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IN VTTRO ANTI SICKLING EFFECT OF *ARTEMISIA HERBA ALBA ASSO* EXTRACT ON SICKLE CELLS

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Abstract

Background: Sickle cell disease (SCD) is one of the major haemoglobinopathies, in which the abnormal haemoglobin altered the cell to sickle shape under low antioxidant status. The patients have increase in the free radicals which damage the cells. It has shown that some medicinal plants which contain antioxidant molecules as phenolics and flavonoids compounds have an anti-sickling activity, which indicates a new therapeutic way to combat and manage this disease. **Objective:** The current study aimed to determine the in vitro anti-sickling effect of *Artemisia herba-alba asso* (Chih) extracts. **Materials and methods:** Ten blood samples were taken from patients known to have Sickle cell disease (HB -SS) attending the Sickle Cell Clinic in Khartoum state used in the evaluation of the anti-sickling effect of the plant extracts in this study (each sample was tested 6 times with different concentrations (250, 500, and 1000 µg/ml) of aqueous and methanolic extracts of the plant and one time with normal saline as a control)). All samples were collected in EDTA container. Emmel test was used to assess anti-sickling activity of this plant. Then data were analyzed by SPSS. **Results:** A high significant increase in the percentage of unsickled Red blood cells with p-value (0.000) was observed in the presence of 1000, 500 and 250µg/ml of *Artemisia herba-alba asso* of both aqueous and methanolic extract. **Conclusion:** The result obtained in this study suggests the using of *Artemisia herba Alba asso* in the management of sickle cell disease complication as well as it's a natural cost effective way for combating the disease. Further in vivo and in vitro studies are recommended to confirm this result.

KEYWORDS: *In vitro, Anti-sickling effect, Artemisia herba Alba asso, Unsickled red blood cells, Sickle cell disease.*

INTRODUCTION

Sickle cell disease or sickle cell anemia is an autosomal recessive disease caused by a single point mutation in the codon of the sixth nucleotide of the beta globin chain.[1] This mutation substitute the glutamic acid amino acid by Valine[2] which alters the hemoglobin affinity toward the oxygen and its solubility in the deoxygenated conditions.[3] The insoluble hemoglobin polymerize and alter the red blood cells to sickle shape,[4] which cause the complication of sickle cell anemia,[4-6] due to the increase in the mortality rate and the dangerous effect of chemotherapy, the development of drugs from natural product is necessary because it is safe ,cheap and available. Plants and fruits contain antioxidants shown beneficial effects when administered by sickle cell anemia patients[7] Antioxidants are the major components of thesis antisickling agents which give the plant this potential property. Thus, the high concentration of the antioxidant is proportional to high antisickling effect.[8]

The genus of Artemisia is a small shrub with hairy leaves belongs to the Asteraceae family. Commonly grow in northern temperature regions, it contain more than 500 species, which produce essential oils used in the traditional and modern medicine.[9]

Artemisia herba-alba Asso (Arabic name chih), commonly known as desert wormwood or white wormwood. This species have a biological and pharmacological activities mentioned by various Researchers, as antifungal activities, antidiabetic effect, antimicrobial activity and antioxidant effect. The sesquiterpene lactones, phenolics compounds, flavonoids and essential oils are the main constituents of this species.[10-11]

MATERIALS AND METHODS

Study design and population

This was experimental study done in Khartoum state from September 2019 to December 2019.Ten blood samples were taken from homozygous haemoglobin S sickle cell disease patients.

Inclusion and exclusion criteria

Samples were collected from sickle cell disease patients, both males and females included in different age. None of the Patients had been diagnosed with other disorder or hereditary hemoglobinopathy.

Plant material

Artemisia Herba Alba Asso raw material (areal parts) were obtained from a local Sudanese shop in Omdurman and then finely milled by pestle into a powder form.

Preparation of the aqueous extract of artemisia herba alba asso

The extract was prepared according to the method described by Sukhdev[12] 20 g of the dried plant sample was soaked in 100 ml hot distilled water for about four hours with continuous steering. After cooled, extract was filtered using filter paper and stored till used. Concentration was calculated by dried 2 ml of the extract in a Petri dish using water as followed: (Weight of dish with extract – empty weight) X 100 / 2.[12]

Preparation of the methanolic extract of artemisia herba alba Asso

100 g of the plant sample was coarsely powdered using mortar and pestle. Coarsely a sample was soaking with methanol 80%. Extraction carried out for five days with daily filtration and evaporation the solvent under reduced pressure using rotary evaporator apparatus. Sample extract was allowed to air in evaporating dish till complete dryness and the yield Percentages (11.32%) were calculated as follows: Weight of extract obtained / weight of plant sample X 100.[12]

METHODOLOGY

Sample collection and preparation

About 4 ml of blood samples were collected in ethylene diamine tetra acetic acid (EDTA) container from each patient, and then centrifuged at 3000 rpm for 10 minutes to remove the plasma. The resulting packed red cells were washed 3 times with normal saline. (5%) suspension from the washed erythrocytes was prepared and used in the test.

Procedure for anti-sickling activity evaluation

Three concentrations were prepared from the stock solution of plant extract (aqueous and methanolic extracts) using normal saline as solvent as follows (250, 500, and 1000 µg/ml). Emmel test: An equal volume from the red blood cell suspension was mixed with each concentration of the plant extract and incubated for 30 minutes, Then 10 µl from each above mixture spotted on the slide and mixed with 10 µl from 2% sodium metabisulfite ($Na_2O_5S_2$), on other slide 10 µl from normal saline was spotted instead of the plant extract to act as a control and mixed with 10 µl from 2% sodium metabisulfite. All the slides had been covered with a cover glass and sealed with paraffin wax to exclude the air then, incubated for 60 minutes at 37°C. All the slides had been tested under oil immersion light microscope, the number of both sickled and unsickled cells had been counted in five different fields, then the percentage of unsickle cells had been determined according to the following formula:

Percentage of unsickling (%) = Number of unsickling cells × 100/total of the cells

Data analysis

The data were analyzed by SPSS using independent T test to compare between mean value of different concentration of extracts and the mean value of the control also to calculated p-value. Results are expressed as mean ± S.D. The P-values less than 0.05 considered statistically significant.

Ethical considerations

Approval of this study was obtained from the Ethical committee of Al-Zaiem Al-azhary University. Research purpose and objective of this study was explained and discussed with the patients and their relatives. Informed consent from patients and hospital were taken to collect the samples.

RESULTS

The result revealed that there was a high significant increase in the mean of percentage of unsickled red blood cells in the three different concentrations (1000, 500 and 250 µg/ml) of the aqueous extract, the mean percentage were (97.1 ± 2.1, 96.3 ± 2.1 and 96.7 ± 2.3) respectively, when compared to the mean of control (63.9 ± 32.3) the P value were (0.000) as shown in table (1). Also in the methanolic extract there was a high significant increase in the mean of percentage of unsickled red blood cells in the three different concentrations (1000, 500 and 250 µg/ml), the mean percentage were (96.4 ± 2.3, 96.5 ± 2.2and 96.8 ±2.3) respectively, when compared to the mean of control (63.9 ± 32.3) the P value were (0.000) as shown in table (2).

In addition, there was no significant difference between the three different concentrations in the aqueous extract when compared with each other (1000, 500 and 250 µg/ml) the P-value were (0.41, 0.67 and 0.69) respectively, table (3). Analysis also showed there was no significant difference between the three different concentrations in the methanolic extract when compared with each other (1000, 500 and 250 µg/ml) with P-value (0.93, 0.79 and 0.72) respectively, table (4). Also, there was no significance difference between the methanolic and aqueous extracts of Artemisia herba Alba for all different concentrations with P -value > 0.05, table (5).

Table (1): Multiple Comparisons of mean of % of un sickle cells in aqueous extraction of artemisia herba alba asso and control.

I	II	Mean (I)	Mean (II)	P. value
Control	A*1000 µg/ml	63.9 ± 32.3	97.1 ± 2.1	0.000
	A500 µg/ml		96.3 ± 2.1	0.000
	A250 µg/ml		96.7 ± 2.3	0.000

***Aqueous extraction**
Mean (I): mean of control
Mean (II): mean of different concentrations of the extract
Independent T test was used to calculate P-value
P-value <0.05: considered significant

Table (2): Multiple Comparisons of mean of % of un sickle cells in methanolic extraction of artemisia herba alba asso and control.

I	II	Mean (I)	Mean (II)	P. value
Control	M*1000 µg/ml	63.9 ± 32.3	96.4 ± 2.3	0.000
	M500 µg/ml		96.5 ± 2.2	0.000
	M250 µg/ml		96.8 ±2.3	0.000

***Methanolic extraction**
Mean (I): mean of control
Mean (II): mean of different concentrations of the extract
Independent T test was used to calculate P-value
P-value <0.05: considered significant

Table (3): Multiple Comparisons of mean of % of un sickle cells in aqueous extractions of artemisia herba alba asso.

I	II	Mean (I)	Mean (II)	P. value
A*1000 µg/ml	A500 µg/ml	97.1 ± 2.1	96.3 ± 2.1	0.416
	A250 µg/ml		96.7 ± 2.3	0.697
A500 µg/ml	A250 µg/ml	96.3 ± 2.1	96.7 ± 2.3	0.670

***Aqueous extraction**
Independent T test was used to calculate P-value
P-value <0.05: considered significant

Table (4): Multiple comparisons of mean of % of un sickle cells in methanolic extraction of artemisia herba alba asso.

I	II	Mean (I)	Mean (II)	P. value
M*1000 µg/ml	M500 µg/ml	96.4 ± 2.3	96.5 ± 2.2	0.930
	M250 µg/ml		96.8 ±2.3	0.725
M500 µg/ml	M250 µg/ml	96.5 ± 2.2	96.8 ±2.3	0.792

***Methanolic extraction**
Independent T test was used to calculate P-value
P-value <0.05: considered significant

Table (5): Multiple comparisons of mean of % of un sickle cells in aqueous and methanolic extraction of artemisia herba alba asso.

I	II	Mean (I)	Mean (II)	P. value
A*1000 µg/ml	M**1000 µg/ml	97.1 ± 2.1	96.4 ± 2.3	0.530
	M500 µg/ml		96.5 ± 2.2	0.590
	M250 µg/ml		96.8 ±2.3	0.787
A500 µg/ml	M1000 µg/ml	96.3 ± 2.1	96.4 ± 2.3	0.861
	M500 µg/ml		96.5 ± 2.2	0.791
	M250 µg/ml		96.8 ±2.3	0.593
A250 µg/ml	M1000 µg/ml	96.7 ± 2.3	96.4 ± 2.3	0.806
	M500 µg/ml		96.5 ± 2.2	0.877
	M250 µg/ml		96.8 ±2.3	0.909

***Aqueous extraction**
****Methanolic extraction**
Independent T test was used to calculate P-value
P-value <0.05: considered significant

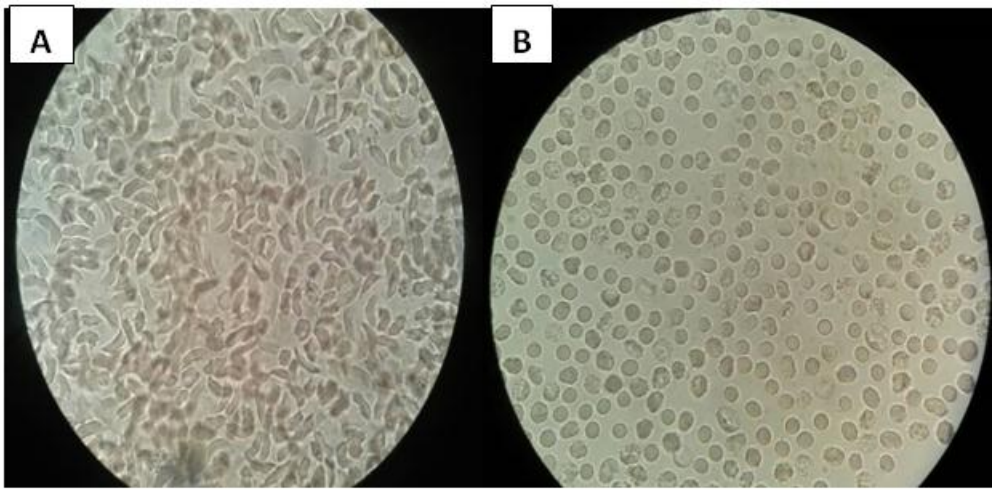

Figure [1]: sickling test; [A] No of un-sickle cells versus sickle cells after addition of control (normal saline)], [B] No of un-sickle cells versus sickle cells after addition of 1000 µg/ml A herba-alba aqueous extract.

DISCUSSION

The antioxidant status in sickle cell anaemia was reported reduced due to increase the release of oxygen free radicals which produced by metabolic processes by the body. It has been shown that antioxidants administration in food and plants would be highly beneficial to the care of Sickle cell disease subjects.[7] Artemisia had been reported to contain a rich store of compounds like flavonoids, phenols and antioxidant nutrients which are thought to be responsible for their observed anti-sickling action.[11] This study demonstrates the anti-sickling activity of aqueous and methanolic extracts of Artemisia herba Alba asso; the effect of plant crude extracts in different concentrations (1000, 500 and 250 µg/ml) of sickle cell after incubation for one hour. The results were highly significant with P-values < 0.05 this result was agreed with study done by Nessrin Ghazi Alabdallat in 2016 which demonstrated the antisickling activity of A. herba alba Asso with significant increase in the percentage of unsickled RBCs in the presence of 1000 and 500 µg/ml, the P-value were 0.0001and 0.0003 respectively, and insignificant increase in the presence of 250 µg/ml, the P-value were 0.901 this was disagreed with the present study which may be due to the sample size difference or ethnic variation.[10]

Also this result concordance with study done by Arthanari Saravanakumar in 2019 demonstrated that Artemisia extractions had ability to inhibit free radicals attributed to their high antioxidant activity. This antioxidant activity had a direct proportion to the antisickling activity.[13]

CONCLUSION

The result obtained in this study suggests the using of Artemisia herba Alba asso in the management of sickle cell disease complication as well as it's a natural cost effective way for combating the disease. Further in vivo and in vitro studies are recommended to confirm this result.

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