Review

Natural Antioxidants in Anemia Treatment

Coralia Cotoraci 1,*,†, Alina Ciceu 2,†, Alciona Sasu 1,† and Anca Hermenean 2,3

1 Department of Hematology, Faculty of Medicine, Vasile Goldis Western University of Arad, Rebreanu 86, 310414 Arad, Romania; alcionasasu@gmail.com
2 “Aurel Ardelean” Institute of Life Sciences, Vasile Godis Western University of Arad, Rebreanu 86, 310414 Arad, Romania; alina_ciceu@yahoo.com (A.C.); anca.hermenean@gmail.com (A.H.)
3 Department of Histology, Faculty of Medicine, Vasile Goldis Western University of Arad, Rebreanu 86, 310414 Arad, Romania
* Correspondence: ccotoraci@yahoo.com
† These authors contributed equally to this work.

Abstract: Anemia, characterized by a decrease of the hemoglobin level in the blood and a reduction in carrying capacity of oxygen, is a major public health problem which affects people of all ages. The methods used to treat anemia are blood transfusion and oral administration of iron-based supplements, but these treatments are associated with a number of side effects, such as nausea, vomiting, constipation, and stomach pain, which limit its long-term use. In addition, oral iron supplements are poorly absorbed in the intestinal tract, due to overexpression of hepcidin, a peptide hormone that plays a central role in iron homeostasis. In this review, we conducted an analysis of the literature on biologically active compounds and plant extracts used in the treatment of various types of anemia. The purpose of this review is to provide up-to-date information on the use of these compounds and plant extracts, in order to explore their therapeutic potential. The advantage of using them is that they are available from natural resources and can be used as main, alternative, or adjuvant therapies in many diseases, such as various types of anemia.

Keywords: anemia; iron deficiency; iron overload; hemolytic anemia; β-thalassemia; sickle cell anemia

1. Introduction

Anemia is defined as a reduction in the number of circulating RBCs [1] or as a condition in which the number of erythrocytes (and subsequently their ability to carry oxygen) is insufficient to meet physiological needs [2]. Anemia is characterized by a decrease of the hemoglobin (Hb) level in the blood (generally less than 13.5 g/dL in men and 12.5 g/dL in women), which results in a reduction in carrying capacity of oxygen. Diseases that decrease Hb production (e.g., iron deficiency, B12, or folate deficiencies) or accelerate its destruction are often the result of a defect in the structure of Hb [3]. Although anemia is frequently diagnosed by a low level of Hb or hematocrit (Htc), it can also be diagnosed by using the number of RBCs, the average mean erythrocyte volume, the number of reticulocytes in the blood, the examination of the blood smear or Hb electrophoresis [4].

Anemia is a major public health problem. It affects people of all ages, especially pregnant women and children. According to statistics, globally, anemia affects 41.8% of pregnant women and 47.4% of preschool children [5]. Moreover, it has negative effects on health and development, including neonatal and perinatal mortality, low birth weight [6], premature birth [7,8], and developmental delays of the children [9]. Clinically, it is characterized by pallor, fatigue, dizziness, difficulty breathing dyspnea, and weakness [10]. In the absence of effective management, anemia can promote decreased cognitive ability, weakened immune system, and increased mortality [11].
Anemia can be classified in terms of pathogenesis and erythrocyte morphology [12]. The pathogenic mechanisms involved in the onset of anemia are inadequate production and loss of erythrocytes as a result of bleeding or hemolysis. Depending on these pathogenic mechanisms, anemia can be divided into hypo-regenerative and regenerative forms. In hypo-regenerative anemia, bone marrow production is low, as a result of impairment of the latter’s function, decreased number of precursor cells, or lack of nutrients [12]. Damage to pluripotent stem cells usually causes pancytopenia (anemia, leukopenia, and thrombocytopenia). All of these can affect normal hematopoiesis or change the microenvironment required for stem cell regeneration, differentiation, and proliferation [13,14]. In the regenerative form, anemia is characterized by an elevated level of erythropoietin in response to decreased Hb and generally reflects a loss of erythrocytes, caused by bleeding or hemolysis. Bone marrow responds appropriately to a low erythrocyte mass by increasing reticulocyte production [12].

Classification of anemias according to erythrocyte morphology is more useful in medical practice [12]. Microcytic anemia is the type of anemia in which circulating RBCs are smaller than usual. The most common cause of this type of anemia is a decrease in the body’s iron reserves, which can have several causes, such as decreased iron in the diet; reduced absorption of iron in the intestine; acute and chronic blood loss; and increased iron demand in certain situations, such as pregnancy and recovery after a major trauma or surgery [15]. The category of microcytic anemias includes iron deficiency anemia (IDA), thalassemia, anemia associated with various chronic conditions (e.g., rheumatoid arthritis, Hodgkin’s lymphoma, chronic infections, and neoplasms), and sideroblastic anemia (e.g., hereditary and intoxication with lead) [12]. Normocytic anemia may be caused by inadequate erythropoietin (EPO) levels or decreased erythropoietin response, reduced erythrocyte survival, and bone marrow infiltration. Most normocytic, normochromic anemias are a consequence of other conditions. A small part of them reflects a primary blood disorder. This may be due to anemia induced by some chronic conditions (inflammation and neoplasia), kidney failure, endocrine failure, bone marrow failure (pure red cell aplasia and aplastic anemia), acute blood loss, and polycystic blood loss [16]. Macrocytic anemia has two forms: megaloblastic (hypersegmented neutrophils) and non-megaloblastic. Megaloblastic anemia is due to impaired DNA synthesis induced by folate and/or vitamin B12 deficiency [17,18].

The main function of RBCs in the blood is the transport of respiratory gases (oxygen (O2) and carbon dioxide (CO2)) to and from tissues, by binding gases to Hb inside erythrocytes. Hb is a tetrameric protein consisting of two α- and two β-polypeptide chains. Each of them contains an iron heme group capable of binding an oxygen molecule [19]. Each heme group contains an (Fe2+) atom, which binds an oxygen molecule. Thus, a Hb tetramer can bind four oxygen molecules [20].

In the human body, iron is mainly found in erythrocytes, in the form of Hb (about 2 g of iron in men and 1.5 g in women) and, to a lesser extent, in storage compounds (ferritin and hemosiderin), and in muscle cells like myoglobin. Iron is also found bound to proteins (hemoproteins) and non-heme enzymes involved in oxidation-reduction reactions and electron transfer (cytochrome and catalase) [21–23].

About 2.2% of the total amount of iron in the body is found in a labile reserve, which forms reactive oxygen species through the Fenton reaction that forms complexes with a class of drugs known as chelators. Iron chelators are used in the treatment of iron overload, a condition often caused by blood transfusions that are used to treat thalassemia and other types of anemia [24,25].

Upon exposure to oxygen, iron forms insoluble oxides that cannot be absorbed into the human gastrointestinal tract. Human enterocytes contain membrane-bound apical enzymes that have the ability to reduce insoluble iron (Fe3+) to an absorbable (Fe2+) form. Iron overload can be particularly harmful to the heart, liver, and endocrine organs. Excess ferrous iron forms hydroxyl free radicals through the Fenton reaction, which causes tissue
damage through oxidative reactions with lipids, proteins, and nucleic acids. Thus, the absorption of iron from the diet and the factors that affect the bioavailability in the body are strictly regulated [26].

The most effective method used to treat anemia is blood transfusion [27]. Oral administration of iron-based supplements is an effective and, at the same time, inexpensive method used to treat patients with iron deficiency anemia. Moreover, this method of treatment is associated with a number of side effects, such as nausea, vomiting, constipation, and stomach pain, which limits its long-term use. In addition, oral iron supplements are poorly absorbed in the intestinal tract, due to overexpression of hepcidin, a peptide hormone that plays a central role in iron homeostasis [28]. If iron supplements are not effectively assimilated by the body, or if their absorption is inhibited, parenteral administration is recommended. Long-term parenteral administration of iron supplements can lead to hyperpigmentation of the skin. Moreover, one of the side effects of taking iron-based supplements is increased free radical production [27]. The use of inappropriate doses of iron-based supplements can induce oxidative stress [29,30], with the formation of oxidation products, and can lead to cardiovascular, neurological, or cancer conditions [31–33]. The presence of an excess of free iron initiates the Fenton reaction, which leads to oxidative damage to cell membranes, proteins, lipids, and nucleic acids, as well as the stimulation of inflammatory mediators [34,35].

Therefore, in the case of patients with anemia, the aim is to reduce the dose of iron and replace it with other complementary treatments.

The therapeutic use of herbal products in common clinical practice is not yet regulated for several reasons, such as the lack of efficacy and toxicity data that are required for their approval by health authorities. To these are added the competition of large pharmaceutical companies that make remarkable profits from the sale of synthetic drugs [36]. Currently, there is an increase in the number of clinical trials with herbal therapeutic products used in various diseases, in order to confirm their therapeutic value and receive the necessary approvals for their marketing [37]. Some phytochemicals or herbs act directly to induce the resolution of anemia, and others act pleiotropically through their antioxidant activity, by increasing oxidative stress resistance or by triggering cellular mechanisms, such as autophagy [38], or, for example, by targeting inflammation in the elderly population and subsequently reducing the anemia associated with chronic inflammation [39].

In this review, we conducted an analysis of the literature on biologically active compounds and plant extracts used in the treatment of various types of anemia. The purpose of this review is to provide up-to-date information on the use of these compounds and plant extracts, in order to explore their therapeutic potential. The advantage of using them is that they are available from natural resources and can be used as main, alternative, or adjuvant therapies in many diseases, such as various types of anemia.

2. Natural Antioxidants in Iron Deficiency Treatment

Iron is essential for the production of Hb. Depletion of iron deposits can be caused by blood loss, decreased iron intake, impaired absorption, or increased demand. Iron deficiency anemia can be caused by occult gastrointestinal bleeding [40] or decreased iron in the diet, or reduced iron absorption [26], accounting for more than half of all types of anemia [11] and require iron supplementation.

Due to the fact that oral iron supplements are associated with side effects, such as gastrointestinal irritations, reduced bioavailability, and lipid peroxidation [41], it is necessary to develop new iron-based supplements without or with minimal side effects. Polysaccharide chelated iron is characterized by high stability, water solubility, and fewer side effects [42]. Other polysaccharide–iron complexes have been used in the treatment of IDA, such as iron–dextran, iron–starch, and Niferex (a combination of ascorbic acid and iron–polysaccharide complex) [43,44].
*Ulva prolifera* is one of the most widespread species of green macroalgae [45]. The sulfate polysaccharides from *Ulva prolifera* (SUE) are a group of sulfated heteropolysaccharides with unique structural features in the form of rhamnose and uronic acid linkages of (1 → 4)-linked β-L-arabinopyranose residues [46]. In the case of rat-induced IDA, the SUE-iron (III) complex induced an increase in the number of RBCs and serum iron levels and contributed to the restoration of normal Hb levels [47].

*Angelica sinensis* has been shown to improve hematopoiesis by increasing the secretion of hematopoietic growth factors, such as erythropoietin, by stimulating hematopoietic cells and muscle tissue [48]. Polysaccharides from *A. sinensis* (ASP) improved serum iron levels and participated in the regulation of iron homeostasis [49]. Moreover, Liu et al. (2012) obtained ASP from the root powder of *A. sinensis* riched in arabinose, glucose, and galactose, with a molar ratio of 1: 5.68: 3.91 [50]. ASP has been shown to decrease hepcidin expression by inhibiting SMAD 4 expression in the liver and stimulating erythropoietin secretion, while other results showed that the decreased hepatic hepcidin expression is due to inhibition of the expression of JAK1/2, phospho-JAK1/2, phospho-SMAD1/5/8, phospho-ERK1/2, and stimulating SMAD7 [51]. Previously, ASPs have been shown to activate erythropoiesis [52,53].

*Beetroot* (*Beta vulgaris*) contains iron, nitrates, sodium, potassium, and betalaine [54]. Among the benefits of beetroot juice are the treatment of anemia by improving the ability of erythrocytes to carry oxygen, lowering blood pressure by dilating blood vessels and relaxing smooth muscles, preventing birth defects by increasing folate levels, etc. [55]. Compared to other vegetables with a high iron content, beets have a low price and are easy to store [56]. Consumption of 8 g of beets for 20 days induced an increase in Hb, ferritin, and serum iron levels, as well as a decrease in transferrin and total iron-binding capacity levels in seven women aged 22–24 years [57]. Consumption of beetroot in the form of juice (100–200 mL) increased the level of Hb [58,59]. Moreover, administration of 200 mL of beet juice for six weeks induced the increase of HTC, RBC, iron, and ferritin levels [58]. The administration of beetroot in the form of powder and iron-based supplements for 14 days in women with anemia led to increased levels of Hb, HTC, and erythrocyte counts [60].

The results of the in vitro and in vivo studies regarding pharmacological effects exerted by the natural antioxidants in iron deficiency anemia are summarized in Table 1.

| Bioactive Compound                  | Doses                        | In Vitro/ In Vivo/ Clinical Study | Model                  | Bioactive Effect                                                                 | References |
|------------------------------------|------------------------------|----------------------------------|------------------------|---------------------------------------------------------------------------------|------------|
| Iron from *Moringa* leaves         | 10% and 20% dehydrated       | in vivo                          | iron-deficient diet in Wistar rats | ↑ serum iron, ferritin, and transferrin concentration                            | [61]       |
| *Moringa* leaves                   | Moringa leaves               |                                  |                        | Dietary iron from *Moringa leaf* was found to be superior compared with ferric citrate (35 mg/kg) in overcoming the effects of iron deficiency in rats |
| Sulfate *Ulva* polysaccharide (SUE)| Low-dose group (SUE-iron (III) with iron concentration of 0.8 mg/mL, 0.7 mg kg⁻¹ BW, Fe) | in vivo                          | iron deficiency anemia in Wistar rats | ↑ RBC number                                                               | [47]       |
|                                   | High-dose group (SUE-iron (III) with iron concentration of 2.3 mg/mL, 2.0 mg kg⁻¹ BW, Fe) |                                  |                        | ↑ serum iron, ↓ TIBC, ↓ IL-4, returns Hb to the normal levels                  |
| *Astragalus membranaceus* polysaccharide-iron (III) complex | APS-iron (III) complex with the iron content 12.5, 25, 50, 75, and 100 mg/kg | in vivo                          | Iron-deficiency anemia rodent model | ↑ Hb, ↑ SOD and CAT, ↓ MDA                                                   | [62]       |
| Ginger + iron supplement           | 215 mg                       | clinical study                   | iron deficiency anemia   | ↑ plasma iron, ↑ plasma ferritin                                              | [63]       |
Quercetin + FeSO₄ 50 mg·kg⁻¹ quercetin + 50 mg·kg⁻¹ FeSO₄   in vivo Iron deficiency anemia rat model ↑ Hb ↑ serum iron level ↑ iron stores in spleen ↑ expression of SLC40 - improve red blood cells level ↓ CD68 macrophage activation ↓ iNOS expression in splenic red pulp [64]

Low-molecular-weight polysaccharide from Enteromorpha prolifera (LPE)-iron (III) complex Low LPE-iron (III) complex group (0.7 mg Fe/kg body weight) in vivo iron deficiency anemia rat model ↑ Hb ↑ RBC numbers ↑ HTC ↑ serum iron ↑ TIBC level ↑ EPO level - improved the growth of rats with IDA - alleviated the hypertrophy of spleen - improved the liver coefficient [65]

Aqueous extract of Mangifera indica stem bark 25, 50, and 75 mg/kg body weight in vivo iron deficiency anemia rat model ↑ PCV, Hb and RCB count ↑ lactase and sucrase activity [66]

Aqueous extract of the stem bark of Theobroma cacao (TC) 25, 50, and 75 mg/kg body weight in vivo iron deficiency anemia model albino rats ↑ PCV, Hb and RCB count ↑ intestinal lactase and sucrase activity [67]

Caulis spatholobi (CS) cells treated with 400 mg/mL of extract in vitro Human hepatocellular carcinoma cell lines HuH7 and HepG2 ↓ HAMP expression in HuH7 and HepG2 cells ↓ BMP-6-induced HAMP expression ↓ IL-6-induced HAMP expression ↓ iron mobilization in mice ↓ phosphorylation of Smad1/5/8 ↓ hepatic iron levels ↑ serum iron levels in mice [68]

Angelica sinensis polysaccharide (ASP) 1 g/kg in vivo iron deficiency anemia in Sprague Dawley rats ↓ hepcidin expression [50]

Angelica sinensis polysaccharide (ASP) 1 g·kg⁻¹ in vivo iron deficiency anemia Sprague Dawley rats ↓ hepcidin expression [51]

Beetroot juice (Beta vulgaris L.) 200 mL clinical study Twenty female soccer players 23.13 ± 0.77 years old ↑ Hb, HTC, RBC, iron, and ferritin levels ↓ TIBC [58]

Beetroot juice (Beta vulgaris L.) 100 mL clinical study adolescent girls ↑ Hb [59]

Beet powder (Beta Vulgaris L.) with Fe supplementation 8 g beetroot powder clinical study 30 pregnant women with anemia ↑ Hb, HTC, ↑ the number of erythrocytes [60]

Carica papaya 110 g of papaya clinical study 42 pregnant women ↑ Hb ↑ HTC [69]

Sweet potato (Ipomoea Batatas L.) 100 g clinical study first trimester pregnant women ↑ Hb [70]

Baobab fruit (Adansonia digitata) 60, 80, and 100 g clinical study 32 pregnant with iron deficiency anaemia ↑ Hb, PCV ↑ serum ferritin [71]
Aqueous extract of *Hibiscus sabdariffa* seeds 400 mg of the extract/kg body weights \[\uparrow \text{HB, PCV, and RBC count of hemorrhagic anemic rats} \]
\[\uparrow \text{the Hb level of the nutritionally iron-deficient rats} \]

| Red beetroot (*Beta vulgaris* L.) | 8 g | clinical study | healthy female volunteers (age range, 22 to 24) | \[\uparrow \text{Hb} \]
|----------------------------------|-----|----------------|-----------------------------------------------|----
|                                  |     |                |                                               | \[\downarrow \text{TIBC} \]
|                                  |     |                |                                               | \[\uparrow \text{ferritin} \]
|                                  |     |                |                                               | \[\downarrow \text{transferrin} \]
|                                  |     |                |                                               | \[\uparrow \text{serum iron levels} \]

3. Natural Antioxidants in Iron Overload Treatment

Iron overload is associated with aplastic anemia, sideroblastic anemia, Blackfan-Diamond anemia, Fanconis anemia, pernicious anemia, congenital dyserythropoietic anemia, hereditary hypochromic anemia, or haemoglobinopathies, as \(\beta\)-thalassaemias or sickle cell anaemia.

Although blood transfusions are important for patients with anemia, chronic transfusions inevitably lead to iron overload, as the body cannot eliminate excess iron. If not treated properly, the cumulative effects of iron overload led to morbidity and mortality [73]. One unit of transfused RBCs contains about 250 mg of iron [74], while the body cannot excrete more than 1 mg of iron per day. A patient who receives 25 units per year accumulates 5 g of iron per year in the absence of chelation [75].

Chelation therapy is used in binding the iron excess and removing it from the body. Synthetic iron chelators are toxic in high doses. Due to the high costs, toxicity and side effects of treatment with synthetic iron chelators, a large number of patients are currently not receiving any iron chelation treatment. There are a number of orally active antioxidants that have the ability to chelate iron and eliminate free radicals. They also have a lower cost and toxicity compared to synthetic drugs. These natural chelators form complexes with metals and could be used in the treatment of iron overload without inducing another micromineral deficiency, being an advantage that synthetic drugs do not have [76].

The natural iron chelators contain a catechol or gallate fragment that acts as a binding site for metals and further is eliminated from the body. In addition to the ability to chelate iron and eliminate free radicals, these antioxidants can be effective in iron overload by reducing the iron load in the liver, increasing iron excretion in feces and urine, reducing serum ferritin, removing iron from ferritin and transferrin, increasing hepcidin expression, reducing iron absorption in the intestine, increasing iron absorption and incorporation into the heme, and inducting osteo- and cardio-protective effects [76].

Flavonoids and polyphenolic compounds with at least two iron binding sites have iron chelating properties. These flavonoids fall into two categories: lipophilic and hydrophilic chelators. Lipophilic chelators increase iron absorption, reduce iron excretion, and increase the deposition of excess iron in tissues. Therefore, they are a possible treatment for iron-deficiency anemia. Hydrophilic chelators, on the other hand, favor the elimination of excess iron, reduce iron absorption, and exert antioxidant and anti-inflammatory activity, without having other side effects [36]. Combining synthetic iron chelators with these antioxidants, or even replacing them with chelators from natural sources, would be a possible treatment for iron overload [76]. By chelating iron, flavonoids decrease high oxygen toxicity, for example, by inhibiting \(\text{HO}^\ast\) production from the Fenton reaction [77]. The mechanism by which certain flavonoids reduce the bioavailability of non-hemic iron...
is not fully understood, but it is assumed that flavonoids have the ability to chelate non-hemic iron [78–82].

Grape seed extract (GSE) contains various polyphenolic compounds, such as gallic acid, catechin, epigallocatechin-3-gallate (EGCG), epigallocatechin, epicatechin-3-gallate, epicatechin, and proanthocyanidins [83]. GSE extracts rich in polyphenols have antioxidant activity due to the phenolic compounds ability to neutralize free radicals and to chelate certain metals, such as iron [84,85]. Moreover, it was found that EGCG and GSE increased absorption on hemic iron on apical side and blocked transepithelial transport [83] or inhibited intestinal absorption of nonhemic iron [86].

Curcumin, the main curcuminoid in Curcuma longa L. (turmeric), is a low-molecular-weight polyphenol, widely used in Ayurveda and Chinese medicine [87]. Turmeric type 97 (77% curcumin, 17% dexethoxycurcumin, and 3% bisdemethoxy curcumin) induced an increase in the level of transferrin receptor 1 (TfR1) and the induction of activated iron-regulatory proteins (IRPs), a decrease of the hepatic ferritin level and its H and L subunits [88]. Other results show that 1000 mg iron/kg body weight and curcumin increased TfR1 and iron-responsive element-binding protein (IRP), favored the appearance of hypochromic RBCs, and decreased the levels of Hb, HTC, serum iron, ferritin, hepcidin, and transferrin saturation, as well as iron levels in the spleen and bone marrow [89].

Quercetin is a flavonol found in onions, broccoli, garlic, tomatoes, black tea, spinach, and apples [90–92], recognized for their antioxidant and anti-inflammatory activity [93]. Quercetin increased the expression of hepcidin, one of the main hormones involved in intestinal absorption of iron, which could involve the Nrf2 pathway [94]. Granado-Serrano et al. (2012) demonstrated that quercetin can activate the Nrf2 pathway by supporting nuclear translocation and its transcriptional activity [95]. Given that the levels of ferroportin (FPN) and ferritin are overexpressed transcriptionally by the Nrf2 pathway, quercetin could affect iron homeostasis and help cells to fight against oxidative stress [96]. Prenatal exposure of mice to quercetin resulted in increased hepatic iron deposits and induced overexpression of hepcidin to adults [77].

Quercetin chelates metal ions in a stable complex, thus preventing the Fenton reaction [97], and protects human erythrocytes from iron-induced oxidative damage [98,99]. Quercetin can also act as a siderophore through glucose transporters [100]. Similar to other flavonoids, it is thought to form a complex with Fe\(^{2+}\) that has greater stability than Fe\(^{3+}\). Even if quercetin initially forms a complex with Fe\(^{2+}\), it will be further self-oxidized, resulting in Fe\(^{3+}\) [101]. Iron chelation by the 3-hydroxyl group of quercetin is an important factor in iron absorption in the duodenum [79]. The decrease in duodenal iron transfer is due to the chelation of iron by quercetin, which increases the apical absorption of iron, but prevents basolateral transport. However, the precise site of iron chelation by quercetin is unknown. It is not known whether chelation occurs in the duodenal lumen or in the cytosol of duodenal enterocytes [96].

Myricetin (3,5,7,3’,4’,5’-hexahydroxyflavonol) is a flavonoid initially isolated from the bark of the Myrica rubra that has been shown to have iron-chelating properties [80,102]. Administration of myricetin to C57BL/6 mice favored an increase in RBCs, Hb levels, and serum iron and decrease in the hepatic expression of hepcidin and splenic iron levels [103].

Silibin is a biologically active compound from silymarin [104], a flavonolignan reported to have iron chelating effect [105], while other studies reported a high affinity for Fe (III) iron-silibin complex in acidic pH [104,106]. Bares et al. (2008) observed that oral administration of silibin for 12 weeks reduced iron deposits in patients with chronic hepatitis C [107].

The main pharmacological effects of the natural antioxidants in iron chelation activities are summarised in Table 2.
Table 2. Pharmacological effects of the natural antioxidants in iron chelation activity.

| Bioactive Compound | Doses | In Vitro/ In Vivo/ Clinical Study | Model | Bioactive Effect | References |
|--------------------|-------|----------------------------------|-------|-----------------|------------|
| EGCG and GSE       | in vitro | cell culture of Caco-2 cells | MEL cell culture | ↓ transepithelial heme iron transpor | [83] |
|                    |        | MEL cell culture | mouse erythroleukemia (MEL) cell line | ↑ apical heme iron uptake (GSE) | |
|                    |        | human Caco-2 (HTB-37TM) cell line | murine erythroleukemia (MEL) cell line | ↓ the cellular assimilation of heme (EGCG) | |
| Curcumin           | 0.5% and 2.0% | in vivo | FVB mice treated with curcumin for 12 weeks | ↓ in the H and L subunits of the iron storage protein ferritin | [88] |
|                    |        |        | C3H/HeNCrImice | ↑ transferrin receptor 1 | |
|                    |        |        | AIN93M basal diet modified to contain various amounts of iron and curcumin: 5, 12, 50, or 1000 mg iron/kg diet plus curcumin at 0% (control), 0.2%, 0.5%, and 2.0% (wt/wt) for 26 weeks | ↓ iron levels in the bone marrow and spleen | |
|                    |        |        | patients with hereditary haemochromatosis | ↓ appearance of hypochromic RBCs | |
|                    |        |        | patients with hereditary haemochromatosis | ↑ transferrin receptor 1 (TfR1) | |
|                    |        |        | patients with hereditary haemochromatosis | ↓ iron-responsive element-binding protein (IRP) | |
|                    |        |        | patients with hereditary haemochromatosis | ↓ ferritin | |
|                    |        |        | patients with hereditary haemochromatosis | ↓ hepcidin | |
| Silybin            | 140 mg silybin (Le-galon Forte) | clinical study | patients with hereditary haemochromatosis | ↓ postprandial iron absorption | [106] |
|                    | 100 mg/kg body weight | in vivo | ethanol-induced iron overload and liver damage in mice | -attenuated the hepatic iron deposition in mice exposed to ethanol or excess iron | [109] |
| Quercetin          | 40-mg/kg myricetin daily for 1 or 5 days | in vitro | mouse primary hepatocytes | -prevented ethanol induced hepatic iron overload by regulating hepcidin expression via the BMP6/SMAD4 signaling pathway | |
|                    | 10-mg/kg myricetin for 5 days, after which the mice were injected with LPS (5 mg/kg, ip) | in vivo | HepG2 an HEK293 cells | ↓ HAMP mRNA levels | |
|                    | 0.2% (v/v) myricetin for up to 30 days | in vivo | CS7BL/6 mice | ↓ SMAD1/5/8 phosphorylation | |

| Black soybean seed coat anthocyanins extract (BSSCE) | 200 mg/mL BSSCE | in vitro | AIN-76A diet containing 2% BSSCE fed to 8-week-old male C57BL/6 mice for 0, 1, 7, 15 or 30d | ↓ hepcidin expression | ↓ splenic Fe concentration | ↑ serum Fe concentrations | ↑ in erythrocyte counts, HB concentrations, HTC values |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Citrus flavonoids-rich extracts from bergamot and orange juices | 2% BSSCE | in vitro | iron overloaded human lung epithelial cells | ↓ ROS | ↓ lipid peroxidation | ↑ mitochondrial function | ↓ iron chelation-prevented DNA-oxidative damage in iron-exposed cells | ↑ catalase activity |
| Baicalein (Scutellaria baicalensis) | in vitro | -zebrafish embryo (Danio rerio) | ↑ hepcidin expression and promoter activity in zebrafish and human and SMAD4-dependent manner |
| Genistein | in vivo | the human hepatocarcinoma cell hepatocytes in a STAT3-dependent and SMAD4-dependent manner |
| Vitamin C | 50 and 100 mg/mL | in vivo | hepcidin-producing HepG2 cell line | -inhibition of hepcidin expression |
| Tucum-Do-Cerrado (Bac tris setosa Mart.) | in vivo | Wistar rats | AIN-93G diet with 150 g of tucum-do-cerrado fruit (pulp and peel)/kg diet | ↓ spleen lipid and protein oxidation; mRNA levels of hepatic Hamp and ferritin | ↑ serum antioxidant capacity | ↑ hepatic mRNA levels of BMP6, Hmox1, Nqo1, and Nrf2 | -abrogated the liver Hamp iron-induced upregulation -prevented intestinal iron accumulation; hepatic lipid peroxidation; splenic protein damage | ↑ of CAT, GR, and GSH-Px activity |
| Angelica sinensis polysaccharide (ASP) | ASP at 25, 50, and 100 mg kg\(^{-1}\) | in vivo | BALB/c mice inoculated with H22 tumor cells | ↓ hepcidin concentration in serum | ↓ levels of serum IL-6 | ↓ serum ferritin | ↓ levels of serum Tf | ↓ levels of serum TIR2 | ↓ levels of serum TIR1 in the ASP 25 mg kg\(^{-1}\) treatment group |
| Hydro-alcoholic extract of Medicago Sativa and Allium Porrum | 200 and 400 mg/kg body weight | in vivo | iron-overloaded rats | ↓ serum ferritin | ↓ serum iron level |
| Methanolic extract of Angel’s wings mushroom (Pleurotus porri gens) | 200 and 400 mg/kg/24 h | in vivo | iron-overloaded mice | -chelation of excess iron | ↓ in plasma Fe\(^{3+}\) content | ↓ in the extent of necrotic hepatocytes, fibrous tissues, and pseudo-lobules |
| Methanol extract of Nerium indicum leaves | 50, 100, and 200 mg/kg b.w. | in vivo | iron-overloaded mice | -antioxidant and iron-chelating properties | ↓ iron overload induced toxicity | -normalized the levels of ALAT, ASAT, ALP, and bilirubin | ↑ levels of SOD, CAT, GST, and nonenzymatic-reduced glutathione | ↓ levels of lipid peroxidation, protein carbonyl, hydroxyproline |
**4. Natural Antioxidants in the Treatment of Hemolytic Anemia**

Hemolytic anemia is a normocytic anemia characterized by low Hb levels due to the destruction of RBCs and increased hemoglobin catabolism. Depending on where the hemolysis occurs, it can be intravascular or extravascular [126]. Destruction can also occur...
in the case of inherited protein deficiencies (membranopathies, i.e., hereditary spherocytosis), fragmentation (i.e., microangiopathic hemolytic anemia, thrombotic thrombocytopenic purpura, and disseminated intravascular coagulation), antibodies that bind to RBC resulting in phagocytosis (immune-mediated), drug-induced hemolysis, infections, or direct trauma [127].

The results of the in vitro and in vivo studies regarding pharmacological effects exerted by the natural antioxidants in hemolytic anemia are summarized in Table 3.

Table 3. Pharmacological effects of natural antioxidants in hemolytic anemia treatment.

| Bioactive Compound | Doses | In Vitro/In Vivo/ Clinical Study | Route of Administration | Model | Bioactive Effect | References |
|--------------------|-------|---------------------------------|-------------------------|-------|-----------------|------------|
| Brassica oleracea var italica aqueous extract | 100 and 200 mg/kg | in vivo | for 14 days | PHZ-induced anaemia in Sprague-Dawley rats | ↑ RBC, ↑ Hb | [128] |
| Hypoestes triflora aqueous extract | 50 mg/kg | in vivo | orally for 30 days | phenylhydrazine hydrochloride-induced anaemia in guinea-pigs | ↑ RBC count | [129] |
| Phyllanthus niruri Linn aqueous extract | 250, 500, and 1000 mg/kg | in vivo | orally using a feeding cannula once daily for 14 consecutive days | 2,4-dinitrophenylhydrazine-induced haemolytic anaemia in Wistar rats | ↑ PCV, Hb, RBC concentration ↓ WBC, MCV, MHC and reticulocytes ↑ catalase and SOD activity | [130] |
| Gossypium hirsutum L. leaf ethanolic extract | 100–400 mg/kg | in vivo | orally | PHZ-induced haemolytic anaemia in rats | ↑ RBC, Hb, PCV, neutrophils and platelets ↓ WBC, lymphocytes, MCV, and MCH | [131] |
| Falcaria vulgaris leaf aqueous extract | 25, 50, 100, and 200 mg/kg | in vivo | Orally | PHZ-induced haemolytic anaemia in rats | ↑ the levels of body weight, WBC, neutrophils, platelet, RBC, Hb, PCV, MCV, MHC, MCHC ↓ the raised levels of ALP, ASAT, ALAT, GGT, urea, creatinine, ferrous, ferritin, and erythropoietin | [132] |
| Beta vulgaris (beet) leaf aqueous extract | 100 and 200 mg/kg | in vivo | Orally daily for 12 days | PHZ-induced haemolytic anaemia model in albino rats | restored the levels of RBCs, WBCs, Hb, and HCT ↓ MCV, MCHC ↓ MDA | [133] |
| Pterocarpus erinaceus stem bark aqueous extract | 250 and 500 mg/kg | in vivo | Oral administration for 14 days | PHZ-induced anaemia in albino rats | ↑ PCV, Hb, RBC, MCV, neutrophiles ↓ WBC, lymphocytes, platelets, MCH, MCHC ↓ ASAT, ALAT | [134] |
| Lophira lanceolata leaves aqueous extract | 200, 400, and 800 mg/kg | in vivo | oral for 3 weeks | PHZ-induced anaemia in wistar rats | ↑ in the RBC count, Hb concentration and PCV | [135] |
| Mangifera indica bark aqueous decoction | 25, 50, and 100 mg/kg | in vivo | once daily for 14 consecutive days by oral feeding cannula | 2,4-dinitrophenylhydrazine-induced haemolytic anaemia in Wistar rats | ↑ PCV and Hb ↓ TLC | [136] |
| Ficus sur bark/fruit methanolic extract | 50, 100, and 150 mg/Kg | in vivo | oral administration for 14 days | PHZ-induced haemolytic anaemia in rats | ↑ Hb, HTC, RBC count | [137] |
| Justicia carnea leaves ethanolic extract | 500 and 1000 mg/kg | in vivo | orally gavage for 28 days | PHZ induced-anemia albino rats | ↑ Hb, RBC, PCV ↓ cholesterol, triacylglycerol, and LDL cholesterol concentrations ↑ HDL-cholesterol | [138] |
| Plant/Extraction Details | PHZ and Malaria Parasites-Induced Anemia | Effect on Hematological Parameters |
|--------------------------|------------------------------------------|-----------------------------------|
| Harungana madagascariensis bark extract | PHZ and malaria parasites-induced anemia | ↑ PCV, RBC and Hb [139] |
| Sorghum bicolor extract 200 or 300 mg/kg in vivo gavage for 15 days | PHZ-induced anaemia in rats | ↑ PCV, RBC and Hb [140] |
| Telfaria occidentalis leaf ethanolic extract 200 mg/kg in vivo orally for 2 weeks | PHZ-induced anaemia in rats | ↑ HCT, Hb, RBCs, lymphocytes, monocytes ↓ serum levels of total protein, albumin, globulin and bilirubin ↑ body weight [141] |
| Spinacia oleracea leaf aqueous extract 100 mg/kg in vivo once daily for 28 days | PHZ-induced anaemia in rats | ↑ Hb, PCV level, RBC count [142] |
| Brilliantsia nitens methanolic extract 400, 800, 1600, 3200 mg/kg in vivo oral intubation daily for 4 weeks | PHZ-induced haemolytic anaemia in rats | ↑ Hb, RBC, WBC and PVC [143] |
| Acacia nilotica ethanolic leaf extract 100 mg/kg and 200 mg/kg in vivo orally for 14 days | PHZ-induced anaemia in rats | ↑ PVC, Hb, RBC, MCV, MCH, PLT ↓ WBC, lymphocytes and neutrophils -exhibited high radical scavenging activity [144] |
| Solanum nigrum leaf methanolic extract 100, 200, 300, and 400 mg/kg in vivo orally by gastric intubation for three weeks | PHZ-induced anaemia in rats | ↑ RBCs, WBCs, Hb, HTC [145] |
| Mangifera indica and Telfairia occidentalis extracts 20 mg kg in vivo oral daily dose | PHZ-induced anaemia in rabbits | ↑ PCV values, RBC counts, Hb, HTC, bilirubin [146] |
| Hibiscus sabdariffa anthocyanins 100 mg/kg in vivo gavage for 4 weeks | 2, 4-dinitrophenylhydrazine (2, 4-DNPH) rabbits | ↑ in blood GSH, RBC counts, PCV and Hb, in MDA and WBC counts [147] |
| Justicia secunda leaves extracts 200 mg/kg in vivo for 21 days | PHZ-induced anaemia in Sprague-Dawley rats | ↑ the number of RBCs, Hb [149] |

PHZ, phenylhydrazine; WBC, white blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; GGT, gamma-glutamyl transferase; TLC, total leucocytes count; 2, 4-DNPH, 2,4-dinitrophenylhydrazine.

5. Natural Antioxidants in the Treatment of Thalassemia

Thalassemias are a group of inherited diseases that lead to a defective hemoglobin production. Patients with thalassemia have a mutation that affects the production of the hemoglobin globin polypeptide chain and is associated with inefficient erythropoiesis. It is characterized by decreased HbA production secondary to low beta-globin chain production and stopping maturation due to apoptosis of erythroid precursors induced by excess alpha chain precipitates [150].

Iron overload is a common complication of thalassemia syndromes, which can lead to organ damage and increased mortality [151,152]. Iron-induced toxicity in β-thalassemia is the leading cause of oxidative stress. Oxidative stress, associated with the formation of reactive oxygen species (ROS), plays an important role in the development of inflamma-
tion, decreased plasma antioxidant levels, depletion of erythrocyte glutathione (GSH), increased lipid peroxidation of RBCs membranes and immunosuppression in the affected patients [153,154]. Moreover, iron overload in patients with β-thalassemia decreases T cell proliferative activity [155,156].

Flavonoids and phenolic compounds have antioxidant properties, ability to neutralize free radicals [157–160] and metal chelation, suggesting that they may have a protective effect under oxidative stress-based pathological conditions caused by iron overload [161]. Therefore, the use of polyphenols as iron chelators has been proposed in clinical practice [162].

Silymarin, isolated from *Silybum marianum*, is a powerful antioxidant and has hepatoprotective and iron chelating activities [163], being introduced as an adjuvant without side effects in numerous clinical trials [164]. Gharagozloo et al. (2013) recommended the use of silymarin as a possible herbal immunomodulatory drug in the treatment of patients with β-thalassemia due to its antioxidant, cytoprotective and iron chelating activity. The study included 59 β-thalassemia patients who received 140 mg of silymarin and desferoxamine (DFO) three times daily for 3 months. Combination therapy has been well tolerated and more effective in reducing serum ferritin levels than administering DFO alone, in increasing GSH level of RBCs and promoting a decrease in serum iron and ferritin [165]. In another clinical study, patients were treated with a combination of DFO and silymarin (420 mg / day) or DFO for 9 months; Silymarin treatment significantly reduced serum iron, ferritin, serum hepcidin, and soluble transferrin receptor (sTfR), demonstrating the beneficial potential of silymarin as an iron chelating agent in reducing the serum ferritin and iron level in β-thalassemia [166]. Similar results were obtained by Hagag et al. (2015) in a clinical study for silymarin in combination with deferiprone (DFP) [167], as well by the combination of deferasirox (DFX) and silymarin [168]. Serum iron levels decreased significantly [168]. Therefore, the effects of iron chelating silymarin are related to its Fe (III) binding capacity.

The results of the studies regarding the pharmacological activities exerted by natural in β-thalassemia treatment are summarized in Table 4.

| Bioactive Compound | Doses | In Vitro/ In Vivo/ Clinical Study | Route of Administration | Model | Bioactive Effect | References |
|--------------------|-------|----------------------------------|-------------------------|-------|-----------------|------------|
| **Resveratrol**    | 5 μM  | in vitro                         | CD34+ cells             | ↑ maturation of erythroid cells ↓ cell proliferation -induces cell differentiation of human β-thalassemic-erythroid cells | [169]    |
|                    | 2.5 mg/kg | in vivo                           | resveratrol incorporated into a standard diet for six months | ↑ ineffective erythropoiesis ↓ anemia | [169]    |
| **Curcumin**       | 500 mg daily for 12 months | clinical study | Beta-thalassemia/Hb E disease | ↑ MDA, SOD, GSH-Px in RBC ↓ serum NTBI ↑ RBC GSH | [170]    |
|                    | 100 μM curcumin | clinical study | Beta-thalassemia patients | ↓ plasma NTBI | [171]    |
| **Fermented papaya preparation (FPP)** | 50 mg/kg daily for 3 months | in vivo | Oral administration | β-thalassemia mouse model daily for 3 months | ↑ GSH, PMN ↓ ROS ↓ lipid peroxidation ↓ externalization of phosphatidylserine | [172]    |
Green tea extract (GTE) 50 mg/kg EGCG in vivo for 2 months β-knockout thalassemic (BKO) mice ↓ MDA, NTBI, and ALAT ↑ plasma hepcidin and insulin ↓ iron accumulation and MDA in pancreas and liver -improved liver and pancreatic β-cell activity by decreasing redox iron/free radicals [173]

Curcumin 200 mg/kg and 50 mg/kg DFP in vivo for 2 months mouse models ↓ plasma NTBI ↓ MDA concentrations ↓ heart iron accumulation -improved HRV [174]

EGCG from green tea in vitro iron-treated erythrocytes ↓ bound Fe³⁺ and iron chelation ↓ oxidative stress [175]

Extracts of green tea (GTE) and curcumin 17.3-35.5 mg/kg EGCG equivalent clinical study daily for 60 days transfusion-dependent β-thalassemia (TDT) patients ↓ of blood urea nitrogen levels ↓ NTBI ↓ LPI -delayed in increasing lipid-peroxidation [176]

Beta-thalassemia major is a hereditary haemolytic anemia in the treatment of which multiple blood transfusions are used [177]. Patients with major thalassemia, also known as Cooley’s anemia, have severe and hypochromic microcytic anemia, associated with an increased number of RBCs and a low level of mean corpuscular volume (MCV) and mean corpuscular Hb (MCH). Peripheral blood smear highlights microcytosis and hypochromia, anisocytosis, poikilocytosis, and nucleated RBCs (e.g., erythroblasts). The number of erythroblasts correlates with the degree of anemia and is significantly increased after splenectomy [178].

Finding natural iron chelators of plant origin that also act as a hepcidin agonist may be useful in the management of excess iron in patients with β-thalassemia major [179].

The results of the studies regarding the pharmacological activities exerted by natural in β-thalassemia major are summarized in Table 5.

Table 5. Pharmacological effects of natural antioxidants in β-thalassemia major treatment.

| Bioactive Compound | Doses | In Vitro/In Vivo/ Clinical Study | Route of Administration | Model | Bioactive Effect | References |
|--------------------|-------|---------------------------------|-------------------------|-------|-----------------|------------|
| Silymarin          | 140 mg/kg + DFO | clinical study | three times/ day | 59 patients with thalassemia major | ↑ GSH levels of RBCs ↑ alkaline phosphatase ↓ ferritin and iron levels ↓ serum iron and ferritin | [180] |
| Silymarin          | Legalon capsules (140 mg) | clinical study | 9 months | 97 patients with β-thalassemia major | ↓ TIBC levels ↓ serum levels of hepcidin and soluble transferrin receptor (sTIR) | [166] |
| Nigella sativa     | 2 g/day | clinical study | Nigella sativa powder added to foods or drinks for 3 consecutive months | 25 blood transfusion-dependent childrens with β-thalassemia major | ↑ Hb, WBCs ↑ neutrophils ↓ MDA ↓ TAC | [181] |
| Fermented papaya   | 3 g | clinical study | 3 g three times a day after meals for three months | patients with β-thalasmas | ↑ GSH in RBCs ↓ ROS in RBCs ↓ lipid peroxidation | [182] |
| Curcumin           | 500 mg capsules (total: 1000 mg) | clinical study | twice daily for 12 weeks | 68 β-thalassemia major patients | ↓ NTBI ↓ ALAT, ASAT -alleviated iron burden and liver dysfunction | [183] |
Green tea extract (GTE) GTE+DFP (50 mg/kg) daily orally for 3 months \(\beta\)-thalassemic mice with iron overload ↓ plasma non-transferrin bound iron concentrations ↓ plasma ALAT activity ↓ tissue iron deposition ↓ plasma NTBI levels ↓ liver oxidative damage [184]

6. Natural Antioxidants in the Treatment of Sickle Cell Anemia

Sickle cell anemia is inherited as an autosomal recessive condition [185]. Sickle cell disease is one of the most notable impairments in the structure of hemoglobin. While the amount of hemoglobin produced may be normal, the substitution of the amino acid valine with glutamic acid results in a structural defect that favors the polymerization of deoxy- genated hemoglobin [186]. It is characterized by the presence of sickle-shaped cells that block blood flow through the spleen, causing splenic sequestration [185]. When deoxyhemoglobin polymerizes, it forms fibers that alter the shape of erythrocytes [186]. Repeated stress caused by sickle cell disease will damage circulating erythrocyte membranes, leading to premature cell death. While sickle cell anemia may remain asymptomatic for a significant period of time, severe hypoxia may cause a seizure, with symptoms of generalized pain, fatigue, headache, jaundice, and repeated vascular occlusion (stroke, etc.) [3].

Given the increasing mortality rate of patients with sickle cell disease, especially in children, and the side effects of chemotherapy, the addition of natural products (phyto- medicines/herbal drugs) in the treatment is beneficial [187]. Several herbal extracts have properties in sickle cell anemia treatment, but there is still no promising drug on the market for the treatment of this condition [188–191]. The active constituents of medicinal plants and natural compounds, known as antisickling agents, are rich in aromatic amino acids, phenolic compounds, and antioxidants [192] and acts as antioxidant therapy to ameliorate the complications of sickle cell anemia [193]. Antioxidants have many beneficial effects, protect against RBC lipid peroxidation, increased glutathione levels (GSH), and reduce ROS levels [194].

Rutin (quercetin-3-rhamnosyl glucoside) is a flavone intensive studied for its antioxidant properties [195–197]. Rutin has antiplatelet and protective effects of the vascular endothelium against oxidative stress in sickle cell anemia [198,199]. Moreover, it restored the integrity of the erythrocyte membrane, prevented and reversed lipid peroxidation, induced increased GSH and CAT levels, and decreased SOD activity. The beneficial effects of rutin in sickle cell anemia may be associated with modulation of deoxy-hemoglobin and alteration of redox homeostasis. Similar results were obtained for chrysin [199].

The main pharmacological effects of the phytochemicals/herbs in sickle cell anemia treatment are summarized in Table 6.

Table 6. Pharmacological effects of natural antioxidants in sickle cell anemia treatment.

| Bioactive Compound | Doses | In Vitro/In Vivo/| Route of Administration | Model | Bioactive Effect | References |
|--------------------|-------|-----------------|--------------------------|-------|-----------------|------------|
| DTT, N-NAC quercetin | 0.25 mM DTT 10 mM N-NAC 100 μM quercetin | in vitro | sickle cell anemia in vitro model | -inhibition of the main cation pathways responsible for dehydration ↓ Ca\(^{2+}\)-induced PS exposure and hemolysis ↓ RBC’s fragility | [194] |
| Rutin | in silico in vitro | sickle erythrocytes induced with 2% metabisulphite | -restored the integrity of erythrocytes membrane -prevented and reversed lipid peroxidation ↑ GSH and CAT levels ↓ SOD activity | [199] |
| Extract/Extract | Concentration | Method | Action | Notes |
|-----------------|--------------|--------|--------|-------|
| Chrysin         | - in silico  | sickling was induced with 2% metabisulphite at 3 h. | - prevented sickling | [200] |
|                 | - in vitro   | | - re-established the integrity of erythrocytes membrane | |
|                 |              | | - prevented and reversed lipid peroxidation | |
|                 |              | | ↑ GSH, CAT | |
|                 |              | | ↓ SOD | |
| Pfaffia paniculata extract | 0.0, 0.2, or 0.5 mg/mL | in vitro | RBCs from patients with sickle cell disease | - improvement of RBC deformability in patients with SCD |
|                 |              | | - the fragility of RBCs of patients with sickle cell disease was not affected | |
| Aqueous extracts of Dennettia tripetala and Physalis angulata leaf extract | in vitro | homozygous sickle cell erythrocyte | ↑ GSH, SOD | |
|                 |              | | ↓ catalase | |
|                 |              | | ↓ ROS | |
|                 |              | | ↓ % of sickled cells | |
|                 |              | | ↓ haemoglobin polymerization rate | |
|                 |              | | ↓ osmotic fragility of human sickle RBCs | |
| Ethanol extract of Terminalia catappa L. (Combretaceae) leaves | in vitro | metabisulphite-induced sickling | - inhibited osmotically-induced hemolysis of human erythrocytes | |
|                 |              | | - prevented and reversed the sickling of human 'SS' erythrocytes | |
| Extracts of the roots of Cissus populnea L. | in vitro | sodium metabisulphite-induced sickling of the HbSS red blood cells | ↓ hemolysis | |
|                 |              | | - protected erythrocyte membrane integrity under osmotic stress conditions | |
| Methanolic leaf extracts of Carica papaya L. (Caricaceae) | in vitro | Hbss red blood cells obtained from non-crisis state sickle cell patients | ↑ for Na+, K+- and Ca2+-ATPases | |
|                 |              | | ↓ for Mg2+-ATPase | |
| Leaves and stem of Parquetina nigrescens L. | in vitro | pre-sickled HbSS blood cell suspensions | - antig-sickling activity | |
|                 |              | | - protected the integrity of the erythrocyte membrane by the reduction in hemolysis of the Hbss cells | |
|                 |              | | - inhibited formation of sickle cells under severe hypoxia | |
| Aqueous extract of Carica papaya leaf | 2, 4, 6, 8, and 10 mg/mL | | | |
|                 |              | | - prevented sickling | |
|                 |              | | - membrane stabilizing effect on HbSS red blood cells | |
| Divanilloylquinic acids isolated from Fagara zanthoxyloides Lam. (Rutaceae) | patients with severe sickle cell anemia | antisickling properties | | |
| Aqueous extract of Zanthoxylum macrophylla | in vitro | sodium metabisulphite-induced sickling in cells | - antisickling activity | |
|                 |              | | - stabilizing the RBC membranes | |
| Aqueous extracts of Zanthoxylum macrophylla roots | in vitro | membrane preparations from human erythrocytes of HbAA, HbAS and HbSS bloods | modulation of the activities of the three membrane-bound ATPases: | |
|                 |              | | ↑ for Na+, K+ and Ca2+ ATPases | |
|                 |              | | ↓ for Mg2+ ATPase | |
| Aqueous and methanolic extracts of leaves, seeds, and stem of Telfairia occidentalis | 10 mg mL−1 | sickled erythrocytes obtained from SCD patients | - aqueous leaves extract exhibited the highest antisickling activity | |
|                 |              | | - methanolic and aqueous stem extracts showed membrane stabilizing effects | |
| Aqueous extracts of Elaeis guineensis Jacq flowers | | | - anti-sickling activity | |
|                 |              | | ↓ in Fe2+/Fe3+ ratio | |
| Extract Source | Concentration | Mode of Administration | Effect |
|----------------|---------------|------------------------|--------|
| Methanol extract of *Mucuna pruriens* leaves | 100, 200, 400, 600, and 800 mg/mL | - | - altered the polymerization of sickle cell Hb - maintained erythrocyte membrane integrity |
| Aqueous extracts of *Zanthoxylum heitzii* | 250, 500, and 1000 μg/mL | in vitro | ↓ % sickle cells - membrane stabilization ↓ haemolysis - scavenging activity of the DPPH and hydroxyl radical |
| *Telera occidentalis, Curcubit maxima, Curcumin sativum and Curcubit lonatus* | | in vitro | - inhibited sickle cell Hb polymerization - improved Fe²⁺/Fe³⁺ ratio |
| *Solanostemon monostachyus* (SolMon), *Carica papaya* seed oil (Cari-oil) and *Ipomoea involucrata* (Ipocrata) | | in vitro | ↓ % sickle cells ↑ RBCs ↓ Fe²⁺/Fe³⁺ ratio ↓ LDH ↑ CAT activity |
| Ethanol extract of *Annona Maricata, Delonix Regia* and *Senna Alata* | | in vitro | ↑ GHS and CAT activities ↓ SOD activity ↓ the sickling activity ↓ the polymerization of sickle cells |
| Ursolic acid isolated from the leaves of *Ocimum gratissimum* L. | | in vitro | - anti-sickling effects ↑ RBCs |
| Extracts of the seed, flower and leaf of *Moringa oleifera* | | in vitro | - anti-sickling effects |
| Aqueous methanol extracts of *Scoparia dulcis* and fractions | 100, 300 and 500 mg/m extract 500 mg/mL fractions | in vitro daily administration of the extract for 30 days | in vivo in Swiss albino mice and Wistar rats - anti-sickling effects |
| Aqueous extract of unripe pawpaw (Carica papaya) | | | sickle cells of patients - prevented sickling of Hb SS red cells and reversed sickled Hb SS red cells |
| Leaves ethanol extracts of *Hymenocardia acidulosa* Tul (Euphorbiaceae) | | | sickle erythrocytes - reversed sickled human RBC - the fractions containing flavonoids, saponins and carboxylic acids were found to be responsible for reversal of the sickled RBC. |
| Leaf and gel extracts of the Aloe vera (Aloe barbadensis) | | | sickle erythrocytes - inhibited sickle cell polymerization - improved of the Fe²⁺/Fe³⁺ ratio of HbSS |
| Aged garlic extract | 5 mL daily clinical study for 4 weeks | | five patients with sickle-cell anemia ↓ number of Heinz bodies - antioxidant activity on sickle RBCs |
| *Moringa Oleifera* Seeds and leaves extracts | | in vitro | erythrocyte cells deoxygenated with 2% sodium metabisulphite - anti-sickling activity in deoxygenated erythrocytes |
| *Amphimas pterocarpoides* | | in vitro | the sickling of RBCs was induced using sodium metabisulphate 2% - anti-sickling effects ↓ the solubility of the deoxy-haemoglobin S - allowed the rehydration of SS cells by reinforcing their capacity to resist against osmotic fragility |
| Isoquercitrin | | in vitro | sickle erythrocytes ↓ % sickle cells ↓ polymerization |
7. Natural Antioxidants in the Treatment of Aplastic Anemia

Aplastic anemia is a condition in which the bone marrow is destroyed and blood cell production is diminished [231]. This usually correlates with a deficiency of erythrocytes (anemia), leukocytes (leukopenia), and platelets (thrombocytopenia) [232,233]. Aplastic anemia refers to the syndrome of chronic primary hematopoietic insufficiency due to lesions, leading to diminution or absence of hematopoietic precursors in the bone marrow [234,235].

Aplastic anemia can be caused either by extrinsic suppression mediated by hematopoietic stem cell immunity or by intrinsic bone marrow progenitor abnormality [236,237]. Damaged hematopoietic stem cells mature into self-reactive T-helper (T1) cells that release cytokines: interferon (IFN) and tumor necrosis factor (TNF) to develop a cytotoxic cascade to kill and suppress other hematopoietic stem cells. The exact antigens of T1 target cells are unclear, but one appears to be one of the glucose inositol phosphate-bound (GPI) proteins on cell membranes. Moreover, genes involved in apoptosis are overexpressed [238].

Strategies recently applied in the treatment of aplastic anemia include immunosuppression and/or hematopoietic stem cell transplantation [239,240]. Numerous lymphocytotoxic agents have been widely used, but some of them have various adverse effects, such as anaphylaxis fever, chest pain, and diarrhea [241].

In recent years, natural herbal products have attracted much attention, being used as an effective and safe alternative treatment for bone marrow failure [242].

For example, saponins extracted from Panax notoginseng (PNS) induced the proliferation of hematopoietic stem/progenitor cells and stromal cells in vitro [243–247], probably by overexpressing genes involved in hematopoiesis, such as GR-NTF [243]. PNS activated the MAPK pathway and GATA transcription factors in hematopoietic cells [245]. Moreover, it has been shown to differentiate the mesenchymal stem cells and NIH3T3 cells [244,246].

The main pharmacological effects of the phytochemicals/herbs in anaplastic anemia treatment are summarized in Table 7.

Table 7. Pharmacological effects of natural antioxidants in aplastic anemia treatment.

| Bioactive Compound | Doses | In Vitro/Route of Administration | Model | Bioactive Effect | References |
|--------------------|-------|---------------------------------|-------|------------------|------------|
| Hyphaene Thebaica (Doum) fruit extract | 1000, 500, and 250 μg/mL in vitro | incubation of RBCs with 2% sodium metabisulphite | ↑ the oxygen affinity | ↑ osmotic fragility of the sickle RBCs | [228] |
| Genistein | in vitro | sickle erythrocytes | ↓ polymerization of Hb S | ↓ % sickle cells | ↑ the osmotic fragility of the erythrocyte cell | [229] |
| Methanol seed extract of Buchholzia coriacea and Mucuna pruriens seed extract | 50%, 25%, 12.5%, and 6.25% seed extracts in vitro | sickle cell blood from sickle cell disease patients with subsequent addition of 2% sodium metabisulphite to cause more sickling | Buchholzia coriacea: | -inhibited sickling | -reversed sickled RBC | [230] |
| | | | Mucuna pruriens: | ↑ the solubility of sickle Hb | ↑ Fe²⁺/Fe³⁺ ratio | |
| | | | | | ↓ osmotic fragility | |

DTT, dithiothreitol; NAC, N-acetylcysteine; 2,3-BPG, 2,3-bisphosphoglycerate; SCD, sickle cell disease; MCF, mean corpuscular fragility; DPPH-2, 2-diphenyl-l-picryl hydrazyl; SolMon, Solenostemon monostachyus; Cari-oil, Carica papaya seed oil; Ipocrata, Ipomoea involucrata; EFCM, Mbalmayo/Ebolowa cocoa bean extract; EFCB, Bertoua cocoa bean extract; FRAP, ferric-reducing antioxidant power.
| In Vivo/ Clinical Study | Leaf Panax notoginseng saponins (LPNS) | 50, 100, and 200 mg/kg | intragastric administration daily for 14 days | Aplastic anemia model in BALB/c mice | ↑ WBC, platelets, RBC, Hb | ↑ hematopoiesis - improve myelosuppression ↓ inflammation | [247] |
|------------------------|---------------------------------------|------------------------|---------------------------------------------|-----------------------------------|------------------------|------------------------------------------------|----|
| Curcumin and baicalein | -curcumin (1 and 4 g/kg) -baicalein (0.6 and 2 g/kg) dissolved in corn oil | intragastric administration once a day for 5 weeks | Aplastic anemia mouse model with iron overload | ↑ WBC ↑ Hb levels ↑ hepcidin and its regulators (BMP-6, SMAD4, and Tfr2) | [248] |
| Ginsenoside Rb1         | 1, 2, and 4 mg/mL                     | intraperitoneally injection daily for 12 days | An immune-mediated aplastic anemia mouse model | ↑ WBC, HGB, PLT, and bone marrow stem cells ↓ T-cell activation by suppressing DC maturation | [249] |
| Panax-adiol saponins component | 20, 40, and 80 mg/kg | intragastric daily for 15 days | Aplastic anemia model mice BALB/c mice | ↑ WBC, platelet, neutrophil counts - enhanced proliferation of hematopoietic progenitor cells ↑ peripheral blood CD3⁺ and CD3⁺CD4⁺ cells and ↓ CD3⁺CD8⁺ cells ↑ CD4⁺CD25⁺FoxP3⁺ cells, alleviated pancytopenia with a hypocellular bone marrow ↑ the percentage of CD4⁺ cells in BMNC ↑ the CD4⁺/CD8⁺ ratio ↓ the pro-inflammatory cytokine concentrations of IL-2 and IFN-γ ↑ the anti-inflammatory cytokine IL-4 - inhibited Fas–FasL-induced BMNC apoptosis - suppressed intracellular apoptosis | [250] |
| Saponins from Dioscorea nipponica | 37.44, 74.88, and 149.76 mg/kg | orally for 14 days | Aplastic anemia model mice | | |

LPNS, leaf Panax notoginseng saponins; BMP-6, bone morphogenic protein 6; HGB, hematocrit and hemoglobin; HPC, hematopoietic progenitor cells; CFU-GM, colony-forming unit for granulocytes and macrophages; CFU-E, colony forming unit-erythroid; BMNC, bone marrow nucleated cells; IFN-γ, interferon-γ; GATA-3, erythroid transcription factor-3.

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