Betulinic acid and its derivatives as anti-cancer agent: A review

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ABSTRACT

Betulinic acid is a known natural product which has gained a lot of attention in the recent years since it exhibits a variety of biological and medicinal properties. This review provides the most important anticancer properties of betulinic acid and its derivatives.
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INTRODUCTION

Cancer is a group of diseases that cause cells in the body to change and grow out of control[1]. Cancer cell growth is different from normal cell growth. Instead of dying, cancer cells keep on growing and form new cancer cells. These cancer cells can grow into (invade) other tissues, something that normal cells cannot do. In most cases the cancer cells form a tumor. But some cancers, like leukemia, rarely form tumors. Instead, these cancer cells are in the blood and bone marrow[2]. Cancer cell escape from many of the normal homeostatic mechanism that control proliferation. They invade surrounding tissues, gets into the body's circulating system and set up areas of proliferation away from the site of their original appearance[3].

Cancer is the second most important disease leading to death in both the developing and developed countries nowadays. The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviors, particularly smoking, in economically developing countries[4]. A substantial proportion of the worldwide burden of cancer could be prevented through the application of existing cancer control knowledge and by implementing programs for tobacco control, vaccination (for liver and cervical cancers), and early detection and treatment, as well as public health campaigns promoting physical activity and a healthier dietary intake. Clinicians, public health professionals, and policy makers can play an active role in accelerating the application of such interventions globally[5].

The naturally occurring pentacyclic triterpenoid, betulinic acid has been shown to possess anti-tumoural activity and overcome resistance by inducing apoptosis in a variety of human cancers. Its selective cytotoxicity against cancer was first described on human melanoma both *in vitro* and *in vivo*. Since that initial study, Betulinic Acid has been reported to be effective on a growing number of human cancers, including those of the lung, colon, prostate, and ovary, whereas normal cells were unaffected by Betulinic Acid treatment. Interestingly, Betulinic Acid has also been successfully applied *in vitro* in childhood cancers, namely medulloblastoma, glioblastoma, Ewing sarcoma, neuroblastoma, and leukaemia.

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Accumulated experimental evidence indicates that Betulinic Acid causes distinct morphological changes in sensitive cells, such as cell shrinkage, DNA fragmentation, nuclear condensation, and membrane blebbing[6].

Betulinic Acid is a candidate for cancer therapy that has been shown to exhibit an antitumor effect without cytotoxicity. The cytotoxicity research on Betulinic Acid showed that it had selective cytotoxicity on tumor cell lines but not on normal cells suggesting that it may have potential for development as a therapeutic agent. Recent evidence indicates that the anticancer activity of Betulinic Acid can be markedly increased when it is used in combination with chemotherapy, ionizing radiation or TRAIL[7]. Its administration reduced the tumor burden of established melanoma and prostate cancer xenografts in nude mice. In particular, Betulinic Acid induces tumor cell death by inducing mitochondria-mediated apoptosis. In addition, Betulinic Acid inhibits prostate cancer tumor growth by degrading a transcription factor, specificity protein (Sp1), which ultimately inhibits angiogenesis[8].

It selectively inhibited melanoma cancer cell and tumor growth and *in vivo* studies this was accompanied by minimal toxic side effects at repeated doses of up to 500 mg/kg. Subsequent studies showed that Betulinic Acid was cytotoxic to many other cancer cell lines and this was associated with different activities. For example, Betulinic Acid induces apoptosis through decreased mitochondrial membrane potential, activation of mitogen-activated protein kinase and modulation of nuclear factor *K*b[9].

Apoptosis induction initiated by Betulinic Acid was not exerted through a ligand/receptor system but it occurs through perturbation of mitochondrial function, such as loss of the mitochondrial transmembrane potential that decreases mitochondrial permeability release of mitochondrial cytochrome C into the cytosol. Moreover, Betulinic Acid-induced apoptosis was found to be independent of p53 in melanoma and neuroectodermal tumor cells. Thus, Betulinic Acid directly altered mitochondria and induced loss of the mitochondrial transmembrane potential associated with inhibition of topoisomerases to provoke cell death of various cancerous cells. Furthermore, betulinic acid was able to improve the effect of tumor radiotherapy under hypoxic condition[10].

A semi-empirical molecular-orbital method (CAChe) demonstrates that the cytotoxicity of betulinic acid derivatives can be predicted by several physical parameters (such as heat of formation, hydrophobicity (log P), water solubility, ionization potential, electron affinity, dipole moment), but not by molecular size (maximum length and width). It induced apoptosis in melanoma and neuroectodermal tumors, independently of the ligand/receptor system, through a reactive oxygen species dependent mitochondrial cytochrome c-caspase pathway[11].

Betulinic acid: The betulinic acid *(3 b-hydroxy-lup-20(29)-en-28-oic acid),* is a known natural pentacyclic lupane type triterpenoid present in many plant species, for example, *Triphyophyllum peltatum, Ancistrocladus heyneanus, Ziziphi fructus, Diospyros leucomelas, Tetracera boliviana and Syzygium formosanum*, and can be obtained in quantity from the bark of the London plane tree, *Platanus acerifolia* and flowering *Eugenia DC*[4]. It is obtained by extraction of barks or core of some plant species or by synthetic processes, e.g. using the betulin (alcohol triterpene) as a synthetic intermediate isolated from the bark of *Betula alba* and *Betula pendula*[12]. This compound and its derivatives possess many favorable biological properties such as anti-cancer, anti-HIV-1 (human immunodeficiency virus type-1), antibacterial, anti-malarial, anti-inflammatory, and anthelmintic activities[13].

Betulinic acid cooperated with anticancer drugs to induce apoptosis and to inhibit clonogenic survival of tumor cells. Combined treatment with Betulinic Acid and anticancer drugs acted in concert to induce loss of mitochondrial membrane potential and the release of cytochrome C and Smac from mitochondria, resulting in activation of Caspases and apoptosis[14].

Betulinic Acid alone was an effective chemotherapeutic drug for the SNU-C5/WT, SNU-C5/5FU-R and SNU-C5/OXT-R cells. The combination of Betulinic Acid with IRT or OXT was effective against SNU-C5/5FU-R cells, and the combination of Betulinic Acid with 5-fluorouracil, IRT or OXT was effective against SNU-C5/OXT-R cells[15].

Betulinic acid was initially known for its high cytotoxicity against human melanoma cancer cells, but later studies also suggest this compound being a broad inhibitor of other cancerous tumors including a neuroectodermal tumors (such as neuroblastoma, medulloblastoma, glioblastoma and Ewing's sarcoma), brain-tumors, human gliomas, leukemia, human colon carcinoma and human prostate adenocarcinoma, head and neck squamous carcinoma cells, lung, colorectal, breast, and cervical cancer[2].

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Physicochemical properties: The pure compound of betulinic acid appears as a white crystalline solid, melts at 295 - 297 ^{o}C. It was isolated from *Melaleuca cajuput* and was chromatographed on a silica gel column using chloroform as eluent[16]. It exhibits limited solubility in organic alcohols such as methanol and ethanol, chloroform, and ether and has low solubility in water, petroleum ether, dimethyl formamide, dimethyl sulfoxide, and benzene[17]. However, it is highly soluble in pyridine and acetic acid. It is not readily visible on thinlayer chromatography plates under UV (254 and 365 nm) but is easily detected following exposure to iodine vapors, anisaldehyde, anisaldehyde – sulphuric acid, or vanillin – sulphuric acid spray reagents[3].

The pharmacokinetics and tissue distribution of betulinic acid was studied in CD-1 mice. The results showed that after i.p 250 and 500 mg/Kg dose, the serum concentrations reached peaks at 0.15 and 0.23h, respectively[18]. The 250 and 500 mg/Kg betulinic acid i.p. doses were found to have elimination half-lives of 11.5 and 11.8h and total clearances of 13.6 and 13.5 l/Kg/h, respectively[19].

Recently, a robust assay based on liquid chromatography/mass spectrometry developed to conduct a quantitative analysis of betulinic acid in mouse, rat and dog plasma. At 15 and 25 mg/mL in mouse, rat or dog plasma, betulinic acid was 99.99% bound to serum proteins, and, at 5 mg/mL, betulinic acid was > or =99.97% bound following i.p. or intravenous administration *in vivo*[18].

Betulinic acid as anti-cancer agent: Betulinic acid (3-beta-hydroxy-lup-20(29)-ene-28-oic acid), is naturally occurring pentacyclic triterpenes discovered in 1995 in the stem bark of the plant *Melaleuca cajuput*; showing cytotoxicity towards a number of cancer cell lines. These compounds can be found in the bark of the many plants and it is an oxidation product of low molecular compound called Betulin, a substance found in the outer bark of white birch tree *Betula alba*[20,21].

Betulinic acid is an experimental antineoplastic agent that induces apoptosis in melanoma cells *in vitro* and *in vivo* , as well as in neuroectodermal tumor cell lines *in vitro*[22]. 3-beta-hydroxy-lup-20(29)-ene-28-oic acid (betulinic acid) (Fig. 1), a C-28 carboxylic acid, derivative of the ubiquitous triterpene betulin, is a member of the class of lupane type triterpenes. However, unlike betulin, the oxidized derivative betulinic acid possesses a number of intriguing pharmacological effects[19].
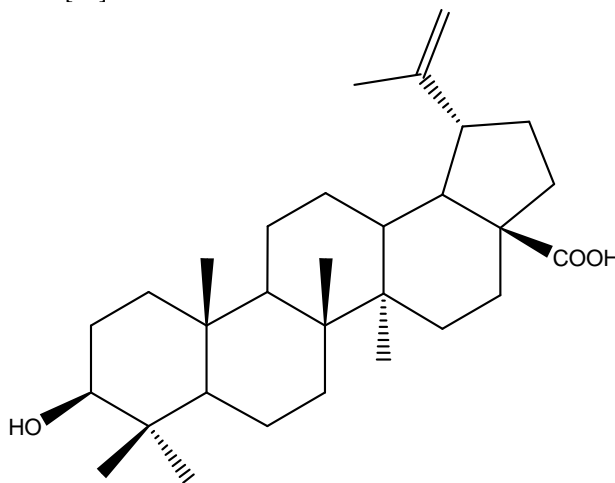

Figure: 1 Structure of Betulinic Acid

Betulinic Acid has been reported to inhibit growth of cancer cells, without affecting normal cells and its lack of cytotoxic activity has been demonstrated in human astrocytes, human dermal fibroblasts, peripheral blood lymphoblasts and animal studies. In 1995, Betulinic Acid was reported as a highly selective growth inhibitor of human melanoma, neuroectodermal and malignant tumor cells and was reported to induce apoptosis in these cells[23]. The cytotoxicity and mode of cell death of Betulinic Acid were determined using the MTT assay and DNA (deoxy-ribonucleic acid) fragmentation analysis, respectively. Cells treated with high concentrations of Betulinic Acid exhibited features characteristic of apoptosis such as blebbing, shrinking and a number of small cytoplasm body masses when viewed under an inverted light microscope after 24hr[24].

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The *in vitro* sensitivity of Betulinic Acid was for broad cell line panels derived from lung, colorectal, breast, prostate and cervical cancer, which are the prevalent cancer types characterized with highest mortalities in woman and men. Multiple assays were used in order to allow a reliable assessment of anti-cancer efficacy of Betulinic Acid. After 48 hr of treatment with Betulinic Acid, cell viability as assessed with MTT and cell death as measured with propidium iodide exclusion showed clear differences in sensitivity between cell lines.
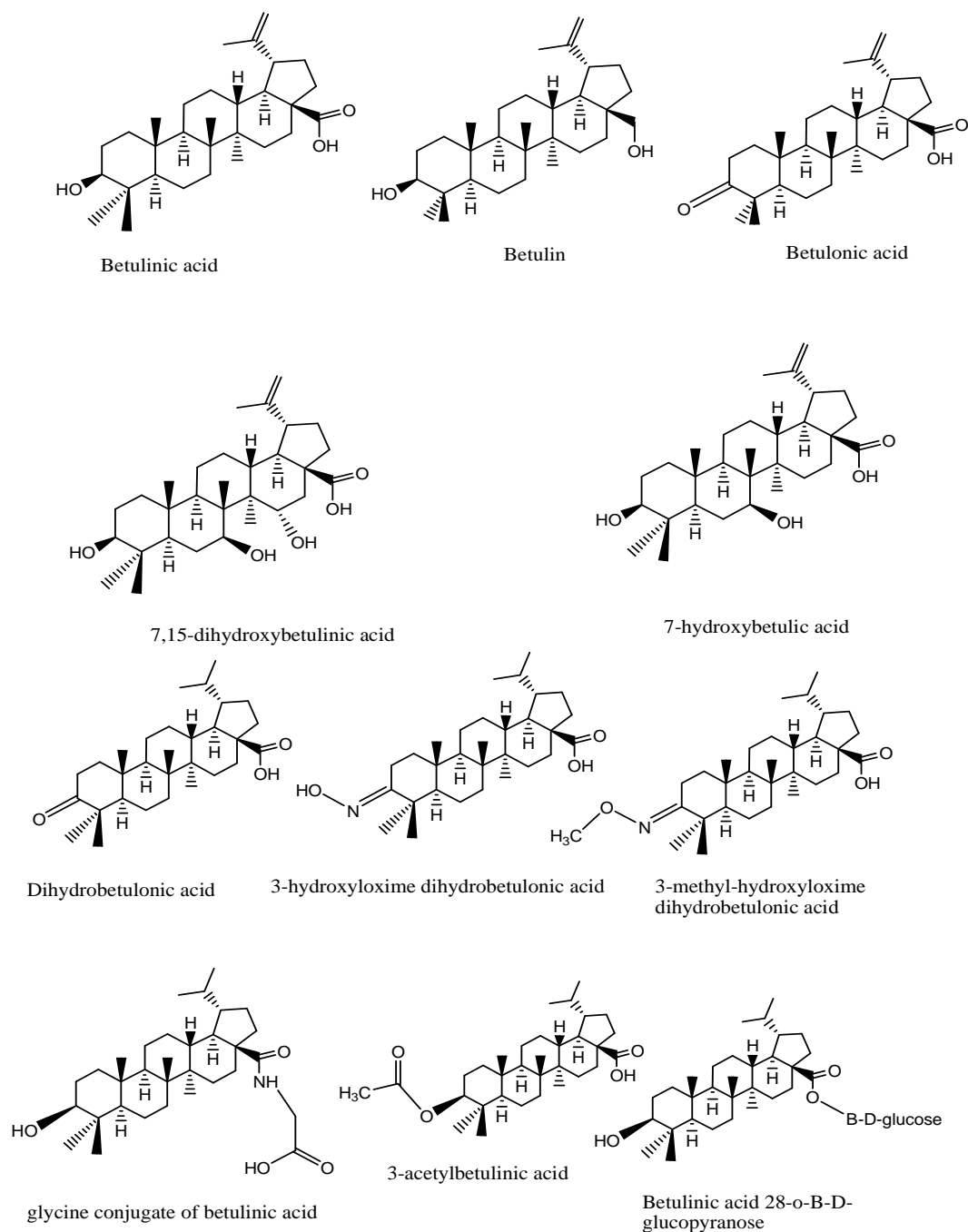
Betulinic acid

Betulin

Betulonic acid

7,15-dihydroxybetulinic acid

7-hydroxybetulic acid

Dihydrobetulonic acid

3-hydroxyloxime dihydrobetulonic acid

3-methyl-hydroxyloxime dihydrobetulonic acid

glycine conjugate of betulinic acid

3-acetylbetulinic acid

Betulinic acid 28-o-B-D-glucopyranose

Figure 2: Betulinic Acid Analogs

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However, in all cell lines tested colony formation was completely halted at remarkably equal Betulinic Acid concentrations that are likely attainable *in vivo*. The results substantiate the possible application of Betulinic Acid as a chemotherapeutic agent for the most prevalent human cancer types[25].

The anti-tumor cytotoxicity of betulinic acid has been extensively studied in a panel of cancer cell lines, primary tumor samples and xenograft mouse models. While initial reports suggested that betulinic acid is selectively cytotoxic against melanoma cell lines, anticancer activity was subsequently also reported against other types of human cancers including neuroblastoma, glioblastoma, medulloblastoma, Ewing tumor, leukemia as well as several carcinoma, i.e. head and neck, colon, breast, hepatocellular, lung, prostate, renal cell, ovarian or cervix carcinom. In addition to tumor cell lines, Betulinic acid was also cytotoxic against primary cancer cells isolated from tumor specimens obtained from neuroblastoma, glioblastoma and leukemia. Also, betulinic acid was cytotoxic in different models of drug resistance, for example primary pediatric acute leukemia samples that were refractory to standard chemotherapeutic agents[4].

Thus, betulinic acid may overcome certain forms of drug resistance. Further, there is evidence that betulinic acid is preferentially cytotoxicity against metastatic over non-metastatic melanoma cell lines. Moreover, betulinic acid cooperated with different cytotoxic stimuli to suppress tumor growth, including ionizing radiation, chemotherapeutic drugs or the death recpetor ligand TRAIL. This suggests that betulinic acid may be used as sensitizer in combination regimens to enhance the efficacy of anticancer therapy. By comparison, normal cells of different origin have been reported to be much more resistant to betulinic acid than cancer cells pointing to some tumor selectivity[2].

Structure-activity relationship studies:
There exists a great deal of interest in probing the structural features responsible for the pharmacological effects of betulinic acid, and to further optimize its activity profile. As a result, numerous derivatization studies have been performed on betulinic acid leading to the production of an array of betulinic acid analogs. Betulinic acid possesses three sites that are highly amenable to derivatization, including the C-3 hydroxyl, C-20 alkene, and C-28 carboxylic acid positions. In addition, betulinic acid has been subjected to solid-phase synthetic modifications for the preparation of combinatorial libraries[26].

This method holds the potential for the production and subsequent pharmacological evaluation of large sets of betulinic acid analogs. Numbers of interesting rearranged betulinic acid analogs have also been prepared using clay catalysts. Furthermore, microorganisms have been utilized as biocatalysts for the preparation of betulinic acid derivatives[27]. Further studies have been performed to derive synthetic betulinic acid analogs in an effort to establish meaningful structure-activity relationships. Three positions in betulinic acid, the C-3 hydroxyl, C-20 alkene, and C-28 carboxylic acid moieties, have served as the target for most derivatization studies (Fig. 2).

The 3b-hydroxyl moiety found in betulinic acid represents a readily available position for chemical modification. However, only a limited number of synthetic C-3 betulinic acid derivatives have been reported and tested for cytotoxicity. Some of these modifications include oxidation to a ketone, acetylation, and formation of various nitrogen-containing analogs (amine; oxime). Despite these efforts, very little can be deduced regarding the role that derivatization of C-3 in betulinic acid may play in controlling its anticancer activity. Kim and co-workers determined that although the introduction of an oxime moiety at C-3 did not exert a considerable impact on the cytotoxicity of betulinic acid, it may result in the potential loss of selectivity against melanoma cells[28].

 Oxidation of the C-3 hydroxyl group in betulinic acid to a ketone yields the highly cytotoxic derivative betulonic acid; however, this also results in a loss of specificity against melanoma cells. An acetylated derivative, 3- O-acetylbetulinic acid, appears to retain the cytotoxicity associated with betulinic acid[29].

An interesting C-3 benzyl ester derivative has also been synthesized. This compound was found inactive against both melanoma and non-melanoma derived cancer cell lines.

It was speculated that the bulky benzyl group was responsible for the loss of activity; however, the concurrent modification of the C-28 position in this molecule to a methyl and the lack of additional related derivatives for comparison hampered efforts to draw further conclusions in this regard. Additional studies are needed to probe the potential for further C-3 modifications that may alter the cytotoxicity and selectivity of betulinic acid[17].

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One of the strategies to increase betulinic acid hydrosolubility is by the synthesis of its glycoside derivatives. Furthermore, the bioactivity of betulinic acid, in some cases can be improved upon the addition of sugar moiety at either C-3 or C-28 or both. Some natural and synthetic betulinic acid glycosides were also reported in the literature.[68] Example, 3-O-β-D-glucopyranoside-betulinic acid (2) was synthesised by chemical reactions and shown some bioactivities. Their synthesis however seems to be difficult in purification procedure. Thus, in connection with their continuous effort on the enzymatic reaction of betulinic acid they now report the preparation of 3-O-β-D-glucopyranoside-betulinic acid by enzymatic glucosidation of betulinic acid (1) and glucose using Novozyme® 435 in organic solvent. Interestingly, it was observed that the reaction was clean and simple, and gave high yield of product. The evaluation of this compound toward some cancer cell lines was also reported herein. The structures of compound (1) and (2) were shown in Fig 3[30].
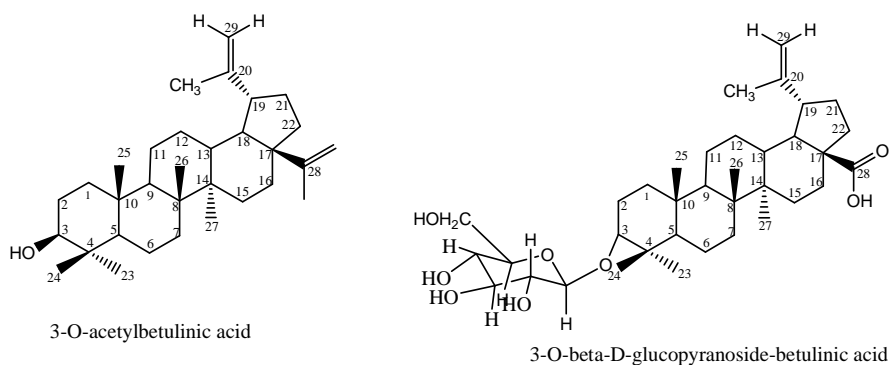

3-O-acetylbetulinic acid

3-O-beta-D-glucopyranoside-betulinic acid

Figure: 3 Glycoside Derivatives of Betulinic Acid

Modifications at C-20 did not enhance cytotoxicity in several cancer cell lines, but derivatives at the C-3 and C-28 position were found to be promising. Amino acid conjugates at the C-28 position enhanced water solubility as well as cytotoxicity. Hydroxylation at the C-3 position gave promising results when tested on murine melanoma cells and another chemical modification at the C-3 position (dimethylsuccinyl Betulinic Acid) turned Betulinic Acid from a proteasome activator into a proteasome inhibitor. Yet another C-3 modification gave better anti-tumor results in a colon cancer xenograft mouse model when compared with Betulinic Acid[31]. The ring skeleton of Betulinic Acid is the platform for many other interesting modifications. One novel, well tolerated Betulinic Acid derivative is NVX-207, which showed significant anti-tumor activity in clinical studies in canine cancer patients with treatment-resistant malignancies[32].

An easy and efficient strategy to prepare betulinic acid esters with various anhydrides was used by the enzymatic Synthesis method. It involves lipase-catalyzed acylation of betulinic acid with anhydrides as acylating agents in organic solvent. Lipase from *Candida Antarctica* immobilized on an acrylic resin (Novozym 435) was employed as a biocatalyst. Several 3-O-acyl-betulinic acid derivatives were successfully obtained by this procedure. The anticancer activity of betulinic acid and its 3-O-acylated derivatives were then evaluated *in vitro* against human lung carcinoma (A549) and human ovarian (CAOV3) cancer cell lines. 3-O-glutaryl-betulinic acid, 3-O-acetyl-betulinic acid, and 3-O-succinyl-betulinic acid showed IC_{50} <10 microg/ml against A549 cancer cell line tested and showed better cytotoxicity than betulinic acid. In an ovarian cancer cell line, all betulinic acid derivatives prepared showed weaker cytotoxicity than betulinic acid[33].

Synthesis of 3-beta-O-phthalic esters from betulinic acid and its esters and Synthesis of phthalic esters from betulin and its monoacetates using classical acylation procedure with phthalic anhydride. The evaluation of cytotoxicity of the prepared compounds was using numbers of tumor cell lines in MTT test. It was discovered that hemiphthalic esters had better cytotoxicity than starting compounds as betulinic acid or quite inactive betulin[34].

The naturally occurring cytotoxic saponin 28-O-beta-d-glucopyranosyl betulinic acid 3beta-O-alpha-l-arabinopyranoside was easily synthesized along with seven bidesmosidic saponins starting from the lupane-type triterpenoids betulin and betulinic acid. As highlighted by the preliminary cytotoxicity evaluation against A549, DLD-1, MCF7, and PC-3 human cancer cell lines, the bidesmosidic betulin saponin , bearing alpha-l-

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rhamnopyranoside moieties at both C-3 and C-28 positions, was determined to be a potent cytotoxic agent (IC$_{50}$ 1.8-1.9 microM)[35].

A series of new imidazole carboxylic esters (carbamates) and N-acylimidazole derivatives of betulin and betulinic acid have been synthesized. The new compounds were screened for *in vitro* cytotoxicity activity against human cancer cell lines HepG2, Jurkat and HeLa. A number of compounds have shown IC$_{50}$ values lower than 2 microM against the cancer cell lines tested and the vast majority has shown a better cytotoxicity profile than betulinic acid, including the betulin derivatives. N-Acylimidazole derivatives and the C-3 carbamate derivatives were the most promising compounds[36].

In 2009 at Japan, sixteen derivatives were synthesized and evaluated in an in vitro EBV-EA activation assay, which is well established to correlate with antitumor-promoting activity. Among them, analogs 4–6 and 17–19 of fig 4 showed significant potency as antitumor- promoters. Compound 6 of fig 4 modified with a prenyl-like ester group was the most effective. Further structural modifications are in progress, and active compounds are being tested in an *in vivo* two-stage mouse skin carcinogenesis assay[37].
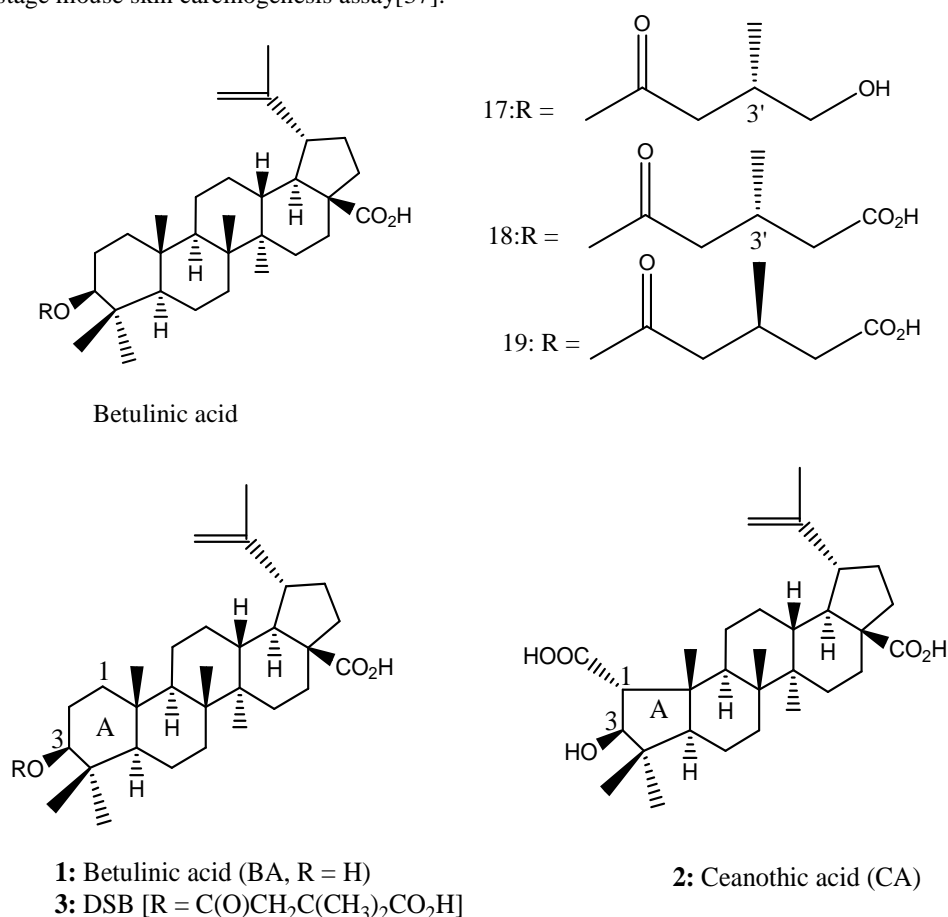

Figure 4. Structural modification of Betulinic Acid

Mechanism of action of Betulinic Acid as anti-cancer Agent:
Numerous studies over the last years aimed at elucidating the molecular mechanisms of betulinic acid-mediated antitumor activity. One characteristic feature of betulinic acid's cytotoxicity is its ability to trigger the mitochondrial pathway of apoptosis in cancer cells. Apoptosis is an intrinsic program of cell death that is present in every cell and regulated by defined signaling pathways[38].

Apoptosis pathways can be initiated at the level of the mitochondria by the release of apoptogenic factors such as cytochrome C, Smac or AIF from the mitochondrial intermembrane space into the cytosol (mitochondrial or

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intrinsic pathway). Smac promotes apoptosis by neutralizing "Inhibitor of Apoptosis Proteins" (IAP)-mediated inhibition of caspase-3 and -9. Alternatively, apoptosis can be triggered by ligation of death receptors (DR) such as CD95 or TRAIL receptors by their cognate ligands, i.e. CD95 ligand or TRAIL (receptor or extrinsic pathway)[4,15,16,18,39].

Apoptosis can be inhibited by at various levels, e.g. by FLIP, Bcl-2 or IAPs (Fig. 5)
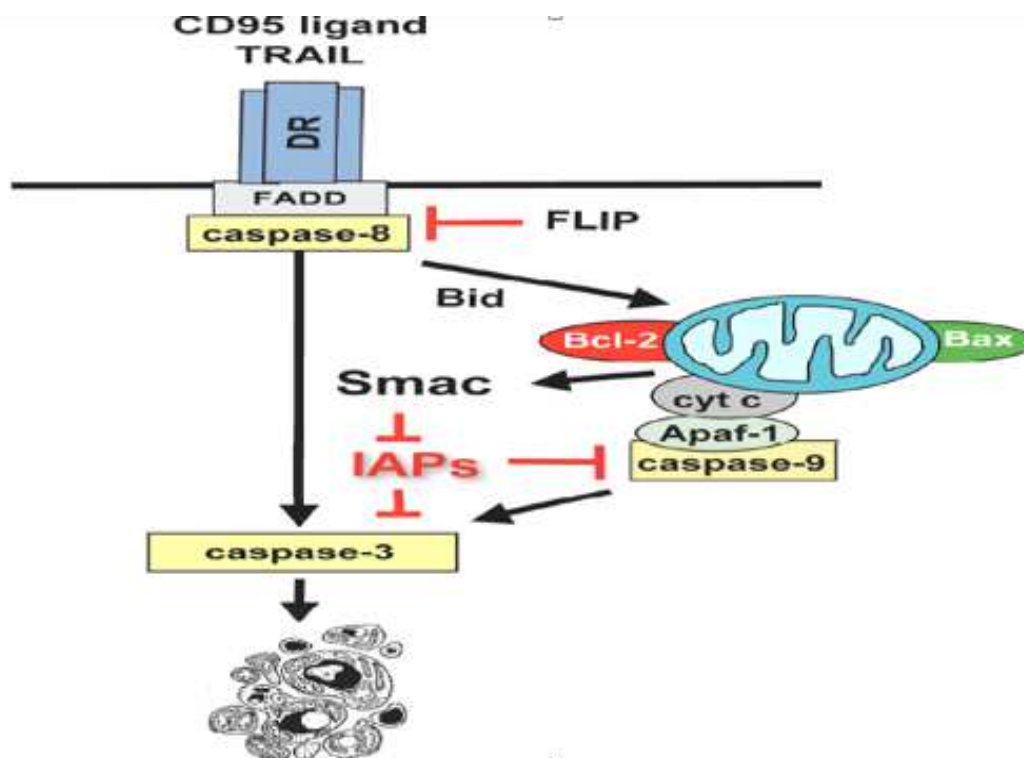

Figure 5. Apoptosis pathways

The main mechanism of anti-cancer action of betulinic acid is known as the induction of apoptosis in cells independent of their p53 status. The Debatin group suggests that betulinic acid could induce mitochondria to undergo permeability transition (PT), which causes the release of cytochrome *c* into the cytosol, the activation of caspases (interleukin 1β-converting enzyme/Ced-3-like proteases), and the DNA fragmentation. Another mechanism indicates betulinic acid acting as the inhibitor of aminopeptidaseN (APN), since APN is closely associated with extracellular matrix components; its inhibition is likely to prevent the melanoma invasion into basement membranes. By its lipophilic character, Betulinic Acid may directly or indirectly influence the lysosomal membrane properties causing lysosomal membrane permeabilization or, alternatively, Betulinic Acid may act as a lysosomotropic agent like siramesine. Siramesine is a lipophilic compound that shares some interesting features with Betulinic Acid such as induction of p53-, Bcl-2- and caspase independent cell death and induction of cytoprotective autophagosomes[32].

Betulinic acid also induces proteasome-dependent degradation of Sp1, Sp3, and Sp4 proteins. Laboratory studies have shown that both basal and hormone-induced expression of VEGF in cancer cell lines is dependent on Sp protein expression), and regulation of survivin in some cells is also dependent on these transcription factors). Moreover, because Sp proteins are up-regulated in many tumors/cancer cells) and are associated with proliferative, angiogenic, and antiapoptotic pathways, it is hypothesized that the anticarcinogenic activity of betulinic acid may be due, in part, to down-regulation of Sp proteins. Betulinic acid also decreased expression of Sp1, Sp3, and Sp4 proteins in SK-MEL2 melanoma cancer cells, and similar results were obtained in other cancer lines[39].

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Betulinic acid inhibits VEGF and survivin promoter expression through proteasome-dependent degradation of Sp proteins. Expression of both VEGF and survivin in some cancer cell lines is regulated by Sp protein interactions with GC-rich promoter sites therefore, the effects of betulinic acid on decreased expression of VEGF and survivin through Sp protein degradation was further investigated. The studies show that betulinic acid decreased luciferase activity in LNCaP cells transfected with pVEGF1 and pVEGF2, and these effects were reversed by the proteasome inhibitor MG132; similar results were observed using the proteasome inhibitor lactacystin. The studies further confirm that betulinic acid–induced degradation of Sp proteins results in decreased VEGF expression in LNCaP cells, and this is consistent with RNA interference studies showing that Sp1, Sp3, and Sp4 regulate VEGF expression in cancer cell lines. Betulinic Acid Perturb Mitochondrial Functions.

It has been found to activate two human apoptotic pathways: the mitochondrial apoptotic pathway and NF-κB pathway. It induces apoptosis independently of CD95 (AP0-1/FAS) and p53. The other apoptosis pathway affected by Betulinic Acid is the NF-κB pathway. DNA damage activates NF-κB. NF-κB leads to inflammation, the synthesis of ROS, cytokines and chemokines including TNF, lymphotoxins, IL-6, and IL-8, and growth and angiogenic factors. NF-κB can lead to malignant proliferation, the prevention of apoptosis, and an increase in metastasis and angiogenesis[4].
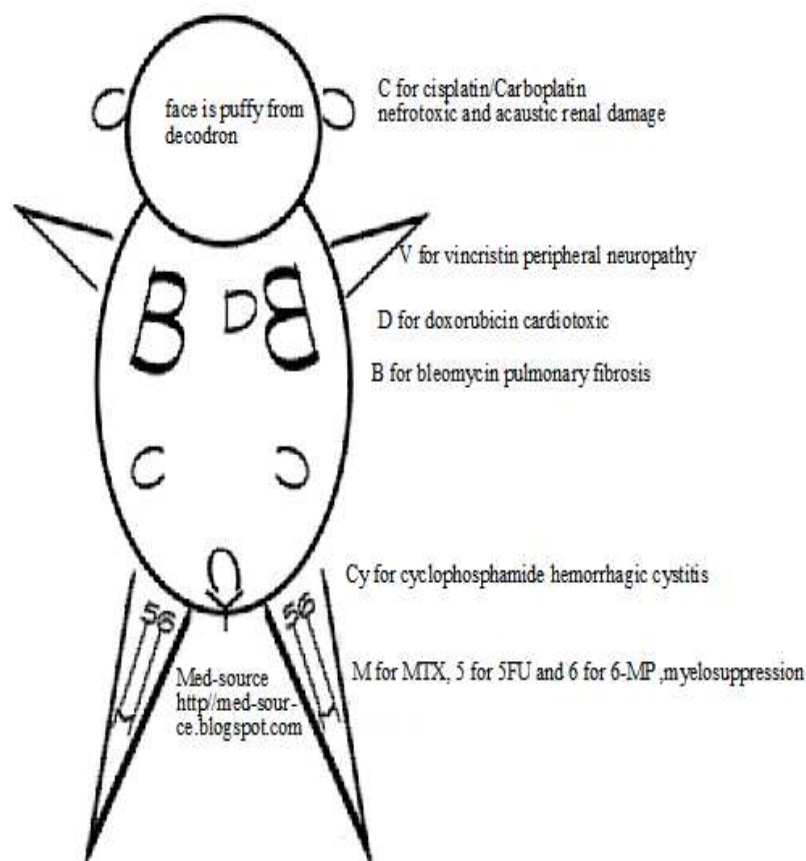

Figure 6. Chemo-men

Advantage of Betulinic Acid over the synthetic ones:
Currently conventional drugs used in cancer chemotherapy includes: - alkylating agents (Mustine, Cyclophosphamide, Chlorambucil, Melphalan, etc); Antimetabolites (methotrexate, 5-fluorouracil, etc) DNA binding agents; topoisomerase inhibitors (camptothecins, etoposide, etc); microtubular inhibitors (vinca alkaloids and taxanes); molecularly targeted agents; small molecules and monoclonal anti-bodies hormones; biological response modifiers[40].

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All of the above agents have their own side effects. Their side effects are represented diagrammatically (Chemo men) as Fig. 6.

Betulinic Acid Derivatives as anti-cancer agent:
Advantage of Betulinic Acid Derivatives over Betulinic Acid: Betulinic Acid is an important natural product, and more and more studies have shown that Betulinic Acid has significant anticancer activity against various kinds of cell lines *in vitro*. In a study, a series of Betulinic Acid derivatives were designed, synthesized and evaluated their antitumor activities against the MGC-803, PC3, A375, Bcap-37, and A431 cell lines and the study suggest that the C-28 amino substituted BetA derivatives possess stronger anti-proliferative ability. The IC_{50} values of compound 3c against the above five cancer cell lines were 2.3, 4.6, 3.3, 3.6, and 4.3 μM, respectively. However, the compounds containing an acyl piperazine moiety at C-28 did not display higher inhibitory activity, and some compounds only had moderate antitumor activity, but still more active than the parent Betulinic Acid[41].

A series of new imidazole carboxylic esters (carbamates) and N-acylimidazole derivatives of betulinic acid have been synthesized. The new compounds were screened for *in vitro* cytotoxicity activity against human cancer cell lines HepG2, Jurkat and HeLa. A number of compounds have shown IC_{50} values lower than 2 lM against the cancer cell lines tested and the vast majority has shown a better cytotoxicity profile than betulinic acid[4].

Betulinic Acid holds great promise as an anti-tumor agent, but as mentioned has a severe drawback in its poor solubility in aqueous solutions and thus its application in vivo. Another non-scientific fact is that as a broadly available product from nature, Betulinic Acid is difficult to patent. For these reasons, and of course in search for even more potent anti-cancer drugs, a lot of effort has been put into developing and testing Betulinic Acid derivatives[32].

Generally, the advantage of betulinic acid derivatives over betulinic acid is the following:-

Betulinic acid is a natural compound with high in vitro cytotoxicity toward many cancer cells. However, the poor water solubility of this compound hampers an effective *in vivo* cancer study. New ionic derivatives of betulinic acid were produced with higher water solubilities, without losing the structural integrity and functionality of this compound.

As a result, these new ionic derivatives have shown much higher inhibitory effects against different cancer cell lines such as melanoma A375, neuroblastoma SH-SY5Y and breast adenocarcinoma MCF7. To improve the potency of Betulinic Acid, dozens of structural modifications have been made to Betulinic acid currently[42].

Recent progress and future perspectives on BetA and its derivative: According to World Health Organization, 80 % of the people living in rural areas depend on medicinal natural products as primary healthcare system. A great deal of pharmaceutical research done in technologically advanced countries like USA, Germany, France, Japan and China has considerably improved quality of the natural products used in the treatment of cancer. Some natural products protect the body from cancer by enhancing detoxification functions of the body. Some of them reduce the toxic side effects of chemotherapy and radiotherapy. Scientists all over the world are concentrating on the natural products to boost immune cells of the body against cancer. By understanding the complex synergistic interaction of various constituents of anticancer of natural products, their formulations can be designed to attack the cancerous cells without harming normal cells of the body[43].

To date, the majority of studies were devoted to the investigation of betulinic acid, as this derivative revealed better bioavailability and cytotoxic effects. However, the need for chemical transformation of betulin into betulinic acid makes the more former compound an interesting medical target, especially because it is also a known natural medicinal product. Betulin and its derivatives were reported to be less active toward some cell lines, but no particular structure activity studies were performed to explain in detail the difference in the activity. One possibility could be much lower solubility of betulin comparing to betulinic acid due to the presence of the much more hydrophilic carboxylic group in the latter, which may adversely affect cell permeability and cell penetration in the case of the betulin[44].

Natural compound betulinic acid shows potent anti-cancer activity through activation of mitochondrial pathway of apoptosis in cancer cells. Betulinic acid may also be used in combination protocols to enhance its antitumor activity,

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for example with chemo- or radiotherapy or with the death receptor ligand TRAIL. Because of its relative reactive cytotoxicity against malignant compared to normal cells, Betulinic acid is a promising new experimental anti-cancer agent for the treatment of human cancer today[38].

Recently, studies showed that substituted BetA analogs can be proteasome inhibitors, and the C-3 and C-30 positions of Betulinic acid are the pharmacophores for improving the proteasome inhibition activity.

Within the C-3 and C-30 substitutions, larger side chains with lipophilic or aromatic side chains are favored for increased inhibition of the chymotrypsin-like activity of 20S proteasome. C-3 and C-30 modified BA compounds showed low toxicity in the previous anti-AIDS studies, and bevirimat, a C-3 substituted BetA, has already succeeded in phase IIb clinical trials. Therefore, development of C-3 and/or C-30 modified Betulinic Acid derivatives as proteasome inhibitors might be able to provide new therapeutic agents for treating cancers and inflammatory diseases[4].

CONCLUSION

Cancer is the second most important disease leading to death in both the developing and developed countries nowadays. This global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviors, particularly smoking, in economically developing countries.

In light of the scientific promise of chemoprevention, there is an overwhelming need to develop new chemopreventive agents that are both effective and safe. One practical approach to this problem is to use natural products as a platform for drug development. Approximately half of the drugs currently used in the clinic are derived from natural products. For thousands of years and nowadays natural products have been used to combat human diseases and play an increasing role in drug discovery and development.

The antitumor activity of natural products has been linked their ability to trigger cell death pathways including apoptosis or programmed cell death in cancer cells. Of natural products Betulinic Acid (a known natural pentacyclic lupane type triterpenoid present in many plant species , obtained by extraction of barks or core of some plant species or by synthetic processes, e.g. using the betulin (alcohol triterpene) as a synthetic intermediate isolated from the bark of *Betula Alba and Betula pendula* treat cancerous cell selectively without harming the normal cell, and this makes it preferable than chemotherapeutic agent. The poor water solubility of this compound hampers an effective in *vivo* cancer study and its new ionic derivatives were produced with higher water solubilities, without losing the structural integrity and functionality of this compound. As a result, these new ionic derivatives have shown much higher inhibitory effects against different cancer cell lines.

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