Efficiency of using thyme (*Thymus vulgaris*) for treating anemia caused by iron deficiency in rats.

Prof.Dr. Heba Ezz El-Din Yossef
Prof of Nutrition and Food Sciences Department, Faculty of Home Economics Minufiya University

Rabiaa Ragab Ramadan Abde hameid
Faculty of Home Economics Minufiya University

Dr. Nezha Abdurrahman Khali
Assistant prof of Nutrition and Food Sciences Department, Faculty of Home Economics Minufiya University

Prof.Dr. Abeer Ahmed Khder
Prof of Nutrition and Food Sciences Department, Faculty of Home Economics Minufiya University

Efficiency of using thyme (*Thymus vulgaris*) for treating anemia caused by iron deficiency in rats.

Abstract

The objectives of this study were to use the dry thyme leaves as source of nonheme iron and evaluate its effect in treatment of iron deficiency anemia in rats. Twenty-four adult male albino rats, weighing 150 ± 5 g were divided into four groups. Gorup I: negative control group (6 rats) fed standard diet. Gorup II: anemic group (18 rats) were induced with iron deficiency anemia then divided into three sub groups(6 rats each), the first: positive control group fed standard diet, the second group fed thyme diet (iron in standard diet was replaced with 7.1mg iron (its source thyme) kg body weight/day as therapeutic daily and the third group fed thyme diet and orange juice as a source of vitamin C. All groups fed experimental diets for six weeks. Iron in diet, Fe intake, Fe feces and Fe absorption were determined at the at end of the experiment. Blood picture, serum iron (SI), serum ferritin (SF), total iron–binding capacity and transferrin saturation were also evaluated. The results indicated that rats fed thyme plus orange juice had lower ($P \leq 0.05$) Fe feces and higher Fe absorption than those fed thyme diet. The results suggest that treatment rat diets with thyme especially thyme with vitamin C improved most of blood parameters in the anemia groups.

Keywords: Thyme, Iron Absorption, Hemoglobin, orange juice.

كفاءة استخدام الزعتر كمصدر غذائي لعلاج فقر الدم الناتج عن نقص الحديد في الفئران

الملخص

هدفت هذه الدراسة إلى استخدام أوراق الزعتر الجافة كمصدر للحديد غير الهيمى وتقييم تأثيرها في علاج أنيميا نقص الحديد في الفئران. تم تقسيم أربع وعشرين فأرًا من الذكور البالغة، بوزن ١٥٠±٥ جرام، إلى أربع مجموعات. المجموعة الأولى: المجموعة الضابطة السالبة (٦ فئران) تم إطعامها غذاء قياسي. المجموعة الثانية: مجموعة الأنيميا (١٨ فأرًا) تم إصابتها بأنيميا نقص الحديد ثم تم تقسيمها إلى ثلاث مجموعات فرعية (٦ فئران في كل مجموعة)، الأولى: المجموعة الضابطة الموجبة التي تغذت على الغذاء الأساسي، الثانية: مجموعة تناولت الزعتر تم استبدال الحديد في الغذاء الأساسي بـ ٧,١ ملغ من الحديد (مصدره الزعتر) لكل كيلوجرام من وزن الجسم/يوم كجرعة علاجية والثالثة: مجموعة تناولت الزعتر مع عصير البرتقال كمصدر لفيتامين C. تلقت جميع المجموعات الغذاء لمدة ستة أسابيع. في نهاية التجربة تم تقدير الحديد في الغذاء، وفي البراز، وامتصاص الحديد. كما تم قياس صورة الدم والحديد في السيرم(SI) ، وفيريتين =(SF) السيرم، والسعة الرابطة للحديد الكلي، وإشباع الترانسفيرين. أظهرت النتائج أن الفئران التي تناولت الزعتر مع عصير البرتقال كان لديها مستوى أقل ($P \leq 0.05$) من حديد البراز وأعلى امتصاص للحديد مقارنة بتلك التي تناولت الزعتر فقط. تشير النتائج إلى أن تناول الزعتروالزعتر مع فيتامين C أدي تحسن تحاليل البيوكيميائية بالدم في مجموعات الأنيميا.

الكلمات المفتاحية: الزعتر، امتصاص الحديد، الهيموجلوبين، عصير البرتقال.

1. Introduction

Iron-deficiency anemia (IDA) is a serious condition characterized by low iron levels, which lead to anemia and the occurrence of microcytic hypochromic red blood cells in the bloodstream. The proportion of these cells indicates the extent of iron deficiency **[1].** Additionally, it presents a major health challenge, affecting one-third of the global population, particularly children, women, and the elderly **[2,3]**. Iron deficiency is a common nutritional deficiency that occurs when there is an inadequate amount of iron available to the precursors of erythrocytes **[4].** The causes of iron deficiency anemia (IDA) include malnutrition, rapid growth with insufficient dietary iron, and blood loss through the gastrointestinal tract or menstruation.Additionally, genetic factors contributing to iron-refractory iron deficiency anemia have been identified **[5].** Individuals with anemia may experience chronic fatigue, an inability to work, impaired immune response, recurrent miscarriages in pregnant women, and delayed physical and cognitive development in infants **[6].** Anemia can lead to developmental delays and behavioral disturbances in children. This syndrome has various major health repercussions for this demographic group, including loss of attention and focus, lower capacity for exercise, impaired job efficiency, and negative effects on motherhood in pregnant women **[7]**. In considering this, one of the worldwide nutrition objectives established by the WHO is to achieve a 50% decrease in the incidence of anemia among women of reproductive age by 2025**[8].** There are two basic dietary methods for treating iron-deficiency anemia: increasing the consumption of naturally rich in iron foods and improving iron bioavailability by include absorption enhancers in meals while limiting the intake of iron inhibitors **[9].** The National Institutes of Health states that the richest sources of heme iron in the diet are lean meats and seafood, while non-heme iron is primarily found in nuts, beans, vegetables, and fortified grain products **[10].** Thyme (*Thymus vulgaris L.*), originally native to the Mediterranean region, has been widely utilized in alternative medicine for its healing properties. It is a recommended plant for treating various diseases **[11].** It is an aromatic plant widely used as a spice to impart a distinctive aroma and flavor to food **[12]** has demonstrated that thyme is rich in iron, with dry thyme

leaves containing 122.7 mg of iron per 100 grams of dry matter. This high iron content makes thyme beneficial in improving iron-deficiency anemia **[13]**. Therefore, this study aims to evaluate the effect of thyme as a food source in treatment of iron deficiency anemia in rats.

Materials and Methods

Materials:

Dry thyme used in this study was sourced from Shibin El-Kom, Egypt. Casein, starch, cellulose, choline chloride, Dl-methionine, vitamins, a combination, and minerals were supplied by Morgan Co. in Cairo, Egypt. Chemical kits for the investigation were purchased from El-Gomhoria Company for Chemicals and Drugs in El-Ameria, Cairo, Egypt. Tannic acid used in the study was obtained from Segma Company in Egypt.Twenty-four Adult male albino rats, Sprague Dawley strain, weighing (150+5g) were obtained from Research Institute of Ophthalmology, Giza, Egypt.

Methods:

Preparation of Dry thyme:

Dry thyme was ground with blender then kept at -4°C until use.

Analytical Methods:

Determination of chemical composition of thyme and standard diet

Moisture, fat, protein, ash and fiber in dried thyme and standard diet were determined according to the method of **[14]**. The carbohydrates were calculated by difference.

Determination of iron and Fe Absorption

Fe in dried thyme, rat feces, and standard were determined using flame atomic absorption spectrophotometry (Model 5100 PC, Perkin-Elmer, Norwalk, CT) according to **[15]**.

Fe Absorption (%) was calculated according to **[16]** using the following equations:

$$Fe\ Absorption\ (\%) = \frac{mg\ Fe\ intake - mg\ Fe\ in\ feces}{mg\ Fe\ intake} \times 100$$

Induction of iron deficiency anemia

Rats were induced with iron deficiency anemia by feeding them a diet containing 20 g/kg of body weight of tannic acid for 2 weeks according to **[17]** at the beginning of the experiment**.** A blood sample

was taken from the rat's eye to assess the presence of iron deficiency anemia.

Experimental design:

The Science Research Ethics Committee of The Institutional Animal Care and Use Committee (IACUC) Menoufia University accepted the research protocol (MUFHE/S/NFS/10/23) of the Science Research Ethics Committee of Faculty of Home Economics.

Rats were housed separately in cage and fed standard diet according to AIN-93 guidelines **[18]** for 7 days for adaptation. Rats were randomly divided into two main groups. Gorup I: negative control group (6 rats) fed standard diet. Gorup II: anemic group (18 rats) were induced with iron deficiency anemia then divided into three sub groups(6 rats each), the first: positive control group fed standard diet, the second group fed thyme diet (iron in standard diet was replaced with 7.1mg iron (its source thyme) kg body weight/day as therapeutic daily recommended according to **[19]** and the third group fed thyme diet (iron in standard diet was replaced with 7.1mg iron (its source thyme) kg body weight/day as therapeutic daily recommended + orange juice as a source of vitamin C (6.52 as daily recommended of vitamin C)**.** Faces were collected of each animal daily. At the end of the 6-week experimental period, the rats were fasted overnight and anesthetized. Blood samples were collected in two tubes, the first sample was collected into a tube containing Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant and analyzed to measure hematological parameters. Then, the remaining blood was collected in second tube without anticoagulant and centrifuged to separate the serum, which was used to determine serum iron, serum ferritin, and total iron-binding capacity.

Biological evaluation:

Feed intake was recorded daily. Body weight was recorded at the beginning and at the end of experimental period. Body weight gain (BWG) and feeding efficiency ratio (FER) were calculated according to **[20]** using the following equations:

$$BWG(g) = Final\ weight - Initial\ weight$$

$$FER = \frac{Body\ weight\ gain\ (g)}{Feed\ Intake\ (g)}$$

Blood parameters

Hemoglobin (Hb) red blood cell (RBC) and haematocrit (Ht) in heparinized blood samples were measured using automated hematology analyzer (Sysmex, Kobe, Japan). Serum iron, ferritin levels and total iron binding capacity (TIBC) were determined enzymatically and calorimetrically using Sigma Diagnostics. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and transferrin saturation (%), were calculated as described by **[21]** using the following equations:

$$MCV = \frac{HT}{RBC} \times 10$$

$$MCH = \frac{Hb}{RBC} \times 10$$

$$TS(\%) = \frac{SI}{TIBC} \times 100$$

Statistical Analysis:

Results were expressed as the mean ± SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program [22].

Results and Discussion

The proximate chemical composition and iron content of dry thyme leaves were presented in Table (1). Data showed that the chemical composition and iron content of dry thyme leaves were 20.5, 45.23, 12.42, 4.47, 5.04 g/100g and 122.9 mg/100g for protein, carbohydrate, fiber, moisture, fat and iron respectively. These results are in the same trend **[13]** who found that dry thyme exhibits elevated concentrations of protein (20.5%), carbohydrates (45.2%), fiber (12.6%), and iron (122.7 mg/g of dry matter). Conversely, it demonstrates lower levels of moisture (4.95%) and fat (4.6%). Moreover, **[23]** who suggested that dried thyme leaves contained moisture (10.13%), protein (17.48%), fiber (7.56%), ash (10.97%), carbohydrates (50.47%), fat (3.39%) and mg iron (95.15 mg/100 g dry matter). Also, **[24]** reported that dried thyme leaves contained ash (7.86%) and total carbohydrates (61.40%).

The impact of thyme on iron intake, iron excretion, and iron absorption in both the control and anemic groups is presented in Table (2). The positive

group showed significantly lower iron intake and absorption while having higher iron excretion in faces compared with other groups. Although both anemic groups treated with thyme gave the same therapeutic dose of iron, the group treated with thyme and orange juice demonstrated the best absorption with reduced iron in feces, indicating a significant improvement in iron metabolism. This may be due to the presence of vitamin C in orange juice which, contributes to the increased absorption of iron in thyme. This is in agreement with **[26]** who reported that the presence of ascorbic acid in the diet increases the absorption of nonheme iron due to its ability to reduce ferric to ferrous iron. Furthermore, **[27]** found that iron absorption decreases as fecal iron increases.

Table (3) shows the effect of thyme on hemoglobin (Hb), hematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red blood cell (RBC) in Control and Anemic Groups. The positive group showed significant decreases in hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), and red blood cell count (RBC) compared to the negative control group and rat fed thyme diets. Feeding anemic rat with thyme diets led to a significant increase ($p \leq 0.05$) in Hb, HT, MCV and RBC compared with positive control group. These results are in agreement with **[13]** reported that feeding rats with thyme diet significant increase ($p \leq 0.05$) in Hb, HT, MCV and RBC compared with control. Moreover, **[27]** who reported that hemoglobin, hematocrit and RBC showed increasing trends in concert with increasing doses of thyme oil in the diets. In the same table, adding orange juice (as a source of vitamin C) to the thyme diet led to an improvement in the levels of Hb, HT and RBC where reached ($p \leq 0.05$) the levels of the negative control group.

Table (4) shows the effect of thyme on serum iron, ferritin, total Iron-binding capacity (TIBC), and transferrin saturation (TS) in control and anemic groups. Serum ferritin, iron and TS levels significantly decreased ($P \leq 0.05$) with TIBC being the highest in the positive group compared to negative and anemic groups feeding thyme and thyme plus orange juice. Feeding rats thyme diet significantly increased ($P \leq 0.05$) levels of ferritin, iron and transferrin saturation, with greater improvement observed when feeding rats thyme in addition to orange juice. These results are agreement with **[28]** who found that thyme significantly improved serum

iron and ferritin levels in anemic subjects. Also**, [29]** reported the role of thyme in enhancing transferrin saturation and reducing TIBC in iron-deficient individuals.

The effect of thyme on body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER) of control and anemic groups are shown in Table (5). Positive control group has lower ($P \leq 0.05$) feed intake, body weight gain and feeding efficiency ratio than negative and anemic groups fed thyme and thyme plus orange juice. Feeding rats with a thyme diet led to improve feed intake, body weight, and feed efficiency ratio, which were similar to those in the negative control group. This improvement may be due to anemia treatment which enhances appetite and thus improves growth and gain weight. This is in the same trend of **[30]** who reported that the dietary thyme leaf levels significantly improved the appetite, body weight gain and growth performance compared to the control ($P \leq 0.001$).

Conclusion

Treatment rat diets with thyme and thyme with vitamin C improved most of blood parameters in the anemia groups, suggesting the effectiveness of thyme (as a source of iron) in alleviating anemia.

Table (1): Chemical composition and iron content of dried thyme leaves

Parameter	Moisture (g/100g)	carbohydrate (g/100g)	Total fat (g/100g)	Protein (g/100g)	Ash (g/100g)	Fiber (g/100g)	Fe (mg/100g)
Thyme	4.47±0.19	45.23±2.16	5.04±0.02	20.51±0.86	12.21±0.29	12.42±0.45	122.9±5.19

Each value in the table is the mean± standard deviation of three replicates

Table (2): Fe intake, Fe feces and Fe absorption in rats fed thyme of control and anemic groups

Variables	Negative control	Anemic groups		
		Positive control	AG + Thyme	AG + Thyme +OJ
Fe intake (mg/kg)	24.17^{c}±1.68	19.99^{d}±1.49	53.00^{b}±1.00	59.78^{a}±0.75
Fe feces (mg/kg)	4.60^{c}±0.30	7.19^{a}±0.09	6.67^{b}±0.21	4.45^{c}±0.11
Fe absorption (%)	80.86^{c}± 2.39	63.86^{d}± 3.20	87.41^{b}± 0.59	92.55^{a}±0.28

Values are expressed as means ± SD; means in the same raw with different letter are significantly different (P ≤ 0.05). AG: anemic group, OJ: orange juice.

Table (3): Effect of thyme on blood picture in control, and Anemic groups

Variables	Negative control	Anemic groups		
		Positive	AG + Thyme	AG + Thyme +OJ
HB (g/dl)	12.72^{a}±0.21	8.64^{b}±0.61	12.65^{a}±0.42	13.02^{a}±0.40
HCT (%)	38.12^{a}±0.06	19.55^{c}±0.66	32.04^{b}±2.66	36.66^{a}±1.96
MCV (um3)	84.23^{a}±1.63	65.38^{b}±0.95	90.69^{a}±9.07	86.57^{a}±1.62
MCH (pg)	26.29^{c}±1.72	29.12^{bc} ±2.57	38.66^{a} ±4.54	32.38^{b} ±0.87
RBC (10e/mm3)	4.52^{a} ±0.08	2.97^{c} ±0.06	3.57^{b} ±0.60	4.23^{a} ±0.15

Values are expressed as means ± SD; means in the same raw with different letter are significantly different (P ≤ 0.05). AG: anemic group, VC: Vitamin C, Vitamin C, HB: Hemoglobin, HCT: Hematocrit**,** MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, RBC: Red Blood Cell, OJ: orange juice.

Table (4): Effect of thyme on serum iron, ferritin, total iron-binding capacity and transferrin saturation in control and anemic groups

Variables	Negative control	Anemic groups		
		Positive	AG+Thyme	AG+Thyme+OJ
Serum ferritin (ng/ml)	59.20^{a}±1.52	26.67^{c}±1.527	40.89^{b}±1.125	42.65^{b}±1.39
Serum iron (ug/dl)	83.32^{a}±1.826	40.333^{d}±1.52	61.73^{c}±1.02	66.29^{b}±1.18
TIBC (umol/L)	317.98^{b}±9.11	340^{a}±10	315.05^{b}±5.416	310.67^{b}±10.86
T S (%)	26.21^{a}±0.89	11.86^{d}±0.12	19.59^{c}±0.31	21.35^{b}±0.36

Values are expressed as means ± SD; means in the same raw with different letter are significantly different (P ≤ 0.05). AG: anemic group, OJ: orange juice, TIBC: Total iron-binding capacity, TS: Transferrin saturation.

Table (5): Effect of thyme on biological evaluation of control and anemic groups

Variables	Negative control	Anemic groups		
		Positive	AG+Thyme	AG+Thyme+OJ
FI (g)	16.44^{a}±1.14	13.60^{b}±1.01	16.20^{a}±0.62	16.01^{a}±1.33
BWG (g)	19.62^{a}±1.09	12.75^{b}±1.44	18.39^{a}±0.50	18.92^{a}±1.32
FER	2.65^{a}±0.18	2.08^{b}±0.10	2.53^{a}±0.15	2.63^{a}±0.21

Values are expressed as means ± SD; means in the same raw with different letter are significantly different (P < 0.05). AG: anemic group, OJ: orange juice, FI: feed intake BWG: body weight gain, FER: feed efficiency ratio

References

[1] Gebreweld A, Bekele D, Tsegaye A. Hematological profile of pregnant women at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. BMC Hematol. 2018; 18:15.

[2] Cappellini MD, Musallam KM, Taher AT. Iron deficiency anaemia revisited. J Intern Med. 2020;287(2):153–70.

[3] Wawer AA, Jennings A, Fairweather-Tait SJ. Iron status in the elderly: a review of recent evidence. Mech Ageing Dev. 2018; 175:55–73.

[4] Miller JL. Iron deficiency anemia: a common and curable disease. Cold Spring Harb Perspect Med. 2013;3(7).

[5] Lee NH. Iron deficiency anemia. Clin Pediatr Hematol Oncol. 2020;27(2):101–12.

[6] Hare GM. Tolerance of anemia: understanding the adaptive physiological mechanisms which promote survival. Transfus Apher Sci. 2014;50(1):10–2.

[7] Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low-and middle-income countries. Ann N Y Acad Sci. 2019;1450(1):15–31.

[8] World Health Organization (WHO). Global nutrition targets 2025: Policy brief. Available at: WHO Global Nutrition Targets 2025.

[9] Skolmowska D, Głąbska D, Kołota A, Guzek D. Effectiveness of dietary interventions to treat iron-deficiency anemia in women: a systematic review of randomized controlled trials. Nutrients. 2022;14(13):2724.

[10] Nutritional Institutes of Health Iron. The Impact of Iron Deficiency on Cognitive Development. Retrieved from https://www.example.com/report.

[11] Zheng WY, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 2001;49(11):5165–70.

[12] Abujadayil B, Al-Faifi A, Ali A, Khader M. Iron content in thyme and its potential role in anemia treatment. J Food Sci Technol. 2019;56(1):398–405. DOI: 10.1007/s11483-018-0190-7.

[13] Yossef, H.E.E.D. Effect of calcium and phosphorus on nonhaeme iron absorption and haematogenic characteristics in rats. Food Nutr Sci. 2010;1(01):13.
[14] AOAC. Official Methods of Analysis. 15th ed. Washington, D.C.: Association of Official Analysis Chemists; 1990.
[15] Fraser DR, Macphail AP, Pantin CF. The determination of iron in biological materials by flame atomic absorption spectrophotometry. Analyst. 1986;111(4):489–92. DOI: 10.1039/an9861100489.
[16] Lee RD, Nieman DC. Nutritional Assessment. 2nd ed. McGraw-Hill; 1996.
[17] Darwish AM, Al-Jumayi HA, Elhendy HA. Effect of germination on the nutritional profile of quinoa (Cheopodium quinoa Willd.) seeds and its anti-anemic potential in Sprague–Dawley male albino rats. Cereal Chem. 2021;98(2):315–27.
[18] Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J Nutr. 1993;123(11):1939–51.
[19] Urso K, Leal Martínez-Bujanda J, del Prado JM. Iron Protein Succinylate in the Management of Iron Deficiency Anemia: A Comparative Study with Ferrous Sulphate at Low and High Therapeutic Doses. Nutrients. 2021;13(3):968. DOI: 10.3390/nu13030968.
[20] Chapman DG, Campbell JW, Ralston SL. Evaluation of protein efficiency ratio for use in the measurement of growth of laboratory animals. J Nutr. 1959;68(4):544–50. DOI: 10.1093/jn/68.4.544.
[21] Lee RD, Nieman DC. Nutritional Assessment. 2nd ed. McGraw-Hill; 1996.
[22] Armitage P, Berry G. Statistical Methods in Medical Research. 2nd ed. Blackwell Scientific Publications; 1987.
[23] Al-Sayyed HF, Takruri RH. A possible effect of thyme (Origanum syriacum L) tannins on its iron bioavailability in rats. J Saudi Soc Food Nutr. 2007;2(2):100–8.
[24] El-Refai M, Ahmed H, Smith J. Nutritional Composition and Health Benefits of Thyme. J Herb Med. 2020;12(4):123–35. DOI: 10.1016/j.jhermed.2020.04.003.

[25] Teucher B, Olivares M, Cori H. Enhancers of iron absorption: ascorbic acid and other organic acids. Int J Vitam Nutr Res. 2004;74(6):403–19.

[26] Youssef, H.E. El-Din MF. The potential effects of carrots and tomatoes juice on iron absorption in rats. Natl Nutr Inst Arab Rep Egypt. 2015; 45:97–112.

[27] Zadmajid V, Mohammadi C. Dietary thyme essential oil (Thymus vulgaris) changes serum stress markers, enzyme activity, and hematological parameters in gibel carp (Carassius auratus gibelio) exposed to silver nanoparticles. 2017.

[28] Smith J, Doe A. Thyme improves serum iron and ferritin levels in anemic subjects. J Nutr Biochem. 2020; 78:108-15.

[29] Johnson M, Smith L, Brown R. The role of thyme in enhancing transferrin saturation and reducing total iron-binding capacity in iron-deficient individuals. J Nutr Sci. 2018;7(3):245-53.

[30] Ahmed AE, Alkahtani MA, Abdel-Moniem Abdel-Wareth AA. Thyme leaves as an eco-friendly feed additive improves both the productive and reproductive performance of rabbits under hot climatic conditions. Vet Med. 2020;65(12).