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Article

Ellagic Acid Exerts Dual Action to Curb the Pathophysiological Manifestations of Sickle Cell Disease and Attenuate the Hydroxyurea-Induced Myelosuppression in Berkeley Mice

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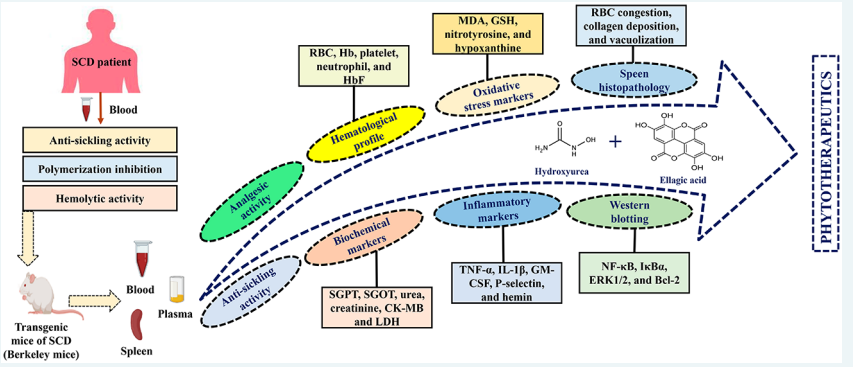

ABSTRACT: The use of adjuvant therapy is an attractive approach to manage sickle cell disease (SCD) symptomatically. The present study aimed to investigate the potential of ellagic acid as an adjuvant therapy with hydroxyurea (HU), a key drug for SCD with myelosuppressive toxic effects. A panel of experiments was performed using SCD patient's blood (ex vivo) and transgenic mice model of SCD (in vivo). Ellagic acid exhibited the following beneficial pharmacological actions: (a) potent anti-sickling, polymerization inhibitory, and inherent non-hemolytic activity; (b) pronounced action to abrogate HU-induced neutropenia and to improve key hematological parameters during SCD (RBC, Hb, platelet levels); (c) considerable action to foster vascular tone (L-proline); (d) marked attenuating effect against oxidative stress (nitrotyrosine, hypoxanthine, MDA, GSH); (e) substantial inhibitory role against inflammation (analgesic activity and regulation of hemin, TNF-α, IL-1β, NF-κB/IκBα); (f) remarkable outcome of declining vaso-occlusive crisis (P-selectin, ERK1/2); (g) notable shielding deed against elevated biochemical marker for organ toxicity (creatinine); (h) noticeably prevented histopathological alterations of the spleen. Additionally, the pharmacokinetic study results of HU in the presence and absence of ellagic acid using a mouse model demonstrate that ellagic acid could be safely co-administered with HU. Overall findings suggest that ellagic acid is a promising candidate for adjuvant therapy in SCD based on its own significant ability against SCD and potentiating capability of HU action via targeting improvement at the various stages of pathophysiological complications during SCD and minimizing HU-induced toxicological manifestations.

KEYWORDS: *sickle cell disease, ellagic acid, anti-sickling agent, hydroxyurea, myelosuppression, adjuvant therapy*

INTRODUCTION

Sickle cell disease (SCD) is congenital hemolytic anemia that arises due to mutation in the β-globin subunit of hemoglobin (Hb), which directs sickle Hb (HbS) production. Under hypoxic conditions, the polymerization of HbS leads to the sickling of red blood cells (RBC). Sickled RBC undergoes hemolysis that triggers vaso-occlusion, ischemia, and pain crises, which are the main pathological features of SCD.[1] This complex disease phenomenon includes severe symptoms such as extreme back pain, acute chest syndrome, cerebral infraction, vascular necrosis, pulmonary hypertension, nephropathy, etc.[2] Hydroxyurea (HU) is the first-ever approved drug (1998) to curb the manifestation of SCD. It increases fetal Hb (HbF) production, which reduces the severe consequences of HbS polymerization.[3] As HU has been initially approved as an anti-cancer drug, its therapy is associated with significant adverse effects like myelosuppression.[4]

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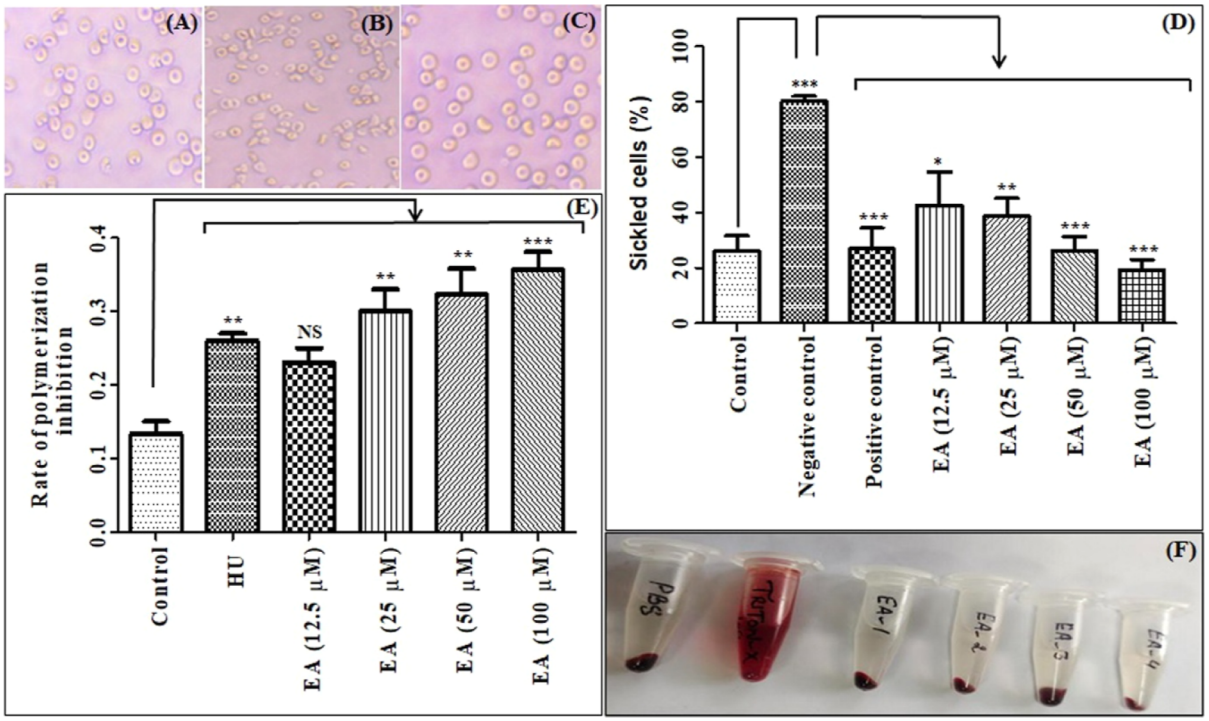

Figure 1. Representative images of anti-sickling activity: control blood (A); control blood after treatment with the inducing agent (negative control) (B); control blood after treatment with EA and subsequently with the inducing agent (C); effect of EA on the anti-sickling activity (D) and the rate of polymerization inhibition (E); representative images for the effect of EA on hemolysis (F) (EA_1, EA_2, EA_3, and EA_4 represent the concentration of EA at 0.01, 0.05, 0.1, and 1 mg/mL, respectively). Data are presented as mean ± SEM ($n = 3$). $p < 0.05/0.01/0.001$ denotes statistically significant (*/**/***). NS denotes not statistically significant.

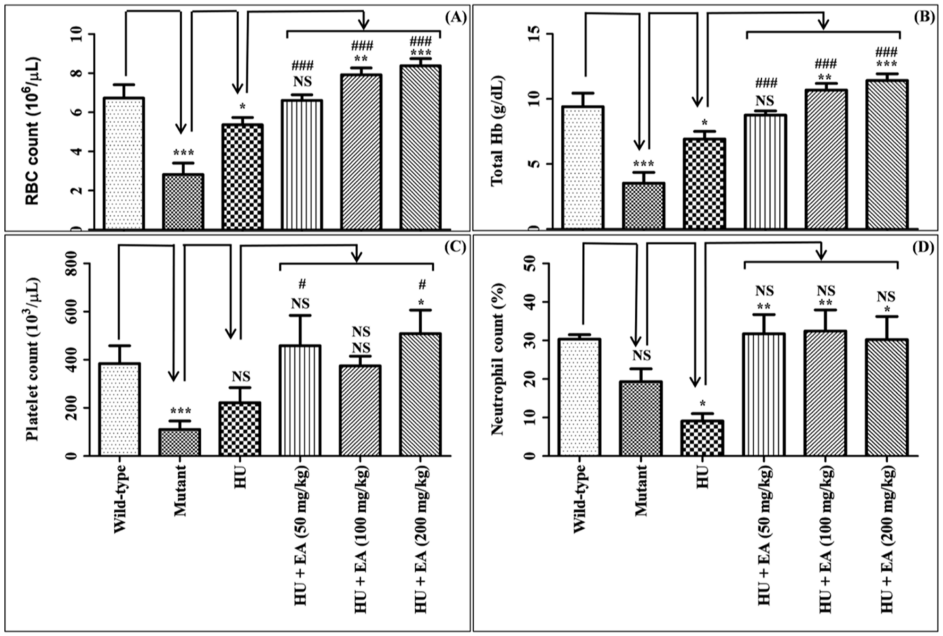

Figure 2. Effect of EA upon concomitant treatment with HU on the following parameters: RBC (A); Hb (B); platelet (C); neutrophil (D). Data are expressed as mean ± SEM ($n = 5$). $p < 0.05/0.01/0.001$ denotes statistically significant (*/**/*** or $^{\#}$/$^{\#\#}$/$^{\#\#\#}$). *Wild-type vs mutant/mutant vs HU/HU vs HU + EA. $^{\#}$Mutant vs HU + EA. NS denotes not statistically significant.

Under these circumstances, recent studies are ongoing to discover new drug modalities and adjuvant/supplementation therapy to counteract the signs/symptoms and, subsequently, improve the survival of SCD patients. In this pursuit, the main pathophysiological targets of SCD that have been explored globally by using drugs/natural products/phytochemicals/botanical drugs are: (a) decline in sickling behavior, (b) reduction in oxidative stress; (c) enhancement in HbF production, (d) lessening of platelet aggregation, (e) lowering of adhesion behavior, (f) reduction in inflammation (ClinicalTrials.gov). In this context, crizanlizumab (2019) and voxelotor (2019) are the two recently approved drugs by the United States

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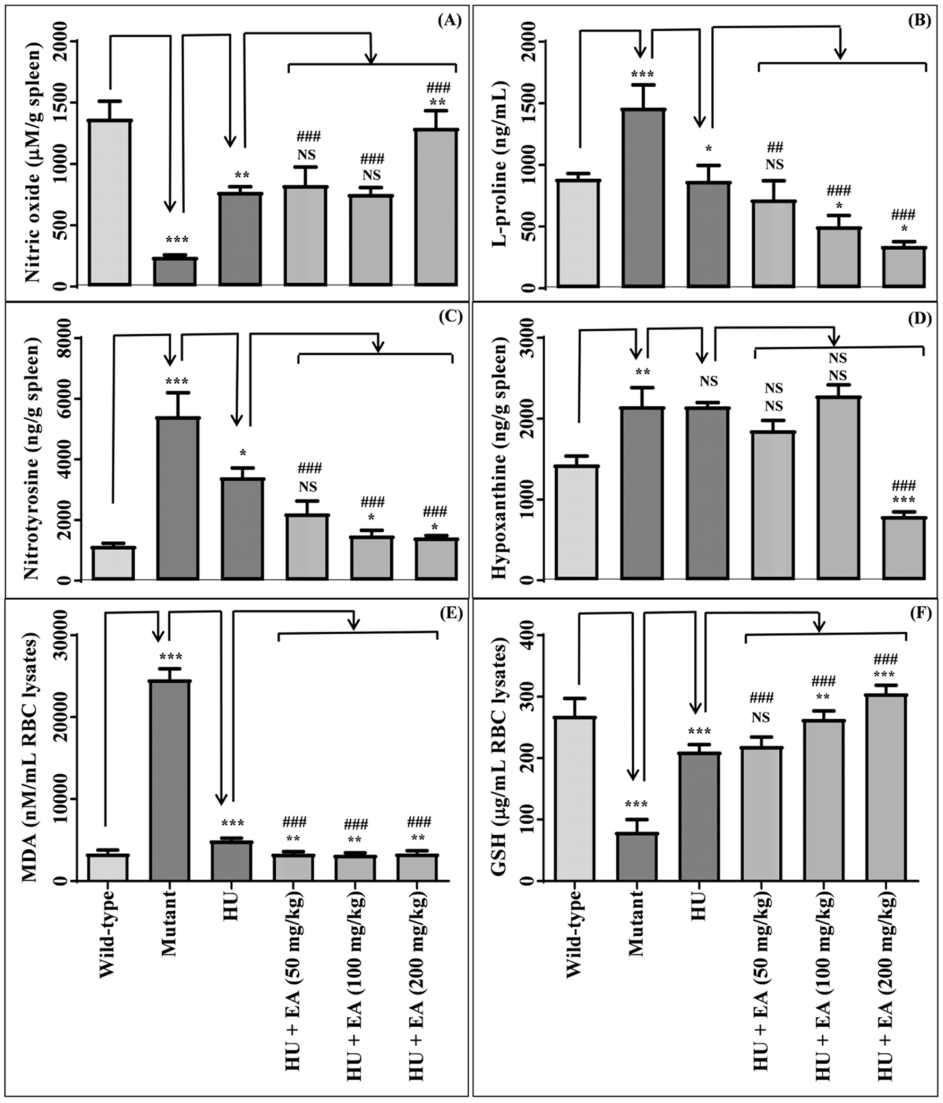

Figure 3. Effect of EA upon concomitant treatment with HU on the following parameters: nitric oxide (A); L-proline (B); nitrotyrosine (C); hypoxanthine (D); MDA (E); GSH (F). Data are expressed as mean ± SEM ($n = 5$). $p < 0.05/0.01/0.001$ denotes statistically significant (*/**/*** or #/##/###). *Wild-type vs mutant/mutant vs HU/HU vs HU + EA. #Mutant vs HU + EA. NS denotes not statistically significant.

Food and Drug Administration (USFDA) to restrict vaso-occlusive crises via blocking P-selectin function and prevent RBC sickling, respectively.[5] Moreover, L-glutamine (2017) has also been approved by USFDA as a supplementation therapy to minimize the signs/symptoms of SCD by reducing oxidative stress.[6]

In the present study, we hypothesized that ellagic acid (EA) might be helpful to combat HU-induced myelosuppression and could counteract symptoms of SCD patients. The main reasons for choosing EA in the present study are: (a) EA is a biologically active phytoconstituent that is predominantly present in a wide range of fruits,[7] and (b) EA is enlisted in the generally recognized as safe (GRAS) substances by USFDA for human consumption.

To date, no report is available for any role of EA alone and in combination with HU to symptomatically manage SCD. Hence, we aimed to explore the same under the purview of improvement in the pathophysiology of SCD using ex vivo (SCD patient's blood) and in vivo (transgenic mice model of SCD) experiments.

RESULTS

EA Displayed Anti-Sickling Action. EA was tested for anti-sickling activity using SCD patients' blood (Figures 1A–D). *p*-Hydroxybenzoic acid as a positive control exhibited significant sickling inhibition corroborating reported findings.[8] EA (12.5–100 μM) displayed a pronounced effect on sickling inhibition.

Influence of EA on polymerization inhibition was explored using SCD patients' blood (Figure 1E). HU showed considerable polymerization inhibition, which aligns with the previous reports.[8] EA (25–100 μM) significantly inhibited the polymerization of HbS.

To check any hemolytic effect of EA, results displayed that it had a negligible impact on hemolysis (≤2%) even at a high concentration (Figure 1F).

The effect of EA on anti-sickling activity was also investigated in the presence of HU using mutant/sickle mice. Mutant mice exhibited considerable sickling of RBC compared to the wild type. Treatment with HU significantly inhibited sickling compared to the mutant group (Figure S1), corroborating the earlier findings.[9] In combination with HU, EA significantly

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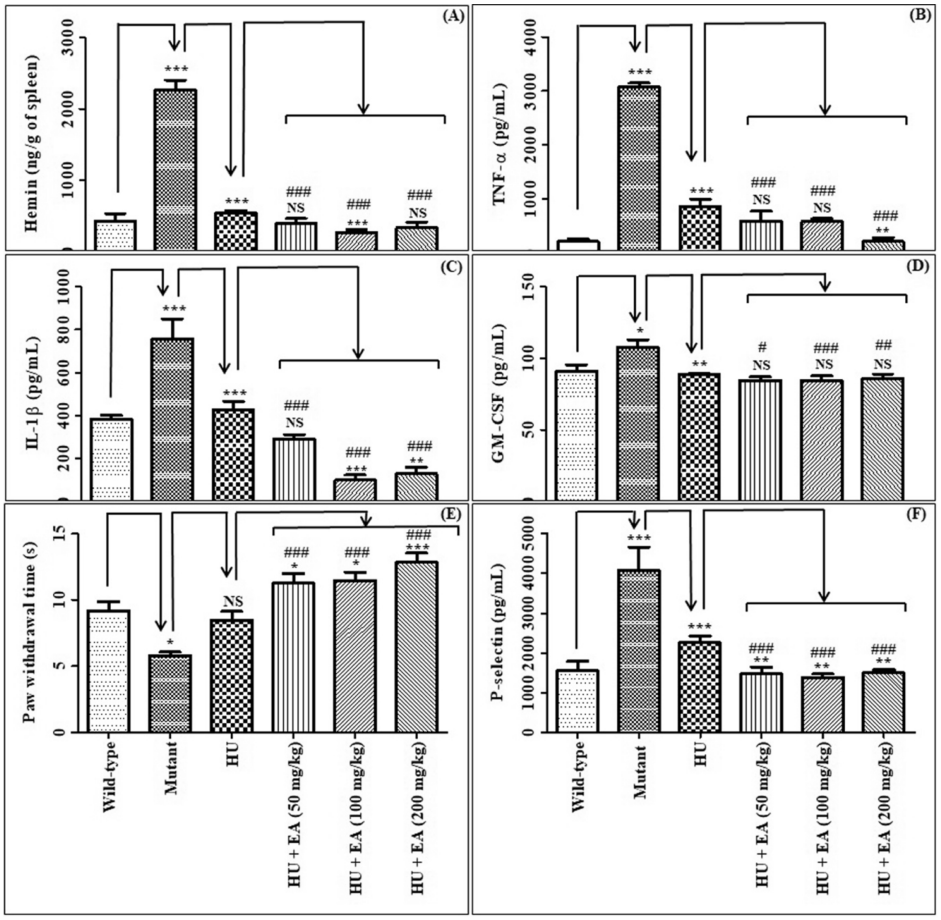

Figure 4. Effect of EA upon concomitant treatment with HU on the following parameters: hemin (A); TNF-α (B); IL-1β (C); GM-CSF (D); paw withdrawal time (E); P-selectin (F). Data are expressed as mean ± SEM (n = 5). p < 0.05/0.01/0.001 denotes statistically significant (*/**/*** or $^{\#}$/$^{\#\#}$/$^{\#\#\#}$). *Wild-type vs mutant/mutant vs HU/HU vs HU + EA. $^{\#}$mutant vs HU + EA. NS denotes not statistically significant.

reduced the sickling of RBC compared to HU alone (100−200 mg/kg)/mutant group (50−200 mg/kg).

EA Abrogated Hematological Profile. Compared to the wild type, we observed a considerable decline in RBC, Hb, and platelet, except the neutrophil count in the mutant group (Figures 2A−D). HU treatment led to a reduction in all the above-mentioned parameters compared to the wild type, demonstrating its myelosuppressive effect. Administration of HU exhibited a significant improvement in RBC and Hb levels compared to the mutant group. Concomitant treatment of EA (100−200 mg/kg) with HU considerably enhanced the same parameters compared to HU.

Although HU lacked any effect on platelet count compared to the mutant group, EA treatment (200 mg/kg) with HU showed notable improvement of platelet count compared to HU alone. Conversely, HU treatment caused a significant reduction in the neutrophil count. However, simultaneous administration of EA (50−200 mg/kg) with HU substantially normalized the neutrophil level.

HbF level was decreased in mutant mice compared to the wild type (Figure S2) in statistically insignificant manner. Compared to the mutant group, HU treatment significantly increased the HbF level, corroborating with the reported literature.[10] EA treatment with HU exhibited a negligible effect on HbF levels compared to HU alone. However, concomitant administration of EA with HU boosted the HbF level compared to the mutant group.

EA Fostered the Vascular Tone. A significant decline in the nitric oxide levels was observed in the mutant group compared to the wild type (Figure 3A), and results align with the reported literature.[4,11] HU treatment was associated with a notable improvement in nitric oxide levels compared to the mutant group, corroborating observed effects in SCD patients.[4,12] EA treatment exhibited a negligible effect on nitric oxide levels except at 200 mg/kg compared to HU alone. However, EA at all the experimental doses with HU substantially enhanced the nitric oxide levels compared to the mutant group.

L-Arginine level was reduced in the mutant group compared to the wild-type group (Figure S3) and improved upon HU treatment that lacks statistical significance. EA, in combination with HU, had a negligible effect on the L-arginine levels compared to HU alone but notably enhanced by EA (200 mg/kg) in combination with HU compared to the mutant group.

L-Proline level was markedly increased in the mutant group and declined upon the treatment of HU (Figure 3B), corroborating with the reported literature.[13] Concomitant administration of EA (100−200 mg/kg) significantly reduced the L-proline level compared to HU alone. Compared to the mutant group, EA at entire dose levels considerably improved the L-proline levels.

EA Attenuated the Oxidative Stress. A significant increase in the nitrotyrosine level of the spleen tissue was observed for the mutant group compared to the wild type (Figure 3C), corroborating with the reported literature.[14] We

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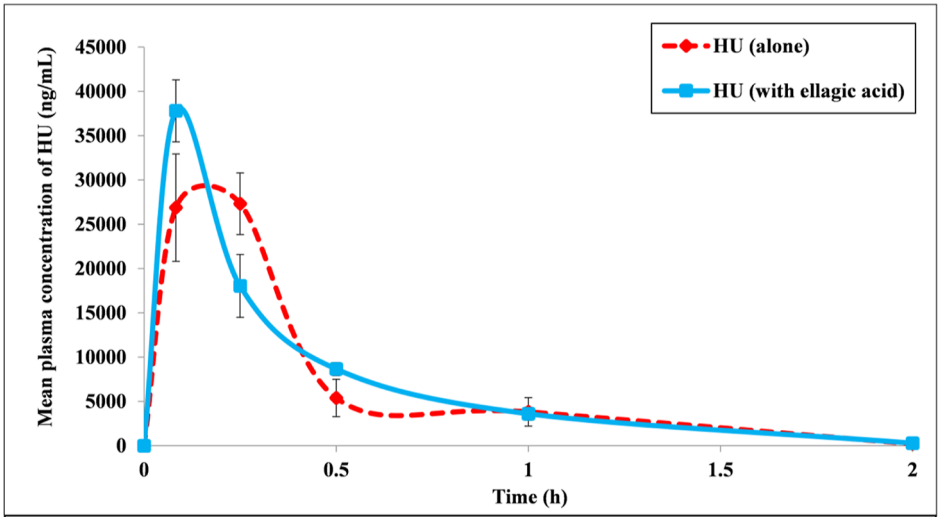

Pharmacokinetic Parameter	HU (alone)	HU (with ellagic acid)
$T_{1/2}$ (h)	0.25 ± 0.01	0.32 ± 0.04
C_{max} (ng/mL)	30381 ± 3497	38688 ± 5634
T_{max} (h)	0.22 ± 0.05	0.14 ± 0.04
AUC_{0-t} (ng.h/mL)	14058 ± 1467	14603 ± 2502
$AUC_{0-\infty}$ (ng.h/mL)	14134 ± 1471	14744 ± 2499
V_d/F (L/kg)	2.67 ± 0.40	3.60 ± 0.80
Cl/F (L/h/kg)	7.46 ± 0.95	7.52 ± 1.10

Figure 5. Mean plasma concentration vs time profiles and main pharmacokinetic parameters of HU after oral administration alone as well as in the presence of EA in mice. Data are expressed as mean ± SEM (n = 5).

noticed a notable reduction in the nitrotyrosine level upon the treatment of HU compared to the mutant group. EA (100−200 mg/kg) in combination with HU remarkably reduced the nitrotyrosine level compared to HU alone. In comparison to the mutant group, nitrotyrosine levels substantially declined upon concomitant administration of EA at all doses with HU.

A significant increase in the hypoxanthine level was observed in the mutant group compared to the wild type (Figure 3D). HU treatment showed a negligible effect on hypoxanthine levels compared to the mutant group. Co-administration of EA (200 mg/kg) with HU significantly reduced the hypoxanthine level in spleen tissue compared to the HU alone/mutant group.

Malondialdehyde (MDA) level in RBC lysates/spleen tissue was significantly elevated in the mutant group compared to the wild type (Figures 3E and S4) and was notably declined upon treatment with HU. HU is reported to decrease MDA levels in SCD patients.[15] Although the treatment of EA in combination with HU remarkably declined the MDA level in spleen tissue at only 200 mg/kg compared to HU alone, it significantly dropped at entire dose levels in the case of the RBC lysate. Moreover, EA at all dose levels improved the MDA level in both RBC lysates and spleen tissue homogenate compared to the mutant group.

Glutathione (GSH) level was markedly reduced in RBC lysate/spleen tissue in the mutant group compared to the wild type (Figures 3F and S4). The same was significantly enhanced upon HU treatment compared to the mutant group, which aligns with the HU effects in SCD patients.[15] Concomitant administration of EA (100−200 mg/kg) with HU further improved the GSH level in only the RBC lysate compared to HU alone. However, EA at entire dose levels upon concomitant treatment with HU significantly augmented the GSH level in RBC lysate/spleen tissue compared to the mutant group.

EA Inhibited Inflammation. Hemin, TNF-α, and IL-1β were significantly elevated in the mutant group and remarkably reduced upon HU treatment (Figures 4A−C). Results corroborate with the reported literature.[2] EA treatment with HU substantially declined hemin (100 mg/kg), TNF-α (200 mg/kg), and IL-1β (100−200 mg/kg) levels compared to HU alone. Compared to the mutant group, all these levels were remarkably reduced upon concurrent EA and HU treatment.

A significant increase in granulocyte-macrophage colony-stimulating factor (GM−CSF) level was observed in the mutant group compared to the wild type (Figure 4D). HU treatment led to a considerable decrease in the GM−CSF level compared to the mutant group, corroborating the previous findings.[16] Concomitant administration of EA with HU exhibited a negligible effect on GM−CSF levels in comparison to HU alone. However, the GM−CSF level substantially declined upon concurrent treatment of EA at all doses in the presence of HU compared to the mutant group.

Mutant mice exhibited a notable decrease in the paw withdrawal time compared to the wild type (Figure 4E). HU treatment did not significantly affect the paw withdrawal time compared to the mutant group. Concomitant administration of EA at entire doses in combination with HU considerably increased the paw withdrawal time compared to the HU alone/mutant group.

Protein expression of nuclear factor-kappa B (NF-κB)/inhibitor of kappa B alpha (IκBα) was significantly elevated in the mutant mice compared to the wild type (Figure S5), which are in line with the reported literature.[17] HU displayed a

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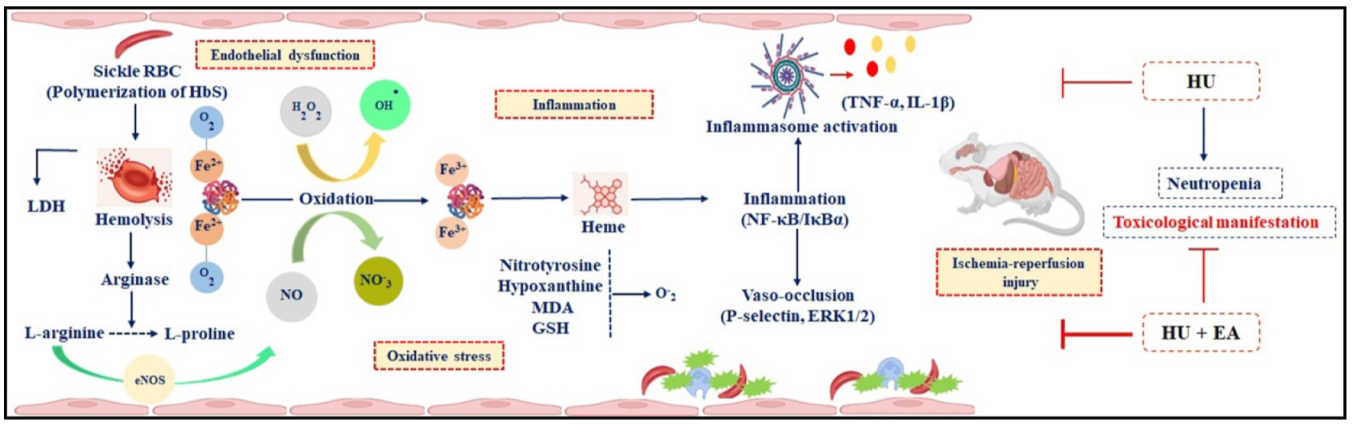

Figure 6. Pictorial presentation for the beneficial action of EA on the pathophysiological targets of SCD.

negligible effect on these proteins compared to the mutant group. EA (200 mg/kg) treatment with HU considerably down-regulated the NF-κB/IκBα levels compared to HU alone. EA (100−200 mg/kg) in combination with HU notably reduced the NF-κB/IκBα levels compared to the mutant group.

EA Declined the Vaso-occlusion Crisis. A significant increase in the P-selectin level in the mutant group was observed compared to the wild type (Figure 4F). HU treatment significantly reduced the P-selectin level compared to the mutant group. EA (50−200 mg/kg) treatment with HU remarkably reduced the P-selectin level compared to the HU alone/mutant group.

Protein expression of extracellular signal-regulated kinase 1/2 (ERK1/2) was significantly enhanced in the mutant group compared to the wild type (Figure S5). Treatment of HU reduced the ERK1/2 expression compared to the mutant group, which is in line with the reported literature.[11] EA (200 mg/kg) with HU significantly reduced the ERK1/2 level compared to HU alone. Additionally, the ERK1/2 level remarkably declined at all doses of EA with HU compared to the mutant group.

EA Prevented Histopathological Changes in the Spleen. Histopathological examination of spleen tissue was done to get better insights into the pathological changes of spleen (Figures S6 and S7). There was a significant increase in RBC congestion, collagen deposition, and vacuolization of histiocytes in the mutant group compared to the wild type. Administration of HU significantly alleviated all the parameters compared to the mutant group, which aligns with the reported literature.[18] EA at all dose levels aided to the preventive effect of HU on the spleen architecture.

EA Unaffected the Pharmacokinetics of HU. We evaluated the effect of EA on the pharmacokinetics of HU. The plasma concentration versus time profile and pharmacokinetic parameters are represented in Figure 5. EA did not alter any pharmacokinetic parameters of HU upon the simultaneous administration of HU with EA.

DISCUSSION

In the quest to explore the potential of EA as an adjuvant therapy for the symptomatic management of SCD, we elucidated the role of EA alone and in combination with a widely prescribed drug for SCD like HU (Figure 6) using SCD patients' blood (ex vivo) and transgenic mice model of SCD (in vivo).

In SCD, polymerization of HbS results in sickling of RBC, which leads to hemolysis. Hemolysis is a hallmark of SCD and the origin of vaso-occlusion, inflammation, and oxidative stress situations.[4,19] Therefore, any candidate having anti-sickling activity, including polymerization inhibition, can restrict the downstream effect of sickling. Voxelotor is the recent USFDA-approved drug to prevent RBC sickling. In the present study, EA is found to be a potent anti-sickling compound, regardless of using blood from SCD patients' or Berkeley mice. Additionally, the proposed adjuvant therapy should be devoid of inherent hemolytic activity. Current study results suggest that EA lacked any effect to trigger further hemolysis in SCD. HU is an anti-cancer agent, and its treatment is associated with the reduced expression of B-cell lymphoma 2 (Bcl-2), which is related to apoptosis.[20] We observed minimal impact of EA to limit the risk of HU treatment linked apoptosis in SCD (Figure S5). Treatment of HU is associated with an increase in γ-globin gene expression, thereby increasing HbF production that helps to prevent RBC's sickling.[21] EA displayed a minor role in the γ-globin gene to potentiate HU action.

Hemolysis in SCD patients causes a decline in the RBC count and Hb level. EvenFlo is reported to increase the Hb level during clinical investigation in SCD patients.[22] Moreover, SCD patients experience life-threatening thrombocytopenia during a prolonged vaso-occlusive crisis. Conversely, HU treatment in SCD patients reduces the severity of the disease, but its chronic treatment causes myelosuppression including neutropenia.[4] Based on the current study results on RBC, Hb, platelet, and neutrophil levels, EA could be beneficial in ameliorating the hematological profile to limit the onset of pain crises in SCD.

Intravascular hemolysis releases free heme and free radicals that diminish arginine and nitric oxide levels. Changes in the normal equilibrium of arginine and its catabolic by-products like ornithine, citrulline, and proline have been associated with pulmonary fibrosis. Supplementation therapy of L-arginine is under clinical exploration for SCD patients to improve the nitric oxide level.[23] Based on our current results on the improvement of nitric oxide, L-arginine, and L-proline levels, EA treatment with HU could be beneficial to improve the vascular tone and minimize pulmonary fibrosis, aiding to combat pathophysiological complications of SCD.

SCD is characterized by producing reactive oxygen species (ROS), resulting in oxidative stress and endothelial dysfunction. L-Glutamine has been recently approved by the USFDA to reduce oxidative stress.[6] Gum Arabic is under clinical exploration to improve anti-oxidant defense in SCD patients.[4] Nitrotyrosine is a marker for nitro-oxidative stress, and our experimental results illustrate that EA could potentiate the HU action to reduce the nitrotyrosine level. Ippoushi et al. reported

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similar results for EA to prevent oxidative damage.[24] Hypoxanthine is metabolized by xanthine oxidase, which contributes to oxidative stress in SCD, leading to multi-organ dysfunction.[25,26] Febuxostat, a xanthine oxidoreductase inhibitor, is reported to improve endothelial dysfunction in sickle mice.[27] In the present study, we observed the influence of EA with HU to reduce the hypoxanthine level. MDA is produced due to lipid peroxidation and can serve as a marker for oxidative stress.[28] Our results suggest that in the presence of HU, EA could restrict the SCD-mediated elevation of MDA. GSH neutralizes ROS and protects cells from oxidative stress-related cell damage.[28] Like the results of MDA, we also observed a similar line of effect by EA with HU on GSH. EA is also reported to reduce MDA and GSH levels in cisplatin-induced nephrotoxicity.[29] Thus, the adjuvant therapy of EA with HU could be beneficial to prevent the exacerbation of oxidative burden in SCD.

Hemolysis causes hemin release into the extracellular space. Hemin activates the adhesion molecules and their adherence to endothelial cells during the active phase of SCD. The increased oxidative burden in SCD patients due to excess quantities of cell-free Hb and heme triggers ROS formation, thereby activating the neutrophil molecules, which can capture circulating sickle RBC and hinder blood flow.[30] Deferasirox and deferoxamine are reported to decline the iron burden in SCD patients.[31] Moreover, curcumin is under preclinical investigation to reduce iron overload.[32] In SCD, sickling of RBC leads to vaso-occlusion, followed by the production of TNF-α and IL-1β, resulting in vasculopathy.[33] A TNF-α blocker (etanercept) and leukotrienes inhibitor (zileuton) are under investigation to treat SCD.[4] Thus, a candidate with the ability to minimize the hemin level and anti-inflammatory activity can assist in decreasing the severity of SCD. Current results reveal that EA could check the inflammatory conditions to prevent the complications of SCD.

GM-CSF is a hematopoietic cytokine that prevents TFr-1 hematopoietic cells to produce γ-globin during erythropoietin-stimulated differentiation. GM−CSF also contributes to leukocytosis in SCD patients.[34] Although EA is reported to reduce the GM−CSF production in LnCaP cells,[35] our current results indicate that EA had a negligible effect on potentiating the HU effect on regulating GM−CSF. Pain crises during SCD are the most common reason for patients to seek medical assistance. SCD patients are generally prescribed acetaminophen with or without codeine or oxycodone, depending on the severity of their pain.[36] Our present results reveal that EA with HU could aid to counteract nociceptive pain in SCD.

Activation of NF-κB involves the phosphorylation of IκBα in the cytoplasm, followed by the subsequent translocation of NF-κB to the nucleus, thereby activating inflammatory genes, which are responsible for the production of cytokines and chemokines.[37] Heme, which is released upon hemolysis, triggers the activation of NF-κB. Sulfasalazine is an anti-inflammatory drug that can inhibit the transcription of NF-κB and interfere with the activation of endothelial cells in sickle mice.[17] The present results of NF-κB/IκBα expression are in line with the results of TNF-α, IL-1β, and hemin levels. Hence, NF-κB/IκBα-mediated regulation of inflammatory pathways by EA could be helpful to attenuate the inflammatory burden of SCD.

In SCD, enhanced expression of P-selectin on the activated endothelium and platelets leads to the progression of vaso-occlusive crises. Crizanlizumab has been recently approved by USFDA to inhibit the action of P-selectin.[38] Current results reveal that EA could augment the action of HU to trim down the P-selectin level. Alves et al. reported similar results for EA in reducing the expression of P-selectin during inflammation.[39] ERK1/2 is aberrantly active in sickle RBC. ERK1/2 activation mediates the adhesion of sickle cells to the vascular endothelium. Inhibition of ERK1/2 in activated neutrophils reduces the expression of adhesion molecules.[40] The effect of EA on ERK1/2 expression is in line with the results of hemin and P-selectin levels. Thus, EA could check both P-selectin and ERK1/2 status (P-selectin > ERK1/2), which can be useful to retard the vaso-occlusion crises in SCD patients.

SCD is associated with splenomegaly and spleen dysfunction in pediatric patients.[17] Results of histopathological examination illustrate that EA, in combination with HU, could exhibit a better protective effect against spleen pathophysiological alterations during SCD. SCD is associated with liver ischemia[41] and kidney impairment.[42] The elevated levels of urea and creatinine in SCD and the effect of HU on these levels are in line with the reported literature.[3,43] Current results indicate that EA had minimal effect for any further improvement in the HU effect on serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and urea levels (Figure S8). Conversely, EA displayed prominent action to trim down the augmented level of creatinine in SCD (Figure S8). In SCD, chronic kidney failure can lead to progressive myocardial damage, and creatine kinase−myocardial band (CK−MB) is reported to increase during SCD.[44] We observed the effect of HU on preventing the elevation of CK−MB levels in SCD, and the results corroborate the previous findings.[45] EA did not aid in any CK−MB lowering effect by HU (Figure S8). Lactate dehydrogenase (LDH) is a direct marker of hemolysis and is elevated during SCD.[21] In the present study, HU restricted the enhanced LDH level due to SCD, and the results are in line with the reported literature.[46] EA treatment with HU exhibited a minimal effect compared to HU alone (Figure S8). Results demonstrate that EA in combination with HU did not exacerbate but could restrict the elevation of hepatic, renal, and cardiac injury markers associated with disease pathophysiology, thereby exerting improvement in the survival of SCD patients.

Adjuvant therapy should be devoid of unintended pharmacokinetic interaction to circumvent any drug interaction that can cause an increase or decrease in plasma exposure, leading to the precipitation of toxic effects of drugs or therapeutic failure of drugs, respectively.[47,48] Lack of any pharmacokinetic interaction between HU (prescribed drug) with EA (adjuvant therapy) was observed in the present study. Hence, our current results dictate safe co-administration of HU with EA for desired pharmacological effects.

CONCLUSIONS

The potential of EA was investigated under the purview of adjuvant therapy in SCD using a battery of ex vivo and in vivo models. Based on the experimental findings, the specific advantageous pharmacological actions are concisely stated as follows: (a) EA has its own pronounced action to combat the disease pathophysiology of SCD, (b) EA can potentiate the HU action to counteract the disease pathophysiology of SCD, (c) EA can prevent HU-induced myelosuppressive effects, and (d) EA can be safely co-administered with HU. EA is found to be a promising candidate for supplementation therapy with HU via targeting various stages of pathophysiological conditions in SCD to prevent finally ischemia-reperfusion injury/organ damage. Results insinuate further exploration of EA in combination with HU at the clinical level.

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MATERIALS AND METHODS

EA was purchased from a commercial source (Sigma-Aldrich). The chromatographic purity of EA was assessed in-house and found to be >99% (Figure S9). The ex vivo studies were carried out using SCD patient's blood (donor). The in vivo experimentations were performed in the Berkeley mice model. Detailed materials and methods are represented in the "Supporting Information".

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsptsci.3c00026.

Detailed information on materials and methods; anti-sickling activity data in mice model; effect on HbF, L-argenine, MDA (spleen), and GSH (spleen); protein expression data; histopathological data; and biochemical data (PDF)

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Author Contributions

A.G. performed the polymerization assay, hemolysis activity, all animal experimentations, LC−MS/MS analysis, data evaluation, and writing—original draft; D.K.: anti-sickling activity, western blotting; R.P.: identification of SCD mice; M.B.: analgesic activity, hematological and biochemical parameter analysis; S.D.S.: resources for funding. A.K.: supervision of anti-sickling activity, western blotting; U.N.: conceptualization, planning and supervision of animal experimentations, writing—review and editing.

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ABBREVIATIONS

SCD, sickle cell disease; Hb, hemoglobin; HbS, sickle hemoglobin; RBC, red blood cells; HU, hydroxyurea; HbF, fetal hemoglobin; USFDA, United States Food and Drug administration; GRAS, generally recognized as safe; MDA, malondialdehyde; GSH, glutathione; GM−CSF, granulocyte-macrophage colony-stimulating factor; NF-κB, nuclear factor-kappa B; IκBα, inhibitor of kappa B alpha; ERK1/2, extracellular signal-regulated kinase 1/2; Bcl-2, B-cell lymphoma 2; ROS, reactive oxygen species; SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; CK−MB, creatine kinase−myocardial band; LDH, lactate dehydrogenase; C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}; AUC_{0-t}, area under the curve for plasma concentration from zero to the last measurable plasma sample time; $AUC_{0-\infty}$, area under the curve for plasma concentration from zero to infinity; V_d/F, volume of distribution after oral administration; Cl/F, clearance after oral administration

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