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Chapter

Nutraceutical Benefits of Green Tea in Beta-Thalassemia with Iron Overload

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Abstract

Secondary iron overload in patients with β-thalassemia is caused by multiple blood transfusions and increased iron absorption. Most of them die from cardiac arrest and infections while others from oxidative tissue damage and organ dysfunction. Under high saturation of transferrin with iron, redox-active iron such as non-transferrin-bound iron, labile plasma iron, and cellular labile iron pool is prone to the production of reactive oxygen species, oxidized biomolecules, oxidative tissue damages, and complications. Iron chelation therapy and antioxidant supplementation are a supportive treatment for patients' better quality of life and life expectancy. Green tea (*Camellia sinensis*) extract (GTE) is abundant with polyphenols, mainly epigallocatechin-3-gallate and nutraceuticals, which are beneficial for cell functions and health. Importantly, GTE possesses antioxidant, free radical scavenging, metal-chelating, anti-hemolysis properties in cell cultures, animals, and humans. This article has reported modes of actions and challenged such wonderful properties of green tea used to remove excessive iron, scavenge harmful radicals, restore malfunctions of vital organs, and treat patients with β-thalassemia with iron overload. Infeasibility and sustainability, the benefits of green tea can be applied for use in other diseases with iron toxicity and oxidative stress.

Keywords: *Camellia sinensis*, catechins, green tea, iron, oxidative stress, thalassemia

1. Introduction

Erythrocytes or red blood cells (RBC) play a crucial role in oxygen carrying and transportation. The main component of RBC is hemoglobin (Hb) which consists of four subunits of globin chain with heme group at the center of each subunit. The types of Hb can be defined by different globin chain compositions. For instances, hemoglobin A (HbA) consists of two α-globin chains and two β-globin chains ($\alpha_2\beta_2$), which is found in high levels of normal human blood. Hemoglobin A_2 (HbA$_2$) consists of two α-globin chains and two δ-globin chains ($\alpha_2\delta_2$), which is found in low levels of normal human blood and may be increased in the blood of patients with β-thalassemia. Fetal hemoglobin (HbF) consists of two α-globin chains and two γ-globin chains ($\alpha_2\gamma_2$), which is present in high levels in fetal and newborn human blood. Likewise, embryonic hemoglobin such as hemoglobin Gower I ($\zeta_2\varepsilon_2$) and Gower II ($\alpha_2\varepsilon_2$) are produced in early life and switched to other Hb types during development.

Human β-thalassemia is characterized by mutations of β-globin gene, resulting in deficient production of the β-globin chains of Hb molecule (ineffective erythropoiesis) and chronic anemia. Over 200 mutations have been identified in this gene, and the type of mutations can influence the severity of the disease. Blood transfusions aim to maintain normal Hb levels to prevent tissue hypoxia, whereas repeated blood transfusions lead to the inevitable consequence of iron accumulation in the body. Iron deposition occurs considerably in almost all tissues but is primarily located in the spleen, liver, heart, and endocrine glands. Besides ferritin iron and hemosiderin, the uncommon forms of iron including labile or transient iron pools (LIP) in the tissues, nonheme iron in RBC membrane, and non-transferrin-bound iron (NTBI) and labile iron pool (LPI) in plasma appear to be redox-active and subsequently generate reactive oxygen species (ROS) via Haber-Weiss and Fenton reactions. The ROS can oxidize biomolecules, causing oxidative tissue damage, organ dysfunctions, complications, and death. Effective iron chelation needs giving to counteract the resulting iron overload and prevent oxidative tissue damage. So far, monotherapy or combined therapy with iron chelators such as desferrioxamine (DFO), deferiprone (DFP), and deferasirox (DFX) has been used for the treatment of patients with β-thalassemia with iron overload, but they present some side effects. Modified medical regimens such as adjunctive iron chelator and antioxidant and drug cocktail are purported to increase the chelation efficacy, minimize the side effects, and achieve additive chelation efficacy. Moreover, commercially available antioxidants such as vitamin C, vitamin E, and *N*-acetylcysteine (NAC) are usually given together with the chelators to relieve the oxidative stress in patients with thalassemia. Herein, an interesting natural product such as green tea extract from tea leaves (*Camellia sinensis*) has been documented in terms of bifunctional antioxidant and iron-chelating properties in iron-overloaded cells and mouse and human thalassemia, rather than its general biological and pharmacological properties.

2. β-Thalassemia

2.1 Etiology and pathophysiology

β-Thalassemia is a quantitative hemoglobinopathy which impairs the production of β-globin chains in Hb due to mutations of the gene located on the short arm of chromosome 11. Accordingly, a reduction of β^+ or an absence of β^0 in β-globin synthesis causes the precipitation of excessive unbound α-globin chains in erythroid precursors due to chain imbalances. The abnormal erythroid precursors are driven into apoptosis pathway during their differentiation and maturation in the bone marrow, consequently leading to erythroid expansion, accelerated extramedullary erythropoiesis, increased dietary iron absorption, and high turnover of RBC. Moreover, impaired β-globin synthesis and ineffective erythropoiesis result in microcytic anemia and progressive splenomegaly. There are three main types of β-thalassemia, in order of decreasing severity, homozygous β-thalassemia major (TM), β-thalassemia intermedia (TI), and heterozygous β-thalassemia minor. Hemoglobin E (HbE)/β-thalassemia is the most prevalent in Southeast Asia where the carrier frequency is around 50%. The interaction of HbE and β-thalassemia results in a clinical spectrum ranging from a severe condition that is indistinguishable from TM to a mild form of TI [1].

Nowadays, β-thalassemia is divided into transfusion-dependent β-thalassemia (TDT) and non-transfusion-dependent β-thalassemia (NTDT). In other mammals, mouse homozygous beta-globin knockout (BKO) thalassemia shows many clinical features of abnormal RBC indices including a decrease in blood Hb concentration,

hematocrit (Hct), RBC numbers, and osmotic fragility and an increase of reticulocyte count. Likely, increased degradation of abnormal RBC is an important consequence of unstable Hb and excessive membrane iron in patients with β-thalassemia. Invasive and noninvasive laboratory investigations reveal systemic and cellular iron overload in TDT and NTDT patients. Large amounts of the irons from enterocytes and reticuloendothelial (RE) macrophages in the spleen can get into plasma compartment and subsequently bind to transferrin (Tf). Accordingly, this can lead to a high saturation of Tf with iron, an appearance of NTBI and LPI, and high levels of ferritin in plasma compartment, together with high iron deposition ferritin (Ft) in several tissues in the body. Consequently, iron accumulation in the vital organs is the cause of susceptibility to infections and immunological abnormalities, liver diseases (e.g., hepatitis, hepatic fibrosis, and hepatocellular carcinoma), cardiomyopathies (e.g., cardiac arrhythmia and heart failure), and endocrine gland dysfunction (e.g., diabetes, growth retardation, hypogonadism, and hypoparathyroidism) [2–4]. Evidentially, most of patients with β-thalassemia with iron overload die of heart failure, while some patients frequently die from infections and suffer from liver diseases and endocrinopathies.

2.2 Iron overload and oxidative stress

2.2.1 Iron transport into cells

In fact, abnormal iron absorption in patients with thalassemia produces an increase in the body iron burden evaluated at 2–5 g per year, and regular blood transfusion (420 mL/U, equivalent to 200 mg of iron) leads to double iron accumulation [5]. Normally, iron is bound to iron-transporting protein in plasma (called transferrin) and in milk (called lactoferrin), forms transferrin-bound iron (TBI), and is transported in blood circulation to target cells. The circulating TBI, which is low saturation in iron-deficiency persons, one-third saturation in normal people, and high saturation in iron overload patients, is taken up into iron-requiring target cells by using ATP-dependent transferrin receptor 1 (TfR1)-mediated endocytosis and delivered in cells for functions and storage in ferritin molecules (H- and L-subunits). When the capacity of transferrin to incorporate iron derived from gastrointestinal (GI) tract and RE cells becomes limited, the transferrin iron-binding capacity (TIBC) has been surpassed [6]. Afterward, two forms of redox-active iron such as NTBI and LPI appear primarily in plasma of patients with β-thalassemia. Pathologically, the NTBI fraction seems to translocate across plasma membrane via specific transporters on specific cell types. NTBI transporter which is originally called divalent metal ion transporter 1 (DMT1) or natural resistance-associated macrophage protein 2 (Nramp2) is proposed to locate on intestinal epithelial cells, erythroid cells and astrocytes, L-type calcium channel (LTCC), T-type calcium channel (TTCC) on cardiomyocytes, T-type calcium channel (TTCC) on hepatocytes, pancreatic islets β cells and kidney tubular cells, anion-exchange protein 2 (AE2) on bronchial epithelial cells, ferrireductases on kidney proximal tubule, and zinc ion protein 18 (ZIP18) on kidney tubular cells and hippocampal neuronal cells [7–14]. Importantly, NTBI and LPI are redox-active and susceptible to chelation [15].

2.2.2 Iron overload

The iron that cells do not require immediately for metabolic processes is stored in ferritin in the liver, enterocytes, and RE macrophages, representing the storage iron pool. The iron that mobilizes transiently inside the cells is called LIP that

is potentially redox-active and increased when the cells are heavily loaded with a large number of extracellular irons, TBI, and NTBI. Iron distribution in the body is strictly regulated by two regulatory systems, systemic and cellular iron homeostasis. Systemic iron homeostasis strictly responds to ensure a balance of iron absorption and iron utilization, which relies on the hepatic hepcidin hormone and the ferroportin actions and occurs in enterocytes, hepatocytes, and splenic macrophages. Hepcidin levels found to decrease in primary hemochromatosis and secondary hemochromatosis such as TI patients due to an acceleration of erythropoietic activity driven by increases of erythropoietin (EPO) production and TfR1 expression. Inversely, hepcidin levels increased in TM patients due to blood transfusion that they do not need to increase erythropoiesis to compensate ineffective erythropoiesis [16]. The regulation will reduce iron efflux from ferroportin at the basolateral part of duodenal epithelial cells and from RE macrophage into the plasma, resulting in iron retention within the cells. Drugs or natural products that increase hepcidin expression and production would be beneficial for the supportive treatment of TI patients with iron overload. Cellular iron homeostasis is dependent on the expression and function of TfR1 and ferritin mediated by iron regulatory element (IRE)/iron regulatory protein (IRP) system. Once TBI is internalized into cells via TfR-mediated endocytosis, iron is mainly stored by ferritin inside the cells [17]. In patients with thalassemia, large amounts of iron from diet and degradation of effete RBC by RE macrophage drain into plasma transferrin and subsequently taken into cells. Consequently, this will result in iron overload, oxidative stress, and depletion of antioxidant defense systems in plasma compartment and many vital organs in the body.

2.2.2.1 Blood

Thalassemia RBC containing large amounts of iron and low protective antioxidant system is prone to be damaged by ROS, leading to chronic hemolytic anemia. In young patients with β-thalassemia, plasma and RBC levels of thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), and ferritin were increased, but the level of catalase (CAT) was decreased when compared with normal children [18]. Interestingly, Aphinives and colleagues have found decreased levels of antioxidants such as reduced glutathione (GSH) and vascular endothelial dysfunction in young Thai patients with β-thalassemia with HbE patients [19]. Additionally, blood levels of CAT, glutathione-*S*-transferase (GST), GSH, and vitamin C were found to decrease in β-thalassemia major patients while blood SOD level was increased [20]. Importantly, the levels of blood antioxidant system including GST, glutathione peroxidase (GPx), glutathione reductase (GR), peroxiredoxin 2 (Prx2), thioredoxin 1, and thioredoxin reductase were decreased in β-thalassemia major patients with regular blood transfusion and iron chelation therapy, whereas the blood levels of CAT and SOD were increased when compared to healthy subjects [21]. Consistently, the levels of red cell SOD, GPx, and CAT activities increased in hemoglobin H (HbH) disease (a moderately severe α-thalassemia) patients when compared to healthy control. However, the Chinese medicine "Yisui Shengxue" granules which have been officially prescribed and clinically used for the treatment of thalassemia for a long time effectively decreased the GPx and CAT activities but increased the SOD activity [22]. Prolonged bleeding times and abnormal platelet aggregation can be found in transfusion-dependent β-thalassemia major patients, possibly due to artifacts caused by in vitro manipulations, oxidative platelets, and circulating procoagulants such as microparticles (MP) tissue factors and platelet-derived MP [23, 24]. Platelet numbers were

approximately 1.5- and 4-fold increase in Thai non-splenectomized and splenectomized patients with HbE/β-thalassemia when compared with normal subjects [25]. In comparison, thrombocytosis, platelet hyperaggregation, and decreased levels of protein S, protein C, and antithrombin III were detected in NTDT (such as TI) patients (9.4% with splenectomy and 90.6% without splenectomy) [26]. Increased oxidative stress of thalassemia platelets was restored by the treatment of iron chelators such as DFO and antioxidants such as NAC and vitamin C [24].

2.2.2.2 Spleen

The spleen is an organ containing some of the RE cells that function to destroy RBC hemoglobin by macrophages and store the released iron in the form of ferritin and hemosiderin. The number of blood transfusions in β-thalassemia major patients seems to correlate with their splenic hemosiderosis and splenic weight [27]. Hemosiderin deposition was found to be greater in the iron-overloaded livers than in the iron-overloaded spleens. Ferritin and hemosiderin increased in hepatocytes and splenic RE cells [28]. Splenectomy is one of the therapeutic options in hyper-transfused β-thalassemia major patients to reduce hyperactivity of RE macrophages; nevertheless, it may increase the iron overload. As a consequence, complications in patients with splenectomized thalassemia have included hypercoagulability, an increased incidence of vascular thrombosis, and an increased risk of infection. Iron overload in the spleen can activate latent nuclear factor-kappa B (NFκB) in alveolar macrophage, reduce immunity, and increase susceptibility to infection [29]. Notably, Prx2, which is a typical-2 cysteine peroxiredoxin and a key antioxidant system, is upregulated during erythropoiesis in patients with β-thalassemia and contributes to the stress erythropoiesis in the patients. Nuclear factor erythroid 2 (Nrf2) is a redox-response transcriptional nuclear factor and cellular adaptive process in response to and protection of oxidative stress [30]. Therefore, the regulation of *Prx2* and *Nrf2* genes results in the upregulation of antioxidant (antioxidant responsive element, ARE) genes required to ensure the survival of iron-overloaded cells.

2.2.2.3 Liver

The liver is one of the main storage organs for iron. Iron overload is considered to be when the ferritin level consistently exceeds 1000 ng/mL (normal range 20–200 ng/mL). Excess free radicals can cause progressive tissue injury and eventually cirrhosis or hepatocellular carcinoma in iron overload patients whose iron is sequestrated predominantly in ferritin or hemosiderin [31]. When plasma transferrin becomes highly saturated, NTBI is detectable and is rapidly transported across the hepatocyte membrane via a specific pathway. Likely, ferroportin 1 is the only protein that mediates the transport of iron out of hepatocytes and is then oxidized by ceruloplasmin and bound to transferrin. Iron deposition affects hepatic parenchymal cells (hepatocytes and bile duct cells) and mesenchymal cells (endothelial cells, macrophage, and Kupffer cells) and often distributes differently from one area to another [32]. As mentioned above, iron overload can induce ROS which can oxidize biomolecules. Lipid peroxidation activates tumor growth factor-beta1 (TGF-β1) expression which is the most potent pro-fibrogenic cytokine, and its expression is increased in almost all of fibrotic diseases. Type I collagen is induced by TGFβ resulting in uncontrolled collagen production and leads to tissue scarring and organ failure. The scar tissue replaces normal parenchyma, increases fibrosis, and blocks the liver portal blood flow consequently generating liver cirrhosis.

2.2.2.4 Heart

Iron overload cardiomyopathy is a common cause of death in patients with β-thalassemia. Biochemically, irons in iron-sulfur clusters and cytochromes are key players for oxidative phosphorylation and superoxide production in the mitochondria. Iron deposition in the heart cells seems to lead oxidative stress and cellular damage. The heart is the most mitochondrial-rich tissue in the body, making iron of particular importance to cardiac function. In cardiac cells, excess iron may result in oxidative stress and alteration of myocardial function because of the DNA damage caused by hydrogen peroxide through the Fenton reaction. Heart failure is the leading cause of death among patients with β-thalassemia with hemosiderosis, of whom around 60% die from cardiac failure. Harmful effects of iron overload on the heart of β-thalassemia major patients can be monitored using noninvasive and invasive techniques. Treatment with iron chelators can protect these patients from cardiac disease induced by iron overload [33–36].

2.2.2.5 Bone marrow

Bone marrow iron deposition (186 µg/g wet weight) increases in proportion to the total body iron stored in a dietary iron overload of African Bantu people and Caucasian patients with idiopathic hemochromatosis [37]. Myelodysplastic syndrome patients who are a heterogeneous group of clonal hematopoietic stem cell malignancies show bone marrow hemosiderosis and also develop systemic iron overload.

2.2.2.6 Endocrine glands

Though hematologic care has improved in patients with homozygous transfusion-dependent β-thalassemia, multi-endocrine dysfunction is still a common complication. Thyroid dysfunction defined as overt hypothyroidism, subclinical hypothyroidism, and exaggerated thyroid-stimulating hormone response was reported in patients with β-thalassemia [38]. In addition, lower serum thyroxine and higher thyrotrophic hormone levels were found in young patients with thalassemia than in normal children. Pituitary siderosis of β-thalassemia major patients causes oxidative tissue damage [39], consequently hypogonadotropic hypogonadism and growth hormone deficiency. Approximately half of the patients' pituitary gland dysfunction associated with iron overload is irreversible. Toxic irons in patients with β-thalassemia major (TDT) and patients with HbE/β-thalassemia are catalytically harmful to adrenal glands and can cause adrenal function insufficiency [40]. A previous study demonstrated that all β-thalassemia major patients were nondiabetic and some of them have decreased oral glucose tolerance test. They showed a normal response of cortisol to insulin and adrenocorticotropic hormone stimulation [41]. Moreover, the β cell pancreatic function and adrenal cortical function are depressed in severe iron loaded. The pancreas is another vital organ for iron accumulation especially pancreatic β cell in the islet of Langerhans. Importantly, TDT patients present iron deposition in the pancreatic beta cells beginning in the early age of life, resulting in decreased insulin secretion [42]. Normal iron level mediates insulin secretion via electron transport chain and endoplasmic reticulum calcium release by ROS triggering. Inversely, highly accumulated iron produces a high level of ROS which damages cell organelles and consequently affects iron-mediated insulin secretion mechanism (**Figure 1**).

As mentioned above, iron overload in thalassemia was assessed with a highly elevated level of plasma iron, and highly accumulated tissue iron concentrations
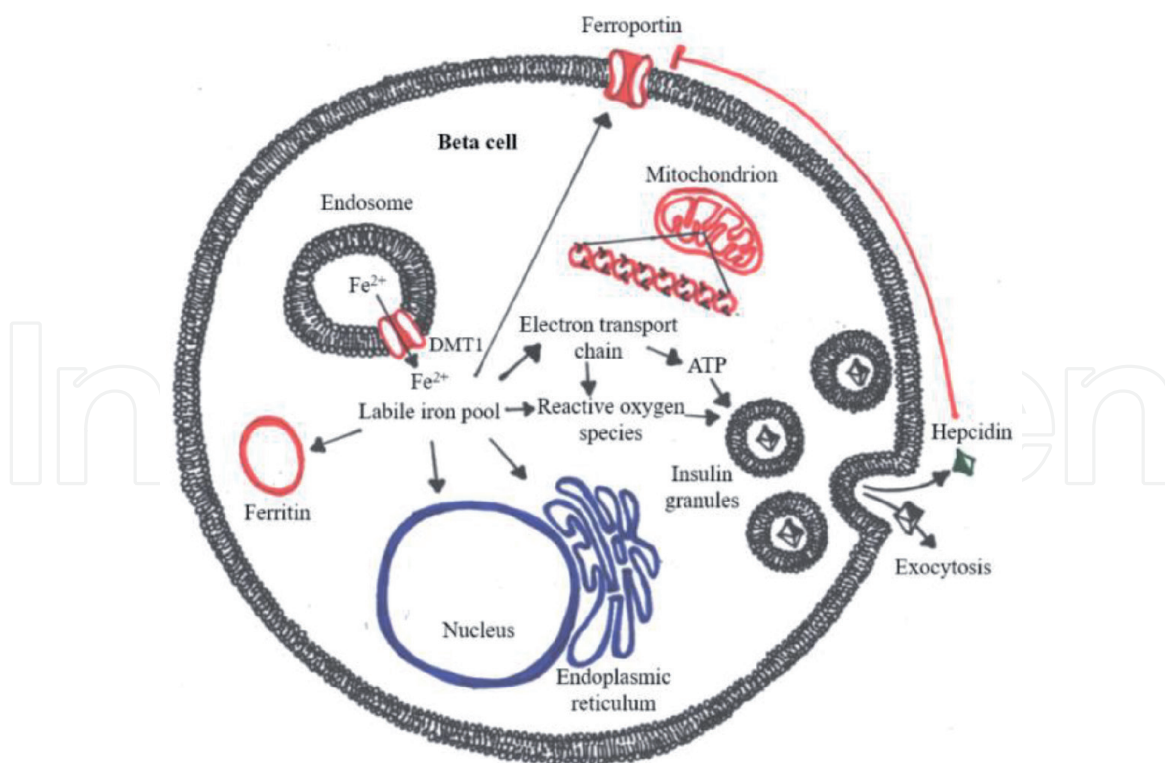
Figure 1.
Iron-mediated insulin secretion in pancreatic β cells (modified from [42]). Abbreviations/symbols:
ATP = adenosine-5′-triphosphate, DMT1 = divalent metal ion transporter 1, Fe²⁺ = ferrous ion.

lead to many complications. Importantly, effective iron chelators are regularly administrated to patients with β-thalassemia for removing the redox iron, ameliorating the complications, and prolonging their life expectancy.
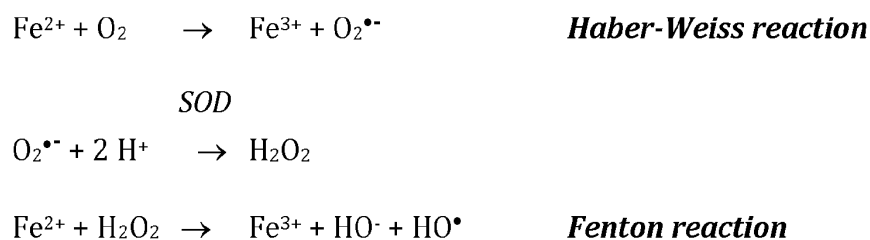
2.2.3 Oxidative stress

Iron can participate in the redox process known as the Haber-Weiss/Fenton reactions to generate ROS including hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) (**Figure 2**) [43]. Subsequently, ROS can oxidize biomolecules such as proteins, carbohydrates, lipids, and nucleic acids in the body causing oxidative tissue damage, organ dysfunctions, complications, and pathogenesis [44, 45]. In addition, ROS can induce cell apoptosis through initiating serial chemical reactions including DNA strand breakage and fragmentation, protein oxidation, and lipids peroxidation [43, 46, 47]. Excessive redox iron can also react with unsaturated lipids to produce alkoxyl and peroxyl radicals [48]. These oxidative reactions result in the impairment of cellular functions and lead to damage of cells, tissues, and organs, which is evident in the iron-loading diseases, β-thalassemia, and Friedreich's ataxia.

2.3 Assessment methods

For blood samples, the levels of serum iron, TIBC, transferrin saturation, ferritin, soluble transferrin receptors, protoporphyrin IX, and routinely measured iron parameters indicate whether the body iron status is normal iron balance, iron deficiency, or iron overloaded. In addition, the levels of redox irons (e.g., NTBI and LPI) and iron-responsive proteins such as EPO, hepcidin, and erythroferrone (ERFE) are determined to distinguish the iron imbalance.

Biopsied liver tissue is a clinical sample that is collected for stoichiometric determination of nonheme iron. However, this invasive technique requires an

$$Fe^{2+} + O_2 \quad \rightarrow \quad Fe^{3+} + O_2^{\bullet-} \qquad \textit{Haber-Weiss reaction}$$

$$\overset{SOD}{O_2^{\bullet-} + 2\,H^+ \quad \rightarrow \quad H_2O_2}$$

$$Fe^{2+} + H_2O_2 \quad \rightarrow \quad Fe^{3+} + HO^- + HO^{\bullet} \qquad \textit{Fenton reaction}$$

Figure 2.
Iron-catalyzed generation of reactive oxygen species. Abbreviations/symbols: Fe²⁺ = ferrous ion, Fe³⁺ = ferric ion, SOD = superoxide dismutase.

expert hepatologist to operate. The liver tissue will be investigated for its iron content using histochemical Pearl's staining, physical atomic absorption spectrometry (AAS), and colorimetric ferrozine methods. Liver iron concentration (LIC) is correlated closely with serum ferritin concentration and is a valuable alternative to assess visceral iron overload in heavily iron-loaded β-thalassemia major patients. In contrast, the collection of other tissues such as the myocardium, liver, pancreas, adrenal glands, anterior pituitary gland, and skin would be too harmful to be collected [49]. Sophisticated noninvasive techniques including magnetic resonance imaging [50], magnetic iron detector susceptometry, superconducting quantum interference device, and nuclear resonance scattering are used to assess iron content in tissues. These methods are valuable for evaluating iron load in the tissues and monitoring the response of different organs to chelation therapy.

2.4 Iron chelation therapy

Iron chelation therapy aims to prevent the accumulation of toxic iron and eliminate the excess iron in patients with transfusion-dependent β-thalassemia. Effective chelation and good management of the patients have correlated with a decline in early deaths and complications. The reduction of plasma and cellular chelatable irons (e.g., NTBI, LPI, and LIP) is a slow process and requires aggressive chelation therapy. The chelation will maintain the iron balance at safe levels to prevent high iron accumulation and oxidative tissue injury. Such non-iron and iron-overloaded models as RBC, cell cultures (e.g., hepatocytes, HepG2 cells, and cardiomyocytes), animals (e.g. mice, gerbils, rats, and transgenic BKO mice), and even human patients with thalassemia are experimentally investigated and clinically tested to assess the safety and efficacy of various iron chelators. Individually, DFO, DFP, and DFX are promising iron chelators widely used for the treatment of patients with β-thalassemia with iron overload. Combined DFO/DFP and DFP/DFX treatments can reverse endocrine complications by improving glucose intolerance and gonadal dysfunction in TD-TM patients [51].

A combination therapy is when two chelators were combined and presented some degree of overlap which pharmacologically lead to improve efficacy and rate of iron removal [52–54]. The most extensively studied combination therapy is a combination between DFO and the oral chelator DFP which has been shown to rapidly reduce liver iron, serum ferritin, and myocardial siderosis compared with DFO monotherapy. Clinical trial data for the combination of DFX and DFO chelation has reinforced the benefits of combination therapy [55]. In addition, the clinical combination of DFX and DFP would be more appealing to patients than other combination therapy regimens involving the parenterally administered DFO. However, concrete data regarding the safety, efficacy, and dosing of each regimen are limited.

In principle, when two chelators are used together, they can act either additively or synergistically. Additivity refers to the predicted level of effect achieved by a drug combination based on individual drug potencies, whereas synergy refers to the exaggerated effect noted upon concurrent use of drugs. Synergism may occur in conditions allowing "iron shuttling." The rate of the chelators' access to cells, both in vitro and in vivo, is determined by size, charge, and lipid solubility of the chelators [55–58]. In plasma, the previous demonstration showed that low concentrations of DFP can act as a "shuttle" by gaining more rapid access to some non-transferrin-bound iron (NTBI) pools than DFO and subsequently donating iron to a DFO as a "sink" [54]. The isobologram analysis was reported to distinguish between synergistic, additive, or a sub-additive response to iron chelator combinations in human hepatic cells (HuH7) [59]. The synergy can imply that the primary chelator can act as a "shuttle" for iron chelated and delivered to the secondary "sink" chelator at low concentrations (**Figure 3**).

2.5 Supplementation of antioxidants

All patients with β-thalassemia have higher oxidative stress levels than normal people. Hitherto, supplementation of antioxidants has been recommended for them to ameliorate oxidative stress, give a better quality of life, and sustain long lives. Many antioxidants such as water-soluble vitamins C (L-ascorbic acid), fat-soluble vitamin E (α-tocopherol), and vitamin A, β-carotenoids, glutathione, curcuminoids, and quercetin are the compounds of interest.

2.5.1 Vitamin C

The levels of AA in leukocytes, platelets, and urine of patients with iron overload such as patients with idiopathic hemochromatosis, TDT patient, and Bantu people were decreased [60, 61]. After treatment of TM patients with AA, the serum levels of iron, transferrin saturation, and ferritin were increased [62]. Possibly, AA would involve mobilization of storage iron from tissue ferritin by reducing ferric oxyhydroxide to ferrous ion, which will subsequently catalyze ROS production via Haber-Weiss reaction and increase oxidative damage in the patients. However,
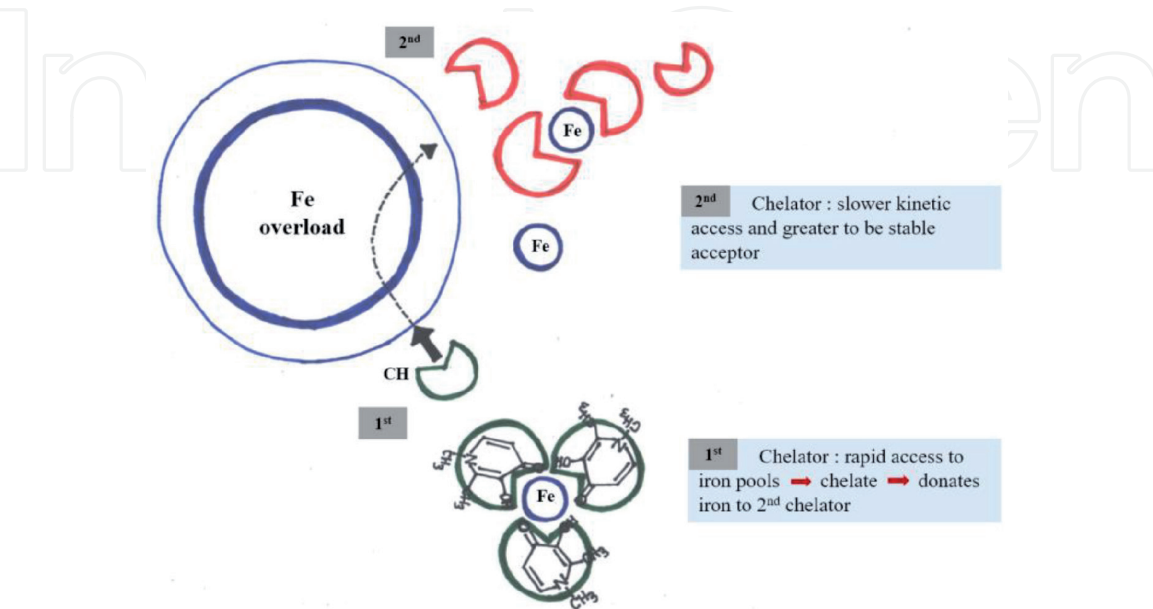

Figure 3.
Iron shuttling mechanism described combination therapy. Abbreviations/symbol: CH = chelator, Fe = iron.

vitamin C plus vitamin E supplementation seemed to be more effective than vitamin E alone for promoting antioxidant activity in patients with β-thalassemia [63].

2.5.2 Vitamin E

Evidently, the serum levels of vitamins A, vitamin E, zinc, selenium, and copper were decreased in young patients with thalassemia, while the serum levels of retinol and selenium inversely correlated with those of iron and ferritin. Moreover, low serum level of vitamin E was found in homozygous β-thalassemia and β-thalassemia/Hb E. Administration of vitamin E and polyphenols together with iron chelators abolished the increase of oxidative stress in patients with thalassemia, suggesting a substantial improvement of their anemia and complications [64]. The results from clinical studies using curcuminoids and vitamin E supported the free radical scavenging activity in the improvement of oxidative stress and platelet functions in patients with thalassemia [65, 66]. Surprisingly, oral administration of high doses of vitamin E effectively decreased plasma lipid peroxidation in patients with β-thalassemia and prolonged RBC survival in some patients. Furthermore, the treatment of TM and TI patients with vitamin E (750 to 1000 IU/day) for 16 months showed a fourfold increase in serum and RBC vitamin E levels and a reduction of serum malondialdehyde (MDA) level when compared with the untreated group [41]. Unchern and colleagues have reported that daily vitamin E supplementation for 3 months significantly increased plasma β-tocopherol levels and decreased plasma oxidant levels in splenectomized β-thalassemia/Hb E patients [66].

2.5.3 N-Acetylcysteine

NAC is a potent antioxidant and free radical scavenger, which involves glutathione biosynthesis and promotes detoxification. It is utilized for the treatment option for sickle cell anemia and diseases involved in the generation of ROS. Under iron overload condition, NAC provides a protective effect on brain dysfunction in iron-overloaded rats; therefore, the compound can diminish ROS production and DNA damage in patients with β-thalassemia [67–69].

2.5.4 Natural products

Quercetin is one of the predominant flavonoids of polyphenolics found in many fruits, vegetables, leaves, grains, red onions, kale, apples, and tea. Flavonoids are commonly present in their glycosylated form in the diet and presence of aglycone, glycoside, as well as methyl, glucuronide, and sulfate conjugates, which have been reported in human plasma. Quercetin has been reported to exert antioxidant and free radical scavenging properties in vitro, while quercetin supplementation has been promoted for the treatment of cancer and other diseases. Surprisingly, dietary quercetin supplementation was found to attenuate liver injury in iron-overloaded mice [70]. As a novel idea, we wish to investigate their role as adjuncts in iron mobilization [71].

Curcuminoids are the phytochemicals from turmeric *Curcuma longa* Linn (family Zingiberaceae), which are composed of three main constituents including curcumin, demethoxycurcumin, and *bis*-demethoxycurcumin, in which the important bioactive molecular structure is diketone moiety. Curcumin was found to suppress the ROS generation and lipid peroxidation in erythrocytes. Surprisingly, curcumin metabolites including di-, tetra-, and hexa-hydrocurcumin exhibit strong antioxidant, free radical scavenging, anti-lipid peroxidative and iron-chelating properties. The other effects are antithrombotic and anti-inflammatory activities. Oxidative

stress condition in red blood cells of β-thalassemia/HbE patients is reduced after treatment with curcuminoids [65], leading to an improvement of their quality of life.

Green tea exhibits many interesting biological, biochemical, and pharmacological properties in health benefits. Normally, tea leaves (*Camellia sinensis*) are processed without fermentation for the preparation of green tea extract (GTE), which is abundant with epigallocatechin-3-gallate (EGCG) and exhibits antioxidative iron-chelating activity. In a recent study, the effect of GTE products on the status of iron overload, oxidative stress, and endocrine hormones was studied in iron overload pancreatic and liver cell lines, β-thalassemic mice, and β-thalassemia/HbE patients.

3. Green tea for thalassemia's health

Green tea is prepared without fermentation from tea leaves and one of the most popular beverages in the world. It is composed of proteins, amino acids (e.g., 5-methylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine), carbohydrates (e.g., cellulose, pectin, glucose, fructose, and sucrose), lipids (e.g., linoleic acid and α-linolenic acids), sterols (e.g., stigmasterol), vitamin B, vitamin C, vitamin E, caffeine, theophylline, chlorophyll, carotenoids, volatile compounds (e.g., aldehydes, alcohols, esters, lactones, and short-chain hydrocarbons), minerals, and essential elements. Importantly, green tea is rich in polyphenols, particularly flavonoids (0.5–1.5%) in forms of catechins (flavan-3-ols). Five main green tea catechins (GTC) are included, EGCG which is the main proportion (59% of total catechins), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC) (**Figure 4**). In addition, green tea contains gallic acid, chlorogenic acid, caffeic acid, kaempferol, myricetin, and quercetin. During the fermentation process, flavonoids in green tea are oxidized into theaflavins and thearubigins, which are the main ingredients in black tea.

In health benefits, green tea plays many important roles in cells and systems in the body. Green tea has been considered a medicine and a healthful beverage since ancient times. Traditional Chinese medicine has recommended this plant for headaches, body aches and pains, digestion, depression, detoxification, as an energizer, and, in general, to prolong life. Caffeine acts mainly upon the central nervous system, stimulating wakefulness, facilitating ideas association, and decreasing the sensation of fatigue [73]. Green tea polyphenols present antioxidant, ROS and reactive nitrogen species (RNS) scavenging, and metal-chelating activities [72, 74, 75]. They may also function indirectly through (1) inhibition of redox-sensitive transcription factors; (2) inhibition of "prooxidant" enzymes including inducible nitric oxide synthase (iNOS), lipoxygenase (LOX), cyclooxygenase (COX), and xanthine oxidase (XO); and (3) induction of antioxidant enzymes including GST and SOD [76].

3.1 Neuronal cells

We have presented that GTE treatment at 0–100 μg/mL was not toxic to the neuroblastoma (SH SY5Y) cells (viability >90%). Nevertheless, the cell viability significantly decreased in iron-loaded condition, and the treatment of the cells with GTE (10–40 μg/mL) was found to improve the viability. GTE monotherapy (10–20 μM) was not effective to reduce LIP levels in SH SY5S cells, whereas combined treatment with GTE and CM1 iron chelator significantly reduced the LIP.
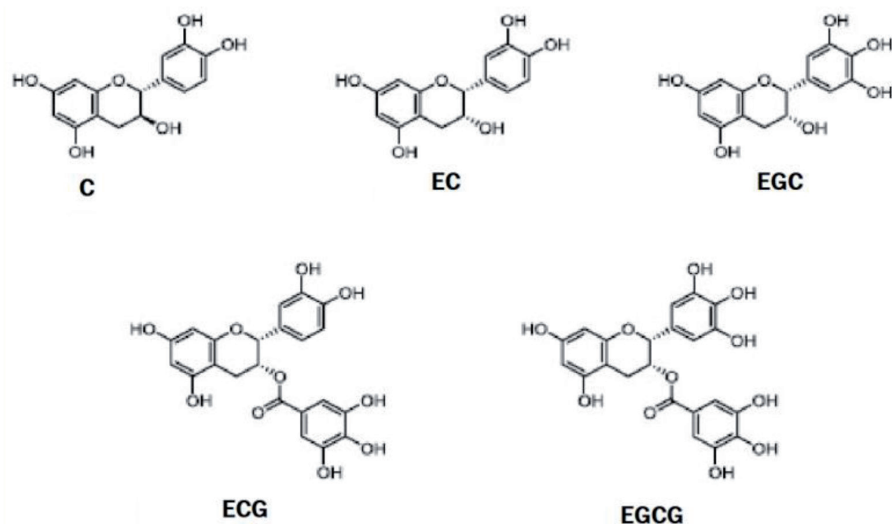
Figure 4.
Chemical structures of five catechins in green tea (modified from [72]). Abbreviations: C = catechin, EC = epicatechin, ECG = epicatechin-3-gallate, EGC = epigallocatechin, EGCG = epigallocatechin-3-gallate.

Moreover, GTE monotherapy (40 μM), as well as the combination of GTE and CM1 chelator, exhibited significant free radical scavenging activity in a concentration-dependent manner [77]. Consistently, Xu and colleagues have reported that the oral administration of EGCG (25 mg/kg/day, 7 days) regulated the iron-export protein ferroportin in the substantia nigra and reduced oxidative stress in Parkinson's disease mice [78].

3.2 Blood cells

Phenolic acids (e.g., chlorogenic acid, caffeic acid, and gallic acid) and flavonols (e.g., kaempferol, myricetin, and quercetin) are present in green tea. GTE and EGCG, which show iron-chelating and antioxidant properties, decrease labile iron (e.g., NTBI and LPI) levels and consequently deplete lipid peroxidation as well as oxidative stress in both iron-loaded rats and thalassemic mice [73, 74]. The compounds were effective in the inhibition of RBC hemolysis, resulting in pro-longed RBC lifespan and decreased iron deposition and oxidative damage in liver [75]. Logically, antioxidative GTE interferes duodenal absorption of dietary iron and iron-chelating properties in vitro and in vivo. The preparation also shows the inhibitory effect on catecholamine secretion from isolated rabbit adrenal glands, possibly by blocking LTCC in the adrenomedullary glands. Active compounds in GTE are effective in the inhibition of red blood cell hemolysis; therefore, red blood cell lifespan can be expanded, and iron deposition in the liver can be decreased [79]. Green tea extract improves the abnormality of sickle red cells [80]. Using fluorochrome labeling and flow cytometry, we found feeding ferrocene diet-induced iron overload and oxidative stress, leading to a significant decrease of RBC survival half-time ($T_{1/2}$) in WT ($T_{1/2}$ = 23 days) and BKO thalassemic mice ($T_{1/2}$ = 13 days) when compared with feeding normal diet ($T_{1/2}$ = 30 days). Surprisingly, the RBC survival rate was approximately 15% increase in GTE-treated WT mice and 27% increase in GTE-treated BKO mice when compared with placebo-treated WT mice (Ounjaijean, S. unpublished data). Antioxidative GTE, particularly EGCG, would possibly scavenge ROS and recycle oxidized glutathione (GSSG) to GSH in RBC cytoplasm, leading to the inhibition of RBC hemolysis and prolonged RBC survival. Tea extracts can be used as a therapeutic agent in case of anemia resulting from red blood cell destruction and alleviate infection-induced inflammation or other

inflammatory diseases [81, 82]. EGCG is presented to ameliorate inflammation via the nuclear factor erythroid-derived 2-like-2 (Nrf2 signaling pathway), leading to prevent osteoblasts [83]. On the other hand, excessive green tea consumption may cause iron-deficiency anemia [67].

3.3 Liver and pancreas cells

By using lactate dehydrogenase assay, Koonyosying and colleagues have found that GTE at 1–10 µM EGCG equivalent was not toxic to rat insulinoma (RINm-5F) cells, whereas GTE doses of 1–30 µM EGCG equivalent were not toxic to human hepatocellular carcinoma (Huh7) cells either, showing 20% inhibitory concentrations (IC20) of 10.6 and 17.4 µM EGCG equivalent, respectively [84]. Interestingly, GTE monotherapy (10 µM EGCG equivalent) revealed an almost twofold reduction of LIP in iron-loaded RINm-5F and Huh7 cells when compared to treatment without GTE, while GTE treatment (1 and 10 µM EGCG equivalent) together with 10 µM DFO showed a synergistic effect of the LIP mobilization. Similarly, the GTE treatments efficiently lowered ROS levels in these two cells in the same manner. Most importantly, the GTE treatments effectively restored the production of insulin from iron-loaded RINm-5F cells and hepcidin production from iron-loaded Huh7 cells, indicating relief of endocrine gland dysfunction from iron-induced oxidative tissue damage [84].

3.4 Hypercoagulability and thrombosis in thalassemia

Oral green tea catechins significantly inhibited arachidonic acid (AA)-induced platelet aggregation in rats [85]. Likewise, EGCG suppressed adenosine diphosphate (ADP)-induced platelet aggregation in a dose-dependent manner and reduced the ADP-induced phosphorylation of p38 mitogen-activated protein kinase and heat shock protein [86]. In addition, EGCG inhibited the ADP-stimulated release of platelet-derived growth factor-AB and soluble CD40 ligand [87]. Moreover, green tea catechins inhibited ATP release from dense granules in washed platelets and AA liberation stimulated by collagen in platelets [85]. Interestingly, GTC and EGCG prevented death due to pulmonary thrombosis, prolonged bleeding time in mice in vivo, and inhibited human platelet aggregation [88]. Nowadays, the life expectancy of TM patients has been improved because of regular blood transfusions and iron chelation therapy. However, patients with thalassemia still suffer from many complications from their chronic disease. Regular transfusion therapy leads to iron overload-related complications including growth retardation, hypoparathyroidism, diabetes and impaired glucose intolerance, osteoporosis, cardiomyopathy, liver fibrosis, and cirrhosis [89]. Heart failure and arrhythmia have been reported as the main cause of death in TM patients, whereas pulmonary hypertension and thrombosis are also the major cardiovascular complications in TI patients possibly due to pro-atherogenic biochemical factors (e.g., iron status and lipid profile) [90–94]. Hypercoagulable pulmonary microthromboembolism in Thai pediatric TE patients was previously investigated [95]. TI patients had thrombosis (9.4%), thrombocytosis (15%), mostly after splenectomy, and lower levels of anticoagulation inhibitors (e.g., protein S, protein C, and antithrombin III) (15%) [26]. Splenectomy also promotes portal vein thrombosis in TM patients [96]. Ineffective erythropoiesis, chronic anemia, iron overload, and polycythemia by erythrocytosis and thrombosis are coincidently occurring in patients with *β*-thalassemia. Signs of cerebrovascular accident (brain ischemia, hemorrhage, and infarct) and heart disease (congestive heart failure and atrial fibrillation) were described in patients with chronically hypercoagulable thromboembolic thalassemia, so anticoagulant and/or anti-platelet therapy is recommended [97].

According to a high incidence of coagulopathy affecting life expectancy, patients with thalassemia have been reported recently to understand contributing factors. Thrombosis related to the hypercoagulable state in thalassemia has been mostly reported. Factors contributing to the hypercoagulable state in patients have been identified [98]. Hypoxia and iron overload are the two major mechanisms of ROS overproduction that can cause oxidative tissue damage and complications [99]. The levels of plasma hemostatic and thrombotic markers including thrombin-antithrombin III complex, β2 thromboglobulin, C-reactive protein, tissue plasminogen activator antigen, protein C, protein S, antithrombin, prothrombin fragments, fibrinogen, and lipoprotein (a) were significantly higher in patients with splenectomized HbE/β-thalassemia than non-splenectomized ones, implying that splenectomy increases platelet hyperactivity, blood hypercoagulability, and risk of thrombosis [100, 101]. ROS-induced activation of vascular endothelial cells can cause vasculitis and thrombosis, showing increased levels of soluble adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), E-selectin (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1), and von Willebrand factor (vWF) in thalassemia blood [102]. Procoagulant activity of circulating damaged RBC or RBC microvesicles may also contribute to thrombotic events in thalassemia hypercoagulability [103]. Carotid artery thrombus is usually associated with severe cardiovascular diseases (CVD), iron-deficiency anemia, and thrombocytosis. Thromboembolic complications are documented in patients with thalassemia, possibly due to the aggregability of abnormal RBC and high amounts of RBC membrane-derived microparticles [104]. The treatment of β-thalassemia/HbE patients with antioxidants can improve oxidative stress and hypercoagulable state [105]. Particularly, NTBI presenting in thalassemia plasma would be one of the risk factors in pulmonary thrombosis and hypertension in splenectomized NTDT patients [106]. DFO and DFP were effective in the amelioration of iron overload and oxidative stress in thalassemic mice, possibly in the prevention of pulmonary thrombosis [107].

Excessive iron and carbon monoxide liberated from heme oxygenase-1 catalyzed heme degradation also contribute to hypercoagulability [108]. Moreover, low arginine bioavailability and elevated arginase I activity in patients with β-thalassemia are possible causes of pulmonary hypertension leading to cardiopulmonary dysfunctions [109]. However, there are no difference in serum arginine between young patients with β-thalassemia and young control children [110]. Anemia (e.g., iron-deficiency anemia) can be linked to cerebral venous thrombosis as well [111]. Ex vivo Hb released from old RBC and in vivo Hb released from thalassemia RBC per se directly bind nitric oxide, whereas arginase will degrade available arginine during hemolysis [112]. Splenectomy, thrombocytosis, and only platelet MP are proposed to be residual hypercoagulable/thrombotic risks in TDT patients [23, 113]. Liver diseases such as liver inflammation and cirrhosis can involve in hypercoagulability, thrombosis, and reduced fibrinolysis [114, 115].

3.5 β-Thalassemic mice

Upanan and colleagues have demonstrated that hepatic hepcidin gene (Hamp1) mRNA levels in heterozygous β-globin knockout thalassemic mice (BKO, muβth-3/+) were significantly lower than that in wild-type mice (WT, muβ+/+) and significantly upregulated with iron loading. However, combined treatment of DFP (50 mg/kg) and GTE (50 mg EGCG equivalent) for 90 days was more effective in the upregulation of the Hamp1 mRNA levels in BKO thalassemic mice significantly. Similarly, monotherapy with the GTE effectively decreased serum levels of alanine aminotransferase in BKO thalassemic mice (Δ11 + 9 U/L), while the combined

GTE/DFP chelation showed greater effect (Δ1 + 9 U/L) (P < 0.05) when compared without GTE (Δ17 + 12 U/L), suggesting protection of oxidative liver damage. Likewise, GTE monotherapy and GTE plus DFP therapy was found to lower the levels of serum NTBI, spleen, and liver iron from iron-loaded BKO mice significantly when compared without GTE treatment [84, 116]. Currently, Al-Basher has shown that oral administration of GTE (100 mg/kg) for two more months lowered iron
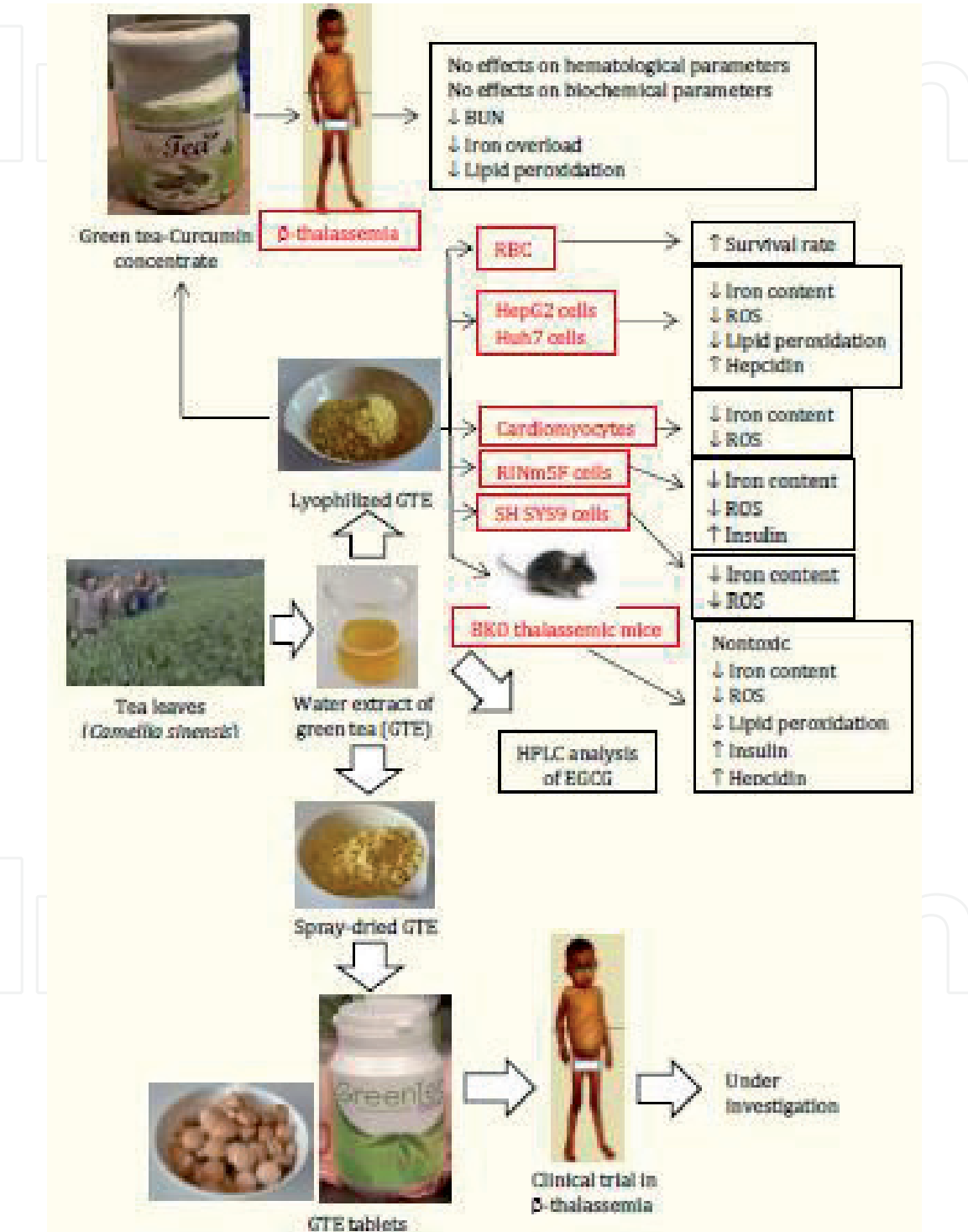

Figure 5.
Challenging with green tea for treatments of iron overload and oxidative: from cells to bedsides. Abbreviations/ symbols: BKO = β-globin knockout, BUN = blood urea nitrogen, EGCG = epigallocatechin-3-gallate, GTE = green tea extract, HepG2 cells = human hepatocellular carcinoma cells, HPLC = high-performance liquid chromatography, Huh7 cells = human hepatocellular carcinoma cells, RBC = red blood cells, RINm5F cells = rat insulinoma cells, ROS = reactive oxygen species, SH SYS9 = human neuroblastoma cells, red alphabet = iron overload.

accumulation, improved antioxidant capacity, and restored the increase of hepcidin in the livers of iron-overloaded rats [117]. Recently, Koonyosying and colleagues have demonstrated that GTE decreased the amounts of iron in the livers (P < 0.05) and pancreas insignificantly from iron-loaded BKO mice; however, combination therapy of DFP and GTE was not efficient superior to the GTE monotherapy in the two cells [84]. Similarly, GTE treatment efficiently lowered the levels of lipid peroxidation products such as plasma TBARS and tissue MDA in liver and pancreatic tissues from BKO mice fed with ferrocene diet, and the combined treatment was not superior to the GTE monotherapy either [84]. Consistent with studies of the cells, GTE restored the production of hepatic hepcidin and pancreatic insulin in iron-loaded BKO mice, and the combined treatment was not more efficient over the GTE monotherapy [84]. The findings suggest protective effects of iron-induced oxidative hepatic and pancreatic damages by GTE, which the action would be mainly free radical scavenging property.

3.6 Patients with β-thalassemia

A recent study of controlled trial in British healthy volunteers has demonstrated that the interval between meal consumption and tea consumption did not affect plasma levels of hepcidin-25 [118]. Surprisingly, the consumption of GTE-curcumin drinks for 60 days tentatively decreased the levels of Hb, Hct, and RBC numbers in TDT patients [84]. Moreover, the drink with the GTE dose of 50 mg EGCG equivalent was found to lower the levels of NTBI and LPI. Taken together, green tea polyphenols and curcuminoids present in the drink could possibly interfere absorption of dietary iron and mobilize delivery of iron to the erythrons [65, 79, 119]. Taken all our studies, we summarize the applications of nutraceutical green tea extract for the amelioration of iron-overloaded cells, β-thalassemic mice, and patients with β-thalassemia (**Figure 5**) [79, 84, 106, 116, 119–128]. In our expectations, we hope the patients will have safer chelation therapy, a better quality of lives, and good health.

4. Conclusions

Patients with β-thalassemia suffer from ineffective erythropoiesis and chronic anemia and from secondary iron overload caused by multiple blood transfusions and increased iron absorption. Iron chelation therapy and antioxidant administration are supportive treatments to ameliorate iron overload and oxidative tissue damage. Green tea (*Camellia sinensis*) abundant with EGCG possesses diuretic, free radical scavenging, and iron-chelating properties. Accordingly, green tea products can decrease the levels of redox-active iron, ROS, and lipid peroxidation products in iron-overloaded cells, β-thalassemic mice, and patients. In addition, green tea can restore pancreatic insulin secretion and hepatic hepcidin production from hepatocytes with iron overload. Furthermore, the nutraceutical properties of green tea products have to be investigated intensively in patients with oxidative stress and neurodegenerative disorders.

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Conflict of interest

The authors declare no conflict of interest.

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Abbreviations

AA	arachidonic acid
ADP	adenosine 5′-phosphate
AE2	anion-exchange protein 2
ARE	antioxidant responsive element
ATP	adenosine-5′-triphosphate
BKO	beta-globin knockout
BUN	blood urea nitrogen
CAT	catalase
CH	chelator
COX	cyclooxygenase
DFO	desferrioxamine
DFP	deferiprone
DFX	deferasirox
DMT1	divalent metal ion transporter 1
EC	epicatechin
ECG	epicatechin-3-gallate
EGC	epigallocatechin
EGCG	epigallocatechin-3-gallate
EPO	erythropoietin
ERFE	erythroferrone
Fe	iron
Fe^{2+}	ferrous ion
Fe^{3+}	ferric ion
GI	gastrointestinal
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
GST	glutathione-*S*-transferase
GTC	green tea catechins
GTE	green tea extract
Hamp1	hepatic hepcidin gene
Hb	hemoglobin
HbA	hemoglobin A
HbA_2	hemoglobin A_2

Hct	hematocrit
HbE	hemoglobin E
HbF	fetal hemoglobin
HbH	hemoglobin H
HepG2	human hepatocellular carcinoma cells
H_2O_2	hydrogen peroxide
HPLC	high-performance liquid chromatography
Huh7	human hepatocellular carcinoma cells
IC_{20}	20% inhibitory concentrations
iNOS	inducible nitric oxide synthase
IRE	iron regulatory element
IRP	iron regulatory protein
LIC	liver iron concentration
LIP	labile iron pools
LOX	lipoxygenase
LPI	labile iron pool
LTCC	L-type calcium channel
MDA	malondialdehyde
MP	microparticles
NAC	*N*-acetylcysteine
NFκB	nuclear factor-kappa B
Nrf2	nuclear factor erythroid 2
NTBI	non-transferrin-bound iron
Nramp2	natural resistance-associated macrophage protein 2
NTDT	non-transfusion-dependent β-thalassemia
OH$^\bullet$	hydroxyl radical
Prx2	peroxiredoxin 2
RBC	red blood cells
RE	reticuloendothelial
RINm5F	rat insulinoma cells
RNS	reactive nitrogen species
ROS	reactive oxygen species
SH SY5Y	neuroblastoma cells
SOD	superoxide dismutase
$T_{1/2}$	half-time
TBARS	thiobarbituric acid-reactive substances
TBI	transferrin-bound iron
TDT	transfusion-dependent β-thalassemia
Tf	transferrin
TfR1	transferrin receptor 1
TGF-β1	tumor growth factor-beta1
TI	β-thalassemia intermedia
TIBC	transferrin iron-binding capacity
TM	β-thalassemia major
TTCC	T-type calcium channel
TTCC	T-type calcium channel
XO	xanthine oxidase

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