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Gum Arabic as fetal hemoglobin inducing agent in sickle cell anemia; in vivo study

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Abstract

Background: High levels of fetal haemoglobin (HbF) decrease sickle cell anaemia (SCA) severity and leads to improved survival. According to in vivo and in vitro studies, butyrate increases HbF production. Its utilization in clinical practice is hampered, however, by its short half-life. Serum butyrate concentrations could be enhanced by colonic bacterial fermentation of Gum Arabic (GA), edible, dried, gummy exudates from Acacia Senegal tree. We hypothesized that regular intake of GA increases serum butyrate levels, thus inducing HbF production and ameliorating symptoms of sickle cell anemia.

Methods: Fourty seven patients (5–42 years) carrying hemoglobin SS were recruited from April 2014 to January 2015. Patients received 30 g/day GA for 12 weeks. HbF, blood count and erythropoietin level were measured. The main outcome of interest was the level of HbF after 12 weeks. The secondary outcomes were improvement in clinical and laboratory results. The study was ethically approved by Alneelain University IRB.

Results: The study revealed significant increase in HbF level P.V:0.000 [95 % CI, 0.43–1.02], MCV P.V:0.000 [95 % CI, 2.312–6.058] and Hematocrit level P.V:0.026 [95 % CI, 0.124–1.902]. No significant difference was encountered in platelets count P.V: 0.346 [95 % CI, –25.76–71.94], and WBCs count P.V:0.194 [95 % CI, –8.035–1.68]. Thirty seven percent of patients experienced minor side effects which resolved within a week.

Conclusion: These findings reveal a novel effect of GA, which may be used to foster fetal hemoglobin production.

Trial registration: ClinicalTrials.gov Identifier: NCT02467257. Registered 3rd June 2015.

Background

Homozygous sickle cell Anemia (SCA) is an autosomal recessive genetic disease that results from the substitution of valine for glutamic acid at position 6 of the β -globin chain, leading to production of hemoglobin S (HbS) [1]. HbS polymerizes in red blood cells upon deoxygenation. This causes the RBCs to change from biconcave disc shape to an irregular sickled shaped. Sickled RBCs can block blood vessels, and thus decrease the delivery of oxygen to organs and tissues. Sickled cells are extremely susceptible to hemolysis and eryptosis [2], causing chronic anemia [3]. Sickle cell disease (SCD) is the most common genetic disorder among people of African descent [4].

Fetal hemoglobin (HbF) expression is a crucial determinant of the clinical severity of SCD [5]. The percentage of HbF (HbF%) influences both laboratory values and clinical features of children and adults with sickle cell anemia [6]. These observations were largely responsible for the shift of therapeutic emphasis and strategies to increase the level of HbF in vivo in patients with sickle cell disease [1]. Hydroxyurea (HU) been approved by the FDA to treat adult sickle cell patients [7]. Still HU is underutilized because of concern regarding safety and lack of availability in many parts of the developing world [8]. In addition HU is expensive [9] and requires regular follow up to assess response and monitor toxicity, which restrict it is usage even more in low resources setting. Both in vivo and in vitro studies demonstrate that butyrate administration similarly increases Hemoglobin F production [10–14]. So far the chemical derivatives of butyrate are of less clinical value because

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of their low bioavailability and rapid metabolism [8]. Arginine butyrate had to be given by continuous intravenous infusion in large volumes, and sodium phenylbutyrate required as many as 40 tablets daily [8]. Butyrate could, however, be generated from Gum Arabic (GA), edible, dried, gummy exudates from the stems and branches of *Acacia Senegal* and *Acacia Seyal*, rich in non-viscous soluble fiber. It is defined by the FAO/WHO Joint Expert Committee for Food Additives (JECFA) as a dried exudation obtained from the stem of *A. Senegal* [15]. GA has wide industrial uses as a stabilizer, thickening agent and emulsifier, mainly in the food industry (e.g. in soft drinks syrup, gummy candies and marshmallows). The US FDA recognized it as one of the safest dietary fibres [15, 16]. GA is indigestible for both human and animals; Its fermentation by colonic intestinal bacteria leads to formation of various degradation products, such as short-chain fatty acids [17]. Gum Arabic ingestion increases serum short chain fatty acid concentration, mainly butyrate and propionate [15, 18]. Serum butyrate concentration increased following administration of GA in healthy subjects [15, 19]. Oral intake of GA has been shown to provide several health benefits [20], such as prebiotic effects [16]. GA significantly increases *Bifidobacteria*, *Lactobacteria*, and *Bacteriodes* in the gut [16]. GA is claimed to have anti-cancer [16], anti-malarial [17] immune-modulatory [17, 21] and antioxidant effects [15, 16, 22]. GA treatment has been shown to favorably influence clinical and laboratory results in rats with adenine-induced chronic renal failure CRF and in humans diagnosed with renal failure [15, 17, 21]. GA shown to increase Erythropoietin level in two separate studies and ameliorated anemia caused by adenine administration [23, 24].

We hypothesized GA degradation delivers short chain fatty acids, which in turn have been shown to stimulate fetal hemoglobin expression in RBCs. Increased levels of erythrocyte fetal hemoglobin are known to hinder the intraerythrocytic HbS polymerization and provide some protection against hemolysis and vaso-occlusive crisis [5]. The present study tested whether Gum Arabic may influence the clinical course of SCD.

To the best of our knowledge this is the first study conducted to investigate the effect of oral administration of GA on fetal hemoglobin production in sickle cell anemia patients.

Methods

This is an experimental study with the aim to produce primary data for hematological efficacy of oral intake of Gum Arabic as fetal hemoglobin inducer in sickle cell anemia patients. The participants were recruited from the out patients clinic of pediatric and adult hematology units in Military hospital-Khartoum-Sudan. Inclusion criteria were: patients homozygous for SCD (SS) as documented by Hemoglobin electrophoreses, aged between 5 and 50 years. The total number of participants recruited mounted to 47. All medications and dosages had been stable for 2 weeks before study entry. All participants received folic acid 5 mg daily to support erythropoiesis. Exclusion criteria: patients received blood transfusion within the last 3 months or admitted to the hospital within 2 weeks because of SCD-related events or crisis. Ethical clearance was obtained from the Institutional Review Board at Alneelain University and from Research Ethics Committee- Khartoum State Ministry of Health. Principal investigator obtained informed consent from each participant or from parents when the patient is less than 18 years old prior to the interview.

Gum arabic administration

GA in powder form, it is a 100 % natural extract powder produced mechanically from the wildy grown *Acacia Senegal* tree with a particle size less than 210 μm . GA in powder form was provided from Dar Savanna Ltd., Khartoum, Sudan. Properties and composition of GA are listed elsewhere [22]. The daily dose was 30 gram. The dose was determined based on previous studies [16, 19]. It was given in one sachet to be consumed early morning dissolved in water for 12 weeks. The GA was provided to the participants every 2 weeks for 3 months (14 sachets per each visit). Empty sachets were retained every visit as indicator of compliance.

Table 1 Demographics and baseline characteristics

Characteristics	Mean	SD	Median	Maximum	Minimum
Age	16.26	8.52	15	42	5
Gender	23(49 %) Male				
Base line weight (Kg)	35.96	14	37.3	63	13
Base line height (Cm)	148.34	20.99	154.5	107	190
Hb g/dL	7.28	1.105	7	11	5.5
Hb F (%)	6.68	5.44	4.80	17.50	00
Hb S (%)	89.99	5.15	91	97.20	79.40
Hb A ₂ (%)	3.33	0.52	3.3	4.4	2.5

Table 2 Comparison between the mean of pre and post intervention values of biomarkers

Variable	Base line value Mean ± SD	Post intervention concentration value Mean ± SD	P.V.	95 % CI
Hb F (%)	6.68 ± 5.44	7.41 ± 5.38	.000 ^b	0.431–1.028
Hb S (%)	90 ± 5.15	89.24 ± 5.10	.000 ^b	0.455–1.043
Hb A ₂ (%)	3.3 ± 0.52	3.33 ± 0.48	0.901	0.064–0.072
Hemoglobin (g/dL)	7.28 ± 1.105	7.2638 ± 1.08	0.777	-0.142–0.188
MCV (fL)	85.2 ± 9.37	89.20 ± 12.33	.000 ^b	-2.312-- 6.058
PCV %	20.56 ± 3.15	21.57 ± 4.29	.026 ^a	0.124–1.902
MCH pg	30.32 ± 3.87	30.01 ± 4.11	0.270	-2.496–0.871
MCHC (g/dL)	35.2 ± 2.22	33.4 ± 2.29	.000 ^b	1.035–2.559
Reticulocyte count %	14.412 ± 4.27	16.27 ± 7.03	.185	-4.855–1.130
Platelets counts 10 ³ /uL	448.27 ± 144.79	471.36 ± 169.41	.346	-71.94–25.76
WBCs 10 ³ /uL	16.72 ± 16.3	13.54 ± 4.68	.195	-1.682–8.035
RBCs 10 ⁶ /uL	2.37 ± 0.41	2.42 ± 0.45	.170	-0.1091–0.197
LDH U/L	717.23 ± 269.95	643.14 ± 244.5	.028 ^a	8.22–139.94
Erythropoietin IU/L	8.74 ± 2.96	8.782 ± 3.92	.926	-944–8617

^aDifference is significant at the 0.05 level (2-tailed)

^bDifference is significant at the 0.01 level (2-tailed)

A pre-coded and pre-tested standardized questionnaire and check list were used to collect data about participants’ physical examination, weight, height, severity of the symptoms and any side effects. Clinical safety assessments and laboratory tests, complete blood count (CBC) every 2 weeks using automated cell counter (Sysmex) were regularly conducted. In addition serum chemistry including renal function tests (RFT) and Liver function test (LFT) were carried out every 4 weeks. Regular follow up was carried by the consultant physician in the unit.

Hemoglobin F was measured by modified fully automated capillary2 flexpiercing hemoglobin electrophoresis

technique (Sepia France) prior to starting GA and then every 4 weeks. Plasma was separated from EDTA sample and used for measurement of Erythropoietin (EPO) level by enzyme-linked immunosorbent assay (ELISA) using “Wkea, USA” EPO kit.

Data were analyzed using SPSS version 20. Paired samples *T* test was used to compare between pre and post intervention results. *P* values equal or less than 0.05 was considered significant.

Results

A total of 47 patients were enrolled (Table 1) between April, 2014 (first subject enrolled) to January, 2015 (last

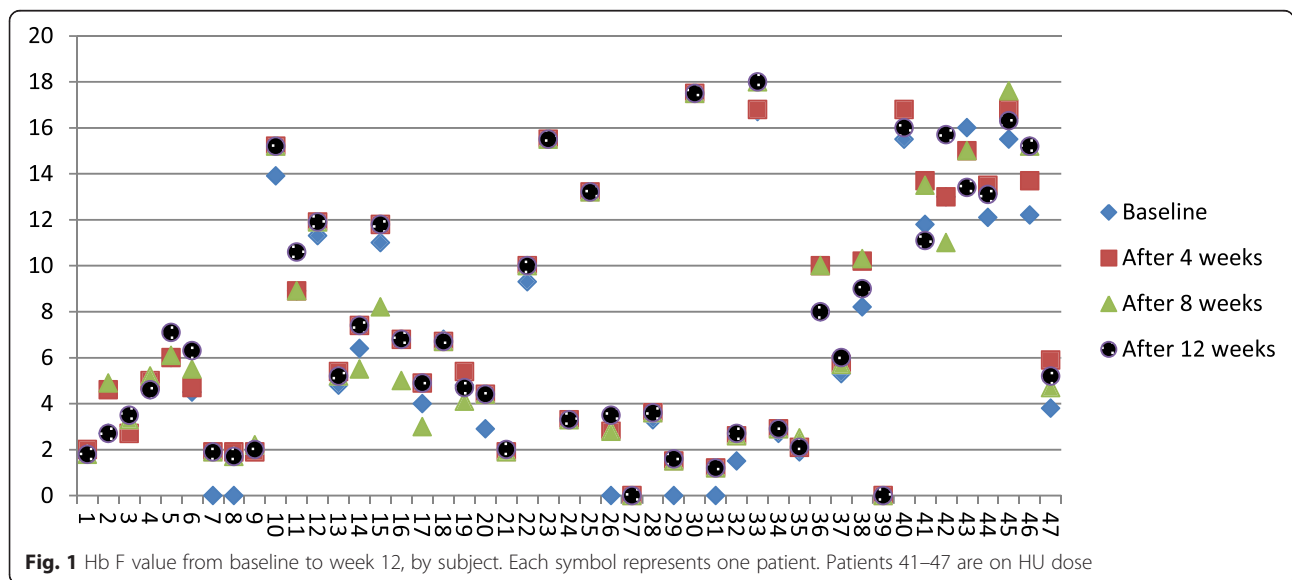


Table 3 Side effects of intervention among study group

Complain	Yes		NO		Total
	N	%	N	%	
Bloating	7	15	40	85	47
Diarrhea	9	19	38	81	47
Vomiting	7	15	40	85	47
Nausea	3	6	44	94	47

patient last visit) when adequate data were collected to allow for planning for further study. All were Sudanese; 23 were males (age 5 to 42 years). Seven patients were on a stable dose of hydroxyurea 500gram per day.

Duration of treatment was for 12 weeks except two patients received GA for 9 weeks and eight patients for 10 weeks. The last recorded results were considered for final analysis as post treatment results. Four patients were excluded because of blood transfusion during first 2 weeks of the study. One patient taking GA for 10 weeks was excluded because he developed severe malaria requiring blood transfusion.

Daily oral intake of GA significantly increased HbF level, MCV and hematocrit (Table 2). Peak HbF was recorded after 4 weeks and sustained till week 12 for most of the patients (Fig. 1 and Additional file 1). GA treatment was not followed by significant increase in hemoglobin concentration or MCH (Table 2). There was no significant change in WBC counts or Reticulocyte count (Table 2) and no significant increase in Erythropoietin level. A positive correlation was observed between absolute change in HbF (ΔF) level and Erythropoietin level (ΔEpo) (Pearson Correlation. 383, P. value 0.04 95 % CI 0.019 to 0.66). Thirteen patients (28 %) have been admitted once to hospital while two patients were admitted twice. All were admitted for 24 h because of painful crisis. One patient has chronic leg

ulcer healed after taking GA. There was a significant increase from base line weight by mean of 1.87Kg (P.V:0.0001 95 % CI 1.24 to 2.49)

GA tolerance and side effects

37 % of patients complained from side effects such as bloating, diarrhea, nausea, and vomiting (Table 3). All these symptoms resolved spontaneously within the first 5 days. The mother of a female 5 year old patient reported that GA treatment of the child was followed by appearance of loose stool; a condition did not require intervention.

Discussion

Sickle Cell Disease is the most common hemoglobin defect around the globe, with a high incidence in sub-Saharan Africa [9]. This necessitates the search for non-toxic oral and cheap therapeutic agents that increase fetal globin expression, and are tolerable for patients with minimal side effects.

According to the present study GA increases the level of HbF and significantly decreases level of HbS (Table 2). Since HbS polymerization depends on its intracellular concentration, a slight reduction is likely to have a beneficial effect on the kinetic of polymerization [25]. GA has no effect on HbA₂ (P.V: 0.9), and this is expected, in spite delta chain is located in chromosome 11 like beta and gamma chains [26]. Butyrate exposure results in true reversal of switch from beta to gamma globin expression [12]. Exposure to GA increases MCV (Table 2, Fig. 2) mimicking the effect of hydroxyurea therapy [4, 6, 27–31]. Increase in MCV is linked to the increase in intracellular HbF [32] and an increase in hemoglobin F is always associated with a concomitant increase in MCV [27, 32].

GA significantly decreased the MCHC value (Table 2 Fig. 3), an effect again mimicking effects of HU [27, 30]. Reduction in MCHC is beneficial in SCA patients since it

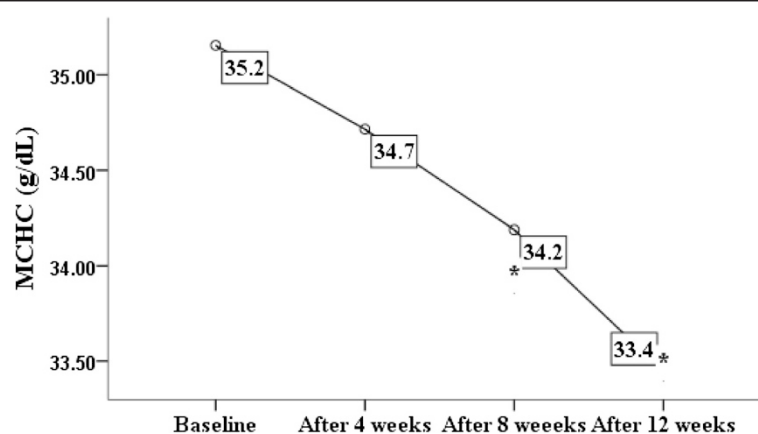


Fig. 2 Effect of GA intake on MCV ($P=0.000$). * indicates significant difference from baseline

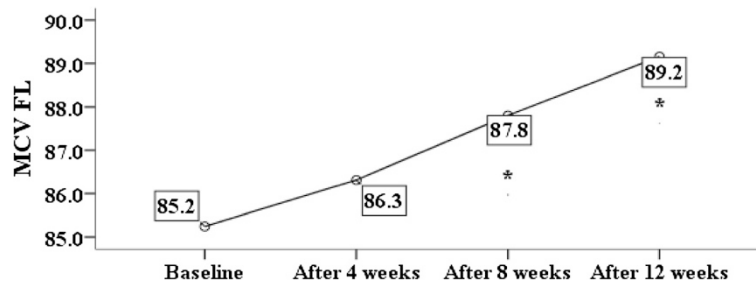


Fig. 3 Effect of GA intake on MCHC ($P=0.000$). * indicates significant difference from baseline

inhibits hemoglobin S polymerization [3, 26]. GA intake did not increase hemoglobin concentration (Table 2). Total hemoglobin is increased in clinical studies following administration of parental drugs such as: 5-azacytidine, decitabine, and butyrate given for longer periods, since they allowed HbF cells to accumulate and survive [33]. Sick cell patients have elevated lactate dehydrogenase enzyme (LDH) levels, a biological marker reflecting intravascular hemolysis [3, 34, 35]. Oral consumption of GA decreased LDH levels (Table 2). GA daily dose had no effect on Erythropoietin level and there was no significant increase in RBCs count. However, the changes in EPO and HbF levels were significantly correlated. Recombinant Erythropoietin alone [36] or in combination with HU [37] is an effective stimulator of fetal hemoglobin production.

This study revealed a novel effect of GA, as oral HbF inducing agent alone or in combination with HU. While HU has a variable therapeutic response [1], in this study 53 % of participants showed response. GA ingestion increase HbF% even in very low baseline HbF% patients. Seven patients with undetectable HbF as measured by electrophoresis, five patients showed elevation of HbF%, absolute change range: (1.2–3.5). The five respondents were less than 10 years old. The other non-respondents aged 36 and 22. This result showed GA intake may have better response among younger patients.

GA can be utilized as natural safe source of short chain fatty acids by sickle cell anemia patients. In spite of mild effect of GA as fetal inducing agent this effect could be substantial. Patients who had HbF values more than 2 % had a 10-year probability of survival of 89 %, compared with 53 % among patients with HbF lower than 2 % [4].

Conclusion

In conclusion GA found to increase the percentage of fetal hemoglobin and MCV and decrease MCHC. GA showed no effect on hemoglobin concentration and leucocytes counts.

One of the limitations in our study is not measuring serum butyrate concentration due to resource limitation.

Another major limitation in this study we didn't analyze the effect of GA on gene expression, again because of resource limitation.

This data sheds light on a new era of SCD management that worth further studies. Long duration clinical trials (more than 6 months) and multi arms will be beneficial to assess the sustainability of increase in HbF and its advantages on clinical events and disease severity.

Additional file

Additional file 1: Percentage of HbF of individual subjects. (PDF 36 kb)

Competing interests

No conflicts of interest, financial or otherwise, are declared by the authors.

Authors' contributions

LK, IF, FL and AS participated in study design. LK, IF and AS were involved in all aspects of the study conduct. LK, AS, IF, FL and MN participated in the writing and review of the manuscript. LK and MN analyzed data. LK and IF performed laboratory studies. OE and HE were significant clinical contributors to the study. All authors approved final version of manuscript.

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