In vitro Studies on SCD-101, A Promising Botanical Extract with Anti-sickling Properties for Sickle Cell Disease Therapy

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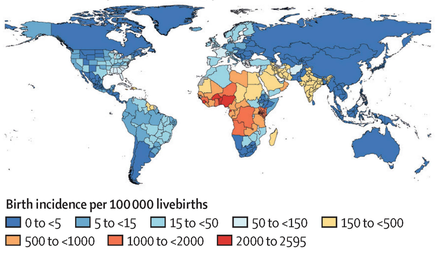
ABSTRACT

Although thousands of people worldwide suffer from sickle cell disease, the most common form of an inherited blood disorder, there are only a handful of available treatments available to combat the significant morbidities, lifelong challenges, reduced quality of life, and early mortality rates in patients. The resources available are also inaccessible to many either due to price or the possibility of adverse effects such as myelosuppression. Thus, to discover more effective and affordable treatments for patients with sickle cell disease, we investigated the anti-sickling effect of SCD-101, a botanical extract often utilized for medicinal purposes in Nigeria and other West African countries. After conducting multiple in-vitro sickling assays, we discovered that there are one or more small molecules located in fractions of SCD-101 that confer the indigenous plant with anti-sickling activity. In view of the outcome of this study, SCD-101 may be a promising option for the treatment of patients with sickle cell disease.

BACKGROUND

Sickle cell disease (SCD) is an inherited genetic disorder that affects approximately 100,000 Americans and 20 − 25 million people worldwide. Caused by a singular substitution of valine for glutamic acid in the sixth position of the ***β***-chain of hemoglobin S (HbS), SCD is a debilitating blood disorder that impairs an individual's ability to live a healthy, pain-free life. The pathophysiology of SCD is based on polymerization of deoxygenated sickle hemoglobin (HbS), which leads to red blood cell (RBC) sickling. This phenomenon occurs under conditions of low oxygen (O_2) saturation known as hypoxia. The consequence of RBC sickling is a systemic illness that manifests primarily as painful crises, leading to progressive organ damage, poor quality of life and a decreased life expectancy in many patients. Although two gene therapy approaches for complete cure of SCD were approved in the US recently, novel therapies that are both effective and cheap to manufacture remain a top priority because the primary disease burden is in the developing world, specifically, in sub-Saharan Africa where the most affected individuals live. This unfortunate reality provides the opportunity for, and justifies the importance of my project, which seeks to investigate SCD-101, a botanical extract from the Sorghum bicolor plant grown in Nigeria. Although its mechanism of action and active substance composition remain unknown, evidence suggests that ≥ 1 small molecules are responsible for its activity.



METHODOLOGY

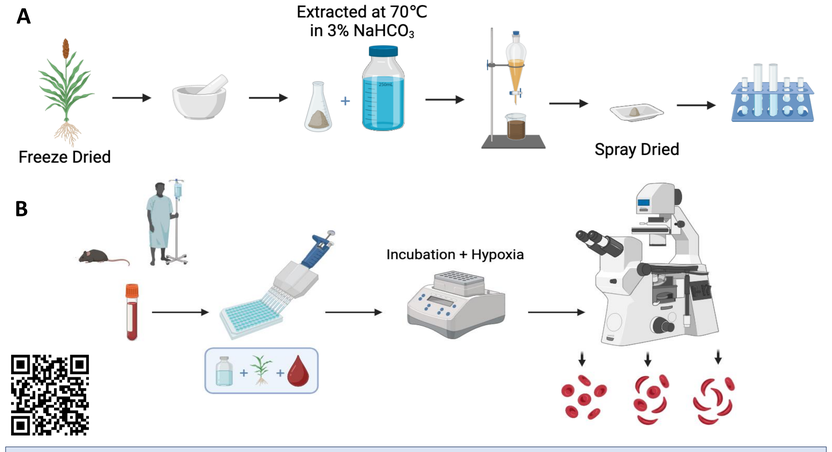


Figure 1: (A) Methodology: SCD-101 Fraction Extraction **(B)** Methodology: Sickling Assay Protocol

PRELIMINARY DATA

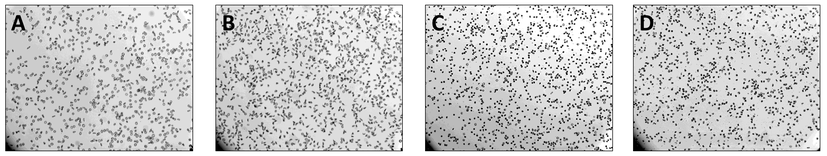


Figure 2: Representative image of (**A)** healthy red blood cells treated with 5% of fraction 5 post incubation and 1 hour hypoxia **(B)** untreated, sickled red blood cells after 1 hour hypoxia **(C)** red blood cells with spherocytosis (**D)** red blood cells with echinocytosis.

	Sample	Image	Sickled Cells	All cells	% Sickled
Patient 1	L1	25	332	490	67.7551
	L2	26	409	531	77.02448
	L3	27	474	644	73.60248
	L4	28	370	551	67.15064
	L5	29	73	253	28.85375
	L6	30	449	627	71.61085
	L7	31	601	1072	56.06343
	L8	32	179	370	48.37838
	H1	33	323	618	52.26537
	H2	34	239	554	43.14079
	H3	35	295	690	42.75362
	H4	36	242	995	24.32161
	H5	37	2	15	13.33333
	H6	38	448	807	55.51425
	H7	39	112	603	18.5738
	H8	40	242	629	38.47377
	Ctrl				70.86331

	Sample	Image	Sickled Cells	All cells	% Sickled
Patient 2	L1	41	262	353	74.22096
	L2	42	723	843	85.76512
	L3	43	817	929	87.94403
	L4	44	573	708	80.9322
	L5	45	174	908	19.163
	L6	46	761	841	90.48751
	L7	47	650	793	81.96721
	L8	48	563	678	83.03835
	H1	49	241	428	56.30841
	H2	50	511	711	71.8706
	H3	51	112	519	21.57996
	H4	52	124	499	24.8497
	H5	53	60	347	17.29107
	H6	54	519	669	77.57848
	H7	55	160	574	27.87456
	H8	56	165	624	26.44231
	Ctrl				81.61926

	Sample	Image	Sickled Cells	All cells	% Sickled
Patient 3	L1	1	342	624	54.80769
	L2	2	291	427	68.14988
	L3	3	266	461	57.70065
	L4	4	450	631	71.31537
	L5	5	34	496	6.854839
	L6	6	375	530	70.75472
	L7	7	281	448	62.72321
	L8	8	268	440	60.90909
	M1	9	104	166	62.6506
	M2	10	182	262	69.46565
	M3	11	317	392	80.86735
	M4	12	172	272	63.23529
	M5	13	75	268	27.98507
	M6	14	196	273	71.79487
	M7	15	301	393	76.59033
	M8	16	244	428	57.00935
	H1	17	122	289	42.21453
	H2	18	176	480	36.66667
	H3	19	242	475	50.94737
	H4	20	69	613	11.25612
	H5	21	118	443	26.63657
	H6	22	372	729	51.02881
	H7	23	61	487	12.52567
	H8	24	145	633	22.90679
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Figure 3: Left) Results obtained using blood samples from Patient 1 **Middle)** Results obtained using blood samples from Patient 2 **Right)** Results obtained using blood samples from Patient 3

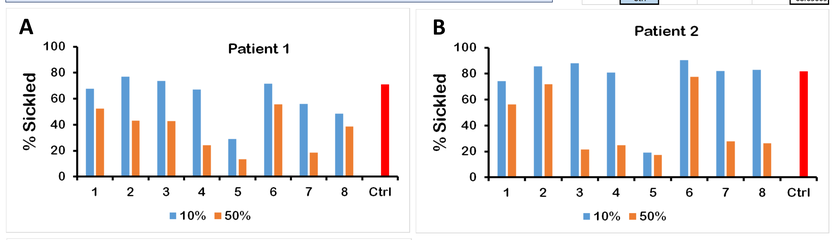


Figure 4: Bar graphs that depicts the effect of various fractions of SCD-101 on the percentage of sickled cells in blood sample from three different patients at low (5%), intermediate (10%), and high (50%) vol/vol suspensions. The percentage of sickled cells was determined using a computer-assisted image analysis system called ImageJ and graphed using Excel.

SECONDARY DATA

	Sample	Image	Sickled cells	All cells	% Sickled
5%	Fraction 5	1	104	500	20.8
	Fraction 5 (pH)	2	295	603	48.92206
	Fraction 1	3	493	732	67.34973
	Fraction 1 (pH)	4	363	628	57.80255
10%	Fraction 5	5	168	575	29.21739
	Fraction 5 (pH)	6	174	526	33.07985
	Fraction 1	7	509	695	73.23741
	Fraction 1 (pH)	8	281	434	64.74654
50%	Fraction 5	9	100	408	24.5098
	Fraction 5 (pH)	10	52	379	13.72032
	Fraction 1	11	281	393	71.50127
	Fraction 1 (pH)	12	324	457	70.89716
Positive Controls	502 (1.5 mM)	13	112	523	21.41491
	VOX (0.75 mM)	14	82	416	19.71154
Negative Controls	Ctrl 1	15	341	567	60.14109
	Ctrl 2	16	301	516	58.33333

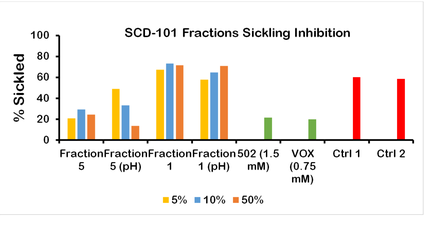
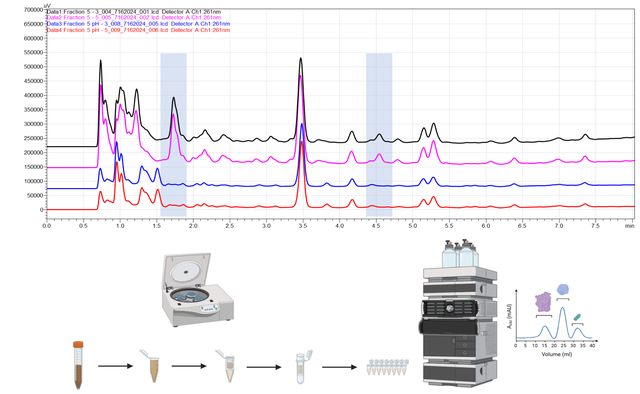


Figure 5: Left) Results collected from sickling assay performed using blood samples from a patient with SCD. Fraction 5, Fraction 1, Fraction 5 (pH =7.4), Fraction 1 (pH = 7.4), positive and negative controls were tested. **Right)** Bar graph that depicts the effect of various suspensions on the % of sickled cells after 1 hour hypoxia.

FRACTION 5 INVESTIGATION



CONCLUSION

- The results of the sickling assay indicate that there are one or more small molecules found in fraction 5 that are responsible for the extract's anti-sickling effects.
- Fraction 5's activity was conserved across blood samples from multiple patients (biological and technical replicates)
- Fraction 5 significantly reduced the percentage of sickled cells at both high and low volumes by at least 50%.
- Although other fractions like fraction 7 showed some promising anti-sickling activity, albeit at high volumes, the morphology of the RBCs was compromised.
- The results of the sickling assay indicate that fraction 5 is the most reliable candidate for further fractionation and investigation as the primary anti-sickling component of SCD-101.

FUTURE DIRECTIONS

If we can definitively identify the fractions that contain one or more small molecules responsible for inhibiting the polymerization of HbS, then we can subsequently concentrate the active components to improve the potency of the extract as well as the dosage requirement if it were to be developed into a drug. In addition, gaining a better understanding about the plant's activity may enable us to learn more about how its anti-sickling agents interact with hemoglobin in vitro and in vivo.

MAIN IMPLICATIONS

The main implication from this experiment is the potential of botanical plants in the treatment of SCD. Botanical plants have long been utilized as a source of medicine by various communities worldwide. Particularly, in developing countries, medicinal plants are heavily relied upon due to their cultural significance as well as accessibility. Unable to afford the cost of treatment offered by traditional hospitals and medical professions, many individuals manage diseases, such as SCD by using crude extracts from plants. Thus, I believe that the investigation of botanical plants could lead to various scientific advances in the drug development industry

ACKNOWLEDGMENTS

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