Review article

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A review on chemistry, source and therapeutic potential of lambertianic acid

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Abstract: Lambertianic acid (LA) is a diterpene bioactive compound mainly purified from different species of *Pinus*. It is an optical isomer of another natural compound daniellic acid and was firstly purified from *Pinus lambertiana*. LA can be synthesized in laboratory from podocarpic acid. It has been reported to have potential health benefits in attenuating obesity, allergies and different cancers including breast, liver, lung and prostate cancer. It exhibits anticancer properties through inhibiting cancer cell proliferation and survival, and inducing apoptosis, targeting major signalling components including AKT, AMPK, NFkB, COX-2, STAT3, etc. Most of the studies with LA were done using *in vitro* models, thus warranting future investigations with animal models to evaluate its pharmacological effects such as antidiabetic, anti-inflammatory and neuroprotective effects as well as to explore the underlying molecular mechanisms and toxicological profile. This review describes the chemistry, source, purification and therapeutic potentials of LA and it can therefore be a suitable guideline for any future study with LA.

Keywords: daniellic acid; lambertianic acid; *Pinus lambertiana*; podocarpic acid; therapeutic potential.

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1 Introduction

Lambertianic acid (LA; $C_{20}H_{28}O_3$) is a naturally occurring bioactive diterpene compound primarily isolated from *Pinus koraiensis* (Family: Pinaceae). It is the (+)-enantiomer of daniellic acid, which can also be obtained from other plants such as *Pinus lambertiana*, *P. armandii*, *P. sibirica*, *Thuja orientalis*,and *Biota orientalis*. Until today, LA has been studied rigorously mainly using different *in vitro* models and reported to have wide spectrum anticancer, anti-obesity and antiallergic activities. Moreover, LA-enriched extracts were also demonstrated to have diverse pharmacological effects including anti-obesity, anti-inflammatory, anticancer, antidiabetes, anti-allergic, stress protective and neuroprotective effects. In view of the wide scope of using bioactive natural compounds as preventive and therapeutic choice, this review focuses on the health beneficial properties of LA. Thus, we here describe the chemistry, source, purification and pharmacological properties of LA with its future therapeutic prospects. This review could therefore be used as a suitable guide for future studies with LA and its natural or synthetic derivatives.

2 Chemistry, source, and purification of lambertianic acid

LA is a naturally occurring bioactive compound with the molecular weight of 316.4 g/mol ($C_{20}H_{28}O_3$). It is the optical isomer of daniellic acid and epimer of (+)-polyalthic acid at C-4 position (Figure 1). According to IUPAC, its chemical name is (1*S*,4*aR*,5*S*,8*aR*)-5-[2-(furan-3-yl)ethyl]-

1,4*a*-dimethyl-6-methylidene-3,4,5,7,8,8*a*-hexahydro-2*H*-naphthalene-1-carboxylic acid. It contains two fused aliphatic rings with a β-substituted furan ring. Presence of an axial carboxylic group in LA facilitates axial methyl or hydrogen interactions. π-Electrons presented in the two double bonds and the lone pair electrons of oxygen atom in the furan ring act as a good attraction for electrophiles. The presence of two quaternary methyl groups can be observed where the methyl of the lower field is most likely attached to the carbon atom that holds the carboxyl group.

Accumulated evidence suggests that LA was mostly purified from different species of *Pinus*. Its different sources are listed in Table 1. LA was firstly purified from *P. lambertiana* [1]. Oleoresin collected from *P. lambertiana* was dissolved in ether and precipitated as salts. The soluble cyclohexylamine salts were separated by acidification. Then the solution was neutralized by potassium hydroxide. The produced acid was crystallized and purified with several recrystallization steps. Although the yield was only 9% in this process, it was almost in pure form.

More recently, LA was purified from the leaves of *P. koraiensis* [2]. Fifty percent methanol (MeOH) extract was prepared from the leaves and concentrated, which was partitioned with ethyl acetate (EtOAc)/distilled water (1:1). A small portion of EtOAc fraction was then taken to a celite column chromatography, treated with chloroform and MeOH (3:1), and finally 15 fractions were obtained. Among those fractions, fraction 6 containing a vivid red–purple spot was yielded LA with more than 98% purity.

Previously in 2003, LA was purified from Siberian cedar gallipot (*P. sibirica*) [3]. The collected sample was used to separate volatile fraction using water vapor. The residue was then dissolved in hot petroleum ether and an organic layer was collected. The hot solution was converted into gel emitting gaseous ammonia bubble. Petroleum ether was added to the gel with vigorous stirring. This resultant precipitate was washed again by petroleum ether. The residue contains ammonium salts and isopimaric and abietic acids. In the next step, it was dried and mixed with HCl–water mixture. Organic layer from this mixture was decanted and then evaporated. Diethyl amine (pH = 9) was added to the mixture and stored overnight. The precipitated crystals were washed with petroleum ether to obtain diammonium salts of resin acids. The resin acid again mixed with petroleum ether to obtain diammonium salt of LA. Then, LA was isolated by treating with *tert*-butyl methyl ester, concentrated HCl, water and then dried over $MgSO_4$. The resultant product was recrystallized to get the pure LA.

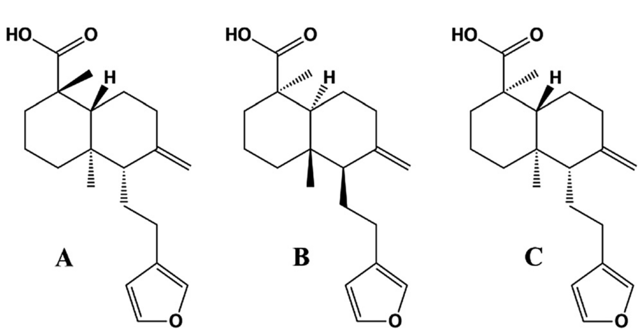

Figure 1: Lambertianic acid (A), its optical isomer daniellic acid (B) and stereoisomer polyalthic acid (C).

Table 1: Sources of lambertianic acid.

Plant name	Plant parts	References
Pinus koraiensis	Leaves	Lee et al 2018
P. lambertiana	Oleoresin	Dauben & German 1966
P. armandii	Leaves	Fang et al 1991
P. bungeana	Oleoresin	Zhan-Qian et al 1998
P. sibirica	Needles and oleoresin	Chernov et al 2005
Thuja orientalis	Leaves	Chae & Chin 2012

3 Synthesis of lambertianic acid

In 1972, Bell and co-workers took an attempt to synthesize LA from another natural compound podocarpic acid [4]. They faced two main problems to accomplish LA synthesis: generation of the exocyclic methylene at C-8 and attachment of the furan ring at C-12. After several trials, they finally resolved the associated problems and got success. The synthetic scheme showing the intermediates is presented in Figure 2. Additionally, LA was used as a starting material to synthesize potential derivatives with specific bioactivities. Chernov et al. [3] proposed efficient methods for the synthesis of several biologically active compounds from LA, such as cantharidin, dihydroisoindole analogs, 14,16-epoxyabietane diterpinoids, furoazocine and furoazonine derivatives.

4 Therapeutic potential of lambertianic acid

4.1 Anti-obesity and hypolipidemic activity

LA-enriched ethanol extract of *P. koraiensis* (EPK) (98.7 µg/mg of LA) has anti-obesity and hypolipidemic effect, as demonstrated by both *in vitro* and *in vivo* studies [5]. Lee

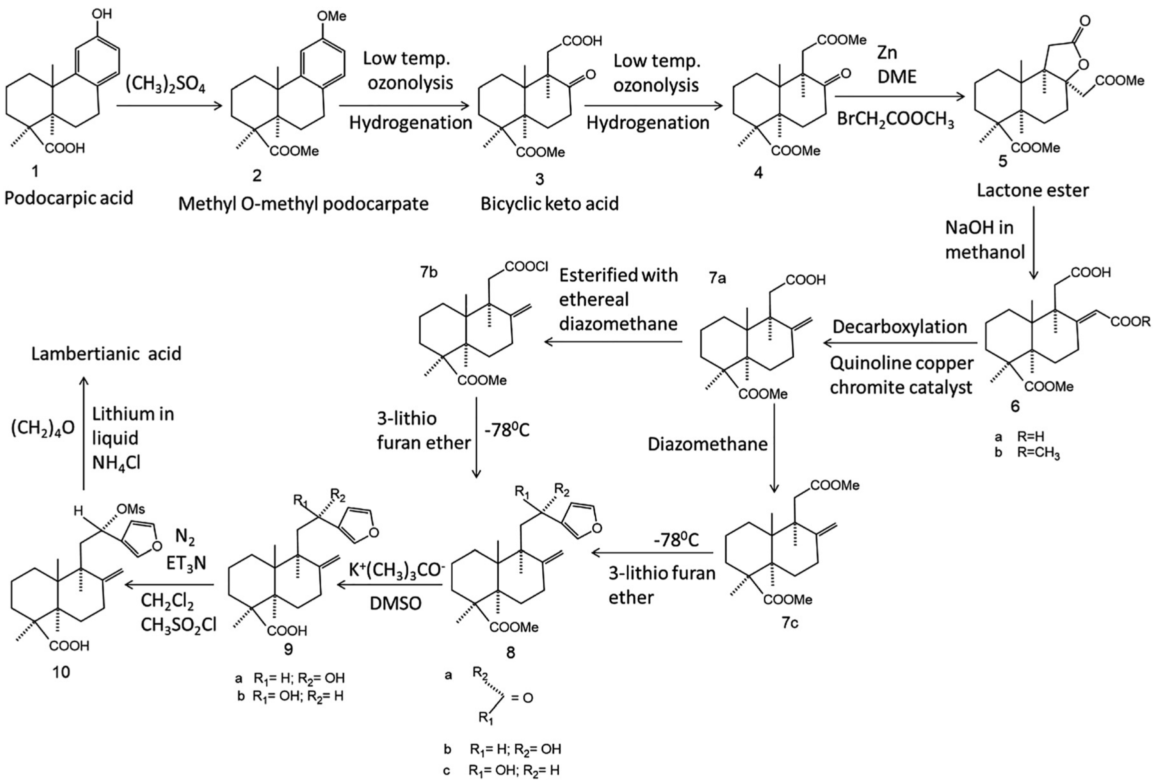

Figure 2: Scheme showing the steps involved in synthesis of lambertianic acid from podocarpic acid.

and colleagues revealed that EPK efficiently attenuates adipocyte differentiation and adipogenesis in 3T3-L1 adipocytes and in rats with high fat diet (HFD)-induced obesity by activating adenosine monophosphate activated protein kinase (AMPK) (Figure 3). AMPK helps in regulating glucose levels and lipid uptake and phosphorylated AMPK activates metabolic enzymes involved in fatty acid and cholesterol synthesis [6–8]. In addition, activated AMPK upregulates PPAR-γ and C/EBPα, suppresses differentiation of preadipocytes into adipocytes, and decreases cellular cholesterol and fatty acids [9–11]. 3T3-L1 preadipocytes cells were treated with EPK for 24 h and it was observed that EPK did not show major effects on cell viability, but it reduced the level of fat concentration after six days of exposure, indicating disturbed adipocytes differentiation. Moreover, EPK reduced the cellular droplets and serum triglycerides levels in 3T3-L1 cells. EPK significantly suppressed the expressions of PPAR-γ, C/EBPα, adiponectin, SREBP-1, HMGCR, FABP and GPDH, and increased phosphorylated AMPK (p-AMPK) in a concentration-dependent fashion, while no change in total protein levels were observed in 3T3-L1 cells. Furthermore, EPK reduced the retroperitoneal and epididymal fat weight as well as serum triglyceride and cholesterol levels while increased the expression of HDL cholesterols compared to those in HFD-fed rats, implying that inhibition of lipid metabolism plays a role in obesity prevention by EPK. Likewise, LA also inhibited fat accumulation in adipocytes, decreased the expression of PPAR-γ, C/EBPα, adiponectin, FAS, SREBP-1 and HMGCR and increased the expression of p-AMPK [5]. In response to LA, lipid accumulation in 3T3-L1 adipocytes has been appeared to be 32% compared with that of the control group. Taken together, the AMPK regulation by LA might be a potent therapeutic approach in

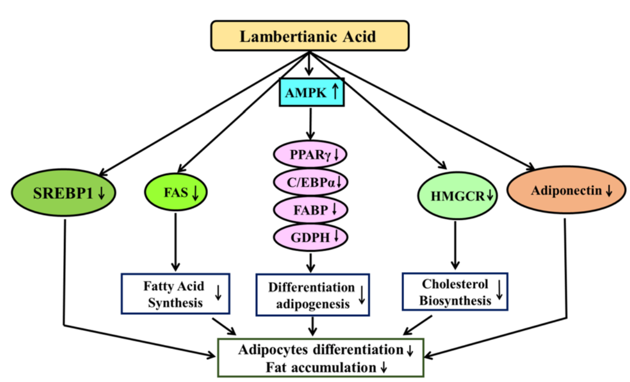

Figure 3: Anti-obesity mechanisms of lambertianic acid (LA) in 3T3-L1 adipocytes. LA downregulates several pathways related to adipocyte differentiation and fat accumulation, including fatty acid and cholesterol synthesis.

preventing obesity in HFD-fed animal models although further investigations are needed to substantiate the anti-obesity effects of LA.

4.2 Anti-allergic activity

LA extracted from *T. orientalis* exhibits a response against allergy and inflammation in bone marrow-derived mast cells (BMMC) collected from male Balb/cj mice [12]. Chae and Chin [12] evaluated the effects of LA on different allergic mediators in phorbol 12-myristate 13-acetate (PMA) plus calcimycin-stimulated BMMCs and showed that LA (15, 30 and 60 μM) remarkably represses the production of prostaglandin D2 (PGD_2). Dose-dependent reduction of leukotriene C_4 (LTC_4) levels was also observed when BMMCs were treated with LA for 30 min. Pretreatment of BMMC with LA (0, 30 and 60 μM) for 30 min before being exposed with PMA plus calcimycin significantly reduces mRNA expression of cyclooxygenase 2 (COX-2) and interleukin 6 (IL-6) cytokine secretion. It was also observed that LA can suppress the release of β-hexosaminidase in a concentration-independent manner in BMMC. Accumulating evidences have proved that the mast cells release different types of pro-inflammatory mediators and allergic stimulators, such as IL-6, histamine, LTC_4 and PGD_2, that mediates acute allergic reaction in response to interaction with allergens [13, 14]. Allergic and inflammatory roles of COX-2 and its metabolites PGD_2 may be potent targets for treating patients with allergen response [15, 16]. Considering all the above facts, LA-induced anti-allergic reactions were consistent with the previous studies related to constraint allergic reactions and LA might be therefore a probable choice for allergic treatment in future.

4.3 Anticancer properties

4.3.1 Breast cancer

Breast cancer is now counted as the most-encountered malignancy found in women. Lee et al. [2] tested LA (0, 7.5, 15, and 30 μM) on two breast cancer cells, MDA-MB-231 and MCF-7, and observed that it shows strong cytotoxic effects on both cells after 24 h with IC_{50} values of 31.8 and 21.1 μM, respectively. It also exhibits apoptotic effects on MDA-MB-231 cells, indicating the cytotoxicity is mainly attributed to apoptosis. Protein assay and cell-cycle analysis of LA-treated MDA-MB-231 cells revealed that LA induces G2/M phase arrest, increases the sub-G1 population and the cleavage of poly (ADP-ribose) polymerase (PARP) and decreases the expression of pro-caspase-3. In addition, LA activates AMPK and acetyl-CoA carboxylase (ACC) through phosphorylation and can suppress protein kinase B (AKT) phosphorylation, suggesting that the AMPK and AKT pathways are associated with LA-induced apoptosis in breast cancer cells (Figure 4). Western blot assay also confirmed that LA actively attenuates the expression of forkhead box protein M1 (FOXM1) and its regulated gene products, including proliferative proteins (Cyclin B1) and anti-apoptotic proteins (X-linked inhibitor of apoptosis protein [XIAP] and B-cell lymphoma 2 [Bcl-2]) in MDA-MB-231 cells. FOXM1 acts as a transcription factor which mediates the transcription of cell cycle-related genes and promotes cell proliferation by the activity of cyclins (Cyclin B1 and D1) in addition with upregulating antiapoptotic proteins Bcl-2 and XIAP [17–20]. Moreover, FOXM1 functions as a downstream protein of AKT signalling pathway [21], and immunoprecipitation of MDA-MB-231 cells with anti-AKT antibody revealed that LA disturbs the binding of FOXM1 with AKT and the binding score of protein-protein interaction is appeared to be 0.805. AMPK inhibitor compound C (Dorsomorphin) can reverse the apoptotic ability of LA, thus suggesting that AMPK regulates the binding of AKT with FOXM1 in LA-induced apoptosis in breast cancer cells. Through inhibiting the AKT/FOXM1 signalling pathway especially by disrupting their binding, LA leads to MDA-MB-231 cells to programmed cell death.

In a recent study with two breast cancer cells, MDA-MB-453 (signal transducer and activator of transcription 3 [STAT3] mutant) and MCF-7 (STAT3 wild type), LA was found to induce cell cytocidal effects more potently in MDA-MB-453 cells than in the MCF-7 cells after incubation for 24 h and its effects on cell viability was dose-dependent [22]. Cell cycle analysis indicated that LA at 30 μM can cause an increase in sub-G1 population and cleavage of PARP in MDA-MB-453 cells more than those in the MCF-7 cells, and the amounts of sub-G1 population were 42.75 and 6.84% in MDA-MB-453 and MCF-7 cells, respectively. Moreover, LA suppresses the phosphorylation of STAT3 and NF-κB, the expression of p300 and RelA/p65 acetylation, subsequently attenuating nuclear translocation of p-STAT3 and NF-κB through their colocalization in MCF-7 cells. Accumulating evidence demonstrates that STAT3 is upregulated in most cancers and NF-κB increases the interaction between STAT3 and p300 [23–25]. STAT3 also plays a significant role in p300-mediated RelA acetylation [26, 27], resulting in accumulation of RelA/p65 in the nucleus [28]. It has been well proved that several inflammatory cytokines, including IL-6, IL-23 and COX-2, activate STAT3 through NF-κB regulated inflammatory responses, and RelA/p65 is conserved through p300-mediated RelA/

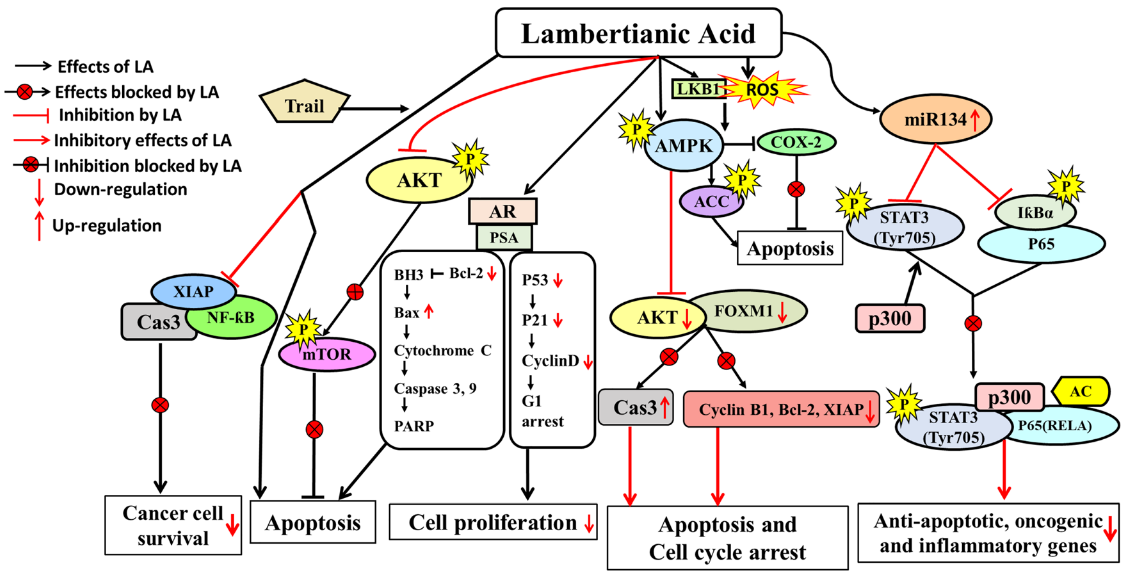

Figure 4: Anti-cancer mechanisms of lambertianic acid (LA). LA acts on different important signalling cascades responsible for cancer cell proliferation, survival and for inducing of apoptosis and cell cycle arrest.

p65 acetylation by STAT3 [26]. Consistently, it was reported that LA blocks the expression of NF-κB regulated genes, including anti-apoptotic proteins (Bcl-2, Bcl-xL, XIAP and survivin), angiogenic protein vascular endothelial growth factor (VEGF), inflammatory protein COX-2, oncogenic genes cellular myelocytomatosis (c-Myc) and inflammatory mediators IL-6 and tumour necrosis factor-alpha (TNF-α) in the MCF-7 cells [22]. In contrast, IL-6 blocked the ability of LA to induce cytotoxicity and PARP cleavage and also suppressed the sub-G1 population to 12.11% relative to that of LA alone in MDA-MB-453 cells. However, the depletion of STAT3 or p300 enhanced the PARP cleavage by LA in MCF-7 cells incubated with LA for 24 h with or without IL-6. In addition, IL-6 downregulated the phosphorylation of STAT3, IkB kinase and p65 and reduced the expression of p300 and Ac-RelA in MDA-MB-453 cells, compared to the untreated control. It is worthy to mention that LA disrupted the binding between phosphorylated STAT3 (p-STAT3), p300 and RelA (p65) and upregulated the level of miRNA134, and miRNA134 mimic disrupted the expression of pro-PARP, p-STAT3, and Ac-RelA. Conversely, the miRNA134 inhibitor reversed the ability of LA reducing the expression of Ac-RelA and pro-PARP in MCF-7 cells, implying that LA can induce apoptosis via miRNA-134-mediated inhibition of STAT3 and RelA/p65 acetylation in breast cancer cells.

4.3.2 Liver cancer

LA exhibits anticancer effects on hepatocellular carcinoma cells (HCC) through induction of apoptotic pathway by ROS-dependent activation of liver kinase B1 (LKB1)/AMPK/ACC signalling cascades [29]. Two hepatocellular carcinoma cells, HepG2 and SK-Hep1, were exposed to different LA concentrations (0, 10, 20, 40 and 80 μM) for 24, 48 or 72 h, and it was found that LA significantly reduces the viability of both cells in a concentration and time-dependent fashion. Cell cycle analyses confirmed that the apoptosis is the main role player behind the cytotoxicity of LA. It was observed that LA dose-dependently increased sub-G1 population, especially at 40 μM it raised the sub-G1 population up to 22.14% compared to the untreated control (2.06%) and also increased the percentage of early apoptotic cells (Annexin V+/PI– staining: 30.1%) for 24 h and late apoptotic or necrosis cells (Annexin V+/PI + staining: 22.9%) for 48 h. However, it induces the cleavage of caspase-3 and PARP along with the suppression of antiapoptotic proteins, Bcl-2 and Bcl-xl. The apoptotic effect of LA was found to be reversed when HepG2 cells were treated with Z-VAD-fmk (10 μM), suggesting that its cytotoxicity was induced by apoptosis through caspase cleavage and inhibition of anti-apoptotic proteins. Furthermore, apoptotic effects of LA are related to AMPK signalling, as evidenced by treating HCC cells with LA (0, 10, 20 and 40 μM) for 24 h. It was also revealed that LA increases the phosphorylation of LKB1, AMPK and ACC while suppressing the expression of phosphatidylinositol 3-kinase (PI3K), p-AKT, p-mTOR and COX-2 in both cells in a dose-dependent manner. The cytotoxic effects of LA on HepG2 cells were significantly reversed by the treatment of AMPK inhibitor compound C. LA also increases the phosphorylation of ERK and p38 and attenuates the phosphorylation of JNK in HepG2 cells, while it decreases the phosphorylation of p38 and JNK and increases the phosphorylation of ERK in SK-Hep1 cells, implying cell-specific activities of LA on mitogen-activated protein kinase (MAPK). Most importantly, LA (40 μM) was found to induce ROS production time-dependently in both HepG2 and SK-Hep1 cells after treating for 1, 3 and 4 h. It is well established that the increased level of ROS is often the cause for apoptosis and damage in a variety of cancer cells via disrupting lipid membranes, proteins and DNA [30, 31]. The involvement of ROS in AMPK-mediated apoptosis was confirmed when LA (40 μM)-treated HCC cells were exposed to AMPK inhibitor compound C (7.5 μM) or ROS scavenger N-acetyl-L-cysteine (NAC) (5 mM). Likewise, the increase of sub-G1 population and Annexin V/PI stained cells by LA was reversed by compound C or NAC in both cells. Compound C can block the ability of LA to induce phosphorylation of AMPK/ACC, PARP cleavage and decreases the expression of Bcl-2 and COX-2 in both HCC cells. NAC also reverses the phosphorylation of AMPK, PARP cleavage and decreases the expression of Bcl-2 and COX-2 in HepG2 cells, clearly indicating that LA generates ROS and subsequently induces AMPK phosphorylation, PARP cleavage and inhibits antiapoptotic proteins such as Bcl-2 and COX-2 leading to apoptosis in HCC cells. Additionally, compared to untreated control, LA increases sub-G1 population to 10.24% in AMPKα wild type MEF cells, but not in AMPK knockout (KO) MEF cells. Similarly, LA induces PARP cleavage, caspase-3 and phosphorylation of AMPK/ACC in AMPKα wild type MEF cells, but not in AMPK KO MEF cells, demonstrating the key role of LKB1/AMPK/ACC signalling in LA-induced apoptosis. It is well documented that ROS has significant involvement in AMPK-mediated apoptosis [32, 33], and AMPK plays a pivotal role in cellular metabolism and apoptosis [34, 35]. Jeong et al. [29] also demonstrated that ROS-dependent LKB/AMPK/ACC activation by LA can be a novel strategy for treating hepatocellular carcinoma.

4.3.3 Lung cancer

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) plays an important role in cancer cells through apoptosis, with low toxicity and less resistance to normal cells [36]. However, its tendency to be resistant to chemotherapeutic agents and cancer cells has made it to be used in limited ways [37, 38], indicating that overcoming the TRAIL resistance might be a promising way for cancer treatment. Ahn et al. [39] demonstrated that LA (20 μM) together with TRAIL (20 ng/ml) shows significant cytocidal effects in TRAIL resistant nonsmall cell lung cancer cell lines (NSCLCs) A549 and H1299, compared to the treatments with TRAIL or LA alone. A549 and H1299 cells undergo apoptosis when LA is used with TRAIL as a combined anticancer treatment. A combination treatment of LA and TRAIL for 24 h significantly increased the early and late apoptosis to 37.50 and 17.88% in A549 cells, and 17.43 and 4.96% in H1299 cells, respectively, when compared to LA or TRAIL alone. Their combined treatment also increases sub-G1 population, cleavage of PARP and caspase-3/8/9 and decreases the expression of pro-PARP and pro-caspase-3/8/9. Most notably, pretreatment of A549 and H1299 cells with pan caspase inhibitor (z-VAD-fmk) (80 μM) and caspase-3 inhibitor (z-IETD-fmk) (50 μM) significantly decreases the elevated sub-G1 population induced by the co-treatment of TRAIL and LA and inhibits the synergistic effect of LA and TRAIL in initiating apoptosis. To undergo TRAIL-mediated apoptotic cell death, TRAIL needs to be activated by binding with death receptors, DR4 and DR5 [40, 41]. In TRAIL resistant cells, the upregulated DR4 helps cells overcome the resistance and then increases response to TRAIL signals, thus leading to apoptosis [42]. The findings were consistent as TRAIL alone fails to show any anticancer effect but together with LA it activates DR4 but not DR5, leading to death receptor-induced apoptotic cell death, and also supress the levels of p-NF-κB, p-IκB and FLICE-inhibitory protein (FLIP) but not decoy receptor 1 (DcR1) and DcR2 in A549 and H1299 cells. Similarly, in combination they suppress the expression of Bcl-2, Bid and XIAP in A549 and H1299 cells compared to the treatment with LA or TRAIL alone. Additionally, combination of TRAIL and LA disrupts the interaction of XIAP with caspase-3 or NF-κB, whereas it is well known that XIAP involves in the activation of NF-κB, which is essential for cancer cell survival [43]. Overall, the findings imply that LA helps NSCLCs overcome the TRAIL resistance and enhances sensitization of the cells to TRAIL-mediated apoptosis through inhibition of XIAP/NF-κB.

4.3.4 Prostate cancer

Androgen receptor (AR) is essential for the development and function of prostate cancer [44–46] which functions through regulating prostate-specific antigen (PSA) expression in prostate cancer cells [47, 48]. Therefore, AR is an important biomarker to confirm the existence of prostate disease and prostate cancer [49]. Several studies also demonstrate that the suppression of AR regulates antispermatogenesis, anticancer and apoptosis in prostate cancer cells [50–52]. Lee and colleagues [53] showed that LA exerts anticancer effects by suppressing AR pathway in AR-sensitive prostate cancer cells LNCaP. LA reduced LNCaP cell viability in dose- and time-dependent fashion with an IC_{50} value of 109 µM. LNCaP cell growth inhibition was accomplished by G1 phase arrest upon LA treatment. Protein analysis assay confirmed that 24 h LA treatment downregulates cell proliferation by reducing several protein levels, including p-53, p-p53, p21, p27, cyclin D1 and cell division kinase 4 (CDK4). Apoptotic effects of LA correspond to increased cleavages of caspase-3/9, PARP and BAX, while the reduced Bcl-2 is found in LA-treated LNCaP cells after 48 h of treatment. LA decreases the expression of AR and PSA in dose- and time-dependently, following the exposure of LNCaP cells for 24 and 48 h, through attenuating the androgen stimulated translocation of AR to the nucleus. These findings were emphasized by the study of transfecting AR siRNA to LNCaP cells treated with LA. It was found that silencing of AR reduced its level together with PSA, blocked inhibitory proteins levels (cyclin D1 and CDK4) and increased tumour suppressor genes p53, p21 and p27. Knockdown of AR in LNCaP cells suppresses their proliferation by 30.1% in comparison with the cells transfected with siRNA, increases apoptosis related proteins BAX, cleaves caspase-3/9 and PARP and inhibits Bcl-2 protein. Overall, AR signalling pathway has crucial role in cell proliferation, apoptosis inhibition and PSA level elevation, all of which are key regulatory mechanistic pathway of AR-dependent prostate cancer cells, and LA exhibits anticancer properties by inhibiting AR expression and PSA in cellular and secretory levels.

In another study, prostate cancer cell line DU145 (STAT3 wild) and PC-3 (STAT3 mutant) cells were used by Sim and co-workers [22] to validate the anticancer activity of LA. They showed that LA induces cytotoxic effects more potently on PC-3 cells than on DU145 cells. However, in a previous study, it was demonstrated that LNCaP cells were affected by LA more than castration resistant cells PC-3 and DU-145 cells [53]. Several studies have revealed that p300-mediated RelA/p65 hyperacetylation by STAT3 is pre-requisite for NF-κB activation and thereby inducing various cancers [25]. Sim et al. [22] demonstrated that LA induces dose-dependent reduction in cell viability and elevates sub-G1 population along with the cleavages of PARP in PC-3 cells better than in DU-145 cells. In DU145 cells, LA reduces the phosphorylation of STAT3 and NF-κB and the expression of p300 and RelA acetylation which are similar with the findings from LA-treated breast cancer cell lines MCF-7. Coimmunoprecipitation performed with DU145 cell lysates using STAT3 and p300 antibodies revealed that it disrupts the binding of p-STAT3, p300 and RelA after 24 h of treatment. Moreover, LA reduces the nuclear translocation of STAT3 and NF-κB, and suppresses several survival genes, including Bcl-2, Bcl-XL, XIAP, survivin, VEGF, Cox-2, C-Myc, and IL-6, and TNF in MCF-7 breast cancer cells. Most importantly, LA activates miRNA134 and the miRNA134-mimic reduces the expression of p-STAT3, pro-PARP, and Ac-RelA. However, miRNA134 inhibitor can reverse the apoptotic effect of LA, clearly demonstrating that LA induces apoptosis via miRNA-134 mediated inhibition of STAT3 and RelA/p65 acetylation (Figure 4). In conclusion, the apoptotic mechanisms of LA correspond to STAT3 phosphorylation and RelA/p65 acetylation in the prostate cancer cell lines.

4.4 Other health benefits

Among the several isolated diterpenes from *Caesalpinia echinata* Lam., LA displayed leishmanicidal activity without showing any toxic side effects to human mononuclear cells derived from peripheral blood *in vitro* at 20 µg/ml [54]. LA derived from *Platycladus orientalis* has been reported to incorporate into the inner membrane of erythrocytes, in turn changes the chemical composition of lipid membrane and alters the erythrocyte cell curvature [55]. This activity is considered as indirect antiplasmodium effect of LA for preventing the proliferation of parasite like *Plasmodium* and as a way to treat malaria. LA-enriched MeOH fraction of *B. orientalis* leaves showed neuroprotective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells [56].

Pinusolide, a labdane type diterpene lactone synthesized from LA, may act as a potent and specific platelet activating factor receptor binding antagonists [57, 58]. An efficient method for gram-scale synthesis and purification of penusolide from LA has been developed and penusolide has been evaluated for its anti-leukemic and apoptotic properties using Burkitt lymphoma cell line and primary lymphoblast and leukaemia cells of children with acute lymphoblastic leukaemia or acute myeloid leukaemia [59]. Tolstikova et al. [60] investigated nootropic activity of LA and its amino derivatives and found them as prominent nootropic agents. They also confirmed the profound nootropic activity of different synthetic LA derivatives using animals [61]. Methyl derivatives (Me-LA) and three amino derivatives of LA exhibited equal low toxicity with having different degrees of influence on central nervous system (CNS) [62]. Me-LA also exhibited a strong antidepressant effect with stimulating action, while amino derivatives led to an antipsychotic and sedative (calming) effect on CNS, without any anticonvulsant action.

4.5 Summary with future perspective

LA has been proved to be a biologically active compound with profound pharmacological interest in attenuating cancer, obesity/diabetes, allergies, etc. Besides, it shows antiparasitic effects. Its anticancer activities are triggered by targeting several important signalling pathways involved in cancer progression such as the pathways for cancer cell proliferation, survival and apoptosis. The signalling components which are mostly affected by LA are AMPK, AKT, AR, mTOR, FOXM1, NFkB, COX-2, etc. If LA is used combinedly with siRNA, it enhances apoptosis pathway, suggesting that LA has not only affect the downstream AR signalling components but also has a potential to inhibit the upstream proteins. It also increases the expression of a micro RNA, miR123, thus inhibiting the expression of downstream anti-apoptotic, oncogenic and inflammatory components. NFkB and COX-2 are involved in generating inflammation. Since LA affects both NFkB and COX-2, it can be successfully used to treat chronic inflammation and other inflammatory diseases/disorders as well. In adipocyte cells, it downregulates different pathways particularly for fat accumulation and adipocyte differentiation. These findings clearly demonstrate that LA can be used for treating obesity and related disorders, especially type 2 diabetes.

In conclusion, LA could be utilized in treating allergies, obesity and different cancers. It can be a promising anticancer drug if it is used in combination with other drugs. Further studies are recommended with LA to determine its anti-inflammatory, antidiabetic and neuroprotective effects especially using *in vivo* models. Before recruiting LA in further clinical studies, its toxicological profile should also be explored. Moreover, LA can serve as a potential chemical platform to synthesize potential derivatives for wide pharmacological/health beneficial properties.

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