International Pharmacy Acta. 2020;3(1):e6
Original Research Article
OPEN ACCESS
ISSN: 2645-3266

Antioxidant and chemopreventive effects of *Asperugo procumbens* in a rat model of hepatocellular carcinoma

Mahdieh Arabsalmani[a], Mahsa Hosni[a], Farshad H. Shirazi[b] ,Sina Andalib[a], Saman Gholami[a], Seyed Hojjat Hosseini[c], Mohammad Kamalinejad[d], Maryam Noubarani[a], Mohadeseh Shamseini[a], Mohammad Reza Eskandari [a*]

a. Department of Pharmacology and Toxicology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.
b. Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
c. Department of Physiology and Pharmacology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.
d. Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Article Info:
Received: December 2019
Accepted: July 2020
Published online: September 2020

*** Corresponding Author:**
Mohammad Reza Eskandari
Email: eskandarimr@zums.ac.ir

Abstract:

Introduction: Hepatocellular carcinoma (HCC) cancer is the fifth most common malignancy, with 0.25–1 million new cases diagnosed annually worldwide. The objective of the present study was to evaluate the antioxidant and chemopreventive effects of aqueous extract of *Asperugo procumbens* L. (AAP) against diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC) in rats.

Methods and Results: The model of hepatocellular carcinoma was induced by a single intraperitoneal injection of DEN (200 mg/kg) as an initiator that after two weeks followed by daily oral administration of 2-acetylaminofluorene (30 mg/kg) as a promoter for two weeks.

AAP-treated rats were pretreated with the extract intragastrically at three different doses two weeks prior to DEN injection. At the end of the experiment, the marked reduction of serum biomarkers of liver damage and cancer, including alfa-fetoprotein (AFP), gamma glutamyl transpeptidase (GGT), alanine transaminase (ALT), and aspartate transaminase (AST) were observed in AAP complemented rats as compared to DEN-treated animals. Besides, the extract exhibited *in vivo* antioxidant activity that evident by increasing GSH concentration along with lipid peroxidation prevention in the liver tissues of HCC animals. In addition, *A. procumbens* showed *in vitro* free radical scavenging activity that determined by 1, 1-Diphenyl-2-picryl hydroxyl (DPPH) antioxidant assay. The relative weight of liver was also reduced in AAP-treaded rats as a prognostic marker in HCC.

Conclusion: Our results obviously confirmed that *A. procumbens* possesses a chemopreventive effect against primary liver cancer induced by DEN in rats as well as *in vitro* and *in vivo* antioxidant activities.

Keywords: *A. procumbens*; Antioxidant; DPPH; Flavonoid; Madwort; Polyphenol.

Please Cite this article as: Arabsalmani M, Hosni M, H. Shirazi F , Andalib S, Gholami S, Hosseini S.H, Kamalinejad M, Noubarani M, Shamseini M, Eskandari M.R. Antioxidant and chemopreventive effects of *Asperugo procumbens* in a rat model of hepatocellular carcinoma. Int. Pharm. Acta. 2020;3(1):e6
DOI: https://doi.org/10.22037/ipa.v3i1.24879

1. Introduction

Primary liver cancer or hepatocellular carcinoma (HCC) is a common and lethal cancer with poor diagnosis [1]. During recent years, estimates of the worldwide mortality from this aggressive tumor were 700,000 annually [2]. Risk factors for HCC include chronic HBV (hepatitis B virus) and HCV (hepatitis C virus) infections, non-alcoholic steatohepatitis (NASH), chronic alcohol use, obesity, chemicals, inborn and acquired metabolic diseases, autoimmune hepatitis, and diabetes mellitus [3]. Chemoprevention is one of the preventive strategies used for the control of HCC incidence that means the use of natural products,

chemicals or their combinations to diminish the risk or delay the onset of cancer [4]. Chemoprevention decreases the risk of tumor formation through interfering with the different stages of carcinogenesis, i.e. initiation, promotion, and progression. Medicinal plants and phytochemicals play a key role in HCC chemoprevention and they have become well-known as a most promising and potentially cost-effective approach to prevent this prevalent cancer [5, 6].

Asperugo procumbens L. (Boraginaceae) commonly called madwort or German-madwort, is a herb with a slender stem that native to Europe but grows in the different part of the world. German-madwort has different pharmacological properties, including antidepressant, sedative-hypnotic, tranquillizer, antispasmodic and mood elevating activities [7-9]. The aerial parts of the herb are being used in Iranian folk medicine for the management of different liver diseases. However, there was no investigation concerning the hepatoprotective effects of *A. procumbens* to support the traditional claims. Hence, the present study was undertaken to assess possible chemopreventive effect of aqueous extract of *A. procumbens* (AAP) against diethylnitrosamine (DEN)-induced HCC in rats.

2. Materials & Methods

2.1. Chemicals

All chemicals were purchased from Sigma- Aldrich Co. (Taufkrichen, Germany) with the highest commercial grade available.

2.2. Plant and extract preparation

The aerial parts of *A. procumbens* obtained from local medicinal herb shops, Tehran, Iran, and were authenticate by Mr. Kamalinejad, a qualified botanist at the Department of Botany, Shahid Beheshti University of Medical Sciences (8004, voucher specimen in Shahid Beheshti University of Medical Sciences Herbarium, Tehran, Iran). The plant was cleaned and the extraction was carried out by the maceration of 100 g plant with 900 mL distilled water for 30 min. The resulting extract was evaporated by placing in water bath 90°C. Finally, the extract was filtered and was kept at –20°C until use. The extract was dissolved in distilled water to receive desired concentrations just before use. The moisture level of the extract was determined by weight loss after placing 2 g of the final extract in an oven at 60–65°C for 72 h. The final extract contained 24% water [10].

2.3. Standardization of Extract

Total polyphenol content was determined by spectrophotometry, using gallic acid as the standard based on the Folin–Ciocalteu method [11]. Total phenolic content was 31.3 ± 1.1 mg gallic acid equivalents (GAE) per gram of AAP (mg of GAE/g of plant extract). Total flavonoid content was measured with the aluminium chloride colorimetric assay [12]. Quercetin was used as the standard and flavonoid contents were expressed as mg of quercetin equivalent per gram of AAP. Flavonoid contents were 26.0 ± 1.3 mg quercetin equivalents (QE) per gram of AAP (mg of QE /g of plant extract).

2.4. DPPH radical scavenging activity

The 1, 1-diphenyl-2-picryl hydroxyl (DPPH) method is based on the free radical scavenging activity [13]. Briefly, 2 mL of DPPH solution in methanol (4×10-5 g/mL) was prepared and added to 1 mL of different concentrations of sample solution in methanol (0.1, 0.5 and 1.0 mg/mL). The mixture was kept in the dark for 30 min at room temperature. The antioxidant activity was measured by Shimadzu, UV/VIS model 160A spectrophotometer at 517 nm. All the measurements were done in triplicate. The IC_{50} values were calculated as means ± SD and butylated hydroxytoluene (BHT) was used as positive control. The antioxidant activity of the extract was: IC_{50} = 383.6±1.34 µg/mL.

2.5. Animals

Male Sprague-Dawley rats weighing 180 to 200 g were housed in ventilated plastic cages over PWI 8-16 hardwood bedding. There were 12 air changes per h, 12 h light photoperiods, an environmental temperature of 21–23°C, and a relative humidity of 50–60%. The animals were fed a standard normal chow diet and given tap water ad libitum. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. All experiments were conducted according to the ethical standards and protocols approved by the Committee of Animal Experimentation of Zanjan University of Medical Sciences, Zanjan, Iran (protocol approval number: (ZUMS.REC.1394.318).

2.6. Hepatocarcinogenesis model

The experimental model of HCC was induced in rats by DEN as the initiator and 2-acetylaminofluorene (2-AAF) as the promoter of hepatocarcinogenesis according to the protocol described previously [14]. Briefly, rats were fasted for 96 hours and then were refed for 24 hours as a proliferative stimulant. Afterward, rats were injected only a single intraperitoneally (i.p.) dose of DEN (200 mg/kg body weight) for initiating hepatocarcinogenesis. After two weeks, liver cancer development was promoted with daily dose of oral 2-AAF (30 mg/kg body weight) for two weeks. HCC was confirmed by histopathological evaluations and the measurement of liver damage and cancer markers, including alfa-

fetoprotein (AFP), gamma glutamyl transpeptidase (GGT), alanine transaminase (ALT), and aspartate transaminase (AST) [15].

2.7. Experimental design

As shown in Figure 1, rats were randomly divided into six different groups of six rats each (n = 6). Group 1 (Control) animals fed with standard diet and served as a normal control, which injected with the single dose of saline. Group 2 were normal animals that treated only with daily oral dose of AAP (400 mg/kg body weight) from the beginning of the experiment. Group 3 (HCC) rats served as untreated HCC animals as described before. Test groups animals (Groups 4- 6) were pretreated with the increasing doses of AAP (100, 200, and 400 mg/kg body weight, respectively) two weeks prior to DEN injection that continued until the end of the study. The different concentrations of the extract were selected based on previous study and were administered orally once daily using an intragastric tube for 8 weeks [16].

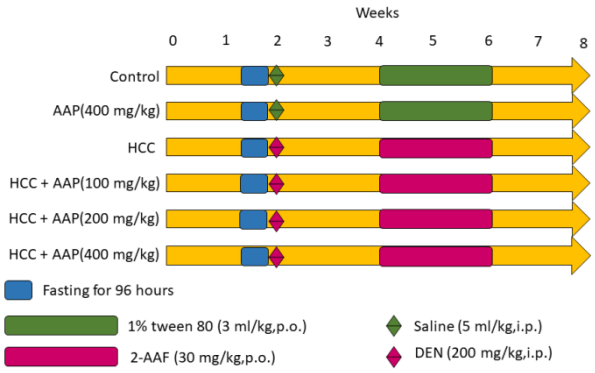

Figure 1. Experimental design. i.p.: intraperitoneal.

2.8. Sample preparations

At the end of the experiment, animals were anaesthetized by ether inhalation and blood was collected from the orbital sinus and centrifuged at 1500Xg for 20 minutes at 4°C to obtain serum for biochemical analysis. In addition, body weights of the animals were recorded and rats were sacrificed. Then, the liver tissues were quickly taken, weighed, and frozen for the determination of hepatic oxidative stress markers. Frozen liver samples were homogenized in ice-cold Tris- HCL buffer (150 mM, pH 7.4). The relative liver weight was calculated as the percentage ratio of liver weight to the body weight.

2.9. Evaluation of biochemical parameters

AFP level has been widely used clinically as a tumor marker for HCC that was measured by Calbiotech AFP ELISA Kit [17]. The activities of biochemical markers of liver damage, including GGT, ALT, and AST were determined by commercially available enzyme kits (Pars Azmoon, Tehran, Iran) [18]. The assays were performed according to the protocols supplied with the kits.

2.10. Determination of protein concentration

The concentration of liver protein was measured by the Coomassie blue protein-binding method using BSA as the standard [19].

2.11. Determination of hepatic oxidative stress markers

Hepatic GSH contents were estimated in liver homogenate by a spectrophotometric method using dithiobis-2-nitrobenzoic acid (DTNB) as the indicator of GSH and expressed as µg/mg protein. The intensity of the yellow color produced in the samples was recorded at 412 nm with a UV spectrophotometer (Infinite M200, TECAN) [20].

The level of malondialdehyde (MDA) as a reliable marker of lipid peroxidation was estimated in liver homogenate by reading the absorbance of the supernatant layer at 532 nm with an ELISA reader instrument (Infinite M200, TECAN). MDA levels were presented as µg/mg protein [21].

2.12. Statistical analysis

The homogeneity of variances was tested using Levene's test. The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD as the post hoc test. The data were presented as the mean±SD (n=6). The results with level of significance (P<0.05) were regarded as significant.

3. Results

3.1. Effects of AAP on serum biochemical parameters

Serum AFP is one of the most frequent diagnostic markers for HCC, which in this investigation was significantly enhanced in HCC rats as compared to normal rats ($P < 0.001$). As can be seen in Figure 2, the oral pretreatment of different concentrations of AAP to HCC rats caused a significant reduction in AFP. In addition, our results showed that the activities of GGT, ALT, and AST were clearly increased in DEN-treated rats compared with normal animals at the end of the experiment (Figure 3-5). The elevations in the serum markers of liver injury, including GGT, ALT, and AST were effectively decreased in AAP-treated rats. Besides, it was shown that the treatment of normal rats (Group 2) with AAP (400 mg/kg body weight daily) did not alter the biochemical markers (Figure 2-5).

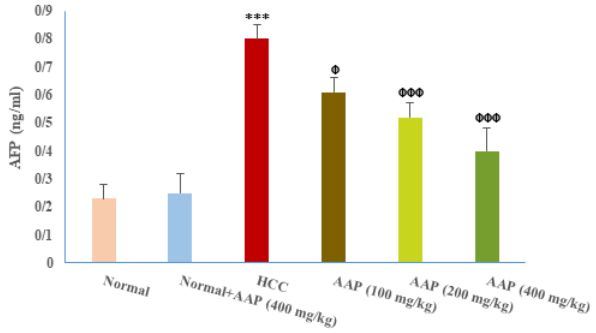

Figure 2. Effects of the German-madwort extract (AAP) on AFP: Values are presented as mean±SD. *** P<0.001 compared to normal group; Φ P<0.05, ΦΦΦ P<0.001 compared to HCC rats.

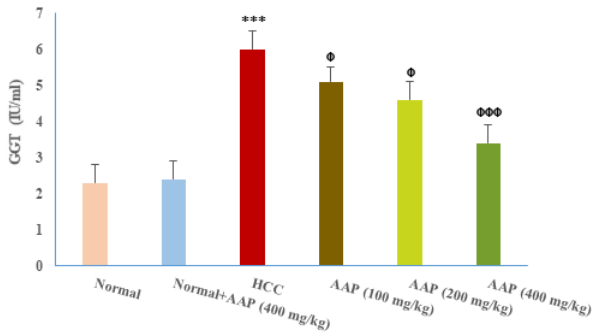

Figure 3. Effects of the German-madwort extract (AAP) on GGT: Values are presented as mean±SD. *** P<0.001 compared to normal group; Φ P<0.05, ΦΦΦ P<0.001 compared to HCC rats.

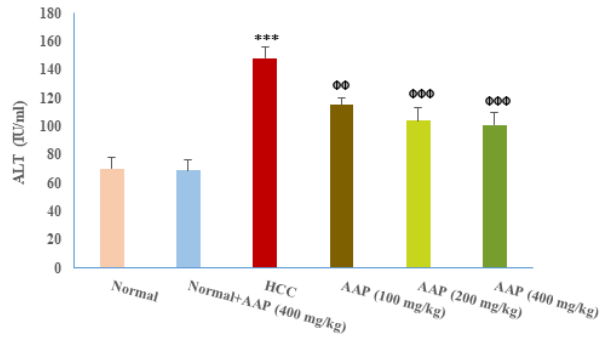

Fig. 4- Effects of the German-madwort extract (AAP) on ALT: Values are presented as mean±SD. *** P<0.001 compared to normal group; ΦΦ P<0.01, ΦΦΦ P<0.001 compared to HCC rats.

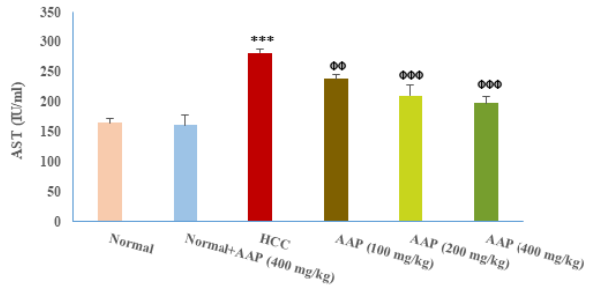

Figure 5. Effects of the German-madwort extract (AAP) on AST: Values are presented as mean±SD. *** P<0.001 compared to normal group; ΦΦ P<0.01, ΦΦΦ P<0.001 compared to HCC rats.

3.2. Effects of AAP on the hepatic markers of oxidative stress

GSH and its related antioxidant defense system are required to neutralize free radicals such as electrophilic carcinogens and to counteract oxidative stress. As indicated in Figure 6, hepatic GSH levels were decreased in DEN-treated rats compared with normal rats (P < 0.001).

Moreover, MDA levels as one of the important markers of oxidative stress were increased in HCC rats compared to normal animals (P < 0.001) (Figure 7). In this study, the pretreatment of the German-madwort extract to HCC rats caused an elevation in GSH concentrations along with a reduction in lipid peroxidation (Figures 6 and 7). It can be also seen that the oral administration of AAP (400 mg/kg body weight/daily) to normal rats did not change the oxidative stress markers (Figures 6 and 7).

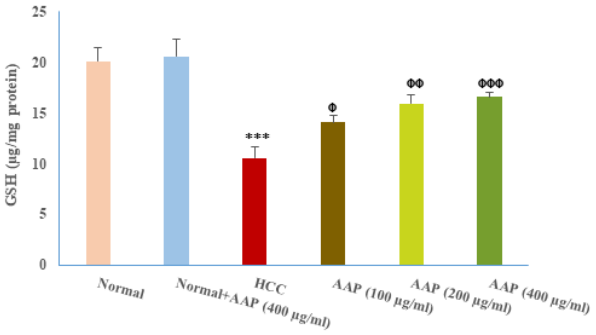

Figure 6. Effects of the German-madwort extract (AAP) on hepatic GSH contents. Values are presented as mean±SD. *** P<0.001 compared to normal group; Φ P<0.05, ΦΦ P<0.01, ΦΦΦ P<0.001 compared to HCC rats.

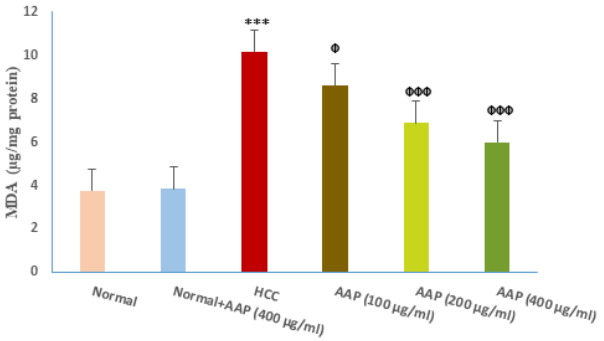

Figure 7. Effects of the German-madwort extract (AAP) on liver lipid peroxidation. MDA formation as the marker of lipid peroxidation was expressed as µg/mg protein. Values are presented as mean±SD. *** P<0.001 compared to normal group; Φ P<0.05, ΦΦΦ P<0.001 compared to HCC rats.

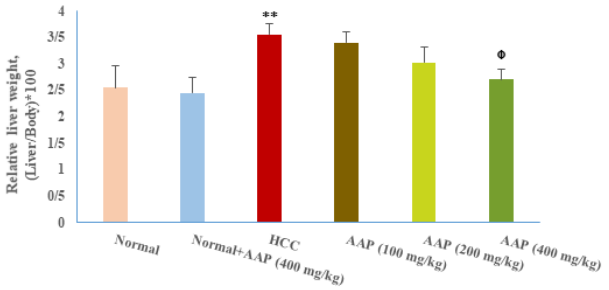

Figure. 8. Effects of the German-madwort extract (AAP) on relative liver weight. Values are presented as mean±SD. ** P<0.01 compared to normal group; Φ P<0.05 compared to HCC rats.

3.3. Effect of AAP on relative liver weight

As presented in Fig. 8, the relative liver weight of HCC rats was improved as compared to the normal animals (P < 0.01). Our results indicated that the oral administration of AAP to DEN-treated rats caused a significant decrease in relative liver weight (P < 0.05). Again, the pretreatment of normal rats (Group 2) with AAP (400 mg/kg body weight daily) did not affect the relative liver weight (Fig. 8).

4. Discussion and Conclusion

In the present study, we investigated the chemopreventive effect of *A. procumbens* against DEN-induced HCC in rats as the end stage of hepatotoxicity. Our results clearly demonstrated that German-madwort possesses a predominant chemopreventive activity in the rat model of HCC as is evident from reduction in the serum markers of liver damage and cancer.

Serum α-fetoprotein or AFP level is considered an important diagnostic marker for HCC [22]. Elevated AFP serum levels are only seen in certain tumors (e.g. HCC), non-tumoral conditions (e.g. cirrhosis), and maternal serum during pregnancy. As shown in Figure 2, the increased concentration of AFP was effectively reduced by the German-madwort extract. In addition, GGT, ALT, and AST are the most widely used HCC tumor markers. GGT expression in tumor cells provides a selective advantage to the cells during tumor promotion and enables them to preserve elevated levels of intracellular GSH. Therefore, it has been suggested that GGT is an independent prognostic indicator in patients with HCC [23]. As shown in Figure 2, the activities of GGT, ALT, and AST were excellently decreased by the German-madwort extract.

Glutathione and glutathione-related enzymes play a pivotal role in the metabolism and detoxification of free radicals and carcinogenic compounds. They are also involved in the regulation of carcinogenic mechanisms and cancer cell death [24]. It has been reported that GSH levels and the activities of GSH-dependent enzymes in the liver tissue of HCC patients were diminished because of the deficiency of antioxidant defense system [25]. Our results clearly point out that the German-madwort extract counteracted DEN-induced oxidative stress and restored GSH concentrations in the liver of rats treated with German-madwort.

Lipid peroxidation, as an important marker of oxidative stress, is a well-defined mechanism of cellular damage in different pathological conditions [26, 27]. MDA is the end product of lipid peroxidation that is involved in the promotion and progression stages of carcinogenesis [28, 29]. In this study, the oral administration of German-madwort to DEN-treated rats decreased MDA levels and liver lipid peroxidation. Finally, our results indicated that the extract prevented increase of relative liver weight caused by DEN treatment.

Recently, there has been a great interest in natural polyphenols potential use as chemopreventive agents. Many *in vitro* and *in vivo* studies have suggested that these natural compounds are promising candidates for HCC prevention by reversing, suppressing or preventing the different steps of hepatocarcinogenesis through various mechanisms [30]. Therefore, it can be suggested that the preventive activity of German-madwort against HCC may be mediated through the antioxidant, anti-inflammation, and anti-carcinogenic effects of polyphenols that are present in the extract. However, further studies to elucidate mechanisms underlying the effect would be complementary to this study. In addition, the authors believe that additional experiments are necessary to explain what active constituents are responsible for the chemopreventive effect of German-madwort against liver cancer and to detect synergy among different compounds of this valuable fruit.

To conclude, our results obviously showed that *A. procumbens* has a preventive effect against DEN-induced liver cancer by decreasing serum biomarkers of liver damage and cancer. Besides, *A. procumbens* displayed *in vitro* as well as *in vivo* antioxidant activity by elevating GSH contents in addition to preventing lipid peroxidation in the liver tissues of HCC rats. The relative weight of liver was similarly diminished in the extract-treaded rats.

Acknowledgements

This study was funded by a Research Grant from Zanjan University of Medical Sciences, Deputy of Research (Grant No: A-12-501-30).

Conflict of interest

None.

Ethics

All experiments were conducted according to the ethical standards and protocols approved by the Committee of

Animal Experimentation of Zanjan University of Medical Sciences, Zanjan, Iran (protocol approval number: (ZUMS.REC.1394.318).

Authors' ORCIDs

Mohammad Reza Eskandari:
https://orcid.org/0000-0003-1817-6912

References

1. Seyed Razi N, Arast Y, Nazemi M, Pourahmad J. The Use of Methanolic Extract of Persian Gulf Sea Cucumber, Holothuria, as Potential Anti-Cancer Agents. Int Pharm Acta. 2018, June1:2: 208-217.
2. Singh AK, Kumar R, Pandey AK. Hepatocellular Carcinoma: Causes, Mechanism of Progression and Biomarkers. Curr Chem Genom Transl Med 2018; 12:9-26.
3. Balogh J, Victor D 3rd, Asham EH, Burroughs SG, Boktour M, Saharia A, Li X, Ghobrial RM, Monsour HP Jr. Hepatocellular carcinoma: a review. J Hepatocell Carcinoma 2016; 3: 41-53. eCollection 2016.
4. Singh S, Singh PP, Roberts LR, Sanchez W. Chemopreventive strategies in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2014; 11: 45-54.
5. Okano J, Fujise Y, Abe R, Imamoto R, Murawaki Y. Chemoprevention against hepatocellular carcinoma. Clin J Gastroenterol 2011; 4:185-197.
6. Stagos D, Amoutzias GD, Matakos A, Spyrou A, Tsatsakis AM, Kouretas D. Chemoprevention of livercancer by plant polyphenols. Food Chem Toxicol 2012; 50: 2155-2170.
7. Mirshafa SA, Azadbakht M, Ahangar N. Study of Antidepressant and Sedative-Hypnotic Activity of Hydroalcoholic Extract of Asperugo procumbens L. Aerial Parts in Mice. Iran J Pharm Res 2013; 12: 529-535.
8. Ahanjan M, Mohana DC, Raveesha KA. Antibacterial activity of Asperugo procumbens L.against some human pathogenic bacteria. African J. Microbiol. Res 2008; 2: 138–140.
9. Bagheri M, Regan MS. Study the status and use of medicinal plants in Iran and in the world. Forest Range Land J 1994; 33: 22–31.
10. Shayesteh R, Kamalinejad M, Adiban H, Kardan A, Keyhanfar F, Eskandari MR. Cytoprotective Effects of Pumpkin (Cucurbita Moschata) Fruit Extract against Oxidative Stress and Carbonyl Stress. Drug Res (Stuttg) 2017; 67: 576-582.
11. Gholami S, Hosseini MJ, Jafari L, Omidvar F, Kamalinejad M, Mashayekhi V, Hosseini SH, Kardan A, Pourahmad J, Eskandari MR. Mitochondria as a Target for the Cardioprotective Effects of Cydonia oblonga Mill. and Ficus carica L. in Doxorubicin-Induced Cardiotoxicity. Drug Res (Stuttg) 2017; 67: 358-365.
12. Salimi A, Motallebi A, Ayatollahi M, Seydi E, Mohseni AR, Nazemi M, Pourahmad J. Selective toxicity of Persian gulf sea cucumber holothuria parva on human chronic lymphocyticleukemia b lymphocytes by direct mitochondrial targeting. Environ Toxicol 2017; 32: 1158-1169.
13. Sabernavaei M, Kobarfard F, Hadjiakhoondi A, Aghaahmadi M, Amin M, Yassa N. Biological Evaluation of the Isolated Compounds from Methanol Fraction of Leutea avicennia Mozaff.. Iran J Pharm Res 2018; 17: 1386-1391.
14. Shirazi FH, Piri M, Keshavarz S, Gholami S, Hosseini SH, Noubarani M, Andalib S, Kamalinejad M, Adiban H, Eskandari MR. Olive fruit (Olea europaea L.): Chemopreventive effect in the rat model of hepatocellular carcinoma. PharmaNutrition 2018; 6: 207-214.
15. Seydi E , Rasekh HR , Salimi A, Mohsenifar Z , Pourahmad J . Myricetin Selectively Induces Apoptosis on Cancerous Hepatocytes by Directly Targeting Their Mitochondria. Basic Clin Pharmacol Toxicol 2016; 119: 249-258.
16. Hamza AA, Ahmed MM, Elwey HM, Amin A. Melissa officinalis Protects against Doxorubicin-Induced Cardiotoxicity in Rats and Potentiates Its Anticancer Activity on MCF-7 Cells. PLoS One 2016; 11: e0167049.
17. Amin A, Hamza AA, Bajbouj K, Ashraf SS, Daoud S. Saffron: a potential candidate for a novel anticancer drug against hepatocellular carcinoma. Hepatology 2011; 54: 857-867.
18. Mirmohammadlu M, Hosseini SH, Kamalinejad M, Esmaeili Gavgani M, Noubarani M, Eskandari MR. Hypolipidemic, hepatoprotective and renoprotective effects of Cydonia oblonga Mill. Fruit in streptozotocin-induced diabetic rats. Iran J Pharm Res 2015; 14: 1207-1214.
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-254.
20. Riener CK, Kada G, Gruber HJ. Quick measurement of protein sulfhydryls with Ellman's reagent and with 4,4'-dithiodipyridine. Anal Bioanal Chem 2002; 373: 266-276.
21. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978; 86: 271-278.
22. Kim DH, Oh HG, Park WH, Jeon DC, Lim KM, Kim HJ, Jang BK, Song KS. Detection of Alpha-Fetoprotein in Hepatocellular Carcinoma Patient Plasma with Graphene Field-Effect Transistor. Sensors (Basel) 2018; 18: 4032.
23. Xia J, Song P, Sun Z, Sawakami T, Jia M, Wang Z. Advances of diagnostic and mechanistic studies of γ-glutamyl transpeptidase in hepatocellular carcinoma. Drug Discov Ther 2016; 10: 181-187.
24. Ortega AL, Mena S, Estrela JM. Glutathione in cancer cell death. Cancers (Basel) 2011; 3: 1285-1310.
25. Lee KT, Tsai SM, Wang SN, Lin SK, Wu SH, Chuang SC, Wu SH, Ma H, Tsai LY. Glutathione status in the blood and tissues of patients with virus-originated hepatocellular carcinoma. Clin Biochem 2007; 40: 1157-1162.
26. Adiban H, Shirazi FH, Gholami S, Kamalinejad M, Hosseini SH, Noubarani M, Eskandari MR. Chemopreventive effect of quince (Cydonia oblonga Mill.) fruit extract on hepatocellular carcinoma induced by diethylnitrosamine in rats. Int Pharm Acta. 2019; 2e2:1-12.
27. Heidari H, Kamalinejad M, Noubarani M, Rahmati M, Jafarian I, Adiban H, Eskandari MR. Protective mechanisms of Cucumis sativus in diabetes-related models of oxidative stress and carbonyl stress. Bioimpacts 2016; 6: 33-39.
28. Bartsch H, Arab K, Nair J. Biomarkers for hazard identification in humans. Environ Health 2011; 10: S11.
29. Sánchez-Pérez Y, Carrasco-Legleu C, García-Cuellar C, Pérez-Carreón J, Hernández-García S, Salcido-Neyoy M, Alemán-Lazarini L, Villa-Treviño S. Oxidative stress in carcinogenesis. Correlation between lipid peroxidation and induction of preneoplastic lesions in rat hepatocarcinogenesis. Cancer Lett 2005; 217: 25-32.
30. Amereh Z, Hatami N, Shirazi FH, Gholami S, Hosseini SH, Noubarani M, Kamalinejad M, Andalib S, Keyhanfar F, Eskandari MR. Cancer chemoprevention by oleaster (Elaeagnus angustifoli L.) fruit extract in a model of hepatocellular carcinoma induced by diethylnitrosamine in rats.