Investigating the Potential Use of Andrographolide as a Coadjuvant in Sickle Cell Anemia Therapy

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ABSTRACT: Andrographolide is one of the main active principles of Andrographis paniculata and has been extensively explored for its therapeutic use. Current studies focus on phytotherapeutics-based adjuvant therapy to symptomatically treat sickle cell anemia (SCA) as there is no specific drug/gene therapy available to date. The present study aimed to explore the potential of andrographolide as an adjuvant therapy for SCA in the presence or absence of hydroxyurea (HU), a key drug for SCA treatment. A panel of ex vivo and in vivo experimentations was performed to explore the antisickling activity of andrographolide, followed by evaluating pharmacokinetic and pharmacodynamic (PK/PD) activities in the presence of HU. Andrographolide showed significant antisickling activity using blood from SCA patients (ex vivo) and did not show any deleterious effect to cause hemolysis using rat blood (ex vivo). It displayed a substantial decrease in HU-induced decline in splenic lymphocyte proliferation and cytokine level (TNF-α and IFN-γ) using rat splenocytes (ex vivo). Concomitant oral administration of andrographolide with HU in rats for 15 days exhibited a noticeable improvement in the RBC count and hemoglobin levels comparable to the efficacy of L-glutamine (in vivo). Simultaneous administration of andrographolide with HU caused no marked effect on any pharmacokinetic parameters of HU except the highest plasma concentration of HU and its corresponding time point, which significantly dropped and delayed, respectively (in vivo). No considerable effect of andrographolide was observed on urease and horseradish peroxidase activity (in vitro). Overall, results suggest that andrographolide has several beneficial actions to be an adjuvant therapy to symptomatically manage SCA, but it should be avoided during the prescribed therapy of HU.

1. INTRODUCTION

Sickle cell anemia (SCA) is congenital hemolytic anemia due to a mutation in the β-globin subunit of hemoglobin (Hb) that directs sickle hemoglobin (HbS) production. In the hypoxia condition, HbS polymerizes and forms fibers, which cause the sickling of red blood cells (RBCs). These sickled RBCs suffer hemolysis to cause vaso-occlusion, ischemia, and pain crisis, which are the main pathological hallmark of SCA. Gene therapy can be an ideal treatment option toward the mitigation of this chronic disease (a monogenic disorder of the blood), but it is unavailable in the market to date. Hydroxyurea (HU) is one of the key drugs for the treatment of SCA. HU treatment is found to be beneficial for SCA patients due to its effect on the augmentation of the fetal hemoglobin (HbF) level that mainly helps to prevent the polymerization of HbS. HU is an anticancer drug, and therefore, myelosuppression is a major adverse effect associated with its treatment. In this context, preclinical as well as clinical studies are ongoing worldwide to improve the pathophysiological conditions of the SCA patients depending upon the different targets, viz. decline in sickling behavior, reduction in oxidative stress, enhancement of HbF production, lessening of platelet aggregation, lowering of adhesion behavior, reduction in inflammation, and so on. Voxelotor and crizanlizumab are the two recently approved drugs that can prevent the sickling of RBC and block the action of P-selectin to restrict vaso-occlusive crisis, respectively. To manage this complex disease phenomenon with severe signs and symptoms leading to mortality, supplementation/adjuvant therapy has been given along with prescription drugs. L-Glutamine (Brand name: Endari) has been recently approved as an adjuvant therapy for SCA management by USFDA because of its potential to reduce oxidative stress. Similarly, several products from plant origin, viz. EvenFlo, NIPRASIN, and gum arabic (NCT04191213) are under different phases of clinical trials. In this pursuit, our current
studies deal with andrographolide, an active principle of *Andrographis paniculata*. It is one of the major plants included in more than 25 Ayurvedic formulations in India and is widely consumed as a traditional medicine all over the world. Moreover, andrographolide is widely consumed by a large population for its several beneficial biological activities. However, there is no report in the literature for its potential use as an adjuvant therapy to manage SCA symptomatically.

Therefore, the present study aimed to evaluate the antisickling potential of andrographolide and then explore its applicability for concomitant use with HU by pharmacokinetic/pharmacodynamic (PK/PD) perspectives using in vitro, ex vivo, and in vivo models. The objectives were to investigate the following: (a) antisickling activity of andrographolide using the blood from SCA patients (ex vivo); (b) hemolytic effect of andrographolide using rat blood (ex vivo); (c) lymphocyte proliferation and cytokine inhibition of HU in combination with andrographolide using rat splenocytes (ex vivo); (d) hematological profile upon repeated-dose of HU in combination with andrographolide using the rat model (in vivo); (e) pharmacokinetics of HU in combination with andrographolide using the rat model (in vivo); and (f) urease and horseradish peroxidase (HRP) inhibitory action of andrographolide (in vitro).

2. RESULTS AND DISCUSSION

2.1. Antisickling Activity of Andrographolide. In SCA patients, polymerization of HbS causes the formation of sickled RBCs that lead to hemolysis and subsequent alterations in the disease pathophysiology. Therefore, the effect of andrographolide on antisickling activity was investigated using SCA patients’ blood. Results of p-hydroxybenzoic acid as a positive control are in line with the literature report. Andrographolide as a test compound showed more than 50% inhibition of sickling at the experimental concentration range (12.5–50 μM) (Figure 1). Results demonstrated that andrographolide has a significant antisickling activity (p < 0.001) that can be further explored as alone or in combination with prescribed drugs. A recently approved drug by USFDA for SCA is Voxelotor (GBT-440), which showed around 66% inhibition of sickling at a concentration of 300 μM. Vanillin, a flavoring agent, has potent antisickling activity, but instability in the gastro-intestinal tract upon oral administration restricts its further development. Thus, our results suggest that andrographolide can limit HbS polymerization to restrict the sickling phenomenon. Thus, andrographolide has the potential to be an adjuvant therapy in SCA. Furthermore, andrographolide may be beneficial to SCA patients for concomitant therapy with HU because the overall decrease in HbS polymerization leads to lower chances of hemolysis and further vaso-occlusion-related complications. However, andrographolide should not cause any potential pharmacokinetic interaction with HU and has to be investigated.

2.2. Hemolytic Activity of Andrographolide. Hemolysis is the main pathological feature of SCA. Therefore, the proposed adjuvant therapy of any candidate should be devoid of inherent hemolytic activity and have a beneficial pharmacological action for SCA treatment to restrict the further aggravation of disease pathophysiology. In the present study, andrographolide displayed a negligible effect (≤2%) to cause the hemolysis in rat blood even at a high experimental concentration (1 mg/mL) (Figure 2). The above experimental results demonstrate that andrographolide is nonhemolytic in nature and has an antisickling activity. Therefore, andrographolide will not cause further hemolysis during adjuvant therapy in the disease conditions.

2.3. Effect of Andrographolide on Lymphocyte Proliferation and Cytokine Inhibition. Vaso-occlusion causes the release of several inflammatory mediators leading to a pain crisis. It is another major hurdle in SCA that needs to be managed symptomatically. Literature reports describe that HU decreased lymphocyte proliferation and inhibited cytokine production. We observed a similar effect of HU on T-lymphocyte proliferation of rat splenocytes. Andrographolide displayed a further reduction of lymphocyte proliferation in comparison to HU alone (Figure 3A). Moreover, andrographolide exhibited a significant decrease in the TNF-α and IFN-γ levels (p < 0.01) in the presence of HU (Figure 3B,C). Therefore, the use of andrographolide in combination with HU may be beneficial to restrict the elevation of cytokine levels, thereby lowering the pain crisis.
The present study results of HU on key hematological parameters in the rat model are similar to the previously reported observations. Concomitant treatment of andrographolide (100 mg/kg, oral) in the presence of HU showed significant boosting of the RBC level \( (p < 0.01) \) when compared with the treatment of HU alone. Moreover, it exhibited a substantial improvement by 1.6 and 1.8 fold in HU-mediated reduction in Hb and platelet level, respectively, in comparison to HU alone, but it lacks statistical significance (Table 1).

2.4. Effect of Andrographolide on HU-Induced Myelosuppression. The main liability during HU therapy is myelosuppression, which is decline in RBC and hemoglobin (Hb) levels. The present study results of HU on key hematological parameters in the rat model are similar to the previously reported observations. Concomitant treatment of andrographolide (100 mg/kg, oral) in the presence of HU showed significant boosting of the RBC level \( (p < 0.01) \) when compared with the treatment of HU alone. Moreover, it exhibited a substantial improvement by 1.6 and 1.8 fold in HU-mediated reduction in Hb and platelet level, respectively, in comparison to HU alone, but it lacks statistical significance (Table 1).

2.5. Impact of Andrographolide on Pharmacokinetics of HU. A pharmacokinetic interaction study is useful to explore any potential of the adjuvant/supplementation therapy to alter the pharmacokinetics of prescribed drugs. The information is generally helpful to assess the possibility of safe co-administration (insignificant interaction), therapeutic failure (significant decrease in plasma exposure), or precipitation of dose-related side effects (significant elevation of plasma exposure). Therefore, our findings showed a significant drop and delay in the \( C_{\text{max}} \) and \( T_{\text{max}} \) of HU, respectively, upon concomitant administration with andrographolide (Figure 4 and Table 2). Gratifyingly, there was no noticeable effect on the \( T_{1/2}, \text{AUC}_{0→0}, \text{AUC}_{0→∞}, V_d/F, \text{and } \text{Cl/F} \) of HU upon simultaneous administration with andrographolide. It is evident in the literature that \( T_{\text{max}} \) may be delayed during co-administration. The prescribed dose of HU is generally very high. Additionally, \( C_{\text{max}} \) and AUC are the critical factors that should always be considered to evaluate the drug interaction potential. Though the AUC of HU was not altered up to a significant extent in the present study, a severe drop in \( C_{\text{max}} \) of HU in the presence of andrographolide is a critical factor considering pharmacokinetic interaction. Similar effects of andrographolide to drop \( C_{\text{max}} \) and/or AUC of etoricoxib and tolbutamide are reported in the literature. Thus, results illustrated that necessary precautions have to be taken to avoid any sub-therapeutic plasma exposure-mediated therapeutic failure of HU treatment during concomitant administration with andrographolide.

2.6. Influence of Andrographolide on Urease and HRP Inhibition. Urease, HRP, and catalase are reported to metabolize HU. In the urease inhibition assay, thiourea as positive control showed profound urease inhibition (IC\(_{50} \) 27.84 \( \mu \)M), which corroborates a previous report. Andrographolide exhibited a high IC\(_{50} \) value (2444 \( \mu \)M) toward urease inhibition (Figure 5A,B). In the present HRP inhibition assay, sodium azide as a positive control showed more than 95% HRP inhibition at 1000 \( \mu \)M. However, andrographolide did not exhibit any considerable inhibitory effect on HRP at the experimental concentration (500 and 1000 \( \mu \)M) (Figure 5C). Thus, we did not observe any substantial effect of andrographolide on urease and HRP activity. Trivedi et al. demonstrated the role of andrographolide to increase catalase levels during oxidative injury. In the current study, the

Table 1. Main Hematological Parameters of Rats After Repeated-Dose Oral Administration of HU Alone as Well as in the Presence of Andrographolide/\( L\)-Glutamine

| parameters                  | total RBC (10\(^6\)/μL) | Hb (g/dL) | platelet count (10\(^3\)/μL) | total WBC count (10\(^3\)/μL) |
|-----------------------------|-------------------------|-----------|-------------------------------|-------------------------------|
| control                     | 7.31 ± 0.16             | 13.42 ± 0.32 | 788.40 ± 23.47               | 5.96 ± 0.50                  |
| HU alone                    | 3.52 ± 0.69***          | 6.84 ± 1.32## | 184.25 ± 53.80###            | 2.38 ± 0.92##                |
| HU + andrographolide        | 6.01 ± 0.43*            | 10.63 ± 0.88 | 334.80 ± 61.58               | 2.90 ± 0.59                  |
| HU + \( L\)-glutamine       | 6.17 ± 0.20*            | 10.73 ± 0.25* | 260.00 ± 20.71               | 4.92 ± 0.42                  |

*Data are expressed as mean ± SEM \( (n = 5) \). Data were compared: control versus treatment of HU alone where \( p < 0.05/0.01/0.001 \) denotes statistical significance \((#/#/#/####)\); treatment of HU alone versus treatment with HU in the presence of andrographolide/\( L\)-glutamine where \( p < 0.05/0.01/0.001 \) denotes statistical significance \((###/###/###)#).
outcome on $C_{\text{max}}$ may be associated with enhanced catalytic activity in the presence of andrographolide. Further mechanistic investigation at multiple-dose administration of andrographolide with HU may provide a better understanding of the altered pharmacokinetic behavior after single-dose administration.

3. CONCLUSIONS

Overall results suggest that andrographolide has antisickling activity, nonhemolytic nature, and anti-inflammatory action as well as can improve the hematological profile. Although andrographolide is devoid of any impact on urease and HRP activities, it can drop $C_{\text{max}}$ and delay $T_{\text{max}}$ of HU, suggesting that it should not be used during HU therapy. However, studies are required to establish its potential as an adjuvant therapy for SCA management.

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents. HU (purity $\geq 98\%$), L-glutamine (purity $\geq 98\%$), p-hydroxybenzoic acid (purity $\geq 99\%$), sodium metabisulphite (purity $\geq 98\%$), urease from Canavalin ensiformis (jack bean), DMEM, Triton X-100, and Con-A were procured from Sigma-Aldrich (St. Louis, USA). Sodium azide and phosphate-buffered saline (PBS) were purchased from Himedia (Mumbai, India). HRP and urea were purchased from Invitrogen (Vienna, Austria) and Ranbaxy (Gurgaon, India), respectively. Formic acid and acetonitrile (MS-grade) were obtained from Thermo Fisher Scientific (Waltham, USA). Other experimental reagents were bio-reagent grade or above. During the analysis, ultrapure water was used (Direct-Q3, Merck-Millipore; Darmstadt, Germany).

4.2. Isolation and Characterization of Andrographolide. Andrographolide was isolated from the leaves of *A. paniculata*, purified through the column chromatographic method, and characterized by NMR and HRMS (Figures S1–S4, Supporting Information). The chromatographic purity of andrographolide was $>99\%$ (Figure S5, Supporting Information).

4.3. Ex Vivo Antisickling Action. The blood sample was collected from SCA patients (Institutional Ethics Committee approval number: IEC/GMC/Cat A/2020/267) in a tube containing citrate-phosphate-dextrose solution with adenine (CPDA buffer) and then diluted with HBSS. Seeding of blood cells ($5 \times 10^4$ cells) was performed in 96-well plates, treated with andrographolide (12.5 to 50 $\mu M$), and incubated in a carbon dioxide incubator for 1 h. Furthermore, a mixture of 1.5 mM cobalt chloride and 0.5% (w/v) sodium metabisulphite, that is, sickling-inducing agent, were added to samples to induce sickling. Cells were analyzed immediately by a Confocal Quantitative Image Cytometer (Make: CQI; Model: Yokogawa; Musashino, Japan). Treatment of cells with the only inducing agent was considered as the negative control (100% sickling) and % inhibition of sickling of a test compound was calculated. The experiment was performed in triplicates, and p-hydroxybenzoic acid (50 $\mu M$) was used as a positive control.

4.4. Ex Vivo Hemolysis Activity. Control rat blood with anticoagulant was taken in microcentrifuge tubes, centrifuged to get settle RBC, then washed, and diluted with PBS (1:10, v/v). After that, diluted RBCs (100 $\mu L$) were mixed with PBS

Figure 4. Mean plasma concentration vs time profile of HU as alone and in the presence of andrographolide in rats. Data are represented as mean ± SEM ($n = 5$).

Table 2. Main Pharmacokinetic Parameters of HU After Oral Administration as Alone and in the Presence of Andrographolide in Rats*

| Treatment arm        | $T_{1/2}$ (h) | $C_{\text{max}}$ (ng/mL) | $T_{\text{max}}$ (h) | AUC$_{0-1}$ (ng h/mL) | AUC$_{0-\infty}$ (ng h/mL) | $V_d/F$ (L/kg) | $Cl/F$ (L/h/kg) |
|----------------------|--------------|--------------------------|----------------------|-----------------------|---------------------------|---------------|---------------|
| HU alone             | 0.29 ± 0.01  | 19152 ± 2239             | 0.17 ± 0.04          | 18831 ± 2262          | 19198 ± 2323              | 1.17 ± 0.17   | 2.83 ± 0.47   |
| HU + andrographolide | 0.66 ± 0.18  | 12170 ± 850*             | 0.75 ± 0.14*         | 15640 ± 1044          | 18409 ± 1208              | 2.55 ± 0.62   | 2.75 ± 0.18   |

*Data are expressed as mean ± SEM ($n = 5$). Data were compared: treatment of HU alone versus treatment with HU in the presence of andrographolide. $p < 0.05$ denotes statistical significance (*). $C_{\text{max}}$: highest plasma concentration; $T_{\text{max}}$: time to reach $C_{\text{max}}$; AUC$_{0-\infty}$: 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; $T_{1/2}$: elimination half-life; $V_d/F$: volume of distribution after oral administration; and $Cl/F$: clearance after oral administration.
(900 μL) containing andrographolide (0.01 to 1 mg/mL) in sample tubes. 1% Triton X-100 and PBS were considered as the positive control and negative control, respectively. The sample tubes were incubated in a shaking water bath (120 rpm, 37 °C, 30 min), centrifuged (8000 rpm, 4 °C, 10 min), taken out the supernatant, and measured by the plate reader at 540 nm (Model: Infinite M200 Pro; Make: Tecan; Grodig, Austria). The % hemolysis was measured using the following equation

\[
\% \text{ hemolysis} = \frac{(\text{ABS}_{\text{sample}} - \text{ABS}_{0})}{(\text{ABS}_{100} - \text{ABS}_{0})} \times 100
\]

where \(\text{ABS}_0\) is the absorbance of the sample in PBS (0% hemolysis) and \(\text{ABS}_{100}\) is the absorbance of the sample in 1% Triton X-100 (100% hemolysis).

4.5. Ex Vivo Splenic Lymphocyte Proliferation and Cytokine Inhibition. Control rat spleens were obtained in incomplete media (RPMI). Cells were counted by a hemocytometer (Model: IS10269/BS749; Make: Rohem; Mumbai India). Seeding of spleen lymphocytes (2.5 × 10^4 cells) was performed in 96-well plates, treated with andrographolide (10 μM), added HU (50 μM), incubated (37 °C, 1 h), and stimulated by Con-A (5 μg/mL). After a gap of 48 h, MTT dye (20 μL of 2.5 mg/mL) was added and incubated again for 4 h. The samples were analyzed at 570 nm by the plate reader. At the end of the study, the cell culture supernatant was taken and analyzed for TNF-α and IFN-γ using ELISA kits (Invitrogen, Waltham, USA).

4.6. In Vivo Effect Against HU-Induced Myelosuppression. Healthy male Wistar rats (140–160 g of body weight) were used in the present study, and necessary approval was obtained prior to experimentation (Institutional Animal Ethics Committee approval no: 73/141/8/2018). Rats were kept under standard laboratory conditions and given a pellet diet and water ad libitum. The effect of andrographolide was investigated to improve the main hematological parameters that altered upon HU treatment. Each study group consisted of five animals: Group-1 (control): vehicle only; Group-2 (diseased control): HU alone; Group-3 (reference): L-glutamine + HU; and Group-4 (test): andrographolide + HU. The optimized HU dose was 300 mg/kg. L-Glutamine and andrographolide were used at 100 mg/kg for each. We used L-glutamine here to observe its effect on hematology irrespective of its mechanism of action to reduce the oxidative stress. Vehicle to prepare dose formulation was Na-CMC (0.5%, w/v) and dose-volume was 10 mL/kg. Each treatment was given through the oral route. The dose was administrated for 15 consecutive days, and blood samples were collected the next day of the last dosing from overnight fasted animals. Blood was analyzed by a hematology analyzer (Model: XT1800i; Make: Sysmex; Kobe, Japan).

4.7. In Vivo Pharmacokinetic Interaction of Andrographolide with HU. Wistar rat model was used to explore the impact of andrographolide on HU pharmacokinetics. HU and andrographolide were used at the dose level of 50 mg/kg and 10 mg/kg, respectively. The dose formulation, route of administration, and dose-volume were the same as mentioned above. Each study group consisted of five animals: Group-1: HU only; Group-2: HU + andrographolide. The dose was administrated to overnight fasted animals where andrographolide was given 30 min before HU. After dosing of HU, blood samples were taken at predose, 0.083, 0.25, 0.5, 1, and 2 h to obtain 50 μL of plasma. Each plasma sample was processed with acetonitrile containing methylurea (internal standard, 100 ng/mL), centrifuged (14000 rpm for 10 min), decanted, filtered, and finally poured into vials for analysis. A matrix match calibration curve (100 to 12800 ng/mL) estimated HU in plasma was done by using our earlier reported LC–MS/MS method. Measured concentration data at particular time points were fitted for the calculation of pharmacokinetic parameters by a noncompartmental method (PK solution software, Summit Research Services, USA).

4.8. In Vitro Effect of Andrographolide on the Inhibition of Urease and HRP Activity. Andrographolide (10 to 4000 μM) and 8 U/mL of urease enzyme were incubated in the assay buffer (100 mM urea, 10 mM LiCl, 1 mM EDTA, 10 mM KH2PO4; pH 8.2) in 96-well plates (room temperature, 10 min). Then, alkali reagent [50 μL; NaOCl (0.1%, v/v) and NaOH (0.5%, w/v) mixture] and phenol reagent [50 μL; phenol (1%, w/v) and sodium nitroprusside
(0.005%, w/v) mixture were added, incubated, and analyzed at 625 nm using a plate reader. The % inhibition was done by the following equation:

\[
\% \text{ Inhibition of urease} = \left( \frac{\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}} \right) \times 100
\]

Thiourea was used as a positive control (1 to 50 μM), and studies were carried out in triplicates. IC₅₀ values were calculated (GraphPad PRISM 5.0) to demonstrate urease inhibition.

For the HRP activity assay, andrographolide (10 μL, 250 or 500 μM) was incubated with TMB (100 μL) and HRP (10 μL) in 96-well plates. Then, hydrogen peroxidase (0.03%, v/v) was added to initiate the reaction, and the blue color that developed due to the TMB converted product was monitored at 655 nm using a plate reader. The % inhibition was done by the equation mentioned above for urease inhibition. Sodium azide was used as a positive control (1000 μM), and studies were carried out in triplicates.

4.9. Statistical Analysis. Statistical evaluation was done by unpaired Student’s t-test (QuickCalcs software, GraphPad Prism, USA). Comparison between two data sets in a particular experiment is mentioned in the respective Table/Figure. Statistical significance was considered at three levels having a p-value of less than 0.05 (*/*#) or 0.01 (**/###) or 0.001 (***/####).

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c07339.

Characterization and purity data of isolated andrographolide (PDF)

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**Author Contributions**
A.G., P.K. and A.D. performed animal experimentation and sample analysis; D.K. and A.K. performed lymphocyte cell proliferation assay and antisickling activity; A.G. and S.D. performed hemolysis assay, cytokines estimation, and MS preparation; A.K. performed isolation and characterization of andrographolide; S.K.D. provided SCA patients’ blood samples and human ethics committee approval formalities; G.S. performed acquisition of chemicals and MS proof read; and U.N. performed the overall study plan and execution including MS correction.

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**Notes**
The authors declare no competing financial interest.

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**REFERENCES**
(1) Pauling, L.; Itano, H. A.; Singer, S. J.; Wells, I. C. Sickle cell anemia, a molecular disease. Science 1949, 110, 543–548.
(2) Cravotto, G.; Boffa, L.; Genzini, L.; Garella, D. Phytotherapeutics: an evaluation of the potential of 1000 plants. J. Clin. Pharmacol. Ther. 2010, 35, 11–48.
(3) Platt, O. S. Hydroxyurea for the treatment of sickle cell anemia. N. Engl. J. Med. 2008, 358, 1362–1369.
(4) Verma, H. K.; Lakkakula, S.; Lakkakula, B. V. K. S. Retrospection of the effect of hydroxyurea treatment in patients with sickle cell disease. Acta Haematol. Pol. 2018, 49, 1–8.
(5) Kapoor, S.; Little, J. A.; Pecker, L. H. Advances in the treatment of sickle cell disease. Mayo Clin. Proc. 2018, 93, 1810–1824.
(6) Sinha, C. B.; Bakshi, N.; Ross, D.; Loewenstein, G.; Krishnamurti, L. Primary caregiver decision-making in hematopoietic cell transplantation and gene therapy for sickle cell disease. Pediatr. Blood Cancer 2021, 68, No. e28749.
(7) Gour, A.; Dogra, A.; Bhatt, S.; Nandi, U. Effect of Natural Products on Improvement of Blood Pathophysiology for Management of Sickle Cell Anemia. In Botanical Leads for Drug Discovery, Springer, 2020; pp 51–65.
(8) Muga, R.; Ajwang, A.; Ouma, J.; Ogijo, J.; Otieno, J.; Okoth, P.; Wafula, C.; Ajwang, S.; Ogolla, D.; Hollist, A.; Adesifoye, J. Efficacy of the Nutritional Supplement, EvenFlo, in the Management of Sickle
Cell Disease: A Randomized Controlled Trial. Nurs. Health Sci. Res. J.
2020, 3, 35−45.
(9) Vetrivka, V.; Vannucci, L. Biological properties of andrographolide, an active ingredient of Andrographis Paniculata: a narrative review. Ann. Transl. Med. 2021, 9, 1186.
(10) Dhiman, A.; Goyal, J.; Sharma, K.; Nanda, A.; Dhiman, S. A review on medicinal prospectives of Andrographis paniculata Nees. J. Pharm. Sci. Innovation 2012, 1, 1−4.
(11) Maiti, K.; Gantait, A.; Kakali, M.; Saha, B.; Mukherjee, P. K. Therapeutic potentials of andrographolide from Andrographis paniculata: a review. J. Nat. Rem. 2006, 6, 1−13.
(12) Conran, N.; Belcher, J. D. Inflammation in sickle cell disease. Clin. Hemorheol. Microcirc. 2018, 68, 263−299.
(13) Atabo, S.; Umar, I. A.; James, D. B.; Mamman, A. I. Sickled erythrocytes reversal and membrane stabilizing compounds in Telluria occidentalis. Scientifica 2016, 2016, 1568061.
(14) Ferrone, F. A. GBT440 increases haemoglobin oxygen affinity, reduces sickling and prolongs RBC half-life in a murine model of sickle cell disease. Br. J. Haematol. 2016, 174, 499−500.
(15) Abraham, D.; Mehanna, A.; Wireko, F.; Whitney, J.; Thomas, R.; Orringer, E. Vanillin, a potential agent for the treatment of sickle cell anemia. Blood 1991, 77, 1334−1341.
(16) Gour, A.; Dogra, A.; Kour, D.; Singh, G.; Kumar, A.; Nandi, U. Effect of Concomitant Hydroxyurea Therapy with Rutin and Gallic Acid: Integration of Pharmacokinetic and Pharmacodynamic Approaches. ACS Omega 2021, 6, 14542−14550.
(17) Gour, A.; Dogra, A.; Wazir, P.; Singh, G.; Nandi, U. A highly sensitive UPLC-MS/MS method for hydroxyurea to assess pharmacokinetic intervention by phytotherapeutics in rats. J. Chromatogr. B 2020, 1154, 122283.
(18) Morton, D.; Reed, L.; Huang, W.; Marcek, J. M.; Austin-LaFrance, R.; Northcott, C. A.; Schelling, S. H.; Enerson, B. E.; Tomlinson, L. Toxicity of hydroxyurea in rats and dogs. Toxicol. Pathol. 2015, 43, 498−512.
(19) Balap, A.; Atre, B.; Lohidasan, S.; Sinnathambi, A.; Mahadik, K. Pharmacokinetic and pharmacodynamic herb−drug interaction of Andrographis paniculata (Nees) extract and andrographolide with etoricoxib after oral administration in rats. J. Ethnopharmacol. 2016, 183, 9−17.
(20) Oga, E. F.; Sekine, S.; Shitara, Y.; Horie, T. Pharmacokinetic herb-drug interactions: insight into mechanisms and consequences. Eur. J. Drug Metab. Pharmacokinet. 2016, 41, 93−108.
(21) Dogra, A.; Gour, A.; Bhatt, S.; Sharma, P.; Sharma, A.; Kotwal, P.; Wazir, P.; Mishra, P.; Singh, G.; Nandi, U. Effect of rutin on pharmacokinetic modulation of diclofenac in rats. Xenobiotica 2020, 50, 1332−1340.
(22) Dogra, A.; Bhatt, S.; Magotra, A.; Sharma, A.; Kotwal, P.; Gour, A.; Wazir, P.; Singh, G.; Nandi, U. Intervention of curcumin on oral pharmacokinetics of daclatasvir in rat: A possible risk for long-term use. Phytother. Res. 2018, 32, 1967−1974.
(23) Kotwal, P.; Dogra, A.; Sharma, A.; Bhatt, S.; Gour, A.; Sharma, S.; Wazir, P.; Singh, P. P.; Kumar, A.; Nandi, U. Effect of natural phenolics on pharmacokinetic modulation of bedaquiline in rat to assess the likelihood of potential food−drug interaction. J. Agric. Food Chem. 2020, 68, 1257−1265.
(24) Scott, J. P.; Hillery, C. A.; Brown, E. R.; Misiewicz, V.; Labotka, R. J. Hydroxyurea therapy in children severely affected with sickle cell disease. J. Pediatr. 1996, 128, 820−828.
(25) Chen, H.-W.; Huang, C.-S.; Liu, P.-F.; Li, C.-C.; Chen, C.-T.; Liu, C.-T.; Chiang, J.-R.; Yao, H.-T.; Lii, C.-K. Andrographis paniculata extract and andrographolide modulate the hepatic drug metabolism system and plasma tolbutamide concentrations in rats. Evidence-Based Complementary Altern. Med. 2013, 2013, 982689.
(26) Yahouédéhou, S. C. M. A.; Adorno, E. V.; da Guarda, C. C.; Ndidi, U. S.; Carvalho, S. P.; Santiago, R. P.; Aleluia, M. M.; de Oliveira, R. M.; Gonçalves, M. d. S. Hydroxyurea in the management of sickle cell disease: pharmacogenomics and enzymatic metabolism. Pharmacogenomics J. 2018, 18, 730−739.