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Review Article

COMPARISON OF THE CYTOTOXIC EFFECTS OF UMBELLIPRENIN AND AURAPTENE

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ABSTRACT

Umbelliprenin and auraptene structurally belong to the class of coumarins. Umbelliprenin can be synthesized chemically or extracted by maceration at room temperature. Various biological effects for both umbel lip renin and auraptene have been reported. One of their most important effects is cytotoxicity. This finding has increased interest in the application of umbelliprenin and auraptene as novel chemotherapeutic agents. In several studies, umbelliprenin and auraptene were successfully evaluated for anti-cancer effect. Moreover, mechanistic studies have attempted to find the mechanism of action of umbelliprenin and auraptene. In this review, we describe the cytotoxic effects of umbelliprenin and auraptene and compare them with each other. Umbelliprenin and auraptene share some cytotoxic and apoptotic induction properties but have differences in the suggested mechanisms.

Keywords: Apoptosis, Auraptene, Cytotoxicity, Cancer, Umbelliprenin.

INTRODUCTION

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 umbeliferae family and order of Apiaceae. *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-prenyloxy chain of UMB is longer and contains 15 instead of 10 carbons (fig. 1) [1].

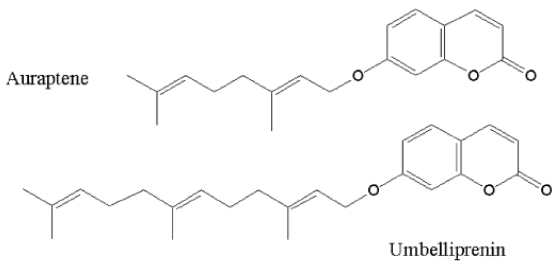

Fig. 1: Structures of UMB and AUR

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 *Ammi 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-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 treatment. Afterwards, procaspase was activated to caspase, and a decrease in procaspase levels was observed in the time range of 3 h to 16 h of treatment. The result showed that UMB activated the intrinsic and extrinsic pathways of apoptosis [12].

Gholami *et al.* showed that UMB could inhibit the expression of myeloid cell leukemia-1 (Mcl-1) gene and protein [13]. Mcl-1 has a key role in the lymphoid development and in the maintenance of B and T lymphocytes.[14]. Kitada *et al.* showed that high levels of Mcl-1 mRNA and protein in CLL inversely correlated with the *in vitro* and *in vivo* responses to chemotherapeutic agents [15].

Gholami *et al.* demonstrated the down-regulation of the Mcl-1 gene expression and protein by real-time polymerase chain reaction (RT PCR) and Western blot, respectively. They found that Mcl-1 mRNA expression increased from 1 h to 3 h incubation, but this increase had a scale-down pattern. Moreover, UMB could inhibit the Mcl-1 protein. Gholami *et al.* concluded that UMB treatment affected the expression of Mcl-1 at the transcriptional and posttranslational levels [13].

Khorramizadeh *et al.* investigated the cytotoxic effect of UMB coated by Fe_3O_4 magnetic nano particles (MNPs). This effect was examined on the human fibrosarcoma cell line (HT-1080) through the MTT method. UMB had moderate antiproliferative effects on the IC50 value of 50 µg/ml. However, the combination of UMB and Fe_3O_4 MNPs produced an IC_{50} value of 9 µg/ml. The results demonstrated that cell proliferation decreased to the low proportion of 45% after treating cells with UMB-coated Fe_3O_4 MNPs [16].

Khaghanzadeh *et al.* evaluated the cytotoxic effect of UMB against QU-DB large cell lung cancer and A549 adenocarcinoma cells. These cells were treated with UMB and IC_{50} was evaluated using the MTT method. The result showed that UMB had a similar IC_{50} against these cell lines ($IC_{50}\approx$ 50 µM). However, the grade of apoptosis induced by UMB was different among these cells. UMB induced significant apoptosis in QU-DB cells at 50 µM and in A549 cells at 88 µM concentrations [17].

The study of Soltani *et al.* on the anti-geno toxicity effects of UMB showed contrasting findings to those of the studies mentioned here. Soltani *et al.* measured the DNA breaks and resistance to H_2O_2-induced damage on human lymphocytes by UMB. Lymphocytes were incubated by different concentrations of UMB. Ascorbic acid was used as the standard antioxidant agent. Surprisingly, UMB protected DNA against the damaging effect of 25 µM H_2O_2 in a concentration-dependent manner [18].

To examine the cancer chemoprevention of UMB, Iranshahi *et al.* assessed this phenomenon both *in vitro* and *in vivo*. In the *in vivo* study, they used a two-stage carcinogenesis model of mouse skin tumors. The tumor induced by peroxynitrite (initiator) and 12-O-tetradecanoylphorbol-13-acetate (TPA; promoter). UMB delayed the formation of papillomas and reduced the number of tumors per mouse compared with the control group. Thus, UMB was hypothesized to be valuable as a chemo preventive agent for cancer [9].

Cytotoxic effects of AUR

AUR inhibited the growth of myelogenous lymphoma cells. Along with four other compounds, AUR isolated from *Citrus aurantium L* in 1996 showed a cell–growth inhibitory effect against mouse lymphocytic leukemia and human myelogenous leukemia cells *in vitro*[19]. Citrus AUR attenuated the *in vivo* inflammatory leukocyte activation that led to the decreased levels of edema formation, H_2O_2 production, leukocyte infiltration, and PCNA-labeling index.

The previous and present results implicate the use of AUR for the prevention and medication of inflammation-related disorders, including cancer, by attenuating the leukocyte activation [20]. The IC_{50} value of AUR against Jurkat T cells and the immortalized line of human T lymphocyte cells used to study acute T-cell leukemia was 16.5 mg/ml [21]. Another reported IC_{50} value for the cytotoxicity of AUR against Jurkat T cells was 55.36 µM [22]. Ziai *et al.* found that the IC_{50} value of UMB against Jurkat cells was 75 µM after 16 h 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-initiation phases, AUR (500 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 biweekly). AUR (250 ppm) was administered for 10 w. Dietary AUR reduced the number of aberrant crypt foci 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 spheres (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-diethylnitrosamine exposure, feeding with a diet containing AUR at 100 ppm and 500 ppm decreased the average number of GST-P-positive and TGF-alpha-positive EAF/cm(2). The number of TGF-alpha-positive EAF at 500 ppm AUR was reduced significantly ($p<0.005$). 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-phenylenebis (methyl-ene) seleno cyanate p-XSC (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-XSC 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 colonic 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

Umbelliprenin	Auraptene
G1 arrest [10]	G1/S arrest [35]
Mithchondria-dependent/-independent [12]	Mithchondria-dependent/-independent [33]
Increased caspase-8, caspase-9 activation	Increased caspase-8 activation
Decreased Bcl-2, Mcl-1 expression [12, 13]	Decreased Bcl-XL expression [33]

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, Nrf2, IL-6, and IL-1b) in the inflamed colon of mice that received AOM and DSS [28]. Dietary AUR decreased the positive rates of PCNA, COX-2, iNOS, and nitro tyrosine 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-1-mediated hypoxic signaling [45].

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

1. Shakeri A, Iranshahy M, Iranshahi M. Biological properties and molecular targets of umbelliprenin-a mini-review. J Asian Nat Prod Res 2014;16:884-9.
2. Safdari H, Neshani A, Sadeghian A, Ebrahimi M, Iranshahi M, Sadeghian H. Potent and selective inhibitors of class A beta-lactamase: 7-prenyloxy coumarins. J Antibiot (Tokyo) 2014; 67:373-7.
3. Jung CG, Uhm KO, Horike H, Kim MJ, Misumi S, Ishida A, *et al.* Auraptene increases the production of amyloid-beta via c-Jun N-terminal kinase-dependent activation of gamma-secretase. J Alzheimers Dis 2015;43:1215-28.
4. Nakamura M, Suzuki T, Takagi M, Tamura H, Masuda T. Stimulation of phosphorylation of ERK and CREB by phellopterin and auraptene isolated from Citrusjunos. Nat Prod Commun 2014;9:1491-4.
5. Zamani Taghizadeh Rabe S, Iranshahi M, Mahmoudi M. *In vitro* anti-inflammatory and immunomodulatory properties of umbelliprenin and methyl galbanate. J Immuno toxicol 2015;25:1-8.
6. Li G, Li X, Cao L, Zhang L, Shen L, Zhu J, *et al.* Sesquiterpene coumarins from seeds of Ferula sinkiangensis. Fitoterapia 2015;103:222-6.
7. Abu-Mustafa EA, el-Bay FK, Fayez MB. Natural coumarins. XII. Umbelliprenin, a constituent of ammi majus L. fruits. J Pharm Sci 1971;60:788-9.
8. Iranshahi M, Arfa P, Ramezani M, Jaafari MR, Sadeghian H, Bassarello C, *et al.* Sesquiterpene coumarins from Ferula szowitsiana and *in vitro* antileishmanial activity of 7-prenyloxycoumarins against promastigotes. Phytochemistry 2007;68:554-61.
9. Iranshahi M, Sahebkar A, Takasaki M, Konoshima T, Tokuda H. Cancer chemo preventive activity of the prenylated coumarin, umbelliprenin, *in vivo*. Eur J Cancer Prev 2009;18:412-5.
10. Barthomeuf C, Lim S, Iranshahi M, Chollet P. Umbelliprenin from Ferula szowitsiana inhibits the growth of human M4Beu metastatic pigmented malignant melanoma cells through cell-cycle arrest in G1 and induction of caspase-dependent apoptosis. Phytomedicine 2008;15:103-11.
11. Ziai SA, Gholami O, Iranshahi M, Zamani AH, Jeddi-Tehrani M. Umbelliprenin induces apoptosis in CLL cell lines. Iran J Pharm Res 2012;11:653-9.
12. Gholami O, Jeddi-Tehrani M, Iranshahi M, Zarnani AH, Ziai SA. Umbelliprenin from ferula szowitsiana activates both intrinsic and extrinsic pathways of apoptosis in jurkat T-CLL cell line. Iran J Pharm Res 2013;12:371-6.
13. Gholami O, Jeddi-Tehrani M, Iranshahi M, Zarnani AH, Ziai SA. Mcl-1 Is Up regulated by prenylated coumarin, Umbelliprenin in jurkat cells. Iran J Pharm Res 2014;13:1385-90.
14. Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. Nature 2003;426:671-6.
15. Kitada S, Andersen J, Akar S, Zapata JM, Takayama S, Krajewski S, *et al.* Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with *in vitro* and *in vivo* chemo response. Blood 1998;91:3379-89.
16. Khorramizadeh MR, Esmail-Nazari Z, Zarei-Ghaane Z, Shakibaie M, Mollazadeh-Moghaddam K, Iranshahi M, *et al.* Umbelliprenin-coated Fe3O4 magnetite nanoparticles: antiproliferation evaluation on human Fibrosarcoma cell line (HT-1080). Mater Sci Eng Proc Conf 2010;30:1038-42.
17. Khaghanzadeh N, Mojtahedi Z, Ramezani M, Erfani N, Ghaderi A. Umbelliprenin is cytotoxic against QU-DB large cell lung cancer cell line but anti-proliferative against A549 adenocarcinoma cells. Daru 2012;20:69.
18. Soltani F, Mosaffa F, Iranshahi M, Karimi G, Malekaneh M, Haghighi F, *et al.* Evaluation of antigenotoxicity effects of umbelliprenin on human peripheral lymphocytes exposed to oxidative stress. Cell Biol Toxicol 2009;25:291-6.
19. Satoh Y, Tashiro S, Satoh M, Fujimoto Y, Xu JY, Ikekawa T. [Studies on the bioactive constituents of aurantii fructus immaturus]. Yakugaku Zasshi 1996;116:244-50.
20. Murakami A, Nakamura Y, Tanaka T, Kawabata K, Takahashi D, Koshimizu K, *et al.* Suppression by citrus auraptene of phorbol ester-and endotoxin-induced inflammatory responses: role of attenuation of leukocyte activation. Carcinogenesis 2000;21:1843-50.
21. Jun DY, Kim JS, Park HS, Han CR, Fang Z, Woo MH, *et al.* Apoptogenic activity of auraptene of Zanthoxylum schinifolium toward human acute leukemia Jurkat T cells is associated with ER stress-mediated caspase-8 activation that

stimulates mitochondria-dependent or-independent caspase cascade. Carcinogenesis 2007;28:1303-13.

22. Min BK, Hyun DG, Jeong SY, Kim YH, Ma ES, Woo MH. A new cytotoxic coumarin, 7-[(E)-3',7'-dimethyl-6'-oxo-2',7'-octadienyl] oxy coumarin, from the leaves of Zanthoxylum schinifolium. Arch Pharm Res 2011;34:723-6.
23. Tanaka T, Kawabata K, Kakumoto M, Matsunaga K, Mori H, Murakami A, *et al.* Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis by citrus auraptene in rats. Carcinogenesis 1998;19:425-31.
24. Kawabata K, Tanaka T, Yamamoto T, Hara A, Murakami A, Koshimizu K, *et al.* Suppression of N-nitroso methyl benzylamine-induced rat esophageal tumorigenesis by dietary feeding of auraptene. J Exp Clin Cancer Res 2000;19:45-52.
25. Tanaka T, Kawabata K, Kakumoto M, Hara A, Murakami A, Kuki W, *et al.* Citrus auraptene exerts dose-dependent chemo preventive activity in rat large bowel tumorigenesis: the inhibition correlates with suppression of cell proliferation and lipid peroxidation and with induction of phase II drug-metabolizing enzymes. Cancer Res 1998;58:2550-6.
26. Mori H, Niwa K, Zheng Q, Yamada Y, Sakata K, Yoshimi N. Cell proliferation in cancer prevention; effects of preventive agents on estrogen-related endometrial carcinogenesis model and on an *in vitro* model in human colorectal cells. Mutat Res 2001;480-481:201-7.
27. Hayashi K, Suzuki R, Miyamoto S, Shin-Ichiroh Y, Kohno H, Sugie S, *et al.* Citrus auraptene suppresses azoxymethane-induced colonic preneoplastic lesions in C57BL/KsJ-db/db mice. Nutr Cancer 2007;58:75-84.
28. Tanaka T, de Azevedo MB, Duran N, Alderete JB, Epifano F, Genovese S, *et al.* Colorectal cancer chemoprevention by 2 beta-cyclodextrin inclusion compounds of auraptene and 4'-geranyl oxy ferulic acid. Int J Cancer 2010;126:830-40.
29. Epifano F, Genovese S, Miller R, Majumdar AP. Auraptene and its effects on the re-emergence of colon cancer stem cells. Phytother Res 2013;27:784-6.
30. Kitano M, Wanibuchi H, Kikuzaki H, Nakatani N, Imaoka S, Funae Y, *et al.* Chemo preventive effects of coumaperine from pepper on the initiation stage of chemical hepatocarcinogenesis in the rat. Jpn J Cancer Res 2000;91:674-80.
31. Sakata K, Hara A, Hirose Y, Yamada Y, Kuno T, Katayama M, *et al.* Dietary supplementation of the citrus antioxidant auraptene inhibits N, N-diethylnitrosamine-induced rat hepato-carcino-genesis. Oncology 2004;66:244-52.
32. Tanaka T, Kohno H, Murakami M, Kagami S, El-Bayoumy K. Suppressing effects of dietary supplementation of the organoselenium 1,4-phenylenebis(methylene)selenocyanate and the citrus antioxidant auraptene on lung metastasis of melanoma cells in mice. Cancer Res 2000;60:3713-6.
33. Tang M, Ogawa K, Asamoto M, Hokaiwado N, Seeni A, Suzuki S, *et al.* Protective effects of citrus nobiletin and auraptene in transgenic rats developing adenocarcinoma of the prostate (TRAP) and human prostate carcinoma cells. Cancer Sci 2007;98:471-7.
34. Krishnan P, Yan KJ, Windler D, Tubbs J, Grand R, Li BD, *et al.* Citrus auraptene suppresses cyclin D1 and significantly delays N-methyl nitrosourea-induced mammary carcinogenesis in female sprague-dawley rats. BMC Cancer 2009;9:259.
35. Zheng Q, Hirose Y, Yoshimi N, Murakami A, Koshimizu K, Ohigashi H, *et al.* Further investigation of the modifying effect of various chemopreventive agents on apoptosis and cell proliferation in human colon cancer cells. J Cancer Res Clin Oncol 2002;128:539-46.
36. Kohno H, Suzuki R, Curini M, Epifano F, Maltese F, Gonzales SP, *et al.* Dietary administration with prenyl oxy coumarins, auraptene, and collinin, inhibits colitis-related colon carcinogenesis in mice. Int J Cancer 2006;118:2936-42.
37. Krishnan P, Kleiner-Hancock H. Effects of auraptene on IGF-1 stimulated cell cycle progression in the human breast cancer cell line, MCF-7. Int J Breast Cancer 2012. doi.org/10.1155/2012/502092. [Article in Press]
38. Murakami A, Kuki W, Takahashi Y, Yonei H, Nakamura Y, Ohto Y, *et al.* Auraptene, a citrus coumarin, inhibits 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion in ICR mouse skin, possibly through suppression of superoxide generation in leukocytes. Jpn J Cancer Res 1997;88:443-52.
39. Murakami A, Shigemori T, Ohigashi H. Zingiberaceous and citrus constituents, 1'-acetoxychavicol acetate, zerumbone, auraptene, and nobiletin, suppress lipopolysaccharide-induced cyclo-oxygenase-2 expression in RAW264.7 murine macrophages through different modes of action. J Nutr 2005;135:2987S-92S.
40. Murakami A, Ohigashi H. Cancer-preventive anti-oxidants that attenuate free radical generation by inflammatory cells. Biol Chem 2006;387:387-92.
41. Onuma K, Suenaga Y, Sakaki R, Yoshitome S, Sato Y, Ogawara S, *et al.* Development of a quantitative bioassay to assess preventive compounds against inflammation-based carcino-genesis. Nitric Oxide 2011;25:183-94.
42. Kawabata K, Murakami A, Ohigashi H. Citrus auraptene targets translation of MMP-7 (matrilysin) via ERK1/2-dependent and mTOR-independent mechanism. FEBS Lett 2006;580:5288-94.
43. Nabekura T, Yamaki T, Kitagawa S. Effects of chemo preventive citrus phytochemicals on human P-glycoprotein and multidrug resistance protein 1. Eur J Pharmacol 2008;600:45-9.
44. de Medina P, Genovese S, Paillasse MR, Mazaheri M, Caze-Subra S, Bystricky K, *et al.* Auraptene is an inhibitor of cholesterol esterification and a modulator of estrogen receptors. Mol Pharmacol 2010;78:827-36.
45. Li J, Mahdi F, Du L, Jekabsons MB, Zhou YD, Nagle DG. Semisynthetic studies identify mitochondrial poisons from botanical dietary supplements--geranyl oxy coumarins from Aegle marmelos. Bioorg Med Chem 2013;21:1795-803.