Dear Editor,

Betulinic acid (3β-hydroxy-lup-20(29)-en-28-oic acid, BA), a pentacyclic lupane-type triterpene, is widely distributed in the plant kingdom (Mukherjee et al., 2006; Fulda, 2008). Johann Tobias Lowitz isolated the reduced form of BA from plants in 1788 and found that it was a prominent outer-bark constituent in white-barked birch trees (Bag and Dash, 2011). BA has a wide range of biological and medicinal properties, including anti-human immunodeficiency virus (HIV), antibacterial, antimalarial, anti-inflammatory, anthelmintic, antinociceptive, anti-herpes simplex viruses-1 (HSV-1), immune-modulatory, antiangiogenic, and anticancer activity (Yogeeswari and Sriram, 2005; Gheorgheosu et al, 2014). Furthermore, the anti-tumor activity of BA can help overcome resistance by inducing apoptosis in a variety of human cancers.

Semi-synthetic derivatives of natural plant products continue to play an important role in drug discovery and development (Pan et al., 2010). To improve the potency of BA, many derivatives have been synthesized and evaluated for biological/medicinal applications (Jonnalagadda et al., 2013; Csuk, 2014). Because of its range of biological properties, BA has attracted much attention in recent years in the pharmaceutical industry. Here, we summarize key recent studies performed to evaluate the biological and pharmacological activities of BA and its derivatives (Table 1).
Table 1: Recent studies on betulinic acid and its biological and pharmacological activities

| Key message                                                                                                                                                                                                                                                                                                                                 | Reference                        |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| BA protects the lungs against inflammation and could be a potential modulator of inflammation in sepsis-induced acute lung injury.                                                                                                                                                                                                                   | Lingaraju et al., 2015            |
| BA induces HeLa cell apoptosis by triggering both the endoplasmic reticulum pathway and the reactive oxygen species (ROS)-mediated mitochondrial pathway.                                                                                                                                                                                                 | Xu et al., 2014                   |
| BA mediates the anti-oestrogenic effects of Proteus vulgaris; this suggests its potential use as a therapeutic agent in estrogen-dependent tumors.                                                                                                                                                                                                        | Kim et al., 2014                  |
| BA dose-dependently inhibits proliferation and induces apoptosis in neuroblastoma and melanoma cell line apoptosis.                                                                                                                                                                                                                              | Tiwari et al, 2014                |
| BA reduces lactase dehydrogenase (LDH) and creatine kinase (CK) release, prevents cardiomyocyte apoptosis, and alleviates the extent of myocardial ischemia/reperfusion injury.                                                                                                                                                                               | Xia et al., 2014                  |
| Combination of BA with cyclodextrin treatment enhances anti-proliferative activity and aids in preventing in vivo tumor development.                                                                                                                                                                                                              | Soica et al., 2014                |
| The betulin (BE) derivative BT06 and BA derivative AB13 may be promising alternatives leishmaniasis therapies, particularly in combination with miltefosine.                                                                                                                                                                                    | Sousa et al., 2014                |
| BA derived from Vitis amurensis root plays a novel role in melanogenesis. This finding has advanced our understanding of cosmetic therapy to reduce skin hyperpigmentation.                                                                                                                                                                                   | Jin et al., 2014                  |
| Spray drying BA is a superior alternative formulation that significantly increases BA oral bioavailability and enhances anticancer efficacy.                                                                                                                                                                                                     | Godugu et al., 2014               |
| BA may prevent bone loss in patients with bone metastases and cancer treatment-induced estrogen deficiency.                                                                                                                                                                                                                                   | Park et al., 2014                 |
| BA may exert hepatoprotective effects by increasing antioxidant capacity, through the improvement of the tissue redox system, maintenance of the antioxidant system, and decreased lipid peroxidation in the liver.                                                                                                                                 | Yi et al., 2014                   |
| BA regulates glycemia through classical insulin signaling by stimulating GLUT4 synthesis and translocation. Additionally, it does not cause hypercalcemia, which is highly significant from a drug-discovery perspective.                                                                                                                                                   | Castro et al., 2014               |
| BA has thyroid-enhancing potential by lowering thyroid-stimulating hormone levels and reducing thyroid tissue damage, thereby minimizing the symptoms of hypothyroidism when used anaphylactically in rats.                                                                                                                                               | Afzal et al., 2014                |
| BA induces eryptosis/erythroptosis in human erythrocytes through Ca^2+ loading and membrane permeabilization.                                                                                                                                                                                                                                 | Gao et al., 2014                  |
| A novel mechanism for BA-mediated ATP-binding cassette transporter A1 (ABCA1) expression has been proposed, which may provide new methods to modulate vascular inflammation and atherosclerosis.                                                                                                                                                                   | Zhao et al., 2013                 |
| Topomer CoMFA studies on 37 BA and BE derivatives confirmed their in vitro anticancer activity (reported as IC50 values) in HT29 human colon cancer cells.                                                                                                                                                                                    | Ding et al., 2013                 |
| Several novel 2,4-dinitrophenyldrazone BA derivatives were prepared by chemical and biotransformation methods using fungi and carrot cells. Some compounds showed significant cytotoxicity and selectivity against tumor cell lines.                                                                                                                        | Baratto et al., 2013              |
Table 1 (cont.): Recent studies on betulinic acid and its biological and pharmacological activities

| Key message                                                                                                                                                                                                 | Reference                      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Histopathological analyses of tumors revealed decreased angiogenesis, proliferation, and invasion in BA-treated animals. This is one of the first studies demonstrating the in vivo antitumor activity of BA in MCF-7 breast cancer tumors in nude mice. | Damle et al., 2013             |
| Lamin B1, which plays a crucial role in pancreatic cancer, was significantly downregulated by BA treatment in pancreatic cancer cells in vitro and in xenograft models.                                      | Li et al., 2013                 |
| BA or BE may ameliorate acute ethanol-induced fatty liver via toll-like receptor 4 (TLR4) and signal transducer and activator of transcription 3 (STAT3) in vivo and in vitro, suggesting it may be a promising agent for ethanol-induced fatty liver therapies.          | Wan et al., 2013               |
| BA modulates the activity of xenobiotic and antioxidative enzymes with putative roles in cancer initiation and proliferation.                                                                                     | Kaur and Arora, 2013           |
| BA ameliorates intracellular lipid accumulation in liver cells, and may be a potential therapeutic for the prevention of fatty liver disease.                                                                       | Quan et al., 2013              |
| BA inhibits deubiquitinases and induces apoptotic cell death in prostate cancer, but not normal, cells and tissues. Thus, it may be an effective, non-toxic, and clinically selective chemotherapeutic.                    | Reiner et al., 2013            |
| BA prevents endothelium-dependent relaxation (EDR) impairment in rat aortas exposed to exogenous superoxide anion, which may closely relate to oxidative stress reduction and endothelial nitric oxide synthase (eNOS)-nitric oxide (NO) pathway activation. | Qian et al., 2012              |
| BA induces apoptosis and blocks autophagic flux in KM3 cells. Both caspase 3 activation and autophagic flux inhibition contribute to BA-mediated apoptosis.                                                        | Yang et al., 2012              |
| BA may exert its anti-tumor effects by inducing tumor cell apoptosis. This process may also improve the immune response.                                                                                          | Wang et al., 2012              |
| BA may serve as an antithrombotic compound via antiplatelet activity. The antiplatelet effect of BA has been suggested to be similar to that prostacyclin (PGI2) receptor agonists; however, this requires further investigation. | Tzakos et al., 2012            |
| BA may be a potent and effective anticancer agent in nasopharyngeal carcinoma (NPG). Further exploration of the mechanism of action could yield novel breakthroughs in anticancer drug discovery.                          | Liu and Luo, 2012             |
| BA has anti-inflammatory and antioxidant properties that protect the lung against the deleterious effects of lipopolysaccharide.                                                                               | Nader and Baraka, 2012         |
| BA significantly inhibits fibrosis by modulating the TLR4/myeloid differentiation factor 88 (MyD88)/nuclear factor (NF)-κB signaling pathway.                                                                   | Wan et al., 2012               |
| BA may have anti-obesity effects by directly inhibiting pancreatic lipase. This may prevent the absorption of lipids from the small intestine. Further, BA may further accelerate fat mobilization by enhancing lipolysis in adipose tissues. | Kim et al., 2012               |
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REFERENCES

Afzal M, Kazmi I, Semwal S, Al-Abbasi FA, Anwar F. Therapeutic exploration of betulinic acid in chemically induced hypothyroidism. Mol Cell Biochem. 2014;386:27-34.

Bag BG, Dash SS. First self-assembly study of betulinic acid, a renewable nano-sized, 6-6-6-6-5 pentacyclic monohydroxy triterpenic acid. Nanoscale. 2011;3:4564-6.

Baratto LC, Porsani MV, Pimentel IC, Pereira Netto AB, Paschke R, Oliveira BH. Preparation of betulinic acid derivatives by chemical and biotransformation methods and determination of cytotoxicity against selected cancer cell lines. Eur J Med Chem. 2013;68:121-31.

Castro AJ, Frederico MJ, Cazarolli LH, Bretanha LC, Tavares Lde C, Buss Zda S, et al. Betulinic acid and 1,25(OH)2 vitamin D3 share intracellular signal transduction in glucose homeostasis in soleus muscle. Int J Biochem Cell Biol. 2014;48:18-27.

Csuk R. Betulinic acid and its derivatives: a patent review (2008-2013). Expert Opin Ther Pat. 2014;24:913-23.

Damle AA, Pawar YP, Narkar AA. Anticancer activity of betulinic acid on MCF-7 tumors in nude mice. Indian J Exp Biol. 2013;51:485-91.

Ding W, Sun M, Luo S, Xu T, Cao Y, Yan X, et al. A 3D QSAR study of betulinic acid derivatives as anti-tumor agents using topomer CoMFA: model building studies and experimental verification. Molecules. 2013;18:10228-41.

Fulda S. Betulinic acid aAid for cancer treatment and prevention. Int J Mol Sci. 2008;9:1096-107.

Gao M, Lau PM, Kong SK. Mitochondrial toxin betulinic acid induces in vitro eryptosis in human red blood cells through membrane permeabilization. Arch Toxicol. 2014;88:755-68.

Gheorgheosu D, Duicu O, Dehelean C, Soica C, Muntean D. Betulinic acid as a potent and complex anti-tumor phytochemical: a minireview. Anticancer Agents Med Chem. 2014;14:936-45.

Godugu C, Patel AR, Doddapaneni R, Somagoni J, Singh M. Approaches to improve the oral bioavailability and effects of novel anticancer drugs berberine and betulinic acid. PLoS One. 2014;9:e89919.

Jin KS, Oh YN, Hyun SK, Kwon HJ, Kim BW. Betulinic acid isolated from Vitis amurensis root inhibits 3-isobutyl-1-methylxanthine induced melanogenesis via the regulation of MEK/ERK and PI3K/Akt pathways in B16F10 cells. Food Chem Toxicol. 2014;68:38-43.

Jonnalagadda SC, Corsello MA, Sleet CE. Betulin-betulinic acid natural product based analogs as anticancer agents. Anticancer Agents Med Chem. 2013;13:1477-99.

Kaur R, Arora S. Interactions of betulinic acid with xenobiotic metabolizing and antioxidative enzymes in DMBA-treated Sprague Dawley female rats. Free Radiol Biol Med. 2013;65:131-42.

Kim HI, Quan FS, Kim JE, Lee NR, Kim HJ, Jo SJ, et al. Inhibition of estrogen signaling through depletion of estrogen receptor alpha by ursolic acid and betulinic acid from Prunella vulgaris var. lilacina. Biochem Biophys Res Commun. 2014;451:282-7.

Kim J, Lee YS, Kim CS, Kim JS. Betulinic acid has an inhibitory effect on pancreatic lipase and induces adipocyte lipolysis. Phytother Res. 2012;26:1103-6.

Liu L, Du Y, Kong X, Li Z, Jia Z, Cui J, et al. Lamin B1 is a novel therapeutic target of betulinic acid in pancreatic cancer. Clin Cancer Res. 2013;19:4651-61.

Lingaraju MC, Pathak NN, Begum J, Balaganur V, Bhat RA, Ramachandra HD, et al. Betulinic acid attenuates lung injury by modulation of inflammatory cytokine response in experimentally-induced polymicrobial sepsis in mice. Cytokine. 2015;71:101-8.

Liu Y, Luo W. Betulinic acid induces Bax/Bak-independent cytochrome c release in human nasopharyngeal carcinoma cells. Mol Cells. 2012;33:517-24.

Mukherjee R, Kumar V, Srivastava SK, Agarwal SK, Burman AC. Betulinic acid derivatives as anticancer agents: structure activity relationship. Anticancer Agents Med Chem. 2006;6:271-9.

Nader MA, Baraka HN. Effect of betulinic acid on neutrophil recruitment and inflammatory mediator expression in lipopolysaccharide-induced lung inflammation in rats. Eur J Pharm Sci. 2012;46:106-13.

Pan L, Chai H, Kinghorn AD. The continuing search for antitumor agents from higher plants. Phytochem Lett. 2010;3:1-8.
Park SY, Kim HJ, Kim KR, Lee SK, Lee CK, Park KK, et al. Betulinic acid, a bioactive pentacyclic triterpenoid, inhibits skeletal-related events induced by breast cancer bone metastases and treatment. Toxicol Appl Pharmacol. 2014;275:152-62.

Qian LB, Fu JY, Cai X, Xia ML. Betulinic acid inhibits superoxide anion-mediated impairment of endothelium-dependent relaxation in rat aortas. Indian J Pharmacol. 2012;44:588-92.

Quan HY, Kim do Y, Kim SJ, Jo HK, Kim GW, Chung SH. Betulinic acid alleviates non-alcoholic fatty liver by inhibiting SREBP1 activity via the AMPK-mTOR-SREBP signaling pathway. Biochem Pharmacol. 2013;85:1330-40.

Reiner T, Parrondo R, de Las Pozas A, Palenzuela D, Perez-Stable C. Betulinic acid selectively increases protein degradation and enhances prostate cancer-specific apoptosis: possible role for inhibition of deubiquitinase activity. PLoS One. 2013;8(2):e56234.

Soica C, Danciu C, Savoiu-Balint G, Borcan F, Ambros R, Zupko I, et al. Betulinic acid in complex with a gamma-cyclodextrin derivative decreases proliferation and in vivo tumor development of non-metastatic and metastatic B164A5 cells. Int J Mol Sci. 2014;15:8235-55.

Sousa MC, Varandas R, Santos RC, Santos-Rosa M, Alves V, Salvador JA. Antileishmanial activity of semisynthetic lupane triterpenoids betulin and betulinic acid derivatives: synergistic effects with miltefosine. PLoS One. 2014;9(3):e89939.

Tiwari R, Puthli A, Balakrishnan S, Sapra BK, Mishra KP. Betulinic acid-induced cytotoxicity in human breast tumor cell lines MCF-7 and T47D and its modification by tocopherol. Cancer Invest. 2014;32:402-8.

Tzakos AG, Kontogianni VG, Tsoumani M, Kyriakou E, Hwa J, Rodrigues FA, et al. Exploration of the antiplatelet activity profile of betulinic acid on human platelets. J Agric Food Chem. 2012;60:6977-83.

Wan Y, Wu YL, Lian LH, Xie WX, Li X, Ouyang BQ, et al. The anti-fibrotic effect of betulinic acid is mediated through the inhibition of NF-κB nuclear protein translocation. Chem Biol Interact. 2012;195:215-23.

Wan Y, Jiang S, Lian LH, Bai T, Cui PH, Sun XT, et al. Betulinic acid and betulin ameliorate acute ethanol-induced fatty liver via TLR4 and STAT3 in vivo and in vitro. Int Immunopharmacol. 2013;17:184-90.

Wang P, Li Q, Li K, Zhang X, Han Z, Wang J, et al. Betulinic acid exerts immunoregulation and anti-tumor effect on cervical carcinoma (U14) tumor-bearing mice. Pharmazie. 2012;67:733-9.

Xia A, Xue Z, Li Y, Wang W, Xia J, Wei T, et al. Cardioprotective effect of betulinic Acid on myocardial ischemia reperfusion injury in rats. Evid Based Complement Alternat Med. 2014;2014:573745.

Xu T, Pang Q, Zhou D, Zhang A, Luo S, Wang Y, et al. Proteomic investigation into betulinic acid-induced apoptosis of human cervical cancer HeLa cells. PLoS One. 2014;9(8):e105768.

Yang LJ, Chen Y, He J, Yi S, Wen L, Zhao J, et al. Betulinic acid inhibits autophagic flux and induces apoptosis in human multiple myeloma cells in vitro. Acta Pharmacol Sin. 2012;33:1542-8.

Yi J, Xia W, Wu J, Yuan L, Wu J, Tu D, et al. Betulinic acid prevents alcohol-induced liver damage by improving the antioxidant system in mice. J Vet Sci. 2014;15:141-8.

Yogeeswari P, Sriram D. Betulinic acid and its derivatives: a review on their biological properties. Curr Med Chem. 2005;12:657-66.

Zhao GJ, Tang SL, Lv YC, Ouyang XP, He PP, Yao F, et al. Antagonism of betulinic acid on LPS-mediated inhibition of ABCA1 and cholesterol efflux through inhibiting nuclear factor-kappaB signaling pathway and miR-33 expression. PLoS One. 2013;8(9):e74782.