ORIGINAL ARTICLE

Synergistic Effects of Red Spinach and Chrysanthemum Flower Ethanol Extracts on Hemoglobin and Hepcidin Levels in Vivo

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ABSTRACT

Introduction: Anemia is a malnutrition condition with high prevalence in the world, especially in developing countries. Adequate intake of macro and micro nutrients is absolutely necessary, in the form of food consumption or extracts. This study aims to analyze the effect of a combination of red spinach and chrysanthemum flower ethanol extracts on hemoglobin and hepcidin levels. **Materials and methods:** This research design uses a laboratory experimental design with *a posttest -only control group design in vivo*. This study used 24 teenage female Wistar rats (*ratus norvegicus*) divided into 6 groups. The treatment was administration of EEBM+EEBK in 4 doses, EEBM 300 mg + EEBK 300 mg, EEBM 600 mg + EEBK 300 mg, EEBM 900 mg + EEBK 300 mg and EEBM 1200 mg + EEBK 300 mg. The evaluation of hemoglobin and hepcidin levels was carried out for nine days. Research data is displayed in the form of Mean ± SD. The research results were analyzed using one-way ANOVA with Tukey's Post-Hoc test. **Results:** The results of the One-Way ANOVA statistical test showed that there were significant differences for Hb (p=0.000) and hepcidin (p=0.007). The most significant dose capable of increasing Hb levels and reducing hepcidin levels was combination of EEBM 600 mg + EEBK 300 mg. **Conclusion:** The combination of red spinach and chrysanthemum flower ethanol extracts shows promise as an alternative for managing hemolytic anemia in adolescents. However, further research directly on human subjects is warranted to validate these findings.
Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP7): 52-57. doi:10.47836/mjmhs.21.s7.7

Keywords: Red spinach, Hemoglobin, Hepcidin, Anemia, Chrysanthemum

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INTRODUCTION

Adequate nutritional intake is needed from childhood to adolescence which is necessary for optimal growth (1). Malnutrition can result not only from inadequate intake of macronutrients, but also from deficiencies in micronutrients. This condition can significantly increase morbidity and mortality rates, particularly in developing countries where limited purchasing power restricts access to nutritious foods and supplements needed to meet essential nutritional requirements (2). Adequate nutrition in terms of micronutrients consists of vitamins and minerals, essential micronutrients needed by the body include iron, vitamins and minerals which cannot be produced by the body and require intake from food or supplements (3).

Iron is essential for producing haemoglobin and the function of many enzymes. One of the impacts of iron deficiency can be disruption of the menstrual process, growth and development disorders, reproductive organ maturation disorders, risk of giving birth to low birth weight babies and anemia where the number of red blood cells and oxygen-carrying capacity does not meet the body's physiological (4). Anemia is a global public health problem that can occur at all ages (5). The incidence of anemia in girls is 21.1%, women of childbearing age 48% (6) . The demographic and health survey in Ethiopia showed that the prevalence of anemia among children under five in 2016 was 57% due to iron deficiency (7) . In Sub-Saharan Africa, 46%-71% of children under 5 years suffer from anemia (80). In Egypt, the Middle East, the prevalence of anemia in children is 39.6% and in adolescents it is around 11.6% (9) . In 2023, prevalence of anemia among adolescence girl in Indonesia as a local study was 28% (10). This high prevalence requires more attention to reduce the negative impacts, especially on the reproduction process.

Heme oxygenase (HOs) are responsible for catalyzing heme into carbon monoxide (CO), biliverdin, and free iron (1) which functions to mediate several

physiological functions by reducing the production of pro-inflammatory cytokines and stimulating the production of anti-inflammatory cytokines. Hepcidin is a type II acute phase inflammatory response protein in response to interleukin (IL)-63, which is the main regulator of iron balance in the blood and bone marrow (11) . Hepcidin deficiency causes a decrease in the number of mature erythrocytes which causes anemia so that iron absorption in the intestine becomes too high and causes hemochromatosis (12) .

Red spinach is one of non-pharmacological treatment to threat and prenevent the occurance of anemia (14). Red spinach is a natural ingredient that is easy to obtain, relatively cheap and has nutritional value and is easy to cultivate in tropical climates (13) . The iron content of 7 mg in red spinach and vitamin C 62 mg can increase hemoglobin levels in the blood (14) . Red spinach contains vitamins (C, A, B2, B6, K, and folate), protein, carbohydrates, fat, minerals, fiber, iron, magnesium, manganese, potassium, and calcium (15) . Red spinach is a source of vitamins C, B6, riboflavin, folate, niacin, soluble fiber, omega-3 fatty acids , and iron, increases blood formation, increases appetite, helps recovery from fatigue, antioxidants, alternative nutritional therapy to increase hemoglobin levels and prevent anemia (16).

Plants in the Chrysanthemum genus belong to the Asteraceae family and have a variety of potential medicinal properties including antibacterial, antiviral, and anti-inflammatory activities (17). Chrysanthemum flowers originate from China since ancient times and have been used as herbal medicine (18). Chrysanthemum flowers as an alternative herbal treatment contain vitamins and minerals including vitamin B, vitamin C and various antioxidants such as polyphenols, flavonoids, lutein and zeaxanthine (19). The vitamin C content in chrysanthemum flower extract can increase the number of hemoglobin and red blood cell it by increasing leads metabolism and preventing deposition (20). This study aims to analyze the effect of a combination of red spinach and chrysanthemum flower ethanol extracts on hemoglobin and hepcidin levels in juvenile female Wistar rats.

MATERIALS AND METHODS

Samples

The red spinach (Amaranthus tricolor L.) used in this study was sourced from Malang City, Indonesia, specifically the leaves and stems, which were extracted. The chrysanthemum flowers (Chrysanthemum morifolium) were obtained from Batu City, Indonesia, and the extraction included the crown, petals, stamens, ovules, and flower base. Both the red spinach ethanol extract (EEBM) and chrysanthemum flower ethanol extract (EEBK) were prepared using maceration method with 70% ethanol as the solvent. The combination of EEBM+EEBK is made in 4 different doses, including: dose 1 (EEBM 300 mg + EEBK 300 mg), dose 2 (EEBM 600 mg + EEBK 300 mg), dose 3 (EEBM 900 mg + EEBK 300 mg), and dose 4 (EEBM 1200 mg + EEBK 300 mg). This research was approved by the Health Research Ethics Committee, Faculty of Medicine, Islamic University of Malang [Reference No: 043/LE.003/VII/01/2022].

Animals

The experimental animals used were female wistar rats (Rattus norvegicus) of juvenile/reproductive age (6-8 weeks), obtained from the Animal Physiology Laboratory, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia. Rats were acclimated for 7 days to water, food and temperature conditions in the laboratory before being used for research.

Induction of anemia in rats was carried out by intraperitoneal injection of phenylhydrazine /PHZ (Merck, Japan) at a dose of 40 mg/kg BW (diluted with Normal Saline). PHZ injection was carried out 2 times in 2 consecutive days with the same dose, namely 40 mg/kg BW (21). The normal hemoglobin range for female rats is 13.9-15.9 g/dL (24).

Research Design and Treatment

This research design uses a laboratory experimental design with an in vivo posttest-only control group design. This study used 24 randomly selected juvenile Wistar rats (Ratus novergicus) divided into 6 groups. The determination of the rat number refers to the Federer formula. Both EEBM and EEBK were prepared using maceration method with 70% ethanol as the solvent. The treatment in this study was the administration of EEBM+EEBK in 4 doses orally (1cc/day). Group 1 (negative control) was not induced by anemia and was not treated. Group 2 (positive control) was induced by anemia and given sterile water. Group 3 was induced anemia and given EEBM 300 mg + EEBK 300 mg (dose 1). Group 4 was induced anemia and given EEBM 600 mg + EEBK 300 mg (dose 2). Group 5 was induced anemia and given EEBM 900 mg + EEBK 300 mg (dose 3). Group 6 was induced anemia and given EEBM 1200 mg + EEBK 300 mg (dose 4). The treatment was administered for 9 days. Since the erythropoiesis process in rats typically takes 5 to 7 days, an increase in hemoglobin levels may be observed after 7 days of treatment (23).

Hemoglobin (Hb) and Hepcidin levels

Hb and Hepcidin levels were checked at 2 times. The first measurement was after the rats were injected with PHZ (day 2). The second measurement is after 7 days of treatment (day 9). Blood samples for testing Hb levels were taken from the lateral tail vein of rats. Hb levels were checked using a Hb meter (EasyTouch GCHb, Bioptik Technology Inc, China) and the results were displayed in g/dL. Blood samples for the hepcidin test are taken through the eye (orbital sinus) with a capillary tube. Hepcidin levels were measured using the Rat

Hepcidin Elisa Kit (BT Lab, China), and then run using ELISA (ChemWell® 2910). Results are displayed in ng/ml.

Statistical test

Research data are presented as Mean + SD. The results were analyzed using one-way ANOVA followed by Tukey's Post-Hoc test (SPSS version 23). A p-value of <0.05 was considered statistically significant.

RESULTS

Hemoglobin (Hb) levels are checked using an Hb meter and the results are displayed in g/dL. The results showed that the combination of EEBM+EEBK was able to increase Hb levels in anemic rats. The mean of Hb levels in rats after treatment are shown in Figure 1. The average Hb level of the positive control group was 11.9 ± 0.53 g/dL. Meanwhile, the average Hb level of the negative control group was 18.37 ± 2.08 g/dL. After being given treatment, Hb levels began to increase at dose 1, namely 18.8 ± 0.79 g/dL. The highest Hb level was obtained when administering dose 2, namely 19.63 ± 1.47 g/dL. Then the Hb level decreased at dose 3, namely 18.27 ± 2.17 g/dL and increased slightly at dose 4, namely 18.87 ± 1.53 g/dL. Even though it decreased, Hb levels at doses 3 and 4 were still within normal limits. The effect of the EEBM+EEBK combination on Hb levels in anemic rats was tested statistically using One-Way ANOVA and Tukey's post-hoc test. Levene's test was conducted first before that and the results showed that the data was homogeneous (p=0.083). Table I shows the results of the One-Way ANOVA statistical test. It showed that there was a significant difference (p=0.000). Based on Tukey's post-hoc test, the most significant dose capable of increasing Hb levels was dose 2 (combination of EEBM 600 mg + EEBK 300 mg) (Table III).

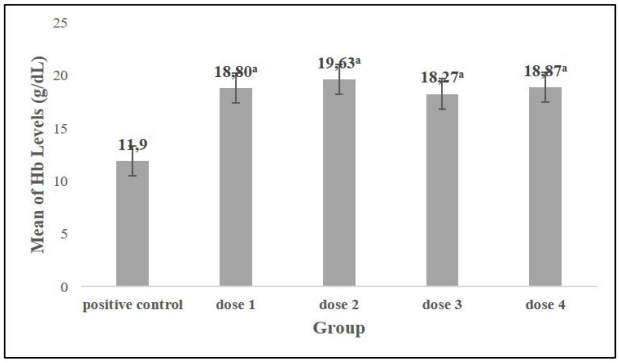

Figure 1: Effect of the RSEE+CFEE combination on Hb levels in anemic rats. Hb levels are expressed in mean±SD (g/dL). The result of the One-Way ANOVA statistic test showed a significant difference (*p*=0.000).

Table I: The results of the One-way ANOVA test for the mean of hemoglobin level in rats after giving treatment in 4 doses

		Sum of Square	df	Mean Square	F	p-value
Hemoglobin Level	Between Groups	120.1693	4	30.0423	14.7992	0.000*
	Within Groups	20.3	10	2.03		
	Total	140.4693	14	10.0335		

Note: (*) p<0.05 was considered to be significant

Table III: The results of the Post-Hoc Tukey test for the mean of hemoglobin level in rats after giving treatment in 4 doses

Group	Group	Difference Mean	p-value
Positive Control	Dose 1	6.90	0.001*
	Dose 2	7.73	0.000*
	Dose 3	6.36	0.001*
	Dose 4	6.96	0.000*
Dose 1	Dose 2	0.83	0.948
	Dose 3	0.53	0.989
	Dose 4	0.06	1
Dose 2	Dose 3	1.36	0.765
	Dose 4	0.76	0.960
Dose 3	Dose 4	0.60	0.984

Note: (*) p<0.05 was considered to be significant

Apart from increasing Hb levels, the combination of EEBM+EEBK can reduce hepcidin levels in anemic rats. Hepcidin levels were measured using ELISA and the results were expressed in ng/ml. The mean of hepcidin levels in rats after treatment are shown in Figure 2. The average hepcidin level of the positive control group was 0.54 ± 0.08 ng/ml. Meanwhile, the average Hb level of the negative control group was 0.44 ± 0.04 ng/ml. Hepcidin levels began to decrease at dose 1, namely 0.31 ± 0.07 ng/ml. These results were the same as dose 4. Meanwhile, hepcidin levels at dose 2 were 0.36 ± 0.05 ng/mL and 0.35 ± 0.07 ng/mL at dose 3. The effect of the EEBM+EEBK combination on hepcidin levels in anemic mice was tested statistically using One-Way ANOVA and Tukey's post-hoc test with Levene's test was performed beforehand. The result of the Levene's test showed that the data was homogeneous (p=0.083). The results of the One-Way ANOVA statistical test showed a significant difference (p=0.007) (Table II). Based on Tukey's post-hoc test, the most significant dose capable of reducing hepcidin levels was dose 1 (combination of EEBM 300 mg + EEBK 300 mg) (Table IV). However, the hepcidin level closest to the negative control (0.44 ng/mL) was 0.36 ng/mL (dose 2) (Figure 2).

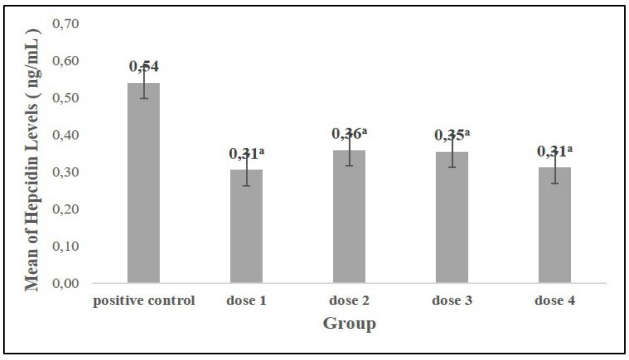

Figure 2: Effect of the RSEE+CFEE combination on hepcidin levels in anemic rats. Hepcidin levels are expressed in mean±SD (ng/mL). The result of the One-Way ANOVA statistic test showed a significant difference (*p*=0.007).

Table II: The results of the One-way ANOVA test for the mean of hepcidin level in rats after giving treatment in 4 doses

		Sum of Square	df	Mean Square	F	p-value
Hepcidin Level	Between Groups	0.1099	4	0.0274	6.6507	0.007*
	Within Groups	0.0413	10	0.0041		
	Total	0.1512	14	0.0108		

Note: (*) p<0.05 was considered to be significant

Table IV: The results of the Post-Hoc Tukey test for the mean of hepcidin level in rats after giving treatment in 4 doses

Group	Group	Difference Mean	p-value
Positive Control	Dose 1	0.23	0.008*
	Dose 2	0.18	0.039*
	Dose 3	0.18	0.034*
	Dose 4	0.22	0.009*
Dose 1	Dose 2	0.05	0.845
	Dose 3	0.04	0.879
	Dose 4	0.01	1
Dose 2	Dose 3	0.00	1
	Dose 4	0.05	0.892
Dose 3	Dose 4	0.04	0.920

Note: (*) p<0.05 was considered to be significant

DISCUSSION

The type of anemia in this study was hemolytic anemia by administering phenilhydrazine (PHZ) to adolescent mice. PHZ is a redox agent that causes hemolytic anemia even in individuals without erythrocyte enzyme deficiency (21). Hemolytic anemia is anemia caused by damage to red blood cells before 120 days. This premature damage can occur intracellularly or extracellularly in the reticuloendothelial system (22). When PHZ interacts with red blood cells, it will produce hydrogen peroxide which oxidizes the suflohydryl group of enzymes, membrane lipid peroxidation occurs , forming Heinzs bodies in erythrocytes and inducing hemolysis (23) (25).

The combination of EEBM and EEBK extracts can significantly improve hemolytic anemia in adolescent mice. The combination of these extracts can increase Hb levels and reduce hepcidin levels with the best dose being 600 EEBM + 300 EEBK. Hb and hepcidin levels have a negative relationship. Hepcidin is known as a hormone that inhibits the absorption of Fe by the body. This hormone ensures that absorption is not excessive, which causes toxicity. From the research results, the decrease in hepcidin levels is a positive thing so that Hb levels can increase. It is crucial to note that excessive extract administration can be detrimental. The optimal combination doses for achieving normal hepcidin levels were found to be 600 EEBM + 300 EEBK and 900 EEBM + 300 EEBK (27).

Hepcidin plays an important role in systemic iron homeostasis (25). High hepcidin levels may lead to iron restriction in inflammatory conditions. Therefore, hepcidin antagonists can increase iron levels to treat inflammatory anemia and hemolytic anemia (26) (31). Through spectrometric tests in previous research, the combination of EEBM and EEBK contains relatively high levels of antioxidants with active compounds of flavonoids and phenols. The combination of EEBM and EEBK produces a total flavonoid content (TFC) of 85.33 mg QE/g and a total phenol content (TFC) of 25.22 mg GAE/g. (28) Flavonoids can increase the action of vitamin C, which plays an important role in preventing and managing anemia. Vitamin C can reduce Ferric (Fe3+) to Ferro (Fe2+) so that it is more easily absorbed in the intestine and increases hemoglobin levels (32).

Flavonoid and polyphenol compounds plays a role in increasing iron absorption, reducing iron excretion, and increasing the deposition of excess iron in tissues. Rutin (quercetin-3-rhamnosyl glucoside) is a flavone that plays a role in protecting vascular endothelium against oxidative stress in sickle cell anemia , restoring erythrocyte membrane integrity, preventing and reversing lipid peroxidation, inducing an increase in GSH and CAT levels, reducing SOD activity and modulating deoxy- hemoglobin and changes in redox homeostasis. The flavonol found in red spinach and chrysanthemum flowers is quercetin which is known for its antioxidant and anti-inflammatory activity. Quercetin affects iron homeostasis and helps combat oxidative stress thereby increasing iron storage and inducing overexpression of hepcidin (33).

The combination of EEBM+EEBK contains flavonoids and vitamin C. Flavonoids function to reduce inflammation, bind iron and reduce oxidative damage and are independent of hepcidin pathway. Vitamin C has almost the same function as flavonoids but through the hepcidin pathway. It makes this combination of extracts effective for treating hemolytic anemia (34). The limitation of this study is that we used rat model with hemolytic anemia. In fact, the most common cases of

anemia are iron deficiency anemia. We recommend that further studies use rat model of iron deficiency anemia.

CONCLUSION

The combination of 600 EEBK and 300 EEBM can significantly increases hemoglobin (Hb) levels while reducing hepcidin levels. This suggests that this combination could serve as an effective alternative for managing hemolytic anemia in teenager. However, further research is necessary to evaluate the effect of direct administration of extract in humans or to develop it in a supplement that is convenient for adolescent consumption.

ACKNOWLEDGEMENT

Thank you to STIKES Widyagama Husada who has provided funding for this research.

REFERENCES

1. Awasthi S, Kumar D, Dixit S, Mahdi AA, Gupta B, Agarwal GG, et al. Association of dietary intake with micronutrient deficiency in Indian school children: a cross-sectional study. J Nutr Sci. 2023;12(e104):1–10. doi:10.1017/jns.2023.83
2. Darnton-hill I, Webb P, Harvey PWJ, Hunt JM, Dalmiya N, Chopra M, et al. Micronutrient deficiencies and gender : social and economic costs. Am J Clin Nutr. 2005;81(5):1198S-1205S. doi: 10.1093/ajcn/81.5.1198
3. Allen LH, Rosado JL, Casterline JE, Lypez P, Mucoz E, Garcia OP, et al. Lack of hemoglobin response to iron supplementation in anemic Mexican preschoolers with multiple micronutrient deficiencies. Am J Clin Nutr. 2000;71(6):1485–1494. doi: 10.1093/ajcn/71.6.1485
4. Fentie K, Wakayo T, Gizaw G. Prevalence of Anemia and Associated Factors among Secondary School Adolescent Girls in Jimma Town , Oromia Regional State , Southwest Ethiopia. Anemia. 2020;2020:1-11. doi: 10.1155/2020/5043646
5. Sari P, Tina R, Judistiani D, Hilmanto D. Iron Deficiency Anemia and Associated Factors Among Adolescent Girls and Women in a Rural Area of Jatinangor , Indonesia. International Journal of Women's Health. 2022;14:1137–1147. doi: 10.2147/IJWH.S376023
6. Wagner KS, Ronsmans C, Thomas SL, Calvert C, Adler A, Ganaba R, et al. Women who experience obstetric haemorrhage are at higher risk of anaemia, in both rich and poor countries. Trop Med Int Heal. 2012;17(1):9–22. doi: 10.1111/j.1365-3156.2011.02883.x
7. Hassen HY, Ali JH, Gebreyesus SH, Endris BS, Temesgen AM. National incidence, prevalence and disability-adjusted life years (DALYs) of common micronutrient deficiencies in Ethiopia from 1990 to 2017: estimates from the global burden of diseases study. Glob Health Action. 2020;13(1):1-11. doi: 10.1080/16549716.2020.1776507
8. Harika R, Faber M, Samuel F, Mulugeta A, Kimiywe J, Eilander A. Are Low Intakes and Deficiencies in Iron , Vitamin A , Zinc , and Iodine of Public Health Concern in Ethiopian , Kenyan , Nigerian , and South African Children and Adolescents?. Food Nutr Bull. 2017;38(3):405-427. doi: 10.1177/0379572117715818
9. Hwalla N, Salem A, Dhaheri A, Radwan H, Alfawaz HA, Fouda MA, et al. The Prevalence of Micronutrient Deficiencies and Inadequacies in the Middle East and Approaches to Interventions. Nutrients. 2017;9(3):1–28. doi: 10.3390/nu9030229
10. Kemenkes R. Survei Kesehatan Indonesia (SKI). Kota Kediri Dalam Angka. 2023;1–68. Available from : https://kedirikota.bps.go.id/id/publication/2023/02/28/c56fb1d4ceef84eb488b8699/kota-kediri-dalam-angka-2023.html
11. Schaetzel T, Sankar R. Effects of micronutrient deficiencies on human health: Its status in South Asia. J Crop Prod. 2008;6(1):55–98. doi: 10.1300/J144v06n01_06
12. Jr RTM. Pathophysiology in Medicine: Hepcidin and iron regulation in health and disease. Am J Med Sci. 2013;345(1):57–60. doi: 10.1097/MAJ.0b013e318253caf1
13. Blanchette NL, Manz DH, Torti FM, Torti S V. Modulation of hepcidin to treat iron deregulation: potential clinical applications. Expert Rev Hematol. 2016;9(2):169–186. doi: 10.1586/17474086.2016.1124757
14. Purba AC. INTERNATIONAL CONFERENCE ON The Role of Red Spinach In Increasing Iron Levels In Adolescent With Anemia : Literature Review. ICMAHS. 2024;2:46–52. Available from : https://ejournal.poltekkes-denpasar.ac.id/index.php/icmahs/article/view/3809
15. Shariff I, Dwiratna S, Yamin BM. Crystallography in agriculture: Green and red spinach (Amaranthus tricolor) grown on soil and hydroponic. IOP Conf Ser Earth Environ Sci. 2020;443(1). doi: 10.1088/1755-1315/443/1/012021
16. Solomons NW, Vossenaar M. Nutrient density in complementary feeding of infants and toddlers. Eur J Clin Nutr. 2013;67(5):501–6. doi: 10.1038/ejcn.2013.46
17. Akib MA, Syatrawati S, Prayudyaningsih R, Antonius S, Kuswinanti T. Effect of AMF propagule dosage forms on the growth and production of Amaranthus tricolor L. E3S Web Conf. 2023;373. Available from : https://doi.org/10.1051/e3sconf/202337303015
18. Rejeki FS, Wedowati ER, Haryanta D. Nutritional quality of spinach (Amaranthus hybridus L.) cultivated using black soldier fly (hermetia illucens)

waste compost. Cogent Food Agric. 2023;9(2):1–13. Available from : https://doi.org/10.1080/23311932.2023.2279742
19. Kim Y, Sung J, Sung M, Choi Y, Jeong H sang, Lee J. Involvement of heme oxygenase-1 in the anti-inflammatory activity of Chrysanthemum boreale Makino extracts on the expression of inducible nitric oxide synthase in RAW264 . 7 macrophages. J Ethnopharmacol. 2010;131(3):550–4. doi: 10.1016/j.jep.2010.07.030
20. Yuan H, Jiang S, Liu Y, Daniyal M, Jian Y, Peng C, et al. The flower head of Chrysanthemum morifolium Ramat. (Juhua): A paradigm of flowers serving as Chinese dietary herbal medicine. J Ethnopharmacol. 2020;261:113043. doi: 10.1016/j.jep.2020.113043
21. Shao Y, Sun Y, Li D, Chen Y. Chrysanthemum indicum L.: A Comprehensive Review of its Botany, Phytochemistry and Pharmacology. Am J Chin Med. 2020;48(4):871–897. doi: 10.1142/S0192415X20500421
22. Liang WL, Gong D, Zhang WK. The Composition of chrysanthemum extracts and their pharmacological functions. STEMedicine. 2020;2(5):e69. doi: 10.37175/stemedicine.v2i5.69
23. Sheth PA, Pawar AT, Mote CS, More C. Antianemic activity of polyherbal formulation, Raktavardhak Kadha, against phenylhydrazine-induced anemia in rats. J Ayurveda Integr Med [Internet]. 2021;12(2):340–5. Available from: https://doi.org/10.1016/j.jaim.2021.02.009
24. Raabe BM, Artwohl JE, Purcell JE, Lovaglio J, Fortman JD. Effects of weekly blood collection in C57BL/6 mice. J Am Assoc Lab Anim Sci. 2011;50(5):680–5. Available from : https://pmc.ncbi.nlm.nih.gov/articles/PMC3189672/pdf/jaalas2011000680.pdf
25. Martin D S and HA 0. H. Reactions of hemoglobin with phenylhydrazine.pdf. Environ Heal Pertpectives. 1985;64(10):265–81. Available from : https://doi.org/10.1289/ehp.8564265
26. Phillips J, Henderson AC. Hemolytic Anemia, Evaluation. Am Fam Physician. 2018;98(6):354–61. Available from : https://www.ncbi.nlm.nih.gov/books/NBK558904/
27. Jain SK, Subrahmanyam D. On the mechanism of phenylhydrazine-induced hemolytic anemia. Biochem Biophys Reaseach Commun. 1978;88(4):1320–4. doi: 10.1016/0006-291x(78)90332-7
28. D'Andrea P, Giampieri F, Battino M. Nutritional Modulation of Hepcidin in the Treatment of Various Anemic States. Nutrients. 2023;15(24):5081. doi: 10.3390/nu15245081
29. Mu M, An P, Wu Q, Shen X, Shao D, Wang H, et al. The dietary flavonoid myricetin regulates iron homeostasis by suppressing hepcidin expression. J Nutr Biochem. 2016;30:53–61. doi: 10.1016/j.jnutbio.2015.10.015
30. Frazer DM, Wilkins SJ, Darshan D, Badrick AC, McLaren GD, Anderson GJ. Stimulated erythropoiesis with secondary iron loading leads to a decrease in hepcidin despite an increase in bone morphogenetic protein 6 expression. Br J Haematol. 2012;157(5):615–26. doi: 10.1111/j.1365-2141.2012.09104.x
31. Adamson JW. The anemia of inflammation/malignancy: mechanisms and management. Hematology Am Soc Hematol Educ Program. 2008;159–65. DOI: 10.1182/asheducation-2008.1.159
32. Kusbandiyah J, Angelia Puspadewi Y, Oktia Maghfiroh D. Analysis of Total Flavonoid and Phenol Content from the Combination of Red Spinach (Amaranthus tricolor L.) Ethanolic Extract and Chrysanthemum Flower (Chrysanthemum morifolium) Ethanolic Extract as a Potential Anti-anemic. J Pendidik Keperawatan Indones. 2023;9(1):37–48. Available from : https://ejournal.upi.edu/index.php/JPKI/article/view/58209/pdf
33. Siregar YI, Adelina A. Pengaruh Vitamin C terhadap Peningkatan Hemoglobin (Hb) Darah dan Kelulushidupan Benih Ikan Kerapu Bebek (Cromileptes altivelis). J Natur Indones. 2012;12(1):75. doi:10.31258/jnat.12.1.75-81
34. Cotoraci C, Ciceu A, Sasu A, Hermenean A. Natural antioxidants in Anemia treatment. Int J Mol Sci. 2021;22(4):1883. doi: 10.3390/ijms22041883
35. Imam MU, Zhang S, Ma J, Wang H, Wang F. Antioxidants mediate both iron homeostasis and oxidative stress. Nutrients. 2017;9(7):671. doi: 10.3390/nu9070671