Gene Reports 20 (2020) 100692

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

The effect of *Cressa Cretica* hydroalcoholic extract on apoptosis and the expression of Bcl_2, Bax and P53 genes in hepatoma cell line HepG2

Mehdi Pouraminaei[a,b], Mohammad Reza Mirzaiey[b], Alireza Khoshdel[c,d], Mohammad Reza Hajizadeh[a,b], Mehdi Mahmoodi[e], Mohammad Ali Fahmidehkar[f,g,*]

[a] Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[b] Molecular Medicine Research Center, Institute of Basic Medical Sciences Research, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[c] Department of Clinical Biochemistry, Faculty of Medicine, Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[d] Department of Clinical Biochemistry, Faculty of Medicine Semnan University of Medical Sciences, Semnan, Iran
[e] Department of Clinical Biochemistry, Afzalipoor Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[f] Department of Clinical Biochemistry, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran
[g] Research Center of Advanced Technologies in Medicine, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran

ARTICLE INFO

Keywords:
Apoptosis
Bax
Bcl_2
Cressa cretica
$HepG_2$
P53

ABSTRACT

Objective: Liver cancer is common worldwide and its occurrence is increasing globally; the most prevalent type is hepatocellular carcinoma. *Cressa cretica* is a halophyte plant that contains flavonoids, which are antibacterial, antioxidant agents. We investigated the effects of *C. cretica* extract on apoptosis and expression of Bcl_2, Bax and P53 genes in the hepatoma cell line, $HepG_2$.

Methods: HepG2 cells were cultured in RPMI 1640 medium, then incubated in various concentrations of hydroalcoholic extract of *C. cretica*. Cell proliferation was investigated using MTT assay and apoptosis was measured using flow cytometry. The expression of apoptosis- related genes were determined by real-time PCR.

Results: Proliferation of $HepG_2$ cells treated with different concentrations of *C. cretica* were diminished significantly in a dose-dependent pattern. The concentration of *C. cretica* required for 50% cell viability was 2300 μg/ml. Expression of P53 increased significantly in the treatment groups compared to control group. Bcl_2 gene expression was decreased significantly compared to the control group, while the Bax gene was over-expressed in the extract treated groups.

Conclusion: *C. cretica* is able to effect on the expression of apoptosis-related genes; therefore, it could play a role in treatment of cancer.

1. Introduction

The liver is one of large organs in a human body and it participates in detoxification and removal of exogenous antigens from the body. Liver is responsible for metabolism of carbohydrates, lipids and amino acids, and also for protein synthesis (Bogdanos et al., 2013). Liver damage and its side effects affect the entire body, so timely treatment is essential (Angulo, 2002).

Liver cancer is the third most common cause of death from cancer; approximately 1 million new cases are reported each year. An especially high incidence of liver cancer occurs in China and middle Africa. Liver cancer is more common in males (Parkin et al., 2005). Liver cancer comprises hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma and fibrolamellar HCC (Sia et al., 2017). The most prevalent primary liver cancer is HCC, which occurs in people with liver disease such as hepatitis B (HB) or hepatitis C (HC) (Xu et al., 2013). Epidemiological studies indicate a relation between HB infection and HC (Donato et al., 1998; Garcia et al., 2002).

Inhibition of apoptosis is a main factor for inducing of cancer which disrupts the balance between apoptosis and differentiation in carcinoma cells and lead to growth of cancer. Bcl_2 family proteins are important for apoptosis; Bax and Bak are pro-apoptotic, while Bcl_2 and Bcl-xl are anti-apoptotic (Al-Shenawy, 2016). The P53 gene is located on short arm of chromosome 17 and could stimulate apoptosis. Mutation of this gene may be related to HCC (Qin et al., 2002).

Hepatectomy, local infusion of drugs such as ethanol into the tumor,

Abbreviations: HCC, hepatocellular carcinoma; HB, hepatitis B; HC, hepatitis C; HepG2, hepatocellular carcinoma cell line

* Corresponding author at: Department of Clinical Biochemistry, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran.
E-mail address: fahmidehkarma@gmail.com (M.A. Fahmidehkar).

https://doi.org/10.1016/j.genrep.2020.100692
Received 16 February 2020; Received in revised form 25 March 2020; Accepted 22 April 2020
Available online 08 May 2020

chemoembolization and chemotherapy are used for treatment of HCC (França et al., 2004). Hepatectomy is a high risk treatment for patient with liver failure and may cause of mortality after resection of liver (Kauffmann and Fong, 2014). No current therapy is a definitive treatment for liver cancer; an effective treatment remains to be found. Many herbal medicines exhibit antioxidant effects and are able to induce apoptosis, therefore, they may be a suitable alternative therapy for inhibiting the progression of HCC (Abdel-Hamid et al., 2018; Rino et al., 2015).

Among plants used in medicine, *Cressa cretica,* a halophyte, has been used for many years. This plant is found in Iran, India and Australia, and belongs to the Convolvulaceae family. The aerial parts of *C. cretica* contain flavonoid compounds including quercetin, kampferol and rutin (Shahat et al., 2004). These phenolic compounds reduce prostaglandin synthesis and enhance superoxide dismutase, catalase and glutathione peroxidase activation (Abdallah et al., 2017). The plant has been used to treat diabetes and Alzheimer's disease. *C. cretica* is used to treat constipation, reduce oxidative stress, and to treat cancer and cardiovascular and neurodegenerative diseases (Afshari and Sayyed-Alangi, 2016). Seeds of *C. cretica* are a source of vegetable oil that is suitable for human consumption (Weber et al., 2007). The extract of the plant is exhibits antibacterial, antifungal, anti-inflammatory and antioxidant properties (Thirunavukkarasu et al., 2014).

We investigated effects of the hydroalcoholic extract of *C. cretica* on apoptosis and the expression of Bcl_2, Bax and P53 genes in the hepatoma cell line, $HepG_2$ to find out if this plant is able to modify apoptosis on cancer cells?

2. Material and methods

C. cretica plants were purchased from Pistachio Research Center and their identity confirmed by a botanical expert. The aerial parts were separated, washed and dried at room temperature. The plants were completely powdered using a laboratory blender (waring products division model, New Hartford, USA). Fifty grams of powdered *C. cretica* was mixed with alcoholic aqueous solvent (30 ml water +70 ml 96% ethanol), then extracted using a Soxhlet extractor (Bakhshi Industries, Tehran, Iran). The extract was dried in freeze drier (VaCo5-D; Zirbus Technology, GmbH, Bad Grund, Germany) at −55 °C.

We used the human hepatocellular carcinoma cell line (HepG2) and L929 cells line (as a control cells) both purchased from Pasteur Institute Cell Bank (Tehran, Iran). The cells were cultured in RPMI 1640 medium (Gibco BRL, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, Karlsruhe, Germany), 1% penicillin/streptomycin (Gibco BRL, Karlsruhe, Germany) and incubated in a 5% CO_2 incubator at 37 °C. At 80% confluence, adherent cells were trypsinized and cultured in 96-well plates at a density of 5×10^3 cells/well. Then both groups of cells were treated with 0–5000 μg/ml concentration of *C. cretica* hydroalcoholic extract for 24, 48 and 72 h. The MTT assay was performed (see below) to determine the effective concentration of extract and $IC_{50,}$ the concentration that kills 50% of cells.

2.1. MTT assay

We performed the MTT assay on $HepG_2$ and L929 cells by MTT kit (Sigma, Deisenhofen, Germany). All cells were cultured at a density of 2500 cells/well in 200 μl growth medium. MTT powder (Sigma, Deisenhofen, Germany)5 mg/ml, was dissolved in RPMI, then added to the wells and incubated for 2–4 h at 37 °C. Then MTT salt is converted into formazan crystals by mitochondrial succinate dehydrogenase enzyme in living cells so, 50 μl DMSO was added to each well and the plates were shaken for 8–10 min to dissolve the formazan crystals (Supino, 1995). The optical density (OD) was measured at 570 nm using a microplate reader. The assay was performed in triplicate and the percentage cell viability (CV) was calculated using the formula, percent cell viability = OD of test well/OD of control well x 100, and used for flow cytometry and real-time polymerase chain reaction (RT-PCR).

2.2. Flow cytometry

Flow cytometry kit (eBioscience, San Diego, California, United States) was used to evaluate apoptosis. $HepG_2$ cells were treated with 2300 μg/ml (IC_{50}) of *C. cretica* hydroalcoholic extract. After 72 h, 5×10^{10} cells were transferred to tubes containing 1 μl PBS (phosphate-buffered saline) and suspended with 195 μl binding buffer. Then 5 μl FITC Annexin V was added to each tube and the solution was incubated at room temperature in dark for 10 min. Finally, 200 μl binding buffer and 10 μl propidium iodide (PI) were added to each tube and analyzed using a flow cytometer (Cyflow; Partec, Münster, Germany).

2.3. Real time PCR

$HepG_2$ cells were seeded in nine 6-well plates at the density of 5×10^4 cells/well. After 24 h cells were divided to three groups: three plates were the control group, three plates were treated with 1000 μg/ml hydroalcoholic extract of *C. cretica* and three plates were treated with 2300 μg/ml hydroalcoholic extract of *C. cretica*. Total RNA of cells was extracted by TRizol reagent (Pars Tous, Tehran, Iran) according to the protocol of the extraction kit (Pars Tous, Tehran,Iran). The quantity of extracted RNA was determined using a spectrophotometer at 260 and 280 nm (Pinto et al., 2009). cDNA synthesis was performed using a cDNA synthesis kit (Pars Tous, Tehran, Iran), oligo (dT) and random hexamer primers (Pars Tous, Tehran, Iran). The reaction was carried out in triplicate for Bax, Bcl_2, P53 and β-actin genes. Primers were evaluated using the BLAST database. The primer sequences are shown in Table 1.

Real-time PCR was performed using 2 μl of each forward and reverse primer, 3 μl normalized cDNA, 10 μl master mix SYBER green and 5 μl of nuclease-free water. Then all plates were placed in ABI-step one plus CFX96 system (Bio-Rad Laboratories Inc., Hercules, CA). For activation of hot start Taq polymerase enzyme and DNA denaturation, one cycle of 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 40 cycles at annealing temperature for 1 min were performed. A melting curve was drawn using the following cycle: 95 °C for 15 s, 60 °C for 30 s, and 95 °C for 15 s, and the temperature was decreased 0.03 °C/s from 95 to 60 °C. The relative quantification was determined using the $2^{-\Delta\Delta Ct}$ formula. All data were analyzed using the ABI-step one plus software version 2.3.

2.4. Statistical analysis

All data were performed in triplicate and are presented as means ± SEM. Our data were analyzed using SPSS statistical software version 21. Differences within groups were analyzed by one-way ANOVA followed by Tukey's test for multiple comparisons. Values for $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Effect of C. cretica extract on cell viability after 72 h treatment

$HepG_2$ cells viability in presence of *C. cretica* hydroalcoholic extract

Table 1
Sequence of primers for real-time PCR.

Gene	Forward primer	Reverse primer
Bcl_2	CTTCTTTGAGTTCGGTGGGG	AAATCAAACAGAGGCCGCAT
P53	TGAAGCTCCCAGAATGCCAG	GCTGCCCTGGTAGGTTTTCT
BAX	TGCCTCAGGATGCGTCCACCAA	CCCCAGTTGAAGTTGCCGTCAG
β-Actin	GGGCATGGGTCAGAAGGATT	CGCAGCTGCTTGTAGAAGGT

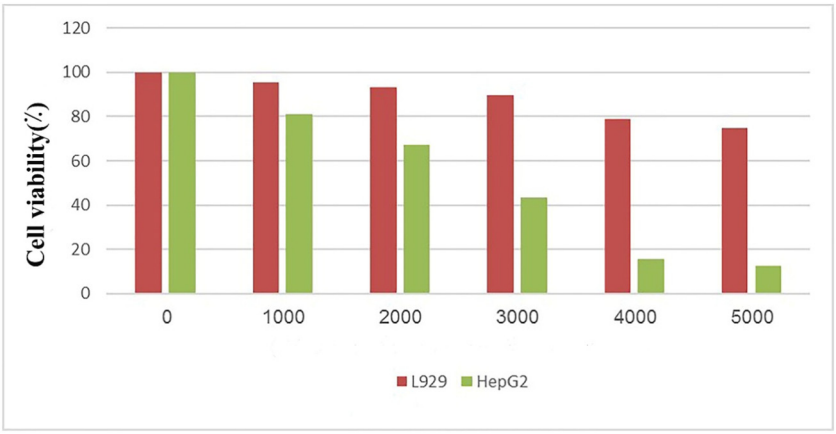


Fig. 1. Average viability of Hepg2 and L929 cells after 72 h exposure to hydroalcoholic extract of *C. cretica* (0–5000 μg/ml concentration) Data are means ± SEM. Cell viability decreased with increasing the concentration of extract.

was evaluated by MTT assay after 72 h of treatment. Comparison of cell viability in different concentrations of *C. cretica* showed that *C. cretica* significantly diminished cell proliferation for all of treatment groups compared to control group ($p < 0.05$); cell viability at 3000 and 4000 μg/ml concentrations was 49 and 17%, respectively. The concentration of *C. cretica* that produced 50% cell viability was 2300 μg/ml (Fig. 1).

3.2. Effect of C. cretica extract on cell apoptosis by flow cytometry

Effect of 1000, and 2300, 3000 μg/ml concentration of hydroalcoholic extract of *C. cretica* on $HepG_2$ cells were evaluated by flow cytometry. We found that *C. cretica* extract induced apoptosis in $HepG_2$ cells (Fig. 2).

3.3. Real time PCR

We performed real time PCR on cultures exposed to 1000 and 2300 μg/ml concentrations of hydroalcoholic extract of *C. cretica* to define the expression of P53, Bcl_2, and Bax genes in $HepG_2$ cells. The expression of P53 gene in control and various extract treated groups are shown in Fig. 3. The expression of P53 gene increased significantly in a dose-depend manner in the extract treated groups compared to controls ($p < 0.05$).

Fig. 4 shows significantly reduced expression of the Bcl_2 gene in 1000 and 2300 μg/ml extract treated groups compared to controls ($p < 0.05$).

The expression of Bax gene was increased significantly in the 1000 and 2300 μg/ml extract treated groups. The increase in the 2300 μg/ml extract treated group was statistically significant compared to controls ($p < 0.05$), while that for the 1000 μg/ml extract treated group was not statistically significant (Fig. 5).

4. Discussion

We investigated the effect of *C. cretica* on the $HepG_2$ cell line. We found that that different concentrations of *C. cretica* reduced cell proliferation significantly after 72 h; the reduction was due to apoptosis. *C. cretica* induced apoptosis in $HepG_2$ cells in a dose-dependent manner.

In 2017 Lee et al. investigated effect of another member of *Convolvulaceae* family on HepG2 cells. They extracted *C. soldanella* with methylene chloride and methanol, and then combined extract was partitioned into the *n*-hexane and 85% aqueous methanol, and reported that solvent fractions of *Calystegia soldanella* stimulate G1 and S phase arrest of the cell cycle and reduce the viability of HepG2 cells (Lee et al., 2017). Another study in 2018 examined the effect of a halophyte plant that called *Juncunol* on human hepatocarcinoma (HepG2) cells and reported that *Juncunol* is able to stimulate the apoptosis in HepG2 cells through loss of the mitochondrial membrane potential ($\Delta\psi m$) (Rodrigues et al., 2018). Wang et al. in 2013 showed that compounds isolated from *S. herbacea* which is a type of halophyte, could have an antiproliferative activities on HepG2 cells (Wang et al., 2013).

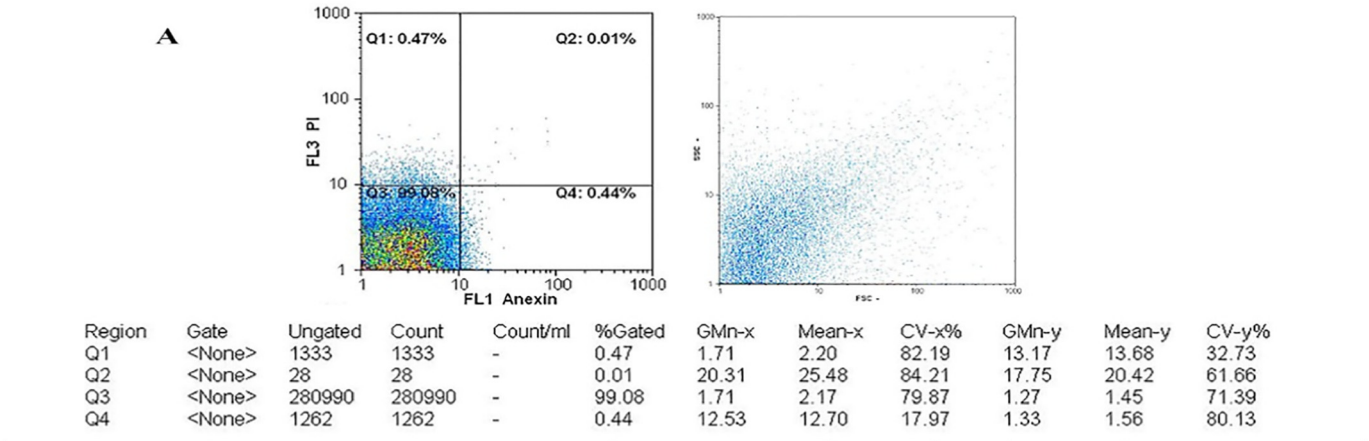
Our real-time PCR findings showed that treatment for 72 h with different doses of *C. cretica* decreased the expression of Bcl_2 gene, while the expression of P53 gene was increased in the extract treated groups compared to controls. The expression of Bax gene increased significantly with the 2300 μg/ml concentration of *C. cretica*.

Several mechanisms including oxidative stress defined for cell apoptosis. Apoptosis depends on the balance between anti-apoptotic (Bcl2) and pro-apoptotic proteins (Bax). The interactions of these two groups of proteins could initiate the caspase cascade to initiate apoptosis (Niewiarowska-Sendo et al., 2018).

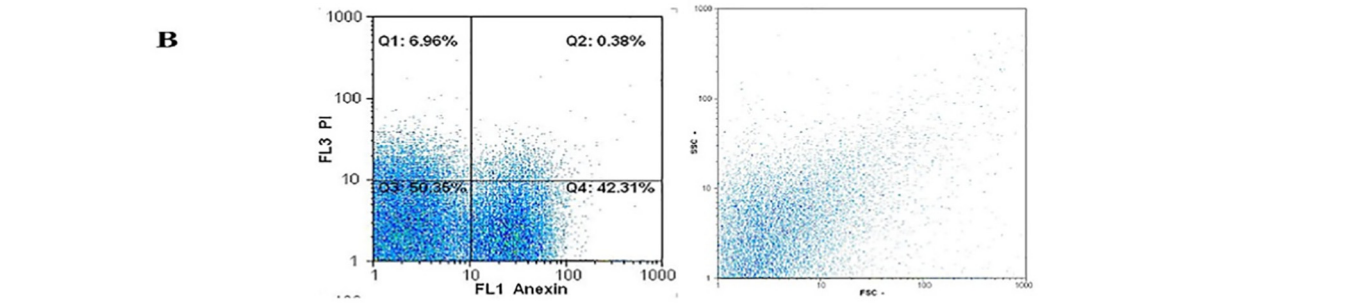
Hashemzaei et al. (2017) reported the effect of quercetin, a flavonoid compound derived from *C. cretica* on tumor cell lines and found it stimulated apoptosis by inhibiting P53 and Bcl_2. These investigators also reported that quercetin reduced the tumor volume in mice with MCF-7 and CT-26 tumors (Hashemzaei et al., 2017). Nishimura et al. (2017) reported the cytotoxic role of quercetin on HepG2 cells. These investigators demonstrated that apoptosis was induced because quercetin increased the Bax level and reduced Bcl2 expression (Nishimura et al., 2017). Liu et al. (2017) reported that quercetin inhibited the proliferation of U251 cells in a dose-dependent manner. These investigators found out that quercetin causes apoptosis by regulating levels of Bcl-2 and Bax (Liu et al., 2017).

Other have reported that quercetin and rutin (a flavonoid compound of *C. cretica*) increased apoptosis by inhibiting the G2/M phase of the cell cycle. Apoptosis could be a way to treat cancer (Liu et al., 2017; Chen et al., 2013; Li et al., 2014). Li et al. (2014) reported that quercetin might prohibit the growth of HepG2 cells. These investigators confirmed that apoptosis, cell death and stimulation of G2/M arrest associate with increase of P53 expression (Li et al., 2014). In our study the expression of P53 increased in extract treated groups, so *C. cretica* may induce the apoptosis and cell death. Deletion of P53 may reduce the apoptotic action which may lead to tumor development (Rangel-Pozzo et al., 2020). Khorsandi et al. (2017) used the MCF-7 human cancer breast cell line to investigate the growth of cells following exposure to quercetin; proliferation of MCF-7 cells decreased in the quercetin-exposed group compared to controls. In quercetin-treated cells, the expression of Bax gene increased, but expression of the Bcl-2 gene decreased (Khorsandi et al., 2017).

Chen et al. (2013) reported that rutin induced apoptosis in LAN-5 cells and Bcl_2 expression was decreased following rutin treatment. LAN-



Region	Gate	Ungated	Count	Count/ml	%Gated	GMn-x	Mean-x	CV-x%	GMn-y	Mean-y	CV-y%
Q1	<None>	1333	1333	-	0.47	1.71	2.20	82.19	13.17	13.68	32.73
Q2	<None>	28	28	-	0.01	20.31	25.48	84.21	17.75	20.42	61.66
Q3	<None>	280990	280990	-	99.08	1.71	2.17	79.87	1.27	1.45	71.39
Q4	<None>	1262	1262	-	0.44	12.53	12.70	17.97	1.33	1.56	80.13



Region	Gate	Ungated	Count	Count/ml	%Gated	GMn-x	Mean-x	CV-x%	GMn-y	Mean-y	CV-y%
Q1	<None>	18290	18290	-	6.96	1.16	1.31	75.64	23.07	36.81	208.51
Q2	<None>	989	989	-	0.38	32.54	40.95	120.09	14.87	18.18	250.17
Q3	<None>	132283	132283	-	50.35	1.54	2.00	94.12	1.55	2.04	93.96
Q4	<None>	111158	111158	-	42.31	37.48	44.54	67.41	1.13	1.26	75.61

Fig. 2. Apoptosis determined by flow cytometry. A) Apoptosis in the control group. B) Apoptosis in group treated with hydroalcoholic extract of *C. cretica* group. Hydroalcoholic extract of *C. cretica* (2300 µg/ml Concentration) increased apoptosis significantly compared to control group.
Q1: %Necrosis. Q2: %Late apoptosis. Q3: %Live cell. Q4: %Premature apoptosis.

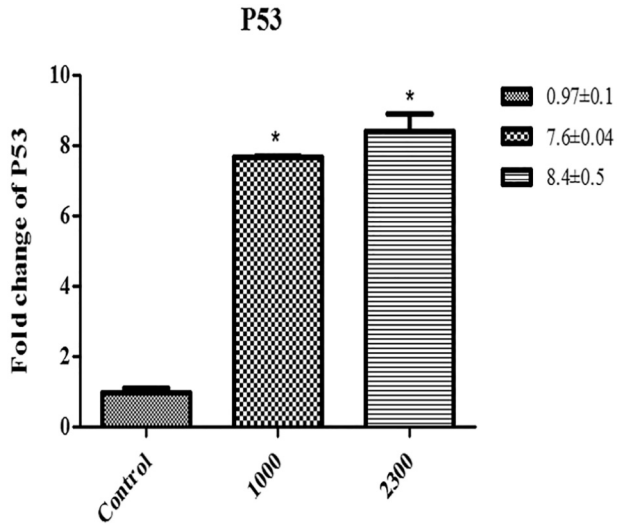


Fig. 3. Expression of P53 gene was increased in the extract treated groups compared to the control group. (*$p < 0.05$). Mean ± SEM.

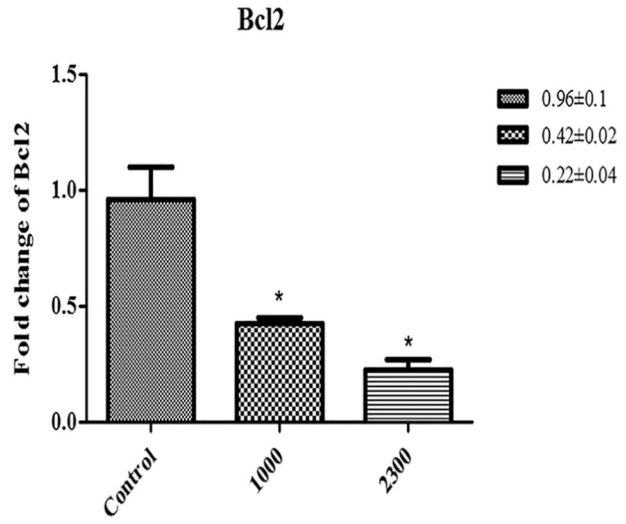


Fig. 4. Expression of Bcl_2 gene in the extract treated groups decreased significantly compared to the control group. (*$p < 0.05$). Mean ± SEM.

5 cells and this flavonoid has a main role in Bcl_2/Bax balance in tumor cells so rutin can improve the unbalanced Bcl_2: Bax ratio so it may be useful for treating apoptosis resistant tumor cells (Chen et al., 2013). Guon and Chung (2016) evaluated the hyperoside and rutin effects on cell apoptosis in HT-29 human colon cancer cells and normal colon epithelium in the FHC cell line. These investigators found that rutin did not affect viability of normal colon epithelium in the FHC cell line, but it decreased the proliferation of HT-29 human colon cancer cells in a dose-dependent manner. They reported that rutin increased expression of the Bax gene and reduced Bcl2 expression (Guon and Chung, 2016). We believe that the flavonoids of may be useful for treating cancer. *C. cretica* extract has effects on the expression of Bax, Bcl2 and P53 genes

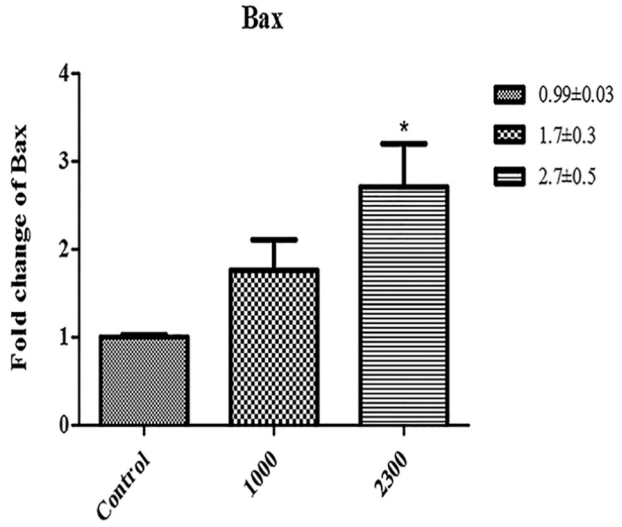


Fig. 5. Expression of Bax gene in the extract treated groups increased significantly compared to control group. (*$p < 0.05$). Mean ± SEM.

and might be able to modify apoptosis in cancer cells. As regards to NF-κB super family involves p50-p65 and has an anti-apoptotic role, hydroalcoholic extract of *C. cretica* may play a role on activation of NF-κB (Ahn et al., 2008; Sawhney et al., 2007). Therefor Further studies in vivo and in vitro are recommended to evaluate the effects of the hydroalcoholic extract *C. cretica* on NF-κB, neoplastic diseases and also other cell lines. It is essential to evaluate acute toxicity of this extract to establish the pharmacological safety of that.

Acknowledgment

We thank Rafsanjan University of Medical Sciences for sincere cooperation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdallah, H.M.I., Elshamy, A.I., El Gendy, A.E.-N.G., El-Gawad, A.M.A., Omer, E.A., De Leo, M., et al., 2017. Anti-inflammatory, antipyretic, and antinociceptive effects of a Cressa cretica aqueous extract. Planta Med. 83 (17), 1313–1320.

Abdel-Hamid, N.M., Abass, S.A., Mohamed, A.A., Muneam Hamid, D., 2018. Herbal management of hepatocellular carcinoma through cutting the pathways of the common risk factors. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 107, 1246–1258.

Afshari, A., Sayyed-Alangi, S.Z., 2016. Antioxidant effect of leaf extracts from Cressa cretica against oxidation process in soybean oil. Food science & nutrition 5 (2), 324–333.

Ahn, K.S., Sethi, G., Chaturvedi, M.M., Aggarwal, B.B., 2008. Simvastatin, 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor, suppresses osteoclastogenesis induced by receptor activator of nuclear factor-κB ligand through modulation of NF-κB pathway. Int. J. Cancer 123 (8), 1733–1740.

Al-Shenawy, H.A., 2016. Expression of Beclin-1, an autophagy-related marker, in chronic hepatitis and hepatocellular carcinoma and its relation with apoptotic markers. APMIS: acta pathologica, microbiologica, et immunologica Scandinavica. 124 (3), 229–237.

Angulo, P., 2002. Nonalcoholic fatty liver disease. N. Engl. J. Med. 346 (16), 1221–1231.

Bogdanos, D.P., Gao, B., Gershwin, M.E., 2013. Liver immunology. Comprehensive Physiology. 3 (2), 567–598.

Chen, H., Miao, Q., Geng, M., Liu, J., Hu, Y., Tian, L., et al., 2013. Anti-tumor effect of rutin on human neuroblastoma cell lines through inducing G2/M cell cycle arrest and promoting apoptosis. TheScientificWorldJournal 2013, 269165 (-).

Donato, F., Boffetta, P., Puoti, M., 1998. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int. J. Cancer 75 (3), 347–354.

França, A.V.C., Elias Júnior, J., Lima, B.L.G.D., Martinelli, A.L., Carrilho, F.J., 2004. Diagnosis, staging and treatment of hepatocellular carcinoma. Braz. J. Med. Biol. Res. 37 (11), 1689–1705.

Garcia, E., Lawson, D., Cotsonis, G., Cohen, C., 2002. Hepatocellular carcinoma and markers of apoptosis (bcl-2, bax, bcl-x): prognostic significance. Appl. Immunohistochem. Mol. Morphol. 10 (3), 210–217.

Guon, T.E., Chung, H.S., 2016. Hyperoside and rutin of Nelumbo nucifera induce mitochondrial apoptosis through a caspase-dependent mechanism in HT-29 human colon cancer cells. Oncol. Lett. 11 (4), 2463–2470.

Hashemzaei, M., Delarami Far, A., Yari, A., Heravi, R.E., Tabrizian, K., Taghdisi, S.M., et al., 2017. Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. Oncol. Rep. 38 (2), 819–828.

Kauffmann, R., Fong, Y., 2014. Post-hepatectomy liver failure. Hepatobiliary surgery and nutrition 3 (5), 238–246.

Khorsandi, L., Orazizadeh, M., Niazvand, F., Abbaspour, M.R., Mansouri, E., Khodadadi, A., 2017. Quercetin induces apoptosis and necroptosis in MCF-7 breast cancer cells. Bratislavske lekarske listy 118 (2), 123–128.

Lee, J.I., Kim, I.-H., Nam, T.-J., 2017. Crude extract and solvent fractions of Calystegia soldanella induce G1 and S phase arrest of the cell cycle in HepG2 cells. Int. J. Oncol. 50 (2), 414–420.

Li, Y., Duan, S., Jia, H., Bai, C., Zhang, L., Wang, Z., 2014. Flavonoids from tartary buckwheat induce G2/M cell cycle arrest and apoptosis in human hepatoma HepG2 cells. Acta Biochim. Biophys. Sin. 46 (6), 460–470.

Liu, Y., Tang, Z.-G., Lin, Y., Qu, X.-G., Lv, W., Wang, G.-B., et al., 2017. Effects of quercetin on proliferation and migration of human glioblastoma U251 cells. Biomed. Pharmacother. 92, 33–38.

Niewiarowska-Sendo, A., Kozik, A., Guevara-Lora, I., 2018. Influence of bradykinin B2 receptor and dopamine D2 receptor on the oxidative stress, inflammatory response, and apoptotic process in human endothelial cells. PLoS One 13 (11), e0206443.

Nishimura, K., Matsumoto, R., Yonezawa, Y., Nakagawa, H., 2017. Effect of quercetin on cell protection via erythropoietin and cell injury of HepG2 cells. Arch. Biochem. Biophys. 636, 11–16.

Parkin, D.M., Bray, F., Ferlay, J., Pisani, P., 2005. Global cancer statistics, 2002. CA Cancer J. Clin. 55 (2), 74–108.

Pinto, F.L., Thapper, A., Sontheim, W., Lindblad, P., 2009. Analysis of current and alternative phenol based RNA extraction methodologies for cyanobacteria. BMC Mol. Biol. 10, 79.

Qin, L.-X., Tang, Z.-Y., Ma, Z.-C., Wu, Z.-Q., Zhou, X.-D., Ye, Q.-H., et al., 2002. P53 immunohistochemical scoring: an independent prognostic marker for patients after hepatocellular carcinoma resection. World J. Gastroenterol. 8 (3), 459–463.

Rangel-Pozzo, A., Booth, S., Yu, P.L.I., Singh, M., Selivanova, G., Mai, S., 2020. p53 CRISPR deletion affects DNA structure and nuclear architecture. J. Clin. Med. 9 (2), 598.

Rino, Y., Yukawa, N., Yamamoto, N., 2015. Does herbal medicine reduce the risk of hepatocellular carcinoma? World J. Gastroenterol. 21 (37), 10598–10603.

Rodrigues, M.J., Vizetto-Duarte, C., Gangadhar, K.N., Zengin, G., Mollica, A., Varela, J., et al., 2018. In vitro and in silico approaches to unveil the mechanisms underlying the cytotoxic effect of juncunol on human hepatocarcinoma cells. Pharmacological reports: PR 70 (5), 896–899.

Sawhney, M., Rohatgi, N., Kaur, J., Shishodia, S., Sethi, G., Gupta, S.D., et al., 2007. Expression of NF-κB parallels COX-2 expression in oral precancer and cancer: Association with smokeless tobacco. Int. J. Cancer 120 (12), 2545–2556.

Shahat, A., Abdel-Azim, N., Pieters, L., Vlietinck, A., 2004. Flavonoids from Cressa cretica. Pharm. Biol. 42 (4–5), 349–352.

Sia, D., Villanueva, A., Friedman, S.L., Llovet, J.M., 2017. Liver cancer cell of origin, molecular class, and effects on patient prognosis. Gastroenterology 152 (4), 745–761.

Supino, R., 1995. MTT Assays. In Vitro Toxicity Testing Protocols. Springer, pp. 137–149.

Thirunavukkarasu, P., Asha, S., Ramanathan, T., Balasubramanian, T., Shanmogapriya, R., Renugadevi, G., 2014. In vitro hepatoprotective activity of isolated fractions of Cressa cretica. Pharm. Chem. J. 48 (2), 121–126.

Wang, X., Zhang, M., Zhao, Y., Wang, H., Liu, T., Xin, Z., 2013. Pentadecyl ferulate, a potent antioxidant and antiproliferative agent from the halophyte Salicornia herbacea. Food Chem. 141 (3), 2066–2074.

Weber, D., Ansari, R., Gul, B., Khan, M.A., 2007. Potential of halophytes as source of edible oil. J. Arid Environ. 68 (2), 315–321.

Xu, X., Fan, Z., Kang, L., Han, J., Jiang, C., Zheng, X., et al., 2013. Hepatitis B virus X protein represses miRNA-148a to enhance tumorigenesis. J. Clin. Invest. 123 (2), 630–645.