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Research Article

Investigation of Protective Effects of Quercetin on Oxidative Stress Induced by Vinblastine in Bone Marrow of Rats

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Abstract

Background: Chemotherapy drugs such as vinblastine cause oxidative stress in the bone marrow resulting changes in blood cell production and anemia. In this study, the antioxidant and therapeutic potential of quercetin was evaluated.

Methods: Twenty-one male Wistar rats were divided into three groups; The Control group received a daily dose of normal saline, group 2 received a single dose of 2 mg/kg b.w. vinblastine intraperitoneally (i.p.) on the first day of study, and group 3 received a single dose of vinblastine (2 mg/kg b.w. i.p.) along with quercetin (20 mg/kg b.w. i.p.) for 14 days. To evaluate oxidative stress in bone marrow; malondialdehyde (MDA), Total Antioxidant Capacity (TAC) and Pro-Oxidant/Antioxidant Balance (PAB) were also measured using specified methods.

Results: The blood analysis showed that the mean level of RBC, Hemoglobin, and Hematocrit were significantly higher in the vinblastine group compared to the control group. Treatment with quercetin could elevate them into the normal range. Administration of vinblastine elevated the levels of bone marrow MDA and PAB significantly ($p<0.05$) compared to the control group but had no effect on total antioxidant capacity. The use of quercetin with vinblastine showed a decrease in the levels of bone marrow MDA and PAB compared to the vinblastine group alone.

Conclusion: The findings of this study showed that quercetin at a dose of 20 mg/kg could improve the anemia induced by vinblastine chemotherapy, and it can also be useful in improving vinblastine-induced lipotoxicity.

Introduction

Vinblastine ($C_{46}H_{58}N_4O_9$) as a natural alkaloid derived from Catharanthus roseus is one of the most effective anticancer drugs currently used in chemotherapy independently or in combination with other drugs. It is used to treat Hodgkin's and non-Hodgkin's lymphomas as well as some solid tumors such as bladder, brain, melanoma, and testicular tumors.[1,2] The action mechanism of vinca alkaloids depends on beta-tubulin and microtubule dysfunction during mitosis, leading to mitosis stoppage at stages of metaphase and cell death.[3,4] Also, vinblastine interferes with spindle formation, causing chromosome segregation.[5] Side effects of this drug include hair loss, white blood cell and platelet loss, anemia, gastrointestinal problems, hypertension, sweating, depression, muscle cramps, dizziness, and headache.[6,7]

As mentioned before, anemia is one of the side effects of taking vinblastine and some other chemotherapy drugs, which is prevalent in about 40% of patients with cancer, and it reaches up to 90% in patients undergoing chemotherapy.[8] Treatment to reduce anemia involves the use of blood-cell-boosting hormones such as erythropoietin, use of iron supplementation, and ultimately blood transfusion.[9] Blood transfusion is not a safe process and can cause many problems for the recipient, especially when this person has other underlying problems.[10,11] Oxidative stress is one of the factors causing and aggravating the anemia. Of course, the underlying cause may not be oxidative stress, but it damages stem cells playing a vital role in hematopoiesis, due to its effect on erythrocyte production in the bone marrow and the life span of red blood cells in a cycle.[12]

Quercetin is a flavonoid found in various food products and plants including fruits, vegetables, beans, and tea; onions and broccoli are common foods containing significant amounts of quercetin.[13,14]

Numerous applications of quercetin have made it a known potent anticancer agent.[15,16] Quercetin, with specific structural features including one hydroxyl group in carbon 3 and a carbonyl group in carbon 4 facilitating binding to iron ions and contributing to antioxidant activity has all the properties required for inhibition of free radical process.[17,18] Also, the presence of catechol in the β-ring of quercetin is

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essential for antioxidant activity and elimination of free radicals.[19] Anti-cancer properties of quercetin have been proven in experiments carried out in the physiological environment of the body and laboratory environment. Quercetin is a potent natural antioxidant because of its role in the elimination of free radicals and bind to metal ions.[20] Accordingly, this study was designed to measure the effectiveness of quercetin in the reduction of oxidative stress associated to anemia in the bone marrow of the rat model that received vinblastine drug.

Materials and Methods

Chemicals and Reagents

Trichloroacetic acid, Thiobarbituric acid, 1-Butanol (Merck KGaA, 64271 Darmstadt, Germany), Quercetin powder (Sigma-Aldrich, St. Louis, Missouri, United States), Vinblastine (Gedeon Richter LTD, Budapest, Hungary), TMB powder (3, 3, 5, 5-tetramethylbenzidine, Fluka), peroxidase enzyme (Applichem: 230 U/mg, A3791, 0005, Darmstadt, Germany), N-chloro 4methylbenzenesulfonamide, sodium salt (chloramine T trihydrate) (Applichem: A4331, Darmstadt, Germany), and hydrogen peroxide (30%) (Merck Company), as well as all the other reagents used were of analytical grade and were prepared in double-distilled water.

Experimental animal groups

Twenty-one male Wistar rats were used in this study with a mean bodyweight of 268.3 ± 9.78 g. The rats were kept in the animal house under the stable physical condition at 25 ± 2 °C and on a 12-hour light-dark cycle with free access to standard food (Javaneh Khorasan Company) and drinking water. The animals were randomly grouped into 3 groups (n=7) 72 hours before starting the study and were placed in appropriate cages to adapt to new conditions. Attempts were made to perform the tests at the same time of day and night within 14 days, as much as possible, to avoid the possible effects of the day-night cycle.

In this study, the rats were randomly divided into the following three groups (n=7). Group I, the control group received 0.5 ml normal saline (0.9%) intraperitoneally. Group II received 2mg/kg vinblastine (dissolved in 0.9% sodium chloride solution) intraperitoneally on the first day of the study. Group III received vinblastine (2mg/kg) intraperitoneally on the first day of study along with 20mg/kg quercetin per day (dissolved in water) intraperitoneally for 2 weeks. The Injection volume never exceeded 0.6 mL. The water and food intake of each group of rats was recorded at the beginning and end of the study.

Collection of the samples

At the end of the study, the rats were anesthetized with ketamine (80 mg/kg) and xylazine (100 mg/kg) after 12-hour fasting, and their blood was collected through cardiac puncture. 2 mL of the blood sample was collected in tubes containing EDTA and the rest was kept in test tubes. Serum samples were obtained by centrifugation of blood samples for 25 minutes at 2500 rpm. Also, bone marrow samples of rats from each group were separated from the femur bone of rats and were transferred to the bone marrow culture medium.

Measurement of blood components through complete blood count (CBC)

The blood samples of rats kept in EDTA-containing CBC tubes were analyzed for hematological parameters including Hemoglobin (Hb in g/dL), Red Blood Cell count (RBC in 10^6/ mm^3), Platelet count (PLT in 10^3/ mm^3), Hematocrit (HCT in %), Mean Cell Volume (MCV n fl), Mean Corpuscular Hemoglobin (MCH in g/dL)), Mean Corpuscular Hemoglobin Concentration (MCHC in %), differential White Blood Cell count (WBC in $10^3/mm^3$), and using auto hematology analyzer system (CBC Mindray BC-3000 Plus).

Evaluation of oxidative stress markers in blood and bone marrow

An FRAP assay based on the ability of plasma to reduce ferric ions (Fe^{3+}) was carried out for measurement of total antioxidant capacity.[21] Following the reduction of ferric ions and converting them to ferrous ions (Fe^{2+}) at acidic pH in the presence of specific reagents, a blue complex was formed which can be measured spectrophotometrically at a wavelength of 593 nm. This reaction is nonspecific, and any molecule capable of reducing ferric ion under the above conditions can participate in this reaction. Malondialdehyde, a product of lipid peroxidation reacted with thiobarbituric acid (TBA) as one of the Thiobarbituric Acid Reactive Substances (TBARS) to form a 1:2 MDA-TBA adduct under acidic and high-temperature conditions to produce a purple complex whose color intensity can be measured at a wavelength of 532 nm. Thus, the quantity of TBARS was proportionate to the amount of MDA. The concentration of TBARS was determined according to the method proposed by Uchiyama and Mihara. The concentration of TBARS was calculated using the MDA standard curve and was expressed as nmol/mg of protein.[22]

Pro-oxidant/antioxidant balance (PAB) assay

Tetramethyl benzidine (TMB) and TMB cation were used as the indicators of oxidation-reduction (due to their electrochemical and optical properties) in order to evaluate the oxidant-antioxidant balance. In this method, the oxidant-antioxidant balance was simultaneously measured in one trial by two different reaction types. In an enzymatic reaction, the TMB chromogen was oxidized to a colored cation, using pro-oxidants, and in a chemical reaction, the TMB cation was converted to a colorless composition, using antioxidants. The photometric adsorption is then compared with the specific adsorption of a series of standard solutions (a mixture of different ratios (0-100%) of hydrogen peroxide and uric acid). The PAB scale was expressed in a contract unit which is called HK (Hamidi-Koliakos) unit.[23]

Statistical analysis

SPSS software version 16.00 was used to analyze data. To compare results in the study groups, the results were subdivided into normal and abnormal variables by one-sample Kolmogorov-Smirnov test. One-way Analysis of Variance (ANOVA) was used for examining normal variables and the non-parametric equivalent of one-way ANOVA-Kruskal-Wallis test was used for evaluating non-normal variables. All tests were used for one-way variance, and a P-value of 0.05 or less was considered statistically significant.

Results

The effects of vinblastine and quercetin on food and water intake, and weight of rats

As shown in Figure 1, a significant decrease was observed in the amount of water intake in the group receiving vinblastine (GII) compared to the control group (GI). Treatment with quercetin (GI) significantly increased the level of water intake compared to the vinblastine group (GII). According to Figure 2, the weight of rats decreased significantly in the vinblastine group. Treatment with quercetin improved weight loss in the treatment group (GIII) ($P < 0.05$). Results showed that treatment with vinblastine and/or quercetin had no effect on food intake level.

The effects of vinblastine and quercetin on blood components

As shown in Table 1, the use of vinblastine elevated the level of WBC compared to the control group (GI). Quercetin reduced the rate increase of WBC in the group receiving quercetin (GIII) compared to the vinblastine group (GII). RBC level was significantly lower in the vinblastine group (GII) compared to the control group (GI). Quercetin was able to increase RBC levels in the vinblastine associated with the quercetin group (GIII) compared to the vinblastine group (GII).

Hemoglobin (Hb) level was significantly lower in the vinblastine group (GII) compared to the control group (GI). Quercetin was able to increase the hemoglobin level in the vinblastine associated with the quercetin group (GIII) compared to the vinblastine group (GII). The level of hematocrit (HCT) was significantly lower in the vinblastine group than the control group. Quercetin also increased hematocrit level in the vinblastine associated with the quercetin group compared to the vinblastine group. This rate was higher in the vinblastine plus the quercetin group than the vinblastine group and had a smaller decrease compared to the vinblastine group.

The level of MCV was significantly lower in the control group (GI) than the vinblastine group (GII).

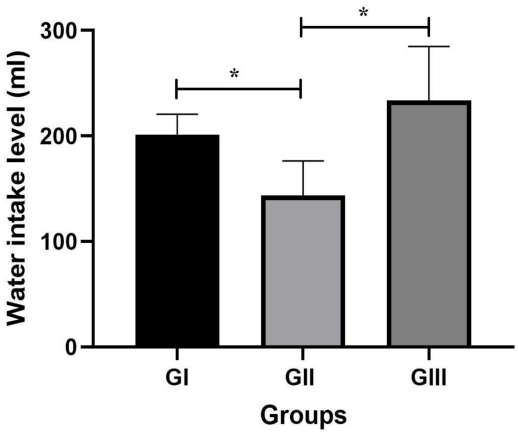

Figure 1. water intake level in control (GI) and treatment groups for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII). Values are means ± SD (n = 7). * P < 0.05.

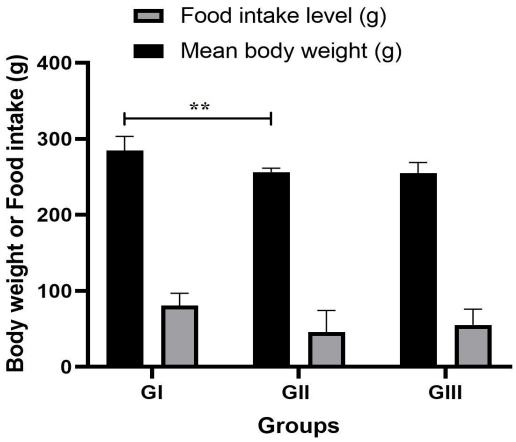

Figure 2. Body weight and food intake in control (GI) and treatment groups for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII). Values are means ± SD (n = 7). **P < 0.01.

Table 1. WBC, RBC, Hemoglobin and Hematocrit levels in controlled (GI) and treated rats for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII).

Test	GI	GII	GIII	F-value	P-value
WBC (10^{-8}.L)	5.15±2.37	8.08±3.78*	3.38±0.90	4.008	0.049
RBC (10^{-12}.L)	7.06±0.44	5.75±1.25**	8.41±0.90	1306	0.001
Hemoglobin ($g.L^{-1}$)	13.80±0.34**	12.68±1.30***	3.38±0.90	17.94	<0.001
Hematocrit (%)	40.83±3.29**	39.48±2.15**	3.38±0.90	11.884	0.002

Data are means ± SD (n = 7): * P < 0.05 compared to control group. ** P < 0.01 compared to control group. *** P < 0.001 compared to control group.

Quercetin was able to slowdown the increase in the rate of MCV in the vinblastine plus quercetin group (GIII) compared to the vinblastine group (GII). Also, MCH level was lower in the control group (GI) than the vinblastine group (GII) and quercetin was able to decline the increase in the rate of MCH in the vinblastine associated with the quercetin group (GII) compared to the vinblastine group (GII) (Table 2).

The effects of vinblastine and quercetin on markers of oxidative stress in blood and bone marrow

Results of the oxidative stress markers shown in Figure 3 indicated that the use of vinblastine increased lipid peroxidation in bone marrow cells, such that a significant increase was found in the level of malondialdehyde in the vinblastine group (GII) compared to the control group (GI). It was shown that the use of quercetin significantly decreased ($p<0.05$) malondialdehyde levels in bone marrow in the vinblastine associated with the quercetin group (GIII). Total antioxidant capacity did not change in the group receiving vinblastine (GII) compared to the control group (GI) in both the serum and the bone marrow. Also quercetin had no effect on total antioxidant capacity in both the serum and the bone marrow (Figure 4).

The effects of vinblastine and quercetin on pro-oxidant-antioxidant balance (PAB)

As shown in Figure 5, the PAB level was significantly higher in the control group (GI) than the vinblastine group (GII). Quercetin was able to decelerate the increase in the level of PAB in the vinblastine associated with quercetin group (GIII) compared to the vinblastine group (GII) and this rate was significantly lower in the vinblastine associated with the quercetin group than the vinblastine group ($p= 0.01$) and increased to a lesser extent.

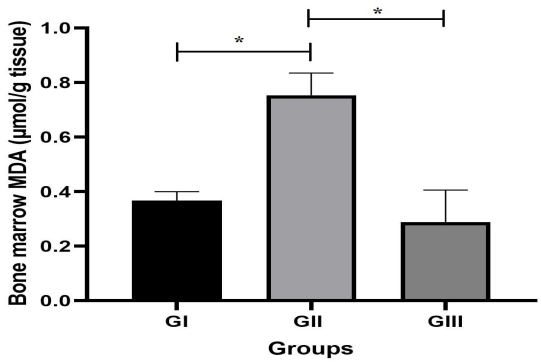

Figure 3. Bone marrow MDA level in control (GI) and treatment groups for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII). Values are means ± SD (n =7). * P < 0.05.

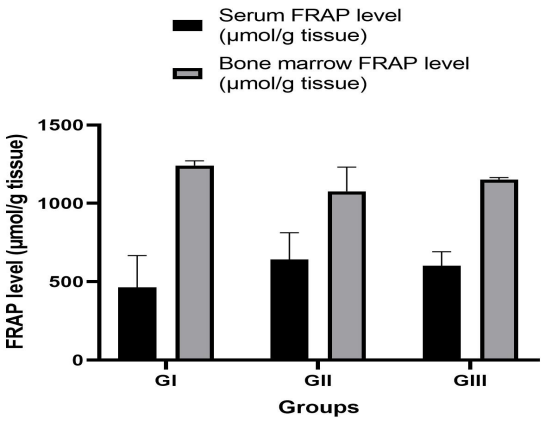

Figure 4. The serum and bone marrow FRAP levels in control (GI) and treatment groups for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII).

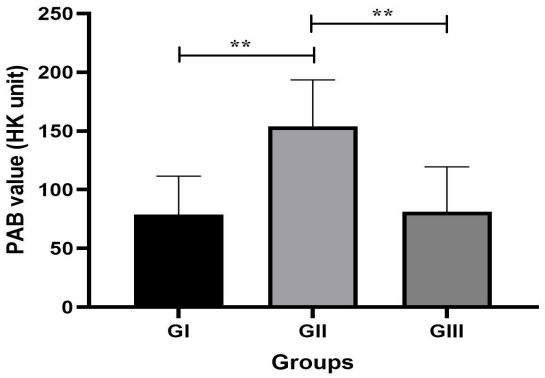

Figure 5. The bone marrow PAB in control (GI) and treatment groups for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII). Values are means ± SD (n =7). ** P < 0.01.

Discussion

Since antioxidant activity of flavonoids attenuates oxidative damage and cell death, in this study, it was attempted to investigate antioxidant and therapeutic effects of quercetin on the reduction of oxidative stress induced by vinblastine. Exposure to vinblastine generates Reactive Oxygen Species (ROS). It has been proved that flavonoids including quercetin neutralized ROS by directly reacting with superoxide anion, nitric oxide (NO), and peroxynitrite.[24,25] Vinblastine related oxidative stress leads to the production of lipid peroxidation in red blood cells as well as oxidative stress in the bone marrow. RBC damage caused by oxidative stress is generally thought to occur as a result of two procedures: oxidation of hemoglobin, followed by conversion of met hemoglobin to hemi chromes, and

Table 2. MCV and MCH levels in control (GI) and treatment groups for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII).

Test	GI	GII	GIII	Chi-square	P-value
MCV (pg)	56±5	71±15*	58±6	5.94	0.05
MCH (%)	19±1	23±3**	20±1	4.91	0.08

the membrane components disrupting due to free radical attack. These procedures can be prevented by the addition of flavonoids.[26] Other effects of oxidative stress include decreasing blood components such as RBC, Hemoglobin, and Hematocrit, and increasing blood components like WBC, MCV, and MCH. Collectively, anemia is one of the side effects of this drug used in chemotherapy.[27,28] The effects of the antioxidant defense mechanism of quercetin as well as its dose and tissue-dependent effects have been reported in previous studies.[29-31] According to the literature preventive and therapeutic potential of quercetin in several research model has been proved. Furthermore, protective activity of quercetin against toxic agents and several drugs has been mentioned.[31]

According to the results of this study, treatment with quercetin increased RBC, Hemoglobin, and Hematocrit. In contrast, these blood components decreased in the group receiving vinblastine compared to the control group, which was in agreement with those reported in the study by Cesquini *et al.*[26] who showed that quercetin effectively prevented red blood cell oxidation, reduced hemoglobin oxidation by 30%, and increased oxyhem oglobin levels. Gargi Sen *et al.*[32] reported that flavonoids were effective in repairing hemoglobin reduction in Leishmania-induced anemia in animals, with quercetin being the most successful compound among the 5 studied compounds regarding prevention of oxidation of cell membrane proteins and lipids in infected animals. It also had a more significant effect on Leishmania-induced anemia, increased life-span of red blood cells, which reduces due to osmotic fragility, and significantly increased hemoglobin levels as well compared to other compounds. It is noteworthy that, splenomegaly was observed in the group receiving quercetin along with vinblastine, indicating the body's attempt for further hematopoiesis and anemia compensation.

Inhibition of lipid peroxidation is one of the other protective effects of quercetin.[33] This observation is in agreement with our findings regarding the inhibition of lipid peroxidation after treatment with quercetin in rats. MDA, as an end-product of lipid peroxidation, may be used as an indicator of oxidative stress that increases in the body after administration of vinblastine. Results also showed that the use of quercetin compound reduced malondialdehyde levels in the bone marrow compared to the group receiving vinblastine, which was similar to the results of previous studies,[34,35] showing that quercetin partially protects blood glutathione and leads to suppression of plasma malondialdehyde levels, as well as nitric oxide metabolites and the production of superoxide anion. Collectively, the results of the present study showed that chronic administration of quercetin in rats caused inhibition of lipid peroxidation. Quercetin also increased total antioxidant capacity (TAC) in the bone marrow compared to the group receiving vinblastine. Kumiko Ishige *et al.*[35] showed that quercetin reduced the levels of free radicals and increased the levels of TAC. However, results of the Ferric Reducing Antioxidant Power (FRAP) tests of bone marrow and serum were not significant, which were in agreement with those obtained in the study by Agnieszka Cierniak *et al.*[36] that elevated FRAP content in the quercetin-treated group might be due to its free radical scavenging property. Oxidative stress-induced by oxygen-derived free radicals is a disturbance in the pro-oxidant antioxidant balance.[37]

Pro-oxidant antioxidant balance is important in the development of oxidative stress-related diseases such as anemia. Results of this study showed that the PAB parameter in the group receiving vinblastine was significantly different from the control group and the group receiving quercetin along with vinblastine, which was similar to the results obtained in the study by Choi *et al.* who stated that quercetin is considered not only as an antioxidant but also as a pro-oxidant in rats.[29] One of the reasons for lack of finding significant results regarding FRAP, which is an oxidative stress marker may have been an inadequate dose of quercetin used or a small sample size, certainly disturbing significance indicators of statistical tests which is among limitations of this study. Therefore, researchers are certainly recommended to consider appropriate and adequate sample sizes in similar studies. Finally one of the side effects of chemotherapy agents is dehydration and our results are in line with the other studies.[38] Treatment with quercetin could improve water intake that could be mentioned for ameliorating chemotherapy side effects.

Conclusion

The findings of the study showed that quercetin at a dose of 20 mg/kg reduced the effects of oxidative stress in rats receiving vinblastine, which could be effective in improving oxidative stress and subsequent anemia. Due to attention to flavonoids such as quercetin, preventive and therapeutic applications of the molecules is noteworthy. The multitude of pre-clinical studies has allowed more in-depth studies, including the development of increasingly specific and targeted clinical studies. To fully understand the mechanism of quercetin function, pre-clinical and clinical studies are suggested to be conducted at whole body, tissue and cell surface.

Acknowledgments

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Ethical Issues

The study was approved by the Deputy of Research and Technology and Ethics Committee of Birjand University of

Medical Sciences (Ethics code: IR.BUMS.REC.1398.156). Ethical considerations were considered at all stages of the research. Ethical considerations related to laboratory animals included choosing right animal species for this study, using a minimum number of animals for the study as much as possible, providing appropriate living conditions for the animals, training researchers to understand the animal's life and physiological conditions (nutrition, health, disease, discomfort and pain, and other physiological and pathological changes in the animal), not using sick animals in the experiment, and finally adhering to ethical protocols in blood sampling and providing conditions for euthanasia of the animals at the end of the study.

Author Contributions

HA and HEZ: Participated in the acquisition, analysis, or interpretation of data for the work. MH: Designed the study and participated in data analysis. All authors participated in drafting the work or revising it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Arnold EJ, Childress MO, Fourez LM, Tan KM, Stwart JC, Bonney PL et al. Clinical trial of vinblastinein dogs with transitional cell carcinoma of the urinarybladder. J Vet Intern Med. 2011;25(6):1385-90. doi:10.1111/j.1939-1676.2011.00796.x
2. Rtibi K, Grami D, Selmi S, Amri M, Sebai H, Marzouki L. Vinblastine, an anticancer drug, causes constipation and oxidative stress as well as others disruptions in intestinal tract in rat. Toxicol Rep. 2017;4:221-5. doi:10.1016/j.toxrep.2017.04.006
3. Cordova E, Morganti L, Odzak A, Arcondo F, Silva M, Zylberman M, et al. Severe hypokalemia due to a possible drug–drug interaction between vinblastine and antiretrovirals in a hiv-infected patient with hodgkin's lymphoma. Intl J STD AIDS. 2017;28(12):1259-62. doi:10.1177/0956462417703026
4. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev Cancer. 2004;4(4):253. doi:10.1038/nrc1317
5. Kirsch-Volders M, Parry E. Genetic toxicology of mitotic spindle inhibitors used as anticancer drugs. Mutat Res-Fund Mol M. 1996;355(1-2):103-28. doi:10.1016/0027-5107(96)00025-5
6. Aksoy M, Erdem S, Dinçol K. Megaloblastic anemia in the course of vinblastine sulphate (velbe) therapy. Blut 1969;19(2):57-63. doi:10.1007/BF01633087
7. Kumar A. Vincristine and vinblastine: A review. Int J Med Pharm Case Reports. 2016;6(1):23-30.
8. Dicato M, Plawny L, Diederich M. Anemia in cancer. Ann Oncol. 2010;21(suppl_7):vii167-72. doi:10.1093/annonc/mdq284
9. Rodgers GM, Becker PS, Bennett CL, Cella D, Khan AAC, Chesney C, et al. Cancer-and chemotherapy-induced anemia. J Natl Compr Canc Ne. 2008;6(6):536-64. doi:10.6004/jnccn.2008.0042
10. Prakash D. Anemia in the icu: Anemia of chronic disease versus anemia of acute illness. Crit Care Clin. 2012;28(3):333-43. doi:10.1016/j.ccc.2012.04.012
11. Roberts DJ, Zygun DA. Anemia, red blood cell transfusion, and outcomes after severe traumatic brain injury. Crit Care. 2012;16(5):154. doi:10.1186/cc11489
12. Fibach E, Rachmilewitz E. The role of oxidative stress in hemolytic anemia. Curr Mol Med. 2008;8(7):609-19. doi:10.2174/156652408786241384
13. Bule M, Abdurahman A, Nikfar S, Abdollahi M, Amini M. Antidiabetic effect of quercetin: A systematic review and meta-analysis of animal studies. Food Chem Toxicol. 2019;125:494-502. doi:10.1016/j.fct.2019.01.037
14. Frankel E, German J, Kinsella J, Parks E, Kanner J. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. The Lancet. 1993;341(8843):454-7. doi:10.1016/0140-6736(93)90206-V
15. Dias TA, Duarte CL, Lima CF, Proenca MF, Pereira-Wilson C. Superior anticancer activity of halogenated chalcones and flavonols over the natural flavonol quercetin. Eur J Med Chem. 2013;65:500-10. doi:10.1016/j.ejmech.2013.04.064
16. Kundur S, Prayag A, Selvakumar P, Nguyen H, McKee L, Cruz C, et al. Synergistic anticancer action of quercetin and curcumin against triple-negative breast cancer cell lines. J Cell Physiol. 2019;234(7):11103-18. doi:10.1002/jcp.27761
17. Bors W, Heller W, Michel C, Saran M. flavonoids as antioxidants: Determination of radical-scavenging efficiencies. Method Enzymol. 1990;186:343-55. doi:10.1016/0076-6879(90)86128-I
18. Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castelli F. Flavonoids as antioxidant agents: Importance of their interaction with biomembranes. Free Radic Biol Med. 1995;19(4):481-6. doi:10.1016/0891-5849(94)00240-K
19. Cotelle N, Bernier J-L, Catteau J-P, Pommery J, Wallet J-C, Gaydou EM. Antioxidant properties of hydroxy-flavones. Free Radic Biol Med. 1996;20(1):35-43. doi:10.1016/0891-5849(95)02014-4
20. Baghel SS, Shrivastava N, Baghel RS, Agrawal P, Rajput S. A review of quercetin: Antioxidant and anticancer properties. World J Pharm Pharmaceutical Sci. 2012;1(1):146-60.
21. Benzie IF, Strain JJ. The ferric reducing ability of plasma (frap) as a measure of "antioxidant power": The frap assay. Anal Biochem. 1996;239(1):70-6. doi:10.1006/abio.1996.0292

22. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86(1):271-8. doi:10.1016/0003-2697(78)90342-1
23. Lapenna D, Ciofani G, Pierdomenico SD, Giamberardino MA, Cuccurullo F. Reaction conditions affecting the relationship between thiobarbituric acid reactivity and lipid peroxidesin human plasma. Free Radic Biol Med. 2001;31(3):331-5. doi:10.1016/S0891-5849(01)00584-6
24. Korkina LG, Afanas' Ev IB. Antioxidant and chelating properties of flavonoids. Adv Pharmacol. 1996;38:151-63. doi:10.1016/S1054-3589(08)60983-7
25. Vanacker SA, Tromp MN, Haenen GR, Vandervijgh W, Bast A. Flavonoids as scavengers of nitric oxide radical. Biochem Biophys Res Commun. 1995;214(3):755-9. doi:10.1006/bbrc.1995.2350
26. Cesquini M, Torsoni M, Stoppa Gt-, Ogo St-. T-booh-induced oxidative damage in sickle red blood cells and the role of flavonoids. Biomed Pharmacother. 2003;57(3-4):124-9. doi:10.1016/S0753-3322(03)00018-0
27. Saka B, Kombaté K, Mouhari-Toure A, Akakpo S, Balaka A, Pitché P, et al. [Evaluation of the treatment of kaposi's sarcoma with vinblastine in togo: A study of 23 cases]. Bull Soc Pathol Exot. 2011;104(5):339-41. French. doi:10.1007/s13149-011-0140-x
28. Skapek SX, Ferguson WS, Granowetter L, Devidas M, Perez-Atayde AR, Dehner LP, et al. Vinblastine and methotrexate for desmoid fibromatosis in children: Results of a pediatric oncology group phase ii trial. J Clin Oncol. 2007;25(5):501-6. doi:10.1200/JCO.2006.08.2966
29. Choi EJ, Chee K-M, Lee BH. Anti-and prooxidant effects of chronic quercetin administration in rats. Eur J Pharmacol. 2003;482(1-3):281-5. doi:10.1016/j.ejphar.2003.09.067
30. Papiez M, Cierniak A, Krzysciak W, Bzowska M, Taha H, Józkowicz A, et al. The changes of antioxidant defense system caused by quercetin administration do not lead to DNA damage and apoptosis in the spleen and bone marrow cells of rats. Food Chem Toxicol. 2008;46(9):3053-8. doi:10.1016/j.fct.2008.06.006
31. Pingili RB, Challa SR, Pawar AK, Toleti V, Kodali T, Koppula S. A systematic review on hepatoprotective activity of quercetin against variou s drugs and toxic agents: Evidence from preclinical studies. Phytother Res. 2020;34(1):5-32. doi:10.1002/ptr.6503
32. Sen G, Mandal S, Roy SS, Mukhopadhyay S, Biswas T. Therapeutic use of quercetin in the control of infection and anemia associated with visceral leishmaniasis. Free Radic Biol Med. 2005;38(9):1257-64. doi:10.1016/j.freeradbiomed.2005.01.014
33. Sorata Y, Takahama U, Kimura M. Protective effect of quercetin and rutin on photosensitized lysis of human erythrocytes in the presence of hematoporphyrin. Biochim Biophys Acta. 1984;799(3):313-7. doi:10.1016/0304-4165(84)90276-9
34. Cebecioglu R, Yildirim M, Akagunduz D, Korkmaz I, Tekin H, Atasever-Arslan B, et al. Synergistic effects of quercetin and selenium on oxidative stress in endometrial adenocarcinoma cells. Bratisl Lek Listy. 2019;120(6):449. doi:10.4149/BLL.2019.072
35. Ishige K, Schubert D, Sagara Y. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radic Biol Med. 2001;30(4):433-46. doi:10.1016/S0891-5849(00)00498-6
36. Cierniak A, Papiez M, Kapiszewska M. Modulatory effect of quercetin on DNA damage, induced by etoposide in bone marrow cells and on changes in the activity of antioxidant enzymes in rats. Rocz Akad Med Bialymst. 2004;49(Suppl 1):167-9.
37. Olisekodiaka M, Igbeneghu C, Onuegbu A, Oduru R, Lawal A. Lipid, lipoproteins, total antioxidant status and organ changes in rats administered high doses of cadmium chloride. Med Prin Pract. 2012;21(2):156-9. doi:10.1159/000333385
38. Manto Chagas C, Alisaraie L. Metabolites of vinca alkaloid vinblastine: tubulin binding and activation of nausea-associated receptors. ACS Omega. 2019;4(6):9784-99. doi:10.1021/acsomega.9b00652