# Ursolic acid: do we need other derivates?

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**Abstract**

**Background:** The nature is a fascinating source of biologic active substances, many of which are showing promising antitumor activities [1, 2]. The triterpenes are an important class of phytochemicals, classified in accordance with isoprene units [3, 4]. These chemicals are synthesized by the plants through cyclic processing of squalen. About 20000 of triterpens, such as cucurbitanes, cycloartanes, friedelanes, holostanes, hopanes, lanostanes, lupanes, oleananes, dammaranes, euphanes, tirucallanes, isomalabaricanes, ursanes and others are identified at the moment. From a wide diversity of triterpenes, the pentacyclic derivatives were the most frequent studied chemicals, due to their anti-inflammatory, analgesic, hepatoprotective, cardiotonic, anti-alergic, anti-microbial and anti-tumor properties [5-9]. Ursolic acid or 3β-hydroxi-urs-12-en-28-oic acid is a triterpen's pentacyclic acid was discovered in plants, such as Ocimum sanctum L.(*Holy Basil*), Prunus laurocerasus L. (*Cherry laurel leaves*), Vaccinum myrtillus L. (*Bilberry*), Crataegus laevigata (*Hawthorn*), Harpagophytum procumbens DC (*Devil's Claw*), Thymus vulgaris L. (*Thyme*), Sambucus nigra L. (*Elder Flowers*), Origanum vulgare L. (*Oregano*), Lavandula augustifolia Mill. (*Lavender*), Vinca minor L. (*Periwinkle*), as well as in the wax from apples, plums and pears peels [10]. It is a pentacyclic triterpenoid which belongs to cyclosqualenoid family [11]. This acid can be determined free or as aglicon of saponins. The recent results are supporting the anti-inflammatory, anti-proliferative, pro-apoptotic, anti-metastatic, anti-angiogenic and anti-parasite functions of this chemical [7,12]. The aim of this study was to highlight in details the anti-tumor activity of ursolic acid, by pointing out its influence on cells proliferating, apoptosis and metastatic property.

**Conclusions:** Ursolic acid is a promising compound in tumor prevention and treatment, with many mechanisms of action on cell's proliferation. Its derivatives usually are more biologically effective than initial compound, so obtaining of new ursolic derivatives makes further investigations in this field have a particular relevance.

**Key words:** triterpenes, ursolic acid, cancer.

## Ursolic acid isolation

This chemical was isolated through different methods [13]. Generally, plants are extracted by two solvents with increasing polarity, hexan and ethyl acetate in Soxhlet. The obtained extract of ethyl acetate is concentrated in rotary evaporator. Until now were purposed many isolation methods in organic solvents by using high pressure liquid chromatography (HPLC), thin layer chromatography (TLC) and gas-chromatography after silylation and methylation [14-16]. Kontogianni et al. (2009) have demonstrated that combination of $^{1}H$-$^{13}C$ HSQC and $^{1}H$-$^{13}C$ HMBC NMR spectroscopies is a fast analytical method which clarifies and quantifies triterpenic acids in plants' extracts [17]. However, today the most frequently used method is bioassay-guided fractionation, based on physico-chemical differences. But this method anyway implies chromatographic techniques.

Finally, a clean isolated ursolic scid (UA) looks as glossy prisms after purification in absolute alcohol or as long threads, hair-like from diluted alcohol. The melting point of this chemical reaches 284-288 $^{0}C$. Ursolic acid is soluble in organic solvents such as ethanol, hot glacial acetic acid, in 2% alcoholic NaOH, dimethyl sulfoxide and dimethyl formamide,

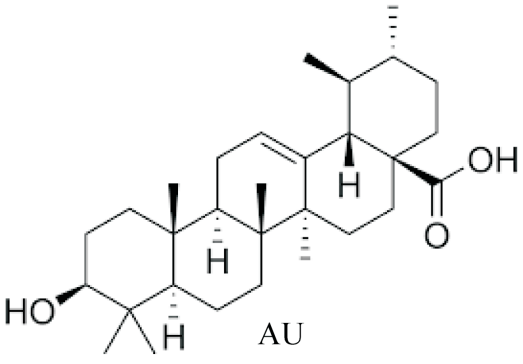


**Fig. 1. Chemical structure of ursolic acid.**

which should be purged with an inert gas. It is insoluble in water (fig. 1).

### Antitumor activity of ursolic acid

Multiple studies have confirmed that tumor progression is stimulated by pro-inflammatory factors: nuclear factor NF-kB, transcriptor activator 3 (STAT3), protein kinase B (AKT), cyclooxygenase-2 (COX-2) [18-20] among them. All these factors showed a pro-tumor activity, by stimulating cell proliferation, angiogenesis and metastatic properties.

Nuclear factor NF-kB is a regulating key implicated almost in all cell's processes [22]. Activation of this factor is often associated with chronic inflammation, tumorigenesys and resistance to chemo/radiotherapy [22, 23]. Many studies in the field support the role of chronic inflammation in tumor formation. Its presence is represented as a high risk in cancer development. Oeckinghaus et al. (2011) demonstrated that phosphorylation of IkB proteins by IkB kinases is a key process finalized with NF-kB coupling DNA and transcription activation of certain genes [24]. Until now have been purposed many agents targeted at this mechanism [7, 25]. Studies in vitro demonstrated the UA ability to block NF-kB activation induced by carcinogenic agents, such as TNF, okadaic acid, H2O2 and tobacco smock. By Shishodia et al. (2003) this action of UA is realized through IkBα kinases suppression and blocking of p65/Re1A phosphorilation [26]. Authors have reported that NF-kB inhibition was supplemented and by NF-KB dependent enzymes blocking, as cyclin D1, COX-2 and MMP-9 (matrix metalloproteinase-9).

Ursolic acid showed a promising activity against multiple types of tumors. Pathak et al. (2007) demonstrated a cytostatic activity of UA in case of multiple myeloma [27]. This action was realized through suppression of a wide series of kinases, such as c-SRC, Janus-activated kinases 1 and 2 (JAK ½ kinases). Doudican et al. (2014) by using a predictive simulation technology demonstrated that UA is very effective in case of multiple myeloma, especially in combination with other anti-cancer agents, such as pan-JNK inhibitor SP600125 [28]. Authors showed that such combination synergistically inhibited proliferation and induced apoptosis, evidenced by an increase in the percentage sub-G1 phase cells, cleavage of caspase 3 and poly-ADP-ribose-polymerase, as well a significant reduction in the expression of cyclin D1 and c-Myc.

Recently, Shanmugam et al. (2011) examined UA action on prostate carcinoma cell lines. This chemical was effective in androgen-independent tumors (DU145), as well androgen-dependent (LNCaP) [29]. This action was realized by suppression of genes regulated by STAT3 and NF-kB [30]. More, Shin et al. (2012) demonstrated that this triterpene has a stimulatory effect on LC3-II (microtubule-associated protein 1A/1B-light chain 3) resulted with activation of autophagy process in PC3 cells [31]. Zhang et al. (2010) consider that UA is beneficial in prostate cancer by its implication in signaling pathway mediated PI3/Akt/mTOR and Beclin-1, finally stimulating apoptosis [32].

Wang et al. (2011) tested UA and its cis-, trans-3-O-p-hydroxycinnamoyl derivatives on prostatic cells clone DU145 [33]. Authors presented an increased inhibitory capacity of UA on metalloproteinases MMP-2 and MMP-9.

Ursolic acid showed anti-tumor effect and in vivo experiments. Shanmugam et al. (2012) tested UA action during 4-36 weeks on mice with DU145 cells implant and with a transgenic prostate adenocarcinoma [7, 34]. Authors demonstrated that UA had an inhibitory action on tumor progression after 8 weeks of UA administration, effect supplemented at 12 weeks with a significant tumor size diminishing. Therewith UA inhibited in prostate a series of pro-inflammatory mediators, as NF-kB, STAT3, IKK α/β and AKT. Systemic effect of UA was expressed by diminishing the TNFα (tumor necrosis factor alpha, cachexin or cachectin) and IL-6 (interleukin 6) levels in peripheral blood.

In accordance with data reported by Teicher et al. (2010), UA has ability to block metastasis development [35]. The mechanisms of action suppose blocking signaling pathway CXCR4/CXCL12 (C-X-C chemokine receptor type 4/C-X-C motif chemokine 12). In Shanmugam et al. (2011) opinion this acid can suppress expression of CXCR4 in prostatic tumor cells, regardless of HER2 (human epidermal growth factor receptor 2) status [30]. Therewith authors support the implication of this natural compound in transcription regulation and blocking of NF-kB activation.

The anti-proliferative effect of UA was confirmed by Zheng et al. (2012) on T24 urinary bladder cancer cells line [36]. Authors confirmed that UA can induce organization of an intracellular signaling complex IRE1-TRAF2-ASK1 (the serine/threonine-protein kinase/endoribonuclease inositol-requiring enzyme 1- TNF receptor-associated factor 2- Apoptosis signal-regulating kinase 1) with pro-apoptotic function.

In accordance with data published by Liu et al. (2012) this triterpene has capacity to improve bronchial epithelium status affected by tobacco extract. More is a promising prophylactic agent able to prevent pulmonary cancer development [37]. Huang's et al. (2011) results demonstrated that UA can induce apoptosis in tumor cells A549, H3255 and Calu-6 [38].

Lung cancer is one the most frequent tumor among smokers [39]. Ursolic acid demonstrated its activity in treating this cancer by blocking invasive properties of series of tumor clones as A549, H3255 and Calu-6. Moreover, this triterpene was able to initiate apoptosis in cancer cells at quite small dosage, of 2μmol/L [38, 40].

Prasad et al. (2012) presented results which denote the efficacy of UA in colorectal cancer [41]. In authors opinion, pro-apoptotic effect is realized by NF-kB inhibition and suppression of proteins with anti-apoptotic function (cFlip (FLICE-like inhibitory protein), survivin, Bcl-2 (B-cell lymphoma 2), Bcl-xl (B-cell lymphoma-extra large)), proliferative (cyclin D1) and pro-metastatic (ICAM-1 (Intercellular Adhesion Molecule 1), VEGF (vascular endothelial growth factor), MMP-9 (Matrix metallopeptidase 9)). This chemical at digestive tract level could stop growing and induce apoptosis in pancreatic tumor cells (PANC-1 (human pancreatic carcinoma, epithelial-like cell line), CAPAN-1 (human pancreatic ductal adenocarcinoma cell line)). In opinion of Li et al. (2012) this action is realized through UA implication in signaling pathways JNK and PI3K/Akt/NF-kB [42].

The anti-tumor activity of UA was demonstrated in vivo mouse models of colorectal cancer [41]. Prasad et al. (2012) reported a considerable decreasing of tumor, ascites, as well diminishing of metastatic properties of cancer cells. Authors support that UA realizes this activity through inhibition of Ki-67 marker of proliferation and CD31. These effects were attended by NF-kB, STAT3 and β-catenin suppression. Andersson et al. (2008) have reported a diminishing of aberrant crypts in colorectal adenoma, after UA oral administration [43].

Ursolic acid presented a promising action in vitro experiment on K562 clone of leukemia cells. Wu et al. (2012) have demonstrated that UA induces apoptosis by stimulating PTEN (Phosphatase and tensin homolog) expression, blocking activation of Akt kinases, alteration of mitochondrial membrane potential, reducing of cytochrome C releasing, stimulating a series of caspases [44]. These data were supplemented by Zhang et al. (2011) who consider that UA can induce differentiation of HL60 promyelocytic leukemia cells to monocytes and stimulate expression of CEBPβ (CCAAT/enhancer-binding protein beta) [45]. Gao et al. (2012) presented promising results in vivo experiments [46]. Ursolic acid, 50 mg/kg administrated 20 days to NOD/SCID mice with U937 implant concluded with impressive blocking of tumor proliferation. These results are in line with Chiang et al. (2003) data, which reported that UA is very effective against P3HR1 cells (2.5 μg/ml) and human immortalised myelogenous *leukemia* line K562 (17.79 μg/ml) [47]. Lauthier et al. (2000) demonstrated that ursolic acid can decrease cell viability in human lymphoma Daudi cells (human Burkitt's *lymphoma* cell line) in a dose-dependent manner [48]. Authors showed that UA also induced morphological changes in cells such as loss of membrane asymmetry, DNA fragmentation and nuclei condensation. In their opinion these changes indicating that the mechanism by which UA induced cell death was through apoptosis. More, authors hypothesized that the binding of UA to glucocorticoid receptors and the Ca2+ currents constituted the first steps of apoptosis. Ovesná et al. (2006) investigated protective effects of UA against H2O2 -induced DNA damage in leukemic L1210, K562 and HL-60 cells [49]. Authors demonstrated that after 24h pre-treatment of cells with UA (2.5-10μmol/l) the incidence of DNA single strand breaks induced by H2O2 decreased significantly.

In mammary carcinoma cells MDA-MB-231 this triterpene initiated apoptosis by stimulating Fas receptor, cleavage of caspases 3, 8 and PARP (*Poly (ADP-ribose) polymerase*), stimulating pro-apoptotic protein Bax and releasing of cytocrome C from mitochondrion in cytoplasm, blocking anti-apoptotic BCL-2 receptor [50]. Subbaramiah et al. (2000) investigated the influence of UA on COX-2 expression in mammary cells treated with PMA (phorbol 12-myristate 13-acetate) [51]. The results attested a long-standing blockage of COX-2, protein-kinases C, c-Jun N-terminal kinases, inhibition of prostaglandin E2 synthesis. An antitumor action presented and UA derivative, 2α-hydroxiursolic acid. This one could block tumor cells MCF-7 proliferation at 20μM concentration, function realized through TNF-α and NF-kB [52]. Plus, De Angel et al. (2010) presented a promising result of UA action on C57BL/6 mice, ovariectomized with transgenic breast carcinoma [53]. Ursolic acid administered during 5 weeks resulted in a significant diminishing of tumor size, effect in authors' opinion exercised by UA involvement in Akt/mTOR signaling pathway and apoptosis inducing. But, this results are contested by Singletary et al. (1996), who did not establish any therapeutic effects after UA administration to the rats with breast tumors induced by 7,12-dimethyl-benz(a)-antracene [54].

A promising, anti-angiogenic action of UA was described after its testing on hepatic cancer cells, as Hep3B, Huh7 and HA22T. Lin et al. (2011) consider, that this function is realized by inhibition of a series of factors, such as HIF-1α (hypoxia inducible factor-1α), bFGF (basic fibroblast growth factor), VEGF (Vascular endothelial growth factor), interleukin 8, urokinazic plasminogen activator (uPA), supplemented by diminishing the levels of reactive oxygen (ROS) and nitric oxide (NO) [55]. Tian et al. (2006) reported that UA has ability to block both hepatic tumor cells HepG2 and their derivatives R-HepG2, resistant to chemotherapy, supplemented by a minor inhibitory effect on normal hepatocites [56]. Authors also demonstrated that COX-2 blocking and HSP (heat shock protein) stimulating, correlated with apoptosis enhance in HepG2 cells. This, pro-apoptotic effect was further determined on other hepatic tumor cells, such as Hep3B, Huh7 and HA22T. But this action was dependent on UA concentration: at high dosage a DNA fragmentation and cells' viability decreasing was attested. Similar results were reported by Ramos et al. (2008), who consider that ursolic acid can prevent DNA damage and has antiproliferative properties applied on HepG2 cells [57].

In accordance with results reported by Yan et al. (2010), treating of hepatic tumor cell with UA lead to Na+, K+-ATP-ase blocking and VEGF reduction [58]. Gayathri et al. (2000) have investigated COX-2 expression in mammary cells, treated with phorbol (PMA), a carcinogenic agent (phorbol 12-myristate 13-acetate). Ursolic acid suppressed effectively the PMA action by blocking COX-2 protein and diminution of E2 prostaglandin synthesis. Likewise, the tumorigenic action of PMA was diminished by blockage of a series of kinases, such protein kinase C, c-Jun-N-terminal-kinase and proten kinase p38 mitogen activated. Administration of

20 mg/kg UA per os during 6 weeks resulted in a significant reducing of oxidative stress markers in hepatic cancer DENA (diethylnitrosamine) induced to Wistar rats [59]. In accordance with authors' opinion, these data support the UA role as prophylactic drug in cancer prevention. The UA activity was examined also in vivo on hepatic tumor H22 [60]. Shao et al. (2011) demonstrated that UA usage at 100 mg/kg could lead to a significant inhibition of tumor growing.

A promising result of UA action was reported and in case of neural origin tumor. Wang et al. (2012) studied the behavior of glioma cells U251 after treating with this triterpene [61]. Authors realized that UA activated caspase-3 and suppressed miR-21(microRNA-21) at concentrations of 5-20 μM. The final effect was expressed by blocking tumor cells proliferation and apoptosis inducing.

The wide chain of UA function is supplemented by Tokuda's et al. (1986) research on skin tumors induced by TPA (tetradecanoyl-phorbol-13-acetate) [62]. Authors concluded that this triterpene could inhibit tumors growing, in a manner similar to retinoic acid, well-known for its anti-tumor activity. Kowalczyk et al. (2009) reported that UA is a very effective agent to prevent skin cancer, because it can block mutation occurrence in 61 codon of Ha-ras oncogene [63].

## The ursolic acid utilization to prevent chemoresistance development

The resistance developed by the tumors to specific therapy, chemo or radio, is one of the main reasons of recurrences and neoplastic progression. In drug resistance development were involved a series of MDR mediators (multi-drug resistance proteins), as well factors with anti-apoptotic function [64]. Shan et al. (2011) demonstrated that UA has ability to block MDR proteins in case of intestine tumor clones (SW480, SW620), leukemia cells HL60, HL60/ADR, K562, K562/ADR and breast carcinoma cell lines (MCF7 şi MCF7/ADR) [48]. Moreover, this compound was very effective and in case of very aggressive HepG2, doxorubicin-resistant clones [65].

## The biological activity of ursolic acid derivatives

A promising biological activity manifest and UA derivatives, frequently more emphasized as incipient chemicals. As we mentioned above, a remarkable anti-proliferative effect demonstrated 2α-hydroxyursolic acid in breast carcinoma cell lines [52]. A series of new derivatives were synthesized on acyl piperazin base. In accordance with Liu et al. (2012) opinion, these chemicals showed an inhibitory action significantly higher than clean UA in case of gastric carcinoma cells (MGC-803) and breast cancer (Bcap-37) [66]. These results are a confirmation of previous data published by Ma et al. (2005), who highlighted the cytotoxic activity of 2α-hydroxyursolic acid on 4 tumor cell lines, as HL-60 (*human promyelocytic leukemia* cells), BGC (*gastric cell line*), Bel-7402 (*hepatic carcinoma* cell *line*) and HeLa (*cervical cancer cell line*) [67].

The recent presentation of Chen et al. (2011) argues the development of new derivatives of UA [68]. Authors demonstrated that UA derivatives obtained on the furoxan (or 1,2,5-oxadiazole 2-oxide) base have a higher cytotoxic potential than native chemical, applied on HepG2 tumors. Tanaka et al. (2012) support the idea that UA derivatives obtained through oxidation with dioxoruthenium-VI- tetraphenylporphyrine had an enhanced cytotoxicity (in comparison to UA) on glioma C6 and skin carcinoma A431 cell lines [69].

A new possibility of UA derivatives obtaining was recently presented by Leipold et al. (2010) [70]. Authors have metabolized UA with 3 clones of gram-positive *Nocardia* bacteria (NRRL 44000, 44822 and 5646). As a result of these biotechnological assays, researches obtained a mixture of UA derivatives: ursolic acid methyl ester, ursonic acid, ursonic acid methyl ester, 3-oxoursa-1,12-dien-28-oic acid and its methyl ester. The acetylating of UA at C-3 position, combined with amino alcohol acetate coupling at C-28 had increased significantly its anti-proliferative activity. In accordance with Meng et al. (2009) these compounds were very effectively applied on different tumor cell lines, such as HeLa, SKOV3 and BGC-823 [71].

A recent study presented by Bai et al. (2012) emphasizes 2 groups of UA derivatives, in accordance with their electrical properties: group I, negatively charged and group II, with positive charge [72]. These derivatives had ability to block cell cycle and stimulate apoptosis in several tumor cell lines: HepG2, AGS, HT-29 and PC-3. It is necessary to mention that cytotoxic effect of group II was more pronounced than group I and UA. Plus, authors synthesized 3β-acetoxy-urs-12-en-28-oyl-1-mono-glicerid derivative, able to induce apoptosis in BGC-823 cells [73].

Shao et al. (2011) tested another UA derivative, N-[3β-acetoxy-urs-12-en-28-oyl]-2-aminodiethanol [60]. This compound showed remarkable pro-apoptotic activities applied on HepG2, BGC-823, SH-SY5Y, HeLa and HELF tumor cells.

Promising effects demonstrated heterocyclic derivatives of UA, obtained by Leal et al. (2012) [74]. New compounds could induce the p53, p21waf1 and NOXA synthesis, effects summarized as anti-proliferative activity in pancreatic carcinoma cells AsPC-1.

An innovative method was purposed by Zhang et al. (2013), which consists in using nanoparticles UA charged (UA-NPs) [75]. In their assays, authors transported effectively this complex into gastric carcinoma cells SGC-7901. The results pointed out a strong inhibition of COX-2 and caspase-3 activation, effects which lead to apoptosis and cytotoxicity.

*Conclusion*: ursolic acid is a promising compound in tumor prevention and treatment, with many mechanisms of action on cell's proliferation. Its derivatives usually are more biologically effective than initial compound, so obtaining of new ursolic derivatives makes further investigations in this field have a particular relevance.

## Conflict of interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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