World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

The effect of fixed oil extracts of *Nigella sativa* on sickle cells: An *in-vitro* study in Khartoum state -Sudan

Eman Ali Abuagla Dafaalla [1,*] and Amira Ahmed Khalid Humeida [2]

[1] *Faculty of Medical Laboratory Sciences, Al-Neelain University, Department of Hematology, Khartoum, Sudan.*
[2] *Faculty of Medicine, Al-Neelain University, Department of Pathology, Khartoum, Sudan.*

World Journal of Advanced Research and Reviews, 2021, 10(03), 317–321

Publication history: Received on 14 May 2021; revised on 20 June 2021; accepted on 24 June 2021

Article DOI: https://doi.org/10.30574/wjarr.2021.10.3.0280

Abstract

In Sudan, sickle cell anemia (SCA) is one of the most common inherited disorders of hemoglobin that has complications and becomes world problem. *Nigella sativa* NS (black seed) has been reported to have calcium antagonist and antioxidant activities, both of which play a role in the management of sickle cell anemia. The aim of the current study was to evaluate of the anti-sickling activity of the NS extracts, forty patients with sickle cell anemia were recruited for the study. A total of 3ml of venous blood was collected from each patient after obtaining the consent and the ethical approval, the blood was treated with *Nigella sativa* (NS) extract, and sickling test was performed. Descriptive study and p-values were used, and the correlation was evaluated, data was analyzed by SPSS. Recent study found that the sickling test after treating blood with *Nigella sativa* extract showed a negative result in 75% of patients and a 25% of patients showed persistently positive sickling test result. The anti-sickling effect in relation to various hemoglobin concentration, gender and age group showed p values of 0.007, 0.672 and 0.853 respectively indicating significant relationship with Hb concentration. Our study concluded that the fixed oil extract from *Nigella sativa* has an *in-vitro* anti-sickling activity on patients with SCA and this finding could indicate the use of this extract in treatment.

Keywords: *Nigella sativa*; Sickle cell anemia; *Nigella sativa* oil extracts; Anti sickling

1. Introduction

Sickle cell disease (SCD) is an inherited autosomal recessive disorder with presence of Hb S in blood. This disease affects millions of peoples globally which results in serious complications due to vasoocclusive phenomenon and hemolysis. Sickle hemoglobin (Hb S) is a structural variant of normal adult hemoglobin (Hb A) caused by a mutation in the HBB gene that leads to the substitution of valine for glutamic acid at position 6 of the β-globin's subunit (βS) of the hemoglobin molecule [1]. SCD It is the most prevalent human hereditary disorder with prominent morbidity and mortality [2]. It is due to the change of an amino acid in position six within the beta globin chain of hemoglobin molecule whereby glutamic acid, a polar amino acid is replaced by valine, a non-polar amino acid [3,4]. The amino acid change is due to the defective gene (mutation) in chromosome 11. At low oxygen tension, the mutant hemoglobin polymerizes inside the RBCs into a gel or further into fibers leading to a drastic decrease in the red cell deformability. Polymerization and precipitation of sickle hemoglobin (HbS) within the erythrocytes cause the change of shape from the normal spherical form into the one resembling a sickle [5]. The presence of sickle shaped RBCs in human blood was first reported by Herrick (1910) [6]. SCA affects millions of people throughout the world [7]. The clinical symptoms of patients suffering from the disease vary widely, some lead a normal life while others suffer from a variety of life-threatening complications. The main clinical symptoms are anemia, mild jaundice, repeated vaso-occlusive crises, hepatosplenomegaly, acute chest syndrome, bone and join pain and growth retardation [8, 2]. SCA widely has no cure, however, treatment can help to relieve symptoms and reduce the complications [9]. Gene therapy is being

* Corresponding author: Eman Ali Abuagla Dafaalla
Faculty of Medical Laboratory Sciences, Al-Neelain University, Department of Hematology, Khartoum, Sudan.

experimentally successful to some extent in transgenic animal models [10]. Among various medicinal plants, *Nigella sativa* (N.*Sativa*) (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential and antioxidant properties. *N. sativa* is commonly known as black seed [11]. Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil. *Nigella sativa* (NS) has been reported to have calcium antagonist and antioxidant activity [12]. The active constituent of *Nigella sativa* principally thymoquinone have potential therapeutic properties; they exhibited anti-inflammatory effect on several inflammatory disorders including encephalomyelitis, colitis, edema and arthritis through suppression of prostaglandin and leukotriene as inflammatory mediator [13]. The protective effect of *N. sativa* against lead acetate induced liver toxicity in male rats was demonstrated [14].

2. Material and methods

The current study is a comparative experimental study, conducted in general pediatrics outpatient clinic and the emergency department at Gafar Ibn Auf Pediatric Hospital**,** Khartoum, Sudan. From January 2019 to November 2020. Ethical approval was obtained from Institutional Review Board (IRB) of Alneelain University, Faculty of Medical Laboratory Sciences. Samples were taken with informed consent from patients and the hospital administration, data was collected using structural interviewing questionnaire, which was designed to collect and maintain all information concerning each case examined. The inclusion criteria included Sudanese males and females affected with sickle cell anemia (SCA) diagnosed by hemoglobin electrophoresis, with ages ranged between (3-15) years, recruited for the study during their consultation at Jafar Ibn Auf Pediatric Hospital. The exclusion criteria included patients with sickle cell anemia who were not diagnosis by hemoglobin electrophoresis and other sickle cell disease. The blood samples used in this study were collected by using dry, plastic syringes, tourniquet, in EDTA from each volunteer under aseptic condition; 3 ml of venous blood was collected from each patient recruited for the study. CBC (by sysmex xp300) and sickling test (before and after treated blood with NS extract) were done to of the forty patients under study; the blood was treated with *Nigella sativa* (NS) extract by mixing with 0.5 ml of the oil extract. Slides were prepared by spreading a drop of treated blood covered with cover glass to ensure complete de-oxygenation condition. The anti-sickling effect, Hb, age and gender were studied, and the p-values were determined. Moreover, Pearson correlation test was used to compare proportions between two groups; data was analyzed by SPSS.

3. Results

Our study of *Nigella sativa* extract on sickle cell anemia showed that gender was distributed as follows; Nearly fifty two percent {n = 21; (52.5 %)} were males and forty-eight percent {n=19;(47.5%)} were females. Nearly thirty-two percent {n=13; (32.5 %)} of patients were aged (less than 5 years), while about thirty eight percent {n=15;(37.5%)} were aged from (5-10 years) and thirty percent {n=12; (30%)} were aged more than 10 years. The SCA population under this study had different Hb concentrations and results found that forty five percent {n=18;(45%)} were at Hb concentration of (5-7 mg /dl), while fifty-five percent{n=22;(55%)} had Hb concentration of (8-11mg/dl) as shown in table 1.

Table 1 Gender, Age and Hb distribution among patients

Variable		**Frequency**	**Percent**
Gender	Males	21	52.5
	Females	19	47.5
Age Group	Less than 5 years	13	32.5
	5 – 10 years	15	37.5
	More than 10 years	12	30
Hb concentration	5- 7 mg/dl	18	45
	8-11mg/dl	22	55

Sickling test was positive in all the patients before adding *Nigella sativa* extract and after treating blood with NS extract, the sickling test showed negative result in 75% of patients and 25% of patients still showed positive results as shown in figure 1.

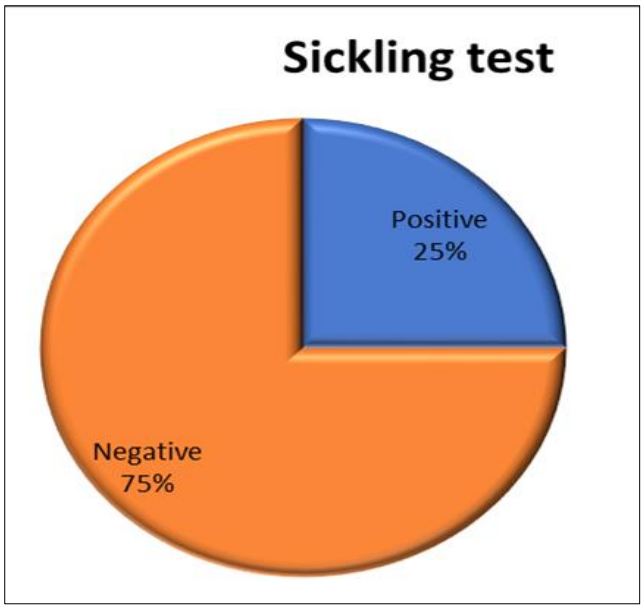

Figure 1 Sickling test after treatment with *Nigella sativa* extract

The anti-sickling effect was studied in correlation with different Hb concentrations, and found that at a Hb concentration (5-7 mg /dl), nearly seventeen percent {n=7 ;(17.5%)} of patients reported negative results while nearly seventeen percent {n=7 ;(17.5%)}of patients showed positive results, at a Hb concentration (8-11mg/dl), nearly fifty eight percent {n=23 ;(57.5%)} from patients gave negative result ,and nearly eight percent {n=3;(7.5%)} of patients gave positive result ,with p value 0.007 and the correlation factor (R) was 0.624 as shown in table2.

Table 2 Anti sickling effect with Hb concentration

Hb concentration	Positive	Negative	P value	R
5-7 mg /dl	7;17.5%	7;17.5%	0.007	0.624
8-11 mg/dl	3; 7.5%	23;57.5%		

The anti-sickling effect in relation to gender showed that out of 23 males, nearly forty two percent{n=17;(42.5%} reported negative results while fifteen {n=6;(15%)} reported positive results. In comparison to females, out of the 17 females, nearly thirty -three percent {n=13;(32.5%)} tested negative in contrast to ten percent {n=4;(10%)} females who tested positive, with P value 0.853 and the correlation factor (R) was 0.140 as shown in table 3.

Table 3 Anti sickling effect with gender

Gender	Positive	Negative	P value	R
Males	6; 15%	17; 42.5%	0.853	0.140
Females	4;10%	13; 32.5%		

The study of anti-sickling effect with age groups found that the age group of less than 5 years reported fifteen percent {n=6;(15%)} negative result and nearly seven percent {n=3;(7.5%)} positive results, while the age group (5-10 years) tested thirty-five percent {n=14;(35%)} negative results and thirteen {n=5;(13%)} positive results, moreover, those aged more than 10 years revealed twenty five percent {n=10;(25%)} negative results and five percent {n=2; (5%)} positive results, with p value 0.672 and correlation factor (R) 0.029 as shown in table 4.

Table 4 Anti sickling effect with age group

Age group	Positive	Negative	P value	R
Less than 5 years	3; 7.5%	6; 15%	0.672	0.029
5-10 years	5; 12.5%	14; 35%		
More than 10	2; 5%	10; 25%		

4. Discussion

Sickle cell disease (SCD) is a painful, lifelong hemoglobinopathy with substantial morbidities and premature mortality. It is inherited as appoint mutation in the hemoglobin (Hb) beta-globin gene where glutamic acid at position 6 is substituted by valine [15]. In Sudan there are many studies were conducted in sickle cell disease regarding diagnosis, causes, pathogenicity, immunological changes and treatment [16-18]. but our current study is first study conducted in Sudan to evaluate the anti-sickling activity of *Nigella sativa* extract on sickle cell anemia. The observed *Nigella sativa* extract had anti sickling activity and sickling test became negative in 75% of patients after adding NS oil extract. Our finding is supported by pervious study done in March 2010 by NK Ibrahim et al who showed that a total of 32 patients with sickle cell anemia were recruited in his study and their mean age was 25±11 years and mean hemoglobin level 9.2± 0.98 mg/dl. The anti-sickling effect of various concentration of *Nigella sativa* extract was investigated using the simple slide method under the deoxygenation condition, the anti-sickling effect began to appear when the concentration of *Nigella sativa* was increased. The use of 0.1%v/v concentration of oil extract of NS resulted in approximately 80% reduction in the formation of sickle cell [19].

The recent study result also is in agreement with a study done by Chloe Jagpal, the results of a one-way ANOVA and Tukey post-hoc statistical analysis showed that there was a statistically significant decrease in % cell sickling of HbS samples to all other treatments ($p<0.01$), and found that black seed oil extract is able to increase antioxidant concentration of HbS sample, this should result in a stronger defense against excessive reactive oxygen species generation and consequently, this could reduce oxidative stress and the HbS cell sickling it exacerbates [20].

The current study found that, anti-sickling effect at Hb concentration 8-11mg/dl is more than in Hb concentration 5-7 mg/dl with p value which is statistically significant, and the correlation factor showed a moderate positive correlation. Anti-sickling effect with gender has no difference in both gender with P value which is statistically insignificant, and the correlation factor showed a negligible correlation. Anti-sickling effect with different age groups showed that there is no difference between age groups with P value which is statistically insignificant, and the correlation factor showed a negligible correlation. Extensive researches using modern scientific technique were carried out by various researchers on *Nigella sativa* since it is believed to be miraculous herpes that can cure multiple ailments and disorder [21]. A study done in 2018 by Saeed Samarghandian, et al studied the effect of *Nigella sativa* and thymoquanine TQ in neurological disease and found that *Nigella sativa* and TQ has protective effect against neurodegenerative disease [22]. Another study done 1n 2009 by Shailendra Kapoor, et al showed that *Nigella sativa* decrease DNA damage and thereby prevent initiation of carcinogenesis in colonic tissue [23].

5. Conclusion

Our study concluded that the fixed oil extract from *Nigella sativa* has an *in-vitro* anti-sickling activity on sickle cells and more studies are required to evaluate the biological effects of *Nigella sativa* in-vivo to initiate the use of the extract to treat SCA.

Compliance with ethical standards

Acknowledgments

The authors wish to acknowledge the efforts and assistance given by staff of Gafar Ibn Auf Pediatric Hospital, Khartoum, for their help and support.

Disclosure of conflict of interest

There is no conflict of interests between authors.

References

[1] Ahmed, Tarig Osman Khalafallah, et al. "Association of XmnI Polymorphism with Fetal Hemoglobin Level in Sudanese Patients with Sickle Cell Disease." International Journal of Contemporary Medicine 9.1 (2021): 31-34

[2] Serjent GR. Serjeant BE. Homozygous sickle cell disease In: Sickle cell disease. 3 rd ed. New York: Oxford University Press. 2001; 429-35.

[3] Pauling L, Itano HA. Sickle cell anemia a molecular disease. Science. 1949; 110: 543-8.

[4] Ingram VM. Gene mutations in human hemoglobin: The chemical difference between normal and sickle cell hemoglobin. Nature. 1957; 180: 326-8.

[5] Eaton WA, Hofrichter J. Hemoglobin S gelation and sickle cell diseaseBlood.1987; 70: 1245-66.

[6] Paul S.Frenette and George F.A.tweh, sickle cell disease :old discovered ,new concept ,and future promise ,J Clin invest. 2 Apr 2007; 117(4): 850-858.

[7] WHO. Sickle cell anaemia. Report by the Secretariate, 117 th session of Executive Board (EB117/34). World Health Organisation Geneva. 2005; 1.

[8] Kar BC. Sickle cell disease in India. J Assoc Physicians India. 1991; 39: 954-60.

[9] Pawliuk R, Westerman KA, Fabry ME, Payen E, Tighe R, Bouhassira EE, et al. Correction of sickle cell disease in transgenic mouse models by gene therapy. Science. 2001; 294: 2368-71.

[10] Wu LC, Sun CW, Ryan TM, Pawlik KM, Ren J, Townes TM. Correction of sickle cell disease by homologous recombination in embryonic.

[11] Khare CP. Encyclopedia of Indian medicinal plants. NewYork: Springes-Verlag Berlin Heidelberg. 2004.

[12] Al-Ali A, Alkhawajah AA, Randhawa MA, Shaikh NA. Oral and intraperitoneal LD50 of thymoquinone, an active principle of Nigella nativa, in mice and rats. J Ayub Med Coll Abbottabad. 2008; 20(2): 25–27.

[13] Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil , as a potent analgesic and anti-inflammatory drug .Phytotherapy Research. 2004; 18(3): 195-199.

[14] Farrag ARH, Mahdy KA, Abdel Raman GH, Osfor MM. Protective effect of *Nigella sativa* seeds against lead-induced hepatorenal damage in male rats . Pakistan Journal of Biological Sciences. 2007; 10(17): 2809-2816.

[15] Mohamed, Elmigdad Abdelgadir, et al. Comparative study of hypercoagulability change in steady state and during vaso-occlusive crisis among Sudanese patients living with sickle cell disease. African Health Sciences. 2020; 20(1): 392-396.

[16] Eldour, Ahmed Abdalla Agab, et al. Red cell alloimmunization in blood transfusion dependent Patients with Sickle Cell Disease in El-Obied city, Sudan. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2015; 14(12): 137.

[17] Bayoumi RA, Abu Zeid YA, Abdul Sadig A, Awad Elkarim O. Sickle cell disease in Sudan. Trans R Soc Trop Med Hyg. 1988; 82(1): 164-8.

[18] Eldour, Ahmed Abdalla Agab, et al. Original Research Article Frequency of Rh D, C, c, E, e and Kell1 Antigens among Sudanese Patients with Sickle Cell Disease: A prospective Study from Khartoum, Sudan. Sch. J. App. Med. Sci. 2016; 4(1A): 1-5.

[19] Ibrahim NK, Ahmed JH, Hassan MK. The effect of fixed oil and water extracts of *Nigella sativa* on sickle cells: an *in vitro* study, Singapore Med J. 2010; 51(3): 230.

[20] Chloe Jagpal, An investigation into the antioxidant effects of plant-based oils, as a model for novel sickle cell anaemia treatments, applied biological and exercise science .September. 2019; 753.

[21] Aftab Ahmad, Asif Husain, Mohd mujeeb, Shah Alam, Abul Kalam Najmi , Nasr Ali Siddique, Zohir A. Damanhouri, and Firoz Anwar. A review on therapeutic potential of *Nigella sativa*: A miracle herb, Asian Pac J Trop Biomed .2013 May; 3(5): 337-352.

[22] Saeed Samarghandian, Tahereh Farkhondeh, Fariborz Samini, A review on possible therapeutic effect of *Nigella sativa* and thymoquinone in neurodegenerative diseases. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders). 2018; 17(6): 412-420.

[23] Shailendra Kapoor. Kristin 24,Schaumburg, Emerging clinical and therapeutic applications of *Nigella sativa* in gastroenterology. World J Gastroenterol. 7 May 2007; 15(17): 2170-2171.