Umbelliprenin (UMB) and auraptene (AUR) belong to a class of compounds named coumarins. They are synthesized by various Ferula species. Ferula species are from the umbelliferae family and order of Apioaceae. Ferula species is a large genus of over 130 species. They are native to the Mediterranean region (Iran, Afghanistan, Turkey and China) and mostly growing in arid climates. 30 species of this genus have been reported from Iran [1]. UMB has a structure close to that of AUR. UMB belongs to the group of sesquiterpene coumarins, and AUR belongs to the class of prenloxy coumarins. The difference between the chemical structures of these compounds is that the length of the 7-prenyloxyl chain of UMB is longer and contains 15 instead of 10 carbons (fig. 1) [1].

INTRODUCTION

A wide range of biological activity has been reported for UMB and AUR, such as inhibitory activity against β-lactamases [2], increased amyloid-β peptide [3], increased phosphorylation of extracellular signal-regulated kinase (ERK) [4], and immunomodulation [5]. One of the most studied effects of these compounds is the cytotoxic effect [6]. In this article, we reviewed the cytotoxic and apoptogenic effects of UMB and AUR and compared them with each other. First, we discussed the cytotoxic effects of UMB, followed by those of AUR, and then we compared their effects with each other.

Cytotoxic effects of UMB

UMB was first extracted from Anmi majus L. fruits by Abu-Mustafa in 1971 [7]. UMB was first extracted as non-furanid coumarin from this source. It can be synthesized chemically or extracted by maceration at room temperature [8, 9]. Briefly, the air-dried roots of Ferula szowitsiana were ground into powder and extracted exhaustively by maceration at room temperature with acetone. The extraction was concentrated under vacuum and was subjected to column chromatography. UMB was extracted by silica gel preparative layer chromatography (PLC). Alternatively, UMB was synthesized by the reaction between 7-hydroxycoumarin and trans-farnesyl bromide in acetone at room temperature. After 24 h, the mixture was concentrated under reduced pressure, and UMB was easily purified by column chromatography.

Barthomeuf et al. first demonstrated the cytotoxicity of UMB. They assessed the cytotoxicity of UMB on M4Beu (metastatic pigmented malignant melanoma), A549 (non-small cell lung carcinoma), PC3 (androgen-resistant prostate carcinoma), PA1 (ovary teratocarcinoma), primary human fibroblasts, MCF7 (breast adenocarcinoma), and DLD1 (colon adenocarcinoma) by flow cytometry [10]. The result showed that UMB had the most cytotoxic effect against M4Beu. It inhibited M4Beu cell proliferation. It induced apoptosis through the caspase-dependent pathway and arrested the cell cycle in G1. Moreover, the cytotoxic effect of UMB was higher in M4Beu cells than in primary fibroblasts; this finding suggested a therapeutic margin. UMB was found to be a more potent inhibitor of M4Beu growth than AUR [10].

Ziai et al. studied the cytotoxic effect of UMB on CLL cell lines. A flow cytometry staining method called annexin V-FITC/PI double staining was used to detect possible apoptosis induced by various concentrations of UMB for different incubation times. The dose- and time-dependent manner of induction of apoptosis in leukemic cells by UMB was demonstrated, and the induced apoptosis in leukemic cells was more pronounced than in normal peripheral blood mononuclear cells (PBMCs). UMB had the most cytotoxic effect on CLL cell lines in a 50 µM concentration (dose-dependent) and had different LC50 in different times of incubation (time-dependent) [11].

Interleukin-4 (IL-4) is an agent that causes resistance to apoptosis in CLL cells. Ziai et al. showed that UMB had a pro-apoptotic effect on CLL cell lines in the presence of IL-4. Interestingly, IL-4 did not increase the drug resistance of CLL cells incubated with UMB compared with other drugs, such as fludarabine, that induce the apoptosis of CLL cells [11].

To more elucidate the pro-apoptotic effects of UMB, Gholami et al. studied the effect of UMB on pro-apoptotic caspases (caspase-3,-8,
and-9) and Bcl-2 family proteins through Western blot. UMB induced a significant increase in the amount of procaspase after 3 h of incubation and 25 µM after 48 h incubation [11]. AUR was reported to suppress gastrointestinal cancers. The dietary administration of AUR significantly inhibited 4-NQO-induced tongue tumorigenesis in conjunction with the reduction of the frequency of dysplastic lesions, as well as the expression of the cell proliferation biomarker and induction of phase II enzymes GST and QR in the liver and tongue [23]. When given during the initiation and post-intubation phases, AUR (100 ppm) suppressed cell proliferation in the esophageal epithelium and inhibited the tumor development induced by N-nitroso methyl benzylamine [24]. Several studies demonstrated the preventive effect of dietary AUR on colon carcinogenesis in azoxymethane-induced colonic carcinoma. The dietary administration of AUR significantly inhibited the development of azoxymethane-induced rat colonic carcinoma [25]. AUR at two dose levels (0.01% and 0.05%) was fed to male CD-1 (ICR) mice for 17 w. The mice were given a single intraperitoneal injection of AOM (10 mg/kg body weight), followed by 1% (w/v) dextran sodium sulfate (DSS) in drinking water for 7 d. AUR in the both doses inhibited the occurrence of colonic adenocarcinoma. [26]. In another study, colonic adenocarcinoma was induced by weekly AOM 3 intraperitoneal injections (10 mg/kg bweekly). AUR (250 ppm) was administered for 10 w. Dietary AUR reduced the number of aberrant crypts and beta-catenin-accumulated crypt in mice with both obese and diabetic phenotypes. [27]. On the basis of the evidence on the chemo preventive effects of AUR, Tanaka et al. developed an inclusion complex of AUR with β-cyclodextrin, and mice were fed (100 ppm and 500 ppm) with this complex. AUR inhibited the development of colonic adenocarcinomas in an AOM/DSS model [28]. AUR suppressed both the wild-type and the chemo-resistant (FOLFOX) colon cancer HT-29 cells at a concentration of 10 µM. It suppressed CD44 and CD166 expression in the chemo-resistant HT-29 cell line. The formation of colon spheroids (proposed surrogate tumors) was suppressed by AUR [29]. As carcinogenesis in the colon in the early stage could be inhibited by dietary AUR, the possible indication of AUR as a chemo preventive agent was suggested. AUR from C. hassaku and rosemary extract exhibited weak inhibitory effects on the number of GST-P-positive foci in rat liver possibly due to suppressed cell proliferation [30]. During N,N-dietethylnitrosamine exposure, feeding with a diet containing AUR at 100 ppm and 500 ppm decreased the average number of GST-P-positive and TGF-α-alpha-positive EAF/cm2 (4.2). The number of TGF-positive EAF at 500 ppm AUR was reduced significantly (p<0.05). The initial feeding with 500 ppm AUR significantly inhibited the incidence and multiplicity of liver cell carcinoma. Moreover, the “post-initiation” feeding with AUR in both doses significantly reduced the development of hepatocellular carcinoma. Feeding with AUR reduced cell proliferation and the apoptotic index in liver cell neoplasms [31]. Apoptotic indices in mice fed with a diet mixed with organo-selenium 1, 4-phenylephrenic (methyl-ene) seleno cyanate p-SC (4, 8, or 15 mg/kg) and AUR (500 mg/kg and 1000 mg/kg) were significantly greater than those in the control group. These results indicated that diet supplementation with p-SC and AUR-induced apoptosis in B16BL6 melanoma cells in mice. Thus, the pulmonary metastasis and growth of these metastatic tumors in lung were inhibited [32]. Transgenic rats with adenocarcinoma of the prostate were protected by the dietary intake of 500 ppm AUR. The intake of AUR also induced apoptosis in androgen-sensitive LNCaP and androgen-insensitive DU145 and PC3 human cells [33]. AUR can reach the target organ for breast cancer treatment (i.e., mammary glands) after dietary administration. AUR significantly delayed the tumor progression and decreased cyclin D1 expression. Cyclin D1 protein contributes to the cell cycle and has been shown to be associated with breast cancer [34].

**Proposed mechanisms**

AUR inhibited cell viability and induced apoptosis in colonic carcinoma, as confirmed by MTS assay, an in vitro assay for cell
proliferation, and a DNA fragmentation assay [26]. The induction of apoptosis by AUR was also confirmed by another study. Using a sensitive and quantitative method (cell death ELISA) and morphological analysis, the apoptosis-inducing effect of AUR and the simultaneous reduction of cell proliferation were demonstrated [35]. Dietary AUR increased the apoptotic index in colon malignancies [36]. AUR-induced apoptosis in Jurkat T cells as a result of the ER stress-mediated activation of caspase-8. Moreover, the apoptosis was suggested to be due to the subsequent induction of the activation of caspase cascade (mitochondria-dependent or-independent) [21]. The treatment by dietary citrus AUR also decreased cell proliferation activity and increased the apoptotic cells of colorectal lesion cancer in mice with both obese and diabetic phenotypes [27].

## Table 1: Comparison of the apoptotic properties between UMB and AUR

<table>
<thead>
<tr>
<th>Umbelliprenin</th>
<th>Auraptene</th>
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<tr>
<td>Mitochondria-dependent/-</td>
<td>Mitochondria-dependent/-</td>
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<tr>
<td>independent [12]</td>
<td>independent [33]</td>
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<tr>
<td>Increased caspase-8, caspase-9 activation</td>
<td>Increased caspase-8 activation</td>
</tr>
<tr>
<td>Decreased Bcl-2, Mcl-1 expression [12, 13]</td>
<td>Decreased Bcl-XL expression [33]</td>
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Cell cycle arrest in the G1/S phase was also proposed as a mechanism for the chemo preventive action of AUR [35]. AUR reduced the MCF-7 cells undergoing the S phase after 24 h of IGF-1 treatment. It reduced the mRNA level of genes that promote G1/S transition and DNA replication [37]. Table 1 shows the comparison of apoptotic properties between UMB and AUR.

The anti-inflammatory effect was suggested for the mechanism of the chemo preventive properties of AUR. The inhibition of TPA-induced intracellular hydroperoxide formation in differentiated HL-60 cells by AUR at a concentration of 50 µM was assessed by flow cytometric analysis [38]. AUR significantly decreased the expression of iNOS/COX-2 and the release of TNF-α [20]. AUR suppressed the expression of COX-2 protein but not mRNA in mouse macrophages [39]. AUR suppressed the production of iNOS protein [40]. It modulated the expression of several pro-inflammatory cytokines (NF-κB, TNF-α, Stat3, NFκB, IL-6, and IL-1β) in the inflamed colon of mice that received AOM and DSS [26]. Dietary AUR decreased the positive rates of PCNA, COX-2, iNOS, and nitrotyrosine in adenocarcinomas [36]. The inflammatory cell–endothelial cell interaction and inflammation-related carcinogenesis in mice were inhibited by AUR [41]. Evidence shows other various pathways that may contribute to the chemo preventive effects of AUR. Citrus AUR markedly reduced pro MMP-7 production through the inhibition of ERK1/2-regulated protein translation pathways [42]. AUR inhibited the functions of P-glycoprotein in KB-C2 cells that resulted in the accumulation of the administered doxorubicin in the cells [43]. The inhibitory effect of AUR on cholesterol esterification through the inhibition of acyl-CoA: cholesterol acyltransferase and the modulation of estrogen receptors were suggested as the other molecular mechanisms underlying the anticancer and chemo preventive effects of AUR [44]. One of the newest proposed mechanisms for the antitumor activity of AUR is its ability to inhibit HIF-1α-mediated by hypoxic signaling [45].

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

## REFERENCES