BETULINIC ACID : A PROMISING ANTI-INFLAMMATORY PHYTOCONSTITUENT, CURRENT AND FUTURE PERSPECTIVES

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Abstract

Betulinic acid is a well-known naturally occurring triterpenoid having lupane-type pentacyclic structure. It is generally found in the bark of birch tree but can also be isolated from other plant sources. It is found as either glycosyl derivative or free aglycon form, in different parts of the plants. This triterpene has gained a lot of attention in the last few years as it exhibits various pharmacological activities, such as anti-neoplastic, anti-inflammatory, anti-HIV, anti-diabetic, anti-malarial, anti-microbial, hepatoprotective. The present review focuses particularly on the anti-inflammatory activity and related mechanism of action of betulinic acid. Conclusions made on the basis of various studies reveal that betulinic acid act as a promising anti-inflammatory drug in the near future.

Keywords: Betulinic acid, Anti-inflammatory, Anti-neoplastic

Introduction

Betulinic acid or 3β-hydroxy-lup-20(29)-en-28-oic acid is a naturally occurring lupane-structured penta cyclic triterpenoid, which is widely distributed in plant kingdom (Moghaddam *et al.,* 2012). Usually, betulinic acid and betulin (3β-lup-20(29)-ene-3,28-diol), a reduced moiety of betulinic acid, were isolated from the bark of birch tree (*Betula* sp., Betulaceae). However, it could also be isolated from various other sources including different species of *Syzygium* (Myrtaceae), *Paeonia* (Paeoniaceae), *Ziziphus* (Rhamnaceae), *Diospyros* (Ebenaceae), *Tetrasera* (Dilleniaceae), *Pseudocydonia* (Rosaceae) (Rios, Manez, *et al.,* 2018). Its molecular formula is $C_{30}H_{48}O_3$ and molecular structure is given in figure 1 along with its various biological effects. It exhibits a variety of pharmacological activities such as anti-neoplastic (Ramadoss *et al.,* 2000; Seneja *et al.,* 2019), anti-inflammatory (Kim *et al.,* 2016), anti-malarial (Silva *et al.,* 2015), anti-HIV (Huang *et al.,* 2015; Li *et al.,* 2016), anti-diabetic (Ajala *et al.,* 2018), hepatoprotective (Liu *et al.,* 2019), anti-oxidant (Nicolov *et al.,* 2019) and anti-microbial effects (Ibrahim, *et al.* 2019).
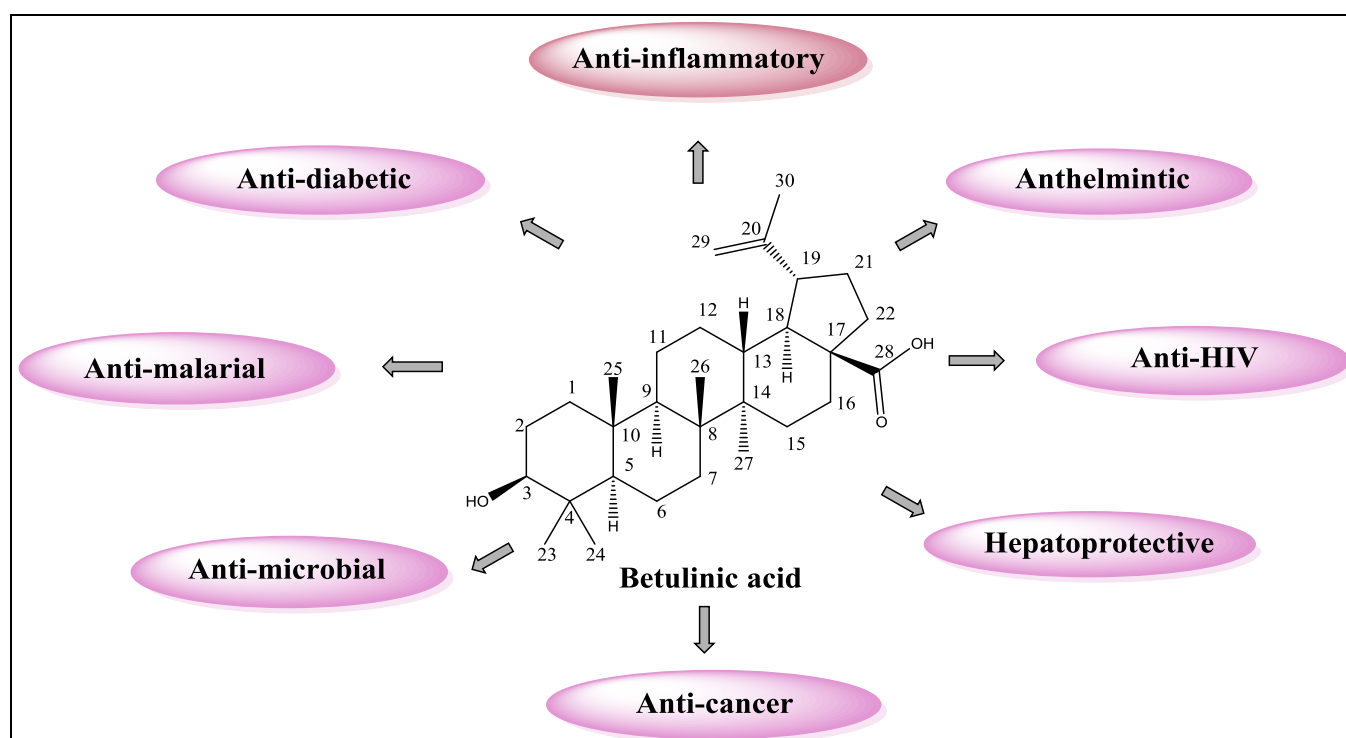

Fig. 1 : Structure and various pharmacological activities of Betulinic acid.

Inflammatory diseases are becoming very common in aging society everywhere in the world. And there are a lot of side effects related to clinically used anti-inflammatory drugs. Natural compounds and traditional medicines offer great hope to identify biologically active products and developing them into drugs for the treatment of inflammatory diseases (Gautam and Jachak, 2009). Betulinic acid, a naturally occurring triterpene, can be isolated from different plant sources and has great potential to act as an anti-inflammatory agent.

Inflammation is the body's attempt to produce a protective immune response against harmful stimuli such as pathogens, irritants, damaged cells. Swelling, redness, pain, and heat are the classical symptoms of an inflammatory process. Inflammation can be either acute or chronic. Acute inflammation is generally initial response of the body against harmful stimuli whereas chronicinflammation can sometimes lead to the growth of several diseases like rheumatoid arthritis, chronic asthma, inflammatory bowel disease, psoriasis, sepsisand neurodegenerative disorders (Ferrero-Miliani, *et al.* 2007). Various experimental and clinical studies have proven that chronic infection and inflammation are the major risk factors for the development of different types of cancers. COX-1, COX-2, interferons- IFN-α_2, IFN-β_1, IFN-γ, cytokines, and cytokine receptors, G-protein coupled receptors, cysteinyl leukotriene-1, histamine-1and cluster of differentiation molecules are some of the major anti-inflammatory targets as shown in figure 2 (Gautam and Jachak, 2009). Macrophages when come in contact with LPS (lipopolysaccharide)- an endotoxin present in the outer membrane of gram-negative bacteria,get activated and produces various pro-inflammatory mediators and cytokines such as TNF-α (tumour necrosis factor-α), IL1-β (interleukin-1), IL-6 (interleukin-6), PGE2(prostaglandin E2) and NO (nitric oxide) (Kim *et al.,* 2016). Some molecular targets of inflammation include NF-κB (Nuclear factor- κB), MAPK (Mitogen-activated protein kinase) and p38 kinases. NF-κBis a major transcription factor that controls the transcription of DNA for the maintenance of the inflammatory responses, cell proliferation and apoptosis (Zhao *et al.*, 2013). It acts as a switch in the body to turn inflammation either on or off. Inactive form of-κBis present in the cytoplasm complexed with inhibitory protein, IkB. On exposure to provoking stimuli, Ikbdegrades and NF-κB get activated. It then translocates to the nucleus and bind to the consensus sequence of pro-inflammatory genes, so to regulate the synthesis of inflammatory cytokines (Maroon *et al.*, 2010). Betulinic acid suppresses the activation of NF-κB thus inhibits the production of inflammatory cytokines and stops inflammation. In this review, we mainly emphasise on the anti-inflammatory activity and related mechanism of action of betulinic acid.
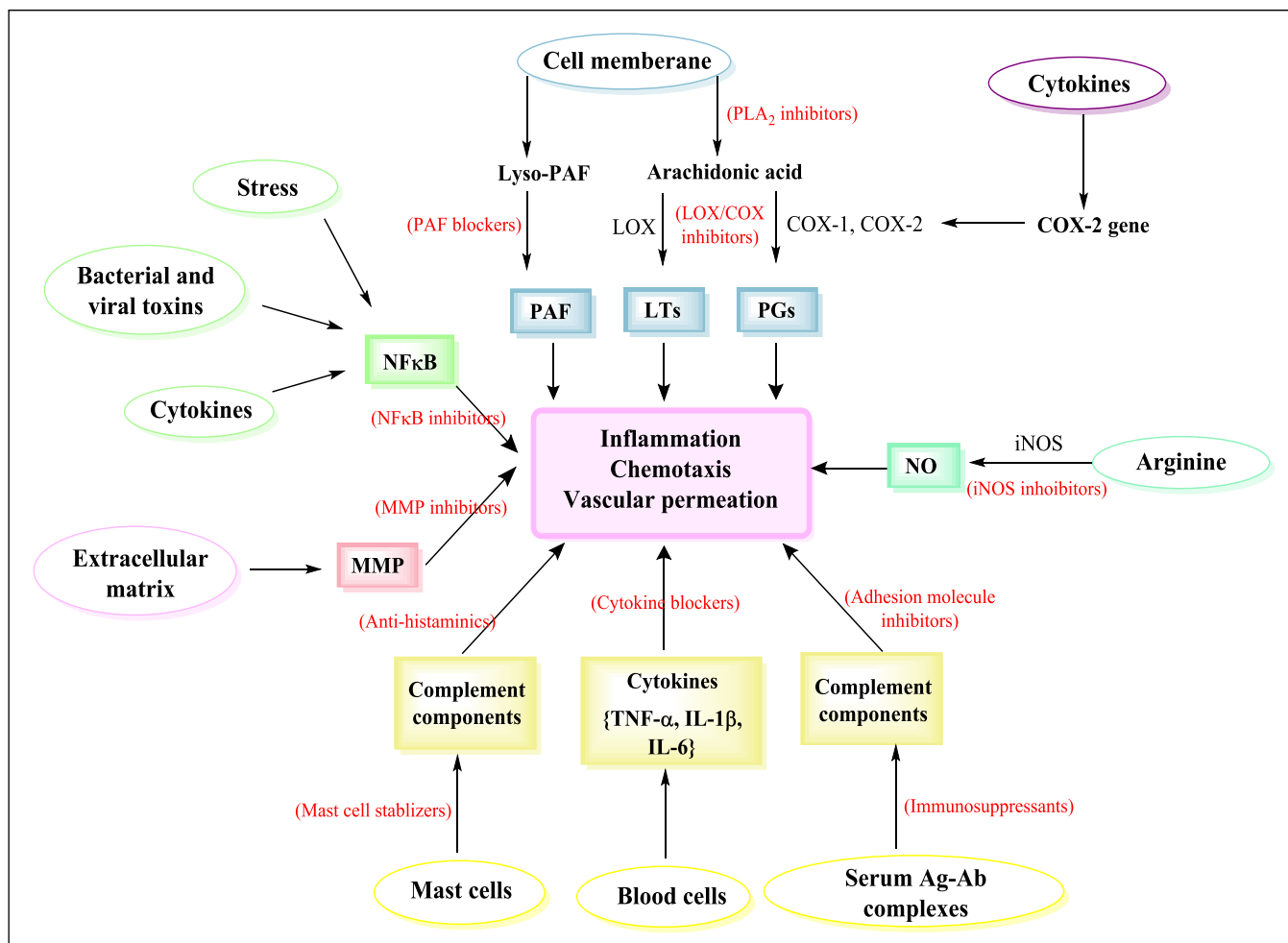

Fig. 2 : Various drug targets involved in the inflammation cascade.
PLA$_2$= phospholipase A$_2$, PGs= prostaglandins, LTs= leukotrienes, PAF= platelet activating factor, LOX= lipoxygenase, COX= cyclooxygenase, IL-1β= interleukin-1β, NF-κB= nuclear factor-κB, TNF-α= tumour necrosis factor-α, IL-6= interleukin-6, MMP= matrix metalloproteinase, NO= nitric oxide, iNOS= inducible nitric oxide synthase.

Anti-inflammatory activity

Betulinic acid obtained from leaf extract *Diospyros kaki*was found to have anti-inflammatory activity. The inhibitory effect of betulinic acid was examined on the inflammatory action in LPS-stimulated RAW 264.7 macrophages. It decreased the levels of COX-2 as well as pro-inflammatory mediators including TNF-α, IL-1β, IL-6, and IL-12 via inhibiting NF-κBas well as Nrf2(Nuclear factor erythroid 2-related factor 2) signaling pathway. In this study, it was investigated that betulinic acid induced HO-1(hemeoxygenase-1) induction via Nrf2 (Nuclear factor erythroid 2-related factor 2) translocation, which was also responsible for their anti-inflammatory properties (Kim, *et al.* 2016; Lee *et al.,* 2003).

Betulinic acid is also effective in case of vascular inflammation and atherosclerosis. (Zhao *et al.,* 2013) investigated the effect of betulinic acid on ABCA1 (ATP-binding cassette transporter A1) expression to find its role in preventing vascular inflammation and atherosclerosis. It was found that betulinic acid suppressed LPS-induced NF-kB activation which leads to down-regulation of miR-33s and promoted ABCA1 expression and cholesterol efflux. This mechanism involved the inhibition of p65 phosphorylation, IkB phosphorylation, nuclear translocation, and NF-kB-dependent gene transcription. Betulinic acid has a potent inhibitory effect on vascular inflammation process also in human umbilical vein endothelial cells. It inhibited the ROS (reactive oxygen species) production and NF-κB activation by blocking the TNF-α-induced expression levels of vascular cell adhesion molecule-1, intracellular adhesion molecule-1and endothelial cell selection as well as gelatinase in TNF-α-activated human umbilical vein endothelial cells (Yoon *et al.,* 2010).

Also, according to the study of (Takada *et al.,* 2003), betulinic acid inhibited NF-κB activation induced by TNFR1, TNFR-associated death domain, TNFR-associated factor 2, NF-Kbinducing kinase, and IkB-α kinase. It also suppressed the NF-κB-regulated gene expressions of cyclooxygenase-2 and matrix metalloproteinase-9induced by carcinogens and inflammatory stimuli.

Betulinic acid significantly decreased the production of nitric oxide in LPS stimulated RAW 264.7 cells in a concentration range of 10-30mM[19] and selectively inhibited COX-2 (IC_{50}= 11.4 μM) over COX--1 (IC_{50}=115 μM) (Su *et al.,* 2002). In previous studies, anti-inflammatory effects of betulinic acid were also observed due to in hibition of bovine prostaglandin synthase by 52% at 200μg/ml and bradykinin induced inflammation by 54% proinflammatory cytokine-induced neutrophil chemoattractant-1 (34%, 1 μM) in stimulated rat macrophages and IL-1β-stimulated rat fibroblast cells (Cichewicz *et al.*, 2004).

Betulinic acid was also responsible for anti-PLA_2 (phospholipase-A_2) activity according to a study based on a combination of ethnopharmacological and bioinformatic information to discover new PLA_2 inhibitors. It inhibited bovine pancreaticPLA_2 by 40% (5 μM) (Rios, 1995). As PLA_2 is a target involved in the pro-inflammatory process, betulinic acid shows its anti-inflammatory effects, here also.

(Bernard, *et al.* 2001) confirmed in their study that an anionic group, eg. carboxylate group is really important for inhibiting PLA_2 by docking betulinic acid into its crystal structure. It was found that, of all the compounds assayed, betulinic acid was the best inhibitor of PLA_2 as it fits into the binding sites of $PLA_2$with good energy values.

In earlier studies, experimental models were used to determine the anti-inflammatory activity of betulinic acid (Gautam and Jachak, 2009). In one such study, betulinic acid along with ten other triterpenoids was assayed on mouse ear oedema induced by the protein kinase C activators, mezerein, TPA (12-*O*-tetradecanoyl phorbol-13-acetate), two 12-de-oxyphorbol-13-monoesters- DPT (13-tetradecanoate) and DPP (13-phenylacetate) and bryostatin 1 and by resiniferatoxin, xylene, and AA (arachidonic acid). Effects of bradykinin-induced paw oedema, as well as rat skin inflammation produced by H_2O_2, were also examined. So, it was found that betulinic acid (0.5mg per ear) inhibited mezerein induced oedema by 48%, TPA induced oedema by 51%, DPT induced oedema by 61% and bryostatin 1 induced oedema by 54% (ID_{50}= 0.77 μmol/ear). Similar results observed in the case of rat skin inflammation induced by glucose oxidase (39% at 0.25mg/site) and bradykinin-induced mouse paw oedema (54% at 10mg/kg). Whereas, in the case of oedema induced by AA, resiniferatoxin and xylene, no effects were observed. As betulinic acid was inactive against the inflammation induced by AA and in neurogenic inflammatory models, it was concluded that this type of inflammation might be dependent on *in-vivo* inhibition of protein kinase C (Huguet *et al.,* 2000; Yueqin *et al.,* 2003). In an *in-vitro* study previously done by (Wang and Poyla, 1996), the effect of betulinic acid was studied on rat liver cyclic AMP-dependent protein kinase (cAK), rat brain Ca^{2+}(protein kinase C) PKC and wheat embryo Ca^{2+} dependent protein kinase (CDPK) with IC_{50} values of 45, 145 and 84μM, respectively. Also, (Recio, *et al.* 1995) investigated the anti-inflammatory activity of betulinic acid as well as some other triterpenoids isolated from *Diospyros leucomelas* on carrageenan and serotonin paw oedema tests and TPA and EPP (ethyl phenyl propiolate) ear oedema tests. They found that betulinic acid (100 mg/kg) inhibited carrageenan-induced mouse paw oedema by 45.6%. EPP induced mouse ear oedema and TPA induced mouse ear oedema was inhibited by 30.3% and 86.2% (0.5 mg per ear), respectively. They indicated that betulinic acid showed its effects through the mechanism related to that of glucocorticoid, so it might be a corticoid-like agent (Mukherjee *et al.,* 1997).

Viji *et al.* (2010) also suggested the anti-inflammatory activity of betulinic acid isolated from *Bacopa monnieri*. According to them, betulinic acid suppresses LPS stimulated IL-6 production in peripheral mononuclear cells by inhibiting the nuclear translocation of NF-κB, indicating that possible mechanism by which betulinic acid exerts anti-inflammatory effect might include inhibition of IL-6 production via inhibition of NF-κB or p38/ERK MAPK (Extracellular signal-regulated kinase, Mitogen-activated protein kinase) pathways.
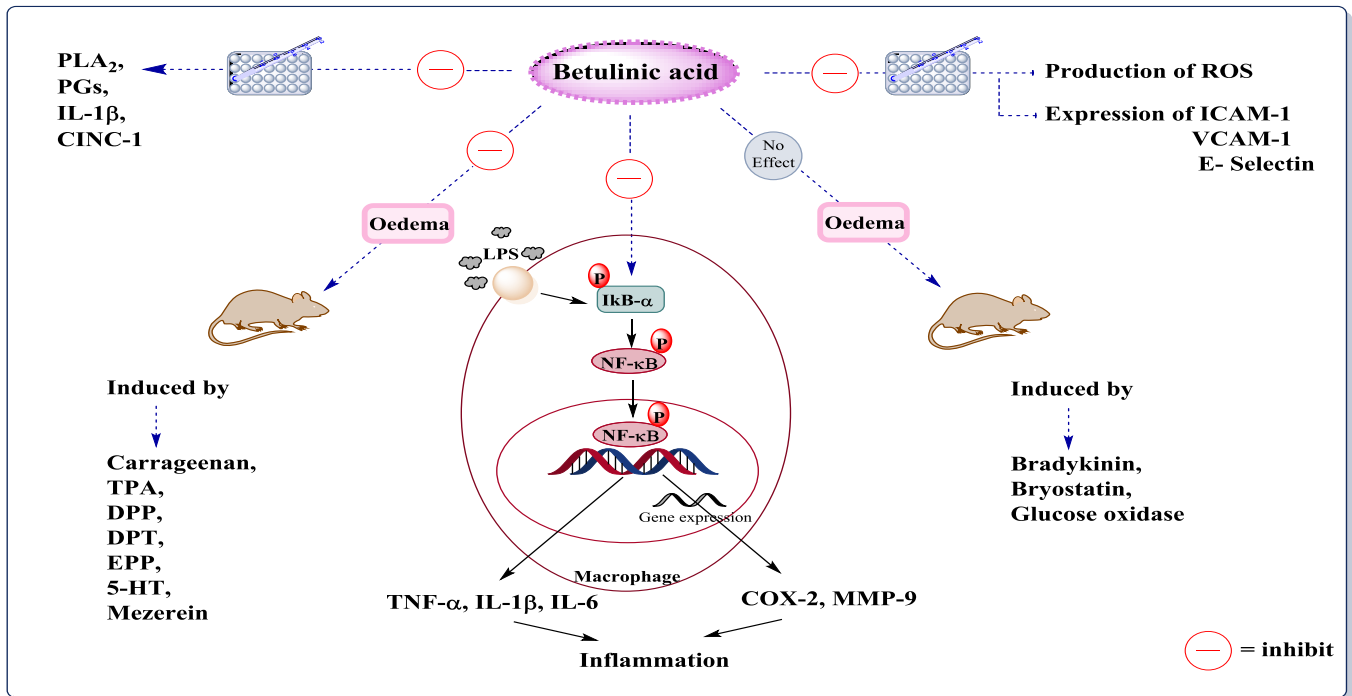
Fig. 3: Mechanism and Anti-inflammatory effect of Betulinic acid against various agents.
PLA$_2$= phospholipase A$_2$, PGs= prostaglandins, IL-1β= interleukin-1β, CINC-1= cytokine- induced neutrophil chemoattractant-1, TPA= 12-O-tetradecanoylphorbol-13-acetate, DPP= 12-de-oxyphorbol-13-phenylacetate, DPT= 12-de-oxyphorbol-13-tetradecanoate, EPP= ethylphenylpropiolate, 5-HT= 5-hydroxy tryptamine, NF-κB= nuclear factor-κB, TNF-α= tumour necrosis factor-α, IL-6= interleukin-6, COX-2= cyclooxygenase-2, MMP-9= matrix metalloproteinase-9, ICAM-1= intracellular adhesion molecule-1 , VCAM-1= vascular cell adhesion molecule-1, E-selectin= endothelial cell selectin, ROS= reactive oxygen species.

Some drawbacks of Betulinic acid

Despite the potential biological activity of betulinic acid, there are some drawbacks which limit its therapeutic application. It is poorly soluble in water which hinders the standard procedures for developing studies related to *in-vitro* assays. It has a low gastrointestinal absorption because of its poor aqueous solubility and short half-life *in vivo,* which also affects its efficacy (Saneja *et al.,* 2018). Poor water solubility is its major problem which limits its application to topical use. To limit this drawback, various derivatives and new forms of administration have been proposed. To increase its solubility, modifications at positions C-3, C-20 and C-28 have been made, without affecting its pharmacological activity (Rios and Manez, 2018). On the other hand, different routes of administration including polymeric and magnetic nanoparticles, liposomes, nanoemulsions, polymeric conjugates, carbon nanotubes and cyclodextrin complexes (Figure-4) have been developed for the delivery of betulinic acid (Saneja *et al.,* 2018).
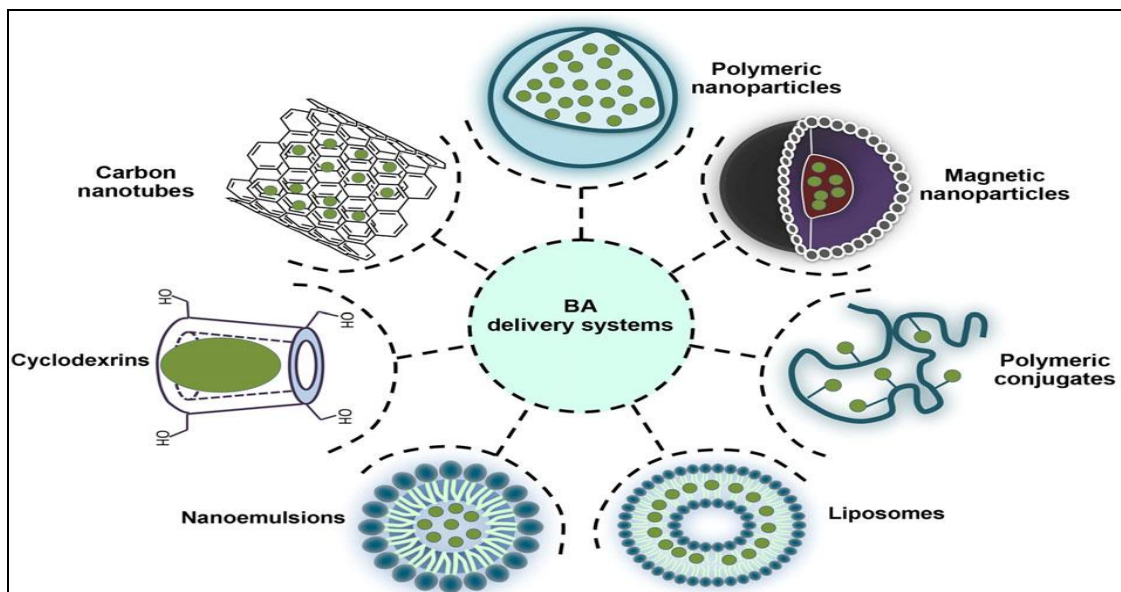

Fig. 4 : Various drug delivery approaches to enhance the therapeutic efficacy of BA (Betulinic acid) (Saneja *et al.,* 2018).

Future perspectives:

Previous studies reveal that betulinic acid, being a natural compound, can be of great interest as an anti-inflammatory agent in the near future, with lesser side effects as compared to synthetic anti-inflammatory drugs. The inhibitory effect of betulinic acid on protein kinase C, PLA$_2$, NF-κB activation and NF-κB related gene expressions of COX-2 and MMP-9, favors it with anti-inflammatory properties. Poor aqueous solubility limits its application to topical use but this can also be prevented by using nano-tube delivery systems or semi-synthetic derivatives prepared by modifying the structure of betulinic acid without any loss of biological activity Further researches are necessary to develop novel drug delivery systems, methods, and formulations to maintain the efficiency and efficacy of this triterpene.

Conclusion

In conclusion, we can say that betulinic acid can act as a potential therapeutic agent for mitigating inflammation. Further research based upon the design and development of drug delivery systems and synthesis of modified derivatives of betulinic acid is needful to enhance its anti-inflammatory activity. Additional studies will be helpful to combat the inflammatory symptoms associated with other diseases also.

Acknowledgment

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