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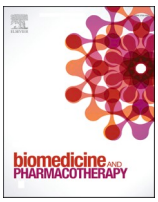

Review

Impacts of oxidative stress and anti-oxidants on the development, pathogenesis, and therapy of sickle cell disease: A comprehensive review

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ABSTRACT

Sickle cell disease (SCD) is the most severe monogenic hemoglobinopathy caused by a single genetic mutation that leads to repeated polymerization and depolymerization of hemoglobin resulting in intravascular hemolysis, cell adhesion, vascular occlusion, and ischemia–reperfusion injury. Hemolysis causes oxidative damage indirectly by generating reactive oxygen species through various pathophysiological mechanisms, which include hemoglobin autoxidation, endothelial nitric oxide synthase uncoupling, reduced nitric oxide bioavailability, and elevated levels of asymmetric dimethylarginine. Red blood cells have a built-in anti-oxidant system that includes enzymes like sodium dismutase, catalase, and glutathione peroxidase, along with free radical scavenging molecules, such as vitamin C, vitamin E, and glutathione, which help them to fight oxidative damage. However, these anti-oxidants may not be sufficient to prevent the effects of oxidative stress in SCD patients. Therefore, in line with a recent FDA request that the focus to be placed on the development of innovative therapies for SCD that address the root cause of the disease, there is a need for therapies that target oxidative stress and restore redox balance in SCD patients. This review summarizes the current state of knowledge regarding the role of oxidative stress in SCD and the potential benefits of anti-oxidant therapies. It also discusses the challenges and limitations of these therapies and suggests future directions for research and development.

1. Introduction

Sickle cell disease (SCD) is the most common pathophysiology associated with red blood cells (RBCs) and involves disruption of hemoglobin (Hb) biochemistry. SCD includes homozygous sickle hemoglobin (HbSS), sickle-hemoglobin C (HbSC), and hemoglobin sickle beta (HbS/β) thalassemia genotypes [1,2] and is prevalent in sub-Saharan Africa, South Asia, the Middle East, India, the Mediterranean, Central America, and Europe [3,4]. The Global Burden of Diseases, Injuries, and Risk Factors Study (2021) reported that the global population living with SCD increased by 41.4% between 2000 and 2021 from 5.46 to 7.74 million. During the same period, the total number of deaths among affected individuals increased by 43.4% from 262,000 to 376,000. In particular, the incidence and prevalence of SCD increased markedly in sub-Saharan Africa. The number of births affected by SCD increased by 27.2% between 2000 and 2021 to reach 405,000, and the all-age prevalence increased by 67.4% over the same period to reach 5.8 million cases [5]. Across all super-regions, prevalences were highest in western and central sub-Saharan Africa and India [6]. On the other hand, the regions with greatest decrease in SCD birth rates were central Europe, eastern Europe, and central Asia, and the Middle East. The African Region of the World Health Organization (WHO) reported that

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SCD affects about 1000 newborns daily in Africa, while more than 50% of them will not survive past 5 years old, which makes SCD the most prevalent genetic disease in the African region [7,8]. On the other hand, India has the largest number of people (more than 20 million) with SCD in South Asia, and a new initiative was adopted by the Indian Ministry of Health and Family Welfare on Nov 15, 2021 to screen newborns for SCD in Madhya Pradesh, Gujarat, and Chhattisgarh. The screening data obtained to date indicates that the prevalence of SCD among new newborns ranges between 2% and 40% and that the number of SCD patients (all ages) will increase in the future [9,10].

One of the possible causes of SCD is a single-point mutation in the Hb-β gene. This mutation leads to the replacement of glutamic acid by valine, which results in the accumulation of abnormal hemoglobin (sickle Hb) [11,12]. Under low oxygen conditions, sickle Hb (HbS) tends to polymerize (a phenomenon known as sickling) and cause intravascular hemolysis, repeated polymerization, vaso-occlusive crisis (VOC), and ischemia-reperfusion injury [13–15]. This hemolysis increases the concentration of cell-free Hb in plasma and causes thrombosis, lipid peroxidation, and oxidative stress [16,17]. These pathophysiological conditions cause a wide range of acute to chronic symptoms, the most common of which are hemolysis, vaso-occlusive episodes (VOEs), and multi-organ damage due to ischemia-reperfusion injury. In addition, SCD increases susceptibility to infections, acute chest syndrome (ACS), renal abnormalities, retinopathy, inflammation, cardiovascular complications, pulmonary hypertension (PH), acute stroke, leg ulcers, and the risk of early mortality [18–20]. SCD has wide-ranging clinical manifestations influenced by various factors. Age, gender, genetic makeup, hematological parameters, and environmental conditions are all known to play a role in the disease's expression [21,22]. Other factors include Fetal hemoglobin (HbF), simultaneous presence of α-thalassemia, Glucose-6- phosphate dehydrogenase deficiency, and *HBB* gene cluster haplotypes [23]. One of the prime clinical indicators of SCD is the pain crisis, which is caused by the occlusion of tiny blood vessels by sickled RBCs. SCD also profoundly affects daily life and patient well-being due to chronic fatigue and organ damage with pain crises, which significantly impact daily functioning and overall well-being [18]. In addition, SCD can adversely affect cognitive performance, academic achievement [24], and employment opportunities by reducing productivity [25]. A recent survey showed that SCD has a significant impact on physical and mental well-being, social life, and work performance. Out of 2145 patients surveyed, 38% reported SCD had a negative impact on their daily household activities and other 62% reported avoiding strenuous activities [26]. Treadwell et al., in a recent study, concluded that holistic management strategies, comprehensive care, and support services are imperative to enhance quality of life and well-being [27]. However, some initiatives, such as penicillin treatment, pneumococcal immunization, newborn screening, and proper disease education, have considerably improved patient health and survival [28–30].

Although there is no cure for SCD, RBC transfusion can provide symptomatic improvement by reducing the HbS percentage, anemia, and hemolysis [31]. Therefore, blood transfusion has an established role in the management of acute and chronic complications of SCD. In addition, oral hydroxyurea can extend and improve patient life, and the randomized BABY HUG trail showed that this agent lowers the risks of VOC and dactylitis in young children [32]. L-glutamine is another promising drug that can lower the risk of VOC in SCD patients by 25% and reduce the number of hospitalizations [33]. L-glutamine has been proposed to protect RBCs from oxidative damage and prevent hemolysis; however, it does not seem to affect hemoglobin levels or reticulocyte counts in SCD patients [34]. The historical timeline and progress made in the treatment of SCD are presented in Fig. 1. The recent advancements in gene therapy and an increased number of clinical trials will change the therapeutic landscape of SCD in the coming years and may eventually lead to an exact cure.

Typically, oxidative stress is elevated in SCD patients due to elevated ROS (reactive oxygen species) levels driven by hemolysis, inflammation, endothelial dysfunction, and other factors [35]. In addition, biological events, such as activation of pro-oxidant enzymes, heme release, and plasma Hb induced by hemolysis, increases the generation of free radicals, which eventually trigger the Fenton reaction, mitochondrial respiratory chain activity, and RBC auto-oxidation [36,37]. Accumulation of these free radicals intensifies the oxidative stress in RBCs, platelets, neutrophils, and endothelial cells, leading to vascular problems in multiple organs [38]. In this review, we summarize mechanisms responsible for oxidative stress in SCD, the precise role of ROS in its pathophysiology, and discus the potential benefits of anti-oxidant therapies.

2. Role of oxidative stress in SCD

Oxidative stress is defined as an imbalance between the intracellular level of oxidizing species, such as ROS and RNS (reactive nitrogen species), and anti-oxidant activity [39]. Usually, the molecular oxygen is reduced to form ROS like superoxide anion radicals (•$O2^-$) and hydroxyl radicals (•OH) along with non-radical oxidants like hydrogen peroxide (H_2O_2) and hydrochlorous acid (HClO). Likewise, RNS include radical species such as nitric oxide (•NO), peroxynitrite (ONOO-), and nitrogen

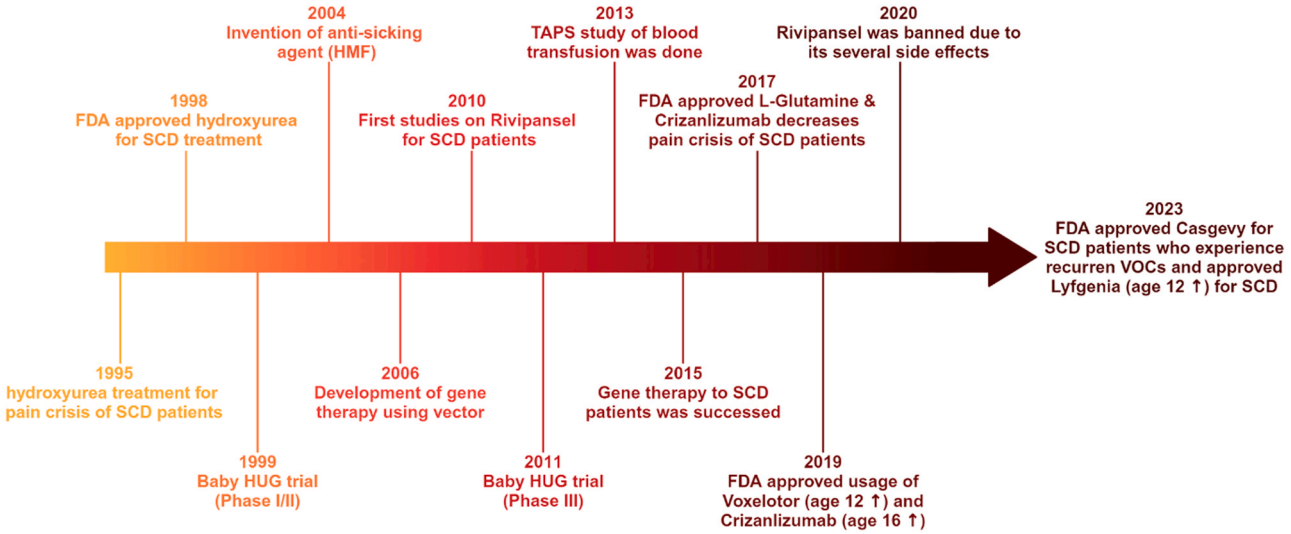

Fig. 1. Chronological overview of the major events and the achievements.

dioxide (•NO_2), which are formed by the reaction among •NO and •$O2^{-}$ [39,40]. Furthermore, ROS accumulation induces oxidation, and thus, damages carbohydrates, proteins, lipids, and DNA [41]. The most frequent ROS generation pathways are shown in Fig. 2.

When defense mechanisms do not neutralize the oxidative conditions of these biomolecules, they can lead to cellular dysfunction that eventually result in a wide range of diseases [42]. Both enzymatic and the non-enzymatic defense mechanisms are implicated in counteracting ROS and may involve multiple anti-oxidants, including SOD, glutathione peroxidase (GPx), thioredoxin/thioredoxin reductase system (TXN/TXNRD1), catalase (CAT), glutaredoxin (GRx), peroxiredoxins (Prx), and glutathione reductase (GR). Non-enzymatic anti-oxidants include reduced glutathione (GSH), tocopherols, zinc, carotenoids, selenium, uric acid, ascorbic acid, riboflavin, ubiquinols, and metal-binding proteins [39,43].

2.1. Mechanism of ROS generation in SCD

Inhibition of ETC (mitochondrial electron transport chain) activity leads to ROS production through the leakage of electrons from complex-I [43] in several ways, such as (a) increased activities of certain oxidases (endothelial xanthine oxidase (XO) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase) [28,44,45], (b) HbS autoxidation [39,45], (c) heme and iron release [28], (d) an increase in asymmetric dimethylarginine (ADMA) [45,46], (e) uncoupling of nitric oxide synthase (NOS) activity [45], and (f) a fall in nitric oxide (NO) bioavailability [45,47]. It was also reported that generation of ROS plays a critical role in both hemolysis and VOC in SCD patients [48].

2.1.1. Increases in the activities of specific oxidases

NADPH oxidase is the the primary O^{2-} producing enzyme in RBCs, WBCs, and vascular endothelial cells [28]. Furthermore, NADPH oxidases are activated by Rac GTPase, protein kinase C, and Ca^{2+} signaling inside sickle RBCs. Certain isolated cytokines, such as transforming growth factor-1 and endothelin-1, in the plasma of SCD patients have been reported to boost NADPH oxidase activity and increase ROS production extracellularly [49]. In addition, ROS-mediated damage of erythrocyte membrane elements can lead to RBC rigidity and membrane fragility [49]. Moreover, hepatic hypoxia-reoxygenation in SCD patients stimulates the release of XO from liver to the circulation, and XO released in this manner immediately binds to vessel luminal cells and disrupts their vascular functions to generate an oxidative environment [50].

2.1.2. HbS autoxidation

The autoxidation of oxy-Hb (oxyhemoglobin) to generate superoxide is the primary source of ROS in RBCs [51]. Normally, methemoglobin (ferric, Hb-FeIII), which can no longer bind oxygen, is produced by the gradual autoxidation of oxygenated Hb (ferrous, Hb-FeII) [52]·H_2O_2 is the product of SOD, and this process can occur spontaneously or be catalyzed by enzymes [51]. Alternatively, these ROS, H_2O_2, and superoxides are neutralized by the RCB intracellular anti-oxidant system, which includes enzymatic and non-enzymatic anti-oxidants, thereby protecting the body from such primary sources of oxidative stress [28,52]. When H_2O_2 contacts methemoglobin, it degenerates Hb and produces iron, which can further react with the residual H_2O_2 to produce the highly reactive and dangerous hydroxyl radical [43]. In RBCs, HbS is 1.7 times more prone to autoxidation than normal Hb [53] and generates twice as many hydroxyl and superoxide radicals, H_2O_2 molecules, and lipid oxidation products [43,54].

2.1.3. Release of heme and iron

Hb binds to haptoglobin (Hp) during mild and moderate hemolysis and produces a complex in plasma that limits free iron release [55]. This complex is internalized and degenerated by CD163 and CD91 receptors, which originate from macrophages and hepatocytes, respectively [28,56]. HbS, a heme variant, is released faster than normal Hb [34]. Furthermore, due to the presence of hydrophobic regions, heme can able to insert into cell membranes to generate intracellular heme-dependent ROS [14]. Moreover, inflammation causes activated cells to release H_2O_2 and O^{2-}, which can interact with heme and trigger the non-enzymatic production of ROS and liberate ferrous ions [57]. This scenario could intensify Fenton-driven reactions and increase oxidative stress [28,57].

2.1.4. Rise in asymmetric dimethylarginine (ADMA)

L-arginine is a substrate for eNOS, but the ADMA is an endogenous inhibitor of eNOS that competes with L-arginine for binding to the active site of eNOS [58]. ADMA causes eNOS uncoupling, which results in the production of superoxide and peroxynitrite, causing oxidative stress and

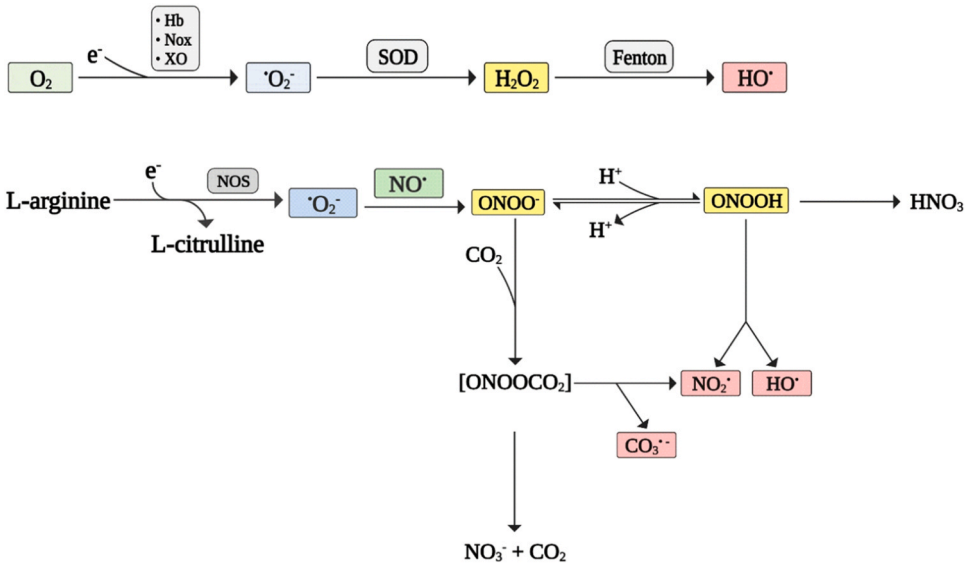

Fig. 2. ROS generation pathways in RBCs involve interaction between oxygen and NO and various enzymes and molecules. The superoxide anion ($^{\bullet}O_2^{-}$) produces H_2O_2 and $OH^{\bullet}$ due to the actions of superoxide dismutase (SOD) and the Fenton reaction respectively, and H_2O_2 and $OH^{\bullet}$, in turn, cause oxidative stress. Normally, L-arginine is converted into nitric oxide by NOS, but the uncoupling of NOS causes the production of superoxides, which combine with NO to produce various ROS species.

limiting the bioavailability of NO (Fig. 2) [59], and interestingly, ADMA levels are related to endothelial dysfunction [60]. Dimethylarginine dimethylaminohydrolase (DDAH) degrades ADMA and is of the therapeutic interest for the indirect regulation of NO [61]. Additionally, children with SCD and high plasma levels of ADMA are at higher risk of having a high tricuspid regurgitated jet velocity [62].

2.1.5. Uncoupling of endothelial nitric oxide synthase (eNOS) activity

eNOS is a vasodilatory enzyme involved in the production of NO, as facilitated by several essential cofactors, including calmodulin (CaM), tetrahydrobiopterin (BH_4), Flavin mononucleotide, Flavin adenine dinucleotide (FAD), and NADPH [63]. When eNOS is not coupled with its substrate/cofactor, superoxide is produced by eNOS uncoupling [41]. Usually, L-arginine is a substrate for eNOS, which catalyzes the conversion of L-arginine and oxygen to NO and L-citrulline. This reaction requires BH_4 as a cofactor, however when the BH_4 to L-arginine ratio is low, eNOS becomes uncoupled and produces superoxide rather than NO [42]. So collectively, uncoupling of eNOS and superoxide formation may lead to oxidative stress in SCD. Although, decline in oxygen concentration and oxidative stress may favor VOC in SCD.

2.1.6. Reduced NO bioavailability

NO bioavailability can be reduced in several ways, but especially by an increase in superoxide anions [64]. The interaction among NO and superoxide is more damaging compared to their actions [65]. When NO reacts with superoxide, it produces peroxynitrite (a highly reactive oxidant), which can decompose to produce •OH and $•NO_2$ (both strong oxidants) [66]. Thus, the reaction between superoxide and NO can directly or indirectly contribute to free radical production. Since Hb is an efficient scavenger of NO, elevated level of plasma Hb can markedly limit NO bioavailability [67]. Methemoglobin and nitrate are produced when NO reacts with oxy-Hb, whereas a stable FeII Hb–NO complex is produced when NO reacts with deoxy-Hb, allowing it to undergo the Fenton reaction [47]. A gender difference in the bioavailability of NO is observed in SCD patients due to the defensive impact of ovarian estrogen on NOS production and activity in females [68]. On the other hand, in males, NO continues its destructive course because of the abundance of cell-free Hb and superoxide.

2.2. ROS-mediated cellular signaling mechanisms in SCD

There are a wide range of ROS-related mechanisms/pathways in SCD, such as (a) hemolysis/endothelial dysfunction, (b) cell adhesion and vascular occlusion, and (c) ischemia–reperfusion injury. The overall mechanism is presented schematically in Fig. 3.

2.2.1. Hemolysis/endothelial dysfunction

SCD is the result of intravascular hemolysis and chronic anemia. Hb released by RBCs to plasma through intravascular hemolysis acts as a NO scavenger [69], and intravascular hemolysis is associated with endothelial dysfunction [70]. Moreover, hemolysis causes RBCs to release large number of red cell DAMPs (damage-associated molecular patterns) into the flow, contributing to endothelial dysfunction and oxidative stress. These conditions can lead to chronic vasculopathy and pulmonary hypertension (PH) [28,71]. Some major DAMPs include heme, IL-33, adenosine 5' triphosphate, and heat shock proteins (Hsp), such as Hsp 70, [72]. Heme can activate the development of neutrophil extracellular traps, toll-like receptor signaling, and inflammasome signaling inflammatory pathways [73]. When this free heme binds to its receptor on cell membranes, it induces apoptosis [74]. Furthermore, by inducing immunological responses in macrophages and monocytes, free heme and iron can also cause inflammatory damage [75]. In addition, hemolysis-associated platelet dysfunction can increase the risk of thrombosis, which, in turn, increases complication rates (e.g., organ damage and stroke) in SCD patients [11].

Arginase (a potential biomarker of hemolysis) is released into plasma from RBCs with Hb and DAMPs, and an increase in plasma reduces plasma arginine levels [76]. After being produced in endothelium by eNOS, NO can rapidly diffuse into neighboring vascular smooth muscle cells and interact with the soluble guanylate cyclase heme moiety, thereby activating the enzyme [77], which then converts GTP (guanosine triphosphate) to cGMP (cyclic guanosine monophosphate) and thus triggers the activations of cGMP-dependent protein kinases [78–80].

2.2.2. Cell adhesion and vascular-occlusion

Sickled RBCs have several types of surface adhesive molecules than normal RBCs. These molecules can cause sickled RBCs to stick to blood vessel walls and obstruct blood circulation, and thus, sickled RBCs adhere to endothelial vascular walls more in SCD patients compared to the healthy people [81]. The adhesion molecules include ICAM-1 (intercellular adhesion molecule 1), VCAM-1 (vascular cell adhesion molecule 1), E-selectin [82], P-selectin [83], and L-selectin [84], which mediate adhesion to vessel endothelium by activating integrin αMβ2 on neutrophils [85]. In addition, VCAM-1 and ICAM-1 are also associated with reduced NO bioavailability and increased hemolysis, favoring RBC adhesion [86]. Lactate dehydrogenase and arginase levels are also positively associated with adhesion [43]. Furthermore, the interaction between sickled RBCs and endothelium can remarkably increase oxidative stress, which again promotes adhesion despite reducing the expression of adhesion molecules. Increased NF-kB (nuclear factor-kB) expression during adhesion and activation are associated with increased oxidative stress [87]. Leucocytes, especially neutrophils, are also involved in cell adhesion [88]. The molecules associated with

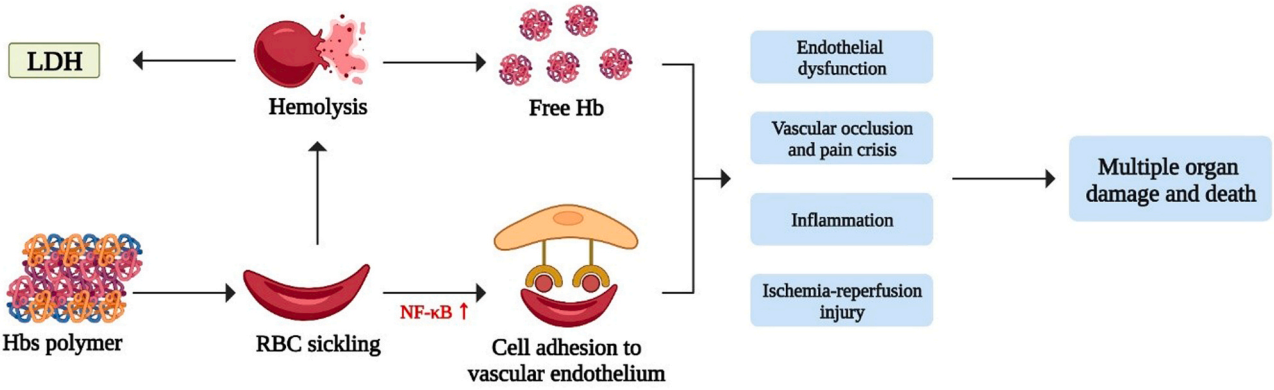

Fig. 3. Overview of the mechanisms involved in SCD-related oxidative stress. Polymerization of deoxy-HbS causes the production of sickle-shaped RBCs, which can self-adhere or undergo hemolysis. The consequences of cell adhesion include endothelial dysfunction, inflammation, vaso-occlusive crisis, and ischemia–reperfusion injury.

leukocyte adhesion to endothelium include α2 Lß2 integrin, αMß2 integrin, CD31 (platelet-endothelial cell adhesion molecule-1), CD36, L-selectin, and PSGL-1 (platelet selectin glycoprotein ligand-1 or CD162) [28]. Moreover, cytokines like IL-6 and TNF-α, which are closely associated with chronic hemolysis, constantly induce the endothelium-derived adhesion molecule to vessel walls, promote VOC, multiple organ damage, and early mortality in SCD patients [89]. Sickled RBC adhesion to vascular endothelium and capillary blockage initiate vascular occlusion of the microcirculation, leading to VOC and episodes of pain crisis [90]. This vessel occlusion obstructs tiny blood vessels and contributes to tissue ischemia, and thus, to reperfusion injury (Fig. 4) [91]. Recently, Simonia et al. [15] described a therapeutic means of reducing platelet activation and thrombus formation in a mouse model of SCD using dietary ALA (alpha-linolenic acid). They found that dietary high-ALA reduced sickle cell count, liver fibrosis, and adhesion molecule (VCAM-1, ICAM-1, and vWF) expressions in aorta, lungs, liver, and kidneys.

2.2.3. *Ischemia–reperfusion injury*

Ischemia is a common consequence of cell adhesion and vascular occlusion in SCD. Interruption of the blood flow to tissues creates ischemic or hypoxic environment [92]. During this ischemic condition, oxygen concentrations are low, and nutrient delivery is inadequate. During this condition, ROS are generated in mitochondria and promote cellular damage and cell death [93]. Reperfusion injury concerns the vascular and tissue damage caused by restoring oxygen levels to tissues after ischemia and causes a spike in free radical concentrations [94]. The level of damage induced is dependent on the degree and duration of ischemia [95]. ATPs in cells breakdown to ADP and AMP during ischemia, and adenosine (a breakdown product of AMP) is then converted into inosine, which further produces hypoxanthine and xanthine by oxidation [96] and results in more ROS ($\bullet O2^-$ and H_2O_2) production. Cellular outcomes depend on the duration and severity of ischemia–reperfusion injury. Prolonged ischemia–reperfusion injury can induce cell damage through mechanisms such as autophagy, necrosis, necroptosis, and apoptosis. On the other hand, brief ischemia–reperfusion injury can trigger survival responses that mitigate ROS production and cellular damage. Moderate ischemia–reperfusion injury can cause cellular dysfunction and activate recovery survival systems, whereas severe ischemia–reperfusion injury can initiate apoptotic and necrotic pathways [97].

3. Clinical implications of genetic polymorphisms in oxidative stress

GSTs (Glutathione S-transferases) are classified into subgroups based on their amino acid sequences: alpha (GSTA), omega (GSTO), theta (GSTT), mu (GSTM), sigma (GSTS), pi (GSTP), and zeta (GSTZ) [98]. The presence of polymorphisms in the GSTM1, GSTP1, and GSTT1 genes are common sources of genetic variation in man [99]. The relationship between these GST polymorphisms and oxidative stress were analyzed by Silva et al. [100], who found a substantial difference for GSTP1 polymorphisms. Whereas, Ellithy et al. [101] noticed a significant association between oxidative stress and GSTM1 in SCD patients. Further, Filho et al. [102] also documented that the GSTM1 gene was found to be the cause of threat in all clinical indicators of SCD.

SOD activity is much higher in SCD patients than in normal controls. Along with extracellular SOD, Cu^-, Zn^-, and Mn^- anti-oxidant enzymes protect against oxidative stress. Sogut et al. [103] investigated the human Mn-SOD gene polymorphisms (Ala-9Val) and its association with SCD and concluded that this polymorphism is not related to SCD. GSH (or γ-L-glutamyl-L-cysteinyl glycine) is a tripeptide that contains cysteine as a key component that is essential for GSH synthesis. Cysteine is derived from dietary sources, protein breakdown, or the trans-sulfuration pathway that converts methionine to cysteine. [104]. Enzymes like MTHFR (Methylenetetrahydrofolate reductase) and CBS (cystathionine β-synthase) plays a vital role in controlling Hcy (homocysteine) concentrations in the trans-sulfuration pathway [105]. Genetic abnormalities in MTHFR and CBS enzyme genes cause changes in Hcy levels, which influence cysteine availability, and ultimately, GSH bioavailability. Silva et al. [106] analyzed the effects of MTHFR C677T and CBS polymorphisms on oxidative stress markers and concluded that a decrease in anti-oxidant capacity was linked to the MTHFR C677T mutation. In addition, the effects of SOD2 rs4880, MPO rs2333227, and XO rs207454, and other common anti-oxidant gene polymorphisms on oxidative stress biomarkers (MDA, AOPP, XO, MPO, CAT, MnSOD, and GPx) have been investigated especially by Tall et al. [107]. Furthermore, several genes are involved in SCD concurrently with oxidative stress conditions. Some genes, their gene locations, and their effects on SCD patients are listed in Table 1.

4. ROS targeted antioxidant therapeutic strategies for SCD

The protective anti-oxidant system is depleted in SCD patients because of the pro-oxidant nature of the disease [117]. The enzymatic defensive system, which includes SOD, GPX, and CAT, and the free radical scavenging molecules, such as vitamin E, vitamin C, and GSH, are generally affected [118]. Some studies have reported that SOD level are reduced in SCD, while others have claimed they are elevated [119]· H_2O_2 is a harmful molecule that normally broken down by CAT or GPX enzymes. Patients with SCD have lower levels of these enzymes, resulting in higher endogenous H_2O_2 levels [120,121]. GSH is the substrate of GPX, which oxidizes it to GSSG (GSH disulfide) and helps to reduce H_2O_2 levels [122]. However, SCD patients have lower total GSH (GSH + GSSG) and GPX levels than normal [123]. Furthermore, SCD patients have high or low CAT levels. High CAT concentration may

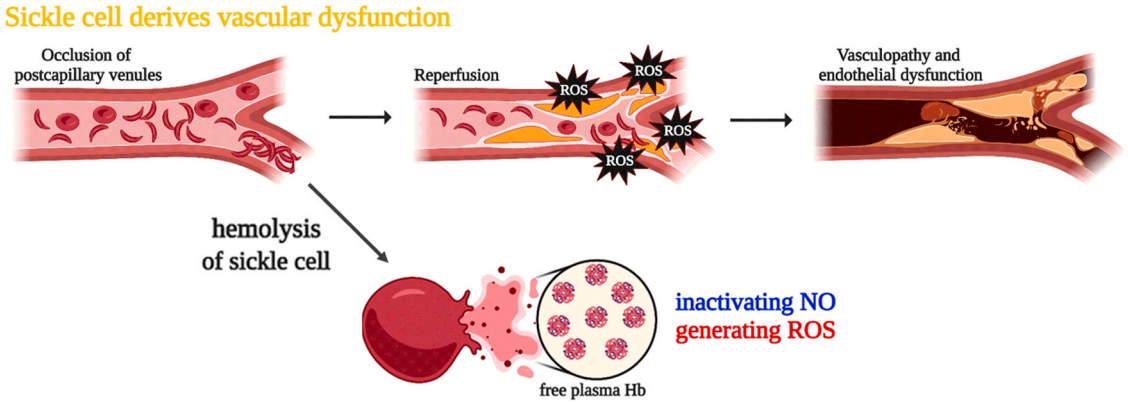

Fig. 4. Schematic illustration of Pathophysiological mechanism of SCD.

Table 1
Summary of genetic variants involved in oxidative stress in SCD patients.

Gene	Protein/Enzyme	Location/ Cytogenic Band	Model (In Vitro/ In Vivo)	Comment/Finding	Reference
NFE2L2	Nrf2	Chr 2: exon 6 2q31.2	Mouse (KW)	Increased ROS and sickling in hypoxic conditions, as well as function loss, resulting in greater splenomegaly	[108,109]
GPX1	GPx1	Chr 3: exon 2 3p21.31	Human	Loss of activity promotes RBC susceptibility to oxidative stress and is allied to hemolysis in SCD patients	[110]
TXN	Thioredoxin	Chr 9: exon 5 9q31.3	Mouse	Exerts an antioxidant effects, guards against oxidative stress damage and damage caused by oxygen-glucose deprivation/reperfusion (OGD/R) in astrocytes	[111]
SOD1	Superoxide dismutase 1	Chr 2: exon 5 21q22.11	Mouse	Poly–ubiquitinated proteins in RBCs accumulate and aggregate	[112]
SOD2	Superoxide dismutase 2	Chr 6: exon 10 6q25.3	Mouse (KW)	SOD2 mRNA levels in SCD patients are considerably lower compared to controls. Since gene expression is connected to hemolysis, iron overload, inflammation, oxidative stress, and SCD cardiopathy, it may be used as a PH biomarker in SCD.	[113]
CAT	Catalase	Chr 11: exon 13 11p13	Human	Catalase depletion might be a consequence of oxidative processes and hemolysis in SCD patients	[114]
HMOX1	Hemeoxygenase 1 (HO1)	Chr 22: exon 5 22q12.3	Mouse	Higher or lower HO1 expression may inversely related with hypoxia/reoxygenation–induced vascular stasis	[115] [116]
NOX1	NADPH oxidase 1	Chr X: exon 14 Xq22.1	Human	NOX activity is linked to RBC stiffness and fragility as an endogenous, erythrocyte-specific process in addition to being the cause of oxidative stress in SCD.	[49]

remove H_2O_2, whereas low concentrations may be caused by high levels of oxidative stress [43]. Fig. 5 depicts how anti-oxidants interact in SCD.

Thus, high levels of oxidative stress may deplete these shielding elements. HO-1 (Heme oxygenase-1) is another anti-oxidant enzyme that protects cells from heme-induced oxidative damage. It breaks down heme into biliverdin, iron, and carbon monoxide, which have anti-oxidant and anti-inflammatory properties [124]. HO-1 also inhibits the expression of VCAM-1, ICAM-1, and NF-kB, which are involved in inflammatory and immune responses [125]. SCD patients exhibited HO-1 upregulation in response to hemolysis. However, this increase in HO-1 is not sufficient to cope with hemolysis-induced heme levels, which results in persistent oxidative damage and VOC produced by ischemia–reperfusion injury [126].

HSCT (hematopoietic stem cell transplantation), gene therapy, and HbF inducers anti-sickling drugs are some of the examples of previous treatments targeting the root cause of SCD. There are two kinds of therapeutic strategies for treating SCD, which are referred to as primary and secondary treatments. Secondary treatments address adhesion, inflammation, oxidative stress, and thrombophilia in SCD [127]. Anti-oxidant therapy provides a promising means of treating oxidative stress in SCD patients, and L-glutamine, NAC (N-acetylcysteine), L-arginine, L-acetylcarnitine, α-lipoic acid, and zinc supplements have been used as anti-oxidant strategies in SCD [128–131]. Some of the most promising anti-oxidants and their effects are presented in Table 2.

4.1. Glutathione (GSH)

GSH is a robust endogenous anti-oxidant that is essential for sustaining cellular redox balance and preventing oxidative stress. It acts as a direct ROS scavenger and aids in the restoration of other anti-oxidants like vitamins E and C [146]. GSH levels are deficient in SCD, and this intensifies oxidative stress and promotes disease development. Exogenous anti-oxidant supplementation and targeting GSH metabolism have emerged as viable treatment options to reduce oxidative stress in SCD [147]. Several studies have been conducted to determine the efficacies of GSH-targeted anti-oxidant therapies on SCD, with the goal of restoring redox equilibrium and alleviating SCD symptoms.

NAC (a precursor of GSH) administration provides a means of enhancing GSH production. NAC administration has been demonstrated to boost intracellular GSH levels and restore the redox equilibrium in SCD patients [148]. In one randomized clinical trial, treatment with oral NAC for 12 weeks resulted in substantial reduction in oxidative stress indicators and enhanced endothelial functions in SCD patients. α-Lipoic acid is a powerful anti-oxidant that may replenish GSH and other

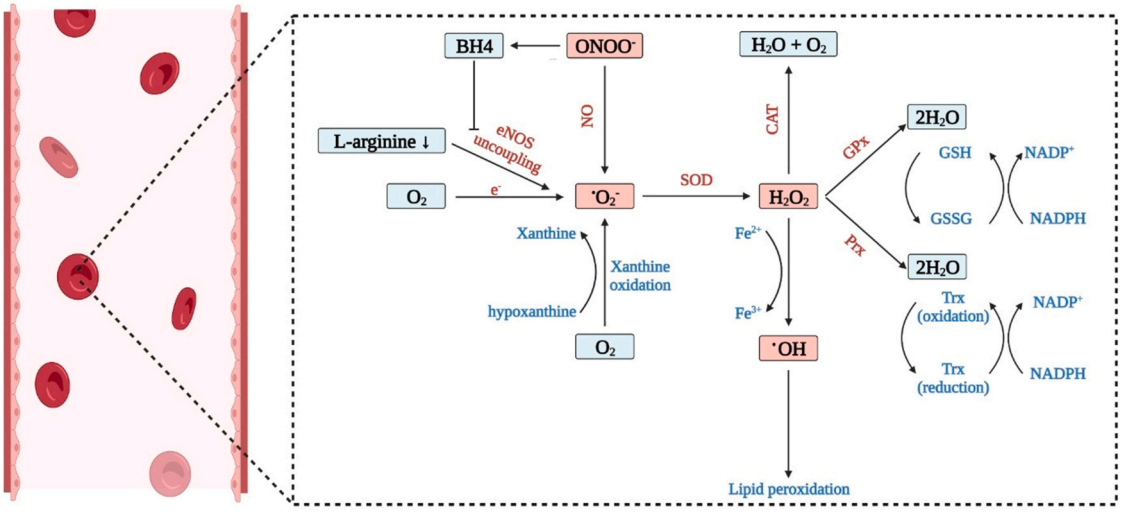

Fig. 5. Schematic representation of anti-oxidants reaction mechanism. Anti-oxidant enzymes, such as SOD, CAT, Prx, and GP_X, protect cells from oxidative stress by scavenging superoxide radicals. These enzymes with SOD convert superoxide to H_2O_2, and then CAT, Prx and GP_X reduce the H_2O_2 to H_2O.

Table 2
Summary of antioxidants and their action mechanisms in SCD patients.

Anti-oxidants	Mechanism of action and Effects	References
Phytochemicals:		
Resveratrol	Lowers oxidative stress and inflammation, enhancing RBC deformability, and alleviating endothelial dysfunction.	[132–136]
Curcumin	Lowers inflammation, oxidative stress, and vaso-occlusive events in SCD.	[132–136]
Quercetin	Lowers inflammation and oxidative stress and enhances RBC function	[137,138]
Essential minerals, Amino acids and their derivatives:		
GSH	Acts as ROS scavenger and restores other anti-oxidants like vitamins E and C.	[139]
Vitamin C	Minimizes oxidative stress markers, escalates RBC deformability and lower the risk of VOCs.	[140]
Vitamin E	Functions as scavenger of the peroxyl radical and helps to block the free-radical propagation and thereby lipid oxidation.	[141]
L-Glutamine	Reduces the endothelial adherence in sickle-shaped RBCs	FDA approved
N-Acetylcysteine	Boost GSH levels by providing cysteine	NCT01800526 phase 2 trial; [142]
Nitric oxide	Reduces discomfort and vaso-occlusion in SCD patients with pulmonary hypertension (PH).	NCT00094887 phase 2 trial; [143]
L-Arginine	Maintains normal NO level and prevent superoxide generation.	NCT02447874 phase 2 trial; [144]
α-Lipoic Acid	Induces the synthesis of GSH.	NCT01054768 phase 2 trial
L-acetylcarnitine	Reduces peroxidative damage.	NCT01054768 phase 2 trial
Zinc	Inhibits lipid peroxidation.	[145]

anti-oxidants *in vivo* [149]. Wang et al. [150] found that α-lipoic acid therapy successfully reduced inflammation, endothelial dysfunction, and oxidative stress in a mouse model of SCD, suggesting α-lipoic acid supplementation has therapeutic potential in SCD. Furthermore, hydroxyurea, a drug used regularly to treat SCD, has been demonstrated to boost intracellular GSH levels and improve anti-oxidant capability. Hydroxyurea benefits the body in various ways, stimulates fetal Hb synthesis, and reduces inflammation and oxidative stress[151]. Several clinical trials have shown that hydroxyurea reduces the incidence of VOC and improves overall clinical outcomes in SCD patients [152].

4.2. Antioxidative Vitamins

There has been a surge of interest regarding the possible effects of antioxidative vitamins in the reduction of oxidative stress and enhancing cellular health in SCD patients [153,154]. Ascorbic acid, generally known as vitamin C, has emerged as a promising anti-oxidant in this context. Vitamin C supplementation was found to minimize oxidative stress markers, improve red blood cell deformability, and thereby lower VOC risk [155]. Furthermore, vitamin C can synergistically boost the activities of other anti-oxidants [140].

Vitamin E is another vital anti-oxidant that can protect against oxidative stress, and was demonstrated in a rat modal [156]. By acting as a chain-breaking anti-oxidant, vitamin E reduces free radical propagation and stabilizes cell membranes. In SCD patients, vitamin E supplementation can improve RBC stability and reduce oxidative stress [157,158]. On the other hand, vitamin E supplementation has not ameliorated anemia and conversely enhanced hemolysis marker levels in SCD patients [159].

Vitamin A also acts as an anti-oxidant and might be useful for the treatment of SCD. Although only a few studies have been conducted in a background of SCD, the anti-oxidant activities of vitamin A are known to contribute to cellular health and decrease oxidative burdens in SCD patents [160,161]. Furthermore, accumulating evidence suggests that vitamin D may play a role in lowering oxidative stress and enhancing clinical outcomes in SCD [162]. Vitamin D supplementation can lower the level of oxidative stress markers, increases immunological functions, and improves bone health, which would reduce hospitalization of people with SCD [163,164]. Further, SCD patients may have low serum B12 levels because of the folate deficiency [165], and thus, these patients require folic acid supplementation to reduce plasma homocysteine levels.

4.3. Phytochemicals

There is rising interest in the possible therapeutic uses of phytochemicals with natural anti-oxidants and anti-inflammatory properties in SCD. These phytochemicals can modulate the expressions of genes and proteins involved in the pathophysiology, which makes them promising options for controlling SCD [166]. Resveratrol, a polyphenol found in grapes and berries, has shown promise as an SCD treatment by lowering oxidative stress and inflammation, enhancing red blood cell deformability, and alleviating endothelial dysfunction [132,133]. Curcumin, a chemical derived from turmeric, has anti-oxidant and anti-inflammatory properties in various disorders and has been reported to lower inflammation, oxidative stress, and VOC events in SCD [134–136]. Quercetin is a type of flavonoid usually found in vegetables and fruits and has anti-inflammatory and anti-oxidant properties [137]. According to one study, quercetin supplementation might lower inflammation and oxidative stress in SCD patients and enhance red blood cell function and overall clinical outcomes [138]. Green tea contains catechins and EGCG (epigallocatechin gallate), which has powerful anti-oxidant and anti-inflammatory properties [167,168]. In particular, EGCG has been proven to reduce oxidative stress, improve red blood cell deformability, and decrease sickle cell adherence to the endothelium [169,170].

The bioavailability of phytochemicals is primarily determined by their solubilities and processing and stability characteristics, and only a fraction of those consumed are absorbed into the systemic circulation [171]. Besides, the body treats phytochemicals as xenobiotics, which explains their transient short half-lives *in vivo*, and even when absorbed, their bioactivities may be limited by processing techniques [172]. Resveratrol has poor bioavailability and is rapidly metabolized in mammals, but nonetheless, has potent therapeutic effects [173]. The poor absorption, rapid metabolism, and rapid elimination of curcumin in humans suggest the limited bioavailability and restricts its clinical application [174]. Although the bioavailability of quercetin is generally low and varies widely between individuals, in vivo administration of quercetin has been shown to antagonize oxidative stress responses [175]. Additionally, phytochemical-functionalized and phytochemical-loaded nanocarriers have been employed for the co-delivery of phytochemicals [176].

4.4. L-glutamine

L-glutamine, the precursor for NAD (nicotinamide adenine dinucleotide), can be helpful for producing NADH (reduced form of NAD) [34]. In SCD patients, NAD production is sufficient, yet there is a remarkable depletion of GSH and glutamine concentrations in the RBC, which increases the ROS burden of sickle cells. In 2007, the FDA (Food and Drug Administration) authorized the oral utilization of L-glutamine in SCD patients [177]. Oral consumption of L-glutamine helps the body produce glutamine and GSH in sufficient amounts, and thus, lowers free radicals in these cells. Other investigators concluded that oral usage of L-glutamine reduced the endothelial adherence of sickle-shaped RBCs, a key element in the pathogenesis of vaso-occlusion. Furthermore, a higher NAD redox potential in RBCs could enhance their ability to cope with oxidative damage and reduce inflammation and adhesion molecule

expression [152].

4.5. N-acetylcysteine (NAC)

Generally, SCD patients have lower levels of GSH, which leads to an increase in ROS levels. NAC is a potent anti-oxidant that has multiple effects on inflammation and vascular functions and can help to boost GSH levels by providing cysteine, a GSH component [142]. GSH levels in the RBCs of SCD patients were found to be 32–36% lower than in healthy controls, but patients had higher levels of other anti-oxidant enzymes (SOD and Gpx) that aid oxidant detoxification. Some clinical investigations (NCT01800526 phase 2 trial; NCT01849016 phase 3 trial) showed a decrease in AGEs (glycoxidation end products), cell-free Hb, and phosphatidylserine expression (a biomarker of erythrocyte inner membrane lipid peroxidation). Hence, NAC supplementation can help increase GSH levels and reduce increased oxidative stress in SCD patients [178].

4.6. Nitric oxide and L-arginine

Inhaling NO, which also has antioxidant properties, can reduce discomfort and VOC in SCD patients with pulmonary hypertension (PH) [143]. When levels of L-arginine are low, superoxide is produced instead of NO, thereby reducing the bioavailability of NO [179]. Thus, L-arginine can be used to maintain normal NO levels and prevent superoxide generation [144]. Unfortunately, the results of multiple clinical trials have inconsistent, though it has been adequately demonstrated that L-arginine treatment can improve NO bioavailability and reduce inflammation in both SCD patients and transgenic knockout sickle mice [180].

4.7. α-lipoic acid and L-acetylcarnitine

α-Lipoic acid and L-acetylcarnitine also possesses anti-oxidant properties [181]. α-Lipoic acid can induce the synthesis of GSH by further including the NRF_2-reliant transcription of GCL (γ-glutamyl cysteine ligase), while L-acetylcarnitine decreases lipid peroxidation. The reason for the beneficial effect of L-acetylcarnitine remains unclear, although it might be due to enhanced mitochondrial metabolism. Generally, mitochondria use fatty acids as fuel, and L-acetylcarnitine helps transport fatty acids into them, thereby reducing tissue lipid peroxidation. According to some studies, the L-acetylcarnitine may help to maintain the normal shape and function of RBCs and reduce peroxidative damage [182].

4.8. Zinc supplementation

Chronic oxidative stress, vaso-occlusion, and inadequate cell-mediated immunity are all linked to zinc deficiency. Zinc acts as an anti-oxidant in SCD patients and can lower the risk of infections. Recent studies have shown that the zinc supplementation can improve the growth and body composition of children with SCD [145]. Furthermore, multiple pathophysiological markers that chronically present were improved by zinc supplementation (25 mg elemental zinc as acetate, 3 times a day up to 3 months) in a placebo-controlled, double-blind trial. Immunity, inflammation, and oxidative stress were all demonstrably improved [128].

4.9. Other antioxidant agents

Some SCD patients may benefit from supplementation with anti-oxidants, such as omega-3 fatty acids, gum arabic, and curcumin [183, 184]. These substances may help to minimize oxidative stress, but they are not widely employed at the moment. Vitamin A, C, and E deficiencies increase the risk of infection and hemolysis in SCD patients. Yet, evidence regarding the impact of these vitamins on the treatment of oxidative stress is conflicting. However, iron chelators, such as deferoxamine, deferiprone, and deferasirox, are crucial for treating transfusion-dependent hemoglobinopathy [185], as they prevent the accumulation of labile iron and ROS formation.

5. Conclusions and future perspectives

The polymerization of deoxy-HbS is the direct cause of oxidative stress and ROS production in SCD patients. This review presents current understanding of the oxidative stress mechanism in SCD and how ROS affects SCD patients. Hemolysis, cell adhesion, endothelial dysfunction, vascular blockage, and ischemia–reperfusion damage are the key effects in ROS production. Currently, antioxidant therapeutic strategies are the only ones capable of neutralizing these effects of ROS and increasing longevity and lowering mortality rates of SCD patients. Thus, attenuating ROS production is critical for the prevention and management of SCD-related complications. While anti-oxidants are effective at reducing oxidative stress within the body, the therapeutic ability of anti-oxidant supplements to address oxidative stress remains a subject of debate. In the context of SCD, oxidative stress balance may be disrupted by excessive anti-oxidant supplementation and potentially exacerbate certain aspects of the condition. Moreover, high doses of certain anti-oxidants may have pro-inflammatory effects and pose additional risks to SCD patients already experiencing chronic inflammation. Hence, it is crucial to emphasize that anti-oxidants cannot restore damage in SCD patients but rather serve as a means of managing disease complications. Exploring the potential of combining traditional treatments with anti-oxidant therapies and investigating specific anti-oxidants represent promising avenues for research aimed at improving treatment SCD treatment outcomes and quality of life. Clinical trials are underway to investigate the efficacy and safety of various anti-oxidant treatments for SCD, and studies are underway to identify novel anti-oxidants or anti-oxidant combinations capable of treating the complications of SCD. The FDA has authorized oral L-glutamine as an alternative to hydroxyurea for the treatment of SCD. Additional studies on oxidative stress markers and anti-oxidant therapies are needed as this could lead to the development of more potent therapeutic approaches and provide valuable information on the condition, its underlying mechanisms, and its treatment.

CRediT authorship contribution statement

Vivek Kumar Gupta: Writing – review & editing, Methodology, Investigation, Formal analysis. **Rakesh Acharya:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Haneul Kang:** Visualization, Software, Formal analysis. **Henu Kumar Verma:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Tarun Sahu:** Writing – review & editing, Formal analysis, Conceptualization. **Jeong-Hwan Lee:** Writing – review & editing, Funding acquisition, Formal analysis. **Ganji Seeta Rama Raju:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **LVKS Bhaskar:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yun Suk Huh:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Eluri Pavitra:** Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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