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In vitro Antisickling Activity of *Zingiber officinale* Roscoe (Ginger) Methanolic Extract on Sickle Cell Disease

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Authors' contributions

This work was carried out in collaboration between both authors. Author NGA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors NGA and IAA managed the literature searches, analyses of the study, performed the spectroscopy analysis, managed the experimental process and identified the species of plant. Both authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Sickle cell disease (SCD) is caused by polymerization of abnormal haemoglobin S when oxygen tension decreases. This lead to changes in the shape of red blood cells and anemia. Previous studies have been indicated that some medicinal plants have shown an antisickling activity, which indicates a new therapeutic way to a range of people who are affected by this hemoglobinopathy. The current study aimed to assess the *in vitro* antisickling activity of ginger. Emmel test was used to assess antisickling activity of ginger. The natural shape of the red blood cells was observed after incubation of red blood cells with ginger extract and 2% sodium metabisulfite as compared to control. A significant increase in the percentage of unsickled red blood cells was observed after incubation of red blood cells with 2% sodium metabisulfite in the presence of 500 µg/ml and 1000 µg/ml of ginger extract. Moreover, the difference between the percentage of unsickled red

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blood cells after 30 min and 60 min incubation time was not significant. In conclusion significant *in vitro* antisickling activity of ginger extract was demonstrated in red blood cells pretreated with 2% sodium metabisulfite.

Keywords: In vitro; antisickling activity; Zingiber officinale Roscoe; SCD.

1. INTRODUCTION

Sickle-cell disease (SCD) is an inherited genetic disorder that affects the haemoglobin within the red blood cells. The recurrent pain and complications caused by the disease can interfere with many aspects of the patient's life, including education, employment and psychosocial development. The sickle-cell trait is now known to be widespread, reaching its highest prevalence in parts of Africa as well as among people in the Mediterranean basin and Saudi Arabia [1].

Zingiber officinale Roscoe is the botanical name for ginger, a tropical herbal plant found in abundance in Asia. It belongs to the family of Zingiberaceae. Generally it is widely used as a spice in traditional and modern cookings [2]. Ginger extract has been extensively studied for its pharmacological and biological activities such as antioxidant and anti-inflammatory properties, anti-fungal, Anti-thrombotic, anti-diabetes, peripheral circulatory stimulant, promotive secretion of saliva and gastric juices, increase the tone of and peristalsis in intestines, and anti-allergen [3-12].

In this study we try to find out the antisickling effect of methanolic extract of the *Zingiber officinale* Roscoe. (F. Zingiberaceae) (rhizomes) for reducing complicated management and cost effective treatment of sickle cell patient.

2. MATERIALS AND METHODS

2.1 Preparation of Methanolic Extract of Ginger

Ginger (dried rhizomes), was purchased from a local herbal store in Majma'ah, Saudi Arabia in June 2014. The dried rhizome was ground in a blender with a particular size to ensure the powder in identical size, and then 100 g of the powder was soaked for 5-7 days with 1000 ml of 80% methanol at 25℃. After filtration, the filtrate was evaporated with a rotary evaporator to remove the methanol under reduced pressure at 50℃. The dry crude extract of ginger was stored in refrigerator in dark glass bottle until use. A stock solution 0.1 g/ml from the crude extract was prepared by dissolving 0.1 g of dry crude extract in 1 ml (DMSO) and then diluted in 9 ml normal saline, this stock solution was stored in refrigerator until use.

2.2 Collection of Blood Samples

The blood samples used in the evaluation of the anti-sickling activity of the plant extract in this study were taken from patients known to have sickle cell disease, attending in the King Khaled Hospital in Majmaah. All these patients were confirmed regarding their SS status using haemoglobin electrophoresis test. The blood samples were collected in sodium EDTA tubes and stored for the experiment. A written informed consent was read and signed by all the patients participating in the study. All research procedures have been approved by the National Ethical Committee, King Abdulaziz for Science & Technology, Kingdom of Saudi Arabia, approval number: MUREC-Jan.06/COM-2015.

2.3 Antisickling Activity

2.3.1 Washing of RBCs

Four milliliters EDTA blood samples obtained from patients were centrifuged at 3,000 rpm for 10 min to remove the plasma. The resulting packed erythrocytes were washed 3 times with 1 ml sterile normal saline per 5 ml of blood. The samples were then centrifuged each time to remove the supernatant. Washed RBC were then re-suspended in remaining suspension and used for the analysis.

2.3.2 Procedure for antisicking activity evaluation

In order to evaluate the antisickling activity of our plant extract, in vitro antisickling assay was performed: Emmel test (Coutejoie and Hartaing, 1992) as the following:

Plant extract a stock solution (10mg/ml) was prepared by dissolving 0.1 g of dry extract in 1 ml of 100% dimethylsulfoxide (DMSO) that was prior diluted to 10 ml with normal saline. Three

different concentrations were prepared as follows (250 µg/ml, 500 µg/ml and 1000 µg/ml).

Washed erythrocyte was mixed with an equivalent volume of 2% sodium metabisulfite ($Na_2O_5S_2$). 10 µl from the above mixture was spotted on a microscope slide then 10 µl from the plant extract was added and mixed with the blood mixture. 10 µl normal saline was added to one of the slides instead of the plant extract which served as control, all the slides were covered with a cover slip. Paraffin was applied to seal the edges of the cover completely to exclude air (Hypoxia) and then slides were incubatedat 37℃ for 2 period interval (30 min and 60 min). Each slide was examined under the oil immersion light microscope and red blood cells were counted in five different fields of view across the slide. The numbers of both sickled and unsickled blood cells were determined and the percentage of unsickled cells was calculated using the formula: {(%) unsickling = Number of unsickling cells × 100/total cells}. All anti-sickling experiments were carried out in triplicate using a fresh blood samples. A high power magnification X1000 was employed to take representative images from different fields to display morphological changes of RBCs during different stages of the experiment using a digital camera.

2.4 Statistical Analysis

All data were reported as the mean ± SD., statistical analysis was performed using SPSS statistics 17. The results were compared by paired t-test with P value ≤ 0.05 considered significant.

3. RESULTS AND DISCUSSION

3.1 Extractive Yield

The extractive yield of studied plant was 9.5%.

3.2 Antisickling Activity of Methanolic Extract of *Zingiber officinale* Roscoe

3.2.1 Effect of plant crude extract on sickle cell morphology

Fig. 1 shows Morphology of red blood cells after incubation of red blood cells with 2% sodium metabisulfite in the presence of 0.9% NaCl (control) while Fig. 2, 3, and 4 show morphology of red blood cells after incubation of red blood cells with 2% sodium metabisulfite in the presence of 250 µg/ml, 500 µg/ml and 1000 µg/ml of crude extract of *Zingiber officinale* Roscoe.

As shown in Fig. 1 almost all red blood cells were sickle-shape which confirmed the nature of sickle red cells which have property to change their normal shape (biconcave shape) to sickling shape under hypoxic condition.

Fig. 2 shows that few RBCs retained their biconcave normal shape while the rest transfigured to sickle-shape.

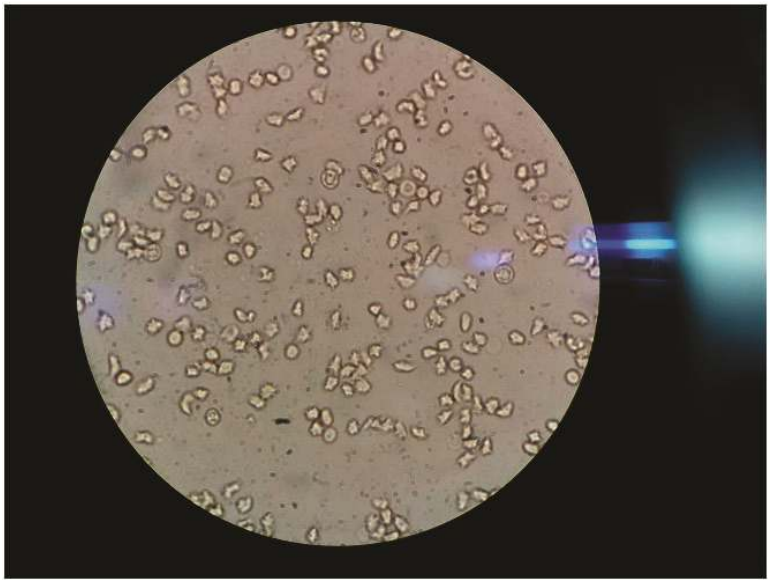

Fig. 1. Morphology of sickle RBCs: Untreated or control, [NaCl 0.9%; Na2s205 2%]

Fig. 3 and 4 show that almost all RBCs retained their biconcavity revealing the antisickling activity of methanolic extract of *Zingiber officinale* Roscoe. This finding points towards anti-sickling activity of the crude methanolic extract of this plant under hypoxic condition, a finding agrees with results of previous similar studies [13-16].

3.2.2 Effect of methanolic extract of *Zingiber officinale* Roscoe on the percentage of unsickled red blood cells

Table 1 shows the percentage of unsickled red blood cells after incubation red blood cells of sickle cells disease patients with 2% sodium metabisulfite in the presence of 250 µg/ml, 500 µg/ml and 1000 µg/ml of methanolic extract of *Zingiber officinale* Roscoe at two different incubation time (30 min and 60 min). As shown in Table 1, compared to control the highest percentage of unsickled red blood cells at 30 min incubation time was observed for 1000µg/ml (99.2%) followed by 500 µg/ml (96.7%) and 250 µg/ml being the lowest (25.3%) and the highest percentage of unsickled red blood cells at 60 min incubation time was observed for 1000 µg/ml (98.0%) followed by 500 µg/ml (96.8%) and 250 µg/ml being the lowest (20.7%). A significant increase in the percentage of un-sickled RBCs was observed at concentrations of 500 µg/ml and 1000 µg/ml of methanolic extract in both incubation time (30 min and 60 min).

These findings coincide with previous studies in which anti-sickling effect of anthocyanins extracts was tested, [13,15,16].

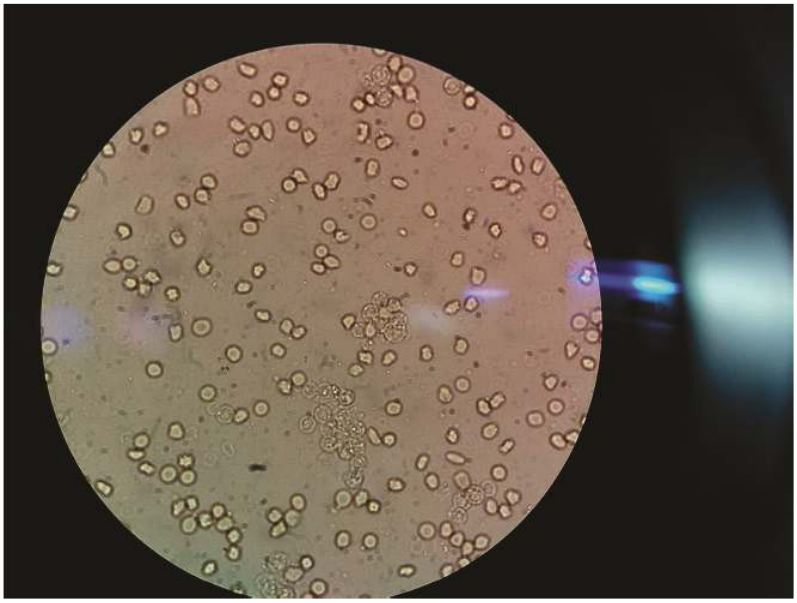

Fig. 2. Morphology of sickle RBCs: Treated with 250 µg/ml of methanolic extract of *Zingiber officinale* Roscoe, [NaCl 0.9%; Na2s205 2%]

Table 1. Antisickling activities of methanolic extract of *Zingiber officinale* Roscoe and normal saline as control. Each value represents the mean value ± S.D., (n =3), P value ≤ 0.05 considered significant, compared to control

Time of incubation (min)	% of unsickled red blood cells			
	Red blood cells+$Na_2O_5S_2$ +250 µg/ml	Red blood cells +$Na_2O_5S_2$ +500µg/ml	Red blood cells+$Na_2O_5S_2$ +1000 µg/ml	Red blood cell+ $Na_2O_5S_2$s + normal saline (Control)
30 min	25.3±8.2	96.7±2.9	99.2±0.68	14.8± 5.9
P value	0.1472	0.0001	0.0001	
60	20.7±12.0	96.8±1.3	98.0±1.0	19.4±12.0
P value	0.9064	0.0004	0.0004	

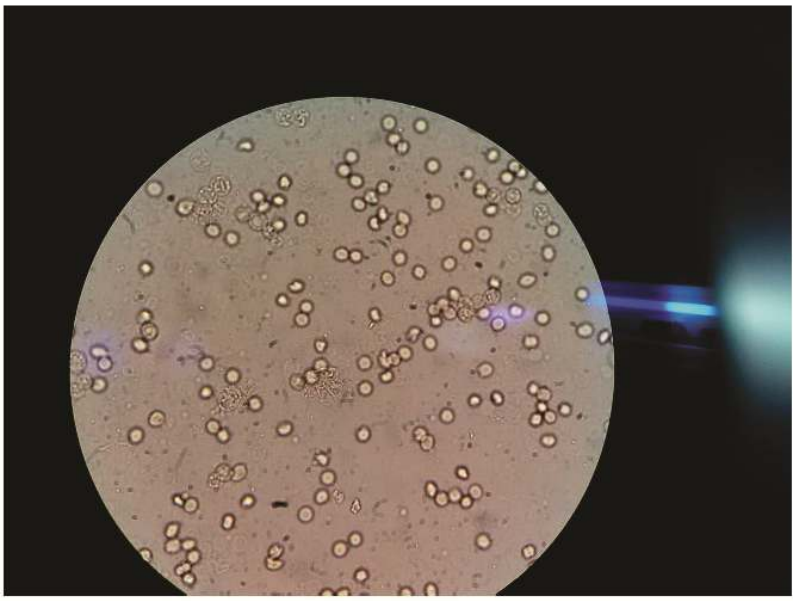

Fig. 3. Morphology of sickle RBCs: treated with 500 µg/ml of methanolic extract of *Zingiber officinale* Roscoe, [NaCl 0.9%; Na2s205 2%]

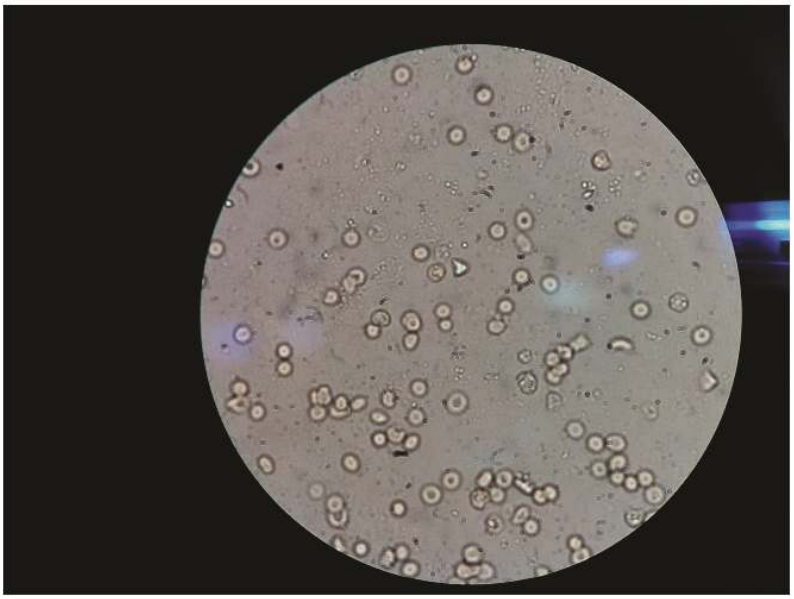

Fig. 4. Morphology of sickle RBCs: treated with 1000 µg /ml of methanolic extract of *Zingiber officinale* Roscoe, [NaCl 0.9%; Na2s205 2%]

3.2.3 Effect of incubation time on the percentage of unsickled red blood cells

As shown in Table 1, after 30 min incubation time of red blood cells of sickle cells disease patients with 2% sodium metabisulfite in the presence of 250 µg/ml, 500 µg/ml and 1000 µg/ml of methanolic extract of *Zingiber officinale* Roscoe the percentages of unsickled red blood cells were 25.3, 96.7 and 99.2, respectively while after 60 min incubation time with same concentration the percentages of unsickled red blood cells were 20.7, 96.8 and 98.0 respectively, no significant difference in the percentage of unsickled red blood cells after 30 min or 60 min incubation time was achieved.

4. CONCLUSION

The results obtained in this paper have shown significant *in vitro* anti-sickling activity of ginger extract. Further studies and data recruitment are required to confirm or deny these findings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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