. SZC017 has been reported against the lung cancer cell line. Previous results exhibit that OA and its analogue have a remarkable potential to arrest the abnormal cell growth of pulmonary metastasis, induce apoptosis and autophagy, regulate cell cycle regulatory proteins, inhibition of cancer cell migration and invasion.

Keywords: Oleanolic acid, triterpenoids, lung, IC₅₀, cancer cell line

Introduction

In the contemporary era, human beings are moving ahead toward natural products. In the past, fauna and flora have played a significant role in the era of chronic disease eradication (Nasim, 2019. Saha S, 2023) ^[25, 30]. Lung cancer is a chronic disaster prevalent globally due to busy routines and genetic reasons; meanwhile, the face of the disease suddenly declines in Western countries compared to developed countries (Jones, 2018). Expensive medicine, painful chemotherapy sessions, Multi Drug Resistance (MDR), and hurdles in early diagnosis are the horrific factors that cause the high mortality rate (Rodriguez-Canales, 2016) ^[29]. Victims of Non-small cell lung cancer (NSCLC) is around 80-85% while small cell lung cancer (SCLC) accounts for negligible but it is more hostile (Brody, 2014) ^[6]. Triterpenoids with a pentacyclic structure have been linked to an extensive range of bioactivities in the last ten years, like anti-inflammatory, hepatoprotective action, hypolipidemia, anti-hyperglycaemic, antioxidant action, nephrotoxic reactions, antiosteoporosis, antiproliferation of prostate cells, anti-muscle atrophy, anti-influenza, and antidepressant effects and cytotoxicity against several cancer cell lines viz, breast cancer, lung cancer, blood cancer, etc. (Bade, 2020) ^[2]. OA is an approved OTC medication for the cure of hepatitis disorder in China (Wu, 2021). It has been reported *in-vitro* anticancer potential against human cancer cell lines viz breast cancer (MCF7 and MDA-MB-231), glioma and glioblastoma (1321N1 astrocytoma), hepatic cancer (HCC cells), leukaemia (HL60), lung cancer (A549 and H460), pancreatic cancer (Panc-28), and skin cancer (A375), human prostate cancer DU145, cytotoxicity in cervical cancer cells (HeLa), human gastric cancer cells MKN-45 and SGC-7901, thyroid carcinoma. *In-vivo* anticancer potential was studied in mice against Balb/C HCC tumours, TPA- induced skin tumours in mice, 1,2 dimethylhydrazine-induced colon carcinomas in rats, and Melanoma induced lung metastasis (Gupta, 2022) ^[16]. It received its name since it was separated from the olive plant (*Olea europaea*) (Pollier, 2012) ^[28].

In situ edible part of plants like fruits, leaves and stem bark of traditional and medicinal plants viz. *Lantana camara*, *Lisgustrum lucidum*, *Olea europaea*, *Achyranthes aspera*, *Aspilia Africana*, *Monothea Buxifolia*, *Borreria stachyde*, *Ocimum sanctum*, *Flaveria Trinervia*, *Satureja Mutica* and *Miconia albicanset* are known as the rich sources of OA and

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sustainably offers the cure of various ailments. Natural pentacyclic triterpenoid OA is used as an over-the-counter drug for acute and chronic hepatitis. However, leaves of *Olea europaea* belongs to oleaceae family, is considered as the commercial source of OA and its content depends on the cultivation method, geographical origin, stage of development and environmental conditions. Beside that routine used spices in cuisine viz. *Thymus vulgaris*, and *Syzygium aromaticum* and fruits plants like apple, loquat, grape, elderberry and sage has also been isolated the OA. Crystallization and filtration, Silica gel column chromatography/CHCl₃/MeOH (99:1), Vacuum Liquid Chromatography and Column chromatography/n-hexane, chloroform and ethyl acetate, Precipitation and crystallization/chloroform and methanol, and Column Chromatography, Thin Layer Chromatography are the various method to isolate the OA. Furthermore Attenuated total Reflection-Fourier transform Infrared spectroscopy (ATR-FTIR), Differential scanning calorimetry (DSC), Electron ionization-mass spectroscopy (ES-MS), Gas chromatography-Flame ionization detector (GC-FID), Gas chromatography-mass spectroscopy (GC-MS), Infrared spectroscopy (IR), Reverse phase-ultra flow liquid chromatography-diode array detector (RP-UFLC-DAD), and Mass Spectroscopy (MS) are the reported characterization techniques for OA. (Wang, 2022; Deshpand, 2022, Dobhal K, 2023) [42, 9, 30, 31].

Lung cancer patients in India were divided among the population as 44% men and 47.6% women, according to data from population-based cancer registries (PBCRs) and hospital-based cancer registries (HBCRs) conducted as part of the National Cancer Registry Programme-National Centre for Disease Informatics and Research of Indian Council of Medical Research. Internationally, 1.80 million deaths were reported since 2020 (Schabath, 2019) [32]. Lung cancer is caused by smoking, second-hand smoke, and environmental pollution (Mao, 2016) [23]. Symptoms are chronic coughing chest pain, short breathing, weight loss, blood coughing, hoarseness, pneumonia, new onset of wheezing, tiredness, difficulty in swallowing, etc. Invasion of lung cancer by TNM, whereas T stands for the location of tumour size, N stands for node expansion to the lymph node near the lungs, and M stand for metastasis to another body part like liver, bone, brain, kidney, adrenal gland, etc. Lung cancer develops because of disruptions in numerous signaling pathways, just like other chronic diseases. Mono-targeted therapies are not the ideal choice because they are commonly associated with drug resistance and are unlikely to be helpful in the treatment or prevention of lung cancer ((Nooreldeen, 2021) [26]. Cancer cells frequently overexpress NF- κ B, a key transcription factor implicated in inflammation, which inhibits tumour cell apoptosis and maintains a persistently inflammatory milieu that is advantageous for cancer proliferation (Petronelli, 2009) [27]. Inflammation serves a crucial part in the development and propagation of cancer cells. Researchers working on the development of novel medications were motivated by OA due to its variety of pharmacological activity processes, low toxicity, and widespread distribution in plants (Mena, 2010) [24]. Asthma is a calamitous candidate to increase the patient of lung cancer, synergistic with inhalation of smoke during smoking. (Garg, R., 2022, Garg, R., 2024) [14, 15]

Chemical constituent diversity of OA

Lupeol, Ursolic acid, β -amyrin, α -amyrin, uvaol, erythrodiol, hederagenin and betulinic acid have been investigated for their therapeutic potential (El-Baba, 2021) [11]. The main issue associated with these that having limited water solubility which causes poor bioabsorption and biotransformation (Bergman, 2019) [4]. Still, the restricted therapeutically potential of pentacyclic terpenoids has been explored in view of biological activity. The critical nucleus can be modified in a variety of ways to identify potential clinical trial candidates for active compounds (Woo, 2021) [43]. OA is chemically 1-(3- β -hydroxyolean-12-en-28-oic acid). Oleanane-type pentacyclic triterpenes include OA, a secondary plant metabolite. Pentacyclic triterpenes have the chemical formula C₃₀H₄₈, which is composed of six isoprene molecules. They are created in plants by cyclizing squalene. It is used as the building block to create novel, semi-synthetic OA compounds with excellent efficacy, decreased side effects, improved bioavailability, and improved solubility (Sultana, 2008) [38]. It is a light-yellow, non-volatile chemical whose solubility rises with temperature. When compared to polar solvents like water, ethanol, 2-propanol, methanol, and acetone, 1-butanol and ethyl acetate are the ones where it is most soluble (Liu, 2005) [21]. Figure 1.

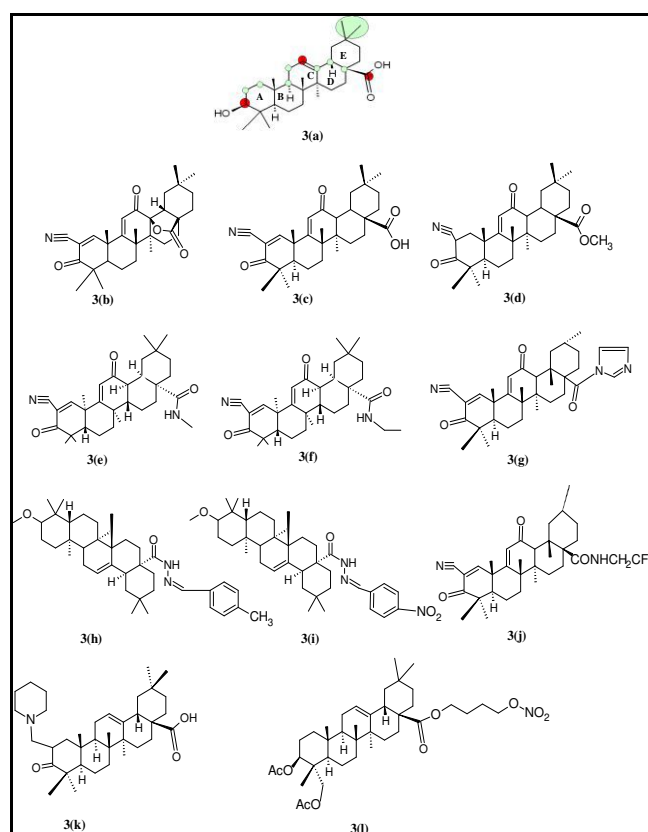


Fig 1: (a): Possible modification at the basic nucleus of OA (Red Highlighted circle modification produces anti-lung cancer active derivative); (b): olean-28,13b-olide 2; (c): 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oic acid; (d): C-28 methyl ester of CDDO (CDDO-Me); (e): Methyl amide ester of CDDO (CDDO-MA); (f): Ethyl amide ester of CDDO (CDDO-EA); (g): C28 imidazole derivative of CDDO (CDDO-Im); (h): Toluyl substituted OA hydrazide-hydrazone hybrid derivative; (i): Nitrophenyl substituted OA hydrazide-hydrazone hybrid derivative; (j): Trifluoromethyl amide of CDDO (CDDO-TFEA); (k): Piperidine derivative of OA (SZC017); (l): Nitric oxide derivative of OA

Therapeutically significance of OA and its analogues to counter the lung cancer

Even though chemotherapy is presently the recommended course of action for advanced NSCLC, most patients experience cross-resistance to different chemotherapeutic medications; also known as MDR. (Wu, 2018) [46]. OA is perceived as a possible starting molecule for future semi synthetic analogues due to its intrinsic-in pharmacological activity, accessibility, and reasonable production (Feng, 2020) [13].

NSCLC and SCLC are the broadly classified status of lung cancer in which NSCLS reported the major population. It further categorized viz. large-cell and squamous cell carcinoma, and adenocarcinoma. After prostate cancer it is the frequently occurring cancer in male and in female followed by the breast cancer. Resection, chemotherapy, radiotherapy, phototherapy, and immunotherapy and combinatorial therapies employed to sustain the lung cancer. (Samanta K, 2023) [31]. Figure 2 explore the possible signalling pathway assist in cancer reduction by OA and their formulation.

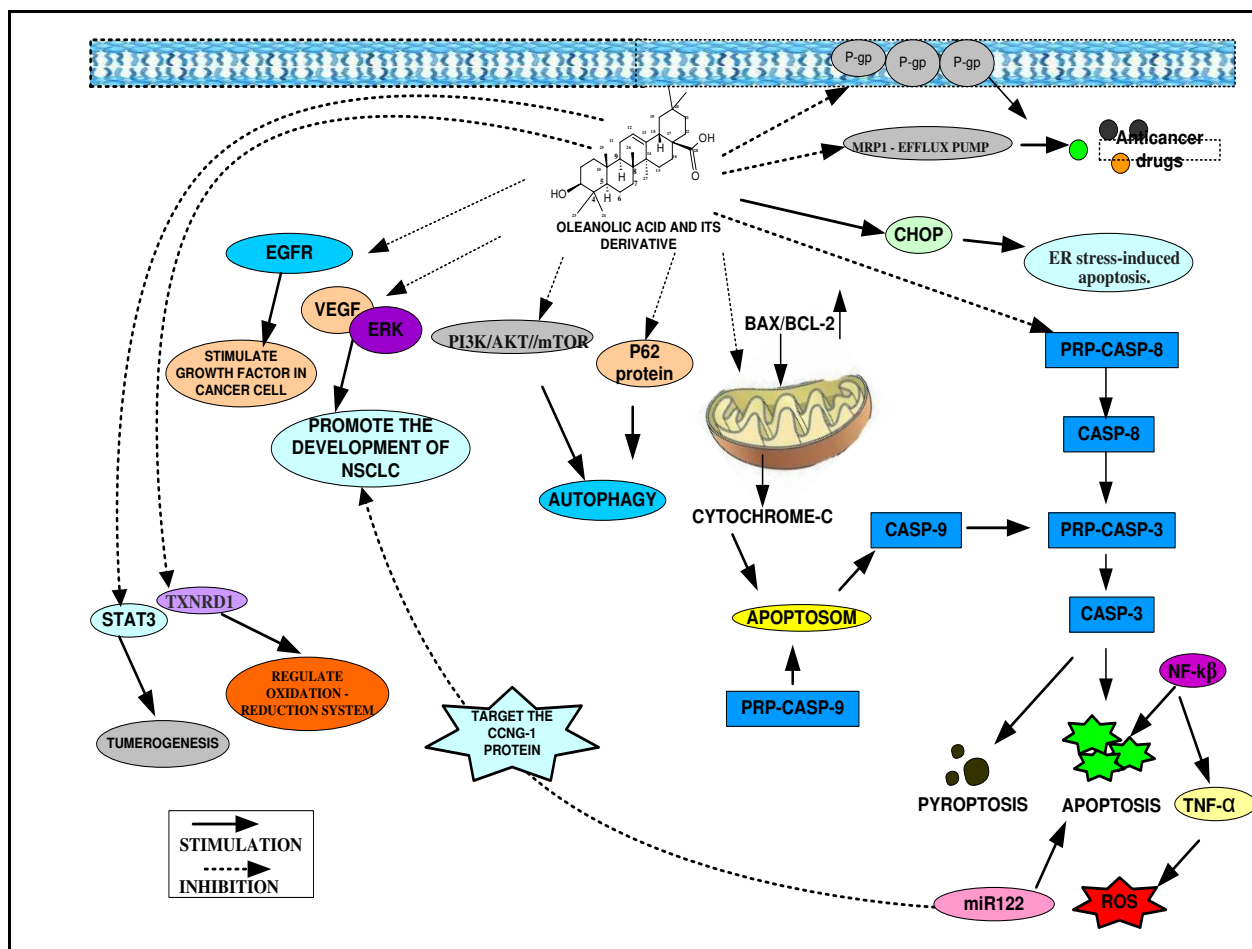


Fig 2: Reported multiple signalling pathway of OA and its analogue against cancer cells. Cysteiny aspartate specific proteinase (CASP); a key protein in the apoptosis and pyroptosis, Vascular endothelial growth factor (VEGF); survival and proliferation of NSCLC tumors; PI3K (phosphatidylinositol-3-kinase)/AKT (protein kinase B)/mTOR (mammalian target of rapamycin) pathway, signalling pathways involved in normal cellular processes; miR-122 is a novel tumour suppressor; induces apoptosis and restrains cell proliferation in multiple cancer cells in NSCLC; The human MDR-1 gene encodes a protein called P-glycoprotein (P-gp); thioredoxin reductases (TXNRD1); imparts a significantly vital role in regulating antioxidant defense, regulating intracellular redox balance, and gene transcription, Signal transducer and activator of transcription 3 (STAT3); promotes tumorigenesis, extracellular-signal-regulated kinase (ERK); regulate cell proliferation and migration, invasion, and metastasis, C/EBP homologous protein (CHOP); conduit to endoplasmic reticulum stress-induced apoptosis, NF- κ B; delaying tumour cell apoptosis and sustaining a microenvironment that is perpetually inflammatory and favourable of cancer growth

Ursolic and OA were subjected to investigate the eradication capability to A549 human lung cancer cells by using trypan blue assay and inducing auto-phagocytosis (self-suicidal pathway) in same by employing confocal microscopy and western blotting. However, Ursolic acid stimulates the AKT/mTOR pathways while OA cascade the AKT independent signalling. Both isomers alter the ultrastructural mitochondrial signalling imparts the essential role in auto-phagocytosis depend on the PINK1 pathway, known as mitophagy. The cristae structure of mitochondria was enlarged or swelled to reveal the gap between the two membranes. When OA treated cell subjected to Mito-tracker

green; caused mitochondria get fragmented in cytoplasm analyzed by confocal microscopy. Western blots interpret the p62 or sequestosome1 (SQSTM1) p62 augmenting when autophagy was blocked play; significant role in inducing mitophagy in A549 cell without or with 5 mM wortmannin (an autophagy inhibitor).

Furthermore, both acids were promising candidate of reactive oxygen species (ROS) production. OA induced a doses-response effect at 24 h and 48 h post-treatment, and only 40 μ g/mL OA caused a notable decline in cell viability (56%). PINK1/Parkin axis is another key regulator of mitophagy, OA subjected cell was analysed by to detect the

magnitude of PINK1 and Parkin proteins. Treatment of OA induced a reduction of p-mTOR independent on p-AKT, signifying the stimulation of auto-phagocytosis induced by OA. Mitophagy can be exerted by ROS production in A549 cells cause impairment of mitochondria membrane by NBT reduction assay. It's obvious that ROS production leads the impairment of healthy cells, to protect the healthy cell due to ROS production adopt the defence mechanism via stimulating of the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response elements (ARE) cascade pathway. There were over expression of Nrf2 expression after OA without or with 5 mM wortmannin was analyzed by Western blot.

A549 cells treated 20 µg/mL of OA and 5 mM wortmannin for 48 h were stained with Giemsa for 20 min and were stained with 1 mg/mL rhodamine 123 for 20 min and a reference histogram of the cells stained with rhodamine 123. OA and wortmannin treatment increased number of cells possessing mitochondrial membrane potential ($\Delta\psi_m$). Then incubated with 1 mg/mL MTT (3-(4, 5-dimethylthiazol-2-yl)- 2, 5-diphenyltetrazolium bromide) for 30 min and precipitated formazan was dissolved with DMS (Dimethyl sulphoxide). The absorbance was measured by using an ELISA spectrophotometer at 570 nm. (Castrejón-Jiménez NS, 2019) [7]

OA were treated to Human lung cancer cell lines, A549, NCI-H460, and NCI-H1299 to find the investigate arrest the cell cycle-related molecular pathway. OA abridged the proliferation rates of lung cancer cells in time- and dose-dependent manner along with over expression of miR-122/Cyclin G1/MEF2D pathway detected by qPCR assay. miR-122 is able to diminish all the cellular mechanism aspects of cancer cells viz. survival, proliferation, invasion, metastasis, drug resistance, metabolism, and angiogenesis. OA extensively abridged the expansion of cancer i.e. A549, 10.7-39%; NCI-H460, 1.3-41.8%; and NCI-H1299 in a dose-dependent manner while manner at the concentration of 30 µg/ml arrest the cancer cell growth in a time-dependent. Restoring their expression counteracted the effect of OA on lung carcinoma cells. CCNG1, a promoter of G0/G1 transition and MEF2D (pcDNA3-CCNG1 and pcDNA-MEF2D) considered as a miR-122 target, and under expressed in lung cancer cells treated with OA in both dose- and time-dependent ways by immunoblot assays. Hepatocyte nuclear factor (HNF) 1a, HNF3b, HNF4a, and HNF6, the considered miR-122-regulating transcriptional factors indicated the miR-122 over expression. Treatment of OA by immunoblot assay showed the up regulation of these transcriptional factors in both dose- and time-dependent manners. High dose of OA drastically arrests the lung cancer invasion xenograft in mice, qPCR assays exposed that miR122 expression was reversed and CCNG1 and MEF2D were depressed by OA. OA was further shown to induce the expression of miR-122-regulating transcriptional factors in lung cancer cells. Collectively, OA induced cell cycle arrest in lung cancer cells through miR-122/Cyclin G1/MEF2D pathway. This finding may contribute to the understanding of the molecular mechanism of OA's anti-tumour activity. (Zhao, X., 2015) [47]

The ethanolic extract of mango peel were subjected to HPLC analysis and disclosed the presence of phenolic bio constituent viz. vanillic aldehyde, caffeic acid, chlorogenic acid, gallic acid, procyanidin B₂ and OA. OA further

explored as the promising candidate against Human NSCLC cell lines A549 with an IC₅₀ value of (4.71±0.30 µM) by using cell counting kit-8 (CCK-8). 5-fluorouracil (3.8 µM) was employed as reference drug. Fluorescence staining procedure confirmed that OA arrest the cell proliferation by destroying the nucleus of A549 cell. (Bai X, 2018) [3]

MDR imparts to combat of lung cancer chemotherapy. OA suppressed the cell viability of the NSCLC cell lines A459 and H460 even with the MDR MRP1/ABCC1 proteins and the anti-apoptotic proteins Bcl-2 and survivin. However, stimulation of NSCLC cell apoptosis by OA cannot be justified due to inhibition of the MDR proteins. Furthermore, OA had no effect on the presence of the anti-apoptotic protein Bcl-2, but over expression observed of the pro-apoptotic protein Bax, altering the Bcl-2/Bax balance towards a pro-apoptotic profile. OA also claimed to decline of the surviving i.e. anti-apoptotic protein, the VEGF and under expressed the melanoma-induced lung metastasis. This study suggested that suggest that OA in the NSCLC repetition could reduce the occurrence of relapses and lessen the expansion of metastases. (Lúcio KA, 2011) [22]

To counter the MDR in cancer, the diverse mechanisms of chemotherapy drugs could be used. Due to high surface area and volume ratio, thermal, optical and magnetic phenomena; nanocarriers offers the high solubility for better penetration, extend the systematic circulation, target the specific site of action, and interaction with receptor substantially produce the potential the therapeutic effect along with minimum toxicity. Mesoporous silica nanoparticles (Nsi) have dragged the attention due to less toxicity and high efficiency. Cisplatin- Nsi was fabricated. Ethanolic solution of OA gradually added the aqueous solution of Cisplatin- Nsi at different stirring rate for one day and was precipitated to collect Cisplatin/OA-Nsi. The particle size and surface potential were characterized by a zetasizer and cisplatin content in the nanocarriers was measured by High-Performance Liquid Chromatography (HPLC). The size distribution of DDP/OA-Nsi was determined using the DLS method.

MTT assay was employed to evaluate cell viability against Cisplatin-resistant human lung adenocarcinoma cell line (A549/Cisplatin cell). Male Balb/c nude mice were employed to evaluate MTT assay. IC₅₀ of Nsi was higher than 300 µM in A549/ Cisplatin cells, indicating the insignificant cytotoxicity effects of Nsi on A549/ Cisplatin cells, which fully proved the biosafety of Nsi. The IC₅₀ of OA-Nsi was 131 µM, proposing that OA exerted certain cytotoxicity effects on A549/Cisplatin cells. It was concluded that Cisplatin /OA-Nsi showed the best anticancer effects on A549/Cisplatin cells, which might ascribe to the synergistic effect between Cisplatin and OA. As for OA and OA-Nsi, due to the drug characteristics of OA, the cytotoxicity of both was low; the cytotoxicity of free OA was the minimal.

The flow cytometry was revealed the apoptosis of A549/ Cisplatin cells after treatment of free OA, free Cisplatin, OA-Nsi, Cisplatin-Nsi, and Cisplatin /OA-Nsi or without treatment. The propidium iodide (PI) and Annexin V were selected as fluorescence dyes. 1.8%, 3.6%, 7.6% apoptosis induced by OA, the OA-Nsi and Cisplatin accordingly. Furthermore, Cisplatin-Nsi induced 9.9% apoptosis comprised by early apoptosis (2.7%) and late apoptosis (7.2%) while Cisplatin /OA-Nsi caused 15.6% apoptosis

comprised by early apoptosis (6.3%) and late apoptosis (9.3%). OA-Nsi and Cisplatin-Nsi produced more potential cytotoxic effect on cancer cell as comparison of cisplatin due to the excellent drug-loading of Nsi offers the high intracellular free drug and improved apoptosis in xenograft tumour model. Moreover, Cisplatin/OA-Nsi exhibited improved apoptosis-inducing capacity than Cisplatin-Nsi or Cisplatin-/OA-Nsi due to the nanocarrier co-delivery of OA and cisplatin possessing synergetic effect on the retention of cisplatin in cancerous cells to exert the apoptosis.

OA acid-loaded Fe₂O₃ magnetic nanoparticles (OMP) were prepared by co-precipitation method and subjected in A549 lung cancer cells for and their cytotoxicity to restore MDR. XRD, FESEM, and EDX were employed to characterize it. The cytotoxicity of pure OA, OMP, and -Fe₂O₃ nanoparticles was determined using the MTT assay. MTT assay revealed that OMP arrest the proliferation of both A549 cells and OA resistant A549 cells in a dose-dependent manner and exaggerated by Fe₂O₃ magnetic nanoparticles. The cancer cells were tested with OMP and Fe₂O₃ magnetic nanoparticles (200 µg/mL) for 24 h, after which the apoptotic cells were quantified by annexin V-FITC staining. The apoptosis was measured by flow cytometry and found that OMP group showed high apoptosis rate in comparison of standard and Fe₂O₃ magnetic nanoparticles. OMP resulted in 39.33% apoptotic cells compared to only 0.06% apoptotic cells (early apoptosis and late apoptosis) observed with the Fe₂O₃ magnetic nanoparticles. (Lee, K. 2016) [19]

Radiotherapy is being the last option to eradicate the malevolent tumour i.e. uninterrupted proliferation of cancer cell. Ionizing radiation works by generating the huge content of ROS and free radical (FR) which offers the DNA strands rupture and cause the cancer cell death. It has remained bottle neck of local resistance due to intrinsic and acquired resistance of tumour cells to radiation treatment. FR is responsible for the production of endogenous antioxidants viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH), while vitamin C and E, carotenoids and polyphenols are exogenous antioxidants. Among of endogenous antioxidants GSH is the tripeptide thiol L-γ-glutamyl-L-cysteinyl-glycine find in traces to maintain intracellular redox equilibrium and cellular defences.

In situ, GSH is synthesized from glutamic acid, cysteine and glycine, catalyzed by γ-glutamylcysteine synthetase (γ-GCS) i.e. rate controlling step and GSH synthase (GSS). Over expression of GSH is disclosed in the numerous tumour cells like bone marrow, breast, glioma, colon, larynx and lung cancers during a refractory response to ionizing radiation via GSH scavenging ROS and free radicals. Conversely, under expression of *in situ* GSH obviously exaggerate the radio sensitivity of cancer cells. Researchers investigated the effect of the OA on the radiosensitivity and the modification *in situ* GSH level on the rat glioma C6 and human lung cancer A549 cell lines. The influence of OA on cell growth was determined using the MTT assay. DMSO was used as blank. The results of the MTT assay showed that the IC₅₀ of OA for C6 cells was 35 µg/ml and for the A549 cells this value was 27 µg/ml, respectively

Irradiation was emitted using a 6-MV X-ray linear accelerator at dose rate of 250 cGy/min and the radiosensitivity of tumour cells was measured by using a clonogenic assay, intracellular levels of GSH were measured

with the glutathione reductase/5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) recycling assay kit. when compared with the cells in the absence of OA, significant decreases in the GSH levels of C6 cells in the presence of OA were noted. Furthermore, the same phenomenon was noted in A549 cells, where intracellular GSH levels showed a gradually declining tendency concomitant with increases in OA concentrations. The results revealed that OA effectively inhibits the synthesis of GSH in tumour cells.

The γ-GCS assay was an adaptation of a previously described method, in which γ-GCS in cell extracts synthesized γ-glutamylcysteine which was then reacted with 2,3-naphthalenedicarboxaldehyde (NDA) to form a highly fluorescent product that was able to be measured fluorometrically at 520 nm. γ-GCS in C6 cells was inhibited by the treatment with 20% IC₅₀ and 30% IC₅₀ of OA. (Wang J, 2013) [41]

Furthermore, tumour hypoxia level and biological modification during OA radio sensitization therapy via 18F-FMISO PET/CT and immunohistochemical experiment on *in vivo* C6 rat glioma cell line. The survival times of tumour-bearing rats in the control group, OA group, IR group, and OA combined with IR group were 43.67±3.3, 44.62±3.1, 53.3±2.2, and 56.8±2.6. TMR growth rates of the control and OA groups were 117.18% and 102.32%, respectively. TMR rates of the IR group and the OA combined with IR group were 3.12% and 11.54%, respectively. Immunohistochemical analysis was carried out to assess tumour biological indicators including HIF-1α, Glut-1, Ki67, P53, and micro vessel density (MVD). The percentages of HIF-1α positively stained cells in the control group, OA group, IR group, and OA combined with IR group were 84.53±4.91, 81.82±6.45, 52.53±6.87, and 38.87±6.32%, respectively. OA combined with radiation could inhibit tumour growth and prolong the survival of tumour-bearing rats. However, tumour volume did not shrink immediately after therapy and there was no significant difference compared with the IR group. Multiple poor prognosis biological markers including HIF-1α, Glut-1, Ki67, P53, and MVD in tumours were under expression in unison after radio sensitization therapy. (Wang H, 2018) [40]

OA reveals the therapeutic significance in cancer invasion, but low hydrophilicity and permeability, and bioavailability suffers in clinical assessments. To improve the bioavailability and decrease the efflux of OA; vitamin E-d-α-tocopheryl polyethylene glycol succinate (vitamin E-TPGS) and PluronicP105 were used. OA-loaded mixed micelles were prepared by ethanol thin-film hydration. OA uptake by two NSCLC cell line (A549 and PC-9) was enhanced by the micelles formulation as compared of free OA.

In vivo imaging showed that OA-micelles could accumulate in the tumours of nude mice. Additionally, smaller tumour size and increased expression of pro-apoptotic proteins were observed in OA-micelle-treated mice, indicating that OA-micelles are more effective than free OA in treating cancer. Cytotoxicity evaluations showed that the half-maximal inhibitory concentrations of free OA and OA-micelles were 36.8±4.8 and 20.9±3.7 µM, respectively, in A549 cells and 82.7±7.8 and 56.7±4.7 µM, respectively, in PC-9 cells. Apoptosis assays revealed that the apoptotic rate of OA-micelle-treated A549 and PC-9 cells was higher than that of cells treated with the same concentration of free OA. Wound

healing and trans well assays showed that migration and invasion were significantly suppressed in OA-micelle-treated cells. Immunofluorescence and Western blot analyses confirmed that the epithelial-mesenchymal transition was reversed in OA-micelle-treated cells. Mixed micelles are a promising nano-drug delivery system for lung cancer treatment. (Wu H, 2016) ^[44]

Nanoparticle-based delivery of anticancer agents accounts the better pharmacokinetics, targeted specific site of action, minimum toxicity profile with improved safety. (Singh A, 2021) ^[35] Researchers developed OA loaded cetuximab (CTX) functionalized albumin nanoparticles (ALB-NPs) to target EGFR in lung cancer therapy by employing molecular docking. Moreover, the in-vitro qualitative and quantitative cellular uptake experiment disclosed the higher uptake of CTX conjugated ALB-NPs than nontargeted ALB-NPs in A549 cells. The *in-vitro* MTT assay revealed that the IC₅₀ value of CTX-OLA-ALB-NPs (4.34±1.90 µg/mL) was significantly reduced ($p < 0.001$) than OLA-ALB-NPs (13.87±1.28 µg/mL) in A-549 cells. CTX-OLA-ALB-NPs caused apoptosis in A-549 cells at concentrations equivalent to its IC₅₀ value and blocked the cell cycle in the G₀/G₁ phases. The hemocompatibility, histopathology and lung safety study confirmed the biocompatibility of the developed NPs. *In vivo* ultrasound and photoacoustic imaging confirmed the targeted delivery of the NPs to lung cancer. (Shukla VN, 2023) ^[34]

Lung carcinoma is a vigorous type of melanoma, having high rate of morbidity and mortality with restricted therapeutic application. Dose dependent anticancer potential of OA conjugated chitosan nanocomplex (OAC) were evaluated on Human lung cancer cells (A-549 and IMR-90) by inducing apoptosis. Chitosan, a multifunctional biocompatible polymer and the bioactive principle, OA was integrated for the effective therapy against cancer was synthesized. They together found to inhibit the cellular breakdown in the treated lung cancer cells in a significant way. The influence of OAC nanocomplex on cell cycle phase distribution and also the mitochondrial membrane potential was assessed using flowcytometry. MTT assay was performed in order to find the IC₅₀ of the synthesized nanocomplex. In order to test the cytotoxic efficacy of the OAC nanocomplex, the treatment groups were established against the lung cancer cell line A549 at different concentrations such as 5, 10, 25, 50 and 100 µg/mL for 24 h. OAC nanocomplex increased the level of cytotoxicity likewise increased, while the normal lung epithelial cells

IMR-90 had not shown any significant toxicity even at higher concentration of 100 µg/mL. The optimum dose of IC₅₀ value was observed at the concentration of 25 µg/mL. The percentage of dead cells by apoptosis was checked by simple staining techniques such as AO/EtBr and DAPI under fluorescent microscope. synthesized nanocomplex decreases the cell count with intact mitochondrial membrane polarity while increases the cell count having low mitochondrial membrane polarity as vice versa after 24 h of treatment. In order to measure the percentage of dead cells using Annexin V/FITC, flow cytometry was performed. The percentage of live cells decreased significantly for the same concentration of 5, 25 and 50 µg/mL. (Abulaiti, A, 2022) ^[1]. Table 1 is the depiction of OA effects on various lung cancer cell line.

Consumption of Methyl amide derivative of CDDO (CDDO-MA) in the diet for two to three weeks; significantly reduced the number, size, and severity of the histopathology of lung tumors developed in female A/J mice with the carcinogen vinyl carbamate (Borella, 2019). The selective ligand for the retinoid X receptor agonist retinoid LG100268 has almost negligible affinity for retinoic acid receptors. Retinoids and triterpenoids signal via several biological pathways, therefore their combined effects on the prevention of lung cancer should be investigated. The CDDO's CDDO-Me and CDDO-EA are both strong inhibitors of lung carcinogenesis. A crucial factor in treating human tumours, which are known to have several genetic abnormalities, is the

multifunctional medications like CDDO-MA that target a variety of pathways. Even though 20 weeks after receiving vinyl carbamate, CDDO-MA significantly reduces tumour load. The CDDO-ME and CDDO are more powerful than the methyl amide. The drug levels of CDDO-EA are 2- to 3-fold higher in the lungs than with CDDO-MA, according to recent tests, indicating that CDDO-ME or CDDO-EA might be more suitable for therapeutic application in the lung (Song, 2019) ^[36]. U937, HL60, Jurkat T, HCT116, HCT116-Bax, and OCIAML3 cancer cells are induced to undergo apoptosis by CDDO-Me through the release of cytochromes from the mitochondria. The apoptosis-inducing compound CDDO-Me was used to either activate JNK and CHOP, which led to the production of Death Receptors (DR 5) and the activation of caspase-8 or to rapidly downregulate FLIP, an endogenous antagonist of caspase-8, in several lung carcinoma cells (Zou, 2017) ^[49].

Table 1: Emphasize OA and modified formulation effects in various lung cancer cell lines

Cancer Cellline	IC ₅₀ Value	Assay	Mode of action
A549 human lung cancer cells (Castrejón-Jiménez NS, 2019) ^[7]	20 µg/mL	Trypan Blue Assay, Confocal Microscopy and Western Blotting	Cascade the AKT independent signalling, PINK1 pathway
A549, NCI-H460, NCIH1299 (Zhao, X., 2015) ^[47]	30 µg/ml, time dependent.	Qpcr Assay	Over expression of mir-122/Cyclin G1/MEF2D pathway, CCNG1 and MEF2D were depressed
Human NSCLC cell lines A549 (Bai X, 2018) ^[3]	4.71±0.30 µM	Cell Counting Kit-8 (CCK-8).	Arrest the cell proliferation by destroying the nucleus of A549 cell
NSCLC cell lines A459 and H460 (Lúcio KA, 2011) ^[22]	50 µg/mL	MTT assay, Western Blot analysis	Over expression observed of the pro-apoptotic protein Bax, altering the Bcl-2/Bax balance towards a pro-apoptotic profile. Decline of the surviving i.e. Anti-apoptotic protein, the vascular endothelial growth factor (VEGF) and under expressed the melanoma-induced lung metastasis
Cisplatin-resistant human lung adenocarcinoma cell line in Male Balb/C Nude Mice (A549/Cisplatin cell).	Apoptosis of A549/ Cisplatin cells Cisplatin-Nsi induced 9.9% apoptosis comprised by early apoptosis (2.7%) and late apoptosis (7.2%) while Cisplatin /OA-Nsi caused 15.6% apoptosis comprised by early apoptosis (6.3%) and late apoptosis (9.3%).	MTT Assay, The Flow Cytometry	The synergistic effect between Cisplatin and OA
A549 lung cancer cells (Lee, K. 2016) ^[19]	Apoptosis induced at 200 µg/mL	MTT Assay, Flow Cytometry	
Rat glioma C6 and human lung cancer A549 cell lines. (Wang J, 2013) ^[41]	C6 cells was 35 µg/ml and for the A549 cells this value was 27 µg/ml,	MTT Assay, The Glutathione Reductase/5,5'-Dithiobis-(2-Nitrobenzoic Acid) (DTNB) Recycling Assay Kit	Intracellular GSH levels showed a gradually declining tendency concomitant with increases in OA concentrations.
<i>in vivo</i> C6 rat glioma cell line (Wang H, 2018) ^[40]	OA group, IR (irradiation) group, and OA combined with IR group were 43.67±3.3, 44.62±3.1, 53.3±2.2, and 56.8±2.6.	Tumour Biological Indicators Including HIF-1α, Glut-1, Ki67, P53, and Micro-vessel Density	OA combined with radiation could inhibit tumour growth and prolong the survival of tumour-bearing rats.
NSCLC cell line (A549 and PC-9) (Wu H, 2016) ^[44]	Free OA and OA-micelles were 36.8±4.8 and 20.9±3.7 µM, respectively, in A549 cells and 82.7±7.8 and 56.7±4.7 µM, respectively, in PC-9 cells.	Wound Healing and Trans Well Assays	To improve the bioavailability and decrease the efflux of OA; vitamin E-d-α-tocopheryl polyethylene glycol succinate (vitamin E-TPGS) and pluronicp105 were used.
A549 cells (Shukla VN, 2023) ^[34]	CTX-OLA-ALB-NPs (4.34±1.90 µg/mL) was significantly reduced (<i>p</i> <0.001) than OLA-ALB-NPs (13.87±1.28 µg/mL)	The <i>in-vitro</i> MTT Assay	Blocked the cell cycle in the G0/G1 phases. <i>In vivo</i> ultrasound and photoacoustic imaging confirmed the targeted delivery of the nps to lung cancer.
Human lung cancer cells (A-549) (Abulaiti, A, 2020) ^[1]	25 µg/mL	MTT, Flowcytometry.	Decreases the cell count with intact mitochondrial membrane polarity

Conclusion

This review has provided numerous findings of the abatement and rehabilitation of lung cancer by using either OA derived from plants or its synthetic and natural derivatives from *in vivo* studies. The CASP, VEGF, PI3K/AKT/mTOR, miR-122, and MDR-1 gene encodes a protein P-gp, TXNRD1, STAT3, CHOP, and NF-kB signalling pathways are targeted by several synthetic OA derivatives, which stimulate autophagy and death in both healthy and malignant cells. It is important to look at how rexinoids and triterpenoids work together to prevent lung cancer because they both signal through various biological pathways. Toxicities, stability, loading capacity, and the ability to cross biological barriers are a few of the issues that still need to be resolved for them to be used in the creation of new drugs. To improve biomedical activities, it is crucial to make use of the right technologies. Researchers are optimistic that exhausted studies and the development of dosage forms will significantly speed up OA research and application; serves people.

Conflict of interest

The authors declare that there is no conflict of interest.

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