

ORIGINAL CONTRIBUTION

Open Access



The protective effect of *Rubus fruticosus* L. on blood composition in cyclophosphamide treated male rats

Naser Mirazi^{1*}, Ida Shahabi Baher¹, Zahra Izadi² and Abdolkarim Hosseini³

Abstract

Background: Some chemotherapy drugs such cyclophosphamide (CP) has destructive effects on hematopoietic cells in the bone marrow tissue. Due to antioxidant and anti-inflammatory features, medicinal herbs have protective effects on the bone marrow tissue. The aim of this experimental study is to examine the protective effects of *Rubus fruticosus* L. extract (RF) on blood parameters in male rats treated with CP.

Methods: In this experimental study, 35 male Wistar rats (220–250 g) were randomly divided into 5 groups ($n = 7$): Control (0.5 mL normal saline), CP (15 mg/kg), positive control (RF per se 200 mg/kg), treatment 1 (CP 15 mg/kg + RF 100 mg/kg), and treatment 2 (CP 15 mg/kg + RF 200 mg/kg). All drugs and extracts were given intraperitoneally for 15 consecutive days. At the end of the intervention, all animals were euthanized and their blood samples were collected by cardiac puncture in anti-coagulant tubes for blood parameters evaluation.

Results: The data analysis showed that CP has decreased significantly in RBC, WBC, Platelets number, hemoglobin and hematocrit in rats ($p < 0.05$). RF could protect hematopoiesis in CP-induced rats ($p < 0.05$).

Conclusion: The use of RF can protect the blood hematopoietic tissue in bone marrow and prevent CP toxic effects.

Keywords: Bone marrow, Cyclophosphamide, Hematopoiesis, *Rubus fruticosus* L.

Introduction

Chemotherapy is an invasive drug treatment that uses chemical drugs lethal for fast-growing cells. Although chemotherapy is an effective way to treat a variety of cancers, the side effects of the drugs used in this method are very hazardous [1]. Cyclophosphamide (CP) is one of the drugs belonging to the group of cytotoxic drugs, which is used in chemotherapy. It is available under the brand name Endoxan and is an anti-neoplastic drug. This drug is converted to the active alkylating metabolite in the body. It adds an alkyl to the cell DNA and prevents it from replicating. CP is absorbed well from the gastrointestinal tract and spreads to body tissues and

fluids. It is metabolized in the liver and excreted by the kidneys [2]. CP has several side effects such as anemia, neutropenia, thrombocytopenia, secondary tumors, and genital abnormalities [3]. CP can prevent the proliferation of hematopoietic and immune cells in connective tissues, especially bone marrow [4]. CP has debilitating effects on the immune system due to its anti-cell proliferative activity in hematopoietic and lymph node tissues [5].

Medicinal herbs with their protective effects are able to prevent cell destruction activities as well as the lethal effects of some chemicals and drugs [6]. In addition, the positive effects of most medicinal herbs on immunization and inhibition of abnormal cell growth have been proven [7]. The raspberry plant, scientifically referred to as *Rubus fruticosus* L. and with a fruit called Blackberry, belongs to the Rosaceae family, which has

* Correspondence: mirazi205@gmail.com

¹Department of Biology, Faculty of Basic Sciences, Bu-Ali Sina University, Hamedan, Iran

Full list of author information is available at the end of the article

various medicinal properties [8]. Glucose, fructose, and sucrose sugars have been reported in the fruit of this plant. Furthermore, vitamins such as A, C, E and folic acid in the raspberry fruit powder have been mentioned in anti-cancer studies [9]. Phenolic acids such as ellagic, gallic, caffeic acid as well as flavonoids such as quercetin, hyperoside, kaempferol, myristicin, catechin, epicatechin, epicatechin gallate, procyanidin B1, and quercetin 3,4'-diglucoside have been identified in the raspberry fruit and leaf [8, 9]. Anthocyanins are a group of flavonoid derivatives and water-soluble pigments giving color to flowers and fruits. Studies show that anthocyanins have anti-cancer, anti-inflammatory, and anti-obesity properties along with their role in preventing diabetes and cardiovascular disease. The primary anthocyanin detected in blackberry is cyanidin-3-O-glucoside. Other anthocyanins have also been identified in the blackberry fruit such as cyanidin 3-xyloside, cyanidin-3-O-dioxaloylglycoside, and cyanidin 3-(600-malonylglucoside) [10]. Carotenoids are an important group of natural fat-soluble pigments and are believed to have properties that boost the immune system and promote health. Various carotenoids including β -cryptoxanthin, lycopene, zeaxanthin, β -carotene, and α -carotene have been also extracted from the raspberry fruit [11]. Blackberries constitute a rich source of natural antioxidants as they contain large amounts of phenols, flavonols, and anthocyanins, including cyanidin, and therefore are free radical inhibitors [12]. The presence of anthocyanins in general and cyanidin-3-glucosides in particular in raspberries indicates their antioxidant ability to inhibit both chemical and intracellular oxidation that is suppressed by peroxy radicals. Cyanidin-3-O-glycoside extracted from blackberries has a strong antioxidant activity and leads to inhibition of neoplastic stimulation, metastasis, neoplastic cell migration and invasion, activation of tumor cell markers (NF- κ B, AP-1, Cox-2, TNF- α and MAPK), the activation of cell migration markers (JNK, p38 and ERK), and the induction of apoptosis in HL-60 neoplastic cells [13]. Due to the antioxidant activity of blackberries, their chemotherapy effects have been shown in rats. All types of raspberries reduced the number of esophageal tumors (papilloma) in animals receiving N-nitrosomethylbenzylamine (NMBA) by 24–56% compared to the control group. This inhibition was accompanied by a decrease in the production of O-6-methyl-guanine induced by NMBA that is added to esophageal DNA. This indicated that raspberries affected NMBA metabolism, reducing DNA damage and thus preventing esophageal cancer in rats [9].

For the reason that the protective effects of the extract of *R. fruticosus* (RF) on rats' bone marrow tissue treated with CP have not been studied thus far, an attempt was made to investigate this issue in this study.

Materials and methods

Chemicals and reagents

Chemicals and reagents used in this study include cyclophosphamide purchased from Baxter Oncology GmbH, Germany, and ethyl alcohol purchased from Sigma company, Germany (grade for molecular biology, purity $\geq 99.45\%$).

Preparation of RF fruit extract

From the orchards around the city of Rasht in the north of Iran, 2 kg of *R. fruticosus* fruit was prepared and after scientific identification with the herbarium code (1036, Malayer University, Iran), the fruits were dried in a suitable place without moisture and in the shade for 20 days. The fruits were then pulverized by a mixer. Afterward, 300 g of the yielded powder was placed in a container containing 80% ethyl alcohol in a refrigerator for one week. The contents of the container were then sifted through a filter paper and concentrated in a rotary apparatus at 60 rpm and at a temperature of 55 °C. The concentrated extract was placed under the hood for 48 h and was dried (the amount of the obtained dried extract was 16.71%). The desired concentrations were prepared and used from this extract.

Animals and ethics

In this experimental study, a total of 35 Wistar rats in the weight range of 220–250 g were purchased from Hamadan University of Medical Sciences. The rats were kept for one week to adapt to the environment at 22 ± 22 °C with a daily cycle of 12 h of light and free access to water and food. All conducted experiments pertaining to animal rights and conservation in this study were in accordance with the standard ethical guidelines (European Communities Directive 2010/63/EU) and were approved by the Local Ethics Committee at the Bu-Ali Sina University (ethic code number: IR.BASU.REC.1397.036).

Experimental design

The rats were randomly divided into 5 groups with 7 rats in each group as follows:

- Control group: Rats that received 1 mL/day normal saline;
- CP group: Rats that received cyclophosphamide dissolved in normal saline at a level of 15 mg/kg body weight/day [14].
- RF 100 group: Rats that received cyclophosphamide 15 mg/kg body weight/day along with *R. fruticosus* fruit extract at a level of 100 mg/kg body weight/day;
- RF 200 group: Rats that received cyclophosphamide 15 mg/kg body weight/day along with *R. fruticosus* fruit extract at a level of 200 mg/kg body weight/day;

- RF 200 per se group: Rats that received only *R. fruticosus* fruit extract at a level of 200 mg/kg body weight/day and served as per se group;

The treatment doses of RF were based upon an earlier report [15]. Injections were administered intraperitoneally for 15 consecutive days.

Blood parameter count

At the end of the experiment, the rats were euthanized using a chamber prefilled with the carbon dioxide (CO₂) gas with a concentration of 70% which, according to previous studies, is a common and safe method for euthanizing laboratory rats [16]. Blood samples were then taken by cardiac puncture and collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were counted using cell counter device (MS9, MS Laboratoires, Germany), and white blood cell by the leucocyte formula.

Statistical analysis

The continuous variables were expressed as mean ± standard deviation (SD) or median (min, max) and analyzed in the GraphPad Prism software. Normal distribution of data was checked using the Kolmogorov-Smirnov test. In case the data passed the test, the one-way analysis of variance (ANOVA) statistical test and Tukey post-hoc test were used to examine the differences between the groups. Alternatively, if the data did not pass the test, then the Kruskal-Wallis test and Dunn test were employed to determine the level of significance. The significance level was set at $p < 0.05$.

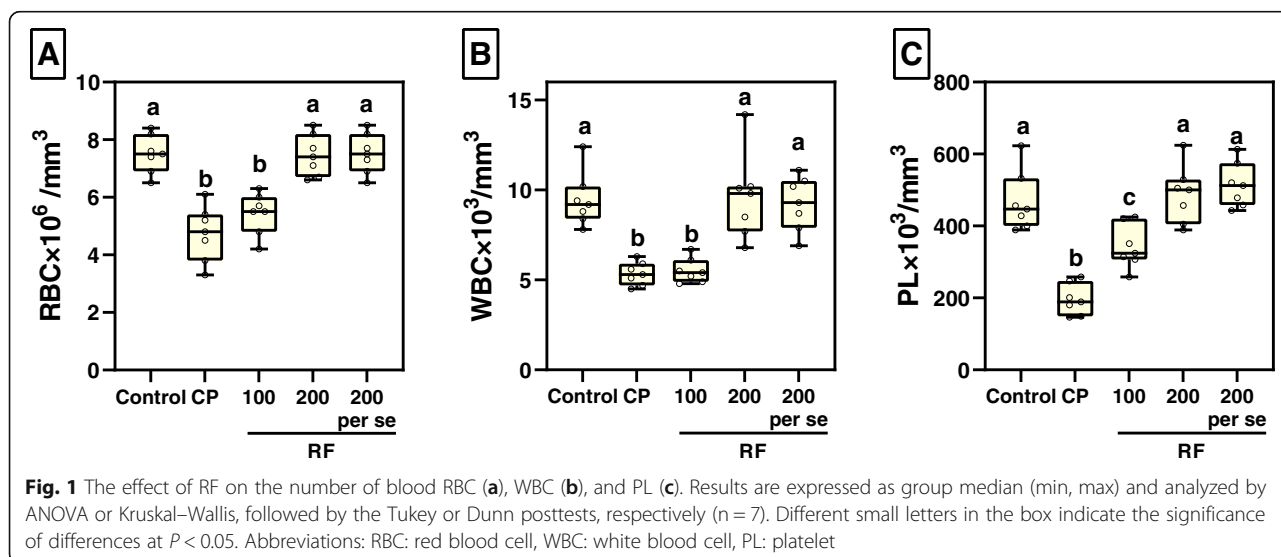
Results

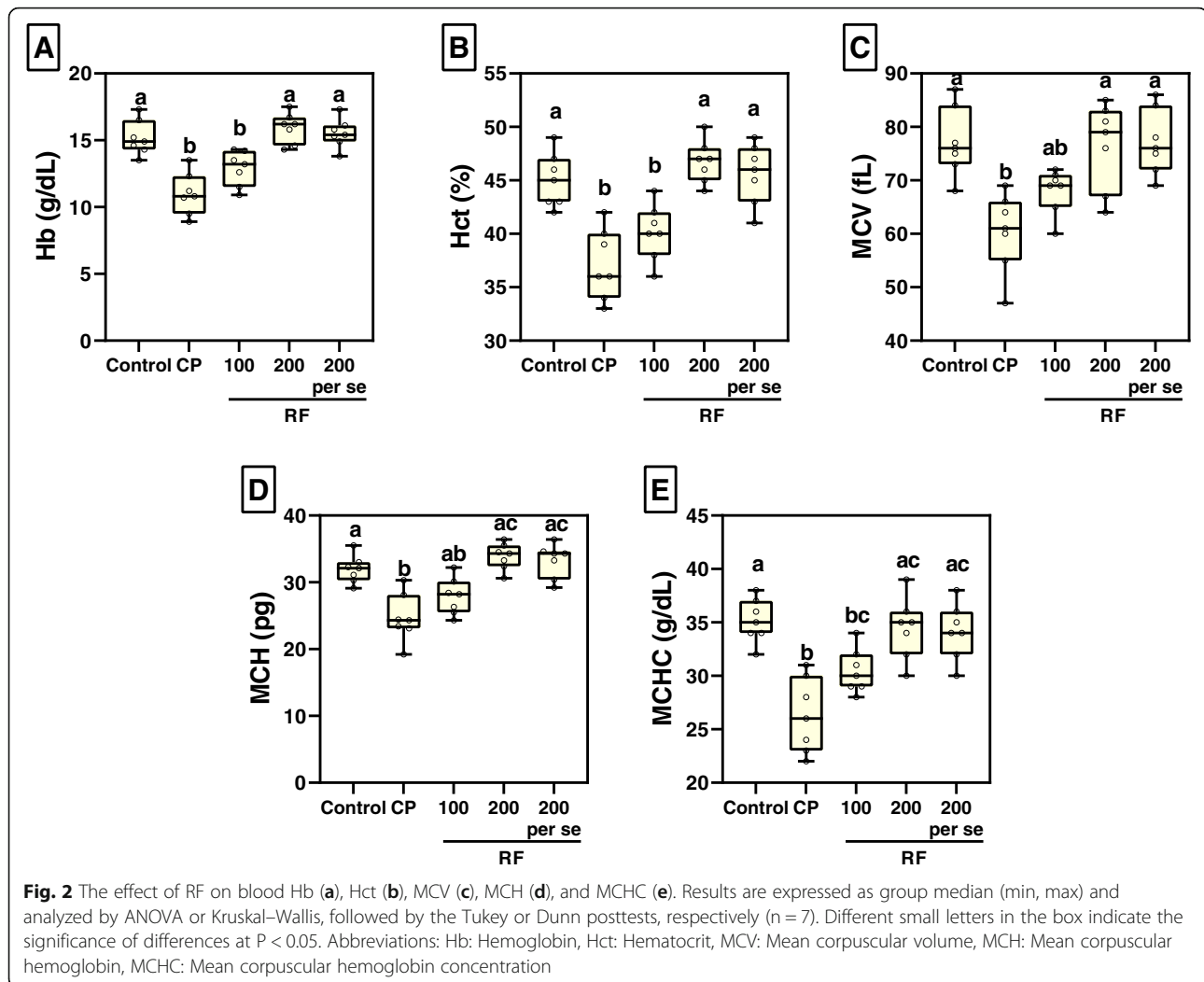
The effect of RF on the number of blood RBC, WBC, and PL

Figure 1 (a-c) shows the effect of different RF treatments on the number of blood red blood cells (RBC), white blood cells (WBC), and platelets (PL). The results of this study showed that CP significantly reduced the number of RBC, WBC, and PL during the experiment compared to the control group ($p < 0.001$). Treatment group RF 100 did not have a positive effect on the process of blood cell formation, and only affected the number of PL when compared with the CP group ($p = 0.002$, Fig. 1c). In the treatment groups, RF 200 was had a very substantial effect in that it led to a significant increase on the blood parameters compared to the CP group ($p < 0.001$). The RF 200 per se group did not change these factors and had no significant difference with the control group

The effect of RF on blood Hb, Hct, MCV, MCH, and MCHC

Figure 2 (a-e) shows the effect of different RF treatments on blood hemoglobin (Hb), hematocrit percentage (Hct), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). CP affected the levels of these parameters and significantly reduced these parameters compared to the control group ($p < 0.001$). Treatment group RF 100 did not have a positive effect on the process of blood cell formation, when compared with the CP group. In the treatment groups, the effect of RF 200 was very substantial as it significantly increased the blood Hb, Hct, MCV, MCH, and MCHC compared to the CP group ($p < 0.001$). The RF 200 per se group did not play a hematopoietic role in bone marrow and had no significant difference with the control group





The effect of RF on indicators of the leukocyte formula

Table 1 shows the effect of different RF treatments on the indicators of the leukocyte formula. CP significantly reduced the percentage of lymphocyte and neutrophil compared to the control group ($p < 0.001$). However, no significant effect of CP on the percentage of monocyte, eosinophil, and basophil was found in comparison to the control group. Treatment with RF 100 played a very constructive role in the process

of making monocyte and neutrophil compared to the CP group ($p = 0.003$ and $p < 0.001$, respectively). However, changes in lymphocyte, eosinophil, and basophil did not differ significantly compared to the CP group. Similarly, there was a significant difference between treatment group RF 200 CP group in term of lymphocyte and neutrophil ($p < 0.001$). Nevertheless, RF 200 did not make significant changes in the percentage of monocyte, eosinophil, and basophil

Table 1 The effect of RF on indicators of leukocyte formula

Groups	Control	CP	RF 100	RF 200	RF 200 per se
Lymphocyte (%)	70.14 ± 5.305 ^a	28.86 ± 3.716 ^b	34.43 ± 3.823 ^b	65.71 ± 5.851 ^a	68.71 ± 4.786 ^a
Monocyte (%)	3.286 ± 2.289 ^a	2.571 ± 0.535 ^a	5.857 ± 1.773 ^b	3.429 ± 0.976 ^{ab}	3.286 ± 0.951 ^{ab}
Neutrophil (%)	22.29 ± 2.870 ^a	60.00 ± 2.887 ^b	50.43 ± 3.101 ^c	24.57 ± 4.429 ^a	24.57 ± 4.392 ^a
Eosinophil (%)	3.00 ± 1.414 ^{ab}	5.429 ± 1.813 ^b	5.571 ± 1.718 ^b	3.286 ± 0.951 ^{ab}	2.571 ± 0.787 ^a
Basophil (%)	1.286 ± 1.113 ^{ac}	3.286 ± 0.951 ^{abd}	3.714 ± 1.496 ^d	2.00 ± 0.817 ^{abcd}	1.143 ± 0.90 ^c

Results are expressed as group mean ± SD and analyzed by ANOVA or Kruskal–Wallis, followed by the Tukey or Dunn posttests, respectively ($n = 7$). Different small letters in the table indicate the significance of differences at $P < 0.05$

compared to the CP group. Finally, RF 200 per se had no incremental effect on the percentage of the leukocyte formula in comparison with the control group.

Discussion

Nowadays, the use of chemical cytotoxic drugs is faced with various side effects in patients with malignant neoplasms [17]. CP is used as an anti-neoplasm drug in the treatment of some tumors. The use of this drug in most patients is associated with several disorders such as anemia, decrease in the number of white blood cells, and weakened immune system [18]. In traditional medicine, medicinal herbs have long been widely used to treat many disorders, especially blood disorders. Being rich in a variety of antioxidants, medicinal herbs are able to prevent cell damage [19]. The presence of vitamins that prevent cell damage and death such as vitamins C, E, A, and D in various medicinal herbs has been proven. These vitamins are an important factor in protecting tissues against oxidizing and destructive factors of DNA [9]. CP reduced the number of red blood cells, white blood cells, and platelets in the bone marrow hematopoietic tissue of the rats used in this study. The raspberry fruit extract could protect the bone marrow tissue against CP due to containing high amounts of various antioxidants and protective vitamins. Colony-stimulating factors involved in cell proliferation and differentiation in bone marrow hematopoiesis are enhanced by chemicals in the raspberry fruit extract. Anthocyanins in the raspberry fruit have been reported to be involved in cell proliferation [10]. The presence of this important substance may have protected hematopoietic tissue against CP. CP significantly reduced the amount of hemoglobin in red blood cells and this effect was significant compared to the control group. As the destructive effects of CP on bone marrow progenitor cells have been proven [20], it appears that the decrease in blood hemoglobin has been due to impaired cell formation and the mechanism of hemoglobin synthesis by this drug.

It has been reported that grapefruit kernel extract inhibits the process of hemoglobin production decrease in mice receiving CP [21]. In line with these results, the raspberry fruit extract was able to reduce the amount of hemoglobin in the blood of the rats receiving CP to its normal amount and inhibit the destructive effect of the drug. The cause of this phenomenon may be related to the effect of the extract on the process of cell proliferation and its antioxidant property to protect the process of protein synthesis. In a study that demonstrated the hematopoietic effect of *Hypericum perforatum* extract, it was shown that specific chemical agents in this plant accelerated the conversion of megakaryocytes to platelets [22]. In the present study, the raspberry fruit extract

could significantly increase the platelets in the rats treated with CP. It is possible that the raspberry fruit was able to prevent the reduction of blood platelets in rats by affecting megakaryocytes or the process of synthesis of interleukin (IL)-6. Moreover, the raspberry fruit is rich in cyanidin called cyanidin-3-O-glycoside. A study showed that this substance plays an effective role in controlling breast adenocarcinoma in humans [23]. Therefore, it is assumed that the presence of this substance in the raspberry extract could have reduced the effects of CP and prevented the destruction of hematopoietic tissue.

Carotenoids have very beneficial effects on the process of protecting cells against tissue damaging agents such as oxidants. Carotenoids are natural color pigments that play a pivotal role in physiological activities. The presence of carotenoids against tissue oxidants, chemical drugs, and some toxic compounds can protect tissues against these hazardous substances [24, 25]. The raspberry fruit is rich in a variety of carotenoids. The presence of these agents in the raspberry fruit is thought to have reduced the hazards of CP in the tested rats. Another anti-cancer agent is a compound called kaempferol, which plays a significant role in protecting tissues from the tumor progression process [26]. Reports indicate the presence of this compound in the raspberry fruit. It appears that the kaempferol in the raspberry fruit extract has been able to prevent the destructive effects of CP in bone marrow. Further, the role of caffeic acid in preventing tissue damage has been suggested in some studies. Caffeic acid is a non-toxic polyphenol found in many foods. It can participate in the modulation of glutathione-S-transferase and glutathione reductase enzymes. The above enzymes are involved in the resistance of cells to cisplatin [27]. The raspberry fruit contains large amounts of caffeic acid [8, 9]. The presence of this compound can play an effective role in protecting the bone marrow tissue against hazardous drugs such as CP. Some medicinal herbs, such as green tea, contain a compound called catechin, which acts as an antioxidant. Some reports suggest that catechins sweep away free radicals and are effective in reducing the impacts of reactive oxygen species (ROS). CP is among the factors producing ROS in tissues [28]. Since catechin in the extracts of some fruits such as raspberries [9] and many medicinal herbs inhibits the effect of CP in the production of ROS, the presence of catechin in the extracts of raspberries in this study might have protected the hematopoietic tissue of bone marrow in the tested rats against this destructive drug. There were some limitations in the present study that should be considered. Our study did not evaluate the effect of RF on the animal model of non-Hodgkin lymphoma and other important biochemical and molecular parameters, such as

antioxidant levels, inflammatory cytokines, gene expression of cell survival pathway, due to limited funding, and sample size. Therefore, supplementary studies need to be conducted to confirm the results and beneficial effect of RF on the CP toxic condition.

In this study, which was conducted on Wistar rats receiving CP, it was demonstrated that CP had the effect of destroying hematopoietic tissue. The number of red blood cells, white blood cells, and platelets was significantly reduced in the rats receiving CP. Simultaneous treatment of rats with the raspberry fruit extract improved their blood parameters. These results indicate that various beneficial compounds in the raspberry fruit extract can protect the bone marrow tissue against the destructive effects of CP in the tested rats.

Acknowledgments

This paper was supported by the Bu-Ali Sina University, Tehran, Iran.

Authors' contributions

Naser Mirazi, Ida Shahabi Baher, Zahra Izadi and Abdolkarim Hosseini Participated in study design, data collection and evaluation, drafting and statistical analysis. Ida Shahabi Baher, and Abdolkarim Hosseini; Contributed extensively in interpretation of the data and the conclusion. Ida Shahabi Baher and Zahra Izadi; Conducted experiments and analyses. All authors performed editing and approving the final version of the paper and also participated in the finalization of the manuscript and approved the final draft.

Funding

This study received no fund from any organization.

Declarations

Ethics approval

All conducted experiments pertaining to animal rights and conservation in this study were in accordance with the standard ethical guidelines (European Communities Directive 2010/63/EU) and were approved by the Local Ethics Committee at the Bu-Ali Sina University (ethic code number: IR.BASU.REC.1397.036).

Competing interests

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

Author details

¹Department of Biology, Faculty of Basic Sciences, Bu-Ali Sina University, Hamedan, Iran. ²Department of Horticulture, Faculty of Agriculture, University of Nahavand, Nahavand, Iran. ³Department of Animal Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.

Received: 23 December 2020 Accepted: 1 April 2021

Published online: 09 April 2021

References

- Zhao CY, Cheng R, Yang Z, Tian ZM. Nanotechnology for cancer therapy based on chemotherapy. *Molecules*. 2018;23(4). <https://doi.org/10.3390/molecules23040826>.
- Rundberg Nilsson A, Soneji S, Adolfsen S, Bryder D, Pronk CJ. Human and murine hematopoietic stem cell aging is associated with functional impairments and intrinsic megakaryocytic/erythroid bias. *PLoS One*. 2016; 11(7):e0158369. <https://doi.org/10.1371/journal.pone.0158369>.
- Hill BT, Rybicki L, Carlstrom KD, Jagadeesh D, Gerds A, Hamilton B, et al. Daily weight-based busulfan with cyclophosphamide and etoposide produces comparable outcomes to four-times-daily busulfan dosing for lymphoma patients undergoing autologous stem cell transplantation. *Biol Blood Marrow Transplant*. 2016;22(9):1588–95. <https://doi.org/10.1016/j.bbmt.2016.06.011>.
- Barnes H, Holland AE, Westall GP, Goh NS, Glaspole IN. Cyclophosphamide for connective tissue disease-associated interstitial lung disease. *Cochrane Database Syst Rev*. 2018;1(1):Cd010908.
- Noh EM, Kim JM, Lee HY, Song HK, Joung SO, Yang HJ, et al. Immuno-enhancement effects of *Platycodon grandiflorum* extracts in splenocytes and a cyclophosphamide-induced immunosuppressed rat model. *BMC Complement Altern Med*. 2019;19(1):322. <https://doi.org/10.1186/s12906-019-2724-0>.
- Xiong F, Guan YS. Cautiously using natural medicine to treat liver problems. *World J Gastroenterol*. 2017;23(19):3388–95. <https://doi.org/10.3748/wjg.v23.i19.3388>.
- Tsang MS, Shaw PC, Chu IM, Cheng L, Wong EC, Lau DT, et al. High-throughput immunological analysis of dictamnii cortex: implication in the quality control of herbal medicine. *Molecules*. 2019;24(16). <https://doi.org/10.3390/molecules24162880>.
- Zia-Ul-Haq M, Riaz M, De Feo V, Jaafar HZ, Moga M. *Rubus fruticosus* L.: constituents, biological activities and health related uses. *Molecules*. 2014; 19(8):10998–1029. <https://doi.org/10.3390/molecules190810998>.
- Stoner GD, Chen T, Kresty LA, Aziz RM, Reinemann T, Nines R. Protection against esophageal cancer in rodents with lyophilized berries: potential mechanisms. *Nutr Cancer*. 2006;54(1):33–46. https://doi.org/10.1207/s15327914nc5401_5.
- Wu X, Prior RL. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. *J Agric Food Chem*. 2005;53(7):2589–99. <https://doi.org/10.1021/jf048068b>.
- Marinova D, Ribarova F. HPLC determination of carotenoids in Bulgarian berries. *J Food Compos Anal*. 2007;20(5):370–4. <https://doi.org/10.1016/j.jfca.2006.09.007>.
- Jiao H, Wang SY. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. *J Agric Food Chem*. 2000;48(11): 5672–6. <https://doi.org/10.1021/jf000765q>.
- Ding M, Feng R, Wang SY, Bowman L, Lu Y, Qian Y, et al. Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. *J Biol Chem*. 2006;281(25): 17359–68. <https://doi.org/10.1074/jbc.M600861200>.
- Choi JS, Jou SS, Oh MH, Kim YH, Park MJ, Gil HW, et al. The dose of cyclophosphamide for treating paraquat-induced rat lung injury. *Korean J Intern Med*. 2013;28(4):420–7. <https://doi.org/10.3904/kjim.2013.28.4.420>.
- Gomar A, Hosseini A, Mirazi N. Preventive effect of *Rubus fruticosus* on learning and memory impairment in an experimental model of diabetic neuropathy in male rats. *PharmaNutrition*. 2014;2(4):155–60. <https://doi.org/10.1016/j.phanu.2014.09.001>.
- Conlee KM, Stephens ML, Rowan AN, King LA. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. *Lab Anim*. 2005;39(2):137–61. <https://doi.org/10.1258/0023677053739747>.
- Zhang QY, Wang FX, Jia KK, Kong LD. Natural product interventions for chemotherapy and radiotherapy-induced side effects. *Front Pharmacol*. 2018;9. <https://doi.org/10.3389/fphar.2018.01253>.
- DeZern AE, Petri M, Drachman DB, Kerr D, Hammond ER, Kowalski J, et al. High-dose cyclophosphamide without stem cell rescue in 207 patients with aplastic anemia and other autoimmune diseases. *Medicine*. 2011;90(2):89–98. <https://doi.org/10.1097/MD.0b013e318210e685>.
- Zhang L, Dou XW, Zhang C, Logrieco AF, Yang MH. A review of current methods for analysis of mycotoxins in herbal medicines. *Toxins*. 2018;10(2). <https://doi.org/10.3390/toxins10020065>.
- Molyneux G, Andrews M, Sones W, York M, Barnett A, Quirk E, et al. Haemotoxicity of busulphan, doxorubicin, cisplatin and cyclophosphamide in the female BALB/c mouse using a brief regimen of drug administration. *Cell Biol Toxicol*. 2011;27(1):13–40. <https://doi.org/10.1007/s10565-010-9167-1>.
- Adeneye AA. Hypoglycemic and hypolipidemic effects of methanol seed extract of *Citrus paradisi* Macfad (Rutaceae) in alloxan-induced diabetic Wistar rats. *Nig Q J Hosp Med*. 2008;18(4):211–5. <https://doi.org/10.4314/nqjhm.v18i4.45040>.
- Leila Y, Naser M. Study of *Hypericum perforatum* L. hydroethanolic leaf s extract on some blood parameters in male rats treated by cyclophosphamide. *Armaghane-danesh*. 2016;21(2):135–47.

23. Strugała P, Loi S, Bażanów B, Kuroopka P, Kucharska AZ, Włoch A, et al. A comprehensive study on the biological activity of elderberry extract and cyanidin 3-o-glucoside and their interactions with membranes and human serum albumin. *Molecules*. 2018;23(10). <https://doi.org/10.3390/molecules23102566>.
24. Tapiero H, Townsend DM, Tew KD. The role of carotenoids in the prevention of human pathologies. *Biomed Pharmacother*. 2004;58(2):100–10. <https://doi.org/10.1016/j.biopha.2003.12.006>.
25. Sathasivam R, Ki JS. A review of the biological activities of microalgal carotenoids and their potential use in healthcare and cosmetic industries. *Mar Drugs*. 2018;16(1). <https://doi.org/10.3390/md16010026>.
26. Imran M, Salehi B, Sharifi-Rad J, Aslam Gondal T, Saeed F, Imran A, et al. Kaempferol: a key emphasis to its anticancer potential. *Molecules*. 2019; 24(12). <https://doi.org/10.3390/molecules24122277>.
27. Sirota R, Gibson D, Kohen R. The timing of caffeic acid treatment with cisplatin determines sensitization or resistance of ovarian carcinoma cell lines. *Redox Biol*. 2017;11:170–5. <https://doi.org/10.1016/j.redox.2016.12.006>.
28. Logsdon AL, Herring BJ, Lockard JE, Miller BM, Kim H, Hood RD, et al. Exposure to green tea extract alters the incidence of specific cyclophosphamide-induced malformations. *Birth Defects Res B Dev Reprod Toxicol*. 2012;95(3):231–7. <https://doi.org/10.1002/bdrb.21011>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- ▶ Convenient online submission
- ▶ Rigorous peer review
- ▶ Open access: articles freely available online
- ▶ High visibility within the field
- ▶ Retaining the copyright to your article

Submit your next manuscript at ▶ [springeropen.com](https://www.springeropen.com)
