Isololiolide, a carotenoid metabolite isolated from the brown alga *Cystoseira tamariscifolia*, is cytotoxic and able to induce apoptosis in hepatocarcinoma cells through caspase-3 activation, decreased Bcl-2 levels, increased p53 expression and PARP cleavage

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**ABSTRACT**

**Background:** Brown macroalgae have attracted attention because they display a wide range of biological activities, including antitumoral properties. In this study we isolated isololiolide from *Cystoseira tamariscifolia* for the first time.

**Purpose:** To examine the therapeutic potential of isololiolide against tumor cell lines.

**Methods/Study design:** The structure of the compound was established and confirmed by 1D and 2D NMR as well as HRMS spectral analysis. The in vitro cytotoxicity was analyzed by colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in tumoral as well as in non-tumoral cell lines. Cell cycle arrest and induction of apoptosis were assessed by flow cytometry. Alteration of expression levels in proteins important in the apoptotic cascade was analyzed by western blotting.

**Results:** Isololiolide was isolated for the first time from the brown macroalga *C. tamariscifolia*. Isololiolide exhibited significant cytotoxic activity against three human tumoral cell lines, namely hepatocarcinoma HepG2 cells, whereas no cytotoxicity was found in non-malignant MRC-5 and HFF-1 human fibroblasts. Isololiolide completely disrupted the HepG2 normal cell cycle and induced significant apoptosis. Moreover, western blot analysis showed that isololiolide altered the expression of proteins that are important in the apoptotic cascade, increasing PARP cleavage and p53 expression while decreasing procaspase-3 and Bcl-2 levels.

**Conclusion:** Isololiolide isolated from *C. tamariscifolia* is able to exert a selective cytotoxic activity on hepatocarcinoma HepG2 cells as well as induce apoptosis through the modulation of apoptosis-related proteins.

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Introduction

Cancer is a major public health problem with an estimated prevalence of about 3% in Europe, increasing to 15% at old age. Moreover, cancer related deaths are estimated to increase to over 11 million in 2030 (WHO, 2010). Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide, after lung and stomach cancer (Ferenci et al. 2010). The current therapeutics used for HCC treatment involves surgical resection, transplantation and/or systemic chemotherapy; however, surgery and transplantation may not be appropriate for many patients and chemotherapy often fails (Liu et al. 2014). Chemotherapy is also constrained by its toxicity, significant resistance to available chemotherapeutic agents and side effects, including neutropenia and myelosuppression (Chau et al. 2006). Current studies involved in developing effective cancer prevention approaches have focused on the use of bioactive natural agents that may have less adverse effects and can exert selective cytotoxicity against cancer cells (Ghate et al. 2014).

The chemical and biological diversity of the marine environment is immeasurable and therefore is an extraordinary resource for the discovery of novel anticancer drugs. Brown algae are a rich source of secondary metabolites displaying a wide variety of bioactivities with important features for pharmaceutical purposes. Cystoseira tamariscifolia has demonstrated interesting biological activities such as antibacterial, antifungal, antiprototozoal, cell division inhibition, anti-inflammatory, antioxidiant and cytotoxic properties (Bennamara et al. 1999; Spavieri et al. 2010; Lopes et al. 2012; Andrade et al. 2013). These properties have been ascribed to the presence of different classes of molecules that were identified in C. tamariscifolia, such as phlorotannins (fucoxanthinol, fucophloroethol, fucophloroethol-7-phloroethol, phlorofucofuroeckol and biectol/dieckol), phloroglucinol, proline, β-sitosterol, fucosterol, and diverse fatty acids (Ferreres et al. 2012; Andrade et al. 2013; Vizetto-Duarte et al. 2015). As C. tamariscifolia extracts have previously demonstrated cytotoxic potential, in this study we describe the identification of isoliolioide, a known carotenoid metabolite, as a selective cytotoxic compound that was isolated from the brown macroalga C.tamariscifolia for the first time. Here we show evidence that exposure of hepatocarcinoma HepG2 cells to isoliolioide is associated with changes in the expression of p53, PARP, Bcl-2 and procaspase-3. These results might explain the dramatic suppression of the S phase as well as the induction of apoptosis caused by this monoterpene.

Material and methods

Chemicals and reagents

Hexane and ethyl acetate were purchased from Prolabo (VWR International, Leuven, Belgium). Merck (Darmstadt, Germany) supplied dimethyl sulfoxide (DMSO). Roswell Park Memorial Institute medium (RPMI), Dulbecco’s Modified Eagle’s medium (DMEM), fetal bovine serum (FBS), L-glutamine and penicillin/streptomycin were obtained from Lonza Ibérica (Barcelona, Spain). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Calbiochem. Primary antibodies for poly (ADP-ribose) polymerase (PARP), p53, Bcl-2, procaspase-3, actin and respective secondary antibodies were from Santa Cruz Biotechnology Inc., Heidelberg, Germany. FITC-conjugated annexin V/propidium iodide (PI) assay kit was acquired from Cayman Chemical Company, USA. Silica gel (Merck, 40–63 μm mesh) was used for column chromatographic separation, while silica gel 60 PF354 (Merck) was used for analytical (0.25 mm) TLC. CDCl3 (Aldrich) was used as solvent for 1H and 13C NMR spectra acquisition and TMS (Aldrich) was used as internal standard. 1D and 2D NMR spectra were recorded at Bruker Digital Avance 800 MHz spectrometer. Additional reagents and necessary solvents were purchased from VWR International (Leuven, Belgium).

Sampling

Cystoseira tamariscifolia was collected in the middle/lower intertidal areas, during the low tide, between May and September 2012 on the Portuguese coast. Biomass was rinsed with distilled water and macroscopic epiphytes and extraneous matter were carefully removed. Identification of specimens was made by Dr Aschwin Engelen (Centre of Marine Sciences, University of Algarve, Portugal) and Dr Javier Cremales Ugarte (Facultade de Ciencias, University of A Coruña) and a voucher specimen of C. tamariscifolia (code number MB016) was deposited at the Centre of Marine Sciences, University of Algarve. Samples were freeze-dried and stored at −20°C until the extraction procedure.

Extraction

Biomass was mixed with hexane (1:10, w/v) and homogenized for 2 min using a disperser IKA T10B Ultra-Turrax at room temperature (RT). The tubes were then vortexed for 1 min, centrifuged (5000 g, 10 min, RT) and the supernatants recovered. The extraction procedure was repeated 3 times and the supernatants combined and filtered. The extract was dried at 40°C under vacuum and dissolved in DMSO for biological activities screening or in the adequate solvent for chemical characterization, aliquoted and stored (−20°C).

Isolation and elucidation of isoliolioide

C. tamariscifolia hexane extract (9 g) was fractionated by column chromatography (2.5 cm x 18 cm) over silica gel (Si02) using increasing amounts of EtOAc in hexane (9:1; 85:15; 4:1; 75:25; 7:3; 3:2; 1:1) and increasing amounts of MeOH in EtOAc (9:1; 8:1; 5:1; 2:1; 1:1) and MeOH (100%) and H2O (100%) as eluents. This procedure afforded 57 fractions, which were analyzed by TLC and pooled together in 21 groups (A – U). Fraction 14 (70 mg) was re-fractionated over Si02 eluted with hexane (100%); hexane/EtOAc (9:1; 8:2; 7:5; 2.5: 2.5; 7:3; 6.5:3.5; 6:4; 5.5:4.1; 1:1; 4:6), EtOAc (100%) and MeOH (100%) to afford 151 fractions which were pooled together in 9 groups after TLC analysis. Group 6–8, obtained from the hexane/ EtOAc elution (6:4 through 1:1), was purified by reverse phase preparative HPLC to afford 3 mg of isoliolioide.

Isoliolioide: Pale yellow oil; 1H NMR (800 MHz, CDCl3, TMS, ppm) δ 5.71 (1H, s, H-7), 4.21 (1H, m, H-3), 2.55 (2H, br d, J = 2.4 Hz, H-4), 2.03 (1H, br d, J = 2.4 Hz, H-2), 1.59 (3H, s, H-11), 1.23 (3H, s, H-10), 1.21 (3H, s, H-9). 13C NMR δ (200 MHz, CDCl3, TMS, ppm): 181.2 (C-6), 171.5 (C-8), 113.3 (C-7), 86.4 (C-5), 65.1 (C-3), 49.8 (C-2), 47.9 (C-4), 35.0 (C-1), 29.9 (C-9), 25.6 (C-11), 25.1 (C-10); HRESIMS m/z 219.0993 [M+Na]+ (calc to C11H16O3Na 219.0997).

Cell culture

HepG2 cells (human hepatocellular carcinoma) were maintained in RPMI-1640 culture media supplemented with glucose (1000 mg/ml), 10% FBS, L-glutamine (2 mM), penicillin (50 U/ml) and streptomycin (50 μg/ml). MRC-5 and HFF-1 human fibroblasts, AGS human gastric cancer, HCT-15 human colon cancer cells were cultured in DMEM culture media supplemented with glucose (1000 mg/ml), 10% FBS, L-glutamine (2 mM), penicillin (50 U/ml) and streptomycin (50 μg/ml). Cell lines were grown in an incubator at 37°C and 5.0% CO2 in humidified atmosphere.
Anti-proliferative assay

In vitro cytotoxic activity of isololiolide was assessed by the MTT colorimetric assay. Hepatocarcinoma HepG2, gastric cancer AGS and colon cancer HCT-15, and also non-tumoral cells (MRC-5 and HFF-1 human fibroblasts) were seeded at a density of $5 \times 10^3$ cells/well on 96-well plates and incubated for 24 h at 37°C in 5.0% CO$_2$. The effect of isololiolide was evaluated on the viability of these cells and the half maximal inhibitory concentration (IC$_{50}$) was calculated upon a 72 h incubation period. Positive control cells were treated with etoposide, while negative control cells were treated with DMSO at the highest concentration used in test wells (0.5%, v/v). The selectivity of the compound was estimated using the following equation: Selectivity = CT/CNT, where CT and CNT indicate the compound-induced cytotoxicity on tumoral cells and on non-tumoral cells, respectively (Oh et al. 2011).

Cell cycle distribution analysis

HepG2 cells were plated at a density of $5 \times 10^4$ cells/ml in 6-well plates and incubated with complete medium only (blank), medium with the solvent DMSO (control, 0.5% v/v) or with isololiolide at IC$_{50}$ concentration (13.15 μM), which was previously determined by the MTT assay. Cells were harvested following 72 h incubation and further processed for cell cycle analysis. Cellular DNA content for cell cycle distribution analysis was evaluated using an Epics XL-MCL Coulter flow cytometer plotting at least 10,000 events per sample. Cell cycle distribution data analysis was subsequently performed using the FlowJo 7.2 software (Tree Star, Ashland, USA).

Apoptosis detection

HepG2 cells were plated at a concentration of $5 \times 10^4$ cells/ml in 6-well plates and incubated with complete medium only (blank), medium with the solvent DMSO (control, 0.5% v/v), or with isololiolide at IC$_{50}$ concentration (13.15 μM) for 72 h. Induction of apoptosis was evaluated by the annexin V-FITC/PI apoptosis kit (Bender MedSystems, Vienna, Austria) according to the manufacturer’s instructions. Measurement of annexin V binding due to phosphatidylserine externalization was analyzed using an Epics XL-MCL Coulter flow cytometer plotting at least 20,000 events per sample. Apoptotic data analysis was subsequently performed using the FlowJo 7.2 software (Tree Star, Ashland, USA).

Protein expression analysis

For the analysis of protein expression, HepG2 cells were treated with complete medium (blank), medium with the solvent (DMSO) or with loliolide at the IC$_{50}$ concentration (13.15 μM), and incubated for 24, 48 and 72 h. After each incubation period cells were lysed in Wimans’s buffer (1% NP-40, 0.1 M Tris–HCl pH 8.0, 0.15 M NaCl and 5 mM EDTA) with EDTA-free protease inhibitor cocktail (Boehringer, Mannheim, Germany). Proteins were quantified using the DC Protein Assay Kit (BioRad, Hercules, CA, USA) and separated in 12% tris-glycine sodium dodecyl sulfate (SDS)–polyacrylamide gel. Proteins were then transferred to a nitrocellulose membrane (GE Healthcare, Madrid, Spain). The membranes were incubated with primary antibodies for PARP (1:4000), actin (1:2000), p53 (1:250), Bcl-2 (1:200) and procaspase-3 (1:200), and further incubated with the appropriate secondary antibodies conjugated with horseradish peroxidase (HRP) diluted 1:2000 in 5% non-fat dried milk in T-TBS. The signal was detected with the Amersham ECL kit (GE Healthcare). Hyperfilm ECL (GE Healthcare) and Kodak GBX developer and fixer twin pack (Sigma).

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) was assessed using the SPSS statistical package for Windows (release 15.0, SPSS Inc.), and significance between means was analyzed by the Tukey HSD test ($p < 0.05$). IC$_{50}$ values were calculated by sigmoidal fitting of the data using GraphPad Prism v. 5.0 (GraphPad Software, Inc., La Jolla, CA). Statistical analysis was performed by the non-parametric Friedman’s test followed by Dunn’s Post-test using GraphPad Prism 5 software. $P$ values < 0.05 were considered as statistically significant.

Results and discussion

Characterization of isololiolide

HRESIMS of the isolated compound showed a [M+Na]$^+$ quasi-molecular ion peak at m/z 219.0993, indicating the molecular formula C$_{15}$H$_{16}$O$_2$, with four unsaturations. Its $^1$H NMR spectrum displayed, despite other signals, peaks assigned to hydrogens of three methyl groups at δ$_H$ 1.21 (s, 3H), 1.23 (s, 3H) and 1.59 (s, 3H), one olefinic hydrogen at δ$_H$ 5.71 (s, 1H) and one oxymethylene hydrogen at δ$_H$ 4.13 (m, 1H). The $^{13}$C and DEPT 135 showed eleven peaks assigned to three methyl, two methylene, two methylene and four quaternary carbons, including one α,β-unsaturated carbonyl group at δC 171.5 (C-8), 113.3 (C-7) and 181.2 (C-8) and one carbinic carbon at δC 65.1 (C-3). HMBC spectrum showed cross peaks between the signals at H-11 with C-4/C-5/C-6, H-9 with C-1/C-6/C-10, H-10 with C-2/C-6/C-9 and H-7 with C-5/C-6/C-8. Isololiolide (Fig. 1) was identified comparing the obtained data with those reported in the literature (Kimura and Maki 2002).

Anti-proliferative activity of isololiolide in tumoral and non-tumoral cell lines

Isololiolide obtained from the C. tamariscifolia hexane extract was tested on human hepatocellular carcinoma cells (HepG2), gastric cancer cells (AGS) and colon cancer cell line (HCT-15). Additionally, the anti-proliferative activity of the compounds was evaluated in human fibroblasts (MRC-5 and HFF-1). The compound proved to be cytotoxic against the different tumoral cell lines, namely AGS (IC$_{50}$ = 32.36 μM), HCT-15 (IC$_{50}$ = 23.59 μM) and especially HepG2 cells (IC$_{50}$ = 13.15 μM; Table 1), showing selectivity indices (SI) of up to 86 and 47 against MRC-5 and HFF-1 fibroblasts, respectively (Table 1). The cytotoxic effect of this molecule toward HepG2 cells was particularly evident, whereas no significant toxic effect was observed in MRC-5 or HFF-1 human fibroblasts. Interestingly, extracts from C. tamariscifolia had previously demonstrated antiproliferative potential against Daudi (human Burkitt’s lymphoma), Jurkat (human leukemic T cell lymphoblast) and K562 (human chronic myelogenous leukemia) cells (Zubia et al. 2009). Isololiolide has been described as a carotenoid metabolite whose precursor is zeaxanthin (Repeta 1989). Carotenoids, such as zeaxanthin, lycopene and astaxanthin have been previously described as modulators of growth factors that play important roles in cell cycle regulation.
Table 1

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>IC₅₀ (µM)</th>
<th>SI: MRC-5</th>
<th>SI: HFF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepG2</td>
<td>13.15 ± 0.56ᵃ</td>
<td>86.07ᵇ</td>
<td>47.94ᵇ</td>
</tr>
<tr>
<td>AGS</td>
<td>32.36 ± 0.20ᶜ</td>
<td>34.97ᶜ</td>
<td>19.12ᵈ</td>
</tr>
<tr>
<td>HCT-15</td>
<td>23.59 ± 0.15ᵇ</td>
<td>47.98ᵇ</td>
<td>26.22ᵈ</td>
</tr>
<tr>
<td>MRC-5</td>
<td>1131.76 ± 1.22ᵉ</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HFF-1</td>
<td>618.62 ± 1.12ᵈ</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The values presented correspond to IC₅₀ values and are the mean ± S.E of at least 3 independent experiments in triplicate. In the same column, values followed by different letters (a-e for IC₅₀ values and A-D for SI) are significantly different (Tukey HSD test, p < 0.05). HepG2, human hepatocarcinoma; AGS, human gastric adenocarcinoma; HCT-15, human colorectal adenocarcinoma; MRC-5, human lung fibroblast derived from healthy tissue; HFF-1, human foreskin fibroblast derived from healthy tissue.

and carcinogenesis (Bi et al. 2013; Alvarez et al. 2014). Moreover, it has been previously demonstrated that breakdown products of carotenoids (e.g. 3-OH-β-apo-10’-carotenal and apo-10’-lycopenal) might act as chemotherapeutic agents against breast and hepatic cancer (Tibaduiza et al. 2002; Ipb et al. 2014). Loliolide, an isolioliolide isomer, is also a well-known carotenoid metabolite derived from the breakdown of fucoxanthin able to inhibit algal growth (Taylor and Burden 1970). On the other hand, isolioliolide has been previously isolated from brown algae namely from Undaria pinnatifida (Kimura and Maki 2002), Dictyopteris divaricata (Song et al. 2004) and Homoeostrichus formosana (Fang et al. 2015). However, this is the first report describing the occurrence of isolioliolide in C. tamariscifolia.

Effects on cell cycle profile

Because of the potential application of carotenoid breakdown products in cancer therapeutics and the observed cytotoxicity in HepG2 cells, we researched the effectiveness of isolioliolide in arresting the cell cycle in the latter hepatocarcinoma cell line. For this purpose, HepG2 cells were incubated with isolioliolide at 13.15 µM (IC₅₀) for 72 h and its effect on cell cycle distribution was studied. Analysis of the cell cycle was performed by flow cytometry and the results showed that this monoterpenoid completely disrupted the normal HepG2 cell cycle. In fact, isolioliolide induced G2/M cell cycle arrest along with a concomitant decrease in the percentage of cells in the S phase (Fig. 2A) and this effect was sustained throughout the 72 h treatment. In fact, the percentage of cells in G2/M phase was 15.09% and 14.91% for the control and DMSO 0.5%, respectively, increasing to 57.95% upon treatment with isolioliolide at 13.15 µM for 72 h (Fig. 2B). In addition, there were virtually no cells in the S phase after the same incubation. Taken together, these results suggest that isolioliolide affects the molecular pathways monitoring and controlling cell cycle progression by arresting the cells at the G2/M checkpoint. The cell cycle checkpoints play an important role in the control system by sensing defects occurring during essential processes, such as DNA replication or chromosome segregation, inducing a cell cycle arrest until the detected defects are repaired (Malumbres 2012).

Apoptosis induction by isolioliolide treatment

The annexin V-FITC/PI flow cytometry assay was used in order to determine if isolioliolide was inducing apoptosis in HepG2 cells. Bivariate staining using annexin V-FITC/PI further demonstrated that isolioliolide induced apoptosis at the IC₅₀ concentration (Fig. 3A). HepG2 cells were treated with isolioliolide for 72 h and a significant increase (P < 0.01) in the percentage of apoptotic cells was observed, from 6.9% in untreated cells, to 29.1% in cells treated with isolioliolide (Fig. 3B). Apoptosis is described as an active process of programmed cellular death that avoids an exacerbated inflammatory response (Fink and Cookson 2005) and is associated

![Fig. 2](image-url)

Fig. 2. HepG2 cell cycle analysis (A) from control (incubation with complete medium only), DMSO (0.5% v/v) or isolioliolide (13.15 µM) treatment for 72 h. Percentage of cells in G1, S and G2/M phases upon the treatments described above (B).
with responses to cancer therapy. In fact, it is widely described that resistance to apoptosis is one of the hallmarks of cancer cells (Hanahan and Weinberg 2011). This resistance enables cancerous cells to survive and divide even in the presence of endogenous proapoptotic stimuli. Therefore, induction of apoptosis is an important mechanism in selecting novel molecules with anti-cancer potential.

**Western blot analysis of apoptosis-related proteins**

Based on the results obtained with annexin V-FITC demonstrating that apoptosis is occurring, the expression of apoptosis-related proteins was evaluated by assessing procaspase-3, PARP, Bcl-2 and p53 protein levels expression in HepG2 cells incubated with complete medium (control), vehicle (DMSO 0.5% v/v) or isololiolide (13.15 μM).

Concerning caspase-3, a decrease in procaspase-3 expression upon isololiolide treatment at 24 h was measured (Fig. 4, P < 0.05). In human cells, apoptosis takes place through a cascade of events involving two main pathways: the intrinsic and the extrinsic pathways (Kroemer et al. 2007). Both pathways ultimately converge on the activation of procaspases (primarily procaspase-3, but also procaspase-7 and procaspase-6) to caspases, which are the cysteine proteases that cleave their protein substrates within the cell. Effector caspase-3zymogen (or procaspase-3) exists within the cytosol as an inactive dimer (Boatright and Salvesen 2003). It is activated by limited proteolysis within the interdomain linker, which is carried out by an initiator caspase or occasionally by other proteases under specific circumstances. At cytosolic concentrations in human cells, the caspase-3zymogens are already dimers, but cleavage within their respective linker segments is required for activation (Boatright and Salvesen 2003). Therefore, a decrease in procaspase-3 levels is due to its proteolysis, leading to caspase-3 activation. Our results showed that incubation of HepG2 cells with isololiolide resulted in a 2-fold decrease of procaspase-3 levels, strongly suggesting that procaspase-3 was processed to caspase-3. In addition, concentrations of procaspase-3 in certain cancerous cells are
significantly higher than those in non-cancerous controls (Putt et al. 2006).

PARP cleavage increased about 4-fold upon isololiolide incubation at 13.15 μM for 24 h (Fig. 5, P < 0.01). Interestingly, this increment seems to be time-dependent as shown in Fig. 5. PARP plays an active role in key biological processes, such as transcription and cell cycle regulation, response to DNA damage, apoptosis and maintenance of genome integrity. The presence of cleaved PARP is one of the most used biomarkers for the detection of apoptosis (Duriez and Shah 1997). Moreover, PARP is a substrate of caspase-3 and its cleavage into two fragments has been considered to be indicative of functional caspase activation (Bressenot et al. 2009). Cleaved PARP was observed after treatment with isololiolide, in a time-dependent manner. In fact, Soldani et al. (2001) reported that PARP proteolysis by caspase is a very early response to the apoptotic stimulus.

Western blot performed in the cell lysates obtained from isololiolide-treated cells showed increased expression of p53 at 24 h (Fig. 6, P < 0.05 ). The tumor suppressor protein p53 acts as a key player in tumor suppression, as it induces apoptosis and cell cycle arrest as well as suppresses angiogenesis (Amaral et al. 2010). p53 is usually responsible for activating DNA repair proteins when DNA has extensive damage, arresting the cell cycle at regulation points or initiating apoptosis if DNA damage shows to be irreparable. Interestingly, p53 not only induces G1 cell cycle arrest, but it is also described to act at the G2/M checkpoint, preventing cells from entering mitosis if DNA damage is found (Taylor and Stark 2001). Furthermore, p53 has the ability to activate the transcription of various pro-apoptotic genes, including those encoding members of the Bcl-2 family (Roo and Kaina 2006).

Our results showed that anti-apoptotic Bcl-2 protein expression remained unchanged after 24 h of incubation with isololiolide, decreasing after 48 h with the same treatment (Fig. 7, P < 0.05 ). Overexpression of anti-apoptotic Bcl-2 family members
have been associated with chemotherapy resistance in various human cancers, and targeting the anti-apoptotic Bcl-2 family members has shown promising results in preclinical studies (Kang and Reynolds 2009). Bcl-2 suppresses apoptosis by binding to Bax or Bak. It is described that inhibiting the anti-apoptotic Bcl-2 could sensitize tumor cells to chemo- and radiotherapy. Therefore, decrease of Bcl-2 levels may be a plan of choice to increase treatment efficacy. Furthermore, it was suggested that p53 also modulates Bcl-2 by downregulation (Kirkin et al. 2004).

In summary, application of isololiolide resulted in the increase on caspase-3 expression, concomitant with increase in PARP cleavage and p53 expression. Corresponding down-regulation of anti-apoptotic/pro-survival Bcl-2 protein was also detected.

Indeed, molecules that activate caspase-3 and p53, cleave PARP or bind to Bcl-2 have shown potential in cell culture and preclinical models of cancer (Peterson et al. 2009).

Taken together, our results strongly suggest that isololiolide is able to exert potent anti-proliferative properties, significantly promoting cell cycle arrest in S phase and inducing cellular apoptosis in a human-derived hepatocarcinoma cell line.

Conclusions

This report demonstrates for the first time the in vitro antitumoral activity of isololiolide obtained from C. tamariscifolia hexane extract against hepatocarcinoma through the induction of apoptosis by altering the expression of proteins important to the apoptotic cascade. As isololiolide exhibited no cytotoxicity on non-tumoral human fibroblasts under the same conditions, it would be important in the future to perform structure–activity relationships (SARs) analysis for further studies. In addition, it may provide novel clues as to how carotenoids and their metabolites play a role in preventing and/or slowing down cancer progression.

Conflicts of interest

The authors declare no conflict of interest.

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